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Conditional quantile regression models of melanoma tumor growth curves for assessing treatment effect in small sample studies

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Tumor growth curves provide a simple way to understand how tumors change over time. The traditional approach to fitting such curves to empirical data has been to estimate conditional mean regression functions, which describe the average effect of covariates on growth. However, this method ignores the possibility that tumor growth dynamics are different for different quantiles of the possible distribution of growth patterns. Furthermore, typical individual preclinical cancer drug study designs have very small sample sizes and can have lower power to detect a statistically significant difference in tumor volume between treatment groups. In our work, we begin to address these issues by combining several independent small sample studies of an experimental cancer treatment with differing study designs to construct quantile tumor growth curves. For modeling, we use a Penalized Fixed Effects Quantile Regression with added study effects to control for study differences. We demonstrate this approach using data from a series of small sample studies that investigated the effect of a naturally derived biological peptide, P28, on tumor volumes in mice grafted with human melanoma cells. We find a statistically significant quantile treatment effect on tumor volume trajectories and baseline values. In particular, the experimental treatment and a corresponding conventional chemotherapy had different effects on tumor growth by quantile. The conventional treatment, Dacarbazine (DTIC), tended to inhibit growth for smaller quantiles, while the experimental treatment P28 produced slower rates of growth in the upper quantiles, especially in the 95th quantile.

Keywords: tumor growth; quantile regression; longitudinal data; combining information; bootstrapping

1. Introduction

Tumor growth curves provide a simple way to understand how tumors change over time and are a useful statistical tool in the development of successful cancer treatments. The traditional approach to fitting such curves to empirical data has been to estimate conditional mean regression functions, which describe the average effect of covariates on growth. However, this method ignores the possibility that tumor growth dynamics are different for different quantiles or percentiles of the possible distribution of growth patterns. In contrast, other areas of growth analysis such as that of human children, individual growth trajectories are routinely measured against a standard group of reference curves that range over several quantiles. This type of comparison is more informative as it gives health care professionals a better understanding of where their patient is on the spectrum of 'normal' and 'abnormal' growth after controlling for important factors such as health history and family characteristics. This suggests that the conditional quantile growth curve can be an invaluable tool in the personalized approach to medical treatment and research, wherein treatments are customized to the individual in order to increase treatment response and minimize side effects.

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In this paper, we take the idea of quantile reference growth curves and apply it to modeling tumor volume growth. In addition to estimating quantile curves, our work also incorporates a novel use of small sample data. In general, study designs with too few animals tend to have lower power to detect a statistically significant difference in tumor volume between treatment groups. To increase power, we pool information across several independent studies by combining the samples together and running our fitting procedure on the combined sample. This research is motivated by a series of small sample studies that investigated the effects of an experimental treatment, P28, on melanoma tumor volumes in mice. The studies differ in terms of sample size, the set of experimental treatment regimes, and melanoma cell line. However, the experimental treatments do overlap between studies, including one dosage of P28 that is included in every study. Our goal is to ascertain what effect treatment has on tumor growth trajectories for different quantiles of the population controlling for study and individual subject effects.

To construct the tumor reference growth curves, we specify a longitudinal regression model for multiple quantiles that incorporates individual subject and study fixed effects. Quantile regression methods [1] extend traditional regression models for the conditional mean to conditional quantiles. Typical regression models posit a functional relationship between the *average* population response and a set of predictors X: $E(Y|X) = f(X, \theta)$. For example, according to the popular linear model of the conditional mean, $E(Y|X = x) = \beta_0 + x'\beta$, the population average changes linearly as a function of x. However, even for the simple linear case, it is not unrealistic to assume that the coefficients will be significantly different for subjects at the extremes of a population and those that resemble the population average. Quantile regression [2] directly models the τ^{th} population quantile as a function of covariates. This allows researchers to assess the quantile effect of various variables on the response function and that is impossible to do with the traditional conditional mean model. Furthermore, quantile regression estimates are more robust than the traditional conditional mean regression estimates, the latter being in general more sensitive to outliers and less accurate and less informative for data arising from skewed or heavy tailed distributions (see [2] for a good introduction).

In a longitudinal study, a random sample of individual subjects is repeatedly measured at different points in time. Although the subjects are independent, usually they are not homogeneous, and care must be taken in controlling for the individual idiosyncratic factors. Finally, observations within subjects can also be correlated, which although does not effect the consistency and robustness of parameter estimates ([3]), it may potentially effect efficiency. The aim of longitudinal analysis is to tease out the general population level response from the individual subject deviations and possible correlations. Several different approaches exist in the literature on applying the quantile regression method to longitudinal data.

Much work exists on methods that control for within subject correlation and attempt to minimize efficiency of parameter estimates. Some proposed methods borrow from the generalized linear model approach to conditional mean regression by specifying a parametric or semiparametric model for the correlation structure of either the responses or of the estimating equations used to fit models. These include a generalized estimating equations type model formulation (see, for example, ([4–6]) and a quadratic inference function type method [7]. In a similar spirit to linear mixed effects models, Geraci and Bottai [8] and Liu and Bottai [9] formulated a mixed effects model quantile regression model that incorporate subject specific random effects. In this approach, the responses are assumed to come from an asymmetric Laplace distribution that depends on the target quantile to be estimated. Further, the random effects are assumed to follow a multivariate distribution that needs to be specified. Alternatively, Karlsson [10] accounts for any possible within subject correlation by using a weighted sum of the usual check function, where the weights are estimated from the correlation of residuals from an initial unweighted median regression estimate.

As we are only concerned with illustrating how the quantile approach can be applied to model tumor growth, considerations on parameter efficiency are outside the scope of the current work. We do, however, aim to construct reference growth curves and thus require a method that can be used to estimate a set of different quantile regressions simultaneously. To the best of our knowledge, the proposed models described earlier are limited to estimating a single quantile at a time and most require specifying either a distribution for the errors or a correlation matrix for the regression estimating equations.

A fully distribution free method that can be used to model multiple quantiles simultaneously is available in [11]. This method, called penalized fixed effects quantile regression (PFEQR), extends the original quantile regression model to longitudinal data by incorporating fixed subject specific model parameters that are penalized to prevent overfitting and decrease model dimension. Under this formulation, it is possible to capture correlation through model parameters allowing for a parsimonious way to account for shifts in within subject correlation across quantiles. In particular, Wei and He [3] and Wei *et al.*

[12] presented a semiparametric quantile regression model for children's conditional growth charts that includes autoregressive terms. In the former paper, this model is shown to be robust for deviations from the assumption of independence and identical distribution assumption for the error structure wherein there is no within subject correlation. Further, and of great importance for researchers, the empirical results in [12] showed that the magnitude of the relationship between current height and past height varies at different points of the conditional distribution of height. In particular, infants at the lower tail of the height distribution had much higher estimated autoregressive coefficients than those in the upper tails—a result that is consistent with a catch-up hypothesis for very small infants but is impossible to test with a conventional conditional mean model.

In our work, we illustrate the potential of quantile regression to study tumor growth. We use PFEQR to estimate a set of quantile exponential growth curves of tumor volume from mice grafted with human melanoma cells. The data arises from multiple independent studies with small sample sizes. We aim to improve the variability of the estimates by combining the data for estimation. The rest of the paper is laid as follows. In Section 2, we give an overview of the PFEQR method for estimating simultaneous quantile regression curves from longitudinal and provide some discussion on tuning parameter choice. In Section 3, we present a quantile regression analysis of treatment efficacy for melanoma tumor growth using an exponential quantile model with fixed study and fixed subject effects.

2. Penalized fixed effects quantile regression for longitudinal data

The quantile tumor growth curves presented in this paper rely on small sample longitudinal studies. Longitudinal data or repeated measurements data are one of the most common types of data collected in pharmacological research. In such studies, samples of individual subjects are followed over time to assess the efficacy of experimental treatments. More formally, let $Y_{ij}, i = 1, \dots, N, j = 1, \dots, t_i$ be the i^{th} subjects response at time j and x_{ij} a p element vector of observed covariates. Assume that each Y_{ij} is distributed according to some unknown cumulative distribution function $F(x)$, whose form may depend on a set of covariates $x \in \mathbb{R}^p$. Following [2], a linear conditional quantile function can then be defined as

$$Q_{Y_{ij}}\left(\tau|x_{ij}\right) = x_{ij}^T\beta(\tau), \tag{1}$$

where $\tau \in (0,1)$, $Q_{Y_{ij}}(\cdot) = F^{-1}_{Y_{ij}}$, and $\beta \in \mathbb{R}^p$ is unknown and depends on τ.

Note that if there is no between-subject heterogeneity, then F is the same for all Y_{ij}. This is not a realistic assumption for longitudinal data, wherein different subjects have their own idiosyncratic responses to the same experimental conditions. Moreover, although subjects may be independent of each other, observations within subjects tend to be correlated. The aim of longitudinal analysis is to tease out the general population level response from the individual subject deviations. A popular approach for the conditional mean models is the use of mixed models. These include subject specific fixed and random effects that account for distributional differences as well as within subject correlation. For quantile regression, an analogous approach has been introduced in [11]. The specification in (1) is augmented as

$$Q\left(Y_{ij}|\tau, x\right) = \alpha_i + x_{ij}^T\beta(\tau), \tag{2}$$

where α_i is the i^{th} subject's individual fixed effect. Arguing from the fact that estimating the usual conditional mean random effects mixed model is equivalent to estimating a penalized fixed effects regression, the author in [11] proposed an analogous estimating procedure for quantile longitudinal regression. Define the piecewise linear 'check function' $\rho_\tau(u) = (\tau - 1\{u < 0\})\,u$. Further, let $(\tau_1, \dots, \tau_K)$ be the quantiles of interest, $(\omega_1, \dots, \omega_K)$ weights that control the influence of individual quantile curve estimates on the overall solution, and λ a shrinkage parameter that controls the size of the estimated subject specific fixed effects. Then, the estimates to (2) are defined as solutions to the following regularization problem that imposes a lasso penalty on the subject fixed effects coefficients:

$$\min_{\beta\in\mathbb{R}} \sum_{k=1}^{K}\sum_{i=1}^{N}\sum_{j=1}^{t_i} \omega_k \rho_{\tau_k}\left(y_{ij} - \alpha_i - x_{ij}^T\beta\left(\tau_k\right)\right) + \lambda\sum_{i=1}^{N}|\alpha_i|. \tag{3}$$

Both the weights and λ must be specified before estimation. Following the suggestion in [11], in this work, we use symmetric weights that are higher for central quantiles and decrease at the upper and

lower tails. In particular, to estimate the five quantiles $\tau = (0.05, 0.25, 0.50, 0.75, 0.95)$, we use $\omega = (0.050, 0.225, 0.450, 0.225, 0.050)$.

Under the model in (2), the fixed effects do not depend on τ and account for a subject specific location shift. It is possible to model the α_i's as functions of τ, which would account for subject specific distributional shifts. However, even the simpler 'location' fixed effects parameterization in (2) includes N extra parameters, which if estimable, tends to increase the variance of estimates. Accounting for distributional subject specific effects via fixed effects would reduce the efficiency of estimates even further if not make the estimation intractable.

2.1. *Selection of tuning parameter λ*

For the choice of the shrinkage parameter, several methods have been proposed in the literature for penalized quantile regression of noncorrelated and single time series data, but little has been done for longitudinal data specifically. In general, shrinkage or regularization parameters are chosen to minimize some sort of optimality criterion that are functions of model accuracy and prediction error. Under the assumption of random subject specific effects, Lamarche [13] derived a form for the tuning parameter that minimizes the asymptotic variance of the estimated quantile fixed effects. In that work, the optimal value of λ depends on plug-in estimators of both the density of the random subject effects and the sparsity function of the data. A more simpler approach relies on the Schwartz Information Criterion (SIC) to choose the tuning parameter. Originally proposed in [14] for penalized quantile regression models for nonlongitudinal data, this was further studied in [15] for linear quantile regression models with many predictors and in [16] for nonparametric quantile regression. However, to our knowledge, no rigorous work has been carried out in using the SIC for the penalized panel quantile regression method.

In addition, nonparametric methods such as the bootstrap [17] are often used in penalty selection for nonparametric regression. However, their use is complicated for the longitudinal data setup. Standard selection methods usually rely on 'accurate' estimates of out-of-sample or the testing sample model prediction error and therefore require out-of-sample prediction of subject specific fixed effects. In addition, the within subject correlation structure precludes the use of simple cross-validation techniques and simple bootstrapping of observations across time. Some work in this area has been carried out in [18] on panel data bootstrapping, but the study did not examine applications to model selection. One alternative can be to take a finite sample approach to the asymptotic selection criterion of Lamarche [13] and take λ as a minimizer of a bootstrap estimate of (total) parameter variance. Possible resampling schemes can be the cross-sectional subject panel bootstrap of Kapetanios [18] or the wild bootstrap as described in [19]. However, the study of bootstrap and other model selection criteria methods for panel quantile regression is beyond the scope of this paper, and we present the results based on a SIC type approach. We do use the cross-sectional subject bootstrap studied in [18] for model inference in Section 3 of this paper.

For the analysis of the melanoma data presented here, we chose the Schwarz Information (SIC) to set the penalty term. SIC measures relative goodness of fit and prediction accuracy. Define $\widehat{f}(x, \tau_k, \lambda)$ as the τ_k^{th} conditional quantile estimate from a model fitted with penalty λ. Let the 'elbow' set be the set of all zero-value residuals:

$$g_\lambda = \left\{ \hat{e}_{ijk} \,|\hat{e}_{ijk} = \, y_{ij} - \widehat{f}\left(x_{ij}, \tau_k, \lambda\right) = 0 \right\}.$$

Then, in the context of longitudinal quantile regression, the SIC is defined as

$$SIC(\lambda) = \log\left(\frac{1}{KT} \sum_{k=1}^{K} \sum_{i=1}^{N} \sum_{j=1}^{t_i} \rho_{\tau_k}\left(y_{ij} - \widehat{f}\left(x_{ij}, \tau_k, \lambda\right) \right) \right) + |g_\lambda|,$$

where $T = \sum_{i=1}^{N} t_i$ is the total number of observations and $|g_\lambda|$ is the number of elements in g_λ. Lower values of SIC indicate better model fit and lower prediction error. A model selection procedure would choose the model fit with λ^* where

$$\lambda^* = \arg\min_{\lambda} SIC(\lambda)$$

In the quantile regression definition of $SIC(\lambda)$, $|g_\lambda|$ is a measure of the effective degrees of freedom (e.d.f.) of the regression fit. In ordinary linear regression, the d.f. is equal to the number of parameters and in general is used to quantify the complexity of a fitting procedure. Overly complex and overfitted

models tend to have worse prediction accuracy and higher parameter variances. The SIC penalizes model complexity through the e.d.f. term. Li *et al.* [16] and Li and Zhu [15] showed that the size of the elbow set $|g_\lambda|$ is a valid estimate for quantile regression e.d.f. However, in our experience with modeling the tumor data, $|g_\lambda|$ proved to be zero or very small for most values of the penalty parameter (we tried λ ranging from 0 to 10,000). Therefore, we modified the criterion for the elbow set to be

$$g_\lambda = \left\{ \hat{e}_{ijk} \; || \hat{e}_{ijk}| = | \, y_{ij} - \widehat{f}\left(x_{ij}, \tau_k, \lambda\right) | \leqslant \epsilon \right\},$$

where ϵ is a very small positive number. We ran the model selection for values of $\epsilon = 0, 10^{-16}, 10^{-14}, 10^{-12}$, on an equally spaced grid of λ values. For the grid spacing, we looked at interval sizes equal to 1, 0.5, and 0.1. In all cases, the optimal λ^* was small, ranging from 0.3 to 2.5. The estimation results presented here are based on $\lambda = 1$ and $\epsilon = 10^{-12}$.

3. Modeling quantile tumor growth curves with combined studies data

3.1. Data

The quantile growth curve method is illustrated using data from xenograft mouse model studies of P28. As opposed to conventional chemotherapies, which destroy cancer and noncancer cells alike, P28 is a novel chemotherapy treatment that preferentially enters a variety of human cancer cells and increases their level of the tumor suppressor protein P53. The latter is an important factor in the body's natural anticancer arsenal—it works by inhibiting cell growth and triggering cell death. Its deficiency has been associated with increased cancer incidence in humans and mice. For more on P53 and melanoma, see [20] for a technical treatment.

For our analysis here, we use data from six independent studies that tested P28 on immunosuppressed mice grafted with human melonoma (MEL) cells that had the P53 gene. The data are comprised of 4 weeks of semi-daily measurement of tumor height, length, and width for each mouse. For each study, the mice were randomly assigned to one of several different chemotherapies, including different doses of P28, which were administered on a daily or semi-daily (three times per week) basis. The studies differ in terms of sample size, the set of experimental treatments, and melanoma cell line. However, the experimental treatments do overlap between studies, including one dosage of P28 that shows up in every design. A summary of the subject counts by study and treatment groups is found in Table I. In addition to P28, different studies included variations to P28—Azurin, which was a precursor to P28, and P28 PEG, which is a biochemical variant with different physiological effects. Finally, five of the six studies included DTIC, which is one of the more common chemotherapy treatments for melanoma. Because of the mechanism by which P28 fights melanoma, it is potentially less toxic than the standard treatment, and therefore, one question for analysis is whether it is as effective in reducing tumor growth as DTIC.

Table I. Mice counts by study and treatment.

	Studies (s)						
	1	2	3	4	5	6	Total
Cell line	MEL2	MEL2	MEL29	MEL23	MEL23	MEL23	
Treatment							
Control	12	20	20	20	9	10	91
Azurin 10 mg/kg daily	—	10	—	—	—	—	10
P28 5 mg/kg daily	8	—	10	10	—	—	28
P28 10 mg/kg daily	8	10	10	10	6	6	50
P28 20 mg/kg daily	—	10	10	10	—	—	30
DTIC 4 mg/kg daily	8	—	10	10	6	—	34
DTIC 5 mg/kg daily	—	—	—	—	—	6	6
P28 10 mg/kg, three times per week	—	—	—	—	—	6	6
P28 PEG 5 mg/kg, three times per week	—	—	—	—	6	6	12
P28 PEG 10 mg/kg, three times per week	—	—	—	—	8	6	14
Margin total	36	50	60	60	35	40	281

MEL2, MEL29, and MEL23 are three different melanoma cell lines with P53 expression.

Figures 1–3 illustrate a selection of the observed tumor trajectories for the one P28 treatment used across all six studies. The data does suggest that there is a treatment effect–the P28 volumes tended to be grow less than control; but the observed pattern of growth and effect size seem to differ between studies. Each of the six studies represents an independent small sample experiment of how well P28 works in suppressing tumor growth relative to untreated tumors. Because the individual study designs used a random assignment of mice to treatment groups, each study should yield an unbiased estimate of the baseline control growth rate and a treatment effect. However, the sample sizes are quite small, ranging between a total of 35 to 60 mice, with each divided among multiple treatment groups. Estimates based on very small sample sizes tend to have higher variances and are thus less informative than those from larger samples. The higher variances lead to a less powerful hypothesis tests and larger confidence regions. This is especially so in the (sparse) panel data context found here, wherein the number of parameters is of the same order as the number of subjects.

For more informative regression estimates, we pool information between studies to estimate the growth curves. For the melanoma data, although the designs are different between studies, there is an overlap in some of the treatments. In particular, all three studies included a group that received a medium daily dose of P28 (10 mg/kg). Instead of estimating separate quantile regression curves by study, the studies are combined, and a single quantile curve is fitted to all the data. The estimated coefficients are then used to construct curves and to assess treatment efficacy.

3.2. Tumor growth model

Popular models of tumor development posit that volume growth follows an S-shaped curve like that of populations in environments with limited resources [21]. During early stages of tumor growth, the collection or population of cells that make up a tumor grows exponentially. However, the rate at which cell numbers and in turn tumor volume grows declines with tumor size, until volume plateaus at what is called the 'carrying capacity' or maximum volume that can be sustained given the limited resources

Table II. Estimated quantile treatment effects.

	Quantile					Mean
	0.05	0.25	0.50	0.75	0.95	(LME)
Intercept	−5.594 (−5.878,−5.293)	−5.196 (−5.353,−5.06)	−4.785 (−4.982,−4.581)	−4.259 (−4.572,−3.799)	−3.630 (−3.88,−3.134)	−4.478 (−4.777,−4.116)
Control	0.065 (0.051,0.082)	0.075 (0.072,0.082)	0.078 (0.072,0.084)	0.081 (0.072,0.087)	0.087 (0.073,0.099)	0.077 (0.069,0.084)
Azurin	0.015 (−0.009,0.04)	0.000 (−0.019,0.01)	−0.001 (−0.023,0.009)	−0.010 (−0.025,0.002)	−0.020 (−0.038,−0.004)	−0.003 (−0.022,0.01)
DTIC, 4 mg	−0.020 (−0.033,−0.008)	−0.028 (−0.036,−0.012)	−0.027 (−0.034,−0.006)	−0.022 (−0.032,0.017)	−0.003 (−0.022,0.02)	−0.013 (−0.021,0.013)
P28, 10 mg	−0.011 (−0.026,0.007)	−0.004 (−0.013,0.003)	−0.006 (−0.016,0.002)	−0.014 (−0.024,−0.002)	−0.023 (−0.041,−0.011)	−0.011 (−0.024,0)
P28, 20 mg	−0.016 (−0.039,0.006)	−0.010 (−0.018,0.004)	−0.004 (−0.017,0.008)	−0.005 (−0.016,0.006)	−0.015 (−0.027,0.001)	−0.005 (−0.013,0.008)
P28, 5 mg	0.001 (−0.019,0.014)	−0.005 (−0.013,0.005)	−0.004 (−0.015,0.01)	−0.008 (−0.022,0.016)	−0.011 (−0.033,0.017)	−0.016 (−0.027,−0.002)
DTIC, 5 mg	0.033 (0.017,0.052)	0.004 (−0.009,0.014)	−0.012 (−0.024,0.016)	−0.002 (−0.038,0.011)	−0.014 (−0.046,0.012)	0.013 (0,0.028)
P28_10, weekly	0.033 (0,0.059)	0.002 (−0.022,0.027)	−0.010 (−0.031,0.015)	−0.008 (−0.042,0.006)	−0.011 (−0.043,−0.001)	0.023 (0.009,0.036)
P28PEG, 10 mg	0.031 (0.005,0.053)	0.021 (−0.002,0.032)	0.007 (−0.011,0.021)	−0.005 (−0.02,0.011)	−0.016 (−0.036,−0.001)	0.030 (0.016,0.041)
P28PEG, 5 mg	0.029 (0,0.047)	0.008 (−0.01,0.024)	0.000 (−0.018,0.012)	−0.012 (−0.028,0.005)	−0.011 (−0.036,0.004)	0.024 (0.011,0.039)

of the organ or physiological system. Common models of this type include the Gompertz and Logistic curves ([21, 22]).

For the melanoma studies, preliminary examination of the data indicates that the study durations were far too short for the sample growth curves to exhibit the S shape reviewed earlier. Rather, tumor volumes increased exponentially over the course of 4 weeks with no apparent inflection point or asymptotic limit to size. Therefore, we model the initial 4-week tumor growth curves using an exponential model.

Let $Y = \log V(t)$ be the natural logarithm of tumor volume at time t. Define the following indices for the observed data: $s = 1, .., 6$ is an index for the different studies of melanoma, $j = 0, \ldots, M$ enumerates the treatment groups, and $i = 1, \ldots, N_{sj}$ is the subject index within groups. Further, let $\tau \in (0, 1)$. Given the observations earlier, an adequate model for the τ^{th} quantile curve for the natural logarithm of tumor volume across time is

$$Q_{Y_{ijs}(t)}(\tau|\cdot, t) = \alpha_i + \beta_0(\tau) + \beta_s(\tau) + \left\{\beta_C(\tau) + \beta_j(\tau)\delta_{ij}\right\} \times t,$$

where α_i is the baseline subject fixed effect, which is constant across quantiles, $\beta_s(\tau)$ is the quantile study effect, $\beta_C(\tau)$ is the quantile change in log volume of control group tumors, $\beta_j(\tau)$ is the quantile treatment effect on the growth rate from the j^{th} treatment, and δ_{ij} is set to 1 if the subject received the j^{th} treatment group and is 0 otherwise.

3.3. Results

Estimates from a PFEQR estimation procedure for five quantiles are presented in Tables II and III. Analogous estimates from a Linear Mixed Effects model for the conditional mean are included as well. For PFEQR, the finite sample distribution of parameter estimates is unknown. To assess statistical significance of the regression coefficients, we used a stratified subject bootstrap to construct confidence intervals. For our bootstrap procedure, we resample subjects with replacement by study and treatment group. This is similar to the cross-section bootstrap for panel data as studied in [18] with the addition of stratification to account for the original study designs.

Our results indicate that the growth rate of untreated tumors increased with higher quantiles. The estimated growth rates for control group tumors are 6.5% per day for the 5th quantile, 7.8% for the median, and 8.7% for the 95th. All the estimated control rates are statistically significant at an $\alpha = 0.05$ level. Here, significance is based on whether the bootstrap confidence interval contains 0. As for treated tumors with P28, only the 10 mg dose of the experimental drug had a statistically significant effect on growth rates and only at the upper quantiles. For tumors treated with the medium daily dose of P28, the 75th quantile growth rate was 1.5 percentage points lower than the same value from the control distribution,

Table III. Estimated quantile growth rates by treatment.

	Quantile					Mean
	0.05	0.25	0.50	0.75	0.95	(LME)
Control	0.065	0.075	0.078	0.081	0.087	0.077
Azurin	0.080	0.075	0.077	0.071	0.067	0.074
DTIC	0.045	0.047	0.051	0.059	0.084	0.064
P28, 10 mg	0.054	0.071	0.072	0.067	0.064	0.066
P28, 20 mg	0.049	0.065	0.074	0.076	0.072	0.072
P28, 5 mg	0.066	0.070	0.074	0.073	0.076	0.061

Table IV. Doubling time by treatment and quantile.

	Quantile					Mean
	0.05	0.25	0.50	0.75	0.95	(LME)
Control	10.664	9.242	8.887	8.557	7.967	9.002
Azurin	8.664	9.242	9.002	9.763	10.345	9.367
DTIC	15.403	14.748	13.591	11.748	8.252	10.830
P28, 10 mg	12.836	9.763	9.627	10.345	10.830	10.502
P28, 20 mg	14.146	10.664	9.367	9.120	9.627	9.627
P28, 5 mg	10.502	9.902	9.367	9.495	9.120	11.363

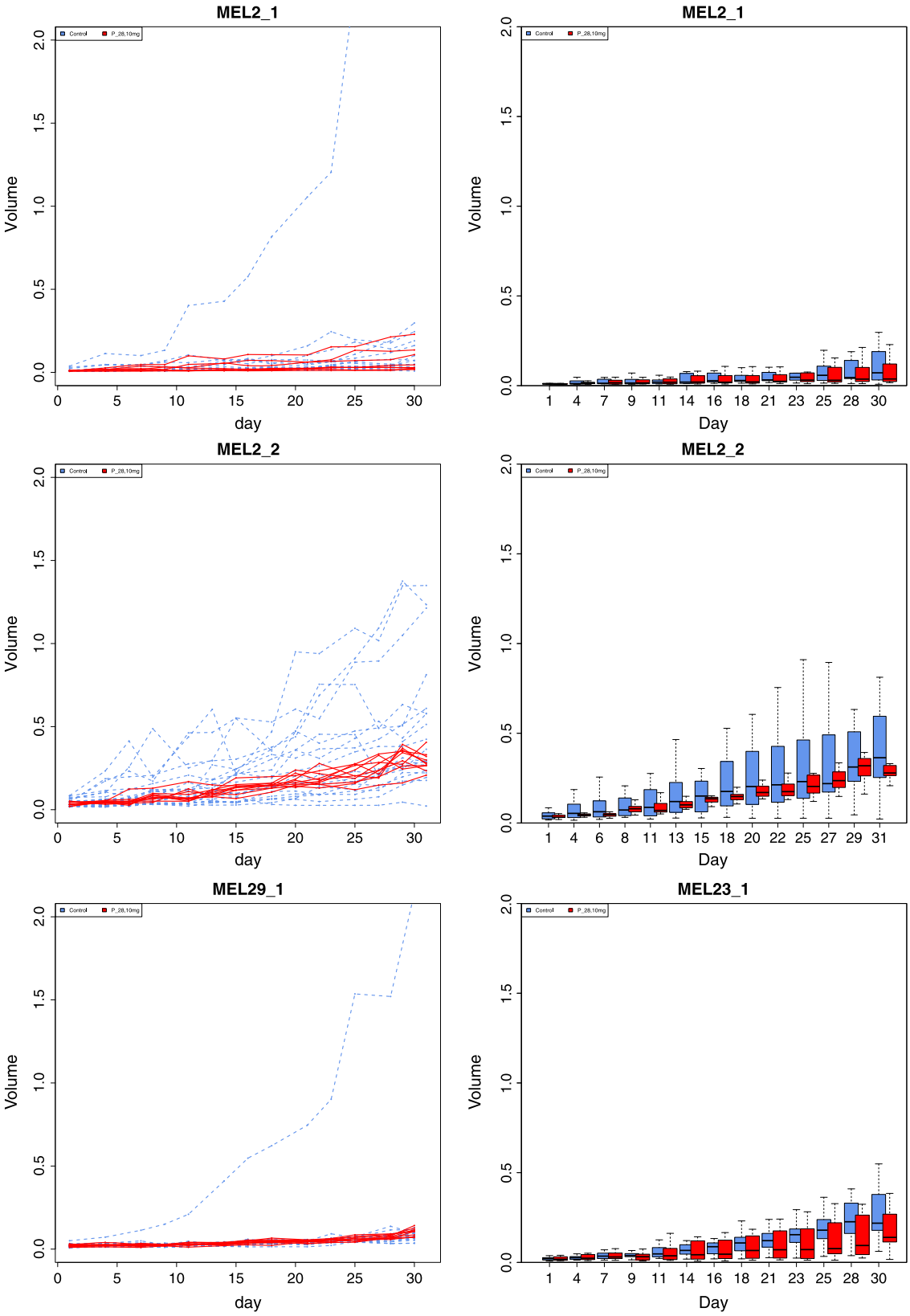

Figure 1. Observed tumor volumes, control and P28, 10 mg daily groups, and MEL2 and MEL 29 cell lines. The blue colored components are the control group values and the red components are the treatment group values.

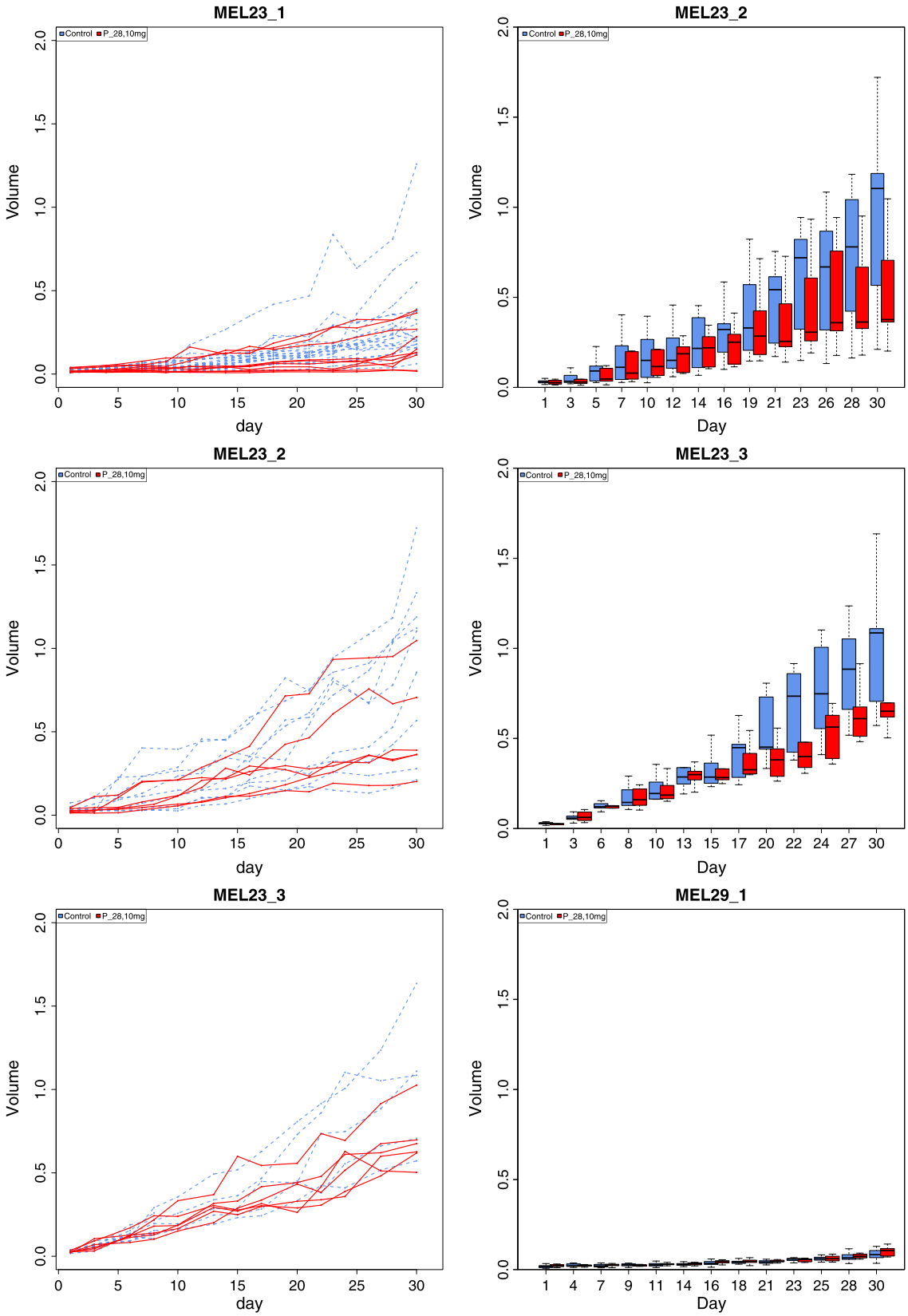

Figure 2. Observed tumor volumes, control and P28, 10 mg daily groups, and MEL23 cell lines. The blue colored components are the control group values and the red components are the treatment group values.

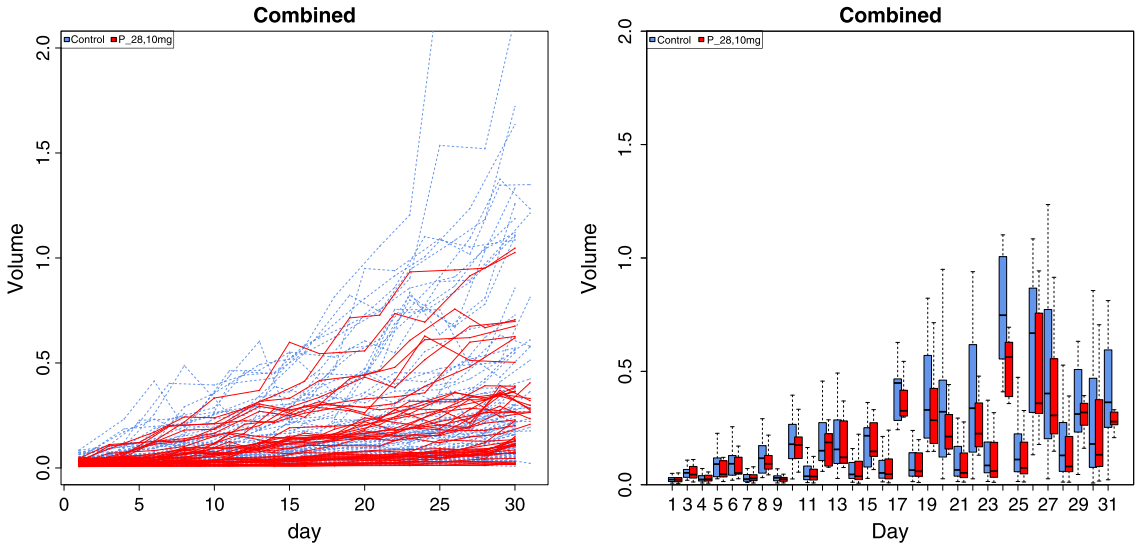

Figure 3. Observed tumor volumes, control and P28, 10 mg daily groups, all studies. The blue colored components are the control group values and the red components are the treatment group values.

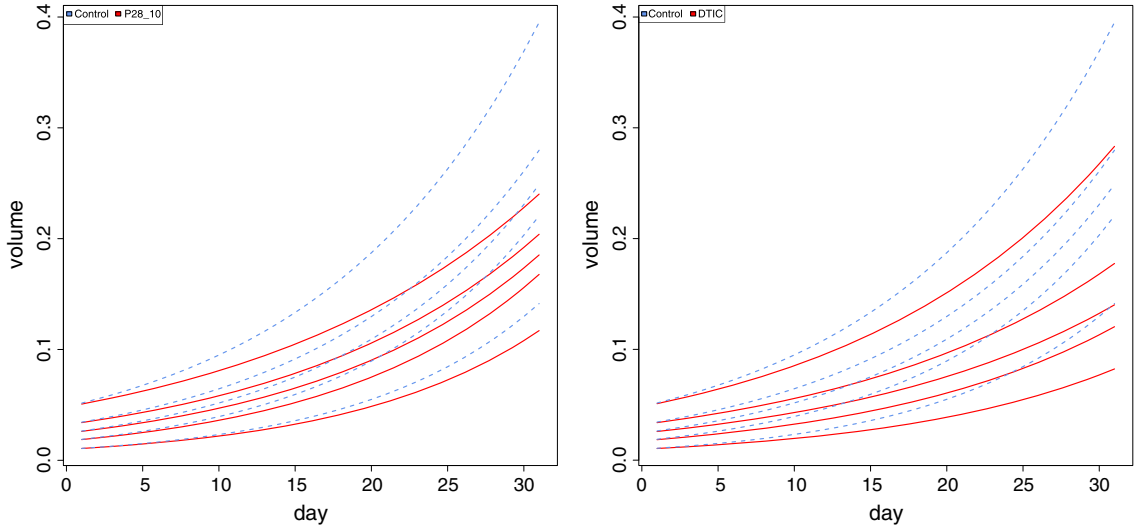

Figure 4. Estimated quantile growth curves for control, P28,10 mg/kg, and DTIC. Fitted curves are presented for the 0.05, 0.25, 0.50, 0.75, and 0.95 quantiles, with higher curves corresponding to higher quantiles. The blue dashed lines are the control group curves and the red solid lines are one of the two treatment groups.

and the 95th rate was 2.7 lower. This yields *upper tail* estimates for the P28, 10-mg tumor growth rate on par with the *lower tail* of the control untreated tumors. In contrast, the standard DTIC chemotherapy had a statistically significant effect on tumor growth in the lower tail and central parts of the distribution. The DTIC effect was not statistically significant at the 95th percentile.

Looking at the corresponding estimates from the conditional mean model, the coefficient values appear to be a rough average of the quantile estimates and mask the differences in growth rates across quantiles. For P28, 10 mg dose, the estimated mean effect is half the estimated effect for the 95th quantile. Similarly, the DTIC mean estimate is again half the value of its effect for lower and central quantiles.

A useful measure of relative tumor growth is the doubling time, T_D, which is the number of days that a tumor takes to double in size. Under the exponential model, this is a fairly easy calculation:

$$T_D = ln(2)/\gamma,$$

where γ is an estimated growth rate. The *quantile* doubling times are presented in Table IV. Looking across quantiles, the control doubling times decline with quantiles. Slow growing tumors of the 5th quantile take 10.7 days to double, those with a median growth trajectory take 8.9 days, and those in the extreme 95th quantile double in 8 days. For mice treated with the medium dose of P28, the doubling times increased by 1.8 days for the 75th quantile and 3.1 days for the 95th over that of the control, with the result that the larger and faster growing tumors treated with P28 take longer to double in size than the smaller and slower growing untreated control tumors.

As a final step, we constructed quantile growth tumor growth curves for the estimates in Table II. Curves for the two significant treatments—P28 10 mg/day and DTIC—are illustrated in Figure 4. In both panels, the dashed blue lines are the control group curves and the red solid lines are the treated tumor curves. Examining the curves shows that the two treatments have different quantile effects. The first panel compares the P28 quantiles to that of the control group. P28 appears to have little effect for the lower quantile trajectories, but it is very effective for the 75th and 95th quantiles. The 75th quantile growth of tumors treated with P28 is on par with the median of untreated tumors, and the 95th quantile curve is on par with the 75th quantile curve of the untreated tumors. The second panel gives a similar comparison for DTIC. In contrast to P28, DTIC appears to have an opposite effect on suppressing growth. The DTIC curves are lower than control for the lower tail, median, and the 75th percentile. However, DTIC appears to not suppress growth of the larger and fast growing tumors in the 95th percentile.

These results have an important implication for melanoma treatment development and personalized patient treatment regimes. Unlike the conclusions that can be drawn from least squares regression, the quantile growth curves show that both the conventional and experimental treatments did not work the same across the entire population of subjects. In particular, mice with faster growing tumors fared better from treatment with the less toxic P28 and not the conventional chemotherapy in the first month of tumor development, while those with smaller tumors did well with DTIC. Thus, the conditional quantile growth curve, and quantile regression in general, can be an invaluable tool in the individualized approach to medical treatment and drug development, wherein treatments are customized to the individual in order to increase treatment response and minimize side effects.

4. Conclusion

In this paper, we combined several small sample studies to construct quantile tumor growth curves. For modeling, we used PFEQR of [11] with added study effects to control for study differences. We demonstrated this approach using data from three studies of xenograft mice models of melanoma cell growth. For inference and hypothesis testing, we used a stratified bootstrap in which we resampled data by study and treatment group. We find that experimental treatment P28 has a different effect on growth rates at different quantiles, with corresponding quantile growth curves much lower than their control group counterparts at the upper tail but are similar to control at the central and lower tail points of the tumor distribution. Similarly, the conventional treatment for melanoma, DTIC, also has a different effect on suppressing tumor growth but is opposite to that of P28. The conventional treatment is more effective in abating growth among smaller tumors but has no significant effect for larger tumors.

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