

Review Article

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

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Received July 6, 2011; accepted July 28, 2011; Epub August 5, 2011; Published August 30, 2011

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.

Keywords: Drug discovery, drug development, unified theory for dose-effect pharmacodynamics, median-effect equation and plot of the mass-action law, drug combination, combination index, synergy definition, econo-green revolution for bio-research, experimental design, computer simulation, diagnostic plot, therapeutic cure of xenograft tumor, ardeemin and immunosuppression, iso-oxazole-fludelonone and epothilones, small size experiment, conservation of animals, pharmacodynamics, dose-reduction index, polygonogram

Introduction

Four decades have passed since the National Cancer Act campaign was signed by President Nixon on December 23, 1971, to declare the campaign for “Conquest of Cancer”, a national crusade. It seems appropriate now to review our progress and rethink the future directions based on past success and especially on the current shortcomings at the fundamental level, as will be illustrated below in several sections.

The accumulation of scientific progress and knowledge building during the past century is broad and magnificent. However, in the cancer therapeutics, the practical progress is, so far, not what we have wished. This is manifested by the fact that the overall age-specific cancer mortality rates have only improved about five per-

cent since 1950s [1]. The concerted efforts for the cancer chemotherapy mainly started about 65 years ago after World War II, beginning with nitrogen-mustard [2]. It is disheartening to see that the major anticancer agents currently in use are still mainly those discovered 30 years or even 50 years ago [3]. Despite of great improvement and tremendous investment on the basic sciences, most of the knowledge acquired is still difficult to translate into practical uses. The biotechnological advancement from double helix of DNA, gene arrays, high throughput screening, combinatory chemistry, human genome project and omics are amazingly impressive. However, the recent targeted therapy which evolved from biotechnology/system biology was found to encounter two major obstacles. One is insufficient efficacy and development of resistance and the other issue is difficult to integrate mathemati-

Table 1. Comparison of conventional divergent approach with the new econo-green convergent approach in bio-sciences

	Divergent Approach	Convergent Approach*
Aim	Knowledge building	Practical application
Philosophy	Individual mechanistic studies	The unified theory as the common denominator
Basis	Diverse techniques and assays	The median-effect equation of the mass-action law/mathematical induction and deduction for convergence
Focus	Intermediary steps for charts with increasing details of branches and arrows	Focus on end-results such as efficacy and toxicity
Time/Effort	Slow and more costly; Variety of methodology mainly at molecular and cellular levels	Econo-green bio-research; Small size experimentation in vitro and in vivo with computer simulation

* For more details, see Refs. 4-6.

cally and translate the massive amount of information acquired into therapeutic use.

Acquiring knowledge is a time consuming, effort intensive and costly endeavor, but cancer patients are dying annually everywhere with over half million in U.S. alone [1] and a quarter of the population eventually inflicted by this dreadful disease during the lifetime. While the basic research at molecular and cellular levels is important for knowledge building, the problem is the most knowledge acquired has little practical applications. This is manifested by the ever complicated pathway maps with increasing number of branches and arrows but the current mathematics or technology still cannot effectively exploit the *divergent* complexity for practical use [4]. It is, therefore, important to focus more efforts on the *convergent* unified theory for integration of knowledge through the common denominator and the quantification of bio-informatics for practical drug discovery approach (Table 1), especially on the econo-green biomedical research and drug development [5].

The author's research happens to transverse the entire past half-century of time and has initiated a unique new approach for a generalized theory for the studies of complicated bio-systems [6-14]. The purpose of this paper is to introduce and to discuss the new idea, concept, and the econo-green theory with broad biomed-

cal communities, especially, in cancer research area for drug discovery and drug development.

This author has introduced the generalized median-effect principle of the mass-action law developed with system analysis via mathematical induction and deduction for the development of the general theory previously [7-10], but was largely unnoticed for nearly two decades (Figure 1). The author would like to emphasize the mass-action law based dynamics and bio-informatics as a guide for the general bio-research applications [4-6, 8]. Using proper experimental design for small size experimentation and amendable with automated computer simulation based on the median-effect equation or plot, one can effectively simplify experimental design and data analysis and quantify the research conclusions [4-6]. This author would like to share personal views and practice on the mass-action law based algorithm as an approach for biomedical research, drug discovery, and drug development (Figure 2). During the past decades, this author has continuously utilized the mass-action law based theoretical algorithms and computerized simulation to conduct vast spectrum of biomedical research and applied it for the new drug discoveries. Although these personal views may not necessarily reflect the contemporary majority opinion, thinking and practice, it serves as a unique case study from the hands-on experience for over 40 years of

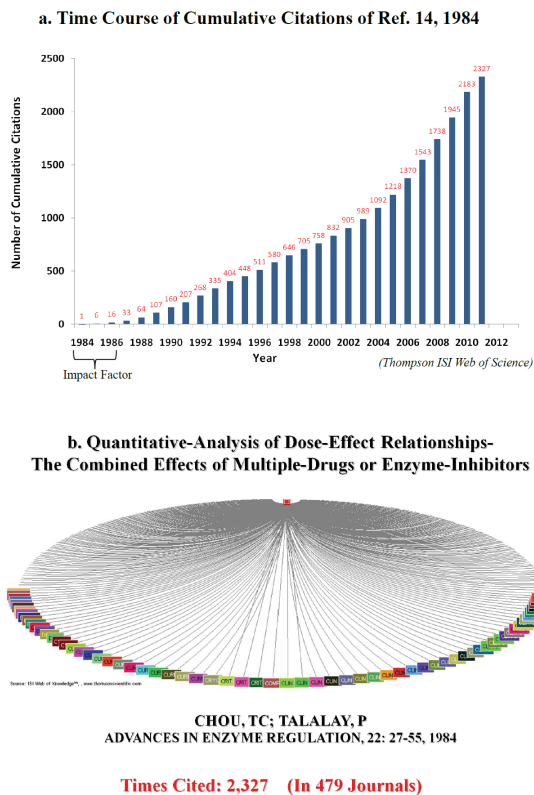


Figure 1. Citation statistics for the Chou-Talalay article [14] introducing the combination-index based on the median-effect principle of the mass-action law. (a) The cumulative citation trend is charted over the period 1984-2010. Note that during the first three years, there were only 16 citations. It takes over two decades to be widely used to become 2,327 citations. (b) The citation map as of June 14, 2011. All data from Thompson ISI Web of Science.

cancer research using a novel physico-chemical and mathematical approach [6] and ideology [5]. The fact that this author is a co-inventor of 30 U.S. Patents for anti-cancer agents or immunosuppressants, and an author/co-author of 250 articles which have been cited 12,450 times with 49.80 citations/ per article and with h-index of 54 (**Figure 3**); clearly indicate a unique case for efficient and low cost approach of bio-research in a small laboratory with limited resources [5].

The author also like to emphasize the importance of collaboration among scientists in different disciplines, for complementing each other, and for maximizing the net research results as exemplified in www.researcherid.com/rid/B-4111-2009 as indicated in **Figure 3** and its sub-

divisional contents in terms of collaborative institutions, major co-authors and the worldwide geographical distributions. The proposed econo-green bio-research is not intended to advocate the reduction in research funding or research staff, but rather, to promote the increase in research efficiency, productivity and competitiveness.

The main purpose of this article is to introduce new concept and new idea in cancer drug discovery and development that is rational, transparent, and frank and at times accompany with critical assessments. It is felt that this is the effective way to promote progress and reform. In this review, the author will discuss the key issues in drug discovery and development in four sections, namely the theoretical basis of the unified theory for pharmacodynamics; the combination index dynamic theorem for multiple entities; the preclinical studies; and the drug clinical development. The theory described here has been cited in over 479 different biomedical journals based on Thompson Reuters ISI Web of Science so it is broadly applicable (**Figure 1**), including cancer research. In this review article, the case study of the discovery of a remarkable microtubule targeting anticancer agent, iso-oxazole-fludelone, has been illustrated.

I. The Theoretical Basis of the Unified Theory for Pharmacodynamics

The Power of Mass-Action Law and Mathematical Induction and Deduction

The mass-action law underlies the physico-chemical principle in biochemistry and biophysics, such as the derivation of the Michaelis-Menten equation for substrate saturation, the Hill equation for higher-order ligand occupancy, the Scatchard equation for receptor binding, and the Henderson-Hasselbalch for pH ionization. Thus, it is prudent to consider that the mass-action law is the fundamental principle of Nature including the biomedical sciences.

This author had attempted to link mass-action law with mathematical induction and deduction for the development of the general theory for biomedical sciences [7-11]. The magic power of mathematical induction and deduction can be illustrated in the following examples:

$$1+2+3+\dots +10 = 55 \quad (\text{Eq. 1})$$

$$1+2+3+\dots +9,999 = 49,995,000 \quad (\text{Eq. 2})$$

$$1+2+3+\dots +123,456 = 7,620,753,696 \quad (\text{Eq. 3})$$

The Unified Theory

Derivation of Major Biochemical and Biophysical Equations from the Median-Effect Equation

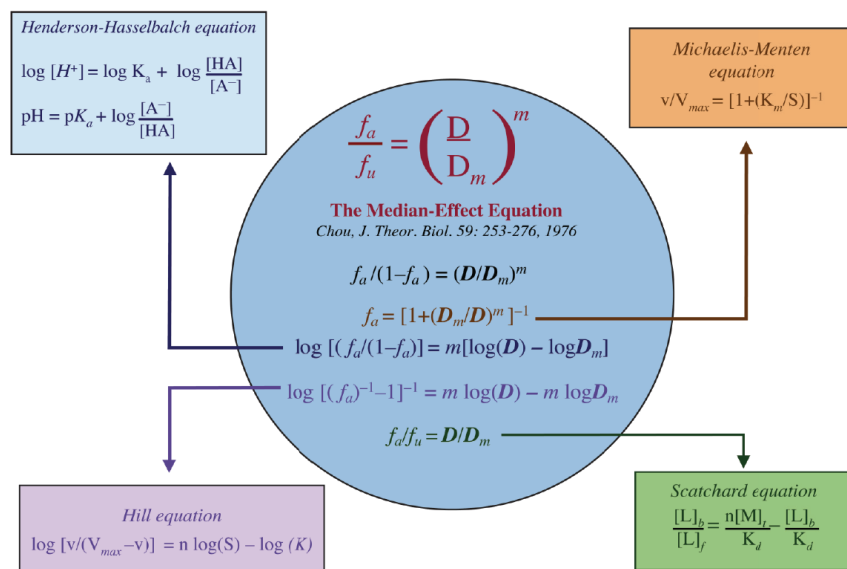


Figure 2. The unified theory based on the median-effect principle of the mass-action law. It can be used to derive the basic physico-chemical equations for broad range of disciplines. The median-effect equation is obtained via mathematical induction and deduction from over 300 mechanism specific equations [6,15].

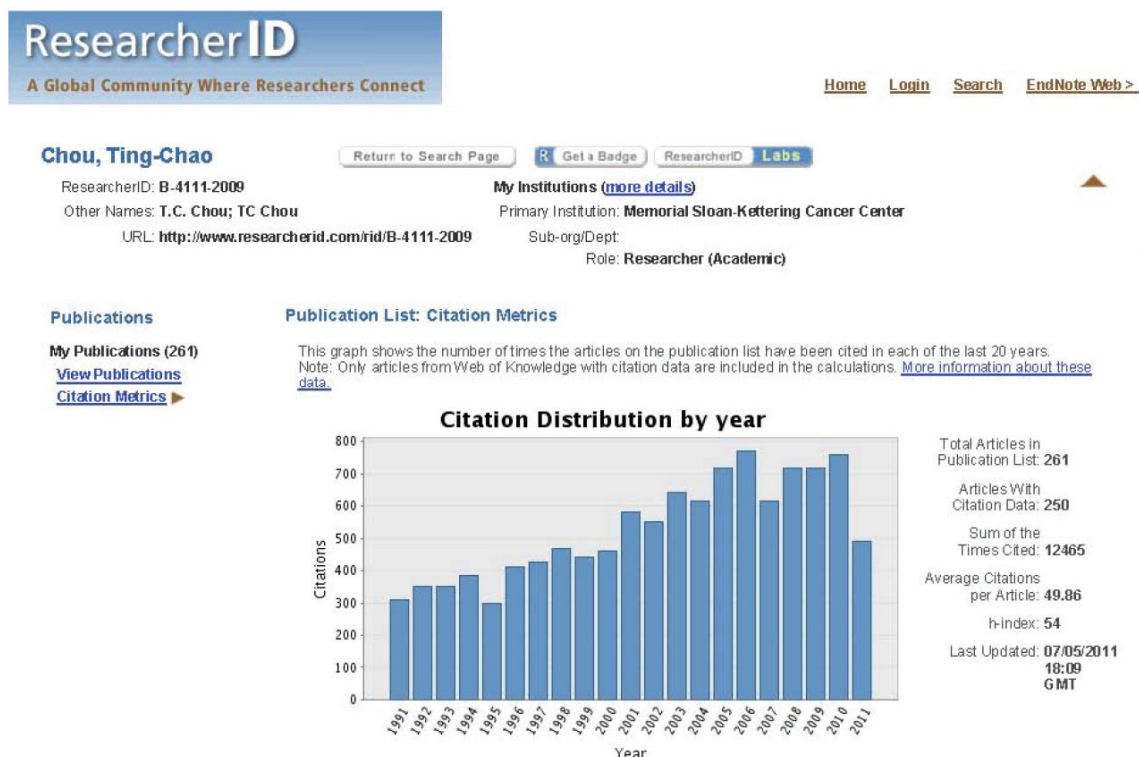


Figure 3. ResearcherID of Ting-Chao Chou for publication/citation record based on www.researcherid.com/rid/B-4111-2009 from Thompson Reuters ISI Web of Science, as of July 1st 2011.

Eq. 1 can be easily proven by the ordinary folks or even a sixth-grade student alike. But Eq. 2 and Eq. 3 would be a difficult proposition for

most individuals. In fact, the validity of Eq. 2 or Eq. 3 can be solved in 20 seconds with a pocket calculator or with a piece of paper and a pen in

about 2-3 minutes. Thus, when this magical power of mathematics is systematically applied to biological systems, it is possible to generate powerful general theory from the mass-action law, as indicated by the median-effect equation (MEE) for single drug [8-11] and for combination index (CI) equation for multiple drug combinations [11-14]. These theoretical innovations plus the extension from the 1st order dynamics to higher order dynamics [12-14] makes this mass-action law based pharmacodynamics (PD) equally applicable in simple biological systems, as well as complicated biological systems from in vitro studies, to animal studies, to human clinical trials [4-6]. This general theory was, in fact, deduced from over 300 mechanism specific equations [7-11]. The flow chart of this new approach for the general theoretical development is illustrated in [Appendix Figure 1](#) and will be elaborated in the sections below.

This seemingly unprecedented approach in biomedical sciences, i.e., the general theory obtained from merging the mass-action law with systematic mathematical induction and deduction, is the central focus underlying the quantitative new drug evaluations to be discussed in this article. Due to the fact that the general theory is mathematics-oriented, its derivation is not easily comprehensible by the general readers. It is pertinent to note here that the theory has been broadly applied in scientific papers in over 50 different biomedical disciplines. The author feels that the repeated reiteration of the theory is essential for each of the major applications.

1.1. The Single Entity Drug-Effect Algorithm

The unified theory of the median-effect principle (MEP) of the mass-action law has been described in details previously [6,8,15]. For the present article, it is necessary to reiterate briefly since it is part of the different applications for the same theory.

The median-effect principle is centered on the median-effect equation (MEE) and the median-effect plot both introduced by Chou in 1976 [8]. It is the general principle for the dose-effect dynamics, for single drug (single entity) solely based on the physico-chemical principle of the mass-action law (**Figure 2**). The median-effect equation (Eq. 4) is derived from over 300 equations through systematic analysis with mathematical induction and deduction [7-12]. Thus, it

is mechanism, unit, and dynamic order independent [5,6,13-15]. Eq. 4 is believed to be the simplest possible way to describe dose and effect relationships, which is given by:

$$\begin{aligned} f_a/f_u &= (D/D_m)^m \\ \text{Or } D &= D_m [f_a/(1-f_u)]^{1/m} \\ \text{Or } f_a &= 1/[1+(D_m/D)^m] \end{aligned} \quad (\text{Eq. 4})$$

where D is dose, f_a is the fraction affected by dose, f_u is the fraction unaffected ($1-f_a$), D_m is the median-effect dose (e.g., IC_{50} , ED_{50} , or LD_{50}) and m is the dynamic-order signifying the shape of the dose-effect curve (**Figure 2**), where $m = 1$, >1 , and <1 indicate hyperbolic, sigmoidal, and flat sigmoidal, respectively [14,15].

The rearrangement of Eq. 4 indicates that dose and effect are interchangeable; when the m and D_m values are determined, the entire dose and effect curve is determined. The most practical significance of the theory is that from the minimum of only two data points, we can draw the entire dose-effect curve, and thus, allow the determination of effect at any given dose [6,15]. More details of its significance for the econo-green bioresearch are to be discussed in Section 1.4 of this article.

The remarkable value of mass-action law based theory is its general applicability [6]. In fact, the algorithm derived from system analysis and mathematical induction and deduction is the general median-effect equation that is proven to be the unified theory (**Figure 2**) for the Michaelis-Menten equation, the Hill equation, the Henderson-Hasselbalch equation, and the Scatchard equation [6,15]. Thus, a system half-affected (D_m) is equivalent to half-saturated (K_m), half-occupied (K), half-ionized (pKa) and half-bound and half-free (Kd), i.e., a cup half full is half empty, and the median-effect equation denotes the dynamic relationship of fullness and emptiness (**Figure 2**). When the basic equations in biochemistry and biophysics can be derived from the median-effect equation, then, the median-effect equation and plot and algorithm should be broadly valid for biochemistry and biophysics. The unified theory as depicted by the median-effect equation is the simplest form of dose-effect relationship that is proven to be mechanism-independent, drug unit independent, and dynamic-order independent, whether it is for single drug or for drug combinations. All the terms in Eq. 4 are ratios, thus they are di-

dimensionless quantities [6].

1.2. The Median-Effect Plot Linearizes the Dose-Effect Curves

Taking logarithm of Eq. 4 and plotting $x = \log(D)$ versus $y = (f_a/f_u)$, as described by Chou is called the *median-effect plot* [8], where

$$\log(f_a/f_u) = m \log(D) - m \log(D_m) \quad (\text{Eq. 5})$$

will linearize all the hyperbolic and the sigmoidal dose-effect curves as shown in **Figure 4**, regardless of the drug's potency (D_m values), the shape of dose-effect curve and the dynamic order in the system (m values), the different drug mechanisms, and the drug units (due to dimensionless quantities). In the median-effect

plot, the D_m value is the anti-log of the x-axis intercept, and the m value is the slope of the median-effect plot. Both can be readily determined quantitatively using a simple computer software, such as CompuSyn [16-18], after entering a theoretical minimum of only two data points or more data points [6]. Thus, when the m and D_m values are determined, the entire dose-effect curve is determined. The fact that the minimum of two data points are required to draw a dose-effect curve, defies the common held belief that from two data points we can draw only a straight line. This is further illustrated in **Figure 4**. Based on this fundamental claim, we are allowed to conduct smaller experimentation using smaller number of data points than previously conceived [6]. Thus, the mass-action law based algorithm has been used as the theoretical basis for econo-green revolution of biomedical research [5] as well as the rationale for the efficient and effective low-cost approach for drug discovery and development, advocated in this article.

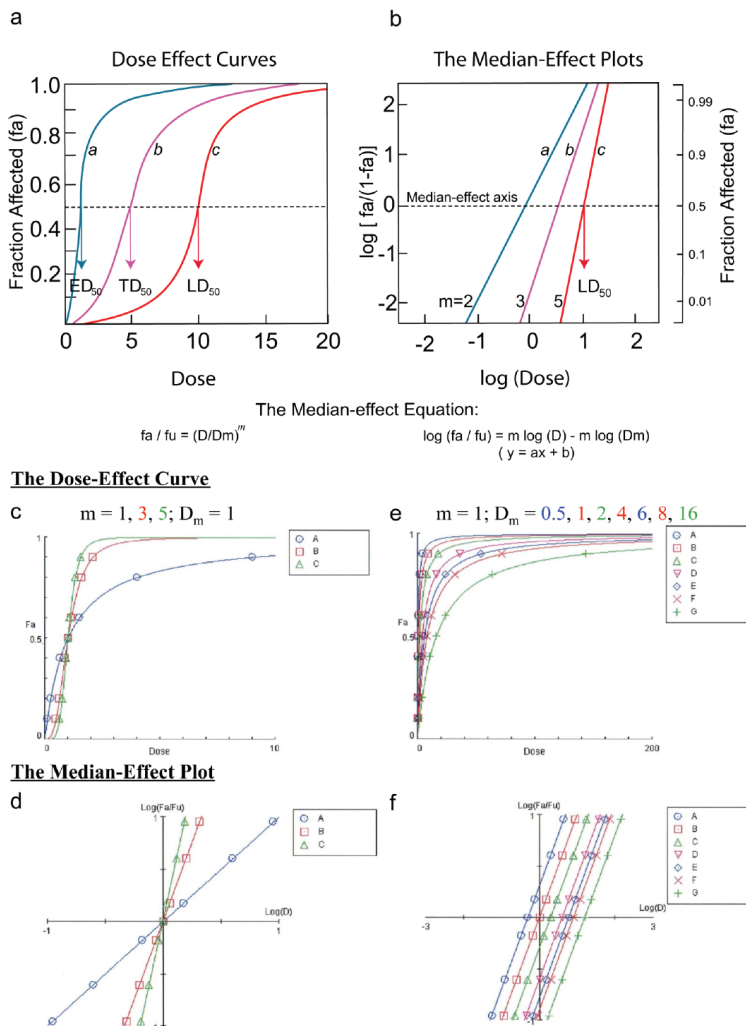


Figure 4. The linearization of dose-effect curves with the median-effect plot of Chou [8]. The dose and effect relations of all data points can be linearized by plotting $\log(\text{dose})$ vs. $\log[\text{fraction affected}/\text{fraction unaffected}]$. The applications are regardless of the potency of drug, the shape of dose-effect curve, the mechanisms of drug action, the unit of drug and the endpoints of measurement (e.g., effect, toxicity, and lethality). Thus, based on the median-effect equation (Eq. 4), the theoretical minimum of drawing a dose-effect curve requires only two data points. This is due to the fact that all dose-effect curves can be linearized by the median-effect plot. The “median” is the universal reference point, and the dose-zero is a default point.

1.3. The Role of Statistics

The relationship between *dose* and *effect* is not statistical random distribution but rather governed by the *unified theory* based on the median-effect principle of the physico-chemical principle of the mass-action law. The “median” is the common-link and common reference points for the first-order dynamics, and from the single entities and complex entities [5,6]. The mass-action law

emphasizes deterministic whereas the statistics focuses on probabilities. In the realistic world, there are biological variations, experimental accuracy and error issues. This is where statistics will set-in. The unified theory described above has nothing to do with statistics *per se*. But rather to stress here how to analyze data efficiently, effectively, and reaches the analytical conclusions quantitatively when the experimental data have already been reasonably, accurately determined. Accurate measurement is the pre-requisite of accurate conclusion for any biological studies. The MEP theory clearly indicates that a minimum of only two data points are required to determine the entire dose-effect curve, if the data are reasonably accurately determined, as indicated in **Figure 4** [i.e., all data points on the dose-effect curve will fall on a straight line after the median-effect plot transformation of Chou [7]]. Due to variability in the measurement, the author never recommends the use of only two data points for biomedical experiments, but rather provides the theoretical basis to indicate econo-green benefits for conducting small size experiments, such as using 5 to 7 data points *in vitro* and 4 to 5 doses *in vivo*, and using the powerful median-effect plot with its simple computerized simulation. Instead of drawing an empirical curve to fit many data points, the author proposes to use fewer data points to fit the mass-action law to achieve econo-green bio-research. The above theory is applied for single entity drugs; however the median-effect equation has also been extended to the theorem for multiple entity drug combination studies [6, 11-18] which will be discussed in Section II. The statistics manages the variability and probability of data, whereas the mass-action law deals with how to analyze the data when the experimental data are reasonably accurately determined.

It is concluded that pharmacodynamics (PD) for single drug as well as multiple drug combinations should be determined based on the mass-action law but not based on statistical analysis [4-6]. More details will be discussed in Section I.6 of this article.

I.4. The Fundamentals for the “Econo-Green” Approach

Recently, this author raised two fundamental questions more “basic” than the contemporary basic bio-research that reached the very root of

biomedical science, and should have far reaching consequences [5] as indicated below.

The first question is how to draw a dose-effect curve? Can we draw a specific dose-effect curve with a minimum of only two data points? The answer is yes by using the median-effect equation. Since two data points are actually four points with the dose zero being the third point, and the “median” dose is the universal reference point as indicated by the median-effect equation (Eq. 4). Since the median-effect plot of Chou as depicted by Eq. 5, linearized all dose-effect curves, all data points from 2 to n all fall on a straight line. For centuries, biologists have been producing a lot of data points and then using a bent ruler or curved mold to draw the empirical dose-effect curve. This empirical, curve fitting including the least square has no theoretical basis for it. As indicated above, the dose-effect curve is not random or arbitrary, but rather followed the physico-chemical principle of the median-effect equation of the mass-action law [6]. All accurately measured data, when entered into the computer, using the mass-action law based software, CompuSyn [18], the dose-effect curve can be constructed in a split second, even with only two data points which is the theoretical minimum. This method has been tested over and over for over 10,000 data sets in biomedical literature (www.researcherid.com/rid/B-4111-2009) but many scientists are still unaware of it, which method takes over a quarter century to be popularized. These lead to far-reaching consequences as has been shown in **Figures 2 and 5**. The main point is that when only two data points can draw the exact dose-effect curve, then, if you have 3, 4, 5, or 6 data points, the method should be extremely powerful. This reduced requirement for the data point and yet access to mass-action law parameters and graphics allow small size experimentation that can be translated to the saving of time, effort and cost, conserving the number of experimental animals and reducing the patients/volunteers in clinical trials. This theoretical approach in conjunction with proper experimental design and with computerized simulation can result in the econo-green bio-research and medical practice.

The second question is what is the additive effect of two drugs? Or n drug? What is synergism? Drug combination has been widely used for treating the most dreadful diseases, includ-

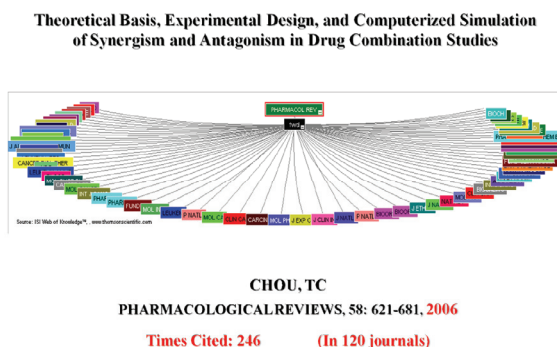


Figure 5. The citation map of multiple entity combinations as illustrated in Pharmacol. Rev. 58: 621-681, 2006 [6]. The fast-growing trend is evident during the past four and half years.

ing cancer and AIDS. Ironically, there are about 20 different definitions flooding the biomedical literature but none supported the others [5,6]. If a single drug dose-effect relationship is drawn by empirical curve, then when two drugs are combined, it will be empirical on top of another empirical that leads to nowhere. It is well known that the additive effect of two drugs is not the simple arithmetic sum of effect of two drugs, nor their multiplied fractions product [4-6]. The confusion, controversy, or ignorance lead to chaos in biomedical sciences, compromising the quality of medical practice, unnecessarily wasting resources, and compromising the best care of the patients.

To dispel this over a century old confusion and controversies, this author with Prof. Paul Talalay of the Johns Hopkins University again using the mass-action law based system analysis, had derived the multiple drug effect equations and, then, introduced the combination index (CI) concept where $CI < 1$, $= 1$, > 1 , quantitatively determines synergism, additive effect, and antagonism, respectively [6,14] (**Figure 5**). This rigorously mathematically “derived” equation and method with automated computer simulation, was largely ignored by the medical community since too much confusions and futile debates among the scientifically ambiguous or unsubstantiated methodologies. It is recently concluded that synergy definition or its determination is a mass-action law issue not a statistical issue [5,6,26].

One theoretical article alone, by Chou and Talalay, published in 1984 [14], received 1, 6, 16

citations during the first three years, but received 404, 705, 1072, 2315 citations in 10, 15, 20 and 27 years (**Figure 1**). The pace of accepting the new idea is astonishingly slow. In any event, the truth shall prevail.

I.5. The Cost-effective Experimental Design and Computerized Analysis

For single entity compounds or multiple drug combinations, this author's laboratory have always used the mass-action law algorithms of the dose-effect curves; the median-effect plot for the mass-action law parameters (D_m for potency IC_{50} , ED_{50} , or LD_{50} ; m for shape; and r for conformity) [6,15]. The theory, equations, and applications has been presented in earlier sections. This analysis can be done in a fraction of a second by using a computer software, CompuSyn [18], after entering dose and effect data from a small size experiment (usually with 5-7 concentrations in vitro or with 4-5 doses with 4-6 animals per dose in vivo). The reason for the small size experiments is due to all dose-effect curves can be linearized with the median-effect plot into a straight line (**Figure 4**). Theoretically, a specific dose-effect curve can be drawn with the mass-action law algorithm even with only two data points. It is stressed here again that this author does not recommend the use of theoretical minimum of data points as indicated above due to the variability in biological measurement [6]. But the theory provides the strong basis for computerized simulation for the econo-green biomedical research [5] that saves tremendous amount of resources and time, and increases the productivity and competitiveness. The cost-effect experimental design for drug combination studies will be illustrated in Section II.2.

I.6. Comparison of Pharmacodynamics (PD) and Pharmacokinetics (PK) and Why PD is Grossly Underdeveloped in Biomedical Research

In biomedical literature, PK and PD are frequently mentioned side-by-side. PD deals with the question of what a drug does to the body, and PK deals with the question of what the body do to the drug. PD is focused on various dose and PK is focused on various time (**Table 2**). While PK is routinely used in clinical studies and preclinical studies, its equations are the conventional empirical-perceived formula for complex systems, such as the determination of absorp-

Table 2. New emphasis of pharmacodynamics (PD) over pharmacokinetics (PK)*

Items	PD	PK
Mode of action	<i>What drug does to the body</i>	<i>What body does to the drug</i>
Characteristics	Mainly vary dose Single Unified Theory	Mainly vary time Multi-factorial mix
Principle	The median-effect principle of the mass-action law	Complex phenomenal observations/measurements
Rigorousness	Explicitly derived equations or theorems	Empirically perceived formula
Applications	Physico/chemical quantitative parameters	Probabilistic empiric parameters
Parameters	D_m , m , r , CI , DRI , IC_{50} , K_m , K_i , K_a and K_d Competitiveness, Exclusivity, Synergism, Antagonism [Mass-action parameters for potency, shape, dynamic order, and interaction indices]	$t_{1/2}$, C_{max} , CI , AUC , V_{dis} Absorption, Distribution, Metabolism, Excretion [Measurement of Parameters without direct physico-chemical law bearing]
Determining Efficacy	Yes	No
Determining Toxicity	Yes	No
Individualized Variability focus	Yes	Yes
Determinant for	<i>What it takes to be a good drug</i>	<i>Proper use of a drug</i>

* This table in slide form was presented at the 3rd Drug Development Summit at Zurich, Switzerland, June 8-9, 2011, sponsored by Oxford Global.

tion, distribution, metabolism, excretion (ADME) and drug levels for the time courses [e.g., half-life ($t_{1/2}$), maximal concentrate (C_{max}), clearance (CI), area under the curve (AUC), volume of distribution (V_{dis}), etc] [19]. PK *per se* determines neither efficacy nor toxicity. Clearly, PD plays much more critical roles in drug discovery and development (Table 2). Unfortunately, there is a lack of clear definition of “pharmacodynamics” in biomedical literature and its equations are empirical without actual derivations [19-21]. The equations in contemporary literature are mainly using the substrate type of enzyme kinetic symbols, such as E_{max} . In fact, most drugs are inhibitor ligands, not substrate ligands. Therefore, the term E_{max} was incorrectly used theoretically and the equations provided [20] were not derivable. The term “pharmacodynamics”, described by Holford and Sheiner in 1981 [20], has no defined meaning since its E_{max} model contradicted the mass-action law. Especially, the “effect” equation as described in Ref. 20, p. 435, Eq. 6 is relevant only for activators not valid for inhibitors. In addition, the “drug-drug interaction” equation described in Ref. 20, p. 446, Eq. 12 or in Ref. 21 is not pos-

sible to be derived mathematically. The unified theory of the median-effect principle derived from the mass-action law (Figure 2) provides theoretical basis for pharmacodynamics with rigorous basis such as the median-effect equation for single drug [6,8] or the combination index equation for drug combinations [6,13]. One article alone [14] that introduced the rigorously derived combination index (CI) in 1984, has been cited 2,315 times in over 479 different journals (Figure 1) which is about one-tenth of all biomedical journals in the world, indicating the broad application and utility in different biomedical disciplines (www.researcherid.com/rid/B-4111-2009). Therefore, it is proposed, that the term of pharmacodynamic bioinformatics shall take the quantitative mass-action law parameters, D_m (the median-effect dose or concentration), m (the shape of the dose-effect curve or dynamic order), r (the linear correlation coefficient of the median-effect plot), K_m (Michaelis-Menten constant), K_i (inhibition constant), K_a (activation constant), K_d (dissociation constant), CI (combination index) and DRI (dose-reduction index) (Table 2). Thus, PD determines what it takes to be a good drug

The Mass-Action Law & Biology

- Based on the Physico-Chemical Principle of the Mass-Action law
- “Median” as the Universal “Common Link” and “Reference Point” (GPS)
- Efficient Pharmacodynamics By-Passing Intermediary

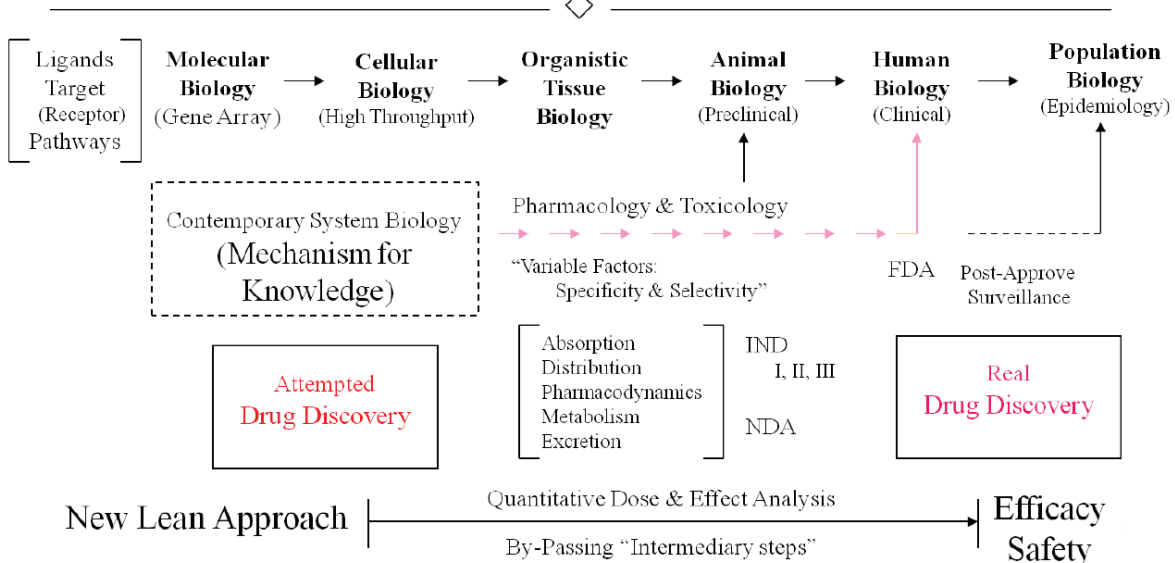


Figure 6. The attempted vs. real drug discovery. The mass-action law based dose-effect dynamic measure the end-points of efficacy and toxicity and by-passing the countless intermediary steps and thus in conjunction with the unified theory as the common denominator and using computer simulation lead to achieving efficient, effective, and low-cost new drug discovery and development.

and PK determines the proper use of a drug. In this author's opinion, the PD studies that determine efficacy and toxicity should be much more emphasized over PK studies (Table 2) in both drug discovery and drug development studies.

For drug discovery investigation, this author has never conducted pharmacokinetic (PK) and ADME (absorption, distribution, metabolism, and excretion) studies until the candidate compound for development has been selected from the lead compounds. Because when a dose is given parentally, orally, or topically, we observe only the endpoint efficacy and/or toxicity. In this process, although the PK and ADME are not determined, they are already included as parts of the intermediate steps, as shown in Figures 6 and 7.

When a drug is given to kill a cell (or an animal) no one really understands exactly how many

“intermediary steps from life to death” are. Overemphasizing the studies on the intermediary steps is an exhaustive attempt and is inefficient. The rationale is to emphasize to the therapeutic effectiveness and usefulness at the end of the final verdict of a new drug. We have limited time and resources for the “intermediary investigations” especially if there are many potential drugs to deal with.

I.7. Excessive Requirements and Costs for the Drug Development?

The pharmaco-economic statistics indicate that research and development spending for the major pharmaceutical companies in 1996, 2000, 2005 and 2008 were 17, 20, 30, and 38 billion dollars, respectively. During these same period of time, the total new drug applications to the FDA were 131, 90, 78 and 89, respectively, in which the new molecular entities were

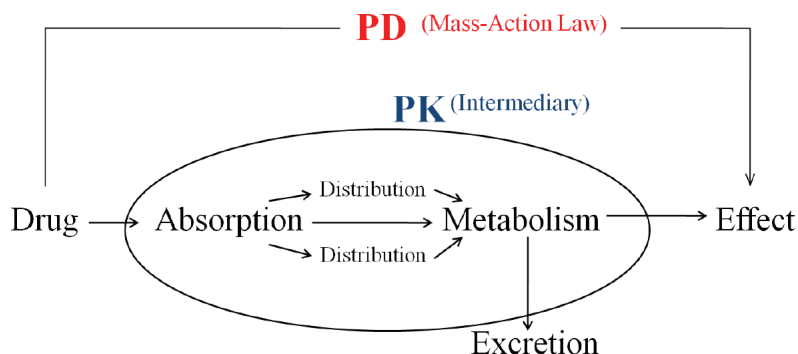


Figure 7. The mass-action law based pharmacodynamic (PD) and the intermediary step-based pharmacokinetics (PK). PD determines efficacy and toxicity of drug toward the target (s) in the body whereas PK determines how body handles the drug in various intermediary steps before it exerts the end-point for the measurement. The intermediary steps are part of the PD determination.

53, 27, 18 and 21, respectively, and biologic license applications were 3, 2, 2, and 6 respectively [22,23]. To develop a new drug from bench work to clinical use can cost over one billion dollars and yet facing high percent of failure rate. Apparently, this trend is not sustainable for the long run and a drastic reform is needed. One formidable task is how this reform be carried out. One approach for reform to ease the sustainability challenge, in the author's opinion, is the econo-green bio-research.

To study the intermediate pharmacological steps, such as ADME, PK and mechanism, are time consuming, costly and frequently difficult to measure or difficult to interpret quantitatively in terms of efficacy and toxicity. These intermediate processes are not part of the drug discovery, *per se*, although they are usually considered as the major part for regulatory approval purposes. Strictly speaking, even the "mechanism" should not be required in drug discovery or patent application or IND (investigational new drug) application since it does not quantitatively predict efficacy or toxicity, although this mechanistic "knowledge" is good to have (Figure 6). High efficacy and low toxicity are the determinants of a good drug. Factors other than the mechanism, such as transport of antifolates or formulation of taxol or the rate of development of resistance or prolonged infusion of epothilones decide the drug's usefulness. The author's laboratory has studied over 100 epothilone analogs, the 16 member-ring macrolides obtained from total synthesis, each compound with the same mechanism of microtubule stabilization. But the knowledge of this mechanism provides no clue as to how to select the candidate compounds for clinical development (see the specific examples given in Section III-3 below). In addition, it is well

known that Aspirin, which has been used worldwide for over a century, we still have little knowledge about its "mechanisms" of antipyretic, anti-inflammatory, analgesic effect, protecting the heart and thinning the blood. Therefore, this author considers that the requirement of mechanism for drug discovery or drug development is only of secondary importance, and overly emphasis may delay the drug developmental process and greatly increase its cost. In the contrary, the author strongly suggests that the priority should be PD over PK studies and the PD should have special emphasis of "Efficacy" and "Safety", as indicated above. Most importantly, PD should be carefully and extensively carried out in animals for efficacy and safety, and PK be mainly carried out in Phase I clinical trials since PK is already the intermediate parts of the preclinical PD studies (Figure 6). In fact, PK usually varies time and PD usually varies dose, and thus, PK and PD are difficult to correlate quantitatively (Table 2). The econo-green theoretical arguments and the practical experimental design and computerized simulation as indicated above can dramatically reduce the drug developmental cost, which can be translated into benefits for the overall medical care. What we really need to emphasize are useful data, *per se*, not the massive amount of speculative or intermediary data. In addition, when conducting PD, the principle of the "dynamics" needs to be clearly understood.

As indicated above, the pharmacodynamic (PD) study is to investigate what the drug does to the body, and the pharmacokinetic (PK) study is to investigate what the body does to the drug (Figure 7 and Table 2). The PK study is safe to the patients (as well as to animals), however, PD study can be potentially harmful to the patients due to toxicities at high doses or due to

non-optimal experimental conditions as well. It is recommended that PK studies should be carried out after the optimal conditions of PD have been established. Otherwise, PK studies would be like fishing expeditions. Thus, the exploratory PD studies should be mainly carried out in animals first by knowledgeable pharmacologists, not just focusing in humans, since animal studies allow greater flexibility and less liability restrictions for explorations. By contrast, the IND approved clinical trial protocol is fixed and to be followed strictly. If clinical trial is not based on "optimal conditions" obtained from extensive animal studies, the clinical results can not reflect the best results. It should be noted that the inter-species variability is well known in biological science, which general issue is not the focus in this article. The optimal conditions can only be established by extensive pharmacodynamic (PD) studies which includes varying the doses [dose range, dose density], route of administration [oral, i.v. injection, i.v. infusion] and schedule [including infusion time], thus both efficacy and toxicity can be determined meaningfully with direct relevance to the clinics.

For the rigorous PD studies, the author established algorithms with computerized simulation based on the median-effect principle of the mass-action law [6-9] that is applicable to virtually any drug with any mechanisms, any units or any dynamic orders [5,6]. The computer program, e.g., CompuSyn, [16-18] greatly facilitates the PD studies.

In essence, the PD general equations can be explicitly derived from the mass-action law, whereas PK formula are empirical and generally lack of rigorous theoretical basis. The PK informatics neither indicates efficacy nor toxicity, which are the two primary criteria for a good drug (**Table 2**). Additionally, the mass-action law for PD is the general principle in biochemistry and biophysics and thus serves as the general "model", whereas PK's formula, although regarded by some as a "model" is, in fact, the empirical formula/parameters or the direct determination of concentrations that have no explicit bearing in biochemical or biophysical theory (e.g., the α and β coefficients are undefined).

Thus, PK is grossly overemphasized in both conventional preclinical and clinical studies that consumed lots of resources and time, whereas,

PD is largely neglected and under-developed especially at the preclinical level despite of its crucial role in both efficacy and safety. The negligence on PD, in this author's opinion, is mainly due to insufficient understanding of pharmacodynamic principle, since the previous PD equation [20] were erroneously obtained from the so-called "E_{max}" model that were for the primary ligands (such as substrates) but not for the reference ligands (such as inhibitors). Furthermore, the drug-drug interaction as described in Ref. 20, p. 446, Eq. 12 or in Ref. 21 is not derivable mathematically from physical or chemical principle [6]. The lack of rigorous development in PD in the past has obviously hindered the overall drug discovery and drug development. No one shall ignore the importance of the variability in biology and the imperfection in measurement of drug effects and these are where statistics set-in. But even when the variability problem is taken care of, the crucial problem of efficacy and safety still remain. The statistics is only an accessory, not the main purpose for the drug discovery or developmental studies. This disparity of overly emphasizing the PK statistics and intermediary ADME steps, and neglecting the PD deterministics in the preclinical studies, has un-intended consequences that would affect not only the meaningful evaluation of new drugs but also increase the failure rate of the new drug development due to the priority of resource allocation.

II. The Combination Index Dynamic Theorem for Multiple Entities

Drug combination, which intends to obtain synergistic effect or reduce toxicity, is of primary importance in treatments of the most dreadful diseases, such as cancer and AIDS [6,24]. Thus, the establishment of multiple drug combination is as important as a new drug development. Unfortunately, the "definition of synergy" is one of the most confusing areas in biomedical sciences since there are about twenty different definitions for synergy in literature, but none supports the others [6,25,26]. The gross negligence and lack of rigorousness have their apparent ramification of consequences for biomedical research as well as for the public health and the medical care to bear. Using the mass-action law and the mathematical induction/deduction, the single entity unified theory discussed above can be extended to combination index theorem for multiple drugs, as de-

scribed previously [6,11-15] and outlined in the [Appendix Figure 1](#). For example, drug 1 and drug 2 can target an enzyme reaction with various mechanisms of actions (e.g., ordered, sequential, ping-pong or random sequence of reaction), and with the permutation of various number of substrates and products. The effects of drug 1 can be competitive with substrate A and non-competitive with substrate B whereas the effects of drug B can be non-competitive with substrate B and uncompetitive with substrate C, etc. These permutations varying orders, varying substrates and/or products can easily lead to the derivation of hundreds of rate equations. By taking the ratio of the equations with the presence and absence of an inhibitor, the common parameters such as K_m , K_i , and V_{max} can be cancelled out and yield the general equation for the dose and effect. Thus, for a two drug combination, in a first-order system ($m=1$), we get the general equation [11,12]:

$$\frac{(f_a)_{1,2}}{(f_a)_{1,2}} = \frac{(f_a)_1}{(f_a)_1} + \frac{(f_a)_2}{(f_a)_2} = \frac{(D)_1}{(D_m)_1} + \frac{(D)_2}{(D_m)_2} \quad (\text{Eq. 6})$$

and when $m \neq 1$, then:

$$\left[\frac{(f_a)_{1,2}}{(f_a)_{1,2}} \right]^{1/m} = \left[\frac{(f_a)_1}{(f_a)_1} \right]^{1/m} + \left[\frac{(f_a)_2}{(f_a)_2} \right]^{1/m} \quad (\text{Eq. 7})$$

$$= \frac{(D)_1}{(D_m)_1} + \frac{(D)_2}{(D_m)_2}$$

Based on Eqs. 6 and 7, in conjunction with Eq. 4, Chou and Talalay in 1983 introduced the term *combination index* (CI) for quantification of synergism ($CI < 1$), additive effect ($CI = 1$), and antagonism ($CI > 1$) [6,13,14], where at $x\%$ inhibition, the general equation for two drugs is given below:

$$CI = \frac{(D)_1}{(D_m)_1} + \frac{(D)_2}{(D_m)_2} = \frac{(D)_1}{(D_m)_1 [f_a / (1-f_a)]^{1/m_1}} + \frac{(D)_2}{(D_m)_2 [f_a / (1-f_a)]^{1/m_2}} \quad (\text{Eq. 8})$$

A typical presentation of algorithms and graphics of CI values as a function of effect (f_a) is illustrated in **Figure 8**. The resulting F_a -CI plot is also called Chou-Talalay plot. The F_a -CI plot and isobologram are two sides of the same coin, where F_a -CI plot is effect-oriented and the isobologram is dose-oriented (**Figure 9**). More details have been given in Reference 6. The algorithm for quantifying synergism or antagonism for three or more drugs have also been derived and the computer software, such as CompuSyn, have been developed [18]. For example, the general equation of a five drug combination at $x\%$ inhibition is:

$$\begin{aligned} {}^5(CI)_x = & \frac{(D_x)_{1,5} [P/(P+Q+R+S+T)]}{(D_m)_1 \{ (f_a)_1 / [1-(f_a)_1] \}^{1/m_1}} + \frac{(D_x)_{1,5} [Q/(P+Q+R+S+T)]}{(D_m)_2 \{ (f_a)_2 / [1-(f_a)_2] \}^{1/m_2}} \\ & + \frac{(D_x)_{1,5} [R/(P+Q+R+S+T)]}{(D_m)_3 \{ (f_a)_3 / [1-(f_a)_3] \}^{1/m_3}} + \frac{(D_x)_{1,5} [S/(P+Q+R+S+T)]}{(D_m)_4 \{ (f_a)_4 / [1-(f_a)_4] \}^{1/m_4}} \\ & + \frac{(D_x)_{1,5} [T/(P+Q+R+S+T)]}{(D_m)_5 \{ (f_a)_5 / [1-(f_a)_5] \}^{1/m_5}} \end{aligned} \quad (\text{Eq. 9})$$

The example of 5-drug combination using automated computerized simulation has been given in the Appendix of Reference 6. This approach in conjunction with polygonogram is particularly useful in evaluating and designing cocktail for anti-HIV therapy as well as herbal therapy in traditional Chinese medicine [5,6]. The general equation of n drug combination at a specified combination ratio for $x\%$ inhibition is given by:

$${}^n(CI)_x = \sum_{j=1}^n \frac{(D)_j}{(D_m)_j} = \sum_{j=1}^n \frac{(D_x)_{1-n} \{ [D]_j / \sum_1^n [D] \}}{(D_m)_j \{ (f_a)_j / [1-(f_a)_j] \}^{1/m_j}} \quad (\text{Eq. 10})$$

where ${}^n(CI)_x$ is the combination index for n drugs at $x\%$ inhibition, $(D_x)_{1-n}$ is the sum of the dose of n drugs that exerts $x\%$ inhibition in combination, $([D]_j / \sum_1^n [D])$ is the proportionality of the dose of each of n drugs that exerts $x\%$ inhibition in combination, and $(D_m)_j [(f_a)_j / (1-f_a)_j]^{1/m_j}$ is the dose

Algorithms for Computerized Simulation of Synergism, Additivism and Antagonism of the Effect of Multiple Drugs

The Median Effect Equation

- (1) $f_a / f_u = (D/D_m)^m$
- (2) $\text{Log}(f_a / f_u) = m \text{Log}(D) - m \text{Log}(D_m)$
- (3) $f_a = 1/[1+(D_m/D)^m]$
- (4) $D_x = D_m [f_a / (1-f_a)]^{1/m}$

$$(5) CI = \frac{(D)_1}{(D_x)_1} + \frac{(D)_2}{(D_x)_2} = \frac{1}{(DRI)_1} + \frac{1}{(DRI)_2}$$

$$(D_x)_{1,2} = (D)_1 + (D)_2$$

$$\text{and } (D)_1/(D)_2 = P/Q$$

$$(D)_1 = (D_x)_{1,2} \times P/(P+Q)$$

$$(D)_2 = (D_x)_{1,2} \times Q/(P+Q)$$

D = Dose

f_a = fraction affected

f_u = fraction unaffected

D_m = median-effect dose

m = slope, Hill-type coefficient or kinetic order

CI : Combination Index

$CI = 1$ (summation)

< 1 (synergism)

> 1 (antagonism)

DRI : Dose-Reduction Index

$$(DRI)_1 = \frac{(D_x)_1}{(D)_1}, \quad (DRI)_2 = \frac{(D_x)_2}{(D)_2}$$

For n Drug Combinations:

$$CI = \sum_{j=1}^n \frac{(D)_j}{(D_x)_j}$$

Figure 8. The computer algorithms. The integration of the median-effect equation and the combination index equation that yields the algorithms for computerized simulation of F_a -CI plot of Chou-Talalay [14] and the F_a -DRI plot of Chou-Martin [18]. It also provide the basis for computerized construction of classic isobologram and the normalized isobolograms of Chou and Chou [16] given in Figure 9. The combination ratio for $(D)_1$ and $(D)_2$ is designated as P/Q . These algorithms can be extended to three or more drug combinations as indicated by Eqs. 9 and 10.

of each drug alone that exerts $x\%$ inhibition, where D_m is the median-effect dose (anti-log of the x -intercept of the median-effect plot), f_a is the fractional inhibition at $x\%$ inhibition, and m is the slope of the median-effect plot, which depicts the shape of the dose-effect curve (where $m = 1$, >1 , and <1 indicate hyperbolic, sigmoidal, and flat sigmoidal curve, respectively). The equations 9 and 10 should be useful for the drug interactions in complicated systems, such as the combination cocktails or the Chinese traditional medicine which almost always involved the combination of multiple components.

II.1. Algorithms for Determining Synergism and Antagonism

The combination index equation as shown in Eqs. 8-10, in conjunction with Eqs. 4 and 5, can be used as algorithms for computerized simulation for the combination index values at different effect levels (i.e., at different f_a 's). This multiple step calculation for simulation is given in **Figure 8**. The computer program for fully automated simulation has been developed by Chou and Chou [16], Chou and Hayball [17] and recently by Chou and Martin [18]. The graphics have been named the *Fa*-CI plot, and later, the *combination index plot* or the *CI plot*. A typical F_a

-CI plot and its interpretation is shown in **Figure 9**, where $CI < 1$, $=1$, and >1 indicates synergism, additive effect, and antagonism, respectively.

As indicated earlier [6], there are about 20 different definitions for synergy or its determination in biomedical literature but none supported the others. These posed serious scientific and regulatory problems since drug combination has been widely used for treating the most dreadful diseases such as cancer and AIDS. It is argued that in the absence of clear and quantitative definition for synergy, the scientific researchers, grant and publication reviewers, and journal editors have no standard to assess synergism; and also the governmental regulatory agencies and the patent offices have no basis to regulate or to determine the synergy claims [5,6,26,27]. Thus, synergism definition rigorously established from the physico-chemical principle of the mass-action law should be used as a standard, as has been used in over 479 biomedical journals internationally (**Figure 1**), unless a better verifiable, rigorous method can be obtained.

II.2. Design of Drug Combination Studies for Maximal Bio-Informatics

Experimental design may dictate the appropriateness of the method of data analysis, and

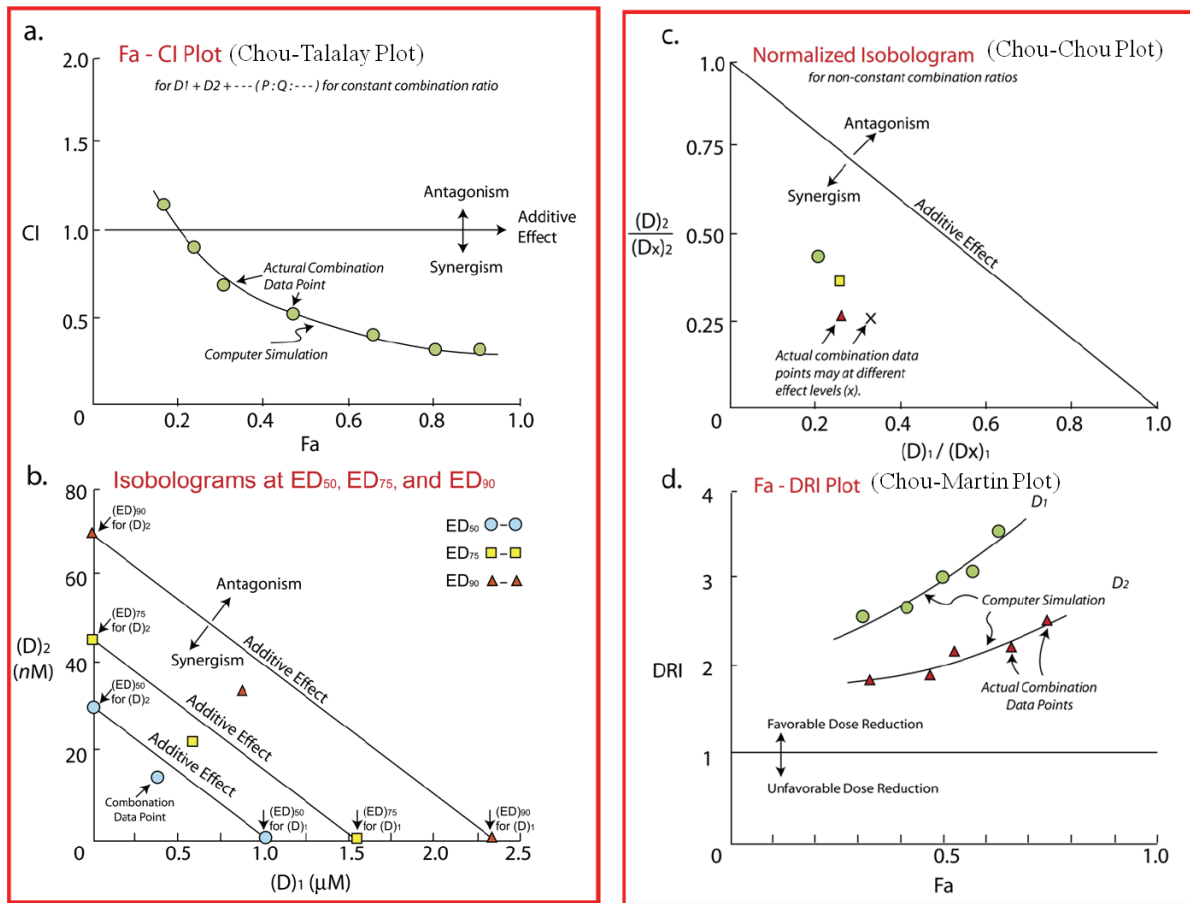


Figure 9. The diagnostic plots. Illustrative examples of computer generated diagnostic plots based on the median-effect equation of Chou [8] and the combination index equation of Chou-Talalay [6] with algorithms given in Figure 8. a. The F_a -CI plot with x =fraction affected (F_a) vs. y =combination index (CI) where $CI < 1$, $=1$, and >1 indicates synergism, additive effect and antagonism, respectively (the Chou-Talalay plot); b. The classic isobologram for the constant-ratio combinations based on the Chou-Talalay's multiple drug effect equation [12] or the combination index equation [14] for iso-effective dose graphics. The combination data points that fall on the hypotenuse indicate additive effect, that fall on the low-left indicate synergism, and that fall on the upper-right indicate antagonism; c. The dose-normalized isobologram for the non-constant ratio combinations for the normalized-dose iso-effective graphics introduced by Chou TC and Chou JH [15,16]; d. DRI is folds of dose-reduction allowed for each drug, at a given degree of effect, in drug combination studies. The F_a -DRI plot is the plot of the dose-reduction index as a function of the fraction affected. $DRI > 1$, $=1$, and <1 indicates favorable dose reduction, no dose-reduction, and negative dose-reduction, respectively. Dose reduction leads to reduction of toxicities in drug combinations.

data analysis may dictate the conclusions [6]. For two drug combinations, A+B, each drug concentrations (or doses) can be varied at a constant ratio, or varied independently at different ratios. Alternatively, Drug A concentration may be varied by the serial dilutions and Drug B concentration be kept at constant (Table 3). The serial dilution usually used 2-fold, 1.5-fold or 3-fold dilutions in pharmacological studies. Serial dilution greater than 3-fold is not recommended since it may result in data point distribution at

too low or too high concentration in which the experimental data points becomes inaccurately measured (e.g., $<0.1\%$ or $>99.9\%$ of inhibition). The recommended concentration range (or dose-range) and dose density (in IC_{50} or ED_{50} unit) is e.g., $0.125x$, $0.25x$, $0.5x$, $1x$, $2x$, and $4x$ of IC_{50} or ED_{50} so that the data points will be distributed below and above IC_{50} or ED_{50} . Similarly, the dose-effect curve for (A+B) can be constructed when the combination ratio is kept constant (Table 3). For the constant ratio combina-

Table 3. A constant ratio experimental design showing the outlay of two drugs for drug combination analysis. The diagonal design results in marked reduction in data points requirement comparing with checker-board or Latin-Square design and yet yields essential bio-informatics via computer simulation [5,6]

		Drug 1					
		0	0.25X (ED ₅₀) ₁	0.5X (ED ₅₀) ₁	(ED ₅₀) ₁	2X (ED ₅₀) ₁	4X (ED ₅₀) ₁
Drug 2	Control	(f _a) ₀	(f _a) ₁	(f _a) ₁	(f _a) ₁	(f _a) ₁	(f _a) ₁
	0	(f _a) ₀	(f _a) ₁	(f _a) ₁	(f _a) ₁	(f _a) ₁	(f _a) ₁
	0.25X (ED ₅₀) ₂	(f _a) ₂	(f _a) _{1,2}				
	0.5X (ED ₅₀) ₂	(f _a) ₂		(f _a) _{1,2}			
	(ED ₅₀) ₂	(f _a) ₂			(f _a) _{1,2}		
	2X (ED ₅₀) ₂	(f _a) ₂				(f _a) _{1,2}	
	4X (ED ₅₀) ₂	(f _a) ₂					(f _a) _{1,2}

tion, it is recommended to use the D_m ratio such as the IC₅₀ ratio [(IC₅₀)_A/ (IC₅₀)_B] or the ED₅₀ ratio [(ED₅₀)_A/ (ED₅₀)_B]. A typical two-drug combination experimental design for automated computer simulation is illustrated in **Table 3**.

For three drug combinations, A+B+C, the pre-requirements are still the dose-effect curve for each drug alone, and then the dose-effect curve of (A+B+C). With these dose-effect parameters, (m)₁, (D_m)₁, (r)₁; (m)₂, (D_m)₂, and (r)₂; (m)₃, (D_m)₃, (r)₃ and (m)_{1,2,3}, (D_m)_{1,2,3}, and (r)_{1,2,3}, the CI values can be calculated and the F_a-CI plot can be constructed automatically by computer. Since (A+B+C) net conclusion is the results of A+B, B+C and A+C, it is recommended that each of these sub-combinations be carried out at the same time, in the same experiment. Thus, the net conclusion of synergism or antagonism can be dissected to improve the understanding [6,24,26]. The explicit example of three drug combination has been illustrated in **Table 2** of Reference 24.

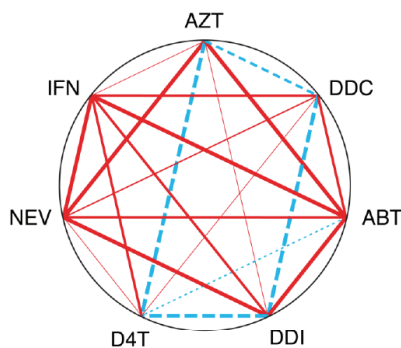
For drug combinations involving more than three drugs, it can be analyzed similar to three drug combinations described above. However, a

new graphics called “polygonogram” has been introduced by Chou and Chou [28] as illustrated in **Figure 10**. The polygonogram provides simple visualization of the complex combinations involving three or more drugs. It also provides semi-quantitative project of the outcome of higher number of drugs from lower number of drugs even before experiments have been carried out (e.g., projection of three or more drug combinations from a series of two-drug combinations as illustrated in **Figure 10**). This approach will be useful for developing anti-HIV cocktails, drug combination regimens, and the traditional Chinese herbal medicine [5,6].

II.3. The Quantitative Diagnostic Plots and Bio-Informatics

This econo-green approach is also vividly illustrated in drug combination studies. For the constant combination ratio for two drugs with a diagonal design allows using only 15 data points (5 points for each drug and their combination including the uninhibited control) (**Table 3**), the fully automated computer analysis can be carried out with the CompuSyn software for synergism, additive, and antagonism quantifica-

a. Two Drug Combinations for Seven Anti-HIV Agents and the Heptagonal Polygonograms



Synergism
 Moderate Synergism
 Slight Synergism
 Nearly Additive Effect
 Antagonism
 Moderate Antagonism
 Slight Antagonism

$$\sum_{r=2}^7 C_r^7 = 120$$

b. Polygonograms for Five Anticancer Agents with Different Mechanisms of Action

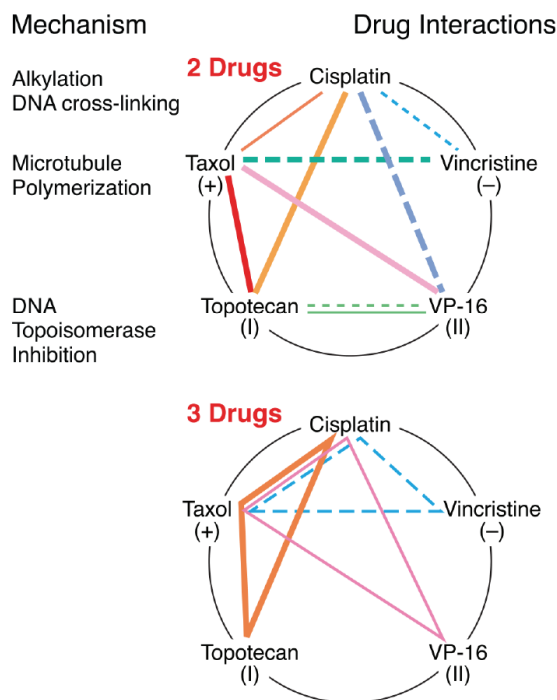


Figure 10. Polygonogram for three or more drug combinations. It provides simple visual inspection for complex combinations with massive amount of data set, and also allows the projection of the outcomes of high component ($n \geq 3$) combinations from low component (e.g, $n=2$) combinations. a. Polygonogram of seven anti-HIV agents with similar or different mechanisms of actions. b. Polygonogram of five anticancer agents with different mechanisms of actions. Solid line indicates synergism, broken line indicates antagonism, heavy line indicates strong interaction, and the thin line indicates weak interaction. Usually, the mechanisms cannot predict synergy. Synergism needs to be quantitatively determined, not to be predicted.

tion (see Ref. 6, Tables 5, 8, 9, 19 and other examples in its Appendices).






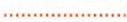





The diagnostic plot and bio-informatics for the dose-effect relationship of a single drug is exhibited by the median-effect plot, $x = \log(D)$ and $y = \log[(f_a)/(1-f_a)]$, the Chou plot [8] as indicated in **Figure 4**. This plot yields the mass-action parameters, D_m for potency, m for dynamic shape, as well as the conformity of the dose-effect data to the mass-action law which is manifested by the r value. The r values are the linear correlation coefficient of the median-effect plot with $r=1.00$ indicates a perfect fit. Following the entry of dose-effect data into a computer, the computer software [16-18] will calculate all the above parameter automatically and instantly, along with automatic generation of dose-effect curve and the median-effect plot.

Interphase of the median-effect equation and the combination index equation create the algorithm (**Figure 8**) for simulation of F_a -CI plot (Chou-Talalay plot) [13,14] and the F_a -DRI plot (Chou-Martin plot) [18], the construction of classic isobologram for constant ratio combination, and the normalized isobologram for non-constant ratio combination (Chou-Chou plot) [16], as indicated in **Figure 9**.

In the F_a -CI plot, the combination index, $CI < 1$, $= 1$, and > 1 indicate synergism, additive effect and antagonism, respectively [6,13,14].

In the F_a -DRI plot (**Figure 9**), the dose-reduction index $DRI > 1$, $= 1$ and < 1 indicate favorable dose reduction for each drug, neutral, and negative dose-reduction, respectively [18]. In practical sense, dose-reduction leads to toxicity reduction

Table 4. How much synergism is synergy? Description and symbols of synergism or antagonism in drug combination studies analyzed with the combination index method

Range of combination index	Description	Graded symbols	Graphic symbols
<0.1	very strong synergism	+++++	
0.1-0.3	strong synergism	++++	
0.3-0.7	synergism	+++	
0.7-0.85	moderate synergism	++	
0.85-0.90	slight synergism	+	
0.90-1.10	nearly additive	±	
1.10-1.20	slight antagonism	—	
1.20-1.45	moderate antagonism	--	
1.45-3.3	antagonism	---	
3.3-10	strong antagonism	----	
>10	very strong antagonism	-----	

The combination index method is based on those described by Chou and Talalay [14] and the computer software of Chou and Martin [18]. The ranges of CI and the symbols are refined from those described earlier by Chou (1991). CI<1, =1, and >1 indicate synergism, additive effect, and antagonism, respectively (modified from Chou and Martin, [18]).

and yet the effect remains the same. Alternatively, under favorable dose-reduction, dose can be somewhat increased to increase efficacy without increasing the toxicity.

The term “isobologram” has been around for over a century but without theoretical derivations [6]. At old time, we need to use graph paper to draw equo-effective curve for two drugs through extrapolation and interpolation manually. Since Chou and Talalay derived the multiple drug-effect equation [13,14], the isobologram, whether for constant-ratio or for non-constant ratio drug combination, for classic and for the normalized isobolograms, respectively, they can be constructed by a click of a computer key [16-18]. A typical examples of computerized analysis printouts are demonstrated in the Appendices of Refs. 6 and 25.

It should be noted that F_a -CI plot and isobologram are two sides of the same coin since both are based on the same multiple drug-effect equation derived by Chou and Talalay [6,14,15]. The F_a -CI plot is effect-oriented and isobologram is dose-oriented. Therefore, both will yield identical quantitative conclusion for synergism or an-

tagonism.

In practice, the F_a -CI plot give the CI values at all effect levels, whereas, isobologram usually give synergism/antagonism bio-informatics at three effect levels since beyond that, the graphics tend to be too crowded to read [6].

CI values provide quantitative designation of synergism (CI<1), additive effect (CI=1), and antagonism (CI>1). For semi-quantitative expression descriptively or visually (symbols, grades, and colors) are given in **Table 4**.

For frequently asked questions, pitfalls or major conceptual errors of drug combination studies have been given in Ref. 26. Some pitfalls and faulty assumptions in drug combinations are summarized in **Table 5**.

With algorithms in hand, the computer software, e.g., CompuSyn [18], can automatically generate instantly the dose-effect curves, the median-effect plot, the F_a -CI plot, the F_a -DRI plot, the F_a -log CI plot, the F_a -log DRI plot, the isobologram, and the polygonogram upon entry of experimental dose and effect data [6]. It is of interest to

Table 5. Examples of frequently asked questions for two-drug combination studies: comments according to Chou, TC*

Issue	Comments
Nature of the issue for synergy determination	Based on the physico-chemical principle of the mass-action law, not based on statistics. Quantitatively determined by the CI value ($CI < 1$); not by the p value.
Multiple doses of each drug alone	A pre-requisite (Required for D_m and m values as well as the CI value)
Multiple doses of combination	Optional (minimum: one dose)
Constant ratio combination	Recommended (but not always required); Using cost-effective diagonal design
Dose range and Dose Density	In vitro usually 5-6 steps of 2-fold serial dilution with median-dose approximately in the middle; The mixture is diluted similarly to form a diagonal scheme
Non-constant ratio combination	Optimal; depend on need for special purposes
Prediction of synergism from knowledge of mechanisms	Rarely predictable. Even if predictable, it is not quantitative Synergism is to be determined, not to be predicted
Theoretical minimum of data points for drawing a dose-effect curve from single drug or their combination	Two data points required when using the median-effect equation and plot (3 rd point is dose zero, 4 th point is the median, the universal reference point)
Are in vitro, in animal and in clinics share the same definition?	Yes, $CI < 1$, $= 1$, and > 1 indicates synergism, additive effect and antagonism, respectively
Can the CI theorem extend to more than two drug combinations?	Yes (but with increased complexity, time and cost)
Can I use all experimental data points for computer simulation?	Not always; use data points only if reasonably accurately determined. Usually do not use data points with $f_a > 0.99$ or $f_a < 0.02$. Inspect before data entries
What are the differences between synergism and potentiation?	Synergism is mutual; both drugs have effects (needs CI value determination) Potentiation is one-sided; one drug has no effect at all (CI calculation cannot be done; present result with percent or fold of enhancement)
Synergy determination in vitro, in animal, and in clinics	Use the same quantitative CI method, but with different costs, time and variability. In vivo may need to use surrogate markers and fractional doses.
Is knowledge of mechanisms required for synergism or antagonism determination?	No, since it depends only on the mass-action law
Time required to determine synergy <i>in vitro</i>	About 1-2 weeks. The econo-green bio-research.
Time required to find out how and why synergy occurs	Usually multiple months or years. The conclusion may be suggestive, speculative (maybe, might be, imply, etc)
Do F_a -CI plot and isobologram provide the same synergism or antagonism determination?	Yes, they are based on the multiple drug effect equation of Chou-Talalay [11,12]. F_a -CI plot is effect-oriented and isobol is dose-oriented. They are two sides of the same coin
What is the major advantage of the F_a -CI plot over isobologram?	F_a -CI plot shows CI at all-effect levels, whereas isobol usually shows synergism or antagonism at three effect levels (e.g., ED_{50} , ED_{75} , and ED_{90}). Beyond three effect levels, the isobols graphic is too crowded or too messy to be read
How to deal with the biphasic dose-effect curve?	Activation and inhibition cannot be analyzed simultaneously, i.e., should be dealt separately, each use only one end-point of measurement.
Can the CI method be used for the combination of activators?	The CI method is developed for inhibitors, i.e., fractional affected (f_a) = $1 - T/C$, $f_u = T/C$ and thus for the median-effect plot $\log(f_a/f_u)$ vs. $\log(D)$ in which $f_a/f_u = (C-T)/T$; For activators, $f_a = C/T$ and $f_u = (T-C)/C$. Alternatively, f_a is the fraction of V_{max} , where V_{max} need to be estimated.

* For more details, see Refs. 5, 6, 14, 15, 18, 24-27.

Table 6. Comparison of two-drug combinations for anti-cancer agents [using econo-green small size experimentation]

	In Vitro	In Animal	In Clinic
Time & Effort	2 weeks	2 months	6 months~1 year
Non-wage Cost	\$200 [cells and chemicals]	\$3,000 [nude mice]	Expensive Trials [Vary]
Sample Size	> 2 x 10 ⁶ [cells]	> 65* [nude mice] [Chou-Talalay method]	> 36§ [vary]
Quantitative "Synergy" Determination	Very Easy [But frequently not done properly in the past]	Not Difficult* [Rarely properly done in the past]	Difficult§ [Use Surrogate Markers and Fractional Doses]

* See Section II.4 and Refs. 29 and 30

§ See Section IV.3 and Refs. 37 and 38

note that quantitative determination of synergism or antagonism of two drugs in vitro with computer simulation will take 1-2 weeks. However, to study the mechanisms of how and why synergism or antagonism occur can easily take several years with the conclusions mainly speculative (Table 5). It can be concluded again that the mass-action law based algorithm and simulation method lead to econo-green approach for cost-effective bio-informatics. The examples of two-drug combination in vitro, in animal, and in clinical trials for small size experimentation are given in Table 6.

II.4. The Case Study for Drug Combinations in Animals and in Humans

The CI method using a small number of data points has also been applied to the *in vivo* studies using the nude mice bearing the xenograft tumors [29,30].

As an illustration for the mass-action law based CI method, the author's laboratory had used only 65 nude mice bearing human mammary carcinoma MX-1 xenografts for the Taxotere, Fludelumone, and their constant ratio (1:5) combinations (each 4 doses and 5 mice at each doses, plus 5 mice for un-treated control) [29,30]. The tumor size and the bodyweight losses were monitored every two days. By computer simulation of experimental data, it is possible to quantitatively determine synergism of antitumor effect and antagonism on bodyweight loss at a given data of measurements. It is also

possible to determine the dose-reduction index (DRI) which means how many fold dose-reduction is allowed as a result of synergism. In addition, isobologram can also be automatically constructed to indicate synergism, additive effect or antagonism. The isobologram can be constructed within one second after the data entries [18].

The CI method is rigorous and quantitative and requires far smaller number of data points (e.g., Table 5) than any other available method known [5]. In fact, this combination index method has been widely applied in biomedical sciences as indicated by over 5,000 citations internationally (www.researcherid.com/rid/B-4111-2009).

III. The Preclinical Studies

III.1. Competitive Nature of Drug Discovery and Drug Development

Basic research is credited for the scientific knowledge; drug discovery is credited for novel idea to have the practical, specific application usually with the right of the intellectual property. In this author's opinion [4], the keys for successful drug discovery are: i) Keep it simple. Too much knowledge is not necessarily helpful since it may hinder the innovation and free spirit. In this electronic information era, the database has overwhelming amount of information but it is humans that makes discoveries, not the machines. The high throughput technology in conjunction with robotics is capable of screening

millions of samples in a short time, but this technology has contributed very little in drug discovery during the past 20 years; ii) Be creative. The best practice is to try to avoid the me-too type of research. The follower may sometimes pull ahead but the chance is very slim, especially in the crowded field; iii) Collaboration. Drug discovery is teamwork. In my opinion, the best team should include the chemists and the pharmacologists; iv) Be practical. Closely observe and always think about practical applications; v) Luck. Louis Pasteur said, "Chance favors the prepared mind". This is an insightful and thoughtful statement. Drug discovery can be a flash of idea or serendipity, for examples, the discovery of insulin or penicillin that are the major milestones in the history of medical sciences.

In basic research, the elucidation of mechanism of action of a drug may take years and yet the conclusions obtained are frequently suggestive, implicative or speculative, since "mechanism" has different expressions, e.g., at chemical, molecular, cellular, and animal levels. In drug discovery, it usually takes six months to one year from synthetic compounds to the initial patent filing, following the entity identification and the reproducible pharmacological evaluations for potential usefulness (**Figure 6**). As indicated above, the mechanism is good to know but it is not necessarily essential for drug discovery. For example, aspirin has been used for over a century, but we still do not understand many of its mechanism of actions [2]. The mechanism, *per se*, cannot be patented since it is a natural process. To be competitive, the drug discovery program should be efficient, practical, focused and mission oriented which is distinct from basic research where the main goal is knowledge building. In many countries, one day later in filing the patent than the others will fail in competition, i.e., loss of intellectual right priority. It is also important to note that the published or disclosed results are in the public domain which cannot be patented. In addition, the international patents are complicated that usually need professional legal counseling due to differences in international and domestic laws.

III.2. The Optimal Conditions for the Best Therapeutics

The pharmacodynamic (PD) studies without sticking to the optimal conditions for dose, schedule, route of administration and duration

of treatment, are practically useless. Thus, any attempt to see therapeutic effect or toxicity at non-optimal conditions is just like a fishing expedition. By chance, one may observe quite good therapeutic effect but there is no indication whether it represents the best result. Thus, neglecting the optimal conditions is a futile practice that waste both time and resources.

Usually before formal PD studies, it is imperative to carry out exploratory "homework". This kind of homework is unlikely carried out in contract laboratories since all these added to the cost and time. Using the fixed protocol for drugs testing with different pharmacological properties, such as doses, schedule, route, and duration of administration can easily compromise the usefulness of the data. A typical example of this using iso-oxazole-fludelone, is illustrated in section III.3.

As a general rule, the best model in vivo is the animals. Statistical, pharmacokinetic formula or the arbitrary assigned "models" have no explicit physico-chemical bearings.

III.3. The Importance of Optimal Conditions for Drug Discovery- A Case Study

A series of synthetic 16-member ring macrolides as anticancer epothilone analogs that stabilize microtubule polymerization, like Taxol, have been studied extensively in the author's laboratory (**Figure 11**). The selected compounds [31-35] have been shown to achieve the "therapeutic cure" against numerous human tumor xenografts in nude mice. The term "cure" has been defined by greater than ten logs of cancer cell kill or complete tumor remission without any relapse for over 10% of animal's life span [34,35]. These 16-member ring macrolides represented by the 3rd generation epothilone, iso-oxazole-fludelone (Iso-flu) [35] has the extraordinary pharmacological properties, comparing with Taxol: i) Iso-flu is 20-fold more potent in inhibiting leukemic CCRF-CEM cell growth, and is 1000-fold more potent against Taxol-resistant, CCRF-CEM/Taxol cells; ii) It is highly water soluble that does not require Cremophor formulation used for Taxol, that induced allergic reactions in patients; iii) Iso-flu at optimal conditions (25mg/kg, Q12Dx3, 6hr-i.v. infusion) leads to therapeutic cure (> 10 log cell kill or complete tumor remission without any relapse for 100-200 days) of five different human xenograft tumors of mammary MX-1

Rational Approach for Molecular Design, Total Synthesis and Pharmacologic Evaluation

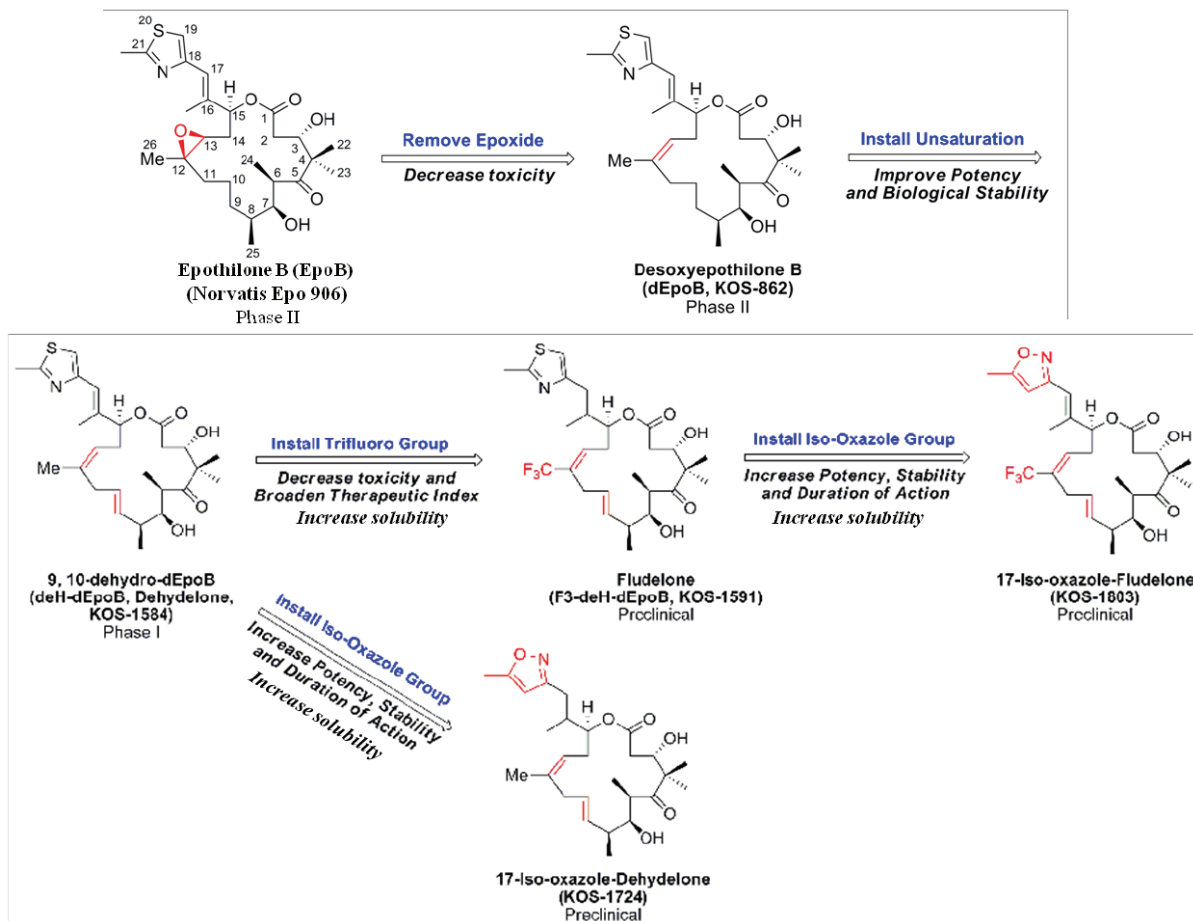


Figure 11. Structures of epothilones and the synthetic analogs. The pharmacology guides the steps lead to the 3rd generation, iso-oxazole-fludelone, as the candidate for clinical development.

(Figure 12), ovarian SK-OV-3, leukemic CCRF-CEM, and Taxol-resistant mammary and leukemic in nude mice, whereas Taxol at its optimal conditions (25mg/kg, Q2Dx6, 6hr-i.v. infusion) leads to inferior therapeutic results [35]; iv) Parts of the data are shown in Figure 12. Iso-flu markedly suppressed refractory xenografts such as lung A549/Taxol, mammary MCF-7/Adriamycin, and neuroblastoma SK-NAS, whereas Taxol has little effects; v) Iso-flu is orally curative against MX-1 whereas Taxol is ineffective. It should be noted that Taxol or the 1st generation epothilone, 12,13-dehydro-epothilone B (dEpoB) are optimally effective for Q2D schedule [31,32], whereas iso-flu requires Q12D or Q14D [35], otherwise too toxic. In addition, while Taxol makes little difference in efficacy whether given i.v. injection or i.v. infusion,

all epothilones requires 6hr i.v. infusion to be maximally effective with the least toxicity. When Iso-flu is given i.v. injection, the toxicity is 6-fold higher than 6hr-i.v. infusion, in terms of dose (Table 7). When the optimal conditions for Taxol and for Iso-flu are interchanged, Taxol has little or no therapeutic effect, and the remarkable “therapeutic cure” exhibited by Iso-flu would be completely missed [35].

Some conclusions from the observations above are the following: i) The mechanism of action (stabilizing microtubule polymerization) of epothilones did not provide any clue as to the usefulness in therapeutic effect since all epothilones tested have the same mechanism; ii) Among epothilones or among microtubule stabilization agents, the optimal conditions for

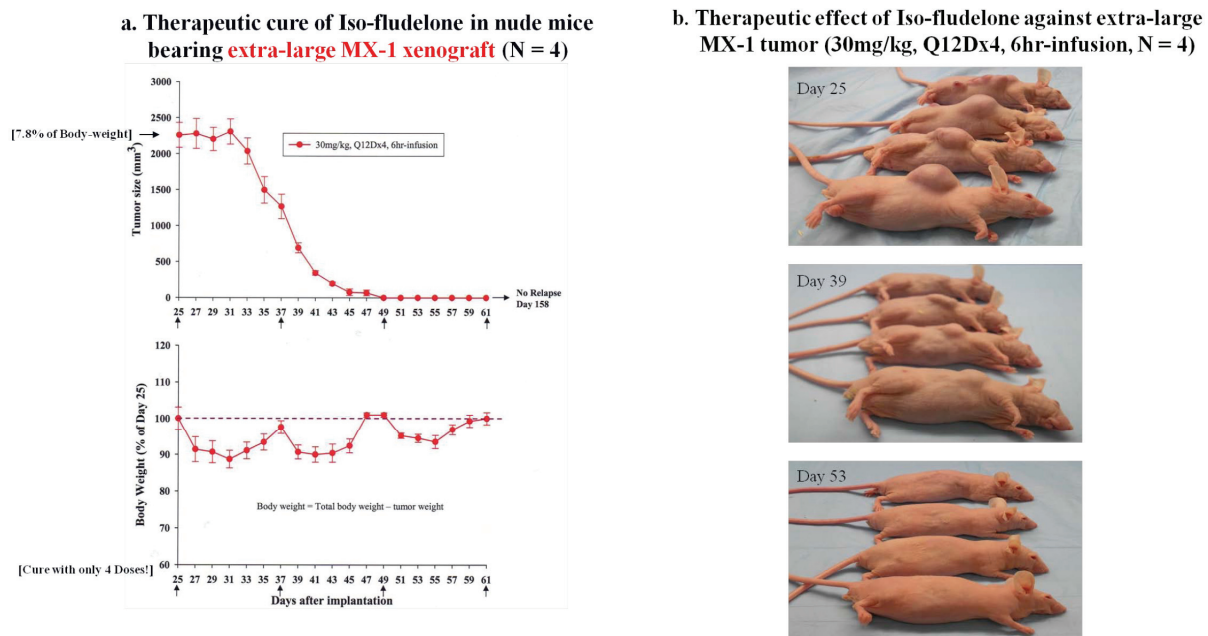


Figure 12. The curative therapeutic effect of iso-oxazole-fludelone against human mammary MX-1 xenograft tumor in nude mice. It took only four doses with 6hr-i.v. infusion to achieve therapeutic cure of extra-large tumor. The photos were taken on Days 25, 39, and 53. (Data reproduced from Ref. 35 with permission).

Table 7. Comparison of “lethal single dose” for i.v. bolus injection vs. 6hr-i.v. infusion*

Drugs	Schedule	Lethal dose (mg/kg)		Lethal dose ratio for
		i.v. bolus inj	6hr-i.v. infusion	6hr-i.v. infusion/i.v. bolus injection [§]
dEpoB	Single dose	60	150*	2.5 fold
Fludelone	Single dose	30	100*	3.3 fold
Iso-Fludelone	Single dose	15	90	6.0 fold
deH-dEpoB	Single dose	30	45	1.5 fold
Iso-deH-dEpoB	Single dose	10	40	4.0 fold
Taxol	Single dose	100	100	1.0 fold

[§] For single dose, i.v. bolus injections for Epos are 1.5-6 fold more toxic than 6hr-i.v. infusion.

* Therefore, infusion should be used. This rule is not applicable to Taxol, although they all have the same mechanisms of microtubule stabilization.

high efficacy and low toxicity are drastically different, e.g., Q2D vs. Q12D for Taxol and iso-flu respectively (**Table 8**); iii) Despite Taxol and Iso-flu share the same mechanism, Taxol can use either i.v. injection or i.v. infusion for the best

therapy whereas long i.v. infusion (e.g., 6hrs) is imperatively required for Iso-flu; vi) The 6hr-i.v. infusion via the tiny tail vein of nude mice, repeatedly, up to 20 mice at a time is highly technical and demanding. In this author’s labora-

Table 8. Dose and schedule on the therapeutic effects of epothilones against MX-1 mammary carcinoma xenograft* in nude mice (6hr-infusion)

Drugs	Dose (mg/kg)	Schedule	Therapeutic Effect		
			Remission	Relapse	Cure*
dEpoB	30	Q2Dx8	4/4	ND	
Fludalone	20	Q2Dx7	4/4	0/4	4/4
Iso-Fludalone	25	Q12Dx3	4/4	0/4	4/4
deH-dEpoB	17	Q6Dx3	3/3	1/3	2/3
Iso-deH-dEpoB	10	Q12Dx2	3/3	0/3	3/3
Taxol	20	Q2Dx7	4/4	ND	

* Therapeutic cure is defined by tumor shrinkage, complete remission and without tumor remission for over 10% of life span (over 100 days for nude mice) or killing tumor cells to below ten billionth remaining based on the log-cell-kill equation of Chou et al [34,35].

Table 9. Determinants for preclinical therapeutic success or failure of epothilones in terms of route and schedule

Drugs	Schedule & interval			Route		
	Q2D	Q6D	Q12D	IV Injection	6hr-infusion	Oral
dEpoB	Yes	Yes [#]	ND	No ^{**}	Yes	No
Fludalone	Yes	Yes [#]	ND	No ^{**}	Yes	Yes
Iso-Fludalone	No [*]	Yes	Yes	No ^{**}	Yes	Yes
deH-dEpoB	No [*]	Yes	Yes	No ^{**}	Yes	Yes
Iso-deH-dEpoB	No [*]	No [*]	Yes	No ^{**}	Yes	No
Taxol	Yes	Yes [#]	ND	Yes	Yes	No

Yes: Choice for therapy

No: Should not be used for therapy

ND: Not determined

* Short schedule intervals lead to neurotoxicity or death

[#] Only at increased doses

^{**} I.V. injection leads to neurotoxicity or death

tory, the custom made mini catheters, infusion device, and restrainer to accomplish the long infusion task [31-35]. Routinely, infusions were carried out 3PM-10PM due to animal room maintenance and early afternoon crowdedness, and the schedule is regardless of the weekends. Thus, in most other laboratories or institutions

(including contract providers) which routinely use i.v. injection protocol and 9AM-5PM working hours would likely abandon the most remarkable compound, Iso-flu, for being too toxic (due to i.v. injections). In addition, a lot of explorations are required for finalizing the schedule of Iso-flu to be Q12D or Q14D (Table 9). Further

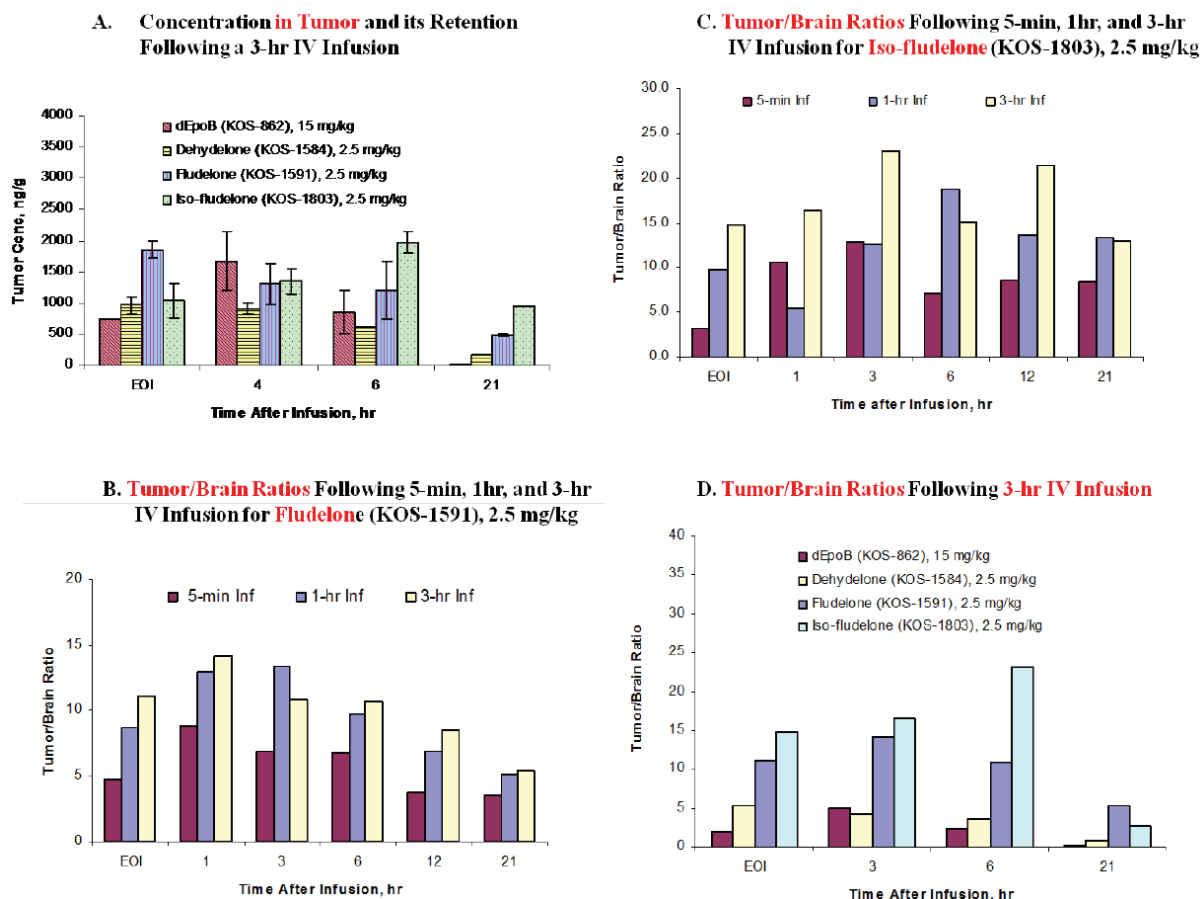


Figure 13. Tissue distribution and retention for iso-oxazole-fludelone in nude mice bearing mammary MX-1 xenograft tumor. The drug combinations in tumor and in brain were determined after 5 min to 3hr i.v. infusion. The results were obtained up to 21hrs. These results indicate that blood/plasma pharmacokinetics has little or no relevance to the actual theory. (Data reproduced from Ref. 35 with permission).

studies also indicated remarkable results: Iso-flu is easily penetrated into tumor tissues and stay there for long period of time. For example, after low dose of Iso-flu (2.5mg/kg, i.v. infusion for 3hrs), the Iso-flu concentration in tumor is several hundred-folds of its IC_{50} in vitro at 21hrs after infusion, and the concentrations of Iso-flu for the tumor/brain ratio is over 10-fold after 21hrs [35] as indicated in **Figure 13**. The case study on Iso-flu illustrated above indicates the unique non-conventional approach for detailed preclinical exploration, extra long i.v. infusion and non-routine experimental schedule, can lead to extraordinary results in the drug discovery process.

As indicated earlier in the section, a computer software [17,18] based on principle of the mass-action law median-effect theory was used in all

data analysis and simulation and usually each dose only used 3-5 nude mice in the xenograft tumor therapeutic experiments [31-35]. The great efficiency and cost-savings is, indeed, proven to be an econo-green bio-research [5].

IV. The Drug Clinical Development

IV.1. Perspective for the Quantitative New Approach

For the invented compounds intended for practical usefulness, very small fraction lead to clinical trials due to the cost, priority and the interested sponsor/developer. About 90% of those entered into clinical trial failed to be developed. There can be many factors attributable for this high risk endeavor: i) Insufficient or lack of properly carried out PD studies for efficacy and

safety; ii) Insufficient interaction between clinicians and the preclinical pharmacologist; iii) Insufficient pharmacological training of the decision maker for candidate compound selection, and iv) Too much requirements of “intermediary steps” that has little relevance to efficacy and safety but has big impact on resources allocation and priority setting. The lesson from this has a very high price tag. Even if passing the regulatory hurdle for commercialization, the consequence and liability of these deficiencies can still make huge damage to patients and the drug company as well. When Vioxx was announced to be withdrawn from the market, the manufacturer’s stock price dropped over 20% in one day fearful of litigations. This loss amount of asset is equivalent to almost half of the NIH annual budget. The regulatory agency tends to have numerous and expensive formal requirements but miss the most important PD studies on efficacy and toxicities that should deserve utmost attention. The consequence of these regulatory practices, research approach and labor cost led to the cost much higher in the Western countries. Taken the high risk and costly nature, some new drug development project is shifting to Eastern countries like China and India, where the development is about one half or even one third of the cost in the West.

IV.2. The Important Roles of Pharmacologists

To improve the success rate in drug discovery and development, the classic pharmacologists should play vital roles, not the basic research scientist nor the marketing director. A useful drug depends on its efficacy and safety. However, there is an increasing shortage of qualified pharmacologist, since the classic pharmacology training program is no longer available in major universities. Many medical schools with pharmacology program no longer provide hands-on experimental pharmacology training. Thus, the worst scenario is that the preclinical pharmacological studies were carried out by those who have not received formal pharmacological training. While pursuing the basic sciences, the fundamental principle of dose-effect dynamics in bio-research are neglected (see below). Furthermore, many trained pharmacologists may have to shift to the more fashionable field such as molecular and cellular biology for career consideration or for acquiring research grant support. The shortage of qualified pharmacologists leads to a vicious cycle. The preclinical PD data for

efficacy and toxicity tend to be insufficient in quantity or quality that cannot convince clinicians, and the clinicians are tied to the fixed approved protocol and with ethical constraints that make exploration of efficacy and safety difficult, especially if the “optimal conditions” (i.e., the proper doses schedule, route, and the duration and frequency of administration, etc.) are not incorporated into the initial trial protocol. The successful drug evaluation and development is usually dependent on human factors.

The widely used pharmacokinetic models are just empirical formula since they are not derivable from the mass-action law (thus, without physico-chemical bearings). The best dose-effect model for preclinical pharmacology is still the animals. Relying on the empirical models and neglect the animal data, or obtain the animal data inappropriately, will lead to objective and relevance issues. It is a general principle and ethics that the exploratory studies should be mainly carried out in animals, not mainly in humans [25].

The essence of preclinical pharmacology is that animal studies are flexible for variety of options for exploration and information gathering. For animal studies, induced toxicities or death by high doses, are acceptable. By no means, the toxicological end-point is allowable in humans. If the non-optimal condition designed protocol is adopted, the results obtained would not only waste the resources and time, but also compromise the well-being of the human subject involved. Therefore, the optimal clinical protocol should be based on preclinical data using animal model. Thus, clinicians and preclinical pharmacologist should have close interactions to design the optimal clinical protocol.

IV.3. The Comparison of Two Clinical Trials

This article proposes the mass-action law based drug combination clinical design and data analysis. Most clinical trials are preoccupied by biological variability. This concern is valid but this should not overlook or neglect the fundamental issues of the basic experimental design and valid data analysis including at the clinical level. In clinical trials field, there has been no shortage of critiques [36]. Here, this author would like to use two similar anti-HIV two-drug combination clinical trials as examples to illustrate the

serious consequences of clinical design and data analysis.

In 1990's, two major and very similar anti-HIV clinical trials carried out in the United States have now been selected as examples for the critical comparison. The trial A for Zidovudin (AZT) plus Lamivudin (3TC), used statistical approach with 366 patients [37], and trial B for Zidovudine (AZT) plus Interferon α (INF) used Chou-Talalay's combination index (CI) method [14] with 36 patients [38]. Both trials A and B used very reliable surrogate markers, such as HIV-RNA, p24 antigen and CD₄⁺ cells, which yielded very accurate measurements.

In trial A, the design used only one dose of AZT and two doses of 3TC and compared the anti-HIV effect among AZT, 3TC, and AZT+3TC and showed that the combined effect was greater than the effects of each drug alone with $p < 0.001$. This conclusion is equivalent to $A+B>A$ or $A+B>B$ which is an axiom that does not need any proof, regardless of the p values. It should be noted that, in trial A [37], single dose of any drug alone cannot determine synergism. By contrast, the trial B design used three doses of for AZT and three doses for IFN and showed that the combination of AZT + IFN was synergistic in efficacy ($CI < 1$) and antagonistic in toxicity toward the host ($CI > 1$) [38].

Since synergy or antagonism determination is the mass-action law issue denoted by $CI < 1$ indicates synergy, $CI = 1$ indicates additive effect, and $CI > 1$ indicates antagonism; synergism or antagonism determination is not a statistical issue as indicated by the p values, thus, it is concluded that trial A did not prove the "synergy claim" for AZT+3TC, whereas the AZT+IFN did, although both combination are obviously more beneficial to the patients than each drug alone. "Synergy claims" in this context, is not only a scientific matter, but also a regulatory and legal intellectual property matter which should not be taken ambiguously.

This is a clear example of the importance of the sound theoretical basis, concise experimental design, and the rational data analysis in clinical trials that are very costly and time consuming. This is also a clear demonstration that the rigorous theoretical approach using the algorithm of the mass-action law with simple computer software can lead to effective, efficient, low cost

green revolution in biomedical research, drug discovery and drug development.

V. Future Directions

Forty years after the launch of the Conquer of Cancer campaign, our hope is more alive than ever. Facing the challenging reality, it is time to critically re-think how to effectively gather informatics, to design efficient experiment and to quantitatively analyze data. We need to learn lessons from the past successes and failures, and most importantly to introduce the new approach for its improvement. This is the frank and transparent way for effectively moving forward to re-think the accustomed medical research where reform is needed.

The current NIH annual budget is about 30 billion dollars for 2011 with furious debate on where and how to cut the expenditure. The main aim of the "econo-green biomedical research" advocated here, on the humanistic dimension, is not cutting research budget or research personnel but rather to increase the efficiency, effectiveness, and competitiveness in medical research, drug discovery and drug development.

The credibility and accountability of cancer research is on the line for critical assessment at the fundamental level. A new approach with the mass-action law based green revolution for bio-research, as discussed in depth in this article, offers an efficient and quantitative bio-informatics and the general direction of applications for bio-medical endeavor.

Furthermore, the drug discovery/drug development in cancer field, and other fields alike is not screening nor the accumulation of massive amount of information for consumption/ digestion, but rather an innovative art and sound judgment. To be efficient, econo-green and sustainable, we need well trained pharmacologists, working closely with innovative chemists and informed clinicians. Science and technology will not make drug discovery or development, but only human does. Nature's law needs to be interpreted logically and logistically in medicine. The aphorism of truth shall prevail for all.

Acknowledgment

I, the author, would like to thank my wife, Dr. Dorothy Chou, for valuable comments and sug-

gestions for this article, and to thank Grace Kang, for the help in preparation of this article. The opinions expressed herewith is entirely my own and in no way represent the institution that I am associated with or the journal that published it.

Declaration of Interest

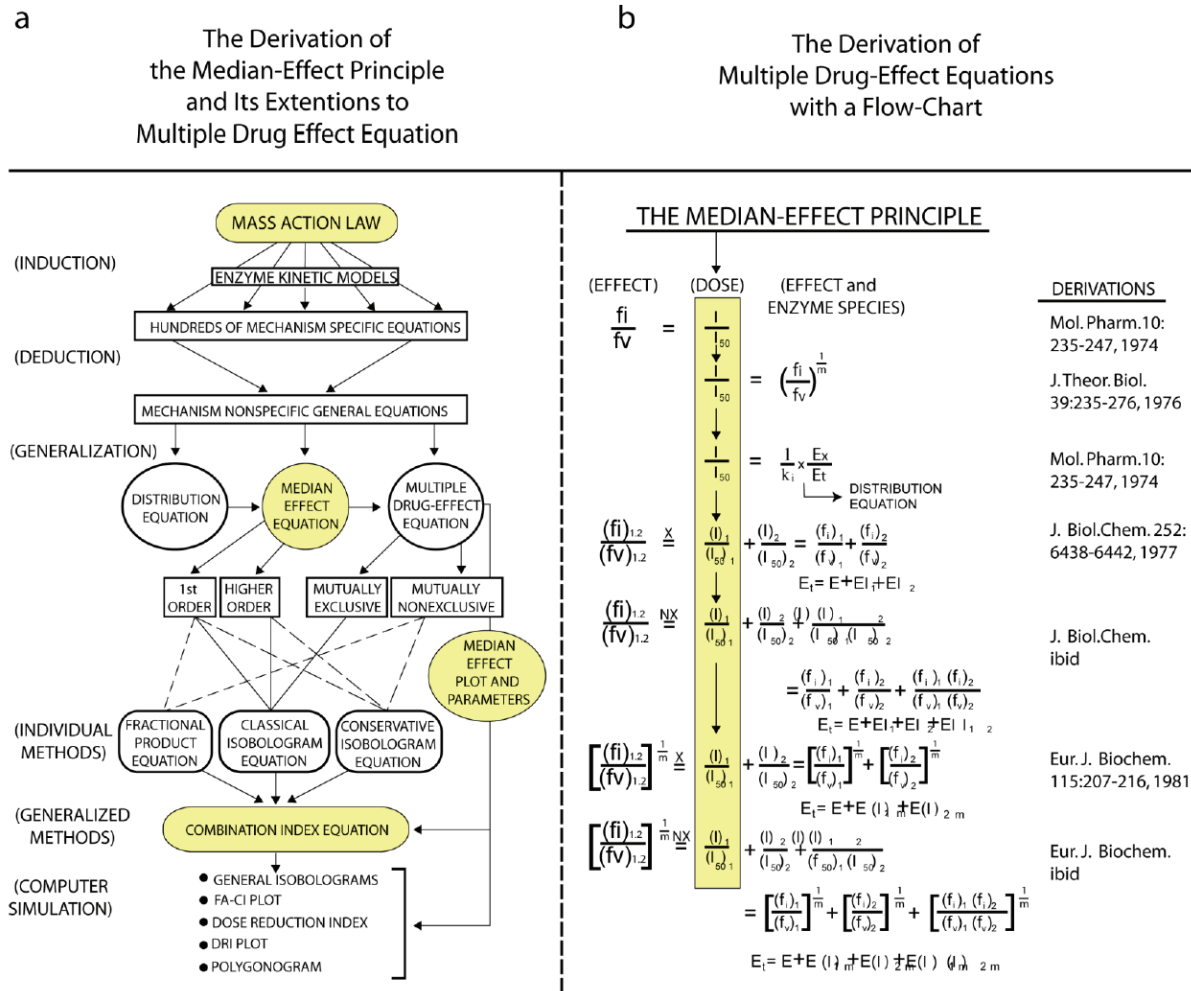
The author is a principal in and holds the copyright to CompuSyn, published by the ComboSyn, Inc., where he serves as a scientific consultant. His son, who contributed to the software's development, is in a position to receive royalties for its publication. T. C. Chou has also received honoraria from seminars and lectures, and consultation fees from universities and pharmaceutical and biotech companies. He has not received a grant support for his three decades of theoretical work and the software, CompuSyn, was developed with his personal funds. The author reports no conflict of interest.

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Appendix Figure 1. Derivation of the median-effect equation as the unified theory for the related theorems. a. Merging the mass-action law with mathematical induction-deduction to derive general equations to create individual method, general methods, and algorithm for the computer software [16-18]. b. The flow chart showing the derivation of the multiple drug effect equation from the single drug-effect equation and the derivation of higher order equation from the first order equation using the median-effect principle as the common link [15]. The pertinent derivations are given in the references on the right column of the figure. In the flow chart: f_i , fractional inhibition; f_v , fractional velocity that is not inhibited; X, mutually exclusive among inhibitors; NX, mutually non-exclusive among inhibitors. For more details, see refs. 6 and 15.