# Mechanisms of drug combinations: interaction and network perspectives

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Abstract | Understanding the molecular mechanisms underlying synergistic, potentiative and antagonistic effects of drug combinations could facilitate the discovery of novel efficacious combinations and multi-targeted agents. In this article, we describe an extensive investigation of the published literature on drug combinations for which the combination effect has been evaluated by rigorous analysis methods and for which relevant molecular interaction profiles of the drugs involved are available. Analysis of the 117 drug combinations identified reveals general and specific modes of action, and highlights the potential value of molecular interaction profiles in the discovery of novel multicomponent therapies.

In recent years, drug discovery efforts have primarily focused on identifying agents that modulate preselected individual targets[1–3]. Although new drugs have continuously been discovered, there is a growing productivity gap, despite major spending on research and development and advances in technology development[4]. This problem arises partly because agents directed at an individual target frequently show limited efficacies and poor safety and resistance profiles, which are often due to factors such as network robustness[5–7], redundancy[8], crosstalk[9–11], compensatory and neutralizing actions[12,13], and anti-target and counter-target activities[14]. With such issues in mind, systems-oriented drug design has been increasingly emphasized as a potentially more productive strategy[15–18]. This approach to drug design has been supported by clinical successes with multicomponent therapies and multi-targeted agents[19–22], and efforts have been directed at the discovery of new multicomponent therapies[7,15–17,22–24].

Knowledge of the molecular mechanisms of existing multicomponent therapies can provide clues to aid the discovery of new drug combinations and multi-targeted agents, and some key characteristics of the modes of these therapies have been outlined[14,17,22,23]. The multiple targets can reside in the same or different pathways and tissues, and their modulation can produce more-than-additive (synergistic) effects triggered by actions converging at a specific pathway site. In addition, effects could be due to negative regulation of network compensatory and neutralizing responses, drug resistance sources, and anti-target and counter-target activities. However, specific mechanisms of action have only been fully elucidated for a few of the explored drug combinations[17,25–30].

Extensive investigations of the molecular basis of drug actions and responses have yielded a substantial amount of information on experimentally determined drug-mediated molecular interaction (MI) profiles and regulatory activities of many drugs and compounds[1,2,31–36]. The MI profile of a drug describes its interactions with individual biomolecules, pathways or processes attributable to its pharmacodynamic, toxicological, pharmacokinetic, and combination effects. Apart from using MI profiles for guiding the development of target discovery technologies[37–43], they might also be explored for gaining further insights into general modes of action of multicomponent therapies and the mechanisms of specific drug combinations. Such a task may be accomplished by analysing the relevant MI profiles from the perspective of coordinated interactions and network regulations[10–12].

In this article, we describe how this possibility was evaluated by comprehensively investigating literature-reported synergistic and other types of drug combinations in which the combination effect has been evaluated by rigorous drug-combination analysis methods and for which relevant MI profiles of the drugs involved are available. Additonal sets of popular drug combinations were also studied. Moreover, pathway analysis was conducted for three of the studied drug combinations. It is cautioned that although connections can be made from literature-described MI profiles to examine why a drug combination may have a particular type of effect, many of these interconnections are likely to be more complicated than those summarized in this article, and their activities are highly dynamic[44–46]. In addition, the activation and level of activity of these connections may be influenced

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Table 1 | **Examples of pharmacodynamically synergistic drug combinations due to anti-counteractive actions***

| Drug A (MoA)‡ | Drug B (MoA)‡ | Reported synergism | Method | Possible mechanism of synergism in anti-counteractive actions |
|---|---|---|---|---|
| ***Different targets of the same pathway*** | | | | |
| Oxaliplatin (DNA adduct, preferably binds to major groove of GG, AG and TACT sites, complex conformation different from that of cisplatin[144], causes DNA strand break and non-DNA initiated apoptosis[145]) | Irinotecan (DNA TOP1 inhibitor, increases EGFR phosphorylation in Lovo and WiDR cells[146]) | Synergistic anticancer effect in AZ-521 and NUGC-4 cells, additive effect in MKN-45 cells[147] | Median drug effect analysis | • Effect of oxaliplatin's DNA adduct formation[144] may be partially reduced by certain mutant DNA TOP1 acting on DNA adduct to generate different topoisomers[148]<br>• Irinotecan inhibition of DNA TOP1 partially offsets this counteractive activity[146] |
| DL-Cycloserine (bacterial cell-wall synthesis inhibitor[149]) | Epigallocatechin gallate (disrupts integrity of bacterial cell wall via direct binding to peptidoglycan[149]) | Synergistic effect on bacterial cell wall[149] | Fractional inhibitory concentration index | • Cell-wall alteration may induce counteractive cell-wall synthesis to restore cell-wall integrity[150]<br>• DL-Cycloserine inhibition of cell-wall synthesis hinders restoration, thereby enhancing epigallocatechin gallate's cell-wall disruption activity |
| ***Different targets of related pathways*** | | | | |
| Paclitaxel (stabilizes microtubules via α-tubulin acetylation[79], distorts mitosis to trigger apoptosis[151], induces p53 and CDK inhibitors[152], activates CASP10, 8, 6 and 3, leading to apoptosis[153], activates ERK[154] and CDK2[155], activates p53 and p38 MAPK[156]) | NU6140 (CDK inhibitor, downregulates anti-apoptotic protein survivin[157]) | Synergistic apoptotic response[157] | Median drug effect analysis | • Use of both drugs promotes complementary apoptosis activities via triple actions of survivin downregulation by NU6140[157], microtubule stabilization[79] and CASP activation[153] by paclitaxel<br>• Paclitaxel's promotion of apoptosis may be partially offset by its counteractive pro-growth activation of ERK[154] and CDK2[155], which may be partially reduced by NU6140's inhibition of CDK[157] |
| ***Different targets of crosstalking pathways*** | | | | |
| Gefitinib (EGFR tyrosine kinase inhibitor, induces CDK inhibitors p27 and p21, decreases MMP2 and MMP9 enzyme activity[158]) | Taxane (disrupts microtubules by binding to β-tubulin[159], induces tumour suppressor gene p53 and CDK inhibitors p21, downregulates BCL-2, leading to apoptosis[152]) | Strong synergistic effect in breast cancer MCF7/ADR cells[160] | Combination index | • Taxane produces anticancer effect by inducing apoptosis[152] and microtubule disruption[159]<br>• Crosstalk between EGFR and HIF1α pathways increases resistance to apoptosis by upregulating survivin[9]<br>• Gefitinib produces anticancer effect via EGFR tyrosine kinase inhibition, which offsets the counteractive EGFR–hypoxia crosstalk in resisting taxane's pro-apoptosis activity |
| ***Different targets in the same pathway that crosstalk via another pathway*** | | | | |
| Gefitinib (EGFR tyrosine kinase inhibitor, induces CDK inhibitors p27 and p21, decreases MMP2 and MMP9 enzyme activity[158]) | PD98059 (MEK inhibitor[161]) | Synergistic antitumour effect in breast cancer MDA-361 and MB-361 cells[108] | Combination index, isobolographic analysis | • An autocrine growth loop crucial for tumour growth is formed in the EGFR–Ras–Raf–MEK–ERK network such that activated MEK activates ERK, which upregulates EGFR ligands, thereby promoting the autocrine growth loop[162]<br>• This loop produces counteractive activity against gefitinib or PD98059 by reducing the effect of MEK or EGFR tyrosine kinase inhibition<br>• Simultaneous use of both drugs helps disrupt this autocrine growth loop, thereby enhancing each other's effect |
| ***Same target (different sites)*** | | | | |
| AZT (HIV-1 RT inhibitor[163]) | Non-nucleoside HIV-1 RT inhibitor[164] | Antiviral synergism[165] | Isobolographic analysis, Yonetani–Theorell plot | • AZT resistance is partly due to phosphorolytical removal of the AZT-terminated primer[166]<br>• NNRTI inhibits RT-catalysed phosphorolysis, thereby reducing AZT resistance[165] |

*In these examples, synergy has been determined by well-established synergy/additive analysis methods and its molecular mechanism has been revealed.
‡MoA, mechanisms of action related to synergy. AZT, azidothymidine; BCL-2, B-cell lymphoma protein 2; CASP, caspase; CDK, cyclin-dependent kinase; EGFR, epidermal growth factor receptor; ERK, extracellular-regulated kinase; HIF1α, hypoxia-inducible factor 1α; MAPK, mitogen-activated protein kinase; MEK, MAPK/ERK kinase; MMP, matrix metalloproteinase; NNRTI, non-nucleoside RT inhibitor; RT, reverse transcriptase; TOP, topoisomerase.

Table 2 | **Examples of pharmacodynamically synergistic drug combinations due to complementary actions***

| Drug A (MoA)‡ | Drug B (MoA)‡ | Reported synergism | Method | Possible mechanism of synergism in promoting complementary actions |
|---|---|---|---|---|
| ***Different targets of related pathways that regulate the same process*** | | | | |
| Aplidin (induces apoptosis[167], activates JNK, EGFR, Src and p38 MAPK[168], inhibits VEGF release and secretion[169]) | Cytarabine (DNA binder[99], inhibits synthesome-associated DNA POLα activity[170], inhibits RNA synthesis and DNA repair[171]) | Aplidin synergizes with cytarabine in exhibiting anticancer activities in leukaemia and lymphoma models *in vitro* and *in vivo*[135] | Chou–Talalay combination index (Calcusyn; Biosoft) | • Both drugs complement each other's activity by inducing apoptosis via the two major apoptotic cascades<br>• Aplidin activates and clusters death receptors of Fas ligand[167], which subsequently activates the receptor-mediated extrinsic cascade[172]<br>• Cytarabine increases cellular stress and reduces survival protein MCL1, which subsequently activates CASPs and apoptosis[171], and triggers the mitochondrial intrinsic cascade[172] |
| ***Different targets of the same pathway that regulate the same target*** | | | | |
| Paclitaxel (stabilizes microtubules via α-tubulin acetylation[79], distorts mitosis to trigger apoptosis[151], and induces p53 and CDK inhibitors[152]) | Tubacin (inhibits HDAC6 and microtubule-associated α-tubulin deacetylase activity[173]) | Synergistically enhances tubulin acetylation[78] | Combination index (Calcusyn) | • Both drugs complement each other's microtubule stabilization effects through enhanced acetylation activity of α-tubulin by paclitaxel[79], and reduced deacetylation activity of α-tubulin deacetylase by tubacin[173] |
| Gefitinib (EGFR tyrosine kinase inhibitor, induces CDK inhibitors p27 and p21, decreases MMP2 and MMP9 activity[158]) | ST1926 (activates MAPKs p38 and JNK, releases cytochrome *c*, activates CASP proteolytic cascade[174]) | Synergistic modulation of survival signalling pathways[175] | Combination Index | • Gefitinib's inhibition of EGFR is complemented by ST1926's activation of p38 MAPK[174] that subsequently mediates internalization of EGFR[176], and by ST1926's activation of CASP proteolytic cascade[174] |
| ***Different targets of related pathways*** | | | | |
| Sildenafil (PDE5 inhibitor[177]) | Iloprost (prostacyclin receptor agonist[178], activates PLC[179], promoting VEGF-induced skin vasorelaxation[180], self-regulates ECAMs[181]) | Synergistic action to cause strong pulmonary vasodilatation[182] | Dose–effect curve surpassed that of individual drug alone combined | • Sildenafil produces vasodilation activity by PDE5 inhibition[177]; iloprost produces vasodilation activity by agonizing the prostacyclin receptor[178] and by activating PLC[179], which promotes VEGF-induced skin vasorelaxation[180]<br>• Targeting of multiple vasodilation regulation pathways — nitric oxide/cyclic GMP[183], Maxi-K channel-mediated relaxation[184], and PLC[179] — contribute to the synergistic actions |
| ***Different target subtypes of related pathways*** | | | | |
| Dexmedetomidine ($\alpha_{2A}$ receptor agonist[185]) | ST-91 (agonist of $\alpha_2$ receptor of other subtypes[186]) | Synergistic antinociceptive action[25,187] | Isobolographic analysis | • ST-91 produces antinociceptive effect via supraspinal receptors and at both spinal and brainstem levels of the acoustic startle response pathway[186] that regulate pain[74]<br>• Dexmedetomidine promotes antinociceptive effect via an endogenous sleep-promoting pathway[185] that sustains reduction in spike activity of spinoreticular tract neurons[73] |
| ***Same target*** | | | | |
| Mycophenolate mofetil (IMPDH inhibitor, drug metabolite mycophenolic acid binds to the site of NAD cofactor[77]) | Mizoribine (IMPDH, drug metabolite mizoribine monophosphate binds to the enzyme in transition state having a new conformation[188]) | Mild synergistic suppression of T and B cell proliferation[189] | Median drug effect analysis, combination index | • Simultaneous inhibition of enzyme reactant-state and transition state have the advantage of covering more conformational space for the inhibitors to better compete with natural substrates for the binding sites |
| Paclitaxel (stabilizes microtubules via α-tubulin acetylation[79], distorts mitosis to trigger apoptosis[151], induces p53 and CDK inhibitors[152]) | Discodermolide (stabilizes microtubule dynamics enhancing microtubule polymer mass[190], resulting in aberrant mitosis that triggers apoptosis[151], induces p53 and CDK inhibitors[152], retains antiproliferative activity against carcinoma cells resistant to paclitaxel due to β-tubulin mutations[191]) | Antiproliferative synergy[192] | Combination index | • Binding sites of both drugs overlap, certain mutations resistant to one drug are ineffective against the other, thus covering a more diverse range of mutant types[15,20,193]<br>• One drug binds and induces a conformational change in tubulin, increasing the binding affinity of the other[15,194]<br>• These drugs may differentially bind to or affect tubulin subtypes, microtubule architectures or microtubule regulators, thereby covering a more diverse range of microtubule dynamics[15,194–196] |

*In these examples, synergy has been determined by well-established synergy/additive analysis methods and its molecular mechanism has been revealed. ‡MoA, mechanisms of action related to synergy. CASP, caspase; CDK, cyclin-dependent kinase; ECAM, endothelial cell adhesion molecule; EGFR, epidermal growth factor receptor; HDAC6, histone deacetylase 6; IMPDH, inosine monophosphate dehydrogenase; MAPK, mitogen-activated protein kinase; MCL1, myeloid cell leukemia sequence 1; MMP, matrix metalloproteinase; PDE5, phosphodiesterase 5; PLC, phospholipase C; POLα, polymerase α; VEGF, vascular endothelial growth factor.

Table 3 | **Examples of pharmacodynamically synergistic drug combinations due to facilitating actions***

| Combination target relationship | Drug A (MoA)‡ | Drug B (MoA)‡ | Reported synergism | Method | Possible mechanism of synergism in promoting facilitating actions |
|---|---|---|---|---|---|
| Different targets of related pathways | Ampicillin (blocks PBP2A and thus bacterial cell-wall synthesis[197]) | Daptomycin (disrupts bacterial membrane structure[198]) | Significant antibacterial synergy[27] | Checkerboard method, fractional inhibitory concentration | • Most PBPs are associated with membrane[199]<br>• Membrane disruption by daptomycin[198] probably hinders the functions of PBPs and further exposes them to ampicillin binding |
| Different targets of related pathways that regulate the same target | Candesartan-cilexetil (angiotensin $AT_1$ receptor antagonist[200]) | Ramipril (ACE inhibitor[201], reduces angiotensin II formation[202]) | Synergistically reduces systolic BP[203] | Dose–response curve shifted 6.6-fold leftwards compared with hypothetical additive curve | • Candesartan-cilexetil reduces systolic BP by antagonizing $AT_1$ receptor[200]<br>• Ramipril reduces systolic BP by inhibiting ACE[201]<br>• Ramipril inhibits $AT_1$ receptor agonist formation[202] thereby facilitating the action of candesartan-cilexetil by reducing $AT_1$ agonist–antagonist competition |

*In these examples, synergy has been determined by well-established synergy/additive analysis methods and its molecular mechanism has been revealed. ‡MoA, mechanisms of action related to synergy. ACE, angiotensin-converting enzyme; BP, blood pressure; PBP, penicillin binding protein.

by genetic variations[47], environmental factors[48], host behaviour[49] and drug scheduling[50]. Therefore, the use of these connections should be more appropriately viewed as a start to a more comprehensive analysis.

## Types of drug combinations

When two drugs produce the same broad therapeutic effect, their combination produces the same effect of various magnitudes compared with the summed effects of the individual drugs. A combination is pharmacodynamically synergistic, additive or antagonistic if the effect is greater than, equal to, or less than the summed effects of the partner drugs[51]. Drug combinations may also produce pharmacokinetically potentiative or reductive effects such that the therapeutic activity of one drug is enhanced or reduced by another drug via regulation of its absorption, distribution, metabolism and excretion (ADME)[51]. A further type of drug combination is a coalistic combination, in which all of the drugs involved are inactive individually but are active in combination[52–55].

Synergistic and potentiative drug combinations have been explored to achieve one or more favourable outcomes: enhanced efficacy; decreased dosage at equal or increased level of efficacy; reduced or delayed development of drug resistance; and simultaneous enhancement of therapeutic actions and reduction of unwanted actions (efficacy synergism plus toxicity antagonism)[17,22,51]. The mechanisms underlying these activities can be better understood by studying the mechanistically contrasting additive, antagonistic and reductive drug combinations. Several rigorous drug-combination analysis methods have been developed and extensively used for analysing combinations from experimental data[15,22,51]. These include checkerboard, combination index, fractional effect analysis, isobolographic analysis, interaction index, median drug effect analysis, and response surface approaches[51–59].

## Literature drug combinations and MI profiles

We searched Pubmed[60] to select literature-reported drug combinations that had been evaluated by rigorous combination analysis methods and for which relevant MI profiles were retrievable from Pubmed. Combinations of the keywords "drug combination", "drug interaction", "multi-drug", "additive", "antagonism", "antagonistic", "infra-additive", "potentiated", "potentiative", "potentiation", "reductive", "supra_additive", "synergism", "synergistic", and "synergy" were used to search publications since 1999. Coalistic drug combinations were not evaluated because few of them are described in the literature. This is partly due to the focus on combinations of drugs that include at least one active drug; indeed, a Medline search using "coalistic" and "coalism" returns only one abstract. In addition, a significantly higher percentage of the studies published before 1999 are based on non-rigorous drug-combination methods. It has been suggested that analysis without using a rigorous method may easily lead to errors in assessing synergism with respect to such effects as enhancement and potentiation[51]. Therefore, to maintain the level of reliability of our assessment without substantially losing statistical significance, we focused on studies published since 1999, which constitute approximately 50% of all abstract entries selected by using our search method.

We collected 315, 88 and 62 abstract entries describing pharmacodynamically synergistic, additive, and antagonistic combinations, respectively, and 56 and 33 abstract

Table 4 | **Examples of pharmacodynamically additive drug combinations***

| Combination target relationship | Drug A (MoA)‡ | Drug B (MoA)‡ | Reported additive effect | Method | Possible mechanism of additive effect |
|---|---|---|---|---|---|
| *Equivalent of overlapping actions* | | | | | |
| Different targets of the same pathways that regulate the same target | Diazoxide (ATP-sensitive $K^+$ channel opener[204], enhances ATPase activity of channel regulatory subunits[205]) | Dibutyryl-cGMP (activates ATP-sensitive $K^+$ channel[204], activated channel[206,207]) | Additive antinociceptive effect[204] | Analysis of variance synergism and dose–effect data analysis | • Diazoxide enhances ATPase activity of channel regulatory subunits of sulphonylurea receptors[205]<br>• Dibutyryl-cGMP activates channel via a cGMP-dependent protein kinase[206,207] |
| Same target (different sites with direct contact with agonist site) | Propofol (interacts with $GABA_A$ receptor[209]) | Sevoflurane (interacts with $GABA_A$ receptor[210]) | Additive action in producing consciousness and movement to skin incision during general anaesthesia[211] | Dixon up–down method | • Propofol binds to TM3 segment of the β2 $GABA_A$ subunit[209]<br>• Sevoflurane binds to Ser270 of the α1 $GABA_A$ subunit[210]<br>• As agonist binding site is located between α1 and β2 subunits[212], both drugs probably hinder agonist activity, thereby producing mutually substitutable actions |
| Same target (same site) | Ampicillin (blocks PBP2A and thus bacterial cell-wall synthesis[197]) | Imipenem (inhibits PBP1A, 1B, 2, 4 and 5 and thus bacterial cell-wall synthesis[213]) | Additive antibacterial effect[27] | Checkerboard method, fractional inhibitory concentration | • Both act at the same active site of PBP2A[214] but at relatively high MICs of ≥32 μg per ml[197]<br>• The relatively high MICs make it less likely for both drugs to saturate target sites, thereby maintaining additive antibacterial effect |
| *Independent actions* | | | | | |
| Different targets of unrelated pathways | Artemisinin (interferes with parasite transport proteins PfATP6, disrupts parasite mitochondrial function, modulates host immune function[215]) | Curcumin (generates ROS and downregulates PfGCN5 HAT activity, producing cytotoxicity for malaria parasites[216]) | Additive antimalarial activities[217] | Fractional inhibitory concentrations | • Artemisinin blocks calcium transport to ER[215]<br>• Curcumin induces DNA damage and histone hypoacetylation[216]<br>• They act at different sites in non-interfering manner |
| Same target (different sites) | Doxorubicin (DNA intercalator[94], prefers AT regions[94]) | Trabectedin (forms covalent guanine adduct at specific sites in DNA minor groove[95], interacts with DNA repair system) | Additive anticancer effect[93] | Isobolographic analysis | • Both bind to DNA in non-interfering manner; one prefers AT regions[94], the other alkylated guanines[95]<br>• Recent progress in designing dual platinum-intercalator conjugates[96] suggests that it is possible for both drugs to act without hindering each other's binding mode |
| *Independent actions at dosages significantly lower than MICs, complementary actions at higher dosages* | | | | | |
| Different targets of unrelated pathways | Azithromycin (hinders bacterial protein synthesis by binding to 50S component of 70S ribosomal subunit[218]) | Imipenem (inhibits PBP1A, 1B, 2, 4 and 5 and thus bacterial cell-wall synthesis)[213] | Additive antibacterial effect[219] | Checkerboard method, fractional inhibitory concentration | • Azithromycin hinders bacterial protein synthesis[218] at MIC of 0.12 μg per ml[220]<br>• Imipenem blocks bacterial cell-wall formation[217] at MICs of ≥32 μg per ml[197]<br>• At dosages significantly lower than MICs for both drugs, azithromycin's reduction of PBPs[213] may be insufficient for imipenem to saturate these proteins, allowing its unhindered inhibition of these proteins[213], thereby these actions proceed in a non-interfering manner |

*In these examples, additive action has been determined by well-established synergy/additive analysis methods and its molecular mechanism has been revealed. ‡MoA, mechanisms of action related to additive effect. $GABA_A$, γ-aminobutyric acid A; ER, endoplasmic reticulum; HAT, histone acetyltransferase; PBP, penicillin binding protein; MIC, minimum inhibitory concentration; PfATP6, sarcoendoplasmic reticulum $Ca^{2+}$ ATPase (SERCA) orthologue of *Plasmodium falciparum*; PfGCN5, *P. falciparum* GCN5 homologue; ROS, reactive oxygen species; TM3, transmembrane 3.

Table 5 | **Examples of pharmacodynamically antagonistic drug combinations***

| Combination target relationship | Drug A (MoA)‡ | Drug B (MoA)‡ | Reported antagonistic effect | Method | Possible mechanism of antagonism of interfering actions |
|---|---|---|---|---|---|
| Different targets of related pathways that regulate the same target | Amphotericin B (forms ion channels in fungal membranes[221]) | Ravuconazole (inhibits biosynthesis of ergosterol, a component of fungal cell membranes[222]) | Antagonism in experimental invasive pulmonary aspergillosis[223,224] | Loewe additivity-based drug-interaction model | • Amphotericin B can form ion channels more easily in the presence of ergosterol[221]<br>• Ravuconazole inhibition of ergosterol synthesis[222] can therefore reduce the activity of amphotericin B in forming ion channels[221] |
| Same target | Aminophylline (adenosine receptor antagonist, phospho-diesterase inhibitor, releases intracellular calcium[97]) | Theophylline (releases intracellular calcium, adenosine receptor antagonist, phospho-diesterase inhibitor[97]) | Antagonism of inhibitory adenosine autoreceptors and release of intracellular calcium[97] | Quantal release measurement | • Adenosine receptor antagonist binding may be associated with non-unique binding site conformations[98]<br>• Aminophylline binding may lock the receptor into a unique conformation that hinders theophylline binding, thereby producing an antagonistic effect |

*In these examples, antagonism has been determined by established methods and its molecular mechanism has been revealed. The antagonism of the listed drug combinations is due to interfering actions of the partner drugs in each combination. ‡MoA, mechanisms of action related to antagonism.

entries describing pharmacokinetically potentiative and reductive combinations, respectively. We then removed 158, 53, 32, 15 and 18 of these entries, respectively, that are redundant (for example, the same combination or the same paper selected by different keyword combinations); ambiguous (for example, synergistic in one report or condition, additive in another report or condition); and involving more than two drugs so as to focus on simpler cases. We further removed 45, 12, 1, 1 and 2 papers, respectively, that described studies using non-rigorous drug-combination methods. For the remaining 217 papers, we searched additional literature for experimentally determined MI profiles related to the mechanism of the claimed combination effects. Our analysis showed that the available literature-reported MI profiles are insufficient or irrelevant to substantiate the claimed combination effects in 110 (59 synergistic, 11 additive, 17 antagonistic, 20 potentiative and 3 reductive combinations) of the 218 remaining papers.

This led to the identification of 107 combinations that can be substantiated by available literature-reported MI profiles. These comprise 53, 12 and 12 sets of pharmacodynamically synergistic, additive and antagonistic combinations, and 20 and 10 sets of pharmacokinetically potentiative and reductive combinations, respectively. Data are summarized in Supplementary information S1 (table), S2 (table), S3 (table), S4 (table), S5 (table), S6 (table) and S7 (table), together with literature-reported mechanisms related to their therapeutic and combination effects. The statistical significance of our assessment can be roughly estimated as follows: for the 110 combination sets not yet substantiated by the available MI profiles, it is reasonable to assume that a high percentage of them may eventually be substantiated by additional experimental findings. If one further assumes that the reported combination effects that are substantiated by MI profiles are at least partly true, then the estimated ratio of truly and falsely reported combinations should be substantially larger than 107 out of 110. Hence, there seems to be a statistically significant number of combinations and sufficient percentages of true claims for supporting a fair assessment of general combination types and mechanisms of drug combinations from the information collected by our search methods.

Examples of our evaluated drug combinations are shown in TABLES 1–7. Many of the MI profiles directly point to a specific biomolecule as the inhibiting, activating or regulating target. So, it is possible to determine the combination effects based on the expected therapeutic and pharmacokinetic consequences of these interactions. Although the molecular target is not exactly specified, some of the profiles identify a specific pathway or process as a target, and provide the pharmacodynamic or pharmacokinetic consequence of the interaction. For instance, in literature reports, arsenic trioxide produces anticancer activity by generating reactive oxygen species, which is partially counteracted by its activation of the AKT survival pathway[61]. The anticancer agent 17-(allylamino)-17-demethoxygeldanamycin (17-AAG) produces its effects by inhibiting the nuclear factor-κB (NF-κB), AP-1 (also known as JUN) and phosphatidylinositol 3-kinase (PI3K)–AKT pathways[61]. Therefore, when used in combination, 17-AAG abrogates arsenic trioxide's counteractive activation of AKT survival pathway[61].

## Pharmacodynamically synergistic combinations

We identified three groups of pharmacodynamically synergistic combinations among the 53 synergistic drug combinations. In the first group (21 combinations), anti-counteractive actions of the drugs involved

Table 6 | **Examples of pharmacokinetically potentiative drug combinations***

| Biochemical class of potentiative effect | Drug A (therapeutic or toxic effects and MoA) | Drug B (MoA related to potentiative effect) | Reported potentiative effect | Possible mechanism of potentiative actions |
|---|---|---|---|---|
| Positive regulation of drug transport or permeation | AZT (anti-HIV; HIV-1 reverse transcriptase inhibitor) | 1,8-Cineole (forms hydrogen bonds with lipid head groups of stratum corneum lipids[225]) | Enhances skin permeation of AZT[226] | Enables drug transport across skin possibly by disrupting absorption barrier via binding to lipid head groups |
| Enhanced drug distribution or localization | Cerivastatin (cholesterol-lowering; HMG-CoA reductase inhibitor) | Gemfibrozil (inhibits CYP2C8-mediated metabolism of statins, inhibits OATP2-mediated uptake of cerivastatin[227]) | Increases plasma concentration of statins by inhibiting their metabolism and uptake[227–229] | Enhances level of drug in plasma by metabolism reduction and uptake inhibition |
| Enhanced drug metabolism | Doxorubicin (anticancer by DNA intercalation; converted to doxorubicinol by NADPH-dependent aldo/keto or carbonyl reductases[230], produces cardiotoxicity by mediating transition from reversible to irreversible damage) | Paclitaxel (stimulates enzymatic activity of NADPH-dependent aldo/keto or carbonyl reductases[230]) | Enhances cardiotoxicity by increasing metabolism of doxorubicin into toxic metabolite[230] | Enhanced metabolism of drug into toxic metabolite |

*In these examples, potentiative effect has been determined by established methods and its molecular mechanism has been revealed. AZT, azidothymidine; CYP2C8, cytochrome P450 2C8; HMG-CoA, 3-hydroxy-3-methyl-glutaryl-CoA; MoA, mechanism of action; OATP2, organic anion transporter 2 (also known as SLCO1B1).

reduce the network's counteractive activities against a drug's therapeutic effect. In the second group (26 combinations), complementary actions positively regulate a target or process by interactions with multiple target/pathway sites, different target subtypes and states, and competing mechanisms[15]. The third group (six combinations) involves facilitating actions: secondary actions of one drug that enhance the activity or level of another drug. The therapeutic and synergistic mechanisms of the combinations in these three groups are summarized in Supplementary information S1 (table), S2 (table) and S3 (table), with selected examples given in TABLE 1, TABLE 2 and TABLE 3, respectively.

***Anti-counteractive actions.*** Anti-counteractive actions may arise from interactions with an anti-target or counter-target[14], and from negative modulations of a network's robustness[5–7], crosstalk[9–11], and compensatory and neutralizing actions[12,13]. These anti-counteractive synergistic combinations act on different targets of related pathways (eight combinations); different targets of crosstalking pathways (four combinations); different targets of the same pathway that crosstalk to each other via another pathway (one combination) or regulate the same (five combinations) or different targets (two combinations); and different sites of the same target (one combination).

An example of actions on different targets is provided by the anticancer combination of cisplatin and topotecan[62–64]. Cisplatin binds to the major groove of GG, AG and TACT sites in DNA[65], which is bypassed by the network's counteractive activity of mutagenic translesional bypass replication across cisplatin–DNA adducts[66]. Topotecan inhibits topoisomerase I, interacts with DNA and stabilizes covalent topoisomerase–DNA complexes to block DNA replication forks[67]. The last function reduces the counteractive effect against cisplatin, therefore resulting in synergism.

An example of actions on the same target is the anticancer combination of cisplatin and trabectedin[68]. Trabectedin interacts with DNA and DNA repair systems in a different manner to that of cisplatin[68] via covalent binding to the 2-amino group of the central guanine of selected DNA pyrimidine-G-G and purine-G-C triplets[69]. This induces the formation of unusual DNA replication intermediates that strongly inhibit DNA replication[70] and subsequently reduces the counteractive effect against cisplatin.

***Complementary actions.*** Complementary actions primarily involve positive regulation of a target or process by targeting multiple points of a pathway[71,72] and its crosstalk pathways[71–75]; interacting with multiple sites[65,76], states[77], conformations[15], and mutant forms[15] of the target; collectively modulating target activity and expression[28]; and simultaneously enhancing the positive and reducing the negative effects of the target[78,79]. These combinations act on different targets of related pathways that regulate the same targets (eight combinations) or the same target/process (five combinations); different targets of related pathways that regulate different targets (six combinations); different targets of the same pathway that

Table 7 | **Examples of pharmacokinetically reductive drug combinations***

| Biochemical class of reductive effect | Drug A (therapeutic or toxic effects and MoA) | Drug B (MoA related to reductive effect) | Reported reductive effect | Possible mechanism of reductive actions |
|---|---|---|---|---|
| Drug transport and permeation | Amphotericin B (antileishmanial, forms aggregate with miltefosine[231]) | Miltefosine (antileishmanial, forms aggregate with amphotericin B[231]) | Reduces miltefosine-induced paracellular permeability enhancement in Caco-2 cell monolayers, inhibits uptake of both drugs, decreases transepithelial transport of both drugs[232] | Reduces drug permeability and transport |
| Drug distribution and localization | Cisplatin (DNA inter- and intra- strand adduct, preferably binds to the major groove of GG, AG and TACT sites[65] thereby inhibiting DNA polymerization and induces DNA damage to trigger apoptosis[120]) | Procainamide hydrochloride (forms cisplatin–procainamide complex[233]) | Reduces cisplatin-induced hepatotoxicity via formation of less toxic platinum complex, leading to inactivation of cisplatin or its highly toxic metabolites and to a different subcellular distribution of platinum[233] | Reduces level of toxic drug by formation of less toxic complex and rearrangement of its subcellular distribution |
| Drug metabolism | Warfarin (anticoagulant and antithrombotic, affects coagulation proteins that act sequentially to produce thrombin, metabolized by CYP3A4[234]) | Quinidine (stimulates CYP3A4-mediated metabolism of warfarin[235]) | Reduces anticoagulant effect of warfarin by stimulating its metabolism[235] | Enhances metabolism of active drug into inactive metabolite |

*In these examples, reductive effect has been determined by established methods and its molecular mechanism has been revealed. CYP3A4, cytochrome P450 3A4; MoA, mechanism of action.

regulate the same target (two combinations); different target subtypes in related pathways (one combination); and the same target at different sites (two combinations), overlapping sites (one combination), and different states (one combination).

An example of actions on different targets is the celecoxib and emodin combination, which synergistically represses the growth of certain cancer cells[80]. Celecoxib is a cyclooxygenase 2 (COX2) inhibitor, which suppresses cancer growth by inactivating protein kinase AKT to stop its suppression of apoptosis[81]. Emodin suppresses cancer growth by inhibiting tyrosine kinases[82] and down-regulating AKT via inhibition of PI3K pathway to reduce AKT suppression of apoptosis[83]. Emodin complements celecoxib's inactivation of AKT[81] to reduce its suppression of apoptosis.

***Facilitating actions.*** Facilitating actions can be illustrated by two examples. The first is the gentamicin and vancomycin combination, which produces synergistic antibacterial action against penicillin-resistant bacterial strains[84]. Gentamicin targets the bacterial ribosome, causes misreading of the genetic code and inhibits translocation to disrupt protein synthesis[85]. Vancomycin inhibits bacterial cell-wall peptidoglycan synthesis[86], selectively inhibits ribonucleic acid synthesis and alters permeability of the cell membrane[87]. The alteration in cell-membrane permeability by vancomycin enhances gentamicin penetration into bacterial cells, thereby increasing its bioavailability.

The second example is the BQ-123 and enalapril combination, which produces synergistic endothelium-dependent vasodilation enhancement[88]. BQ-123 is an endothelin A ($ET_A$) receptor antagonist that mediates vasodilation[89]. Enalapril upregulates the $ET_B$ receptor and inhibits angiotensin-converting enzyme, leading to vasodilation[90,91]. BQ-123 antagonism of the $ET_A$ receptor[89] displaces endogenous ET1 from the $ET_A$ receptor on to the upregulated $ET_B$ receptor to enhance its activity by effectively increasing $ET_B$ agonist concentration[88].

## Pharmacodynamically additive combinations

Investigation of additive and antagonistic combinations provides contrasting perspectives for facilitating the study of synergistic combinations. Additive combinations (see Supplementary information S4 (table), with selected examples in TABLE 4) result from equivalent or overlapping actions (nine combinations) and independent actions (four combinations) of the drugs involved.

Equivalent and overlapping actions involve interactions with different targets of the same pathways that equivalently regulate the same target (seven combinations), or interactions that directly or indirectly affect the same site of the same target (two combinations). For example, retinoic acid and trichostatin A additively inhibit cell proliferation by overlapping actions of upregulation of retinoic acid receptor β and reactivation of its mRNA expression[92].

Independent actions involve interactions with different targets of unrelated pathways (three combinations),

Table 8 | **Assessment of clinically widely used drug combinations (part 1)***

| Suggested combination type | Drug A (MoA)‡ | Drug B (MoA)‡ | Reported combination effect (method) | Possible mechanism of combination actions |
|---|---|---|---|---|
| ***Different targets of unrelated pathways*** | | | | |
| Potentiative combination by enhancing drug distribution or localization | Amoxicillin (inhibits bacterial cell-wall synthesis[236], destroyed by β-lactamase[237]) | Clavulanate (β-lactamase inhibitor[117]) | Antibacterial synergy[103] (comparison of inhibitory activity) | • Clavulanate maintains level of amoxicillin at bacterial cell wall by inhibiting its degradation enzyme β-lactamase[117], thereby potentiating the antibacterial activity of amoxicillin |
| ***Different targets of related pathways that regulate the same target*** | | | | |
| Synergistic combination due to facilitating actions | Salmeterol ($\beta_2$-adrenoceptor agonist[238] that activates T-cell subtypes[186], promotes apoptosis via adrenoreceptor- and cAMP-independent, $Ca^{2+}$-dependent mechanism[239]) | Fluticasone (glucocorticoid receptor binder[240] that induces apoptosis[241], upregulates $\beta_2$- adrenoceptor[112]) | Synergistic *in vitro* T-cell activation and apoptosis induction in asthma[110] (comparison of activity and protein levels) | • Salmeterol's agonistic activity on the $\beta_2$-adrenoceptor[238] is facilitated by fluticasone's upregulation of the $\beta_2$- adrenoceptor[112], leading to synergistic T-cell activation and apoptosis induction |
| ***Different targets of the same pathway (upstream – downstream relationship)*** | | | | |
| Redundant combination in targeting upstream and downstream targets of the same single-route pathway | Sulphamethoxazole (DHPS inhibitor[118], metabolite covalently haptenates human serum proteins[242]) | Trimethoprim (DHFR inhibitor[119]) | No synergy detected against *E. coli*[111] and *S. somaliensis* strains[104], therapeutic effect due to sulphamethoxazole alone, clinical use of combination discontinued and converted to single drug[104] (chequerboard) | • Both drugs target the same single-route folate metabolism pathway<br>• Sulphamethoxazole targets the upstream DHPS[118] and trimethoprim targets the downstream DHFR[119]<br>• Redundant combination if sulphamethoxazole effectively inhibits DHPS<br>• Trimethoprim inhibition of DHFR serves as a backup when sulphamethoxazole becomes less effective |
| ***Different targets of related pathways*** | | | | |
| Unclear | Rifampicin (bacterial DNA-dependent RNA polymerase inhibitor[243]) | Fusidic acid (interferes with bacterial protein synthesis by inhibiting the translocation of peptide elongation factor G from the ribosome[244]) | Synergistic effect against *S. somaliensis* strains *in vitro*[104] (chequerboard) | • Mechanism unclear<br>• A report suggests that transcribing activity of DNA-dependent RNA polymerase from *E. coli* is inhibited *in vitro* by addition of preparations of elongation factor T purified to homogeneity[245] |
| Synergistic combination due to facilitating action | Erythromycin (binds to bacterial 70S ribosomal complex to inhibit bacterial protein synthesis[114]) | Penicillin (binds to DD-transpeptidase that links peptidoglycan, which weakens bacterial cell wall[246]) | Combination inhibits 80% of the *S. somaliensis* strains both synergically and additively[104] (chequerboard) | • Weakening of bacterial cell wall by penicillin, which enhances erythromycin penetration into bacterial cells, thereby enhancing its bioavailability[114] |
| Potentiative combination by enhancing drug distribution or localization | Ergotamine ($5\text{-}HT_{1B}$/$5\text{-}HT_{1D}$ receptor agonist[247], agonist of presynaptic dopamine receptors and $\alpha_2$-adrenoceptors, postsynaptic $\alpha_1$ and $\alpha_2$-adrenoceptors, and antagonist of the postsynaptic $\alpha_1$-adrenoceptors[248]) | Caffeine (adenosine receptor antagonist[249] that increases dopamine and GABAergic activities[250], cAMP-PDE inhibitor[251]) | Symptomatic treatment of chronic vascular headache by the combination[105] (comparison of activity) | • Caffeine increases water solubility of ergotamine to enhance its absorption[122], producing potentiative effect<br>• Possible synergy may occur at dopamine receptor, which requires further investigation |
| Additive combination due to equivalent action | Niacin (niacin receptor HM74A agonist that inhibits hepatocyte DGAT and triglyceride synthesis leading to increased intracellular ApoB degradation[252]) | Simvastatin (HMG-CoA reductase inhibitor[123]) | Combination reduces LDL and VLDL, and increases HDL cholesterol[106] (comparison of activity and protein levels) | • Niacin reduces secretion of VLDL and LDL cholesterol[252]<br>• Simvastatin reduces synthesis of LDL cholesterol and triglycerides, and increased HDL-cholesterol[123]<br>• Both drugs equivalently reduce the level of LDL cholesterol |

*These combinations, which were not collected by our literature search procedure, have primarily been studied by less rigorous combination analysis methods and the relevant studies have been published before 1999. ‡MoA, mechanisms of action related to combination effect. 5-HT, 5-hydroxytryptamine (serotonin); ApoB, apolipoprotein B; COX, cyclooxygenase; DGAT, diacylglycerol acyltransferase; DHFR, dihydrofolate reductase; DHPS, dihydropteroate synthase; *E. coli*, *Escherichia coli*; GABA, γ-aminobutyric acid; HDL, high density lipoprotein; HM74A, G protein-coupled receptor HM74a (also known as GPR109A) HMG-CoA, 3-hydroxy-3-methyl-glutaryl-CoA; LDL, low density lipoprotein; PDE, phosphodiesterase; *S. somaliensis*, *Streptomyces somaliensis*; TYMS, thymidylate synthase; VLDL, very-low density lipoprotein.

Table 8 | **Assessment of clinically widely used drug combinations (part 2)***

| Suggested combination type | Drug A (MoA)‡ | Drug B (MoA)‡ | Reported combination effect (method) | Possible mechanism of combination actions |
|---|---|---|---|---|
| ***Same target (different binding sites)*** | | | | |
| Synergistic combination due to complementary action | Cisplatin (DNA inter- and intra-strand adduct, preferably binds to the major groove of GG, AG and TACT sites[65] thereby inhibiting DNA polymerization and induces DNA damage to trigger apoptosis[120]) | Cyclophosphamide (metabolite forms DNA adduct at phosphoester[121] and at G N-7 positions[253], thereby inhibiting DNA polymerization and induces DNA damage to trigger apoptosis[208]) | Combination produces response rates of 60–80% in patients with small-cell lung cancer[107] (comparison of activity) | • Cisplatin and cyclophosphamide form DNA adducts at different sites[120,121], possibly at mutually compatible binding conformation because of the small size of the drugs<br>• The two drugs thereby complement each other's actions on DNA |
| ***Same target*** | | | | |
| Synergistic combination due to facilitating action | Methotrexate (DHFR inhibitor[134]) | Fluorouracil (anticancer, metabolite inhibits TYMS that stops DNA synthesis[254], stabilizes and activates p53 by blocking MDM2 feedback inhibition through ribosomal proteins[255]) | Synergism in inhibiting viability of L1210 murine tumour cells[113] (comparison of activity) | • Apart from methotrexate's anticancer DHFR inhibitory activity[134], methotrexate metabolite forms reversible ternary complexes with fluorouracil on one site of TYMS to enhance its binding to the enzyme[113]<br>• Fluorouracil's anticancer TYMS inhibitory activity is therefore enhanced |
| Synergistic combination due to complementary action | Diclofenac (non-selective COX inihibitor[115], COX1 inhibition increases formation of kynurenic acid in brain to produce analgesic effect[115]) | Paracetamol (metabolite agonizes cannabinoid receptors to produce analgesic effect[212,256], selective COX2 variant inhibitor[257]) | Synergy in treatment of acute pain in humans[109] (isobolographic analysis) | • Apart from its analgesic action via cannabinoid receptors[212,256], paracetamol reduces active oxidized form of COX to resting form[116] to complement diclofenac's analgesic action of COX1 inhibition[115] |

*These combinations, which were not collected by our literature search procedure, have primarily been studied by less rigorous combination analysis methods and the relevant studies have been published before 1999. ‡MoA, mechanisms of action related to combination effect. 5-HT, 5-hydroxytryptamine (serotonin); ApoB, apolipoprotein B; COX, cyclooxygenase; DGAT, diacylglycerol acyltransferase; DHFR, dihydrofolate reductase; DHPS, dihydropteroate synthase; *E. coli*, *Escherichia coli*; GABA, γ-aminobutyric acid; HDL, high density lipoprotein; HM74A, G protein-coupled receptor HM74a (also known as GPR109A) HMG-CoA, 3-hydroxy-3-methyl-glutaryl-CoA; LDL, low density lipoprotein; PDE, phosphodiesterase; *S. somaliensis*, *Streptomyces somaliensis*; TYMS, thymidylate synthase; VLDL, very-low density lipoprotein.

or different sites of the same target (one combination). For instance, doxorubicin and trabectedin produce an additive anticancer effect via equivalent action of DNA intercalation and covalent guanine adduct formation at specific sites in the DNA minor groove[93]. Both drugs bind to DNA in a non-interfering manner; doxorubicin prefers AT regions[94], whereas trabectedin alkylates guanines[95]. Recent progress in designing dual platinum-intercalator conjugates[96] suggests that it is possible for both drugs to act without hindering the binding mode of each other.

### Pharmacodynamically antagonistic combinations

Antagonistic drug combinations (see Supplementary information S5 (table), with selected examples in TABLE 5) involve interfering actions at the same target (two combinations), or different targets of related pathways that regulate the same target (two combinations). One possible mechanism for antagonistic drug combination against the same target is mutual interference at the same site, which can be illustrated by the aminophylline and theophylline combination[97]. Both aminophylline and theophylline are adenosine receptor antagonists and phosphodiesterase inhibitors, and are involved in the release of intracellular calcium[97]. Adenosine receptor antagonist binding may be associated with non-unique binding site conformations[98]. Therefore, aminophylline or theophylline binding probably locks the receptor into a unique conformation that hinders theophylline or aminophylline binding, leading to antagonism. Similarly, inhibitor–activator, antagonist–agonist, blocker–substrate, and other mutually interfering pairs of drugs that bind to the same site may also produce antagonism.

One mechanism for antagonistic drug combination against different targets of related pathways is counteractive actions that hinder the normal actions of the partner drug, which can be illustrated by the cytarabine and 17-AAG combination[99]. Cytarabine is a DNA binder[99] and 17-AAG is a heat-shock protein antagonist that abrogates the AKT survival pathway[61,100]. 17-AAG antagonizes the cytotoxic activity of cytarabine, which is partly due to the induction of G1 cell-cycle arrest, which subsequently prevents the incorporation of cytarabine into cellular DNA[99].

### Pharmacokinetically potentiative combinations

Potentiative drug combinations (see Supplementary information S6 (table), with selected examples in TABLE 6) involve positive modulation of drug transport or permeation (seven combinations), distribution or localization (eight combinations), and metabolism (three combinations). Potentiative modulation of drug transport or permeation enhances drug absorption via disruption of transport barrier, delay of barrier recovery, or inhibition

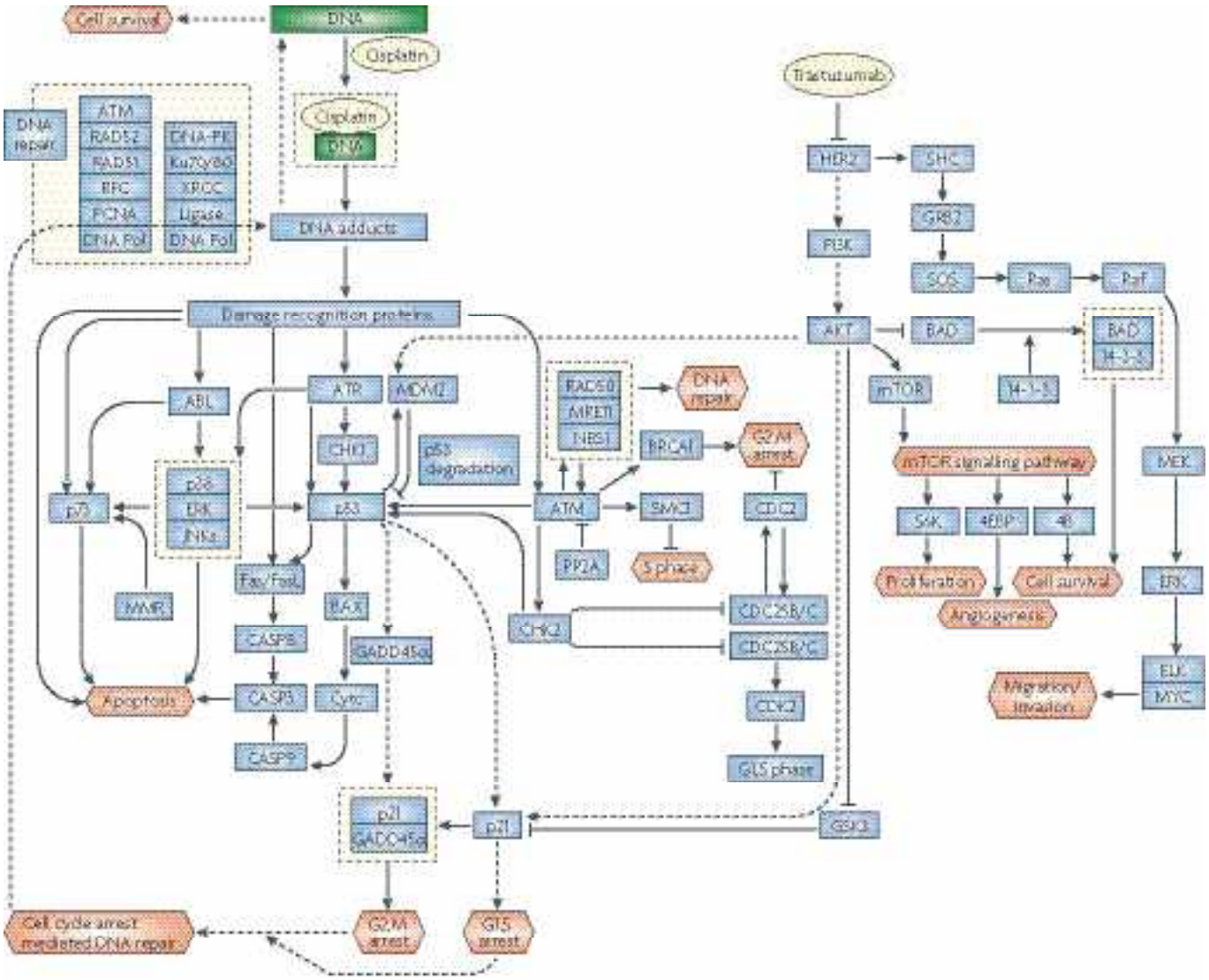


Figure 1 | **Pathways affected by the cisplatin and trastuzumab combination.** Cisplatin forms adducts with DNA that inhibit DNA polymerization and induce DNA damage to trigger apoptosis[120] (via p53– BCL-2-associated X protein (BAX), p53–Fas, p38– Jun N-terminal kinase (JNK), and p73 pathways). Trastuzumab is an anti-HER2 (also known as ERBB2) antibody that inhibits HER2-mediated proliferation, angiogenesis, survival and migration[130] (via phosphatidyl-inositol 3-kinase (PI3K)–AKT–mammalian target of rapamycin (mTOR) and Ras– extracellular signal-regulated kinase (ERK) pathways). Induction of DNA damage and apoptosis by cisplatin may be attenuated by DNA repair systems in certain cell types[120] (via p53–p21 pathways). This counteractive DNA repair action may be reduced by the anti-HER2 activity of trastuzumab, which suppresses the DNA repair pathway[131] and inhibits the PI3K–AKT pathway[132] to enhance apoptosis[133]. The corresponding pathways (dashed lines) involve the inhibition of HER2–PI3K–AKT-mediated activation of p21, which reduces the activity of p21 in facilitating checkpoint kinase 1 (CHK1)–p53–p21 and CHK1–p53–growth arrest and DNA-damage-inducible, alpha (GADD45α)–p21 mediated induction of cell-cycle arrest that is important for ataxia telangiectasia mutated (ATM)-mediated DNA repair. Reduction of AKT activity by trastuzumab's inhibition of HER2 also lowers the activity of p53 binding protein homologue (MDM2) in facilitating p53 degradation, which enhances p21 activation to counterbalance the reduced AKT activation of p21. We were unable to identify another counterbalancing pathway, and it is unclear to what extent the MDM2-mediated counterbalance pathway affects the overall state of p21 activation. 4EBP, eukaryotic initiation factor 4E (eIF4E)-binding protein; ATR, ataxia telangiectasia and Rad3 related; BAD, BCL-2-associated agonist of cell death; BRCA1, breast cancer 1, early onset; CASP, caspase; CDC2, cell division cycle 2, G1 to S and G2 to M; CDC25, cell division cycle 25 homologue; CDK2, cyclin-dependent kinase 2; Cyt *c*, cytochrome *c*; DNA PK, DNA protein kinase; DNA Pol, DNA polymerase; FasL, Fas ligand; GRB2, growth factor receptor-bound protein 2; GSK3, glycogen synthase kinase 3; KU70, also known as XRCC6; KU80, also known as XRCC5; MEK, mitogen-activated protein kinase/ERK kinase; MMR, mismatch repair; NBS1, Nijmegen breakage syndrome 1; PCNA, proliferating cell nuclear antigen; PP2A, protein phosphatase 2A; RFC, replication factor C; S6K, S6 kinase (also known as RPS6KB1); SHC, Src homology 2 domain containing; SMC1, structural maintenance of chromosomes 1A; XRCC, X-ray-repair-cross-complementing.

of drug efflux. Potentiative modulation of drug distribution or localization increases drug concentration in plasma or a specific tissue by blocking drug uptake and inhibiting metabolic processes that convert drugs into excretable forms. Potentiative metabolism modulation stimulates the metabolism of drugs into active forms, or inhibits the metabolism of drugs into inactive forms.

Typical potentiative effects can be illustrated by two examples. One is the enhanced absorption of antithrombotic low-molecular-weight heparin (LMWH) by chitosan[101]. LMWH is an antithrombin binder that inhibits activated coagulation factors. Chitosan reversibly interacts with components of tight junctions to widen paracellular routes, which increases the permeability of LMWH across mucosal epithelia and therefore enhances its absorption. The second example is 2′-deoxyinosine enhancement of antitumour activity of 5-fluorouracil in human colorectal cell lines and colon tumour xenografts[102]. 5-Fluorouracil is metabolized by thymidine phosphorylase and other enzymes into a metabolite that stabilizes p53 due to RNA-directed effects. 2′-Deoxyinosine enhances thymidine phosphorylase activity and thus the metabolism of 5-fluorouracil into its active metabolite.

### Pharmacokinetically reductive combinations

Seven reductive drug combinations were identified, which involve negative modulation of drug transport or permeation (two combinations), distribution or localization (one combination), and metabolism (four combinations), respectively (see Supplementary information S7 (table), with selected examples in TABLE 7). Reductive modulation of drug transport or permeation typically blocks drug absorption or promotion of first-pass elimination by actions such as drug–drug aggregation to reduce the permeability and inhibition of drug transport into plasma or target site. Reductive modulation of drug distribution/localization decreases the drug concentration in plasma or a specific tissue, which typically involves stimulation of metabolic processes for converting drugs into excretable forms and inhibition of metabolic processes for increasing drug concentration. Drug activity can also be reduced by metabolism modulation to convert drugs into inactive forms.

### Further assessment of popular drug combinations

Several drug combinations have been extensively used for clinical applications for many years[103–109]. For some of these classical drug combinations, the studies of their combination effects have been primarily conducted and published before 1999, and are frequently based on non-rigorous combination analysis methods. Therefore, some of these classical combinations were not selected by our search procedure. Nonetheless, their popular use is a strong indication of their possible beneficial combination effects in comparison with those of individual drugs, and so it is of interest to assess the effects and mechanisms of these classical drug combinations.

We identified ten sets of classical drug combinations that were missed by our search procedure and contain no drug of abuse or withdrawn drug. TABLE 8 summarizes literature-described modes of actions of individual drugs, suggested combination type and possible mechanism of these combinations. The ten combinations include five synergistic[103,104,109,110,113], one dual synergistic/additive[104], and one non-synergistic[104,111] combinations. The clinical use of the non-synergistic combination has been replaced by single-drug therapy[104]. For the remaining three combinations, we were unable to find a literature report indicating their possible types of combination. It is also noted that four of the ten combinations have been studied by rigorous drug combination analysis methods.

Literature-described MI profiles seem to provide some clues to the possible mechanisms for nine of the ten combinations. The synergistic salmeterol and fluticasone, methotrexate and fluorouracil, and erythromycin and penicillin combinations probably involve facilitating actions[112–114]. The diclofenac and paracetamol synergism may arise from complementary action[115,116], and amoxicillin and clavulanate synergism possibly stems from potentiative enhancement of drug distribution[117]. We were unable to find information for assessing the reported synergism of the rifampicin and fusidic acid combination[104]. The reported non-synergistic sulphamethoxazole and trimethoprim combination seems to involve redundant actions in targeting upstream and downstream targets of a single-route pathway, with the downstream drug acting as a second line of defence[118,119]. For the three combinations without reported types of combination actions, the cisplatin and cyclophosphamide combination probably produces synergistically complementary action[120,121]; caffeine in the ergotamine and caffeine combination may involve the potentiation of ergotamine's action by enhancing its distribution[122]; and the niacin and simvastatin combination possibly produces an additive effect due to their equivalent actions[123].

### Pathway analysis

Pathway analysis is an effective approach for a more comprehensive assessment of drug combination effects[124], as well as other drug activities and responses[125,126]. Advances in systems biology and other areas of biomedical and pharmaceutical research have enabled the integration of biomolecular network information, individual MI profiles, 'omics' data, and disease information for drug validation and for understanding the mechanism of drug actions[127–129]. It is therefore of interest to explore pathway analysis approaches for further study of some of the drug combinations evaluated by MI profiling.

FIGURE 1 shows the related pathways of the cisplatin and trastuzumab combination (see Supplementary information S1 (table)), and describes potential mechanisms underlying the effects of the combination[120,130–133]. In addition to protein–protein, protein–substrate and protein–nucleic acid interactions, pathway analysis also needs to take into consideration drug metabolism, transport, drug–drug interactions and complex formation. This can be illustrated by comparative analysis of the anticancer combination of methotrexate and fluorouracil[113,134], and the antibacterial combination of sulphamethoxazole and trimethoprim[118,119] (TABLE 8), which

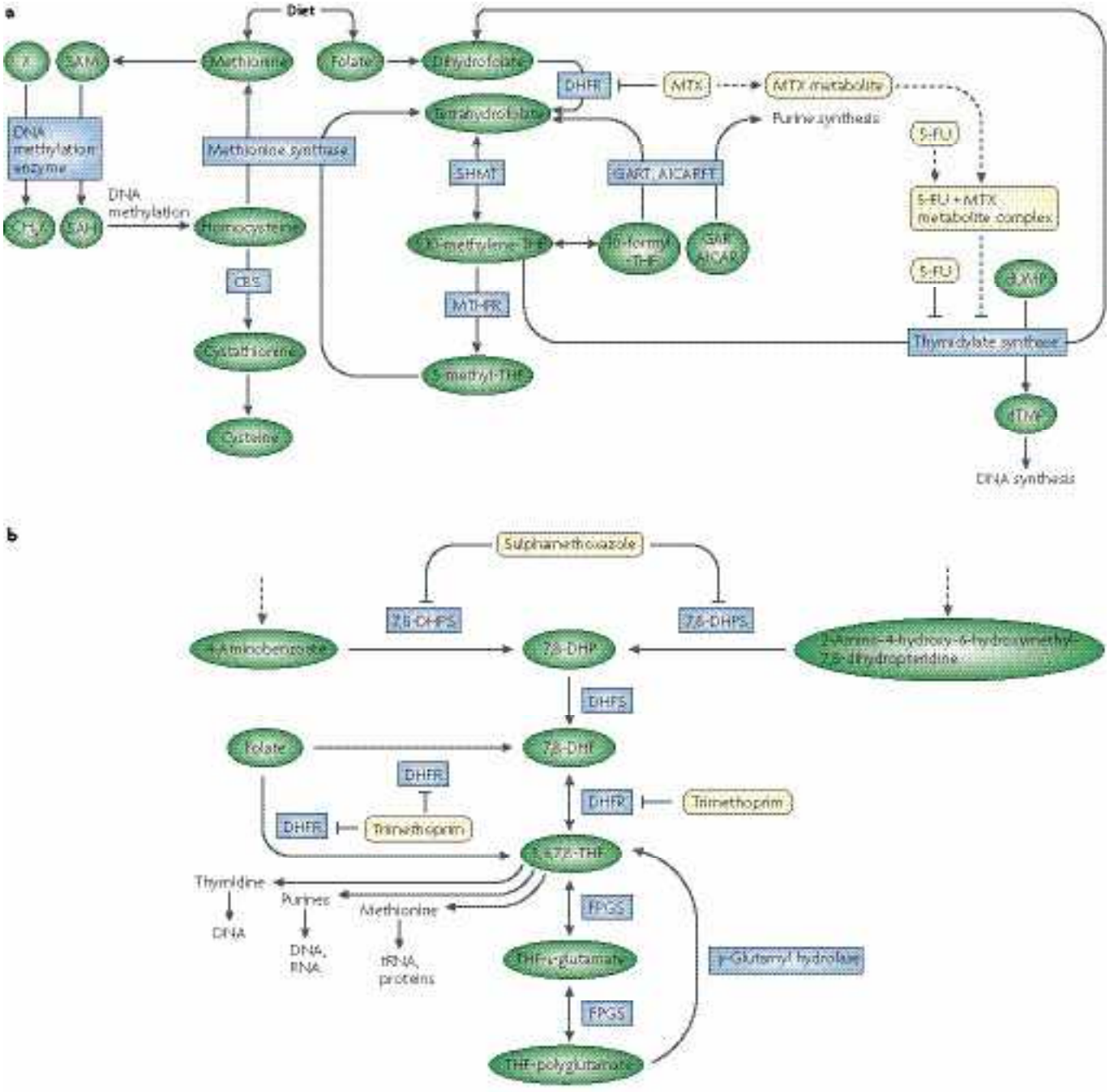


Figure 2 | **Constrasting effects of drug combinations on folate metabolism pathways.** The human folate metabolism pathway affected by the combination of methotrexate (MTX) and fluorouracil (5-FU) is shown in panel (**a**), and the *Escherichia coli* folate metabolism pathway affected by the sulphamethoxazole and trimethoprim drug combination is shown in panel (**b**). Although both combinations target upstream and downstream targets in a single pathway leading to DNA synthesis (assuming that synthesis of 7,8-dihydropteroate is essential for bacterial growth), only the sulphamethoxazole and trimethoprim combination shows the expected redundant effect such that effective inhibition of 7,8-dihydropteroate synthase (7,8-DHPS) by sulphamethoxazole renders trimethoprim inhibition of dihydrofolate reductase (DHFR) unnecessary for reducing DNA synthesis[118,119]. The unexpected MTX–5-FU synergism arises because the MTX metabolite forms reversible ternary complexes with 5-FU on one site of thymidylate synthase to enhance its binding to the enzyme[113,134] (dashed line in part **a**), which synergistically facilitates the anticancer thymidylate synthase inhibitory activity of 5-FU. AICARFT, 5-amino-imidazole-4-carboxamide ribonucleotide transformylase; CBS, cystathionine β-synthase; dTMP, deoxythymidine monophosphate; dUMP, deoxyuridine monophosphate; FPGS, folypolyglutamate synthase; GART, glycinamide ribonucleotide transformylase; MTHFR, methylene tetrahydrofolate reductase; SAH, S-adenosyl homocysteine; SAM, S-adenosyl methionine; SHMT, serine hydroxymethyl transferase; THF, tetrahydrofolate.

target human and bacterial folate metabolism pathways, respectively, but produce contrasting combination effects. The pathways affected by these two combinations are shown in FIG. 2a and FIG. 2b, respectively.

## Perspectives

Analysis of the selected drug combinations suggests that knowledge of MI profiles of individual drugs, network crosstalk and regulation, and modes of actions of drug combinations are useful starting points for investigating the effects of drug combinations. For the analysed cases of synergistic, potentiative, additive, antagonistic and reductive combinations, and probably many others, the literature-described MI profiles of the drugs involved seem to offer useful clues to the mechanism of combination actions from the perspectives of coordinated molecular interactions and network regulation. Clues to other aspects of pharmacodynamic, toxicological and pharmacokinetic effects may also be obtained from the relevant MI profiles.

Discovery of efficacious drug combinations may be facilitated by targeting key efficacy and toxicity regulating nodes of positive[72,74] and negative regulations[7,9–11], anti-targets and counter-targets[14], compensatory and neutralizing actions[12,13], and transporter-mediated and enzyme-mediated pharmacokinetic activities[101]. Both the discovery and the analysis of drug combinations can be facilitated by the collective use of different approaches and methods. For instance, signs of MI profiles as well as genes, pathways affected by or responsive to drug combinations[135] and individual drugs[136–138] may be detected from gene-expression or proteomics profiles by using unsupervised hierarchical clustering and supervised machine learning methods[135,136,139,140]. These, combined with knowledge of the characteristics and activities of targets[3] and proteins involved in ADME and toxicology[36], enable the prediction of responses and markers[136–138], unknown therapeutic actions[139], targets and characteristics[139,141,142], efficacy[143], toxicological effects[139], and resistance profiles[140] of drug combinations and individual drugs.


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**CORRIGENDUM**

# Mechanisms of drug combinations: interaction and network perspectives

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There are two errors in the author names: Zhiwei W. Cao should be Zhiwei Cao and Yixue X. Li should be Yixue Li.