

PubH 7470: STATISTICS FOR TRANSLATIONAL & CLINICAL RESEARCH



COMBINATION THERAPIES : Part II: Measuring Synergistic Effects

BASIC QUESTION

- When both of the agents in a combination are active, that is to produce positive tumor response, frequently we wish to compare the therapeutic result of the combination with the results achieved by the component agents.
- **Is the effect of the combination equivalent to greater than the sum of the individual effects?**

When the addition of one agent apparently increases the effect of the other, so that the effect of a combination appears to be greater than would be expected; the term synergism is used to describe these situations with enhancement of tumor response

- The term antagonism is used when the effect of the combination less lethal than the sum of the individual effects
- Without synergism and antagonism, the two individual effects are additive

Experiments must be done:

- (1) Stage 1: to characterize ability of each drug to kill cancer cells or to shrink tumors; before**
- (2) Stage 2: to see if the drug combination is more lethal than the sum of individual effects; Both are often in the form of early, pre-clinical experiments**
 - either “In Vitro” or “In Vivo”.**

A STAGE 1 IN VITRO DESIGN

Cells from a tumor-derived cell line are deposited in wells of a cell culture dish in complete growth medium. After phase growth is established (say, 72 hrs in a typical cell line), wells are treated with **different concentrations of a test agent – including a control** (i.e. vehicle) well. Doses are spread over a wide range from very low to very high.

The endpoint is “cell survival” and the aim is to establish “**potency parameters**”.

IN VITRO MODEL

Cells in a well; some die, some survive the treatment – could consider “**Logistic Regression**”.

“d” be one of the doses; $x = \log(d)$

n_0 = # of surviving/viable cells @ control well

n_x = # of surviving/viable cells @ dose “d”;

$p_x = n_x/n_0$ % of surviving cells @ dose “d”.

$$\ln \frac{p_x}{1 - p_x} = \alpha + \beta x + \varepsilon$$

(1) Logistic Model & (2) Drug on log scale

A STAGE 1 IN VIVO DESIGN

A group of **mice with induced tumors**, say $n=50$, 10 mice are selected and sacrificed to measure baseline tumor volumes. The other 40 mice are randomized into **10 groups of 4 mice each treated with 10 different doses** of a test agent; **doses are spread over a wide range from very low to very high.**

The endpoint is “tumor volume” and the aim is to establish “potency parameters”.

IN VIVO MODEL

Endpoint is “tumor volume” – continuous scale

“d” be one of the doses; $x = \log(d)$

v_0 = average tumor volume of control group

v_x = average tumor volume treated with dose “d”;

$p_x = v_x/v_0$; $(1-p_x)$ is % tumor reduction for dose d;

$$\ln \frac{p_x}{1-p_x} = \alpha + \beta x + \varepsilon$$

(1) Logistic Model (but analyzed as Linear Regression) & (2) Drug on log scale?

DETERMINATION OF ED50

$$\ln \frac{p_x}{1 - p_x} = \alpha + \beta x + \varepsilon$$

In both In Vitro and In Vivo experiments, we can fit the above model and obtain estimates of “intercept”, “a”, and “slope”, “b”, by MLE (In Vitro) or least squares (In Vivo). Then log of ED50 is obtained by setting $p = .5$

$$\text{ED50} = \exp(-a/b)$$

Summary/Points to emphasize:

- (1) Logistic Regression Model with drug on log scale; but there could be exceptions – could simply be checked with a “Scatter Diagram”**
- (2) Ideally, experiments in this stage 1 (single drugs) should be done with a larger number of doses so that ED50's are obtained with negligible or minimum errors.**
- (3) Next Question: How to illustrate synergistic or antagonistic effects? And, in the presence of synergism, how to measure its strength?**

THE ISOBOLOGRAM

- Steel & Peckham (International Journal of Radiation Oncology, 1979) use a graphical device called “Isobologram” to evaluate and to illustrate two agents used in combination
- Effect doses (EDs) of two drugs are put on axes
- A straight line joining two points of the same effect level, say from the ED_{50} of drug 1 on the x-axis to the ED_{50} of drug 2 on the y-axis, is called an iso-effect line: e.g. the **50% iso-effect line**.

SYNERGISTIC REGION

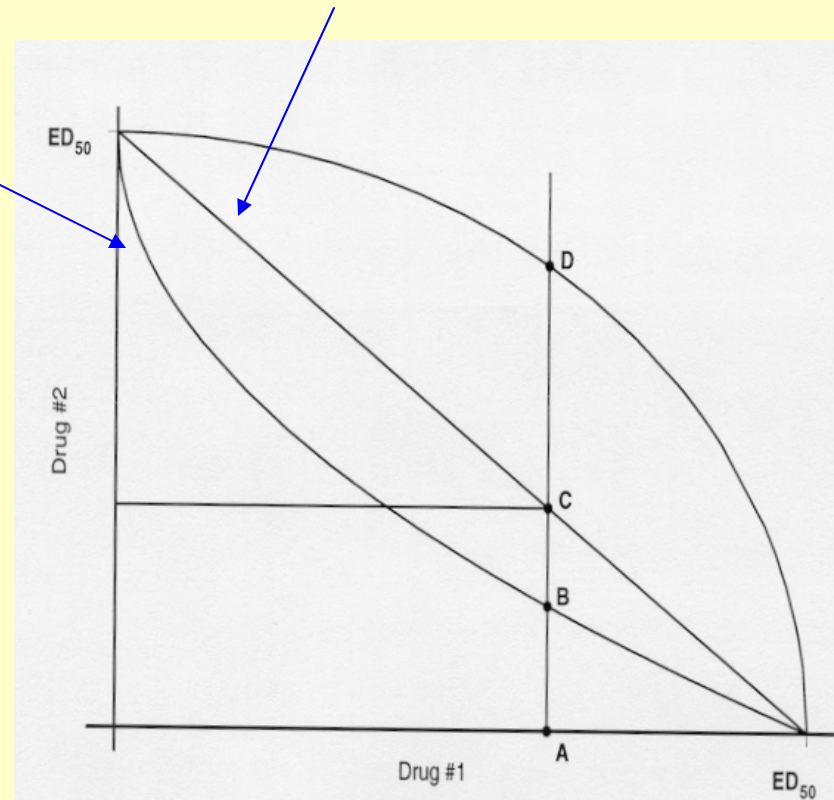
You fix dose D of drug 1 and increase the dose of the other drug so as to reach the same response, say 50%:

synergism

If you need less Drug #2 in the combination (stop below the line, say point “B”), to reach the same effect; that’s synergism.

By varying the chosen level “A”, the endpoint trace a concave curve.

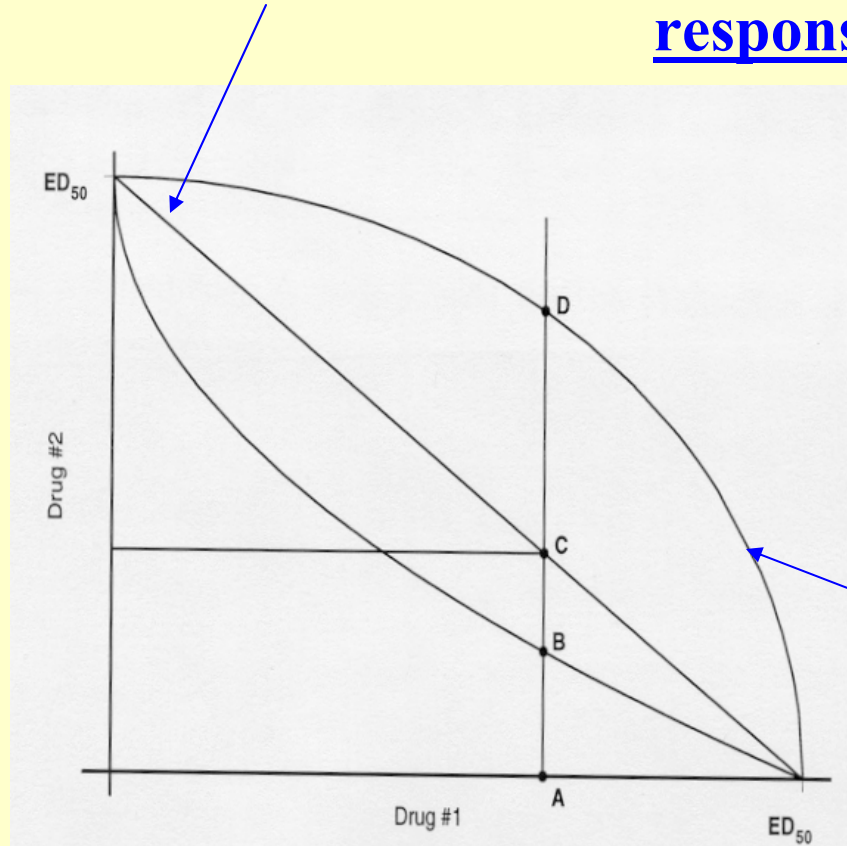
Additive line



ANTAGONISTIC REGION

additive

You fix dose “A” of drug 1 and increase the dose of the other drug so as to reach the same response, say 50%:



If the two drugs in the combination act antagonistically, one needs more drug 2 to reach the same effect and the resulting curve is convex.

antagonism

There are 2 categories of agents:

- (1) For category A, measurements (i.e. the doses) on two axes of the isobologram are on regular scale;**
- (2) For category M, measurements (i.e. the doses) on two axes of the isobologram are on the log scale**

“COMBINATION INDEX”

- Chou & Talalay define a “**combination index**” CI as follows; if $CI < 1$, it's synergism, if $CI > 1$, it's antagonism, and if $CI = 1$, effects are additive (Chou & Talalay, *Advances in Enzyme Regulation*, 1984)

$$CI = \left[\frac{d_1}{ED50_1} \right] + \left[\frac{d_2}{ED50_2} \right]$$

- The authors maintain that it is based on the “**mass-action law**”; it has become “**the standard**” to evaluate combination therapies

The “Combination Index” (CI) has been very popular and very dominant among basic scientists. By early 2010, it has been cited in more than 1900 scientific articles published on over 400 biomedical journals internationally. However, most published results are based on rather small sample sizes, using mostly In Vivo experiments – perhaps, In Vivo trials are expensive and time-consuming (plus the lack of tumor-induced animal models)

The problems are:

- (2) In order to understand and characterize drug interactions, we need to define or model “additive effects” of drug combinations. It is not simple: Chou claimed that it took him “about 10 years to figure out what an additive effect is” (Chou, 2010). Unfortunately, we believe, the issue is still unresolved.**
- (2) Even with some concept of additively, “synergism” is still not a well-defined term. In one review article by Golden and Mantel in 1957, 7 definitions of “synergism” were given and in a more recent review by Greco et al. (1995), 13 different methods for determining synergism were listed and not even two methods agree with each other and a few more might have been added.**

HOW TO PROCEED?

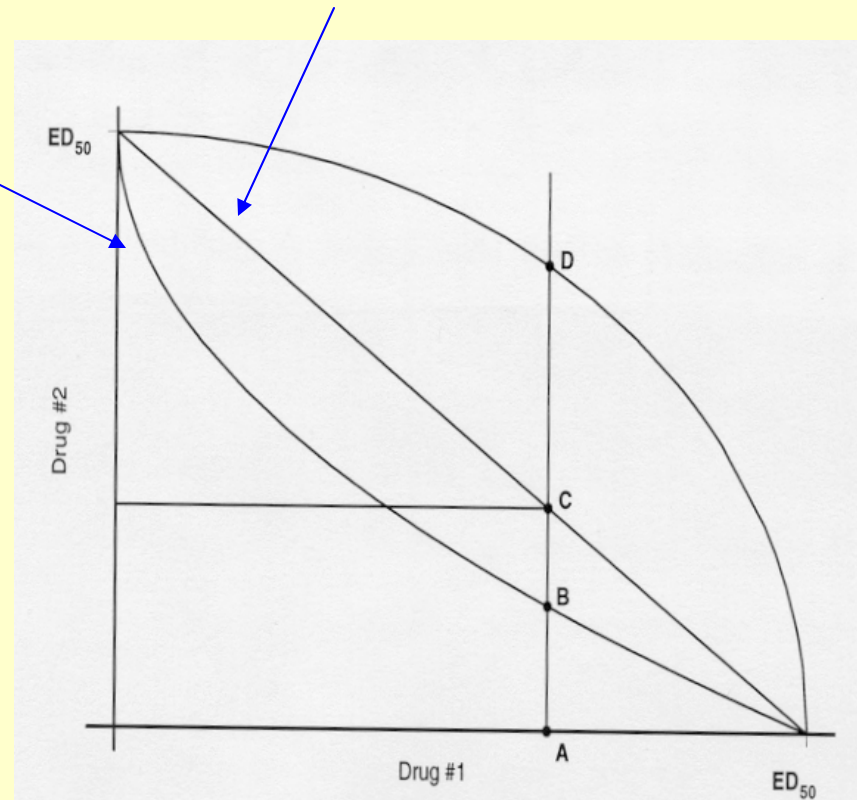
You fix dose “A” of drug 1 and increase the dose of the other drug so as to reach 50% (point B)

Additive line

synergism

Question:

How do we get to point “B” in the diagram? i.e. how to “calculate” the dose of Drug 2 – the distance from “A” to “B”



Issue #1: EXPERIMENT DESIGN

Should it be a real “Trial by Error”: You fix a dose of Drug 1 and increase the dose of the other drug so as to reach a preset response, 50%? & do again if needed ? This is very time-consuming and un-practical.

Experiments are usually done in one of two possible ways:

(1) Non-constant drug ratio, and

(2) Constant drug ratio

Say, we can fix the dose of Drug #1 at “dose = a” and combine with varying doses of drug #2: $b_1, b_2, \dots b_k$; the combined doses are: $\{d_1 = a+b_1, d_2 = a+b_2, \dots, d_k = a+b_k\}$. Given this series of combined doses and the corresponding responses (e.g. percentages of cells killed), we then fit the same “logistic model” and obtain $ED50_c$ of the drug combination. Note that the “ratio” of individual doses in a combined dose, say a/d_i , is not constant across the combined doses.

- (1) After one such experiment with a series of k doses, we have one CI value
- (2) Then you can vary the “ a ”, the fixed dose of Drug #1, to create another series, or
- (3) One can systematically preset a “grid” with different doses of both drugs
- (4) Fitting the model to each row and each column to obtain an CI value;
- (5) Still, in each series – row or column – the ratio of drug doses is not constant across combined doses.

	Dose of Drug 1						
Dose of Drug 2	0	6	12.5	25	50	100	
0	100.00	90.30	77.99	56.72	11.19	4.66	
3	98.51	84.33	68.28	50.19	8.96	4.66	
6	71.83	55.97	44.40	25.93	9.14	2.43	
12.5	19.40	19.98	16.23	12.31	5.60	1.68	
25	4.48	8.58	7.65	6.16	7.65	0.00	
50	2.24	6.16	7.09	3.92	3.54	0.00	

Entries are proportions of surviving cells.

This experiment was kind of poorly designed, i.e. over-dosed; in many configurations, doses of drugs #1 and/or #2 might exceed its/their ED50.

	Dose of Drug 1						
Dose of Drug 2	0	6	12.5	25	50	100	ED50
0	100.0	90.30	77.99	56.72	11.19	4.66	21.68
3	98.51	84.33	68.28	50.19	8.96	4.66	
6	71.83	55.97	44.40	25.93	9.14	2.43	
12.5	19.40	19.98	16.23				
25	4.48	8.58	7.65				
50	2.24	6.16	7.09				
ED50	9.85						

Investigators should try first with first row (Drug 1 alone) and first column (Drug 2 alone) – **ideally with many more doses** - before deciding the dose of each to “fix”. As it turns out several combinations are not eligible (because the dose of #1 or/and the dose of #2 already exceeds its own ED50 (contents are now deleted)).

In establishing the two ED50; we should compare the “fit” of the following models to data of each drug – at least visibly – to decide whether drug doses are on regular or log scale (most/all data we have fit the second model but **the other is a possibility**)

$$\ln \frac{p}{1-p} = \alpha + \beta d$$

$$\ln \frac{p}{1-p} = \alpha + \beta x; \quad x = \log(d)$$

FIRST STEP

	Dose of Drug 1						
Dose of Drug 2	0	6	12.5	25	50	100	ED50
0	100.0	90.30	77.99	56.72	11.19	4.66	21.68
3	98.51	84.33	68.28	50.19	8.96	4.66	
6	71.83	55.97	44.40	25.93	9.14	2.43	
12.5	19.40	19.98	16.23				
25	4.48	8.58	7.65				
50	2.24	6.16	7.09				
ED50	9.85						

- (1) Data on column #2 are what we have when dose of Drug 1 is fixed at $d_1=6$ and varying the dose of Drug 2 (Data on row #2 are what we have when dose of Drug 2 is fixed at $d_2=3$ and varying the dose of Drug 1)
- (2) In fitting the model to each row (or column), “dose” corresponding to each response percentage is the “total” of two drug doses; e.g. $(6+12.5)$ for that 44.4%

NEXT STEPS

	Dose of Drug 1						
Dose of Drug 2	0	6	12.5	25	50	100	ED50
0	100.0	90.30	77.99	56.72	11.19	4.66	21.68
3	98.51	84.33	68.28	50.19	8.96	4.66	
6	71.83	55.97	44.40	25.93	9.14	2.43	
12.5	19.40	19.98	16.23				
25	4.48	8.58	7.65				
50	2.24	6.16	7.09				
ED50	9.85						

(1) Data on column #2 are what we have when dose of Drug 1 is fixed at $d_1=6$ and varying the dose of Drug 2. We fit data of column #2 to the model and obtain $ED50_c$: This is the combined dose; dose of Drug #2 which would added to $d_1 = 6$ of Drug #1 to achieve 50% response is $(ED50_c - 6)$.

(2) This is the “distance from A to B” we are looking.

CALCULATION OF CI

The “amounts” of individual drugs in the combination (needed to get 50% response) are: “6” and “ED_c- 6”; therefore:

$$CI = \frac{6}{ED50_1} + \frac{ED50_c - 6}{ED50_2}$$

(Note that if “k”, the number of combined doses, is larger one would get ED50c, and hence CI, with negligible or small standard error.)

NATURE OF SYNERGISTIC EFFECTS

You fix dose “A” of drug 1 and increase the dose of the other drug so as to reach 50% (point B)

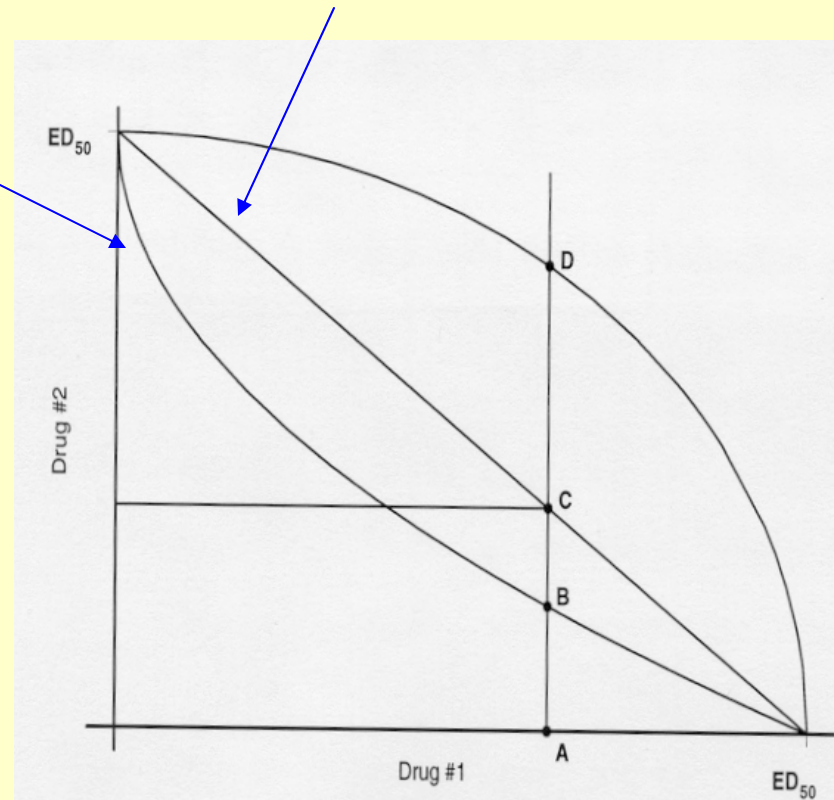
Additive line

A Possibility:

Point “B” may be further away from the “additive line” in the middle part than at the ends:

Synergistic effects may vary with drug ratio

synergism



In designing experiments with non-constant drug ratio, data in each series carry different levels of synergistic effects; they may not fit the model well. Even if data still fit the model; the resulting CI value would have a larger standard error. In a review article (2010), Chou recommended only experiments with constant-ratio drug combinations.

Experiments with constant-ratio drug combinations can be designed as follows: **Drugs are pre-mix at certain ratio**, say p-to-q (p units of Drug #1 to q units of Drug #2) before dispensing into k combined doses from low to high. The ratio in all k doses is the same, p-to-q. The “units” are not conventional dose units (say, milligrams) because different drugs have different strengths which makes it hard to know how to set the ratio. **One can use an unit the ED50 of each Drug.**

For example, one can try 5 series of doses (each will yield one CI value):

**3 ED50s of Drug #1 to 1 ED50 of Drug #2; and
2 ED50s of Drug #1 to 1 ED50 of Drug #2; and
1 ED50 of Drug #1 to 1 ED50 of Drug #2; and
1 ED50 of Drug #1 to 2 ED50s of Drug #2; and
1 ED50 of Drug #1 to 3 ED50s of Drug #2**

**i.e. The five ratios are 3:1, 2:1, 1:1, 1:2,
and 1:3 in ED50 units.**

CALCULATION OF CI

For each series, after the $ED50_c$ of the combination is obtained; the needed amount of each drug and the CI is calculated as follows:

$$d_1 = \left(\frac{p}{p+q}\right)ED50_c$$

$$d_2 = \left(\frac{q}{p+q}\right)ED50_c$$

$$CI = \frac{d_1}{ED50_1} + \frac{d_2}{ED50_2}$$
$$= \left\{ \frac{p}{(p+q)ED50_1} + \frac{q}{(p+q)ED50_2} \right\} ED50_c$$

Issue #2: PRECISION OF CI

An important statistical question is how to determine the Variance or Standard Error of CI (for experiment)? Chou, Talalay and most basic scientists try to avoid the issue (of sampling variation) by calculating the Index only from data sets which fit the model well; those with very high correlation coefficient (say exceeding .995):

$$\ln \frac{p_x}{1 - p_x} = \alpha + \beta x + \varepsilon$$

In general one can obtained Variance of CI using “Delta Method” (Error Propagation), especially more simple in cases where individual ED50 were obtained from larger samples (so their standard errors can be considered negligible):

$$\begin{aligned} \text{CI} &= \frac{d_1}{\text{ED50}_1} + \frac{d_2}{\text{ED50}_2} \\ &= \left\{ \frac{p}{(p+q)\text{ED50}_1} + \frac{q}{(p+q)\text{ED50}_2} \right\} \text{ED50}_c \\ \text{Var}(\text{CI}) &\cong \left\{ \frac{p}{(p+q)\text{ED50}_1} + \frac{q}{(p+q)\text{ED50}_2} \right\}^2 \text{Var}(\text{ED50}_c) \end{aligned}$$

Issue #3: IS CI AN “INDEX”?

No matter how we do the experiment, the resulting value of CI is dose-dependent; It depends on the “drug dose ratio”. Should we consider such a number an “Index”?

An “Index” is a summarized figure, a statistic representing a system or a phenomenon. CI is a “data point”. If we have a sample of size one (one experiment with a series of doses) then a data point would serve as an index; that was in the early days of combination therapies. If the sample size is greater than one (say, 5 series or more), one must combine all data points to form an “Index”.

A STATISTICAL MODEL

One possible approach is to assume a “**Model for Isoboles**” (Hewlett, 1969; Machado and Robinson, 1994). There are many possibilities, the following fits in very well with Chou and Talalay’s index:

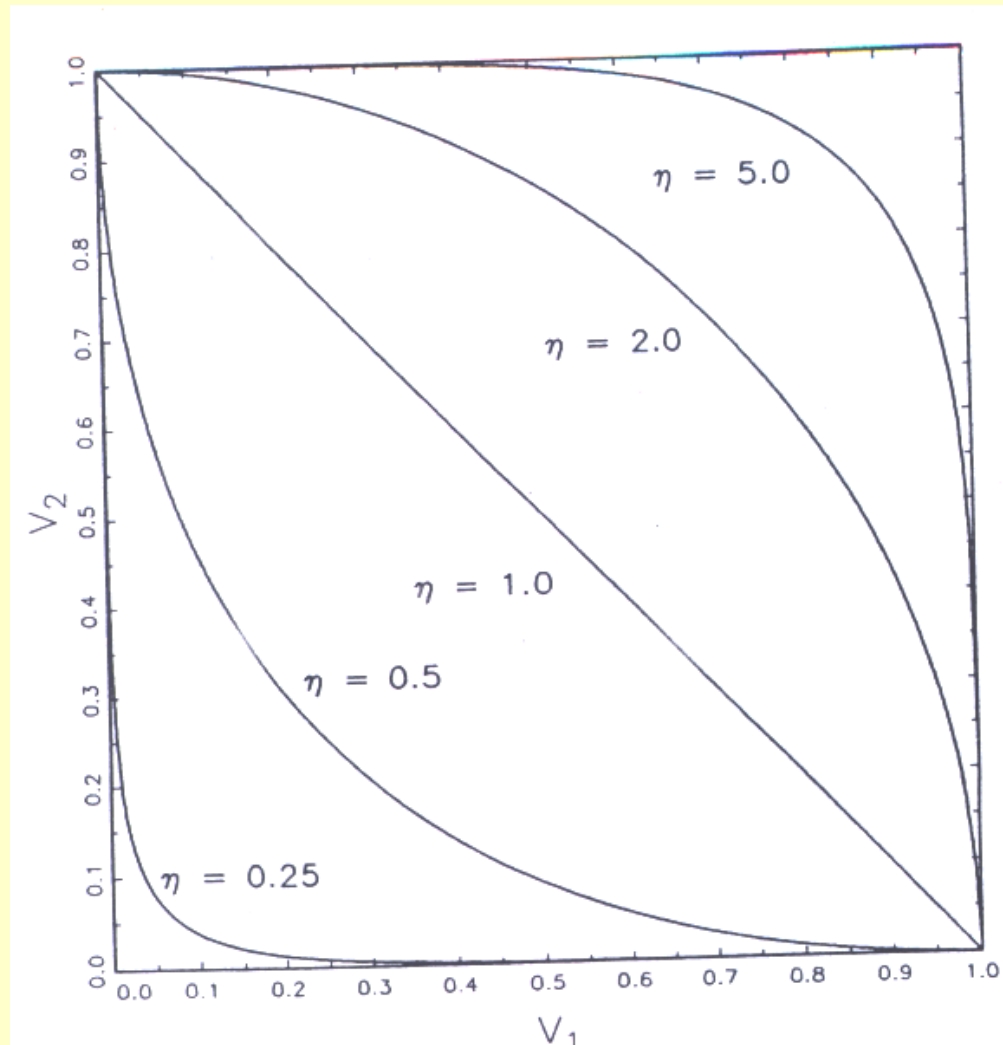
Let consider the "Model for Isoboles":

$$\left(\frac{d_1}{ED50_1} \right)^\eta + \left(\frac{d_2}{ED50_2} \right)^\eta = 1$$

η is the overall "Combination Index":

$\eta < 1$ for Synergism, $\eta > 1$ for Antagonism,

$\eta = 1$ if Effects are Additive



Various values of η

$$CI = \frac{d_1}{ED50_1} + \frac{d_2}{ED50_2}$$

No matter how we design the experiment, each series of doses not only giving us a CI value but a point with coordinates (x,y) = (d1,d2); we can “fit” the “isobole model” by “Least Squares” to obtain a value for the Combination Index, a point estimate of η .

$$\left[\frac{x}{ED50_1} \right]^\eta + \left[\frac{y}{ED50_2} \right]^\eta = 1$$

Issue #4: MEASUREMENT SCALE

- Chou & Talalay define a “combination index” CI as

$$CI = \left[\frac{d_1}{ED50_1} \right] + \left[\frac{d_2}{ED50_2} \right]$$

- The authors maintain that it is based on the “mass-action law”; but acceptance of that “proof” has not been universal.
- The more accepted origin is the **Median Effect Principle** of Pharmacology; and there are even exceptions to this “empirical principle”

MEDIAN EFFECT PRINCIPLE

When a dose d of an agent is applied to a pharmacological system, the fractions f_a and f_u of the system affected and unaffected satisfy the so-called “median effect principle” (Chou, Journal of Theoretical Biology, 1976):

$$\frac{f_a}{f_u} = \left\{ \frac{d}{ED_{50}} \right\}^m$$

If we set “ $p = f_a$ ”, the median effect principle and the logistic regression model are completely identical with a slope $\beta_1 = m$ i.e. Drug Dose is on Log scale in a Linear Logistic Model; If this is the case, shouldn't terms (i.e. drug doses) in the CI formula be expressed on log scale?

Perhaps Chou and Talalay wrongly expressed the “Median Effect Principle” for drug combinations?

One Drug :

$$\frac{f_a}{f_u} = \left\{ \frac{d}{ED_{50}} \right\}^m$$

Multiple Drugs (Chou & Talalay) :

$$\left[\frac{f_a}{f_u} \right]_{A,B}^{\frac{1}{m}} = \left[\frac{f_a}{f_u} \right]_A^{\frac{1}{m}} + \left[\frac{f_a}{f_u} \right]_B^{\frac{1}{m}}$$

Or it should be *multiplicative*?

$$\left[\frac{f_a}{f_u} \right]_{A,B} = \left[\frac{f_a}{f_u} \right]_A \left[\frac{f_a}{f_u} \right]_B$$

Unlike the popular Median Effect Principle for single drugs, Chou and Talalay model for multiple drugs is an additive model. The “Additive Model” has been so dominating and no one has **challenged or improved** the “Combination Index” for 25 years; investigators have adopted this Index to report their findings on almost 2000 peer-reviewed publications. But I believe that **it is an “open issue”**.

For statisticians,

- (1) Is there a problem in our usual logic that “the odds to response” follows a Multiplicative Model?**
- (2) The extension from Simple Regression to Multiple Regression has always been so natural; could this be a case where that extension breaks down?**

As we mentioned earlier, the “additive effect” of drug combination (the result without synergism or antagonism) is not a simple concept and we believe that the issue is still unresolved. According to Lee et al. (2007; Greco et al, 1995), it narrows down to two models:

- (1) **The Loewe Additive Model:** The combination effect is neutral (our new term for additive) if $E(d_1, d_2) = E(d_1) + E(d_2)$; and
- (2) **The Bliss Multiplicative Model:** The combination effect is neutral (our new term for additive) if $E(d_1, d_2) = E(d_1)E(d_2)$,

where $E(d)$ is the effect of d . We proposed to use the term “neutral”; combination effects could be additively neutral or multiplicatively

THE MEASUREMENT SCALE IS MATTER

But it might be matter for Chou and Talalay and for us; their “additivity requirement” ($CI = 1$) is the equation of the 50% iso-effect line when axes are on regular scale . Another possibility is the equation of the same line when both axes are on log scale.

POSSIBILITIES ?

- (1) For agents in category M where the response is linearly related to the log of the dose, the combination effect follows the Bliss Multiplicative Model and we use the term “Multiplicative Synergism” with a Combination Index defined by:

$$\text{CIM} = \left(\frac{\log(d_1)}{\log(\text{ED50}_1)} \right) + \left(\frac{\log(d_2)}{\log(\text{ED50}_2)} \right)$$

- (2) For agents in category A where the response is linearly related to the dose itself, the combination effect follows the Loewe Additive Model and we use the term “Additive Synergism” with a Combination Index:

$$\text{CIA} = \left[\frac{d_1}{\text{ED50}_1} \right] + \left[\frac{d_2}{\text{ED50}_2} \right]$$

Example 1:

For example, $(ED50)_1 = 50$, $(ED50)_2 = 100$, and we used 3.23 units of agent A mixed with 25.12 units of agent B to obtain 50% response ($ED50_c = 3.23 + 25.12 = 28.35$),

$$CIM = \frac{\log(3.23)}{\log(50)} + \frac{\log(25.12)}{\log(100)} = 1.0$$

$$CIA = \frac{3.23}{50} + \frac{25.12}{100} = 0.316$$

It is obvious that the combination under investigation is multiplicatively neutral but additively synergistic. An appropriate conclusion would depend on the nature of the response: (a) if the response is linearly related to the dose, a conclusion of synergistic effects would be justified, but (a) if the response is linearly related to the log of the dose, i.e. the Median Effect Principle applies, then synergistic effects should not be implicated.

Example 2:

For the data in Chou and Talalay's Example 1 (pp. 36-38, 1984), we have $I_{90} = 1511$ for ADP-ribose, $I_{90} = 13.62$ for ADP, and a mixture of 946.6 units of ADP-ribose and 4.998 units of ADP would attain 90% inhibition. These results lead to:

$$CIM = \frac{\log(946.6)}{\log(1511)} + \frac{\log(4.998)}{\log(13.62)} = 1.553$$

$$CIA = \frac{946.6}{1511} + \frac{4.998}{13.62} = 0.996$$

Since the data fit perfectly a linear model with dose on the log scale, a conclusion of antagonism would be more appropriate than neutral effects (Chou & Talalay).

According to Lee et al., the literature indicates that the Loewe additive model works in the setting of mutually exclusive drugs while the Bliss multiplicative model works in the setting of mutually nonexclusive drugs. However, the true mechanism of drug interactions often remains unknown and “there is no generally accepted agreement as to which of the two models is more appropriate”.