Drug Interaction Assessment with Repeated Measurements in Fixed Ray Design

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Abstract

We considered the problem of assessing the joint effects of combined therapies in *in-vitro* studies with repeated measurements. To better estimate the confidence interval of combination index at a combination dose with observed effect, we proposed the mixed effects linear regression in the estimation of the dose-effect curve for each single drug and their combination. With unbiased estimation of variance components in median effect model, our approach improves the accuracy in construction of confidence interval of combination index for hypothesis testing of synergistic effects.

Key Words: Loewe additivity model; Mixed Effects Linear Regression; Repeated Measurements; Synergy.

1. Introduction

Chou and Talalay (1984) and Chou (1991) proposed a procedure to characterize a two-drug interaction. Assuming that both the marginal dose-effect curves for single agents and the dose-effect curve for the combination doses at a fixed ray (i.e., $d_1/d_2 = c$, where c is a constant forming a ray in the $d_1 \times d_2$ dose plane), they assessed the drug interaction at the observed combinations using combination indices, which estimated a statistic derived from loewe's additivity model (Berenbaum, 1985, 1989; Greco et al., 1995; and Tallarida, 2000, Lee et al., 2007). Lee and Kong (2009) proposed an analytic approximation to construct the confidence intervals for the combination indices, which was similar to what was generated using Monte Carlo techniques (Belen'kii and Schinazi 1994).

Although combination index can be estimated with only one observation at each combination dose, there were new development in the design of the experiments for drug combination assessment nowadays. Given the relatively low cost to conduct *in-vitro* study, repeated measurements were collected to improve the accuracy in estimation of interaction indices. For example, a guideline "In vitro synergy characterization Design and methodology" proposed by Sanofi recommended to perform experiment 3 times sequentially with 10 concentrations in triplicates to have robust estimations on synergy for the ray design to investigate synergy for different ratio of the compounds in the mixture. However, the construction of the combination index and its confidence interval were simply implemented by applying the same method after averaging over the effects (for example, the percentage of inhibition, or proportion of cell surviving) at each single or combination dose.

To gain efficiency, the ray design was used to pool data at various combination doses to form a better estimate of the combination index. The objective of the following work is

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to improve the accuracy in the construction of confidence interval for combination index in a ray design, by taking into account variability between experiments and replicates of the repeated measurements.

2. Combination Index and its Confidence Interval for Median Effects Model

Chou and Talalay (1984) used a ray design to assess drug interactions. The advantage of their method is that it used all observations with the component doses at a fixed ray. For that fixed ray, Chou and Talalay's median-effect equation has the following form

$$E = \text{logit}^{-1}\left(\frac{d}{D_m}\right)^m = \frac{\left(\frac{d}{D_m}\right)^m}{1+\left(\frac{d}{D_m}\right)^m} \tag{1}$$

where d is the dose of a drug eliciting effect E, D_m is the median effective dose of a drug, and m is Hill coefficient, a slope parameter depicting the shape of the curve. When E describes the proportion of cell surviving, m is negative, and the curve described by Equation (1) falls with increasing drug concentration; when E describes the percentage of inhibition, m is positive, and the curve rises with increasing drug concentration.

A transformation of Chou and Talalay's median-effect equation can be written as

$$\text{logit}(E) = \log\frac{E}{1-E} = m(\log(d) - \log(D_m)) = \beta_0 + \beta_1 \log(d), \tag{2}$$

where $\beta_0 = -m\log(D_m)$ and $\beta_1 = m$. Suppose model (2) has the form

$$\text{logit}(E) = \beta_0 + \beta_1 \log(d) + \epsilon, \tag{3}$$

with model error ϵ following $N(0, \sigma^2)$, the marginal dose-effect curve for drug i can be estimated by $\text{logit}(E) = \hat{\beta}_{0,i} + \hat{\beta}_{1,i}\log(d)$, for $i = 1, \cdots, k$. Considering a drug combination on a fixed ray as a special drug with dose-effect curve $\text{logit}(E) = \hat{\beta}_{0,c} + \hat{\beta}_{1,c}\log(d)$ where $d = \sum_{i=1}^{k} d_i$ is the total dose of the combination, the combination index for a single observation $\mathbf{d} = (d_1, \cdots, d_k)$ on that ray can be estimated by

$$\hat{\tau}(\mathbf{d}) = \sum_{i=1}^{k} \frac{d_i}{\hat{D}_{y,i}} = \sum_{i=1}^{k} \frac{d_i}{\exp\left(-\frac{\hat{\beta}_{0,i}}{\hat{\beta}_{1,i}}\right)\left(\frac{y}{1-y}\right)^{1/\hat{\beta}_{1,i}}} = d\sum_{i=1}^{k} \frac{p_i}{\exp\left(-\frac{\hat{\beta}_{0,i}}{\hat{\beta}_{1,i}}\right)\left(\frac{y}{1-y}\right)^{1/\hat{\beta}_{1,i}}}, \tag{4}$$

where $p_i = d_i/d$.

Under the assumption that the dose-effect curves follow Chou and Talalay's median-effect equation, Lee and Kong (2009) investigated the characteristics of the combination index and its logarithmic transformation, and propose a procedure to construct the confidence interval for the estimated combination index by approximating the variance of $\text{Var}(\hat{\tau}(\mathbf{d}))$ using delta method (Bickel and Doksum, 2001).

3. Combination Index and its Confidence Interval for Median Effects Model with Repeated Measurements

Suppose a plate-wise bias α_j existed in repeated measurements for model (2)

$$\text{logit}(E_{i,j}) = \beta_0 + \alpha_j + \beta_1 * \log(d_i) + \epsilon_{i,j}, \tag{5}$$

where α_j follows $N(0, \sigma_\alpha^2)$ with n_c and n_i being the number of observations when k drug combination was used or drug i used alone, $i = 1, \cdots, k$. The combination index for a single observation $\mathbf{d} = (d_1, \cdots, d_k)$ can be adapted to

$$\hat{\tau}(\mathbf{d}) = d\sum_{i=1}^{k} \frac{p_i}{\hat{D}_{\bar{y},i}} = d\sum_{i=1}^{k} \frac{p_i}{\exp\left(-\frac{\hat{\beta}_{0,i}}{\hat{\beta}_{1,i}}\right)\left(\frac{\bar{y}}{1-\bar{y}}\right)^{1/\hat{\beta}_{1,i}}}. \tag{6}$$

To estimate $\mathrm{Var}(\hat{\tau}(\mathbf{d}))$ with normal approximation, we first derived an approximate variance of $\mathrm{Var}(\log(\hat{\tau}(\mathbf{d})))$ when $\log(\hat{\tau}(\mathbf{d}))$ is more symmetric than $\hat{\tau}(\mathbf{d})$. Let $\log(\hat{\tau}(\mathbf{d})) = \log(d) + h(\hat{\boldsymbol{\beta}}, \bar{y})$ with

$$h(\boldsymbol{\beta}, \bar{y}) = \log\left(\sum_{i=1}^{k} \frac{p_i}{\hat{D}_{\bar{y},i}}\right)$$

and $\mathrm{Cov}(\hat{\boldsymbol{\beta}})$ the variance-covariance matrix of $2k$ parameters $\hat{\boldsymbol{\beta}} = (\hat{\beta}_{0,1}, \hat{\beta}_{1,1}, \cdots, \hat{\beta}_{0,k}, \hat{\beta}_{1,k})$. Because the combination index (6) was a function of $\bar{y}$ at constant dose $\mathbf{d}$ on a fixed ray, $\mathrm{Var}(\log(\hat{\tau}(\mathbf{d}))) = \mathrm{Var}(h(\hat{\boldsymbol{\beta}}, \bar{y}))$.

Denote

$$H(\boldsymbol{\beta}, y) = \exp(h(\boldsymbol{\beta}, y)) = \exp\left(\sum_{i=1}^{k} \frac{p_i}{\hat{D}_{y,i}}\right).$$

Using multivariate delta method, we can approximate the variance of $\log(\hat{\tau}(\mathbf{d}))$,

$$\begin{aligned}
\log(\hat{\tau}(\mathbf{d})) &= \mathrm{Var}(h(\hat{\boldsymbol{\beta}}, \bar{y})) \qquad (7)\\
&\approx \frac{1}{H(\hat{\boldsymbol{\beta}}, \bar{y})}\mathrm{Var}(H(\hat{\boldsymbol{\beta}}, \bar{y}))\\
&= \sum_{i=1}^{k}\left(\frac{p_i}{H(\hat{\boldsymbol{\beta}}, \bar{y})D_{\bar{y},i}}\right)^2\left(\frac{1}{\hat{\beta}_{1,i}^2}\mathrm{Var}(\hat{\beta}_{0,i}) + 2\frac{\log\frac{y}{1-y} - \hat{\beta}_{0,i}}{\hat{\beta}_{1,i}^3}\mathrm{Cov}(\hat{\beta}_{0,i}, \hat{\beta}_{1,i})\right.\\
&\quad \left. + \frac{(\log\frac{y}{1-y} - \hat{\beta}_{0,i})^2}{\hat{\beta}_{1,i}^4}\mathrm{Var}(\hat{\beta}_{1,i})\right)\mathrm{Var}(\log\frac{\bar{y}}{1-\bar{y}}). \qquad (8)
\end{aligned}$$

The last equation holds because (a) $p_1, \cdots, p_k$ are constant on the fixed ray; (b) $\mathrm{Cov}(\hat{\boldsymbol{\beta}})$ is indeed blocked diagonal with each block being a 2×2 matrix when the pairs $(\hat{\beta}_{0,i}, \hat{\beta}_{1,i})$ and $(\hat{\beta}_{0,j}, \hat{\beta}_{1,j})$ are independent for $i \neq j$; and (c) $\bar{y}$ is only dependent on the pair $(\hat{\beta}_{0,c}, \hat{\beta}_{1,c})$ but independent of $\hat{\boldsymbol{\beta}}$.

A natural estimate for the last multiplier $\mathrm{Var}(\log\frac{\bar{y}}{1-\bar{y}})$ in (8) is the mean square root of the model variance σ^2 in (5). As we would tell from the simulation study in next section, the model variance estimator in (5) has much smaller bias than that in (2).

Once (8) the variance for $\log(\hat{\tau}(\mathbf{d}))$ is obtained, a $(1-\alpha)\times 100\%$ confidence interval for $\log(\tau)$ can be constructed as

$$\left[\log(\hat{\tau}) - z_{\alpha/2}\sqrt{\mathrm{Var}(log(\hat{\tau}))}, \log(\hat{\tau}) + z_{\alpha/2}\sqrt{\mathrm{Var}(log(\hat{\tau}))}\right],$$

where $z_{\alpha/2}$ is the $1-\alpha/2$ percentile of standard normal distribution. Note the large sample approximation using normal distribution is considered especially reasonable in our setting of repeated measurements when the total number of observations $n = n_c + \sum_{i=1}^{k} n_i$ is much larger than $2k+2$.

Thus, a $(1-\alpha)\times 100\%$ confidence interval for τ can be approximated by

$$\left[\hat{\tau}\exp\left(z_{\alpha/2}\sqrt{\mathrm{Var}(log(\hat{\tau}))}\right),\hat{\tau}\exp\left(z_{\alpha/2}\sqrt{\mathrm{Var}(log(\hat{\tau}))}\right)\right]. \quad (9)$$

4. Simulation Study

To examine whether the confidence intervals proposed in last section 3 have proper characteristics, we simulated two drugs that followed the median-effect Equation (5) with the same slope $m = 2$ and median effective doses: $Dm_1 = Dm_2 = Dm_3 = 0$. That setting was identical to the "sham combination" in Berenbaum (1989), giving us the additive effects at an arbitaray dose combination with a constant combination index of 1.

We took a fixed ray with ratio $d_2/d_1 = 1/1$ and constant standard deviation in model error $\sigma = 0.25$. Two settings of within-group standard deviation σ_α, 0.2 and 0.4, were tested for the sensitivity of random effects to estimation of combination index. The replicates of dose effects were generated on three doses 0.8, 1.6 and 3.2, for each of the single drug, and three doses, (0.6,0.6), (1.2, 1.2) and (2.4, 2.4), for the mixture (d1, d2) at the fixed ray, using the median effects model

$$\log\frac{E_{i,j}}{1-E_{i,j}} = \beta_0 + \alpha_j + \beta_1 * \log(d_i) + e_{i,j},$$

with $e\ N(0,\sigma^2)$.

As a function of the model error $\mathrm{Var}(\log\frac{\bar{y}}{1-\bar{y}})$, the estimate of the 95% confidence interval for the combination indices at observed combination dose strongly depends on the estimation accuracy of model variance σ^2. In Table 4 we compared the standard deviation and root mean square error (RMSE) of the estimated σ^2 using the proposed method or the median effects model in Chou and Talalay (1984). In both scenarios, our method not only unbiasedly estimates the σ^2, but also significantly reduced the variance and RMSE of the estimate.

	$\sigma = 0.2$		$\sigma = 0.4$	
	ZS16	CT84	ZS16	CT84
mean	-0.001	-0.145	-0.005	-0.291
median	-0.001	-0.153	-0.006	-0.308
st.dev	0.035	0.042	0.066	0.084
RMSE	0.035	0.151	0.066	0.303

Table 1: The mean, median, standard deviation and root mean square errors (RMSE) for the estimation of model error $\hat{\sigma} - \sigma$. We compared the proposed method (ZS16) with the median effects model in Chou and Talalay (CT84) using 1000 simulations.

Because the construction of the 95% confidence interval was under $H_0 : II = 1$, we next assess the probabilities to conclude synergistic, additive, antagnistic effects based on 95% confidence intervals of combination indices under H_0. In both scenarios, our proposed method has the probability of additive effects reasonably close to 95%. The 95% confidence intervals constructed based on Lee and Kong (2009) for Chou and Talalay (1984) under-estimates the variance of combination indices, leading to a larger than expected probabilities of false conclusions of synergistic or antagnisitic effects.

	ZS16			CT84		
Dose	0.6	1.2	2.4	0.6	1.2	2.4
$\sigma = 0.2$						
Synergistic	0.009	0.003	0.006	0.161	0.123	0.125
Additive	0.975	0.962	0.953	0.671	0.702	0.762
Antagnisitic	0.016	0.035	0.041	0.168	0.175	0.113
$\sigma = 0.4$						
Synergistic	0.015	0.006	0.008	0.163	0.124	0.125
Additive	0.958	0.923	0.921	0.674	0.699	0.752
Antagnisitic	0.027	0.071	0.071	0.163	0.177	0.123

Table 2: The probabilities to conclude synergistic, additive, antagnistic effects based on 95% confidence intervals of combination indices under H_0. We compared the proposed method (ZS16) with Chou and Talalay (1984, CT84) using 1000 simulations. The implementation of the 95% confidence interval estimation for CT84 was based on Lee and Kong (2009).

5. Applications

In this section we will apply our methods into two experimental studies conducted for different diseases at UT–MDACC. Dependent on the design of the experiment, the mixed-effects regression model varies in the structure of random effects.

5.1 Combination of Targeted Therapies for Aggressive B-cell Lymphomas

Aggressive B-cell lymphomas, including diffuse large B-cell lymphoma (DLBCL) and mantle cell lymphoma (MCL) continue to be clinically challenging to treat. The fundamental clinical problem is that standard frontline combination chemotherapy for both type of lymphomas achieve lasting remissions but is not curative, highlighting an urgent need for better rational therapy. The objective of the experiment is to explore the combination of targeted therapies for better treatment of aggressive B-cell lymphoma patients.

The experiment at Dr. Lan Pham's lab was conducted on a cell line derived from a primary mantle cell lymphoma tumor sample. A novel inhibitor ABT-199, was investigated together with Ibrutinib, a FDA-approved anticancer drug targeting B-cell malignancies, for their drug interaction effects in combination doses at the fixed ray with $d_2/d_1 = 2/1$. Dose effects were measured in 3 replicates for each dose in either combinations or single agents.

We first obtained the dose-effect curves for ABT-199 and Ibrutinib by a linear mixed-effects regression of $\text{logit}(E)$ on $\log(d)$ based on the cell viability on single agents. The median-effects plot indicates that the data follow the median-effect Equation (5) reasonably well (Figure 1A and 1C). Based on the fitted median-effect equations, we calculated the combination indices based on (6) for varied effects for combination doses at the fixed ray with $d_2/d_1 = 2/1$ and constructed their associated confidence bounds based on (9). Figure (1D) shows the plot of the combination indices (on the logarithm scale) versus effects (solid line) for combination doses at this fixed ray with the point estimates and 95% confidence intervals for observed combinations. Based on the confidence bound (dotted line), we conclude that the combination doses at the fixed ray with with effect below 0.5 are synergistic.

5.2 Combination of Targeted Therapies for Soft Tissue Sarcoma in a Hierarchical Experimental Design

Soft tissue Sarcoma (STS) was a disease derived from connective or supportive tissues, accounting for 1% of adult cancers and 15% of pediatric cancers. Among 70 different histological subtypes of STS, 5–10% are Undifferentiated Pleomorphic Sarcoma (UPS), which typically represents large and fast growing tumors. Patients are still at risk for both recurrence and metastasis after standard treatments, including radiation therapy and chemotherapy. The objective of the experiment is to explore the combination of targeted therapies for better treatment of UPS patients.

A cell line UPS-186, which was derived from a sporadic UPS tumor sample, was used to test the effectiveness of treatment regimens at Dr. Keila Torres's lab. Two novel inhibitors, AEW541 and BGT226, were investigated for their drug interaction effects in combination doses at the fixed ray with $d_2/d_1 = 3/20$. The experiment was repeated for three time, each with triplicates of dose in combination or single agents.

We first obtained the dose-effect curves for AEW541 and BGT226 by a linear mixed-effects regression of logit(E) on log(d) based on the cell viability on single agents. The random effects in the mixed-effects regression had a nested structure to accommodate the specific design feature. The median-effects plot indicates that the data follow the median-effect Equation (5) reasonably well (Figure 2A and 2C). Based on the fitted median-effect equations, we calculated the combination indices based on (6) for varied effects for combination doses at the fixed ray with $d_2/d_1 = 3/20$ and constructed their associated confidence bounds based on (9). Figure (2D) shows the plot of the combination indices (on the logarithm scale) versus effects (solid line) for combination doses at this fixed ray with the point estimates and 95% confidence intervals for observed combinations. Based on the confidence bound (dotted line), we conclude that the combination doses at the fixed ray with with effect between 0.4 and 0.65 are synergistic.

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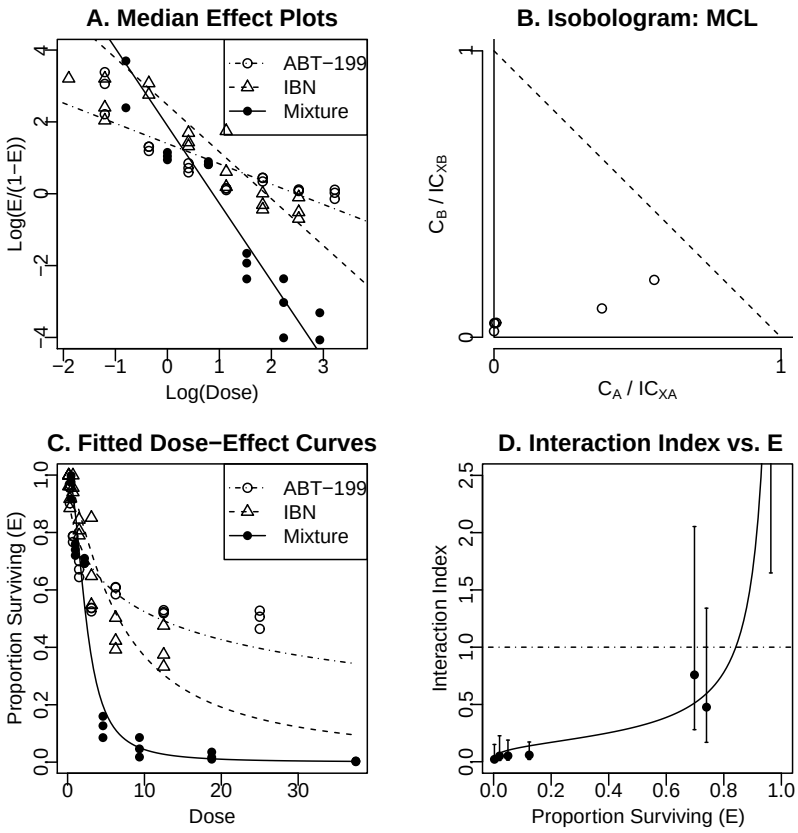

Figure 1: Median-effect plot (Panel A), isobologram (Panel B), plots of dose effect curves (Panel C) and combination indices versus effects (Panel D) for the combination doses at the fixed ray with for ABT-199 and Ibrutinib. In Panel D, the solid line is the plot of the estimated combination indices versus effects (proportion cell surviving). The circles in Panel B from right to left, which gives the point estimates of the combinations in the isobologram, and the vertical bars in Panel D from left to right , which gives the 95% confidence intervals of the combination indices for observed combinations, correspond to the combination doses of (25, 12.5), (12.5, 6.25), (6.25, 3.1), (3, 1.5), (1.5, 0.75), (0.75, 0.37) and (0.3, 0.15), respectively.

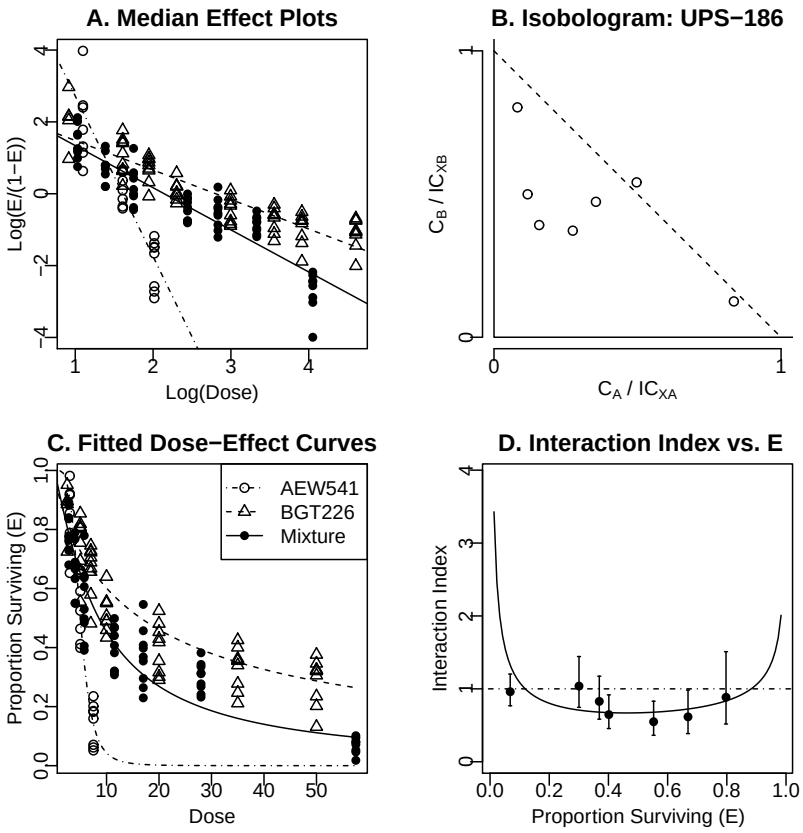

Figure 2: Median-effect plot (Panel A), isobologram (Panel B), plots of dose effect curves (Panel C) and combination indices versus effects (Panel D) for the combination doses at the fixed ray with for AEW541 and BGT226. In Panel D, the solid line is the plot of the estimated combination indices versus effects (proportion cell surviving). The circles in Panel B from right to left, which gives the point estimates of the combinations in the isobologram, and the vertical bars in Panel D from left to right , which gives the 95% confidence intervals of the combination indices for observed combinations, correspond to the combination doses of (7.5, 50), (3, 20), (2, 13.3), (1.5, 10), (0.75, 5), (0.5, 3.3) and (0.3, 2), respectively.