

# Compusyn Report Examples and Applications (Cont'd)

C. Select References [Summaries and Abstracts] for MAL-PD/BD/CI/BI *Theory and Commentary*

# Comparison of Mass-Action Law Dynamic Theory-based “Top-Down” Approach with Observation/Statistics-based “Bottom-Up” Approach in Biomedical R&D

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Poster Board 555

The traditional dose-effect dynamics (DED) research and development (R&D) in biomedical sciences usually start with proposed specific aim, defined rationale and select methods/procedures. The observations and dose-effect data are then analysis using chosen method with statistical analysis for plausible conclusions. This approach is considered as the open “*bottom-up*” approach with exploratory features. However, in the absence of universal fundamental principle, this open approach frequently encounters variability and diversity issues thus require large sample size, many doses for empirical curve fittings, and may involve subjective decisions. By contrast, the general Mass-Action Law (MAL) theory-based biodynamics/pharmacodynamics/bioinformatics (BD/PD/BI) is a deterministic unified “*top-down*” approach, which is opposite to the traditional “*bottom-up*” approach, yet provides complementary alternative for ultimate goals of biomedical R&D, with excellent features of cost-effectiveness and quantitative computer simulation for digital MAL informatics. The MAL-BD/PD/BI unified theory consists of (i) Median-effect eq. (MEE), (ii) Combination Index eq. (CIE) and (iii) Dose-reduction Index eq. (DRIE), and their respective algorithms for diagnostic simulations. All *terms* of MEE, CIE and DRIE are *dimensionless relativity ratios*. Thus, the applications of MAL-unified theory is independent from: units, mechanisms, physical states; in molecular, cellular, tissue, organ, and diseases studies; and in animals, humans, clinical trials; in marine, agricultural, food and environmental sciences. Recently, the applications also extended to radiation, UV, microwave, photo- and thermo- dynamics studies. As of December 2022, the applications of MAL- BD/PD/CI/BI and its mathematical quantitative *definitions* of “PD”, synergism ( $CI < 1$ ) additive effect ( $CI = 1$ ) and antagonism ( $CI > 1$ ), and DRI have garnered over 22,000 citations in over 1,488 journals in biomedical sciences and beyond. The Michaelis-Menten, Hill, Henderson-Hasselbalch, Langmuir, and Scatchard equations are all *specific general equations*, the special cases within the domain of the MAL *unified general equation*. It worth noting here, the MAL unified theory was derived from over 300 reaction rate-equations, subjecting to the pattern analysis, combinatorial analysis, and mathematical induction and deduction, which is a completely different approach from the other five theoretical derivations as indicated above. It is noted that Top-Down and Bottom-Up approaches are opposite and complementary alternative, in *unity*, just like Yin-Yang concept in ancient philosophy, and like two sides of the same coin, or front and rear views of the same entity. Interestingly, the signals of central nerve systems are descending and the peripheral nerve systems are ascending, yet they are coordinating superbly well. It is intriguing that in the standard model of elementary particle physics, the six quarks are: top, down, bottom, up, charm and strange. This terminology of 4/6 coincidence may be by chance since it has no direct evidence yet that the mass-action law basic principle have any interplays in it. What apparent is that MAL-principle and algorithm have proven utilities in quantitative precision medicine and in digital translational medicine for biomedical R&D.

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For a related subject, please see: Chou TC. Mass-action law dynamic theory/algorithm-based *top-down general bioinformatics*. Am. Soc. Biochem. Mol. Biol. 2023 Annual Meeting. Discover BMB-2023. Seattle, WA, March 25-28. **Journal of Biological Chemistry** 2023;299(3):S201. Abstract No 1195. <http://dx.doi.org/10.1016/j.jbc.2023.103419>

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**Perspective**

# Drug Combination Studies and Their Synergy Quantification Using the Chou-Talalay Method

Ting-Chao Chou

Volume 70, Issue 2, 15 January 2010

<https://doi.org/10.1158/0008-5472.CAN-09-1947>

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## Abstract

This brief perspective article focuses on the most common errors and pitfalls, as well as the do's and don'ts in drug combination studies, in terms of experimental design, data acquisition, data interpretation, and computerized simulation. The Chou-Talalay method for drug combination is based on the median-effect equation, derived from the mass-action law principle, which is the unified theory that provides the common link between single entity and multiple entities, and first order and higher order dynamics. This general equation encompasses the Michaelis-Menten, Hill, Henderson-Hasselbalch, and Scatchard equations in biochemistry and biophysics. The resulting combination index (CI) theorem of Chou-Talalay offers quantitative definition for additive effect ( $CI = 1$ ), synergism ( $CI < 1$ ), and antagonism ( $CI > 1$ ) in drug combinations. This theory also provides algorithms for automated computer simulation for synergism and/or antagonism at any effect and dose level, as shown in the CI plot and isobologram, respectively. *Cancer Res*; 70(2); 440–6. ©2010 AACR.

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REVIEW PAPER

# Frequently asked questions in drug combinations and the mass-action law-based answers



Ting-Chao Chou \*

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## KEYWORDS

Median-effect equation;  
Combination index;  
Dose-reduction index;  
Isobologram;  
Polygonogram;  
Computer synergy  
simulation

**Summary** Drug combinations have been widely used in the treatment of the most dreadful diseases, such as cancer and AIDS. In the search for synergistic combinations for therapy, numerous articles have been published during the past century. However, the term “synergy” has at least 20 different definitions in literature but none supports others. The confusion on synergy claims has far reaching consequences in biomedical research, drug discovery and development, regulation, and medical care of patients. This article reviews the current status and enlists the frequently occurred pit-falls, misconceptions and common errors in drug combination studies. The questions and issues are contemplated to be answered and clarified with the physico-chemical algorithms of the mass-action law, specifically with the unified theory of the median-effect equation and its combination index theorem for drug combinations. The derived theory, algorithm and its computer simulation lead to a quantitative indexed bioinformatics, and econo-green bio-research using small number of data points.

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# The mass-action law based algorithms for quantitative econo-green bio-research

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Ting-Chao Chou ✉

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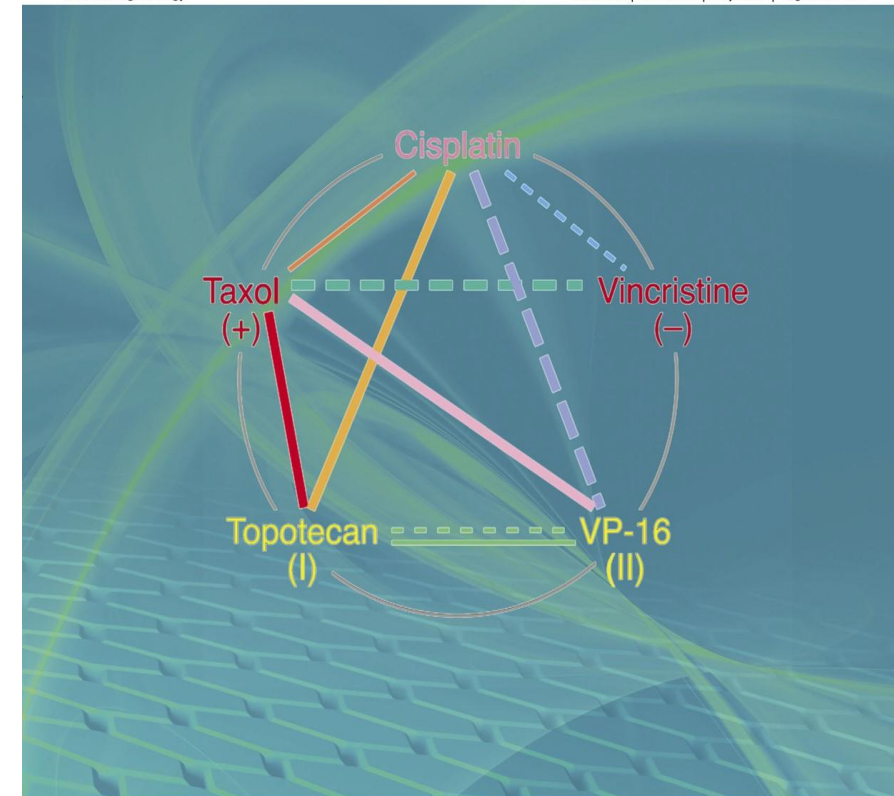
**Abstract:** The relationship between dose and effect is not random, but rather governed by the unified theory based on the median-effect equation (MEE) of the mass-action law. Rearrangement of MEE yields the mathematical form of the Michaelis–Menten, Hill, Henderson–Hasselbalch and Scatchard equations of biochemistry and biophysics, and the median-effect plot allows linearization of all dose-effect curves regardless of potency and shape. The “median” is the universal common-link and reference-point for the 1st-order to higher-order dynamics, and from single-entities to multiple-entities and thus, it allows the all for one and one for all unity theory to “integrate” simple and complex systems. Its applications include the construction of a dose-effect curve with a theoretical minimum of only two data points if they are accurately determined; quantification of synergism or antagonism at all dose and effect levels; the low-dose risk assessment for carcinogens, toxic substances or radiation; and the determination of competitiveness and exclusivity for receptor binding. Since the MEE algorithm allows the reduced requirement of the number of data points for small size experimentation, and yields quantitative bioinformatics, it points to the deterministic, efficient, low-cost biomedical research and drug discovery, and ethical planning for clinical trials. It is concluded that the contemporary biomedical sciences would greatly benefit from the mass-action law based “Green Revolution”.

# Integrative Biology

Quantitative biosciences from nano to macro

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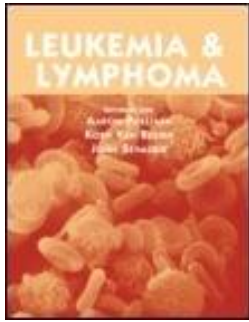
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PERSPECTIVE  
Chou  
The mass-action law based algorithms for quantitative econo-green bio-research



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# Preclinical versus clinical drug combination studies

**Ting-Chao Chou**

Leukemia & Lymphoma Volume 49, 2008 - Issue 11 | Pages 2059-2080 | Accepted 15 Jul 2008 <https://doi.org/10.1080/10428190802353591>

**Abstract:** This brief review provides a practical guide for drug combination studies and delineates its essence in terms of the mass-action-based theory, experimental design and automated computerised data analysis. The combination index (CI) method of Chou-Talalay is based on the multiple drug effect equation derived from the median-effect principle of the mass-action law. It provides quantitative determination for synergism ( $CI < 1$ ), additive effect ( $CI = 1$ ) and antagonism ( $CI > 1$ ), and provides the algorithm for computer software for automated simulation for drug combinations. It takes into account both the potency (the  $D_m$  value) and the shape of the dose-effect curve (the  $m$  value) of each drug alone and their combination. The best feature is that it allows for small size experiments. The automated computer simulation reveals whether there is a synergism, determines how much synergism (the CI value) at any effect levels (the  $F_a$ -CI plot), or at any dose levels (the isobologram), provides the information regarding how many folds of dose-reduction is allowed for each drug, at a given effect for a synergistic combination, comparing with the dose required for each drug alone (the  $F_a$ -DRI plot), and the optimal combination ratio and schedule dependency for synergy. The 'polygonogram' dissects the component drug interactions or projects the make-ups of cocktails in complicated combinations. Based on scientific, practical and ethical reasons, it is not possible to 'determine' synergism in humans, and thus prior to the drug combination clinical trials, preclinical drug combination studies in vitro and/or in animals should be carried out to obtain the basis and rationale for studies in humans.

# The mass-action law based algorithm for cost-effective approach for cancer drug discovery and development

Ting-Chao Chou

Am J Cancer Res. 2011; 1(7): 925–954. Published online 2011 Aug 5.

PMCID: PMC3196289. PMID: 22016837

<http://www.ncbi.nlm.nih.gov/pmc/articles/pmc3196289/>

**Abstract:** The mass-action law based system analysis via mathematical induction and deduction lead to the generalized theory and algorithm that allows computerized simulation of dose-effect dynamics with small size experiments using a small number of data points in vitro, in animals, and in humans. The median-effect equation of the mass-action law deduced from over 300 mechanism specific-equations has been shown to be the unified theory that serves as the common-link for complicated biomedical systems. After using the median-effect principle as the common denominator, its applications are mechanism-independent, drug unit-independent, and dynamic order-independent; and can be used generally for single drug analysis or for multiple drug combinations in constant-ratio or non-constant ratios. Since the “median” is the common link and universal reference point in biological systems, these general enabling lead to computerized quantitative bio-informatics for econo-green bio-research in broad disciplines. Specific applications of the theory, especially relevant to drug discovery, drug combination, and clinical trials, have been cited or illustrated in terms of algorithms, experimental design and computerized simulation for data analysis. Lessons learned from cancer research during the past fifty years provide a valuable opportunity to reflect, and to improve the conventional divergent approach and to introduce a new convergent avenue, based on the mass-action law principle, for the efficient cancer drug discovery and the low-cost drug development.

# Functional biodynamics theory and algorithms for inhibitor or activator effectors and their interactions, by computer simulation bioinformatics for translational medicine

Ting-Chao Chou

The FASEB Journal / Volume 36, Issue S1 / 13 May 2022

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**Abstract:** The conventional *bottom-to-up approach* of R&D is usually: aims, design, findings, data analysis, statistics, and conclusions or suggestive hypothesis. The experimental *findings need to fitting empirical curves, formula, or model*. The *alternative nonconventional, top-to-down approach, shown* here, is the mathematical system analysis to derive the unified general pharmacodynamics theory/equation/algorithm (**PD**) based on mass-action law (**MAL**) for automated computer simulation. Thus, the approach allows using small number of dose-data-points *to fit the unified MAL-PD theory/algorithm*, for the efficient, cost-effective automated computer simulation to achieve quantitative/ indexed bioinformatics (BI) diagnostic simulations and conclusions. **The median-effect equation (MEE) or its plot (MEP)** is the unified general theory of the **MAL** for dose-effect biodynamics/pharmacodynamics (**BD/PD**), where “dose” can be drugs, biologicals, hormones, modulators, infecting agents, radiation, or UV; and the “effect” can be inhibitors, activators, in cells, tissues, organs, diseases, animals or in clinical trials. The MAL algorithms also leads to the derivation of the general **combination index equation (CIE)** with its Fa-CI plot and Isobologram for BI, that allow the *quantification* of Synergism (CI<1), Additive effect (CI=1) and Antagonism (CI>1) at different-effect, or different effect levels. The unique features of MAL-PD approach are: **(A) All terms of the MEE and CIE** of the MAL-PD equations are “**dimensionless relativity ratio**”, therefore, **MAL-theory is valid** regardless of units, mode or mechanism of actions of effector(s), and the target systems. **(B) The MEE with its MEP, linearizes dose-effect curves into straight lines**, and thereby MEP leads to the “**Two Dose-Data-Points Minimum Theory**”, where the 3rd point is dose zero, and the 4th dose-point is  $D_m$ , which is the universal reference-point and dynamic-order common-link. Thus, particularly useful in animal studies or clinical trials which rarely tolerated over 3 (or more) “dose-data points” with acceptable dose-range and dose density; **(C) MAL theory/algorithm** allows the *same* general MAL-principle to bridge between the basic in vitro/animal studies and the clinical trials. **In conclusion**, the MAL-BD/PD/BI/CI approach/theory/algorithm/method serves as the general largest-common-denominator *for* simplifying the complexity and diversity of biological systems, with automated simulations. This MAL “top to down” innovation provides a new avenue *for* biomedical R&D, and for drug evaluations, which is simple, quantitative, efficient and cost-effective.

# MAL-pharmacodynamics theory based small-size experimental-design and analysis, in vitro, in animals and in clinical trial, with bioinformatics algorithm, for automated computer simulation to achieve quantitative/digital/indexed conclusions

Ting-Chao Chou

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<https://doi.org/10.1096/fasebj.2022.36.S1.R2801>



**Abstract:** The conventional *bottom-to-up* approach of R&D is usually from aims to design, to experimental *findings*, to *fitting empirical curves, formula, or model*, to statistical analysis, to drawing conclusion/hypothesis. The *alternative nonconventional, top-to-down approach* is the mathematical system analysis to derive the unified general pharmacodynamics theory/equation/algorithm (PD) based on mass-action law (MAL) for automated computer simulation. Thus, the approach allows using small number of dose-data–points *to fit the unified MAL-PD theory/algorithm*, for the efficient, cost-effective automated computer simulation to achieve quantitative/digital/indexed conclusions. The MAL-PD indicates: **(I) The median-effect eq. (MEE)** “dose-effect dynamics” defines PD-parameters:  $D_m$  (median-effect-dose) for potency, and  $m$  value for the dynamic-order signifying the shape of dose-effect curve (DEC), where  $m=1$ ,  $>1$  and  $<1$  indicate hyperbolic, sigmoidal and flat sigmoidal, respectively, and **(II) Computer simulation of combination index eq. (CIE)** with Fa-CI plot, allow the quantification of Synergism ( $CI<1$ ), Additive effect ( $CI=1$ ) and Antagonism ( $CI>1$ ) at different effect levels. The advantages of MAL-PD approach are: **(A)**In animal studies or clinical trials rarely tolerated *over 3* (or more) “dose-data points” with acceptable dose-range and dose density; **(B)**MAL theory/algorithm allows the *same* general MAL-principle to bridge between the basic in vitro/animal studies and the clinical trials; **(C)**The MEE with its plot (MEP), linearizes DEC’s into straight lines, and thereby MEP leads to the “Two Dose-Data-Points *Minimum Theory*”, where the 3rd point is dose zero, and the 4th dose-point is  $D_m$ , which is the universal reference-point and dynamic-order common-link; **(D)**Since **all terms** of the MEE and CIE of the MAL-PD equations are “dimensionless ratio”, therefore, MAL-PD/CI is generally valid/applicable in R&D, regardless of nature of the effectors (e.g., drugs, biologicals, radiation, UV, pH) or units (e.g.,  $\mu\text{g}$ , nM, IU, Rad, Gy,  $\mu\text{g/ml}$ , mg/kg,  $\text{mg/M}^2$ ), or mechanisms of action (competitive, noncompetitive, uncompetitive, sequential, ordered, ping-pong, or random); and regardless of in vitro, in animals, or in clinical trials. **In conclusion**, the MAL-PD/CI theory serves as the general largest-common-denominator *for* simplifying the complexity and diversity of biological systems, *for* biomedical R&D, and for drug evaluations, with automated simulations. Specific examples for real data analysis/simulation in vitro, and in animals will be illustrated for single drugs, and their combinations, for anticancer and antiviral studies.

## New Paradigm, Equations, Algorithm, and Computer software of Mass-action Law Based Biodynamics, Pharmacodynamics and Bioinformatics (MAL-BD/PD/BI) for Econo-Green Biomedical R&D and Regulatory Guidance

Ting-Chao Chou,

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**Abstract:** The prevailing divergent, empirical biomedical R&D heavily rely on statistics for variation, probability, correlation, and significance that may lead to inefficiency or high cost-effectiveness. An alternative precision/quantitative approach with Nature's biochemical, biophysical and bio-mathematical principle, leads to the unified fundamental Doctrine of the Median (DoM), that serves as the as the largest common denominator to simplify biomedical complexity and diversity. Instead of using many data points to fit the empirical curves or models, we can reversely, using only few dose data-points to fit DoM, with MAL equations, algorithms and auto-computer simulations of diagnostic plots using CompuSyn software, to achieve quantitative, automated, digitalized or indexed conclusions, in vitro, in animals and in clinical trials. Thus, experimental observations-first priority has become MAL theory-first compliance endeavor. From MAL to DoM to MAL-BD/PD/BI, took over 17 years to complete that involve the derivation of over 300 bio-reaction rate equations via the un-precedent pattern analysis, combinatory analysis and mathematical induction-and-deduction. MAL-BD/PD/BI reveals three main paradigms: (1) The Median-effect equation (MEE) as the unified theory that indicates i. Dose and Effect are interchangeable, ii. Median dose ( $D_m$ ) is the universal reference point and dynamic-order common link, iii.  $K_i$  can never greater than  $IC_{50}$ , iv. Dose-effect curves (DEC) can be linearized into straight lines, with slope ( $m$ ) and x-intercept ( $\log D_m$ ) of the Median-effect plot (MEP), and v. The DEC can be generated via MEP with a minimum of only two data points (the MTDP theory) by computer simulation, where the 3rd point is dose-zero, and the 4th point is the universal  $D_m$  point. Thus, for data-points availability, 2 becomes 4, 3 becomes 5, and 4 becomes 6. This is a breakthrough for in vivo study/trials. (2) The Combination Index equation (CIE) where  $CI < 1$ ,  $= 1$ , and  $> 1$  indicates, synergism, additive effect, and antagonism, respectively. (3) The Dose reduction index equation (DRIE), where  $DRI > 1$ ,  $= 1$ , and  $< 1$ , indicate favorable, no dose-reduction, and unfavorable dose-reduction, respectively, in drug combinations. The diagnostic plots provide needed BI. The terms of DoM, MEE and CIE are all dimensionless ratios, these indicating that the theory is independent to drug's physical entities (chemicals, biologicals, natural products, radiation, temperature, oxygen-tension, and pH, etc), units ( $\mu g$ , nM, mg/ml, mg/kg, IU, Rad, multiple of infections, etc) or mechanisms of actions (competitive, noncompetitive, uncompetitive, sequential, ordered, ping-pong, and random mechanisms). As of Jan 1, 2021, the article introduced MAL-BD/PD/BI algorithms (Chou TC, Pharmacol. Rev. 58:621-681, 2006) received 3,813 citations in 1,030 biomedical journals. In addition, the article introducing the CI algorithm and computer software (Chou TC and Talalay P. Adv. Enz. Regul. 22:27-55, 1984) received 6,937 citations in 1,338 biomedical journals globally. These confirmed the board applications to nearly all disciplines of biomedical R&D.

## Biophysical, Biochemical and Mathematical *Doctrine of the Median*: The Algorithms of the Unified Bio-Dynamics Theory for Basic Biomedical R&D and Regulatory Guidance

Ting-Chao Chou,

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**Abstract:** System analysis via pattern-combinatory analysis and mathematical induction-deduction on the mass-action law (**MAL**), yields the Median-Effect Equation (**MEE**),  $fa/fu = (D/Dm)^m$  where the *Ratio* of fraction affected (*fa*)/fraction unaffected (*fu*) equals to the *Ratio* of mass dose (*D*)/median mass dose (**Dm**); *m* is the *dynamic order* of action. MEE describes the general “Dose and Effect” or “Mass and Action” mathematical relationship and algorithm, and indicates that Dose and Effect are *interchangeable*. This leads to the *Doctrine of the Median (DoM)*, where *Dm* signifies *potency*, and *m* signifies the *shape* (of the Dose-Effect Curve, **DEC**). It further reveals that *Dm* is the universal reference-point and the common dynamic-link at different dynamic orders. In addition, *Dm* is the Harmonic Mean of kinetic constants, *K<sub>ii</sub>* and *K<sub>is</sub>*; and *K<sub>i</sub>* can be calculated from *Dm* (*IC<sub>50</sub>*), and *K<sub>i</sub>* can never greater than *IC<sub>50</sub>*. Further, MEE of DoM is the Unified Pharmacodynamics (**PD**), Biodynamics (**BD**) and Bioinformatics (**BI**) Theory [**MAL-PD/BD/BI**], encompassing Michaelis-Menten Eq. of enzyme substrate half-saturation, Henderson-Hasselbalch Eq. of pH half-ionization, Hill Eq. of ligand higher-order half-occupancy, and Scatchard Eq. of receptor half-bound and half-free.

MAL-MEE reveals two major paradigms: **(i)** All DEC can be linearized by the **Median-Effect Plot [MEP]**,  $x = \text{Log}(D) \underline{vs} \text{Log}(fa/fu)$ , where  $fa+fu = 1$ , and when  $fa=fu$ , it is “*Dm*”. By computer simulation (e.g., CalcuSyn or CompuSyn), MEP yields PD-parameter values, *m* as slope, and the x-intercept as [antilog of *Dm*] (and thus, for the *Dm* value), instantly. Therefore, DEC is instantly simulated. **(ii)** Since MEP linearizes all DEC, therefore, based on MEE, we need a minimum of only two data points to simulate the entire dose effect curve instantly. This “Two-Data Point Theory” (**TDPT**) should have great impact for animal studies and clinical trial protocol design, since in vivo cannot use too many doses, to avoid severe toxicity or very weak effect to measure. TDPT simplicity also raises the issue that single-dose clinical trials are *not the PD Studies*. Extension of MEE leads to **Combination Index Equation (CIE)** and diagnostic **Fa-CI plot**, where,  $CI < 1, = 1$  and  $> 1$ , indicates/quantitates Synergism, Additive Effect, and Antagonism, respectively, at different dose or effect levels, by computer simulation for 2 to *n* drug combinations. It also leads to **Dose-Reduction Index Equation (DRIE)** and diagnostic **Fa-DRI plot**, to calculate how much dose reductions for each drugs as the results of synergy combinations. Also leads to computerized auto-construction of “**Isobologram**”, “**Dose-Normalized Isobologram**” and “**Polygonogram**” for drug synergy graphic analysis.

The MAL-PD/BD/BI theory, based on MEE, CIE, and its computer-software, has been applied for single drug or drug combinations, in vitro, in vivo, in animals study and in clinical trial protocol design. Two most relevant references are: **1.** Chou TC. *Pharmacology. Rev.* 58: 621–681, 2006 (Cited 3,119 times in 914 journals) and **2.** Chou TC and Talley P. *Adv. Enz. Regal.* 22: 27–55, 1984 (Cited 6,275 times in 1,249 journals, as of 10.3.2019).

**D.** Select References for MAL-PD/BD/CI  
Applications [Summaries & Abstracts]  
*in Vitro* and *in Vivo*

# Computerized Quantitation of Synergism and Antagonism of Taxol, Topotecan, and Cisplatin Against Human Teratocarcinoma Cell Growth: a Rational Approach to Clinical Protocol Design

Ting-Chao Chou, Robert J. Motzer, Youzhi Tong, George J. Bosl

JNCI: Journal of the National Cancer Institute, Volume 86, Issue 20, 19 October 1994

Pages 1517–1524

<https://doi.org/10.1093/jnci/86.20.1517>



**Abstract:** Cisplatin-based induction chemotherapy may achieve a complete response (i.e., no sign of tumor following treatment) in 70%-80% of patients with germ cell tumors. However, only a minority of patients in whom the first-line regimens fail are cured with the salvage regimens. *Purpose* : The aim of these studies was to identify new agents or new regimens for the treatment of germ cell tumors by carrying out quantitative assessment in vitro of two promising new antitumor agents (paclitaxel [Taxol] and topotecan) and three more established agents (cisplatin, vincristine, and etoposide). These agents were used singly or in two- and three-drug combinations and were selected because they represent five distinct categories of antineoplastic mechanisms. *Methods* : The combination index-isobologram method, which is based on the median-effect principle developed by Chou and Talalay, was used for computerized data analysis. This method was selected because it takes into account both the potencies of each drug and combinations of these drugs and the shapes of their dose—effect curves. *Results* : Synergism against the growth of teratocarcinoma cells resistant to cisplatin (833K/64CP10 cells) was greater than against the growth of parent 833K cells. The degrees of synergism were in the following order: cisplatin + topotecan  $\geq$  paclitaxel + cisplatin + topotecan  $>$  paclitaxel + topotecan  $\geq$  paclitaxel + etoposide  $<$  paclitaxel + cisplatin + etoposide  $>$  paclitaxel + cisplatin. All other combinations showed nearly additive effects or moderate antagonism. The degrees of antagonism were as follows: cisplatin + etoposide  $\geq$  paclitaxel + vincristine  $>$  paclitaxel + cisplatin + vincristine  $>$  cisplatin + vincristine. The combination of paclitaxel and cisplatin was synergistic against 833K/64CP10 cells and moderately antagonistic against 833K cells. Since the combination of paclitaxel, cisplatin, and topotecan and the two-component combinations of these drugs (cisplatin + topotecan and paclitaxel + topotecan) showed synergism stronger than that of other combinations, these three drugs were selected for illustrating detailed data analysis, using a computer software program developed in this institute. *Conclusions and Implications* : Our findings suggest that, as a result of synergy, the doses of these agents needed to achieve an antitumor effect may be reduced by twofold to eightfold when these agents are given in combination. The present quantitative data analyses for synergism or antagonism provide a basis for a rational design of clinical protocols for combination chemotherapy in patients with advanced germ cell tumors.

*Specific Drug Combo Example in Animals for Illustration:  
A Model for Clinical Trial Protocol Design & Data Analysis with Only 10 Dose-Data Points*



Drug combination *in vivo* using combination index method: Taxotere and T607 against colon carcinoma HCT-116 xenograft tumor in nude mice

Volume 3, Issue 3, September 2016, Pages 15-30

Jianing Fu<sup>a,1</sup>, Ning Zhang<sup>b,1</sup>, Joseph H. Chou<sup>c</sup>, Hua-Jin Dong<sup>d</sup>, Shu-Fu Lin<sup>e</sup>,  
Gudrun S. Ulrich-Merzenich<sup>f</sup>, Ting-Chao Chou<sup>g,\*</sup> <https://doi.org/10.1016/j.synres.2016.06.001>

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Taxotere

ABSTRACT

The median-effect equation (MEE) of the mass-action law and the combination index (CI) theorem have been used for quantitative determination of synergism (CI < 1), antagonism (CI > 1) and additive effect (CI = 1) in animals *in vivo*. Experimental design, the theoretical algorithm and the CompuSyn software simulation have been used to illustrate step-by-step for the combination of two anti-cancer agents, Taxotere and T607 compound, with similar mode of actions of targeting microtubule polymerization, but with distinct chemical structures. These two compounds acted synergistically against human colon carcinoma HCT-116 xenograft tumor in athymic nude mice. In all, only 78 nude mice have been used. The synergy is especially significant ( $p < 0.01-0.05$ ) following Q3Dx4, x3 *i.v.* treatments, at higher doses and at later stages of treatment. The MEE and the CI theorem of Chou-Talalay quantitatively determined synergism or antagonism at different doses and different effect levels as indicated by the Fa-CI plot and by isobolograms in CompuSyn simulation and automated graphics. The practical logistics on pre-experimental planning, scheme/design/layout, and precautions in terms of dose number, dose range, dose density, drug combination ratio, conservation of laboratory animals as well as regulatory and cost-effective considerations have been presented. The mass-action law based CI algorithm has been proven to be simple to use, economy to practice, even for *in vivo* experimentations. Most significantly, the mass-action law based algorithm provides quantitative indexed conclusions.

## Multifaceted cytoprotection by synthetic polyacetylenes inspired by the ginseng-derived natural product, panaxytriol

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**Abstract:** We describe herein the discovery of a series of panaxytriol (PXT)-derived polyacetylene small molecules with promising cytoprotective activity. In mouse xenograft models, we have demonstrated the capacity of our synthetic analogs to mitigate a range of cancer therapeutic agent-induced toxicities, including body weight loss, lethality, neurotoxicity, and hematotoxicity. Our PXT analogs have also been found to reduce radiation-induced body weight loss and lethality in mouse models. Moreover, several PXT analogs appear to exhibit moderate in vivo antiinflammatory activity as well as in vitro immunoenhancing capabilities. These compounds appear to derive their activity through induction of cancer preventive phase 2 enzymes. The studies described herein suggest that coadministration of a PXT-derived agent with cancer chemotherapeutics or radiation therapy may serve to mitigate a range of therapy-associated toxicities.

## **Therapeutic effect against human xenograft tumors in nude mice by the third generation microtubule stabilizing epothilones**

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**Abstract:** The epothilones represent a promising class of natural product-based antitumor drug candidates. Although these compounds operate through a microtubule stabilization mechanism similar to that of taxol, the epothilones offer a major potential therapeutic advantage in that they retain their activity against multidrug-resistant cell lines. We have been systematically synthesizing and evaluating synthetic epothilone congeners that are not accessible through modification of the natural product itself. We report herein the results of biological investigations directed at two epothilone congeners: iso-fludelone and iso-dehydellone. Iso-fludelone, in particular, exhibits a number of properties that render it an excellent candidate for preclinical development, including biological stability, excellent solubility in water, and remarkable potency relative to other epothilones. In nude mouse xenograft settings, iso-fludelone was able to achieve therapeutic cures against a number of human cancer cell lines, including mammarian-MX-1, ovarian-SK-OV-3, and the fast-growing, refractory, subcutaneous neuroblastoma-SK-NAS. Strong therapeutic effect was observed against drug-resistant lung-A549/taxol and mammary-MCF-7/Adr xenografts. In addition, iso-fludelone was shown to exhibit a significant therapeutic effect against an intracranially implanted SK-NAS tumor.

# Ribavirin Antagonizes the Effect of Azidothymidine on HIV Replication

Markus W. Vogt, Kevan L. Hartshorn, Phillip A. Furman, Ting-Chao Chou, James A. Fyfe Leslie A. Coleman, Clyde Crumpacker, Robert T. Schooley and Martin S. Hirsch  
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<https://doi.org/10.1126/science.2435003>



**Abstract:** Azidothymidine and ribavirin both inhibit replication of human immunodeficiency virus in vitro and show promise of clinical utility in patients infected with this virus. In this study, the possible interactions of these drugs were examined in vitro, and a reproducible antagonism between azidothymidine and ribavirin was found to occur under a variety of experimental conditions. The mechanism responsible for this antagonism appeared to be inhibition of azidothymidine phosphorylation by ribavirin. Because similar effects may occur in vivo, clinical trials of these two drugs in combination must be performed only under carefully controlled conditions.

# Human neutralizing monoclonal antibodies of the IgG1 subtype protect against mucosal simian–human immunodeficiency virus infection

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**Abstract:** Although maternal human immunodeficiency virus type 1 (HIV-1) transmission occurs during gestation, intrapartum and postpartum (by breast-feeding), 50–70% of all infected children seem to acquire HIV-1 shortly before or during delivery<sup>1</sup>. Epidemiological evidence indicates that mucosal exposure is an important aspect of intrapartum HIV transmission<sup>2,3</sup>. A simian immunodeficiency virus (SIV) macaque model has been developed<sup>4</sup> that mimics the mucosal exposure that can occur during intrapartum HIV-1 transmission. To develop immunoprophylaxis against intrapartum HIV-1 transmission, we used SHIV–vpu+ (refs. 5,6), a chimeric simian–human virus that encodes the env gene of HIV-IIIB. Several combinations of human monoclonal antibodies against HIV-1 have been identified that neutralize SHIV–vpu+ completely in vitro through synergistic interaction<sup>7</sup>. Here, we treated four pregnant macaques with a triple combination of the human IgG1 monoclonal antibodies F105, 2G12 and 2F5. All four macaques were protected against intravenous SHIV–vpu+ challenge after delivery. The infants received monoclonal antibodies after birth and were challenged orally with SHIV–vpu+ shortly thereafter. We found no evidence of infection in any infant during 6 months of follow-up. This demonstrates that IgG1 monoclonal antibodies protect against mucosal lentivirus challenge in neonates. We conclude that epitopes recognized by the three monoclonal antibodies are important determinants for achieving substantial protection, thus providing a rational basis for AIDS vaccine development.

## Desoxyepothilone B is curative against human tumor xenografts that are refractory to paclitaxel

Ting-Chao Chou, Xiu-Guo Zhang, Christina R. Harris, and Samuel J. Danishefsky

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**Abstract:** The epothilones are naturally occurring, cytotoxic macrolides that function through a paclitaxel (Taxol)-like mechanism. Although structurally dissimilar, both classes of molecules lead to the arrest of cell division and eventual cell death by stabilizing cellular microtubule assemblies. The epothilones differ in their ability to retain activity against multidrug-resistant (MDR) cell lines and tumors where paclitaxel fails. In the current account, we focus on the relationship between epothilone and paclitaxel in the context of tumors with multiple drug resistance. The epothilone analogue Z-12,13-desoxyepothilone B (dEpoB) is >35,000-fold more potent than paclitaxel in inhibiting cell growth in the MDR DC-3F/ADX cell line. Various formulations, routes, and schedules of i.v. administration of dEpoB have been tested in nude mice. Slow infusion with a Cremophor-ethanol vehicle proved to be the most beneficial in increasing efficacy and decreasing toxicity. Although dEpoB performed similarly to paclitaxel in sensitive tumors xenografts (MX-1 human mammary and HT-29 colon tumor), its effects were clearly superior against MDR tumors. When dEpoB was administered to nude mice bearing our MDR human lymphoblastic T cell leukemia (CCRF-CEM/paclitaxel), dEpoB demonstrated a full curative effect. For human mammary adenocarcinoma MCF-7/Adr cells refractory to paclitaxel, dEpoB reduced the established tumors, markedly suppressed tumor growth, and surpassed other commonly used chemotherapy drugs such as adriamycin, vinblastine, and etoposide in beneficial effects.