

UCSF

UC San Francisco Previously Published Works

Title

The path of least resistance: aggressive or moderate treatment?

Permalink

<https://escholarship.org/uc/item/7566128t>

Journal

Proceedings of the Royal Society B, 281(1794)

ISSN

0962-8452

Authors

Kouyos, Roger D
Metcalf, C Jessica E
Birger, Ruthie
[et al.](#)

Publication Date

2014-11-07

DOI

10.1098/rspb.2014.0566

Peer reviewed



CrossMark
click for updates

Review

Cite this article: Kouyos RD *et al.* 2014 The path of least resistance: aggressive or moderate treatment? *Proc. R. Soc. B* **281**: 20140566. <http://dx.doi.org/10.1098/rspb.2014.0566>

Received: 20 March 2014

Accepted: 22 August 2014

Subject Areas:

evolution, health and disease and epidemiology, theoretical biology

Keywords:

drug resistance, evolution, treatment strategies

Author for correspondence:

Ruthie Birger

e-mail: rbirger@princeton.edu

[†]These authors contributed equally to this study.

Electronic supplementary material is available at <http://dx.doi.org/10.1098/rspb.2014.0566> or via <http://rspb.royalsocietypublishing.org>.

The path of least resistance: aggressive or moderate treatment?

Roger D. Kouyos^{1,2,†}, C. Jessica E. Metcalf^{1,3,†}, Ruthie Birger^{1,†}, Eili Y. Klein^{1,8}, Pia Abel zur Wiesch⁴, Peter Ankomah⁵, Nimalan Arinaminpathy^{1,16}, Tiffany L. Bogich^{1,15}, Sebastian Bonhoeffer⁶, Charles Brower^{1,20}, Geoffrey Chi-Johnston⁷, Ted Cohen⁴, Troy Day⁹, Bryan Greenhouse¹⁰, Silvie Huijben¹⁹, Joshua Metlay¹³, Nicole Mideo¹⁴, Laura C. Pollitt^{11,12,18}, Andrew F. Read^{11,12,15}, David L. Smith³, Claire Standley¹⁷, Nina Wale^{11,12} and Bryan Grenfell^{1,15}

¹Department of Ecology and Evolutionary Biology, Princeton University, Princeton, NJ, USA

²Division of Infectious Diseases and Hospital Epidemiology, University Hospital Zürich, University of Zürich, Zürich, Switzerland

³Department of Zoology, Oxford University, Oxford, UK

⁴Division of Global Health Equity, Brigham and Women's Hospital and Department of Epidemiology, Harvard School of Public Health, Boston, MA, USA

⁵Department of Biology, Emory University, Atlanta, GA, USA

⁶Institute of Integrative Biology ETH Zurich, Zurich, Switzerland

⁷Department of Epidemiology, Bloomberg School of Public Health, and ⁸Center for Advanced Modeling, Department of Emergency Medicine, Johns Hopkins University, Baltimore, MD, USA

⁹Departments of Mathematics and Biology, Queen's University, Kingston, Ontario, Canada

¹⁰Department of Medicine, Division of Infectious Diseases, University of California, San Francisco, CA, USA

¹¹Centre for Infectious Disease Dynamics, and ¹²Departments of Biology and Entomology, The Pennsylvania State University, University Park, State College, PA, USA

¹³General Medicine Division, Massachusetts General Hospital, Boston, MA, USA

¹⁴Department of Ecology and Evolutionary Biology, University of Toronto, Toronto, Ontario, Canada

¹⁵Fogarty International Center, National Institutes of Health, Bethesda, MD, USA

¹⁶Department of Infectious Disease Epidemiology, Imperial College London, London, UK

¹⁷Department of Health Policy, George Washington University, Washington, DC, USA

¹⁸Centre for Immunology, Infection and Evolution, University of Edinburgh, Edinburgh, UK

¹⁹Barcelona Centre for International Health Research, Hospital Clínic, Universitat de Barcelona, Barcelona, Spain

²⁰Center for Disease Dynamics, Economics & Policy, Washington, DC, USA

 RB, 0000-0002-2960-5084

The evolution of resistance to antimicrobial chemotherapy is a major and growing cause of human mortality and morbidity. Comparatively little attention has been paid to how different patient treatment strategies shape the evolution of resistance. In particular, it is not clear whether treating individual patients aggressively with high drug dosages and long treatment durations, or moderately with low dosages and short durations can better prevent the evolution and spread of drug resistance. Here, we summarize the very limited available empirical evidence across different pathogens and provide a conceptual framework describing the information required to effectively manage drug pressure to minimize resistance evolution.

1 . Introduction

Since the first introduction of anti-infectives (antibiotics, anti-malarials, anti-virals, anthelmintics) the evolution of resistance to chemotherapy has threatened clinical care, and continues to be a serious global health problem [1,2]. Although almost every anti-infective that has been introduced and regularly used has eventually had its effectiveness diminished by the emergence and spread of drug-resistance, the lag time until drug-resistance evolves differs considerably across drug-pathogen combinations. Consequently, a key question is which strategies are optimal for minimizing or delaying drug resistance for each specific pathogen. Generally, slowing the evolution of resistance in a population is best achieved by

treating as few patients as possible, thereby minimizing the selective pressure for resistance [3,4]. This must be balanced against the benefits of treatment, which can reduce morbidity, mortality and the spread of infections by curing individuals faster (treatment as prevention). Maximizing the good achieved with a drug thus involves a trade-off between curing infections and avoiding the spread of resistance. To attempt to balance these two aims, the traditional recommendation has been to use a drug only when the patient's condition necessitates, but then to treat an infection as aggressively as possible, using the highest possible dose for at least as long as it takes to eliminate the pathogen [5]. This approach, which we refer to as *aggressive chemotherapy*, is fundamentally motivated by the need to cure the patient, but there is also some limited empirical evidence demonstrating that *aggressive chemotherapy* can prevent the de novo evolution of resistance by ensuring clearance of partially resistant strains that are able to persist at lower drug levels.

In contrast, recent theory and experimental data have suggested that reducing the dosage or the length of treatment may slow the spread of resistance under some conditions [6–9]. This approach, which we refer to as *moderate chemotherapy* (see glossary) recommends that drug treatment should aim to optimize clinical outcomes but not necessarily to clear the infection, and in fact, has a long history within the literature (e.g. see the concept of premunition in malaria [10]). Moderate chemotherapy may be successful if the host immune system is able eventually to clear the infection [11,12], or if complete eradication of the pathogen from the host is not essential for treatment of the acute illness. In fact, evolutionary ecology suggests that evolution of tolerance is a common strategy of hosts to cope with pathogens [13]. The key concept underlying this approach for resistance management is that the strength of selection for resistance is given by the difference in the relative fitness (see glossary) of drug-sensitive and drug-resistant pathogens, and that this quantity increases with the dosing of the drug. Thus, moderate chemotherapy reduces the advantages of drug-resistant pathogens by being less restrictive to drug-sensitive pathogens that compete against the resistant strains.

Here, we compare these two alternate approaches of aggressive versus moderate chemotherapy from a broad ecological perspective, discuss the factors and mechanisms that can favour one over the other approach, and summarize the still very scarce empirical evidence supporting either moderate or aggressive chemotherapy.

2. Optimal treatment from an evolutionary perspective

The reason that two such different recommendations exist (moderate or aggressive) is that the spread of resistance depends on two processes that react in opposite ways to increasing drug pressure (see glossary) in individual patients. On the one hand, the rate at which resistant mutants are generated depends on the abundance of the pathogen, and is therefore a *decreasing function of drug pressure*, since drug pressure is generally expected to correlate with numbers of pathogens killed. Furthermore, sufficiently high drug pressures can kill partially resistant strains and thereby prevent the further accumulation of resistance mutations. On the other hand, once resistance mutations are present, the rate with which they increase in frequency in the human population is a function of the selective advantage for resistant

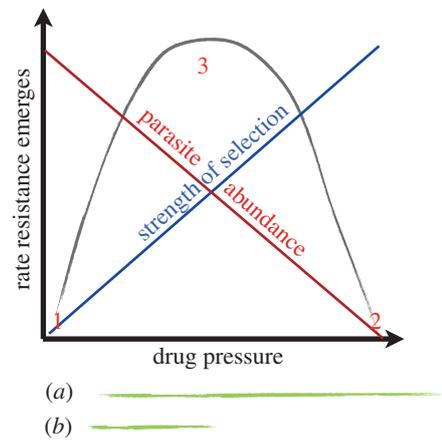


Figure 1. The curve defining resistance evolution as a function of drug pressure. The x-axis is the possible range of drug pressures, measured as either drug dosage, or duration of treatment, with the realistic/neutral range of drug pressures highlighted in green, showing (a) a case where aggressive chemotherapy is likely to be optimal, as there is a level of drug pressure for which the pathogen can be completely cleared and (b) a case where moderate chemotherapy is likely to be optimal for managing resistance, as there is no realistic degree of drug pressure that can clear the pathogen. The y-axis is the rate of resistance emergence, or the inverse of time from introduction of treatment until a resistant strain is established. Numbers 1–3 refer to the three qualitative evolutionary regimes: no evolution of resistance because selection is too weak (1), no evolution of resistance because pathogen cannot replicate (2), maximal speed of resistance evolution (3).

organisms, which *increases with drug pressure*. The reason for this is that more aggressive treatment will be more likely to remove susceptible competitors either at the primary site of the infection or at colonization sites. These drug-sensitive competitors might otherwise limit the spread of the resistant pathogens at these sites [14]—e.g. by depleting resources such as nutrients or space, by directly interfering with the growth of the resistant strain, or by changing the context of immune response—and thus their removal can benefit the resistant strains.

One way to frame the interplay of these two effects is a simple conceptual curve describing the speed of resistance evolution in relation to drug pressure in individual hosts (see [15] for an analogous framework developed in the context of immune escape). Consider the effect of the extremes of drug pressure on a single mutation that confers some finite degree of resistance (for example, it may increase the drug dosage that can be tolerated by the pathogen by a finite amount). In the absence of drug pressure, there is no selection favouring this resistance mutation, which will therefore not increase in abundance (regime 1 in figure 1). At the other extreme, drug pressure is so intense that even the marginally resistant pathogens are cleared and therefore cannot be transmitted (regime 2 in figure 1). This effect will be compounded by a reduced mutational input, because higher drug levels lead to an increased kill rate, which reduces the number of pathogen cell divisions, and thus the probability of resistance emergence during treatment. Since at the extremes of very high and very low drug pressure, resistance mutations do not spread, the rate at which resistance spreads either within a patient or in a population must be maximized at an intermediate level of drug pressure (regime 3 in figure 1). For resistance evolution in *in vitro* systems or in individual patients, this conceptual curve is related to the pharmacodynamical concept of the mutation-selection window [16–18] (MSW, see glossary).

However, our conceptual curve also applies to the epidemic spread of resistance: all other factors being equal, the speed of resistance spread in a population will depend on the dosing with which the drug is typically administered and the spread will be fastest for intermediate levels of dosing. Moreover, the MSW typically considers the effect of dosing on a single strain, whereas the curve in figure 1 is modulated by co-infection with different strains and even by co-infection with different species (bystander selection, see below).

Medical necessity requires drug pressure to be high enough to guarantee clinically successful treatment of the patient, imposing a lower bound to drug pressure; while avoiding toxic outcomes, imposing an upper bound to drug pressure. It is within this 'clinically neutral' range, that drug pressure might be optimized to minimize resistance. As several studies have demonstrated comparable clinical outcomes for short-course ('moderate') treatment versus long-course ('aggressive') treatment for several infections [19–21], the existence of such 'clinically neutral' ranges might be relatively common. The crucial question therefore is how this clinically neutral range of drug pressures maps onto the conceptual curve (figure 1).

- If the clinically neutral range extends to include the range that would clear those resistant strains that are currently present in the human population or that can evolve from them in the short-term, then aggressive chemotherapy is likely to be the best strategy to minimize resistance (scenario *a* in figure 1).
- Conversely, if levels necessary to clear those strains are considerably beyond the threshold for toxicity or bioavailability (which is, for example, the case if fully resistant strains already exist in the population) then moderate chemotherapy could be the best strategy to minimize the spread of resistance at both the individual and population scale (scenario *b* in figure 1).

A key challenge consists in assessing the generalizability of a broad clinically neutral range beyond the diseases studied so far. Moreover, it is unclear whether this clinically neutral range includes treatment durations that are so short that treatment does not completely clear the pathogen from the site of infection (but the immune system completes the clearance). Finally, resistant pathogen strains might require higher doses or longer durations of treatment and thereby exhibit a substantially narrower neutral range. Determining the quantitative effects of resistance on the clinically neutral range remains however an open challenge.

The key difficulty in applying this logic is that lack of empirical data and a poor theoretical understanding of the processes underlying the conceptual curve imply that its exact shape is not known for most pathogen–drug combinations (see below). If the conceptual curve is applied to the speed with which resistance evolves at the epidemic (or population) level, there is the additional difficulty that the curve links different scales of pathogen population biology—the *x*-axis corresponds to drug pressure at the within-host scale, whereas the *y*-axis corresponds to the speed of evolution at the epidemic scale—and there is currently a very limited empirical and theoretical understanding of how such within host effects translate into epidemic scale effects. Nonetheless, framing the problem in this way allows us to identify the key elements likely to affect optimal treatment relative to resistance.

3. Determinants of optimal treatment relative to resistance

Several factors determine the optimal choice of treatment (moderate or aggressive) to meet the goals of patient treatment while avoiding the emergence of resistance. These include: the genetic architecture underlying resistance; community levels of resistance, which may be related to the length of time an anti-infective agent has been in use; and patient adherence to therapy.

If the genetic architecture underlying resistance implies that a large number of mutations are required to achieve full resistance, i.e. if the genetic barrier to resistance is high, then the first mutation to spread will probably confer only a moderate level of resistance, meaning that clinically relevant levels of drug pressure will clear the partially resistant mutant [22]. Thus, aggressive chemotherapy may be more advantageous if full resistance is a quantitative trait requiring many mutations, and full resistance has yet to appear within the population. A corollary of this is that combination therapy, which raises the genetic barrier, could increase the advantages of aggressive therapy. Identifying the magnitude of the genetic barrier for a particular drug is, however, a major difficulty, in part because the correspondence between the genetic barrier *in vivo* and *in vitro* is not perfect. In the context of combination therapy against bacteria, for example, many mechanisms, such as biofilm formation, states of quiescence, efflux-pumps, or multi-drug-resistant plasmids can confer resistance to many drugs at once [23]. As these mechanisms may play a different role in different settings (*in vitro* versus *in vivo*; human versus animal models), their effect will be hard to infer from *in vitro* tests, or even from small-scale *in vivo* tests (indeed even such small-scale *in vivo* tests are extremely scarce, see section *Empirical evidence*).

The dynamic context of evolution may also mean that the benefit of aggressive or moderate treatment may change as a function of the number of years since the drug has been in use. Drugs that are approved tend to have a high genetic barrier to resistance initially (at least *in vitro*), and clear infections rapidly. This implies that the range of neutral drug pressures incorporates clearance of the first emerging partial resistance mutations (i.e. scenario *a* in figure 1), which recommends aggressive treatment. Over time, pathogens are likely to accumulate resistance mutations and the drug pressure necessary to eliminate all pathogens (including the ones that have acquired new mutations) will increase (i.e. scenario *b* in figure 1). This reflects a shift of the maximum of the conceptual curve to higher drug pressures, thereby broadening the drug pressure range in which moderate treatment is optimal. This implies that adaptive management strategies, which alter the degree of 'aggressiveness' through time (e.g. depending on the level of resistance found in cross-sectional surveys), could extend the lifespan of a drug beyond what can be achieved with a uniformly aggressive or moderate treatment strategy.

The benefits of moderate versus aggressive treatment will also depend on the epidemiological context. If the presence of susceptible pathogens within a host limits the replication and transmission of resistant pathogens, then the frequency of co-infection with different pathogen strains will affect the strength of competition and hence the optimal treatment strategy for reducing the spread of resistance. For example, for malaria in high-transmission areas, where co-infection is more frequent [24–27] moderate treatment may be more

beneficial than it would be in low-transmission areas, because in the former case resistant and sensitive strains compete more often within an individual host and hence aggressive treatment is then more likely to cause the removal of a sensitive competitor. However, rapid reinfection by susceptible strains in high-transmission areas may mitigate this disadvantage of aggressive chemotherapy.

Similarly, the complex relationship between colonization and infection with bacteria creates a situation in which any chemotherapy will select for resistance in entire microbial communities occupying a range of tissue types ('bystander' selection) [28]. In particular, such bystander treatment might affect microbial communities both in the tissue occupied by the focal pathogen and in other tissues (for example, orally administered antibiotics can affect the gut microbiota irrespective of the site occupied by the focal pathogen [29]). These unintended consequences might increase the advantages of moderate chemotherapy by reducing the amount of time non-target organisms are exposed to a drug, especially given the possibility of horizontal gene transfer [30].

Finally, optimal treatment with respect to resistance minimization might also depend on variation in patient adherence. Non-compliance with recommended treatment courses by some patients is a general feature of chemotherapy. In addition, there is often substantial variation in the absorption and metabolism of drugs across patients. This suggests that moderate treatment may occur unintentionally even in populations where aggressive chemotherapy is recommended. If low levels of unintentional moderate treatment are a driving force in the emergence of resistance then an aggressive chemotherapy policy's main benefit (of inhibiting the accumulation of resistance mutations) will be hampered [31]. Accordingly, this could change the shape of the conceptual curve to favour moderate chemotherapy. Alternatively, it might imply that even more aggressive chemotherapy should be recommended because higher drug doses might be more robust to imperfect adherence.

4. Empirical evidence

There is surprisingly limited empirical evidence describing how treatment regimes can affect the emergence and spread of resistance (electronic supplementary material, table S1). Even worse, the evidence might be biased because most empirical studies are based on the effect of drug pressure on the *de novo* evolution of resistance—i.e. on the emergence, establishment and increase in resistance in a single infection or *in vitro* culture founded by a susceptible strain—and this scenario tends to favour aggressive therapy.

Clinical data for the impact of drug pressure on *de novo* drug resistance evolution stem mostly from infections requiring long-lasting treatment such as HIV [32–34] and TB [35]. For HIV-1, studies considering resistance evolution in relation to patient adherence suggest that treatment that does not completely suppress pathogen replication facilitates *de novo* evolution of resistance [32–34,36]. There is some evidence that resistance evolution is maximized at intermediate adherence [37], but the clinical needs of HIV therapy exclude moderate chemotherapy as a strategy. For TB, the main objective of long treatment duration is to prevent relapse of the infection, and the clinical evidence for an increased risk of resistance evolution with short treatment duration is mixed [35]. However, as for HIV, residual replication due to non-

compliance or PK/PD variability is considered a risk factor for resistance evolution in TB [38,39].

For a broad range of bacterial pathogens, experimental studies done *in vitro* or in animal models support the view that aggressive chemotherapy can contribute to resistance management, with most studies finding that high drug pressure/doses prevent the *de novo* evolution of resistance mutations (see [40], references therein, and electronic supplementary material, table S1). These results support the notion that for concentrations above the mutant prevention concentration (MPC, see glossary), *de novo* resistance cannot evolve in the target pathogen [41,42]. Interestingly, it has also been shown in animal models that intermediate drug-concentrations can maximize the abundance of resistant strains [43,44] (specifically, Tam *et al.* [44] found an 'inverted-U'-shaped relation similar to figure 1). Overall, most experimental findings from infections with drug-sensitive strains indicate that concentrations above the MPC can be reached *in vivo* and hence the *de novo* evolution of antimicrobial resistance should be curbed 'by administering the highest tolerated doses of antibiotic' [40]. It should be noted, however, that the advantage of high doses might be non-existent if a single and probably point mutation leads to full resistance (e.g. resistance to pyrimethamine or atovaquone in malaria [45]) or if fully resistant mutants are expected to pre-exist even in infections founded by a susceptible strain (e.g., in the past in HIV monotherapy with low-genetic barrier drugs [46]); because in this situation whatever the dose administered, resistant pathogens may persist and cause therapy failure.

The effect of drug pressure/dosing on transmitted resistance (i.e. if an individual infection is founded by a resistant strain, or a combination of susceptible and resistant strains) has received much less attention in empirical studies, even though it constitutes a large part of the global health burden. One study on *Streptococcus pneumoniae* found that low doses of beta-lactams increase the risk of carrying transmitted penicillin-resistant strains [47] (but note: long duration was also associated with resistance in this study). By contrast, experiments where drug-resistant and drug-susceptible malaria pathogen strains are inoculated into mice either singly or in co-infections indicate that the presence of a competitor considerably slows the rate of increase in the resistant pathogens, but this disadvantage disappears in the presence of drugs, and the stronger the drug treatment, the greater the benefit to resistant pathogens [6,9]. Furthermore, there were no health benefits for the mice in aggressive relative to moderate chemotherapy. This suggests that aggressive chemotherapy can promote the spread of resistance once fully resistant strains are present in the population; and moderate chemotherapy may not be associated with any health costs. These two studies consider the effect of treatment strategies on transmitted resistance in individual hosts, but what is completely lacking are studies to assess the comparative effect of aggressive versus moderate in entire transmission chains.

Broadening the focus to the whole pathogen community, there is evidence that chemotherapy will unavoidably affect any other organisms in the vicinity of the targeted pathogen at least for bacterial infections [48] (i.e. bystander selection, see above), and that the prevalence of resistance in the microflora increases with antibiotic consumption [49] and the duration of treatment [50]. This evidence suggests that use of aggressive chemotherapy with the aim of minimizing mutational inputs into target pathogen populations only makes sense as a resistance management strategy if

mutational inputs are a more important source of de novo resistance than horizontal transfer of resistance factors from non-target microflora such as commensal bacteria.

5. Future directions

Overall, clear unambiguous empirical evidence for either aggressive or moderate chemotherapy in the context of resistance management is still largely lacking. Moreover, all empirical examples concern resistance evolution at the level of the individual host, and it is unclear how dynamics from the within-host scale links to the epidemic level (cross-scale dynamics). The key missing elements for understanding how treatment strength shapes the emergence and spread of resistance are (i) experimental data on both the shape of the conceptual curve (discussed above) and the location of the clinically neutral range of treatments on the drug pressure axis; (ii) a more thorough understanding of the cross-scale dynamics of anti-microbial resistance (see, e.g. [51]); and (iii) consideration of the different pathogen ecologies that determine how much competition between resistant and sensitive pathogens is likely to occur within a host. Quantitative predictions of resistance evolution, including the outcome of greatest public health interest, i.e. the disease burden and the proportion of infections that is no longer treatable due to resistance, require all these elements. Such predictions must be based on models of cross-scale dynamics, encompassing the effect of dosing strategies on within-host dynamics and medical outcomes through to transmission across populations [52]. The following research directions have most potential to engage with these issues.

Given the broad use of chemotherapy in agriculture, these systems could provide unique opportunities for testing the effect of dosing on the evolution of resistance. Experimental animal transmission systems are also a promising direction for testing evolutionary outcomes of dosing strategies [53]. More generally, there is a lack of good animal models to test the *in vivo* effect of chemotherapy in bacteria (although some progress has been made using other infections, such as malaria models in mice [6,9]). A consequence of this is that genetic barriers to resistance are generally evaluated *in vitro*. By necessity such *in vitro* studies do not incorporate the immune system, which is likely to be a key element in the success of moderate chemotherapy [8]. Moreover, it is often unclear how evolutionary processes [54] and drug dosing [55] can be translated from *in vitro* to *in vivo* systems. Measurement of resistance evolution in animal models and in semi-realistic animal model populations (e.g. farms) might narrow the gap between experimental predictions and expected genetic barriers in treatment of humans.

Ethical issues generally prevent direct observation of the effects on resistance of a range of drug pressures in humans. However, our understanding of the effect of optimal dosing on resistance could be improved by measuring the effect in clinical trials that are already testing varying drug dosages from a position of equipoise. This is an outcome that is rarely measured [56], and can be crucial in assessing why certain patients do not respond to certain dosages. Moreover, tests of the effects of different chemotherapy strengths in human populations might be ethically implemented in the case of prophylactic use of drugs (e.g. in malaria trials such as [57] or HIV trials such as [58]). A failure to explore the effects of

strength of treatment on emergence of resistance via randomized population level trials that are already being conducted for the purposes of prophylaxis would be a missed opportunity.

At the individual scale, generally, decisions on better treatment regimens to contain resistance will be greatly aided by diagnostic tests that can distinguish between drug-sensitive and drug-resistant strains of a pathogen. In the case of mono-infection with either a sensitive or resistant strain, such diagnostic tests can help dictate which therapies have the potential to be effective. In the case of initial co-infection with sensitive and resistant strains, advanced diagnostics could help inform the decision as to whether to use moderate chemotherapy or which drugs to use in combination if aggressive chemotherapy is likely to be more effective [59]. The experience with HIV suggests that baseline resistance testing reduces the problem of transmitted resistance (provided that treatment can be adapted to the infecting strain). Since transmitted resistance is the main reason for moderate therapy, this implies that improved diagnostics can potentially increase the benefits of aggressive therapy. It is, however, unclear whether this effect applies to infections other than HIV. Among the challenges to such an approach are the availability of alternative treatments, the timely determination of the resistance profile (delays caused by resistance testing in bacterial infections often mean that optimal treatment is not administered until resistance is proven), and resistance in the microflora.

Observational studies comparing variability in the effect of the dosing of chemotherapy at different spatial scales (e.g. states, cities) on the emergence and spread of resistance may shed light on the optimal treatment for managing resistance. Most observational/ecological studies so far focus on the correlations between bulk quantities (such as daily defined doses) of drugs used in a given region and the prevalence of resistance [3]. Despite the general limitations of ecological approaches, a key extension of these analyses would be to include information on the dose and timing of treatment in individual patients in addition to the bulk quantities.

Apart from its public health relevance, the question of optimal treatment strength is a real-world illustration of eco-evolutionary interactions. In particular, it illustrates the concept of evolutionary rescue, which deals with the question of how evolutionary adaptation can prevent extinction after environmental changes. Related issues have been discussed in the evolution of virulence, e.g. the work of Gandon *et al.* [60] on pathogen escape from imperfect vaccines. While little is known about the effect of treatment strength on evolution of resistance, even less is known about the consequence of treatment strategy on the evolution of virulence, though this would be a key direction for future research.

The question of how treatment can be used to minimize the spread of resistance (while achieving goals of patient health) is not purely academic. Recent reports have documented the emergence of malaria parasites with delayed clearance from artemisinins [61]. This delayed-clearance phenotype, while not of clinical significance yet, is the first indication that resistance to artemisinin is beginning to emerge, and may be spreading [62]. Artemisinins are an essential component of combination treatments necessary to clear malaria in many parts of the world, which makes understanding the effects of treatment on artemisinin-resistant strains a particular urgency. The drug policies to manage this and other resistance problems will necessarily consist of several components, including switching to new drugs (if available), combining available drugs, reducing

unnecessary treatment, and improving compliance with treatment recommendations. The success of any such strategy will, however, eventually depend on the adequacy of the recommended dosing; and both extremes of too high and too low dosing may needlessly accelerate the spread of resistance.

References

- Hughes JM. 2011 Preserving the lifesaving power of antimicrobial agents. *JAMA* **305**, 1027–1028. (doi:10.1001/jama.2011.279)
- Walsh TR, Toleman MA. 2012 The emergence of pan-resistant Gram-negative pathogens merits a rapid global political response. *J. Antimicrob. Chemother.* **67**, 1–3. (doi:10.1093/jac/dkr378)
- Goossens H, Ferech M, Vanderstichele R, Elseviers M. 2005 Outpatient antibiotic use in Europe and association with resistance: a cross-national database study. *Lancet* **358**, 718–723.
- Bronzwaer SL, Cars O, Buchholz U, Molstad S, Goetsch W, Veldhuijzen IK, Kool JL, Sprenger MJ, Degener JE. 2002 A European study on the relationship between antimicrobial use and antimicrobial resistance. *Emerg. Infect. Dis.* **8**, 278–282. (doi:10.3201/eid0803.010192)
- Ehrlich P. 1913 Address in pathology, on chemotherapy: delivered before the Seventeenth International Congress of Medicine. *Br. Med. J.* **2**, 353–359. (doi:10.1136/bmj.2.2746.353)
- Huijben S, Nelson WA, Wargo AR, Sim DG, Drew DR, Read AF. 2010 Chemotherapy, within-host ecology and the fitness of drug-resistant malaria parasites. *Evolution (NY)*. **64**, 2952–2968.
- Read AF, Day T, Huijben S. 2011 The evolution of drug resistance and the curious orthodoxy of aggressive chemotherapy. *Proc. Natl. Acad. Sci. USA*. **108**(Suppl.), 10 871–10 877. (doi:10.1073/pnas.1100299108)
- Geli P, Laxminarayan R, Dunne M, Smith DL. 2012 ‘One-size-fits-all’? Optimizing treatment duration for bacterial infections. *PLoS ONE* **7**, e29838. (doi:10.1371/journal.pone.0029838)
- Huijben S, Bell AS, Sim DG, Tomasello D, Mideo N, Day T, Read AF. 2013 Aggressive chemotherapy and the selection of drug resistant pathogens. *PLoS Pathog.* **9**, e1003578. (doi:10.1371/journal.ppat.1003578)
- Sergent E, Parrot L, Donatien A. 1925 On the necessity of having a term to express the resistance of carriers of germs to superimposed infections. *Trans. R. Soc. Trop. Med. Hyg.* **18**, 383–385. (doi:10.1016/S0035-9203(25)90394-X)
- Handel A, Longini IM, Antia R. 2009 Antiviral resistance and the control of pandemic influenza: the roles of stochasticity, evolution and model details. *J. Theor. Biol.* **256**, 117–125. (doi:10.1016/j.jtbi.2008.09.021)
- Mouton JW, Ambrose PG, Canton R, Drusano GL, Harbarth S, MacGowan A, Theuretzbacher U, Turnidge J. 2011 Conserving antibiotics for the future: new ways to use old and new drugs from a pharmacokinetic and pharmacodynamic perspective. *Drug Resist. Updat.* **14**, 107–117. (doi:10.1016/j.drup.2011.02.005)
- Råberg L, Graham AL, Read AF. 2009 Decomposing health: tolerance and resistance to parasites in animals. *Phil. Trans. R. Soc. B* **364**, 37–49. (doi:10.1098/rstb.2008.0184)
- Lipsitch M, Samore MH. 2002 Antimicrobial use and antimicrobial resistance: a population perspective. *Emerg. Infect. Dis.* **8**, 347–354. (doi:10.3201/eid0804.010312)
- Grenfell BT, Pybus OG, Gog JR, Wood JLN, Daly JM, Mumford JA, Holmes EC. 2004 Unifying the epidemiological and evolutionary dynamics of pathogens. *Science* **303**, 327–332. (doi:10.1126/science.1090727)
- Zinner SH, Gilbert D, Greer K, Portnoy YA, Firsov AA. 2013 Concentration–resistance relationships with *Pseudomonas aeruginosa* exposed to doripenem and ciprofloxacin in an *in vitro* model. *J. Antimicrob. Chemother.* **68**, 881–887. (doi:10.1093/jac/dks463)
- Negri M-C, Lipsitch M, Blazquez J, Levin BR, Baquero F. 2000 Concentration-dependent selection of small phenotypic differences in TEM beta-lactamase-mediated antibiotic resistance. *Antimicrob. Agents Chemother.* **44**, 2485–2491. (doi:10.1128/AAC.44.9.2485-2491.2000)
- Firsov AA, Vostrov SN, Lubenko IY, Arzamatsev AP, Portnoy YA, Zinner SH. 2004 ABT492 and levofloxacin: comparison of their pharmacodynamics and their abilities to prevent the selection of resistant *Staphylococcus aureus* in an *in vitro* dynamic model. *J. Antimicrob. Chemother.* **54**, 178–186. (doi:10.1093/jac/dkh242)
- El Moussaoui R, Roede BM, Speelman P, Bresser P, Prins JM, Bossuyt PMM. 2008 Short-course antibiotic treatment in acute exacerbations of chronic bronchitis and COPD: a meta-analysis of double-blind studies. *Thorax* **63**, 415–422. (doi:10.1136/thx.2007.090613)
- Li JZ, Winston LG, Moore DH, Bent S. 2007 Efficacy of short-course antibiotic regimens for community-acquired pneumonia: a meta-analysis. *Am. J. Med.* **120**, 783–790. (doi:10.1016/j.amjmed.2007.04.023)
- Altamimi S, Khalil A, Khalaiwi KA, Milner RA, Pusic MV, Al Othman MA. 2012 Short-term late-generation antibiotics versus longer term penicillin for acute streptococcal pharyngitis in children. *Cochrane database Syst. Rev.* **8**, CD004872.
- Lindsey HA, Gallie J, Taylor S, Kerr B. 2013 Evolutionary rescue from extinction is contingent on a lower rate of environmental change. *Nature* **494**, 463–467. (doi:10.1038/nature11879)
- Blahna MT, Zalewski CA, Reuer J, Kahlmeter G, Foxman B, Marrs CF. 2006 The role of horizontal gene transfer in the spread of trimethoprim-sulfamethoxazole resistance among uropathogenic *Escherichia coli* in Europe and Canada. *J. Antimicrob. Chemother.* **57**, 666–672. (doi:10.1093/jac/dkl020)
- Gupta V, Dorsey G, Hubbard AE, Rosenthal PJ, Greenhouse B. 2010 Gel versus capillary electrophoresis genotyping for categorizing treatment outcomes in two anti-malarial trials in Uganda. *Malar. J.* **9**, 19. (doi:10.1186/1475-2875-9-19)
- Babiker HA, Walliker D. 1997 Current views on the population structure of *Plasmodium falciparum*: implications for control. *Parasitol. Today* **13**, 262–267. (doi:10.1016/S0169-4758(97)01075-2)
- Arnot D. 1998 Clone multiplicity of *Plasmodium falciparum* infections in individuals exposed to variable levels of disease transmission. *Trans. R. Soc. Trop. Med. Hyg.* **92**, 580–585. (doi:10.1016/S0035-9203(98)90773-8)
- Dye C, Williams BG. 1997 Multigenic drug resistance among inbred malaria parasites. *Proc. R. Soc. Lond. B* **264**, 61–67. (doi:10.1098/rspb.1997.0009)
- Karami N, Nowrouzian F, Adlerberth I, Wold AE. 2006 Tetracycline resistance in *Escherichia coli* and persistence in the infantile colonic microbiota. *Antimicrob. Agents Chemother.* **50**, 156–161. (doi:10.1128/AAC.50.1.156-161.2006)
- Zhang L, Huang Y, Zhou Y, Buckley T, Wang HH. 2013 Antibiotic administration routes significantly influence the levels of antibiotic resistance in gut microbiota. *Antimicrob. Agents Chemother.* **57**, 3659–3666. (doi:10.1128/AAC.00670-13)
- Salyers AA, Gupta A, Wang Y. 2004 Human intestinal bacteria as reservoirs for antibiotic resistance genes. *Trends Microbiol.* **12**, 412–416. (doi:10.1016/j.tim.2004.07.004)
- Lipsitch M, Levin BR. 1998 Population dynamics of tuberculosis treatment: mathematical models of the roles of non-compliance and bacterial heterogeneity in the evolution of drug resistance. *Int. J. Tuberc. Lung Dis.* **2**, 187–199.
- Lima VD, Harrigan R, Murray M, Moore DM, Wood E, Hogg RS, Montaner JS. 2008 Differential impact of adherence on long-term treatment response among naive HIV-infected individuals. *AIDS* **22**, 2371–2380. (doi:10.1097/QAD.0b013e328315cdd3)
- Harrigan PR *et al.* 2005 Predictors of HIV drug-resistance mutations in a large antiretroviral-naive cohort initiating triple antiretroviral therapy. *J. Infect. Dis.* **191**, 339–347. (doi:10.1086/427192)
- Sethi AK, Celentano DD, Gange SJ, Moore RD, Gallant JE. 2003 Association between adherence to antiretroviral therapy and human immunodeficiency virus drug resistance. *Clin. Infect. Dis.* **37**, 1112–1118. (doi:10.1086/378301)

35. Menzies D, Benedetti A, Paydar A, Martin I, Royce S, Pai M, Vernon A, Lienhardt C, Burman W. 2009 Effect of duration and intermittency of rifampin on tuberculosis treatment outcomes: a systematic review and meta-analysis. *PLoS Med.* **6**, e1000146. (doi:10.1371/journal.pmed.1000146)
36. Maggiolo F, Airoldi M, Kleinloog HD, Callegaro A, Ravasio V, Arici C, Bombana E, Suter F. 2007 Effect of adherence to HAART on virologic outcome and on the selection of resistance-conferring mutations in NNRTI- or PI-treated patients. *HIV Clin. Trials* **8**, 282–292. (doi:10.1310/hct0805-282)
37. Bangsberg DR, Moss AR, Deeks SG. 2004 Paradoxes of adherence and drug resistance to HIV antiretroviral therapy. *J. Antimicrob. Chemother.* **53**, 696–699. (doi:10.1093/jac/dkh162)
38. Van den Boogaard J, Boeree MJ, Kibiki GS, Aarnoutse RE. 2011 The complexity of the adherence–response relationship in tuberculosis treatment: why are we still in the dark and how can we get out? *Trop. Med. Int. Heal.* **16**, 693–698. (doi:10.1111/j.1365-3156.2011.02755.x)
39. Srivastava S, Pasipanodya JG, Meek C, Leff R, Gumbo T. 2011 Multidrug-resistant tuberculosis not due to noncompliance but to between-patient pharmacokinetic variability. *J. Infect. Dis.* **204**, 1951–1959. (doi:10.1093/infdis/jir658)
40. Roberts JA, Kruger P, Paterson DL, Lipman J. 2008 Antibiotic resistance—what’s dosing got to do with it? *Crit. Care Med.* **36**, 2433–2440. (doi:10.1097/CCM.0b013e318180fe62)
41. Zhao X, Drlaca K. 2008 A unified anti-mutant dosing strategy. *J. Antimicrob. Chemother.* **62**, 434–436. (doi:10.1093/jac/dkn229)
42. Olofsson SK, Cars O. 2007 Optimizing drug exposure to minimize selection of antibiotic resistance. *Clin. Infect. Dis.* **45**(Suppl. 2), S129–S136. (doi:10.1086/519256)
43. Stearne LET, Goessens WHF, Mouton JW, Gyssens IC. 2007 Effect of dosing and dosing frequency on the efficacy of ceftizoxime and the emergence of ceftizoxime resistance during the early development of murine abscesses caused by *Bacteroides fragilis* and *Enterobacter cloacae* mixed infection. *Antimicrob. Agents Chemother.* **51**, 3605–3611. (doi:10.1128/AAC.01486-06)
44. Tam VH, Louie A, Deziel MR, Liu W, Drusano GL. 2007 The relationship between quinolone exposures and resistance amplification is characterized by an inverted U: a new paradigm for optimizing pharmacodynamics to counterselect resistance. *Antimicrob. Agents Chemother.* **51**, 744–747. (doi:10.1128/AAC.00334-06)
45. White NJ. 2004 Antimalarial drug resistance. *J. Clin. Invest.* **113**, 1084–1092. (doi:10.1172/JCI21682)
46. Ribeiro RM, Bonhoeffer S. 2000 Production of resistant HIV mutants during antiretroviral therapy. *Proc. Natl Acad. Sci. USA* **97**, 7681–7686. (doi:10.1073/pnas.97.14.7681)
47. Guillemot D, Carbon C, Balkau B, Geslin P, Lecoer H, Vauzelle-Kervroëdan F, Bouvenot G, Eschwège E. 1998 Low dosage and long treatment duration of beta-lactam: risk factors for carriage of penicillin-resistant *Streptococcus pneumoniae*. *JAMA* **279**, 365–370. (doi:10.1001/jama.279.5.365)
48. Malhotra-Kumar S, Lammens C, Coenen S, Van Herck K, Goossens H. 2007 Effect of azithromycin and clarithromycin therapy on pharyngeal carriage of macrolide-resistant streptococci in healthy volunteers: a randomised, double-blind, placebo-controlled study. *Lancet* **369**, 482–490. (doi:10.1016/S0140-6736(07)60235-9)
49. Gustafsson I, Sjölund M, Torell E, Johannesson M, Engstrand L, Cars O, Andersson DI. 2003 Bacteria with increased mutation frequency and antibiotic resistance are enriched in the commensal flora of patients with high antibiotic usage. *J. Antimicrob. Chemother.* **52**, 645–650. (doi:10.1093/jac/dkg427)
50. Raum E, Lietzau S, von Baum H, Marre R, Brenner H. 2008 Changes in *Escherichia coli* resistance patterns during and after antibiotic therapy: a longitudinal study among outpatients in Germany. *Clin. Microbiol. Infect.* **14**, 41–48. (doi:10.1111/j.1469-0691.2007.01841.x)
51. Murcia PR *et al.* 2010 Intra- and interhost evolutionary dynamics of equine influenza virus. *J. Virol.* **84**, 6943–6954. (doi:10.1128/JVI.00112-10)
52. MacLean RC, Hall AR, Perron GG, Buckling A. 2010 The population genetics of antibiotic resistance: integrating molecular mechanisms and treatment contexts. *Nat. Rev. Genet.* **11**, 405–414. (doi:10.1038/nrg2778)
53. Atkins KE, Read AF, Savill NJ, Renz KG, Islam AF, Walkden-Brown SW, Woolhouse MEJ. 2012 Vaccination and reduced cohort duration can drive virulence evolution: Marek’s disease virus and industrialized agriculture. *Evolution (NY)*. **67**, 851–860. (doi:10.1111/j.1558-5646.2012.01803.x)
54. Govorkova EA. 2013 Consequences of resistance: *in vitro* fitness, *in vivo* infectivity, and transmissibility of oseltamivir-resistant influenza A viruses. *Influenza Other Respir. Viruses* **7**(Suppl 1), 50–57. (doi:10.1111/irv.12044)
55. Jiang ZD, DuPont HL. 2005 Rifaximin: *in vitro* and *in vivo* antibacterial activity—a review. *Chemotherapy* **51**(Suppl. 1), 67–72. (doi:10.1159/000081991)
56. Harrington WE, Mutabingwa TK, Kabyemela E, Fried M, Duffy PE. 2011 Intermittent treatment to prevent pregnancy malaria does not confer benefit in an area of widespread drug resistance. *Clin. Infect. Dis.* **53**, 224–230. (doi:10.1093/cid/cir376)
57. Tiono AB *et al.* 2013 A controlled, parallel, cluster-randomized trial of community-wide screening and treatment of asymptomatic carriers of *Plasmodium falciparum* in Burkina Faso. *Malar. J.* **12**, 79. (doi:10.1186/1475-2875-12-79)
58. Kibengo FM *et al.* 2013 Safety, adherence and acceptability of intermittent tenofovir/emtricitabine as HIV pre-exposure prophylaxis (PrEP) among HIV-uninfected Ugandan volunteers living in HIV-serodiscordant relationships: a randomized, clinical trial. *PLoS ONE* **8**, e74314. (doi:10.1371/journal.pone.0074314)
59. Van Rie A *et al.* 2005 Reinfection and mixed infection cause changing *Mycobacterium tuberculosis* drug-resistance patterns. *Am. J. Respir. Crit. Care Med.* **172**, 636–642. (doi:10.1164/rccm.200503-4490C)
60. Gandon S, Mackinnon MJ, Nee S, Read AF. 2001 Imperfect vaccines and the evolution of pathogen virulence. *Nature* **414**, 751–756. (doi:10.1038/414751a)
61. Dondorp AM, Fairhurst RM, Slutsker L, Macarthur JR, Breman JG, Guerin PJ, Wellems TE, Ringwald P, Newman RD, Plowe CV. 2011 The threat of artemisinin-resistant malaria. *N. Engl. J. Med.* **365**, 1073–1075. (doi:10.1056/NEJMp1108322)
62. Phy AP *et al.* 2012 Emergence of artemisinin-resistant malaria on the western border of Thailand: a longitudinal study. *Lancet* **379**, 1960–1966. (doi:10.1016/S0140-6736(12)60484-X)
63. Gullberg E, Cao S, Berg OG, Ilbäck C, Sandegren L, Hughes D, Andersson DI. 2011 Selection of resistant bacteria at very low antibiotic concentrations. *PLoS Pathog.* **7**, e1002158. (doi:10.1371/journal.ppat.1002158)

Glossary

Full resistance

resistance levels such that drug levels that can be safely administered do not lead to clinical benefits or cannot clear the pathogen. Conversely, partial resistance indicates resistance levels such that realistic drug levels can clear the pathogen, but this occurs more slowly than it would do in fully susceptible strains

De novo evolution of resistance

Genetic barrier of resistance of a given drug or treatment
MSW

appearance of a mutation that confers resistance within a single host, and its transmission to subsequent hosts

number of mutations the pathogen needs to accumulate in order to achieve full resistance

the mutation-selection window (MSW) has been defined as the range between the minimal inhibitory concentration (MIC)

and the mutant prevention concentration (MPC), where the MIC is the minimal concentration at which wild-type growth is inhibited and the MPC is the minimal concentration at which growth of resistant single point mutants of the wild-type is inhibited. The MSW is very similar in concept to the curve in figure 1. However, the MSW is typically restricted to *in vitro* or within-host systems and it specifies the range for which growth of the resistant strain is possible (rather than the value for which it is maximized). Recent work has moreover contested the MIC as the lower limit of the MSW as it has been shown that the range in which drug resistance is selected may go considerably beyond this traditional MSW [63]

Moderate chemotherapy

treatment where the aim is to maximize host health outcomes while trying to minimize drug doses. This might not mean eliminating the pathogen during drug treatment. Note that moderate treatment may still recommend doses above the MIC as long as they are not sufficiently high or taken for sufficiently long to completely clear the pathogen. Moderate treatment aims to optimize the dosage and timing of treatment of individual patients rather than at coordinating anti-infective use at the population scale (e.g., antibiotic cycling in hospitals). However, the outcome being optimized is typically the same (minimizing the spread of resistance at the

population level) in both cases. The approach of moderate chemotherapy contrasts aggressive chemotherapy, which aims to completely clear the pathogen from the patient

Clinically neutral range	range of drug pressures with comparable clinical outcomes for an individual patient (but potentially different outcomes with regard to resistance evolution)
Break points	MICs used to identify the degree of resistance possessed by a particular strain (often within the classes 'susceptible', 'intermediate', 'resistant')
Emergence	first appearance of a drug-resistant mutation in a focal population
Spread	following emergence, the drug-resistant pathogens increasing in frequency within the population
Establishment	drug-resistant mutation maintains a consistent equilibrium frequency in the population
Cost of resistance	reduction in fitness experienced by resistant pathogens relative to susceptible pathogens in the absence of the drugs; often caused by mutations in genes key to metabolic processes that are drug targets
Drug pressure	the relative degree to which treatment can reduce abundance of the susceptible pathogens; which can be achieved either through high concentration of treatment or long duration of treatment (provided that the concentration is not too low)
Fitness	quantity that quantifies the ability to survive and reproduce and contribute to the gene pool in the next generations