Benefits of using multiple first-line therapies against malaria

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Despite the availability of many drugs and therapies to treat malaria, many countries' national policies recommend using a single first-line therapy for most clinical malaria cases. To assess whether this is the best strategy for the population as a whole, we designed an evolutionary-epidemiological modeling framework for malaria and compared the benefits of different treatment strategies in the context of resistance evolution. Our results show that the population-wide use of multiple first-line therapies (MFT) against malaria yields a better clinical outcome than using a single therapy or a cycling strategy where therapies are rotated, either on a fixed cycling schedule or when resistance levels or treatment failure become too high. MFT strategies also delay the emergence and slow the fixation of resistant strains (phenotypes), and they allow a larger fraction of the population to be treated without trading off future treatment of cases that may be untreatable because of high resistance levels. Earlier papers have noted that cycling strategies have the disadvantage of creating a less temporally variable environment than MFT strategies, making resistance evolution easier for the parasite. Here, we illustrate a second feature of parasite ecology that impairs the performance of cycling policies, namely, that cycling policies degrade the mean fitness of the parasite population more quickly than MFT policies, making it easier for new resistant types to invade and spread. The clinical benefits of using multiple first-line therapies against malaria suggest that MFT policies should play a key role in malaria elimination and control programs.

drug resistance | epidemiology | evolution | treatment strategies | cost of resistance

Prompt treatment with effective antimalarials is a crucial element of malaria control, but the effectiveness of antimalarial drugs can be seriously compromised by the evolution of drug resistance in the *Plasmodium* spp. that cause malaria. The evolution and worldwide spread of resistance to chloroquine (CQ) over the past 50 years (1) and the subsequent failure of sulfadoxine-pyrimethamine (SP) (1, 2) have created a crisis for many African nations and other malaria-endemic countries (3). A number of these countries have adopted the highly effective artemisinin-based combination therapies (ACTs) as their first-line therapy for uncomplicated malaria, but ACTs are not yet widely available. Extensive resistance has yet to be detected to artemisinin drugs, but there is concern that resistance could emerge and spread rapidly (4). Efforts to delay resistance to artemisinin have included a call to end production of artemisinin monotherapies and plans to subsidize ACTs to displace artemisinin monotherapies (5). As ACTs become available and affordable in the postchloroquine era, there will be a need to adopt sustainable treatment strategies that will further extend the useful therapeutic life of these and other new antimalarials.

Current and historical practice in Africa has been to recommend a single first-line therapy or drug (we use the terms interchangeably) for uncomplicated malaria and, when drug-resistance levels rise, to replace the officially recommended drug with a new one to which resistance has not yet emerged. In Kenya, for example, CQ was replaced by SP as the first-line treatment in 1998 and SP by artemether-lumefantrine (an ACT) during 2004–2006 on the basis of high levels of resistance and treatment failure (6, 7). This "wait-and-switch" strategy burdens the surveillance networks and public health systems of developing countries and, as we show here, yields a suboptimal morbidity and mortality outcome. A more sustainable strategy would be to prospectively deploy existing antimalarials to minimize mortality and morbidity and delay resistance emergence and treatment failure for as long as possible.

In this study, we evaluate the use of multiple first-line therapies (MFT) in slowing the evolution of resistance. MFT is defined as a drug policy in which several therapies are made available in both the public and the private sectors, and patients and clinicians can choose which therapy to use. For most of the analysis presented here, we assume that the different first-line therapies are used in equal amounts in the host population; an important area of further study will be to determine how a particular drug-use distribution can be achieved in a given population. Just as combination therapies, wherein multiple drugs are coformulated into a single treatment, delay the evolution of resistance (8–15), deploying multiple therapies slows down resistance evolution compared with using a single therapy for the whole population. An MFT strategy with n drugs has two main benefits. First, it increases the variability of drugs in the parasite's environment, making it difficult for the parasite to adapt to any one part of the environment. If on every new infection, the parasite has a small chance of encountering the same drug it saw in its previous host, evolving resistance to any particular drug is difficult. Second, when using n drugs in equal amounts, the rate each drug is used is cut to $1/n$ of total use, thus reducing the overall selection pressure for resistance to that drug. Rationing treatment could achieve a similar reduction in selection pressure, but an MFT strategy reduces each drug's use without denying treatment. This must be balanced against the alternative of holding the drugs in reserve under a wait-and-switch policy; such comparisons have been done in the context of bacterial infections and antibiotic resistance (16–19), but malaria presents unique scientific and operational challenges. In making this comparison for malaria, we may be able to answer a question of historical interest: would CQ and SP still be useful if they had been deployed together? Today, the relevant policy question that requires attention is how to deploy the current ACTs to best effect.

We focus our analysis on the case when three different therapies are available, and we show that using more first-line therapies (*i*) significantly delays resistance emergence and treatment failure, (*ii*) slows the evolution of resistance, (*iii*) lowers the total clinical burden of malaria, and (*iv*) reduces the effect of the classic resistance-disease tradeoff, wherein treating a single case now and generating drug resistance may trade off with the ability to treat several cases in the future (20, 21). In addition, we show that MFT

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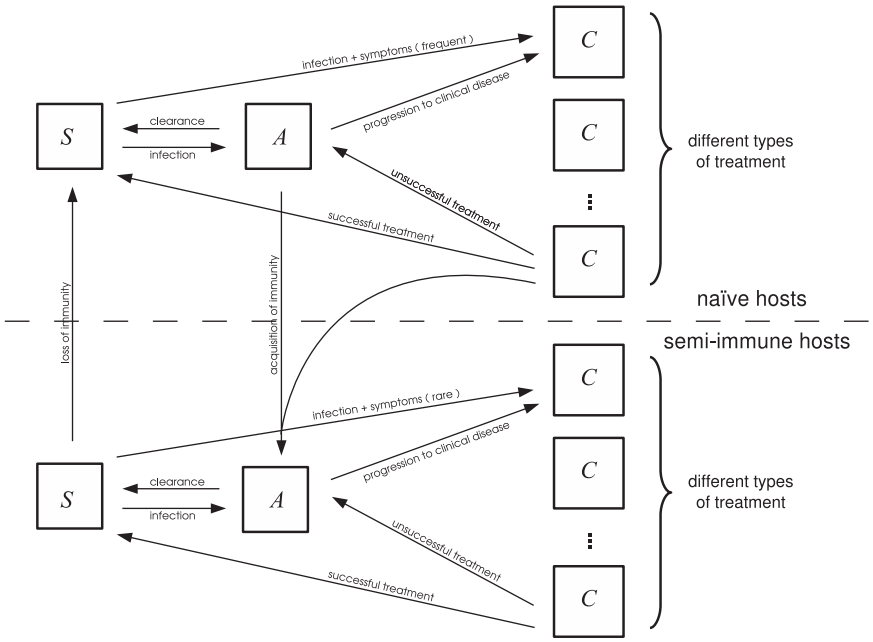

Fig. 1. Schematic of basic model dynamics; see *SI Appendix* for full equations. The model allows for multiple types of resistant strains to be circulating in the population; for simplicity, the model diagram shows the dynamics for only one strain. Susceptible individuals (S) can become asymptomatic (A) or symptomatic/clinical (C) after receiving an infectious bite from a mosquito. Unsuccessful treatment results from drug resistance; successful treatment occurs when the infecting parasites do not have resistance to the drug(s) being used. When hosts in the C classes are undergoing treatment, parasites can evolve *de novo* resistance to the drugs being used (not shown on diagram). The curved arrow indicates that hosts can acquire immunity as a result of clinical disease.

strategies have better clinical outcomes and delay treatment failure longer than the currently practiced strategies of switching among first-line therapies, whether the switching is done on a predetermined schedule or when treatment failures increase, and we reveal a new feature of parasite ecology that makes switching strategies disadvantageous in general.

Modeling and Theory

Basic ecological and epidemiological theory provides a sound rationale for using multiple first-line therapies. Below, we present the simplest general disease model that allows for treatment with n different drugs. Let x denote the number of susceptible individuals and y_i the number of infected individuals who are being treated with drug i; y_0 is the number of individuals who are treated with no drug. The dynamic equations

$$\dot{x} = \lambda - dx - x\sum_{j=0}^{n}\beta_j y_j + \sum_{j=0}^{n}\nu_j y_j$$

$$\dot{y}_i = f_i x\sum_{j=0}^{n}\beta_j y_j - (\nu_i + d)y_i \qquad [1]$$

describe a simple SIS-like model where λ is the immigration/birth of new susceptibles, d is the hosts' death rate, and f_i is the fraction of the population that receives drug i as treatment. The parameters β_j and ν_j are, respectively, the transmissibility and recovery rate of the disease in a host being treated with drug j. For a particular drug distribution described by the f_i, the pathogen will have basic reproductive number (22, 23):

$$R_0 = \frac{\lambda}{d}\sum_{j=0}^{n}\frac{f_j\beta_j}{\nu_j + d}. \qquad [2]$$

In a population of hosts where n drugs are used in equal amounts ($f_i = 1/n$ for $i > 0$), the basic reproductive number of a parasite that evolves resistance to drug 1 will be

$$R_0^* = \frac{\lambda}{d}\cdot\frac{1}{n}\cdot\frac{\beta_1}{\nu_1 + d}, \qquad [3]$$

which illustrates why increasing n, the number of drugs, is a good way of preventing the emergence and spread of resistance. When many drugs are used simultaneously in a population, resistant strains (phenotypes) can appear via mutation but will have a difficult time spreading and establishing because of their low R_0. In fact, if a particular threshold value were reached, $R_0^* < 1$ or $R_0^* < R_0^{\text{sens}}$, the resistant type would not be able to sustain a chain of transmission and would die out. The reduction in resistance spread by a factor of n can also be derived [see supporting information (SI) *Appendix*] in models that allow for multiple infections per host (24, 25) and for standard population-genetic models of malaria evolution (26). One of the earliest malaria population-genetic models, by Hastings (24), can be modified to include multiple therapies and, when either the number of therapies n is large enough or the fitness cost of resistance s is high enough, the equilibrium frequency of a resistant type settles to

$$\frac{f}{f + (1-f)ns}, \qquad [4]$$

where f is the fraction of malaria cases that are treated by some drug or therapy; $f = \Sigma_{j=1}^{n} f_j$. In the Hastings model, increasing n lowers the frequency of resistant phenotypes, an effect that is strongest when s is large and f is small (see *SI Appendix*).

In this article, we introduce a malaria-specific modeling framework, based on Eqs. **1**, that includes immunity, asymptomatic and symptomatic (clinical) infections, the evolution of multidrug resistance, a fitness cost for resistant strains, and *de novo* mutation by which parasites can acquire resistance. We assume no cross-resistance among the drugs. Hosts are divided into susceptible individuals (S), asymptomatically infected individuals (A), and infected individuals manifesting clinical disease (C); see Fig. 1 for a diagram of the model. Individuals in the C classes are infected with a particular strain (resistant to one or more drugs or sensitive to all drugs) and are treated by a particular drug. Hosts are either immunologically naïve or semi-immune; semi-immune hosts are 40% as infective as naïve hosts (27) and progress from an asymptomatic state to clinical disease less often than naïve hosts (28, 29). Untreated asymptomatic infections are cleared in 200 days on average for sensitive strains (30, 31) and more quickly for resistant strains, depending on how costly resistance is to the parasite; clinical episodes last a few days to a few weeks, depending on the effec-

tiveness of drug treatment (30). The full set of equations and parameter values for our model is available in *SI Appendix*.

We let our model settle to the drug-sensitive strain's stable endemic equilibrium in the absence of drug treatment, and we evaluate the outcomes of deploying drugs in different ways. Minimizing resistance alone is a meaningless objective, because that is best achieved by not using any drugs, but the total amount of illness or death prevented over a fixed time period is a consistent measure of success that captures the value of both treating patients today and minimizing resistance levels in the future (20). Model results are usually sensitive to the length of the planning horizon; in general, the planning horizon should be chosen carefully, the main considerations being when new classes of drugs may become available and how long a particular treatment strategy will be financially and operationally sustainable. Here, we use a planning horizon of 20 years.

We consider four criteria for evaluating drug policies. The first is the average daily percentage of hosts who are experiencing a clinical case of malaria (daily percentage clinical, or DPC). Clinical case days are counted over the length of the treatment period and discounted at an annual rate of 3%. The second criterion is the total number of discounted clinical cases that remain untreated or receive a failing treatment (defined as treatment of a host whose parasites are resistant to the drugs being used). We call this the number of treatment failures (NTF) and present it in units per year per 100 population. NTF can be viewed as a proxy for mortality, whereas DPC can be viewed as a proxy for morbidity. Our last two criteria relate to the lifespan of the drugs being used: we measure the time until the total level of resistance to the drug treatment has reached 5% ($T_{.05}$) and the time until the fraction of treatments failing has reached 10% ($T^F_{.10}$). The 5% resistance threshold is a danger zone for resistance evolution (32); 10% treatment failure is the World Health Organization-recommended level at which a national first-line therapy should be replaced (33). $T^F_{.10}$ determines the useful therapeutic life (UTL) of the drug(s) being deployed.

Results and Discussion

Using our four evaluation criteria, we compared a single, two, and three first-line therapies over a randomly sampled set **P** of 5,000 parameter combinations spanning a range over the basic reproductive number ($1.1 \le R_0 \le 100$; see ref. 29), *de novo* mutation rate ($10^{-6} \le \sigma_i \le 10^{-1}$), fraction of clinical cases that are treated ($0.2 \le f \le 1.0$), the cost of resistance ($0.05 \le s_i \le 0.20$; see ref. 34), and the parasite population's inbreeding coefficient ($0.0 \le F \le 1.0$); drug-specific parameters are subscripted by i. Using three first-line therapies minimized failed treatments and overall clinical disease for 92.6% of the parameter combinations tested; this means that, without any knowledge of mutation rates or costs of resistance for particular resistant phenotypes, the odds are 14:1 that we would obtain a better clinical outcome by simply treating one-third of the population with drug 1, one-third with drug 2, and the final one-third with drug 3. When the σ_i are equal, an MFT strategy with three therapies is optimal under all four criteria 99.7% of the time (a single first-line therapy could be better in the comparisons on **P** when σ_1 is low but σ_2 and σ_3 are high). See Fig. 2 for a typical picture of the dynamics of resistance evolution under single and multiple therapies.

In attempting to understand resistance evolution in a multidrug context, we must first understand the features of our system that have the most important effects of drug-resistance evolution in general.

One of the key epidemiological variables that drives resistance evolution in malaria is the fraction φ of infected hosts that have clinical malaria (Fig. 2 *Bottom*), because only hosts with symptoms receive drug treatment; the fraction φ is a measure of the selection pressure on the parasite population to evolve resistance. When φ is low, the high level of asymptomatic hosts causes natural selection to work against the resistant phenotypes, because competition between sensitive and resistant parasites occurs in the absence of drugs among asymptomatic hosts; in addition, when φ is low, there is little selection pressure for resistance because of the low probability that an individual parasite will encounter a drug. The effect of a low fraction of hosts with clinical disease was hypothesized to explain the regional absence of the *dhfr* Leu-164 mutation, which confers antifolate resistance (35).

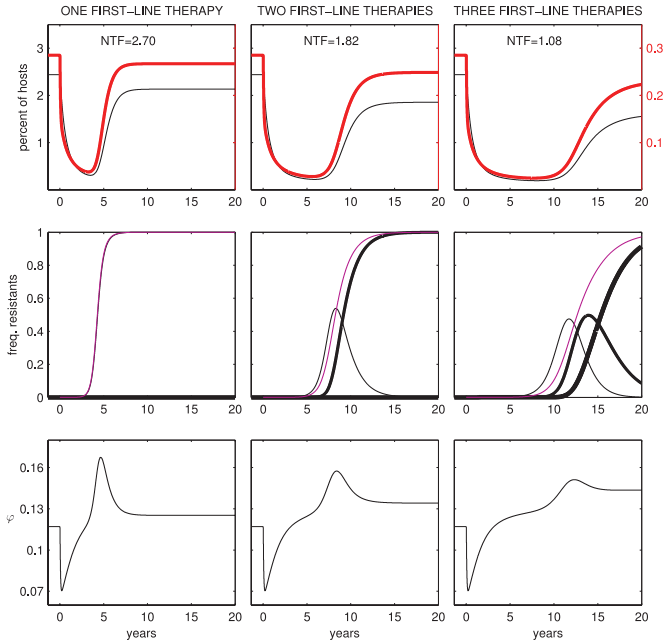

Fig. 2. Differences between single and multiple first-line therapies. Here, $R_0 = 3$, $s_i = 0.1$, $\sigma_i = 10^{-5}$, $f = 0.6$, and F = 1.0. System is started at its endemic equilibrium, and treatment is begun at time 0. (*Top*) Percentage of hosts undergoing a clinical episode of malaria (red line) and percentage of all hosts that are infected (black line); red axis labels correspond to red line. (*Middle*) Frequency of single- (thin black line), double- (medium black line), and triple-resistant strains (thick black line); a thicker line indicates more resistance. The magenta lines in the second row indicate the fraction of incident treated cases that receive a failing treatment. (*Bottom*) φ the fraction of infections that are currently in a clinical state and possibly being treated by drugs. As in a classic resistance epidemic, a quick initial decrease in disease prevalence and clinical cases is followed by a period of low prevalence, which is followed by a period when prevalence creeps back up almost to pretreatment levels; disease prevalence will not attain its full pretreatment level as long as there is some cost to resistance. The major difference among the treatment strategies is the pattern of fixation in the middle row. Here and in the model in general, resistance evolution begins later and occurs more slowly when more first-line therapies are used.

The influence of malaria transmission intensity on φ is one of two key relationships that drives the dynamics of our model. As R_0 increases, φ decreases because of an increased level of host immunity (Fig. 3 *Right*), which may help explain why resistance is more likely to arise in low transmission areas (36–38). The second relationship is that between R_0 and the equilibrium number of clinical cases $\hat{C}$ (Fig. 3 *Left*); $\hat{C}$ is maximized at an intermediate R_0, because $\hat{C}$ increases with higher prevalence but decreases with higher immunity, both of which increase with R_0. This relationship between the clinical burden of malaria and the transmission intensity is in general agreement with field data (28, 39).

Increasing R_0 has two basic effects on resistance evolution: it shortens the parasites' generation time, thus making any type of evolution faster, and it lowers φ weakening the selection pressure for resistance. Fig. 3 *Right* shows how these two opposing effects balance. In general, decreasing R_0 when it is large will lead to earlier treatment failure and resistance emergence, as suggested elsewhere (40). In our model, this behavior is explained by an immunogenic mechanism (as opposed to a recombination mechanism in other

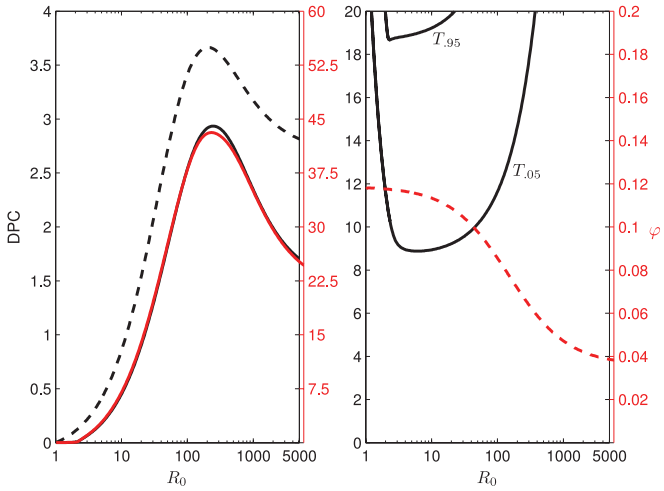

Fig. 3. In these graphs, $s_i = 0.1$, $\sigma_i = 10^{-5}$, F = 1.0, and the model was run for 20 years; $f = 0.6$ for the solid lines and $f = 0.0$ for the dashed lines. *Left* shows the clinical outcomes DPC (black lines) and NTF (red line) as a function of R_0. *Right* shows the time to emergence ($T_{.05}$) and time to fixation ($T_{.95}$) as a function of R_0; the dashed red line shows the equilibrium value of φ when there is no treatment or resistance. Red lines correspond to red axis labels. For higher R_0, φ is lower; thus, there is less selection pressure favoring the resistants and their time to emergence/fixation is longer. This relationship breaks down for very low R_0 where the parasites' generation time is long and evolution is slow. The inner axis labels correspond to NTF (red numbers) and years until resistance reaches 5% or 95% (black numbers).

models), whereby lowering R_0 decreases population-wide immunity and increases the proportion of clinical cases among infecteds (φ). However, any increased risk of resistance with lower R_0 needs to be balanced against the benefit of reduced transmission. As Fig. 3 *Left* shows, reducing transmission below $R_0 = 200$ yields a reduction in clinical cases and failed treatments even when resistance evolution is taken into account. Note that reducing R_0 from 5,000 to 200 causes an increase in clinical cases and failed treatments; this occurs in our model because (*i*) lowering R_0 in this range reduces immunity but has little effect on prevalence, and (*ii*) for the 20-year timeframe we have chosen, resistance barely emerges for these extremely high R_0 values, and the clinical outcomes for these situations can be compared in the absence of resistance evolution.

In addition to the transmission intensity described by R_0, a second influential parameter in our model is the fraction f of clinical cases that are treated by drugs. Depending on the price of treatment and the local population's access to clinics and pharmacies, f will vary greatly from region to region. When more cases are treated, resistance and treatment failure arrive more quickly (see Fig. 4), and we obtain the standard result that treating the fewest patients results in the least resistance.

However, when considering the clinical outcomes DPC and NTF, it is no longer optimal to treat the fewest patients. Fig. 4 *Lower* shows that it is optimal to treat all patients when the cost of resistance is high ($s > 0.2$) and approximately one-half of patients when the cost of resistance is low ($s < 0.2$); the optimal treatment fraction f^* is sensitive to the length of the outlook period. Consider Fig. 4 where $s = 0.1$. Here, when the treatment fraction is low ($f < 0.2$), resistance does not emerge, and NTF depends only on the fraction of cases treated and is unaffected by treatment strategy; if there is no resistance, there is no benefit to using MFT over a single first-line therapy or vice versa. For $f > 0.4$, it becomes clear that MFT strategies result in fewer failed treatments (and fewer clinical cases; data not shown). When everyone receives treatment, using three first-line therapies results in a 49% reduction in failed treatments (and clinical cases) compared with a single first-line therapy. Note that, as we move from a single first-line therapy to two and three first-line therapies, the optimal treatment fraction f^* moves rightward on the graph, which means that, as we add more first-line therapies into our treatment strategy, we will be able to treat a higher proportion of clinical cases without "overtreating" and driving the evolution of resistance too strongly.

In comparing the useful therapeutic lives of treatment strategies in Fig. 4 *Upper*, it becomes apparent that MFT strategies have longer UTLs, because they use more drugs. A more relevant comparison measures the UTL per drug rather than the UTL of the treatment strategy as a whole; at $s = 0.1$ and $f = 0.6$, for example, the per-drug UTL for a strategy of three first-line therapies is 3.32 years (one-third of the 9.95-year UTL of an MFT strategy with three therapies), whereas the per-drug UTL of a single first-line therapy is 3.54 years. Thus, it would seem optimal to use our single first-line therapies in sequence for a total UTL of $3.54 \times 3 = 10.62$ years. However, this simple calculation obscures the fact that when n drug therapies are used in sequence, their total UTL cannot be calculated by summing the UTLs of the individual therapies. In our example, using the drugs in sequence, the UTL of the first therapy would be 3.54 years, the second therapy 2.92 years, and the third therapy 2.83 years, for a total UTL of 9.29 years.

When using therapies in sequence, there is a particular aspect of parasite ecology that reduces the UTL of each subsequent therapy, even when there is no cross-resistance among the drugs. During the first treatment period, resistant parasites emerge and spread by competing for hosts with drug-sensitive wild-type parasites. During the second treatment period, parasites resistant to therapy 2 compete against a mixture of wild types and resistants left over from the previous treatment period, hence they emerge into a less

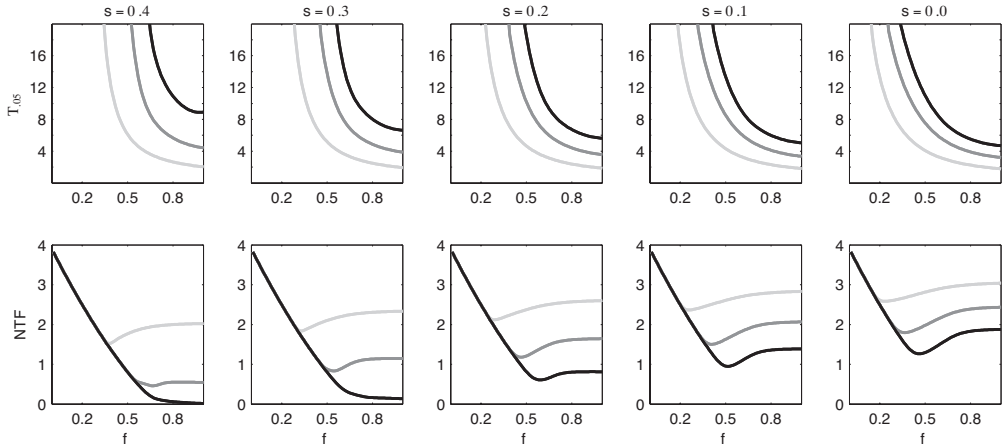

Fig. 4. In these graphs, $R_0 = 3$, $\sigma_i = 10^{-5}$, F = 1.0, and the model was run for 20 years. The black line corresponds to MFT with three drugs, the medium gray line to MFT with two drugs, and the light gray line to a single first-line therapy. Note that using more therapies has a bigger advantage when the cost of resistance is higher. Fig. S2 in *SI Appendix* shows the bottom row without discounting.

POPULATION BIOLOGY

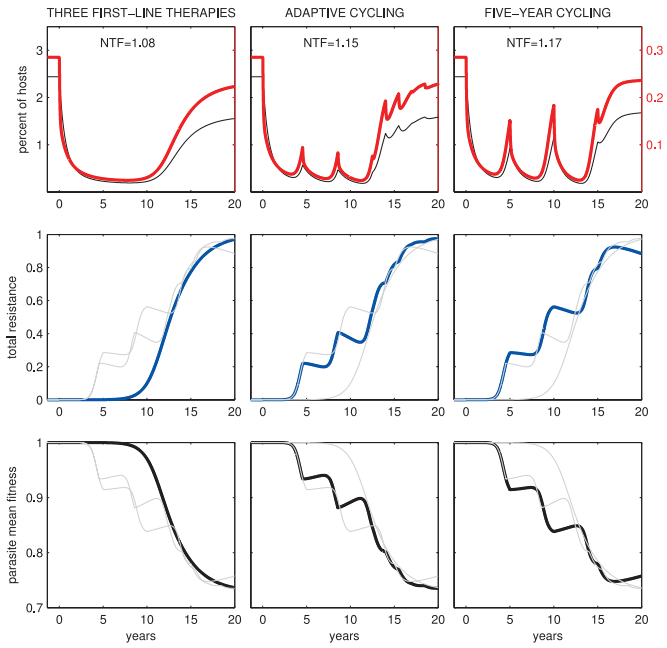

Fig. 5. Differences between MFT and cycling strategies; ''adaptive cycling'' means switching drugs at 10% treatment failure with a 1-year switch delay. Here, $R_0 = 3$, $s_i = 0.1$, $\sigma_i = 10^{-5}$, $f = 0.6$, and F = 1.0. *Top* is as in Fig. 2. *Middle* tracks the total level of resistance in the parasite population (triple resistants count as fully resistant, double resistants count as 2/3 resistant, and so on); light gray lines show the ''total resistance'' line from the other two columns for comparison. *Bottom* tracks the parasites population's mean fitness, calculated in the absence of drug treatment, with a fitness of one assigned to drug-sensitive parasites; the light gray lines show the mean-fitness line from the other two columns for comparison.

competitive environment where they increase in frequency more quickly and cause high levels of treatment failure more rapidly. During the third treatment period, parasites resistant to therapy 3 emerge into an even less competitive environment than was present at the beginning of period 2, and treatment failure arrives even more quickly. From the perspective of parasite ecology, each resistant strain degrades the mean fitness of the parasite population (see Fig. 5) and constructs a niche in which it is easier for subsequent resistant strains to invade (41, 42). This fundamental ecological interaction among drug-resistant strains in a parasite population is a key reason why MFT strategies outperform drug-cycling strategies.

In fact, compared with MFT policies, cycling policies have two fundamental disadvantages that can be understood in the light of evolution. Organisms evolve in response to environmental pressures, and cycling policies are more likely to create environments favorable to drug-resistant malaria in two ways. First, cycling lowers the mean fitness of the parasite population more quickly than MFT, thereby creating a less competitive environment that is more conducive to the invasion and spread of new resistant types. Second, from the perspective of the pathogen, cycling creates a less temporally variable environment that makes resistance evolution easier in the long term, as described by Bergstrom *et al.* (16). These are two distinct ecological-evolutionary effects: at the beginning of the second cycling period, newly emerged resistant parasites can invade and spread easily, because (*i*) they can count on a temporally invariable environment for some time; and (*ii*) they encounter weak competition, the mean fitness of the parasite population having been degraded in the previous cycling period.

To compare the health outcomes of cycling policies and MFT strategies, we ran our model for 20 years over the parameter set **P** and compared MFT with 10 variants of cycling policies (see *SI Appendix*); one such cycling strategy approximates the status quo pattern of switching drugs and is described here. For 75.1% of parameter combinations, MFT outperformed a cycling policy that switched first-line therapies at 10% treatment failure with a 1-year switch delay. Quantitatively, the NTF values under both strategies were quite close, within 5% of each other for 64.4% of parameter values. For 20.9% of parameter values, MFT enjoyed a 5–10% advantage, and for 9.5% of parameter values, MFT enjoyed a >10% advantage. For 3.6% of parameter values, MFT had a 5–10% disadvantage, and for 1.6% of parameter values, a >10% disadvantage. The most noticeable quantitative benefit of using multiple first-line therapies over cycling strategies was the increased delay in time to resistance emergence and treatment failure, which ranged from 2- to 4-fold (for equal σ_i). The longest delay in resistance emergence is achieved under an MFT policy with an even drug distribution, but some diversity is better than no diversity (see Fig. 6). For example, using three drugs in a 50/25/25 ratio almost doubles the time until resistance reaches 5%, compared with a single first-line therapy. Note in Fig. 6 that time to emergence is determined primarily by the frequency of the most frequently used drug.

In addition to the clinical and drug lifespan benefits of deploying multiple first-line therapies, MFT strategies may enjoy some operational advantages, because they do not incur the economic costs

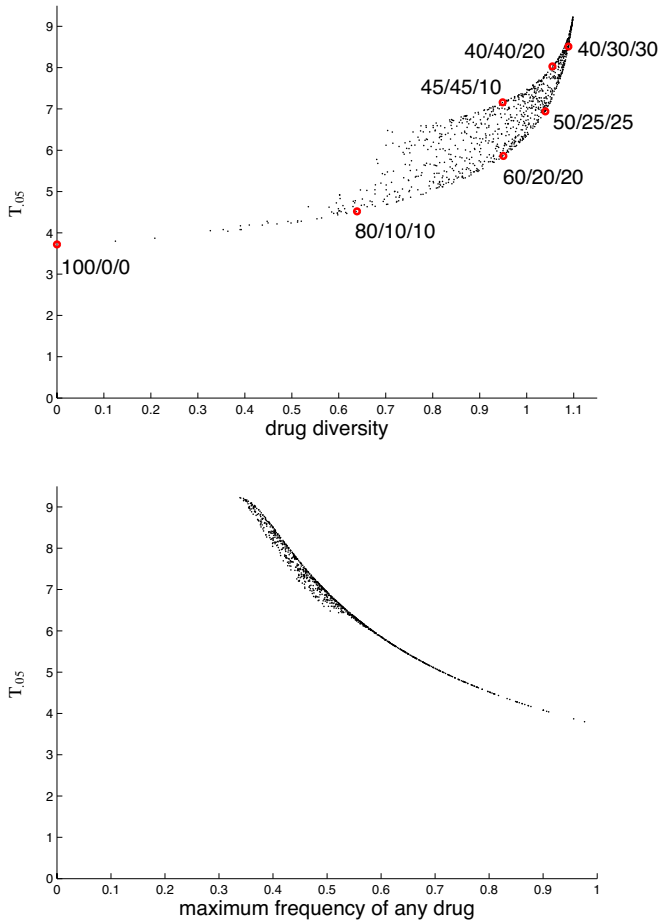

Fig. 6. Results of 1,000 simulations, with $R_0 = 3$, $\sigma_i = 10^{-5}$, $f = 0.6$, $s_i = 0.1$, and F = 1.0, where the drug distribution of three drugs was chosen randomly. Certain drug distributions are highlighted in *Upper*. *Lower* shows the same simulations plotted against the frequency of the most used drug. Drug diversity is measured as $-\,p_i \Sigma \log p_i$, where p_i is frequency of use of drug *i*. The 60/20/20 strategy has the same diversity measure as the 45/45/10 strategy, but resistance arrives sooner under the former because one drug has such a high frequency of use.

involved in switching therapies and maintaining a high level of surveillance (43). Moreover, cycling strategies are sensitive to the length of the cycling period and implementation delay (Fig. S1 in *SI Appendix*), and they may incur a higher level of treatment failure than noted in the modeling if there are delays in phasing out a failed first-line therapy. Note that the MFT strategy analyzed in this study is a nonadaptive strategy. In the context of the same surveillance networks available to cycling strategies, the clinical benefits of an adaptive MFT over cycling strategies could be much greater.

An important challenge in applying this type of theoretical work is that we will usually not know the *de novo* mutation rate to resistant strains (σ_i) or the resistant types' fitness cost of resistance (s_i), and we may have to rely on our best judgment to make a conservative but effective recommendation. Moreover, not all factors in malaria control—imperfect compliance with treatment regimens, delays in implementing policy switches, recommended use of second-line drugs, likely use of cheap but ineffective drugs such as chloroquine, varying levels of surveillance available to detect resistance or treatment failure–have been included in our model; model-influenced regional recommendations should take into account regional variations in drug-use patterns and malaria epidemiology.

The pharmacokinetic/pharmacodynamic (PK/PD) aspect of malaria treatment has also been omitted from the model presented here. In reality, some drugs remain in the system for days, whereas others linger for weeks. When designing MFT strategies, drugs with longer half-lives should be used more sparingly to distribute the drugs as uniformly as possible in the parasites' environment; this way, the parasite has the lowest probability of encountering the same drug twice in a row. More importantly, the increasing use of ACTs makes it critical to analyze the effects of mismatched half-lives in combination therapies (4). The short half-life of the artemisinin component in ACTs may make it possible to use multiple ACTs in an MFT strategy, even though each therapy contains an artemisinin derivative. These suggestions should be evaluated with detailed within-host PK/PD modeling.

Our results suggest an important general principle for malaria treatment that holds across a broad parameter range and in other model formulations: compared with a single first-line therapy, multiple first-line therapies reduce total clinical cases and failed treatments, significantly delay resistance emergence and treatment failure, and slow resistance evolution once resistance emerges. Extrapolating our conclusions to use of multiple ACTs may yield even stronger results. Because resistance to the nonartemisinin partner drugs typically arises and spreads quite easily, the evolution of artemisinin resistance is likely to be followed in rapid succession by the evolution of resistance to all ACTs. Thus, in theory, the optimal strategy would be to deploy all available ACTs to reduce selection pressure on the partner drugs; protecting the partner drugs may extend the useful therapeutic lives of all ACTs. As new drugs are deployed, and as we are able to assess the levels of use in different countries, we should look toward identifying locations with drug-use patterns that would allow us to quantify the population-wide benefits of using multiple first-line therapies to treat malaria infections.

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