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The efficacy of antiretroviral therapies for human immunodeficiency virus (HIV) infection can be assessed by studying the trajectory of the changing viral load with treatment time, but estimation of viral trajectory parameters by using the implicit function form of linear and nonlinear parametric models can be problematic. Using longitudinal viral load data from a clinical study of HIV-infected patients in Taiwan, we described the viral trajectories by applying a nonparametric mixed-effects model. We were then able to compare the efficacies of highly active antiretroviral therapy (HAART) and conventional therapy by using Young and Bowman's (1995) test.

Key words: AIDS clinical trial, HIV dynamics, longitudinal data, kernel regression, nonparametric mixed-effects model, viral load trajectory

Introduction

Surrogate viral markers, such as the amount of HIV RNA in the plasma (the amount of HIV RNA in the patient's plasma represents the patient's viral load), currently play important roles in clinical research evaluating antiviral therapies for the acquired immunodeficiency syndrome (AIDS). Before HIV RNA assays were developed in mid-1990s, CD4+ cell counts served as the primary surrogate marker in AIDS clinical trials. Later, the amount of HIV RNA in the patient's plasma (viral load, measured as the copy number of the viral RNA) was shown to better predict the clinical outcome (Mellors et al., 1995; Mellors et al., 1996; Saag et al., 1996), and thus replaced CD4+ cell counts as the primary surrogate marker used in most AIDS clinical trials.

It is, therefore, important to characterize the trajectory that describes the change in viral load that occurs during antiviral treatment, because it is this trajectory that is commonly used to evaluate the efficacy of the treatment. For example, if the viral load reduces, we may infer that the treatment has successfully suppressed the replication of the virus. The differences between the viral loads resulting from different antiviral treatments may be used to compare the antiviral activities of the treatments. Appropriate analysis of the viral load

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is therefore very important in HIV/AIDS drug development. In general, it is believed that the replication of the virus is suppressed at the beginning of an antiviral treatment, but recovery of the virus (called rebound) can occur in later stages of treatment, because of drug resistance or treatment failure. Some parametric models have been developed to describe the progression of AIDS phenomenologically; among the best known of these models are the exponential models (Ho et al., 1995; Wei et al., 1995). More recently, biomathematicians and biologists have proposed a variety of complicated models that include the use of differential equations. The use of these models has led to a deeper understanding of the pathogenesis of AIDS (e.g., Perelson & Nelson, 1999; Wu and Ding, 1999).

In recent years, the necessity for appropriate models has gained more importance with the widespread use of highly active antiretroviral therapy (HAART) to treat HIV/AIDS (Ghani et al., 2003). Numerous studies have shown that HAART is effective in extending the time taken from the diagnosis of HIV-infection to AIDS or death in HIV-infected patients (e.g., Detels et al., 1998; Tassie et al., 2002) as well as reducing the likelihood of perinatal HIV transmission (Cooper et al., 2002). However, in many clinical practices, combination antiviral therapy has failed to completely and durably suppress HIV replication (e.g., Deeks et al., 1999).

To determine the efficacy of treatments in suppressing HIV replication in patients, the present study focuses on the following questions: (i) Given longitudinal viral load data, how can one identify a common feature of the antiviral activities of each treatment? (ii) How can we compare the antiviral efficacies of two different treatments? If we can answer question (ii), we may be able to demonstrate that the better treatment should be evaluated in a large-scale clinical study. However, it may be difficult to answer these questions by using existing parametric or semi-parametric methods. To sufficiently consider all of the information available from the observations, and to avoid the misspecification of parametric modeling, we will use a nonparametric mixed-effects model to analyze the longitudinal viral load data, and we will incorporate the local linear approximation technique developed by Wu and Zhang (2002). The test statistic proposed by Young and Bowman (1995) will then be used to answer question (ii).

The remainder of this paper is organized as follows. In Section 2, we give details of the proposed model, with the method of estimation, and use the test statistic of Young and Bowman (1995) to determine whether there is a difference between the effects of two treatments. In Section 3, we illustrate the use of the proposed methodology with longitudinal viral load data from 30 HIV-infected patients treated with HAART alone and another 30 patients treated with monotherapy or dual therapy. Some discussion is given in Section 4.

Methodology

Nonparametric Models and Estimation Methods

We fit the viral load trajectory data of HIV-infected patients receiving a treatment by using a nonparametric mixed-effects (NPME) model:

$$y_i(t) = \log_{10}\{V_i(t)\} = \eta(t) + v_i(t) + \varepsilon_i(t), \quad i = 1,2,...,n \qquad (2.1)$$

where $V_i(t)$ is the number of copies of HIV-1 RNA per mL of plasma at treatment time t for the ith patient and $y_i(t)$ is the corresponding value in $\log_{10}$ scale; $\eta(t)$ is the population mean function, also called the fixed-effects or population curve; $v_i(t)$ are individual curve variations from the population curve $\eta(t)$ and these variations are called random-effects curves; and $\varepsilon_i(t)$ are measurement errors. We assume that $v_i(t)$ and $\varepsilon_i(t)$ are independent in which $v_i(t)$ can be considered as realizations of a mean 0 process with a covariance function $\gamma(s, t) = \mathrm{E}(v_i(s)\, v_i(t))$, and $\varepsilon_i(t)$ can be considered as realizations of an uncorrelated mean 0 process with variance $\sigma^2(t)$. The population curve $\eta(t)$ reflects the overall trend or progress of the

treatment process in an HIV-infected population and, hence, can provide an important index of the population's response to a drug or treatment in a clinical or biomedical study, so in this paper we are mainly interested in estimating $\eta(t)$. In addition, an individual curve $s_i(t) = \eta(t) + v_i(t)$ can represent an individual's response to a treatment in a study, so a good estimate of $s_i(t)$ would help the investigator to make better decisions about an individual's treatment management and would enable us to classify subjects on the basis of individual response curves. Similar models have been proposed by Shi et al. (1996) and Zeger and Diggle (1994) to describe CD4+ cell counts.

Let t_{gij}, $j = 1,2,...,n_{gi}$, be the design time points for the *i*th individual in treatment group *g*. Then, NPME model (2.1) becomes

$$y_{gi}(t_{gij}) = \eta_g(t_{gij}) + v_{gi}(t_{gij}) + \varepsilon_{gi}(t_{gij}),$$
$$j = 1,2,...,n_{gi};\ i = 1,2,...,n_g;\ g = 1,2 \qquad (2.2)$$

Here, n_g is the number of subjects in treatment group *g*, and n_{gi} is the number of measurements made from subject *i* in treatment group *g*. We now wish to estimate $\eta_g(t)$ and $v_{gi}(t)$ simultaneously, via a local approximation of the NPME model (2.2), by using the local linear mixed-effects model approach of Wu and Zhang (2002), which combines linear mixed-effects (LME) models (Laird & Ware, 1982) and local polynomial techniques (Fan & Gijbels, 1996). For this purpose, we assume the existence of the second derivatives of $\eta_g(t)$ and $v_{gi}(t)$ at *t*, which are then approximated locally by a polynomial of order 2 as follows:

$$\eta_g(t_{gij}) \approx \eta_g(t) + \eta'_g(t)(t_{gih} - t) \equiv X_{gij}^T \boldsymbol{\beta}_g$$

and

$$v_{gi}(t_{gij}) \approx v_{gi}(t_{gij}) + v'_{gi}(t)(t_{gij} - t) \equiv X_{gij}^T \boldsymbol{b}_{gi}$$

where

$X_{gij} = (1, (t_{gij} - t))^T$, $\boldsymbol{\beta}_g = (\eta_g(t),\ \eta'_g(t))^T$, and $\boldsymbol{b}_{gi} = (v_{gi}(t),\ v'_{gi}(t))^T$.

Consequently, the NPME model (2.2) can be approximated by the following model:

$$y_{gij} = X_{gij}^T(\boldsymbol{\beta}_g + \boldsymbol{b}_{gi}) + \varepsilon_{gij},\quad j = 1,2,...,n_{gi};$$
$$i = 1,2,...,n_g;\ g = 1,2 \qquad (2.3)$$

which is called a LME model. Note that, for simplicity of notation,

$y_{gij} = y_{gi}(t_{gij})$, $\varepsilon_{gij} = \varepsilon_{gi}(t_{gij})$, $\boldsymbol{\varepsilon}_{gi} = (\varepsilon_{gi1},...,\varepsilon_{gin_{gi}})^T \sim N(\mathbf{0}, \boldsymbol{\Sigma}_{gi})$, and $\boldsymbol{b}_{gi} \sim N(\mathbf{0}, D_g)$ for $\boldsymbol{\Sigma}_{gi} = \mathrm{E}(\boldsymbol{\varepsilon}_{gi}\boldsymbol{\varepsilon}_{gi}^T)$ and $\mathrm{D_g} = \mathrm{E}(\boldsymbol{b}_{gi}\boldsymbol{b}_{gi}^T)$.

To estimate $\eta_g(t)$ and $v_{gi}(t)$, which are the first element of $\boldsymbol{\beta}_g$ and $\boldsymbol{b}_{gi}$, respectively, under the standard normality assumptions for $\boldsymbol{b}_{gi}$, we can minimize the following objective function:

$$\sum_{i=1}^{n_g} \{(\boldsymbol{y}_{gi} - \boldsymbol{X}_{gi}(\boldsymbol{\beta}_g + \boldsymbol{b}_{gi}))^T \boldsymbol{K}_{gi\lambda}^{1/2} \boldsymbol{\Sigma}_{gi}^{-1} \boldsymbol{K}_{gi\lambda}^{1/2}$$
$$(\boldsymbol{y}_{gi} - \boldsymbol{X}_{gi}(\boldsymbol{\beta}_g + \boldsymbol{b}_{gi})) + \boldsymbol{b}_{gi}^T D_g^{-1} \boldsymbol{b}_{gi} +$$
$$\log|\boldsymbol{\Sigma}_{gi}|\}$$

where

$$y_{gi} = (y_{gi1},...,y_{gin_{gi}})^T;\ \boldsymbol{X}_{gi} = (X_{gi1},...,X_{gin_{gi}})^T$$
$$;\ \boldsymbol{K}_{gi\lambda} = \mathrm{diag}\{K_\lambda(t_{gij} - t),...,K_\lambda(t_{gin_{gi}} - t)\}$$

is the kernel weight of the residual term for $K_\lambda(\cdot) = K(\cdot/\lambda)/\lambda$, in which $K(\cdot)$ is a kernel function; λ is a bandwidth selected by a leave-one-subject-out cross-validation approach (Wu & Zhang, 2002); and the term $\boldsymbol{b}_{gi}^T D_g^{-1} \boldsymbol{b}_{gi}$ is a penalty term to account for the random effects $\boldsymbol{b}_{gi}$, taking between-subject variation into account.

Thus, for given $\boldsymbol{\Sigma}_{gi}$ and D_g, the resulting estimators can be obtained as follows:

$$\hat{\boldsymbol{\beta}}_g = (\sum_{i=1}^{n_g} \boldsymbol{X}_{gi}^T \boldsymbol{\Omega}_{gi} \boldsymbol{X}_{gi})^{-1} (\sum_{i=1}^{n_g} \boldsymbol{X}_{gi}^T \boldsymbol{\Omega}_{gi} \boldsymbol{y}_{gi})$$

$$\hat{\boldsymbol{b}}_{gi} = (\boldsymbol{X}_{gi}^T \boldsymbol{K}_{gi\lambda}^{1/2} \boldsymbol{\Sigma}_{gi}^{-1} \boldsymbol{K}_{gi\lambda}^{1/2} \boldsymbol{X}_{gi} + D_g^{-1})^{-1} \boldsymbol{X}_{gi}^T \boldsymbol{K}_{gi\lambda}^{1/2} \boldsymbol{\Sigma}_{gi}^{-1} \boldsymbol{K}_{gi\lambda}^{1/2} (\boldsymbol{y}_{gi} - \boldsymbol{X}_{gi} \hat{\boldsymbol{\beta}}_g) \quad (2.4)$$

where

$\boldsymbol{\Omega}_{gi} = \boldsymbol{K}_{gi\lambda}^{1/2} (\boldsymbol{K}_{gi\lambda}^{1/2} \boldsymbol{X}_{gi} D_g \boldsymbol{X}_{gi}^T \boldsymbol{K}_{gi\lambda}^{1/2} + \boldsymbol{\Sigma}_{gi})^{-1} \boldsymbol{K}_{gi\lambda}^{1/2}$. As a result, the estimators of $\eta_g(t)$ and $v_{gi}(t)$ are $\hat{\eta}_g(t) = (1, 0)\hat{\boldsymbol{\beta}}_g$ and $\hat{v}_{gi}(t) = (1, 0)\hat{\boldsymbol{b}}_{gi}$.

The unknown variance-covariance parameters in D_g and $\boldsymbol{\Sigma}_{gi}$ can be estimated by using maximum or restricted maximum likelihood, implemented by using the EM algorithm or the Newton-Raphson method (Davidian & Giltinan, 1995; Vonesh & Chinchilli, 1996).

Of particular interest are the comparative effects of the two treatments. Therefore, we need to compare the equality of the two population curves $\eta_1(t)$ and $\eta_2(t)$. To do this, we fit the model $\eta_c(t) + v_{cgi}(t)$ to all data, where $\eta_c(t)$ is the fixed-effects (population) curve for the data and $v_{cgi}(t)$ are random-effects curves that deviate from $\eta_c(t)$. As is done when estimating $\eta_g(t)$ and $v_{gi}(t)$, we can use the local linear approximation approach of Wu and Zhang (2002) to obtain the estimators, $\hat{\eta}_c(t)$ and $\hat{v}_{cgi}(t)$, of $\eta_c(t)$ and $v_{cgi}(t)$.

Our main concern is how to justify that the difference between the two population curves is statistically significant. To compare the effects of two treatments, we apply the following test statistic (Young & Bowman, 1995):

$$\text{TS} = \sum_{g=1}^{2} \sum_{j \in T_g} \frac{\{\hat{\eta}_g(t_{gj}) - \hat{\eta}_c(t_{gj})\}^2}{\hat{\sigma}^2} \quad (2.5)$$

where T_g = {all distinct times t_{gi} in treatment g} and

$\hat{\sigma}^2 = \sum_{g=1}^{2} \sum_{i=1}^{n_g} (n_{gi} - 1)\hat{\sigma}_{gi}^2 / (n - \sum_{g=1}^{2} n_g)$ is an estimator of the variance of the measurement error with $n = \sum_{g=1}^{2} \sum_{i=1}^{n_g} n_{gi}$; $\hat{\sigma}_{gi}^2$ are obtained by using the first-order difference approach proposed by Rice (1984), as follows:

$$\hat{\sigma}_{gi}^2 = \frac{1}{2(n_{gi} - 1)} \sum_{j=1}^{n_{gi}-1} (y_{gi[j+1]} - y_{gi[k]})^2,$$

$i = 1,2,...,n_g$; $g = 1,2$

If the two population curves are equal; that is, under the null hypothesis H_0: $\eta_1(t) = \eta_2(t)$, the distribution of the test statistic TS in (2.5) is then approximated by $a\chi^2(b) + c$, where $\chi^2(b)$ is a chi-squared distribution with b degrees of freedom. Moreover, a, b, and c are constants such that the mean, variance, and skewness of $a\chi^2(b) + c$ are equal to the corresponding quantities of the test statistic TS, which can be calculated directly. The distribution of $a\chi^2(b) + c$ is then used to calculate the p-value. The standard error of the difference between the estimates for the two population curves can be computed as

$$\text{se}_{\text{diff}}(t) = \text{se}\{\hat{\eta}_1(t) - \hat{\eta}_2(t)\} = \sqrt{\text{se}_1^2(t) + \text{se}_2^2(t)}$$

where $\text{se}_1(t) = \text{se}\{\hat{\eta}_1(t)\}$ and $\text{se}_2(t) = \text{se}\{\hat{\eta}_2(t)\}$ are the standard errors of the estimates of the population curves, respectively. A reference band whose width is centered at the average of the two estimated curves $\pm 2 \times \text{se}_{\text{diff}}(t)$ can be

used to see how much difference there is between the two treatment groups (Young and Bowman, 1995). Note that, theoretically we should consider correlation when using the approach of Young and Bowman (1995), but we do not just because of mathematical simplicity. Ignoring the correlation may lose some efficiency, however, as you will see, for the real-life data analysis given in the next section there is significant difference between the treatment effects of the two groups even using independent structure. Considering correlation may increase power but seems unnecessary.

Results

The Analysis of Longitudinal Viral Load Data

In this section, we illustrate the practical use of the proposed methodology with longitudinal viral load data from HIV-infected patients. The data set we are using includes the longitudinal viral load data obtained from 30 HIV-infected patients who received monotherapy or dual therapy and 30 HIV-infected patients who received HAART in several hospitals in Taipei, Taiwan, between 1997 and 2002. These data are subsets of data from a much larger cohort data of 1,195 HIV-infected patients in Taipei. Among the 1,195 HIV-infected patients, most of them received diverse treatments, so, to ensure the validity of the comparison, we chose to use data from the patients treated with HAART who had never been given any other treatment regimen and non-HAART patients who had never been treated with HAART. Treatment durations varied, because patients began receiving treatment at different times during the study period. Figure 1 presents scatter plots of viral load (in $\log_{10}$ scale) against treatment durations for the HIV-1-positive patients.

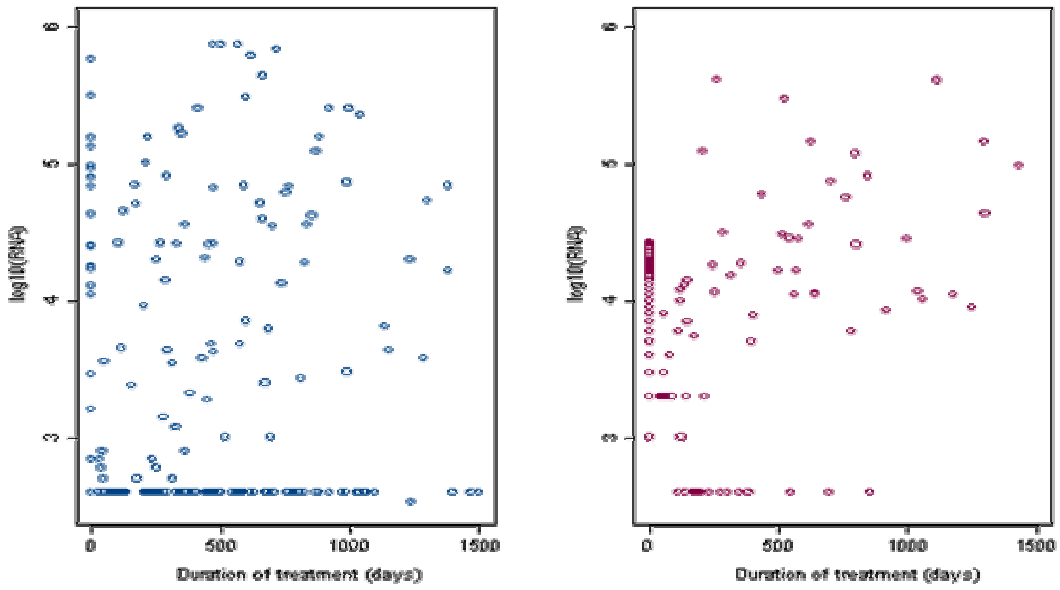

Figure 1: Scatter plot of viral load ($\log_{10}$ of copy number of HIV RNA in plasma) versus duration of treatment with HAART (left) or non-HAART (right).

After excluding missing data, we have 208 complete viral load observations in the HAART group, of which 108 have a value less than 400; and we have 164 complete viral load observations in the non-HAART group, of which 69 have a value less than 400. If we use the criterion that a treatment is considered successful in its antiviral effect when the viral load is below 400, the success rates in the HAART and non-HAART groups are 51.9% and 42.1%, respectively.

For data analysis, we used the quartic kernel, $K(u) = (15/16)(1 - u^2)^2 I_{(|u| \leq 1)}$. The estimates of the two population curves are depicted in Figure 2. From Figure 2, we can see that the estimates of the two population curves have different patterns although both decrease at the beginning of treatment. The estimated curve for the HAART group shows that the viral load is maintained at a constant level until the end of the treatment, whereas that for the non-HAART group shows that the viral load decreases sharply during the first 480 days, reaching its lowest point on day 480. However, after 480 days, the viral load increases, remains constant for a short time, and increases again at the end of the treatment.

A Chi-squared test for the success rates of the two treatments gives a p-value of 0.07. It is hard to say that there is a significant difference between the effects of the two treatments, although the success rate in the HAART group is greater than that in the non-HAART group. Therefore, to look more closely at the difference between the effects of the two treatments, we use the principle described in Section 2. The p-value obtained by using this method is less than 10^{-4}, which indicates that the two population curves for each treatment are substantially different. To confirm this conclusion, we obtained a range of reference values and plotted them with our viral load trajectory estimates in Figure 2. The two estimated population curves deviate from the reference band, and the efficacy of the HAART is seen to be almost significantly superior to that of the conventional therapy that does not include HAART.

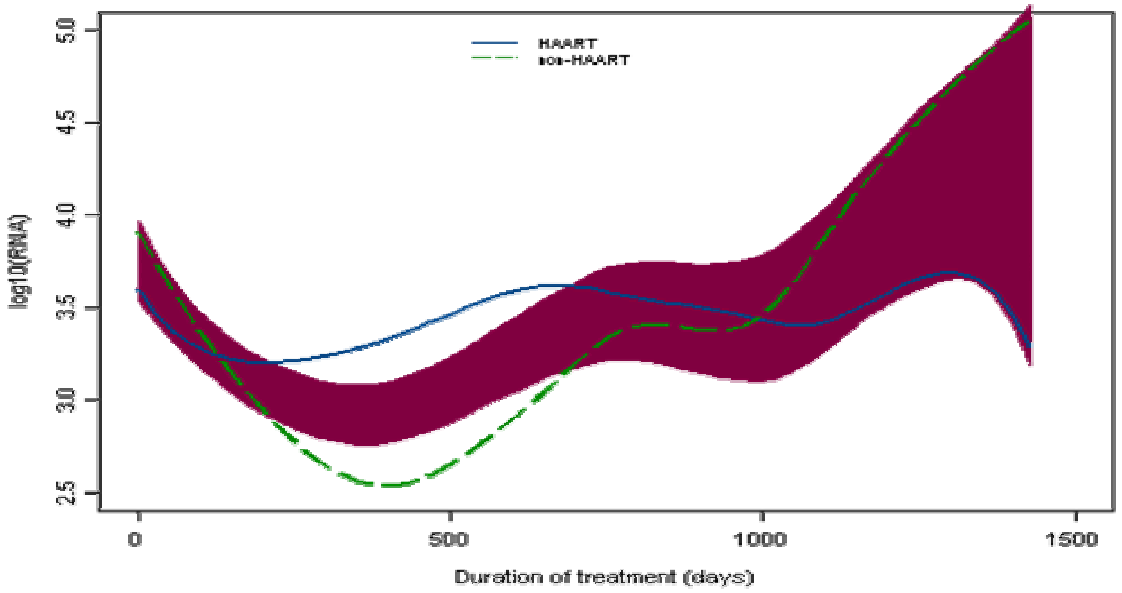

Figure 2. Estimate of two population curves.

Discussion

To determine the efficacy of antiviral treatments by using longitudinal viral load data, we applied nonparametric mixed-effects models to estimate the patterns of the viral trajectories in the two sampled populations. This approach avoids misspecification and, thus, the occurrence of an artificial bias. By combining the between-subject and within-subject information, the models we have proposed can parsimoniously capture the features of viral response to an antiviral therapy, such that the estimated curve is able to show common features of the antiviral activity.

In implementing the estimation of population curves, we used local linear regression and the bandwidth selection method proposed by Wu and Zhang (2002) to select the bandwidth. Besides the local linear methods applied in this article, the method of regression splines may also be implemented for parameter estimation. The approach of regression splines transforms the models to standard linear mixed-effects models and is easy to implement by using existing software such as SAS and SPLUS.

The result of our illustrative example indicates that HAART has effects that are significantly different from those of treatment that did not include HAART. At the beginning of treatment, non-HAART has strong antiviral activity, which is lacking with HAART. However, during the course of the treatment, the superiority of non-HAART lessens, and this therapy ultimately fails, whereas HAART maintains a constant effect throughout treatment. This maintenance of the viral load at a constant level confirms previous findings and is preferable to the fluctuation of load resulting from non-HAART. This result confirms that HAART is worth continuing, despite its inability to suppress viral replication completely (Deeks & Martin 2001).

Finally, the reference band covers a wider range of viral loads at the end of treatment, despite the increasing difference between the two estimated curves. This is not surprising because of the smaller sample size resulting from a shorter treatment duration for some patients at that time.

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