COMBINATION VERSUS SEQUENTIAL MONOTHERAPY IN CHRONIC HBV INFECTION: A MATHEMATICAL APPROACH

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Abstract. Sequential monotherapy is the most widely used therapeutic approach in the treatment of HBV chronic infection. Unfortunately, under therapy, in some patients the hepatitis virus mutates and gives rise to variants which are drug resistant. We wonder whether those patients would have benefited from the choice of combination therapy instead of sequential monotherapy. To study the action of these two therapeutic approaches and to explain the emergence of drug resistance, we propose a stochastic model for the infection within a patient who is treated with two drugs, either sequentially or contemporaneously, and who, under the first kind of therapy develops a strain of the virus which is resistant to both drugs. Our stochastic model has a deterministic approximation which is a slight modification of a classic 3-strain model. We discuss why stochastic simulations are more suitable than the study of the deterministic approximation, when modelling the rise of mutations (this is mainly due to the amplitude of the stochastic fluctuations). We run stochastic simulations with suitable parameters and compare the time when, under the two therapeutic approaches, the resistant strain first reaches detectability in the serum viral load. Our results show that the best choice is to start an early combination therapy, which allows to stay drug resistance free for a longer time and in many cases leads to viral eradication.

Keywords: viral dynamics stochastic modelling, deterministic approximation, ordinary differential equations model, mutation, drug resistance.

1. Introduction

Hepatitis B virus is the cause of one of the most common infections in the world. Its consequences, such as the liver cirrhosis and the hepatocellular carcinoma, affecting approximately 25% of patients with chronic HBV infection, are important causes of morbidity and mortality worldwide [1, 27, 31, 35]. Antiviral therapy has demonstrated to have an important role in reducing both the markers of liver disease progression and, above all, the evolution to cirrhosis and hepatocellular carcinoma [35]. It has been shown that high serum levels of HBV-DNA ($> 10^4$ copies/ml) are predictive of an increased risk of hepatocellular carcinoma, regardless of the transaminase level and the presence of liver cirrhosis [11]: therefore it is clear that the chronic patient with high viral loads needs to be treated with antiviral therapy (which should be considered a preventive and anti-cancer therapy [35]).

So far antiviral drugs are mainly used in a sequential monotherapy (that is, only one drug is employed at a time and if an increase in viral load is observed, then the drug is substituted with another one). Some unsolved problems remain today: viral eradication (certified by the disappearance of HBsAg or cccDNA), which would allow the discontinuation of the therapy, is obtained in only 10% of treated patients [28]. On the other hand, the long term treatment with

sequential monotherapy [10, 21, 52], is likely to select drug-resistant strains, because of the onset of viral breakthrough and mutant escape (that is the emergence of variant viruses) under the selective pressure of medications [24, 46].

As stated in the international guidelines, the combination therapy (that is, therapy with a cocktail of drugs) has a well-defined role in the treatment of chronic hepatitis B, in selected categories of patients, such as those with decompensated cirrhosis, HIV co-infection, pre-existing mutations and post-liver transplantation [10, 21, 52, 57]. To date, only a few studies have attempted to compare the effectiveness of the two different approaches (combination versus monotherapy) in treatment-naive patients. These studies did show promising results in favor of combination therapy ([22, 43, 59, 60]).

We conjecture that combination therapy, as a therapy for chronic HBV infection, is preferable to sequential monotherapy. Our conjecture is based on the following observation: both in in vitro and in vivo studies, the antiviral drugs for treatment of hepatitis B have shown a non-competitive but rather additive and potentially synergistic mechanism of action ([15, 17, 43, 47, 64]). These observations reveal potential advantages of combination therapy, in terms of strength of antiviral effects and diminished or delayed resistance. Moreover, despite being a stable DNA virus, hepatitis B virus replicates in the host cell through an RNA phase [39], which makes it similar to the Human Immunodeficiency Virus (HIV). The natural history of HIV infection has been radically transformed by the introduction of combination antiviral therapy (Highly Active Antiretroviral Therapy, HAART) [9]. Therefore we believe that this kind of therapy should also be beneficial for HBV treatment. In particular we infer that under combination therapy the appearance of drug resistance is less likely, or at least, occurs after a longer time.

The behaviour of drug-resistant strains is a priori unpredictable. Therefore, we believe that it is important to use a mathematical model, which takes into account the emergence of mutants of the virus. The usual pattern of viral load under treatment, that has been captured by mathematical models introduced for instance in [45] and [58], is a rapidly decaying phase followed by a slower decaying phase (see for instance Figure 1 and Figure 2 in [58]). The standard mathematical model for viral dynamics within a single patient is given by a three ordinary differential equations system (in short, ODEs system):

$$\begin{cases} \dot{T} = \lambda - \delta' T - \alpha V T; \\ \dot{Y} = \alpha V T - \delta Y; \\ \dot{V} = pY - cV; \end{cases} \tag{1.1}$$

where T denotes target (uninfected) hepatocytes which are produced at rate λ, die at rate $\delta' T$ and are infected at a rate αVT; Y denotes infected hepatocytes which die at rate δY (δ possibly larger than δ') and V denotes free virus. Virions are produced from infected cells at rate pY and are cleared from bloodstream at rate cV. The system has no exact solutions but approximate solutions may be obtained under different hypotheses.

If the patient undergoes treatment, the drug acts against reproduction and/or infection. In the model this is explained by introducing the efficacy parameters $\varepsilon \in [0,1]$ ([45, 58]) and $\eta \in [0,1]$ ([33, 40]). In (1.1) one must replace α by $(1-\eta)\alpha$ and p by $(1-\varepsilon)p$. The parameters ε and η respectively indicate the ability of treatment to block viral production or infection (the extremal values 0 and 1 refer to no ability at all and complete ability, respectively). Of course the behaviour under no treatment is retrieved setting $\eta = \varepsilon = 0$. This model has been used in several papers (for chronic HBV infection, HIV infection or acute HBV infection) to fit experimental data under treatment with different drugs, leading to estimates for some of the parameters involved, mainly for ε, c and δ ([13, 14, 29, 33, 40, 44, 58, 62, 63]).

Unfortunately, in a certain fraction of patients, after an initial decrease, the serum viral load starts increasing and one may eventually face secondary therapeutic failure, which is conventionally defined as a rebound of at least 1 $\log 10$ in viral load above the lowest level previously reached. This failure is named secondary as it happens after a more or less prolonged time during which the viral load drops, as opposed to primary therapeutic failure, which is when the drug is not able, from the beginning of the cure, to determine a decay in the viral load. Secondary failure is due to the emergence of drug-resistant strains (which are usually not observed without therapy). In the literature one can find several attempts to model drug resistance: in [37] and [61] there are two HIV strains infecting CD4+ cells (one susceptible and one resistant to therapy). In [49] and [50] the standard model (1.1) is mimicked: there are separate equations for the free susceptible virus and the free resistant one; also infected cells are considered separately when infected with the resistant or with the susceptible strain. Each of these models is deterministic: a fixed fraction of the reproductions of the susceptible virus leads to mutation (reverse mutations, from the resistant strain to the susceptible one, are considered as negligible). Nevertheless the true nature of the interactions between target cells, infected cells, free virus and immune system is stochastic. It is well-known that the use of deterministic models instead of stochastic ones is justified by the large number of interacting particles. We recall in Section 2 the theorems which are the basis of this approximation. It has already been observed (see [48]) that in reality even if a particular strain is generated by mutation, there is some probability that it will disappear due to stochasticity. Moreover stochastic fluctuations are far from being negligible and often frequencies of mutants are very small (see Theorem 2.4 and the comments thereafter). We therefore develop a stochastic 3-strain model (Section 2) to which we devote our study.

The idea is that the mutations are pre-adaptive, linked to random errors of the replicative process. Hence, the antiviral therapy does not determine any resistant strains, but simply selects them [36, 39, 42, 46, 48, 49, 50]. Our main question is whether patients who during sequential monotherapy develop drug resistance, leading to secondary treatment failure, would have benefited from the choice of combination therapy instead (that is, drug resistance would not have appeared, or at least it would have at a later time). Thus we model the situation where the physician has two

drugs at hand: drug A and drug B, drug A being the one which is known to be the more efficient. Note that this is the simplest case where the dilemma between sequential and combination therapy arises (two being the minimum number of drugs possible in combination therapy).

We suppose that the patient is treatment-naive and has a high serum viral load of HBV virions, of a type (usually the wild-type) that we call *type 1*. By hypothesis, if cured with drug A, the patient will develop a resistant strain, *type 2* virus, whose serum viral load will increase. Let us stress here that by definition strain 2 of the virus is the one that first emerges during therapy, as resistant to drug A. Its genotype is a priori unknown. Therapy with drug B will prove effective in striking type 2 down, but eventually another mutation will arise (*type 3* mutants, again a priori of unknown genotype). Our main question is whether providing drug A and drug B together can significantly delay the instant when, the concentration of the variant 3 in serum exceeds the limit of detection (that is, with current tests at hand, approximately 20 copies/ml). If so, that would be an argument in favour of combination therapy.

Here is the outline of the paper. In Section 2 we recall the mathematical results which allow us to approximate the average behaviour of stochastic models with deterministic models. We introduce our stochastic model (Definition 2.1) and discuss why, when mutants viral loads are low, the description by means of the stochastic model is more accurate than the one with the deterministic model.

In Section 3 we analyze the equilibrium points of the ODEs system (2.3), which is the deterministic approximation of our stochastic model (the system has no exact solutions). The stability of the equilibria, depending on the reproductive ratios of the three types, is discussed (see Table 1). The emergence under therapy of drug-resistant strains which are not otherwise observed is explained assuming that when present together with the wild type, the viral loads of type 2 and type 3 are small (in absolute value or at least if compared to the viral load of type 1).

In Section 4 we first discuss how we can derive the unknown parameters of the infection. The idea is to make some assumptions on the equilibrium viral loads of the 3 strains and to associate to any plausible set of viral loads a corresponding parameter set. This leads to 46 different parameter sets which are tested with stochastic simulations, under sequential or combination therapy. Different initial conditions are considered (see Tables 4, 5, 6).

Section 5 is devoted to the discussion of the consequences of our results onto the choice of the best therapeutic approach in the cure of chronic HBV infection. In particular our results suggest that combination therapy is the best choice if started at the early stages of the chronic HBV infection.

2. Stochastic model and deterministic approximation

The simplest stochastic model for viral reproduction is the branching process ([23]). This model is very rough (the behaviour of the average number of particles is either exponential growth, stationarity or exponential decrease), therefore to add complexity, one introduces space and/or

interaction between particles, see [18]. Adding space one obtains the branching random walk where the reproductive capacity of the virus depends on its "location" (which may represent not only position but also type): its behaviour on complex networks has been studied for instance in [5, 6, 65]. In order to obtain a more realistic behaviour, where only a certain maximal viral load can be achieved (or at least, growth slows down when the viral population is too large), we can put interaction into play (logistic competition is perhaps the simplest interaction). Spatially displaced interacting particle systems have been studied for instance in [2, 3, 4].

The model that we introduce here is not spatial, and is essentially a modification of the stochastic model which lies underneath (1.1). We first reduce the number of unknowns in (1.1) to two. Indeed we assume that, by homeostasis, the total number of hepatocytes is constant, that is $T + Y = H_0$, where H_0 is fixed. Namely we suppose that, whenever an infected hepatocyte dies, it is replaced (almost immediately) by an uninfected one. This means that infected hepatocytes are not destroyed by the immune system at a too fast rate. This is in agreement with the common belief that cytotoxic T lymphocytes may directly inhibit viral replication and thus inactivate HBV without killing the infected hepatocyte, and that in chronically infected patients, infected hepatocytes escape immune recognition, sparing massive liver damage (see [12, 19, 32]). This is particularly true during the first stage of the disease, the compensated phase (which lasts years under a correct antiviral therapy) or in the large group of HBV infected people called *inactive carriers* [53]. This assumption allows us to deal only with the dynamics of Y and V and to replace T with $H_0 - Y$. Now we partition V into the three types of virions V_1, V_2, V_3 and Y into Y_1, Y_2 and Y_3 (according to the type of infection).

The process is characterized by the following positive parameters:

(a) the infection parameters α_1, α_2, α_3, where α_i corresponds to the rate at which virions of type i infect target hepatocytes ($i = 1, 2, 3$);
(b) the production parameters p_1, q_2, r_3, i.e. the rates at which hepatocytes which are infected with type 1, type 2 and type 3 virus respectively, produce virions of the same type;
(c) the mutation parameters p_2 and q_3, i.e. the rates at which hepatocytes which are infected with type 1 and type 2 virus respectively, produce virions of type 2 and type 3;
(d) the hepatocytes clearance rates δ_1, δ_2, δ_3, where δ_i corresponds to the rate at which hepatocytes carrying type i infection are removed and thus replaced by target hepatocytes ($i = 1, 2, 3$);
(e) the free virions clearance rates c_1, c_2, c_3, where c_i corresponds to the rate at which virions of type i are cleared from bloodstream ($i = 1, 2, 3$).

Definition 2.1 (Stochastic model)**.** *Given the positive parameters α_i, δ_i, c_i ($i = 1, 2, 3$) and p_1, q_2, r_3, p_2, q_3, the stochastic process $(Y_1(t), Y_2(t), Y_3(t), V_1(t), V_2(t), V_3(t))_{t\geq 0}$ is a continuous time Markov chain with values in $\mathbb{N}^6$. The transition rates $q_{k,k+l}$ ($k \in \mathbb{N}^6$, $l \in \mathbb{Z}^6$) are as follows, for all*

$k \neq \mathbf{0}$,

$$\begin{cases} \alpha_i \left(1 - \frac{Y_1}{H_0} - \frac{Y_2}{H_0} - \frac{Y_3}{H_0}\right) V_i & \text{if } l = \mathbf{e}_i, i = 1,2,3; \\ \delta_i Y_i & \text{if } l = -\mathbf{e}_i, i = 1,2,3; \\ p_1 Y_1 & \text{if } l = \mathbf{e}_4; \\ p_2 Y_1 + q_2 Y_2 & \text{if } l = \mathbf{e}_5; \\ q_3 Y_2 + r_3 Y_3 & \text{if } l = \mathbf{e}_6; \\ c_i V_i & \text{if } i = 1,2,3 \text{ and } l = -\mathbf{e}_4, -\mathbf{e}_5, -\mathbf{e}_6, \text{ respectively}; \\ 0 & \text{otherwise}; \end{cases}$$

($\mathbf{e}_i$ being the elements of the natural base of $\mathbb{R}^6$).

Although this is a random process, results by Kurtz prove that its dynamic behaviour is approximated by the solution of an ODEs system ([25], [26], see also [51] for an example of application in epidemic models). These results apply to density dependent processes, hence we must consider a modification of our process.

Definition 2.2 (Density dependent process and density process)**.** *A one-parameter family of continuous time Markov chains $(X^{(N)}(t))_{t\geq 0}$ with state space $E \subseteq \mathbb{Z}^d$ and transition rates $(q_{ij}^{(N)})$ is called density dependent if there exists a continuous function $f : \mathbb{R}^d \times \mathbb{Z}^d \to \mathbb{R}$, such that*

$$q_{k,k+l}^{(N)} = N f\left(\frac{k}{N}, l\right), \qquad l \neq 0 \text{ and } k, l \in \mathbb{Z}^d.$$

Suppose $(X^{(N)}(t))_{t\geq 0}$ is a density dependent process. By rescaling with N we get the density process $(X_N(t))_{t\geq 0} := (\frac{1}{N} X^{(N)}(t))_{t\geq 0}$.

Under certain conditions $(X_N(t))_{t\geq 0}$ converges to a deterministic process that is the solution of a system of first order ODEs that is governed by the following function F:

$$F(x) = \sum_{l \in \mathbb{Z}^d} l f(x, l),$$

as stated in [25, Theorem 3.1] (a slightly different version of the statement, claiming almost sure convergence, can be found in [20, Theorem 2.1, p.456]). We recall the theorem here.

Theorem 2.3 (Deterministic Approximation)**.** *Suppose that there exists an open set $E \subseteq \mathbb{R}^d$ such that the function F is Lipschitz continuous on E and*

$$\sup_{x\in E} \sum_l |l| f(x, l) < \infty; \qquad \lim_{r\to\infty} \sup_{x\in E} \sum_{|l|>r} |l| f(x, l) = 0.$$

Then, for every trajectory $(x(s, x_0), s \geq 0)$ satisfying the following system of ODEs

$$\begin{cases} \frac{d}{ds} x(s, x_0) = F(x(s, x_0)) \\ x(0, x_0) = 0 \end{cases}$$

(where $x(s,x_0) \in E$, $0 \le s \le t$), $\lim_{N\to\infty} X_N(0) = x_0$ implies that for every $\delta > 0$,

$$\lim_{N\to\infty} \mathbb{P}(\sup_{0\le s\le t} |X_N(s) - x(s,x_0)| > \delta) = 0.$$

This theorem implies that the process $(X_N(t))_{t\ge 0}$ can be approximated to first order by a deterministic process, for large N. If the density process $(X_N(t))_{t\ge 0}$ is initially close to x_0, it will tend to stay close to the trajectory $(x(s,x_0), s > t)$ in some appropriate time-interval. The behaviour of the random fluctuations of the density process $(X_N(t))_{t\ge 0}$ around its deterministic approximation is given by the following theorem ([20, Ch.11], see also [51]).

Theorem 2.4 (Fluctuations)**.** *If for all compact set $K \subset E$ we have $\sum_l |l|^2 \sup_{x\in K} f(x,l) < \infty$ and $f(\cdot,l)$ and ∂F are continuous, then the process $\{\sqrt{N}(X_N(s) - x(s,x_0))\}_{s\ge 0}$ converges in law to a fixed process V.*

This result implies that order of magnitude of the stochastic fluctuations of X_N around its deterministic approximation x, is $1/\sqrt{N}$.

In our case, taking $x_i = Y_i/N$, $i = 1,2,3$, $x_i = V_{i-3}$, $i = 4,5,6$, $N = H_0$, we have

$$f(x,l) = \begin{cases} \alpha_i (1 - x_1 - x_2 - x_3)\, x_{i+3} & \text{if } l = \mathbf{e}_i, i = 1,2,3; \\ \delta_i x_i & \text{if } l = -\mathbf{e}_i, i = 1,2,3; \\ p_1 x_1 & \text{if } l = \mathbf{e}_4; \\ p_2 x_1 + q_2 x_2 & \text{if } l = \mathbf{e}_5; \\ q_3 x_2 + r_3 x_3 & \text{if } l = \mathbf{e}_6; \\ c_i x_i & \text{if } l = -\mathbf{e}_i, i = 4,5,6; \\ 0 & \text{otherwise.} \end{cases}$$

Thus F has the following expression and it is easily checked that the hypotheses of Theorem 2.3 are satisfied:

$$F(y_1,y_2,y_3,v_1,v_2,v_3) = \begin{pmatrix} \alpha_1 (1 - y_1 - y_2 - y_3)\, v_1 - \delta_1 y_1 \\ \alpha_2 (1 - y_1 - y_2 - y_3)\, v_2 - \delta_2 y_2 \\ \alpha_3 (1 - y_1 - y_2 - y_3)\, v_3 - \delta_3 y_3 \\ p_1 y_1 - c_1 v_1 \\ p_2 y_1 + q_2 y_2 - c_2 v_2 \\ q_3 y_2 + r_3 y_3 - c_3 v_3 \end{pmatrix}. \tag{2.2}$$

An estimate for H_0 is $2 \cdot 10^{11}$ ([54], also in accordance with the result of $139{\cdot}10^6$ cells/g in [55] and an average weight for a human liver of $1.5{\cdot}10^3$g). Thus we may infer that, in a time interval of at least 30-40 years, the trajectories of the solution of the following system of ODEs

$$(\dot{y}_1, \dot{y}_2, \dot{y}_3, \dot{v}_1, \dot{v}_2, \dot{v}_3)^T = F(y_1,y_2,y_3,v_1,v_2,v_3). \tag{2.3}$$

approximate the trajectories of the stochastic process $\left(\frac{Y_1(t)}{H_0}, \frac{Y_2(t)}{H_0}, \frac{Y_3(t)}{H_0}, \frac{V_1(t)}{H_0}, \frac{V_2(t)}{H_0}, \frac{V_3(t)}{H_0}\right)_{t\ge 0}$. Nevertheless, by Theorem 2.4, the fluctuations are of order $1/\sqrt{2\cdot 10^{11}} \approx 2.2 \cdot 10^{-6}$. Thus when a fraction of infected hepatocytes or $V_i(t)/H_0$ is smaller than 10^{-6} then the approximation is far

from being accurate. This is particularly true at the early stages of the infection (all the V_is are low) or when a mutation has just appeared (V_2 and/or V_3 are low).

Remark 2.5 (The model under therapy)**.** *The stochastic model we defined in* (2.1) *and its approximation, that is the solution of* (2.3) *are not only models for the case where there is no therapy, but also where there is therapy with drug A or B or both. We only have to change the parameters involved by therapy. To be precise, we denote by ε_A, η_A the efficacies parameters of drug A against strain 1, by ε_{B1}, η_{B1} and ε_{B2}, η_{B2} the efficacies parameters of drug B against strain 1 and strain 2 respectively, and by ε_C, η_C the efficacy parameters of the combination of drug A and B against strain 1.*

If therapy with drug A only is in act, then we must replace α_1, p_1 and p_2 by $(1-\eta_A)\alpha_1$, $(1-\varepsilon_A)p_1$ and $(1-\varepsilon_A)p_2$ respectively; if we use drug B only then we replace α_1, p_1, p_2, α_2, q_2 and q_3 by $(1-\eta_{B1})\alpha_1$, $(1-\varepsilon_{B1})p_1$ $(1-\varepsilon_{B1})p_2$, $(1-\eta_{B2})\alpha_2$, $(1-\varepsilon_{B2})q_2$ and $(1-\varepsilon_{B2})q_3$ respectively; in the case of combination therapy, then α_1, p_1 and p_2 must be replaced by $(1-\eta_C)\alpha_1$, $(1-\varepsilon_C)p_1$ and $(1-\varepsilon_C)p_2$ respectively, while α_2, q_2 and q_3 are modified as in the case of the cure with B only (by hypothesis, drug A has no impact on the type 2 infection).

3. Analysis of the deterministic model

The ODEs system (2.3) has no exact solution, thus in order to understand its behaviour, we start by analyzing its equilibria. Before going into the details of the analysis, we make some assumptions on the parameters:

(1) The viral clearance parameters c_i do not depend on the type, namely $c := c_1 = c_2 = c_3$.

(2) $p_2 \ll p_1$, $q_3 \ll q_2$ and also p_2 and q_3 have small absolute values.

For the first assumption we note that the c_is depend only on the lifetime of virions in bloodstream, thus on the antibodies production of the infected patient. We assume that this production does not depend on the variant (this may of course not be the case, but here we want to avoid a large number of unknown parameters). In (2), p_1+p_2 and q_2+q_3 represent the reproduction rates of type 1 and type 2 infected hepatocytes respectively; p_1 and q_2 are the rates of conformal reproductions, while p_2 and q_3 are the rates of non-conformal reproductions. Assumption (2) follows, since the probability of mutation is very small.

An important role in the stability of the equilibria is played by the basic reproductive ratios of the three variants

$$R_1 = \frac{\alpha_1 p_1}{c\delta_1}, \qquad R_2 = \frac{\alpha_2 q_2}{c\delta_2}, \qquad R_3 = \frac{\alpha_3 r_3}{c\delta_3}. \tag{3.4}$$

Note that if the patient is undergoing therapy the corresponding infection and production parameters have to be changed according to Remark 2.5. For instance, under therapy with drug A, the basic reproductive ratio of the type 1 variant becomes $(\alpha_1 p_1(1-\eta_A)(1-\varepsilon_A))/(c\delta_1)$.

There are four equilibrium points, that is, solutions of $F(x) = 0$ (F taken from (2.2)): the disease-free state and three infected equilibria X_1 (with all variants present), X_2 (where the first variant is cleared) and X_3 (with only the third mutant present). An equilibrium point x is locally asymptotically stable if all the eigenvalues of $JF(x)$ are negative, where $JF(x)$ is the Jacobian matrix of F.

$$JF(x) = \begin{pmatrix} -(\alpha_1 v_1 + \delta_1) & -\alpha_1 v_1 & -\alpha_1 v_1 & \alpha_1\beta & 0 & 0 \\ -\alpha_2 v_2 & -(\alpha_2 v_2 + \delta_2) & -\alpha_2 v_2 & 0 & \alpha_2\beta & 0 \\ -\alpha_3 v_3 & -\alpha_3 v_3 & -(\alpha_3 v_3 + \delta_3) & 0 & 0 & \alpha_3\beta \\ p_1 & 0 & 0 & -c & 0 & 0 \\ p_2 & q_2 & 0 & 0 & -c & 0 \\ 0 & q_3 & r_3 & 0 & 0 & -c \end{pmatrix},$$

where $\beta = 1 - y_1 - y_2 - y_3$.

3.1. **Disease-free equilibrium.** $X_0 = (0, 0, 0, 0, 0, 0)$. $JF(X_0)$ has six eigenvalues:

$$\frac{-\delta_1 - c \pm \sqrt{4\alpha_1 p_1 + (\delta_1 - c)^2}}{2}, \frac{-\delta_2 - c \pm \sqrt{4\alpha_2 q_2 + (\delta_2 - c)^2}}{2}, \frac{-\delta_3 - c \pm \sqrt{4\alpha_3 r_3 + (\delta_3 - c)^2}}{2}.$$

These eigenvalues are real and they are all negative (hence X_0 is locally asymptotically stable) if $R_i < 1$ for all $i = 1, 2, 3$. If at least one R_i is larger than 1, then there is at least one eigenvalue which is positive and X_0 is locally asymptotically unstable.

3.2. **The infected equilibrium without type 1 and type 2.** $X_3 = (0, 0, y_{3,3}, 0, 0, v_{3,3})$. It has biological meaning (that is, $y_{3,3}$ and $v_{3,3}$ are positive) if and only if $R_3 > 1$, since

$$v_{3,3} = \frac{R_3 - 1}{R_3}\frac{r_3}{c}, \qquad y_{3,3} = 1 - \frac{1}{R_3} = \frac{c}{r_3} R_3 v_{3,3}.$$

$JF(X_1)$ has six eigenvalues:

$$\frac{-\alpha_3 r_3(c + \delta_1) \pm \sqrt{(\alpha_3 r_3(c - \delta_1))^2 + 4\alpha_1\alpha_3 c\delta_3 p_1 r_3}}{2\alpha_3 r_3},$$
$$\frac{-\alpha_3 r_3(c + \delta_2) \pm \sqrt{(\alpha_3 r_3(c - \delta_2))^2 + 4\alpha_2\alpha_3 c\delta_3 q_2 r_3}}{2\alpha_3 r_3},$$
$$\frac{-\alpha_3 r_3 - c^2 \pm \sqrt{4c^3\delta_3 + (\alpha_3 r_3 - c^2)^2}}{2c}.$$

Easy computations show that X_3 is locally asymptotically stable if and only if $R_3 > 1$, $R_3 > R_1$ and $R_3 > R_2$.

3.3. **The infected equilibrium without type 1 infection.** $X_2 = (0, y_{2,2}, y_{3,2}, 0, v_{2,2}, v_{3,2})$. It has biological meaning if and only if $R_2 > 1$ and $R_2 > R_3$, since

$$v_{2,2} = \frac{q_2}{c}\frac{(R_2-1)(R_2-R_3)}{R_2(R_2-R_3+R_3 q_3/r_3)}, \qquad v_{3,2} = \frac{q_3(R_2-1)}{c(R_2-R_3+R_3 q_3/r_3)} = \frac{q_3}{q_2}\frac{R_2}{R_2-R_3} v_{2,2}$$
$$y_{2,2} = \frac{c}{q_2} v_{2,2}, \qquad y_{3,2} = \frac{c}{r_3}\frac{R_3}{R_2} v_{3,2}.$$

Two eigenvalues are

$$\frac{1}{2}\left(-c - \delta_1 \pm \sqrt{(\delta_1 - c)^2 + 4\alpha_1 p_1(1 - y_{2,2} - y_{3,2})}\right),$$

which are negative if and only if $R_1 < 1/(1-y_{2,2}-y_{3,2}) = R_2$. The other four eigenvalues are the solutions of a quartic equation and have expressions which are too involved to be studied here. We prefer to study the approximated solutions obtained substituting $q_3 \approx 0$ (and $v_{3,2} \approx 0$, which is plausible if $R_2 - R_3$ is not too small compared with $R_2 - 1$) in the characteristic polynomial:

$$\frac{1}{2}\left(-\alpha_2 v_{2,2} - \delta_2 - c \pm \sqrt{(\alpha_2 v_{2,2} + \delta_2 - c)^2 + 4\alpha_2 q_2(1 - y_{2,2} - y_{3,2})}\right)$$
$$\frac{1}{2}\left(-c - \delta_3 \pm \sqrt{(\delta_3 - c)^2 + 4\alpha_3 r_3(1 - y_{2,2} - y_{3,2})}\right).$$

Computations show that these approximate eigenvalues are all negative if and only if $v_{2,2} > 0$ and $R_2 > R_3$ respectively. Thus X_2 is locally asymptotically stable if and only if $R_2 > 1$, $R_2 > R_1$ and $R_2 > R_3$.

3.4. **The infected equilibrium with type 1.** $X_1 = (y_{1,1}, y_{2,1}, y_{3,1}, v_{1,1}, v_{2,1}, v_{3,1})$, where

$$\begin{array}{lll} v_{1,1} = \frac{p_1}{c} y_{1,1}, & v_{2,1} = \frac{R_1}{R_2}\frac{q_2}{c} y_{2,1}, & v_{3,1} = \frac{R_1}{R_3}\frac{r_3}{c} y_{3,1}, \\ y_{2,1} = \frac{R_2}{R_1 - R_2}\frac{p_2}{q_2} y_{1,1}, & y_{3,1} = \frac{p_2 q_3}{q_2 r_3}\frac{R_2 R_3}{(R_1 - R_2)(R_1 - R_3)}. & \end{array} \tag{3.5}$$

As for $y_{1,1}$, it has a complicated expression which is of no interest here (but can be obtained from the relation $y_{1,1} + y_{2,1} + y_{3,1} = 1 - 1/R_1$). Here is its approximation if one substitutes $q_3 \approx 0$:

$$y_{1,1} \approx \frac{(R_1 - 1)(R_1 - R_2)}{R_1(R_1 - R_2 + R_2(p_2/q_2))}.$$

Thus X_1 has biological meaning if $R_1 > 1$, $R_1 > R_2$ and $R_1 > R_3$. The eigenvalues of $JF(X_1)$ have too complicated expressions, which make them untreatable. We study the approximate solutions obtained with the substitutions $q_3 \approx 0$, $p_2 \approx 0$ (and $v_{2,1} \approx 0$, which is plausible if p_2 is small and $R_1 - 1$ is not too small compared with $R_1 - R_2$) in the characteristic polynomial. The approximated eigenvalues are

$$\frac{1}{2}\left(-\alpha_1 v_{1,1} - \delta_1 - c \pm \sqrt{(\alpha_1 v_{1,1} + \delta_1 - c)^2 + 4\alpha_1 p_1(1 - y_{1,1} - y_{2,1} - y_{3,1})}\right),$$
$$\frac{1}{2}\left(-c - \delta_2 \pm \sqrt{(\delta_2 - c)^2 + 4\alpha_2 q_2(1 - y_{1,1} - y_{2,1} - y_{3,1})}\right),$$
$$\frac{1}{2}\left(-c - \delta_3 \pm \sqrt{(\delta_3 - c)^2 + 4\alpha_3 r_3(1 - y_{1,1} - y_{2,1} - y_{3,1})}\right).$$

Substituting $y_{1,1} + y_{2,1} + y_{3,1} = 1 - 1/R_1$, we get that X_1 is locally asymptotically stable if $R_1 > 1$, $R_1 > R_2$ and $R_1 > R_3$ and unstable otherwise.

The conditions for stability can be summarized by Table 1.

3.5. **Drug resistance explained by the model.** Let us discuss how the presence of these equilibria and their stability can be biologically interpreted, and how this interpretation reflects onto the parameters. We are modelling the infection within a chronic patient, therefore with no treatment all three variants have reproductive ratios larger than 1. Moreover we believe that type 1 has largest "fitness" and that type 2 has larger "fitness" than type 3, in the sense that variants are more

R_1	R_2	R_3	**Equilibria**
$R_1 < 1$	$R_2 < 1$	$R_3 < 1$	X_0 stable, other X_is unstable
$R_1 > 1$	$R_2 < R_1$	$R_3 < R_1$	X_1 stable, other X_is unstable
$R_1 < R_2$	$R_2 > 1$	$R_3 < R_2$	X_2 stable, other X_is unstable
$R_1 < R_3$	$R_2 < R_3$	$R_3 > 1$	X_3 stable, other X_is unstable

TABLE 1. Stability of the equilibria as a function of R_1, R_2, R_3.

fit if, when present together at equilibrium, they have prevailing serum viral loads. We also define the fittest variant to be the one with larger reproductive ratio (in a moment we will show that this assumption leads to prevailing viral loads). Therefore in the beginning $R_1 > R_2 > R_3 > 1$. With no cure, after an appropriate amount of time, the system tends to lie in a neighbourhood of X_1. The effect of therapy is to lower the corresponding R_i, namely the new R_i becomes $R_i(1-\eta)(1-\varepsilon)$, where η and ε are the efficacy parameters of the drug acting against variant i. Thus, if the patient is given drug A, then type 1 is cured and the system moves towards equilibrium X_2, where variant 2 emerges and variant 1 disappears. If also type 2 is cured, with drug B (R_2 is lowered), the new equilibrium will be X_3, that is, the patient will show drug resistance. The disease-free equilibrium represents the desirable state where the patient is permanently cured, and can be reached, according to the deterministic model, only if we have a drug at hand which acts also against the third variant (by assumption, here X_0 cannot be reached).

We believe that the reason why drug-resistant mutants are not observed unless under therapy is the fact that, even if mutations do happen, nevertheless in the equilibrium together with the wild type, mutants are numerically negligible. In other words, we assume that $v_{2,1}$ and $v_{3,1}$ are negligible if compared to $v_{1,1}$. From (3.5) we have

$$\frac{v_{2,1}}{v_{1,1}} = \frac{R_1}{R_1 - R_2} \cdot \frac{p_2}{p_1};$$
$$\frac{v_{3,1}}{v_{1,1}} = \frac{R_1 R_2}{(R_1 - R_2)(R_1 - R_3)} \cdot \frac{p_2 q_3}{p_1 q_2},$$

whence the negligibility of the variants at X_1 follows when p_2 and q_3 are small (with respect to p_1 and q_2 respectively) and $R_1 - R_2$ and $R_1 - R_3$ are not too small.

In the following figures we can see the behaviour of the viral loads of the three variants with no therapy (Figure 1, converging towards X_1), when curing only variant 1 (Figure 2, converging towards X_2) and with the combination therapy (Figure 3, converging towards X_3). Note that the scales are different for the three variants and that on the vertical axis we have the total number of virions divided by H_0.

The plots are obtained with MatLab R2013b, by numerical solutions of the differential equations system with set of parameters (measured in days^{-1}): $c = 0.65$, $\delta_1 = \delta_2 = \delta_3 = 0.0143$, $p_1 = 2.6$, $p_2 = 8.0997 \cdot 10^{-10}$, $q_2 = 0.2889$, $q_3 = 10^{-7}$, $r_3 = 0.26$, $\alpha_1 = 4.7668 \cdot 10^{-3}$, $\alpha_2 = 4.29 \cdot 10^{-2}$,

$\alpha_3 = 4.7666 \cdot 10^{-2}$ (this is one of the sets obtained as plausible in Section 4). In Figure 1 the initial condition is $v_1(0) = 0.8$, $v_2(0) = 10^{-7}$, $v_3(0) = 10^{-9}$, $y_1(0) = 0.2$, $y_2(0) = v_2(0) \cdot \frac{cR_2}{q_2 R_1}$, $y_3(0) = v_3(0) \cdot \frac{cR_3}{r_3 R_1}$. The values for $v_1(0)$ and $y_1(0)$ are taken from the literature on untreated chronic patients, $v_2(0)$ and $v_3(0)$ are arbitrarily chosen (smaller than $v_{2,1}$ and $v_{3,1}$ respectively), $y_i(0)$ is taken multiplying $v_i(0)$ by $y_{i,1}/v_{i,1}$, $i = 2, 3$. This means that we believe that the ratio of infected hepatocytes to viral load is the same before reaching equilibrium and at equilibrium (this hypothesis was confirmed by the stochastic simulations run in Section 4). We used the *ode15s* command (due to the stiffness of the solution, other more accurate methods did not produce smooth plots). In Figure 2 the initial condition is $v_1(0) = 0.8$, $v_2(0) = 10^{-5}$, $v_3(0) = 10^{-7}$, $y_1(0) = 0.2$, $y_2(0) = v_2(0) \cdot \frac{cR_2}{q_2 R_1}$, $y_3(0) = v_3(0) \cdot \frac{cR_3}{r_3 R_1}$ (we suppose that cure intervenes after some time of infection and variants have had the time to develop). The healing parameters are $\varepsilon_A = 0.9$ and $\eta_A = 0.5$. We used the *ode45* command (which is usually the most accurate method). In Figure 3 the initial condition is $v_1(0) = 0.8$, $v_2(0) = 10^{-1}$, $v_3(0) = 10^{-4}$, $y_1(0) = 0.2$, $y_2(0) = v_2(0) \cdot \frac{cR_2}{q_2 R_1}$, $y_3(0) = v_3(0) \cdot \frac{cR_3}{r_3 R_1}$ (we cure type 1 and 2, thus they are both strong at the beginning). The healing parameters are $\varepsilon_A = 0.9$, $\eta_A = 0.5$, $\varepsilon_B = 0.85$, $\eta_B = 0.5$. Again we used the *ode45* command.

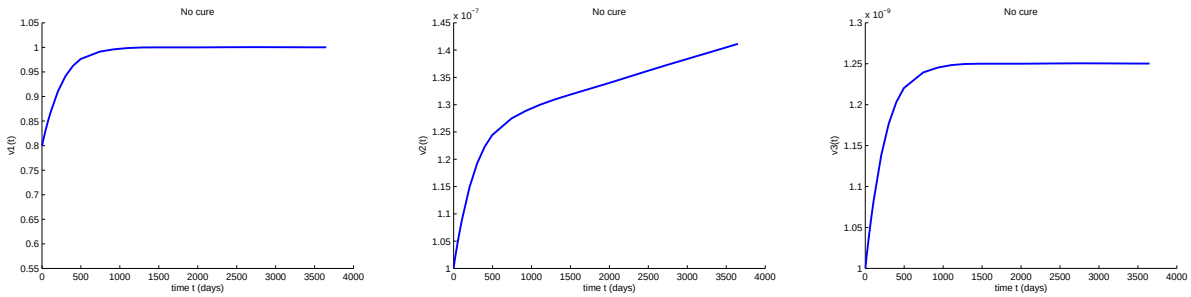

FIGURE 1. Viral loads of the three types with no therapy.

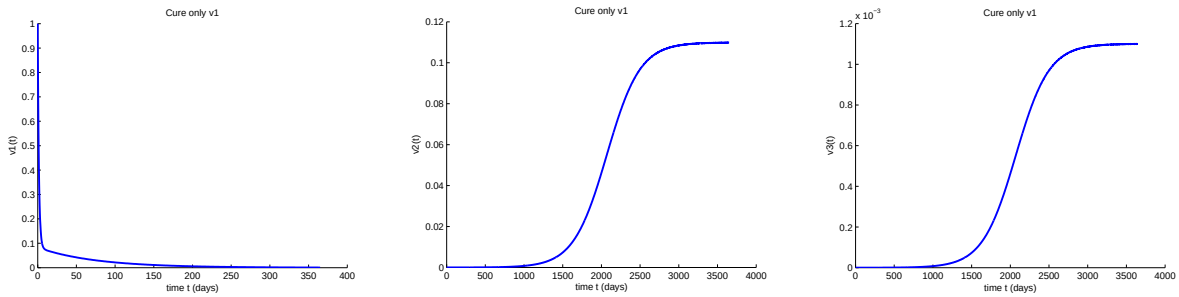

FIGURE 2. Viral loads of the three types curing only type 1.

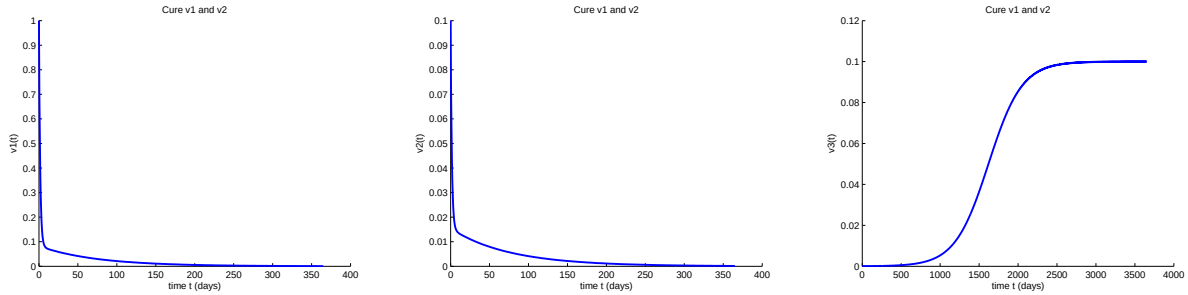

FIGURE 3. Viral loads of the three types with therapy against types 1 and 2.

4. Numerical comparison under sequential or combination therapy

4.1. **Parameters and initial conditions.** In order to understand the behaviour of the viral loads of the three variants of the virus, with or without therapy, we need to perform numerical simulations of the stochastic model. We stress that we believe that the true nature of the problem is stochastic. The deterministic approximation, which is traditionally employed, may not be accurate for low viral loads, due to the amplitude of the stochastic fluctuations (Theorem 2.4). Moreover, according to the deterministic model, it suffices that some virions of type 3 (which has reproductive ratio larger than 1) are produced by mutation, and the infection cannot be stopped unless one finds a cure against these mutants. In reality, by stochasticity, sometimes mutants are produced but then also wiped out. We therefore devote our study mainly to stochastic simulations.

We need to know the numerical values of the parameters involved, and of the initial condition. The twelve parameters are: the infection parameters α_1, α_2 and α_3; the clearance parameters c, δ_1, δ_2 and δ_3; the production parameters p_1, p_2, q_2, q_3, r_3.

From [58] we have $c = 0.65$ day^{-1} and $\delta_1 = 0.0143$ day^{-1}. We assume that the clearance parameter of the infected hepatocytes does not depend on the type of virus which infected the cell, that is $\delta_1 = \delta_2 = \delta_3 = 0.0143$ day^{-1}. The production parameter p_1 can be obtained from the relation $p_1 = cv_{1,1}/y_{1,1}$ (see (3.5)). The values of $v_{1,1}$ and $y_{1,1}$ differ in different patients, we take average values in chronic patients. The order of magnitude of the initial viral load in the patients of [58] ranged from 10^7 to 10^9 copies/ml: for simplicity's sake, we take a viral load $\widetilde{v}_{1,1} = 0.67 \cdot 10^8$ copies/ml. Note that this corresponds to $v_{1,1} = 1$ (or a total of $2 \cdot 10^{11}$ free virions in the blood – assuming $3 \cdot 10^3$ml of serum), since the coordinates of the equilibria of Section 3 represent the total number of infected hepatocytes and of free virions, rescaled by dividing by $H_0 = 2 \cdot 10^{11}$). It is usually assumed that a percentage varying between 5% and 40% of the total number of hepatocytes is productively infected in chronic HBV patients (see [8]). We take $y_{1,1} = 0.25$, whence $p_1 = 2.6$ day^{-1}.

We are still missing seven parameters: α_1, α_2, α_3, p_2, q_2, q_3, r_3. We observe that different set of parameters lead to different equilibria, in particular to different viral loads corresponding to $v_{1,1}$,

c	δ_1	p_1	$v_{1,1}$	$y_{1,1}$
0.65 day^{-1}	0.0143 day^{-1}	2.6 day^{-1}	1	0.25

TABLE 2. Parameters from the literature.

$v_{2,1}$, $v_{3,1}$, $v_{2,2}$, $v_{3,2}$ and $v_{3,3}$. Indeed these viral loads are functions of the parameters (see equations in Section 3). With some algebraic effort, we can write six of the missing parameters as functions of the six equilibrium viral loads and of the seventh parameter. Thus we derive expressions for α_1, α_2, α_3, p_2, q_2 and r_3, depending on $v_{1,1}$, $v_{2,1}$, $v_{3,1}$, $v_{2,2}$, $v_{3,2}$, $v_{3,3}$ and q_3. We choose q_3 arbitrarily: we assume that it is equal to 10^{-7} (it must be very small). Besides $v_{1,1}$, which has been observed in the literature, the other viral loads are unknown. They depend on the strains involved, which are by definition a priori unknown. Therefore we let the equilibrium viral loads vary among plausible values. The choice of the plausible values is made according to some criteria:

(1) type 2 and type 3 have, at X_1, negligible viral loads with respect to type 1, that is $v_{1,1} \gg v_{2,1} \geq v_{3,1}$;
(2) viral loads of a fixed type increase when the competitors are erased, that is, $v_{3,3} > v_{3,2} > v_{3,1}$, $v_{2,2} > v_{2,1}$;
(3) we believe that the fact that type 1 has the largest fitness is also reflected by the fact that when type 1 is removed from the system, the other two types can never exceed type 1's initial viral load, that is $v_{1,1} \geq v_{i,j}$ for all i, j.

In principle, we let $v_{2,1}$ and $v_{3,1}$ vary from 9 to 4 orders of magnitude less than $v_{1,1}$; $v_{2,2}$ and $v_{3,2}$ from 7 to 2 orders of magnitude less than $v_{1,1}$; $v_{3,3}$ from 7 to 1 order of magnitude less than $v_{1,1}$.

As a second step, we compute the corresponding parameters α_1, α_2, α_3, p_2, q_2 and r_3. A set of viral loads is possible only if the corresponding parameters have the correct biological meaning, namely we require that

(1) all the parameters are positive;
(2) $p_1 \geq q_2 \geq r_3$ (type 1 has a faster production rate, and type 2 is faster than type 3);
(3) p_2/p_1 and q_3/q_2 are small (error-free replications are less likely than mutations);
(4) at equilibria, the percentage of infected hepatocytes lies between 5% and 40%.

These requirements allow us to drop many of the plausible values. In Table 3 we write the range of the viral loads $\widetilde{v}_{i,j}$ (recall that $\widetilde{v}_{i,j} = v_{i,j} \cdot (2 \cdot 10^{11})/(3 \cdot 10^3)$). We let the loads vary from the

	$\widetilde{v}_{2,1}$	$\widetilde{v}_{3,1}$	$\widetilde{v}_{2,2}$	$\widetilde{v}_{3,2}$	$\widetilde{v}_{3,3}$
min	6.7	$6.7 \cdot 10^{-2}$	$6.7 \cdot 10^2$	$6.7 \cdot 10^3$	$6.7 \cdot 10^2$
max	$6.7 \cdot 10^2$	6.7	$6.7 \cdot 10^5$	$6.7 \cdot 10^6$	$6.7 \cdot 10^6$

TABLE 3. Range of equilibrium viral loads (in copies/ml).

minimum to the maximum, with a multiplicative step of 10 (thus, for instance the possibilities for

$\widetilde{v}_{2,1}$ are 6.7, 67 and 670). Among the $3^3 \cdot 4^2 \cdot 5$ plausible cases from Table 3, only 46 satisfy all our requirements. Moreover, for all these 46 parameter sets we got $p_2/p_1 \leq 10^{-5}$ and $q_3/q_2 \leq 10^{-3}$. We performed stochastic simulations for all the 46 parameter sets (named "cases" hereafter).

The aim is to compare sequential monotherapy (drug A followed by drug B) with combination therapy (drug A together with drug B), thus the efficacy parameters are needed. From [58] we have $\varepsilon(\text{adefovir}) = 0.993$, while from [45] we have $\varepsilon(\text{lamivudine})$ ranging from 0.87 to 0.96 depending on the dosage. In our numerical tests, we choose $\varepsilon_A = 0.97$ and $\varepsilon_{B1} = \varepsilon_{B2} = 0.85$: we assumed that the first drug is highly efficient against the wild type, while the second drug, which acts also on type 2, is less efficient. Although some papers assume that η is neither 0 nor 1 (see for instance [33, 40]), there are no explicit estimates of this parameter for the various drugs, hence we picked $\eta_A = \eta_{B1} = \eta_{B2} = 0.5$ ([33] used as a plausible value 0.5).

As for the combination efficacies, we observe that combination therapy has a larger efficacy in reducing the production rates (compared to single drug therapy); for instance [29] showed that, at the dosage of their trial, lamivudine only has $\varepsilon = 0.94$, while combined with famciclovir it gets $\varepsilon = 0.988$. Therefore it seems reasonable to rule out antagonism in combination therapy, and consider only synergism, where $\varepsilon_C \geq \max(\varepsilon_A, \varepsilon_{B1})$ and $\eta_C \geq \max(\eta_A, \eta_{B1})$. The simplest way to model synergism is to suppose that whenever a virus is the target of two drugs together, the effects add in an independent manner. Namely, if drug A prevents a fraction η_A of virions of type 1 from infecting target hepatocytes, drug B will prevent a fraction η_{B1} of the remaining virions from infecting target hepatocytes, and the effect on replication will be analogous (just taking the εs). In mathematical words, the combined efficacies of the cocktail drug A + drug B against production of type 1 and against infection of new cells respectively, are $\varepsilon_C = 1 - (1 - \varepsilon_A)(1 - \varepsilon_{B1})$ and $\eta_C = 1 - (1 - \eta_A)(1 - \eta_{B1})$. If the patient is given drug A and drug B in combination, then the viral load of type 1 decays more rapidly (R_1 is multiplied by $(1 - \eta_C)(1 - \varepsilon_C)$) and the system moves directly from X_1 to X_3.

4.2. **Simulations.** We simulated the course of the infection during a time lapse of 20 years (with a six-hour step), monitoring the random time T when the viral load of type 3 exceeds 20 copies/ml comparing therapy with only drug A, sequential monotherapy and combination therapy. We used MatLab R2013b: we discretized time with a step *tstep* of 6 hours. At the beginning of each of these time intervals v_1, v_2, v_3, y_1, y_2 and y_3 are updated. The removal of virions from bloodstream and the recovery of infected hepatocytes is simulated through a binomial distribution: for instance if at a certain time we have V virions of type 1, after a time lapse *tstep* there will be a remaining number equal to $\mathcal{B}(V, \exp(-c \cdot tstep))$. In order to simulate reproductions, for each type of virus, we divided the infected hepatocytes in random subpopulations $I_0, \ldots, I_l$, according to a multinomial distribution. Indeed, I_j, $j = 0, \ldots, l-1$, is the set of infected hepatocytes which produce j virions during the time interval and I_l is the set of those producing at least l virions (in this case we update

the production with exactly l virions for each hepatocyte in I_l). The error is kept minimal, since l is chosen such that the probability of producing $l-1$ virions is smaller than 10^{-11}. Similarly, to update the number of infected hepatocytes, the target hepatocytes are randomly partitioned into those which will not be infected in the time interval and those which get infected by one of the virus types. It is worth noting that MatLab is able to simulate binomials $\mathcal{B}(n,p)$ up to $n = 10^7$ and requires long machine time when $n > 10^6$. Thus we created a new command which uses the normal or Poisson approximation when n is large.

In sequential therapy, drug B is prescribed when $\widetilde{v}_2(t) > 667$ cp/ml (recall that, according to [11], one wishes to keep serum viral loads below 10^4 cp/ml). In all simulations, $\widetilde{v}_1(0) = 0.67 \cdot 10^8$ cp/ml, $y_1(0) = 0.25$. The behaviour of the system highly depends on the initial values of $\widetilde{v}_2(0)$ and $\widetilde{v}_3(0)$. In Table 4, $\bar{T}$ is the average of the observed values of T among the 46 possible cases, in Table 5 the cases were only 42 since in the remaining 4 the viral load of type 2 at equilibrium together with the other strains was 6.7 cp/ml and therefore the system (which supposedly started some time in the past with no mutants) cannot reach $\widetilde{v}_2 = 67$. Moreover, in the last column of Table 5, $\bar{T}$ is the average of T among the 6 cases where combination therapy did not eradicate the disease.

It appears that, if we want to delay the appearance of type 3 virions and we start therapy before that third type mutations have taken place, then sequential monotherapy is slightly better than monotherapy with drug A, but the advantage is small and is not even present in all cases. Indeed the delay obtained with sequential therapy, with respect to monotherapy, ranges from -2% to 6% if $\widetilde{v}_2(0) = 6.7$ cp/ml and from -1% to 5% if $\widetilde{v}_2(0) = 67$ cp/ml. On the other hand, in these situations combination is strikingly better than sequential therapy. Combination therapy reaches the goal of eradicating the disease in most cases and when it does not, the delay obtained, with respect to monotherapy, ranges from 32% to 128%.

only A	sequential	combination
$T = 5.17$ years	$T = 5.28$ years	disease always eradicated

TABLE 4. Comparison with $\widetilde{v}_2(0) = 6.7$ cp/ml and $\widetilde{v}_3(0) = 0$ cp/ml.

only A	sequential	combination healed	combination
$T = 4.67$ years	$T = 4.76$ years	36/42 cases	$\bar{T} = 7.84$ years

TABLE 5. Comparison with $\widetilde{v}_2(0) = 67$ cp/ml and $\widetilde{v}_3(0) = 0$ cp/ml.

The advantage of combination therapy becomes less clear if simulations are started with $\widetilde{v}_3(0) > 0$. For instance if $\widetilde{v}_3(0) = 1$ cp/ml, then combination therapy was better than sequential therapy only in 10 out of 46 cases and worse in 32 out of 46 cases (they were substantially equivalent in the

remaining 4 cases). It is not a priori clear how long it would take for a patient which is chronically infected with the wild type virus, to develop mutating strains with a sufficiently high viral load to make combination therapy a worse choice. We simulated chronic patients with no mutants which start developing mutations but undergo no therapy whatsoever for 10 years and, starting from the viral loads reached after these 10 years, we simulated other 10 years of therapy. The results are stated in Table 6, where the two therapeutic approaches are considered equivalent if the delay ranges in $\pm 2\%$. It is important to note that there is complete healing in 11 of the 28 cases where combination is preferable and even when sequential therapy is preferable the delay is at most 25% of the corresponding time with combination therapy.

sequential	combination	seq. better	comb. better	equiv.
$\bar{T} = 2.97$ years	$\bar{T} = 3.90$ years	13/46	28/46	5/46

TABLE 6. Comparison with $\widetilde{v}_2(0)$ random.

$\widetilde{v}_2(0)$	min delay when better	max delay when better
7	always healing	always healing
67	32%	128%
Random	2%	209%

TABLE 7. Delays with combination compared to sequential therapy.

Here are the plots of the viral load of type 3 in a case where combination therapy leads to healing. The values of the parameters are (as usual, in day^{-1} units) $c = 0.65$, $\delta_1 = \delta_2 = \delta_3 = 0.0143$, $p_1 = 2.6$, $p_2 = 3.6459 \cdot 10^{-7}$, $q_2 = 5.5182 \cdot 10^{-4}$, $q_3 = 10^{-7}$, $r_3 = 5.7630 \cdot 10^{-5}$, $\alpha_1 = 4.7732 \cdot 10^{-3}$, $\alpha_2 = 19.3360$, $\alpha_3 = 181.7917$. Figure 4 has initial conditions $\tilde{v}_1(0) = 0.67 \cdot 10^8$ cp/ml, $\tilde{v}_2(0) = 67$ cp/ml, $\tilde{v}_3(0) = 0$. Figure 5 corresponds to the initial conditions $\tilde{v}_1(0) = 0.67 \cdot 10^8$ cp/ml, $\tilde{v}_2(0) = 67$ cp/ml, $\tilde{v}_3(0) = 1$ cp/ml. The plots of the viral loads of type 1 and 2 are not qualitatively different from the ones in Section 3.

5. Discussion

Drug resistance is a well-known cause of therapeutic failure. The simplest case where one can test the ability of combination therapy (versus sequential monotherapy) to fight drug resistance, is the case of a 3-strain infection with two drugs available (which act only against two variants of the virus). The drug-resistant strains that will emerge under therapy are a priori unknown. This gives the need of a general mathematical model for viral dynamics. The usual deterministic models do not take into account stochastic fluctuations, which are not negligible when the viral loads of some of the variants are low.

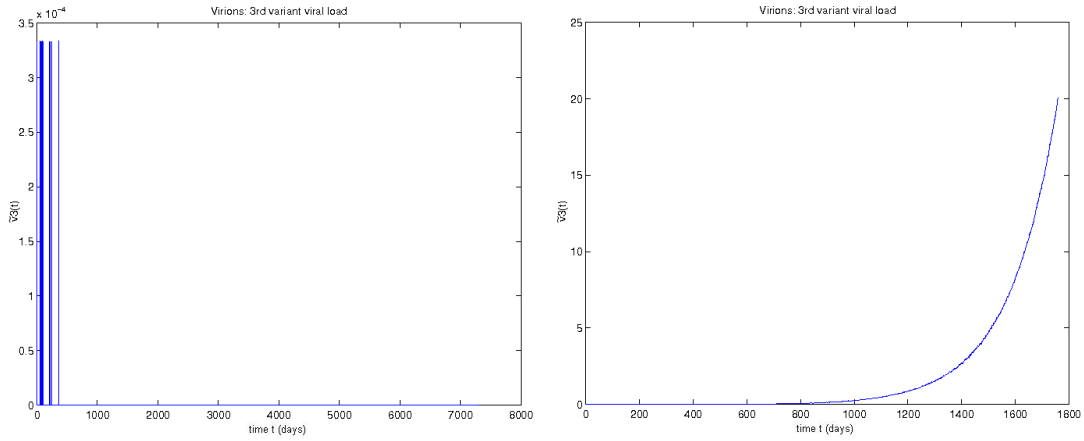

FIGURE 4. Viral loads of type 3, under combination (left) or sequential therapy (right).

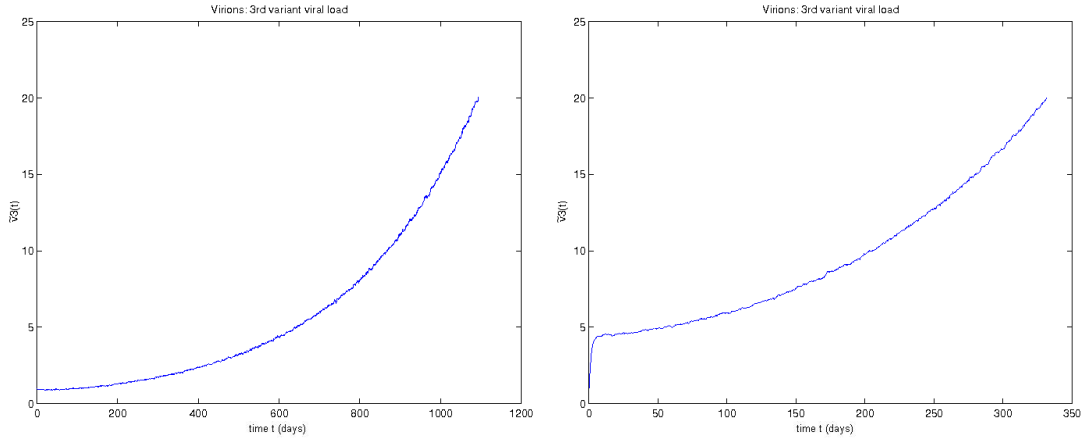

FIGURE 5. Viral loads of type 3, under combination (left) or sequential therapy (right).

In this paper we propose a stochastic model and simulate it with MatLab R2013b. We assume that the reason why drug-resistant strains only appear during therapy is competition: the resistant strains are less fit than the wild-type (together with the wild-type strain, even if present, mutants remain numerically negligible). This assumption is considered the most likely explanation of drug resistance, see [48]. We also believe that the drug-resistant variants emerge quite slowly, due to a reduced speed of replication. The assumptions are reflected by some constraints on the infection, production and mutation parameters of type 2 and type 3 strains. In order to run simulations we need the numerical values of these parameters: the ones relative to the wild-type are taken from the literature (Table 2). A mutation parameter (q_3) is chosen arbitrarily, while the others are given as functions of q_3 and of the equilibrium viral loads that type 2 and type 3 can reach, with or without therapy. Letting the equilibrium viral loads vary among plausible values (Table 3) we get 46 possible parameter sets (corresponding to 46 possible couples of type 2 and type 3 strains).

Our numerical analysis shows that combination therapy is by far the best choice if started at the early stages of the chronic infection, while it seems that the advantage is not striking if therapy is in act when drug-resistant mutants are already numerous and have started to significantly infect the hepatocytes. This difference is not surprising, since if type 3 mutants are absent, they are generated only by mutation from type 2, hence the need of lowering the number of type 2 virions and infected cells. On the other hand, if type 3 mutants are already present, in some cases the competition with other strains might slow the proliferation of the type 3 infection. In this model, if drug-resistant strains are already present in abundance, the cure may open the way for their proliferation. Nevertheless, we believe that in reality early therapy is always convenient, since the probability that drug-resistant strains are already near equilibrium (together with the wild-type) is very low, being these drug-resistant strains quite slow at reproduction. This is confirmed by stochastic simulations run starting from a configuration with no mutants at the beginning: in typical cases the mutants take years to reach equilibrium (which is another reason why drug-resistant strains are seldom observed before therapy).

Moreover, we want to stress that our result are robust with respect to the arbitrary parameters. Indeed we checked with simulations also $q_3 = 10^{-6}$, $\eta_A = 0.25$, $\eta_B = 0.25$ (one change at a time) and got the same qualitative results (what changed was the actual time of detection of type 3, not which was the best therapy).

It is worth noting that, even if we were forced to make some arbitrary parameter choices and the model is not fitted to any data (which is intrinsic in the problem itself since we want to address unknown variants of the HBV), nevertheless the order of magnitude of the time before drug resistance that we obtained is in accordance with the time observed in case trials. For instance in [30, Table 1] many patients report, with different drugs, resistance after a treatment period ranging from 3 to 6 years.

The biological and clinical importance of this model is to show a clear advantage in favor of the combination strategy, as it could lead to a greater delay in the development of drug-resistant variants, which have less fitness than the wild type (drug sensitive) strain, but are able eventually to escape from the therapy. The analysis of the model also puts emphasis on the need of an early antiviral therapy.

By the way, there is also clinical evidence that the best choice is a broad spectrum early therapy: for instance in [56] the authors observed that the emergence of entecavir-resistant strains is more likely to appear in patients which were already lamivudine-resistant (rather than in treatment-naive patients).

Another important point in favor of this regimen in the clinical practice is that the use of a combination therapy could allow treating physicians to retrieve older and less expensive molecules in developing countries, which truly are the settings where hepatitis B is most prevalent. In addition, this approach could be a valid alternative in case of toxicity/intolerance to newer drugs,

such as for people with renal diseases unable to take tenofovir (which to date appears to be the most promising drug in HBV therapy).

Concerning the type of combination therapy, ideally the best combination therapy for HBV infection should consider the association between a *high power* drug and a *high genetic barrier* drug. Power is the speed with which a drug causes the suppression of viral replication hence it is proportional to the efficacy parameter ε introduced in Section 4). The genetic barrier represents the number of mutations needed by the virus to replicate effectively in the presence of the therapy: the higher is the genetic barrier of a drug, or of a combination regimen, the smaller set of mutants are resistant to the treatment. Of course, nowadays entecavir or tenofovir are molecules which are both powerful and show a low incidence of mutations. Indeed, after 4 years of treatment [30] only 1.2% and 0% of the patients respectively, developed drug-resistance. Nevertheless, in principle sooner or later drug-resistant mutants may appear with any drug, which is one argument in favour of combination therapy even with these drugs at hand. Recent results show the appearance of drug resistance in coinfected HBV-HIV patients, treated with tenofovir ([38]). The authors identify in poor compliance to therapy the reason of this failure; it is our opinion that this is another aspect to keep in mind.

In conclusion, we suggest to intervene in the natural history of HBV infection immediately and with a broad-spectrum approach: a combination of powerful and high genetic barrier drugs. We believe that combination therapy is less likely to select resistant strains, especially in patients with a suboptimal compliance.

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