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Combinatorial drug therapy for cancer in the post-genomic era

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Over the past decade, whole genome sequencing and other 'omics' technologies have defined pathogenic driver mutations to which tumor cells are addicted. Such addictions, synthetic lethalities and other tumor vulnerabilities have yielded novel targets for a new generation of cancer drugs to treat discrete, genetically defined patient subgroups. This personalized cancer medicine strategy could eventually replace the conventional one-size-fits-all cytotoxic chemotherapy approach. However, the extraordinary intratumor genetic heterogeneity in cancers revealed by deep sequencing explains why *de novo* and acquired resistance arise with molecularly targeted drugs and cytotoxic chemotherapy, limiting their utility. One solution to the enduring challenge of polygenic cancer drug resistance is rational combinatorial targeted therapy.

Most malignant diseases, collectively referred to as cancer, are treated with some combination of surgery, radiation therapy and/or drug treatment[1]. Surgery and radiation are used to treat cancer that is confined locally, whereas drug therapy is essential to kill cancer cells that have spread (metastasized) to distant sites in the body. Until recently, drug treatment mainly involved cytotoxic chemotherapy that kills all rapidly dividing cells, both tumor and normal. Accumulated empirical clinical experience, supported by animal models, showed that cytotoxic drugs are most effective when given in combination to achieve additive or synergistic effects[2,3]. A caveat has been that success requires the ability to combine drugs at their respective effective doses without unacceptable side-effects. The rationale underlying combination cytotoxic chemotherapy has been to co-administer drugs that work by different molecular mechanisms, thereby increasing tumor cell killing while reducing the likelihood of drug resistance and minimizing overlapping toxicity. This approach followed the successful precedent of using combinatorial drug therapies to treat tuberculosis and other microbial infections[4] and was the strategy that proved highly effective in antiretroviral treatment for HIV[5].

Clinical success with combination chemotherapy was first achieved with co-administration of the antifolate methotrexate, the Vinca alkaloid tubulin inhibitor vincristine, the purine nucleotide synthesis inhibitor 6-mercaptoturine and the steroidal agent prednisone in childhood acute lymphoblastic leukemia—subsequently extended to lymphomas with the combination regimen of vincristine and prednisone plus the DNA-damaging agents nitrogen mustard and procarbazine and then to testicular cancer and epithelial malignancies, notably colorectal, breast and many others[3] (**Fig. 1**). Early, pregenomic research in the 1950s and 1960s established the major mechanisms of *de novo* and acquired resistance to cytotoxic drugs. These mechanisms include the following: decreased metabolic activation or enhanced degradation of the drug; increased expression of the drug target; alteration of the target or pathway to reduce sensitivity; and reduced uptake as well as chemical and enzymatic protection mechanisms to deal with reactive DNA alkylating agents[6]. The mechanisms of drug action and resistance were particularly well defined for the dihydrofolate reductase inhibitor methotrexate, still used today, for which selective amplification of the drug target was first defined as a mode of resistance in 1978 (ref. 7). Further mechanistic understanding of the development of the multidrug-resistant (MDR) state in the 1970s and 1980s led to treatments aimed at overcoming resistance due to drug transporters; however, combination strategies with modulators of the MDR1 (P-glycoprotein) efflux pump[8] proved disappointing, envisaged at that time to result from the common involvement of multiple resistance mechanisms, including insensitivity to drug-induced apoptosis and induction of drug-detoxifying mechanisms[9]. Most recently, post-genomic identification of the extensive intratumoral genetic heterogeneity present in human cancers, and the clonal evolution and polygenic resistance that occurs, especially under the Darwinian selective pressure of therapy, provides us with a more complete molecular explanation for the formidable clinical challenge of tumor resistance to all available therapies[10–13].

The enduring principles that emerged from the pregenomic, cytotoxic chemotherapy era have remained applicable, even as the exciting new generation of molecularly targeted drugs have become available as a direct result of the genomic elucidation of the pathogenic drivers of different cancers[14]. Cancer drug discovery and development is now firmly focused on exploiting pathogenic oncogene and nononcogene addiction, synthetic lethalities, and other vulnerabilities and dependencies that can result in impressive selective therapeutic effects in specific malignancies[15,16].

Despite the progress in moving from a one-size-fits-all cytotoxic approach to a new era of genetically targeted personalized molecular medicine, many challenges remain. The most important challenge is how to tackle the interrelated problems of genetic heterogeneity and drug resistance in cancer using intelligent drug combinations.

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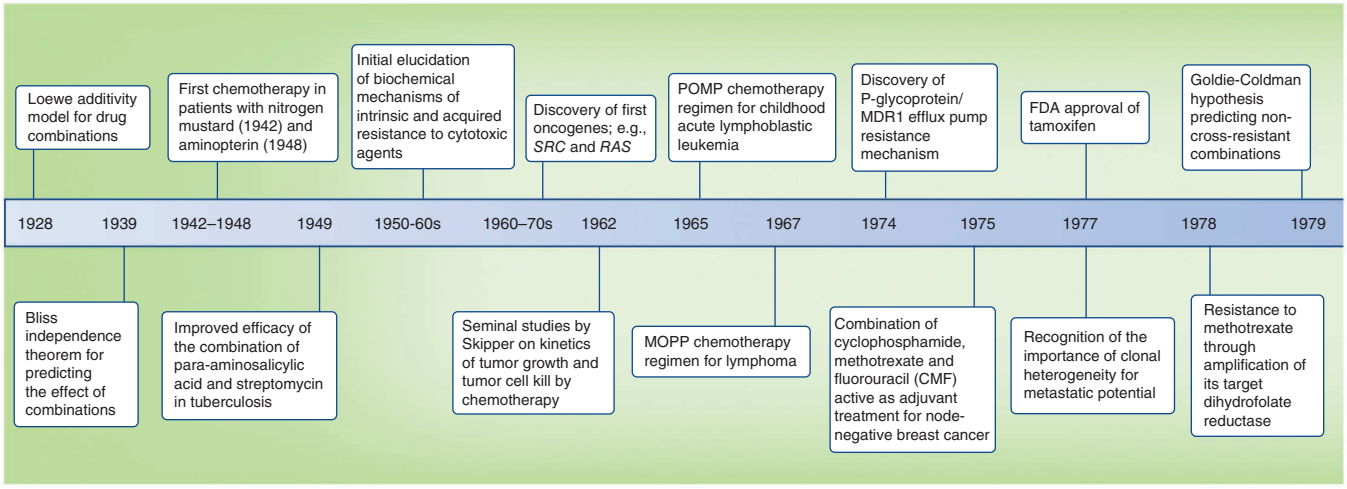


Figure 1 History of combination therapy for cancer. POMP, procarbazine, vincristine (Oncovin), nitrogen mustard (mustine) and prednisone. MOPP, nitrogen mustard, vincristine, prednisone and procarbazine.

Here we assess the need for combination therapy, evaluate how combinatorial therapies are progressing in the clinic and review how to identify new drug combinations using *in silico*, *in vitro* and animal models that are likely to achieve valuable clinical benefit.

Successful targeted therapies in the clinic

Much of the success with targeted therapies has arisen from the exploitation of the state of oncogene addiction, in which cancer cells become 'addicted to'—that is, physiologically dependent upon—the continued activity of specific activated or overexpressed oncogenes (or the loss of tumor suppressor genes) for maintenance of their malignant phenotype[17,18]. There have been encouraging advances in the treatment of hematological malignancies and solid tumors, as revealed in hypothesis-testing clinical trials employing predictive as well as proof-of-target-engagement pharmacodynamic biomarkers[19], following the administration of molecularly targeted drugs as single agents in well-defined oncogene-addicted subsets of cancers[20]. Use of the BCR-ABL inhibitor imatinib (Gleevec)[21,22] in chronic myeloid leukemia, for example, has led to an 80% decrease in disease mortality[23]. Imatinib also has activity in gastrointestinal stromal tumors by inhibiting mutant KIT, which is a frequent driver oncoprotein in this cancer. Before imatinib, addition of all-*trans* retinoic acid to the treatment of acute promyelocytic leukemia harboring translocations in the retinoic acid receptor α (*RARα*) gene led to curative responses in most patients[24].

Another key example is the ERBB2/HER2 antibody trastuzumab (Herceptin) that was approved initially for the treatment of patients with metastatic breast cancer whose tumors overexpress and are dependent upon the HER2 protein and who had received one or more chemotherapy regimens for their metastatic disease, or alternatively in combination with paclitaxel (Taxol) for HER2-positive metastatic patients who had not received prior chemotherapy[25]. Yet other examples are small-molecule tyrosine kinase inhibitors, such as the epidermal growth factor receptor (EGFR) inhibitors gefitinib (Iressa) and erlotinib (Tarceva) in EGFR-mutant non-small cell lung cancer (NSCLC)[26] and more recently the BRAF inhibitor vemurafenib (Zelboraf) in mutant *BRAF*-driven melanoma[27] and the anaplastic lymphoma kinase (ALK) inhibitor crizotinib (Xalkori) in *ALK*-translocated NSCLC[28]. Some molecularly targeted drugs have modest single-agent activity but nevertheless show significant clinical benefit—and are now integrated into clinical practice—when administered with standard-of-care cytotoxic drug treatment; examples include the EGFR antibody cetuximab (Erbitux) in combination with irinotecan (Camptosar)/5-fluorouracil in metastatic colorectal cancer[29] and the HER2 tyrosine kinase inhibitor lapatinib (Tykerb) combined with capecitabine (Xeloda) in advanced breast cancer[30].

In addition to >100 currently approved molecularly targeted agents, several hundred are in preclinical and clinical development[16]. These include not only those that target oncogene addiction, but also agents that exploit other cancer-associated mechanisms. For example, through their activity in *BRCA*-deficient cancers lacking homologous-recombination DNA repair, small-molecule inhibitors of poly(ADP-ribose) polymerase (PARP) such as olaparib, that block repair of DNA single-strand breaks, provide the first clinical validation of therapeutic synthetic lethality, mediated through enhanced DNA damage[31]. In addition, drugs acting on the heat shock protein 90 (HSP90) molecular chaperone exemplify exploitation of nononcogene addiction[32].

Resistance to therapies targeting addiction

There is a growing unease in academia and industry that well-validated targets will become increasingly harder to find[33]. Moreover, the frequently transient nature of responses to novel molecular therapeutics in many solid cancers has been linked to multiple mechanisms of resistance. Resistance mechanisms commonly comprise alterations in the addiction pathway that enable cancers to remain dependent on the original oncogenic process. But resistance can also involve bypass mechanisms that activate a parallel signaling track as well as pathway-independent routes mediated by epithelial-mesenchymal transition or the gain of stem cell-like features, together with effects mediated through cancer-host cell interactions in the tumor microenvironment, including changes in angiogenesis drivers (for a review, see ref. 15).

Notable examples of resistance mechanisms include the development of secondary mutations, such as in gatekeeper residues, in kinase targets such as ABL and EGFR, which confer resistance to imatinib and gefitinib/erlotinib[34,35], respectively. These may be overcome by second-generation agents, such as the multikinase inhibitor dasatinib (Sprycel), that can bind to and block the resistant allele[36]. Other examples of tumor resistance include decreased sensitivity to vemurafenib through *NRAS* mutation, receptor tyrosine kinase activation and overexpression of *CRAF* or *COT*/*MAP3K8* (which allow ERK1/2 MAP kinase pathway reactivation) and expression of *BRAF* splice variants that dimerize in a RAS-independent manner, all of which attenuate or prevent ERK blockade by RAF

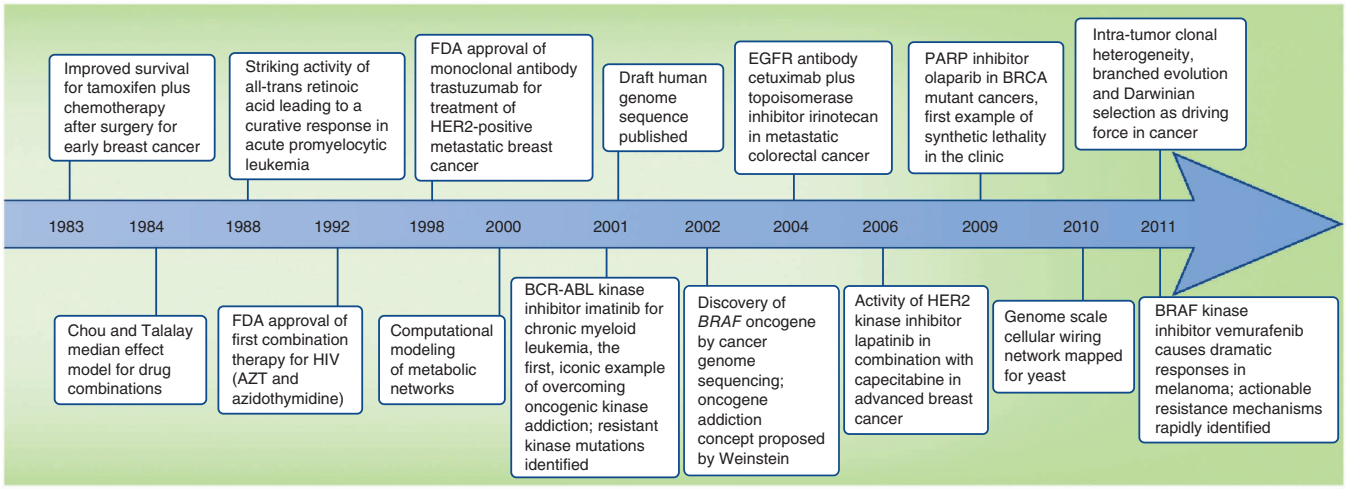


Figure 1 (Continued)

inhibitors[37–39]. Alternatively, vemurafenib resistance can occur through loss of PTEN (phosphatase and tensin homolog) or RB1 (retinoblastoma 1), despite effective inhibition of ERK signaling by the BRAF drug[40]. Furthermore, deep sequencing has revealed the huge extent of preexisting or induced molecular and phenotypic heterogeneity that exists in individual tumors (see above) and demonstrated the simultaneous presence of multiple driver mutations within the same tumor[11]. All of these potential resistance mechanisms and others could explain why well-designed drugs developed against well-validated cancer targets nevertheless fail to deliver sustained benefit in the clinic.

Combinatorial targeted therapy

Realization of the full potential of molecularly targeted cancer therapeutics is dependent on identifying the best possible drug combinations. This will require use of new technologies, including large-scale genomics and systems or network biology with associated computational approaches[41]. The scale of the challenge is illustrated by the sheer number of mathematically possible drug combinations. If we consider the set of ~250 approved cancer drugs, there are 31,125 possible two-way combinations and 2,573,000 three-way combinations. For the estimated 1,200 cancer drugs currently in development the respective numbers rise to 719,400 and 287,280,400. Or, we can consider the possible combinations of cancer genes that might be targeted. Based on analyses using our integrated drug discovery platform canSAR[42] (http://canSAR.icr.ac.uk/) of genes listed in the Cancer Genome Census[43] (http://www.sanger.ac.uk/genetics/CGP/Census/), we estimate that there are around 124 conventionally druggable targets that are reported to harbor causative mutations in cancer. This gives us the potential for 7,626 two-way target combinations and 310,124 three-way combinations. Although these numbers are theoretical and not all combinations make mechanistic sense, the potential combinations would increase further if we included targets representing nononcogene addiction, synthetic lethality and other considerations. Clearly, it would be prohibitively expensive to evaluate such large numbers of drug and/or target combinations in animal models let alone in a clinical setting. We therefore need methods to evaluate and prioritize the best potential combinations, either using large-scale unbiased *in silico* or experimental biology methods or by taking hypothesis-driven approaches based on new genomics, proteomics and other omics technologies[44–46].

Despite the theoretical and practical challenges, many drug combinations are already being evaluated in the clinic. In the following sections, we review progress using selected examples of clinical combinatorial drug treatments.

Current combinations of targeted drugs with chemotherapy

Combining molecularly targeted agents with chemotherapy has generally been pragmatic. Most new molecular cancer therapeutics generally have modest efficacy with responses that are not rapid or durable. Thus, targeted drugs might benefit from being combined with cytotoxic agents or radiation. This strategy is also influenced by consideration of how to gain regulatory approval for new drugs in settings where cytotoxic therapies are already marketed. Examples include the monoclonal antibodies trastuzumab in combination with paclitaxel[25] in breast cancer, rituximab (Rituxan) in combination with cyclophosphamide/doxorubicin/vincristine/dexamethasone in non-Hodgkin's lymphoma[47] or cetuximab in combination with irinotecan in colon cancer[48] together with receptor tyrosine kinase inhibitors like lapatinib combined with capecitabine in breast cancer[30]. Key factors underpinning such combinations include tolerability and avoidance of possible pharmacokinetic interactions, and there is often no compelling biological rationale underpinning them[49,50]. Although it may seem reasonable to combine a targeted agent with the standard of care, the experience from breast cancer teaches us that the concomitant administration of a hormonal agent with chemotherapy might show a trend toward an inferior result; in such cases these agents may be better used in sequence rather than in combination[51].

There are exceptions where the molecularly targeted drug is specifically intended not to have single-agent activity (at least in most cancers) but rather to enhance the activity of co-administered cytotoxic chemotherapy. An example is the use of checkpoint kinase 1 (CHK1) inhibitors[52] in combination with chemotherapy drugs, such as DNA damaging agents gemcitabine (Gemzar) or irinotecan, where the therapeutic modulator is designed to exploit defective cell cycle checkpoints in cancer.

Current combinations of molecularly targeted drugs

There are multiple potential approaches to the combination of targeted drugs (**Table 1**). Targeting more than one related oncogenic receptor tyrosine kinase, or inhibiting the same receptor in more than one way, is a rational approach to increase activity or overcome resistance. An interesting example is the case of trastuzumab resistance in HER2-amplified

Table 1 Clinical trials evaluating combinations of molecularly targeted agents

Drug combination	Biological rationale	Patient population likely to benefit	Stage
Targeting same family of RTKs in more than one way			
Pertuzumab + trastuzumab + docetaxel versus placebo + trastuzumab + docetaxel (CLEOPATRA; NCT00567190)	Resistance to trastuzumab (targeting HER2) is mediated by continued HER2/HER3 dimerization; pertuzumab acts at different site on HER2 from trastuzumab to inhibit dimerization.	*HER2* amplified breast cancer	Approved June 2012
Trastuzumab + AUY922 (NCT01271920)	Resistance to trastuzumab (targeting HER2) is mediated by truncated p95HER2; by inhibiting HSP90, AUY922 will cause degradation of all forms of HER2 and also overcome other resistance mechanisms.	*HER2* amplified breast cancer	Phase 1
'Vertical' targeting of RTK and downstream effectors			
Everolimus + trastuzumab + vinorelbine versus placebo + trastuzumab + vinorelbine (BOLERO-3; NCT01007942)	Resistance to trastuzumab (HER2 inhibitor) is mediated by *PIK3CA* mutation or *PTEN* loss; everolimus is an mTOR/PI3 kinase pathway inhibitor.	*HER2* amplified breast cancer	Randomized phase 3
GSK2118436 + GSK1120212 (NCT01072175)	MEK activation by COT and CRAF is a mechanism of resistance to BRAF inhibitors e.g., GSK2118436; this can be overcome using MEK inhibitors such as GSK1120212.	*BRAF* mutant melanoma and colorectal cancer	Phase 1
'Horizontal' targeting of parallel pathways			
MK2206 + selumetinib (NCT01021748)	AKT activation is a mechanism of resistance to selumetinib and other MEK inhibitors; MK2206 is an AKT inhibitor.	*KRAS* mutant NSCLC	Phase 1
GDC-0941 + GDC-0973 (NCT00996892)	PI3K pathway activation is a mechanism of resistance to GDC-0973 and other MEK inhibitors; GDC-0941 can overcome this by inhibiting PI3 kinase.	Dual *PIK3CA/KRAS* mutant colorectal cancer	Phase 1

Trials listed above are examples of combinations of molecularly therapeutic agents that target the same receptor tyrosine kinase, target a signal transduction pathway at different nodes or target multiple signal transduction pathways. RTK, receptor tyrosine kinases.

breast cancer. Mechanisms of resistance include continued ligand-induced signaling due to HER2–HER3 dimerization[53], which has been targeted by combining the two differentially acting HER2 antibodies trastuzumab and pertuzumab (Perjeta)[54] in the randomized phase 3 CLEOPATRA trial involving over 800 patients (NCT00567190) which led to the recent approval of the triple combination of the two antibodies plus docetaxel. Alternatively, the expression of the truncated p95-HER2, which lacks the trastuzumab binding site, also results in trastuzumab resistance. This and other resistance mechanisms could be targeted by the combination of trastuzumab with HSP90 inhibitors that potently degrade HER2 (and additional oncogenic client proteins), which is highly dependent on the molecular chaperone for its function and stability[55]. This approach has been used successfully in HER2-positive breast cancer with the first-generation HSP90 inhibitor 17-AAG (tanespimycin)[56] and is now being investigated in current clinical trials with improved non-geldanamycin HSP90 inhibitors, such as AUY922 (ref. 57) (NCT01271920). Although the pleiotropic effects of HSP90 inhibitors are expected to reduce the opportunity for resistance to occur, *de novo* and acquired resistance to tanespimycin is associated with reduced metabolic activation of its quinone group to produce the potent hydroquinone form, mediated by loss of expression or selection for an inactive polymorphic form of NQO1/DT-diaphorase, a possibility absent with nonquinone chemotypes like AUY922 (refs. 58,59).

Activation of oncogenes and inactivation of tumor suppressor genes downstream of a membrane receptor frequently leads to pathogenic activation of oncogenic signal transduction pathways. Efficacy of targeted therapies may be enhanced, or resistance overcome, by simultaneous combinatorial targeting of the receptor and a downstream signal transduction pathway. Combinatorial options are sometimes described as 'vertical' (within an oncogenic pathway) or 'horizontal' (across two such 'parallel' pathways), although in reality signaling outputs operate in complex networks that need to be understood more fully (see below).

An example of vertical oncogenic pathway targeting is the discovery of mechanisms of trastuzumab resistance through activating mutations of the *PIK3CA* oncogene, which encodes the p110α isoform of phosphoinositide-3 (PI3) kinase[60], or loss of expression of the opposing phosphatase, the tumor suppressor PTEN[61]. The combination of an mTOR (mammalian target of rapamycin) inhibitor, which is one of several possible PI3 kinase pathway inhibitors (e.g., the rapamycin analog everolimus), with trastuzumab is currently being evaluated in HER2-amplified breast cancer in the ongoing BOLERO-3 trial (NCT01007942). Because the androgen receptor pathway remains the major oncogenic driver in metastatic castrate-resistant prostate cancer, as demonstrated by the efficacy of the CYP17 inhibitor abiraterone (Zytiga)[62] and the highly potent androgen receptor antagonist enzalutamide (MDV3100)[63], combinatorial vertical targeting of the pathway is now recognized to be important.

One example of the rational, horizontal, combinatorial targeting of parallel oncogenic signaling pathways is based on the observation of increased phosphorylation of the kinase AKT (functioning downstream of PI3 kinase), in preclinical NSCLC models as a mechanism of resistance to allosteric inhibitors of another kinase MEK (MAP kinase kinase), which operates downstream of RAS and RAF in the ERK1/2 MAP kinase pathway[64,65]. Preliminary efficacy of the combination of the MEK inhibitor AZD6244 and the allosteric AKT inhibitor MK2206 (ref. 66) has been demonstrated in NSCLC in ongoing hypothesis-testing phase 1 trials (NCT01021748). Indeed, combining drugs to overcome feedback loops that are activated by a given molecularly targeted agent has attracted considerable interest. Another topical example of this is the co-administration of monoclonal antibodies targeting insulin-like growth factor receptor 1 (IGFR-1) to prevent the insulin receptor substrate 1 (IRS1)-mediated feedback loop activating AKT that occurs after treatment with an mTOR inhibitor[67,68]. This approach is being explored clinically in promising ongoing studies with combinations such as dalotuzumab plus ridaforolimus[69] (NCT01234857).

Although therapeutic benefit may be observed, the ever-present problem of additive on-target toxicity has led to the failure of combinations of two different anti-angiogenic vascular endothelial growth factor (VEGF) targeting agents, namely bevacizumab (Avastin) and sunitinib (Sutent), which caused hypertension and hemolytic anemia[70]. In contrast, the vertical pathway combination of BRAF (GSK2118436) and MEK (GSK1120212) inhibitors—which

has a sound rational basis in seeking to overcome several of the identified mechanisms of resistance to BRAF inhibitors as well as blocking the undesirable paradoxical activation of CRAF in healthy cells (through RAF dimer formation) following treatment with BRAF inhibitors[71,72]—has led to additive activity against melanoma with surprisingly manageable skin toxicity and encouraging efficacy[73].

Targeting multiple pathways and hallmark cancer traits

Individual targeted agents that simultaneously affect multiple oncogenic signal transduction pathways could be used either as single agents or in combination with other anti-signaling drugs to yield even more powerful antitumor effects and block the induction of resistance mechanisms. A range of receptor kinase inhibitors, such as sorafenib (Nexavar), vandetanib (Caprelsa), foretinib and regorafenib have more than one target (by serendipity or design) and are known to be effective in clinical settings[74–77]. The polypharmacology of kinase inhibitors can be revealed by kinase profiling technology although *de novo* design of a required kinase selectivity profile can be challenging[78]. Of considerable current interest is the optimal selectivity profile of class I PI3 kinase inhibitors and in particular the potential therapeutic advantage and side effects of simultaneously inhibiting mTOR as well as PI3 kinase[79].

As mentioned previously HSP90 inhibitors are good examples of targeted pleiotropic drugs, as they were designed to deplete multiple oncogenic client proteins and thus combinatorially block many cancer-supporting signal transduction pathways and cancer hallmarks[57,80,81]. Clinically, these agents have shown utility when used particularly to target the relevant driver HSP90 client oncoprotein, including amplified HER2 in breast cancer, mutant EGFR and ALK fusions in NSCLC, mutant BRAF in melanoma, and the androgen receptor in prostate cancer, in addition to other signaling proteins such as AKT[32]. Moreover, HSP90 inhibitors have major advantages in targeting drug-resistant alleles and overcoming or preventing resistance through multiple mechanisms, and might be used in combination with other targeted agents, especially those acting on the same driver HSP90 clients in the above cancers[32].

Combination approaches might also include drugs that act on epigenetic processes, including histone deacetylase inhibitors[82,83], and other chromatin-modulating enzymes, such as demethylases, that could counteract the resistance that is associated with a chromatin-mediated, reversible drug-tolerant state in cancer cell subpopulations that have stem cell–like features[84].

Most cancer drug discovery and development is focused on targeted agents that induce apoptosis or cell cycle arrest in malignant cells. However, combinations of agents that individually modulate the multiple malignant hallmarks of cancer, including invasion, angiogenesis and metastasis, together with tumor metabolism and proteostasis, as well as those that affect other aspects of the tumor microenvironment or stimulate the immune response (e.g., CTLA-4 inhibitor ipilimumab, Yervoy, in melanoma[85]), could also be very powerful when added to drug combinations by striking simultaneously at the multiple malign phenotypic behaviors of cancer cells[86–88].

Unbiased approaches to drug combinations

Although hypothesis-driven and candidate empiric approaches are important, systematic high-throughput unbiased screening strategies provides a complementary approach to identifying effective drug combinations. Combination high-throughput screening of all licensed drugs has been carried out in an attempt to discover unexpected synergistic interactions[89]. This is exemplified by the combination of the antiparasitic agent pentamidine (NebuPent) and the phenothiazine antipsychotic chlorpromazine (Thorazine) which exert dual synergistic anti-mitotic effects in cancer cells[90,91]. Such an unbiased approach can reveal unexpected and promising combinations of already licensed agents that can be progressed rapidly to the clinic.

A recent chemical genomic screen identified transcriptional repressors, including anthracyclines like doxorubicin (already in clinical use), that selectively downregulated the anti-apoptotic protein MCL1[92]. The gene encoding MCL1 is frequently amplified in human cancer. This apoptosis-modulating activity might explain the activity of anthracyclines in clinical drug combinations. In the same study, high BCL-xL expression was identified as a potential predictive biomarker for use in patient selection.

As an alternative to chemical screening, another unbiased strategy is to conduct systematic genome-wide loss- or gain-of-function screens in tumor cells where the objective is to identify genes for which RNA interference (RNAi) silencing or cDNA overexpression results in sensitivity or resistance to cancer drugs[93,94]. Extensive efforts have been focused on genetic interaction screens with gene knockouts or RNAi in microbial systems, with implications for cancer targets[95,96]. Success has also been achieved using similar screens in cancer cells. For example, the PI3K pathway was identified as a determinant of trastuzumab resistance in breast cancer[97], and CDK10 was identified as an important determinant of resistance to tamoxifen[98] using large-scale screening of RNAi libraries.

Recently, another unbiased large-scale RNAi screen identified feedback activation of EGFR as a cause of resistance of colon cancer cells to BRAF inhibition, suggesting the use of a combination of BRAF and EGFR inhibitors in *BRAF* mutant, *EGFR*-expressing colon tumors[99]. Additionally, an overexpression kinome screen identified *COT*, *CRAF* and genes encoding receptor tyrosine kinases as genes able to cause resistance to BRAF inhibition, again suggesting actionable combination strategies, including the use of MEK, tyrosine kinase and HSP90 inhibitors[37,38].

We are now seeing an increasing use of massively parallel next-generation sequencing of whole exomes and genomes to discover genes that are likely to be involved in resistance, both in tumor-derived cancer cell lines and in cancer tissue obtained from patients treated with targeted agents[12,14]. Automated large-scale screening of cancer drugs in extensive panels of hundreds of genetically annotated cell lines could also be adapted to identify promising combinatorial regimens and the mechanistic underpinnings of such effective drug combinations that would inform patient selection using predictive biomarkers[100,101].

Computational approaches to identify targeted combinations

Computational methods are being applied to explain and predict both therapeutic resistance and potential drug combinations, particularly to maximally exploit the full depth of high-throughput experimental data. Historically, the Loewe additivity model[102] allowed the prediction of the maximum effect of combined drugs based on their individual drug-response functions. Later, the Goldie-Coldman hypothesis was used to explain the emergence of drug resistance based on the genetic instability of the cancer cells, and predicted that this problem can only be tackled by combining non-cross-resistant chemotherapies[103]. Loewe's model was expanded in Chou and Talalay's combination index, which allowed quantifying synergy, additivity or antagonism[104]. Based on the median-effect principles of the mass action law, enzyme kinetics were incorporated into the model to allow prediction of the degree of inhibition by the combined drugs, assuming that they acted through the same mechanism with no allostery. Additionally, a general solution by Berenbaum[105]

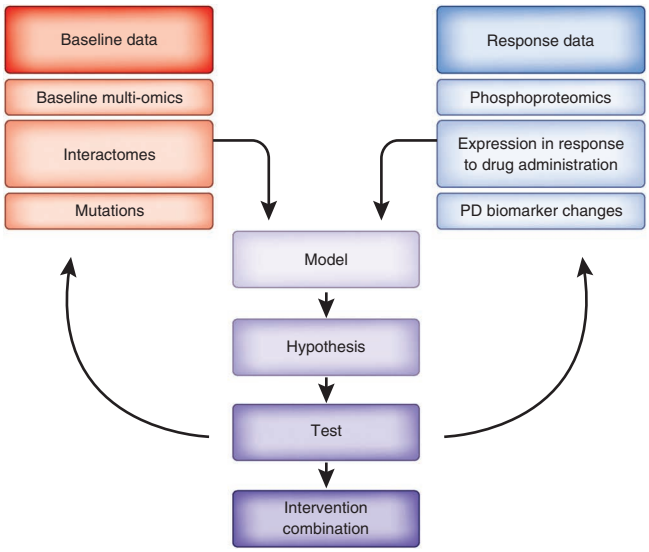
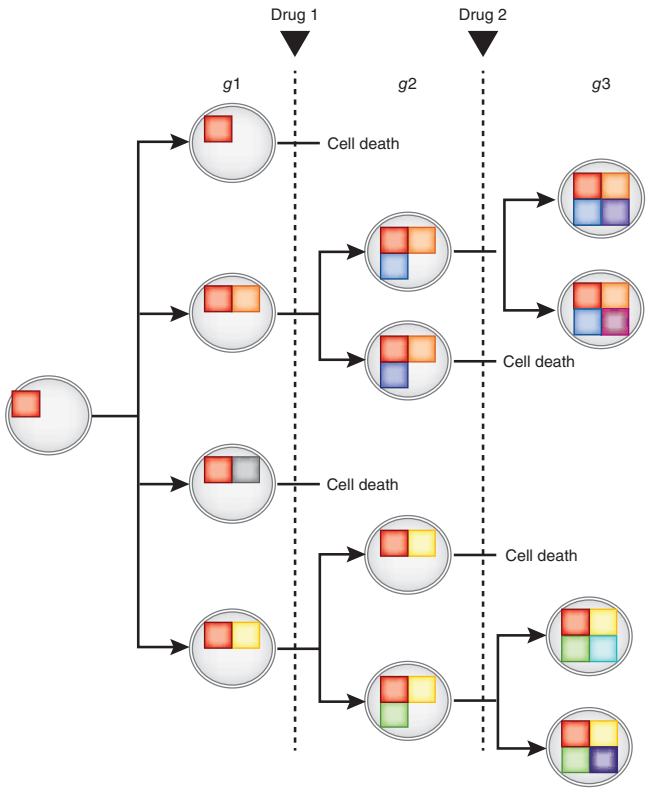


Figure 2 Components of iterative computational approaches for identifying drug combinations. Baseline or static multi-omics data, including gene expression, mutation, DNA copy number and proteomics information, provide inputs for the generation of an initial model of the system. Cell or protein network dynamic and kinetic data measure the altered abundance, activity or cellular location of proteins over time and in response to perturbations such as drug interventions. These data are fed into the mathematical model and can be used to generate hypotheses and simulate likely outcomes. Hypotheses are then tested in the laboratory and data from these tests can be used to refine the model, eventually resulting in a data-driven drug combinations. PD, pharmacodynamics.

represented dose-effect responses in a multidimensional hyperplane. This scalable solution allowed the identification of synergy or antagonism for any number of drugs.

Although early methodologies primarily focused on the dose-response relationships of drugs, understanding the molecular mechanisms of drug action and including them in the mathematical model became necessary. Simulation of the kinetics of folate metabolism[106], and later the more complex biochemical pathways of folate and nucleotide metabolism[107], represented the first computational modeling of a biological pathway and the effect of drugs upon it. Synergy models were used to deduce likely underlying biological interaction networks from drug combination effects[108]. Meanwhile, computational models identified patterns in the pathway topology downstream of EGFR, which can explain the observed combinatorial effects of drugs acting on this pathway[109].

To enable mechanistic understanding of drug resistance and predict potential drug combinations, researchers now use two interconnected disciplines: first, cellular connectivity and interaction networks; and second, the Darwinian evolution of cancer cell populations. New models make use of massive data inputs from next-generation technologies, such as deep exome and genome sequencing. In the following sections, we focus on these two classes of new computational modeling strategies: evolutionary or Darwinian modeling and network and/or systems modeling. These two classes comprise a multitude of different mathematical models. Despite their diversity, the approaches have the same fundamental components (**Fig. 2**). An initial model is built from prior knowledge and baseline data, such as individual mutations, whole exome and/or genome sequencing data, protein interaction networks and cell population statistics[110,111]. The model is enriched where possible with dynamic/kinetic information—including changes in cell-growth rates, enzyme kinetics, phosphoproteomic patterns, mutations, gene expression profiles and epigenetic marks—followed over time and in response to perturbation by drug treatments. After internal validation based on known test data, the model can be used to predict outcomes in cells. Experimental testing can then be used confirm the predictions and/or provide additional data to improve the model. Incorporation of observed biomarker changes will facilitate responsive data-led enhancements to the *in silico* models.



Evolutionary modeling for drug combinations

Evolutionary or Darwinian models use population statistics[112] to explain tumor heterogeneity and clonal evolution[10]. Cancer cells present in a tumor are treated as a heterogeneous population and cell lineage can be defined based on genetic similarity. This allows identification of the probable cell of origin, and prediction of likely trends in tumor growth and drug resistance based on the clonal genetics of the tumor[113].

An evolutionary tree is constructed using genomic analyses of tumor cells based on mutation status or epigenetic marks[11,114,115].

Figure 3 Evolutionary model of clonal heterogeneity. Darwinian evolution of a heterogeneous tumor in response to selection pressure from drug intervention is shown. Each circle represents a cell; *g*1–*g*3 are three cell generations. At the time of administration of the first-line drug (Drug 1), there are four discrete populations with distinct genomic changes, such as somatic mutations (represented by colored squares). Only two of the populations survive Drug 1, presumably due to advantages conferred by mutations. These surviving populations constitute the majority of the tumor (*g*2), which is now resistant to Drug 1. The majority of cells in g2 acquire new mutations as represented by the light blue, dark blue and green squares. Selective pressure from a second-line treatment (Drug 2) results in a third generation (*g*3) that is multi-drug resistant. Evolutionary models based on population genetics can be used to mathematically represent this process. Such models can be used to assess potential outcomes of hypothetical drug combinations or different dosing schedules *in silico*.

Table 2 Major public repositories of protein pathway and interaction network data

Resource	Comment	URL	Reference
Database of Interacting Proteins (DIP)	Experimentally identified physical interaction between proteins. No directionality information.	http://dip.doe-mbi.ucla.edu/dip/Main.cgi	159
IntAct	Manually curated from 5,000 publications plus some individual user entries. No directionality information.	http://www.ebi.ac.uk/intact/	160
MINT	Interactions between biological entities including DNA/RNA as well as protein. Protein interaction data mined from literature. No directionality information.	http://mint.bio.uniroma2.it/mint/	161
Pathway commons	Contains both pathways and interactions. Some directionality information available.	http://www.pathwaycommons.org/pc/home.do	162
PICOLO	Three-dimensional structure derived protein-protein interactions. Limited to structurally characterized proteins but accurate and curated.	http://www-cryst.bioc.cam.ac.uk/databases/piccolo	163
Reactome	Curated pathways. Contains directionality information.	http://www.reactome.org/	164
ROCK	A breast cancer resource that also contains a large set of protein interaction and pathway data compiled from many sources and independent of disease. Contains transcriptional effects as well as physical interactions. No directionality information.	http://rock.icr.ac.uk/	165
STRING	Known and predicted protein interactions. Three-dimensional structural annotation enhances the interaction networks. No directionality information.	http://string-db.org/	166
BioGrid	Curated protein-interaction and transcriptional data.	http://thebiogrid.org/	167

Several commercial products are also available, such as Thomson Reuters' MetaCore (http://www.genego.com/metacore.php), Ingenuity IPA (http://www.ingenuity.com/) and KEGG (Kyoto Encyclopaedia of Genes and Genomes; http://www.genome.jp/kegg/).

In such evolutionary models, the administration of drugs confers selection pressure on the population, resulting in the survival of the fittest cells: that is, in the present therapeutic context, those with the genetic features that make them drug resistant[116] (**Fig. 3**). The models identify clonal evolutionary branch points and associate them with genetic events that coincide with, and may pathogenically drive, downstream differences such as acquired drug resistance[113].

Evolutionary models have been useful for understanding drug effects and drug resistance, and are being used to predict better dosing schedules and combinations of cancer drugs. Studies using evolutionary models of acquired drug resistance to protein kinase inhibitors acting on the EGFR pathway[117] have shown that the likelihood of resistant clones emerging can be predicted based on the resulting downstream cellular effects of the drug together with tumor size. If a drug acts by causing cell death, then the likelihood of resistant clones arising is low for small tumors and high for large tumors. On the other hand, drugs that act by inhibiting cell growth rather than cell death have a smaller chance of driving emergence of resistant clones regardless of tumor size, provided that resistant clones do not already exist. This finding described the nuances of kinase inhibitor drug action and highlighted the importance of understanding and addressing these when considering effective drug dosing and scheduling. Evolutionary approaches have been applied to identify optimal combinations and administration schedules for EGFR inhibitors gefitinib and erlotinib in NSCLC cell lines to prolong the likely clinical benefit through delaying the evolution of resistant mutants[118]. Furthermore, the application of evolutionary models in drug-resistant NSCLC, together with cell-based studies, has identified that sequential therapy using cytotoxic agents with either erlotinib or gefitinib was more effective than monotherapy or concurrent combination dosing[119].

Network modeling for drug combinations

Network-based modeling is a parallel and complementary approach to evolutionary modeling and can be applied regardless of the availability of in-depth data on oligoclonality. This approach focuses on the mathematical modeling of the complex pathways and protein interaction networks underpinning the hallmarks of cancer, and uses genomic and proteomic data—from patient samples or cancer cells in culture—to model the networks in the context of specific genetic backgrounds[45,120,121]. These network models require several factors: a computational representation of the protein interaction network in question; the ability to mathematically represent its kinetic changes; and the ability to predict likely outcomes of perturbation.

Baseline omics data, ideally derived from patient samples, are often a first step in identifying the molecular signature of a specific malignancy or responsive gene group[23,122]. Once a set of genes is identified, protein set enrichment and pathway analysis is typically done to identify the underlying processes represented by these genes[123]. Major canonical pathways in cancer are increasingly well established[86,87];

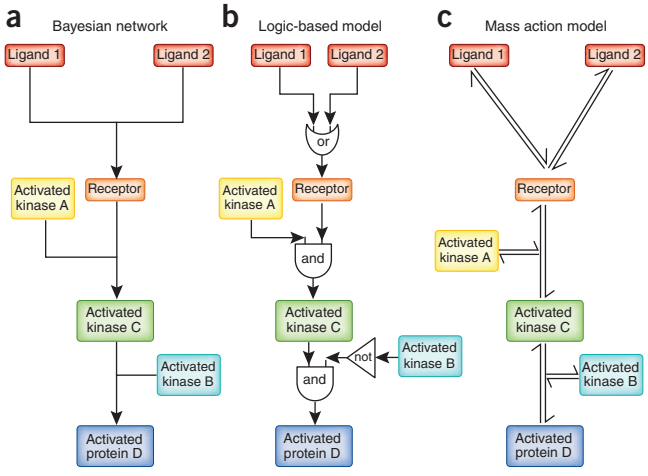


Figure 4 Network-based computational models. In this hypothetical pathway, a receptor can be activated on binding either ligand 1 or ligand 2. Upon activation, the receptor recruits activated kinase A resulting in the activation of kinase C. Activated kinase C can activate protein D provided that a third kinase (kinase B) is not activated. (**a**) A Bayesian network, where every connection in the network is represented by a set of probabilities. Probabilities are dependent on previous events. (**b**) A logic-based model where logic gates, with underlying truth tables, represent each of the connections in the database. (**c**) A mass action model where all interactions in the network are represented as reaction equilibriums with underlying kinetics.

however, these canonical pathways do not capture complex and context-dependent cellular wiring patterns. Several resources exist that allow users to retrieve protein interaction maps or pathways for a set of proteins. **Table 2** highlights major public databases. These resources overlap to some degree and have strengths and weakness. A major weakness across all resources (**Table 2**) is the scarcity of directionality data. For example, the databases provide information that kinase A interacts with protein B, rather than kinase A activates protein B. Because of this, a concerted effort by the research community is required to address the lack of directionality and temporal data in order to improve the utility of the databases for therapeutic applications, such as computational prediction of drug combinations.

Input data for network models can be of any type that capture temporal changes in samples or changes in response to biological or therapeutic intervention[45,124]. An example is the monitoring of changes in protein phosphorylation in IGFR interaction networks from breast cancer cell lines, before and after IGF-1 stimulation[45]. Where available, proteomic and phosphoproteomic biomarkers from patient samples are an extremely valuable kinetic data source[125], allowing capture of changes to cellular networks before patient treatment and at different stages after treatment, including in response to drug combinations[126]. This facilitated the identification of synergies between MET and EGFR inhibitors as a novel approach to address chemoresistance in patients with glioblastoma multiforme expressing EGFRvIII[126]. Epigenetic changes, such as DNA methylation or histone modification at different disease stages or in response to drug treatment, are also used[127].

Pathway and protein-protein interactions benefit from extensive public resources as described above and in **Table 2**; however, no such resources exist to capture network kinetic data. Although useful information can be gleaned from sources such as PhosphoSite[128], which captures curated data from phosphoproteomics studies, kinetic data are not collated into public databases, and are usually produced in the laboratory for a specific study. Hence, there is a need to create such a public resource.

Modeling cancer networks

A long-term goal of systems/network biology is the production of a responsive mathematical model describing the behavior of cells or even whole organisms (e.g., see the E-cell project; http://www.e-cell.org/). Clearly, this is an ambitious goal that is being met in part through modeling causal protein interaction-network pathways or defined interaction networks. An example is the modeling of causal protein interaction networks in individual human immune cells from multiparameter data obtained experimentally through flow cytometry and proteomics experiments[129] as well as the modeling of the MAPK pathway[130]. **Figure 4** shows representations of a simple signaling pathway as characterized by the major model types adopted in the field.

Logic-based models have been applied successfully to describe cancer network kinetics[46,131,132], to identify differential signaling networks between normal and cancer tissue[120], and to define likely drug synergies[46]. A model of the nuclear factor kappa B (NFκB) pathway using dose-response data for drugs targeting molecules in the pathway identified synergies between certain agents, such as the (I kappa B kinase) IKK inhibitor PS-1145 when combined with the HSP90 inhibitor geldanamycin[46]. A study of 231 unique pairwise combinations of 22 receptor-specific ligands in the RAW 264.7 mouse leukemic monocyte macrophage cell line showed that whereas treatment with individual ligands demonstrated limited measurable effect (e.g., cytokine release), combinations of ligands nearly all had synergistic or additive effects. For example, the authors identified synergy between ligands inducing cAMP production, such as the beta-adrenergic agonist isoproterenol, with Ca^{2+}-mobilizing ligands, such as the pyrimidinergic receptor P2Y agonist 2-methylthioATP. The study mapped the potential cellular wiring to explain the observed synergies and hypothesized that limited mechanisms of cross-talk could potentially be exploited to maximize the synergy[133]. Later, a logical model of the metabolic circuits from the same cells showed that the circuits could be simplified into semi-independent transduction units that lie downstream of signal receptors[134]. This model, if experimentally verified with emerging interactome data, would simplify the design of drug combinations using co-administered agents that target individual units or multiple units depending on the genetics of the disease.

A mass action model of the IGF-1 signaling network, using phosphoproteomic analysis before and after IGF-1 stimulation in MDA-MB231 human triple-negative breast cancer cells[45] identified that the combination of inhibitors of the MAP4K and PI3K/AKT pathways provides a synergistic effect in reducing cell viability, whereas combinations of inhibitors of the MAPK and mTOR pathways show the opposite effect in activating AKT and increasing cell viability[45].

Although we are some way away from achieving a human cellular wiring map, such as the one achieved for *Saccharomyces cerevisiae*[95], network modeling will benefit from the expanding efforts to map the human interactome, including the impact of cancer and drugs on the model. Using novel systematic approaches, such as genome-wide synthetic lethality screens[93,94,135] and chemical genetics[91,136], should provide complementary data that can help to explain the interactions between cellular pathways and inform drug combinations through systematic, unbiased experimental determination of the effects of the combinatorial inhibition of targets.

Other computational approaches

In other work, a completely computational and unbiased approach[137] exploited a series of drug features, such as molecular targets, pharmacological data and toxicity profiles, to develop a novel algorithm to predict effective synergies using 184 pairwise combinations of US Food and Drug Administration (FDA)-approved drugs. This approach identified 16 high-scoring drug combinations, not previously tested, that could be of potential use in the clinic.

As with all computational methods, the integration of alternative models to generate combination hypotheses and identify consensus findings will be advantageous. Computational protocols, such as CytoSolve[138], allow the combination of alternative models and generation of consensus hypotheses. In addition, the BioModels database[139] captures curated published biological models that can be used for specific molecular processes and will eventually be a very useful training resource for the field. Sophisticated and data-responsive computational approaches are evolving alongside the evolution of new screening and omics technologies. The alliance of new generation computational and experimental technologies will pave the way for identifying data-driven real-time drug combinations.

Preclinical testing and biomarker-led clinical trials

The high failure rate of cancer drugs, especially in large and expensive late-stage human clinical trials, indicates the urgent need for improved translation of therapies from preclinical models, particularly so for drug combinations. The computational and *in vitro* cell culture–based models that are frequently used in the drug combination studies described in this article are very useful for prioritizing options. The value of very large annotated cancer cell line panels to systematically link drug response to genetics has been demonstrated, indicating potential for future combination studies[100,101,140]. However, *in vivo* studies in mice are normally needed to ensure relevance to cancer in the whole animal

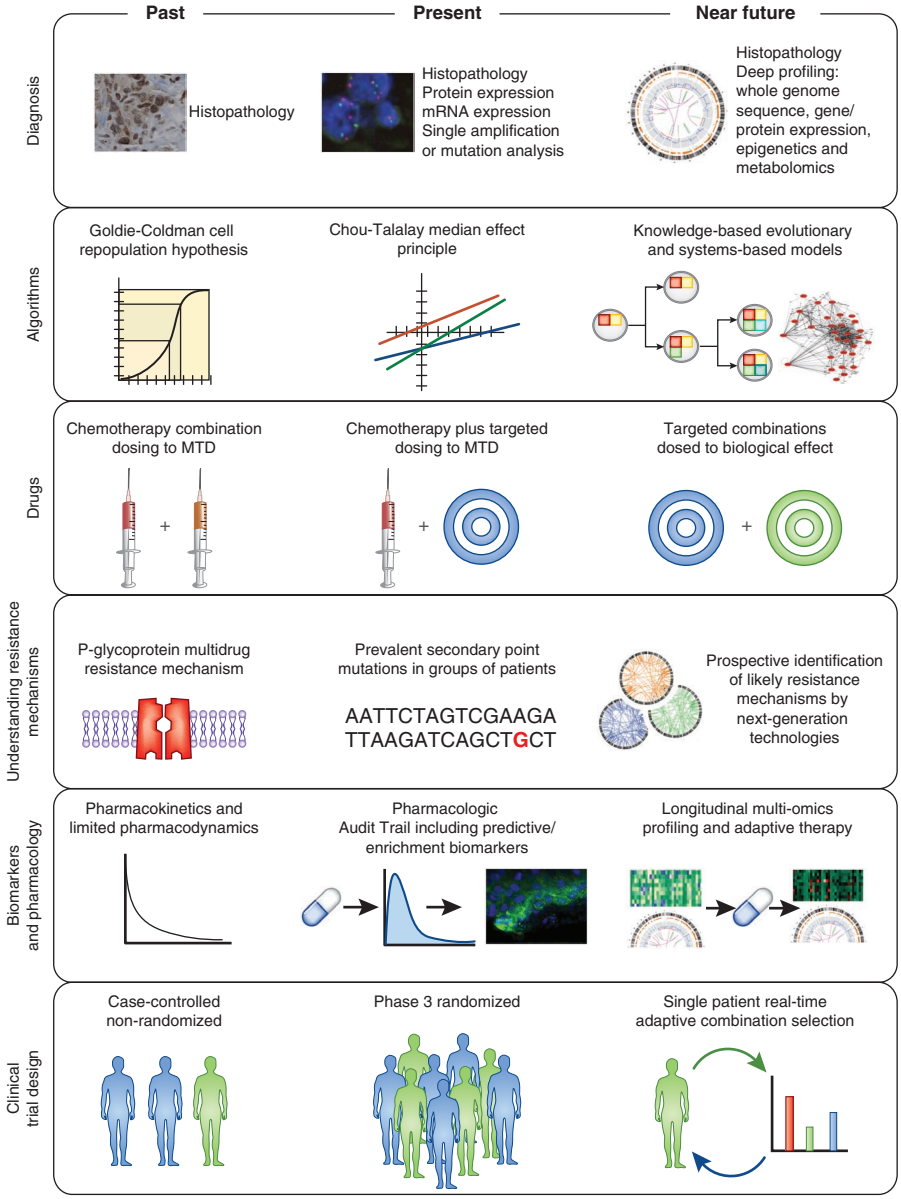


Figure 5 The evolution of strategies and technologies for evaluating drug combinations. The near future will see the advent of cocktails of molecularly targeted combinations that are rationally defined based on deep profiling of the patient and adapted in response to longitudinal molecular follow-up. The syringe symbol indicates cytotoxic chemotherapy and the target symbol indicates molecularly targeted therapy. MTD, maximum tolerated dose.

setting, including host-stromal interactions and immune responses. Human tumor xenografts molecularly characterized for genomic drivers and vulnerabilities are very useful, and their value can potentially be increased with early passage and orthotopic refinements[141].

The new generation of genetically engineered mouse models is proving increasingly relevant and useful because they mimic spontaneous and autochthonous cancer progression[142,143]. Recent studies of such models with oncogenic *KRAS*-driven lung and pancreatic adenocarcinoma showed that they can closely phenocopy human therapeutic responses to standard-of-care treatment regimens[144]. Genetically engineered mouse models have clear potential for discovering predictive biomarkers, gaining mechanistic insights into drug resistance in human cancers and predicting clinical outcomes, particularly for drug combinations[143]. As an example, studies using mutant *Kras* G12→D and *PIK3CA* H1047→R showed that inhibitors of the PI3K-mTOR pathway may be active as single agents in cancers with *PIK3CA* mutations but will need to be combined with MEK inhibitors to treat *KRAS*-mutated lung cancers, leading to clinical trials such as the AKT-MEK inhibitor combination discussed earlier[145] Proteomic and gene expression studies using triple-negative breast cancer cell lines and GEMMs showed that cancer cells remodel the kinome in response to MEK inhibition as single inhibitors, which eventually gives rise to drug resistance and rescue from cell arrest by upregulation of receptor tyrosine kinase inhibitors as a result of c-Myc degradation[146]. Combination of MEK inhibitors with receptor tyrosine kinase inhibitors showed rapid response and increased apoptosis, in contrast to the response resulting from single agents.

In view of the many opportunities and high costs of clinical trials (from $50–100 million for a large randomized phase 3 study), it is important that prioritized drug combinations are evaluated in carefully planned, hypothesis-testing, biomarker-rich clinical studies. All early trials of new agents should, wherever possible, involve the use of predictive and/or enrichment biomarkers for patient selection together with pharmacodynamic biomarkers to assess target and pathway modulation in a Pharmacologic Audit Trail[16,19]. Moreover, special considerations apply to drug combinations, because careful attention to principles of patient selection, dose, schedule, exposure and target engagement alongside tolerability and efficacy is crucial to success. For example, such careful studies might in some cases predict the relative benefits of intermittent or pulsatile dosing rather than simultaneous co-administration[147]. It is critical to know if the molecular targets and pathways being modulated are hit hard enough and for long enough, as indicated from previous preclinical and clinical data[148].

Whereas phase 1 combination studies have been typically based on individual single-agent trials that defined the maximum tolerated and recommended doses together with optimal pharmacokinetic-pharmacodynamic profiles, clinical investigators carrying out early trials with drug combinations often now employ flexible designs that allow creative escalation or de-escalation of the doses of the component drugs, again with careful attention to drug exposures and target engagement[148]. Daily, weekly or intermittent dosing, potentially with drug holidays if required, can be used to manage toxicity. The overall aim is to get a dose and schedule that is not only effective in terms of optimal pharmacokinetic-pharmacodynamic effects, but also well tolerated. Based on modeling and network biology approaches, we are likely to have to carry out trials where individual agents demonstrate therapeutic activity only when used in appropriate combinations.

The potential to avoid known resistance mechanisms (e.g., evading efflux pumps and gatekeeper mutations) and the likely use in drug combinations (e.g., eliminating cytochrome P450-mediated and other metabolic drug-drug interactions and hERG liabilities) is now routinely factored into the chemical design and selection of small-molecule drug candidates[149]. Despite this, addictive toxicity still frequently limits the development of successful combinatorial regimens for cancer drugs. Early success with combinations of molecularly targeted agents featuring monoclonal antibodies was due at least in part to their excellent specificity, whereas small-molecule therapeutics commonly exhibit off-target as well as on-target effects[25,29]. Regimens combining multiple molecularly targeted agents are currently fraught with actual or potential additive on-target and off-target toxicity. For example, mechanistically attractive combinations of MEK and AKT inhibitors could cause additive toxicity involving skin rash and diarrhea. These issues need to be managed by careful target engagement and pathway biomarker evaluation, adaptive changes of administration schedule, and an appreciation that it may not always be necessary to administer both drugs at their single-agent maximum-tolerated doses. Attention to such factors has led to successful implementation of several combinations, resulting in clinical benefit that is not observed with the constituent single agents alone[66].

Detection of pathogenic driver mutations or amplifications, for example, with inhibitors of HER2, EGFR, BRAF and ALK, has led to considerable success in stratifying subsets of patients who are likely to respond to single, targeted agents or combinations thereof. However, there are currently very few scientifically validated and clinically qualified predictive response biomarkers[12]. Even where preclinical evidence indicates the usefulness of a predictive biomarker, there is less clarity as to whether molecular subtyping has led to prediction of a response in the clinic. For example, *PIK3CA* mutations were not necessarily predictive biomarkers of response for an mTOR inhibitor in the setting of estrogen receptor–positive breast cancer[150]. Next-generation sequencing is providing a more comprehensive assessment of mutational profiles that will facilitate the further refinement of patient stratification strategies in clinical trials of drug combinations[20].

It is now clear that combination treatment strategies will require longitudinal genetic assessment of the tumor state—ideally in the first instance by unbiased mutation and gene expression profiling—so that the therapy can be adapted as the tumor reacts dynamically to the perturbation and evolves under the selective pressure of treatment. Also valuable would be the broad assessment of signaling output at the protein level—both to choose the best drug combination and then to monitor network effects, for example, using phosphoproteomics[126]. Although repeat assessments currently require multiple tumor biopsies, ongoing and future developments in noninvasive functional imaging or refinement of technology to assess circulating tumor cells and other blood-borne markers, such as tumor DNA[151], could make the aspiration of real-time monitoring and therapeutic adaption a reality for clinical trials and potentially for routine use[41].

One important question that remains to be answered is whether it is preferable to use a full battery of combinatorial agents up-front or to administer simpler cocktails in a sequential fashion, with fewer drugs in each combination. Addressing this issue will require more work in preclinical models and in careful clinical trials. Past work from cancer and other diseases, together with computational and systems biology analysis, suggests that up-front treatment with the maximum combination of agents will commonly have greater impact. Sequential therapy may be valuable as demonstrated by the example of adjuvant endocrine treatment for breast cancer, which does not show an improved outcome when hormonal agents are administered concomitantly[152] but may be superior when agents are administered in sequence[153]. We should increasingly be able to predict adaptive responses and genetic resistance mechanisms from preclinical models, thereby informing clinical drug combinations.

Traditionally, the clinical development of new drugs has been pursued by evaluating one agent at a time—even for drugs that will almost inevitably end up in combinations—with the new drug often tested in large randomized phase 3 studies in combination with the standard of care as compared with the standard of care (usually cytotoxic agents) alone[154]. It has become clear that traditional approaches to phase 2 and 3 studies may no longer be appropriate in many situations. Moreover, such large inflexible clinical trials compete for patients and resources with other opportunities. There is increasing use of adaptive clinical trial designs, in which learning from initial cohorts informs subsequent decision making (**Fig. 5**). A Baysian perspective can be taken that facilitates the building of an efficient and accurate trial, as in the I-SPY2 study (NCT01042379), which evaluates drugs from several companies in a phase 2 screening process.

Although there is evidence of different companies working together on clinical trials of two unapproved drugs, as with the AKT and MEK study mentioned earlier[66], such cooperation still seems to be limited by commercial factors including intellectual property claims. One possible solution is to expand precompetitive, open-access drug discovery and clinical development ideas to incorporate drug combinations[155].

There is now evidence that the regulatory agencies are conscious of the current challenges and dilemmas inherent in combination studies and are evolving to accommodate the need for greater flexibility while at the same time ensuring that medicines are safe and effective[156]. Guidance on the development of novel combination therapies has been issued by the FDA[156] (http:/www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM236669.pdf). With the trend toward the inclusion of an expansion cohort in phase 1 studies comprising patients predicted to be sensitive, it can be argued that seeing a high response rate could lead directly to a New Drug Application or to a randomized phase 3 study[23]. The idea is to make active agents and effective combinations available to patients as early as possible in areas of high unmet medical need. Any risks can be managed by subsequent pharmacovigilance. Finally the cost of drug combinations must also be considered in health systems that are increasingly financially constrained[157] (http://asco.org/topfive).

Conclusions

Very recent research predicts a global surge in new cancer cases from 12.7 million in 2008 to 22.2 million by 2030, indicating a huge increase in unmet medical need for which effective drug combinations will be essential[158]. Powered by genomic technologies, astonishing progress has been made in our understanding of the genetics and biology of human cancers and in the discovery of molecularly targeted therapies for personalized medicine. This means that clinical trials can and should be underpinned by sound biological hypotheses. At the same time, the major challenges of extensive tumor heterogeneity, clonal selection and adaptive feedback loops have now been recognized, providing a new genetic and biochemical explanation for the enduring and shape-shifting nature of drug resistance. It seems that some things never change. To the pessimistic, the personalized cancer medicine glass suddenly looks emptier than it did with imatinib in chronic myeloid leukemia. A more optimistic view is that the glass is half full, but that we need to redouble our efforts to be more sophisticated

in our use of the powerful technologies at our disposal, including smart drugs, validated biomarkers and next-generation sequencing, other omics technologies and molecular imaging, to overcome the redoubtable foe that is cancer. It is clear that drug combinations currently provide a route, and possibly the only route to overcome the complexity of human cancers. The drug combinations field has moved on considerably from the previous dominance of empirical clinical studies that were based on ad hoc preclinical investigations seeking to identify synergistic or additive interactions with existing agents, or on pragmatically combining clinically active drugs with nonoverlapping toxicity, to the use of rational approaches driven by biological hypotheses based on preventing or overcoming known polygenic-resistance mechanisms and feedback loops. Clinical trials featuring adaptive designs and the Pharmacologic Audit Trail with predictive and molecular response biomarkers will increasingly be underpinned by computational and experimental network biology science. Combining drugs based on increasingly well-understood molecular interactions and attacking complementary cancer hallmarks or distinct cell populations in heterogeneous tumors is now imperative. Emphasis over the last few years has been on combining addiction-blocking drugs with each other, or combining these molecularly targeted agents with cytotoxic chemotherapy, in patient subgroups that are increasingly defined by predictive biomarkers. We now need to understand better how to include drugs targeting synthetic lethal mechanisms (and other nononcogene addiction drugs) within drug combinations, and to determine the extent to which they can be combined successfully with chemotherapy drugs, other targeted agents and immunotherapy without increasing toxicity. There is no question that success in defeating cancer depends on sophisticated weaponry that includes a battery of rationally designed drug combinations, constructed and administered according to the molecular pathology and network biology of the particular tumor, in the individual patient, at the particular time in question (**Fig. 5**). In this way we can achieve truly personalized, precision medicine for individual cancer patients. We face a major challenge, but we have powerful tools to tackle it.

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COMPETING FINANCIAL INTERESTS

The authors declare competing financial interests: details are available in the online version of the paper.

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1. Mukherjee, S. *The Emperor of All Maladies* (Scribner Book Company, 2011).
2. DeVita, V.T. Jr., Young, R.C. & Canellos, G.P. Combination versus single agent chemotherapy: a review of the basis for selection of drug treatment of cancer. *Cancer* **35**, 98–110 (1975).
3. Chabner, B.A. & Roberts, T.G. Jr. Timeline: chemotherapy and the war on cancer. *Nat. Rev. Cancer* **5**, 65–72 (2005).
4. Espinal, M.A. *et al.* Standard short-course chemotherapy for drug-resistant tuberculosis: treatment outcomes in 6 countries. *J. Am. Med. Assoc.* **283**, 2537–2545 (2000).
5. Hammer, S.M. *et al.* Treatment for adult HIV infection: 2006 recommendations of the International AIDS Society-USA panel. *J. Am. Med. Assoc.* **296**, 827–843 (2006).
6. Brockman, R.W. Mechanisms of resistance to anticancer agents. *Adv. Cancer Res.* **7**, 129–234 (1963).
7. Schimke, R.T., Kaufman, R.J., Alt, F.W. & Kellems, R.F. Gene amplification and drug resistance in cultured murine cells. *Science* **202**, 1051–1055 (1978).
8. Juliano, R.L. & Ling, V. A surface glycoprotein modulating drug permeability in Chinese hamster ovary cell mutants. *Biochim. Biophys. Acta* **455**, 152–162 (1976).
9. Gottesman, M.M. Mechanisms of cancer drug resistance. *Annu. Rev. Med.* **53**, 615–627 (2002).
10. Greaves, M. & Maley, C.C. Clonal evolution in cancer. *Nature* **481**, 306–313 (2012).
11. Gerlinger, M. *et al.* Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. *N. Engl. J. Med.* **366**, 883–892 (2012).
12. Yap, T.A., Gerlinger, M., Futreal, P.A., Pusztai, L. & Swanton, C. Intratumor heterogeneity: seeing the wood for the trees. *Sci. Transl. Med.* **4**, 127ps110 (2010).
13. Anderson, K. *et al.* Genetic variegation of clonal architecture and propagating cells in leukaemia. *Nature* **469**, 356–361 (2011).
14. Garraway, L.A. & Janne, P.A. Circumventing cancer drug resistance in the era of personalized medicine. *Cancer Discov.* **2**, 214–226 (2012).
15. de Bono, J.S. & Ashworth, A. Translating cancer research into targeted therapeutics. *Nature* **467**, 543–549 (2010).
16. Yap, T.A. & Workman, P. Exploiting the cancer genome: strategies for the discovery and clinical development of targeted molecular therapeutics. *Annu. Rev. Pharmacol. Toxicol.* **52**, 549–573 (2012).
17. Weinstein, I.B. Cancer. Addiction to oncogenes–the Achilles heal of cancer. *Science* **297**, 63–64 (2002).
18. Weinstein, I.B. & Joe, A. Oncogene addiction. *Cancer Res.* **68**, 3077–3080, discussion 3080 (2008).
19. Yap, T.A., Sandhu, S.K., Workman, P. & de Bono, J.S. Envisioning the future of early anticancer drug development. *Nat. Rev. Cancer* **10**, 514–523 (2010).
20. MacConaill, L.E. & Garraway, L.A. Clinical implications of the cancer genome. *J. Clin. Oncol.* **28**, 5219–5228 (2010).
21. Druker, B.J. *et al.* Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia. *N. Engl. J. Med.* **344**, 1031–1037 (2001).
22. Joensuu, H. *et al.* Effect of the tyrosine kinase inhibitor STI571 in a patient with a metastatic gastrointestinal stromal tumor. *N. Engl. J. Med.* **344**, 1052–1056 (2001).
23. Sellers, W.R. A blueprint for advancing genetics-based cancer therapy. *Cell* **147**, 26–31 (2011).
24. Huang, M.E. *et al.* Use of all-trans retinoic acid in the treatment of acute promyelocytic leukemia. *Blood* **72**, 567–572 (1988).
25. Slamon, D.J. *et al.* Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. *N. Engl. J. Med.* **344**, 783–792 (2001).
26. Mok, T.S. *et al.* Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. *N. Engl. J. Med.* **361**, 947–957 (2009).
27. Chapman, P.B. *et al.* Improved survival with vemurafenib in melanoma with BRAF V600E mutation. *N. Engl. J. Med.* **364**, 2507–2516 (2011).
28. Kwak, E.L. *et al.* Anaplastic lymphoma kinase inhibition in non-small-cell lung cancer. *N. Engl. J. Med.* **363**, 1693–1703 (2010).
29. Cunningham, D. *et al.* Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer. *N. Engl. J. Med.* **351**, 337–345 (2004).
30. Geyer, C.E. *et al.* Lapatinib plus capecitabine for HER2-positive advanced breast cancer. *N. Engl. J. Med.* **355**, 2733–2743 (2006).
31. Fong, P.C. *et al.* Inhibition of poly(ADP-ribose) polymerase in tumors from BRCA mutation carriers. *N. Engl. J. Med.* **361**, 123–134 (2009).
32. Neckers, L. & Workman, P. Hsp90 molecular chaperone inhibitors: are we there yet? *Clin. Cancer Res.* **18**, 64–76 (2012).
33. Prinz, F., Schlange, T. & Asadullah, K. Believe it or not: how much can we rely on published data on potential drug targets? *Nat. Rev. Drug Discov.* **10**, 712 (2011).
34. Yun, C.H. *et al.* The T790M mutation in EGFR kinase causes drug resistance by increasing the affinity for ATP. *Proc. Natl. Acad. Sci. USA* **105**, 2070–2075 (2008).
35. Shah, N.P. *et al.* Multiple BCR-ABL kinase domain mutations confer polyclonal resistance to the tyrosine kinase inhibitor imatinib (STI571) in chronic phase and blast crisis chronic myeloid leukemia. *Cancer Cell* **2**, 117–125 (2002).
36. Choi, H.G. *et al.* A type-II kinase inhibitor capable of inhibiting the T315I "gatekeeper" mutant of Bcr-Abl. *J. Med. Chem.* **53**, 5439–5448 (2010).
37. Catalanotti, F. & Solit, D.B. Will Hsp90 Inhibitors Prove Effective in BRAF-Mutant Melanomas? *Clin. Cancer Res.* **18**, 2420–2422 (2012).
38. Johannessen, C.M. *et al.* COT drives resistance to RAF inhibition through MAP kinase pathway reactivation. *Nature* **468**, 968–972 (2010).
39. Poulikakos, P.I. *et al.* RAF inhibitor resistance is mediated by dimerization of aberrantly spliced BRAF(V600E). *Nature* **480**, 387–390 (2011).
40. Xing, F. *et al.* Concurrent loss of the PTEN and RB1 tumor suppressors attenuates RAF dependence in melanomas harboring (V600E)BRAF. *Oncogene* **31**, 446–457 (2012).
41. Chen, R. *et al.* Personal omics profiling reveals dynamic molecular and medical phenotypes. *Cell* **148**, 1293–1307 (2012).

42. Halling-Brown, M.D., Bulusu, K.C., Patel, M., Tym, J.E. & Al-Lazikani, B. canSAR: an integrated cancer public translational research and drug discovery resource. *Nucleic Acids Res.* **40**, D947–D956 (2012).
43. Futreal, P.A. *et al.* A census of human cancer genes. *Nat. Rev. Cancer* **4**, 177–183 (2004).
44. Hawkins, R.D., Hon, G.C. & Ren, B. Next-generation genomics: an integrative approach. *Nat. Rev. Genet.* **11**, 476–486 (2010).
45. Iadevaia, S., Lu, Y., Morales, F.C., Mills, G.B. & Ram, P.T. Identification of optimal drug combinations targeting cellular networks: integrating phospho-proteomics and computational network analysis. *Cancer Res.* **70**, 6704–6714 (2010).
46. Yan, H., Zhang, B., Li, S. & Zhao, Q. A formal model for analyzing drug combination effects and its application in TNF-alpha-induced NFkappaB pathway. *BMC Syst. Biol.* **4**, 50 (2010).
47. Coiffier, B. *et al.* CHOP chemotherapy plus rituximab compared with CHOP alone in elderly patients with diffuse large-B-cell lymphoma. *N. Engl. J. Med.* **346**, 235–242 (2002).
48. Sobrero, A.F. *et al.* EPIC: phase III trial of cetuximab plus irinotecan after fluoropyrimidine and oxaliplatin failure in patients with metastatic colorectal cancer. *J. Clin. Oncol.* **26**, 2311–2319 (2008).
49. Jackman, A., Kaye, S. & Workman, P. The combination of cytotoxic and molecularly targeted therapies - can it be done? *Drug Discov. Today* **1**, 445–454 (2004).
50. Rodon, J., Perez, J. & Kurzrock, R. Combining targeted therapies: practical issues to consider at the bench and bedside. *Oncologist* **15**, 37–50 (2010).
51. Albain, K.S. *et al.* Adjuvant chemotherapy and timing of tamoxifen in postmenopausal patients with endocrine-responsive, node-positive breast cancer: a phase 3, open-label, randomised controlled trial. *Lancet* **374**, 2055–2063 (2009).
52. Garrett, M.D. & Collins, I. Anticancer therapy with checkpoint inhibitors: what, where and when? *Trends Pharmacol. Sci.* **32**, 308–316 (2011).
53. Sergina, N.V. *et al.* Escape from HER-family tyrosine kinase inhibitor therapy by the kinase-inactive HER3. *Nature* **445**, 437–441 (2007).
54. Nahta, R., Hung, M.C. & Esteva, F.J. The HER-2-targeting antibodies trastuzumab and pertuzumab synergistically inhibit the survival of breast cancer cells. *Cancer Res.* **64**, 2343–2346 (2004).
55. Chandarlapaty, S. *et al.* Inhibitors of HSP90 block p95–HER2 signaling in Trastuzumab-resistant tumors and suppress their growth. *Oncogene* **29**, 325–334 (2010).
56. Modi, S. *et al.* Combination of trastuzumab and tanespimycin (17-AAG, KOS-953) is safe and active in trastuzumab-refractory HER-2 overexpressing breast cancer: a phase I dose-escalation study. *J. Clin. Oncol.* **25**, 5410–5417 (2007).
57. Eccles, S.A. *et al.* NVP-AUY922: a novel heat shock protein 90 inhibitor active against xenograft tumor growth, angiogenesis, and metastasis. *Cancer Res.* **68**, 2850–2860 (2008).
58. Kelland, L.R., Sharp, S.Y., Rogers, P.M., Myers, T.G. & Workman, P. DT-Diaphorase expression and tumor cell sensitivity to 17-allylamino, 17-demethoxygeldanamycin, an inhibitor of heat shock protein 90. *J. Natl. Cancer Inst.* **91**, 1940–1949 (1999).
59. Gaspar, N. *et al.* P. Acquired resistance to 17-allylamino-17-demethoxygeldanamycin (17-AAG, tanespimycin) in glioblastoma cells. *Cancer Res.* **69**, 1966–1975 (2009).
60. Kataoka, Y. *et al.* Association between gain-of-function mutations in PIK3CA and resistance to HER2-targeted agents in HER2-amplified breast cancer cell lines. *Ann. Oncol.* **21**, 255–262 (2010).
61. Nagata, Y. *et al.* PTEN activation contributes to tumor inhibition by trastuzumab, and loss of PTEN predicts trastuzumab resistance in patients. *Cancer Cell* **6**, 117–127 (2004).
62. de Bono, J.S. *et al.* Abiraterone and increased survival in metastatic prostate cancer. *N. Engl. J. Med.* **364**, 1995–2005 (2011).
63. Scher, H.I. *et al.* Prostate Cancer Foundation/Department of Defense Prostate Cancer Clinical Trials Consortium. Antitumour activity of MDV3100 in castration-resistant prostate cancer: a phase 1–2 study. *Lancet* **375**, 1437–1446 (2010).
64. Meng, J. *et al.* High level of AKT activity is associated with resistance to MEK inhibitor AZD6244 (ARRY-142886). *Cancer Biol. Ther.* **8**, 2073–2080 (2009).
65. Meng, J. *et al.* Combination treatment with MEK and AKT inhibitors is more effective than each drug alone in human non-small cell lung cancer *in vitro* and *in vivo*. *PLoS ONE* **5**, e14124 (2010).
66. Tolcher, A. *et al.* A phase I dose escalation study of oral MK-2206 (allosteric AKT inhibitor) with oral selumetinib (AZD6244; MEK inhibitor) in patients with advanced or metastatic solid tumours. *J. Clin. Oncol.* **29** (suppl.), Abstract 3004 (2011).
67. Shah, O.J., Wang, Z. & Hunter, T. Inappropriate activation of the TSC/Rheb/mTOR/S6K cassette induces IRS1/2 depletion, insulin resistance, and cell survival deficiencies. *Curr. Biol.* **14**, 1650–1656 (2004).
68. Rodrik-Outmezguine, V.S. *et al.* mTOR kinase inhibition causes feedback-dependent biphasic regulation of AKT signaling. *Cancer Discov.* **1**, 248–259 (2011).
69. Cosimo, S. *et al.* A phase I study of the mTOR inhibitor ridaforolimus (RIDA) in combination with IGFR-1R antibody dalotozumab (DALO) in patients with advanced tumours. *J. Clin. Oncol.* **28** (suppl.), Abstract 3008 (2010).
70. Falchook, G. *et al.* A phase I study of bevacizumab in combination with sunitinib, sorafenib and erlotinib plus cituximab and trastuzumab plus lapatinib. *J. Clin. Oncol.* **28** (suppl.), Abstract 2512 (2010).
71. Heidorn, S.J. *et al.* Kinase-dead BRAF and oncogenic RAS cooperate to drive tumor progression through CRAF. *Cell* **140**, 209–221 (2010).
72. Poulikakos, P.I., Zhang, C., Bollag, G., Shokat, K.M. & Rosen, N. RAF inhibitors transactivate RAF dimers and ERK signalling in cells with wild-type BRAF. *Nature* **464**, 427–430 (2010).
73. Infante, J. *et al.* Phase I/II study to assess safely, pharmacokinetics and efficacy of the oral MEK 1/2 inhibitor GSK1120212 (GSK212) dosed in combination with the oral BRAF inhibitor GSK2118436 (GSK436). *J. Clin. Oncol.* **29** (suppl.), Abstract CRA8503 (2011).
74. Escudier, B. *et al.* Sorafenib in advanced clear-cell renal-cell carcinoma. *N. Engl. J. Med.* **356**, 125–134 (2007).
75. Wells, S.A. Jr. *et al.* Vandetanib in patients with locally advanced or metastatic medullary thyroid cancer: a randomized, double-blind phase III trial. *J. Clin. Oncol.* **30**, 134–141 (2012).
76. Eder, J.P. *et al.* A phase I study of foretinib, a multi-targeted inhibitor of c-Met and vascular endothelial growth factor receptor 2. *Clin. Cancer Res.* **16**, 3507–3516 (2010).
77. George, S. *et al.* Efficacy and safety of regorafenib in patients with metastatic and/or unresectable GI stromal tumor after failure of imatinib and sunitinib: a multicenter phase ii trial. *J. Clin. Oncol.*, published online, doi: 10.1200/JCO.2011.39.9394 (21 May 2012).
78. Apsel, B. *et al.* Targeted polypharmacology: discovery of dual inhibitors of tyrosine and phosphoinositide kinases. *Nat. Chem. Biol.* **4**, 691–699 (2008).
79. Shuttleworth, S.J. *et al.* Progress in the preclinical discovery and clinical development of class I and dual class I/IV phosphoinositide 3-kinase (PI3K) inhibitors. *Curr. Med. Chem.* **18**, 2686–2714 (2011).
80. Pearl, L.H., Prodromou, C. & Workman, P. The Hsp90 molecular chaperone: an open and shut case for treatment. *Biochem. J.* **410**, 439–453 (2008).
81. Banerji, U. Heat shock protein 90 as a drug target: some like it hot. *Clin. Cancer Res.* **15**, 9–14 (2009).
82. Lane, A.A. & Chabner, B.A. Histone deacetylase inhibitors in cancer therapy. *J. Clin. Oncol.* **27**, 5459–5468 (2009).
83. Moffat, D. *et al.* Discovery of 2-(6-{[(6-fluoroquinolin-2-yl)methyl]amino}bicyclo[3.1.0]hex-3-yl)-N-hydroxypyrim idine-5-carboxamide (CHR-3996), a class I selective orally active histone deacetylase inhibitor. *J. Med. Chem.* **53**, 8663–8678 (2010).
84. Sharma, S.V. *et al.* A chromatin-mediated reversible drug-tolerant state in cancer cell subpopulations. *Cell* **141**, 69–80 (2010).
85. Hodi, F.S. *et al.* Improved survival with ipilimumab in patients with metastatic melanoma. *N. Engl. J. Med.* **363**, 711–723 (2010).
86. Hanahan, D. & Weinberg, R.A. The hallmarks of cancer. *Cell* **100**, 57–70 (2000).
87. Hanahan, D. & Weinberg, R.A. Hallmarks of cancer: the next-generation. *Cell* **144**, 646–674 (2011).
88. De Palma, M. & Hanahan, D. The biology of personalized cancer medicine: facing individual complexities underlying hallmark capabilities. *Mol. Oncol.* **6**, 111–127 (2012).
89. Keith, C.T., Borisy, A.A. & Stockwell, B.R. Multicomponent therapeutics for networked systems. *Nat. Rev. Drug Discov.* **4**, 71–78 (2005).
90. Lee, M.S. *et al.* The novel combination of chlorpromazine and pentamidine exerts synergistic antiproliferative effects through dual mitotic action. *Cancer Res.* **67**, 11359–11367 (2007).
91. Lehar, J., Stockwell, B.R., Giaever, G. & Nislow, C. Combination chemical genetics. *Nat. Chem. Biol.* **4**, 674–681 (2008).
92. Wei, G. *et al.* Chemical genomics identifies small-molecule MCL1 repressors and BCL-xL as a predictor of MCL1 dependency. *Cancer Cell* **21**, 547–562 (2012).
93. Iorns, E., Lord, C.J., Turner, N. & Ashworth, A. Utilizing RNA interference to enhance cancer drug discovery. *Nat. Rev. Drug Discov.* **6**, 556–568 (2007).
94. Mullenders, J. & Bernards, R. Loss-of-function genetic screens as a tool to improve the diagnosis and treatment of cancer. *Oncogene* **28**, 4409–4420 (2009).
95. Costanzo, M. *et al.* The genetic landscape of a cell. *Science* **327**, 425–431 (2010).
96. Giaever, G. *et al.* Chemogenomic profiling: identifying the functional interactions of small molecules in yeast. *Proc. Natl. Acad. Sci. USA* **101**, 793–798 (2004).
97. Berns, K. *et al.* A functional genetic approach identifies the PI3K pathway as a major determinant of trastuzumab resistance in breast cancer. *Cancer Cell* **12**, 395–402 (2007).
98. Iorns, E. *et al.* Identification of CDK10 as an important determinant of resistance to endocrine therapy for breast cancer. *Cancer Cell* **13**, 91–104 (2008).
99. Prahallad, A. *et al.* Unresponsiveness of colon cancer to BRAF(V600E) inhibition through feedback activation of EGFR. *Nature* **483**, 100–103 (2012).
100. Garnett, M.J. *et al.* Systematic identification of genomic markers of drug sensitivity in cancer cells. *Nature* **483**, 570–575 (2012).
101. Barretina, J. *et al.* The Cancer Cell Line Encyclopedia enables predictive modelling of anticancer drug sensitivity. *Nature* **483**, 603–607 (2012).
102. Loewe, S. Die quantitativen. Probleme der Pharmakologie. *Ergeb. Physiol.* **27**, 47–187 (1928).
103. Goldie, J.H. & Coldman, A.J. A mathematic model for relating the drug sensitivity of tumors to their spontaneous mutation rate. *Cancer Treat. Rep.* **63**, 1727–1733 (1979).
104. Chou, T.C. & Talalay, P. Quantitative analysis of dose-effect relationships: the combined effects of multiple drugs or enzyme inhibitors. *Adv. Enzyme Regul.* **22**, 27–55 (1984).

105. Berenbaum, M.C. The expected effect of a combination of agents: the general solution. *J. Theor. Biol.* **114**, 413–431 (1985).
106. Harrap, K.R. & Jackson, R.C. Enzyme kinetics and combination chemotherapy: an appraisal of current concepts. *Adv. Enzyme Regul.* **13**, 77–96 (1975).
107. Jackson, R.C. Kinetic simulation of anticancer drug interactions. *Int. J. Biomed. Comput.* **11**, 197–224 (1980).
108. Lehar, J. *et al.* Chemical combination effects predict connectivity in biological systems. *Mol. Syst. Biol.* **3**, 80 (2007).
109. Peifer, M. *et al.* Analysis of compound synergy in high-throughput cellular screens by population-based lifetime modeling. *PLoS ONE* **5**, e8919 (2010).
110. Hood, L., Heath, J.R., Phelps, M.E. & Lin, B. Systems biology and new technologies enable predictive and preventative medicine. *Science* **306**, 640–643 (2004).
111. Lage, K. *et al.* A human phenome-interactome network of protein complexes implicated in genetic disorders. *Nat. Biotechnol.* **25**, 309–316 (2007).
112. Little, M.P. Cancer models, genomic instability and somatic cellular Darwinian evolution. *Biol. Direct* **5**, 19 (2010).
113. Gerlinger, M. & Swanton, C. How Darwinian models inform therapeutic failure initiated by clonal heterogeneity in cancer medicine. *Br. J. Cancer* **103**, 1139–1143 (2010).
114. Navin, N. *et al.* Tumour evolution inferred by single-cell sequencing. *Nature* **472**, 90–94 (2011).
115. Campbell, P.J. *et al.* Subclonal phylogenetic structures in cancer revealed by ultra-deep sequencing. *Proc. Natl. Acad. Sci. USA* **105**, 13081–13086 (2008).
116. Komarova, N.L. & Wodarz, D. Evolutionary dynamics of mutator phenotypes in cancer: implications for chemotherapy. *Cancer Res.* **63**, 6635–6642 (2003).
117. Foo, J. & Michor, F. Evolution of resistance to anti-cancer therapy during general dosing schedules. *J. Theor. Biol.* **263**, 179–188 (2010).
118. Chmielecki, J. *et al.* Optimization of dosing for EGFR-mutant non-small cell lung cancer with evolutionary cancer modeling. *Sci. Transl. Med.* **3**, 90ra59 (2011).
119. Mumenthaler, S.M. *et al.* Evolutionary modeling of combination treatment strategies to overcome resistance to tyrosine kinase inhibitors in non-small cell lung cancer. *Mol. Pharm.* **8**, 2069–2079 (2011).
120. Saez-Rodriguez, J. *et al.* Comparing signaling networks between normal and transformed hepatocytes using discrete logical models. *Cancer Res.* **71**, 5400–5411 (2011).
121. Hughey, J.J., Lee, T.K. & Covert, M.W. Computational modeling of mammalian signaling networks. *Wiley Interdiscip. Rev. Syst. Biol. Med.* **2**, 194–209 (2010).
122. Clarke, P.A., te Poele, R., Wooster, R. & Workman, P. Gene expression microarray analysis in cancer biology, pharmacology, and drug development: progress and potential. *Biochem. Pharmacol.* **62**, 1311–1336 (2001).
123. Haw, R., Hermjakob, H., D'Eustachio, P. & Stein, L. Reactome pathway analysis to enrich biological discovery in proteomics data sets. *Proteomics* **11**, 3598–3613 (2011).
124. Lu, Y. *et al.* Kinome siRNA-phosphoproteomic screen identifies networks regulating AKT signaling. *Oncogene* **30**, 4567–4577 (2011).
125. Geho, D.H., Petricoin, E.F., Liotta, L.A. & Araujo, R.P. Modeling of protein signaling networks in clinical proteomics. *Cold Spring Harb. Symp. Quant. Biol.* **70**, 517–524 (2005).
126. Huang, P.H. *et al.* Quantitative analysis of EGFRvIII cellular signaling networks reveals a combinatorial therapeutic strategy for glioblastoma. *Proc. Natl. Acad. Sci. USA* **104**, 12867–12872 (2007).
127. Mulero-Navarro, S. & Esteller, M. Epigenetic biomarkers for human cancer: the time is now. *Crit. Rev. Oncol. Hematol.* **68**, 1–11 (2008).
128. Hornbeck, P.V., Chabra, I., Kornhauser, J.M., Skrzypek, E. & Zhang, B. PhosphoSite: a bioinformatics resource dedicated to physiological protein phosphorylation. *Proteomics* **4**, 1551–1561 (2004).
129. Sachs, K., Perez, O., Pe'er, D., Lauffenburger, D.A. & Nolan, G.P. Causal protein-signaling networks derived from multiparameter single-cell data. *Science* **308**, 523–529 (2005).
130. Takahashi, K., Tanase-Nicola, S. & ten Wolde, P.R. Spatio-temporal correlations can drastically change the response of a MAPK pathway. *Proc. Natl. Acad. Sci. USA* **107**, 2473–2478 (2010).
131. Huang, S. & Ingber, D.E. Shape-dependent control of cell growth, differentiation, and apoptosis: switching between attractors in cell regulatory networks. *Exp. Cell Res.* **261**, 91–103 (2000).
132. Morris, M.K., Saez-Rodriguez, J., Clarke, D.C., Sorger, P.K. & Lauffenburger, D.A. Training signaling pathway maps to biochemical data with constrained fuzzy logic: quantitative analysis of liver cell responses to inflammatory stimuli. *PLoS Comput. Biol.* **7**, e1001099 (2011).
133. Natarajan, M., Lin, K.M., Hsueh, R.C., Sternweis, P.C. & Ranganathan, R. A global analysis of cross-talk in a mammalian cellular signalling network. *Nat. Cell Biol.* **8**, 571–580 (2006).
134. Folger, O. *et al.* Predicting selective drug targets in cancer through metabolic networks. *Mol. Syst. Biol.* **7**, 501 (2011).
135. Luo, J. *et al.* A genome-wide RNAi screen identifies multiple synthetic lethal interactions with the Ras oncogene. *Cell* **137**, 835–848 (2009).
136. Lee, M.J. *et al.* Sequential application of anticancer drugs enhances cell death by rewiring apoptotic signaling networks. *Cell* **149**, 780–794 (2012).
137. Zhao, X.M. *et al.* Prediction of drug combinations by integrating molecular and pharmacological data. *PLoS Comput. Biol.* **7**, e1002323 (2011).
138. Ayyadurai, V.A. & Dewey, C.F. CytoSolve: a scalable computational method for dynamic integration of multiple molecular pathway models. *Cell Mol. Bioeng.* **4**, 28–45 (2011).
139. Li, C. *et al.* BioModels Database: An enhanced, curated and annotated resource for published quantitative kinetic models. *BMC Syst. Biol.* **4**, 92 (2010).
140. Workman, P., Clarke, P.A. & Al-Lazikani, B. Personalized medicine: patient-predictive panel power. *Cancer Cell* **21**, 455–458 (2012).
141. Workman, P. *et al.* Guidelines for the welfare and use of animals in cancer research. *Br. J. Cancer* **102**, 1555–1577 (2010).
142. Dow, L.E. & Lowe, S.W. Life in the fast lane: mammalian disease models in the genomics era. *Cell* **148**, 1099–1109 (2012).
143. Singh, M., Murriel, C.L. & Johnson, L. Genetically engineered mouse models: closing the gap between preclinical data and trial outcomes. *Cancer Res.* **72**, 2695–2700 (2012).
144. Singh, M. *et al.* Assessing therapeutic responses in Kras mutant cancers using genetically engineered mouse models. *Nat. Biotechnol.* **28**, 585–593 (2010).
145. Engelman, J.A. *et al.* Effective use of PI3K and MEK inhibitors to treat mutant Kras G12D and PIK3CA H1047R murine lung cancers. *Nat. Med.* **14**, 1351–1356 (2008).
146. Duncan, J.S. *et al.* Dynamic reprogramming of the kinome in response to targeted MEK inhibition in triple-negative breast cancer. *Cell* **149**, 307–321 (2012).
147. Solit, D.B. *et al.* Pulsatile administration of the epidermal growth factor receptor inhibitor gefitinib is significantly more effective than continuous dosing for sensitizing tumors to paclitaxel. *Clin. Cancer Res.* **11**, 1983–1989 (2005).
148. Yap, T.A., Omlin, A. & de Bono, J.S. The development of therapeutic combinations targeting major cancer signaling pathways. *J. Clin. Oncol.* (in the press).
149. Hoelder, S., Clarke, P.A. & Workman, P. Discovery of small molecule cancer drugs: Successes, challenges and opportunities. *Mol. Oncol.* **6**, 155–176 (2012).
150. Baselga, J. *et al.* Everolimus in postmenopausal hormone-receptor-positive advanced breast cancer. *N. Engl. J. Med.* **366**, 520–529 (2012).
151. Higgins, M.J. *et al.* Detection of tumor PIK3CA status in metastatic breast cancer using peripheral blood. *Clin. Cancer Res.* published online, doi:10.1158/1078-0432 (15 March 2012).
152. Baum, M. *et al.* Anastrozole alone or in combination with tamoxifen versus tamoxifen alone for adjuvant treatment of postmenopausal women with early breast cancer: first results of the ATAC randomised trial. *Lancet* **359**, 2131–2139 (2002).
153. Coombes, R.C. *et al.* Survival and safety of exemestane versus tamoxifen after 2–3 years' tamoxifen treatment (Intergroup Exemestane Study): a randomised controlled trial. *Lancet* **369**, 559–570 (2007).
154. Berry, D.A. Adaptive clinical trials in oncology. *Nat. Rev. Clin. Oncol.* **9**, 199–207 (2012).
155. Edwards, A.M., Bountra, C., Kerr, D.J. & Willson, T.M. Open access chemical and clinical probes to support drug discovery. *Nat. Chem. Biol.* **5**, 436–440 (2009).
156. Woodcock, J., Griffin, J.P. & Behrman, R.E. Development of novel combination therapies. *N. Engl. J. Med.* **364**, 985–987 (2011).
157. Brody, H. From an ethics of rationing to an ethics of waste avoidance. *N. Engl. J. Med.* **366**, 1949–1951 (2012).
158. Bray, F., Jemal, A., Grey, N., Ferlay, J. & Forman, D. Global cancer transitions according to the Human Development Index (2008–2030): a population-based study. *Lancet Oncol.* published online, doi:10.1016/S1470-2045(12)70211-52012 (1 June 2012).
159. Salwinski, L. *et al.* The Database of Interacting Proteins: 2004 update. *Nucleic Acids Res.* **32**, D449–D451 (2004).
160. Aranda, B. *et al.* The IntAct molecular interaction database in 2010. *Nucleic Acids Res.* **38**, D525–D531 (2010).
161. Ceol, A. *et al.* MINT, the molecular interaction database: 2009 update. *Nucleic Acids Res.* **38**, D532–D539 (2010).
162. Cerami, E.G. *et al.* Pathway Commons, a web resource for biological pathway data. *Nucleic Acids Res.* **39**, D685–D690 (2011).
163. Bickerton, G.R., Higueruelo, A.P. & Blundell, T.L. Comprehensive, atomic-level characterization of structurally characterized protein-protein interactions: the PICCOLO database. *BMC Bioinformatics* **12**, 313 (2011).
164. Croft, D. *et al.* Reactome: a database of reactions, pathways and biological processes. *Nucleic Acids Res.* **39**, D691–D697 (2011).
165. Sims, D. *et al.* ROCK: a breast cancer functional genomics resource. *Breast Cancer Res. Treat.* **124**, 567–572 (2010).
166. Szklarczyk, D. *et al.* The STRING database in 2011: functional interaction networks of proteins, globally integrated and scored. *Nucleic Acids Res.* **39**, D561–D568 (2011).
167. Stark, C. *et al.* The BioGRID Interaction Database: 2011 update. *Nucleic Acids Res.* **39**, D698–D704 (2011).