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ASSESSMENT OF ANTITUMOR ACTIVITY FOR TUMOR XENOGRAPH STUDIES USING EXPONENTIAL GROWTH MODELS

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Abstract

In preclinical tumor xenograft experiments, the antitumor activity of the tested agents is often assessed by endpoints such as tumor doubling time, tumor growth delay (TGD), and log₁₀ cell kill (LCK). In tumor xenograft literature, the values of these endpoints are presented without any statistical inference, which ignores the noise in the experimental data. However, using exponential growth models, these endpoints can be quantified by their growth curve parameters, thus allowing parametric inference, such as an interval estimate, to be used to assess the antitumor activity of the treatment.

Keywords

Exponential growth curve; log₁₀ cell kill; Tumor doubling time; Tumor growth delay; Xenografts

1. INTRODUCTION

The assessment of antitumor activity of the tested agents in preclinical tumor xenograft experiments is important for finding promising new compounds for human cancer treatment. Several endpoints may be chosen to characterize the effectiveness of the treatment, such as tumor doubling time, tumor growth delay (TGD), and log₁₀ cell kill (LCK) (Demidenko, 2004, 2010). In tumor xenograft literature, the values of these endpoints are presented without any statistical inference, which ignores the noise in the experimental data (Corbett et al., 2003; Houghton et al., 2007). Recently, Wu and Houghton (2009) developed a bootstrap interval of the LCK to assess the antitumor activity. The proposed interval estimation is purely nonparametric without tumor growth modeling. However, in short-term animal experiments, tumor growth often can be fitted by a growth curve. For example, an untreated tumor growth is usually well approximated by an exponential curve

$$V(t) = V_0 e^{bt}, \quad b > 0 \quad (1)$$

where $V(t)$ is the tumor volume at time t , V_0 is the initial volume, and b is a parameter characterizing the rate of tumor growth. The growth of a treated tumor is more complicated

and difficult to model through its entire growth curve. However, the regrowth curve of a treated tumor often follows an exponential curve, too. To quantify the antitumor activity of a treatment based on these endpoints, it is only necessary to model the exponential regrowth curve for the treated tumor.

Tumor growth curve theory is well established, with a long history in biomathematics and biostatistics. One of the pioneer investigations in tumor growth kinetics was made by Skipper et al. (1964), who described the first model of tumor growth kinetics—an exponential growth. The Gompertz growth curve was introduced by Gompertz (1825) and has proven to be well suited to describe the growth of an unperturbed tumor (Lloyd, 1975; Norton, 1988). However, both exponential and Gompertz curves often do not fit the growth curve of the treated tumor. Norton and Simon (1977) and Hanfelt (1991) proposed some generalized Gompertz curves to describe treated tumor growth. Demidenko (2004) and Liang and Sha (2004) applied a biexponential curve to treated tumor growth. Demidenko (2006) proposed a linear-exponential regrowth curve analysis. Liang (2005) proposed a spline growth curve model and applied it to tumor xenograft data analysis. However, these proposed models are only applied to a certain of specific tumor growth curves.

In general, the treated tumor growth curve is difficult to model. Therefore, in our approach, only tumor regrowth is modeled by an exponential curve. The tumor doubling time, TGD, and LCK then can be formulated by curve parameters. Therefore, point estimates and confidence intervals of these endpoints can be derived from the growth curve analysis.

2. TUMOR DOUBLING TIME

A useful measure of tumor growth rate is tumor doubling time t_D , which is defined as the time it takes for a tumor to double its starting volume. For an exponential growth curve as in Eq. (1), the doubling time t_D starting volume at time t_0 is determined by the following equation:

$$2=V(t_0+t_D)/V(t_0)=e^{bt_D}$$

The doubling time t_D is then solved as

$$t_D=\frac{\ln(2)}{b} \quad (2)$$

which is independent of the starting time t_0 and inversely proportional to the tumor growth rate b . Therefore, measuring the tumor doubling time is equivalent to measuring the tumor growth rate for an exponential growth curve. Since tumor volumes are measured at certain time points during the study period, the exact tumor doubling time t_D of an individual tumor is often not observed. However, one can fit the following linear regression line:

$$y_j=a+bt_j+e_j, \quad j=1, \dots, n \quad (3)$$

where $y_j = \ln\{V(t_j)\}$ is the natural logarithm tumor volume at time t_j for the j th time point and $\{e_j\}$ are the independent $N(0, \sigma^2)$ variates of measurement errors. The model slope parameter b can be estimated by $\hat{b}$ with standard error $\widehat{se}(\hat{b})$ from the well-known regression theorem (Weisberg, 2005). Therefore, the tumor doubling time t_D can be estimated by

$$\hat{t}_D = \frac{\ln(2)}{\hat{b}}$$

and its standard error is derived by the delta method,

$$\widehat{se}(\hat{t}_D) = \ln(2) \hat{b}^{-2} \widehat{se}(\hat{b})$$

Since $\hat{t}_D$ is the inverse of normal variate $\hat{b}$, the distribution of $\hat{t}_D$ is skewed. To construct the interval estimate, taking a logarithm transformation of the $\hat{t}_D$, the standard error of $\ln(\hat{t}_D)$ can be derived by the delta method,

$$\widehat{se}\{\ln(\hat{t}_D)\} = \hat{b}^{-1} \widehat{se}(\hat{b})$$

Therefore, a $100(1 - \alpha)\%$ confidence interval of t_D is given by

$$\left[\hat{t}_D e^{-z_{1-\alpha/2} \hat{b}^{-1} \widehat{se}(\hat{b})}, \hat{t}_D e^{z_{1-\alpha/2} \hat{b}^{-1} \widehat{se}(\hat{b})} \right] \quad (4)$$

where $z_{1-\alpha/2}$ is the $100(1 - \alpha/2)$ percentile of the standard normal distribution. An alternative $100(1 - \alpha)\%$ confidence interval of t_D can be obtained by constructing the confidence interval of b and taking the inverse of it (Hanfelt, 1997):

$$\left[\frac{\ln(2)}{\hat{b} + t_{n-2, 1-\alpha/2} \widehat{se}(\hat{b})}, \frac{\ln(2)}{\max\{0, \hat{b} - t_{n-2, 1-\alpha/2} \widehat{se}(\hat{b})\}} \right] \quad (5)$$

where $t_{n-2, 1-\alpha/2}$ is the $100(1 - \alpha/2)$ percentile of the t -distribution with degrees of freedom of $n - 2$.

To assess the treatment effect, the ratio doubling times of treated versus untreated may be of interest:

$$\gamma = \frac{t_D(T)}{t_D(C)} = \frac{b_C}{b_T}$$

where $t_D(T)$, b_T and $t_D(C)$, b_C represent the tumor doubling times and growth rates of the treated and untreated tumors, respectively. The growth rates b_T and b_C can be estimated by fitting the following regression model:

$$y_{ij} = a_C x_i + a_T (1 - x_i) + b_C t_{ij} x_i + b_T t_{ij} (1 - x_i) + e_{ij}, \quad j = 1, \dots, n_i \quad (6)$$

where $y_{ij} = \ln\{V(t_{ij})\}$, x_i takes value of 0 and 1 for $i = T$ and C , respectively, and $\{e_{ij}\}$ are the independent $N(0, \sigma^2)$ variates of measurement errors. By noting the independence between $\hat{b}_C$ and $\hat{b}_T$, the interval estimate of γ can be obtained by the delta method and Fieller's theorem (Fieller, 1954). The standard error of $\gamma = \hat{b}_C / \hat{b}_T$ can be derived by the delta method:

$$\widehat{se}(\hat{\gamma}) = \frac{1}{\hat{b}_T} \left\{ v_{11} + \hat{\gamma}^2 v_{22} \right\}^{1/2}$$

where $v_{11} = \widehat{var}(\hat{b}_C)$ and $v_{22} = \widehat{var}(\hat{b}_T)$. Therefore, a $100(1 - \alpha)\%$ delta interval of the ratio γ is given by

$$\hat{\gamma} \pm z_{1-\alpha/2} \widehat{se}(\hat{\gamma}) \quad (7)$$

Since γ is expressed as a ratio, applying Fieller's theorem, the Fieller's interval can be obtained by solving the following equation for γ :

$$(\hat{b}_C - \gamma \hat{b}_T)^2 \leq t_{df, 1-\alpha/2}^2 (v_{11} + \gamma^2 v_{22})$$

where $t_{df, 1-\alpha/2}$ is the percentile of the t -distribution with degrees of freedom $df = n_T + n_C -$

4. A $100(1 - \alpha/2)\%$ Fieller's interval is then given by

$$\left[\hat{\gamma} \pm \frac{t_{df, 1-\alpha/2}}{\hat{b}_T} \left\{ (1-g)v_{11} + \hat{\gamma}^2 v_{22} \right\}^{1/2} \right] / (1-g) \quad (8)$$

where

$$g = t_{df, 1-\alpha/2}^2 \frac{v_{22}}{\hat{b}_T^2}$$

3. LOG₁₀ CELL KILL

The effectiveness of a tested agent is often assessed by the number of cells killed by the treatment. Lloyd (1975) provided a formal definition of log₁₀ cell kill (LCK), which is defined as the negative log₁₀ fraction of tumor cells surviving treatment (F), that is,

$$\text{LCK} = -\log_{10}(F) \quad (9)$$

The quantification of the LCK for a tumor growth model is based on the following assumptions: (a) Growth of the untreated tumor follows an exponential growth curve, (b)

mass (and corresponding volume) of a tumor is directly proportional to the number of malignant cells in the mass, and (c) the treated tumor regrowth curve is the same as that of an untreated control tumor. Under these assumptions, the regrowth curve of the treated tumor is identical to that of the untreated tumor, separated in time by amount τ .

$$V(t) = V_0 e^{b(t-\tau)}$$

Let t_* represent the treatment starting time; then the fraction of the tumor cells surviving after treatment may be formulated as

$$F = \frac{V(t_* - \tau)}{V(t_*)} = e^{-b\tau}$$

Therefore, the LCK defined by Eq. (9) is given by

$$\text{LCK} = \frac{b\tau}{\ln(10)} = \frac{T-C}{3.32t_D} \quad (10)$$

where $T-C$ is the difference in times to the predetermined size between treated and untreated tumors and 3.32 is an approximate value of $\log_2(10)$. The final expression of Eq. (10) of the LCK has been widely used in the tumor xenograft literatures (Corbett et al., 2003).

To express the LCK by the regrowth curve parameter θ defined next, let $y_t = \ln\{V(t)\}$ be the logarithm tumor volume, and suppose the untreated tumor grows according to $y_t = a + bt$ and the treated tumor regrows according to $y_t = a - \theta + bt = a + b(t - \tau)$, where θ is the difference of the intercepts between untreated curve, and treated regrowth curve which quantifies the number of cells killed by the treatment. Then $\theta = b\tau$, and the LCK of Eq. (10) is expressed as

$$\text{LCK} = \frac{\theta}{\ln(10)}$$

The parameter θ can be estimated by fitting the following regression model:

$$y_{ij} = a - \theta(1 - x_i) + bt_{ij} + e_{ij}, \quad j = 1, \dots, n_i, \quad (11)$$

where $y_{ij} = \ln\{V(t_{ij})\}$, x_i takes a value of 0 and 1 for $i = T$ and C , respectively, and $\{e_{ij}\}$ are the independent $N(0, \sigma^2)$ variates of measurement errors. An estimate of LCK is then given by

$$\widehat{\text{LCK}} = \frac{\hat{\theta}}{\ln(10)} \quad (12)$$

and its standard error is

$$\widehat{se}(\widehat{LCK}) = \frac{\widehat{se}(\hat{\theta})}{\ln(10)}$$

A $100(1 - \alpha)\%$ confidence interval of LCK is given by

$$[\hat{\theta} \pm z_{1-\alpha/2} \widehat{se}(\hat{\theta})] / \ln(10) \quad (13)$$

4. TUMOR GROWTH DELAY

Tumor response to treatment is also often assessed by TGD, which is defined as the difference in times from initial treatment to the predetermined size between treated and untreated tumors. To make parametric inference for the TGD, two scenarios are next discussed.

Scenario 1: Parallel growth and regrowth curves

To estimate the TGD, using the same notations as given in the previous section, suppose the untreated tumor grows (in logarithmic scale) according to $y_t = a + bt$ and the treated tumor regrows according to $y_t = a - \theta + bt = a + b(t - \tau)$. Then the TGD is expressed as (also see Demidenko, 2006, 2010) as

$$\text{TGD} = \tau = \frac{\theta}{b}$$

The model parameters b and θ can be estimated by fitting regression model (11). The TGD can be estimated by

$$\widehat{\text{TGD}} = \hat{\tau} = \frac{\hat{\theta}}{\hat{b}}$$

The standard error of $\widehat{\text{TGD}}$ can be estimated by the delta method:

$$\widehat{se}(\hat{\tau}) = \frac{1}{\hat{b}} \left\{ v_{11} - 2\hat{\tau}v_{12} + \hat{\tau}^2 v_{22} \right\}^{1/2}$$

where $v_{11} = \widehat{\text{var}}(\hat{\theta})$, $v_{12} = \widehat{\text{cov}}(\hat{\theta}, \hat{b})$, and $v_{22} = \widehat{\text{var}}(\hat{b})$. Therefore, a $100(1 - \alpha)\%$ delta interval of the TGD is given by

$$\hat{\tau} \pm z_{1-\alpha/2} \widehat{se}(\hat{\tau}) \quad (14)$$

Since the TGD is also expressed in a ratio form, Fieller's interval can be obtained by solving the following equation for τ :

$$(\hat{\theta} - \tau \hat{b})^2 \leq t_{df, 1-\alpha/2}^2 (v_{11} - 2\tau v_{12} + \tau^2 v_{22})$$

where $df = n_T + n_C - 3$. A $100(1 - \alpha/2)\%$ Fieller's interval is given by

$$\left[\hat{\tau} - \frac{g v_{12}}{v_{22}} \pm \frac{t_{df, 1-\alpha/2}}{\hat{b}} \left\{ v_{11} - 2\hat{\tau} v_{12} + \hat{\tau}^2 v_{22} - g \left(v_{11} - \frac{v_{12}^2}{v_{22}} \right) \right\}^{1/2} \right] / (1-g) \quad (15)$$

where

$$g = t_{df, 1-\alpha/2}^2 \frac{v_{22}}{\hat{b}^2}$$

Scenario 2: Nonparallel growth and regrowth curves

The growth rate of a tumor regrowing after treatment is usually slower than that of the untreated controls. Although this may not be universally true, it occurs in most experimental tumors due to the “tumor bed effect” (Begg, 1980). In such nonparallel growth cases, the procedure for determining tumor cell kill is questionable; however, the TGD may still provide useful information (Lloyd, 1975).

Suppose an untreated tumor grows (in logarithmic scale) according to the curve $y_t = a_C b_C t$ and a treated tumor regrows according to the curve $y_t = a_T + b_T t$ and $b_C < b_T$. An F -test derived from regression analysis can be used to test the equal slopes of two growth curves. Let c be the prespecified tumor size (in logarithm scale) for the TGD endpoint. Then the TGD is $\tau = t_2 - t_1$, where t_1 and t_2 are the solutions of the following equations:

$$a_C + b_C t_1 = c, \quad a_T + b_T t_2 = c$$

Therefore,

$$\text{TGD} = \tau = \frac{c - a_T}{b_T} - \frac{c - a_C}{b_C}$$

The model parameters a_C , b_C , a_T , and b_T can be estimated by fitting regression model (6). The TGD can be estimated by

$$\widehat{\text{TGD}} = \hat{\tau} = \frac{c - \hat{a}_T}{\hat{b}_T} - \frac{c - \hat{a}_C}{\hat{b}_C}$$

Let

$$f(x, y) = \frac{c-x}{y}$$

Using the delta method, the variance of $f(\hat{a}, \hat{b})$ is given by

$$\widehat{var}\{f(\hat{a}, \hat{b})\} = \frac{1}{\hat{b}^4} \{ \hat{b}^2 \widehat{var}(\hat{a}) + 2\hat{b}(c-\hat{a})\widehat{cov}(\hat{a}, \hat{b}) + (c-\hat{a})^2 \widehat{var}(\hat{b}) \}$$

The standard error of $\widehat{T_{GD}}$ can be estimated by

$$\widehat{se}(\hat{\tau}) = [\widehat{var}\{f(\hat{a}_C, \hat{b}_C)\} + \widehat{var}\{f(\hat{a}_T, \hat{b}_T)\}]^{1/2}$$

Therefore, a 100 (1 - α)% delta interval of the TGD is given by

$$\hat{\tau} \pm z_{1-\alpha/2} \widehat{se}(\hat{\tau}) \quad (16)$$

5. SIMULATION STUDY

Simulations were carried out to study the 95% coverage probabilities of intervals discussed in previous section under various scenarios. The time points of tumor volume measurement were set up to be $n = 7$ and 11 weeks (in unit days) starting from day 0, and the standard deviation σ of the measurement error ranges from 0.01 to 0.5. The growth rates were set to be $b = 0.1$ for single growth curve or parallel growth curves and $b_C = 0.2$ and $b_T = 0.1$ for untreated and treated tumor of nonparallel growth curves, respectively. For each parameter configuration, 5,000 independent Monte Carlo samples were used to ensure that the Monte Carlo error for estimating a 95% coverage probability is about $0.003 = (0.05 \times 0.95/5000)^{1/2}$. The empirical coverage probability of each interval is the frequency of number of intervals including the true parameter of interest. The simulation results are given in Table 1. Since the inverse interval and Fieller's intervals are exact, the simulated coverage probabilities, of these intervals are closer to the nominal level of 0.95. All delta intervals have lower coverage probabilities, which indicates that the delta intervals are liberal. However, as the number of measurements increases, the coverage probabilities of the delta intervals are improved.

6. EXAMPLES

Three data sets were selected from a published Pediatric Preclinical Testing Program (PPTP) study (Houghton et al., 2007). The first data set listed the volumes of an untreated tumor from a Wilms tumor line KT-11 xenograft experiment. The data show that the tumor followed an exponential growth curve (Figure 1). Fitting the linear regression model (3) on the logarithm tumor volume, the rate of growth and its standard error are $\hat{b} = 0.0466$ (day^{-1})

and $\widehat{se}(\hat{b})=0.001$, respectively, with residual standard error $\hat{\sigma}=0.037$ and model $R^2=0.998$. Then the tumor doubling time is $t_D = \ln(2)/0.0466 = 14.87$ (days), and its standard error is $\widehat{se}(\hat{t}_D)=0.32$. Therefore, the 95% confidence intervals of t_D are [14.26, 15.51] and [14.19, 15.63] for delta method (4) and inverse method (5), respectively.

The other two data sets describe the osteosarcoma tumor line OS-2 xenografts. Each data set describes an untreated tumor and a treated tumor. The treated mouse was given cyclophosphamide at 150mg/kg intraperitoneally every 7 days for 6 weeks. Tumor volumes were measured every 2 or 5 days. The growth curves are plotted in Figures 2 and 3.

For the parallel group data (Figure 2), since initial volumes of the two tumors are not equal, relative tumor volume (tumor volume divided by initial volume) was used. Let y_t be the logarithm of the relative tumor volume. After fitting the regression model (11), the untreated tumor grows according to $y_t = b\hat{t}$ with growth rate (standard error) $b = 0.071(0.003)$ and the treated tumor regrows starting on day 16 according to $y_t = -\theta\hat{b}\hat{t}$ with $\theta = 1.44(0.086)$ and $\widehat{cov}(\hat{\theta}, \hat{b})=0.00024$.

Therefore, the estimate of LCK (standard error) is

$$\widehat{\text{LCK}} = \frac{\hat{\theta}}{\ln(10)} = 0.63(0.049)$$

The 95% confidence interval of the LCK is [0.57, 0.69], which shows that cyclophosphamide does not have high antitumor activity against the tumor line OS-2 based on the cutoff point of $\text{LCK} > 0.7$ (Corbett et al., 2003). The estimate of TGD (standard error) is

$$\widehat{\text{TGD}} = \frac{\hat{\theta}}{\hat{b}} = 20.46 (0.84) \text{ days}$$

The 95% confidence intervals of the TGD are [18.81, 22.10] and [18.64, 22.40] for delta method (14) and Fieller's method (15), respectively.

For nonparallel group data (Figure 3), fitting the regression model (6) to the logarithm relative tumor volume data shows that the untreated tumor grows according to $y_t = \hat{a}_C \hat{b}_C t$ with $\hat{a}_C = 0.224(0.141)$ and $\hat{b}_C = 0.103(0.017)$, and the treated tumor regrows starting at day 16 according to $y_t = \hat{a}_T \hat{b}_T t$ with $\hat{a}_T = -1.568(0.292)$ and $\hat{b}_T = 0.061(0.010)$. For the prepecified relative tumor size 2, the estimate of TGD is given by

$\widehat{\text{TGD}} = (\log(2) - (-1.568))/0.061 - (\log(2) - 0.224)/0.103 = 32.3$ (day), with standard error of 2.2 (day). The 95% confidence interval of the TGD is [27.9, 36.7].

From these examples, we observed that the delta interval is narrower than the Fieller's interval. The lower coverage probability of the delta interval from simulation study explains its narrow interval compared to the Fieller's interval.

7. DISCUSSION AND CONCLUSION

Using exponential growth and regrowth curve models, tumor doubling time, TGD, and LCK can be expressed by model parameters. Therefore, parametric inference can be made for these endpoints by a simple regression analysis. The confidence intervals obtained using Fieller's method are exact. Simulation study shows that the interval obtained using delta method is liberal for small sample. Therefore, for small sample data, Fieller's interval is recommended. Finally, for group data, more comprehensive analysis would involve a mixed model (Demidenko, 2010). If tumor growth follow Gompertz curves, following Lloyd's derivation (Lloyd, 1975), one can make statistical inference on these endpoints by applying a nonlinear mixed model.

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Figure 1. Exponential growth curve of an untreated tumor from a Wilms tumor line KT-11 xenograft model.

Figure 2. Parallel growth curves for an untreated tumor and a treated tumor from a osterosacoma tumor line OS-2 xenograft model. The dots/solid line and circles/broken line represent the observed log relative tumor volumes and fitted regression line for the untreated tumor and treated tumor, respectively.

Figure 3. Nonparallel growth curves for an untreated tumor and a treated tumor from a osterosacoma tumor line OS-2 xenograft model. The dots/solid line and circles/broken line represent the observed log relative tumor volumes and fitted regression line for the untreated tumor and treated tumor, respectively.

Table 1
Empirical coverage probabilities of 95% confidence intervals based on 5,000 Monte Carlo samples

<i>n</i>	Method ^a	Standard deviation (σ)				
		0.01	0.05	0.1	0.5	
7	Delta (4)	0.888	0.887	0.889	0.890	
	Inverse (5)	0.948	0.948	0.948	0.948	
	Delta (7)	0.911	0.913	0.913	0.917	
	Fieller (8)	0.945	0.946	0.946	0.946	
	Delta (14)	0.929	0.930	0.929	0.930	
11	Fieller (15)	0.950	0.950	0.950	0.954	
	Delta (16)	0.923	0.923	0.925	0.931	
	Delta (4)	0.920	0.921	0.920	0.920	
	Inverse (5)	0.951	0.951	0.951	0.951	
	Delta (7)	0.936	0.937	0.938	0.937	
	Fieller (8)	0.951	0.952	0.952	0.952	
	Delta (14)	0.936	0.935	0.936	0.937	
	Fieller (15)	0.951	0.951	0.951	0.951	
	Delta (16)	0.937	0.937	0.937	0.938	

^aWhere delta ($\times$), inverse ($\times$), or Fieller ($\times$) means the delta, inverse, or Fieller interval given by equation number of ($\times$) in the paper.