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Multi-target therapeutics: when the whole is greater than the sum of the parts

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Drugs designed to act against individual molecular targets cannot usually combat multigenic diseases such as cancer, or diseases that affect multiple tissues or cell types such as diabetes and immunoinflammatory disorders. Combination drugs that impact multiple targets simultaneously are better at controlling complex disease systems, are less prone to drug resistance and are the standard of care in many important therapeutic areas. The combination drugs currently employed are primarily of rational design, but the increased efficacy they provide justifies *in vitro* discovery efforts for identifying novel multi-target mechanisms. In this review, we discuss the biological rationale for combination therapeutics, review some existing combination drugs and present a systematic approach to identify interactions between molecular pathways that could be leveraged for therapeutic benefit.

Introduction

Recent decades have seen considerable research efforts invested in the discovery and development of therapeutics that modulate individual disease-modifying targets. Although this approach has led to growth in the industry and numerous successful drugs reaching the market, unfortunately few new drugs act at novel molecular targets. Successful development of first-in-class drugs is challenging, in part because agents directed against individual molecular targets are often found to be less effective at treating disease and, therefore, reach the market later than hoped [1]. In some cases, the poor efficacy of these agents can be attributed to buffering effects in which the biological system utilizes a redundant mechanism or a drug-mitigating response [2]. Consequently, many single-target drugs cannot fully correct a complex disease condition such as cancer [3].

The limitations of many monotherapies can be overcome by attacking the disease system on multiple fronts [4]. Multi-target therapeutics can be more efficacious and less vulnerable to adaptive resistance because the biological system is less able to compensate for the action of two or more drugs simultaneously. Indeed, multi-component drugs are now standard in therapeutic areas such as cancer, diabetes and infectious disease; paradoxically composed of agents that were initially developed as single-target drugs. Unfortunately, the standard approach of combining monotherapies at the clinical stage limits the number of drug pairs that can be tested and bypasses the opportunity to find therapeutically relevant interactions between novel targets.

The systematic pursuit of combination drugs *in vitro* can identify these unexpected multi-target mechanisms, but necessitates large-scale searches of a vast space of possible target combinations using cell-based experiments that preserve the essential elements of the disease network. Combination searches using active pharmaceutical ingredients can be especially valuable because potential synergies identified by these screens can rapidly move into preclinical and clinical development [5]. In addition, combination effects between compounds with known biomolecular targets can reveal unexpected interactions between disease-relevant pathways [6]. One day, this pathway-focused approach to target discovery could help lead to a greater understanding of disease biology [7].

This article will review some of the multi-target therapeutics that are currently on the market or in development, and outline some of the important aspects of the discovery of multi-target therapeutics using compounds and cell-based *in vitro* assays.

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Rationale for multi-target therapeutics

Disease systems are characterized by the dysregulation of biological pathways, and it is challenging to identify drugs with molecular targets that can restore the healthy state of an individual when modulated in isolation. Disease-causing dysregulation of cellular processes can result from genetic mutations and from environmental factors that lead to epigenetic changes in gene expression [8,9]. Collectively, these changes alter the expression of proteins in multiple cellular pathways, leading to changes at the individual cell level (e.g. growth, differentiation or apoptosis), the tissue level and, ultimately, the disease state. The strategy behind many modern pharmaceuticals is to restore the healthy state by inhibiting a molecular target that is central to the disease mechanism. Drug discovery efforts are, therefore, crucially dependent on identifying individual molecular targets and validating their relevance to a human disease. This target validation is followed by identification of specific chemical- or antibody-based modulators of the target. Although the industry is adept at identifying and optimizing inhibitors, validation of the disease-relevance of biological targets remains extremely difficult and can often take decades to complete [10]. In addition, there might be few disease-relevant targets that are druggable using currently available chemistry [11,12]. These circumstances have resulted in few first-in-class inhibitors reaching the market in any given year. An alternative drug discovery approach that targets the complex systems biology [13] that underlies a disease might be more successful for identifying novel therapeutic opportunities [7]. A greater understanding of the disease network could also reveal that inhibition of an individual target is, in many cases, insufficient to restore the system to the healthy state. In these cases, modulating the activity of multiple targets might be required to achieve optimal therapeutic benefit [4,14].

The rationale for a multi-target approach can be especially applicable to oncology. Efficacious and durable responses in cancer can require multi-target therapeutics because the process of oncogenesis is known to be multigenic, and most cancers have four to seven independent mutations [15,16]. Interestingly, most tumorigenic viruses encode proteins that block the activity of the tumor suppressor genes p53 and Rb [17]. Evolution has favored viral strains that can simultaneously block redundant mechanisms and safeguards that the cell uses to prevent inappropriate cell-cycling and -proliferation. These viruses use a multi-target approach to drive their own proliferation. Given that multiple nodes in the system must be modified to induce cancer, many researchers have proposed that multiple interventions will be required to counter this process.

Existing multi-target therapeutics

Increasingly, drug combinations are the standard of care for the treatment of diseases including cancer, type 2 diabetes mellitus (T2DM), viral and bacterial infection, and asthma. Often, these combinations are applied as co-therapy regimens, but in many cases the individual components of the combination are co-formulated as a single pill or injection. A new generation of multi-target drugs is currently emerging from clinical development: single chemical entities that act simultaneously at multiple molecular targets. There are several categories of multi-target therapeutics that can be defined on the basis of target relationship (Box 1). In the first class, the therapeutic effect occurs at separate molecular targets that can reside within individual signaling pathways, between pathways within a cell or at separate tissues in the body. In the second category, modulation of one target facilitates action at a second target, for example by altering compound metabolism, inhibiting efflux pumps or blocking other resistance mechanisms (Figure 1). Third, a coordinated action at multiple sites on a single target or macromolecular complex (e.g. prokaryotic ribosome) yields the therapeutic effect. Note that the set of targets in each of these three cases can be modulated either by a mixture of separate chemical entities or by a single compound designed to have multiple actions. Although multi-target action can be achieved in several ways, it is the coordinated effect at the set of targets that results in the biological and, hopefully, therapeutic effect [18]. Table 1 lists some examples of multi-target therapeutics from various indications, and select examples are discussed.

BOX 1

Multi-target therapeutic types

(i) Components impact separate targets to create a combination effect. The targets can reside in the same or separate pathways within an individual cell, or in separate tissues. Intracellular examples include Bactrim®, which impacts two targets in the folate biosynthesis pathway in bacteria, or cyclosporine-A with steroid, as shown in Figure 1. Vytorin® is an example of an inter-tissue combination where one agent blocks cholesterol synthesis in the liver and the other prevents uptake of dietary cholesterol in the small intestine.

(ii) One component alters the ability of another to reach its target. In this type of combination one agent can alter the metabolism of the pharmaceutically active component, or one agent can block an efflux pump or other resistance mechanism (e.g. β-lactamase, Figure 1) to increase the activity of the other.

(iii) The components bind separate sites on the same target to create a combination effect and increase the pharmacological action. For example, the components of the combination Synercid® bind two separate sites on the prokaryotic ribosome.

The benefits of multi-target action are well established in cancer. Traditional chemotherapeutic agents have been routinely applied as co-therapies. For example, adjunctive agents can sensitize cancer cells to DNA-damaging drugs, and newer co-therapy protocols including 5-FU, leucovorin and oxaliplatin (i.e. FOLFOX) are now applied in colorectal cancer. Currently, the molecularly targeted agents such as Herceptin® (trastuzumab) and Erbitux® (cetuximab) are being developed in combination protocols with traditional antineoplastics, estrogen blockade and other targeted agents [19,20]. For example, Herceptin®, which targets ErbB2 (HER-2/neu), is being applied in combination with the anti-VEGF (vascular endothelial growth factor) antibody Avastin® to treat breast cancer, and Erbitux® (which targets ErbB1) is applied in combination with irinotecan for the treatment of colorectal cancer. A novel class of receptor tyrosine kinase (RTK) inhibitors that possess multi-target action in a single chemical entity are currently in clinical development. Lapatinib and canertinib are examples of a new class of pan-ErbB inhibitors [21,22]. These new agents with multi-target action will almost certainly be applied in

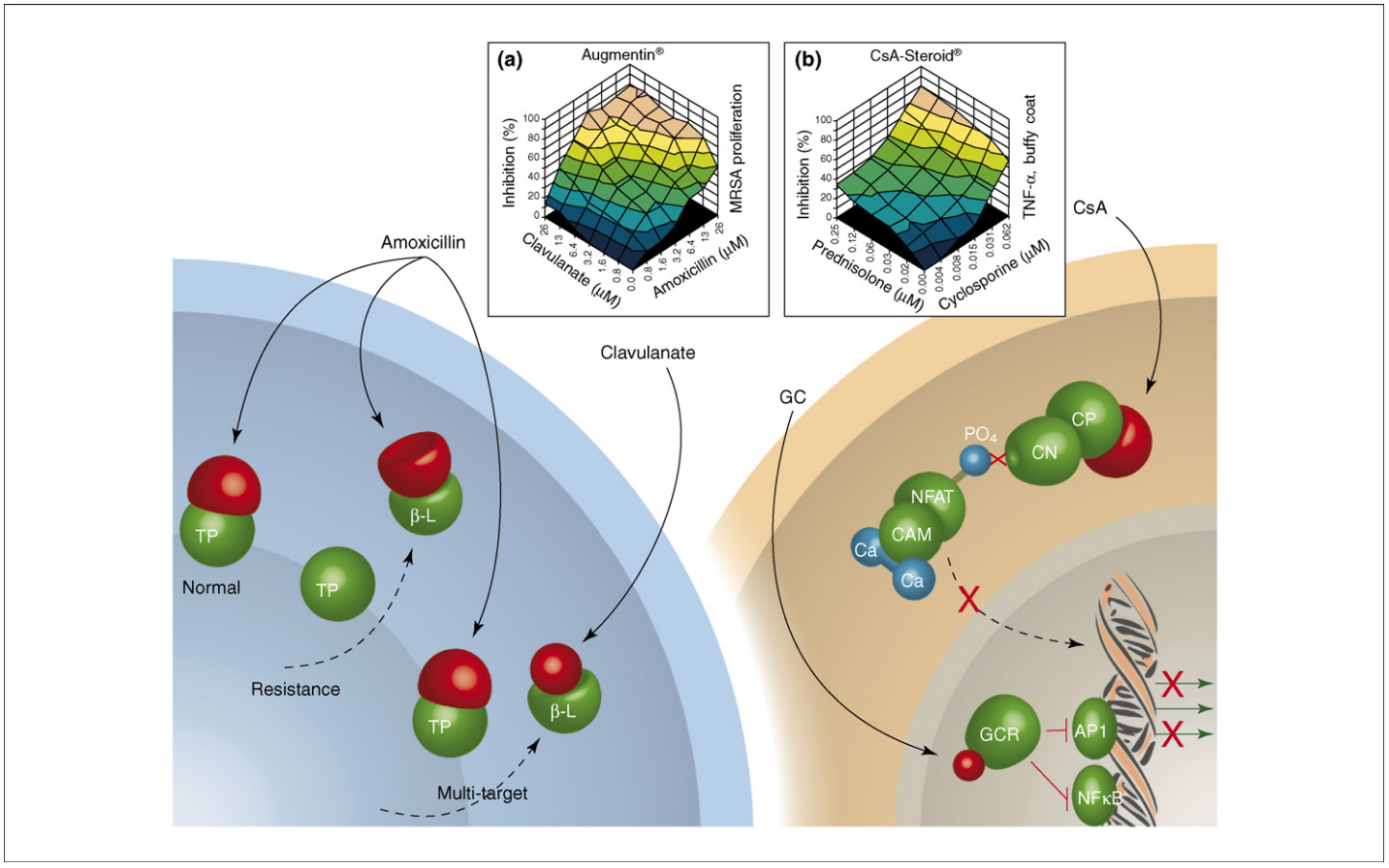

FIGURE 1

The multi-target mechanisms underlying two combination therapies and the synergistic responses they produce *in vitro*. The common antibacterial combination Augmentin® **(a)** uses a decoy compound (clavulanate), which irreversibly inactivates the β-lactamase (β-L) resistance mechanism to maintain the efficacy of amoxicillin against bacterial cell-wall transpeptidases (TPs). The combination of steroid and cyclosporine-A is a potent immunosuppressive **(b)**. Steroids bind the glucocorticoid receptor (GCR) and negatively regulate pro-inflammatory response genes under the control of nuclear factor κB (NFκB) and activating protein-1 (AP1) promoters. Additional pro-inflammatory genes are suppressed by the binding of cyclosporine-A to cyclophilin (CP), which forms an inhibitory complex with calcineurin (CN), blocking dephosphorylation and nuclear translocation of the transcription factor nuclear factor of activated T cells (NFAT). The *in vitro* dose-response matrices for each combination are shown as Figure insets. The component agents are serially diluted along each axis and the response over all concentration pairs is shown as a 3D inhibition surface.

combination with other molecularly targeted or traditional chemotherapeutic agents once they reach the market.

Use of combination therapies for T2DM is widespread and increasing with the recent launch of several co-formulated products [23]. Metformin (Glucophage®), which suppresses gluconeogenesis in the liver and improves glucose uptake in peripheral tissue through AMP-activated protein kinase (AMPK), is effective for glucose control and is the standard first-line therapy in T2DM. Patients who do not achieve adequate glycemic control with metformin alone receive combination drugs in which metformin has been co-formulated with either the secretagogue glyburide (Glucovance®), which increases insulin secretion from the pancreas, or the peroxisome-proliferative activated receptor gamma (PPARγ) agonist rosiglitazone (Avandamet®), which improves insulin sensitivity. These combinations contain modulators that act at multiple tissues to improve glucose homeostasis and integrate their multi-target action at the level of the patient. This is in contrast to many anticancer and anti-infective multi-target therapeutics that integrate their effect at the individual cell level.

Combinations are routinely used as anti-infective agents to treat bacterial and viral infections. Hepatitis-C-infected patients are given Rebetron®, a combination therapy of polyethylene glycol (PEG)–interferon-2α and ribavirin. The highly active antiretroviral therapy (HAART) for HIV infection is a cocktail of reverse-transcriptase inhibitors and, more recently, protease inhibitors. The combination drugs Epzicom®, Truvada® and Combivir® contain two nucleoside reverse-transcriptase inhibitors (NRTI), and a new triple combination (Atripla®) including tenofovir, emtricitabine and efavirenz (i.e. two NRTIs and one non-nucleoside RTI) was recently approved for the treatment of HIV. These combination drugs are superior in controlling viral load and preventing emergence of resistant strains of HIV when compared with their component single-target drugs. The widely used antibacterial combination Bactrim® contains the dihydrofolate reductase inhibitor trimethoprim and the weak dihydropteroate synthase inhibitor sulfamethoxazole. This combination exhibits a strikingly synergistic combination effect by impacting two nodes in the folate biosynthesis pathway.

Discovery of multi-target therapeutics

The success of the combination drugs discussed justifies efforts to identify novel multi-target therapeutics early in the discovery

TABLE 1

Examples of combination-drug products or candidates

Trade name	Indication	Compound 1	Compound 2	Target or mechanism 1	Target or mechanism 2
Drug combinations					
Vytorin®	Hyperlipidemia	Ezetimibe	Simvastatin	Dietary cholesterol	HMG-CoA reductase
Caduet®	CHD	Amlodipine	Atorvasatin	Calcium-channel antagonist	HMG-CoA reductase
Lotrel®	Hypertension	Amlodipine	Benezapril	Calcium-channel antagonist	ACE inhibitor
Glucovance®	T2DM	Metformin	Glyburide	Gluconeogenesis	Insulin secretagogue
Avandamet®	T2DM	Metformin	Rosiglitasone	Gluconeogenesis	PPARγ agonist
Truvada®	Antiviral (HIV)	Emtricitabine	Tenofovir	RT inhibitor	RT inhibitor
Kaletra®	Antiviral (HIV)	Lopinavir	Ritonavir	Protease inhibitor	Protease inhibitor
Rebetron®	Antiviral (Hepatitis C)	PEG–interferon	Ribavirin	Interferon-α2B	Antimetabolite
Bactrim®	Antibacterial	Trimethoprim	Sulfamethoxazole	DHFR	DHPS
Advair®	Asthma	Fluticasone	Salmeterol	Glucocorticoid receptor	β2-Adrenergic
Multi-target drugs					
Cymbalta®	Depression	Duloxetine	NA	SRI	NRI
Sutent®	Cancer	Sunitinib	NA	PDGFR	VEGFR
Nexavar®	Cancer	Sorafenib	NA	BRAF	VEGFR
Sprycel®	Cancer	Dasatinib	NA	BCR–ABL	SRC
Tykerb®	Cancer	Lapatinib	NA	EGFR (ErbB1)	HER-2 (ErbB2)

Abbreviations. HMG-CoA reductase, hydroxymethylglutaryl-coenzyme A reductase; CHD, coronary heart disease; ACE, angiotensin-converting enzyme; T2DM, type 2 diabetes mellitus; PPAR, peroxisome proliferative activated receptor; RT, reverse transcriptase; DHFR, dihydrofolate reductase; DHPS, dihydropteroate synthase; SRI, serotonin reuptake inhibitor; NRI, norepinephrine reuptake inhibitor; VEGFR, vascular endothelial growth factor receptor; PDGFR, platelet-derived growth factor receptor; BCR–ABL, breakpoint cluster region–abelson kinase; SRC, sarcoma virus kinase; EGFR, epidermal growth factor receptor; PEG, polyethylene glycol; BRAF, v-raf murine sarcoma viral oncogene homolog B1; HER-2, human epidermal growtgh factor receptor 2; NA, not applicable.

process, but the systematic pursuit of combination drugs presents unique experimental challenges. First, multi-target therapies rely upon complexity in the disease system, which must be reproduced *in vitro* for discovery screening. Second, without *a priori* knowledge of target pairs that interact synergistically, the vast space of possible target combinations needs to be covered by an agnostic search. Finally, the sensitivity of synergistic interactions to dosing ratios requires substantial experimental investment and specialized analyses for each combination tested.

Cell-based phenotypic assays: better models of disease systems

The first experimental requirement for multi-target drug discovery is an appropriate assay for combination testing. The efficient cell-free assays used for high-throughput molecular-target screening are not suitable because they do not adequately model the systems biology of an intact cell. Therefore, cell-based phenotypic assays are employed because they maintain reasonable experimental efficiency while preserving disease-relevant molecular-pathway interactions [5]. For oncology-focused screens, proliferation assays using tumor cell lines are monitored using metabolic reduction assays, ATP quantification, DNA content or detecting apoptosis-associated cleavage reactions. Measuring insulin-sensitive glucose-uptake is an example of a phenotypic assay that can be employed as a model of T2DM, in contrast to a molecular-focused assay such as PPARγ agonism or glucose transporter-4 (GLUT-4) translocation. Broad phenotypic measures are preferred because they probe a wide variety of disease-relevant mechanisms, increasing the likelihood of discovering unexpected beneficial synergies. Primary tissue assays that contain multiple cell types can model higher levels of systems integration to uncover multi-target mechanisms. For example, inflammatory drug screening using mixed cultures of lymphocytes enables the assay system to model paracrine effects between various cell types. *In vivo* screening using a whole organism model such as the zebrafish [24] could identify multi-target therapeutics that integrate their effect at the level of the organism.

A library of agents that probes diverse molecular targets can produce novel combination effects

Agnostic screening of compound combinations requires efficient search methods and a library of chemical and biological agents that perturb a diverse set of molecular targets. Using *in vitro* methods to test combinations of drugs provides an opportunity for large, agnostic surveys of molecular mechanisms that can combine to produce synergistic combination effects. By contrast, clinicians can only test a few drug combinations based on knowledge of disease biology, drug mechanism or intuition. Theoretically, testing combinations *in vitro* enables all possible combinations to be tested. For example, the complete set of anti-infective drugs could be tested in clinically relevant bacterial strains to identify optimal antibacterial combinations. To expand the search, approved drugs from other therapeutic classes could be tested in combination with these anti-infective drugs, and with each other, to identify novel anti-infective therapies. The logical extrapolation of this approach is a chemical combination screen in which compounds able to perturb all known molecular targets are tested in pairwise, or higher order, combinations using cell-based assays that model disease.

The compound library for conducting these searches, therefore, needs to contain a highly diverse set of agents including: all active pharmaceutical ingredients from the USA, EU and Japan; over-the-counter drugs, generally-regarded-as-safe (GRAS) drugs and food additives; and non-druglike small-molecules and biologics that antagonize or agonize diverse biological targets relevant to human disease. Such a library has two primary advantages over a traditional combinatorial chemistry library. First, by definition, the compounds are druglike and largely free of chemically unstable or

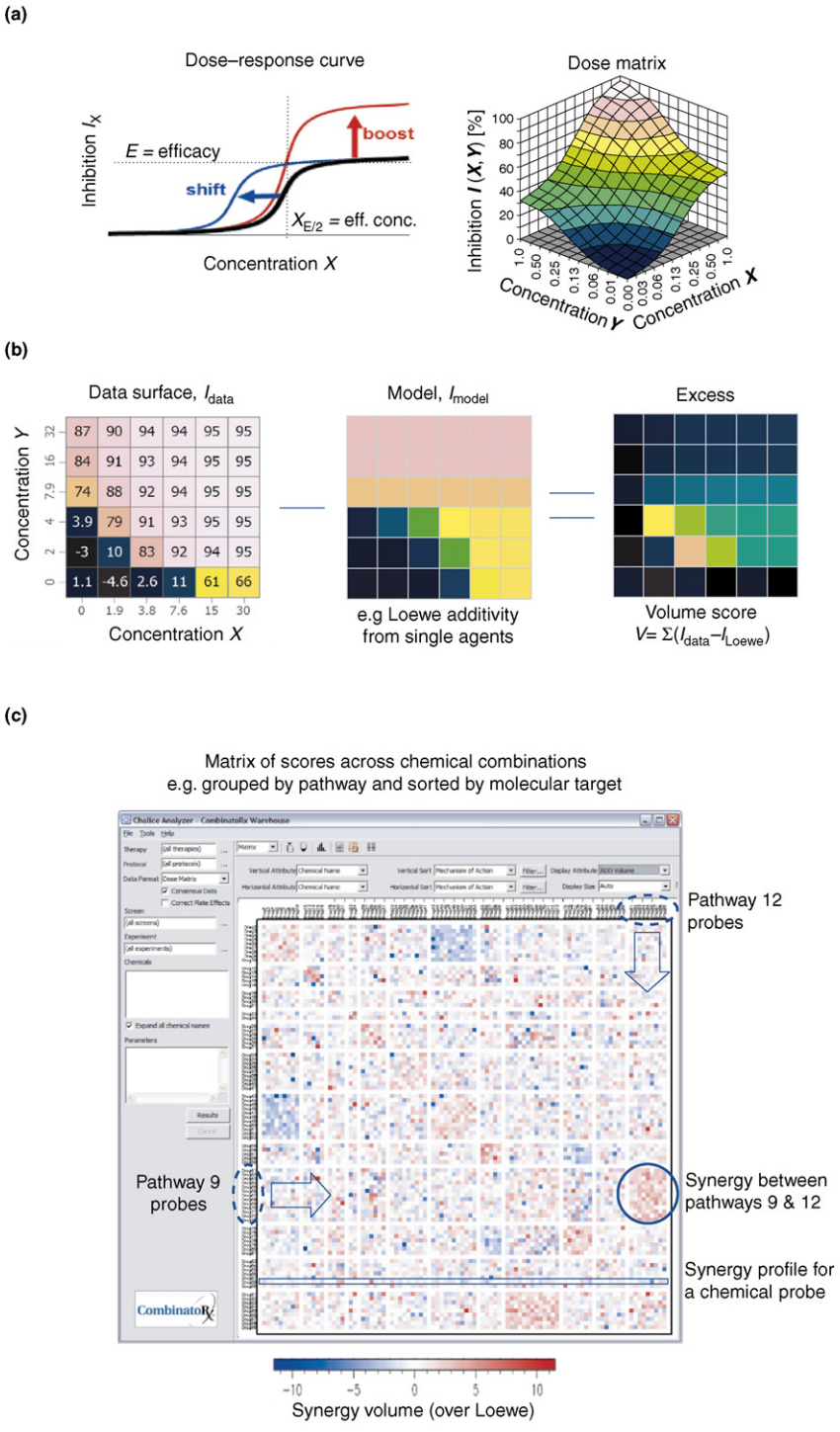

FIGURE 2

Systematic discovery of multi-target mechanisms. (a) Covering possible synergies: combination effects between compounds can produce potency shifts (blue curve) or boosts in the maximal effect (red curve) in combination, relative to the single agent response (black curve), and these effects can occur at any agent dosing ratio. Dose–response-matrix experiments can detect such combination effects over a wide range of concentrations and ratios, and can be represented as a 3D inhibition surface or by using color to represent the inhibition observed at each dosing pair. **(b)** Scoring for synergy: the dose–response matrix for each pair of agents in a screen can be compared with a reference model, and the resulting difference surface can be integrated into a numerical combination effect score. **(c)** Analyzing a collection of scores: synergy scores from large screens can be collected into 'synergy profiles' for each agent, and displayed to emphasize mechanistic relationships between pathways. Here, the scores from a hypothetical combination screen are displayed on a grid where axes are sorted by the molecular mechanism for each agent grouped by pathway designation. Broad patterns of synergy, for example as seen between pathways 9 and 12, can reveal novel, functional interactions and relationships.

reactive compounds; and, second, the library components are all biologically active and are, therefore, more likely to yield multi-target effects. However, because the number of combinations expands quadratically with the number of agents being tested, multi-target discovery efforts are usually constrained by the efficiency of the screening platform available. For example, even a small set of 2000 agents generates almost two million unique pairwise combinations.

Synthetic screens uncover unappreciated biological network connections

Systematic searches for multi-target mechanisms, using combinations of drugs, can be considered a form of conditional screening [25]. Treatment with one component of the combination induces a state or change in the system that sensitizes it to the action of the second agent. This is the equivalent of a synthetic screen from the field of classical genetics. An important advantage of inducing a synthetic state with a small molecule or drug is that the temporal aspect of the combination effect can be characterized. For example, the effect of pre-treating cells with one agent, before adding a second agent, can be compared with the measured effect seen when the order of addition is reversed. In addition to treatment with small-molecule drugs, the approach can be extended to searches where the synthetic state is induced by treatment with a biological agent such as a small-inhibitory RNA (siRNA) [26], a therapeutic antibody or a tumor-specific growth factor. Treating cells with endogenous factors such as insulin-like growth factor-1 (IGF-1) can mimic the micro-environment of a tumor, where stromal cells secrete various factors that can alter the state of the cancerous cells within the tumor [27]. In some cases, the agent used to induce the synthetic state can lack a measurable response in the particular assay employed. However, this silent effect might become measurable in the context of a combination. This type of response is the chemical genetic equivalent of a synthetic-lethal interaction in classical genetics. This type of synthetic interaction can reveal previously unappreciated interactions or associations between molecular targets or the signaling networks in which they reside. Synthetic-effect combination screening is a method that uncovers these silent effects in the context of treatment with a second agent [28]. Examples of this kind of synthetic-effect combination include interactions between glycogen synthase kinase-3β (GSK3β) and tumor necrosis factor (TNF)-related apoptosis inducing ligand [29], and between breast cancer type 2 susceptibility gene (BRCA2) and poly (ADP-ribose) polymerase-1 (PARP1) [30]. Multi-target therapeutics discovered using this type of conditional screening can have better efficacy and more selectivity *in vivo* than compounds identified in molecular-target-focused screens.

Recognizing multi-target effects *in vitro* requires combination analytics

A key requirement for discovering multi-target therapeutics is the ability to compare efficiently the activity of a drug combination with the activity of the component agents in isolation. Combination effects between drugs can be seen as an increase in potency or as a boost in efficacy measured by the assay (Figure 2a). Because synergistic interactions between compounds can occur over a range of concentrations, systematic searches for synergistic combinations require testing various ratios of the component drugs. In practice, this can be achieved using a dose-response matrix design [5], with measurements taken at all possible pairings of serially diluted single agents (Figure 2a). The resulting dose–response surface shows the combination effect produced for every pair of single agent doses and can be easily compared with the single agent effects at corresponding concentrations along the edges of the dose–response matrix (Figure 2b). The observed response surfaces can be evaluated using a null-hypothesis reference-model such as Loewe additivity (Box 2), and a score based on the excess volume between the experimentally observed and model surfaces can be calculated to identify synergistic or antagonistic multi-target mechanisms (Figure 2b). This score emphasizes the average level of synergy or antagonism, minimizing the effects of outlying data spikes, and identifies combinations with a robust combination effect across a broad concentration range.

Combinations as tools for probing systems biology

Global analysis of combination screens can reveal patterns of synergy between particular drug classes or mechanisms, as recently shown for antibacterial combinations [31]. The synergy scores for each combination can be displayed on a color scale in a grid format (Figure 2c) where the scores in each row or column represent the synergy profile of an individual drug with the rest of the library. The drugs on each axis of the grid can be sorted by mechanistic class or primary target, and clusters of high synergy scores show pairs of molecular targets that interact synergistically (Figure 2c). Alternatively, the synergy profiles for the components of the combination screen can be clustered or bi-clustered [32] to identify regions of synergy that can reveal multi-target interactions between alternate

BOX 2

The Loewe additivity standard

The most widely used combination reference is Loewe additivity [40,41], which describes the trade-off in potency between two agents when both sides of a dose matrix contain the same compound. For example, if 50% inhibition is achieved separately by 1 μM of drug A or 1 μM of drug B, a combination of 0.5 μM drug A and 0.5 μM drug B should also inhibit by 50%. Synergy over this level is especially important for justifying the clinical use of proposed combination therapies because it defines the point at which the combination can provide additional benefit over simply increasing the dose of either agent. Synergy, relative to Loewe additivity, can be illustrated graphically using an 'isobologram' [38], where experimental dose–response-matrix data are used to draw a contour, as a function of the component concentrations $\boldsymbol{X}$ and $\boldsymbol{Y}$, at which the combination achieves the chosen inhibition level $\boldsymbol{I}$. Loewe additive combinations produce linear contours connecting the single agent effective concentrations $\boldsymbol{X_I}$ and $\boldsymbol{Y_I}$, and synergy occurs where the data contour falls between the additivity line and the origin. Mathematically, this can be expressed in terms of a combination index (**CI**) [42], $\mathbf{CI} = \boldsymbol{X}/\boldsymbol{X_I} + \boldsymbol{Y}/\boldsymbol{Y_I}$, which can be thought of as the total ratio of drug required in combination to achieve $\boldsymbol{I}$ over the corresponding single agent concentrations needed to achieve the same effect (**CI**<1 implies synergy). An alternative way to measure synergy, without requiring a predefined effect level, is to iteratively calculate [43] the Loewe additive response $\boldsymbol{I_{Loewe}}(\boldsymbol{X},\boldsymbol{Y})$ at each dose matrix point from the single-agent response curves, and then sum the differences between $\boldsymbol{I_{Loewe}}$ and the experimental data.

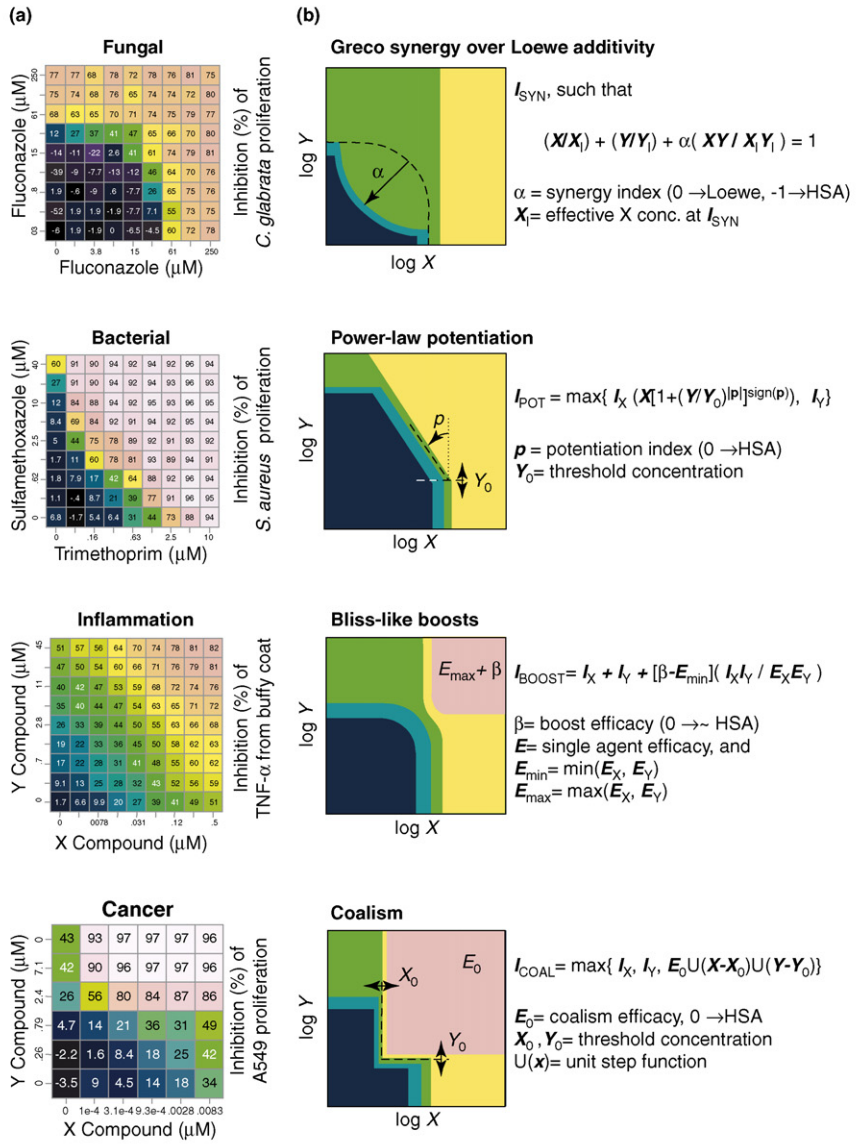

FIGURE 3

Shape models to describe the observed diversity of combination effects. (a) Examples: dose–response-matrix measurements produce a wide variety of response surface shapes that depend upon target connectivity in the disease network. To the left are four examples of dose matrices observed using cHTS disease-model assays, using a similar color scheme as in Figure 2. Agents themselves (e.g. fluconazole) produce dose-additive responses, and synergies can take the form of dose-shifts (e.g. Bactrim®: trimethoprim with sulfamethoxazole), effect boosts (e.g. inflammation example) or coalisms (e.g. cancer). **(b)** Models for surface shape: such combination effects can be quantitatively described using parametric shape models of inhibition $I(X,Y)$ as a function of the single agent concentrations X and Y, which are calculated in reference to the single-agent response curves $I_X(X)$ and $I_Y(Y)$. Greco synergy [38] describes potency shifts measured by the synergy index α, ranging from HSA (i.e. the activity of the highest single agent) through Loewe additivity to strong synergies between active agents. Extreme potency shifts such as those seen with Bactrim® are best described using a power-law model with a potentiation index p to measure the degree of synergy. Boosts in efficacy are quantifiable using a function based on the Bliss model for independent elimination probabilities [39], with a parameter β added to describe varying boost levels. The coalism model describes cases where there is a strong combination effect at a constant level, E_0, that is above threshold concentrations X_0 and Y_0, independent of all single-agent activity. This latter model is the chemical genetic equivalent of a synthetic-lethal interaction. Information about the mechanism of an observed combination can be obtained by comparing an observed dose-response matrix to this set of models.

targets of the components. A large collection of synergy profiles for chemical probes with diverse targets can also be used to identify novel mechanisms for uncharacterized agents, analogous to diverse phenotypic profiling screens for target identification [33]. For example, the molecular target of a probe with a combination-effect profile most closely matching the test-compound profile becomes a probable target candidate for the test compound.

Detailed mechanistic relationships between drug targets can be inferred from the dose–response surface shapes. Combination screens produce a wide variety of response morphologies (Figure 3a) that can be quantitatively characterized using shape models derived from the single-agent curves (Figure 3b). Predictions from simulated pathways, and empirical evidence from combination screens using agents with known molecular targets,

TABLE 2

Various approaches to create multi-target therapeutics
Mixture of monotherapies
Advantages
Straightforward to tailor ratio of agents in the mixture – to account for differential potency at target and/or target stoichiometry
Opportunity for sequenced action or independently varying target exposure (e.g. using immediate versus extended release formulations of the components)
Speed to proof-of-concept clinical trials and cost advantage over multi-targeted single agent
Disadvantages
Might need to align pharmacokinetics and/or pharmacodynamics of the component agents in co-formulated product
Approval might require 'combination versus parts' factorial trial design
Must look for drug–drug interaction (DDI) relative to single agent
Individual multi-targeted single agent
Advantages
Standard new chemical entity intellectual property position
Standard development program and regulatory approval process
Easier manufacture and formulation of an individual active pharmaceutical ingredient (API) compared with a mixture
Disadvantages
Challenge to achieve multi-selective action without becoming nonselective
Challenge to optimize potency at two targets simultaneously in a single chemical structure – might only be able to achieve low potency at the targets
Difficult to achieve sequenced action at the targets

Some molecular target structures might not be druggable with a single chemical scaffold – necessitating a challenging linking or 'dumbbell' approach [35].

have shown that the observed response morphology depends on the biological connectivity of the drug targets. This information can be used to elucidate the mechanism of action of the therapeutic combination, by comparing the observed response to predictions for various mechanistic scenarios. Alternatively, response surfaces from combinations of agents with known targets can be used to improve network models of biological systems, by providing constraints on how their targets can be connected. For example, as already shown for genetic mutations [34], a combination screen in yeast proliferation using inhibitors of metabolic enzymes could be used to improve a network model of yeast metabolism by highlighting inconsistencies between the observed shapes and predictions from the model using simulated inhibitors.

Future embodiments of multi-target therapeutics

The favorable efficacy of existing combination therapeutics shows that searches specifically designed to identify multi-target mechanisms can provide a new path forward in drug discovery. Most multi-target therapeutics will be developed as a mixture of agents with selectivity for individual targets, but in some cases it might be possible to build multi-target action into a single chemical entity [35]. The advantages and disadvantages of these two approaches for creating multi-target therapeutics are discussed in Table 2.

Multi-target action in a single chemical entity can be achieved by linking two agents, or by simultaneously optimizing dual specificity into a single low-molecular-weight compound [35]. The compounds with multi-target action simplify clinical trial design, compared with mixtures, and could reduce the risk of drug–drug interactions. Unfortunately, the advantages of simplified development and a standard new-chemical-entity approval process can be outweighed by the technical difficulties of optimizing a multi-targeted single agent (Table 2). For example, it can be challenging to match potencies at two separate targets with a single chemical entity. Using a mixture of monotherapies enables straightforward optimization of potencies by adjusting the ratio of agents in the mixture. Finally, a single chemical entity with specificity for more than one target could be less effective than a combination if the multi-target effect requires sequenced action at the individual targets. For example, if pre-treatment with the first agent induces expression of the target of the second agent [36]. Multi-target action using mixtures or using a multi-targeted single agent can, in fact, be considered complementary approaches. The ease of optimizing potency at the various targets, and speed to proof-of-concept clinical trials for the mixture approach, could justify the more challenging, costly and time-consuming process of engineering multi-target action into a single chemical entity.

Concluding remarks

The so-called 'one drug one target' drugs have revolutionized modern medicine and, in many cases, can be considered wonder drugs. Unfortunately, many patients are unable to benefit from these therapies because of pharmacogenomic effects [37]. For example, some patients could have differences in key disease-relevant biological pathways compared with the majority of the population, and this could alter the contribution of a particular target to the disease in these individuals. In such cases, the action of an alternative target might predominate and these patients could benefit from a combination that simultaneously impacts the principal and alternative targets. Creating multi-target therapeutics that address pharmacogenomic differences in this way could, therefore, provide benefit to more patients than monotherapies. This approach might represent a first step toward breakthrough medicines that attack the systems biology of disease, and also might provide medical benefit for a greater proportion of patient populations.

The success of multi-targeted agents in therapeutic areas such as cancer, depression, diabetes and infectious diseases justifies systematic efforts to identify new combination therapeutics in diverse indications. Dose–response matrix screening of combinations using cell-based phenotypic assays provides a basic approach for the discovery of multicomponent therapeutics, and can also be used to probe how molecular targets are connected within biological networks. Finally, therapeutics leveraging synergistic multi-target mechanisms could produce greater levels of efficacy and could be less prone to resistance than monotherapies. The 'whole' of these synergistic combinations is, by definition, greater than the sum of the action of the parts and could provide a better mechanism for impacting the complex systems biology of human disease.

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