Optimal Drug Synergy in Antimicrobial Treatments

Citation

Published Version
doi:10.1371/journal.pcbi.1000796

Permanent link
http://nrs.harvard.edu/urn-3:HUL.InstRepos:4553093

Terms of Use
This article was downloaded from Harvard University’s DASH repository, and is made available under the terms and conditions applicable to Other Posted Material, as set forth at http://nrs.harvard.edu/urn-3:HUL.InstRepos:dash.current.terms-of-use#LAA

Share Your Story
The Harvard community has made this article openly available. Please share how this access benefits you. Submit a story.

Accessibility
Optimal Drug Synergy in Antimicrobial Treatments

Joseph Peter Torella¹,², Remy Chait³, Roy Kishony¹,³*

¹ Department of Systems Biology, Harvard Medical School, Boston, Massachusetts, United States of America, ² Department of Physics, University of Oxford, Oxford, United Kingdom, ³ School of Engineering and Applied Sciences, Harvard University, Cambridge, Massachusetts, United States of America

Abstract

The rapid proliferation of antibiotic-resistant pathogens has spurred the use of drug combinations to maintain clinical efficacy and combat the evolution of resistance. Drug pairs can interact synergistically or antagonistically, yielding inhibitory effects larger or smaller than expected from the drugs’ individual potencies. Clinical strategies often favor synergetic interactions because they maximize the rate at which the infection is cleared from an individual, but it is unclear how such interactions affect the evolution of multi-drug resistance. We used a mathematical model of in vivo infection dynamics to determine the optimal treatment strategy for preventing the evolution of multi-drug resistance. We found that synergy has two conflicting effects: it clears the infection faster and thereby decreases the time during which resistant mutants can arise, but increases the selective advantage of these mutants over wild-type cells. When competition for resources is weak, the former effect is dominant and greater synergy more effectively prevents multi-drug resistance. However, under conditions of strong resource competition, a tradeoff emerges in which greater synergy increases the rate of infection clearance, but also increases the risk of multi-drug resistance. This tradeoff breaks down at a critical level of drug interaction, above which greater synergy has no effect on infection clearance, but still increases the risk of multi-drug resistance. These results suggest that the optimal strategy for suppressing multi-drug resistance is not always to maximize synergy, and that in some cases drug antagonism, despite its weaker efficacy, may better suppress the evolution of multi-drug resistance.

Introduction

As antibiotic-resistant pathogens become more common, clinicians increasingly turn to multi-drug treatment to control infection [1–5]. The inhibitory effect of two drugs in combination can be larger or smaller than expected from their individual effects, corresponding to synergetic or antagonistic interactions between the drugs respectively [6–9]. Synergistic interactions are usually thought of as advantageous since, for a given amount of drug, they more effectively inhibit the growth of drug-sensitive pathogens. However, in vitro studies have suggested that, for the same level of inhibition, more synergistic drug pairs may foster antibiotic resistance [10–12]. Antagonistic drug combinations, on the other hand, are less effective at inhibiting drug-sensitive pathogens, but can reduce and even invert the selective advantage of single-drug resistant mutants, causing selection against resistance [13].

These recent observations point to a possible tradeoff in the choice of synergetic versus antagonistic drug combinations with respect to their effects on treating infection and suppressing antibiotic resistance. However, while antagonistic drug combinations increase selection against resistance, and should therefore minimize resistance, they also kill the infection more slowly, giving resistance more time to emerge. Antagonism therefore has two contradicting effects on the evolution of resistance: on one hand, it increases the risk of resistance by decreasing antibiotic inhibition and allowing more time for resistance to evolve; on the other hand, it decreases the risk of resistance by decreasing the selective advantage of single drug resistant mutants. We ask which of these opposing effects is stronger, and therefore which type of drug interaction – synergetic or antagonistic – best prevents the overall chance of emergence of multi-drug resistance.

We frame this problem in the context of a clinical infection, formalizing the two main factors in the success of an antibiotic treatment as “treatment efficacy” and “prevention of multi-drug resistance.” Treatment efficacy is the rate at which the infection is cleared by the treatment, and can be defined as the reciprocal of the time, \( t_{\text{clear}} \), at which the total infection is eliminated, \( \frac{1}{t_{\text{clear}}} \). Prevention of multi-drug resistance is defined as the reciprocal of the number of double-drug resistant mutants expected to arise during the course of treatment, \( \frac{1}{N_{\text{double}}} \). In real infections, multi-drug resistance can arise either through a single mutation conferring cross-resistance to both drugs simultaneously, or through the sequential acquisition of mutations conferring resistance to each drug individually [10,14–16]. Furthermore, resistance to a single drug can develop in several small steps or in one large step [13,17–19]. For simplicity, and to emphasize the role of drug interactions, we concentrate here on an idealized case in which resistance to the two-drug combination evolves through sequential acquisition of two spontaneous mutations, each conferring strong resistance specific to one of the two antibiotics (Fig. 1).

We asked what level of drug interaction (ranging from strong synergy to strong antagonism) maximizes treatment efficacy \( (\frac{1}{t_{\text{clear}}}) \) and prevention of multi-drug resistance \( (\frac{1}{N_{\text{double}}}) \). Maximizing \( \frac{1}{t_{\text{clear}}} \) is straightforward: as more synergetic drug...
pairs have increased killing potency and clear the infection more quickly, maximally synergistic drug pairs should maximize $1 / t_{\text{clear}}$ [4,20]. In attempting to maximize $1 / N_{\text{double}}$, however, the best choice of drug interaction is less clear. Assuming sequential acquisition of resistance to each drug, the rate at which multi-drug resistance arises will depend on the size of the single-drug resistant mutant population. The size of this single-mutant population, in turn, depends on two factors: the rate at which such mutants arise, and their selective advantage over the wild-type. Synergistic drug pairs decrease the first factor because they more quickly kill the source wild-type population from which single mutants arise. However, synergistic drug pairs also increase the second factor: single-drug resistant mutants will have a strong selective advantage in a synergistic treatment because resistance removes both the burden of one drug, and its enhancing effect on the other drug [13] (Fig. 1B). Synergistic drug pairs therefore decrease the rate at which single-drug resistant mutants appear, but increase their selective advantage. Antagonistic drug pairs do the opposite: though they allow a larger number of single-drug resistant mutants to arise, they also diminish the selective advantage of these mutants. The net effect of a given drug interaction on the evolution of multi-drug resistance is therefore not obvious, and requires a quantitative model to determine the overall impact of mutation and selection's countervailing effects.

To better understand how drug interactions affect the risk of multi-drug resistance, we used a population genetic model of microbial infection previously applied to predict single-drug resistance in vivo in mice [21], and modified it to account for the sequential acquisition of mutations leading to multi-drug resistance. We used this model to ask what level of drug interaction maximizes treatment efficacy ($1 / t_{\text{clear}}$) and prevention of multi-drug resistance ($1 / N_{\text{double}}$).

**Results**

**Simple model for the evolution of resistance in multi-drug treatment**

We based our model on work by Jumbe et al. [21], which investigated a mouse-thigh *P. aeruginosa* infection model [22–24] and provided a mathematical model that quantitatively described the relationship between exposure to the fluoroquinolone antibiotic levofloxacin, and changes in drug-susceptible and drug-resistant bacterial subpopulations over time. This mathematical model was successful both in reproducing the observed changes in drug-susceptible and resistant subpopulations over time, and in predicting the dose of levofloxacin needed to suppress amplification of levofloxacin-resistant (efflux-pump-expressing) mutants. To investigate the effect of antibiotic interactions on...
treatment efficacy and the prevention of multi-drug resistance in a simple scenario, we modified the Jumbe et al. model in four ways: we include a second antibiotic in our model; we assume a constant antibiotic dose; we assume a low hill coefficient, consistent with the mechanisms of a range of antibiotics [25]; and we assume no cost for antibiotic resistance. The consequences of these assumptions are discussed throughout the text.

Our model incorporates treatment with two antibiotics, A and B. It uses a set of ordinary differential equations (ODEs) to follow the population sizes of the drug-sensitive wild-type strain ($N_{WT}$), the total single-drug resistant population ($N_{single}$), the total single-drug resistant population ($N_{AR}$), and the expected number of multi-drug resistant mutants ($N_{double}$) arising over time (Fig. 1A; Methods):

$$N_{WT} = N_{WT}[G - K(D_{WT})] - 2\mu GN_{WT}$$

$$N_{single} = N_{single}[G - K(D_{single})] + 2\mu GN_{single} - \mu GN_{single}$$

$$N_{double} = \mu GN_{single}$$

Populations are affected by growth, antibiotic killing and mutation, where $G$, $K$ and $\mu$ are the growth rate, antibiotic killing rate and frequency of resistance mutations per generation, respectively, and $D_{WT}$ and $D_{single}$ are the effective doses of antibiotic felt by the wild-type and single-drug resistant mutant populations. We assume for simplicity that antibiotic resistance imposes no fitness cost, so that the growth rates of the sensitive and resistant populations are the same. To account for competition for resources, we assume this growth rate is given by the logistic equation,

$$G = g\left(1 - \frac{N_{tot}}{N_{max}}\right),$$

where $g$ is the maximal growth rate, $N_{tot} = N_{WT} + N_{single}$ is the total population size, and $N_{max}$ is the maximal carrying capacity (Fig. S1B). This competition for resources was included in the in vivo murine infection model [21], and has been observed in or inferred from a range of infections [26], including S. pneumoniae [27], and Methicillin-Resistant S. aureus (MRSA) [28].

While we assume the growth rates of the wild-type and single-drug resistant mutants are the same, the rates $K$ at which they are killed by antibiotic are different and depend on the effective antibiotic dose, $D$, felt by each population:

$$K(D) = \frac{k}{1 + D^{-H}}$$

where $k$ is the maximal killing rate and $H$ is the Hill coefficient, which determines the steepness of the killing rate as a function of drug dose. In contrast to Jumbe et al., we set $H = 1$, which is representative of many common antibiotics [25], although different values of $H$ give rise to similar overall model behavior (Fig. S2). The effective drug dose, $D$, depends on the dosage of the two drugs and on their interaction (Fig. 1B, Text S1, Fig. S1A). For simplicity we assume both drugs are administered at the same dose, $D$, defined in units of their minimum inhibitory concentra-

**Antibiotic interactions create a saturable tradeoff between treatment efficacy and prevention of multi-drug resistance**

To determine the impact of drug interaction on treatment outcome, we first looked at the differences in treatment efficacy ($1/t_{clear}$) and prevention of multi-drug resistance ($1/N_{double}$) over a range of drug interaction values ($\epsilon = -1.5$ to $1.5$) while holding drug dosage fixed (Fig. 2, $D = 7$ MIC). We observed two distinct and robust (Fig. S2) behaviors, depending on whether $\epsilon$ falls above or below a critical value, $\epsilon^*$ (Fig. 2; for the parameters used, $\epsilon^* \approx 0.14$).

For $\epsilon < \epsilon^*$, we observed a tradeoff between treatment efficacy and prevention of resistance. In this regime, increasing synergy yields greater $1/t_{clear}$ (Fig. 2, unshaded region); this is expected, as increasing the synergistic interaction between the drugs kills the wild-type more quickly. Despite faster infection clearance, however, greater synergy actually decreases $1/N_{double}$; namely, it increases the risk of multi-drug resistance. Conversely, more antagonistic drug pairs increase $1/N_{double}$, albeit at the expense of reduced efficacy.

This tradeoff between efficacy and prevention of resistance breaks down at a critical threshold, $\epsilon^*$, above which increasing synergy no longer increases $1/t_{clear}$, but still decreases $1/N_{double}$ (Fig. 2, shaded region). Above this “synergy ceiling,” further increasing synergy therefore has only undesirable effects, since it
increases the risk of multi-drug resistance without increasing efficacy. Optimal drug pairs for treating the given infection must therefore have a level of drug interaction lower than \( \varepsilon^* \) and, due to the tradeoff between \( 1/t_{\text{clear}} \) and \( N_{\text{double}} \), the optimal value of \( \varepsilon \) will depend on the relative importance assigned to these two conflicting goals.

The frequency of resistance mutations determines the "synergy ceiling" \( \varepsilon^* \)

To understand what determines the level of the synergy ceiling, \( \varepsilon^* \), we ask what causes the transition from tradeoff behavior at \( \varepsilon < \varepsilon^* \), to plateau behavior at \( \varepsilon > \varepsilon^* \) (Fig. 2). Due to the steep biphasic behavior of efficacy (\( 1/t_{\text{clear}} \)) around \( \varepsilon^* \), we looked to population time courses to determine how the time of clearance, \( t_{\text{clear}} \), was affected by drug interactions below, at or above \( \varepsilon^* \) (\( \varepsilon = -1.5, 0, 1, 1.5 \), respectively; Fig. 3A). For \( \varepsilon < \varepsilon^* \) (Fig. 3A, top), the wild-type subpopulation outlives the single-drug resistant mutants and \( t_{\text{clear}} = t_{\text{WT}} \). Since wild-type killing is stronger for more synergistic drug pairs, increasing \( \varepsilon \) decreases \( t_{\text{clear}} \) and, explaining why efficacy increases with \( \varepsilon \) in this region (Fig. 2, unshaded region). For \( \varepsilon > \varepsilon^* \) (Fig. 3A; bottom), the wild-type is eliminated before the single-mutant population, and \( t_{\text{clear}} = t_{\text{single}} \). Because the killing rate of the single-drug resistant mutants is independent of \( \varepsilon \), \( t_{\text{clear}} \) becomes effectively independent of \( \varepsilon \), causing \( 1/t_{\text{clear}} \) to plateau for \( \varepsilon > \varepsilon^* \) (Fig. 2, shaded region). \( \varepsilon^* \) is therefore the level of drug interaction for which wild-type and single-mutant populations are cleared simultaneously (\( t_{\text{WT}} = t_{\text{single}} \); Fig. 3A, middle).

Since \( \varepsilon^* \) represents the level of drug interaction for which \( t_{\text{clear}} = t_{\text{WT}} \), parameters that differentially alter \( t_{\text{clear}} \) and \( t_{\text{WT}} \) will alter \( \varepsilon^* \). While we found that a number of model parameters had some effect on \( \varepsilon^* \) (Fig. S2), the strongest effect was due to changes in the frequency of resistance mutations, \( \mu \). \( \mu \) differentially affects \( t_{\text{WT}} \) and \( t_{\text{clear}} \) because, although it has virtually no effect on \( t_{\text{WT}} \), the single-mutant population size at the onset of treatment increases linearly with \( \mu \), \( N_{\text{single}} = 2\mu N_0 \), thereby increasing \( t_{\text{clear}} \). For \( t_{\text{clear}} \) to match this increase in \( t_{\text{clear}} \), the wild-type killing rate must decrease; namely, \( \varepsilon^* \) must be reduced. We therefore expected \( \varepsilon^* \) to decrease with increasing \( \mu \) and, indeed, increasing the frequency of resistance mutations gave rise to consistent decreases in \( \varepsilon^* \) (Fig. 3B). Interestingly, for high frequencies of resistance (\( \mu > 2 \times 10^{-6} \)) the synergy ceiling \( \varepsilon^* \) falls below zero (representing an antagonistic drug interaction); in this case mildly synergistic and even additive interactions fall in the undesirable regime where the risk of multi-drug resistance increases without any corresponding gain in treatment efficacy.

**Competition for resources underlies the tradeoff between treatment efficacy and prevention of multi-drug resistance**

Why does synergy, despite clearing the infection faster, increase the risk of multi-drug resistance (Fig. 2)? Since synergistic drug pairs clear the infection more quickly than antagonistic drug pairs, the rate at which they generate double mutants must also be higher. The overall rate at which double mutants arise, \( N_{\text{double}} = \mu g N_{\text{single}} (1 - N_{\text{WT}} / N_{\text{max}}) \), is affected by two variables: it increases with the size of the single-mutant population, \( N_{\text{single}} \), and decreases with total population size, \( N_{\text{tot}} \), due to the inhibitory effect of resource limitation on growth and mutation (Fig. 4A). The total number of double mutants expected to arise is simply the integral of this instantaneous rate over the treatment course. In order to determine why synergistic treatments increase \( N_{\text{double}} \), we therefore analyzed the trajectories of synergistic and antagonistic treatments through the space of \( N_{\text{single}} \) versus \( N_{\text{tot}} \) (Fig. 4A; \( \varepsilon = 1.5 \), solid line, \( \varepsilon = -1.5 \), dashed line). The initial slopes of these trajectories (Fig. 4A, arrows) are determined by the relative fitness of the wild-type and single-drug resistant populations under antibiotic treatment. The synergistic treatment selects strongly against the wild-type population, producing a trajectory with a steep slope that drives treatment into a region of high \( N_{\text{double}} \). In fact, for \( \varepsilon = 1.5 \), solid line, \( \varepsilon = -1.5 \), dashed line), the rate of increase in \( N_{\text{double}} \) is therefore the level of drug interaction for which wild-type and single-mutant populations are cleared simultaneously (\( t_{\text{clear}} = t_{\text{WT}} \); Fig. 3A, middle).

Since \( \varepsilon^* \) represents the level of drug interaction for which \( t_{\text{clear}} = t_{\text{WT}} \), parameters that differentially alter \( t_{\text{clear}} \) and \( t_{\text{WT}} \) will alter \( \varepsilon^* \). While we found that a number of model parameters had some effect on \( \varepsilon^* \) (Fig. S2), the strongest effect was due to changes in the frequency of resistance mutations, \( \mu \). \( \mu \) differentially affects \( t_{\text{WT}} \) and \( t_{\text{clear}} \) because, although it has virtually no effect on \( t_{\text{WT}} \), the single-mutant population size at the onset of treatment increases linearly with \( \mu \), \( N_{\text{single}} = 2\mu N_0 \), thereby increasing \( t_{\text{clear}} \). For \( t_{\text{clear}} \) to match this increase in \( t_{\text{clear}} \), the wild-type killing rate must decrease; namely, \( \varepsilon^* \) must be reduced. We therefore expected \( \varepsilon^* \) to decrease with increasing \( \mu \) and, indeed, increasing the frequency of resistance mutations gave rise to consistent decreases in \( \varepsilon^* \) (Fig. 3B). Interestingly, for high frequencies of resistance (\( \mu > 2 \times 10^{-6} \)) the synergy ceiling \( \varepsilon^* \) falls below zero (representing an antagonistic drug interaction); in this case mildly synergistic and even additive interactions fall in the undesirable regime where the risk of multi-drug resistance increases without any corresponding gain in treatment efficacy.
function of \( N_{\text{single}} \) alone. Because synergistic drug pairs better limit \( N_{\text{single}} \) by quickly killing the wild-type population from which single mutants arise, they therefore also better limit multi-drug resistance in cases of weak competition. Indeed, this advantage of synergy disappeared entirely when we artificially turned off wild-type to in infections frequently exhibiting competition, due to a scarcity of resources, thereby preventing the emergence of multi-drug resistance. Whereas antagonistic drug pairs kill bacterial populations more slowly, and therefore allow more time for resistance to emerge, they also decrease the selective advantage of resistant mutants. Which of these two opposing effects of antagonism dominates in determining its overall impact on the chance of evolving multi-drug resistance? Framing this problem in the context of a clinical infection, we asked how two measures of treatment outcome, treatment efficacy and prevention of multi-drug resistance, depend on drug interaction.

We found that the optimal drug interaction can be determined primarily as a function of two infection parameters: population size at the outset of treatment, and the frequency of resistance mutations (see summary of our results in Fig. S3). For clinically relevant scenarios where initial population sizes are well below the carrying capacity, competition for resources is weak and synergy, which is typically preferred in clinical settings for its superior treatment efficacy [3,4,20], is also expected to best prevent the emergence of multi-drug resistance.

Where resource competition is significant, however, strong synergy may not always be the optimal treatment strategy. Real infections frequently exhibit competition, due either to a scarcity of

**Figure 3.** The synergy ceiling \( \varepsilon^* \) is determined by clearance of the wild-type population before the single-mutant subpopulation. (A) Population sizes of the wild-type \( (N_{\text{WT}}, \text{black}) \) and single-drug resistant mutants \( (N_{\text{single}}, \text{blue}) \) over treatment courses with levels of interaction below, at or above the critical value \( \varepsilon^* \) \( (\varepsilon = -1.5, 0.14, 1.5) \). Populations start with sizes \( N_{\text{WT}}^0, N_{\text{single}}^0 \) and are killed by antibiotics until they are cleared at times \( t_{\text{clear}}^{\text{WT}}, t_{\text{clear}}^{\text{single}} \) respectively; the overall time of clearance of the infection is simply \( t_{\text{clear}} = \max \left( t_{\text{clear}}^{\text{WT}}, t_{\text{clear}}^{\text{single}} \right) \) (orange markers). The interaction level \( \varepsilon \) affects the order in which the wild-type and the single-drug resistant subpopulations are eliminated: below the synergy ceiling \( (\varepsilon < \varepsilon^*, \text{top}) \), the wild-type is eliminated after the single-drug resistant mutant and \( t_{\text{clear}} = t_{\text{clear}}^{\text{WT}} \); at the synergy ceiling \( (\varepsilon = \varepsilon^*, \text{middle}) \), the two populations die simultaneously and \( t_{\text{clear}} = t_{\text{clear}}^{\text{WT}} = t_{\text{clear}}^{\text{single}} \); above the synergy ceiling \( (\varepsilon > \varepsilon^*, \text{bottom}) \), the single-drug resistant mutant outlives the wild-type, such that \( t_{\text{clear}} = t_{\text{clear}}^{\text{single}} \). Because increasing \( \varepsilon \) increases the wild-type killing rate but has no effect on the single-mutant killing rate, efficacy increases with \( \varepsilon \) below the synergy ceiling \( (t_{\text{clear}} = t_{\text{clear}}^{\text{WT}} \text{, but plateaus at and above it (} t_{\text{clear}} = t_{\text{clear}}^{\text{single}} \text{, vertical dashed line: notice that } t_{\text{clear}} = t_{\text{clear}}^{\text{single}} \text{ is the same both at and above the synergy ceiling). (B) Increased mutation rates, \( \mu \), give rise to lower \( \varepsilon^* \). Inset: treatment efficacy, \( 1/t_{\text{clear}} \text{, plateaus at lower levels of drug interaction } \varepsilon \text{ for higher mutation rates } (\mu = 10^{-7}, \text{blue;} \mu = 10^{-6}, \text{orange;} \mu = 10^{-5}, \text{green; blue and green lines are shifted slightly along y-axis for clarity); } \varepsilon^* \text{ values for each line are indicated by vertical dashed lines, and by circles in the main panel. Orange markers indicate the treatment efficacy achieved for different values of } \varepsilon \text{ when } \mu = 10^{-6}, \text{corresponding to the } t_{\text{clear}} \text{ values in panel A. doi:10.1371/journal.pcbi.1000796.g003.} \)
carbon or iron [26,31,32], or saturation of available adhesion sites (e.g. in biofilm formation [33,34]). In our model, such competition is predicted to give rise to a tradeoff between treatment efficacy and resistance prevention: increased synergy leads to greater efficacy, but at the expense of an increased risk of multi-drug resistance. Importantly, this tradeoff saturates for levels of synergy greater than a critical value \( e^*/C^3 \), above which greater synergy does not further increase efficacy, but still increases the risk of multi-drug resistance. If the goal is to minimize multi-drug resistance, then choosing drug interactions above this “synergy ceiling” may be counterproductive. This is especially important given the dependence of \( e^*/C^3 \) on the frequency of resistance mutations: our model predicts that for infections where resistance rates are high (\( N_0^0 \approx 2 \times 10^6 \)) \( e^*/C^3 \) may be negative (antagonistic), favoring the use of antagonistic drug pairs over mildly synergistic or even purely additive antibiotic combinations. Indeed, for the modified Jumbe model, such competition is predicted to give rise to a tradeoff between treatment efficacy and resistance prevention: increased synergy leads to greater efficacy, but at the expense of an increased risk of multi-drug resistance.
et al. model that we study, ε* is nearly additive; and while the resistance frequency we use may be an overestimate (Jumbe et al. determined this as the rate of all mutations conferring only a 3-fold increase in the MIC), these and higher mutation rates have been identified in human pathogens [35,36]. Together, the potential for strong competition and high mutation rates in infection suggest that the tradeoff and synergy ceiling behaviors observed in our model – as well as the ability of antagonistic drug pairs to minimize multi-drug resistance – may describe the properties of some clinical infections.

We emphasize that drawing concrete therapeutic conclusions from this study would be beyond its scope. Our model incorporates many simplifying assumptions: we assume ε* to be a fixed value, although it has been observed to change with both the absolute and relative doses of the antibiotics administered [37,38]; drug administration and pharmacokinetics are not considered, although they may significantly impact the evolution of resistance [23,39–42]; resistance mutation rates per generation are assumed to be independent of growth and antibiotic-killing rates; and while we consider an idealized case in which multi-drug resistance arises from strong, sequential mutations conferring resistance to each antibiotic, real mutations may confer cross-resistance to both drugs simultaneously, or only partial resistance to a single drug [10,14,16]. One consequence of partial resistance is antibiotic killing of drug-resistant mutants for drug interactions above ε*; while for strong resistance this killing would be minimal, weak resistance may allow strong killing to undermine synergy ceiling behavior (Fig. S4). Finally, we note that this model does not consider the impact of host immune defenses, which may substantially impact microbial growth and death rates in clinical infections [43,44]; whether the influence of host defenses favors the use of some drug combinations over others, however, remains to be seen.

While these caveats indicate the limitations of this simple model and suggest important avenues for future study, our results make a number of novel predictions about the relationship between drug interaction and multi-drug resistance: that there exist conditions under which antagonistic drug pairs may better prevent multi-drug resistance despite their weaker efficacy; that there is a synergy ceiling to how much efficacy can be achieved by modulating drug dose with smaller or larger drug doses, respectively. All model simulations fall on the dashed line, where drug doses are equal: Da = Db. (B) Logistic growth model. As the population size, Ntot increases, competition causes the growth rate, G, to fall from its maximal value, g, to 0 at the carrying capacity, Nmax (Eq. 4). Unless otherwise indicated, in model simulations Ntot = Nmax at the outset of treatment (black circle).

Found at: doi:10.1371/journal.pcbi.1000796.s001 (0.11 MB TIF)

**Figure S2** Prevention of resistance, and the synergy ceiling ε*, are robust to changes in model parameters. To test the robustness of the model to parameter changes, we varied each parameter independently and measured its effect on both the relative ability of strongly synergistic and antagonistic drug pairs (ε = 1.5, –1.5) to prevent multi-drug resistance, σsyn/σant (solid black line), and the level of the synergy ceiling ε* (solid blue line). All lines have undergone 5-point smoothing. Dashed lines indicate the points at which synergistic and antagonistic drug pairs prevent resistance equally well (σsyn/σant = 1, black), or the synergy ceiling is additive (ε* = 0, blue). In each panel, those points corresponding to the original set of model parameters are indicated by circles. (A) σsyn/σant and ε* vary little with changes in drug dose D, (B) carrying capacity Nmax, or (C) maximal growth rate g. (D) As previously discussed, increases in the frequency of resistance mutations µ decrease ε* substantially (Fig. 3), while having no significant effect on σsyn/σant. (E) Likewise, increases in the initial population size, N0, significantly decrease σsyn/σant (Fig. 4), but also decrease ε*. (F) Changes in the Hill coefficient, H, of antibiotic killing had a more complex effect on σsyn/σant and ε*; while σsyn/σant was consistently less than 1 over a wide range of H (antagonism better prevents resistance), its magnitude was parabolic with H, with antagonistic drug pairs having the greatest advantage for \( H \approx 3 \). ε* also appeared parabolic with H and was lowest (most antagonistic) at \( H \approx 2 \). In the limit of large H, maximal antibiotic killing rates are achieved for both wild-type and single-drug resistant populations, regardless of drug interaction. Synergistic and antagonistic drug pairs therefore fail to differentially impact wild-type killing rates, and σsyn/σant = 1 at

**Methods**

**Model details**

Our model consists of 3 ODEs (Eq. 1–3) describing the population sizes of the wild-type and single-drug resistant mutants (NWT, NWT), as well as the number of double mutants expected to arise during a treatment course (Ndouble). Parameter values for this model include first-order maximal growth (g) and death rate (k) constants, carrying capacity Nmax and mutation rate µ (per individual per generation), which were taken from the in vivo murine model investigated in Jumbe et al. [21] (Table S1). Initial population sizes (NWT, NWT) were determined by assuming that, prior to treatment, the infections grew from a single cell to the initial population size N0 while mutating, such that NWT = \( 2\mu N_{tot} \) and NWT = \( (1-2\mu)N_{tot} \); unless otherwise indicated, NWT = Nmax. ODEs were solved in MATLAB (Version 7.1, MathWorks, Natick, MA) using a built-in, numerical ODE solver (ODE45). To avoid artifacts associated with using continuous ODEs to describe finite populations, each step was modified with the assumption that the wild-type or single-drug resistant population is eliminated (size decreases to zero) if its size drops below one.

**Supporting Information**

**Figure S1** Models of drug interaction and logistic growth. (A) Model of drug interaction. The effective drug dose for the wild-type strain, DWT, is a function of three variables: the doses of drugs A and B (DA, DB) and the interaction parameter ε (Text S1). Isolates of the wild-type effective dose (DWT = 1 MIC), are shown for additive (ε = 0, black), synergistic (ε > 0, red) and antagonistic (ε < 0, blue) drug pairs. While for additive drug pairs the effective dose is a simple sum of the drugs’ individual doses, synergistic or antagonistic drug pairs achieve the same effective dose with smaller or larger drug doses, respectively. All model simulations fall on the dashed line, where drug doses are equal: DA = DB. (B) Logistic growth model. As the population size, Ntot increases, competition causes the growth rate, G, to fall from its maximal value, g, to 0 at the carrying capacity, Nmax (Eq. 4). Unless otherwise indicated, in model simulations Ntot = Nmax at the outset of treatment (black circle).

![Figure S1](https://www.ploscompbiol.org/figure/S1)

![Figure S2](https://www.ploscompbiol.org/figure/S2)
high $H$ (black line). Furthermore, saturation of killing rates causes $t_{clear}^{opt}$ to be greater than $t_{clear}^{single}$ for all values of $\varepsilon$, $\varepsilon^*$ is therefore undefined for $H > 3$ (blue line), though saturation of the wild-type killing rate still causes efficacy to effectively plateau for low values of $\varepsilon$.

Found at: doi:10.1371/journal.pcbi.1000796.s002 (0.37 MB TIF)

**Figure S3** Optimal drug interactions as a function of resistance frequency and initial population size. The contour map shows the synergy ceiling, $\varepsilon^*$, for a given combination of resistance mutation frequency, $\mu$, and population size at the start of treatment, $N_{tot}$. $\varepsilon^*$ decreases monotonically with increasing $\mu$ (as in Fig. 3), but is nearly unaffected by $N_{tot}$. The black line is a single contour above which antagonistic drug pairs prevent multi-drug resistance better than synergistic drug pairs ($\sigma_{syn}/\sigma_{ant} < 1$). Above this contour, greater synergy increases the chance of multi-drug resistance; the optimal drug interaction must therefore fall below $\varepsilon^*$, with its specific value depending on the priority assigned to treatment efficacy versus prevention of multi-drug resistance. Below the contour, however ($\sigma_{syn}/\sigma_{ant} > 1$), greater synergy decreases the chance of multi-drug resistance; the optimal drug interaction is therefore maximal synergy (region below the black contour is colored dark red), regardless of $\varepsilon^*$. The magenta circle indicates the combination of $\mu$ and $N_{tot}$ corresponding to the original set of model parameters.

Found at: doi:10.1371/journal.pcbi.1000796.s003 (0.10 MB TIF)

**Figure S4** Partial antibiotic resistance weakens synergy ceiling behavior. In the model we assume strong antibiotic resistance, such that the antibiotic-resistant subpopulation feels the effect of only a single drug; effectively, this makes the MIC of the drug to which it is resistant infinite and produces the familiar synergy ceiling, in which efficacy increases with $\mu$ up to a critical level $\mu^*$ and plateaus above it (blue line, Fig. 2; all lines have been shifted on the vertical axis for clarity). As previously discussed (Fig. 3), this plateau is due to the resistant subpopulation dying after the wild-type when $\varepsilon > \varepsilon^*$. When we weaken the assumption of strong resistance, however (MIC $< \mu^*$), drug interactions still affect drug-resistant mutants, even when such mutants are killed after the wild-type. This results in efficacy increasing over all $\varepsilon$, but retaining its characteristic biphasic profile (red, green, orange lines). This biphasic behavior is due to the stronger killing of the wild-type than the resistant mutant, which persists even with only partial resistance. While stronger resistance produces behavior similar to the typical synergy ceiling (MIC increases 100-fold, red line), weaker resistance (MIC increases 4-fold, orange line) yields a still-biphasic curve, but one in which increases in $\varepsilon$ improve efficacy substantially in all cases. The synergy ceiling behavior is therefore most relevant in cases of strong antibiotic resistance.

Found at: doi:10.1371/journal.pcbi.1000796.s004 (0.08 MB TIF)

**Table S1** Parameters used in this study.

Found at: doi:10.1371/journal.pcbi.1000796.s005 (0.03 MB DOC)

**Table S1** Found at: doi:10.1371/journal.pcbi.1000796.s006 (0.02 MB DOC)

**Acknowledgments**

For helpful comments and discussion, we thank R. Jajoo, J.-B. Michel, R.C. Moellering, and R. Ward.

**Author Contributions**

Conceived and designed the experiments: JPT RC RK. Performed the experiments: JPT. Analyzed the data: JPT RC RK. Wrote the paper: JPT RK.

**References**