# ANTIMICROBIAL PHARMACODYNAMICS: CRITICAL INTERACTIONS OF 'BUG AND DRUG'

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Antimicrobial pharmacodynamics is the discipline that integrates microbiology and pharmacology, with the aim of linking a measure of drug exposure, relative to a measure of drug potency for the pathogen in question, to the microbiological or clinical effect achieved. The delineation of such relationships allows the drug dose to be chosen in a rational manner, so that the desired effect (for example, the maximal bactericidal effect) can be achieved in a large proportion of the intended patient population. Ultimately, the goal of any anti-infective therapy is to administer a dose of drug that has an acceptably high probability of achieving the desired therapeutic effect balanced with an acceptably low probability of toxicity. Appropriate use of the latest pharmacodynamic modelling approaches can minimize the emergence of resistance and optimize the outcome for patients.

PHARMACODYNAMICS
The study of the biochemical and physiological effects of drugs and the mechanisms of their actions.

MINIMAL INHIBITORY CONCENTRATION
(MIC). The lowest drug concentration that results in stasis.

EFFECTIVE CONCENTRATION$_{90}$ ($EC_{90}$). Usually applied to virus susceptibility — the drug concentration resulting in a decrease in replication of 90%.

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Antimicrobial PHARMACODYNAMICS is the discipline that attempts to link measures of drug exposure to the microbiological or clinical effects that are observed once an anti-infective drug has been administered. The general area of pharmacodynamics is a large field, in which exposure to all classes of drugs is linked to observed effects. However, the realm of antimicrobial pharmacodynamics is special. Here, we have the important advantage of having some measure of the activity of the drug (for example, the MINIMAL INHIBITORY CONCENTRATION (MIC) or EFFECTIVE CONCENTRATION$_{90}$ ($EC_{90}$)) for the pathogen in question. In other areas of pharmacodynamics, the aim is to 'dock' a molecule into a receptor that is normally or abnormally expressed on a cell surface or that is intracellular. At present, true between-patient differences in the affinity of the drug for the receptor are not measured. By contrast, in the field of antimicrobial pharmacodynamics, we can directly measure the ability of the anti-infective agent to dock into its 'receptor' (the pathogen) and cause the effect for which it was designed. This difference allows us to link drug exposure to the observed effect more directly.

To understand antimicrobial pharmacodynamics, it is necessary to be conversant with four important ideas. First, administering a fixed dose of drug to a large number of patients, even on a mg $kg^{-1}$ basis, will result in substantially different profiles of the changing concentration of the drug over time (concentration–time profiles) among these patients (variability in exposure). Second, the shape of the curve describing the concentration–time profile (the concentration–time curve) can have an impact on the effect of a particular drug dose in some instances (different pharmacodynamic variables are linked to microbiological effect). Third, only free (non-protein-bound) drug is microbiologically active. Finally, the higher the value of the measure of the potency of the drug (for example, the MIC or $EC_{90}$) for the pathogen in question, the less effect a fixed drug exposure will have. It is also important to recognize that to truly understand the relationship between drug exposure and the response, these ideas must be viewed in an integrated fashion.

## Starting the investigation

When starting a pharmacodynamic investigation, the most important decision is the endpoint that is to be measured and linked to drug exposure. This endpoint

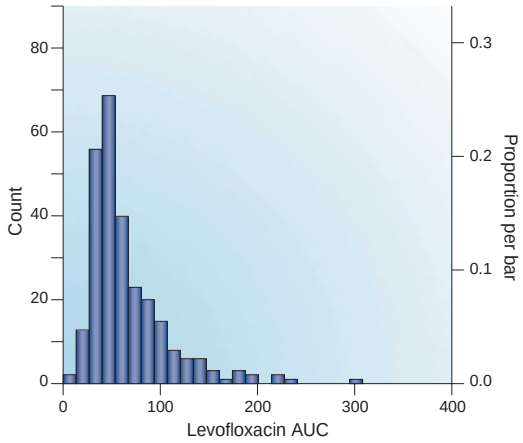



Figure 1 | **True between-patient variance.** The area under the concentration–time curve (AUC) distribution from a 252-patient study of levofloxacin in the treatment of community-acquired infections. The range of exposures generated with a drug dose of 500 mg is broad, and mainly reflects between-patient variance in clearance.

differs, depending on the study, the setting and the pathogen. Whether the study is an *in vitro* examination, a study using an animal model or a clinical study has a significant impact on the endpoint chosen. In many *in vitro* systems or animal models in which the effects of antibacterial or antifungal agents are examined, the outcome that is measured most often is a direct determination of the size of the bacterial population. For HIV therapeutics, the change in viral load, as measured by PCR or an equivalent technology, is the most common endpoint examined. For clinical trials, the dichotomous outcomes of patient success or failure — microbiologically or clinically — are the most frequently measured outcomes. Once an endpoint has been chosen, the pharmacodynamic investigation attempts to link some measure of drug exposure to this endpoint. Different data-analysis tools are appropriate for each of these scenarios, and will be discussed further below.

### The first idea: true between-patient variance

For decades, attempts to generate exposure–response relationships used the dose of the drug as the independent variable. These attempts were almost always unsuccessful, for several reasons. Arguably, the most important of these is the fact that the dose is a particularly poor measure of drug exposure for populations of patients. There is true between-patient variability in important PHARMACOKINETIC parameters, such as DRUG CLEARANCE and VOLUME OF DISTRIBUTION. Some of these differences between patients can be explained by differences in physiological factors, such as the glomerular filtration rate, weight, age or gender. However, even when all these physiological factors are accounted for, true between-patient variability still exists. Therefore, when administering a fixed dose of drug to large numbers of patients in a target patient population, a relatively large range of drug exposures will be observed.

An example of this is shown in FIG. 1. Our group studied the fluoroquinolone levofloxacin in 252 patients with community-acquired infections[1,2]. Levofloxacin is a very well-behaved drug in a pharmacokinetic sense: it has linear clearance, which is mostly accounted for by renal processes, and, at least in normal volunteer populations, has a relatively small standard deviation for the value of clearance[3]. In the clinical trial, the patients all had values of serum creatinine less than 2.0 mg $dl^{-1}$, so the physiological differences in glomerular filtration rate among patients was not the main cause of the observed differences in clearance. Nonetheless, the actual range of exposures generated by a drug dose of 500 mg was broad (FIG. 1), reflecting mostly the true between-patient variability in clearance.

Although most of the observations are clustered around AREA UNDER THE CONCENTRATION–TIME CURVE (AUC) values of 50–70 (mg hr)$L^{-1}$, the range is broad, and extends from <20 (mg hr)$L^{-1}$ to >200 (mg hr)$L^{-1}$. It is not surprising that attempts to use dose as a surrogate for drug exposure were not successful in trying to identify the relationships between drug exposure and patient response.

It is also important to recognize that this discussion refers to drug concentrations in serum or plasma. These serve as a surrogate for drug concentrations at the infection site. In most cases, these measurements serve us quite well in our aim to link drug exposure to effect. However, there are instances, such as meningitis, when the pathological process has a major impact on the drug concentration–time profile at the infection site. In such circumstances, the actual drug concentrations at the infection site must be measured. Here, too, there will be true between-patient variability in drug concentrations at the infection site. Some infections occur intracellularly and again, serum or plasma drug concentrations are usually good surrogates. However, much more investigation in this area is needed.

### The second idea: curve shape can be important

*β-lactams: the shape of the concentration–time curve has an impact.* The realization that the shape of the concentration–time curve is important, which is truly one of the central ideas of antimicrobial pharmacodynamics, should be directly credited to Harry Eagle[4]. Eagle and his group carried out a seminal series of studies with penicillin in the late 1940s and 1950s. In a small-animal model system (the mouse thigh infection model) they were able to clearly show that the amount of time that the penicillin concentrations exceeded the MIC for the strain of pathogen chosen had a direct impact on the degree of microbiological effect observed.

Although this was shown convincingly in several publications, the idea was lost until the early 1980s when it was rediscovered by Craig and colleagues[5]. This approach was ultimately fairly successful in explaining the differences in effect among different classes of anti-infective agents. For the β-lactam class of agents, the clearest demonstration that the shape of the concentration–time curve significantly affects the microbiological effect was developed by the laboratory of Gerber[6]. These

PHARMACOKINETIC
The study of the bodily absorption, distribution, metabolism and excretion of drugs.

DRUG CLEARANCE
The volume of plasma that is completely cleared of drug per unit time.

VOLUME OF DISTRIBUTION
The apparent volume in the patient relating dose and observed plasma concentration.

AREA UNDER THE CONCENTRATION–TIME CURVE (AUC). A measure of the total exposure to drug — the integral of the concentration–time curve.

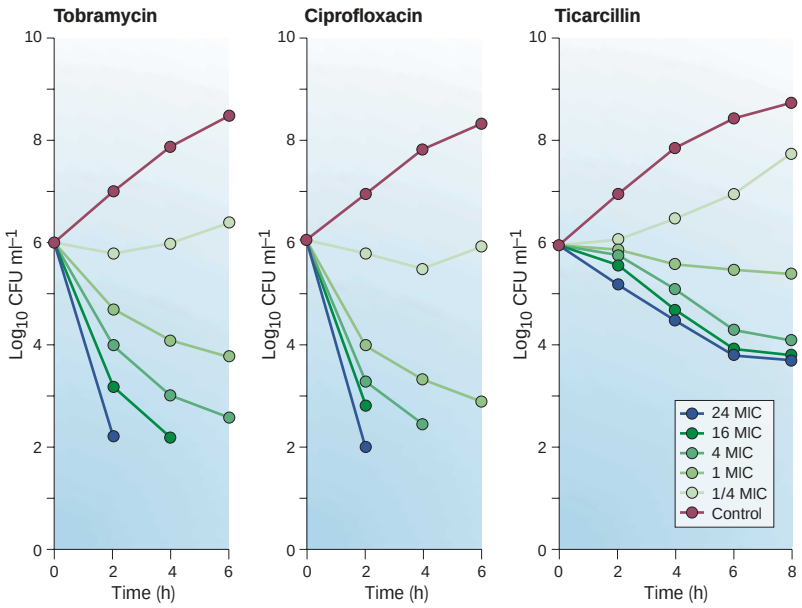



Figure 2 | **Different pharmacodynamic variables are important for different drugs.** The rates of cell killing change greatly with concentration for tobramycin (an aminoglycoside) and ciprofloxacin (a fluoroquinolone). By contrast, for ticarcillin (a β-lactam), the rates of killing change when the concentration of drug increases from the minimal inhibitory concentration (MIC) to approximately 4–6 times the MIC. Greater increases in drug concentration cause little or no further change in the kill rate. Reproduced with permission from REF. 9 © (1998) The University of Chicago Press.

investigators used the carbapenem antibiotic imipenem in a mouse thigh infection model. The same total dose of drug was administered to two groups of mice, one of which had the whole dose administered at time zero, while the other had fractions of the total dose administered every few minutes for 6 hours. The fractionated dosing was calculated to result in a drug profile that mimicked the half-life of the drug in humans (~1 hour), while in the other group, the PEAK CONCENTRATION was much higher and the half-life shorter, thereby reflecting murine physiology. Consequently, the shapes of the curve would be quite different.

The aim of this experiment was to observe the microbiological effect over time. The human pharmacokinetic profile gave the greater microbiological effect, even though the murine profile achieved much higher peak concentrations. The total AUC was approximately equivalent in both groups. This shows that the rate at which organisms are killed at the primary infection site is not very sensitive to drug concentration, as long as the drug concentration exceeds the MIC for the target organism. This finding is applicable to the β-lactam class of agents. It is the time that the concentration of free β-lactam remains above the MIC (TIME > MIC) for the organism that is linked to the microbiological effect. Other classes of anti-infective agents (for example, fluoroquinolones and aminoglycosides), do not demonstrate this property, as Craig's laboratory has shown[7,8].

In addition to the idea that time > MIC is important for β-lactam drugs, it is also important to recognize that the concentration of free drug does not have to remain above the MIC for the entire dosing interval to achieve the maximal antimicrobial effect. Different types of β-lactam agents, such as penicillins, cephalosporins and carbapenems, require free drug to be present for differing fractions of the dosing interval to achieve a BACTERIOSTATIC effect or maximal BACTERICIDAL effect. Again, Craig and colleagues have demonstrated this convincingly[9]. For bacteriostasis, the concentration of free drug must exceed the MIC for 35–40%, 30% and 20% of the dosing interval for cephalosporins, penicillins and carbapenems, respectively. Achievement of the maximal bactericidal effect requires 60–70%, 50% and 40% coverage, respectively, for these β-lactam classes.

*Aminoglycosides & quinolones: the shape of the concentration–time curve has no impact.* Aminoglycosides and quinolones behave quite differently from β-lactams. For these agents, the shape of the concentration–time curve does not have a significant impact on the microbiological effect observed. Indeed, it is the AUC relative to the organism's MIC for the drug (the AUC/MIC ratio) for 24 hours that is most closely linked to the microbiological effect observed[7,8].

For aminoglycosides and quinolones, a different index of exposure is linked to outcome, probably because of the methods by which these agents kill most target pathogens. Once the drug concentration exceeds 4–6 times the MIC, the rate at which pathogens are killed by β-lactam drugs is relatively concentration-independent, whereas the rates at which pathogens are killed by aminoglycosides and quinolones are relatively concentration-dependent over a broad concentration range (FIG. 2). This explains why, for the different classes of drugs, different measures of exposure are linked to the microbiological effect (BOX 1).

There is another group of drugs for which the AUC/MIC ratio is linked to outcome, but the agents are not concentration-dependent in their effect. Examples of this group include drugs such as azithromycin and vancomycin[10,11]. These agents are either poorly bactericidal or slowly bactericidal. In both cases, these drugs cause a profound POST-ANTIBIOTIC EFFECT or PAE[12], that is, after exposure to the drug and complete drug removal, the bacteria take a much longer time to regrow 1 $\log_{10}$ colony-forming units (CFU) $ml^{-1}$ than do similarly treated, but non-drug-exposed bacteria. This is probably related to the bacterial injury that is caused by the drug exposure. For the agents cited, the duration of the PAE is prolonged by increasing the AUC/MIC ratio. Of interest, for these drugs, as the MIC increases and the AUC/MIC ratio decreases, the duration of the PAE is reduced and these agents become more similar to time > MIC drugs.

## β-lactams: kill concentration independence

Two important questions that arise are why the killing rate that is engendered by the β-lactams is less concentration-dependent than that seen with fluoroquinolones and aminoglycosides, and why it is not necessary to keep concentrations in excess of the MIC for the whole dosing interval. Although these questions are complex, there are two main reasons for these findings and it is clear that they are interrelated.

PEAK CONCENTRATION
The highest concentration attained in a dosing interval.

TIME > MIC
The time that drug concentrations exceed the MIC, often stated as a percentage of the dosing interval.

BACTERIOSTATIC
An antibiotic that inhibits the growth of a bacterial population.

BACTERICIDAL
An antibiotic that kills 99.99% of a bacterial population.

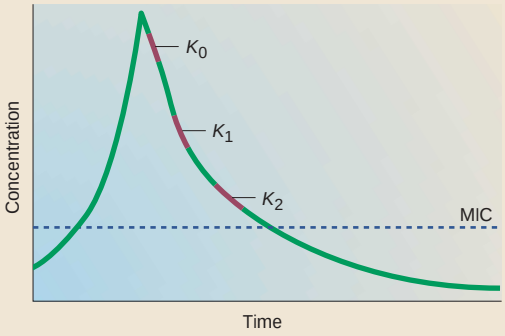
AUC/MIC
Ratio of the area under the concentration–time curve to the MIC.

POST-ANTIBIOTIC EFFECT (PAE)
Persistent inhibitory effect on a microorganism that results from drug exposure after the drug has been completely removed.

**Box 1 | Why are different pharmacodynamic variables important for different drugs?**

The effect of anti-infective agents in improving the health of patients (or animals) is mediated through their ability to prevent the growth of, or kill, the infecting pathogen at the primary site of infection. If one takes as a hypothesis that the number of organisms killed at the primary infection site is closely linked to the outcome, then it becomes clear why different measures of exposure are linked to outcome for the different drug classes.

The figure shows a concentration–time profile and how this relates to cell killing. For the β-lactam drugs, as they are relatively concentration-independent in their kill rate, the rate of killing reaches its maximum very quickly as the drug concentration increases from the MIC to 4–6 times the MIC.




The number of organisms killed is approximated by equation 1:

$$\text{Cell kill} \approx \int_{t1}^{t2} K(c)dt \qquad (1)$$

where $K$ is the kill rate engendered by the drug, $t1$ is the time where the concentration of free drug first exceeds the MIC and $t2$ is the point in time at which the drug ceases to engender an appreciable kill rate (around the MIC). Because the kill rate for the β-lactams is nearly independent of concentration (that is, in the figure $K_0 \approx K_1 \approx K_2$), it can be approximated as a constant and can move across the integral sign, giving equation 2:

$$\text{Cell kill} \approx K\int_{t1}^{t2} dt = K_{t1}^{t2}[t] = K(t2 - t1) \qquad (2)$$

This tells us that for a drug where the kill rate engendered is relatively independent of concentration and falls precipitously when the drug concentration declines below the MIC, the number of organisms killed is approximated by the relatively constant killing rate multiplied by the time that the drug concentration remains above the MIC. Therefore, for drugs with such a relationship between concentration and effect, like the β-lactams, time > MIC is the pharmacodynamically linked variable. The shape of the concentration–time curve can then have an impact on the microbiological effect, if the concentrations remain in excess of the MIC. This can be accomplished through the use of short dosing intervals (for example, every 4 hours) and also by the use of continuous[45] or prolonged[46] infusions.

The situation is different for those agents for which the kill rate engendered is dependent on concentration, such as aminoglycosides and fluoroquinolones. The same relationship as that in equation 1 pertains. However, because the kill rate changes with concentration (that is, in the figure, $K_0 > K_1 > K_2$), it cannot be taken across the integral sign. The total number of organisms killed can then be approximated as an expectation, as shown in equation 3:

$$\text{Cell kill} \approx \sum_{i=1}^{n} K_i(c) \times t(c) \qquad (3)$$

where the kill rate changes with concentration and $t$ (c) is the time period over which the kill rate is appropriate (that is, where not enough concentration change has taken place to change the kill rate appreciably). It should be noted that the integral of the concentration–time curve is the AUC. It is then straightforward to understand why the AUC/MIC ratio is the pharmacodynamically linked variable for drugs that are concentration-dependent killers.

The link between time > MIC and the microbiological effect has its origin in the mechanism of action of the β-lactam drugs, which is related to the acylation of their targets, the β-lactam-binding proteins. Williamson and Tomasz examined the binding of benzylpenicillin to the β-lactam-binding proteins in a lysis-defective mutant of *Streptococcus pneumoniae*[13]. In this investigation, they showed that inhibition of peptidoglycan synthesis and, after a lag time, organism stasis, occurred only after a relatively high proportion of the available β-lactam-binding proteins had been acylated. Death by lysis and alteration of culture turbidity could not be observed in these experiments because a lysis-deficient mutant was used. Experiments over a longer time scale demonstrated the maximal acylation of the different binding proteins. This was not significantly different to the acylation that was seen in the shorter (150 minute) experiments.

These findings explain the relative lack of concentration-dependence in the killing rate for β-lactams. There is a maximum proportion of the targets (β-lactam-binding proteins) that can be acylated. A large proportion of the β-lactam-binding proteins are already acylated by the time bacterial stasis is achieved. As the percentage of target binding proteins that are acylated increases above the level of acylation that is associated with bacteriostasis, a bactericidal effect is seen. Once maximal acylation is achieved, the killing rates cannot increase any further. This explains why the killing rates for β-lactam drugs are maximal at a low multiple of the MIC. The phrase 'concentration-independent in kill rate' has been popularized for the β-lactams. Ironically, because there is little difference between the proportion of the target that is acylated for stasis and for maximal rates of killing, the rate of killing actually

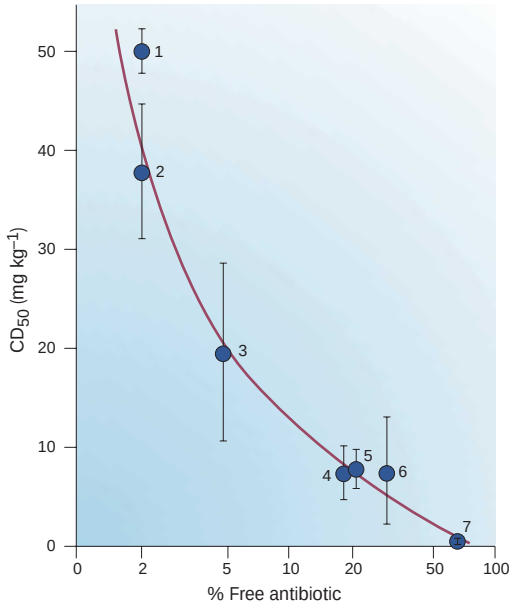



Figure 3 | **Only non-protein-bound drug is microbiologically active.** The $CD_{50}$ (the dose protecting 50% of infected animals) was determined for seven isoxazolyl penicillins (numbers 1–7) and related to the proportion of the drug that was free (non-protein bound). Increasing the proportion of free drug translated into lower doses required to protect 50% of the animals. Modified with permission from REF. 15 © (1983) British Society for Antimicrobial Chemotherapy.

increases markedly with increasing drug concentration. However, the maximal rate of killing is attained at a low multiple of the MIC, which is probably the best description of the phenomenon.

Finally, the ability to achieve a particular level of acylation of the β-lactam-binding proteins does not occur instantaneously. Rather, this chemical reaction proceeds with time. This explains why the drug concentrations do not need to exceed the MIC for the full dosing interval. The level of acylation that is required for stasis or maximal cell killing occurs over a period of time that is shorter than the dosing interval, but probably varies among different types of β-lactams. There is a deacylation rate, but this is relatively slow and the PAE (where applicable) and the POST-ANTIBIOTIC SUB-MIC EFFECT (PA-SME) prevent organism regrowth until the next dosing interval, assuming that this is less than the sum of the time > MIC and the persistent effects, such as the PAE and PA-SME[12,14].

### The third idea: only free drug is active

Protein binding and its effect on microbiological activity has always been a contentious issue. However, there are considerable data from both antibacterial and antiviral research to indicate that protein binding has a reasonably predictable adverse impact on the microbiological activity that is observed[15,16].

Merriken, Briant and Rolinson performed an important experiment examining the impact of protein binding on the anti-staphylococcal effect of isoxazolyl penicillins[15]. These investigators used a lethal *Staphylococcus aureus* mouse infection model with an intraperitoneal challenge of 100 median lethal doses. They examined seven isoxazolyl penicillins: oxacillin, cloxacillin, dicloxacillin and four experimental compounds. All compounds had identical MIC values for the challenge strain used. The half-lives were short and ranged from 6.5 minutes to 18.8 minutes. Protein binding ranged from 36% (64% free drug) to 98% (2% free drug). Values of the PROTECTIVE DRUG DOSE ($PD_{50}$; $CD_{50}$ in FIG. 3) — the dose of drug that is necessary to protect 50% of the animals — are shown in FIG. 3 as a function of the percentage of free antibiotic. Clearly, protein binding is an important factor in determining the $PD_{50}$. The same idea is true for anti-HIV therapeutics[16]. The effect of the binding protein α-1-acid glycoprotein (AAG) on the antiviral activity of the experimental HIV-1 aspartyl protease inhibitor A-80987 has been examined[16], and it was shown that protein binding and the consequent effect on free drug concentrations had a marked impact on the microbiological effect of A-80987.

### The fourth idea: a high MIC can lessen effect

It is trivial to say, but no less important to understand, that the higher the value of the measure of potency (for example, a higher MIC or $EC_{90}$), the lower the measure of drug exposure relative to the measure of potency (the time > MIC is shorter, and the AUC/MIC ratio and the PEAK CONCENTRATION/MIC ratio are reduced) and the lower the level of the expected microbiological effect. This was demonstrated in a GRANULOCYTOPENIC rat model of *Pseudomonas aeruginosa* sepsis in which survivorship was the endpoint (FIG. 4). Drusano *et al.* examined the effect of a fluoroquinolone antimicrobial[17]. Two isogenic mutants of the parent strain were created that had higher MICs for the drug being examined. The MICs of the three strains (the parent and two mutant strains) for the drug were 1, 4 and 8 mg $L^{-1}$, respectively. With a standard dose of 80 mg $kg^{-1}$, it is clear that survivorship decreases as the MIC increases. The AUC remains constant, but the MIC increases, leading to decreased AUC/MIC ratios. Indeed, when a 20 mg $kg^{-1}$ drug dose is administered to the parent strain (MIC of 1 mg $L^{-1}$) so that the resulting AUC/MIC ratio is identical to that for the treatment of one of the mutant strains (MIC of 4 mg $L^{-1}$) with the standard 80 mg $kg^{-1}$ dose, the resulting survivorship curves are virtually superimposable. This indicates that either the exposure or the MIC can be changed, but that the outcome is related to the pharmacodynamically linked variable — in this instance, the AUC/MIC ratio. Also, with the same drug exposure, increasing the MIC leads to reduced response rates.

### Integrating the four ideas

To integrate these ideas, it is first necessary to decide what magnitude of effect is desired from the drug dose chosen. This choice is usually based on clinical circumstances. For instance, treatment of a minor community-acquired

POST-ANTIBIOTIC SUB-MIC EFFECT
(PA-SME). Persistent inhibitory effect on a microorganism that results from drug exposure after the drug has been diluted to a fraction of the MIC.

PROTECTIVE DRUG DOSE
($PD_{50}$ or $CD_{50}$). The drug dose resulting in protection of 50% of challenged animals.

PEAK CONCENTRATION/MIC
The ratio of the peak concentration to the MIC.

GRANULOCYTOPENIC
A blood disorder that is characterized by a severe reduction in the number of granulocytes in blood.

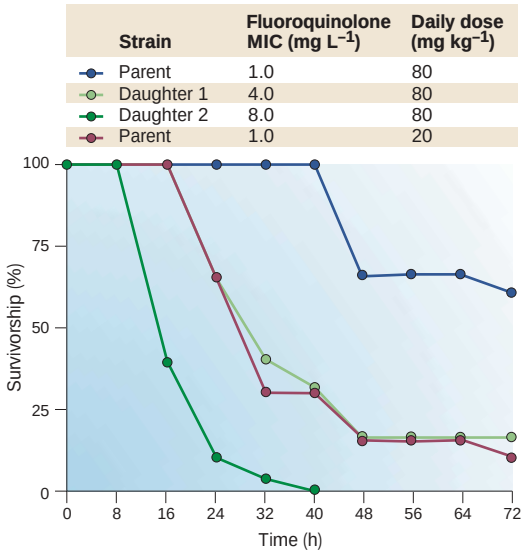



Figure 4 | **The higher the measure of potency, the lower the measure of drug exposure relative to the measure of potency.** In this study, groups ($n$ = 20) of neutropenic rats infected with $10^9$ colony-forming units (CFU) per ml of three isogenic strains of *Pseudomonas aeruginosa* were treated with a fluoroquinolone (lomefloxacin). One group was infected with the parent *P. aeruginosa* strain, with a fluoroquinolone minimal inhibitory concentration (MIC) of 1.0 mg $L^{-1}$ and was treated with 80 mg $kg^{-1}$ lomefloxacin once daily. Another group was infected with a daughter strain that had a fluoroquinolone MIC of 4.0 mg $L^{-1}$ and was treated with 80 mg $kg^{-1}$ once daily (Daughter 1). A third group was infected with a second daughter strain with a fluoroquinolone with a MIC of 8.0 mg/L and also treated with 80 mg $kg^{-1}$ (Daughter 2). The final group was infected with the parent strain (MIC of 1.0 mg $L^{-1}$), but treated with 20 mg $kg^{-1}$ once daily. This provides the same AUC/MIC ratio as being infected with a strain with an MIC of 4.0 mg $L^{-1}$ and receiving an 80 mg $kg^{-1}$ dose of lomefloxacin daily. If the treatment stays constant at 80 mg $kg^{-1}$, as the MIC increases, survivorship decreases. By contrast, when different doses are used but the AUC/MIC ratio remains the same, survivorship is not significantly different. AUC, area under the concentration–time curve. Modified with permission from REF. 17 © (1993) American Society for Microbiology.

infection might only require that the drug achieve a static effect, so that the patient's intact immune system can clear the infection. However, treatment of a severely granulocytopenic patient with sepsis will require a near-maximal bactericidal effect. The relationship between measures of drug exposure and the microbiological effect that is achieved, as determined in a NEUTROPENIC mouse lung infection model, is shown in FIG. 5. In this analysis[9], different measures of exposure are displayed on the $x$-axes. It is clear that time > MIC provides the best link between exposure and effect as would be expected for a β-lactam agent (cefotaxime). It is also clear that bacteriostasis is achieved when the drug concentration exceeds the MIC for 35–40% of the dosing interval and near-maximal effect is achieved when 60–70% of the dosing interval is covered. It should be emphasized that these exposure targets were developed in neutropenic animals. Restoring the granulocytes will decrease the magnitude of the exposure targets[18]. The size of the change will, however, differ between drug classes.

Once a target is selected, various questions can be posed. First, what is the highest MIC that will reliably achieve the target for a specific drug dose in a population of patients? This is a method by which a breakpoint MIC value can be determined for the effect desired. Second, how useful will a specific drug dose be, both in the population of patients for which the drug is intended and over the full range of MIC values that are likely to be encountered by that patient population?

The four ideas can then be integrated through the use of a MONTE CARLO SIMULATION (see the online links box) and by collecting a large number of isolates against which it is intended to use the drug and determining the MIC distribution. In this way, the questions can be addressed. The Monte Carlo simulation allows delineation of the full spread of values (for example, peak concentrations and AUCs) that would be seen in a large population after the use of a specific drug dose (FIG. 1). These total drug values can then be corrected for any protein-binding differences between the animal or *in vitro* model in which they were developed and those seen in humans.

For each MIC value in the distribution of a large collection of target pathogens, the target attainment rate can be determined in the population of simulated subjects. This provides an answer to the first question. Because the fraction of the organism collection at each MIC value is known, a weighted average (expectation) of the target attainment rates can be taken. This value provides an answer to the second question of how useful a specific drug dose will be for the population for which it is to be used. Our laboratory first described this application of the Monte Carlo simulation[19,20].

This technique has been validated clinically several times[20–22]. Indeed, our group has published a prediction of drug effectiveness with validation provided by a prospective, randomized, double-blind clinical trial in the area of HIV therapeutics[22]. Furthermore, it has been used to provide the basis for changing the National Committee for Clinical Laboratory Standards (NCCLS) susceptibility breakpoints for several cephalosporins for *S. pneumoniae* as well as not changing the breakpoint for penicillin G for this pathogen[23,24].

## Suppression of resistance as an endpoint

Microbiological effects, such as those described in the examples above, and clinical outcomes (see below) are the most common endpoints used in pharmacodynamic modelling. However, it is also possible to model other important endpoints, such as suppression of the emergence of resistance (BOX 2). Resistance to antimicrobial agents can occur through the acquisition of new DNA or through point mutations in chromosomes. The selective pressure of the antimicrobial agent allows the resistant clone to amplify. Horizontal transmission of the resistant isolate magnifies the problem. It is possible to choose a dose of drug that prevents amplification of the resistant clone. This technique is

NEUTROPENIC
An abnormal decrease in the number of white cells in the blood.

MONTE CARLO SIMULATION
An analytical technique for solving a problem by performing a large number of simulations and inferring a solution from the collective results that can be used to calculate the probability distribution of possible outcomes.

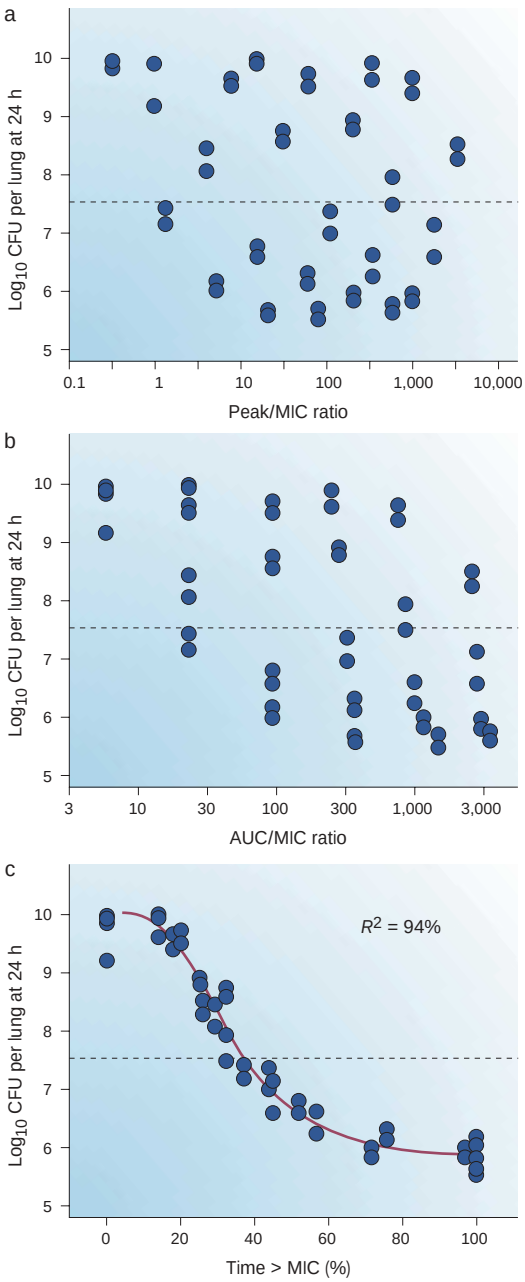



Figure 5 | **The relationship between three pharmacodynamic parameters: peak concentration/MIC ratio, AUC/MIC ratio and time > MIC.** In this experiment, the peak concentration/MIC ratio (**a**); the AUC/MIC ratio (**b**); the percentage of time the serum levels exceed the MIC (% time > MIC) (**c**), and the number of colonies of *Klebsiella pneumoniae* ATCC 43816 in the lungs of neutropenic mice after 24 hours of therapy with cefotaxime were measured. Each point represents data obtained for one mouse. The dashed line reflects the number of bacteria at the beginning of therapy. The $R^2$ value in part **c** represents the percentage of variation in bacterial numbers that could be attributed to differences in the time > MIC. The target exposures (time > MIC) for stasis and near-maximal cell killing are ~40% and ~65%, respectively. Target exposures can vary by isolate and have an associated confidence limit. AUC, area under the concentration–time curve; CFU, colony-forming units; MIC, minimal inhibitory concentration. Modified with permission from REF. 9 © (1998) The University of Chicago Press.

most applicable to the situation where a random point mutation occurs in the drug target site (for example, mutation of topoisomerase II or IV in the case of fluoroquinolone resistance) or, perhaps, where a mutation occurs that allows overexpression of a resistance factor (for example, efflux pumps).

As we are in a crisis of resistance in the therapy of both nosocomial and community-acquired infections, the ability to choose doses and schedules of drugs to help suppress the emergence of resistance could be a key step in preventing this trend. Such an approach could help extend the utility of older drugs as well as optimize the development of new agents (BOX 2).

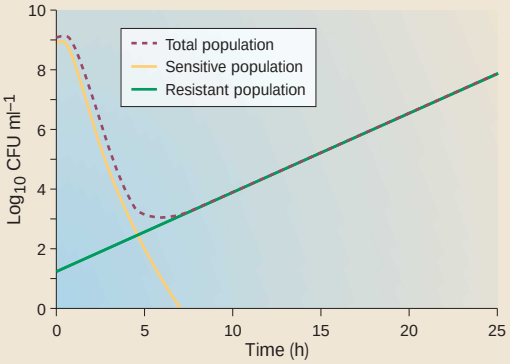
Our group has used this approach, with a non-neutropenic mouse thigh infection model with *P. aeruginosa* as the pathogen[25]. Both the total *P. aeruginosa* population and the resistant population (resistant to at least three times the baseline MIC) in the mouse thigh were determined. A model was fit to the data, with good results (FIG. 6a,b). The AUC/MIC ratio associated with suppression of the resistant clones was identified. A subsequent prospective validation experiment was performed using two doses of drug administered over a longer period of time than the initial study so that, in effect, the future was being predicted. One dose studied was expected to amplify the resistant subpopulation to near its maximum level, whereas the other was meant to keep the number of mutants in the population constant at the number present at baseline. The validation experiment worked well, as can be seen in FIG. 6c,d. Both drug doses performed as predicted. This implies that it is possible to choose a drug dose for use in a population of interest that can help suppress the amplification of resistant clones.

## The transition to the clinic

Determining which pharmacodynamic variable is most closely linked to microbiological effects in preclinical systems is important, but this link is elucidated so that the drug doses and schedules used provide the highest probability of a good clinical or microbiological outcome for patients. Also, the Monte Carlo simulation is useful in extending the insights obtained from the preclinical environment to the clinic, but it is also crucial to determine whether the findings from our preclinical work can be validated in patients.

Over the past ten years, several studies have been published, both retrospective and prospective, linking exposure to an anti-infective drug with a response. The first modern publication in this area was by Forrest and colleagues and was a retrospective evaluation of ciprofloxacin from several clinical trials in which concentration–time data had been collected[26]. These authors were able to successfully link the AUC/MIC ratio to both clinical and microbiological outcomes. Indeed, they also examined the time to pathogen clearance from the lower respiratory tract by sampling through the endotracheal (ET) tube on a daily basis. Unfortunately, this sampling was carried out without quantitative microbiology and without the use of a protected specimen brush or other instrument that

**Box 2 | The emergence of resistance**




When large populations of organisms exist at the primary infection site, as is often the case in patients with nosocomial pneumonia, for example, the size of the population often exceeds the inverse of the mutational frequency to resistance. This means that the total population consists of a large population of wild-type organisms combined with a smaller population of mutant organisms, the MIC of which is increased relative to the wild-type parent strain.

The presence of this smaller, more resistant bacterial population has important implications for chemotherapy. When a fixed dose of drug is administered, the resultant drug exposure will frequently have a differential effect on the two populations. The wild-type population will often be readily killed by the drug exposure. The mutant population, even when the MIC is only slightly increased (for example, 2–4-fold), is often not killed by the drug exposure, allowing this population to amplify over time. With time, the total population decreases the number of wild-type clones and increases the mutant clones, thereby increasing the level of resistant isolates in the population. This is shown schematically in the figure, which shows the differential effects of drug exposure on the two populations. The wild-type (sensitive) population is efficiently killed, whereas the mutant (resistant) population is often amplified. The change in profile of the total population is explained by the differential effects of the drug exposure on the two populations.

would prevent contamination from the inside of the ET tube. Several laboratories have shown that the inside of the ET tube is frequently colonized by the same pathogen causing the nosocomial pneumonia[27,28]. Therefore, if the pathogen is not found on sampling, one can have a degree of faith in the finding. However, if the causative pathogen is found on culture, one does not know whether the pathogen was acquired from the ET tube or the lungs. Consequently, this part of the Forrest trial cannot be evaluated. Nonetheless, this retrospective evaluation was in agreement with previous animal model and *in vitro* data that indicated that either the peak concentration/MIC ratio or the AUC/MIC ratio is linked to the microbiological effect for fluoroquinolones.

The first prospective, multicentre trial designed specifically to delineate the pharmacodynamically linked variable for an experimental anti-infective agent was published by Preston *et al*.[1] In this study, the fluoroquinolone levofloxacin was used to treat community-acquired infections. Limited plasma sampling was guided by stochastic optimal design theory[29] (see below). The concentration determinations for the whole population were analysed by population pharmacokinetic modelling and estimates of drug exposures for individual patients were generated using maximal *A-posteriori* probability (MAP) Bayesian estimation (see below). Both clinical and microbiological outcomes were determined for the patients. As these were dichotomous outcomes, logistic regression analysis was used to link measures of drug exposure to the probability of a good clinical or microbiological outcome. The logistic regression curve for AUC/MIC ratio is shown in FIG. 7. In this study, both the peak concentration/MIC and AUC/MIC ratios could be linked to both clinical and microbiological outcomes.

Recently, a relatively large number of pharmacodynamic analyses of anti-infective agents have been published. Among the antibacterial agents, these studies include fluoroquinolone, aminoglycoside and β-lactam relationships[1,26,30–34], and are shown in TABLE 1. The question that arises is why there should be such an explosion of pharmacodynamic studies in clinical populations. The answer is probably related to the ready availability of tools to design pharmacokinetic studies and analytical and statistical tools to allow drug exposure to be linked to effect. These pharmacokinetic and statistical tools can be thought of as those that allow drug exposure to be estimated (PK tools) and those that allow measures of exposure to be linked to response (statistical tools). These are discussed below.

*Identifying informative sampling times: stochastic optimal sampling.* To link drug exposure to effect, it is important to have a robust estimate of the drug exposure in a particular patient. Owing to true between-patient variability (see above), it is necessary to measure the concentration of drug in the serum to obtain an accurate estimate of drug exposure. The advent of population pharmacokinetic modelling in the late 1970s allowed estimates to be made of how target populations of patients handle a drug[35]. Initially, such population studies were carried out with randomly obtained serum samples. If the number of samples obtained was large enough, this would result in reasonably accurate estimates of the pharmacokinetic parameter values for the population. However, for the development of pharmacodynamic relationships, it is important to have an accurate estimate of drug exposure in individual patients. Random sampling can provide sufficiently accurate estimates for some patients, but not others.

In traditional pharmacokinetic studies, normal volunteers are often studied and large numbers of blood samples are obtained from each subject. This approach cannot be justified in most clinical studies, as a large number of blood sampling times would be insupportable in ill patient populations and impossible in certain subpopulations, such as children or the very elderly. In addition, the interruption of clinical care would not allow such invasive schedules for blood sampling.

Optimal sampling theory[36] identifies a limited number of sampling times that allow precise pharmacokinetic parameters to be estimated following sample analysis. This smaller, but more informative, set of sampling times is much more congruent with the clinical circumstances. Originally, optimal sampling theory only generated a number of sampling times that was the same as the number of model parameters, owing to the underlying assumption that there is only one true set of population pharmacokinetic parameters and that measures of dispersion in these parameters do not affect the calculations. As indicated above, true between-patient variability in pharmacokinetic

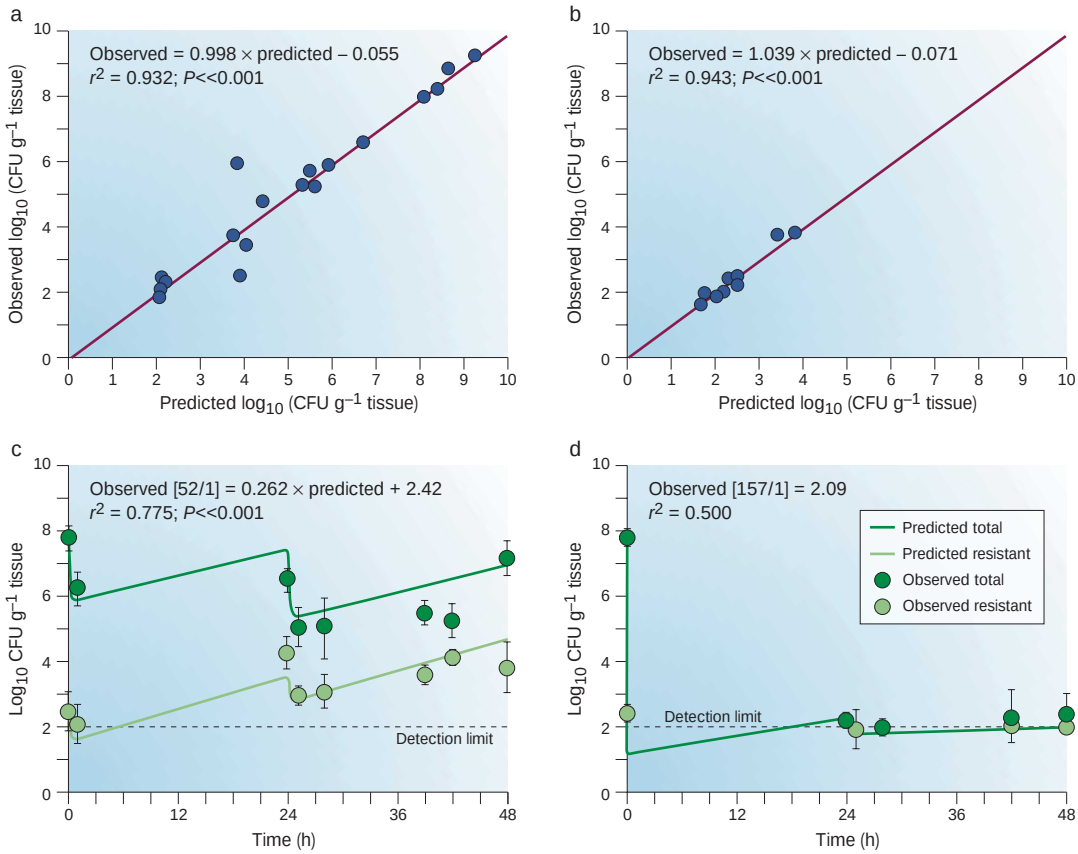



Figure 6 | **Suppressing the emergence of resistance.** Four treatment regimens of levofloxacin were evaluated against *Pseudomonas aeruginosa* in a non-neutropenic mouse thigh infection model. The effect of the four drug doses on the total population (**a**) and the resistant subpopulation (**b**) was determined over time. Five differential equations were fitted to the data. The graphs show the predicted–observed plot for the total and resistant subpopulations following maximum *A-posteriori* probability (MAP) Bayesian estimation. This model explained well the survival and bactericidal kinetics for both populations ($r^2 \geq 0.93$ for total and resistant populations). The emergence of resistance model that was developed was then validated prospectively by generating response predictions for doses not previously studied that would (**c**) encourage selection of resistance or (**d**) suppress the emergence of resistance. An exposure of an AUC/MIC ratio of 157 was calculated to prevent emergence of resistance. Experiments were carried out for 48 hours, not 24 hours as in the studies performed to generate the parameter estimates. Levofloxacin dosing occurred at 0 and 24 hours. The lines are model predictions (not best-fit curves). The model predicted changes in the resistant mutant population well at both exposures. AUC, area under the concentration–time curve; CFU, colony-forming units; MIC, minimal inhibitory concentration. Modified with permission from REF. 25 © (2003) American Society for Clinical Investigation.

parameter values does exist. As patients' parameter values move away from the population mean values, the information provided in the sampling schedule decreases. Several laboratories addressed this problem by explicitly recognizing true between-patient variability in the calculations[29,37,38]. This allows relatively small sets of sampling times (usually 6–7) to be generated that are sufficiently robust for most of the patient populations to be studied. In so doing, accurate estimates of drug exposures can be calculated, allowing better estimation of the relationships between drug exposure and response.

*Identifying the parameter values for a patient group: population pharmacokinetic modelling.* Population pharmacokinetic modelling differs from traditional pharmacokinetic modelling in that it deals explicitly with populations of patients rather than with an individual patient. Sheiner *et al.* produced the first software for this purpose and named it NONMEM, for nonlinear mixed effects models[35]. This (among other) population modelling approach(es) allows the observed variance to be explained as being due to the model chosen (for example, a two-compartment model versus a one-compartment model), certain fixed effects (for example, the differing weights and glomerular filtration rates of the patients) and explicitly recognizing that true between-patient variability in pharmacokinetic parameter values does exist (for example, that the estimate of serum clearance has a standard deviation associated with it). The rest of the variance not explained by these factors is often called residual variance.

NONMEM and many other population modelling programs explicitly assume a distribution for the parameter values, for example, that the clearances of the population have a normal or log-normal distribution. Another group of population modelling programs, including NPAG (non-parametric adaptive grid) and NPML (non-parametric maximum likelihood), make no

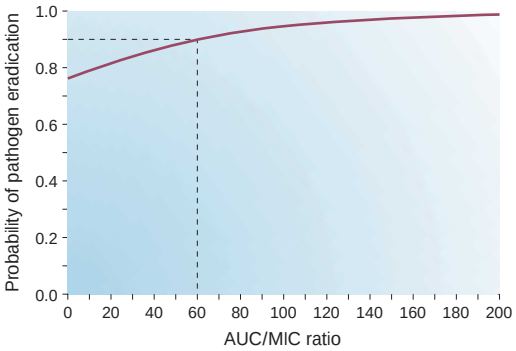



Figure 7 | **Transferring to the clinic: linking the AUC/MIC ratio to clinical and microbiological outcomes.** A pharmacokinetic profile for levofloxacin was obtained for 252 patients with community-acquired infections. For 116 patients, an outcome (microbiological response: pathogen eradication or pathogen persistence) was measured, and an MIC determined for the pathogen to levofloxacin. The curve was generated from a logistic regression analysis ($p < 0.0012$). The AUC/MIC ratio required for a 90% probability of eradication was 60. This is equivalent to a free-drug AUC/MIC ratio of 42. AUC, area under the concentration–time curve; MIC, minimal inhibitory concentration.

explicit assumptions about the shape of the parameter distribution[39,40]. There are positive aspects and limitations for both parametric and non-parametric programs, but both have played a central role in generating pharmacodynamic relationships.

Population pharmacokinetic modelling allows an insight into how specific populations of patients that can differ in their physiology handle drugs. Not surprisingly, ill patient populations handle drugs quite differently from normal volunteer populations, mostly owing to issues of age and altered physiology. For the agent levofloxacin, a normal volunteer population demonstrated a serum clearance of approximately 11 L $hr^{-1}$ for a 750 mg dose, whereas a population of patients with nosocomial pneumonia demonstrated a serum clearance of approximately 6 (population median) to 7 (population mean) L $hr^{-1}$ (REFS 29,41).

Although this information is helpful in designing studies and in choosing doses to obtain a specific amount of exposure, it does not solve the problem of obtaining a patient-specific estimate of drug exposure.

*Identifying the parameter values for individual patients: maximal* A-posteriori *probability (MAP) Bayesian estimation.* To obtain patient-specific estimates of drug exposure, we use MAP-Bayesian estimation. This estimation process explicitly balances information about the specific patient (the drug concentrations obtained from that patient) with prior knowledge about how a specific patient population handles a specific drug. This prior knowledge is codified as a measure of central tendency of the parameter values (usually the mean value of the parameter) and a measure of dispersion (usually the standard deviation) of the parameter values in the population[42]. This process is often done automatically in population modelling programs. In this way, the best point estimates of the pharmacokinetic parameter values for each patient in the data set can be obtained. This allows calculation of the peak concentration/MIC ratio, AUC/MIC ratio or time > MIC.

*Linking exposure to response.* Once a complete data set has been obtained, including the endpoint that has been chosen for analysis, drug exposure measures, the MIC for the pathogen that has been isolated from the patient, other factors that can affect outcome (for example, intubation status) and, perhaps, protein binding, attempts can be made to delineate a pharmacodynamic relationship.

The choice of the statistical tool will depend explicitly on the endpoint that has been chosen for analysis. Microbiological outcome and clinical outcome are dichotomous variables. Consequently, a tool such as logistic regression that estimates the probability of the occurrence of an outcome as a function of the independent variable(s) could be the most appropriate way to attempt to link exposure to response in this instance (FIG. 7).

Sometimes, as in HIV disease or cytomegalovirus (CMV) disease, the time to an event is the outcome that is measured. For example, in the case of HIV disease, the time to loss of viral control has been examined[43]. The ability of the therapeutic regimen to drive the viral load below the level of detection of the currently available assay for HIV copy number in serum was shown to have a significant impact on the time to the loss of viral control. Likewise, in CMV retinal disease in HIV-positive

Table 1 | Recent studies delineating pharmacodynamic relationships for antibacterial agents

| Drug | Patient population | References |
|---|---|---|
| ***Fluoroquinolones*** | | |
| Ciprofloxacin | Nosocomial infections (mostly pneumonia) | 26 |
| Levofloxacin | Community infections | 1 |
| Gatifloxacin/levofloxacin | Community infections | 30 |
| Grepafloxacin | Community infections | 31 |
| Levofloxacin | Nosocomial pneumonia | 32 |
| ***Aminoglycosides*** | | |
| Gentamicin/tobramycin | Nosocomial pneumonia | 33 |
| ***β-lactams*** | | |
| Cefipime | Infections requiring hospitalization | 34 |

patients, the AUC of the drug foscarnet was shown to affect the time to expansion of the lesion in the fundi[44]. In these cases, stratified Kaplan–Meier analysis and Cox proportional hazards modelling were the statistical tools used.

For endpoints that are continuous variables, such as the number of organisms at a primary infection site, measures of exposure can be linked to the effect through a sigmoid-Emax effect model (FIG. 5c).

Classification and regression tree (CART) analysis is an exploratory data-analysis technique that is a powerful way to examine how different factors interact and can influence outcome. However, in the area of anti-infective pharmacodynamics, this tool is particularly useful for determining exposure breakpoint values. For example, in the studies of levofloxacin in both community-acquired infections and nosocomial pneumonia, breakpoint values were determined by CART analysis[1,31]. Likewise, Ambrose found the breakpoint AUC/MIC value for the fluoroquinolone therapy of pneumococcal respiratory tract infections by using CART analysis[30].

These and many other statistical techniques allow us to link measures of exposure to the outcomes that were chosen at the start of the analysis.

## Overall summary

Antimicrobial pharmacodynamics — the field that integrates microbiology and pharmacology — is an area that has seen a huge growth in knowledge over the past two decades. This area is different from all other areas of pharmacodynamic investigation owing to the ability to isolate the pathogen — be it a bacterium, virus, parasite or fungus — and determine a measure of potency of the drug in question for this pathogen. Once measures of exposure can be linked to effect, it is possible to choose drug doses that have a high likelihood of achieving the desired goals of therapy. These relationships are now being elucidated more frequently owing to the broad availability of sophisticated pharmacokinetic software and the application of appropriate statistical techniques to the data sets. The true aims of anti-infective therapy are to administer a dose of drug to a patient that will have an acceptably high probability of attaining the desired therapeutic effect, while also having an acceptably low probability of concentration-related toxicity. Owing to our ability to develop such relationships, we now have the ability to approach this long-desired goal of therapy and improve the outcomes of infection for our patients. As the development of concentration–effect and concentration–toxicity relationships becomes more common, the main regulatory agencies responsible for overseeing drug development (for example, the US Food and Drug Administration and the European Agency for the Evaluation of Medicinal Products) should incorporate this process into the regulatory development requirements. In this way, the best balance between risk and benefit for the ill patient requiring drug administration can be attained.

Finally, where does the field need to go in the future? Hopefully, more clinical studies will show that the techniques developed provide an adequate bridge from the bench to the bedside. More investigation is needed on the concentrations of free drug at the infection site and their impact, as well as on the therapy of resistant organisms and rare infections, including pathogens that are potential agents of bioterrorism. The choice of exposure target and the impact of clinical signs and symptoms on this choice is an area in which little is known, but much could be gained by investigation. The greatest need, however, is in the broader availability of mathematical modelling software to take the field forward. Newer techniques in population modelling (for example, approaches using Markov chain Monte Carlo techniques) and stochastic optimal design must come to the fore and a new generation of investigators must be trained to bring the pharmacodynamic approach to all areas of anti-infective chemotherapy.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## Author's note
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## Competing interests statement
The author declares that he has no competing financial interests.

## Online links

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