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Assessing Interactions for Fixed-Dose Drug Combinations in Subcutaneous Tumor Xenograft Studies

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Abstract

Drug combinations in preclinical tumor xenograft studies are often assessed using fixed doses. Assessing the joint action of drug combinations with fixed doses has not been well developed in the literature. Here, an interaction index is proposed for fixed-dose drug combinations in a subcutaneous tumor xenograft model. Furthermore, a boot-strap percentile interval of the interaction index is also developed. The joint action of two drugs can be assessed based on confidence limits of the interaction index. Tumor xenograft data from actual two-drug combination studies are analyzed to illustrate the proposed method.

Keywords

Bootstrap; Confidence interval; Drug-drug combination; Log10 cell kill; Interaction index; Right-censoring

1. Introduction

The study of the joint action of drugs administered in combination is important for the treatment of advanced cancer. The joint action of drug combinations is usually divided into three types: additive, synergistic, and antagonistic. The goal of the study is to detect a synergistic effect of the drug combination. Statistical methods to assess the joint action of drug combinations have been developed over many years. A dose-response surface model is often used to assess the joint action by applying Loewe's simple similar joint action principle [1]. Such methods have been extensively studied by Hewlett [2], Finney [3], Chou and Talaly [4], Greco et al. [5], Giltinan et al. [6], Machado and Robinson [7], Plummer [8], and more recently by Tan et al. [9], Straetmans et al. [10], Kong and Lee [11]–[12], and others. However, dose-response surface methods are not applicable to a fixed-dose drug combination study. Therefore, testing the superiority of the combination over its components is often conducted for a fixed-dose drug combination study with a normally distributed response. Examples include Snapinn [13], Laska and Meisner [14], Hung [15]–[16], Hung et al. [17], and others. But the superiority of the drug combination does not reflect the interaction of the drugs. In cancer therapy development, drug combinations are first tested in preclinical animal models to determine whether the combination therapy should be further investigated in a clinical trial. Drug combinations are often tested in preclinical tumor xenograft models with fixed doses to minimize the number of animals required per drug combination. To study the joint action of drug combinations in such experiments, an

interaction index of a relative-effect multiplicative model is proposed for an orthotopic tumor xenograft model [18]. However, for a subcutaneous tumor xenograft model, there is little literature available to study the joint action of drug combinations. Here, an interaction index of the surviving fraction multiplicative model is defined to assess the joint action of a two-drug combination with a fixed dose of each drug. Furthermore, a bootstrap percentile interval of the interaction index is also proposed. The joint action of two drugs can be assessed based on confidence limits of the interaction index. Tumor xenograft data from actual two-drug combination studies are analyzed to illustrate the proposed method.

2. Subcutaneous Tumor Xenograft Model

In a subcutaneous tumor xenograft model, human cancer cells are engrafted into SCID mice, typically on the flank. Researchers monitor the growth of these tissue fragments into tumors of measurable size. Once tumors have reached a certain size, the mice are randomized into different treatment groups and receive a drug or drug combination. Tumor volume is measured for each tumor at the initiation of the study and weekly throughout the study period. Each mouse is euthanized when the tumor reaches four times its initial volume or at the end of study, thus resulting in incomplete longitudinal tumor volume data. Therefore, the time to event (e.g., tumor quadrupling), rather than tumor volume, is often the endpoint for assessing antitumor activity [19]. The exact time of tumor quadrupling in such experiments is not observed because tumor volume is recorded at evenly spaced intervals. However, the following interpolation formula can be used to calculate the tumor quadrupling time [19]–[20],

$$t_e = t_1 + (t_2 - t_1) \log(V_e/V_{t_1}) / \log(V_{t_2}/V_{t_1}), \quad (1)$$

where t_e is the interpolated quadrupling time, t_1 and t_2 are the lower and upper observation times bracketing the time of quadrupling tumor volume $V_e = 4V_0$, where V_0 is the initial tumor volume. Tumor quadrupling times are often subjected to right-censoring because of the death of the experimental mice, limitations of the follow-up period, or the number of cured tumors. Therefore, survival analysis should be used to analyze such time-to-event data. For example, the median tumor quadrupling time of a treatment group should be estimated from its Kaplan-Meier survival distribution. Specifically, letting $t_1 < t_2 < \dots < t_k$ be the unique ordered tumor quadrupling times of the mice, and d_j be the number of tumor quadruplications that occur by t_j , and n_j the number of individuals at risk immediately prior to t_j , then the Kaplan-Meier estimator [21] of the survival function is

$$\hat{S}(t) = \prod_{t_j \leq t} \left(1 - \frac{d_j}{n_j}\right).$$

The median tumor quadrupling time can be estimated as

$$\hat{m} = \min\{t_j, \hat{S}(t_j) < 0.5\}. \quad (2)$$

In a case of where the estimated survival function $\hat{S}(t)$ is exactly equal to 0.5 on the interval $[t_j, t_{j+1})$, the median is taken to be $m \hat{=} (t_j + t_{j+1})/2$.

3. Interaction Index

Lloyd [22] formulated the relationship between tumor cells surviving and tumor growth using a tumor growth curve theory and showed how the volume of tumor cells surviving after treatment can be estimated from the tumor volume data if both untreated and treated cells grow following Gompertz functions. In a short period in *in vivo* tumor xenograft studies, untreated tumor cells often grow exponentially, say $V(t) = V_0 e^{bt}$, and after a certain period of time, treated tumor cells regrow exponentially too. Lloyd's tumor cell survival theory [22] can be used to estimate tumor cells surviving for such experiments. Assume two identical exponential curves are separated in time by ε units (which represents tumor growth delay). One represents the growth curve $V(t)$ of an untreated tumor, and the other represents the growth curve of a treated tumor. Let t^* represent the time of starting the treatment. The tumor volume surviving after treatment can be found by extrapolation of the regrowth curve to the time of starting the treatment, which is $V(t^* - \varepsilon)$. The fraction of the tumor surviving treatment may be formulated as [22]

$$F = \frac{V(t^* - \varepsilon)}{V(t^*)}.$$

The log₁₀ cell kill (LCK) is defined formally as

$$\text{LCK} = -\log_{10}(F) = \frac{\varepsilon}{3.32t_d}, \quad (3)$$

which represents the number of log₁₀ units by which the tumor is reduced, where ε is the tumor growth delay and $t_d = \log 2/b$ is the doubling time of the untreated tumor.

In a fixed-dose two-drug combination tumor xenograft experiment, the goal is to assess the joint action of the combination. Let two agents be represented by A and B and their combination by AB , and let F_g be the corresponding tumor cells surviving fraction of group g after treatment, where $g = A, B, AB$. The joint action is additive only if the surviving fraction of the drug combination equals the multiplicative of the surviving fractions of the two single drugs, that is $F_{AB} = F_A \times F_B$. Otherwise, the interaction is either supra-additive or sub-additive, which is summarized as follows:

Supra-additive: $F_{AB} < F_A \times F_B$

Additive: $F_{AB} = F_A \times F_B$

Sub-additive: $F_{AB} > F_A \times F_B$

This is called a surviving fraction multiplicative model or Bliss independent model [23]. Based on this multiplicative model, an interaction index can be defined on as

$$I = \text{LCK}_{AB} - \text{LCK}_A - \text{LCK}_B \quad (4)$$

where $\text{LCK}_g = -\log_{10}(F_g)$, $g = A, B, AB$. Therefore, $I > 0$, $I = 0$, or $I < 0$ indicates a supra-additive, additive, or sub-additive joint action of the two-drug combination, respectively. Because the joint action for fixed-dose drug combinations is only defined locally, the terms

supra-additive and sub-additive are used rather than synergistic and antagonistic, respectively, to distinguish the global joint action definition.

4. Confidence Interval

For a tumor xenograft model with treatment and control groups, let $m_{\hat{C}}$ and $m_{\hat{T}}$ be the corresponding estimated median tumor quadrupling times of the control and treatment groups defined by (2) and $m_{\hat{d}}$ be the estimated median tumor doubling time of the control group via interpolation formula (1) with $V_e = 2V_0$. Then an estimate of the log10 cell kill of (3) is given by

$$L\hat{CK} = \frac{\hat{\varepsilon}}{3.32\hat{m}_d},$$

where $\hat{\varepsilon} = m_{\hat{T}} - m_{\hat{C}}$ is the estimated tumor growth delay and $m_{\hat{d}}$ is the estimated median tumor doubling time of the control group. Then the interaction index I of (4) can be estimated by

$$\hat{I} = L\hat{CK}_{AB} - L\hat{CK}_A - L\hat{CK}_B.$$

To construct the confidence interval of the interaction index I , let the observed tumor quadrupling times consist of n pairs $(t_{1g}, \delta_{1g}), \dots, (t_{ng}, \delta_{ng})$ of group $g = A, B, C, AB$, where $t_{ig} = \min(t_{ig}^0, x_{ig})$ is an observed tumor quadrupling time t_{ig}^0 or an observed censoring time x_{ig} , and $\delta_{ig} = I(t_{ig} = t_{ig}^0)$ is the event indicator, and the observed tumor doubling times of the control group are $(t_{1d}, \dots, t_{nd})$. Then the bootstrap percentile interval of I can be obtained by using the following bootstrap procedures for the right-censored data [24]–[25]:

1. Independently draw a large number of bootstrap samples, $\{(t_{1g}^{*b}, \delta_{1g}^{*b}), \dots, (t_{ng}^{*b}, \delta_{ng}^{*b}), b = 1, \dots, M\}$, with each bootstrap sample obtained by sampling with replacement from $\{(t_{1g}, \delta_{1g}), \dots, (t_{ng}, \delta_{ng})\}$, $g = A, B, C, AB$.
2. Independently draw a large number of bootstrap samples, $\{t_{1d}^{*b}, \dots, t_{nd}^{*b}, b = 1, \dots, M\}$, with each bootstrap sample obtained by sampling with replacement from $\{t_{1d}, \dots, t_{nd}\}$.
3. Calculate median estimates of Kaplan-Meier curves for bootstrap samples obtained in step 1) and 2) above, say $\hat{m}_g^{*b}$ and $\hat{m}_d^{*b}$, $g = A, B, C, AB$ and $b = 1, \dots, M$.
4. Calculate $L\hat{CK}_g^{*b} = \{\hat{m}_g^{*b} - \hat{m}_C^{*b}\} / 3.32\hat{m}_d^{*b}$, $g = A, B, AB$ and $b = 1, \dots, M$.
5. Calculate the interaction index $\hat{I}^{*b} = L\hat{CK}_{AB}^{*b} - L\hat{CK}_A^{*b} - L\hat{CK}_B^{*b}$.
6. The bootstrap standard error estimate of $\hat{I}$ is given by

$$\widehat{se}(\hat{I}) = \left\{ \sum_{b=1}^M (\hat{I}^{*b} - \bar{\hat{I}}^*)^2 / M \right\}^{1/2}$$

$$\text{where } \bar{\hat{I}}^* = \sum_{b=1}^M \hat{I}^{*b} / M.$$

7. Let $\hat{G}_*(s) = \#\{\hat{I}^{*b} < s\} / M$ be the bootstrap distribution of $\{\hat{I}^{*b}, b = 1, \dots, M\}$, then a $100(1 - \alpha)\%$ bootstrap percentile interval is given by

$$(\hat{G}_*^{-1}(\alpha/2), \hat{G}_*^{-1}(1 - \alpha/2)). \quad (5)$$

5. Simulation Studies

It is important to know whether the proposed bootstrap percentile interval of the interaction index I is appropriate for practical use. Simulation studies were performed to investigate the coverage probabilities under small samples of 10, 20, and 30 per group. In the simulation, tumor doubling and quadrupling times were generated from a Weibull distribution with a survival function $S(t) = \exp(-(t/\lambda)^\beta)$ where the scale parameter λ and shape parameter β were obtained from fitting the Weibull distribution for each group to real tumor xenograft data (Example 1, Section 6) (Table 1). This model yielded an interaction index of $I = -0.3123$. The censoring times were generated from a uniform distribution $U(0, c_i)$ with $c_i, i = 1, 2$, which was determined by prespecified censoring proportions of 10%, 20%, and 30% for each group except for the control group, for which no censoring was assumed in the simulation studies. Table 2 shows the simulated empirical coverage probabilities of the bootstrap percentile interval based on 10,000 independent Monte Carlo samples and 2,000 independent bootstrap samples. The simulation results show that the coverage probabilities of the proposed bootstrap percentile interval are reasonably close to the nominal level for practical use.

6. Examples

In this section, we will illustrate the proposed method using two actual subcutaneous tumor xenograft models generated in the Pediatric Preclinical Testing Program [26]. In the first example, the pediatric alveolar rhabdomyosarcoma cell line Rh30 was used to study the joint action between rapamycin (5 mg/kg, 5 times per week) and IMC-A12 (1 mg, twice weekly). In the study, Rh30 tumor cells were subcutaneously implanted into 40 female SCID mice. After tumors reached certain size (between 200–500 mm³), tumor-bearing mice were then equally randomized into treatment groups and received a single drug or a drug combination for 4 weeks of treatment and 8 weeks of follow-up. The tumor volumes were measured at the initiation of the study (week 0) and weekly thereafter. Mice were euthanized when their tumor volumes quadrupled due to ethical reasons, thus resulting in incomplete longitudinal tumor volume data. The observed tumor growth profiles are shown in Figure 1. The tumor doubling and tumor quadrupling times were calculated and are given in Table 3. The median tumor doubling time of the control group was 3.97 days. The median tumor quadrupling times were 8.53, 14.37, 16.0, and 19.61 days for control, IMC-A12, rapamycin,

and IMC-A12+rapamycin groups, respectively. Therefore, the combination did not significantly prolong the tumor quadrupling time compared with that of the two single drugs. The estimate (standard error) of the interaction index was $\hat{I} = -0.169(0.262)$. The 95% bootstrap percentile interval was $(-0.813, 0.176)$. The combination showed only additive interaction for the Rh30 cell line.

In the second example, the same drugs and drug combination shown in example 1 were studied in the pediatric Ewing sarcoma cell line EW5 for 4 weeks of treatment and 8 weeks of follow-up. The observed tumor growth profiles are shown in Figure 2. The tumor doubling and quadrupling times were calculated and are given in Table 4. The median tumor doubling time of the control group was 3.50 days. The median tumor quadrupling times were 7.07, 19.66, 17.78, and 67.22 days for the control, IMC-A12, rapamycin, and IMC-A12+rapamycin groups, respectively. Therefore, the combination did significantly prolong the tumor quadrupling time compared with that of the two single drugs. The estimate (standard error) of the interaction index was $\hat{I} = 3.167(0.479)$. The 95% bootstrap percentile interval was $(1.908, 3.688)$. Therefore, the combination showed supra-additive interaction for the EW5 cell line.

7. Conclusions

An interaction index is defined to quantify the joint action of a two-drug combination for fixed-dose subcutaneous tumor xenograft experiments. Furthermore, a bootstrap percentile interval of the interaction index is proposed. The simulation results show that the coverage probability of the bootstrap percentile interval is reasonably close to the nominal level for practical use.

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Figure 1.
Tumor volume growth in four groups for pediatric alveolar rhabdomyosarcoma cell line Rh30.

Figure 2.
Tumor volume growth in four groups for pediatric Ewing sarcoma cell line EW5.

Table 1

Parameters of Weibull distribution used in simulation studies

	Quadrupling time			Doubling time	
	A	B	C	D	A
λ	10.3713	16.3722	22.0164	21.5891	4.5603
β	3.7827	5.0991	2.6609	8.0925	4.5140

A: control, B: IMC-A12, C: rapamycin, D: IMC-A12+rapamycin.

Table 2
Empirical coverage probabilities of 95% bootstrap percentile interval of the interaction index

Censoring proportions						
n	(0,0,0)	(.1,.1,.1)	(.1,.1,.2)	(.1,.1,.3)	(.1,.2,.2)	(.2,.2,.3) [†]
10	0.968	0.967	0.967	0.968	0.963	0.960
						0.964
20	0.967	0.968	0.973	0.972	0.963	0.955
						0.966
30	0.965	0.963	0.966	0.965	0.955	0.944
						0.958

[†] (.2,.2,.3) represents 20%, 20% and 30% censoring for treatment group A, B and AB, respectively. No censoring was assumed for control group.

Table 3
Tumor doubling and quadrupling times (days) for Rh30 IMC-A12+rapamycin model

Group	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10
Doubling times										
A	5.28	3.37	2.33	4.31	3.91	6.18	4.03	3.87	4.57	3.87
Median										
A	13.21	6.75	4.63	8.67	8.17	13.96	8.25	8.40	11.67	9.91
B	12.26	17.53	16.16	10.81	20.82	13.69	12.37	12.61	19.35	15.05
C	14.95	22.34	11.95	16.18	15.83	38.89	24.17	22.80	14.56	13.84
D	19.68	20.45	19.54	20.27	19.53	24.52	18.44	18.25	18.51	25.43
Quadrupling times										
A	13.21	6.75	4.63	8.67	8.17	13.96	8.25	8.40	11.67	9.91
B	12.26	17.53	16.16	10.81	20.82	13.69	12.37	12.61	19.35	15.05
C	14.95	22.34	11.95	16.18	15.83	38.89	24.17	22.80	14.56	13.84
D	19.68	20.45	19.54	20.27	19.53	24.52	18.44	18.25	18.51	25.43
Median										

A: control, B: IMC-A12, C: rapamycin, D: IMC-A12+rapamycin.

Table 4
Tumor doubling and quadrupling times (days) for EW5 IMC-A12+rapamycin model

Group	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10	
Doubling times										Median	
A	3.19	7.89	3.43	4.76	4.57	3.08	3.09	3.55	3.97	3.46	3.50
Quadrupling times										Median	
A	6.42	10.67	6.84	8.98	8.68	6.16	6.19	7.22	8.54	6.93	7.07
B	18.95	15.60	17.99	32.01	34.24	17.29	28.24	17.07	33.47	20.37	19.66
C	12.65	28.60	16.15	18.32	19.57	33.19	17.24	16.00	15.74	18.56	17.78
D	69.35	58.73	69.74	67.22	21.00*	67.69	66.86	64.55	62.30	69.70	67.22

A: control, B: IMC-A12, C: rapamycin, D: IMC-A12+rapamycin.

* indicates a censoring - mouse died after day 21 due to toxicity.