

Interaction Analysis of Three Combination Drugs via a Modified Genetic Algorithm

WEN WAN¹, XIN-YAN PEI², STEVEN GRANT², JEFFREY B. BIRCH³, JESSICA FELTHOUSEN², YUN DAI², HONG-BIN FANG⁴, MING TAN⁴, SHUMEI SUN¹

¹*Department of Biostatistics, Virginia Commonwealth University, Richmond, VA 23298*

²*Department of Internal Medicine, Virginia Commonwealth University, Richmond, VA 23298*

³*Department of Statistics, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061*

⁴*Department of Statistics, Georgetown University, Washington, DC 20057*

Few articles have been written on analyzing and visualizing three-way interactions between drugs. Although it may be quite straightforward to extend a statistical method from two-drugs to three-drugs, it is hard to visually illustrate which dose regions are synergistic, additive, or antagonistic, due to a four-dimensional (4-D) problem of plotting three-drug dose regions plus a response. This problem can be converted and solved by showing some dose regions of our interest in a 3-D, three-drug dose regions. We propose to apply a modified genetic algorithm (MGA) to construct the dose regions of interest after fitting the response surface to the interaction index (II) by a semiparametric method, the model robust regression method (MRR). A case study with three anti-cancer drugs in an *in vitro* experiment is employed to illustrate how to find the dose regions of interest. For example, suppose researchers are interested in visualizing where the synergistic areas with $II \leq 0.4$ are in 3-D. After fitting a MRR model to the calculated II , the MGA procedure is used to collect those feasible points that satisfy the estimated values of $II \leq 0.4$. All these feasible points are used to construct the approximate dose regions of interest in a 3-D.

KEY WORDS: Genetic Algorithm (GA); Interaction Index (II); Model Robust Regression (MRR); Synergism; Three-drug combination; Viability.

1 Introduction

Studies of interactions among drugs have become increasingly important and interesting in many areas of biomedical research including oncology [1]. An effective and accurate evaluation of drug interaction for *in vitro* and/or *in vivo* studies can help to determine whether a combination therapy should be further investigated in clinical trials.

Many different methods have been proposed in the literature on how to evaluate drug interactions. A review by Berenbaum [2] included eight approaches, and another review by Gerco et al [3] categorized 13 approaches for determining drug interactions. A recent review by Lee [4] compared seven models in the anesthetic area. Zhao et al [5] compared four methods: the three popular ones, the isobologram [6], the combination index analysis [7], the universal response surface method [3, 8, 9], and the one developed by Zhao’s lab, the curve shift analysis. They concluded that all the methods were useful and provided complimentary information.

Although there is no uniform agreement on the terminology of drug interaction [4], the Loewe additivity model is considered as the “gold standard” when defining drug interactions [1, 2, 3, 4, 10, 11].

For a dose mixture of a three-drug combination at (x_1, x_2, x_3) , based on the Loewe additivity model, Berenbaum (1977) defined the interaction index (II) at (x_1, x_2, x_3) as

$$II = \frac{x_1}{X_1} + \frac{x_2}{X_2} + \frac{x_3}{X_3}, \quad (1)$$

where X_k represents the dose of the k^{th} drug alone that yields the same response as observed one at the combination (x_1, x_2, x_3) and $k = 1, 2, 3$. Since the value for each X_k is unknown, it typically is estimated from a dose-response curve fit to data resulting from an experiment where each drug is tested alone. Consequently, II must be estimated at each drug combination. This method will be illustrated in Section 4.3. The three drugs are called additive at the combination if the interaction index $II = 1$, or synergistic (antagonistic) if $II < 1 (> 1)$. An appropriate statistical model may then be used for modeling a response surface of the calculated interaction indexes.

In a case of a two-drug combination, a three-dimensional (3-D) surface may provide a complete description on dose effect [2, 3, 13, 14, 15] and may be estimated parametrically, non-parametrically, or semi-parametrically [1, 14, 16]. As mentioned in Fang et al [14], the interpretation of interactions can be easily and visually inspected on either contours of the response surface of interaction indexes [17] or by using the 3-D surface graphical method [15]. Then departures from the theoretical additive surface with the actual estimated surface can be used to identify regions of synergy and/or antagonism.

Previous literature on evaluating drug interactions has focused on the two-drug combination case. One reason may be that a two-drug analysis is easy to illustrate. Another reason for this may be that three-or-more-drug combination is much more complicated to describe and illustrate than the two-drug combination since there may exist not only second order interactions but third order and higher interactions as well. In addition, while it can be quite straightforward to extend a statistical model from a two-drug to a three-drug combination, the graphical display of the response surface

and the contour plot are much more difficult to obtain due to the 4-D nature of the three-drug dose plus a response region.

It is not possible to obtain a 4-D response surface plot but we can obtain a 3-D figure showing some desirable region(s) of interest. In this study, we use the three modeling methods, the parametric (a common response surface method), nonparametric, and semiparametric methods, to model the interaction index surface and the viability surface, respectively. After finding the best method, based on a certain model comparison criteria, for fitting the interaction index surface, we apply a modified genetic algorithm (MGA) [18] to construct dose region(s) of interest which can be visualized in a 3-D plot.

The remainder of this paper is organized as follows. First, we briefly introduce s semiparametric method, the model robust regression (MRR) technique; a nonparametric method; and a more traditional parametric method. Second, we introduce the modified genetic algorithm (MGA) and apply the MGA to construct dose region(s) of interest based on the best model fit. Finally, a real example based on three anti-cancer drugs is introduced to compare the three methods and illustrate how to find interesting region(s) including those representing synergism and/or antagonism.

2 Semiparametric Approach: Model Robust Regression

Semiparametric approaches combine a parametric method with a nonparametric method. One semiparametric method, model robust regression (MRR), proposed by Mays, Birch and Starnes [19], was originally developed for situations when there is partial knowledge about the underlying model, a situation very common in real life. It has been shown that MRR can improve estimates of mean response by combining both the parametric and nonparametric estimates into one estimate, simultaneously reducing both bias and variance of estimation [19, 20].

Suppose we want to estimate the true relationship between the k explanatory variables, $x_{1i}, x_{2i}, \dots, x_{ki}$, and the response variable, y_i , $i = 1, \dots, n$, where n is the sample size. We assume that a random measurement error is independent, identically distributed, with mean zero and constant variance.

To obtain a MRR fit [20], first, obtain a parametric fit to the raw data \mathbf{y} , which can be estimated by the ordinary least squares (OLS) method. Second, obtain a nonparametric fit to the residuals \mathbf{r} that result from fitting the parametric fit to the raw data. Any reasonable nonparametric method can be used such as spline-based methods or kernel-based methods. We used local linear regression (LLR), a kernel-based method, for this application. Third, choose an appropriate mixing parameter λ , where $\lambda \in [0, 1]$ which is used to combine the parametric fit with the nonparametric fit so that the MRR fit can achieve minimum of a prediction error criterion.

Thus, the MRR fit at $\mathbf{x}'_0 = (x_{10}, x_{20}, \dots, x_{k0})$ can be given by

$$\hat{y}_0^{(MRR)} = \check{\mathbf{x}}'_0 (\mathbf{X}'\mathbf{X})^{-1} \mathbf{X}'\mathbf{y} + \lambda \tilde{\mathbf{x}}'_0 (\tilde{\mathbf{X}}'\mathbf{W}_{r_0}\tilde{\mathbf{X}})^{-1} \tilde{\mathbf{X}}'\mathbf{W}_{r_0}\mathbf{r} = \mathbf{h}_0^{(OLS)'} \mathbf{y} + \lambda \mathbf{h}_{r_0}^{(LLR)'} \mathbf{r}, \quad (2)$$

where in its parametric portion, $\check{\mathbf{x}}'_0 = (1, x_{10}, \dots, x_{k0}, x_{(k+1)0}, \dots, x_{(q-1)0})$ with q potential regressors and the matrix $\mathbf{X}' = [\check{\mathbf{x}}'_1, \dots, \check{\mathbf{x}}'_n]'$. In its nonparametric portion, the residual

vector $\mathbf{r} = \mathbf{y} - \hat{\mathbf{y}}^{(OLS)} = \mathbf{y} - \check{\mathbf{x}}_0'(\mathbf{X}'\mathbf{X})^{-1}\mathbf{X}'\mathbf{y}$ is considered as a new response, $\check{\mathbf{x}}_0' = (1 \ x_{10} \ \dots \ x_{k0}) = (1, \mathbf{x}'_0)$, the matrix $\check{\mathbf{X}}' = [\check{\mathbf{x}}_1, \check{\mathbf{x}}_2, \dots, \check{\mathbf{x}}_n]'$, the local weight matrix $\mathbf{W}_{r0} = \text{diag}(w_{r01}^{(KER)}, \dots, w_{r0n}^{(KER)})$, and $w_{r0i}^{(KER)} = \frac{K(\mathbf{x}_0, \mathbf{x}_i)}{\sum_{i=1}^n K(\mathbf{x}_0, \mathbf{x}_i)}$ is a kernel weight associated with

the distance of \mathbf{x}'_i to \mathbf{x}'_0 . We use the so-called Gaussian kernel function in this work, $K(\mathbf{x}_0, \mathbf{x}_i)$, given by

$$K(\mathbf{x}_0, \mathbf{x}_i) \propto K\left(\left\|\frac{\mathbf{x}_0 - \mathbf{x}_i}{b_r}\right\|\right) \quad \text{or} \quad \prod_{j=1}^k K\left(\frac{x_{0j} - x_{ij}}{b_r}\right), \quad (3)$$

where $K\left(\frac{x_{0j} - x_{ij}}{b_r}\right) = e^{-\left(\frac{x_{0j} - x_{ij}}{b_r}\right)^2}$, $j = 1, \dots, k$. The bandwidth parameter b_r , also called the smoothing parameter, is utilized to determine the smoothness of the estimates. The b_r is chosen first and the mixing parameter λ second to achieve a minimum of a type of the prediction error sum of squares (an adjustment to the PRESS statistic, referred to as PRESS** by Wan and Birch [20]). The details of the choice of the bandwidth and the mixing parameter can be found in Wan and Birch [20].

In addition, equation (2) can be written in terms of ‘‘HAT’’ vectors: in the parametric portion $\mathbf{h}_0^{(OLS)'} = \check{\mathbf{x}}_0'(\mathbf{X}'\mathbf{X})^{-1}\mathbf{X}'$, and in the nonparametric portion $\mathbf{h}_{r0}^{(LLR)'} = \check{\mathbf{x}}_0'(\check{\mathbf{X}}'\mathbf{W}_{r0}\check{\mathbf{X}})^{-1}\check{\mathbf{X}}'\mathbf{W}_{r0}$.

Two other methods can be used to fit a model to the response. Based on the settings above for the MRR fit, the parametric method, based on the OLS fit at $\mathbf{x}'_0 = (x_{10}, x_{20}, \dots, x_{k0})$ is

$$\hat{y}_0^{(OLS)} = \check{\mathbf{x}}_0'(\mathbf{X}'\mathbf{X})^{-1}\mathbf{X}'\mathbf{y} = \mathbf{h}_0^{(OLS)'}\mathbf{y}, \quad (4)$$

exactly the same as the parametric proportion of the MRR fit. And for the nonparametric method, the LLR fit is

$$\hat{y}_0^{(LLR)} = \check{\mathbf{x}}_0'(\check{\mathbf{X}}'\mathbf{W}_0\check{\mathbf{X}})^{-1}\check{\mathbf{X}}'\mathbf{W}_0\mathbf{y} = \mathbf{h}_0^{(LLR)'}\mathbf{y}. \quad (5)$$

Instead of using the residuals \mathbf{r} as a response variable as in the nonparametric proportion of the MMR fit, the LLR fit uses the raw data \mathbf{y} directly. The LLR local weight matrix \mathbf{W}_0 is similar to \mathbf{W}_{r0} but with different weights due to using a different bandwidth. The LLR model chooses a bandwidth, b , by minimizing PRESS** of the raw data instead of the residual data.

In this study, the vector \mathbf{x}' is 1×3 , representing dose levels of three drugs. The response variable y is the interaction index or viability which is defined below in equation 6.

3 Using a modified genetic algorithm to find synergistic regions of interest

After building a fitted model in Section 2, we would often like to visualize its entire response surface. However, as previously mentioned, we cannot display a 4-D plot. In this study, instead of showing the entire response surface, we can display those regions that are of interest. For example, those regions displaying synergistic, additive, and

antagonistic interaction behavior, where, for example, whose $\hat{II} \leq 0.4$, $0.9 \leq \hat{II} \leq 1.1$, and $\hat{II} \geq 1.8$, respectively. Here \hat{II} refers to the estimated interaction index that is obtained by one of the three estimation methods described in Section 2 used to fit the II data. This actually becomes an optimization problem, whose objective function is either equation (2), (4) or (5). We use a modified genetic algorithm (MGA) by Wan and Birch[18] to find the region(s) of interest.

A genetic algorithm (GA) and its MGA both are adaptive heuristic global optimization algorithms. In GA terminology, a set of multiple concurrent search points or a set of chromosomes (or individuals) is called a *population*. These chromosomes are evaluated for their performance with respect to an objective function. Each iterative step where a new population is obtained is called a *generation*. The three main GA operations, utilized to generate a new population from the current population in each iterative step, consist of selection, crossover, and mutation. Each GA operation involves randomness. Therefore, if a GA is repeatedly many times, we can imagine that solutions at each generation at each time are different from each other due to its randomness and its heuristic search [18].

The idea of the MGA [18] is to incorporate the Nelder-Mead (NM) simplex algorithm, a local directional search method, into a traditional genetic algorithm process. During each GA iteration cycle, once the GA finds a point of interest, a simplex local search is implemented with the point considered as a starting point for the simplex algorithm. The local search is halted when the objective function fails to either increase or decrease, depending on the optimization goal. During the MGA process, all the points of interest are recorded as long as their \hat{II} values satisfy the requirements (for example, $\hat{II} \leq 0.4$, $0.9 \leq \hat{II} \leq 1.1$, or $\hat{II} \geq 1.8$). Such points are referred to “feasible points.” The MGA combines the advantage of both the GA and NM methods. In the MGA process, the GA component moves quickly throughout the combination mixture space to locate points of interest and the NM simplex component examines the local region around each point of interest in greater detail to collect as many feasible points as possible in those region(s). A basic MGA procedure is provided in Appendix.

Wan and Birch [18] also provided a procedure of using the MGA to determine the approximate feasible regions with the following three stages. Step 1 below is set to find the feasible synergistic area. This step can be easily altered by adjusting the II_{cutoff} if the feasible additive and/or the feasible antagonistic regions are desired.

1. Based on the priority from the experimenters, the II 's feasible solutions are determined. That is, an appropriate cutoff value, II_{cutoff} , is chosen so that all locations found by MGA which achieve corresponding II values less than or equal to this cutoff are regarded as feasible points.
2. When MGA is repeated many times with different random seeds, feasible points are collected. As the number of repetitions of MGA is increased, the approximate feasible regions approach the true feasible regions.
3. Plot these feasible points in a 3-D dose region. Then, based on these plots, calculate the feasible dose regions for each drug.

4 Example: Three Anti-cancer Drugs in *In Vitro* Experiment

In our case study, three anti-cancer drugs were considered CEP-3891 (denoted by CEP), a cell-cycle checkpoint kinase (Chk1) inhibitor, PD-184352 (PD), a MEK1/2 inhibitor, and HA14-1 (HA), a B-cell lymphoma-2 (Bcl-2) inhibitor. The goal of the experiment was to determine whether there are synergism effects of the three drugs against human Multiple Myeloma cells. The doses of these three drugs were denoted by x_{CEP} , x_{PD} , and x_{HA} , respectively. The primary outcome measurement was 7-Aminoactinomycin D (7-AAD) staining, used to measure percentage of killed cancer cells. To adjust the effect of negative control (saline), viability for percentage of survival cells, the response variable of interest, was calculated as follows:

$$\text{viability} = 100 \times (100 - \text{percentage of killed cells}) / (100 - \text{percentage of killed cells at control}). \quad (6)$$

4.1 Study design

Due to the need to reduce developmental time and costs, especially important in cancer drug development [11], for *in vitro* studies, we used the Tan and Fang’s method [11, 14, 21, 22] to determine doses of mixtures of three drugs and sample sizes to detect departures from additivity. The Tan and Fang’s method is designed for the case of non-constant potency of one drug to another and for the presence of nonparallel single dose-response slopes between drugs, a situation very common in practice. The main idea of their experimental design method is to select dose combinations that are uniformly scattered in the experimental domain that maximizes the power of an F-test through a semiparametric model whose nonparametric portion is expected to capture any departures from additivity.

Based on preliminary single drug dose-response data collected initially, the dose mixtures were obtained by the Tan and Fang’s method. There were 30 mixtures ($m = 30$) of the three-drug combinations with eight replicates. The dose ranges of the three drugs in the mixtures are $x_{PD} \in [1.73, 14.78]\mu M$, $x_{HA} \in [1.48, 14.77]\mu M$, and $x_{CEP} \in [0.18, 1.77]\mu M$. The experimental domain in this case was a rectangular cuboid where the lengths of the three dose ranges would be the dimensions of the cuboid. All the dose mixtures obtained in this case were uniformly scattered in the cuboid including the surfaces of the cuboid.

The experimental data was collected and provided by Dr. Steven Grant’s lab, at Virginia Commonwealth University.

4.2 Results on single drug dose response curves

Figure 1 shows the observed single drug dose-response data (red cycles) and three fitted blue lines representing their linear relationships for the three drugs respectively. The response variable is viability. The fitted models can be found in the titles of each sub-figure. The degree-of-freedom-adjusted coefficient of determination (denoted by R_{adj}^2 [24]), a measure of goodness-of-fit of the linear models, was 97.17%, 97.45%, 91.53% for

PD, HA and CEP, respectively. All the three single drug dose-response linear models can explain over 90% variability of the observed data, indicating that all the estimated models fit the data quite well.

[Insert Figure 1 about here.]

Both potency of HA relative to PD and potency of CEP relative to PD are not constant due to their different slopes and intercepts. Definition of potency can be found in Tan et al [11].

4.3 Results on response surfaces of interaction index and viability in mixtures of three-drug combinations

We can calculate the interaction index at m^{th} mixture combination $(x_{PD}^{(m)}, x_{HA}^{(m)}, x_{CEP}^{(m)})$ using (1), once we know the single drug dose-response curves given by the estimated linear models as mentioned in Section 4.2. More specifically, a value of II at the m^{th} mixture and the j^{th} replicate can be given by

$$II_{mj} = \frac{x_{PD}^{(m)}}{(y_{mj} - \hat{\alpha}_{PD})/\hat{\beta}_{PD}} + \frac{x_{HA}^{(m)}}{(y_{mj} - \hat{\alpha}_{HA})/\hat{\beta}_{HA}} + \frac{x_{CEP}^{(m)}}{(y_{mj} - \hat{\alpha}_{CEP})/\hat{\beta}_{CEP}}, \quad (7)$$

where y_{mj} is the viability at the m^{th} combination $(x_{PD}^{(m)}, x_{HA}^{(m)}, x_{CEP}^{(m)})$ and the j^{th} replication, $m = 1, \dots, 30$, $j = 1, \dots, 8$. The $\hat{\alpha}$ s and $\hat{\beta}$ s are the estimated intercepts and slopes, respectively, in the fitted linear models, shown in Figure 1. In the case study, II is considered as a random variable, depending on values of the combination dosages, $(x_{PD}, x_{HA}, \text{ and } x_{CEP})$, all of which are random explanatory variables. We use \hat{II} to represent the estimate of mean response at each dose combination from a fitted model to the II values.

The maximum of the observed viability is 101.62% at $(x_{PD} = 0.0, x_{HA} = 0.0, x_{CEP} = 0.6)$ and its minimum is 5.45% at $(x_{PD} = 13.88, x_{HA} = 12.94, x_{CEP} = 1.61)$. The maximum of the calculated II per definition (7) is 1.81 at $(x_{PD} = 13.88, x_{HA} = 12.94, x_{CEP} = 1.61)$ and its minimum is 0.54 at $(x_{PD} = 3.98, x_{HA} = 1.94, x_{CEP} = 0.56)$. Obviously, there exist synergistic, antagonistic, and additive areas of the three drugs in the dose range of the 30 mixtures.

To discover where the synergistic, antagonistic, and additive areas are, modeling the response surfaces of the random variables II and viability respectively is the next step. As mentioned in Section 2, we apply the MRR/OLS/LLR approaches to model the II and the viability, respectively.

Table 1 shows the results of model comparisons of the OLS, LLR, and MRR methods in II and viability with respect to the four criteria: 1) DF_{error} , the degrees of freedom of error, is given by $DF_{error} = n - tr(\mathbf{H})$ [23], where \mathbf{H} is the "hat" matrix appropriate for each method, MRR, LLR, and OLS. See Wen and Birch [20] for complete definitions; 2) s^2 , estimate of error variance, computed as sum of squares for error divided by DF_{error} ; 3) R_{adj}^2 ; 4) PRESS**, a type of prediction error sum of squares, as mentioned in Section 2. Criteria 1-3 focus on describing how well a model is fit by the observed data, while Criteria 4 focuses on describing the prediction capability associated with

the fitted model. Criteria 2-4 are standard criteria for comparing models [24]. We hope to find a model with the largest DF_{error} and R_{adj}^2 and the smallest s^2 and PRESS**.

[Insert Table 1 about here.]

In Table 1, all adjusted coefficients of determination, R_{adj}^2 , are over 95% indicating that all methods fit very well. The MRR method fits the best in describing II and the LLR method best fits viability in terms of s^2 , R_{adj}^2 , and PRESS**. The MRR fits actually are quite competitive to the LLR fits in both the response surfaces for II and viability. Therefore, we use the MRR fits to obtain the dose regions of interest, which will be mentioned in Section 4.4.

The parametric OLS model may be the easiest way to interpret the interactions between the drugs in the three methods. The OLS fits, also the parametric proportions of the MRR fits, are chosen by the highest R_{adj}^2 as follows.

$$\begin{aligned} \hat{II}^{(OLS)} = & 1.46 - 0.39x_{PD} + 0.11x_{HA} - 1.13x_{CEP} + 0.0014x_{PD}x_{HA} + 0.29x_{PD}x_{CEP} \\ & - 0.016x_{HA}x_{CEP} + 0.0013x_{PD}x_{HA}x_{CEP} + 0.048x_{PD}^2 - 0.0043x_{HA}^2 + 0.44x_{CEP}^2 - 0.0017x_{PD}^3 \\ & + 0.00017x_{HA}^3 - 0.11x_{CEP}^3 - 0.00020x_{PD}^2x_{HA} - 0.034x_{PD}^2x_{CEP} + 0.0013x_{PD}^3x_{CEP}, \end{aligned} \quad (8)$$

and

$$\begin{aligned} \text{viability}^{(OLS)} = & 127.46 - 14.67x_{PD} - 8.60x_{HA} - 57.73x_{CEP} + 0.93x_{PD}x_{HA} - 4.62x_{PD}x_{CEP} \\ & + 5.20x_{HA}x_{CEP} - 0.13x_{PD}x_{HA}x_{CEP} + 1.04x_{PD}^2 + 0.31x_{HA}^2 + 16.14x_{CEP}^2 - 0.029x_{PD}^3 - 0.017x_{HA}^3 \\ & - 0.045x_{PD}^2x_{HA} + 0.30x_{PD}^2x_{CEP} + 0.095x_{HA}^2x_{CEP} + 1.41x_{CEP}^2x_{PD} - 2.35x_{CEP}^2x_{HA}. \end{aligned} \quad (9)$$

Both OLS models are hierarchical lower-order polynomial with interactions between the three drugs. Only one term is not significant with p-value = 0.31 (the interaction term $x_{PD}x_{HA}$ in $\hat{II}^{(OLS)}$) and all the other terms in both models above are significant with p-values < 0.05.

Both OLS models have three regular two-way interactions, a three-way interaction, second-order polynomials, third-order polynomials, and interactions between one drug and second-order polynomial of another drug. The two regressors $x_{PD}^3x_{CEP}$ and $x_{PD}^3x_{CEP}$ in the II OLS model are not in the viability OLS model, while $x_{HA}^2x_{CEP}$, $x_{CEP}^2x_{PD}$, and $x_{CEP}^2x_{HA}$ in the viability model are not in the II model. There exist a total of 14 items in both models, although a few of signs of coefficients in the two models were opposite.

The signs of the estimated coefficients in the viability model (9) indicate that the viability response surface is not a monotonic function of the three drugs. Therefore, unlike II , smaller values of viability may not represent the synergism and larger values of viability may not represent the antagonism, both depending on mixture dosages. Only values of II can tell whether its corresponding mixtures are synergistic, additive, or antagonistic, per the definition in equation (1).

In the estimated OLS model of II in (8), values of II could be $> 1 (= 1, < 1)$, depending on the combination values of dose mixture (x_{PD}, x_{HA}, x_{CEP}). That is, in the dose region with $0 \leq x_{PD} \leq 14.78$, $0 \leq x_{HA} \leq 14.77$, and $0 \leq x_{CEP} \leq 1.77$, there exist some synergistic, antagonistic, and additive areas of the three drugs.

To discover the synergism (antagonism) of two drugs (say, PD and HA), we can set the dose level of the third drug to zero, i.e., $x_{CEP} = 0$ in (8). Then the OLS model becomes

$$\hat{II}^{(OLS)}(PD, HA) = 1.46 - 0.39x_{PD} + 0.11x_{HA} + 0.0014x_{PD}x_{HA} + 0.048x_{PD}^2 - 0.0043x_{HA}^2 - 0.0017x_{PD}^3 + 0.00017x_{HA}^3 - 0.00020x_{PD}^2x_{HA}.$$

Obviously, an estimated \hat{II} value of the model with $x_{CEP} = 0$ could be $> 1 (= 1, < 1)$, therefore, there exist some synergistic, additive, and antagonistic areas of the drugs PD and HA in the region of $0 \leq x_{PD} \leq 14.78$ and $0 \leq x_{HA} \leq 14.77$. Of course, these areas for drugs PD and HA would likely change with values x_{CEP} different from zero as the presence of a significant 3-way interaction indicates that any 2-drug interaction is dependent on the third one.

The next section will show by using MGA how to visualize the synergistic and antagonistic areas of interest of the three drugs.

4.4 Results on regions of interest

The range of the calculated values of II based on observed data is $[0.54, 1.81]$. Suppose researchers are interested in the synergistic areas with $II \leq 0.4$, the additive areas with $0.9 \leq II \leq 1.1$, and the antagonistic areas with $II \geq 1.8$. Using the MRR fit to II , the MGA is used to find the synergistic, additive, and antagonistic areas by those feasible points satisfying the objective functions as follows, respectively:

$$\hat{II}^{(MRR)} \leq 0.4, \tag{10}$$

$$0.9 \leq \hat{II}^{(MRR)} \leq 1.1, \tag{11}$$

$$\hat{II}^{(MRR)} \geq 1.8. \tag{12}$$

Using the procedure in Section 3, we are able to find all feasible points, which satisfy our requirements in (10 - 12). Using recommendations from Wen and Birch (2011), we set each MGA with 300 iterations and we run a MGA about 50-200 times with different random seeds. Once the feasible points are found, we plot them in a 3-D dose region.

Figure 2 shows the two separate synergistic areas of the three-drug dose regions with $II \leq 0.4$ in the two different angles: the left figure is for the regions of PD vs HA vs CEP and the right one is for CEP vs PD vs HA. The colors of dots are changed from red at the front of the cuboid to black at the back. The two separate regions are one with the dose ranges of $0.0 \leq x_{PD} \leq 0.79$, $0.0 \leq x_{HA} \leq 1.47$, $1.56 \leq x_{CEP} \leq 1.77$ and the other with $4.47 \leq x_{PD} \leq 6.77$, $0.0 \leq x_{HA} \leq 0.22$, $0.22 \leq x_{CEP} \leq 0.81$. The smallest II value found by MGA is 0.29 at mixture (0.0, 0.0004, 1.77).

[Insert Figure 2 about here.]

Figure 3 shows the additive three-drug dose areas with $0.9 \leq II \leq 1.1$ in two different angles, which are the same as in Figure 2. From the mixture at about (0.0, 14.77, 1.77), as both HA's and CEP's dose levels decrease and PD's gradually increases,

the additive area become more wider. Comparing the two right-side figures in Figures 2 and 3, the region under the additive surface is likely to be synergistic, while the region above the surface is likely to be antagonistic.

[Insert Figure 3 about here.]

Figure 4 shows four antagonistic areas with $II \geq 1.8$ in two different angles. The biggest area is a triangular based pyramid, whose apex is at $(0.0, 14.77, 0.0)$. As levels of PD and CEP increase and level of HA decreases simultaneously, the base of the pyramid becomes wider. This pyramid reaches out an “arm” toward CEP. That is, as level of CEP increases with fixed levels of $x_{PD} = 0.0$, and $x_{HA} = 14.77$, it is also antagonistic. There are two small antagonistic areas like two “rain drops” from the pyramid: one is with both x_{PD} and x_{CEP} around 0.0 and x_{HA} in about $[2.5, 4.0]$; and the other is with x_{CEP} around 0.0, x_{HA} around 14.77, and x_{PD} in about $[10.0, 13.5]$. The fourth antagonistic area is another pyramid whose apex is at about $(14.78, 14.77, 1.77)$, the largest dose combination. The largest II value found is 3.0 at $(14.78, 14.77, 1.73)$. All these areas are above the additive surface shown in the right graph of Figure 3.

[Insert Figure 4 about here.]

5 Conclusion and Discussion

Studies of interactions among drugs have become increasingly important and interesting, though little research exists that describes interactions for three or more drug combinations. Through a case study of three anti-cancer drugs, we propose to apply the MGA procedure to construct combination regions of interest after fitting a MRR model of interaction index (II). This method can be easily extended to a case of four or more drug combinations for finding combination regions of interest, by using conditional plots, that is, 3-D plots conditioned on a specific value or range of values of the other drugs.

In this study, the Loewe additivity model is used as the “gold standard” to define interactions of three-drug combinations: synergistic ($II < 1$), additive ($II = 1$), and antagonistic ($II > 1$). Since II is considered as a random variable with a certain variability, its range $[0.9, 1.1]$ was recommended as “nearly additive” by the computer software Calcsyn [25].

A semiparametric method, MRR, was used to model the response surface of estimated values of II . The main idea of MRR is to account for deficiencies in a possibly misspecified parametric model utilizing any systematic structure contained in the residuals resulting from parametric fit. In this study, our parametric model, a lower-order polynomial model with interactions, is fit to the II values via the OLS method. The resulting residuals are then smoothed with an appropriate nonparametric method (LLR is used in this paper). The resulting smoothed fit to the residuals is then combined with the parametric fit via a mixing parameter, λ , chosen to minimize an adjusted prediction oriented criteria. MRR was designated for those situations when there is partial knowledge about the underlying model. In the case study, the three methods OLS,

LLR, and MRR for fitting the II response surface are compared. All three methods fit the calculated II values very well with their adjusted coefficients of determination all over 95%. In terms of prediction error sum of squares and the estimate of error variance, MRR fits are the best in describing II and very competitive to LLR in describing viability. The estimated II values ranged from 0.54 to 1.81 indicating that in the combination regions, some areas are synergistic, some are additive, and others are antagonistic. In other words, combinations of the three drugs (PD, HA and CEP) are not always synergistic.

After fitting the response surface to the calculated II response values, we apply the MGA procedure to construct combination dose regions of interest such as the synergistic areas with $II \leq 0.4$, the additive areas with $0.9 \leq II \leq 1.1$, and the antagonistic areas with $II \geq 1.8$. The MGA procedure, introduced by Wan and Birch (2011), is actually a improved Hybrid Genetic Algorithm (HGA). Its main idea is that with benefits from the stochastic property of a GA, we let GA quickly locate the feasible regions and then utilize the Nelder-Mead simplex method, a local optimization method, to collect the feasible points. This process should be repeated many times with different random seeds so that the collected feasible points can approximately generate the regions of interest. In addition, MGA or HGA are global optimization tools, which can deal with any high-dimensional nonlinear model. Wan and Birch [18] mentioned that MGA could be a good choice for finding all feasible regions, even for very high dimensional, complicated problems.

During the process of collecting feasible points of interest, we found that sometimes too many feasible points were collected resulting in a very nice looking smooth shape but one so large as to be too hard to display in a timely manner. For such cases, we remove all redundancy points (i.e., duplicates) and even randomly remove some feasible points. This remedy does little to alter the shape of the region but greatly enhances the ability to display the plot.

Whether a combination dose region is synergistic or not completely depends on a value of II . Therefore the accuracy of the II values is very important. Following its definition, the value of II depends on the doses of single drugs alone that yield the same response as observed at a particular combination of drugs. Therefore, improving the accuracy of the single drug dose response curves may improve the quality of II . In the future work, we plan to use the MRR method to fit the single drug dose response curve. The results may be a better description of the data though it will likely complicate the computation required to compute II .

A possible limitation of the study is that the viability values of a single drug dose response of CEP are all $> 70\%$ in Figure 1, which may be unrealistic. Another possible limitation is determining the boundaries of \hat{II} for classifying the dose combinations for additivity, synergistic, and antagonistic regions, especially for additivity region. In our case study, the boundaries of the additivity region were chosen based on the recommendation of the software Calcsym [25] and they may not be appropriate for other case studies. There likely exist more formal quantitative methods for determining these boundaries. One possible method is for each dose combination selected by the MGA, \mathbf{x}_0 , compute not only the estimated interaction index there, \hat{II}_0 , but its 95% confidence interval. If this interval contains one, supporting the hypothesis that $II_0 = 1$, then \mathbf{x}_0 is considered as a feasible point in the additivity region. If the upper

confidence limit is substantially below (above) one, then \mathbf{x}_0 is considered as a feasible in the synergistic (antagonistic) region. Other reasonable methods exist as well.

R code and C++ code are available upon request from the authors.

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References

- [1] Kong M and Lee JJ. A Generalized Response Surface Model with Varying Relative Potency for Assessing Drug Interaction. *Biometrics*. 2006; 62(4):986-995.
- [2] Berenbaum MC. What is synergy? *Pharmacological Reviews*. 1989; 41:93-141.
- [3] Greco WR, Bravo G, and Parsons JC. The search for synergy: a critical review from a response surface perspective. *Pharmacological Reviews*. 1995; 47:331-85.
- [4] Lee S. Drug interaction: focusing on response surface models. *Korean Journal of Anesthesiology*. 2010 May; 58(5):421-434.
- [5] Zhao L, Au JLS, and Wientjes MG. Comparison of method for evaluating drug-drug interaction. *Frontiers in Bioscience*. 2010; 2:241-249.
- [6] Fraser TR. The antagonism between the actions of active substances. *British Medical Journal*. 1872; 2(618):485487.
- [7] Chou TC and Talalay P. Quantitative analysis of dose-effect relationships: the combined effects of multiple drugs or enzyme inhibitors. *Advances in Enzyme Regulation*. 1984; 22:2755. [PubMed: 6382953]
- [8] Faessel HM, Slocum HK, Jackson RC, Boritzki TJ, Rustum YM, Nair MG, and Greco WR. Super in vitro synergy between inhibitors of dihydrofolate reductase and inhibitors of other folate-requiring enzymes: the critical role of polyglutamylation. *Cancer Research*. 1998; 58:30363050. [PubMed: 9679969]
- [9] Greco WR, Park HS, and Rustum YM. Application of a new approach for the quantitation of drug synergism to the combination of cis-diamminedichloroplatinum and 1-beta-Darabinofuranosylcytosine. *Cancer Research*. 1990; 50:53185327. [PubMed: 2386940]
- [10] Lee JJ, Kong M. Confidence Intervals of Interaction Index for Assessing Multiple Drug Interaction. *Statistics in Biopharmaceutical Research*. 2009; 1(1): 4-17.
- [11] Tan MT, Fang HB, and Tian GL. Dose and Sample Size Determination for Multi-Drug Combination Studies. *Statistics in Biopharmaceutical Research*. 2009; 1(3): 301-316.

- [12] Berenbaum MC. Synergy, additivism and antagonism in immunosuppression. *Clinical and Experimental Immunology*. 1977; 28:118.
- [13] Copenhaver TW, Lin TL, Goldberg KM. Joint drug action: a review. *American Statistical Association, Proceedings of the Biopharmaceutical Section*, San Francisco, 1987; 160164.
- [14] Fang HB, Ross DD, Sausville E, and Tan M. Experimental design and interaction analysis of combination studies of drugs with log-linear dose responses. *Statistics in Medicine*. 2008. 27:30713083
- [15] Prichard MN, Shipman Jr C. A three-dimensional model to analyze drugdrug interactions. *Antiviral Research*. 1990; 14:181206.
- [16] White DB, Faessel HM, Slocum HK, Khinkis L, Greco WR. Nonlinear response surface and mixture experiment methodologies applied to the study of synergism. *Biometrical Journal*. 2004; 46:5671.
- [17] Suhnel J. Evaluation of synergism or antagonism for the combined action of antiviral agents. *Antiviral Research*. 1990; 13:2340.
- [18] Wan W, Birch JB. Using a Modified Genetic Algorithm to Find Feasible Regions of a Desirability Function. *Quality and Reliability Engineering International*. 2011; 27(8); 1173-1182.
- [19] Mays JE, Birch JB, Starnes BA. Model robust regression: combining parametric, nonparametric, and semiparametric methods. *Journal of Nonparametric Statistics*. 2001; 13: 245-277.
- [20] Wan W, Birch JB. A Semiparametric Technique for the Multi-Response Problem. *Quality and Reliability Engineering International*. 2011; 27(1): 47-59.
- [21] Tan, M., Fang, H.B., Tian, G. L., and Houghton, P. J. Experimental Design and Sample Size Determination for Testing Synergy in Drug Combination Studies Based on Uniform Measures. *Statistics in Medicine*. 2003; 22: 20912100.
- [22] Fang HB, Tian GL, Li W, Tan M. Design and Sample Size for Evaluating Combinations of Drugs of Linear and Loglinear Dose-Response Curves. *Journal of Biopharmaceutical Statistics*. 2009. 19: 625640.
- [23] Ruppert D, Wand M, Carroll R. *Semiparametric Regression*. Cambridge University Press, Cambridge. 2003.
- [24] Myers RH. *Classical and Modern Regression with Applications*. Duxbury, Thomson Learning. 1990.
- [25] Chou TC and Hayball MP. CalcuSyn: Windows software for dose effect analysis, *Cambridge(U.K.): Biosoft*. 1996. <http://www.biosoft.com/w/calculusyn.htm>

APPENDIX: Modified Genetic Algorithm Used to Find Feasible Points

A basic MGA procedure has the following steps (Wan and Birch, 2011).

1. Define an objective/fitness function, and its variables. Set GA operations (such as population size, parent/offspring ratio, selection method, number of crossovers and mutation rate).
2. Randomly generate the initial population. That is, obtain $\mathbf{x}_1, \dots, \mathbf{x}_n$, where n is the number of chromosomes in a population, also called the population size.
3. Evaluate each chromosome in the initial population by the objective function. That is, compute $II(\mathbf{x}_1), \dots, II(\mathbf{x}_n)$, where $II(\mathbf{x}_i)$ is the objective function evaluated at the i^{th} chromosome \mathbf{x}_i .
4. Generate an offspring population, by GA operations (such as selection/mating, crossover, and mutation). That is, generate a new set of n settings of the three drugs, using the operations of selection, crossover, and mutation of $\mathbf{x}_1, \dots, \mathbf{x}_n$ to obtain $\mathbf{x}_1^*, \dots, \mathbf{x}_n^*$.
5. Evaluate each individual in the offspring population by the objective function.
6. Decide which individuals to include in the next population. This step is referred to as “replacement” in that individuals from the current parent population are “replaced” by a new population, whose individuals come from the offspring and/or parent population.
- D. Is the best chromosome in the offspring population also the best over the current parent population and does the best offspring have a II value satisfying the requirement, say $II \leq 0.4$?
 - D-1. If no, directly go to Step 6.
 - D-2. If yes, then define and implement a NM local direction search with that chromosome as a starting point. The local search will be ended when the objective function fails to progress. Find the chromosome with the best II value and replace the best chromosome with the best II value in the offspring population with this point. Then go to Step 6.
7. If a stopping criterion is satisfied, then the procedure is halted. Otherwise, go to Step 4.

In this study there are three ($k = 3$) variables, i.e., there are $k = 3$ genes in each chromosome. We also make the following assumptions. The (parent) population size n is $2k$ and the offspring population size is also $2k$. The type of selection we utilize is random pairing. The blending crossover is utilized and the number of crossover points is one. Random uniform mutation is utilized and the mutation rate is set around or equal to $1/k$. The type of replacement over both parent and offspring populations is tournament. Details about the settings can be found in Haupt and Haupt (2004).

Table 1: Results on model comparisons of the OLS, LLR, and MRR methods in \hat{II} and viability. Best values in bold.

		b	$\hat{\lambda}$	DF_{error}	s^2	R_{adj}^2	PRESS**
\hat{II}	OLS	—	—	222.000	0.00190	0.9786	0.5019
	LLR	1.000	—	209.045	0.00176	0.9801	0.4821
	MRR	1.000	0.73	212.541	0.00175	0.9802	0.4753
viability	OLS	—	—	284.000	27.1399	0.9617	8764.24
	LLR	1.000	—	257.975	9.8163	0.9862	3535.98
	MRR	0.785	0.95	258.883	9.9995	0.9859	3569.50

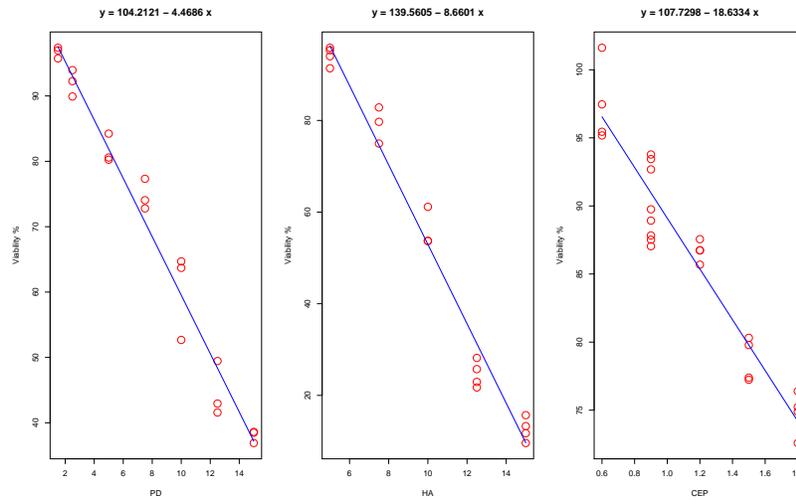


Figure 1: Plots of the single drug dose-response curves: Left: PD; Middle: HA; Right: CEP, respectively.

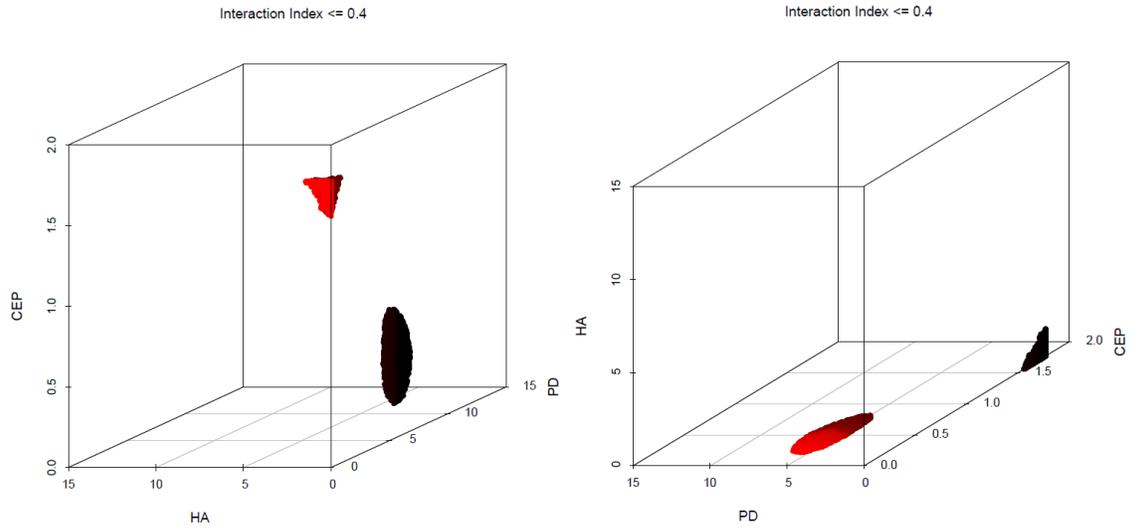


Figure 2: Plot of three-drug dose regions of synergistic areas with $II \leq 0.4$: Left: PD vs HA vs CEP; Right: CEP vs PD vs HA, respectively. The colors of dots are changed from red at the front of the cuboid to black at the back.

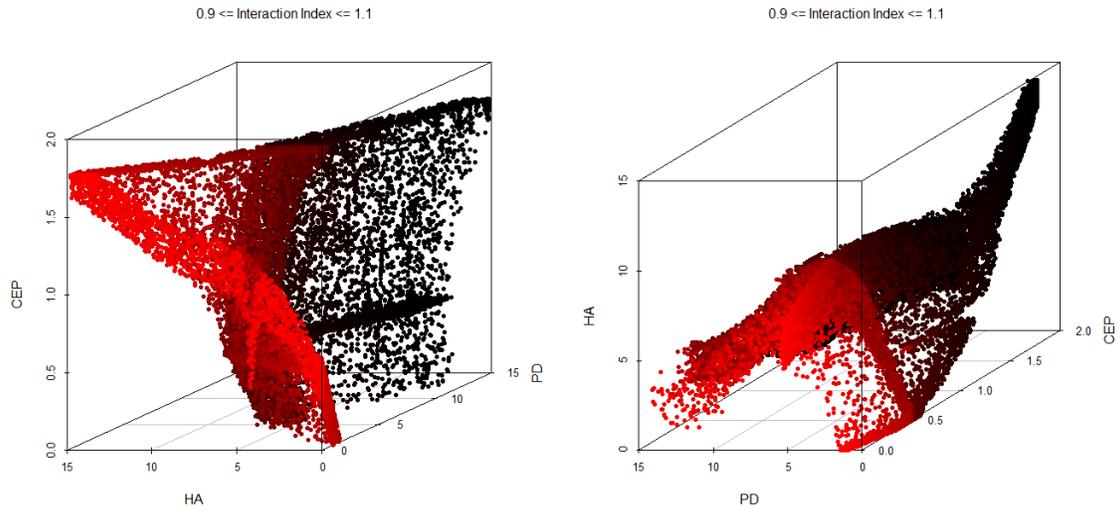


Figure 3: Plots of three-drug dose regions of additive areas with $0.9 \leq II \leq 1.1$: Left: PD vs HA vs CEP; Right: CEP vs PD vs HA, respectively. The colors of dots are changed from red at the front of the cuboid to black at the back.

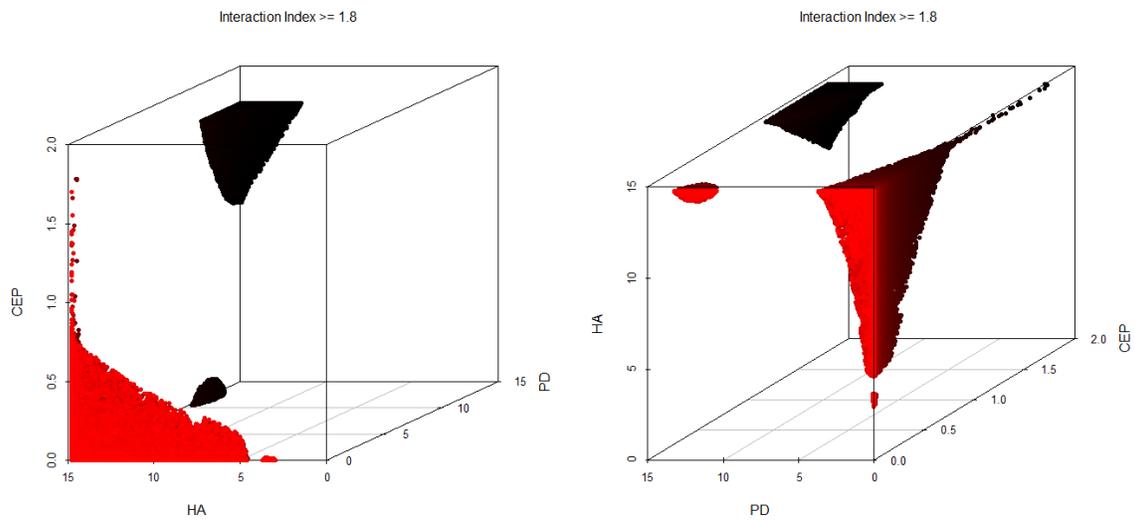


Figure 4: Plots of three-drug dose regions of antagonistic areas with $II \geq 1.8$: Left: PD vs HA vs CEP; Right: CEP vs PD vs HA, respectively. The colors of dots are changed from red at the front of the cuboid to black at the back.