## S1 Table | Literature-reported pharmacodynamically synergistic drug combinations due to anti-counteractive actions, in which synergy has been determined by well established synergy/additive analysis methods and its molecular mechanism has been revealed.

<table>
<thead>
<tr>
<th>Combinational target relationship</th>
<th>Drug A (mechanism of actions related to synergy)</th>
<th>Drug B (mechanism of actions related to synergy)</th>
<th>Reported synergistic effect</th>
<th>Synergism determination method</th>
<th>Possible mechanism of synergism in anti-counteractive actions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Different targets of the same pathway</td>
<td>17-AAG (heat-shock protein antagonist, induced cell cycle inhibition and apoptosis by inhibiting NF-kappaB, AP-1 and PI3K/Akt pathways(^a), Hsp90/FLT3 inhibitor(^b))</td>
<td>Arsenic trioxide (degraded aberrant PML-retinoic acid receptor alpha fusion protein, generated reactive oxygen species, and activated Akt survival pathway(^c))</td>
<td>Synergistic anticancer effect(^d)</td>
<td>Median dose effect analysis ((\text{Calcusym}))</td>
<td>Arsenic trioxide’s anticancer generation of reactive oxygen species is partially off-set by its own counteractive activation of Akt survival pathway(^d). 17-AAG abrogated arsenic trioxide’s activation of Akt survival pathway(^c) to reduce the counteractive effect</td>
</tr>
<tr>
<td>Different targets of the same pathway that regulated the same target</td>
<td>Cisplatin (DNA inter- and intra-strand adduct, preferably bind to the major groove of GG, AG and TACT sites(^9), thereby inhibited DNA polymerization and induced DNA damage to trigger apoptosis(^10))</td>
<td>Trabectedin (bind covalently to central G in the minor groove of selected DNA pyrimidine-G-G and purine-G-C triplets(^11), formed unusual DNA replication intermediates thereby inhibited DNA replication(^12), interacted with DNA and DNA repair systems in a way different from cisplatin(^13))</td>
<td>Synergistic antitumor activity(^13)</td>
<td>Interaction index method of Berebbbaum</td>
<td>Trabectedin inhibition of DNA replication(^12) reduced the counteractive activity of DNA polymerase mediated mutagenic translesional bypass replication across cisplatin-DNA adducts(^14)</td>
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<td>Oxaliplatin (DNA adduct, preferably bind to major groove of GG, AG and TACT sites, complex conformation different from that of cisplatin(^4), caused DNA strand break and non-DNA initiated apoptosis(^5))</td>
<td>Irinotecan (DNA topoisomerase I inhibitor, increased EGFR phosphorylation in Lovo &amp; WiDR cells(^6))</td>
<td>Synergistic anticancer effect in AZ-521 and NUGC-4 cells, additive effect in MKN-45 cells(^7)</td>
<td>Median drug effect analysis</td>
<td>Effect of oxaliplatin’s DNA adduct formation(^4) may be partially reduced by certain mutant DNA topoisomerase I acting on DNA adduct to generate different topoisomers(^8). Irinotecan inhibition of DNA topoisomerase I(^6) partially off-sets this counteractive activity</td>
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<td>Topotecan (topoisomerase I inhibitor, interacted with DNA, stabilized a covalent topoisomerase-DNA complex, thereby blocked DNA replication forks(^15))</td>
<td>Topotecan blocking of DNA replication(^15) reduced the counteractive activity of DNA polymerase mediated mutagenic translesional bypass replication across cisplatin-DNA adducts(^14)</td>
<td>Synergistic cytotoxic activity(^16-18)</td>
<td>Multi-drug effect equation, combination index, median-drug effect method</td>
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<tr>
<td>Compound</td>
<td>Description</td>
<td>Synergistic/Combination Effect</td>
<td>Cell Wall Alteration</td>
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<td>Cisplatin</td>
<td>(DNA inter- and intra-strand adduct, preferably bind to the major groove of GG, AG and TACT sites, thereby inhibited DNA polymerization and induced DNA damage to trigger apoptosis)</td>
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<tr>
<td>Sabarubicin</td>
<td>(topoisomerase II inhibitor)</td>
<td>Synergistic cytotoxic effect in tumour cell lines NSCLC H460 and SCLC GLC4</td>
<td>Sabarubicin blocking of DNA replication reduced the counteractive activity of mutagenic translesional bypass replication across cisplatin-DNA adducts</td>
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<tr>
<td>DL-cycloserine</td>
<td>(bacterial cell wall synthesis inhibitor)</td>
<td>Synergistic effect on bacterial cell wall integrity</td>
<td>Cell wall alteration may induce counteractive cell wall synthesis to restore cell wall integrity, DL-cycloserine inhibition of cell wall synthesis hindered the restoration thereby enhanced Epigallocatechin gallate’s cell wall disruption activity</td>
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<tr>
<td>Epigallocatechin gallate</td>
<td>(disrupted integrity of bacterial cell wall via direct binding to peptidoglycan)</td>
<td>Synergistic effect on bacterial cell wall</td>
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<td>Gefitinib</td>
<td>(EGFR tyrosine kinase inhibitor, induced cyclin-dependent kinase inhibitors, inhibited p27 and p21, decreased MMP-2 and MMP-9 enzyme activity)</td>
<td>Synergistic inhibitory effect on colorectal cancer Lovo &amp; WiDR cells</td>
<td>Irinotecan produced anticancer effect via DNA topoisomerase inhibition, but promoted proliferation by increased phosphorylation of EGFR in certain cell types. Gefitinib produced anticancer effect via EFR tyrosine kinase inhibition and others, which offsets the counteractive effect of increased EGFR phosphorylation</td>
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<tr>
<td>Etoposide</td>
<td>(topoisomerase II inhibitor, induced DNA double-strand breaks during DNA replication, increased expression of DNA repair-related protein Rad51)</td>
<td>Synergism between etoposide and 17-AAG in leukemia cells</td>
<td>The effect of etoposide’s DNA strand break is partially offset by its own counteractive increase of expression of DNA repair-related protein Rad51. Higher levels of Rad51 and its interacting partner Chk1 are associated with presence of FLT3. Inhibition of Hsp90/FLT3 by 17-AAG may reduce Rad51 and Chk1 to reduce the counteractive effect</td>
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<td>17-AAG</td>
<td>(heat-shock protein antagonist, induced cell cycle inhibition and apoptosis by inhibiting NF-kappaB, AP-1 and PI3K/Akt pathways)</td>
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<tr>
<td>Drug</td>
<td>Mode of action</td>
<td>Method</td>
<td>Synergistic effect</td>
<td>Notes</td>
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<td>Artemisinin (interacted with heme to mediate its decomposition into free radicals that alkylate essential malarial proteins)</td>
<td>Synergistic antimalarial effects in both chloroquine - sensitive and - resistant P. falciparum strains. Artemisinin's antimalarial activity possibly arise from its interaction with heme that facilitates heme conversion into free radical, which can be off-set by parasite's counteractive actions of heme polymerization into insoluble hemozoin and heme degradation by glutathione. These counteractive actions are partially reduced by methylene blue's inhibition of heme polymerization and glutathione reductase, resulting in synergistic antimalarial effect.</td>
<td>Isobologram method</td>
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### Combination index

<table>
<thead>
<tr>
<th>Combination index</th>
<th>Tamoxifen (estrogen receptor antagonist)</th>
<th>Trastuzumab (herceptin) (anti-HER-2/neu antibody)</th>
<th>Synergistic growth inhibition in ER-positive, HER-2/neu-overexpressing BT-474 breast tumor cells</th>
</tr>
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<tr>
<td>ER crosstalks with EGFR and HER-2/neu, signaling via EGFR and HER-2/neu can activate ER and its coactivator AIB1, ER of cell membrane can activate EGFR/HER-2. Anti-HER-2/neu antibody trastuzumab stopped HER-2/neu induced activation of ER and AIB1. ER antagonist tamoxifen stopped ER induced activation of EGFR/HER-2. Use of both drugs reduced the counteractive crosstalks.</td>
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<tr>
<th>Combination index</th>
<th>Rapamycin or deforolimus (mTOR inhibitor)</th>
<th>CI-1040 or PD0325901 (MEK inhibitor)</th>
<th>Synergistic antitumor efficacy in animal models of human lung cancer and in K-RAS mutant, non-V600EB-RAF, B-RAFV600E mutant cell lines</th>
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<tr>
<td>Effects of the inhibition of mTOR by rapamycin or its analogue deforolimus may be partially offset by NPM/ALK-induced mTOR activation that is transduced through the MEK-ERK signaling pathway. This counteractive action may be reduced by CI-1040 or PD0325901’s inhibition of MEK.</td>
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<th>Combination index</th>
<th>Paclitaxel (stabilized microtubules via alpha-tubulin acetylation, distorted mitosis to trigger apoptosis, induced p53 and CDK inhibitors, activated caspase-10, caspases-8, -6, and -3, leading to apoptosis, activated ERK and CDK, activated p38 MAP kinase and p53)</th>
<th>NU6140 (CDK inhibitor, down-regulated antiapoptotic protein survivin)</th>
<th>Synergistic apoptotic response</th>
</tr>
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<tr>
<td>Median drug effect analysis</td>
<td>Use of both drugs promoted complementary apoptosis activities via triple actions of surviving down-regulation by NU6140, microtubule stabilization, and caspase activation by paclitaxel. Paclitaxel’s promotion of apoptosis may be partially offset by its counteractive pro-growth activation of ERK and CDK, which may be partially reduced by NU6140’s inhibition of CDK.</td>
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| Combination index | Different targets of cross-talking pathways | Gefitinib (EGFR tyrosine kinase inhibitor, induced cyclin-dependent kinase inhibitors p27 and p21, decreased MMP-2 and MMP-9 enzyme activity) | Taxane (disrupted microtubule by binding to beta-tubulin, induced tumor suppressor gene p53 and cyclin-dependent kinase inhibitors P21, down) | Strong synergistic effect in breast cancer MCF7/ADR cells |
|-------------------|------------------------------------------|-----------------------------------------------|--------------------------------------------------------------------------------------------------|
| Taxane produced anticancer effect by inducing apoptosis and microtubule disruption. Crosstalk between EGFR and hypoxia-inducible factor-α pathways increased resistance to apoptosis by up-regulating survivin. Gefitinib produced anticancer effect via |

**Notes:**
- SUPPLEMENTARY INFORMATION
- In format provided by Jia et al. (FEBRUARY 2009)
- Tamoxifen (estrogen receptor antagonist)
- Trastuzumab (herceptin) (anti-HER-2/neu antibody)
- Synergistic growth inhibition in ER-positive, HER-2/neu-overexpressing BT-474 breast tumor cells
- Combination index
- ER crosstalks with EGFR and HER-2/neu, signaling via EGFR and HER-2/neu can activate ER and its coactivator AIB1, ER of cell membrane can activate EGFR/HER-2. Anti-HER-2/neu antibody trastuzumab stopped HER-2/neu induced activation of ER and AIB1. ER antagonist tamoxifen stopped ER induced activation of EGFR/HER-2. Use of both drugs reduced the counteractive crosstalks.
- Rapamycin or deforolimus (mTOR inhibitor)
- CI-1040 or PD0325901 (MEK inhibitor)
- Synergistic antitumor efficacy in animal models of human lung cancer and in K-RAS mutant, non-V600EB-RAF, B-RAFV600E mutant cell lines
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- Paclitaxel (stabilized microtubules via alpha-tubulin acetylation, distorted mitosis to trigger apoptosis, induced p53 and CDK inhibitors, activated caspase-10, caspases-8, -6, and -3, leading to apoptosis, activated ERK and CDK, activated p38 MAP kinase and p53)
- NU6140 (CDK inhibitor, down-regulated antiapoptotic protein survivin)
- Synergistic apoptotic response
- Median drug effect analysis
- Use of both drugs promoted complementary apoptosis activities via triple actions of surviving down-regulation by NU6140, microtubule stabilization, and caspase activation by paclitaxel. Paclitaxel’s promotion of apoptosis may be partially offset by its counteractive pro-growth activation of ERK and CDK, which may be partially reduced by NU6140’s inhibition of CDK.
- Different targets of cross-talking pathways
- Gefitinib (EGFR tyrosine kinase inhibitor, induced cyclin-dependent kinase inhibitors p27 and p21, decreased MMP-2 and MMP-9 enzyme activity)
- Taxane (disrupted microtubule by binding to beta-tubulin, induced tumor suppressor gene p53 and cyclin-dependent kinase inhibitors P21, down)
- Strong synergistic effect in breast cancer MCF7/ADR cells
- Combination index
- Taxane produced anticancer effect by inducing apoptosis and microtubule disruption. Crosstalk between EGFR and hypoxia-inducible factor-α pathways increased resistance to apoptosis by up-regulating survivin. Gefitinib produced anticancer effect via...
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<tr>
<th>Compound</th>
<th>Bioactivity</th>
<th>Notes</th>
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<tr>
<td>Gleevec (selective inhibitor of c-Abl, p210bcr-abl, c-Kit, and PDGF-R tyrosine kinases)</td>
<td>L744,832 or LB42918 (farnesyltransferase inhibitor, inhibited Ras farnesylation)</td>
<td>Synergistically promoted apoptosis in different imatinib-sensitive and -resistant BCR-ABL-positive CML cells</td>
</tr>
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<td>Cisplatin (DNA inter- and intra-strand adduct, preferably bind to the major groove of GG, AG and TACT sites)</td>
<td>Trastuzumab (herceptin) (anti-HER-2/neu antibody)</td>
<td>Synergistic growth inhibition in SNU-216 as an HER2-amplified cell line among gastric cancer cell lines</td>
</tr>
<tr>
<td>Dasatinib (inhibitor of c-abl, src, fyn, lck and kit)</td>
<td>PKC412 (inhibitor of Fli, PKC, VEGFR2, PDGFR, c-kit)</td>
<td>Synergistic apoptotic effects in HMC-1.2 cells</td>
</tr>
<tr>
<td>Different targets in the same pathway that crosstalk via other pathway</td>
<td>Gefitinib (EGFR tyrosine kinase inhibitor, induced cyclin-dependent kinase inhibitors p27 and p21, decreased MMP-2 and MMP-9 enzyme activity)</td>
<td>Synergistic antitumor effect in breast cancer MDA-MB-361 cells</td>
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</table>

The table above summarizes the bioactivities and effects of various compounds in the context of regulating Bcl-2, leading to apoptosis, and their interactions with other targets such as EGFR, HER-2/neu, and Ras. The table also includes notes on the methods used for analysis, such as median dose effect analysis and isobologram analysis. The compounds listed include Gleevec (selective inhibitor of c-Abl, p210bcr-abl, c-Kit, and PDGF-R tyrosine kinases), Cisplatin (DNA inter- and intra-strand adduct), Trastuzumab (herceptin) (anti-HER-2/neu antibody), Dasatinib (inhibitor of c-abl, src, fyn, lck and kit), and Gefitinib (EGFR tyrosine kinase inhibitor). The table also includes notes on the potential for counteractive activity against various targets, such as EGFR-hypoxia crosstalk and Ras farnesylation.
<table>
<thead>
<tr>
<th>Same target (different sites)</th>
<th>AZT (HIV-1 reverse transcriptase inhibitor(^9^0))</th>
<th>Non-nucleoside HIV-1 reverse transcriptase inhibitor(^9^9)</th>
<th>Antiviral synergism(^9^0)</th>
<th>Isobolographic analysis, Yonetani &amp; Theorell plot</th>
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<tr>
<td>PD98059 by reducing the effect of MEK or EGFR tyrosine kinase inhibition. Simultaneous use of both drugs helps disrupting this autocrine growth loop, thereby enhancing each other’s effect</td>
<td>AZT resistance is partly due to phosphorolytical removal of the AZT-terminated primer(^9^1), NNRTI inhibited RT catalyzed phosphorolysis, thereby reduced AZT resistance(^9^0)</td>
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</tbody>
</table>
References:


11. Marco, E. & Gago, F. DNA structural similarity in the 2:1 complexes of the antitumor drugs trabectedin (Yondelis) and chromomycin A3 with an oligonucleotide sequence containing two adjacent TGG binding sites on opposing strands. Mol Pharmacol 68, 1559-67 (2005).


75. Pietras, R. J., Pegram, M. D., Finn, R. S., Maneval, D. A. & Slamon, D. J. Remission of human breast cancer xenografts on therapy with humanized monoclonal antibody to HER-2 receptor and DNA-reactive drugs. Oncogene 17, 2235-49 (1998).