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Nonlinear response surface in the study of interaction analysis of three combination drugs

Wen Wan^{1,*}, Xin-Yan Pei², Steven Grant², Jeffrey B. Birch³, Jessica Felthousen², Yun Dai², Hong-Bin Fang⁴, Ming Tan⁴, and Shumei Sun¹

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

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

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

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

Abstract

Few articles have been written on analyzing three-way interactions between drugs. It may seem to be quite straightforward to extend a statistical method from two-drugs to three-drugs. However, there may exist more complex nonlinear response surface of the interaction index (II) with more complex local synergy and/or local antagonism interspersed in different regions of drug combinations in a three-drug study, compared in a two-drug study. In addition, it is not possible to obtain a four-dimensional (4D) response surface plot for a three-drug study. We propose an analysis procedure to construct the dose combination regions of interest (say, the synergistic areas with $II \geq 0.9$). First, use the model robust regression method (MRR), a semiparametric method, to fit the entire response surface of the II , which allows to fit a complex response surface with local synergy/antagonism. Second, we run a modified genetic algorithm (MGA), a stochastic optimization method, many times with different random seeds, to allow to collect as many feasible points as possible that satisfy the estimated values of $II \geq 0.9$. Last, all these feasible points are used to construct the approximate dose regions of interest in a 3D. 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.

Keywords

Genetic algorithm (GA); Interaction index (II); Model robust regression (MRR); Synergism; Three-drug combination; Viability

*Corresponding author: wwan@vcu.edu, Phone: +1-804-827-0220, Fax: +1-804-828-8900.

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1 Introduction

Studies of more-than-two-drug combination become increasingly important to avoid cancer recurrence that is a critical problem in oncology (Kong and Lee, 2006; Luo et al., 2009). 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.

In a mixture experiment, it is especially important to detect the extent to which doses of the mixture interact with one another to enhance (synergistically) and/or which doses reduce (antagonistically) from an observed effect. Although there is no uniform agreement on the terminology of drug interaction (Lee, 2010), the Loewe additivity model is considered as a widely accepted method to quantify drug interactions (Berenbaum, 1989; Greco et al., 1995; Kong and Lee, 2006; Lee and Kong, 2009; Tan et al., 2009; Lee, 2010).

For a dose mixture of a three-drug combination at (x_1, x_2, x_3) , based on the Loewe additivity model, the interaction index (II) at (x_1, x_2, x_3) is defined 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 calculated/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).

Few articles in the literature have analyzed and visualized three-way interactions between drugs. Many methods focused on two-drug interaction have been compared and reviewed (Berenbaum, 1989; Greco et al., 1995; Lee, 2010; Zhao et al., 2010). The isobologram analysis method (Loewe and Muischnek, 1926; Greco et al., 1995; Tallarida, 2007) provides a visual assessment of the interaction of two drugs but obviously it cannot work for a three-drug study. The median-effect method by Chou and Talalay (1984) has been widely used to model a dose-effect curve. However, a fixed-ratio ray design that is required for the median-effect method may miss some interesting regions of the full concentration-effect surface (Prichard and Shipman, 1990; Greco et al., 1995). The ray design does not satisfy the exploratory purpose of discovering any synergistic effects in the full concentration-effect surface for a three-drug study. The third method with various response surface models (RSMs) (Lee, 2010) is used to model the entire surfaces of a measured response and/or calculated Loewe interaction index. The RSMs may be easily extended from a two-drug to a three-drug combination, although some RSMs may not be inadequate to describe the presence of pockets of local synergy or local antagonism (Greco et al., 1995; White et al., 2004; Kong and Lee, 2006).

In a case of a two-drug combination, a three-dimensional (3D) surface may provide a complete description on dose effect by some RSMs (Copenhaver et al., 1987; Berenbaum, 1989; Prichard and Shipman, 1990; Greco et al., 1995; Fang et al., 2008) and may be estimated parametrically, nonparametrically, or semiparametrically (White et al., 2004; Kong and Lee, 2006; Fang et al., 2008). As mentioned in Fang et al. (2008), the interpretation of interactions can be easily and visually inspected on either contours of the response surface of interaction indexes (Suhnel, 1990) or by using the 3D surface graphical method (Prichard and Shipman, 1990). Then departures from the theoretical additive surface with the actual estimated surface can be used to identify regions of synergy and/or antagonism.

It may seem to be quite straightforward to extend a statistical RSM from a two-drug to a three-drug combination. However, there may exist more complex nonlinear response surface with more complex local synergy and/or local antagonism interspersed, due to various two-drug interactions and one three-drug interaction. In addition, it is not possible to obtain a 4D response surface plot for a three-drug study, due to the 4D nature of the three-drug dose plus a response region.

In this three-drug study, we propose an analysis procedure to construct the dose combination regions of interest: say, the synergistic areas with $II \leq 0.9$, the additive areas with $0.9 < II < 1.1$, and the antagonistic areas with $II \geq 1.1$. First, use the model robust regression method (MRR) (Wan and Birch, 2011A), a semiparametric method, to model the interaction index surface and the measured response surface, respectively. This MRR model allows to fit a complex response surface with irregularly shaped local synergy/antagonism. We will compare the MRR method with the parametric (a common response method) and the local linear regression method (LLR), a nonparametric method, based on a certain model comparison criteria. Second, after finding the best model, we run a modified genetic algorithm (MGA), a stochastic optimization method, many times with different random seeds (Wan and Birch, 2011B), which allows to collect as many as possible feasible points that satisfy the estimated values of II , say, $\hat{II} \leq 0.9$ for synergistic areas. Wan and Birch (2011B) have shown that the MGA method performs the most computationally efficiently than the GA, the grid method, and the Nelder–Mead simplex method, especially in a highly nonlinear surface. Last, all these feasible points are used to construct dose region(s) of interest that can be visualized in a 3D plot.

The remainder of this paper is organized as follows. First, we briefly introduce a 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 in an *in vitro* study of three drugs against human Multiple Myeloma cell lines 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. The MRR, one semi-parametric method, proposed by Mays et al. (2001), 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 (Mays et al., 2001; Wan and Birch, 2011A).

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 (Wan and Birch, 2011A), 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 $\lambda \in [0, 1]$ that is used to combine the parametric fit with the nonparametric fit so that the MRR fit can achieve minimum of a prediction error criterion.

Two other methods can be used to fit a model to the response. The parametric method, based on the OLS fit, is exactly the same as the parametric proportion of the MRR fit. For the nonparametric method, 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.

More details and the key formulas in the MRR, OLS, and LLR fits can be found in Appendix I.

In this study, the $k = 3$ explanatory variables represent mixture dosages of three drugs. The response variable y is the interaction index or viability that is defined below in Eq. (2). The n is the total number of viability/interaction index, including all the experimental replicates.

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 4D 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 $\hat{II} = 0.9$, $0.9 < \hat{II} < 1.1$, and $\hat{II} = 1.1$, 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. We use a modified genetic algorithm (MGA) by Wan and Birch (2011B) to find the region(s) of interest. Wan and Birch (2011B) have shown that the MGA

method performs the most computationally efficiently than the GA, the grid method, and the Nelder–Mead simplex method, especially in a highly nonlinear function.

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, cross-over, and mutation. Each GA operation involves randomness. Therefore, if a GA is repeated 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 (Wan and Birch, 2011B).

The idea of the MGA (Wan and Birch, 2011B) 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 $\hat{f}$ values satisfy the requirements (e.g., $\hat{f} \leq 0.9$, $0.9 < \hat{f} < 1.1$, or $\hat{f} \geq 1.1$). Such points are referred to as “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 as possible feasible points in those region(s). A basic MGA procedure is provided in Appendix II.

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 mixture 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:

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

(2)

4.1 Study design

Before the experiment, we were uncertain of the dose-mixture-response relationship and would like to explore the full mixture dose regions. Therefore, we used the Tan and Fang's method to determine a sample size and doses of mixtures of three drugs to detect departures from additivity (Tan et al., 2003; Fang et al., 2008; Fang et al., 2009; Tan et al., 2009). The Tan and Fang's method is designed for the case of nonconstant 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 total number of observations was 240. The dose ranges of the three drugs in the mixtures are $x_{PD} \in [1.73, 14.78]\mu\text{M}$, $x_{HA} \in [1.48, 14.77]\mu\text{M}$, and $x_{CEP} \in [0.18, 1.77]\mu\text{M}$. The experimental domain in this case was a rectangular cuboid where the lengths of the three dose ranges would be the edges 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 circles) and three fitted blue lines representing their linear relationships for the three drugs, respectively. As dose of each individual drug increases, the response variable viability linearly decreases. The fitted models can be found in the titles of each subfigure. The degree-of-freedom-adjusted coefficient of determination (denoted by R_{adj}^2 (Myers, 1990)), 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. There were a total of 63 values of viability: 21 from PD, 18 from HA and 24 from CEP.

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. (2009).

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 calculated based on

$$II_{mj} = \frac{x_{PD}^{(m)}}{(y_{mj} - \hat{\alpha}_{PD})/\hat{\beta}_{PD}} + \frac{x_{HA}^{(m)}}{(y_{mj} - \hat{\alpha}_{HA})/\hat{\beta}_{HA}} + \frac{x_{CEP}^{(m)}}{(y_{mj} - \hat{\alpha}_{CEP})/\hat{\beta}_{CEP}}, \quad (3)$$

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 Fig. 1. Therefore, we can calculate 240 II values based on observed viability and estimated coefficients $\hat{\alpha}$ s and $\hat{\beta}$ s. However, there was an outlier in viability, confirmed by Dr. Grant's lab. Therefore, the actual total number of calculated II values based on (3) was 239.

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 interaction index at each dose combination from a fitted model to the calculated II values.

The maximum of the calculated II per definition (3) is 1.81 at $(x_{PD} = 13.88, x_{HA} = 12.94, x_{CEP} = 1.61)$ and its minimum is 0.54 at $(x_{PD} = 3.98, x_{HA} = 1.94, x_{CEP} = 0.56)$. Per the range of the calculated II , very likely there exist synergistic, antagonistic, and additive areas of the three-drug combinations.

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. To model the viability, we include both 239 values from mixtures experiment and 63 from the three individual drugs experiments.

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 $DF_{error} = n - tr(H)$ (Ruppert et al., 2003), where H is the "hat" matrix appropriate for each method, MRR, LLR, and OLS. See Wen and Birch (2011A) 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 prediction error sum of squares. 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 (Myers, 1990). We hope to find a model with the largest DF_{error} and R_{adj}^2 and the smallest s^2 and PRESS.

In Table 1, all adjusted coefficients of determination, R_{adj}^2 , are over 90% indicating that all methods fit very well in II . The estimated error variances and R_{adj}^2 by MRR and LLR are almost equal to each other, both better than OLS. The MRR method fits the best in describing II and the LLR method best fits viability in terms of 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, also the parametric proportion of the MRR fits, is chosen by the highest R_{adj}^2 as follows.

$$\hat{II}^{(OLS)} = 1.142 - 0.199x_{PD} + 0.445x_{HA} + 0.030x_{CEP} - 0.033x_{PD}x_{HA} + 0.080x_{PD}x_{CEP} - 0.023x_{HA}x_{CEP} + 0.127x_{PD}^2 - 0.031x_{HA}^2 + 0.072x_{CEP}^2, \quad (4)$$

and

$$\widehat{\text{viability}}^{(OLS)} = 116.61 - 41.74x_{PD} - 6.54x_{HA} - 9.64x_{CEP} + 1.64x_{PD}x_{HA} - 0.21x_{PD}x_{CEP} + 0.36x_{HA}x_{CEP} + 8.31x_{PD}^2 + 0.06x_{HA}^2 + 0.31x_{CEP}^2. \quad (5)$$

Both OLS models are hierarchical lower order polynomial with interactions between the three drugs. Only one term is not significant with p -values = 0.303 (the interaction term $x_{PD}x_{CEP}$ in $\widehat{\text{viability}}^{(OLS)}$) and all the other terms in both models above are significant with p -values < 0.05.

The signs of interactions between drugs may indicate some interactions between drugs but cannot indicate whether or not there are any synergistic or antagonistic effects between drugs. The signs of the estimated coefficients (some positive and others negative) indicate that both response surface models are not monotonic functions of doses of the three drugs. More specifically, 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 $\hat{II}$ can quantify whether its corresponding mixtures are synergistic, additive, or antagonistic, per the definition in Eq. (1).

In the estimated OLS model of $\hat{II}$ in (6), values of $\hat{II}$ 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 x_{PD} 14.78, 0 x_{HA} 14.77, and 0 x_{CEP} 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, that is $x_{CEP} = 0$ in (6). Then the OLS model becomes

$$\hat{II}^{(OLS)} = 1.142 - 0.199x_{PD} + 0.445x_{HA} - 0.033x_{PD}x_{HA} + 0.127x_{PD}^2 - 0.031x_{HA}^2, \quad (6)$$

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 x_{PD} 14.78 and 0 x_{HA} 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

With consideration of data variability, the software Calcsym (Chou and Hayballcite, 1996) recommended that the additivity region is defined in $0.9 < II < 1.1$. In addition, the estimated error variance is quite small, < 0.002 , shown in Table 1. Therefore, we are interested in the synergistic areas with $II \leq 0.9$, the additive areas with $0.9 < II < 1.1$, and the antagonistic areas with $II \geq 1.1$. 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:

$$\hat{II}^{(MRR)} \leq 0.9, \quad (7)$$

$$0.9 < \hat{II}^{(MRR)} < 1.1, \quad (8)$$

$$\hat{II}^{(MRR)} \geq 1.1. \quad (9)$$

Using the procedure in Section 3, we are able to find all feasible points, which satisfy our requirements in (7)–(9). Using the recommendations from Wan and Birch (2011B), we set each MGA with 300 iterations and we run a MGA about 500 times with different random seeds. Once the feasible points are found, we plot them in a 3D dose region.

Figure 2 shows the synergistic area of the three-drug dose regions with $\hat{II} \leq 0.9$ in the two different angles: the left figure is for the regions of CEP versus PD versus HA and the right one is for HA versus PD versus CEP. The colors of dots are changed from red at the front of the cuboid to black at the back. The major regions are synergistic when the dose of HA is mild or low, $< 7.5\mu\text{M}$, with wide dose ranges of PD and CEP. As PD's dose level increases, the synergistic area gets thinner. There is a small valley region with synergistic effects (in the left figure), when PD's dose level is close or equal to zero, CEP's is at about $0\text{--}0.5\mu\text{M}$, and HA's dose range at about $4\text{--}7\mu\text{M}$. The smallest $\hat{II}$ value found by MGA in the experimental domain is 0.50 at mixture $(x_{PD}, x_{HA}, x_{CEP}) = (1.73, 1.48, 0.81)$. Overall, the synergistic dose regions are quite irregular and complicated.

Figure 3 shows the additive three-drug dose areas with $0.9 < \hat{II} < 1.1$ in two different angles, which are the same as in Fig. 2. The left figure shows that the additive area hangs over there covering the synergistic region shown in Fig. 2 with a rough surface.

Figure 4 shows the antagonistic area with $\hat{II} = 1.1$ in two different angles. The surfaces of collected antagonistic points by MGA match well with the ones of collected additive and synergistic points. Comparing the three left-side figures in Figs. 2, 3, and 4, the region under the additive surface is synergistic, while the region above the surface is antagonistic. The additive ones are between the synergistic and antagonistic ones.

5 Conclusion and discussion

Studies of interactions among drugs have become increasingly important and interesting. Little research exists that describes interactions for three or more drug combinations. Through a case study of three anti-cancer drugs, we propose an analysis procedure to construct the dose combination regions of interest. First, we use MRR, a semiparametric method, to fit the entire response surface of the II , which allows to fit a complex response surface with local synergy/antagonism. Second, we run a MGA, a stochastic optimization method, many times with different random seeds, to allow to collect as many feasible points as possible that satisfy the estimated values of $II = 0.9$. Last, all these feasible points are used to construct the approximate dose regions of interest in a 3D. This procedure 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, 3D plots conditioned on a specific value or range of values of the other drugs.

In this study, the Loewe additivity model is used to quantify 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 (Chou and Hayballcite, 1996).

Many articles in the literature that were focused on a case of two-drug combination to model a response surface of interaction index/measured outcome may be extended to the three-drug case study. In this study, 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. Importantly, the high $R^2_{adj} > 95\%$, small $s^2 < 0.002$, and the constructed irregular synergistic/antagonistic dose regions indicate that the MRR model fits the response surface of the II very well and captures the local synergy/antagonism very well.

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, in this case study, combinations of the three drugs (PD, HA, and CEP) are not always synergistic. These results are consistent with the statement of some agent combinations that it may demonstrate different types of interaction at different local regions of the concentration-effect surface (Greco et al., 1995; White et al., 2004; Kong and Lee, 2006) and with the results in Fang et al. (2008) and Fang et al. (2009).

After fitting the response surface to the calculated II response values, we apply the MGA procedure to construct combination dose regions of interest: the synergistic areas with $\hat{II} < 0.9$, the additive areas with $0.9 < \hat{II} < 1.1$, and the antagonistic areas with $\hat{II} > 1.1$. The MGA procedure, introduced by Wan and Birch (2011B), is actually an 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. Using the recommendations from Wan and Birch (2011B), we set each MGA with 300 iterations and we run a MGA about 500 times with different random seeds. In addition, MGA or HGA are global optimization tools, which can deal with any high-dimensional nonlinear model. Wan and Birch (2011B) shown that MGA performs the most computationally efficiently than the GA, the grid method, and the Nelder–Mead simplex method, especially for very high dimensional, complicated problems.

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 Fig. 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 Calculusym (Chou and Hayball, 1996) 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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Appendix I: Semiparametric Approach: Model Robust Regression

Before fitting a MRR model, each independent variable (regressor) needs to be converted into a coded variable so that its range becomes $[-1, 1]$. For example, a raw variable can be

converted to a coded variable x by $x = \frac{\text{raw} - (\max + \min)/2}{(\max - \min)/2}$, where $\max$ and $\min$ are the maximum and minimum of the raw variable, respectively.

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

$$\hat{y}_0^{(MRR)} = \tilde{\mathbf{x}}_0' (\mathbf{X}'\mathbf{X})^{-1} \mathbf{X}'\mathbf{y} + \lambda \tilde{\mathbf{x}}_0' (\tilde{\mathbf{X}}' \mathbf{W}_{r0} \tilde{\mathbf{X}})^{-1} \tilde{\mathbf{X}}' \mathbf{W}_{r0} \mathbf{r} = \mathbf{h}_0^{(OLS)'} \mathbf{y} + \lambda \mathbf{h}_{r0}^{(LLR)'} \mathbf{r}, \quad (\text{A1})$$

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

$\mathbf{r} = \mathbf{y} - \hat{\mathbf{y}}^{(OLS)} = \mathbf{y} - \tilde{\mathbf{x}}_0' (\mathbf{X}'\mathbf{X})^{-1} \mathbf{X}'\mathbf{y}$ is considered as a new response,

$\tilde{\mathbf{x}}_0' = (1, x_{10}, \dots, x_{k0}) = (1, \mathbf{x}'_0)$, the matrix $\tilde{\mathbf{X}}' = [\tilde{\mathbf{x}}'_1, \tilde{\mathbf{x}}'_2, \dots, \tilde{\mathbf{x}}'_n]'$, 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

$$K(\mathbf{x}_0, \mathbf{x}_i) \propto K\left(\left\|\frac{\mathbf{x}_0 - \mathbf{x}_i}{b_r}\right\|\right) \text{ or } \prod_{j=1}^k K\left(\frac{x_{0j} - x_{ij}}{b_r}\right), \quad (\text{A2})$$

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 (2011A)). The details of the choice of the bandwidth and the mixing parameter can be found in Wan and Birch (2011A).

In addition, Eq. (A1) can be written in terms of “HAT” vectors: in the parametric portion

$\mathbf{h}_0^{(OLS)'} = \tilde{\mathbf{x}}_0' (\mathbf{X}'\mathbf{X})^{-1} \mathbf{X}'$, and in the nonparametric portion $\mathbf{h}_{r0}^{(LLR)'} = \tilde{\mathbf{x}}_0' (\tilde{\mathbf{X}}' \mathbf{W}_{r0} \tilde{\mathbf{X}})^{-1} \tilde{\mathbf{X}}' \mathbf{W}_{r0}$.

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

$$\hat{y}_0^{(OLS)} = \tilde{\mathbf{x}}_0' (\tilde{\mathbf{X}}' \tilde{\mathbf{X}})^{-1} \tilde{\mathbf{X}}' \mathbf{y} = \mathbf{h}_0^{(OLS)'} \mathbf{y}, \quad (\text{A3})$$

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

$$\hat{y}_0^{(LLR)} = \tilde{\mathbf{x}}_0' (\tilde{\mathbf{X}}' \mathbf{W}_0 \tilde{\mathbf{X}})^{-1} \tilde{\mathbf{X}}' \mathbf{W}_0 \mathbf{y} = \mathbf{h}_0^{(LLR)'} \mathbf{y}. \quad (\text{A4})$$

Instead of using the residuals $\mathbf{r}$ as a response variable as in the nonparametric proportion of the MRR 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.

Appendix II: 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 cross-overs, 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 $I(\mathbf{x}_1), \dots, I(\mathbf{x}_n)$, where $I(\mathbf{x}_j)$ is the objective function evaluated at the j^{th} chromosome $\mathbf{x}_j$.
4. Generate an offspring population, by GA operations (such as selection/mating, cross-over, and mutation). That is, generate a new set of n settings of the three drugs, using the operations of selection, cross-over, 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 0.9?

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, that is 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 cross-over 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).

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

Figure 2.

Plots of three-drug dose regions of synergistic areas with $\hat{IT} = 0.9$: Left: CEP versus PD versus HA; Right: HA versus PD versus CEP, 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 $0.9 < \hat{H} < 1.1$: Left: CEP versus PD versus HA; Right: HA versus PD versus CEP, 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 $\hat{H} = 1.1$: Left: CEP versus PD versus HA; Right: HA versus PD versus CEP, respectively. The colors of dots are changed from red at the front of the cuboid to black at the back.

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

	b	$\hat{\chi}$	DF_{error}	s^2	R^2_{adj}	PRESS
$\hat{H}$	OLS	—	—	229.000	0.00252	0.9715
	LLR	0.310	—	210.132	0.00176	0.9802
	MRR	0.314	0.87	212.647	0.00176	0.9802
$\widehat{viability}$	OLS	—	—	292.000	70.7216	0.9003
	LLR	0.259	—	259.180	9.8828	0.9861
	MRR	0.255	1.00	258.949	9.8807	0.9861