Assessing joint action of combination therapy in vivo pre-clinical experiments using Global-Two-Stage regression

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1 Abstract

In a fixed-dose two drug combination treatment PDX experiment, tumor-bearing mice were randomly assigned to one of the four treatment arms: control group (denoted as C), one of the single drug treatment group (denoted as A or B), and combination treatment group (denoted as AB). For each mouse, the tumor volume was measured at the initial day of treatment, and then twice a week till the end of the study. Nowadays, PDX models become a golden standard for in vivo drug screening. Increasing evidence has shown a high association between drug responses in PDX models and patients' responses[Gao et al., 2015, Izumchenko et al., 2017]. Combination therapy is a popular approach in cancer treatment and new drug development to overcome the resistance of single therapy or to improve the effectiveness of treatment [Dawson and Carragher, 2014, Wright, 2016]. The combination effect is usually categorized into three types: synergistic, additive, and antagonistic. The additive effect simply means that the combination of two drugs only shows the additive effect with no interaction presented; the synergistic effect represents than when two drugs applied together, they produce an enhanced effect; and the antagonistic effect is the opposite of the synergistic effect, it represents the combined effect is reduced when two drugs present together. While the synergistic effect of combination therapy is a promising area for cancer treatment, it remains a challenging problem to estimate the joint action of fixed-dose two-drug combination in PDX models due to limited literature available in preclinical in vivo experiments. Lopez [Lopez et al., 1999] proposed a distance based hypothesis testing approach to test the joint action of combination therapy by quantifying the deviation between the expected tumor growth curve under the null hypothesis and observed tumor growth curves of mice in combination therapy group. However, our simulation study shows that the proposed method has high type I error rate and tends to conclude that the combination drug is antagonistic.

2 Method

2.1 Growth/Drug effect model

We assume that log tumor growth rate consists of two parts: natural growth of tumor and growth decay inhibited by drug/treatment. In math notation, it can be represented as

$$\frac{dL(t)}{dt} = (\text{tumor growth}) - (\text{drug effect})$$

where L is the logarithm of tumor volume and t is the time. Let $V = exp(L)$ to be the tumor volume, then the change in tumor size over time dV/dt can be calculated as

$$\frac{dV(t)}{dt} = \frac{dV(t)}{dL(t)}\frac{dL(t)}{dt} = (\text{tumor growth})V(t) - (\text{drug effect})V(t)$$

This equation is in line with Ribba' review [Ribba et al., 2014], where a series of mixed-effect models are presented to quantify the effects of anticancer drug treatment. There can be various options of modeling tumor growth after cancer treatment. Two most popular tumor growth models are exponential growth and Gompertz growth. Additionally, drug effect term can be chosen based on the knowledge of dose/response kinetics for specific agents. Here, we follow the formulation proposed by Lopez [Lopez et al., 1999], that assumes the delay of tumor growth is proportion to the drug concentration at a constant rate. Besides, we will show that the additive model is in line with Bliss independence definition.

Choosing the drug induced effect as $\beta_g exp(-k_g t)$ in equation (13) in [Ribba et al., 2014]

$$\frac{dL(t)}{dt} = \begin{cases} \alpha_1 - \alpha_2(L - L_0) & \text{control} \\ \alpha_1 - \alpha_2(L - L_0) - \beta_g exp(-k_g t) & g = A \text{ or} B \end{cases}$$

which is equivalent to

$$\frac{dL(t)}{dt} = \alpha_1 - \alpha_2(L - L_0) - \beta_A exp(-k_A t)I(g = A) - \beta_B exp(-k_B t)I(g = B)$$

Integrating with respect to t,

$$L(t) = \begin{cases} L_0 + \frac{-\alpha_1 exp(-\alpha_2 t)+\alpha_1}{\alpha_2} & \text{g = C} \\ L_0 + \frac{-\alpha_1 exp(-\alpha_2 t)+\alpha_1}{\alpha_2} - \frac{\beta_g exp(-k_g t)}{\alpha_2 - k_g} + \frac{\beta}{(\alpha_2 - k_g)exp(\alpha_2 t)} & g = A \text{ or } B, k_g \neq \alpha_2 \\ L_0 + \frac{-\alpha_1 exp(-\alpha_2 t)+\alpha_1}{\alpha_2} - \frac{\beta t}{exp(\alpha_2 t)} & g = A \text{ or } B, k_g = \alpha_2 \end{cases}$$

Let y_{ij}, ith individual and jth data point within individual i. t_{ij} is the time

$$y_{ij} = L(\psi_i, t_{ij}) + \epsilon_{ij}$$
$$\psi_i = \bar{\psi} + \eta_i$$

, where $\psi_i = (\alpha_{1i}, \alpha_{2i}, \beta_{Ai}, k_{Ai}, \beta_{Bi}, k_{Bi}) \sim N(\bar{\psi}, \Omega)$, $\bar{\psi}$ is the vector of the population parameters, $\eta_i \sim N(0, \Omega)$ in the random effect for each individual and $\epsilon_{ij} \sim N(0, \sigma^2)$.

Using the probability model:

$$y_{ij} \sim N(L(\psi_i, t_{ij}), \sigma^2)$$
$$\psi_i \sim N(\bar{\psi}, \Omega)$$

2.2 Estimation of Population parameters

1. Stochastic approximation EM algorithm (saemix)

Let $\theta = (\bar{\psi}, \Omega, \sigma^2)$. Maximum likelihood estimation of θ consists of maximizing with respect to θ:

$$\mathcal{L}(\theta, y) = p(y; \theta) = \int p(y, \psi; \theta) d\psi = \prod_{i=1}^{N} \int p(y_i|\psi_i; \theta) p(\psi_i; \theta) d\psi$$

Stochastic approximation EM algorithm (SAEM) can be used maximize the likelihood function.

2.3 Hypothesis Testing

2.3.1 Normalized prediction distribution errors

We are testing the H0: the fitted model is adequately to capture tumor growth curve in the combination group (The two drugs are independent under bliss independence). We use normalised prediction distribution errors (npde) [Mentré and Escolano, 2006, Comets and Mentré, 2021, Comets et al., 2010] to check the model fitting on combination drug. The predictive distribution can be obtained as

$$p(y_i; \hat{\theta}) = \int p(y_i|\psi_i; \hat{\theta}) p(\psi_i; \hat{\theta}) d\psi$$

where $\hat{\theta}$ is the maximum likelihood estimator. The predictive distribution can be computed by Monte-Carlo simulation. For each replication k, we draw a individual parameters ψ_i from the estimated parameters $\hat{\theta}$, and the generate $y_{ij}^{sim(k)}$ from the distribution $p(y_i|\psi_i; \hat{\theta})$. The prediction discrepancy for an observation y_{ij} is defined as the cdf of the posterior predictive distribution

$$pd_{ij} = F_{ij}(y_{ij}) = \int^{y_{ij}} p(y_i; \hat{\theta}) dy_i = \int^{y_{ij}} \int p(y_i|\psi_i; \hat{\theta}) p(\psi_i; \hat{\theta}) d\psi_i dy_i \approx \frac{1}{K} \sum_{k=1}^{K} I[y_{ij}^{sim(k)} < y_{ij}]$$

The pd_{ij} is the percentile of the observation y_{ij} in the marginal distribution of the posterior predictive distribution under H_0. When using Monte-Carlo approximation pd_{ij} can be calculated as proportion of the simulated value is less then the observed value y_{ij}. If the model and the population parameters are correct, by construction, these prediction discrepancies should follow a uniform

distribution over $[0, 1]$. Then, it was transform to standard normal distribution using the normal inverse cumulative density function.

$$npde_{ij} = \Phi^{-1}(pde_{ij})$$

For repeated measurements, there's correlation between a given individual's observations, which leads to an inflated type I error rate. To account for the correlation, we can use the empirical variance-covariance matrix to decorrelate the simulated and observed observations. The empirical mean is obtained as:

$$a = E[Y_i] = \frac{1}{K}\sum_{i=1}^{K} Y_i^{sim(k)}$$

and the empirical variance is:

$$var[Y_i] = \frac{1}{K-1}\sum_{i=1}^{K}(Y_i^{sim(k)} - a)(Y_i^{sim(k)} - a)^T$$

Decorrelation is performed simulataneously for simulated data:

$$Y_i^{sim(k)\star} = var[Y_i]^{-1/2}(Y_i^{sim(k)} - a)$$

Then we can use the decorrelated data to calculate npde and compared it with standard normal distribution. To test the distribution of the npde, there tests are used: 1) rank test to test the mean is 0, 2) fisher variance test to test its variance is 1, 3) Shapiro-Wilks test to test normality. The global test is combining three tests together with a Bonferreoni correction. Alternative is to use Kolmogorov–Smirnov test to test whether the npde is normally distributed with mean 0 and varaince 1.

2.3.2 Distance-based statistics

A distance measure is introduced to measure how close are the expected value and the observed value. Distance between observed values $\mathbf{y}_j$ and expected value $L(\mathbf{t}, \theta_j)$ for one sample across n_j points is defined

$$D_j = \frac{1}{n_j}\left[\mathbf{y}_j - L(\bar{\psi}, \mathbf{t}_j)\right]^{\mathrm{T}}\left[Cov(\mathbf{y}_j)\right]^{-1}\left[\mathbf{y}_j - L(\bar{\psi}, \mathbf{t}_j)\right]$$

asymptotically $D_j n_j \sim \chi^2_{n_j}$, and $\sum_{j=1}^{M} D_j n_j$ values for all mice in each group and compared this sum to a χ^2 distribution with df equal to the $\sum_{j=1}^{M} n_j$.

- $\sum_{j\in A,B,C} D_j n_j$ is used to assess the goodness of the non-linear mixed effect model.
- $\sum_{j\in AB} D_j n_j$ is used to test that whether the additive model is correctly specified.

2.3.3 Prediction Interval

use binomial test to test the proportion of observations lies outside the 90% prediction interval.

3 Simulation

log tumor growth under null hypothesis:

$$y_i j = L(\phi_i, t_{ij}) + \epsilon_{ij} \;\; \epsilon_{ij} \sim N(0, 0.1^2)$$

log tumor growth under alternative:

$$y_i j = L(\phi_i, t_{ij}) + \epsilon_{ij} + \eta_{ij} \;\; \epsilon_{ij} \sim N(0, 0.1^2), \eta_{ij} \sim N(0.1, 0.2^2)$$

Parameter	Population Mean	Standard Deviation
α_0	0.4	0.05
α_1	0.05	0.01
β_A	0.3	0.05
k_A	0.05	0.01
β_B	0.8	0.1
k_B	0.1	0.02

Table 1: Simulation values for population parameters

4 Archive

4.0.1 Growth Model - Control

In control group, there is no drug in use, so there's no drug term in the equation.

$$\frac{dL(t)}{dt} = (\text{tumor growth})$$

We assume that the tumor growth without perturbation follows a Gompertizian growth model [Gompertz, 1825], where the relative growth rate decrease with increasing tumor size .

$$\frac{dL(t)}{dt} = \alpha_1 - \alpha_2(L - L_0)$$

where t is time, L is the natural logarithm of tumor volume and L_0 is the logarithm of tumor volume at $t = 0$, α_1 is the relative growth rate dL/dt when $L = L_0$, so $\alpha_1 \geq 0$, and α_1/α_2 is the maximum capacity of tumor volume growth. Note that when $\alpha_2 = 0$ tumor volume follows a exponential growth. $\alpha_2 > 0$ indicates growth rate decrease as tumor volume increases.

After integration:

$$\Rightarrow L(t) = \begin{cases} L_0 + \alpha_1 t & \alpha_2 = 0 \\ L_0 + \frac{-\alpha_1 exp(-\alpha_2 t) + \alpha_1}{\alpha_2} & \alpha_2 \neq 0 \end{cases}$$

4.0.2 Drug Effect Model - Single Drug

Compare to the control group, single drug is injected to mice so there's one more drug effect term.

$$\frac{dL(t)}{dt} = (\text{tumor growth}) - (\text{drug g effect})),$$

where $g = A, B$.

We assume that the drug concentration decay exponentially and the delayed growth rate is proportional to drug concentration. In mathematical notation, it is:

$$\frac{dL(t)}{dt} = \alpha_1 - \alpha_2(L - L_0) - \beta_g k_g (t - t_g) exp\left(-k_g(t - t_g)\right) \boldsymbol{I}_{[t \geq t_g]}$$

where $g = A, B$ represents which mono-therapy is in use, β_g is the inhibition effect of the drug, $k_g(t - t_g)exp(-k_g(t - t_g))$ shows the drug concentration in mice with time, t_g is the drug administration time, and k_g is rate of decay of the drug concentration. Note that $\beta_g \geq 0, k_g \geq 0$

$$L(t) = \begin{cases} L_0 + \alpha_1 t & \alpha_2 = 0, k_g = 0 \\ L_0 + \alpha_1 t + \frac{\beta_g}{k_g} + \frac{\beta_g}{k_g} exp\left(-k_g(t - t_g)\right)\left(-k_g(t - t_g) - 1\right) & \alpha_2 = 0, k_g \neq 0 \\ L_0 + \frac{\alpha_1 - \alpha_1 exp(-\alpha_2 t)}{\alpha_2} - \frac{1}{2}\beta_g \alpha_2 (t - t_g)^2 exp\left(-\alpha_2(t - t_g)\right) & \alpha_2 = k_g, k_g \neq 0 \\ L_0 + \frac{\alpha_1 - \alpha_1 exp(-\alpha_2 t)}{\alpha_2} + \frac{\beta_g k_g exp\big(-k_g(t-t_g)\big)\big((k_g - \alpha_2)(t-t_g)+1\big)}{(k_g - \alpha_2)^2} - \frac{\beta_g k_g exp(-\alpha_2 t)}{(k_g - \alpha_2)^2} & \alpha_2 \neq k_g, \alpha_2 \neq 0 \end{cases}$$

where $t \geq t_g$

Denote D_g be the term related to drug g in the logarithm of tumor volume equation, and it can be calculated as:

$$D_g = \begin{cases} 0 & k_g = 0 \\ \frac{\beta_g}{k_g} exp\left(-k_g(t-t_g)\right)\left(-k_g(t-t_g)-1\right) & \alpha_2 = 0, k_g \neq 0 \\ -\frac{1}{2}\beta_g k_g (t-t_g)^2 exp\left(-k_g(t-t_g)\right) & \alpha_2 = k_g, k_g \neq 0 \\ \frac{\beta_g k_g exp\left(-k_g(t-t_g)\right)\left((k_g-\alpha_2)(t-t_g)+1\right)}{(k_g-\alpha_2)^2} - \frac{\beta_g k_g exp(-\alpha_2 t)}{(k_g-\alpha_2)^2} & \alpha_2 \neq k_g, \alpha_2 \neq 0 \end{cases}$$

where $t \geq t_g$

Then the log tumor volume can be expressed with respect to

$$L(t) = \begin{cases} L_0 + \alpha_1 t + D_g & \alpha_2 = 0 \\ L_0 + \frac{\alpha_1 - \alpha_1 exp(-\alpha_2 t)}{\alpha_2} + D_g & \alpha_2 \neq 0 \end{cases}$$

4.0.3 Combined Effect Model under additive

In two fixed-dose drug combination, two drugs are administrated. Under the null hypothesis of additive, the growth rate are expected to be:

$$\frac{dL(t)}{dt} = \text{(tumor growth)} - \text{(drug A effect)} - \text{(drug B effect)}$$

Assume two drugs act independently, then the expected growth rate follows the equation below:

$$\frac{dL(t)}{dt} = \alpha_1 - \alpha_2(L - L_0) - \beta_A k_A (t - t_A) exp\left(-k_A(t-t_A)\right) \boldsymbol{I}_{[t \geq t_A]} - \beta_B k_B (t - t_B) exp\left(-k_B(t-t_B)\right) \boldsymbol{I}_{[t \geq t_B]}$$

Then under the null hypothesis, we have the following formula for combined drug group

$$L(t) = \begin{cases} L_0 + \alpha_1 t + D_A + D_B & \alpha_2 = 0 \\ L_0 + \frac{\alpha_1 - \alpha_1 exp(-\alpha_2 t)}{\alpha_2} + D_A + D_B & \alpha_2 \neq 0 \end{cases}$$

where $t \geq max(t_A, t_B)$

4.1 Relation to Bliss Independence Model

Bliss independence approach bases the definition of independence on its probabilistic interpretation, where the presence of one drug does not affect the probability of another drug's effect on tumor growth decay. We note that the additive model is in concordance with Bliss independence model using the Treatment-to-control ratio. Let $E(A)$ and $E(B)$ represent the probability of cell growth inhibited by drug A and B respectively. Under Bliss independence,

$$E_{AB} = E_A + E_B - E_A E_B$$

where the effect $E_g = 1 - V_g(t)/V_C(t)$, indicating the percent of tumor being inhibited, and $V_g(t)(L_g(t))$ is the tumor volume (log tumor volume) in $g, g \in A, B, C, AB$ denote drug A, B, control group C and combined therapy AB. This formula is equivalent to

$$\begin{aligned}
&\frac{V_{AB}(t)}{V_C(t)} = \frac{V_A(t)}{V_C(t)}\frac{V_B(t)}{V_C(t)} \\
\iff & log(V_{AB}(t)) = log(V_A(t)) + log(V_B(t)) - log(V_C(t)) \\
\iff & L_{AB}(t) = L_A(t) + L_B(t) - L_C(t) \\
\iff & L_{AB}(t) = \begin{cases} L_0 + \alpha_1 t + D_A(t) + D_B(t) & \alpha_2 = 0 \\ L_0 + \frac{\alpha_1 - \alpha_1 exp(-\alpha_2 t)}{\alpha_2} + D_A(t) + D_B(t) & \alpha_2 \neq 0 \end{cases}
\end{aligned}$$

4.2 Parameter Estimation and Hypothesis Testing in GTS

4.2.1 Parameter Estimates For Individual Mouse

We begin by obtaining estimates of model parameters for each individual mouse receiving at most one drug. Assume that for mouse j and $j = 1, \ldots, M, j \in A, B, C$

$$\mathbf{y}_j = L(\mathbf{t}, \theta_j) + \epsilon_j$$

where

- $\mathbf{y}_j = (y_{j,t_1}, y_{j,t_2}, \ldots, y_{j,t_{n_j}})^{\mathrm{T}}$ an $n_j \times 1$ vector of observed log tumor volume of mouse j across n_j time points,
- $L(\mathbf{t}, \theta_j) = (L(t_1, \theta_j), L(t_2, \theta_j), \ldots, L(t_{n_j}, \theta_j))^{\mathrm{T}}$ an $n_j \times 1$ vector of expected log tumor volume of mouse j across n_j time points,
- θ_j is $k \times 1$ parameters for mouse j; eg. in control group $\theta_j = (\alpha_{1,j}, \alpha_{2,j})^{\mathrm{T}}$; in one drug group $\theta_j = (\alpha_{1,j}, \alpha_{2,j}, \beta_{A,j}, k_{A,j})^{\mathrm{T}}$ or $(\alpha_{1,j}, \alpha_{2,j}, \beta_{B,j}, k_{B,j})^{\mathrm{T}}$
- $\epsilon_j \sim N_{n_j}(0, \sigma^2 I_{n_j})$ an $n_j \times 1$ vector of measurement error.

We use nonlinear least square method to estimate individual specific $\hat{\theta}_j$ for θ_j, which is to minimize the objective function:

$$Q(\theta_j) = \frac{1}{2}\left[\mathbf{y}_j - L(\mathbf{t}, \theta_j)\right]^{\mathrm{T}}\left[\mathbf{y}_j - L(\mathbf{t}, \theta_j)\right] = \frac{1}{2}\sum_{i=1}^{N}\left(y_{j,t_i} - L(t_i, \theta_j)\right)^2$$

Asymptotically,

$$\sqrt{n_j}(\hat{\theta}_j - \theta_j) \sim N_k\left(0, \hat{\sigma}^2\left(\frac{1}{n_j}\left[\nabla_{\hat{\theta}_j} L(\mathbf{t}, \hat{\theta}_j)\right]\left[\nabla_{\hat{\theta}_j} L(\mathbf{t}, \hat{\theta}_j)\right]^{\mathrm{T}}\right)^{-1}\right)$$

where $\hat{\sigma}^2 = \frac{1}{n_j - k}\sum_{i=1}^{n_j}(y_{j,t_i} - L(t_i, \hat{\theta}_j))^2$ and $\nabla_{\hat{\theta}_j} L(\mathbf{t}, \hat{\theta}_j) = (\nabla_{\hat{\theta}_j} L(\mathbf{t}_1, \hat{\theta}_j), \ldots, \nabla_{\hat{\theta}_j} L(\mathbf{t}_{n_j}, \hat{\theta}_j))$ an $k \times n_j$ matrix of gradients. The hessian matrix $\nabla^2_{\hat{\theta}_j} Q(\hat{\theta}_j) \approx \left[\nabla_{\hat{\theta}_j} L(\mathbf{t}, \hat{\theta}_j)\right]\left[\nabla_{\hat{\theta}_j} L(\mathbf{t}, \hat{\theta}_j)\right]^{\mathrm{T}}$. For each individual mouse, we have estimated $\hat{\theta}_j$ along with it's information matrix $\hat{S}_j^{-1}$.

4.2.2 Population Distribution of model parameters

We use modified global two-stage (GTS) method to estimate the population distribution of parameters [Steimer et al., 1984]. The basic assumptions are: (1) The model's structure is common to all individuals; (2) The parameter values may be different from one subject to another; (3) The measurement noise has same structure across all individuals. Assume that the parameters are random variables $\theta_j \sim_{i.i.d} N_k(\theta_0, C_0)$ and that there are total M mice in one drug group and control group. The maximum likelihood estimates $\hat{\theta}_0$ and $\hat{C}_0$ can be obtained by minimize the following objective function:

$$O(\xi) = \sum_{j=1}^{M} [(\hat{\theta}_j - \theta_0)^{\mathrm{T}} (\hat{S}_j + C_0)^{-1} (\hat{\theta}_j - \theta_0) + ln\ det(\hat{S}_j + C_0)]$$

The iterative scheme for finding $(\hat{\theta}_0, \hat{C}_0)$ is as follows. At iteration $(n+1)$, given $(\hat{\theta}^{(n)}, \hat{C}^{(n)})$

1. Produce refined estimates of individual parameters:

$$\hat{\theta}_j^{(n+1)} = \left(\hat{S}_j^{-1} + (\hat{C}^{(n+1)})^{-1}\right)^{-1} \left(\hat{S}_j^{-1}\hat{\theta}_j + (\hat{C}^{(n+1)})^{-1}\hat{\theta}^{(n)}\right)$$

2. Produce a refined estimate of the population characteristics:

$$\hat{\theta}^{(n+1)} = \frac{1}{M}\sum_{j=1}^{M} \hat{\theta}_j^{(n+1)}$$

$$\hat{C}^{(n+1)} = \frac{1}{M}\sum_{j=1}^{M} (\theta_j^{(n+1)} - \hat{\theta}^{(n+1)})(\theta_j^{(n+1)} - \hat{\theta}^{(n+1)})^{\mathrm{T}} + \frac{1}{M}\sum_{j=1}^{M} (\hat{S}_j^{-1} + (\hat{C}^{(n+1)})^{-1})^{-1}$$

The number of parameters for each mouse are not the same, i.e we have two parameters in control group and four parameters in one-drug group. To make the parameters have the same dimension, we fill in zeros to the inestimable parameters in 6×1 vector $\theta_j = (\alpha_{1,j}, \alpha_{2,j}, \beta_{A,j}, k_{A,j}, \beta_{B,j}, k_{B,j})^{\mathrm{T}}$ and $\theta_j \sim N_6(\theta_0, C_0)$. We augment all the information matrix by setting to zero those rows and columns of the information matrix and zeros in parameter vector corresponding to the inestimable parameters. The estimation of population parameters are insensitive to the inestimable parameters. For example, for control group,

$$\hat{\theta}_j = (\alpha_{1,j}, \alpha_{2,j}, 0, 0, 0, 0)^{\mathrm{T}}$$

, and

$$\hat{S}_j^{-1} = \begin{pmatrix} s_{11} & s_{12} & 0 & 0 & 0 & 0 \\ s_{21} & s_{22} & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \end{pmatrix}$$

Another example, for drug B group,

$$\hat{\theta}_j = (\alpha_{1,j}, \alpha_{2,j}, 0, 0, \beta_B, k_B)^{\mathrm{T}}$$

, and

$$\hat{S}_j^{-1} = \begin{pmatrix} s_{11} & s_{12} & 0 & 0 & s_{15} & s_{16} \\ s_{21} & s_{22} & 0 & 0 & s_{25} & s_{26} \\ 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \\ s_{51} & s_{52} & 0 & 0 & s_{55} & s_{56} \\ s_{61} & s_{62} & 0 & 0 & s_{65} & s_{66} \end{pmatrix}$$

4.2.3 Hypothesis Testing

Assume that for mouse j,

$$\mathbf{y}_j = L(\mathbf{t}, \theta_j) + \epsilon_j$$

where

- $\mathbf{y}_j = (y_{j,t_1}, y_{j,t_2}, \ldots, y_{j,t_{n_j}})^{\mathrm{T}}$ an $n_j \times 1$ vector of observed log tumor volume of mouse j across n_j time points,
- $L(\mathbf{t}, \theta_j) = (L(t_1, \theta_j), L(t_2, \theta_j), \ldots, L(t_{n_j}, \theta_j))^{\mathrm{T}}$ an $n_j \times 1$ vector of expected log tumor volume of mouse j across n_j time points,
- $\epsilon_j \sim N_{n_j}(0, \sigma^2 I_{n_j})$ an $n_j \times 1$ vector of measurement error.

When estimating the population parameters, we have the following assumptions: $\theta_j = (\alpha_{1,j}, \alpha_{2,j}, \beta_{A,j}, k_{A,j}, \beta_{B,j}, k_{B,j})^{\mathrm{T}}$ and $\theta_j \sim N_6(\theta_0, C_0)$. By first order taylor approximation,

$$L(\mathbf{t}, \theta_j) \approx L(\mathbf{t}, \theta_0) + \nabla_{\theta_0} L(\mathbf{t}, \theta_0)(\theta_j - \theta_0)$$

then

$$E[L(\mathbf{t}, \theta_j)] \approx L(\mathbf{t}, \theta_0)$$

and

$$cov(L(\mathbf{t}, \theta_j)) \approx [\nabla_{\theta_0} L(\mathbf{t}, \theta_0)] C_0 [\nabla_{\theta_0} L(\mathbf{t}, \theta_0)]^{\mathrm{T}}$$

we have

$$L(\mathbf{t}, \theta_j) \sim N_{n_j}\left(L(\mathbf{t}, \theta_0), [\nabla_\theta L(\mathbf{t}, \theta_0)]\, C_0\, [\nabla_\theta L(\mathbf{t}, \theta_0)]^{\mathrm{T}}\right)$$

Since $\epsilon_j \sim N_{n_j}(0, \sigma^2 I_{n_j})$, we have

$$\mathbf{y}_j = L(\mathbf{t}, \theta_j) + \epsilon_j \sim N_{n_j}\left(L(\mathbf{t}, \theta_0), [\nabla_\theta L(\mathbf{t}, \theta_0)]\, C_0\, [\nabla_{\theta_0} L(\mathbf{t}, \theta_0)]^{\mathrm{T}} + \sigma^2 I\right)$$

We substitute θ_0, C_0, σ^2 are by their estimator $\hat{\theta}_0, \hat{C}_0, \hat{\sigma}^2$, then

$$\mathbf{y}_j \sim N_{n_j}\left(L(\mathbf{t}, \hat{\theta}_0), \left[\nabla_{\hat{\theta}_0} L(\mathbf{t}, \hat{\theta}_0)\right] \hat{C}_0 \left[\nabla_{\hat{\theta}_0} L(\mathbf{t}, \hat{\theta}_0)\right]^{\mathrm{T}} + \hat{\sigma}^2 I\right)$$

A distance measure is introduced to measure how close are the expected value and the observed value. Distance between observed values $\mathbf{y}_j$ and expected value $L(\mathbf{t}, \theta_j)$ for one sample across n_j points is defined

$$D_j = \frac{1}{n_j} \left[\mathbf{y}_j - L(\mathbf{t}, \theta_j)\right]^{\mathrm{T}} \left[Cov(\mathbf{y}_j)\right]^{-1} \left[\mathbf{y}_j - L(\mathbf{t}, \theta_j)\right]$$

asymptotically $D_j n_j \sim \chi^2_{n_j}$, and $\sum_{j=1}^{M} D_j n_j$ values for all mice in each group and compared this sum to a χ^2 distribution with df equal to the $\sum_{j=1}^{M} n_j$.

- $\sum_{j \in A,B,C} D_j n_j$ is used to assess the goodness of the non-linear mixed effect model.
- $\sum_{j \in AB} D_j n_j$ is used to test that whether the additive model is correctly specified.

5 Simulation

We conducted a simulation study to evaluate the Type I error of the Global-Two-Stage method. We generated individual parameters for each subject from the independent normal distribution with mean and standard deviation from the Table 2. We generate data under 9 different scenarios: with number of samples to be 5, 10, 50 and number of observations to be 10, 25 or 50 3. Tumor Growth curves were simulated according to the Gompertz function formulated in Method section, with additional measurement error added to the growth curve. We assume that measurement error for each observation is independently, identical sampled from normal distribution with mean and variance to be 0 and 0.05 respectively.

Parameter	Population Mean	Standard Deviation
α_0	0.1247	0.0077
α_1	0.0234	0.0046
β_A	0.8124	0.0433
k_A	0.3279	0.0203
β_B	0.4133	0.0311
k_B	0.2453	0.0131

Table 2: Simulation values for population parameters

N Samples	N Observations	Type I error rate
5	10	0.739
5	25	0.807
5	50	0.956
10	10	0.762
10	25	0.924
10	50	0.968
50	10	0.782
50	25	0.999
50	50	0.951

Table 3: Type I error rate

5.1 One realization

We began by obtaining estimates of model parameters for each individual subject in non-treatment, or one drug groups, using nonlinear least square (NLS) with nonegative constraints on all the parameters. Overall, NLS provides good individual trajectory estimations that captures the general trend of stimulated growth curves Figure 1. However, there is convergence issue when estimating individual parameters, and 10 out of 15 fits do not converge. This result may be explained by the fact that number

of observations is small compared to the large number of parameters. As in the control group, when only two parameters are needed to be estimated, the optimization algorithm converges better. Though, the algorithm does not converge, the outputs of hessian matrix and estimated values are still available. We still utilize those outputs to move over to the next step of population estimation.

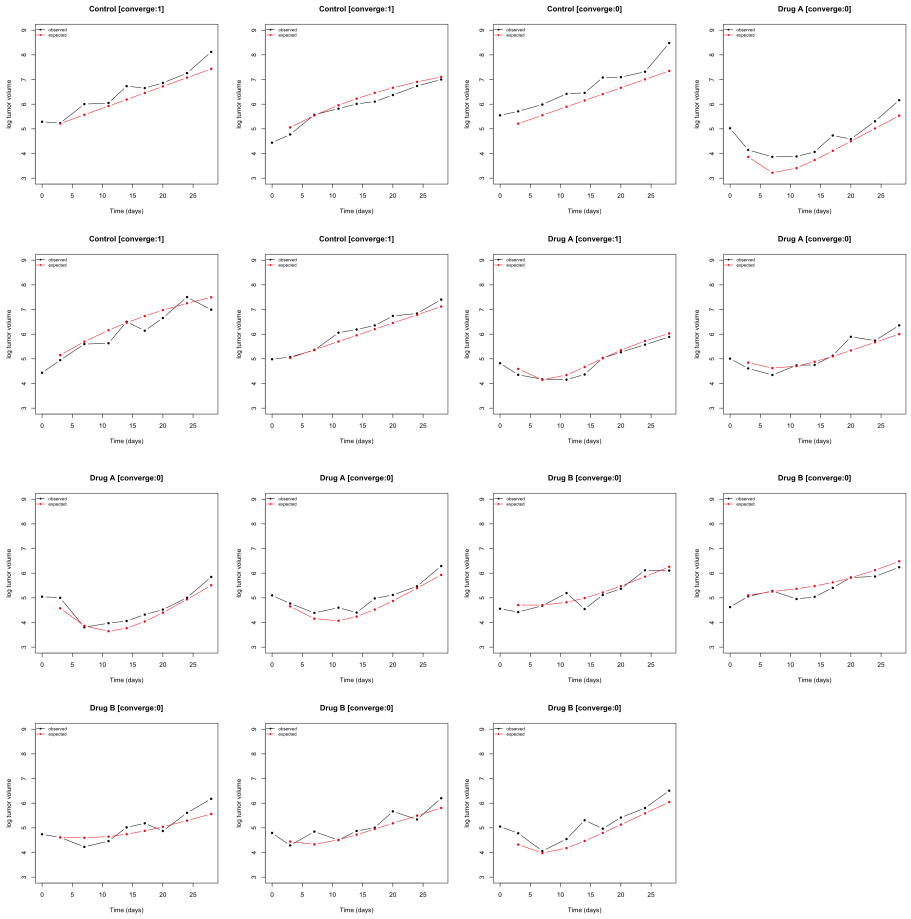

Figure 1: The black dotted lines represent the simulated trajectories for mice, while the red dotted lines represent the fitted values using nonlinear least square. The convergence code 0 indicates the optimization algorithm failed, and code 1 indicates the optimization algorithm converged.

To obtain the population distribution of parameters, we combined individual parameters estimates from the 15 mice receiving at most one drug treatment by using a modified GTS. Using the estimated population distribution of our model parameters, we plotted the population distribution of trajectories for the tumor growth in each treatment group 2. For the combination therapy group, the expected population tra-

jectory is under the null hypothesis. We found out that for the control and one drug groups, the population curve can capture the mean trend. However, for the combination group, the simulated tumor growth curves are constantly above the expected curve. The proposed method does not provide reasonable prediction for combination treatment under additive. This inconsistency may be due to the small sample size (n=15) in the training set or the convergence issues in individual parameter estimation.

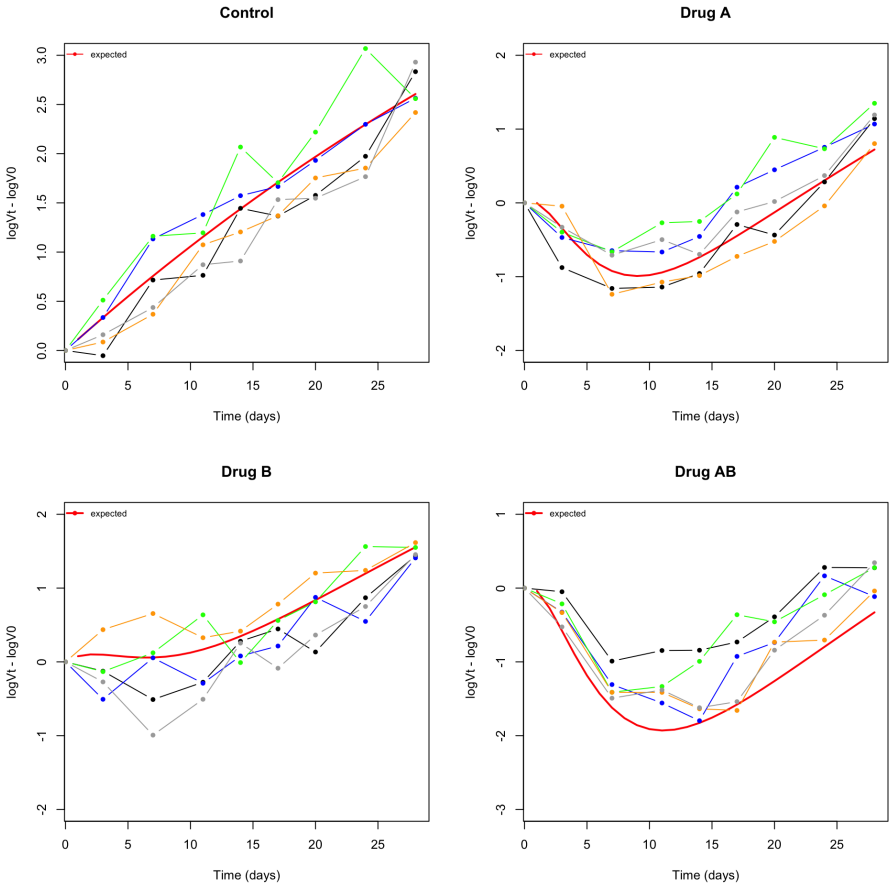

Figure 2: The dotted lines are the simulated observed trajectories and the red solid lines represent the fitted population curve from GTS

In the final part of the method, the proposed distance is used to measure the similarity between observed and predicted trajectories. For each mouse in training set, this statistics is used as a measure of goodness-of-fit of the growth curve, while for combination treatment group, this is used as a statistical test for the null hypotheses. Figure 3 shows the distance for each mouse and the significance of such distance is

color coded: red represents p-value < 0.05 and black otherwise. This hypothesis results illustrate that, the proposed method will lead to high type I error rate, since our dataset is all simulated from null hypothesis. 3 out of 5 mice is combination group is concluded having a antagonistic combination effect which does not in line with our simulation scenario. The proposed method is prone to conclude that the combination drug is antagonistic. This result is expected due to the poor population estimation in combination group.

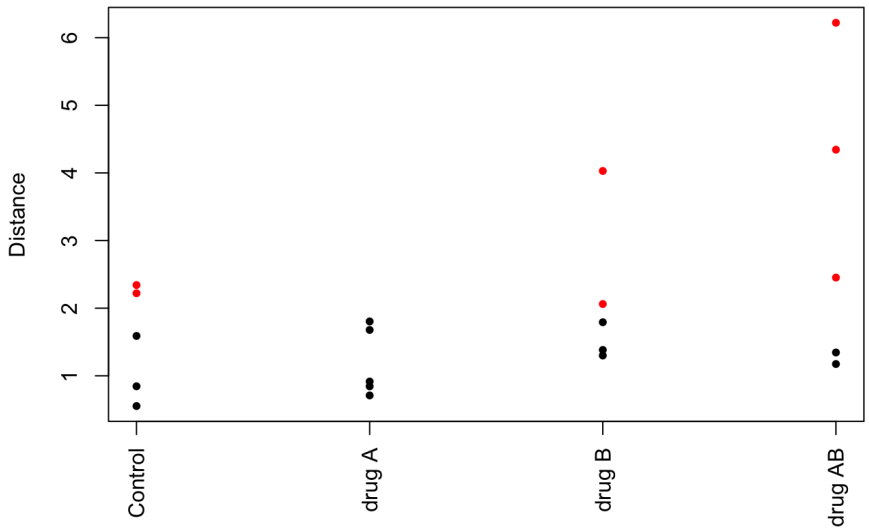

Figure 3: Distance values shows the deviation between individual simulated tumor volume. The red dot illustrates that the simulated data for such mouse is significantly far from the fitted population trajectory. In combination therapy group, it indicate distance for such mouse are significant under null hypothesis.

6 Simplest Scenario: constant growth rate and drug effect

6.1 Growth Model

In the Simplest Scenario of tumor growth, we assume that the tumor growth term and drug effect term to be constants, which is the same model as Demidenko proposed [Demidenko and Miller, 2019].

The models are:

$$\frac{dL_C(t)}{dt} = \alpha_1 \text{ (Control)}$$
$$\frac{dL_A(t)}{dt} = \alpha_1 - \beta_A \text{ (Treatment A)}$$
$$\frac{dL_B(t)}{dt} = \alpha_1 - \beta_B \text{ (Treatment B)}$$
$$\frac{dL_{AB}(t)}{dt} = \alpha_1 - \beta_A - \beta_B \text{ (Treatment AB)}$$

We Reparametrize the above equations and get,

$$\frac{dL_C(t)}{dt} = \alpha_C \text{ (Control)}$$
$$\frac{dL_A(t)}{dt} = \alpha_A \text{ (Treatment A)}$$
$$\frac{dL_B(t)}{dt} = \alpha_B \text{ (Treatment B)}$$
$$\frac{dL_{AB}(t)}{dt} = \alpha_{AB} \text{ (Treatment AB)}$$

Then the null hypothesis of additive effect is equivalent to

$$H_0 : \alpha_C + \alpha_{AB} = \alpha_A + \alpha_B$$

which is the same as

$$H_0 : exp\left((\alpha_C + \alpha_{AB})t\right) = exp\left((\alpha_A + \alpha_B)t\right)$$

Calculating AUC for each group:

$$\int exp(\alpha t)dt = exp(\alpha t)/\alpha$$

$$AUC_C AUC_{AB} = exp(\alpha_C t)/\alpha_C exp(\alpha_{AB} t)/\alpha_{AB}$$
$$AUC_A AUC_B = exp(\alpha_A t)/\alpha_A exp(\alpha_B t)/\alpha_B$$

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