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# Intermittent treatment of severe influenza

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ABSTRACT

Severe, long-lasting influenza infections are often caused by new strains of the virus. The long duration of these infections leads to an increased opportunity for the emergence of drug resistant mutants. This is particularly problematic since for new strains there is often no vaccine, so drug treatment is the first line of defense. One strategy for trying to minimize drug resistance is to apply drugs periodically. During treatment phases the wild-type virus decreases, but resistant virus might increase; when there is no treatment, wild-type virus will hopefully out-compete the resistant virus, driving down the number of resistant virus. A stochastic model of severe influenza is combined with a model of drug resistance to simulate long-lasting infections and intermittent treatment with two types of antivirals: neuraminidase inhibitors, which block release of virions; and adamantanes, which block replication of virions. Each drug's ability to reduce emergence of drug resistant mutants is investigated. We find that cell regeneration is required for successful implementation of intermittent treatment and that the optimal cycling parameters change with regeneration rate.



## 1. Introduction

The influenza virus causes a potentially fatal illness that appears in both annual seasonal outbreaks and in occasional pandemics. While there are vaccines that can prevent infection, they must be re-formulated for every new strain (Jang and Seong, 2014; Soema et al., 2015), causing a delay in the availability of an adequate vaccine when a new strain of influenza arises. Unfortunately, influenza mutates rapidly (Drake, 1993), causing genetic drift of strains, and can also undergo re-assortment events (Qiao et al., 2014; Westgeest et al., 2014), creating entirely new strains. This means that vaccines are not a good first line of defense against new strains of influenza.

Influenza antivirals are typically effective against a wide variety of strains of influenza (Spanakis et al., 2014), making them a better choice for controlling spread of a new strain of influenza. Unfortunately, the rapid mutation rate of influenza also causes problems with the use of antivirals. Influenza resistance to antivirals arises through a single amino acid mutation (Abed et al., 2005; Baz et al., 2006; Bright et al., 2006; Gubareva et al., 2000), so resistance to antivirals can emerge quickly (Bright et al., 2006; Dharan et al., 2009; Zaraket et al., 2010). There are currently two classes of antivirals used for treatment of influenza. Adamantanes prevent uncoating of the virion after it has entered the cell by blocking the action of the M2 matrix protein (Abed et al., 2005). Unfortunately, resistance to adamantanes in circulating strains is already high (Bright et al., 2006; Dong et al., 2015), limiting its usefulness. Neuraminidase inhibitors prevent release of the virion from the cell by blocking the action of the neuraminidase surface protein (Abed et al., 2002; Gubareva et al., 2000). Most circulating strains are still sensitive to neuraminidase inhibitors (Spanakis et al., 2014), making them the antiviral of choice for pandemic stockpiles.

Given the rapid mutation rate of influenza and the limited number of antivirals available to treat influenza, it is important to investigate treatment strategies that might limit the emergence of resistance during the course of an infection. One strategy used in other infectious diseases is intermittent treatment (de Bree et al., 2017; Goujard et al., 2012). Intermittent treatment involves periodic switching between antiviral treatment and no treatment. If a drug resistant mutation arises during the treatment phase, its replication will not be suppressed by the antiviral, so the drug-resistant virus will multiply. Once treatment is stopped, however, any remaining wild-type virus can also freely multiply, and will hopefully out-compete the drug-resistant strain, driving down the number of drug-resistant virions. If the cycles of treatment and no treatment periods are correctly optimized, then both wild-type and drug-resistant virions can be eradicated (de Bree et al., 2017). Note that this strategy will only work consistently if the drug-resistant strain is less fit than the wild-type strain, which seems to be the

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case for at least some influenza drug-resistant mutations (Abed et al., 2016; Baek et al., 2015; Burnham et al., 2015; Butler et al., 2014; Paradis et al., 2015; Pascua et al., 2016).

While drug resistance can emerge during the course of a typical short duration seasonal infection (Dobrovolny and Beauchemin, 2017; Perelson et al., 2012), there is little time for it to be transmitted to other people. The bigger concern for transmission and spread of drug-resistant influenza is long-lasting, severe infections which allow for shedding of drug resistant influenza over several weeks or even months (Bruminhent et al., 2014; Eshaghi et al., 2014; Ghedin et al., 2012; Hurt et al., 2013). While severe influenza infections are long compared to seasonal infections, they are still much shorter than human immunodeficiency virus (HIV) or hepatitis B virus (HBV) infections, which sometimes use intermittent treatment, and offer more limited choices for the length of the treatment on and treatment off periods.

In this paper, we study the emergence of drug resistance during severe infections by combining two models of within-host influenza, one which models severe infections (Dobrovolny et al., 2010) and one which models the emergence of drug resistance (Dobrovolny and Beauchemin, 2017). We apply intermittent treatment to the model via a switching function that either applies a constant drug treatment, or leaves the system untreated. We find that cell regeneration is critical for intermittent treatment to work and that when cell regeneration is fast enough, the periodicity of switching between treatment and no treatment phases does not affect the effectiveness of the treatment.

## 2. Methods

### 2.1. Modeling influenza infections

To capture the dynamics of severe influenza infections, the single cell population differential equation model with delayed viral production, as proposed in Baccam et al. (2006), was extended to a two target cell model in Dobrovolny et al. (2010). In terms of the nomenclature, we separated the two target cells into default (subscript d) and secondary (subscript s) cells with each containing a wild-type (subscript wt) and a mutant (subscript $\mu$) subpopulation. In the model, the default cells represent the preferred target for human influenza, while the secondary population represents cells that can be infected by human influenza, but with more difficulty. The key parameters that control the differences between the two cell populations are the relative susceptibility to infection ($r_\beta \in \mathbb{R}^+$), the relative viral production rate ($r_p \in \mathbb{R}^+$) and the fraction of initial secondary target cells ($r_\mathrm{T} \in [0, 1]$). Initial allocation of a secondary cell population is a crucial step that makes our model capable of reproducing the dynamics of long-lasting influenza infections. Note that $r_\mathrm{T}$ only appears in the initial conditions and therefore does not explicitly appear in the system of differential equations.

The initial amount of wild-type virus ($V_\mathrm{wt}$) and mutant virus ($V_\mu$) proceed to infect primary target cells ($T_\mathrm{d}$) at rate $\beta$ and secondary target cells ($T_\mathrm{s}$) at rate $r_\beta\beta$. Once infected, cells migrate into their eclipse phase ($E$), where they are producing viral proteins and RNA, but not yet releasing new virus, and then turn into productively infected cells $I$ at rates $\tau_\mathrm{E}^{-1}$ and $\tau_\mathrm{I}^{-1}$, respectively. There are four distinct types of cells: any combination of default or secondary with wild-type or mutant are possible. Once primary (secondary) target cells have reached their productive stage, they will produce virus at rate $p$ ($r_p p$) while slowly dying off at rate $c$. When target cells die they accumulate as $D$, from which they may regenerate back to available target cells $T$ at rate $\ell$.

An infection is medicated with drugs of two types. Drugs based on adamantanes prevent the virus from infecting available target cells and the drug's efficacy on wild-type and mutant strains is controlled via parameters $m_\mathrm{wt}$ and $m_\mu$. Neuraminidase inhibitor based drugs (NAI) do not prevent cell infection but prevent production of new virions. The drug's efficacy is controlled by the parameters $n_\mathrm{wt}$ and $n_\mu$. All efficacies assume values between 0 and 1 and represent the relative reduction in infection rate (for adamantanes) or production rate (for NAIs) caused by the antiviral. We make the assumption that the efficacy remains constant during treatment, even though antivirals are taken as pills which causes a time-varying drug concentration. Recent work has shown that the assumption of constant drug efficacy adequately approximates time-varying drugs (Palmer et al., 2017).

We additionally would like to allow the mutation of each virus entity from its wild-type into a drug-resistant strain (and vice versa). In the model, this is incorporated via the choice of mutation rate $\mu_\mathrm{nt}$ that fixes the probability with which either virus type will mutate. A number of different mutations have been reported for adamantanes (Abed et al., 2005; Bright et al., 2006; Hay, 1996; Hayden, 1996) and NAIs (Baz et al., 2006; Gubareva et al., 2000), but in our model we restrict ourselves to the most common type of mutation (S31N in the M2 protein for amantadines and H275Y in the N1 protein for the NAI-based drug oseltamivir) and assume that they occur at the average mutation rate of influenza A, namely $\mu_\mathrm{nt} = 7.3 \times 10^{-5}$ per nucleotide per replication (Drake, 1993).

The model resulting from these contemplations is a system comprised of 14 differential equations. These can be compacted by means of an index $j$ that assumes a wildtype or mutant stance,

$$\dot{\boldsymbol{T}} = \begin{pmatrix} \dot{T}_\mathrm{d} \\ \dot{T}_\mathrm{s} \end{pmatrix} = -\left(\beta_\mathrm{wt} V_\mathrm{wt} + \beta_\mu V_\mu\right) \begin{pmatrix} 1 & 0 \\ 0 & r_\beta \end{pmatrix} \boldsymbol{T} + \ell \boldsymbol{D} \tag{1a}$$

$$\dot{\boldsymbol{E}}_j = \begin{pmatrix} \dot{E}^j_\mathrm{d} \\ \dot{E}^j_\mathrm{s} \end{pmatrix} = (1 - m_j)\beta_j V_j \begin{pmatrix} 1 & 0 \\ 0 & r_\beta \end{pmatrix} \boldsymbol{T} - \tau_E^{-1} \boldsymbol{E}_j \tag{1b}$$

$$\dot{\boldsymbol{I}}_j = \begin{pmatrix} \dot{I}^j_\mathrm{d} \\ \dot{I}^j_\mathrm{s} \end{pmatrix} = \tau_E^{-1} \boldsymbol{E}_j - \tau_I^{-1} \boldsymbol{I}_j \tag{1c}$$

$$\dot{V}_j = (1 - n_j) p_j \begin{pmatrix} 1 - \mu_\mathrm{nt} \\ r_p - r_p \mu_\mathrm{nt} \\ \mu_\mathrm{nt} \\ r_p \mu_\mathrm{nt} \end{pmatrix} \cdot \begin{pmatrix} \boldsymbol{I}_\mathrm{wt} \\ \boldsymbol{I}_\mu \end{pmatrix} - c V_j \tag{1d}$$

$$\dot{\boldsymbol{D}} = \begin{pmatrix} \dot{D}_\mathrm{d} \\ \dot{D}_\mathrm{s} \end{pmatrix} = \tau^{-1}\left(\boldsymbol{I}_\mathrm{wt} + \boldsymbol{I}_\mu\right) - \ell \boldsymbol{D}. \tag{1e}$$

The different compartments of the model and their interactions are shown in Fig. 1. In the absence of cell regeneration, this is a target cell limited model where the infection terminates when all target cells have been infected. This does not equate to death of the patient however, since not all cells in the respiratory tract are target cells for influenza (Chan et al., 2013; Hui et al., 2017). We include cell regeneration as proportional to the number of dead cells which represents stimulation of reproduction by cell death (Beers and Morrisey, 2011). Since the two target cells of the model represent two different types of cells, we assume that death of default cells stimulates regeneration of default cells and death of secondary cells stimulates regeneration of secondary cells.

All of the differential equations that describe our model are fully deterministic and can be solved by choice of a stable integration method. In doing so, the observables will assume non-discrete values (along the positive real axis), which is a behavior that we would like to restrict, due to the discrete nature of the underlying cell model. ODEs produce the mean-field dynamics and are not representative of the course of the infection in a single patient. Some patients will clear the wild-type virus before a drug resistant mutant appears and not have any infection at all. In other patients,

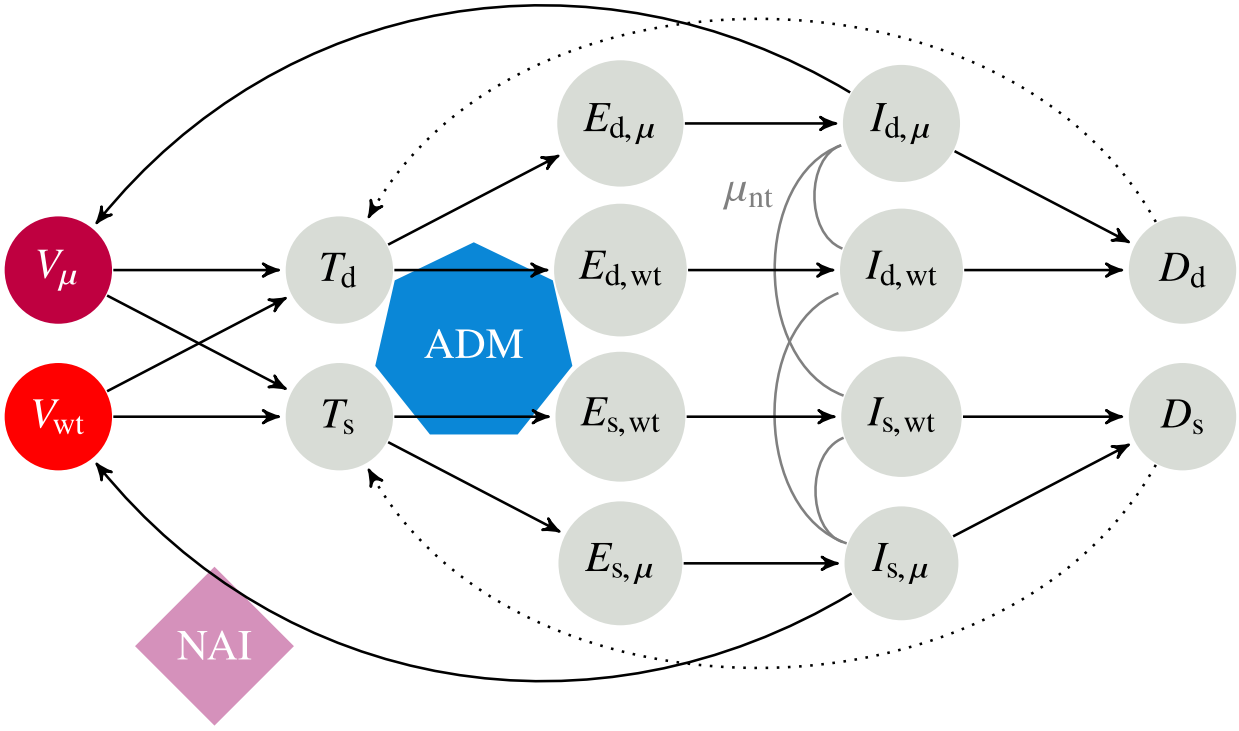


**Fig. 1.** An illustration of the cell model used to study severe influenza infections, as described in Eqs. (1a)–(1e). When infected, target cells enter an intermediate eclipse phase. Once fully infected, they start to produce new virus, then die out eventually. Depending on the choice of regeneration rate dead cells may come back as target cells, which is indicated by their dotted connection. An adamantane drug stops cells from entering into the eclipse phase altogether, while NAI-based drugs hinder the production of new virus. Since we allow virus cells to mutate (which they will do at rate $\mu_{nt}$), infected cells may produce both wild-type and mutant virus, indicated by the connections between infected subpopulations.

the drug resistance mutation appears and an infection results. Use of the ODE will result in an infection every time since ODEs allow fractions of virus to infect fractions of cells, when in reality we need at least one virion to infect one cell in order for the infection to proceed. To ensure this, we corrected the result of each timestep via implementation of the Euler-Maruyama method (Kloeden and Platen, 1992).

### 2.2. Model parameters

Throughout simulations, the initial number of overall target cells was set to $T_0 = 4 \times 10^8$ cells, which coincides with anatomical estimates for the human upper respiratory tract (Baccam et al., 2006). We assumed that the initial viral inoculum consists entirely of wild-type virus. We also fixed $r_T$ at 70%, as in (Dobrovolny et al., 2010), which was based on estimates of the proportion of ciliated to non-ciliated cells in the human lung. The remaining parameters are fixed to values determined in Baccam et al. (2006) from model fits to patient data. Default parameters used in the simulations are listed in Table 1.

The two target cell population model is able to capture both severe and seasonal infections, depending on the choice of parameters $r_p$ and $r_\beta$. In a recent work, the parameter space of this model was studied in detail for the specific parameters that are able to induce a severe influenza infection (Dobrovolny et al., 2010). Since we are interested in modeling a long-lasting infection, we chose $(r_\beta, r_p) = (10^{-4}, 2 \times 10^3)$. For a comparison of a severe influenza infection and a common infection in the absence of cell regeneration as they evolve over the days post infection (dpi), see Fig. 2.

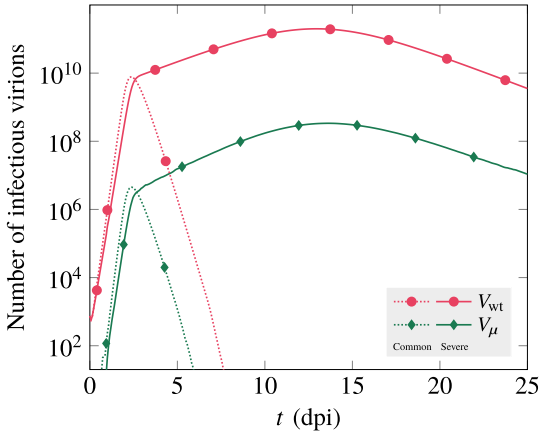


**Fig. 2.** Shown are wild-type (circles) and mutant (diamonds) virus curves for a common (dotted) and a severe (solid) influenza infection. In the common infection, the virus is at peak value after about 3 dpi and is then quickly cleared from the system. Severe infections are inherently different: the virus arrives at the same high levels early on but then continues to rise from there and even after as many as 25 dpi the infection has not withdrawn. The severe infection uses values of $r_p$ and $r_\beta$ given in Table 1, while the seasonal infection uses the same $r_\beta$, but uses $r_p = 10^2$.

### 2.3. Relative fitness

To study the efficacy of an adamantane-based drug on different types of cell compositions, we introduced a parameter that characterizes the relative fitness $\beta_{fit}$ between mutant and wild-type infection rates. When studying the efficacy of a NAI-based drug (that blocks the release of viral particles), we varied instead how the production rate of mutant and wild-type virus compare (denoted

**Table 1**
Initial conditions and parameter values of the model. Viral titer amounts are measured in units of $[V] = \mathrm{TCID}_{50}/mL$. Parameter values are from Baccam et al. (2006), except for [a], [b], and [c] which are from Dobrovolny et al. (2010), Drake (1993), and Beers and Morrisey (2011), respectively.

| Symbol | Description | Default value |
|---|---|---|
| $T_0$ | Total number of target cells | $4 \times 10^8$ |
| $(E_0, I_0, D_0)$ | Initially eclipsed, infected, dead cells | (0, 0, 0) |
| $V_0$ | Initial viral inoculum | $7.5 \times 10^{-2}[V]$ |
| $\tau_E$ | Length of eclipse phase | $6\,h$ |
| $\tau_I$ | Length of virus production phase | $4.6\,h$ |
| $c$ | Virus clearance rate | $1/4.6\,h^{-1}$ |
| $\beta_{\mathrm{d,wt}}$ | Cell infection rate (default, wild-type) | $3.2 \times 10^{-5}[V]^{-1} \cdot d^{-1}$ |
| $p_{\mathrm{d,wt}}$ | Virus production rate (default, wild-type) | $4.6 \times 10^{-2}[V] \cdot d^{-1}$ |
| $r_\beta$ [a] | Ratio of secondary to default $\beta$ | $10^{-4}$ |
| $r_p$ [a] | Ratio of secondary to default $p$ | $2 \times 10^3$ |
| $\mu_{\mathrm{nt}}$ [b] | Mutation rate | $7.3 \times 10^{-5}$ |
| $\ell$ | Cell regeneration rate | $0.03\,d^{-1}$ |

by $p_{\mathrm{fit}}$).

$$\beta_{\mathrm{fit}} = \frac{\beta_\mu}{\beta_{\mathrm{wt}}}, \qquad p_{\mathrm{fit}} = \frac{p_\mu}{p_{\mathrm{wt}}}. \tag{1}$$

Experimental measurement of viral fitness is not yet well-defined (Wargo and Kurath, 2012), and experimental measurements of relative fitness are not based on the definition of fitness used here (Wu et al., 2006), so there are no good estimates for relative fitness. Further complicating matters, relative fitness for a specific drug-resistance mutation seems to depend on the strain in which it occurs (Butler et al., 2014; Paradis et al., 2015; Pinilla et al., 2012) and experiments sometimes give contradictory results on whether a particular mutation increases or decreases fitness (Brookes et al., 2011; Herlocher et al., 2004; Ives et al., 2002; Paradis et al., 2015; Pinilla et al., 2012; Wong et al., 2012). Additional compensatory mutations will also alter the fitness of the mutant virus (Bloom et al., 2010; Govorkova et al., 2010). For intermittent treatment to work, the fitness of the mutant must be less than that of the wild-type, but large enough to cause some breakthrough infections. Without experimental guidance as to an exact value, and based on our simulation results, we decided to use a relative fitness of 0.5.

### 2.4. Intermittent treatment

We apply intermittent treatment through a switching function that changes the value of $m_j$ for adamantanes or $n_j$ for NAIs from zero (no treatment) to some fixed non-zero value (treatment). The duration of treatment and no treatment phases is governed by the parameters $t_{\mathrm{on}}$ and $t_{\mathrm{off}}$, respectively. Simulations start with either $m_j$ or $n_j$ non-zero for a duration of $t_{\mathrm{on}}$, followed by both $m_j = n_j = 0$ for a duration of $t_{\mathrm{off}}$. This switching scheme with fixed $t_{\mathrm{on}}$ and $t_{\mathrm{off}}$ is then continued for the remainder of the simulation. Note that our assumption that drug treatment starts at $t = 0$ models post-exposure prophylaxis.

## 3. Results

### 3.1. Drug resistance during a severe infection

We first used our model to examine the dynamics of drug resistance during traditional continuous treatment. We assume that antiviral treatment starts at the same time as the infection ($t = 0$), immediately affecting viral replication in all cell types, and remains constant over its entire duration. As a measure of the likelihood of an infection impairing its host, we simulated an ensemble of 1000 identical cell models and monitored the number in which the virus exceeded the symptomatic threshold, fixed at a value of $10^4[V]$ (Dobrovolny et al., 2010). Clinically, these are known as breakthrough infections, since patients get sick despite undergoing prophylactic treatment. The measurements were repeated for both adamantane- and NAI-based drugs with varying efficacies, allowing us to identify similar general trends that hold regardless of the precise potency of the drug (Fig. 3). For both drugs, the relative fitness has to surpass a threshold value for a finite probability of breakthrough infections. For a given relative fitness, adamantanes are more effective at preventing breakthrough infections. Unfortunately, adamantane resistance is already widespread (Bright et al., 2006; Dong et al., 2015), but this would also apply to new influenza antivirals that target the entry phase of the infection (Lin et al., 2017). These results are similar to previous modeling results using a seasonal influenza model (Dobrovolny and Beauchemin, 2017).

Not only is it important to examine how many drug-resistant mutants are present during an infection, but we are also interested in understanding how quickly they can be detected. We expect the virus levels in an infection to surpass detection levels (which we fixed at 10 [V] (Dobrovolny and Beauchemin, 2017)) quicker if the mutant virus were to be fitter than the wild-type virus. We studied this by monitoring the times of detection ($t_{\mathrm{det}}$) as a function of $\beta_{\mathrm{fit}}$ and $p_{\mathrm{fit}}$, respectively. From Fig. 3 we observe that higher efficacies result in a suppression of the development of the infection, making times of detection become greater. In addition, when the efficacy approaches zero, the measured times of detection stop fluctuating around their mean value.

### 3.2. Intermittent treatment of severe infections without cell regeneration

It is customary to neglect cell regeneration in influenza models since the infection is short compared to typical cell regeneration times (Crosby and Waters, 2010). Severe infections last longer, but as this might still be a valid assumption, we first apply intermittent treatment to a model with $\ell = 0$, as described in the Methods. Examples of infection curves that result from different choices of the parameters that control the periodic switching between treatment phases are shown in Fig. 4. For adamantanes, the initial drug treatment period keeps wild-type virus from growing, but fails to completely eliminate it, so that viral titer quickly increases once treatment is turned off. At some point, the wild-type virus produces a drug-resistant mutant which continues to thrive once treatment is resumed. For treatment with NAIs, we see a similar trend, although in this case, virus grows slowly even during antiviral treatment. In all cases, for both antivirals, treatment eliminates wild-type virus and allows the mutant virus to thrive, resulting in a severe infection with a drug-resistant strain. Since there is no cell regeneration in the model depicted, the wild-type virus does not have any cells left to infect in order to out-compete the drug resistant virus whenever the treatment is paused. Therefore, the intermittent treatment is not able to suppress the infection in these examples.

### 3.3. Intermittent treatment with cell regeneration

Since cell regeneration appears to be crucial for the success of intermittent treatment, we vary $\ell$ which controls the regeneration of dead cells. Once cells die, new target cells are generated to take their place. For different choices of regeneration rates, we monitored the total infection time (TIT), i.e. the total time the virus population was larger than the symptomatic threshold of $10^4$ $[V]$ viral particles (Dobrovolny et al., 2010). If intermittent treatment works effectively, the total infection time for a treated infection should be shorter than the total infection time for an untreated infection. An untreated infection using these parameters has a symp-

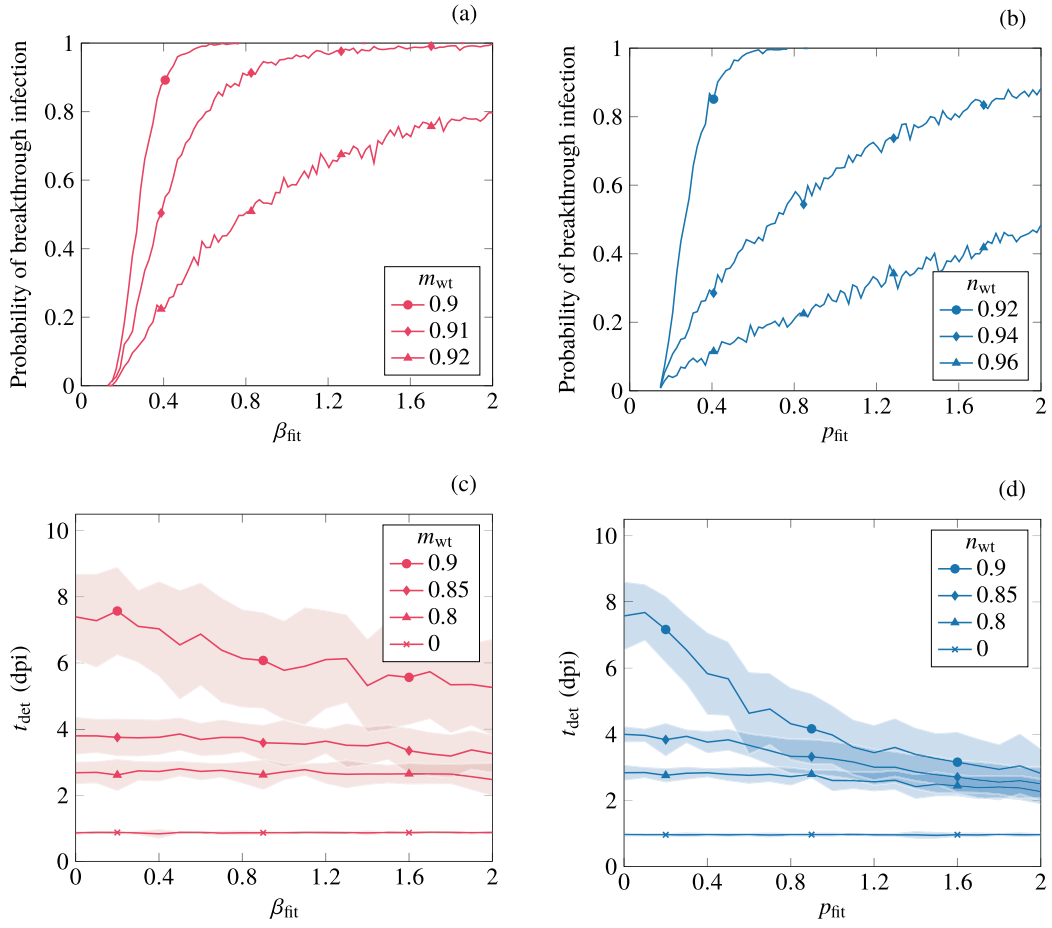


**Fig. 3.** Shown in (a) is how likely an adamantane-based drug is to suppress an infection, for a varying ratio between the wild-type and mutant virus' abilities to infect available target $\beta_{\text{fit}}$ for a variety of drug efficacies $m_{\text{wt}}$. In (b) the suppressing behavior of an NAI-based drug for varied virus production rates in mutant and wild-type virus populations $p_{\text{fit}}$ is shown for different drug efficacies $n_{\text{wt}}$. In (c), times of detection $t_{\text{det}}$ are plotted against changing values of $\beta_{\text{fit}}$ while an adamantane-based treatment is in effect. Similarly, in (d) the times of detection are evaluated for a given $p_{\text{fit}}$, while an NAI-based drug is being applied. The shaded areas that surround measurements indicate the standard deviation over all realizations.

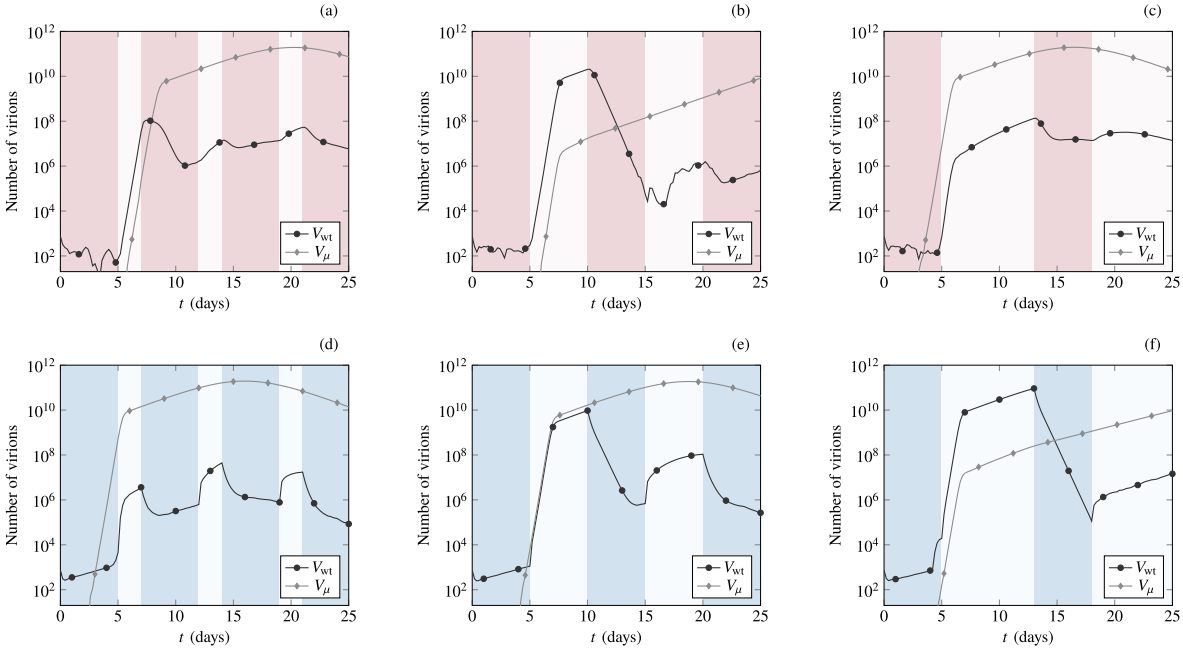


**Fig. 4.** Shown are examples of how wild-type (circles) and mutant (diamonds) influenza virus populations develop stochastically under intermittent treatment in the absence of regeneration. In (a), (b) and (c) an adamantane is applied with an efficacy of $m_{\text{wt}} = 0.94$. Treatment in (d), (e) and (f) is NAI-based, with an efficacy of $n_{\text{wt}} = 0.92$. The switching of treatment is highlighted, in dark regions drugs are being applied, in light regions treatment is paused. From left to right, the switching period is changed gradually and $t_{\text{off}}$ is increased from 2 to 5 and up to 8 d while treatment durations are kept constant at $t_{\text{on}} = 5$ d.

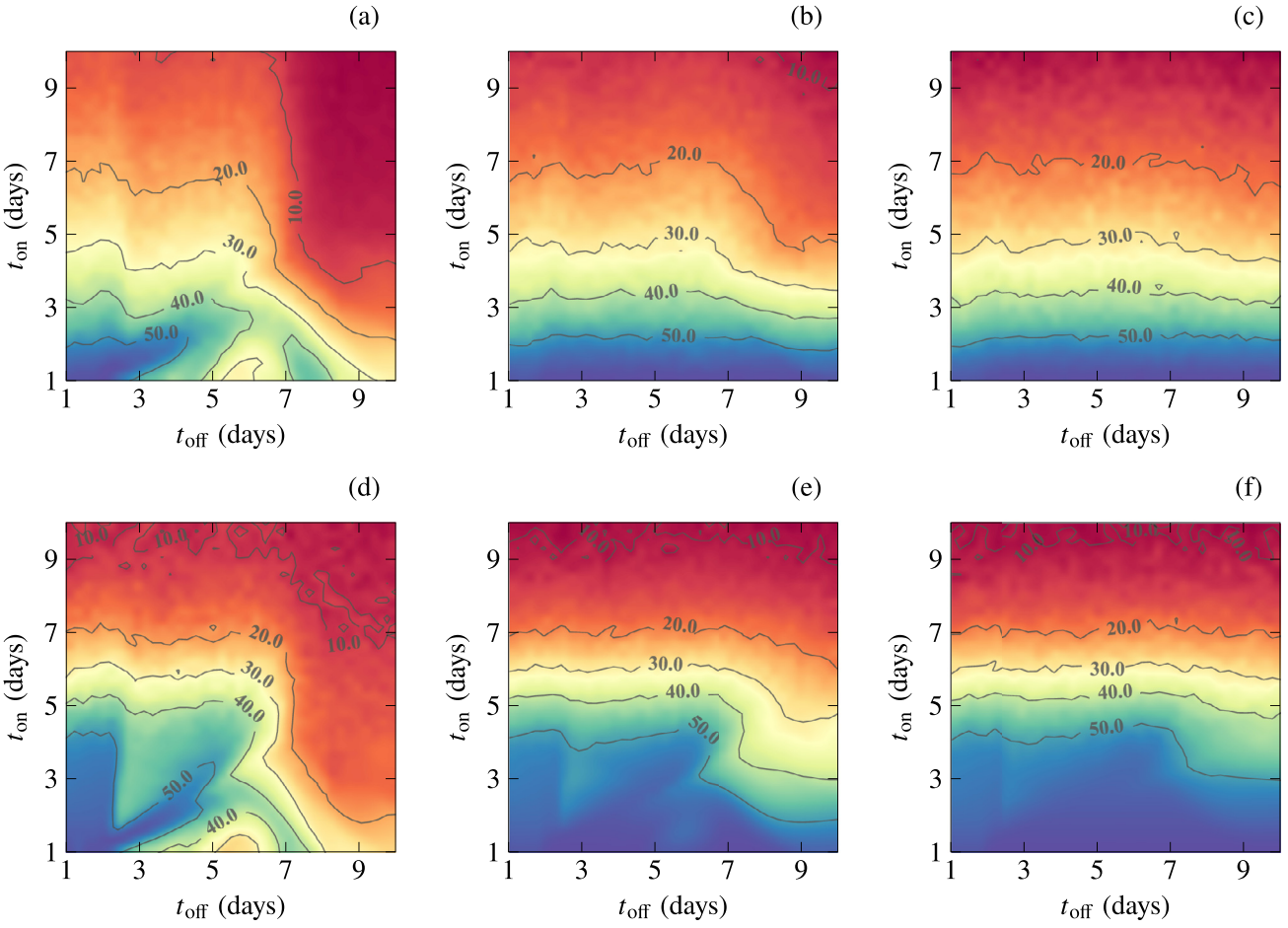


**Fig. 5.** Mean total infection times of a thousand ensembles for adamantane-based treatment (top row) and NAI-based treatments (bottom row). Periodic drug application was varied in terms of how long it was paused ($t_{off}$) and applied ($t_{on}$). Regeneration rates increase from left to right. In (a) and (d) $\ell = 160^{-1}$ d$^{-1}$, in (b) and (e) $\ell = 40^{-1}$ d$^{-1}$, in (c) and (f) $\ell = 20^{-1}$ d$^{-1}$.

tomatic duration of about 56 d. We examined a variety of pairings of $(t_{on}, t_{off})$, with both variables in the range 1–10 d. Example results are shown in Fig. 5. Note that infections generally did not dip below the threshold and rebound as treatment was turned on and off since as the amount of one type of virus (wild-type or mutant) decreased, the amount of the other would increase such that the total amount of virus remained above the threshold until final resolution of the infection. Thus the TIT is typically a continuous period of time, although it takes, on average, about 1.5 d after the start of the infection to reach the symptomatic threshold.

When regeneration is included, there are many combinations of $(t_{on}, t_{off})$ that will lead to shorter infections. As the regeneration rate increases, total infection time shows a decreasing dependence on $t_{off}$, particularly for adamantanes, and total infection time is largely a function of $t_{on}$. If there is a sufficiently high regeneration rate, then the wild-type virus has enough available target cells to rise to levels higher than the mutant virus within a one day $t_{off}$ and longer $t_{off}$ will not affect the final outcome. When regeneration is low, levels of wild-type virus rise more slowly and if $t_{off}$ is too short, wild-type virus will not rise to levels high enough to drive down the levels of drug-resistant, so multiple on-off cycles might be needed to eliminate the drug resistant virus. Thus for a particular $t_{on}$, the total infection time will increase when $t_{off}$ is short. In Fig. 5(a) and (d), we see that there is a steep increase in the total infection time at $t_{off} \sim 7$–8 d which indicates the $t_{off}$ long enough to allow wild-type virus to surpass the drug resistant mutant during the first treatment cycle. The shortest infections occur when treatment periods are long. We also examined the fraction of drug resistant mutants in the viral titer above the symptomatic threshold, finding that intermittent treatment was effective at reducing the amount of drug resistant mutants in the viral titer. The model predicts that the fraction of drug resistant mutants falls to below 1% once $t_{off}$ is greater than 2 d.

### 3.4. Variability of infection times

In order to capture in a single number the variability in total infection times as we vary treatment schemes, we look at how strongly the average of the total infection time (for a fixed $t_{on}$) varies when we change the duration for which the drug is paused ($t_{off}$). From this motivation, we compute the unbiased estimator of the variance, where we explicitly denote the remaining dependence on $t_{on}$, as that has not been averaged out:

$$s^2(t_{on}) = \frac{1}{N-1} \sum_{\{t_{off}\}} \left[ \mathrm{TIT}_\ell(t_{on}, t_{off}) - \overline{\mathrm{TIT}}_\ell(t_{on}) \right]^2,$$

where $\mathrm{TIT}_\ell(t_{on}, t_{off})$ is the TIT for a specific combination of $(t_{on}, t_{off})$, $\overline{\mathrm{TIT}}_\ell(t_{on})$ is the mean TIT for a particular $t_{on}$ averaged over values of $t_{off}$, and $N$ is the number of different $t_{off}$ values examined. From these quantities we can estimate the coefficient of variation for a given regeneration rate $\ell$ and $t_{on}$:

$$c_{v,\ell}(t_{on}) = \frac{\sqrt{s^2(t_{on})}}{\overline{\mathrm{TIT}}_\ell(t_{on})}$$

The coefficient of variation provides us with a natural measure of the amount of influence that the variation of $t_{off}$ has. Thus, the

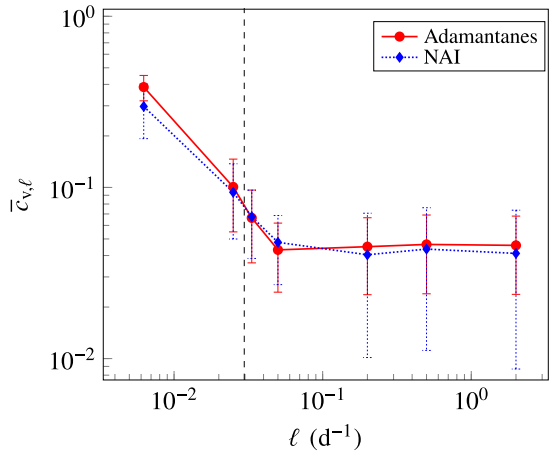


**Fig. 6.** The coefficients of variation for different $\ell$ are shown for adamantane (solid, circles) and NAI (dotted, diamonds). The higher the value of the coefficient of variation, the more susceptible the virus population is to periodic applications of the drug. For small regeneration rates, intermittent treatment becomes effective and switching between treatment and no treatment induces changes in the total infection time.

larger $c_{v,\ell}(t_{on})$, the more susceptible we assume the infection to be to variations in the periodic treatment scheme. When we are only interested in studying the coefficient of variation as a function of the regeneration rate, we can compute $\bar{c}_{v,\ell}$ by further averaging over all $t_{on}$. If this quantity is large, then the system is sensitive to the choice of $t_{on}$ and $t_{off}$; if the parameter is small, then all choices of $t_{on}$ and $t_{off}$ will shorten the infection by the same amount. For both adamantanes and NAIs, we observe similar behavior of $\bar{c}_{v,\ell}$ as a function of regeneration rate (Fig. 6). The regeneration rate of epithelial cells in the healthy lung is estimated at 0.03 $d^{-1}$ (Beauchemin and Handel, 2011; Beers and Morrisey, 2011), however, for the model, we are interested in the regeneration rate of cells that can participate in the infection. Cells only become target cells when they have the surface receptors that virus can use to bind to and enter the cell. Since surface receptors are a property of terminally differentiated cells (Crystal and West, 1991), we do expect this regeneration rate to be lower than the cited estimate. This places human infection in the regime where choices of $t_{on}$ and $t_{off}$ are significant. This means that the efficacy of treatment will depend on the choice of $t_{on}$ and $t_{off}$, with our simulation results suggesting that values of $t_{off}$ greater than 2 d will greatly reduce the amount of drug resistant virus and that longer values of $t_{off}$ help reduce TIT.

## 4. Discussion

By means of a combination of two mathematical models we captured the stochastic dynamics of severe influenza infections that can arise in hosts through an interaction between populations of mutant and wild-type virus. When the infection is caused by a new strain of the virus often no vaccine exists, and drugs (such as amantadine and neuraminidase inhibitors that were studied here) are used to hinder the infection from spreading. We showed that intermittent treatment can be used successfully to aid the efficacy of these drugs and help prevent the emergence of drug resistance when faced with long lasting instances of the infection. We looked at the dependence of the total infection time on the two parameters of intermittent treatment, $t_{on}$ and $t_{off}$, and found that a range of combinations of these parameters were effective in shortening the duration of the infection. We proposed a simple measure to capture the relative changes in the total infection time that are induced by variations in the intermittent treatment parameters, the averaged coefficient of variation. We found that $\bar{c}_{v,\ell}$ depends on the regeneration rate of host cells, with slow regeneration being particularly sensitive to the choice of $t_{on}$ and $t_{off}$.

Our findings suggest that intermittent treatment is a possible strategy for shortening the duration of severe influenza infections and that it can substantially limit the amount of drug resistant virus, although there are some practical difficulties in implementing the protocol. There is the obvious issue of optimizing intermittent treatment parameters, which may vary from patient to patient. Our study specifically shows that the combinations of $t_{on}$ and $t_{off}$ that reduce the duration of the infection are altered by cell regeneration rates, which are known to change over time due to aging (Paxson et al., 2011). There is also some error in the estimated human regeneration rate, since the only estimate we could find is the average turnover rate for healthy epithelial cells (Beauchemin and Handel, 2011; Beers and Morrisey, 2011). It is possible that during an infection this rate could be different. Additionally, the quoted regeneration rate is based on the time for a new cell to appear; the cell receptors necessary for participation in the infection are a feature of terminally differentiated cells and will lead to an additional lengthening of the time for regeneration. There is also the problem of delaying the onset of treatment. Our implementation of the drug is equivalent to post-exposure prophylaxis, but this will not be possible for most individuals. For a typical uncomplicated infection that lasts about 7 days (Beauchemin and Handel, 2011), it does not make sense to apply intermittent therapy since the infection duration is too short. In many cases, however, physicians can't tell in the first few days of an infection whether it is uncomplicated or will turn into a severe infection. The latter cases are unlikely to be identified until 5–7 days into the infection, at which point intermittent therapy can be applied. This investigation assumed treatment started at the onset of the infection, so further investigation is needed to determine if delayed start of intermittent treatment will be as effective and whether optimal treatment parameters are dependent on the time delay.

Since our model suggests that intermittent treatment could be beneficial for severe influenza, it should also be seriously considered for chronic viral infections such as HIV or hepatitis. There has been some clinical investigation of intermittent treatment for HIV (Lundgren et al., 2008; Martinez-Picado et al., 2003) or of treatment interruptions (Fox et al., 2008; Serwanga et al., 2011; Walker, 2008) and modeling studies of drug resistance emergence during drug holidays (a single interruption of treatment) for HIV (Luo et al., 2011). However, there are other differences between HIV and hepatitis and influenza. For example, both HIV and hepatitis are thought to have reservoirs of infected cells, in the form of latently infected cells for HIV (Chun et al., 1997) and extrahepatic replication sites for hepatitis (Revie and Salahuddin, 2011), that can continue or re-initiate an infection that appears to have been cleared. There is also evidence that in both HIV and hepatitis infected cells can replicate (Dahari et al., 2005; Maldarelli et al., 2014), so our model assumption of only uninfected target cells regenerating is violated. Intermittent treatment might allow viral load to rise to levels that are easily transmissible for short durations even if viral load is eventually controlled (Hamlyn et al., 2012). Finally, intermittent treatment schedules are more complicated than continuous treatment, possibly affecting patient compliance with the treatment regimen (Guy et al., 2013).

Our study found that there are some slight dynamical differences in the effect of the two antivirals. We observed that adamantanes generally shortened infections more than NAIs when $t_{on}$ was short, but that NAIs were more effective than adamantanes when $t_{on}$ was long. A previous modeling study also noted differences in the emergence of drug resistant mutants during a seasonal in-

fection under treatment with the two antivirals (Dobrovolny and Beauchemin, 2017). These arise because adamantanes prevent infection of the cell, blocking the infection cycle at its start, whereas NAIs block release, allowing cells to become infected and possibly creating a drug resistant mutant that is then unaffected by the drug blocking viral release. This model of intermittent treatment is an example of a non-linear switched system (Liberzon and Morse, 1999) where we have applied the switching function to two different parameters for the modeling of two different antiviral treatments. There are other systems in which switching can be applied to different parameters, such as parasite control (Xiang et al., 2014; Xiang et al., 2016) and microbial fermentation (Hu et al., 2016). Studies of these systems also suggest that control of the systems is possible with switching on different parameters, although the optimal switching parameters vary under different control schemes. Although there are slight differences in the dynamics, the dependence of $\bar{c}_{v,\ell}$ on regeneration rate is strikingly similar for both drugs, suggesting that the importance of regeneration in driving dynamics during intermittent treatment might be independent of antiviral mechanism of action.

The model used in this study has limitations. It does not include an explicit immune response, which plays a role in terminating influenza infections (Beauchemin and Handel, 2011; Dobrovolny et al., 2013), and can help control the emergence of drug resistance (Canini et al., 2014; Dobrovolny and Beauchemin, 2017). It is possible, then, that intermittent treatment would not be necessary in immunocompetent patients, but would be more useful in immunocompromised patients. Since the model parameters are derived from infections in healthy adults (Baccam et al., 2006), parameter values implicitly reflect the contribution of an immune response. However, it would be informative to explicitly examine the role of different immune components in controlling the emergence of drug resistant mutants. Our model also assumes exponential transitions between cell states which is known to be biologically unrealistic (Beauchemin et al., 2017; Holder and Beauchemin, 2011; Kakizoe et al., 2015), although the effect of this assumption is most strongly observed in single cycle experiments, and not in multiple cycle infections such as the ones studied here (Holder and Beauchemin, 2011). We limited this study to a specific value of mutant fitness. In reality, drug-resistance mutations in different backgrounds, or drug-resistance mutations at different sites, will result in different mutant fitness (Butler et al., 2014; Holder and Beauchemin, 2011; Paradis et al., 2015; Pinilla et al., 2012). Additionally, mutant fitness could evolve (Kryazhimskiy et al., 2011; 2009) as other mutations accumulate (Bloom et al., 2010; Govorkova et al., 2010). This means that different strains of influenza will respond differently to intermittent treatment with the response possibly changing over the course of the infection. While this could be captured by varying the relative fitness parameter of the model, this study was limited to examining the role of cell regeneration. We also used a specific value of the mutation rate, although estimates of the mutation rate vary (Nobusawa and Sato, 2006; Sanjuan et al., 2010). Mutation rate, like fitness of the virus, is a model parameter that should be explored in more detail, but is not the focus of this paper. A slower mutation rate will make it less likely for mutants to emerge, but once they have emerged, the number of mutants is driven by their replication rate rather than by the mutation rate. This means that the mutation rate is unlikely to affect the efficacy of intermittent treatment. These shortcomings should not, however, affect our findings on the importance of cell regeneration in enabling intermittent treatment.

The model predictions presented here suggest that intermittent treatment deserves further investigation, though possibly not application to patients or clinical trials at this point. Not all of the parameters of the model have been investigated yet, relative fitness of the mutant virus being a particularly important parameter. Additionally, the model has not been parameterized or validated with human data, which is an important step in building confidence in the model predictions. We are also limited by a lack of knowledge of some of the important parameters. For example, viral fitness is still ill-defined and difficult to measure (Wargo and Kurath, 2012; Wu et al., 2006), making it difficult to decide which strains of influenza should be considered for intermittent treatment. While cell regeneration rate was shown to vary with age (Paxson et al., 2011), the study was done in mice, and similar studies have not been done in humans, so we don't know the range of possible cell regeneration rates in humans. Thus, it is not clear whether the variation in regeneration rate will limit who can be considered for treatment.

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