Significance testing of clinical data using virus dynamics models with a Markov chain Monte Carlo method: application to emergence of lamivudine-resistant hepatitis B virus

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Bayesian analysis using a virus dynamics model is demonstrated to facilitate hypothesis testing of patterns in clinical time-series. Our Markov chain Monte Carlo implementation demonstrates that the viraemia time-series observed in two sets of hepatitis B patients on antiviral (lamivudine) therapy, chronic carriers and liver transplant patients, are significantly different, overcoming clinical trial design differences that question the validity of non-parametric tests. We show that lamivudine-resistant mutants grow faster in transplant patients than in chronic carriers, which probably explains the differences in emergence times and failure rates between these two sets of patients. Incorporation of dynamic models into Bayesian parameter analysis is of general applicability in medical statistics.

Keywords: Markov chain Monte Carlo; hypothesis testing; hepatitis B; virus dynamics; drug resistance

1. INTRODUCTION

Therapy development for fatal diseases presents major challenges for medical statistics. Reliable significance tests based on minimal clinical data are required to make informed decisions about ongoing treatments and clinical trials. Such data sets may be unique and irreproducible because therapies can turn out to be unjustifiable over the course of a clinical trial; however, such trials may be highly informative, especially as they represent direct consequences of risk factors. For instance, some of the participants in a clinical trial of high viraemia patients for the treatment of end-stage hepatitis B virus (HBV)-associated liver disease using the anti-viral drug lamivudine and liver transplantation had HBV re-emerge. Because reinfection following liver transplantation has potentially fatal consequences this treatment has since been modified; the original patient cohort thus provides a unique set of data which may never be duplicated. Analysis of clinical data sets normally use non-parametric statistics such as the Mann–Whitney rank test. However, the small size of the data sets may mean that such tests have insufficient power. More powerful tests can be constructed using virus dynamic models that can be tailored individually to the trial. With the development of Markov chain Monte Carlo (MCMC) methods (Gilks *et al.* 1996) these tests are realistic and feasible. MCMC is an ideal method for such analyses because of its immense flexibility, and has already been

used in a variety of instances in medicine (Gilks *et al.* 1993; Spiegelhalter *et al.* 1996).

In this paper we compare antiviral treatment of HBV between liver transplant and non-transplant patients, examining the hypothesis that the rate of mutant growth is different in the two groups, and propose that this disparity is responsible for the differences in the observed emergence times. Traditional non-parametric analysis is of questionable validity because of differences in experimental design between the two data sets that may lead to a high type I error in these tests. Our analysis using a novel MCMC method demonstrates that HBV lamivudine-resistant mutants have a significantly faster replication cycle in transplant patients, and is a contributing factor in the differences between transplant and non-transplant patient prophylaxis failure. Such an analysis is vital for the informed use of immunosuppressive drugs in patients with HBV, the adaptation of therapies between these patient groups, and the development of new drug targets through a greater understanding of the virus life cycle and viral ecology.

2. CLINICAL DATA

Liver transplantation offers the only treatment option for patients with end-stage HBV-associated liver disease. However, this was once restricted to low viraemia patients because those with high virus titres (greater than 10^6 copies ml^{-1}) have a poor prognosis resulting from a high probability of HBV recurrence. Re-emergence under immune suppression (required for transplant) causes

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Figure 1. Viraemia of three transplant patients (log base 10). The lines shown are the best fit for the growth and decay phases. Transplantation occurred at day 0, and lamivudine was administered before transplantation as indicated by the arrow. Bars are 95% confidence intervals; half-bars indicate readings above or below the detection limit. Under immune suppression, final viraemia (for those patients that acquired resistant mutants) was higher than the initial chronic state because nearly all hepatocytes were infected (Davies *et al.* 1991), and thus all patients had a final viraemia above 10^7 copies ml^{-1} . HBV DNA was measured by ligand hybridization (Abbott, Abbott Diagnostics Division, Maidenhead, Berkshire, UK) and quantitative PCR (Roche Amplicor, Roche Diagnostics Ltd, Lewes, East Sussex, UK). Abbott was used to extrapolate readings above 4×10^7 copies ml^{-1} , the limit of the Roche assay.

severe hepatitis (Davies *et al.* 1991). The high efficiency of the antiviral drug lamivudine in reducing virus levels in non-transplant patients suggested that treatment of high viraemia patients may be possible. However, in a cohort of nine patients treated with lamivudine, three developed lamivudine-resistant mutants (figure 1 and table 1) as verified by sequencing (Cane *et al.* 1999). Such a study raises a number of questions (Burroughs *et al.* 1998; Mutimer *et al.* 1999), in particular why re-emergence appears to be more frequent in transplant patients than non-transplant patients (30% and 14% failure over one year of treatment, respectively; Lai *et al.* 1998).

To examine the problem, this data set (together with data pertaining to a transplant patient from another study) was compared with data from a set of six chronic HBV carriers who had developed lamivudine-resistant mutants while on lamivudine monotherapy (figure 2). Five of these patients had previously received combination therapy (interferon- α + lamivudine) for a short time (less than 140 days); however, the time interval between these treatments is believed to be sufficient not to cause any significant change in the HBV-infected hepatocyte population. Emerging populations are all verified to be lamivudine-resistant mutants by sequencing and are either dominated by a single mutant strain, or are an apparently stable mixture. Data from these patients are summarized in table 2. We use the prefixes NTX and TX to distinguish chronic and transplant patients, respectively.

Comparison of these two data sets indicates that the mutant emerges (relative to start of therapy) significantly earlier in transplant patients (average 374 days, s.d. = 44 days, three patients) relative to non-transplant patients (average 570 days, s.d. = 150 days, six patients), ($p = 0.056$ rank test). This earlier emergence has also been observed in lamivudine therapy of transplant patients where therapy starts a significant period after transplant (Perrillo *et al.* 1999). The simplest explanation of this effect is a faster growth rate of mutants in transplant patients as suggested by a linear regression analysis on the data sets (table 2) ($p < 0.005$ Mann-Whitney). However, such an analysis is of questionable validity, because the time resolution of the non-transplant data is insufficient to guarantee that the emergence of the mutant has, in fact, been captured. A typical time-series consists of a viraemia plateau corresponding to the wild-type virus under lamivudine before emergence, a higher plateau corresponding to the lamivudine-resistant mutant steady state after emergence, and an intermediate value at a single time-point (figures 2 and 3). In five of the chronic patients (patients 1–5, figure 2) there are insufficient time-points to perform a linear regression because there is no guarantee that the mutant dominated at the lowest time-point, i.e. was just about to emerge, or had just finished exponential growth at the top point. Sequencing at these data points was not able to provide any further information, and invariably suggested that the mutant was a minority species at a relative frequency of less than 5% (detection limit) at the time-point at the base of the supposed emergent growth phase. This contrasts with the transplant data in which growth has been captured principally because of the higher time

Table 1. *Lamivudine-resistant mutant growth characteristics in vivo: transplant patients*

patient	sampling interval at emergence (days)	mutant growth rate ^a (day ⁻¹)	maximum observed growth rate (day ⁻¹)	time (days) since start of therapy when viraemia reaches ^b	
				400 copies ml ⁻¹	10 ⁶ copies ml ⁻¹
1	28	0.12 (0.007)	0.11	260	324
2	40	0.31 ^c	0.31	383	408
3	28	0.12 (0.005)	0.12	327	391
— ^d	28	0.166 ^c	0.17	210 ^d	260 ^d

^a Standard deviations are given in parentheses.^b Computed by interpolation from linear regression analysis.^c Based on two data points; therefore standard deviation cannot be calculated.^d The final patient is from a different study who had a pre-treatment viraemia below 400 copies ml⁻¹, and lamivudine therapy started a week after transplant. Therefore this patient is not included in the emergence time analysis, but only used for growth rate comparison.Table 2. *Lamivudine-resistant mutant growth characteristics in vivo: non-transplant patients*

patient ^a	sampling interval at emergence (days)	mutant growth rate (day ⁻¹)			time (days) since start of therapy when viraemia reaches 10 ⁶ copies ml ⁻¹ ^{b,d}
		linear regression ^b	maximum observed	mode ^c (MCMC)	
1	100	> 0.054 (two points)	0.054	0.057	600 ^c
2	60	0.048 (0.003) (three points)	0.060	0.060	290 (3)
3	50–100	0.035 (0.002) (three points)	0.038	0.034	730 (4)
4	50–100	0.029 (0.002) (four points)	0.033	0.023	613 (5)
5	90	0.025 (0.001) (four points)	0.037	0.021	621 (8)
6	90–100	0.0154 (0.0004) (six points)	0.049	0.015	564 (7)

^a Patients are ordered by mutant growth rate as estimated by linear regression.^b Standard deviations are given in parentheses.^c Mode of Bayesian posterior probability distribution for growth rate r using uniform prior on r in $[0, 0.35]$, and normal priors $L \sim \mathcal{N}(10, 10)$ and $H \sim \mathcal{N}(20, 3)$. Based on 800 000 steps.^d Computed by interpolation from linear regression analysis.^e Based on two data points; therefore standard deviation cannot be calculated.

resolution and greater difference in initial and final viraemia (figure 1).

An improvement on a linear regression analysis is based on the maximum observed growth between any two data points for which emergence is evident (i.e. viraemia remains higher than that at the earlier time-point). This does not assume that there are three data points on the emerging mutant growth phase. Such an analysis indicates that there are significant differences between the data sets ($p < 0.005$ Mann–Whitney rank test), using the values given in tables 1 and 2. However, this test is also questionable because the significance of this result may be due to the different time intervals between observations or the viraemia range differences of the two data sets. Both of these effects bias towards rejecting the null hypothesis when it is true, i.e. high type I error, because the estimated growth rate between two points may be less than the actual mutant growth rate (those two points are not necessarily on the growth phase).

3. ANALYSING THE GROWTH RATE

The high correlation coefficients observed in the linear regression analysis of the growth phase in the non-transplant patients suggests that the time-series is, in fact,

representative of the growth of the mutant (table 3). To confirm that this is the case, and that the growth rate is not as high as that observed in the transplant patients, we constructed a hypothesis test for observing time-series patterns. The basic idea of the test is that if the emerging strain has a high growth rate it is very unlikely that a time-point in the exponential growth phase of the mutant would actually be observed. For instance, if it takes ten days to increase the viraemia from the lower to a higher plateau, a time resolution of 60 days only has a one in six chance of an observation lying on the growth phase. Therefore the probability of observing a significant point on the growth phase is less than one in six in this example.

The viraemia time-series can be described to a good approximation by a piecewise linear growth model (figure 3) ($y = \log_e v$ the natural logarithm of the viraemia),

$$y(t) = L, \quad t < t^*; \quad y(t) = L + r(t - t^*), \\ t^* < t < t^* + \frac{1}{r}(H - L); \quad y(t) = H \text{ otherwise,} \quad (1)$$

with the mutant numerically dominating from time t^* , and r is the mutant growth rate. Here L and H are the (natural logarithms of the) lower and higher steady states. The variable y is the expectation of the log

Figure 2. Viraemia of non-transplant patients (log base 10). Only patients 1 (a), 3 (b) and 6 (c) are shown (one other patient had a similar viraemia profile to patient 1, and two others to patient 3, although only patient 3 displayed a continued decline; the others plateaued soon after the start of therapy). Patient 6 was the only patient with large gaps in the time-series which caused problems in reconstructing the dynamics. Bars are 95% confidence intervals. The upper limit of the Roche assay is 4×10^7 copies ml^{-1} .

viraemia. Although a logistic growth model would be more natural, this approximation is easier to implement in the MCMC algorithm (see Appendix A), and both capture the exponential growth phase that dominates the

Figure 3. Piecewise linear model of mutant emergence.

dynamics (figure 1). Under growth model (1) the predicted viraemia of a time-point intermediate between two measurements that encircle the growth phase can be computed. This is a Bayesian calculation of the observed viraemia at a time-point $t \in [t_L, t_H]$, conditional on the fact that the mutant was a minority population at time t_L , numerically dominated at time t_H and the mutant grew with rate r . This is a suitable model for the emergence of a mutant, because the time interval $[t_L, t_H]$ is short compared with the time since the start of therapy; therefore a uniform distribution is appropriate. In practice, the actual interval $[t_L, t_H]$ is not important because the data determine the time-frame of the plateaus and emergence is trapped between observations at these two levels. Thus, in five patients we take t_L within 200 days of the start of treatment because the pre-resistance plateau is established by that time. In patient NTX3 the viraemia continued to decay after an initial rapid exponential decline (figure 2) presumably caused by the clearance of infected hepatocytes (Nowak *et al.* 1996; Burroughs *et al.* 1998), and thus the viraemia failed to settle to a steady state. This means that only a small number of time-points near the end of this decay phase can be used in the analysis (those which conform approximately to the model of figure 3), or the model should be adapted to include such a decay phase. We adopt the former method for simplicity, and this reduces the power of our test on this patient.

The likelihood of observing an intermediate viraemia value is best illustrated by the probability density function (PDF) of a single measurement η at time t ,

$$P(\eta|r, t^*) = \frac{1}{\sqrt{(2\pi)\sigma}} \exp - \frac{(\eta - y(t))^2}{2\sigma^2}, \quad (2)$$

where $y(t)$ is given by equation (1). Imposing the prior $t^* \in [t_L, t_H]$, the variable t^* can be integrated out to give

$$\begin{aligned} P(\eta|r) &= \frac{1}{t_H - t_L} \int_{t_L}^{t_H} \frac{dt^*}{\sqrt{(2\pi)\sigma}} \exp - \frac{(\eta - y(t))^2}{2\sigma^2} \\ &\equiv \frac{t - t_L - w}{t_H - t_L} \frac{1}{\sqrt{(2\pi)\sigma}} \exp - \frac{(\eta - H)^2}{2\sigma^2} \\ &\quad + \frac{1}{t_H - t_L} \frac{1}{r} \int_{(L-\eta)/\sigma}^{(H-\eta)/\sigma} \frac{dz}{\sqrt{(2\pi)}} \exp - \frac{z^2}{2} \\ &\quad + \frac{t_H - t}{t_H - t_L} \frac{1}{\sqrt{(2\pi)\sigma}} \exp - \frac{(\eta - L)^2}{2\sigma^2}. \end{aligned}$$

Table 3. Time-series pattern hypothesis test: $r \geq 0.12 \text{ day}^{-1}$

patient	time-point	observed viraemia ($\log_e$)	viraemia CI ($\log_e$) 95% central minimum	p^a	regression coefficient ^b	
					observed	upper 95% CI
1	586	13.7	13.3–17.4	0.03	0.934	0.950
2	308	14.2	13.0–16.6	0.01	0.990	0.947
3	748	14.7	12.6–15.7	0.006	0.999	0.960
4	622	14.3	11.8–15.0	0.01	0.9998	0.977
5	691	15.0	14.4–17.6	0.01	0.977	0.934
6	489	13.8	10.4–17.7	< 0.01	0.922	0.986

^a Distribution lying around central minima within boundaries determined by height of PDF at observed value.

^b Regression coefficient of the time-point estimated and the two surrounding values.

Figure 4. Predicted viraemia 748 days after the start of lamivudine monotherapy for non-transplant patient 3 assuming a mutant growth rate of 0.12 per day conditional on the observed data (except that at day 748). Based on 800 000 steps. The predicted viraemia is bimodal with an approximately uniform distribution between the peaks. The PDF values for L and H are also shown, demonstrating their overlap with the peaks. The observed viraemia (arrow) lies in the uniform region.

Here we have defined $w = (H - L)/r$, and our expression assumes $t \in (t_L + w, t_H)$. In the limit $\sigma \rightarrow 0$ this expression reduces to the expected time-partition result: $\eta = H$ with probability $(t - t_L - w)/(t_H - t_L)$, $\eta = L$ with probability $(t_H - t)/(t_H - t_L)$, and uniform in $[L, H]$ with probability $w/(t_H - t_L)$. For non-zero σ , the PDF is a convolution of this distribution with the Gaussian distribution. Thus for high growth rates r , the distribution for η is peaked at L and H , while for low r the distribution is broad with Gaussian decay above H and below L . Such a probability density allows assessment of the probability of observing a viraemia lying in the central uniform valley.

Incorporation of additional observations effectively limits the likely values of t^* , i.e. an observation η' close to L at t_1 gives a weighting $\exp(-(\eta_1 - L)^2/2\sigma^2)$: $\exp(-(\eta_1 - H)^2/2\sigma^2)$ in favour of $t^* > t_1$. In order to compute the probability distribution for the viraemia at time t_k , we use the following likelihood:

$$\mathcal{L}_k = \prod_{i \neq k} \exp\left(-\frac{(\eta_i - y(t_i))^2}{2\sigma^2}\right), \quad (3)$$

where the observed viraemia at t_i is η_i , $y(t)$ is defined in equation (1), and the measurement at time t_k is omitted. A Markov chain for the variables t^* , L and H computes the Bayesian probability $\mathbb{P}(t^*, L, H, y(t_k)|r, \{\eta_i\}_{i \neq k})$ for an assumed growth rate r ; see Appendix A. The PDF of the predicted observation at time t_k , $\mathbb{P}(\eta_k|r, \{\eta_i\}_{i \neq k})$, now follows. This can be performed either for a fixed r , or using a given PDF for r , e.g. estimated from the transplantation data.

The priors used for L and H are $\mathcal{N}(10, 10)$ and $\mathcal{N}(20, 3)$. The former is very weak because the data strongly determine L . However, the higher plateau H is determined only by 1–3 data points depending on the patient; thus we use a stronger prior that reflects the fact that all patients have an initial steady-state viraemia in the range 10^7 – 10^9 copies ml^{-1} and the final viraemia is expected to be of this order.

We assume measurement errors are normally distributed: calculating the standard deviation from the fact that half a $\log_{10}$ difference between two independent measurements is normally considered significant for the polymerase chain reaction (PCR). We interpret this as a 90% significance level, giving a standard deviation for

Figure 5. Estimated PDF for the mutant growth rate r in non-transplant patients, their average, and the transplant patient population average (modelled as a normal distribution $N(0.179, 0.045)$). The PDF for r is calculated with a uniform prior on r between zero and 0.35 per day, and priors $L \sim N(10, 10)$, $H \sim N(20, 3)$. Based on 800 000 steps.

the (natural logarithm of the viraemia) of 0.493, i.e. $[1/(2^{1.5} \times 1.65)] \log_e 10$. This compares well with our estimate of 0.505 (21 d.f. over three patients) from the data.

(a) Results

The posterior distribution for viraemia of patient NTX3 748 days after the start of lamivudine therapy is shown in figure 4, assuming that the growth rate for the mutant is 0.12 day^{-1} , the slowest growth rate observed in the transplant patients (table 1). The observed viraemia is within the central (uniform) valley of the distribution, within the central 0.6% of the distribution (table 3). This clearly demonstrates that the time-series observed for this patient is significantly different from that of a mutant that grows at 0.12 day^{-1} . The observed time-series becomes more probable as the assumed growth rate is reduced. Choosing appropriate time-points for the other non-transplant patients we find that all six patients have time-series that are significantly different ($p < 0.05$) from those expected if the mutants grow at a rate as high as 0.12 day^{-1} (table 3). This result has only a weak dependence on the priors. The regression coefficient PDF is also calculated for the predicted point and the two surrounding observations. This PDF confirms the conclusions in most of the patients (table 3), and supports the earlier comment that these regression coefficients are high.

(b) Estimating the growth rate

The previous test has demonstrated that the data strongly support the hypothesis that the growth rate of lamivudine-resistant mutants is higher in transplant patients compared with chronic carriers (no significant liver disease). In this section we consider direct estimation of resistant mutant growth rates in the chronic carriers. The earlier Markov chain can be modified to incorporate the growth rate r and therefore calculate the probability

distribution $\mathbb{P}(r, t^*, L, H | \{\eta_i\}_i)$, and thus the desired distribution $\mathbb{P}(r | \{\eta_i\}_i)$. The low resolution of the data in the growth phase means that these distributions are only defined within the context of priors on r which limit high growth rates. The distribution $\mathbb{P}(r | \{\eta_i\}_i)$ is predicted to have a behaviour $a + b/r$ for large r ; the $1/r$ decay occurs when there is a single datum point on the growth phase which restricts the location of the growth phase but not the growth rate, which can be arbitrarily large, while the constant asymptotic value as r tends to infinity derives from gaps in the time-series which separate observations that are basically L from those that are basically H . In such a region, the growth rate can be arbitrarily large and growth initiated (t^*) anywhere in that interval. If an intermediate viraemia is observed, this asymptotic value is very low because the intermediate value contributes the smaller of $\exp(-(\eta - L)^2/2\sigma^2)$ or $\exp(-(\eta - H)^2/2\sigma^2)$. For low r , the distribution declines rapidly below the mode as in normal regression analysis, because the data strongly restrict low growth rates (figure 5).

Because the distributions $\mathbb{P}(r | \{\eta_i\}_i)$ are normalized through a prior, it is important that the prior is sufficiently weak. We choose a uniform prior $r \in [0, 0.35]$, the upper limit being larger than any of the growth rates observed in the transplant patients. A uniform distribution is preferable (compared to a normal prior) because it does not distort the probability distribution and allows the tails to be compared with the predicted form. These distributions are shown in figure 5 and have asymptotic tails $a + b/r$ as required when r tends to infinity. The mode is prior independent and agrees with the linear regression gradient estimate (table 3). Distribution moments depend strongly on the prior for r since the asymptotic tail is not normalizable, and thus the estimated growth rates must be viewed within the context of this prior.

The PDF values for r can be used to compute an estimate of the population mean by averaging, $r_{\text{pop est}} = (1/n) \sum r_i$.

Strictly, this distribution should be computed within MCMC using a Markov chain incorporating the six non-transplant data sets (Gilks *et al.* 1996). We obtain a population growth rate of 0.105 (s.d. = 0.035) day⁻¹, with a PDF that is approximately normal (figure 5). This population growth rate is significantly different from the transplant estimate at 90% significance, $p < 0.10$ by a direct comparison of the PDF of $r_{\text{pop est}}$ and the transplant estimate $N(0.179, 0.045)$. There are a number of factors that can affect the significance of this result, principally the measurement error standard deviation (increasing this by 15% takes $p \sim 0.25$) and the prior r , because the underlying conditional is not normalizable. However, our estimate of the measurement error supports the traditional error estimates to within 2.5%, and $r \in [0, 0.35]$ is very reasonable because growth rates higher than 0.35 day⁻¹ are unrealistic and have not been observed.

4. CONCLUSIONS

Our primary aim in this analysis was the general demonstration that MCMC can be used to construct tests on difficult clinical data where non-parametric methods may be of questionable validity. This was illustrated through the specific construction of a test to compare the growth rates of lamivudine-resistant mutants in transplant and non-transplant HBV patients, regression analysis and non-parametric tests in this case possibly being prone to large type I errors originating from differences between the data sets in the time-periods between observations and viraemia ranges. MCMC methodology allowed model-specific hypotheses to be constructed which incorporated virus growth dynamics and overcame these clinical design differences. This method is of general applicability to time-series analysis and/or comparisons where the underlying dynamics are understood. Incorrect dynamic model hypotheses would invalidate the conclusions; however, the method can be extended to also compare possible models (Gelman & Meng 1996).

Our analysis was conclusive in determining that the viraemia time-series of each chronic patient was not consistent with a mutant growth rate as high as that observed in transplant patients (0.12 day⁻¹), $p < 0.05$. This was extended to a population comparison by controlling large growth rates with suitable priors, giving similar but less significant results, $p < 0.10$. We conclude that lamivudine-resistant mutants have significantly higher growth rates in transplant patients compared with non-transplant patients, resulting either from an increase in infection pressure caused by immunotherapy as observed *in vitro* (increased virion productivity; McMillan *et al.* 1995), the reduction in clearance of infected cells because of immunotherapy, or an increase in the number of susceptible cells at transplant. The difference in growth rates is probably significant in explaining the difference in emergence times (tables 1 and 2) and the higher failure rate (30%) in transplant patients over the first year compared with non-transplant patients (14%; Lai *et al.* 1998). This is because the virus growth cycle is faster in transplant patients and therefore failure occurs on a faster time-scale. In confirmation, over a two year time-frame non-transplant patients also have a failure rate of 20–30% (Leung *et al.* 1999).

Our Bayesian analysis could be improved through incorporation of the sequence data. Sequencing indicates that at the time-points at the base of the emergent phase the mutant had a relative frequency of less than 5%. Such information could easily be incorporated into the Markov chain. Improvements in the resolution of the data, and especially of the emerging phase, would be very valuable. However, given the probability of emergence (30% over two years) and distribution of emergence times (mean 570 days, s.d. = 150 days), obtaining three data points on the growth phase will be expensive, difficult and will take two to three years. This justifies the use of powerful statistical parametric tests to maximize the use of existing clinical trials. Use of PCR techniques to detect YMDD mutants at low concentrations (Chayama *et al.* 1998) may allow resolution to be appropriately adjusted in future trials.

APPENDIX A

In this appendix we outline the construction of the Markov chain and its update algorithm. The Markov chain consists of the two steady-state ($\log_e$) viraemias L and H , the time after which the mutant numerically dominates t^* , and the growth rate of the mutant r . The observed viraemia (natural logarithm) η_i at time t_i has mean $y(t_i)$, (1) and standard deviation (measurement error) σ , i.e. $\eta_i \sim N(y(t_i), \sigma)$. The likelihood is given by

$$\mathcal{L} = \text{priors} \times \prod_i \exp\left(-\frac{(\eta_i - y(t_i))^2}{2\sigma^2}\right). \quad (\text{A1})$$

Gibbs algorithms for updating the variables can be expressed in terms of cut and pastes of normal and uniform distributions, i.e. a continuous distribution that has sections that are normal or uniform. Drawing from such distributions requires the probability of being in each section, and then drawing from the appropriate truncated distribution.

To update H , define the boundaries $h_i = L + r(t_i - t^*)$, then

$$\begin{aligned} \pi(H|\cdot) &\propto \exp\left(-\frac{(H - H_0)^2}{2\sigma_H^2}\right) \prod_{H < h_i} \exp\left(-\frac{(\eta_i - H)^2}{2\sigma^2}\right) \\ &\times \prod_{H > h_i} \exp\left(-\frac{(\eta_i - h_i)^2}{2\sigma^2}\right), \end{aligned} \quad (\text{A2})$$

where products over i are restricted to those satisfying $t_i > t^*$. A weak prior $N(H_0, \sigma_H)$ is used, and we further restrict $H > L$. Similarly, to update L , define the boundary $l_i = H - r(t_i - t^*)$ when $t_i > t^*$, then

$$\begin{aligned} \pi(L|\cdot) &\propto \exp\left(-\frac{(L - L_0)^2}{2\sigma_L^2}\right) \prod_{l_i < t^*} \exp\left(-\frac{(\eta_i - L)^2}{2\sigma^2}\right) \\ &\times \prod_{L < l_i} \exp\left(-\frac{(\eta_i - r(t_i - t^*) - L)^2}{2\sigma^2}\right) \prod_{L > l_i} \exp\left(-\frac{(\eta_i - H)^2}{2\sigma^2}\right). \end{aligned} \quad (\text{A3})$$

A weak prior $N(L_0, \sigma_L)$ is used, and we further restrict $L < H$. Because L and H are logarithms there are no positivity restrictions. The distributions $\pi(L|\cdot)$, $\pi(H|\cdot)$

consist of sections of normal distributions with boundaries at l_i and h_i respectively. They are continuous as demonstrated by examination of equations (A2) and (A3).

Define the window $\tau_i = [t_i - (H - L)/r, t_i]$. The conditional for t^* is then

$$\begin{aligned} \pi(t^*|\cdot) &\propto \prod_{t^* > \tau_i} \exp\left(-\frac{(\eta_i - L)^2}{2\sigma^2}\right) \\ &\times \prod_{t^* \in \tau_i} \exp\left(-\frac{(t^* - t_i + (\eta_i - L)/r)^2}{2\sigma^2/r^2}\right) \\ &\times \prod_{t^* < \tau_i} \exp\left(-\frac{(\eta_i - H)^2}{2\sigma^2}\right), \end{aligned} \quad (\text{A4})$$

where we restrict $t^* \in [t_L, t_H]$ by a uniform prior, where t_H and t_L are suitably chosen to safely encircle the mutant exponential growth phase. Here we have used the notation $t^* < \tau_i$ to mean $t^* < t \forall t \in \tau_i$. The distribution $\pi(t^*|\cdot)$ therefore consists of sections of uniform and normal distributions with boundaries at end-points of τ_i , and at t_L, t_H , and is continuous as demonstrated by examination of equation (A4).

These three distributions are sufficient to calculate the distribution of any observation at a given time, for specified growth rate r . Thus the probability of observing an intermediate viraemia on the growth phase can be computed, testing whether the observed value is likely directly from the confidence interval of the PDF.

To calculate the PDF of the growth rate r , an update for r must be determined. Consider the set of observations i with $t_i > t^*$, and define $\rho_i = (H - L)/(t_i - t^*)$. Then the conditional probability is a cut-paste distribution with boundaries at ρ_i given by

$$\begin{aligned} \pi(r|\cdot) &\propto \prod_{r < \rho_i} \exp\left(-\frac{((\eta_i - L)/(t_i - t^*) - r)^2}{2\sigma^2/(t_i - t^*)^2}\right) \\ &\times \prod_{r > \rho_i} \exp\left(-\frac{(\eta_i - H)^2}{2\sigma^2}\right). \end{aligned} \quad (\text{A5})$$

We restrict $r \in [0, r_0]$ through a uniform prior.

Two properties of the PDF for r can be deduced from the likelihood (A1) for the case of two observations that generalize to the general case (fixed L, H). First, for large r the asymptotic form

$$\mathbb{P}(r|\{\eta_i\}) \sim a + \frac{b}{r}, \quad (\text{A6})$$

follows by integration over t^* . Second, if the points lie on the growth phase

$$\mathbb{P}(r, t^*|\{\eta_i\}) \sim \frac{1}{r} \exp - \frac{1}{2} \sum_i (\Delta\eta_i - r\Delta t_i)^2, \quad (\text{A7})$$

where $\Delta\eta_i = \eta_i - \bar{\eta}$, $\bar{\eta}$ being the mean of the observed values, and similarly for Δt_i . Thus the mode for two observations is approximately $(\eta_2 - \eta_1)/(t_2 - t_1)$ as expected, with an approximately Gaussian distribution. The pre-factor of $1/r$ arises because of the parametrization of the regression line as $y = r(t - t^*)$ instead of the more common $y = rt + a$. The latter is unsuitable because the time-region includes the emergence event, and is

therefore conditional on growth occurring from L to H in the interval $[t_L, t_H]$.

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