Published in final edited form as:

Stat Biopharm Res. 2009 February 1; 1(1): 4–17. doi:10.1198/sbr.2009.0001.

Confidence Intervals of Interaction Index for Assessing Multiple Drug Interaction

J. Jack Lee and

Department of Biostatistics, The University of Texas M. D. Anderson Cancer Center, Houston, TX (jjlee@mdanderson.org).

Maiying Kong

Department of Biostatistics, The University of Texas M. D. Anderson Cancer Center, Houston, TX, and Department of Bioinformatics and Biostatistics, University of Louisville, Louisville, KY (maiying.kong@louisville.edu).

Abstract

Studies of interactions among biologically active agents have become increasingly important in many branches of biomedical research. We consider that the Loewe additivity model is one of the best general reference models for defining drug interactions. Based on the Loewe additivity model, synergy occurs when the interaction index is less than one, and antagonism occurs when interaction index is greater than one. Starting from the Loewe additivity model and the marginal dose–effect curve for each drug involved in a combination, we first present a procedure to estimate the interaction index and its associated confidence interval at a combination dose with observed effects. Following Chou and Talalay's method for assessing drug interaction based on the plot of interaction indices versus effects for combination doses at a fixed ray, we then construct a pointwise $(1-\alpha)\times 100\%$ confidence bound for the curve of interaction indices versus effects. We found that these methods work better on the logarithm transformed scale than on the untransformed scale of the interaction index. We provide simulations and case studies to illustrate the performances of these two procedures, and present their pros and cons. We also provide S-Plus/R code to facilitate the implementation of these two procedures.

Keywords

Antagonism; Loewe additivity model; Synergy

1. Introduction

Studies of interactions among biologically active agents, such as drugs, carcinogens, or environmental pollutants, have become increasingly important in many branches of biomedical research. Our research group reviewed the literature (Lee, Kong, Ayers, and Lotan 2007) and agree with many researchers (e.g., Berenbaum 1985, 1989; Greco, Bravo, and Parsons 1995) that the Loewe additivity model should be considered as the “gold standard” for defining drug interactions

For a combination of k drugs ($k \geq 2$) at $(d_1, \dots, d_k)$, based on the Loewe additivity model, drug interactions at this combination can be characterized as

$$\frac{d_1}{D_{y,1}} + \dots + \frac{d_k}{D_{y,k}} \begin{cases} < 1, & \text{synergy;} \\ = 1, & \text{additivity;} \\ > 1, & \text{antagonism.} \end{cases} \quad (1)$$

Here $d_1, \dots, d_k$ are doses of each drug in the mixture of the k drugs resulting in effect y , and $D_{y,1}, \dots, D_{y,k}$ are the doses of drugs that result in the effect y for each respective drug given

alone. The summation, $\frac{d_1}{D_{y,1}} + \dots + \frac{d_k}{D_{y,k}}$, is called the interaction index, which is denoted as τ . Based on the Loewe additivity model, the combination dose $(d_1, \dots, d_k)$ is said to be synergistic if the interaction index is less than the constant number of 1, and additive or antagonistic if the index is equal to or greater than 1, respectively. To give an intuitive idea about the interaction index, we illustrate its meaning in the special case of $k = 2$. Note that the combination dose (d_1, d_2) produces the same effect y as drug 1 alone at dose level $D_{y,1}$, and drug 2 alone at dose level $D_{y,2}$, which implies that 1 unit of drug 2 will produce the same effect as $\frac{D_{y,1}}{D_{y,2}}$ units of drug

1. Thus, the amount of dose at the combination (d_1, d_2) equals to $d_1 + d_2 \frac{D_{y,1}}{D_{y,2}}$ in terms of drug 1 dose. By definition, $\tau = \frac{d_1}{D_{y,1}} + \frac{d_2}{D_{y,2}}$ which implies that $d_1 + d_2 \frac{D_{y,1}}{D_{y,2}} = \tau D_{y,1}$. $\tau < 1$ implies

$d_1 + d_2 \frac{D_{y,1}}{D_{y,2}} = \tau D_{y,1} < D_{y,1}$, therefore, the amount of the combination dose (d_1, d_2) to produce the same effect y is less than the amount of dose when single drug is applied, hence, indicating synergy. The smaller τ is, the more is the reduction of the amount of dose in the combination,

and the stronger is the synergy. Similarly, $\tau > 1$ implies that $d_1 + d_2 \frac{D_{y,1}}{D_{y,2}} = \tau D_{y,1} > D_{y,1}$, that is, the amount of the combination dose (d_1, d_2) producing the same effect y is more than each single drug dose, hence indicating antagonism. The geometric interpretation of the interaction index can be best shown graphically in Figure 1, panels (A) and (B), where $P = (D_{y,1}, 0)$, $Q = (0, D_{y,2})$, $U = (d_1, d_2)$, all yield the same effect y . If we draw a line $\overline{RS}$ passing through U and parallel to the line $\overline{PQ}$, and draw a line $\overline{OU}$ intercepting with $\overline{PQ}$ at V , then, from the basic

$$\frac{\text{length}(\overline{OU})}{\text{length}(\overline{OV})}$$

geometric properties, the interaction index can be expressed as the ratio of $\text{length}(\overline{OU})$. In Figure 1(A), the closer the point U is toward the origin, the less is the amount of combination dose required to produce the same effect as drug 1 alone at $D_{y,1}$ or drug 2 alone at $D_{y,2}$, hence, the stronger is the synergy. By the same token in Figure 1(B), the further the point U is away from the origin, the larger is the amount of combination dose required to produce the same effect as drug 1 alone at $D_{y,1}$ or drug 2 alone at $D_{y,2}$, hence, the stronger is the antagonism. From Figure 1, we conclude that the interaction index can be used to measure the mode and magnitude of drug interactions.

Given the combination dose $(d_1, \dots, d_k)$ and its effect y , and the marginal dose–effect curves $f_i(D_i)$ for drug i ($i = 1, \dots, k$), the calculation of the interaction index at a combination dose $(d_1, \dots, d_k)$ is straightforward. One simply replaces $D_{y,i}$ by $f_i^{-1}(y)$, where f_i^{-1} is the inverse function of f_i ($i = 1, \dots, k$). However, since the dose–effect curves are usually estimated and the effect y is observed with error, to make valid inferences for drug interactions, one needs to account for all these variabilities. In other words, one needs to consider the estimated interaction indices along with their variances to make valid statistical inferences on drug interaction.

In most settings, the functional form of the marginal dose response curves may not be known and need to be estimated from the data. Chou and Talalay (1984) proposed the median-effect equation which has been widely used to model the dose–effect curve with good success (Chou 2006). In our cell line study (Kong and Lee 2006; Lee et al. 2007), we also find that the median-

effect equation fits the data well. Chou and Talalay's median-effect equation has the following form

$$E = \frac{\left(\frac{d}{D_m}\right)^m}{1 + \left(\frac{d}{D_m}\right)^m}, \quad (2)$$

where d is the dose of a drug eliciting effect E , D_m is the median effective dose of a drug, and m is a slope parameter depicting the shape of the curve. When m is negative, the curve described by Equation (2) falls with increasing drug concentration; when m is positive, the curve rises with increasing drug concentration. The median-effect Equation (2) is independent of the drug's mechanisms of action and does not require knowledge of conventional kinetic constants (Chou 2006; Greco et al. 1995). Under the assumption that the dose–effect curves follow Chou and Talalay's median-effect equation, in Section 3 we investigate the characteristics of the interaction index and its logarithmic transformation, and propose a procedure to construct the confidence interval for the estimated interaction index.

Although interaction index can be estimated at each combination dose separately, this approach is not efficient. The result tends to be more varying as it depends on only measurement at a single combination dose level. To gain efficiency, one can assume a model and pool data at various combination doses to form a better estimate of the interaction index. One commonly used approach is applying the ray design. Chou and Talalay (1984) and Chou (1991) proposed a procedure to characterize a two-drug interaction by first fitting marginal dose–effect curves and a dose–effect curve for the combination doses with their components at a fixed ray (i.e., $d_1/d_2 = \text{a constant}$, forming a ray in the $d_1 \times d_2$ dose plane), then assessing drug interaction based on the plot of their combination indices versus effects for combination doses at this fixed ray. The confidence intervals for the combination indices were constructed by Monte Carlo techniques (*CalcuSyn* at <http://www.biosoft.com/w/calculusyn.htm>; Belen'kii and Schinazi 1994; *CompuSyn* at <http://www.combosyn.com/>). The combination index has the same form as the interaction index when the combined drugs are mutually exclusive. However, Chou and Talalay's mutual exclusiveness and nonexclusiveness criteria are difficult to evaluate, and the combination index has been criticized by many researchers (Berenbaum 1989; Greco et al., 1995). In Section 3, by adopting the interaction index for Chou and Talalay's method, we extend their method to assess drug interactions among $k (\leq 2)$ drugs, and propose a procedure to construct a pointwise $(1 - \alpha) \times 100\%$ confidence bound for the estimated curve of interaction indices versus effects by accounting for all the variabilities in estimating the dose–effect curves. In Section 4, we present the results from simulations and case studies to show that our proposed procedure performs at least as well as the Monte Carlo procedure in terms of covering the underlying curves and shortening the confidence bound, and performs better in terms of taking less time to compute. The last section is devoted to discussion.

2. Interaction Index and its Confidence Interval at a Combination Dose

In this section, we will present how to construct a confidence interval for interaction index at a combination dose $(d_1, \dots, d_k)$ with an effect y . Here the dose–effect curve for drug i ($i = 1, \dots, k$) is estimated from the marginal data with only i th drug being applied. Note that Chou and Talalay's median-effect equation (2) also can be rewritten as

$$\log \frac{E}{1-E} = m (\log d - \log D_m) = \beta_0 + \beta_1 \log d, \quad (3)$$

where $\beta_0 = -m \log D_m$ and $\beta_1 = m$. The dose producing effect E can be written as either

$$d = D_m \left(\frac{E}{1-E} \right)^{\frac{1}{m}}, \quad (4)$$

or

$$d = \exp \left(-\frac{\beta_0}{\beta_1} \right) \left(\frac{E}{1-E} \right)^{\frac{1}{\beta_1}}. \quad (5)$$

Suppose model (3) has the form $\log \frac{E}{1-E} = \beta_0 + \beta_1 \log d + \epsilon$ with ϵ following $N(0, \sigma^2)$, then we may regress $\log \frac{E}{1-E}$ on $\log d$ to get the marginal dose–effect curve $\log \frac{E}{1-E} = \widehat{\beta}_{0,i} + \widehat{\beta}_{1,i} \log d$ for drug i with $i = 1, \dots, k$. Meanwhile we may get the variances and covariances for the estimates $\widehat{\beta}_{0,i}$ and $\widehat{\beta}_{1,i}$ for $i = 1, \dots, k$. If the observed mean effect at a combination dose $(d_1, \dots, d_k)$ is y , then, based on (5), the associated interaction index can be estimated by

$$\widehat{\tau} = \sum_{i=1}^k \frac{d_i}{\widehat{D}_{y,i}} = \sum_{i=1}^k \frac{d_i}{\exp \left(-\frac{\widehat{\beta}_{0,i}}{\widehat{\beta}_{1,i}} \right) \left(\frac{y}{1-y} \right)^{\frac{1}{\widehat{\beta}_{1,i}}}}. \quad (6)$$

The simulations in Section 4 indicate that the distribution of $\log(\widehat{\tau})$ is approximately normal, while $\widehat{\tau}$ deviates from a normal distribution for large σ 's. Thus, we should apply the delta method (Bickel and Doksum 2001) to $\log \tau$ instead of τ , then we take the exponential transformation to get the confidence interval for τ . By applying delta method to $\log(\widehat{\tau})$, we get

$$\begin{aligned} \text{var}(\log(\widehat{\tau})) &\leq \frac{1}{\widehat{\tau}^2} \text{var}(\widehat{\tau}) \\ &\leq \frac{1}{\widehat{\tau}^2} \left(\frac{\partial \widehat{\tau}}{\partial \beta_{0,1}}, \frac{\partial \widehat{\tau}}{\partial \beta_{1,1}}, \dots, \frac{\partial \widehat{\tau}}{\partial \beta_{0,k}}, \frac{\partial \widehat{\tau}}{\partial \beta_{1,k}}, \frac{\partial \widehat{\tau}}{\partial y} \right) \\ &\quad \times \Sigma \left(\frac{\partial \widehat{\tau}}{\partial \beta_{0,1}}, \frac{\partial \widehat{\tau}}{\partial \beta_{1,1}}, \dots, \frac{\partial \widehat{\tau}}{\partial \beta_{0,k}}, \frac{\partial \widehat{\tau}}{\partial \beta_{1,k}}, \frac{\partial \widehat{\tau}}{\partial y} \right)^T, \end{aligned} \quad (7)$$

where

$$\frac{\partial \widehat{\tau}}{\partial \widehat{\beta}_{0,i}} = \frac{d_i}{\widehat{D}_{y,i} \widehat{\beta}_{1,i}}, \quad \frac{\partial \widehat{\tau}}{\partial \widehat{\beta}_{1,i}} = \frac{d_i}{\widehat{D}_{y,i}} \frac{\log \frac{y}{1-y} - \widehat{\beta}_{0,i}}{\widehat{\beta}_{1,i}^2}$$

for $i = 1, \dots, k$, and

$$\frac{\partial \widehat{\tau}}{\partial y} = -\frac{1}{y(1-y)} \left(\frac{1}{\widehat{\beta}_{1,1}} \frac{d_1}{\widehat{D}_{y,1}} + \dots + \frac{1}{\widehat{\beta}_{1,k}} \frac{d_k}{\widehat{D}_{y,k}} \right),$$

Σ is the variance–covariance matrix of the $2k$ parameters $(\widehat{\beta}_{0,1}, \widehat{\beta}_{1,1}, \dots, \widehat{\beta}_{0,k}, \widehat{\beta}_{1,k})$ and the observed mean effect y at $(d_1, \dots, d_k)$. Any two pairs of parameters, $(\widehat{\beta}_{0,i}, \widehat{\beta}_{1,i})$ and $(\widehat{\beta}_{0,j}, \widehat{\beta}_{1,j})$ when $i \neq j$ are independent since typically, different experimental subjects was used for drug i alone and for drug j alone, respectively. Further, all those subjects are different from the subjects administrated the combination dose $(d_1, \dots, d_k)$. Thus, the estimates $(\widehat{\beta}_{0,i}, \widehat{\beta}_{1,i})$ are independent of the estimates $(\widehat{\beta}_{0,j}, \widehat{\beta}_{1,j})$ when $i \neq j$, and all of them are independent of the observed mean effect y at $(d_1, \dots, d_k)$. Therefore, Σ is a block diagonal matrix with the block being a 2×2 matrix except for the last diagonal element $\text{var}(y)$. An approximate variance of

$\log(\widehat{\tau})$ can be obtained by $\text{var}(\log(\widehat{\tau})) \simeq \frac{1}{\widehat{\tau}^2} \text{var}(\widehat{\tau})$, where

$$\begin{aligned} \text{var}(\widehat{\tau}) &\simeq \sum_{i=1}^k \left(\frac{d_i}{D_{y,i}} \right)^2 \\ &\times \left(\frac{\text{var}(\widehat{\beta}_{0,i})}{\widehat{\beta}_{1,i}^2} + \frac{2\text{cov}(\widehat{\beta}_{0,i}, \widehat{\beta}_{1,i}) \left(\log \frac{y}{1-y} - \widehat{\beta}_{0,i} \right)}{\widehat{\beta}_{1,i}^3} \right. \\ &\quad \left. + \frac{\text{var}(\widehat{\beta}_{1,i}) \left(\log \frac{y}{1-y} - \widehat{\beta}_{0,i} \right)^2}{\widehat{\beta}_{1,i}^4} \right) \\ &+ \left(\frac{1}{\widehat{\beta}_{1,1}} \frac{d_1}{D_{y,1}} + \dots + \frac{1}{\widehat{\beta}_{1,k}} \frac{d_k}{D_{y,k}} \right)^2 \\ &\times \left(\frac{1}{y(1-y)} \right)^2 \text{var}(y). \end{aligned} \quad (8)$$

We can estimate $\text{var}(y)$ in two ways. When there are replicates at the combination dose $(d_1, \dots, d_k)$, $\text{var}(y)$ can simply be estimated by the sample variance at (d_1, d_2) . Otherwise, we may borrow the information from estimating the marginal dose–effect curves. Note that

$\text{var} \left(\log \frac{y}{1-y} \right) \simeq \left(\frac{1}{y(1-y)} \right)^2 \text{var}(y)$, thus, we may substitute $\left(\frac{1}{y(1-y)} \right)^2 \text{var}(y)$ by the average of the squared residuals obtained from fitting the median-effect Equation (3) for all drugs involved assuming a constant variance for both the single and combination drug effects. Once the variance is obtained, a $(1 - \alpha) \times 100\%$ confidence interval for $\log(\tau)$ can be formed

$$\left[\log(\widehat{\tau}) - t_{n-2k, \frac{\alpha}{2}} \sqrt{\text{var}(\log(\widehat{\tau}))}, \log(\widehat{\tau}) + t_{n-2k, \frac{\alpha}{2}} \sqrt{\text{var}(\log(\widehat{\tau}))} \right],$$

where $t_{n-2k, \frac{\alpha}{2}}$ is the $1 - \frac{\alpha}{2}$ percentile of t -distribution with $n - 2k$ degree of freedom, and

$n = \sum_{i=1}^k n_i$ with n_i ($i = 1, \dots, k$) being the number of observations when drug i is used alone. $2k$ is the total number of estimated parameters involved in estimating the interaction index (6). Thus, a $(1 - \alpha) \times 100\%$ confidence interval for τ can be constructed as:

$$\left[\widehat{\tau} \exp \left(-t_{n-2k, \frac{\alpha}{2}} \sqrt{\text{var}(\log(\widehat{\tau}))} \right), \widehat{\tau} \exp \left(t_{n-2k, \frac{\alpha}{2}} \sqrt{\text{var}(\log(\widehat{\tau}))} \right) \right]. \quad (9)$$

When $\text{var}(\log(\widehat{\tau}))$ is small, we have

$$\begin{aligned}
& \widehat{\tau} \exp\left(\pm t_{n-2k, \frac{\alpha}{2}} \sqrt{\text{var}(\log(\widehat{\tau}))}\right) \\
& \simeq \widehat{\tau} \exp\left(\pm \frac{t_{n-2k, \frac{\alpha}{2}}}{\widehat{\tau}} \sqrt{\text{var}(\widehat{\tau})}\right) \\
& \simeq \widehat{\tau} \pm t_{n-2k, \frac{\alpha}{2}} \sqrt{\text{var}(\widehat{\tau})}.
\end{aligned}$$

Therefore, if the error in (3) is small, the confidence interval for τ based on (9) is essentially the same as the confidence interval constructed by directly applying the delta method to $\widehat{\tau}$, which is

$$\left[\widehat{\tau} - t_{n-2k, \frac{\alpha}{2}} \sqrt{\text{var}(\widehat{\tau})}, \quad \widehat{\tau} + t_{n-2k, \frac{\alpha}{2}} \sqrt{\text{var}(\widehat{\tau})} \right]. \quad (10)$$

In Section 4, we illustrate that, for a large error in (3), the confidence interval (9) behaves better than (10) in two aspects: (i) the lower limit is greater than zero all the time; and (ii) the confidence interval has a coverage rate that is closer to the nominal rate. Therefore, the confidence interval (9) is preferred. When $n - 2k$ is large, say $n - 2k \geq 20$, one may use $z_{\frac{\alpha}{2}}$

instead of $t_{n-2k, \frac{\alpha}{2}}$ in estimating the confidence intervals (9) and (10), where $z_{\frac{\alpha}{2}}$ is the $1 - \frac{\alpha}{2}$ percentile of the standard normal distribution.

3. Interaction Indices and Their Confidence Bound at a Fixed Ray

3.1 Two-Drug Combination

Note that the confidence interval (9) is based on a single observation and the marginal dose–effect curves, and the estimated interaction index and its confidence interval are greatly influenced by this single observation. Chou and Talalay (1984) used a ray design to assess drug interactions. The advantage of their method is that it uses all observations with the component doses at a fixed ray. We adopt the interaction index instead of the combination index when

using their approach. The basic idea (Chou 1991) is to regress $\log \frac{E}{1-E}$ on $\log D$ for each of the two drugs used alone and regress $\log \frac{E}{1-E}$ on $\log(d_1 + d_2)$ for the combination doses (d_1 , d_2) with $\frac{d_2}{d_1} = \frac{\omega_2}{\omega_1}$, say, $\log \frac{E}{1-E} = \beta_{0,c} + \beta_{1,c} \log D_c$. Then for each fixed effect y , one may estimate the interaction index by

$$\widehat{\tau}_{CT} = \frac{\widehat{D}_{y,c} \frac{\omega_1}{\omega_1 + \omega_2}}{\widehat{D}_{y,1}} + \frac{\widehat{D}_{y,c} \frac{\omega_2}{\omega_1 + \omega_2}}{\widehat{D}_{y,2}}, \quad (11)$$

where $\widehat{D}_{y,1} = \exp\left(-\frac{\widehat{\beta}_{0,1}}{\widehat{\beta}_{1,1}}\right) \left(\frac{y}{1-y}\right)^{\frac{1}{\widehat{\beta}_{1,1}}}$, $\widehat{D}_{y,2} = \exp\left(-\frac{\widehat{\beta}_{0,2}}{\widehat{\beta}_{1,2}}\right) \left(\frac{y}{1-y}\right)^{\frac{1}{\widehat{\beta}_{1,2}}}$, and

$\widehat{D}_{y,c} = \exp\left(-\frac{\widehat{\beta}_{0,c}}{\widehat{\beta}_{1,c}}\right) \left(\frac{y}{1-y}\right)^{\frac{1}{\widehat{\beta}_{1,c}}}$. Commercial software *CalcuSyn* and *CompuSyn* are available for estimating the interaction indices and their confidence intervals. The confidence intervals for interaction indices in (11) were constructed based on Monte Carlo techniques and the normal assumption on the parameters (Belen'kii and Schinazi 1994). Briefly, the parameters, $(\widehat{\beta}_{0,i}, \widehat{\beta}_{1,i})$ for $i = 1, 2, c$, and the interaction index (11) are estimated from the observed data,

then certain number of random samples (say, 500) of the parameters, $(\widehat{\beta}_{0,i}, \widehat{\beta}_{1,i})_j$ for $j = 1, \dots, 500$ and $i = 1, 2, c$, are generated based on their estimated values and covariances under the normal assumption on each pair of these parameters. Thus, 500 interaction indices, $\widehat{\tau}_{CT,j}$ ($j = 1, \dots, 500$), can be calculated and its standard deviation can be estimated as

$\widehat{\sigma}_{\tau}^2 = \frac{1}{500} \sum_{j=1}^{500} (\widehat{\tau}_{CT,j} - \widehat{\tau}_{CT})^2$. Consequently, the confidence interval can be constructed as $[\widehat{\tau}_{CT} - z_{\frac{\alpha}{2}} \widehat{\sigma}_{\tau}, \widehat{\tau}_{CT} + z_{\frac{\alpha}{2}} \widehat{\sigma}_{\tau}]$. In the simulation and case studies in Section 4, we used $t_{n_1+n_2+n_c-6, \frac{\alpha}{2}}$ instead of $z_{\frac{\alpha}{2}}$ since the number of observations is small.

In the following subsection, we extend Chou and Talalay's method to $k(\geq 2)$ drugs, estimate drug interaction at a fixed ray, say $d_1 : d_2 : \dots : d_k = \omega_1 : \omega_2 : \dots : \omega_k$, and construct a $(1 - \alpha) \times 100\%$ confidence interval for the constructed interaction index at each effect y . Thus, by varying y , a pointwise confidence bound for the curve of interaction indices versus effects with combination doses at the fixed ray can be constructed by using the delta method.

3.2 k -drug combination

Again, we assume that the fitted dose–effect curve is $\log \frac{E}{1-E} = \widehat{\beta}_{0,i} + \widehat{\beta}_{1,i} \log D_i + \epsilon$ for drug i with $i = 1, \dots, k$. The fitted dose–effect curve for the mixture with their component doses at

a fixed ray with $d_1 : d_2 : \dots : d_k = \omega_1 : \omega_2 : \dots : \omega_k$ is $\log \frac{E}{1-E} = \widehat{\beta}_{0,c} + \widehat{\beta}_{1,c} \log D_c + \epsilon$ with $D_c = d_1 + d_2 + \dots + d_k$. Then, for each fixed effect y , one may estimate interaction index by

$$\widehat{\tau}_{CT} = \frac{\widehat{D}_{y,c} \frac{\omega_1}{\omega_1 + \dots + \omega_k}}{\widehat{D}_{y,1}} + \dots + \frac{\widehat{D}_{y,c} \frac{\omega_k}{\omega_1 + \dots + \omega_k}}{\widehat{D}_{y,k}}, \quad (12)$$

where $\widehat{D}_{y,i} = \left(\frac{y}{1-y} \right)^{\frac{1}{\widehat{\beta}_{1,i}}} \exp \left(-\frac{\widehat{\beta}_{0,i}}{\widehat{\beta}_{1,i}} \right)$ for $i = 1, \dots, k, c$. Again, $(\widehat{\beta}_{0,i}, \widehat{\beta}_{1,i})$ and $(\widehat{\beta}_{0,j}, \widehat{\beta}_{1,j})$ are independent as long as $i \neq j$ for $i, j = 1, \dots, k, c$. Based on the delta method (Bickel and Doksum 2001), we can obtain an approximate variance for $\widehat{\tau}_{CT}$

$$\begin{aligned} \text{var}(\widehat{\tau}_{CT}) &= \sum_{i=1}^k \left(\frac{\partial \widehat{\tau}_{CT}}{\partial \widehat{D}_{y,i}} \right)^2 \text{var}(\widehat{D}_{y,i}) \\ &\quad + \left(\frac{\partial \widehat{\tau}_{CT}}{\partial \widehat{D}_{y,c}} \right)^2 \text{var}(\widehat{D}_{y,c}) \\ &= \sum_{i=1}^k \left(-\frac{\omega_i \widehat{D}_{y,c}}{\left(\sum_{j=1}^k \omega_j \right) \widehat{D}_{y,i}^2} \right)^2 \text{var}(\widehat{D}_{y,i}) \\ &\quad + \left(\frac{1}{\sum_{i=1}^k \omega_i} \left(\sum_{i=1}^k \frac{\omega_i}{\widehat{D}_{y,i}} \right) \right)^2 \text{var}(\widehat{D}_{y,c}) \end{aligned} \quad (13)$$

with

$$\begin{aligned}\text{var}(\widehat{D}_{y,i}) &= \begin{pmatrix} \frac{\partial \widehat{D}_{y,i}}{\partial \widehat{\beta}_{0,i}}, \frac{\partial \widehat{D}_{y,i}}{\partial \widehat{\beta}_{1,i}} \end{pmatrix} \Sigma_{\widehat{\beta}_{0,i}, \widehat{\beta}_{1,i}} \begin{pmatrix} \frac{\partial \widehat{D}_{y,i}}{\partial \widehat{\beta}_{0,i}} \\ \frac{\partial \widehat{D}_{y,i}}{\partial \widehat{\beta}_{1,i}} \end{pmatrix} \\ &= \widehat{D}_{y,i}^2 \begin{pmatrix} -\frac{1}{\widehat{\beta}_{1,i}}, \frac{\widehat{\beta}_{0,i} - \log \frac{y}{1-y}}{\widehat{\beta}_{1,i}^2} \end{pmatrix} \\ &\quad \times \Sigma_{\widehat{\beta}_{0,i}, \widehat{\beta}_{1,i}} \begin{pmatrix} -\frac{1}{\widehat{\beta}_{1,i}} \\ \frac{\widehat{\beta}_{0,i} - \log \frac{y}{1-y}}{\widehat{\beta}_{1,i}^2} \end{pmatrix}\end{aligned}$$

for $i = 1, \dots, k, c$, respectively. Thus, replacing $\text{var}(\widehat{D}_{y,i})$ in (13), we can obtain the estimated variance for $\widehat{\tau}_{CT}$:

$$\begin{aligned}\text{var}(\widehat{\tau}_{CT}) &= \sum_{i=1}^k \left(\frac{\omega_i \widehat{D}_{y,c}}{\sum_{j=1}^k \omega_j \widehat{D}_{y,j}} \right)^2 \\ &\quad \times \left(\frac{\text{var}(\widehat{\beta}_{0,i})}{\widehat{\beta}_{1,i}^2} + \frac{2\text{cov}(\widehat{\beta}_{0,i}, \widehat{\beta}_{1,i}) \left(\log \frac{y}{1-y} - \widehat{\beta}_{0,i} \right)}{\widehat{\beta}_{1,i}^3} \right. \\ &\quad \left. + \frac{\text{var}(\widehat{\beta}_{1,i}) \left(\log \frac{y}{1-y} - \widehat{\beta}_{0,i} \right)^2}{\widehat{\beta}_{1,i}^4} \right) \\ &\quad + \left(\frac{\widehat{D}_{y,c}}{\sum_{i=1}^k \omega_i} \left(\sum_{i=1}^k \frac{\omega_i}{\widehat{D}_{y,i}} \right) \right)^2 \\ &\quad \times \left(\frac{\text{var}(\widehat{\beta}_{0,c})}{\widehat{\beta}_{1,c}^2} + \frac{2\text{cov}(\widehat{\beta}_{0,c}, \widehat{\beta}_{1,c}) \left(\log \frac{y}{1-y} - \widehat{\beta}_{0,c} \right)}{\widehat{\beta}_{1,c}^3} \right. \\ &\quad \left. + \frac{\text{var}(\widehat{\beta}_{1,c}) \left(\log \frac{y}{1-y} - \widehat{\beta}_{0,c} \right)^2}{\widehat{\beta}_{1,c}^4} \right).\end{aligned}\tag{14}$$

Here, we prefer the confidence interval based on the delta method on $\log(\widehat{\tau}_{CT})$ since $\log(\widehat{\tau}_{CT})$ is more approximately normally distributed than $\widehat{\tau}_{CT}$. A $(1 - \alpha) \times 100\%$ confidence interval for $\widehat{\tau}_{CT}$ can be constructed by

$$\left[\widehat{\tau}_{CT} \exp\left(\frac{-t_{n+n_c-2k-2, \frac{\alpha}{2}}}{\widehat{\tau}_{CT}} \sqrt{\text{var}(\widehat{\tau}_{CT})}\right), \widehat{\tau}_{CT} \exp\left(\frac{t_{n+n_c-2k-2, \frac{\alpha}{2}}}{\widehat{\tau}_{CT}} \sqrt{\text{var}(\widehat{\tau}_{CT})}\right) \right].\tag{15}$$

Again $n = \sum_{i=1}^k n_i$ and n_i ($i = 1, \dots, k$) is the number of observations when drug i is used alone, n_c is the number of observations for the combination doses at a fixed ray. By varying y in different values, we can construct a pointwise $(1 - \alpha)100\%$ confidence bound for the curve of interaction indices versus effects. Thus, we can assess drug interactions for combination doses at the fixed ray while considering the stochastic uncertainty in obtaining the observations.

Remark—Comparing the variances of estimated interaction indices in (8) and (14), we note that the first terms in both equations are approximately the same, while the second terms are markedly different. In Sections 2 and 3, the k dose–effect curves for all the drugs involved are estimated. The first terms in both equations describe the uncertainty contributed by estimating the k marginal dose–effect curves. In Section 2, we estimate the interaction index based on the observed mean effect at a single combination dose, and oftentimes, we assume the combination dose is measured without error. Under this setting, the second term in (8) describes the variability contributed by the mean of the observed effects at the combination (d_1, d_2) . In

Section 3, we have the observations for combination doses at a fixed ray, then we fit the dose–effect curve for this ray. We estimate the interaction index for each fixed effect, where the combination dose producing such an effect is estimated. Thus, the second term in (14) describes the uncertainty contributed by the variance of the estimated combination dose $\widehat{D}_{y,c}$, which could be split into the estimated combination dose

$$(\widehat{d}_1, \dots, \widehat{d}_k) = \left(\frac{\omega_1}{\sum_{i=1}^k \omega_i} \widehat{D}_{y,c}, \dots, \frac{\omega_k}{\sum_{i=1}^k \omega_i} \widehat{D}_{y,c} \right).$$

4. Simulations and Case Studies

4.1 Simulations

To examine whether the confidence intervals proposed in Sections 2 and 3 have proper characteristics, we performed simulations in the following two scenarios.

Scenario 1: three drugs, at a single combination dose—In the first scenario, we simulated three drugs that followed the median-effect Equation (2) with the same slope $m = -1$ and different median effective doses: $Dm_1 = 1$, $Dm_2 = 2$, and $Dm_3 = 4$, respectively. We took the combination dose (d_1, d_2, d_3) with each component being one third of its associated median

effective dose, that is, $(d_1, d_2, d_3) = \left(\frac{Dm_1}{3}, \frac{Dm_2}{3}, \frac{Dm_3}{3} \right) = \left(\frac{1}{3}, \frac{2}{3}, \frac{4}{3} \right)$. If the combination dose is additive, the expected effect will be 0.5. Let us denote the interaction index at this combination dose as τ , the effect as E , then based on Equation (4), we have

$$\frac{d_1}{Dm_1 \left(\frac{E}{1-E} \right)^{\frac{1}{m}}} + \frac{d_2}{Dm_2 \left(\frac{E}{1-E} \right)^{\frac{1}{m}}} + \frac{d_3}{Dm_3 \left(\frac{E}{1-E} \right)^{\frac{1}{m}}} = \tau.$$

Thus, the effect at (d_1, d_2, d_3) can be explicitly expressed as

$$E = \frac{\left(\tau^{-1} \left(\frac{d_1}{Dm_1} + \frac{d_2}{Dm_2} + \frac{d_3}{Dm_3} \right) \right)^m}{1 + \left(\tau^{-1} \left(\frac{d_1}{Dm_1} + \frac{d_2}{Dm_2} + \frac{d_3}{Dm_3} \right) \right)^m}.$$

We vary τ among (0.2, 0.4, 0.6, 0.8, 1, 1.25, 1.67, 2.5, 5). The corresponding effect E will be (0.167, 0.286, 0.375, 0.444, 0.5, 0.556, 0.625, 0.714, 0.833), respectively. Note that the slope m is negative, so the dose–effect curve is decreasing. If the effect is less than 0.5, then the combination dose will be synergistic and the interaction index will be less than 1; whereas if the effect is greater than 0.5, the combination dose will be antagonistic and the interaction index will be greater than 1. It is obvious that the farther the interaction index moves away from 1, the stronger is the interaction effect.

Under the above setting, we first generated six equally spaced doses, ranging from 0.1 to three-fold of the associated median effective dose for each drug. We then generated the effects based

on the model $\log \frac{E}{1-E} = \beta_0 + \beta_1 \log d + \epsilon$ with $\epsilon \sim N(0, \sigma^2)$ for each drug, where $\beta_0 = -m \log$

D_m and $\beta_1 = m$. We generated the observed effect at the combination dose $\left(\frac{1}{3}, \frac{2}{3}, \frac{4}{3} \right)$ with the

same size of the stochastic variation. The total sample size for each simulated experiment was 19 (six observations for each single drug and one observation for the combination dose effect). We fitted each dose–effect curve based on the generated data. Then, for each τ , we estimated the interaction index based on (6), constructed its 95% confidence intervals based on (9) and (10), respectively, calculated the length of the confidence intervals, and counted whether the true τ lies in the respective confidence intervals based on (9) and (10), and whether the confidence interval based on (9) lies below 1, contains 1, or lies above 1. We repeated this procedure 1,000 times, and averaged all the above quantities. We summarized the results in Table 1 under different settings for σ : $\sigma = 0.1$ and $\sigma = 0.4$, respectively. From Table 1, we conclude that (a) the estimation for τ (mean($\hat{\tau}$) in Table 1) is close to the true value and the accuracy decreases as σ increases; (b) the resulting coverage rates (Cov.rate.log) based on confidence interval (9) are closer to the nominal coverage rate of 95% than those (Cov.rate) based on (10), particularly, when σ is larger; (c) the average lengths of the confidence intervals (Len.ci.log) based on (9) and the average lengths of the confidence intervals (Len.ci) based on (10) increase as σ increases with Len.ci.log slightly larger than Len.ci to provide the nominal coverage rate; and (d) the percentage of times the model correctly assesses drug interaction based on (9) as synergy (Pct.syn.log), additivity (Pct.add.log), or antagonism (Pct.ant.log) decreases as σ increases. For each underlying interaction index among (0.2, 0.6, 1, 1.67, 5), we obtained the Q-Q plot of the 1,000 estimated interaction indices (Figure 2, Columns B1 and B3) as well as the Q-Q plot of the 1,000 logarithms of the estimated interaction indices (Figure 2, Columns B2 and B4) under the settings $\sigma = 0.1$ and $\sigma = 0.4$, respectively. From Figure 2, it is clear that for small σ (e.g., $\sigma = 0.1$), both the estimated interaction index and the logarithm of the estimated interaction index are approximately normally distributed. But, when σ becomes large, say $\sigma = 0.4$, the estimated interaction indices deviate from a normal distribution, while the logarithms of the estimated interaction indices are still approximately normally distributed. Therefore, one would expect that the delta method on the logarithm of the interaction index would work better for constructing confidence intervals for interaction indices. This assertion has been verified by the results of the current simulation studies. Thus, we prefer using confidence interval (9) over (10) for the interaction index in Section 2, and using the confidence interval (15) in Section 3.

Scenario 2: Two drugs, with a ray design—The second scenario involves two drugs that have the same dose–effect curves as drug 1 and drug 2 in the first scenario. That is, the two dose–effect curves follow the median-effect Equation (2) with the same slope $m = -1$, and median effective doses: $Dm_1 = 1$ and $Dm_2 = 2$, respectively. We assume that the dose–effect

curve—for the combination doses (d_1, d_2) at the fixed ray, say, $\frac{d_2}{d_1} = \frac{2}{1}$ follows the median-effect Equation (2) with $Dm_{12} = 1.5$ and $m_{12} = -2$.

We generated five equally spaced doses, ranging from 0.1 to three-fold of the associated median effective dose for each of the single drug, and five equally spaced doses, ranging from 0.5 to three-fold of the associated median effective dose for the mixture (d_1, d_2) at the fixed ray with

$\frac{d_2}{d_1} = \frac{2}{1}$ and with the dose in the median effect Equation (2) being $d_1 + d_2$. We then generated the effects associated with these generated doses based on the model

$\log \frac{E}{1-E} = \beta_0 + \beta_1 \log d + \epsilon$ with $\epsilon \sim N(0, \sigma^2)$ for the two drugs and their mixture under the settings $\sigma = 0.2$ and $\sigma = 0.4$, respectively. The total sample size for each simulated experiment is 15 (five observations for each single drug and five observations for combination doses). In addition, under each setting for σ , we generated seven samples for illustration. For each sample, we first fitted the dose–effect curves for the two drugs and the mixture, and then performed the following steps: we (a) estimated the interaction indices based on (12) for 42 equally spaced

effect levels between the range of 0.1 to 0.95; (b) constructed their confidence intervals based on (15) and on a Monte Carlo simulation proposed by Belen'kii and Schinazi (1994), respectively; and (c) estimated interaction index (6) and constructed the confidence interval (9) for each observed combination dose. Figure 3 illustrates plots of the underlying curve of the interaction index versus effect (solid line), the pointwise 95% confidence bound for this curve based on (15) (dotted lines), the confidence bound based on the Monte Carlo simulation (dashed lines), and the estimated interaction indices (dots) and their confidence intervals (vertical bars) for the observed doses based on (9) for the seven illustrative samples under each setting for σ . From Figure 3, we conclude that (a) the pointwise 95% confidence bound (dotted lines) embrace the true curve (solid line) well; (b) the pointwise confidence bounds based on (15) (dotted lines) are similar to those based on Monte Carlo simulations (dashed lines) when σ is small, but perform better when σ is large; and (c) the confidence intervals based on single observations (vertical bars) are generally wider than both confidence bounds, and the conclusions based on confidence bounds are more accurate than those based on the confidence intervals (vertical bars) for single observations. In addition, for each sample, we calculated the ratio of the length of the confidence interval based on (9) and the confidence interval based on Monte Carlo simulation described in Section 3 for each of the observed effects at the combination doses at the fixed ray. Under $\sigma = 0.2$, the ratios for the seven samples have a mean 2.05 with standard deviation 0.58, and range from 1.12 to 3.28. Under $\sigma = 0.4$, the ratios for the seven samples have a mean 2.33 with standard deviation 0.92, and range from 1.01 to 4.15 after removing an extreme of the observation with effect close to one (Figure 3, Panel B7). Therefore, when several observations for combination doses at a fixed ray are available, the confidence bound derived in Section 3 using more available information is more efficient, thus preferred. In addition, when we ran this simulation to get the 14 confidence bounds in the 14 panels in Figure 3 by separately using the confidence interval based on (15) and Monte Carlo procedure on an Intel 1.83 GHz computer, the time it took was 10 seconds and 17 minutes, respectively. It is clear that the calculation for the confidence bound based on (15) is much faster than that based on Monte Carlo simulations.

4.2 Case Studies

Our research group (Lee et al. 2007; Kong and Lee 2006) investigated drug interactions between two novel agents, SCH66336 and 4-HPR, in a number of squamous cell carcinoma cell lines (Chun et al. 2003). Here we present the dataset and results from cell line UMSCC22B

in Table 2 for investigating drug interaction in combination doses at the fixed ray with $\frac{d_2}{d_1} = \frac{1}{1}$.

We first obtained the dose–effect=curves for SCH66336 and 4-HPR by a linear regression of

$\log \frac{E}{1-E}$ on $\log d$, based on the data in Table 2. Recall that

$\log \frac{E}{1-E} = m(\log d - \log D_m) = \beta_0 + \beta_1 \log d$. The estimates of β_0 , β_1 , D_m , and $\hat{\sigma}$ for drug 1, drug 2, and the mixture of the drugs with equal concentrations are summarized in the same table.

The transformed data $\log \frac{E}{1-E}$ versus $\log d$ and the median-effect plots are shown in Figure 4(A). This median effect plot indicates that the data follow the median-effect Equation (2) reasonably well. Based on the fitted median-effect equations, we calculated the interaction

indices based on (12) for varied effects for combination doses at the fixed ray with $\frac{d_2}{d_1} = \frac{1}{1}$ and constructed their associated confidence bounds based on (15) and on Monte Carlo simulations (Belen'kii and Schinazi 1994), respectively. Figure 4(B) shows the plot of the interaction

indices (on the logarithm scale) versus effects (solid line) for combination doses at this fixed ray and the 95% pointwise confidence bounds based on (15) (dotted lines) and on Monte Carlo simulations (dashed lines). Based on the confidence bound (dotted line), we conclude that the

combination doses at the fixed ray with $\frac{d_2}{d_1} = \frac{1}{1}$ with effect less than 0.52 are synergistic, and the combination doses at the fixed ray with effect greater than 0.52 are additive. The conclusions based on the confidence bounds obtained from Monte Carlo simulations (dashed lines) are slightly different. We also calculated four interaction indices based on (6) and their confidence intervals based on (9) at the four observed data points (d_1, d_2) as being (0.1, 0.1), (0.5, 0.5), (1, 1), and (2, 2). The four interaction indices were 0.791, 0.609, 0.256, and 0.103, and their corresponding 95% confidence intervals were [0.202, 3.091], [0.169, 2.193], [0.060, 1.087], and [0.018, 0.581], respectively. These pairs of interaction indices versus effects, along with their 95% confidence intervals are shown as vertical bars in Figure 4(B). From these four interaction indices and their confidence intervals, we conclude that the combination doses at

the fixed ray with $\frac{d_2}{d_1} = \frac{1}{1}$ are synergistic for doses $\geq 2 \mu\text{M}$ for each single drug, and additive for doses $\leq 1 \mu\text{M}$ for each single drug. The conclusions from the two procedures in Section 2 and 3 are slightly different: the combination doses (1, 1) and (0.5, 0.5) with respective observed effect 0.3551 and 0.4919 were identified as additive based on the second and third vertical bar (reading from left to right), while based on the confidence bound (dotted lines), the combination doses were identified as synergistic as each effect was less than 0.52. The relative length of the confidence interval based on (9) versus the monte Carlo confidence interval ranges from 1.27 to 3.72 for the four combination doses at the fixed ray. Once again, this example shows that the confidence interval estimation based on a single observation (9) is not as efficient as the corresponding confidence interval based on model (15) which used more data.

We also examined another dataset from a drug combination study for o-phenanthroline and ADP on the inhibition of horse liver alcohol dehydrogenase, which was analyzed by Chou and Talalay (1984) and by Belen'kii and Schinazi (1994). The dataset and the estimated median-effect parameters are shown in Table 3. The median-effect plots for the two drugs and their

mixture (d_1, d_2) at the fixed ray with $\frac{d_2}{d_1} = \frac{1}{17.4}$ are shown in Figure 5(A). The plot of interaction indices versus the fractional inhibitions at this fixed ray are shown as a solid line in Figure 5 (B). In the same panel, we illustrate the point-wise confidence bound (dotted lines) based on (15) and the pointwise confidence bound (dashed line) based on Monte Carlo simulations for this curve, and the estimated interaction indices (dots) and their associated confidence intervals (vertical bars) based on (9) for the combination doses having observed effects. Again, the two 95% confidence bounds are almost the same, the vertical bars are wider than the confidence bound, and the conclusions on drug interactions based on vertical bars and those based on confidence bounds are consistent.

We developed two S-PLUS/R programs. One is used to estimate the interaction index and its confidence interval for a single combination dose of multiple drugs, and the other is used to estimate the pointwise confidence bound for the curve of interaction index versus effect for combination doses at a fixed ray. The S-PLUS/R code and the data example are available in *CI of Interaction Index*, which can be downloaded from <http://biostatistics.mdanderson.org/SoftwareDownload/>.

5. Discussion

We proposed a procedure in Section 2 to estimate the interaction index and constructed its associated confidence interval for a multiple drug combination. In most cases, the dose–effect for a single agent is known, and investigators are interested in assessing whether drug

combinations are synergistic. When resources are limited, the experiment can be conducted in only a limited number of combination doses. We can assess drug interactions for those combination doses based on the procedure provided in Section 2. Note that although the dose–effect curves follow Chou and Talalay's median-effect equation work reasonably well, the model may not work in certain cases. In these cases, other dose–effect models must be sought. For example, Lee et al. (2009) found that the $E_{\max}$ model describes the experimental data there better than Chou and Talalay's median-effect equation; thus, the $E_{\max}$ model was used there. Upon finding the dose–effect curves of any parametric form which fits the data, one may use the same philosophy to estimate the interaction index and construct its associated confidence interval based on the delta method. However, using this “at a combination dose” method, one can assess drug interactions only at combination doses having observed effects, and the drug interaction tends to be predicted as additivity due to lack of efficiency (i.e., wide confidence intervals) even with nonadditive drug interactions.

Chou and Talalay's method based on a ray design is widely used. We provided a procedure to construct point-wise confidence bound for Chou and Talalay's curve of interaction index versus effect in Section 3. The procedure we provided avoids extensive calculations used in Monte Carlo techniques, which were required in the software *CalcuSyn* and *CompuSyn* and in the method provided by Belen'kii and Schinazi (1994). From the simulations and case studies in Section 4, we find that the confidence bounds provided in Section 3 are at least as good as the confidence bounds constructed using Monte Carlo techniques, while the confidence bounds in Section 3 are much faster to compute. Our limited simulation studies also show that the approximation based on the logarithm transformation and t -statistic works reasonably well when sample size was as low as 19 in one case and 15 in another case.

From simulations and case studies in Section 4, it is clear that the confidence intervals based on single observations (vertical bars in Figures 3, 4, and 5) are wider than the pointwise confidence bounds based on a ray design which use more data. In a ray design, the constructed confidence bound used all the information on this ray, therefore, the estimates based on Section 3 will be more efficient and more accurate. By examining the curve of interaction indices versus effects on several rays and examining their associated confidence bounds, one may obtain an overall picture of the drug interactions. The limitation is that one can only assess drug interactions for combination doses at these fixed examined rays. When a factorial design or a uniform design (Tan, Fang, Tian, and Houghton 2003) is used, a good strategy is to use response surface models, which use all the information presented in the observed data. We have proposed a generalized response surface model (Kong and Lee 2006) and a semiparametric model (Kong and Lee 2007) to capture drug interaction for all combination doses. However, response surface models for more than three drugs are difficult to construct. Therefore, to assess drug interactions among multiple drugs, the directly calculated interaction index and the plots of interaction indices versus effects at several fixed rays are still feasible and remain appealing methods to use. The confidence intervals we provided in Sections 2 and 3 are easy to calculate, and have a desirable coverage rate. Hence, it suggests that it is not necessary to run extensive Monte Carlo simulations for obtaining these confidence intervals. Based on the result of this article, Lee et al. (2009) constructed the simultaneous confidence interval for interaction indices over a range of treatment effects. The simultaneous confidence interval is also easy to calculate but is more conservative. The proposed confidence intervals can help us to gauge the uncertainties of the interaction indices for combination doses for two or more drugs and can also be used to provide more in-depth assessment for drug interactions.

Acknowledgments

The research was supported in part by grants from the National Cancer Institute CA106451, CA97007, CA16672, and Department of Defense W81XWH-04-1-0142 and W81XWH-05-2-0027. The authors are thankful to Lee Ann

Chastain for editorial assistance, to the Editor, Associate Editor, and the referee for their constructive comments. The authors also thank Professor Paul Lewi for pointing out an error in an earlier version of the article.

References

- Belen'kii MS, Schinazi RF. Multiple Drug Effect Analysis With Confidence Interval. *Antiviral Research* 1994;25:1–11. [PubMed: 7811057]
- Berenbaum MC. The Expected Effect of a Combination of Agents: The General Solution. *Journal of Theoretical Biology* 1985;114:413–431. [PubMed: 4021503]
- Berenbaum MC. What is Synergy? *Pharmacological Reviews* 1989;41:93–141. [PubMed: 2692037]
- Bickel, PJ.; Doksum, KA. *Mathematical Statistics: Basic Ideas and Selected Topics*. Prentice Hall; New Jersey: 2001. p. 306-314.
- Chou, TC. The Median-Effect Principle and the Combination Index for Quantitation of Synergism and Antagonism. In: Chou, TC.; Rideout, DC., editors. *Synergism and Antagonism in Chemotherapy*. Academic Press; San Diego: 1991. p. 61-101.
- Chou TC. Theoretical Basis, Experimental Design, and Computerized Simulation of Synergism and Antagonism in Drug Combination Studies. *Pharmacological Reviews* 2006;58:621–681. [PubMed: 16968952]
- Chou TC, Talalay P. Quantitative Analysis of Dose Effect Relationships: The Combined Effects of Multiple Drugs or Enzyme Inhibitors. *Advances in Enzyme Regulation* 1984;22:27–55. [PubMed: 6382953]
- Chun KH, Lee JJ, Ayers GD, Lotan R. Synergistic Induction of Apoptosis in Human Head and Neck Squamous Cell Carcinoma (HNSCC) Cell Lines by the Combination of the Synthetic Retinoid 4HPR and the Farnesyltransferase Inhibitor SCH66336. *Proceedings of American Association for Cancer Research* 2003;44.
- Greco WR, Bravo G, Parsons JC. The Search of Synergy: A Critical Review from a Response Surface Perspective. *Pharmacological Reviews* 1995;47(2):331–385. [PubMed: 7568331]
- Kong M, Lee JJ. A General Response Surface Model with Varying Relative Potency for Assessing Drug Interactions. *Biometrics* 2006;62(4):986–995. [PubMed: 17156272]
- Kong M, Lee JJ. A Semiparametric Response Surface Model for Assessing Drug Interactions. *Biometrics* 2008;64:396–405. [PubMed: 17900314]
- Lee JJ, Kong M, Ayers GD, Lotan R. Interaction Index and Different Methods for Determining Drug Interaction in Combination Therapy. *Journal of Biopharmaceutical Statistics* 2007;17:461–480. [PubMed: 17479394]
- Lee JJ, Lin HY, Liu DD, Kong M. Applying E_{max} Model and Interaction Index for Assessing Drug Interaction in Combination Studies. *Frontiers in Biosciences*. 2009 in press.
- Tan M, Fang H, Tian G, Houghton PJ. Experimental Design and Sample Size Determination for Testing Synergism in Drug Combination Studies Based on Uniform Measures. *Statistics in Medicine* 2003;22:2091–2100. [PubMed: 12820275]

A. Synergy

B. Antagonism

Figure 1.

Illustration of the interaction index. $P = (D_{y,1}, 0)$ represents the drug 1 dose producing effect y , $Q = (0, D_{y,2})$ represents the drug 2 dose producing effect y , and $U = (d_1, d_2)$ represents the combination dose producing the same effect y . $\bar{RS}$ is the line passing by U and parallel to $\bar{PQ}$, and V is the intercept of $\bar{OU}$ and $\bar{PQ}$. The interaction index can be expressed as the ratio

$$\frac{\text{length}(\bar{OU})}{\text{length}(\bar{OV})}$$
. Panel A illustrates the case for synergy while Panel B for antagonism.

A1: $\Pi=0.2$ A2: $\Pi=0.6$ A3: $\Pi=1$ A4: $\Pi=1.67$ A5: $\Pi=5$ **Figure 2.**

Q-Q plots of estimated interaction indices (Column B1 and B3) and Q-Q plots of the logarithms of the estimated interaction indices (Column B2 and B4) for 1000 samples under Scenario 1. Columns B1 and B2 show the Q-Q plots under $\sigma = 0.1$, and Column B3 and B4 show the Q-Q plots under $\sigma = 0.4$.

Figure 3.

Simulated results under Scenario 2. The solid line is the plot of the underlying true interaction

indices versus effects for combination doses at the fixed ray with $\frac{d_2}{d_1} = \frac{2}{1}$, the dotted lines and the dashed lines are the 95% pointwise confidence bounds for the curve of the interaction index versus effect based on the delta method and Monte Carlo simulation under the settings $\sigma = 0.2$ (Panels A) and $\sigma = 0.4$ (Panel B), respectively, and the dots and the vertical bars are the estimated interaction indices and their confidence intervals for observed combinations.

Figure 4.

Median-effect plots (Panel A) and the plot of interaction indices versus effects (Panel B) for

the combination doses at the fixed ray with $\frac{d_2}{d_1} = \frac{1}{1}$ for SCH66336 and 4HPR. In Panel B, the solid line is the plot of the estimated interaction indices versus effects, the two dotted lines and the two dashed lines are the pointwise 95% confidence bounds for the curve of interaction index versus effect based on the delta method in Section 3 and Monte Carlo simulation, respectively, and the dots and the vertical bars are the estimated interaction indices and their confidence intervals for observed combinations. The four vertical bars from left to right correspond to the combination doses of (2, 2), (1, 1), (0.5, 0.5), and (0.1, 0.1), respectively.

Figure 5.

Median-effect plots (Panel A) and the plot of interaction indices versus effects (Panel B) for

the combination doses at the fixed ray with $\frac{d_2}{d_1} = \frac{17.4}{1}$ for o-phenanthroline and ADP. In Panel B, the solid line is the plot of the estimated interaction indices versus effects, the two dotted lines and the two dashed lines are the pointwise confidence bounds for the curve of interaction index versus effect based on the delta method and Monte Carlo simulation, respectively, and the dots and the vertical bars are the estimated interaction indices and their confidence intervals for observed combinations.

Simulation results for Scenario 1: a fixed combination dose $(d_1, d_2, d_3) = \left(\frac{1}{3}, \frac{2}{3}, \frac{4}{3}\right)$ but with varying interaction indices. The three dose-effect curves follow the median-effect equation with $m = -1$ and $(Dm_1, Dm_2, Dm_3) = (1, 2, 4)$.

Table 1

τ	0.2	0.4	0.6	0.8	1	1.25	1.67	2.5	5
mean($\hat{\tau}$)	0.202	0.402	0.601	0.804	1.001	1.266	1.676	2.503	5.022
Cov.rate	0.950	0.952	0.946	0.947	0.939	0.958	0.951	0.947	0.948
Cov.rate.log	0.954	0.955	0.950	0.950	0.941	0.953	0.951	0.951	0.954
Len.ci	0.093	0.180	0.266	0.353	0.439	0.555	0.737	1.109	2.276
Len.ci.log	0.094	0.181	0.268	0.357	0.443	0.560	0.744	1.119	2.298
Pct.syn.log	100	100	99.4	51.7	3.4	0	0	0	0
Pct.add.log	0	0	0.6	48.2	94.1	46.4	0.2	0	0
Pct.ant.log	0	0	0	0.1	2.5	53.6	99.8	100	100
mean($\hat{\tau}$)	0.220	0.435	0.644	0.879	1.080	1.419	1.850	2.753	5.664
Cov.rate	0.930	0.933	0.918	0.923	0.916	0.945	0.941	0.920	0.931
Cov.rate.log	0.956	0.959	0.952	0.952	0.945	0.956	0.953	0.952	0.956
Len.ci	0.397	0.777	1.151	1.562	1.926	2.552	3.381	5.108	10.966
Len.ci.log	0.463	0.901	1.336	1.812	2.237	2.971	3.972	6.018	13.122
Pct.syn.log	94.6	52.3	20.8	6.5	3.1	0.5	0.1	0	0
Pct.add.log	5.4	47.7	79.2	92.2	94.5	91.1	79.2	48.4	5.9
Pct.ant.log	0	0	0	1.3	2.4	8.4	20.7	51.6	94.1

Table 2

Fractions of squamous cell carcinoma cells (UMSCC22B) surviving after 72 hours of treatment by single and combination dose levels of SCH66336 and 4-HPR and the fitted median-effect parameters.

SCH66336 dose (μM)	4-HPR dose dose (μM)	Fractional survival	Median-effect parameters
0.1		0.6701	$\hat{\beta}_{0,1} = 0.094(0.085)$
0.5		0.6289	$\hat{\beta}_{1,1} = -0.335(0.066)$
1		0.5577	$\hat{D}m_1 = 1.326$
2		0.4550	$\hat{\sigma}_1 = 0.187$
4		0.3755	
	0.1	0.7666	$\hat{\beta}_{0,2} = 0.217(0.073)$
	0.5	0.5833	$\hat{\beta}_{1,2} = -0.398(0.058)$
	1	0.5706	$\hat{D}m_2 = 1.726$
	2	0.4934	$\hat{\sigma}_2 = 0.129$
0.1	0.1	0.6539	$\hat{\beta}_{0,12} = -0.225(0.092)$
0.5	0.5	0.4919	$\hat{\beta}_{1,12} = -0.596(0.082)$
1	1	0.3551	$\hat{D}m_{12} = 0.686$
2	2	0.2341	$\hat{\sigma}_{12} = 0.182$

Note: The number inside the parentheses in the last column is the standard error of the estimate.

Table 3

Inhibition of horse liver alcohol dehydrogenase by o-phenanthroline and ADP alone and in combination (Chou and Talalay 1984; Belen'kii and Schinazi 1994).

o-phenanthroline	ADP	Fractional Inhibition	Median-effect parameters
8.7		0.132	$\hat{\beta}_{0,1} = -4.696(0.145)$
17.4		0.267	$\hat{\beta}_{1,1} = 1.302(0.046)$
26.1		0.411	$\hat{D}m_1 = 36.803$
34.8		0.476	$\hat{\sigma}_1 = 0.058$
43.5		0.548	
	0.5	0.175	$\hat{\beta}_{0,2} = -0.601(0.079)$
	1.0	0.400	$\hat{\beta}_{1,2} = 1.178(0.127)$
	1.5	0.492	$\hat{D}m_2 = 1.666$
	2.0	0.542	$\hat{\sigma}_2 = 0.161$
	2.5	0.592	
$9.2 \times \frac{17.4}{18.4}$	$9.2 \times \frac{1}{18.4}$	0.507	$\hat{\beta}_{0,12} = -3.843(0.038)$
$18.4 \times \frac{17.4}{18.4}$	$18.4 \times \frac{1}{18.4}$	0.769	$\hat{\beta}_{1,12} = 1.739(0.012)$
$27.6 \times \frac{17.4}{18.4}$	$27.6 \times \frac{1}{18.4}$	0.872	$\hat{D}m_{12} = 9.117$
$36.8 \times \frac{17.4}{18.4}$	$36.8 \times \frac{1}{18.4}$	0.919	$\hat{\sigma}_{12} = 0.015$
$46.0 \times \frac{17.4}{18.4}$	$46.0 \times \frac{1}{18.4}$	0.944	

Note: The number inside the parentheses in the last column is the standard error of the estimate.