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An Application of a Hill-based Response Surface Model for a Drug Combination Experiment on Lung Cancer

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Abstract

Combination chemotherapy with multiple drugs has been widely applied to cancer treatment due to enhanced e cacy and reduced drug resistance. For drug combination experiment analysis, response surface modeling has been commonly adopted. In this paper, we introduce a Hill-based global response surface model and provide an application of the model to a 512-run drug combination experiment with three chemicals, namely AG490, U0126, and indirubin-3'-monoxime (I-3-M), on lung cancer cells. The results demonstrate generally improved goodness of fit of our model from the traditional polynomial model, as well as the original Hill model based on fixed-ratio drug combinations. We identify different dose-effect patterns between normal and cancer cells based on our model, which indicates the potential effectiveness of the drug combination in cancer treatment. Meanwhile, drug interactions are analyzed both qualitatively and quantitatively. The distinct interaction patterns between U0126 and I-3-M on two types of cells uncovered by the model could be a further indicator of the efficacy of the drug combination.

Keywords

drug combination; drug interaction; factorial design; Hill model; response surface model

1 Introduction

Drug combinations have been widely applied in disease treatment, especially chemotherapy for cancer [1]. Among the benefits of drug combinations are improved effectiveness and inhibited drug resistance due to multiple targets, and enhanced efficiency due to synergistic drug interactions [1–5]. In order to understand the pathological mechanisms of the combination, and the interactions between the combined drugs, as well as to select the optimal combination, preclinical trials *in vitro* are usually conducted. However, due to cost and efficiency considerations, both the number of runs and the range of drug dosages in the experiments are limited. Therefore, the design of drug combination experiments and the follow-up statistical analysis are of great importance, and have been continuously studied.

To analyze the data obtained from drug combination experiments, response surface modeling is an effective method, where the dose-effect curve is statistically fitted and the optimal drug combination and the drug interaction patterns are thus determined. For two-drug combinations, Hill models based on ray designs are the most commonly used due to the clear practical bearings of their parameters and the boundedness of the predicted effect values [3, 6]; for multiple drug combination studies, polynomial models accompanied by full factorial designs or fractional factorial designs have been effectively applied [5, 7]. However, both models bear their respective limitations: restricted applicability for Hill models and unboundedness for polynomial models.

In this paper, we introduce a Hill-based global response surface model, originally proposed by Minto *et al.* [8] for an anesthetics study. The model, derived from both Hill and polynomial models, combines the strengths of the two originating models while avoiding their shortcomings. Brun *et al.* [9] applied the Hill-based model in an anti-fungal study with a ray design of three drugs. Here, we further apply the model to data from a three-drug combination experiment on lung cancer with a full factorial design. One advantage of the full factorial design over a ray design is its e ciency in terms of ratio coverage. In fact, the ray design in Brun *et al.*'s study concerns with 91 rays of fixed ratios with 11 dilution levels each ray, while our 8-level full factorial design includes more fixed ratios (roughly 140) with various dilution levels from 2 to 7. The fitting results of the lung cancer data demonstrate improved goodness of fit of the Hill-based model from polynomial models. Based on the predictions by the model, effectiveness of the drug combination is indicated and types of drug interactions are identified.

The paper is organized as follows. In Section 2, we first review two traditional models, the Hill model and the polynomial model, and then introduce how the two models are combined to a global model for drug combination analysis. In Section 3, we present our application of the model to the drug combination experiment on lung cancer cells and follow-up analysis on the drug interactions. Section 4 contains our conclusions.

2 Response Surface Modeling

2.1 The Hill Model

In complex systems such as cellular, multicellular or *in vivo* systems, dose-effect relationship usually follows a sigmoidal curve, most commonly modeled by Hill models [3]. For two-drug (denoted by *A* and *B*) combination studies, each combination with a fixed ratio between *A* and *B* is assumed to be a “new” drug following the Hill model [6], as in:

$$Y_r = \frac{1}{1+\left(\frac{C_r}{IC_{50,r}}\right)^{\gamma}} + \varepsilon_r, \quad (1)$$

where index *r* represents the fixed ratio; C_r, the explanatory variable, represents the concentration or the dose of the combination; Y_r, the response variable of C_r, represents the effect level; and $IC_{50,r}$ are two parameters to be estimated from data; and $g=e_r$ is the random error.

The most notable strength of the Hill model is the self-explanatory feature of its parameters. The numerator of the formula is a constant 1, which corresponds to the effect level under no drug treatment (i.e., concentration 0). Here the effects are normalized between 0 and 1. The parameter $IC_{50,r}$ is the dosage of the drug combination that yields 0.5 effect level, and the slope parameter describes the changing rate of the curve. The model is bounded as the concentration increases to infinity, thus eligible for prediction outside the experimental range.

2.2 The Polynomial Model

For multiple drug studies, however, the original Hill model can only address fixed ratio combinations, thus less applicable when a global response surface of the drug combination across various ratios is concerned and of interest. The polynomial model, instead, is more commonly used to fit the global response surface in accompany with a full factorial design and, to reduce cost, a fractional factorial design [5, 10]:

$$Y=\beta_0+\sum_i \beta_i x_i+\sum_{i,j} \beta_{ij} x_i x_j+\varepsilon, \quad (2)$$

where, p drugs assumed in the combination, x_i represents the dose level, usually coded, of drug i; Y represents the effect level; β_i and β_{ij} are the parameters of main e ects and interactions; and ε is the random error. Note that higher order interactions between drugs are assumed to be negligible due to the effect hierarchy principle [10].

However, the predicted effect level by the polynomial model is boundless as the dose level increases to infinity, which is an obvious deviation from actualities. Thus the polynomial model lacks prediction power beyond the dose range of the experiment. In addition, the dose levels for a certain drug, commonly designed as a geometric sequence in practice, are often transformed to coded levels by logarithm before fitting, yet with the exception of the control run where 0 is assigned. This transformation may lead to inaccuracy in the estimation due to the alternated quantitative relations between the dose levels.

2.3 The Combined Model

To take advantage of both the Hill model and the polynomial model while excluding their respective limitations, we here introduce a global dose-response surface model for multiple drug combinations by combining these two classic models [8, 9]. To inherit the basic principle from the Hill model for two-drug combinations, we assume a combination with a fixed proportion between multiple drugs as a “new” drug whose dose-effect curve follows a distinct Hill model. Thus, each fixed drug proportion corresponds to a sigmoidal Hill-based curve with a different set of parameters. Thereby we can build a global model by assuming the parameters of the original Hill model as functions of the drug proportion variables:

$$Y=\frac{1}{1+\left(\frac{C}{IC_{50}\left(\vec{\theta}\right)}\right)^{\gamma\left(\vec{\theta}\right)}}+\varepsilon. \quad (3)$$

Here, assuming a combination of p drugs and C_i as the concentration of drug i, $C=\sum_{i=1}^{p} C_i$ is the explanatory variable representing the total concentration of the drug combination; $\overrightarrow{\theta}=(\theta_1,\theta_2,\ldots,\theta_p)$ is the proportion variable vector, where $\theta_i = C_i/C$ represents the proportion of drug i in the combination; $IC_{50}\left(\overrightarrow{\theta}\right)$ and $\gamma\left(\overrightarrow{\theta}\right)$, the original parameters in the Hill model, are now functions of $\overrightarrow{\theta}$; Y represents the effect level; and ε is the random error.

To incorporate the polynomial model, we further assume $IC_{50}\left(\overrightarrow{\theta}\right)$ and $\gamma\left(\overrightarrow{\theta}\right)$ as polynomial functions of $\overrightarrow{\theta}$:

$$IC_{50}\left(\overrightarrow{\theta}\right)=b_0+\sum_{i=1}^{p-1} b_i\theta_i+\sum_{1\le i\le j\le p-1} b_{ij}\theta_i\theta_j; \quad (4)$$

$$\gamma\left(\overrightarrow{\theta}\right)=a_0+\sum_{i=1}^{p-1} a_i\theta_i+\sum_{1\le i\le j\le p-1} a_{ij}\theta_i\theta_j. \quad (5)$$

Here b_i, b_{ij}, a_i, a_{ij} are parameters. Note that we only take $p-1$ components of the proportion $\overrightarrow{\theta}$ to ensure the identifiability of the parameters, as with the constraint $\sum_{i=1}^{p}\theta_i=1$. In particular, for a three-drug combination with $p = 3$, we only include θ_1 and θ_2 because $\theta_3 = 1 - \theta_1 - \theta_2$ is implicitly included in the model. It is possible to include all θ_i explicitly with reparameterization; see [9].

Combining the Hill model and the polynomial model to address response surface modeling has a number of advantages. One of the most significant is its global applicability. The model is able to address all different combinations among multiple drugs, overcoming the restricted applicability of the traditional Hill model to only fixed ratios of combined drugs. Also, by setting the proportions of other drugs as zero, the model corresponds to the original one- or two-drug Hill model, demonstrating its consistency. For instance, when we assign $\theta_1 = \theta_2 = 0.5$ and $\theta_i = 0$, $i = 3, \ldots p$ in (4) and (5), the combined model (3) is equivalent to the traditional Hill model (1) with fixed ratio $r = 1$ between drug A and B. Further, the parameters in the model still hold strong practical bearings, inheriting the strengths of both the Hill and polynomial models. When the proportion vector of the drug combination is fixed as $\overrightarrow{\theta}=\overrightarrow{\theta}_0$, $IC_{50}\left(\overrightarrow{\theta}_0\right)$ indicates the concentration yielding 50% of the maximum effect under such a fixed combination. In addition, the model does not depend on any presumption on the interaction types between the drugs involved, thus enabling a full analysis on drug interaction patterns.

3 Results and Analysis

3.1 Drug Combination Experiment on Lung Cancer

We focus on the drug combination experiment on lung cancer cells led by Al-Shyoukh *et al.* [11]. As inhibition of cell survival and proliferation has been widely applied in cancer

treatment [12], three inhibitors targeting distinct but connected cellular signaling pathways for cell survival and proliferation, namely AG490 (*A*), U0126 (*B*), and indirubin-3'-monoxime (I-3-M) (*C*), were chosen in the experiment. With the objective of identifying difference in responses between cancer and non-cancer cells, both A549, a non-small cell lung cancer cell line, and AG02603, a normal fibroblast cell culture, derived from normal healthy tissues, were selected as subjects.

Cellular ATP is one of the most common and essential markers for live cells, and measuring ATP is a generally accepted quantitative and sensitive assay for assessing the inhibition of cellular growth, proliferation, and induction of cell killing by drugs [11, 13]. Therefore, total cellular ATP levels of both lung cancer A549 cells and primary lung fibroblast AG02603 cells were measured 72 hours after drug treatment, and normalized by untreated cellular ATP levels as the responses or effect levels in the experiment. Individual treatments of the three drugs were first conducted to determine the concentration ranges covering the minimal to the maximal inhibitory effects, from which 8 levels of each drug were chosen for the combination experiment, given in Table 1. A full factorial design of 512 runs was then adopted with each drug taking these 8 different dose levels. All 512 combinations were applied to both lung cancer A549 cells and primary lung fibroblast AG02603 cells, and ATP levels were experimentally measured and scaled as in individual drug pre-experiment.

3.2 Model Fitting and Comparison

We fit the data to our model (3)-(5) with $p = 3$ with respect to the results of both normal and cancer cell experiments with the nonlinear least squares methods in the free statistical software *R* (http://www.r-project.org). In search of best-fitted model, we variate our two parameter functions (4) and (5) with linear terms only, linear and quadratic terms without interactions, and full quadratic expressions. We apply F tests to compare these models and conclude that the use of the full quadratic model is necessary for both normal and cancer cells. We also fit the data to the quadratic polynomial model (2) directly for comparison.

Table 2 gives the estimated parameters of the Hill-based models. The estimates of b_2, b_{22}, a_1, a_2, a_{11} and a_{22} take different signs (as bolded) for normal and cancer cells, which could indicate significant difference in the dose-effect pattern between cancer and normal cells. As the ultimate goal of drug combinations in cancer treatment is to kill most cancer cells while leaving normal cells intact, such distinct response patterns could be a good indicator of the e ectiveness of this drug combination.

Next we compare the Hill-based model with the traditional polynomial model. Table 3 shows the mean squared errors (MSE) and the coe cient of multiple determination R^2. Both models fit the data very well with MSE under 0.0031 and R^2 over 0.97 for both normal and cancer cells. Figures 1 and 2 show the scatterplots of fitted effect values vesus observed values ($\hat{y} \sim y$). Majority of the points cluster closely to the $\hat{y} = y$ line. Yet, on the plots of the polynomial models there are a few obvious outliers and several unrealistic negative fitted e ect levels (with estimated ATP level < 0). The Hill-based model is more stable and therefore preferred.

To further validate the Hill-based global model, we analyze the estimated parameter functional values of IC_{50} and in (4) and (5) by Model (3) based on the data in comparison to the estimated parameters by the original Hill model (1), shown in Table 4. Here we select 10 fixed-ratio combinations among three drugs (including single-drug combinations) and, in respect to such fixed ratios, obtain the estimated IC_{50} and in (4) and (5) and their standard errors (SE) based on the fitting results of the Hill-based model in Table 2. For reference, we sort out those specific fixed-ratio experiment runs from the 512-run experimental data and treat them as independent ray designs to fit the original Hill model (1) respectively. The corresponding estimated parameters IC_{50} and from the Hill model (1) are compared with their estimated functional values by Model (3).

Table 4 compares the estimated parameters and their standard errors. The estimates of IC_{50} from the global Hill-based model (3) are quite consistent with estimates from the original Hill model (1) among all selected fixed-ratio combinations, mostly within one standard error interval. The estimates of from Model (3) are of less proximity, but all within one to three standard error intervals. Such proximity between the estimates gives convincing evidence on the validity of our assumptions on the parameter function forms (4) and (5), in turn the validity of our proposed Hill-based model (3). Note that the standard errors from the original Hill model (1) are comparatively larger in all cases, because fewer runs are used to fit Model (1) (6–8 runs) than Model (3) (512 runs).

3.3 Drug Interaction Analysis

We are also able to analyze the drug interaction patterns with our model. Here we adopt the Loewe additivity model as our reference model [14, 15], which was widely advocated as the most appropriate definition for two drug interactions [6, 16], and further extended by Chou and Talalay [17]:

$$I=\frac{C_{A,r}}{IC_{X,A}}+\frac{C_{B,r}}{IC_{X,B}}, \quad (6)$$

where X is the reference effect level for analysis, usually taking 50%; r is the fixed ratio between drug A and B for the combination; $C_{A,r}$ and $C_{B,r}$ represent the respective concentrations of drug A and B for the combination to yield the effect level of X; $IC_{X,A}$ and $IC_{X,B}$ represent concentrations of drug A and B respectively, when applied individually to result in X level effect. If the interaction index, I, is equal to 1, we call the mixture additive, meaning no interaction between drug A and B; if $I < 1$, Loewe synergism, meaning these two drugs work cooperatively; if $I > 1$, Loewe antagonism, meaning the two drugs inhibit each other.

Based on our fitted dose-effect model for normal and cancer cells, we report the pairwise Loewe interaction index among the three drugs from the experiment at the fixed e ect level of 50% and the fixed ratio of 1:1 with their respective standard errors as examples, given in Table 5. Note that, except for the interaction between drug B and C on cancer cells, all the other interactions are identified as synergism. The difference in interaction types between drug B and C on normal and cancer cells is also a good indicator of the distinct dose-effect

patterns of this drug combination on these two groups of cells, implicating the e ectiveness of the combination.

A more thorough yet less quantitative analysis of drug interaction could be achieved by investigating the contour plots. Here we report the contour plots of the effect levels predicted by the polynomial model (2) and the Hill-based model (3) based on pairwise drug combinations with the third drug dose fixed at 0, given by Figures 3 and 4, on two types of cells respectively. As drug combinations that yield greater efficacy thus result in lower effect levels bear more significant pharmaceutical and clinical values, we only report plots with the range of normalized effect y 0.5 with interval of 0.1. Note that convex plots indicate Loewe synergism and concave plots indicate Loewe antagonism.

Comparing the plots given by both models within each row of Figures 3 and 4, especially $A \sim B$, $A \sim C$ normal cells and $A \sim B$, $B \sim C$ on cancer cells, the model gives more sensible predictions. The plots by the polynomial model appear in ellipsoidal shapes, where the drug effect first decreases and then increases with respect to dose increase for some fixed ratios. Such unrealistic predictions may well result from the structural defect of unboundedness of the linear model stated earlier.

Generally, throughout the range of effect levels under evaluation, all pairwise drug interactions are identified similarly by both models as synergistic, except between $B \sim C$ on cancer cells. In fact, the most remarkable contradictory reports given by two models are in this case. Our Hill-based model demonstrates a distinctively strong and stable antagonistic interaction between $B \sim C$ on cancer cells while the polynomial model reports slightly synergistic interaction on effect from 0.5 to 0.3, yet insensible results less than 0.2.

We can also evaluate the interactions as well as the response surfaces across types of cells with corresponding plots from both Figures 3 and 4. The plots rea rm the di erent interaction types between $B \sim C$ on two types of cells, not only on 0.5 effect level, but all levels lower than 0.4. Furthermore, significantly di erent dose-effect patterns are demonstrated by the Hill-based model. The plots show a significant higher dose levels required to yield same effect level on normal cells than cancer cells, especially between $B \sim C$. Such di erence is another good indicator of the effectiveness of the drug combination on eliminating cancer cells while maintaining normal cells.

4 Conclusion

By assigning the original parameters of a Hill model as polynomial functions of proportion variables of the drug combination, we are able to extend the Hill model for global response surface modeling of multiple drug combinations. We have also provided an application of the model to a 512-run three-drug (AG490, U0126, and I-3-M) combination experiment on lung cancer. The fitting results show generally improved goodness of fit from traditional polynomial model for response surface, especially for cancer cells on lower e ect levels where greater clinical and pharmacological importance lies. The Hill-based model is further validated by comparing the parameter estimates of IC_{50} and with those fitted by the original Hill model based on fixed-ratio combinations. A di erent dose-e ect pattern between normal

and cancer cells is also identified, which indicates potential e ectiveness of the drug combination in cancer treatment.

We then analyzed the interaction patterns among the three drugs based on our model both quantitatively with Loewe interaction index and qualitatively with contour plots. Except for the antagonism between U0126 and I-3-M on cancer cells, all other pairs of interactions are identified as synergism at 50% e ect level. A comparison between the contour plots also indicates a more reliable and sensible prediction by our Hill-based model than the polynomial model. In addition, a reduction in dose level to yield same effect level on cancer cells from normal cells shown by our contour plots further verifies the effectiveness of the drug combination.

With a growing demand for drug combination experiment analysis, the Hill-based model could be used as an effective alternative to traditional polynomial models for response surface modeling in full factorial or fractional factorial designs. We are also currently working on an antiviral drug combination experiment with six drugs and six levels each [7, 18]. It is possible that further exploration and improvement on the model are to be addressed in future research.

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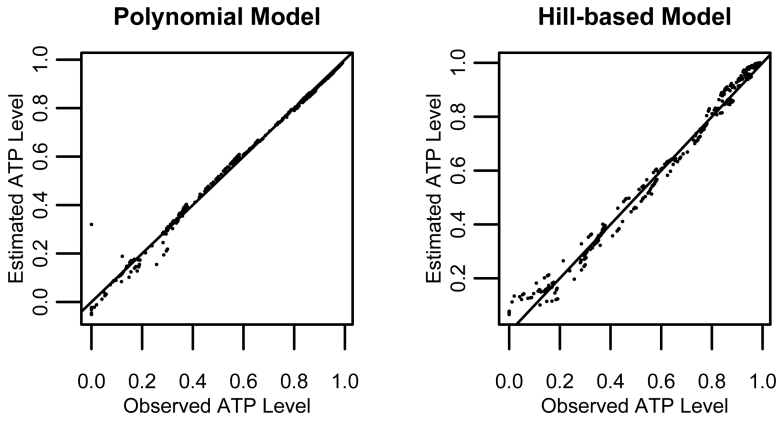

Figure 1.
Scatterplot of fitted vs. observed ATP level on normal cells

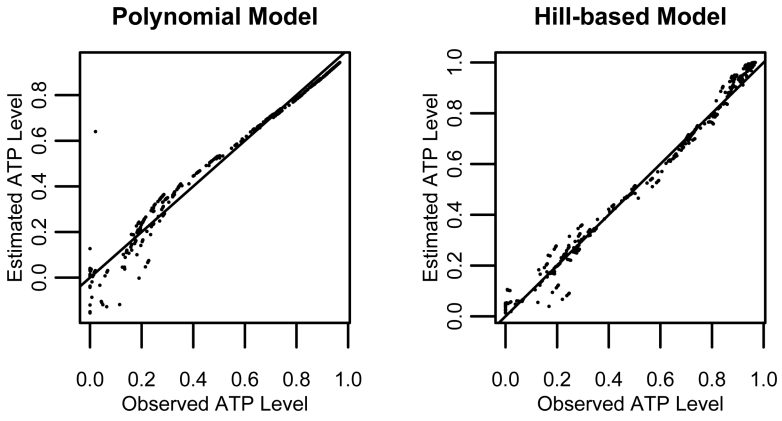

Figure 2.
Scatterplot of fitted vs. observed ATP level on cancer cells

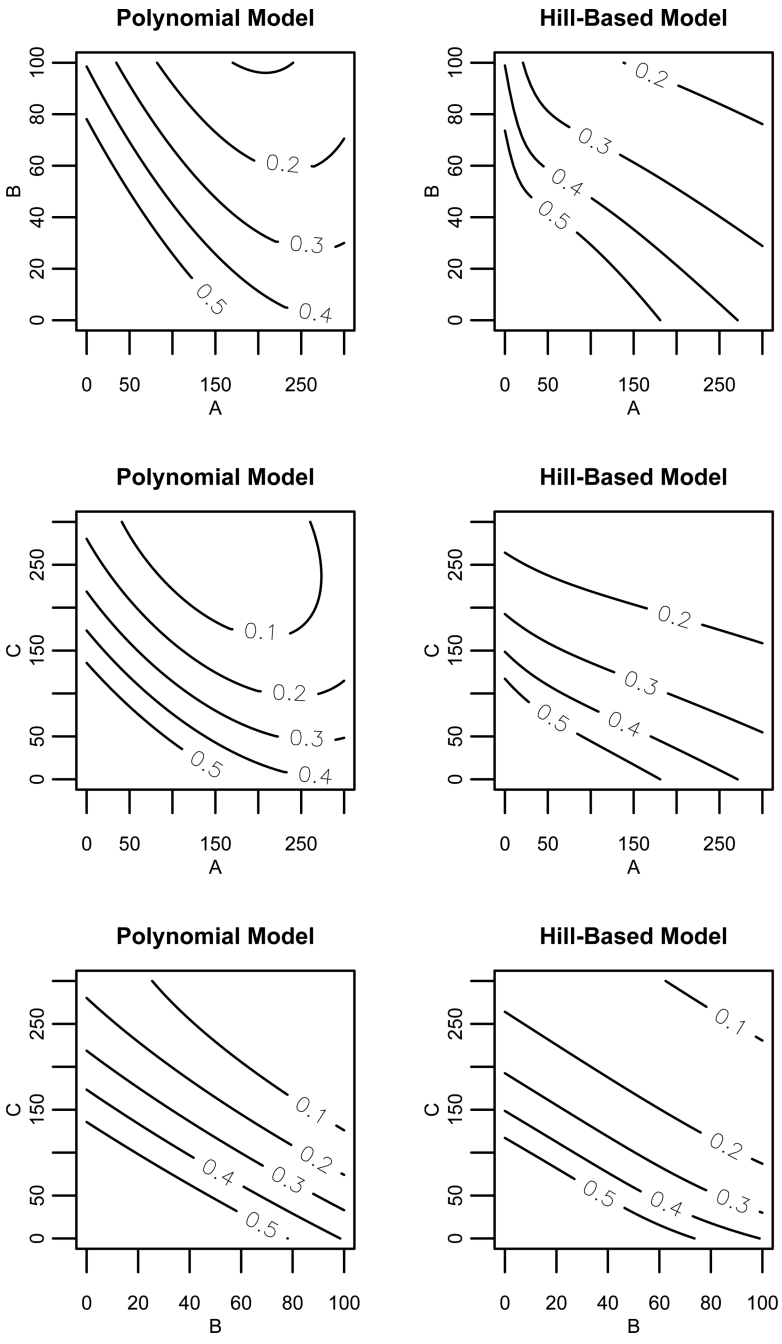

Figure 3.
Contour plots of predicted results on normal cells

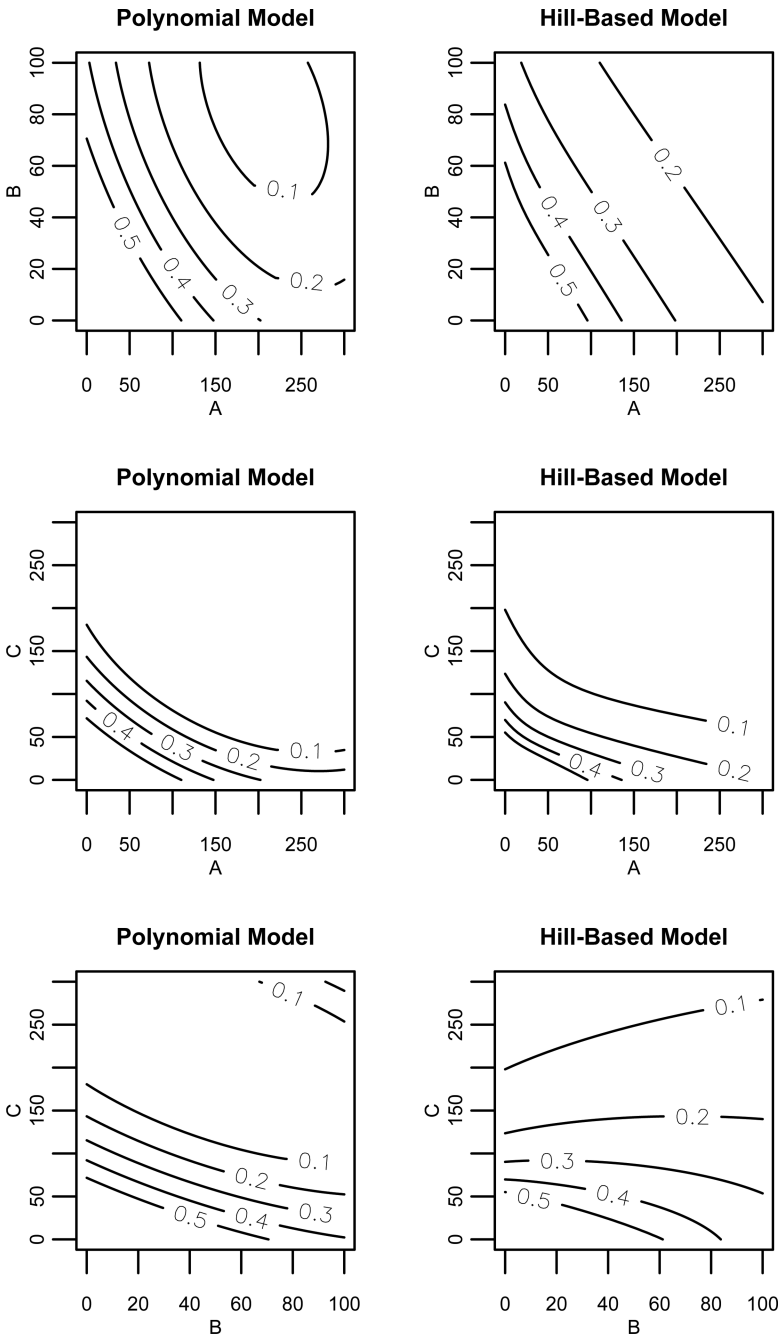

Figure 4.
Contour plots of predicted results on cancer cells

Table 1

Dose levels of the drug combination experiment on lung cancer

Drug	Dose Levels (units: μM)							
AG490	0	0.3	1	3	10	30	100	300
U0126	0	0.1	0.3	1	3	10	30	100
I-3-M	0	0.3	1	3	10	30	100	300

Table 2

Comparison of Estimated Parameters for Normal and Cancer Cells

parameter	b_0	b_1	b_2	b_{11}	b_{22}	b_{12}
normal	117.11***	−15.71	**− 86.15*** **	79.55***	**42.67*** **	−33.75*
cancer	55.11***	−2.98	**43.72** **	43.72***	**− 22.07***	−17.09
parameter	a_o	a_1	a_2	a_{11}	a_{22}	a_{12}
normal	1.70***	**− 1.02*** **	**0.41**	**0.31**	**− 0.74** **	1 21***
cancer	1.72***	**1.39*** **	**− 2.50*** **	**− 194*** **	**2.08*** **	0.25

Note: Significance levels are coded as 0

*** 0.001

** 0.01

* 0.05.

Table 3

Comparison of MSEs and R^2 of the fitted models

	Polynomial Model (2)		Hill-based Model (3)	
	MSE	R^2	MSE	R^2
normal	0.000497	0.995	0.000891	0.991
cancer	0.00302	0.974	0.00142	0.988

Table 4

Estimated IC_{50} and γ on fixed ratio combinations between Model (1) and (3)

fixed ratio	cell	IC_{50} (SE)		γ (SE)	
A:B:C	group	Model(1)	Model(3)	Model(1)	Model(3)
Single A	normal	182.47(4.92)	180.94(2.57)	1.06(0.03)	1.00(0.02)
1:0:0	cancer	98.92(6.93)	95.85(1.75)	1.16(0.09)	1.17(0.03)
Single B	normal	70.96(2.55)	73.62(1.31)	1.37(0.07)	1.37(0.04)
0:1:0	cancer	60.86(5.04)	61.20(1.50)	1.12(0.11)	1.29(0.05)
Single C	normal	115.73(5.95)	117.11(1.42)	1.48(0.11)	1.70(0.03)
0:0:1	cancer	54.14(5.11)	55.11(1.05)	1.72(0.20)	1.29(0.04)
1:1:0	normal	84.67(5.31)	88.29(1.84)	1.78(0.17)	1.60(0.04)
	cancer	67.90(5.61)	68.84(2.01)	1.35(0.14)	1.26(0.05)
0:1:1	normal	83.43(4.77)	84.70(1.69)	1.61(0.13)	1.72(0.05)
	cancer	63.81(4.47)	63.67(1.95)	1.09(0.08)	0.99(0.04)
1:0:1	normal	132.51(10.95)	129.14(2.39)	1.21(0.11)	1.28(0.03)
	cancer	65.37(7.09)	64.55(1.42)	2.07(0.47)	1.93(0.06)
10:1:0	normal	152.99(4.35)	158.30(1.99)	1.13(0.04)	1.17(0.02)
	cancer	88.80(6.39)	89.50(1.41)	1.20(0.10)	1.19(0.03)
0:1:10	normal	106.73(8.32)	109.63(1.16)	1.62(0.19)	1.74(0.03)
	cancer	55.18(4.40)	57.49(0.94)	1.47(0.15)	1.51(0.03)
1:1:1	normal	92.79(8.90)	92.98(1.40)	1.96(0.37)	1.59(0.03)
	cancer	64.40(4.59)	64.01(1.26)	1.30(0.11)	1.39(0.03)
10:1:10	normal	119.60(6.86)	122.89(2.14)	1.38(0.09)	1.34(0.03)
	cancer	64.97(7.75)	64.51(1.27)	1.98(0.46)	1.83(0.05)

Hill-based model (3). Note that the standard errors from the original Hill model (1) are comparatively larger in all cases, because fewer runs are used to fit Model (1) (6-8 runs) than Model (3) (512 runs).

Table 5

Loewe Interaction Index based on Model (3) Interaction Index (SE)

Drugs	*A* ~ *B*	*B* ~ *C*	*A* ~ *C*
Normal	0.844(0.023)	0.937(0.024)	0.908(0.020)
Cancer	0.922(0.034)	**1.096(0.040)**	0.922(0.016)

Note: Effect level X = 50%, fixed ratio 1:1 for pairwise drugs