nature microbiology

REVIEW ARTICLE

https://doi.org/10.1038/s41564-019-0357-1

Drug repurposing for antimicrobial discovery

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Antimicrobial resistance continues to be a public threat on a global scale. The ongoing need to develop new antimicrobial drugs that are effective against multi-drug-resistant pathogens has spurred the research community to invest in various drug discovery strategies, one of which is drug repurposing—the process of finding new uses for existing drugs. While still nascent in the antimicrobial field, the approach is gaining traction in both the public and private sector. While the approach has particular promise in fast-tracking compounds into clinical studies, it nevertheless has substantial obstacles to success. This Review covers the art of repurposing existing drugs for antimicrobial purposes. We discuss enabling screening platforms for antimicrobial discovery and present encouraging findings of novel antimicrobial therapeutic strategies. Also covered are general advantages of repurposing over de novo drug development and challenges of the strategy, including scientific, intellectual property and regulatory issues.

One of the greatest challenges to global public health today is antimicrobial resistance. Antimicrobial resistance is currently responsible for over 700,000 deaths annually around the world and has been predicted to exponentially rise to above 10 million deaths annually by 2050, with an estimated economic cost of $100 trillion[1]. As antimicrobial resistance continues to rise, the effective treatment of an ever-increasing range of infectious diseases caused by drug resistant parasites, viruses, fungi and bacteria will be threatened[2,3]. In recent years, there have been several outbreaks of severe infectious diseases, for example, those caused by the artemisinin-resistant *Plasmodium falciparum* malaria[4], the fungus *Exserohilum rostratum*[5], *Candida auris*[6], Ebola virus[7], Zika virus[8], carbapenem-resistant *Klebsiella pneumoniae*[9] and plasmid-mediated colistin-resistant Enterobacteriaceae[10]. In common is a lack of susceptibility in these microorganisms to some of the most common and potent therapeutic options available.

The medical need for novel antimicrobial treatment options globally is undeniable, and efforts to discover and develop novel antimicrobial drugs should be intensified. Modern drug discovery and development, however, is a lengthy and arduous process that inevitably struggles to deliver new therapies in a timely manner. To blame are the long intervals and high costs associated with research and development that most often culminate in high failure rates due to safety and toxicity issues[11,12], among many other sources of attrition[13]. Thus, strategies to improve the efficiency of drug development are urgently needed to enable effective drugs to enter the clinic. One creative strategy for the identification of novel antimicrobials, which is gaining momentum in publicly funded research institutions and increasingly being leveraged by pharmaceutical companies, is that of drug repurposing.

Drug repurposing, repositioning and redirecting are common terms used to describe the process of generating novel clinical opportunities for known approved drugs, whether through new indications or new commercial opportunities for already marketed drugs[14]. In general, the promise of drug repurposing comes from two very different principles. The first is that many drugs have cryptic biological activities[15], a phenomenon that is made evident by the many side-effects of drugs. The second is that different diseases often share common molecular pathways and/or genetic factors[16]. The latter notion has been frequently leveraged in cancer therapy, where drug repurposing has been validated with the successful application of a number of non-cancer drugs for cancer treatment[17,18]. Repurposing has been common practice in the pharmaceutical industry for years and is by no means a recent approach; however, repurposing success stories and companies leveraging repurposing strategies are increasing in number. Reviews of the field indicate that at least 46 approved drugs have already been repurposed for new therapeutic uses[19,20]. Some classical examples of these are discussed in Box 1. In recent years, interest in new approaches to drug research and development, especially for antimicrobial drug discovery, has brought drug repurposing to the forefront as a promising strategy. In this context, drug repurposing is often contingent on uncovering cryptic antimicrobial activities in existing drugs. Faced with the threat of antimicrobial resistance, options for treating infectious diseases are increasingly limited. Further, in recent years the pharmaceutical industry has reduced in-house antimicrobial research in favour of therapeutic areas that offer stronger returns on research and development investments[21]. Thus, drug repurposing is gaining traction as a strategic approach in antimicrobial research with the potential to reduce costs and expedite approval timelines. Encouraging in vitro and in vivo results from drug repurposing efforts to uncover novel antimicrobial agents are being published with increasing frequency, offering hope that this strategy can also be used to uncover new treatments for microbial infection.

In this Review, we focus on drug repurposing as an alternative to conventional antimicrobial drug discovery and development. We provide a conceptual introduction to drug repurposing, with examples, success stories and common pitfalls. In addition, we outline some modern strategies to antimicrobial drug discovery in the repurposing space, as well as important translational and regulatory hurdles typically encountered during the process.

An accelerated drug development process

The prospective advantages of drug repurposing over de novo drug development for antimicrobial drug discovery are manifold; however, drug repurposing primarily allows for an accelerated drug development process as compared to the typical route for de novo drug development (Fig. 1a)[19,20]. Repurposing efforts bypass much of the discovery and preclinical stages, since pharmacokinetic, pharmacodynamic and toxicity profiles of the drugs are often already

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Box 1 | Classical repurposing success stories

Historically, drug repurposing has largely stemmed from unintentional and serendipitous findings that took place when a drug was found to have an off-target effect or a previously unrecognized effect that could be used towards a new indication. Perhaps the most celebrated example of successful drug repurposing is sildenafil. Originally intended to treat hypertension, sildenafil was repurposed for the treatment of erectile dysfunction and pulmonary arterial hypertension. Other examples of successfully repurposed drugs include thalidomide, originally developed for morning sickness and now used to treat symptoms of leprosy and multiple myeloma, and bupropion, originally developed for depression and repurposed for smoking cessation a decade later. Minoxidil is an example of an old drug repurposed with a new indication and reformulation. Originally recognized for hypertension, minoxidil was later reformulated and indicated for hair loss. These classical repurposing success stories offer great promise for the feasibility and effectiveness of the drug repurposing strategy. Importantly, repurposed drugs have largely leapfrogged time-consuming research hurdles of toxicity profiling, target validation, hit-to-lead optimization, and/or in vivo drug metabolism and pharmacokinetic studies. While these examples were due to serendipitous observations, researchers are increasingly searching for drugs to repurpose, including for antimicrobial purposes.

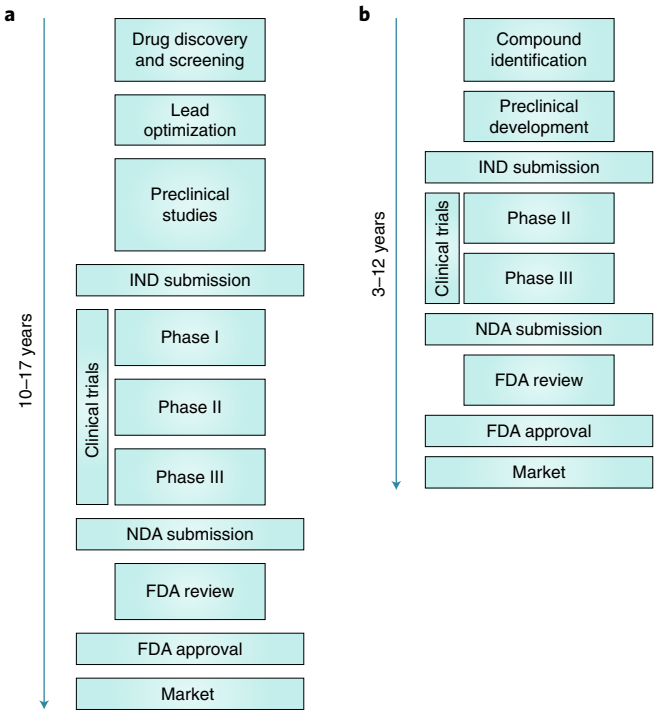

Fig. 1 | Schematic representation of the regulatory process for licensing conventional versus repurposed compounds. a, The typical route to the clinic for de novo drug development first involves intellectual property filing and preclinical studies, where success brings about filing of an Investigational New Drug Application (IND) with the FDA in preparation, for example, for the US marketplace. The drug candidate is then evaluated in humans through a series of clinical trials (Phase I–III). Success here leads to a New Drug Application (NDA) that allows entry into the marketplace for prescription and sales to patients/consumers. **b**, Repurposing efforts bypass much of the discovery and preclinical stages and typically forgo extensive Phase I studies for safety, entering clinical trials at Phase IIa. Successful clinical trials lead to an NDA and the repurposed drug enters the market.

known[20]. Because the lead candidate has likely been subjected to safety and toxicological evaluation for the original indication, the pre-clinical phase only requires a demonstration of efficacy for the new indication in either a cell or animal model system. Success here can lead to filing of an Investigational New Drug Application with the U.S. Food and Drug Administration (FDA) and is uniquely subject to the 505(b)(2) process, which allows information from previous studies to be used in the evaluation of the drug candidate[22]. Thus, the lead candidate can typically forgo extensive Phase I studies for safety and enter clinical trials at Phase IIa, where safety is evaluated with considerably less rigor and cost[23,24]. Successful clinical trials lead to a New Drug Application and the repurposed drug can enter the market (Fig. 1b). Not only can this mean faster development times and reduced costs, but also development risks are reduced, as safety, the major limitation in drug discovery, is well established[19]. From an economic perspective, the bypassed developmental stages (for example, Phase I) allow savings of ~15% of the overall cost[20], which has been estimated at upwards of $800 million for de novo drug development[25]. This also means an estimated 3–12 year process for repurposed drugs versus a 10–17 year process for de novo drug development[19]. A more rapid route to the clinic is also possible because key parameters, such as chemical optimization, manufacturing and formulation, have often also been completed and can be bypassed[19]. Moreover, drug repurposing can further reduce the costs of developing new therapies when patent protection expires, allowing generic manufacturing. Thus, savings for consumers are potentially enormous. These are all advantageous when it comes to antimicrobial drug discovery and, accordingly, efforts to repurpose drugs for infectious diseases are becoming increasingly attractive.

Challenges of the drug repurposing process

While repurposing involves lower risks and costs compared to de novo development of novel antimicrobial agents, a large limitation when it comes to antimicrobial applications is clinical pharmacology. For instance, the process is only feasible if effective concentrations for the new usage are in a similar range as those of the original drug[26]. Indeed, dosing of a drug and, in turn, potential toxicity concerns, are arguably the most complex and unpredictable areas to overcome in drug repurposing efforts. A particular challenge in antimicrobial drug discovery is that antibiotics are typically dosed at levels considerably higher than non-antibiotic drugs. Thus for a non-antibiotic drug repurposed for infectious disease, efficacy is often seen only at doses far in excess of those specified in the original registration, and the concern is that this may lead to toxicity and adverse events. This is a common drawback in the repurposing process[26,27]. Such a compound may well provide a new lead chemical scaffold for new drug development, however, low efficacy ultimately hampers the potential for true repurposing, as any such compounds would have to go through an entire regulatory process.

Similarly, limitations in the pharmacokinetic profiles of existing drugs, which were of course optimized for their original target product profile, can also hamper repurposing efforts where a new target product profile is sought. Half-life requirements, tissue distribution and plasma protein binding, for example, may be unsuitable for the new purpose[26]. Particularly in the case of antibacterial compounds, plasma protein binding can play a major role and is often well recognized to impair antimicrobial activity[28,29]. Indeed, since higher concentrations of bioavailable drugs are often needed for antimicrobial efficacy, protein binding may further narrow the therapeutic index for the antimicrobial indication. It is thus imperative to take into account the known human pharmacokinetic profile as well as pharmacokinetic properties when considering credible

candidates for drug repurposing. Here, the literature and curated compilations can be very useful[30,31].

Some strategies to circumvent the limitations of insufficient drug concentrations in human plasma could include combinatorial therapies (discussed in the 'Screening for drugs that synergize with antimicrobials' section below). Further, in the case that higher doses are required and adverse effects may take place, combinations may have an advantage due to the dose-sparing impact of synergy. In other cases, where repurposed drugs cannot be used systemically, topical or inhaled indications may be viable options. Although this would result in a slower process since a development pathway may still be required, the repurposed drug could be modified to enhance the antimicrobial activity through medicinal chemistry to achieve concentrations within the applicable clinical range in humans. Certainly, since repurposed drugs generally have known physiochemical and pharmacokinetic properties, they provide a more attractive starting point in optimization studies. Finally, the ever-growing field of bioinformatics can be used to screen FDA-approved drug libraries to identify more potent antimicrobial inhibitors. With approved drugs increasing over the years—on average 20 to 30 new molecular entities (NMEs) each year have been approved by the FDA[32]—the space for drug repurposing is ever expanding.

Another principal disadvantage of drug repurposing concerns intellectual property rights and patents. Market exclusivity remains an important principle in the repurposing process, where utility or 'method of use' patents are issued and exist for a period of 20 years. A limitation of the repurposing process is that this form of intellectual property is considered more contestable than 'composition of matter' patents that characterize the conventional process. The latter have proven to be the strongest patent protection[33] and are more easily attainable from de novo drug development. 'Method of use' patents that cover a new indication for a product or a new dosing method can be challenged as merely incremental advances. However, given the right circumstances, a 'method of use' patent can be as effective as a 'composition of matter' patent in protecting a repositioned drug product. This will largely depend on the availability of generic products that can be substituted through off-label use to achieve the same therapeutic results as the repurposed drug. The main concern is that off-label prescription of existing drugs can obviate any market exclusivity afforded by 'method of use' patent for the repurposed drug[19]. Nonetheless, while the FDA does not prohibit physicians from prescribing drugs off-label, it does prevent pharmaceutical companies from marketing their drugs for off-label uses[34]. Without clinical-trial evidence to support the new use, physicians are less likely to accept them as appropriate medical treatments. Moreover, 'composition of matter' protection may be available for repurposed drugs (for example, where the repurposed drug incorporates a new patentable chemical entity or formulation), patentable delivery mechanism or drug combinations[19]. Hence, from a legal perspective, thoughtful strategy predicated on intellectual propery and regulatory considerations is imperative.

Finally, another limitation in the drug repurposing process involves the high cost of clinical trials. Clinical trials are conventionally sponsored by pharmaceutical companies seeking a return on investment. Since most repurposed drugs are often generics or toward the end of patent lifetime, there is little interest from pharmaceutical companies in investing in clinical trials. Proposals for solutions to this problem have included increasing support from public funding sources[35], which are centred more on patient and public health outcomes than commercial interests. Another solution is smaller clinical trials[36]. These are more likely for repurposed drugs and are often designated as Phase II trials, but these have lower status in the eyes of many clinicians, even though the trials may be of a high quality. Further, while efficacy may be apparent during Phase II for antimicrobials, non-inferiority is often difficult to prove in a small trial with a small number of patients, and superiority is rarely testable. Overall, while drug repurposing may be a very practical approach, the lack of funding and interest from the pharmaceutical industry has the potential to limit clinical development prospects.

Drug repurposing approaches

In recent years, the need for the development of new antimicrobials coupled with advancements in screening techniques and ready access to pertinent libraries has positioned researchers for success when it comes to drug repurposing. Drug repurposing campaigns rely on having access to compounds that have either been approved for clinical use or for which clinical data is available (for those abandoned by pharma). Access to such chemical libraries was once very limited and often complicated by commercial concerns, particularly around intellectual property. Increasingly, researchers are gaining access to a range of assets available off the shelf from commercial vendors and pharma partners, or through libraries compiled by, for example, the National Institutes of Health (NIH; www.nih-clinicalcollection.com; Box 2). Further, databases are increasingly being created to capture lists of all approved small molecules as freely available electronic resources (Box 3). Coupled with screening techniques, such innovations could deliver on the long-sought discovery of known and approved drugs for antimicrobial purposes. Below, we describe some of the approaches that have been described to hasten the identification of the most promising candidates in repurposing efforts. These include traditional empirical screening, unconventional screening assays, screening for drugs that synergize with existing antimicrobials and in silico strategies.

Empirical screening. A common approach for drug repurposing is the systematic screening of non-antimicrobial FDA-approved drugs for those with antimicrobial activity in cell-based models. These phenotypic-based screens are empirical in nature and ask a straightforward question: which molecules inhibit growth of the microorganism of interest? Such approaches are in contrast to conventional repurposing strategies that often require a priori knowledge of disease or binding characteristics, as well as the mechanism of action (MOA) of the drug. Indeed, past strategies have heavily relied on hypothesis-driven approaches, usually involving computational matching of compounds to specific viral or human proteins[37]. When it comes to drug repurposing for antimicrobial discovery, the original drug screening paradigm of phenotypic-based screening has dominated, likely owing to its ability for fast discovery, which, when coupled with access to libraries of drugs already approved in humans, has the potential to lead to rapid clinical development.

Several studies have highlighted the discovery of approved, non-antimicrobial drugs uncovered by cell-based high-throughput screening (HTS) for antimicrobial purposes. Indeed, multiple academic groups have screened ~1,000–2,000 drugs in various whole-cell assays against diseases caused by parasites[38–43], viruses[44–51], fungi[52–55] and bacteria[56–60] to identify approved non-antimicrobial drugs that possess antimicrobial activity, indicating that these drugs have a potential alternative use for the treatment of infection. Recent advances in HTS have transformed empirical screening into an increasingly simple and rapid high-technology process, using the most advanced methods available in bioassays, robotics, computation and data-handling. The approach of phenotype-based screening, however, provides little or no insight as to the MOA, a significant impediment to modern drug discovery efforts[61], and thus requires additional studies into mechanistic analysis to determine precisely how the drug is working to achieve the desired effect. In some cases, the known functions of the identified drugs may provide clues for the study of the MOA. In-depth characterization of the MOA and molecular targets can also aid in the design of improved next-generation compounds. Nevertheless, very few molecules identified by empirical screening cited herein have a

Box 2 | Potential sources of compounds for drug repurposing

- Compound library vendors such as Microsource, Prestwick and Sigma-Aldrich offer libraries of already approved drugs, the majority of which are off patent and have especially high potential for drug repurposing.
- Large pharma companies are increasingly opening up their compound libraries to external parties[178]. For example, AstraZeneca and Lilly are allowing access of their compound collections to the wider scientific community through programs such as Open Innovation[178,179]. These chemical collections include compounds suited to drug repurposing.
- The NIH Clinical Collection (www.nihclinicalcollection.com) consists of approximately 450 compounds that have a history of use in human clinical trials and are available for a nominal fee.
- The Johns Hopkins Clinical Compound Screening Initiative (www.jhccsi.org) has assembled a collection of over 3,100 existing FDA-approved drugs known as the JHCCL[42]. The long-term goal of this initiative is to acquire each of the approximately 11,000 drugs ever used in medicine[180].
- One of the first comprehensive databases for drug repurposing came from the NCGC[139]. The NCGC Pharmaceutical Collection is a publicly available resource including a complete and non-redundant list of all approved molecular entities. Further, it has been created as a physical collection of small molecules amenable to HTS.
- A recent creation is repoDB, a comprehensive database of approved and failed drugs and their indications[107]. This database includes drugs that have reached the clinic as well as those tested in clinical trials that did not reach approval. It is particularly difficult to access such compounds and their annotations, both as a complete list and for purchase. Indeed, while some chemical vendors offer a subset of approved drugs, most of these commercial libraries overlap in their content and include only a small fraction of the approximate 11,000 drugs that have reached the clinic.
- Most recently, the Drug Repurposing Hub was introduced, containing extensively curated annotations for each of 3,422 drugs marketed around the world or that have been tested in human clinical trials[172]. Meticulous steps were taken to create this hub resulting in a robust information resource and comprehensive drug-screening library to encourage drug repurposing. The Hub (http://www.broadinstitute.org/repurposing) includes such information as literature-reported targets and details about commercial sources of all compounds with prior testing of purity[172].

Box 3 | Database and analysis tools freely available for pre-screening strategies

- DrugBank[105]: detailed drug (that is, chemical, pharmacological and pharmaceutical) data with comprehensive drug target (that is, sequence, structure and pathway) information for ~8,000 drugs.
- PubChem[104]: biological activity of >90 million unique compounds.
- ChEMBL[106]: curated chemical database of bioactive molecules with bioactivity against drug targets.
- NCGC Pharmaceutical Collection (NPC)[139]: a comprehensive resource of clinically approved drugs enabling repurposing and chemical genomics.
- TDR Targets[181]: database of genomic and chemical datasets to facilitate the identification and prioritization of drugs and drug targets in neglected disease pathogens.
- PROMISCUOUS[182]: an exhaustive resource of protein–protein and drug–protein interactions with applicability in drug repositioning.
- cMap[111]: a catalogue of gene expression data collected from human cells treated with chemical compounds and genetic reagents.
- repoDB[107]: database of approved and failed drugs for drug repositioning.
- The Drug Repurposing Hub[172]: detailed curated annotations for existing drugs, including details about commercial sources for all compounds.
- SIDER[183]: an information portal on marketed medicines and their recorded adverse drug reactions.
- SuperDRUG2[184]: database of approved and marketed drugs, including PK and drug–drug interactions.

defined MOA in microorganisms. Some may argue that, in the case of repurposing campaigns, characterization of MOA may not be critical as the drugs have been proven safe. Indeed, regulatory agencies around the world will approve a new drug without requiring the precise MOA or molecular target, as long as the drug is efficacious and safe for patients[62]. In fact, many early FDA-approved drugs were discovered using phenotypic screens and were approved by regulatory agencies before their precise MOA or protein targets were identified[63]. Thus, growth inhibition screens of FDA-approved drugs have dominated repurposing strategies, whereas this approach has mostly been eschewed in recent de novo drug discovery efforts using naïve synthetic libraries. The latter have largely been replaced by target-based strategies because of the ultimate need to characterize MOA[62,64]. Nevertheless, especially in the case of antimicrobial discovery, MOA studies can be critical, as they allow for an understanding of the biochemical and genetic basis of growth inhibition as well as how microorganisms will resist such perturbation. Such an understanding is of paramount importance to the design of strategies to curtail resistance.

Unconventional screening. Increasingly, researchers are turning to unconventional screening strategies that go far beyond in vitro assays that measure growth inhibition of pathogens. These include platforms such as target-based phenotypic screens and screens in more clinically relevant conditions[64]. Specifically, for antimicrobial drug repurposing, these approaches have included target-based phenotypic screens, whole-animal screens and screening under conditions that better reflect the environment pathogens experience during infection.

Target-based phenotypic screening strategies have utilized clever screens exploiting specific changes in phenotypes caused by blocking a particular pathway[65–67], changes in gene expression of a specific reporter gene to identify direct or indirect inhibitors of a pathway of interest[68,69], or image-based assays[70]. Whole-animal screening strategies bypass the traditional in vitro approach of drug discovery and move into whole-organism models, as increasingly more in vivo screening assays are being developed with relatively high screening throughput. For instance, antimicrobial repurposing campaigns have been commonly conducted in *Caenorhabditis elegans* worms[71,72]. Further, unconventional screening strategies have made use of conditions that better reflect the host during infection, ignoring the more traditional yet artificial conditions that support robust microbial growth[73]. For example, these repurposing efforts have used human serum and lung surfactant[74], host defence constituents such as macrophages[75], or whole host cells[76].

Overall, such integrative approaches can be exploited to preferentially uncover inhibitors of certain pathways. Importantly, these platforms streamline target characterization and enable new target discovery[64]. Moreover, the use of host-like conditions or models not only allows the discovery of antimicrobial agents, but also host immunomodulatory compounds, which could further expand repurposing efforts. While newer to repurposing efforts, these approaches have been at the forefront of recent drug discovery efforts[64]. While it was empirical screening that brought the most success during the Golden Age of antibiotic discovery (1950–1990)[77], researchers are increasingly altering screening strategies to more unconventional ones to open up new opportunities to identify novel antimicrobial leads. Accordingly, when it comes to repurposing efforts, the use of such innovative platforms is also increasing, as approved libraries (which are limited in size) become exhaustively screened by the traditional route. The main advantages of adopting less traditional methods for screening are an expansion of targets and the ability to streamline MOA studies. Here, clever designs of screens lend to testable hypotheses as to the MOA of the uncovered compounds and render the important step of target identification more feasible[64]. Target identification can then be approached by direct biochemical methods, genetic interactions or computational inference[61]. In many cases, however, a combination of approaches is often required to fully characterize and understand on-target and off-target effects[61]. Overall, when done in a thoughtful and disciplined manner, unconventional screening platforms represent a sound approach to provide avenues to new targets and easier target identification. Moreover, platforms that take into account host-environments may result in better translation from in vitro to in vivo conditions, further accelerating this approach. Given that approved drug libraries are limited in size and empirical screening for growth inhibition will eventually be limiting to the discovery of novel compounds, unconventional screening platforms will further enable repurposing efforts.

Screening for drugs that synergize with antimicrobials. A third screening approach for drug repurposing that is increasing in popularity is the identification of drugs that modulate the activity of known antimicrobials. The combination of antibiotics with non-antibiotic drugs as potentiators is an attractive option for novel drug development, extending the lifespans of antimicrobial drugs and overcoming antibacterial drug resistance. This approach is exemplified by the long-standing, successful and widespread co-administration of β-lactamase inhibitors, such as clavulanic acid, with β-lactam antibiotics, such as amoxicillin (augmentin). Accordingly, a number of groups have recently screened libraries of off-patent medications for agents that potentiate the action of common antimicrobials. Three main strategies have been adopted: i) the search for synergizing compounds, where repurposed drugs have related MOAs that enhance the action of an antimicrobial agent[78–81]; ii) the search for drugs that re-sensitize resistant microorganisms by targeting resistance mechanisms (such as reversing efflux and inhibiting resistance enzymes)[65,82–85]; and iii) the search for drugs that allow for gain of activity, where both agents are ineffectual alone but one uncovers the target for another[82,86].

The first strategy looks for repurposed drugs that enhance the action of an antimicrobial agent. Often, researchers select a candidate antimicrobial agent and screen it in combination with a library of previously approved drugs, looking for a boost in efficacy against a strain of interest. A limitation of the approach is such evaluation often takes place against a single microbial strain, and the resulting chemical matter may be of limited value clinically where there is diversity among pathogenic strains. Other groups have looked for combinations of various approved drugs, identified in previous repurposing screens, to improve efficacy. Particularly in repurposing screening campaigns, identified molecules often have insufficient potency on their own and thus researchers have turned to looking for other drugs to augment these compounds[80,81]. Importantly, individual drug concentrations in the combinations were reduced to clinically relevant levels in these studies. Where approved drugs may not be clinically useful because of potency concerns, synergistic combinations are, by definition, dose-sparing. Plus, synergistic combinations have been shown to be highly efficacious and therapeutically more specific[87].

The second screening strategy looks for drugs that can be repurposed as antibiotic adjuvants that inhibit resistance mechanisms in order to sensitize otherwise resistant microorganisms. Indeed, many groups have screened libraries of FDA-approved drugs for those that modulate resistance mechanisms in order to salvage conventional antibiotics. For example, recent developments towards traditional adjuvants include studies where FDA-approved drugs were assessed for their ability to inhibit efflux pumps to potentiate fluoroquinolones against *Staphylococcus aureus*[83], eradicate *S. aureus* biofilms to potentiate vancomycin[84] and eradicate *Candida albicans* biofilms to increase the activity of the antifungals amphotericin B and caspofungin[85]. The clinical success of antibiotic–adjuvant combinations, such as augmentin, makes the adjuvant approach an extremely attractive avenue to the development of novel therapeutics. Importantly, restoring or potentiating the activity of existing antibiotics adds a treatment option at a time where therapies are limited.

The third combinatorial strategy involves HTS of previously approved drugs to identify compounds with unanticipated adjuvant activity. This type of search uncovers synergistic interactions among two agents that are ineffectual alone. Often, the identified repurposed drug targets a non-essential pathway that now exposes the target of the antimicrobial agent. An example is the search for adjuvants that enhance entrance of other antimicrobials into target cells by increasing the permeability of the outer membrane of Gram-negative bacteria[86,88,89], which are intrinsically insensitive to a number of antibiotic classes due to the low permeability of their outer membrane. Importantly, such strategies can enable the use of antibiotics that are not traditionally considered treatment options for Gram-negative bacteria. This combinatorial strategy has particular appeal from the perspective of novelty. Where the focus of conventional antibiotic discovery has been on essential physiological functions, targeting non-essential pathways presents the opportunity to broaden the search to an uncharted target base. This framework also gives potential new utility for a large repertoire of yet-to-be-discovered drugs. While slowing the evolution of drug resistance is a key motivation for using combinations, a disadvantage of the strategy of combining a molecule that targets a non-essential pathway is that it may place more selection pressure on the bacteria to mutate and become resistant. When resistance arises to a drug targeting an essential pathway, the resistance mutation has to first prevent the antibiotic from inhibiting the target and, second, must ensure that the essential function of the drug target can still be performed. In the case of a non-essential pathway, however, the mutational target size is less limited and thus may select for more rapid resistance[90,91].

Overall, synergy-based screens for repurposing efforts offer the ability to increase the utility of antimicrobials for which resistance has emerged. Also, for those approved drugs that do not possess activity at a concentration achievable at sites of infection, their combinations may achieve efficacy[26]. Further, combinatorial approaches are powerful in their dose-sparing abilities for those drugs that may exhibit toxicity. Notably, combination therapy is the current standard of care regularly used by clinicians when faced with infections caused by difficult-to-treat, multi-resistant organisms[92]. Such an approach has also been suggested to reduce the risk of selection of antimicrobial-agent-resistant strains[93,94]. It is noteworthy, however, that synergy can have two conflicting effects on resistance: it may

reduce the evolution of resistance by clearing the infection faster, thereby limiting the time available for resistant mutations to arise, but it may also increase the selective advantage of single-drug-resistant mutants[95]. Other hurdles unique to combination therapies include drug–drug interactions, as well as optimized drug ratios and dosing regimens to match the pharmacological properties of each compound[96]. Indeed, the relative pharmacokinetic properties of compounds are key in choosing combinations. In the case of drug repurposing, an appreciable edge is the knowledge of the latter early in the process, which may help improve success rates. Finally, a fundamental problem in exploring the effects of drug combinations is the enormous number of experiments that are required to systematically explore all possible combinations of a set of drugs[91].

In silico screening to identify repurposing opportunities. In addition to whole-cell and target-based screens of chemical libraries, in silico profiling or pre-screening of compound libraries may also be a useful approach to identify new drug leads for infectious diseases. These methods take advantage of modern high-performance computing as well as the vast amount of publicly available pharmacological, biological and chemical data. By incorporating the latter into large databases and developing analytical tools to sift through the data, researchers can perform virtual screens that can uncover previously unrecognized connections between drugs and targets. In the case of drug repurposing, in silico screening is advantaged by a wealth of pharmacological and clinical information available for approved drugs. Typically, in silico screening may be ligand- or network-based.

A common computational platform for drug discovery relies on molecular docking, predicting a molecule's orientation within a protein target's binding site, based on the assertion that similar binding sites bind similar molecules[37]. This ligand-based method has been adopted for repurposing efforts for antimicrobial discovery. For example, entecapone, prescribed for the treatment of Parkinson's disease, was uncovered as a potential antibacterial compound against multiple drug-resistant *Mycobacterium tuberculosis*, based on the high degree of binding site similarity between human catechol-*O*-methyltransferase and the bacterial enoyl-acyl carrier protein reductase (InhA), an enzyme essential for fatty acid synthesis[97]. The prediction was validated by in vitro and InhA kinetic assays. Of note, the minimum inhibitory concentration (MIC) of entacapone was well below the toxicity threshold determined by an in vitro cytotoxicity model using a human neuroblastoma cell line, suggesting that entecapone is a promising lead compound for antitubercular therapeutics. Nevertheless, serum binding in the range of 98–99% may hinder its antimicrobial therapeutic potential[98]. Other ligand-based pharmacophore-modelling studies using libraries of previously-approved drugs have identified virulence inhibitors of methicillin-resistant *S. aureus* (MRSA)[99], efflux pump inhibitors in *S. aureus*[100], and an inhibitor that affects galactose metabolism and lipopolysaccharide biosynthesis in drug-resistant Gram-negative pathogens[101,102].

Pre-screening plays an important role in drug repurposing and involves compound prioritization based on in silico assessment[103]. The approach provides a cost-effective alternative to screening entire libraries. Databases, such as PubChem[104], DrugBank[105], ChEMBL[106] and others (Box 3), contain information retrieved and manually curated from the literature that is freely available to guide compound selection. Increasingly, with the advent of repurposing, databases are released that focus on repurposed drugs and failed drugs, including their therapeutic indications as well as pharmacological, biological and epidemiological factors that facilitate drug repurposing[107,108]. In a recent study by Sateriale et al., data mining was used to identify drug repurposing possibilities for protozoan parasites[109]. The authors developed an automated approach to screen the predicted proteomes of 13 parasitic organisms against databases of known drug targets to enrich libraries for potential bioactive compounds, with validation from a cell-based screen for inhibitors of *Cryptosporidium parvum* growth. The approach resulted in significantly higher hit rates in the pre-screened library than in the un-screened cohort of compounds.

Network-based in silico approaches use the methods of systems biology and bioinformatics to directly compare host responses to pathogens and drugs. The methods used in this paradigm vary in complexity from lists of over- and under-expressed genes in a biological system, to more complicated interaction networks. Such data from a variety of high-throughput techniques can then be compared to establish therapeutic relationships between known drugs and new disease indications. Network-based computational approaches have recently been employed for drug repurposing screens for antimicrobials. A rather straightforward example in this regard is the work of Chavali et al., who used metabolic modelling to generate a list of 15 genes and 8 double-gene combinations predicted to be relevant targets for the neglected tropical disease caused by the parasite *Leishmaniasis major*[110]. The authors were able to associate these genes with 254 FDA-approved compounds based on drug–target interactions, and found validation for 14% of these compounds that overlapped with an independent HTS screen against leishmaniasis. Another group used the host transcriptional response to influenza virus to query the cMap database, a public database that holds over 6,000 transcriptome profiles on the treatment of human cell lines with over 1,300 compounds, most of which are FDA-approved drugs[111], to identify novel broad spectrum antivirals[112]. Other groups are creating models to enable network-based in silico screening. For example, Coelho et al. developed a pipeline to predict the probability of drug–target interactions using the MRSA interactome as a case-study[113]. By predicting the probability of each drug–target pair to interact, this computational pipeline can enable the discovery of putative leads for drug repurposing. Overall, efforts to repurpose drugs for infectious diseases using network-based computational methods are becoming increasingly attractive[114,115].

While computational drug repurposing is still waiting to see its first success story with a compound reaching the market, experimental evidence is accumulating in support of the feasibility of this approach. The ongoing need to discover novel antimicrobial drugs will continue to spur the research community to tackle drug discovery through in silico screening, with ever-growing data stores and computational tools for analysis making it possible for scientists to identify likely candidates for repurposing. The appeal of virtual screening is the potential for cost-effective processing of compound and data collections, orders of magnitude larger than what can be achieved in the laboratory. On the same token, this approach relies heavily on the availability of quality data for predictions. As such, it may miss many compounds of high potential. Where false positive results can be detected in follow-up experiments and secondary screens, false negative results are more difficult to detect and can obstruct efforts to identify drug interactions when screening small libraries. The number of false negative results can be reduced with more replicate experiments and rigorous statistical analysis[116], or with a variation of biological assays (for example, testing in more cell lines), although these options will be restricted by experimental time and cost[117]. Further, druggable target selection is of crucial importance for in silico efforts and early considerations to validate target are imperative. Additionally, targets are restricted to those proteins that have been computationally modelled, lessening the potential to uncover inhibitors of novel targets. Finally, each computational method discussed here has its own field of applicability, drawbacks and limitations. Thus, none of these methods alone will likely be sufficient enough to disclose or model the complex interplay between drugs, targets and diseases. Therefore, it is thought that repurposing efforts would greatly benefit from better integration and use of various computational methods[118]. Nevertheless, the possibility to

Table 1 | Examples of drugs repurposed for antimicrobial uses along with their developmental stage from discovery to FDA approval

Drug	Initial use	Repurposed use	Stage
Amphotericin B	Antifungal	Visceral leishmaniasis	FDA approved (1997)[119]
Astemizole	Allergic rhinitis	Malaria	Discovery[42]
Auranofin	Rheumatoid arthritis	Amebiasis	Orphan Drug Status (2012)[39]; Phase IIa (NCT02736968)
Chlorcyclizine	Allergy	Antiviral	Phase I trial completed[47]
Doxycycline	Antibacterial	Malaria	Off-label use[122]
Eflornithine	Antitumour agent	Human African trypanosomiasis	Approved (1990)[175]
Miltefosine	Skin metastases	Visceral leishmaniasis	Approved (2014)[176]
Niclosamide	Anthelmintic	Antiviral, Antibacterial	Discovery[67,69]
Paromomycin	Antibiotic	Visceral leishmaniasis	Approved (1994)[123]
Pentamidine	Antiprotozoal	Antibacterial	Discovery[86]
Sertraline	Depression	Antifungal	Phase III completed[156]
Spiramycin	Antibiotic	Congenital toxoplasmosis	Experimental drug status[124]
Tamoxifen	Anticancer	Antifungal	Discovery[53]
Zidovudine	Anticancer	Antiviral	Approved (1987)[177]

save time and funds in order to narrow in on a shorter drug list may ultimately improve the success of drug repurposing campaigns.

Drug repurposing successes

Drug repurposing has come to the forefront in recent years to address the global situation of antimicrobial resistance. So far, however, despite various repurposing initiatives for new antimicrobials, only few have been successfully approved for new indications and are largely for parasitic and protozoal diseases (Table 1). An example is the approval of antifungal amphotericin B for treatment of visceral leishmaniasis[119]. The anti-malarial drugs chloroquine[120] and pyrimethamine[121] have been repurposed for amoebiasis and toxoplasmosis, respectively. To date, not a single drug has been repurposed for use as an antibacterial. Interestingly, however, a few antibiotics have been repurposed, including doxycycline for malaria[122], paromomycin for visceral leishmaniasis[123] and spiramycin for toxoplasmosis[124]. Where repurposing human-targeted drugs to other human targets can benefit from polypharmacology of the drugs[125], this is not the case for antibacterial discovery, which may explain the lack of success to date. In fact, with antibacterial compounds, selectivity for the microorganism over the host is typically important to success. Interestingly, fungi and parasites, for example, have targets that are more similar to humans. Indeed, eukaryotic microorganisms are more similar to their hosts than prokaryotic pathogens in terms of their biochemistry and metabolism, genetic composition, cell architecture and biology[126]. Thus it is tempting to assume that underlying polypharmacology—35% of active compounds are thought to hit more than one target[15]—has helped enable repurposing opportunities for eukaryotic microbial pathogens, more so than prokaryotic pathogens. Nevertheless, there is still considerable interest in drug repurposing and, while many are still in their infancy, several potential candidates have been investigated for new indications, such as antiparasitic, antiviral, antifungal and antibacterial purposes (Table 1).

Antiparasitic. A number of whole-cell-based HTS campaigns have recently been carried out by different groups looking to repurpose compounds for antimalarial activity[41,42,127]. In 2016, a total of 216 million cases of malaria were reported[128], and the widespread resistance of the human malaria parasite *P. falciparum* to commonly used drugs, like chloroquine, contributes to treatment failure and increased mortality[129]. In 2006, a screen of the Johns Hopkins Clinical Compound Library (JHCCL) for inhibitors of *P. falciparum* identified the antihistamine astemizole as effective against chloroquine-sensitive and multidrug-resistant parasites, and showed efficacy in mouse models of malaria[42]. Astemizole was introduced in 1983 as a non-sedating selective H1-histamine receptor antagonist for the treatment of allergic rhinitis. Although astemizole demonstrated very potent inhibition of malaria parasites and is used in the developed world, clinical trials to have it repositioned as an antimalarial in the developing world were complicated by its withdrawal from the United States market for safety concerns[130]. These included its ability to block human ether-a-go-go-related gene (HERG) K^+ channels. Interestingly, although withdrawn from the USA in 1999, astemizole is still produced for sale generically as an antihistamine in over 30 countries, including many countries that are malaria endemic. It is noteworthy that astemizole was shown to inhibit *P. falciparum* with submicromolar potency and its main metabolite, demethylastemizole, was effective at 100 nM concentrations[42]. Both were also active in mouse models of malaria at doses below that used in humans to treat allergic rhinitis[42]. Astemizole has since been shown to also have activity against *Plasmodium* liver stages[131] and is being pursued as a starting point for new antimalarial compounds[132].

Another success story comes from a recent repurposing campaign for inhibitors of *Entamoeba histolytica*, a protozoan intestinal parasite and causative agent of human amoebiasis. Amoebiasis affects an estimated 50 million people and causes 100,000 deaths globally each year[133]. Large-scale screening of this anaerobic parasite has been hindered by the low throughput of traditional assays and lack of efficient readout assays. Debnath et al. devised and validated a suitable HTS that identified auranofin, an FDA-approved oral, gold-containing drug that has been in clinical use to treat rheumatoid arthritis for 25 years[39] (Fig. 2a). Notably, auranofin was granted orphan-drug status by the FDA for the treatment of human amoebiasis in 2012. The main MOA of auranofin is through the inhibition of reduction/oxidation enzymes that are essential for maintaining intracellular levels of reactive oxygen species[134]. However, auranofin surely has other activities, as it has been uncovered in a wide number of antibacterial screens[135]. Even though auranofin is rapidly metabolized, its efficacy in animal models of amoebic colitis and amoebic liver abscess were significant. Importantly, auranofin exhibited anti-*Entamoeba* activity, with a half-maximal effective concentration of 0.338 μg ml^{-1}, which is seven-fold lower than the clinically achievable blood concentration of the drug (2.37 μg ml^{-1})[39]. Further, auranofin was ten-fold more potent against *E. histolytica* than the control amoebiasis drug, metronidazole. More recently, auranofin

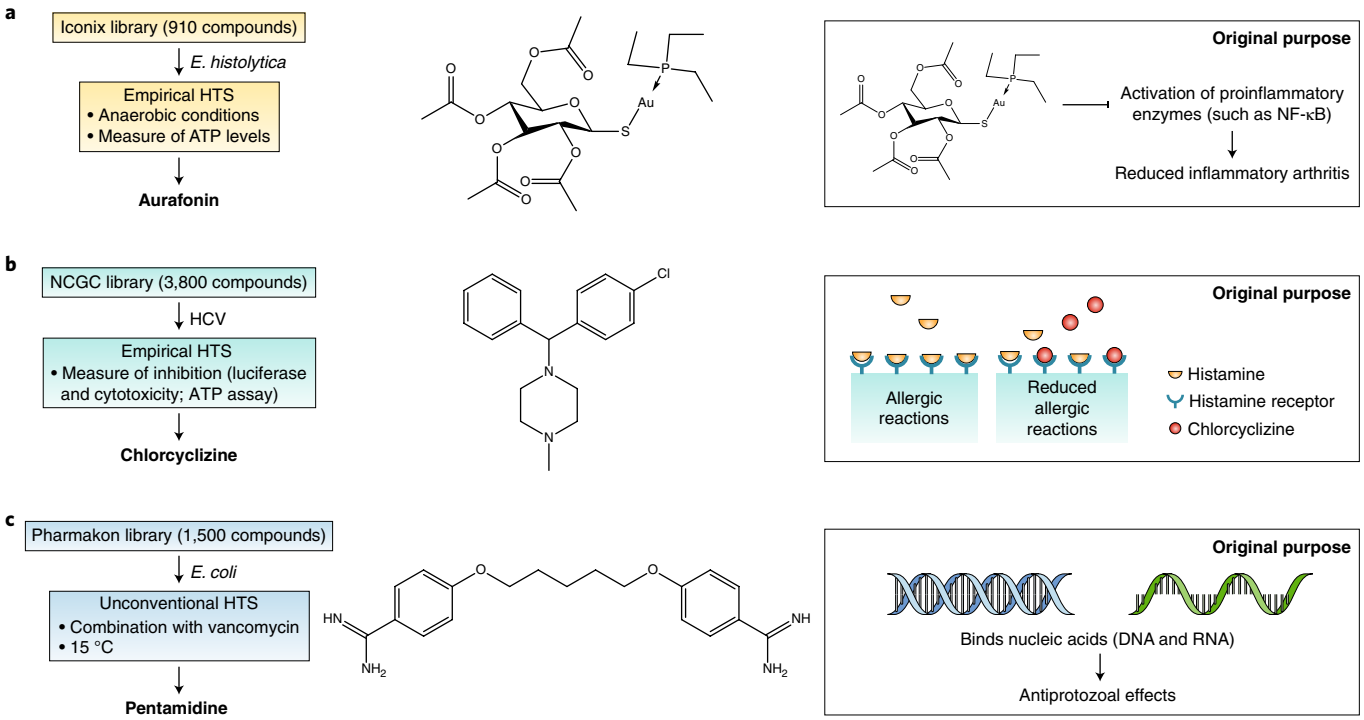

Fig. 2 | Examples of the road to success for some novel antimicrobials identified through repurposing screening campaigns. a–c, Aurafonin[39], (**a**) chlorcyclizine[47] (**b**) and pentamidine[86] (**c**) have been identified as novel antimicrobials through repurposing screening campaigns. Summarized is the library screened for each discovery, as well as the screening methodology employed (left). The original purpose for each FDA-approved drug is also represented (right).

has also been shown to be effective against metronidazole-resistant parasitic isolates[136]. However, it is noteworthy that auranofin does not attain sufficient blood levels quickly, taking over 12 weeks for its concentrations in the blood to gradually increase[137], which may hinder any further progress for auranofin as an antiparasitic drug.

Antiviral. The identification of new classes of hepatitis C virus (HCV) life cycle inhibitors has been a particularly active area in the repurposing arena. Several independent campaigns have screened various libraries of approved drugs for the rapid discovery of new classes of HCV life cycle inhibitors, including the NIH clinical compound collection[46] (a library of pharmacologically active compounds)[44] and the NIH Chemical Genomics Center (NCGC) Pharmaceutical Collection[47]. HCV affects an estimated 185 million people worldwide, with an unmet need for the development of effective and affordable treatment options[138]. A cell-based screen of the NCGC Pharmaceutical Collection[139] that detects viral infection uncovered chlorcyclizine as a potent inhibitor of HCV infection[47] (Fig. 2b). Chlorcyclizine is an over-the-counter drug for allergy symptoms, first approved in the 1940s. The antiviral MOA of chlorcyclizine was shown to be mediated by inhibition of an early stage of HCV infection, likely targeting viral entry into host cells. This is in contrast to the more common antiviral mechanism of inhibition of viral replication. Chlorcyclizine also displayed synergy with various approved anti-HCV drugs in vitro, including ribavirin[47], which has been the cornerstone of HCV therapy for many years. Notably, chlorcyclizine significantly inhibited infection of HCV without evidence of emerging drug resistance in animal models, placing chlorcyclizine as a promising candidate for drug repurposing for the treatment of HCV infection[47]. Accordingly, a Phase 1 trial was recently completed, which assessed the antiviral activity of chlorcyclizine alone or in combination with ribavirin in patients with chronic hepatitis C (ID: NCT02118012). With a good safety profile, oral delivery and global affordability, chlorcyclizine may well be a promising candidate for drug repurposing for treatment of HCV infection. A roadblock to overcome, as chlorcyclizine is further developed in combination with ribavirin, will be the need for matching pharmacokinetic parameters.

Other repurposing successes have come from the urgent need to identify therapies against Zika virus (ZIKV), a mosquito-borne flavivirus that recently spread across the Western Hemisphere[140]. To this end, Barrows et al. screened a library of FDA-approved drugs for the ability to block ZIKV infection, identifying more than 20 therapeutics that decreased ZIKV infection in human liver cells[51]. Among these were drugs previously shown to have anti-flaviviral activity, and others with no previous reports of antiviral activity. Importantly, multiple drugs reduced ZIKV infection in human cell lines, providing the opportunity for rapid drug development, which is especially critical for quickly spreading infectious diseases. In another study to identify inhibitors of ZIKV, Xu et al. designed a rational screening approach using caspase-3 activity as a primary screening readout. This was based on the group's previous finding that ZIKV infection of human cortical neural progenitors results in an increase in caspase-3 activation[141]. The authors performed a drug repurposing screen of ~6,000 compounds that included approved drugs, clinical trial drug candidates and pharmacologically active compounds[67]. Interestingly, niclosamide potently inhibited ZIKV replication. Niclosamide was approved by the FDA, in 1982, for use in humans to treat tapeworm infection and is included in the World Health Organization (WHO)'s list of essential medicines. The authors further demonstrated the effectiveness of niclosamide in human neural progenitor cells and astrocytes, which are target sites for ZIKV infection in the foetal brain[67]. Notably, where niclosamide can be clinically delivered at micromolar levels[142], its potency on inhibition of ZIKV replication is in the submicromolar range, showing great potential for anti-ZIKV repurposing. Further, niclosamide has low toxicity in mammals, with an oral median lethal dose (LD_{50}) value of 5,000 mg per kg body weight in rats[142], showing great potential as a repurposing candidate.

Antifungal. A repurposing success story for novel antifungal candidates comes from a HTS campaign looking for molecules that cause yeast cell lysis. Krysan et al. screened a library of FDA-approved drugs and identified tamoxifen as a fungilytic molecule[53]. Tamoxifen, an oestrogen receptor antagonist, is primarily used to treat breast cancer. Introduced in the 1970s, tamoxifen has been prescribed to millions of patients with oestrogen-receptor-positive breast cancer, which accounts for ~70% of all breast cancer patients[143]. Now, tamoxifen is available as a generic drug worldwide, which has made it the single most prescribed drug in the world for the treatment of any cancer[143]. Tamoxifen has also been shown to target a number of proteins in mammalian cells, including calmodulin, protein kinase C, phospholipase C, phosphoinositide kinase, P-glycoprotein and swell-induced chloride channels[144,145]. An important distinguishing factor is that the oestrogen receptor effects require only nanomolar concentrations of tamoxifen, while the oestrogen-receptor-independent effects occur at concentrations approximately 10-fold higher[144]. Interestingly, tamoxifen has been well recognized for its antifungal properties, established decades ago[146], but the activity and MOA of tamoxifen had not been fully characterized. It soon became evident that the antifungal properties of tamoxifen were related to its oestrogen-receptor-independent mechanisms. Some reported mechanisms have included inhibition of membrane peroxidation[147], membrane-damaging effects[148] and interference with calcium homeostasis[149,150]. Based on these studies, it may be the case that the antifungal activity of tamoxifen is the result of its effects on multiple physiologic processes in yeast. More recently, tamoxifen was shown to reduce kidney fungal burden in a mouse model of disseminated candidiasis and shown to interfere with calmodulin function as part of its MOA[145]. Tamoxifen shows potential for further antifungal drug discovery and development. Attractive features of this drug include its high oral bioavailability and fungicidal activity. Nevertheless, the risk of adverse effects often associated with tamoxifen[151] warrants further optimization of molecules structurally related to it, to improve its improve antifungal and pharmacologic properties, which will ultimately delay its repurposing into an antifungal.

A more advanced repurposing attempt for antifungals is the adjunctive use of sertraline in cryptococcal meningitis. Sertraline is a selective serotonin reuptake inhibitor that is primarily used to manage depression. Sertraline was first identified, during a screen of the JHCCL, as having modest growth-inhibitory effects against the filamentous fungus *Aspergillus nidulans*[55]. Interestingly, potential antifungal activity of this antidepressant was first observed in a clinical setting where three patients with premenstrual dysphoric disorder (PMDD) and recurrent vulvovaginal candidiasis (VVC) underwent sertraline therapy for PMDD[152]. During sertraline intervention, patients had no recurrent episodes of acute VVC. Indeed, antifungal activity was observed for sertraline against various isolates of *Candida* species[152]. Follow-up studies demonstrated that the MICs of sertraline required to inhibit *Aspergillus* and *Candida* strains[152,153] were much higher than the serum concentrations achievable in standard therapeutic regimens (55–250 ng ml^{-1}). As such, the clinical value of sertraline in treating infections caused by the latter fungi was not initially evident. Only later was sertraline shown to potentiate the anti-cryptococcal activity of azoles[154]. After a successful exploratory Phase II study with sertraline as adjunctive therapy for cryptococcal meningitis[155], a Phase III study to determine the value of adding this compound to a standard induction therapy for cryptococcal meningitis (ID: NCT01802385) was just recently completed, where a lack of efficacy was noted[156], putting into question its promise as adjunctive antifungal therapy.

Antibacterial. Target-based phenotypic repurposing screens for antibacterial compounds have recently been on the rise. For instance, Imperi et al. devised a target-based phenotypic screen designed to search for inhibitors of quorum-sensing in *P. aeruginosa* among a library of FDA-approved compounds. This strategy uncovered the anthelmintic drug niclosamide as an inhibitor of *P. aeruginosa*'s production of acyl-homoserine lactone, as well as quorum-sensing signalling molecules and regulated virulence factors[69]. As noted earlier, niclosamide is a WHO essential medicine and was approved by the FDA for use in humans to treat tapeworm infection in 1982. In the aforementioned repurposing screen, niclosamide affected the transcription of 250 genes in *P. aeruginosa* with a high degree of target specificity towards quorum-sensing-dependent genes. In accordance with the strong antivirulence activity observed in vitro, niclosamide prevented *P. aeruginosa* pathogenicity in insects. Importantly, niclosamide displays overall low toxicity, however its pharmacokinetic properties predict potential drawbacks for systemic administration[157]. Indeed, recent efforts to improve systemic exposure to the drug have been focused on employing nanotechnology approaches and novel chemistry[158,159]. Nevertheless, in terms of antibacterial therapy, these drawbacks can be obviated with the topical use of this compound for superficial *P. aeruginosa* infections[69]. Interestingly, niclosamide has proven to be a multifunctional drug, inhibiting multiple signalling pathways and biological processes, including cancer[160,161], rheumatoid arthritis[162] and various infectious diseases[67,72,163]; as such, it is often uncovered in repurposing screening efforts. There are currently four clinical trials of niclosamide-use in colon cancer and prostate cancer in the clinical trials registry (ClinicalTrials.gov). Others, perhaps for the antibacterial properties of this compound, will surely follow as the beneficial effects of niclosamide are appreciated in specific diseases. Further, improvement of the pharmacological and -kinetic properties of niclosamide through re-formulation strategies, for example, may also contribute to the more widespread use of this drug.

Emergent approaches in antibacterial discovery that have drawn great interest recently include adjuvants, which enhance the activity of other antimicrobials, for example by increasing the permeability of the outer membrane[88]. The latter is most relevant to Gram-negative bacteria, which are intrinsically insensitive to a number of antibiotic classes, including the macrolides and aminocoumarins, as a result of the low permeability of the outer membrane. One novel approach to circumvent this lack of antibiotics that are active against Gram-negative bacteria is the identification of adjuvant molecules that potentiate the effects of Gram-positive antibiotics, typically large-scaffold molecules, against these strains. A recent screen by Stokes et al., of 1,500 previously approved drugs, identified a cryptic activity for the drug pentamidine. It displayed synergy with large-scaffold antibiotics against *E. coli*[86] (Fig. 2c). Mechanistic studies revealed that pentamidine altered the integrity of the outer membrane of *E. coli*, providing a physical basis for this synergy. Pentamidine is an essential medicine for the treatment of pneumocystis pneumonia and West African trypanosomiasis. Most notably, activity of pentamidine in combination with conventional antibiotics in murine models of infection was shown, including in a colistin-resistant *A. baumannii* infection, displaying a promising dose-sparing effect for pentamidine and novobiocin[86]. Interestingly, during the 1940–1950s, pentamidine and other aromatic diamidines were investigated as potential antibacterial compounds[164–166] but were not pursued clinically due to adverse side effects and the abundant availability of other antimicrobials. Further, anecdotally, an immunocompromised patient was treated for presumed *P. carinii* pneumonia with intravenous pentamidine that resulted in marked clinical improvement and successful clearing of an *S. aureus* infection[167]. Nonetheless, the antimicrobial MOA of pentamidine has remained largely elusive, with a plethora of various MOA described[168–170]. The report by Stokes et al. was the first to highlight the adjuvant capability of pentamidine and, importantly, showed the potential for considerable dose-sparing[86]. Indeed, the doses required to treat these animals were at concentrations that

should be safely tolerated by the animals and significantly lower than equivalent human therapeutic doses. Overall, this study demonstrated the feasibility of repurposing existing drugs to face novel threats and highlighted the dose-sparing power of combinations. Although early days, the in vitro and in vivo activity of pentamidine against multi-drug resistant strains of *A. baumannii* at doses with desirable pharmacological properties paves the way for future clinical trials on the antibacterial efficacy of pentamidine.

Future

The initiative and insight to screen existing drugs for novel uses has largely come from small biotech companies or academic research groups[19]. Indeed, in all likelihood it will require a widespread effort from public–private partnerships, non-profits, academic researchers and companies to successfully investigate and approve drugs for other indications. Some companies even specialize in developing proprietary screening, and computational and data-mining technologies to aid in the repurposing process, such as Biovista, NuMedii and Excelra, among others. Arguably, both academia and small biotech companies, while having exciting new screening platforms, may be constrained by a lack of resources and the wherewithal to pursue clinical development. Further, repurposing is unlikely to happen in a big pharmaceutical company context, notwithstanding rare examples in late-stage development, such as Viagra. For these reasons, partnerships among academic groups, biotechnology companies and larger pharmaceutical companies are surely the likely path to successful commercialization in this space. Moreover, there is clearly a role for public health. Indeed, new momentum is coming from government-led initiatives such as the NIH program, the National Center for Advancing Translational Science[139], and more recently, the Medical Research Council in the United Kingdom[171] have invested in this area. The explosion of repurposing databases, such as the recent initiative by the Broad Institute and the Drug repurposing Hub[172], is an indication that this approach is gaining momentum. Overall, these initiatives lend credence to the potential of drug repurposing and should encourage repurposing in academia and large pharmaceutical companies alike.

A major hurdle to overcome, however, remains the seeming lack of incentives for repurposing drugs compared to those for de novo drug development. Incentives, such as better patent protection, more market exclusivity and tightly regulated conditions for entry by generic pharmaceutical manufacturers, would surely help in further promoting repurposing activities among for-profit drug developers[173]. The success engendered by the Orphan Drug Act of 1983 (implemented by the United States Congress), which qualifies the sponsor for, among other incentives, a period of seven-year market exclusivity from the date of approval as well as the waiving of many other fees, is an example of this. Indeed, since the passage of the act and until the end of 2014, the FDA has approved 511 orphan drugs, whereas previously only 38 drugs were approved for the treatment of orphan diseases[174]. It is tempting to think that deploying the kinds of incentives used for de novo drug development will generate the kinds of repurposing activity society needs. It is certainly enticing to think that, given the right legal and monetary incentives, firms may behave as they do with de novo drug development; the impact on antimicrobial drug discovery could be transformative.

Concluding remarks

Increasing interest in drug repositioning has occurred due to sustained high failure rates and costs required to bring new drugs to market. In the case of antimicrobials, the risk is amplified by the looming threat of antimicrobial resistance and the pressing need for strategies to tackle the absence of new antimicrobial drugs. While repurposing efforts for this class of drugs have had limited success, recent headway, particularly in other therapeutic areas, should encourage this path. Where will progress ultimately come from? Collaboration will surely be key. Academic laboratories and small biotech companies have frequently discovered new activities for existing drugs, but translation of these discoveries to the clinic requires additional sophistication, commonly resident in large pharmaceutical companies. Where academics contribute to screening, develop new screening technologies, and perform the basic science that can inform on properties and mechanisms of repurposed drugs, pharma companies have a vital role to play in providing unparalleled knowledge and resources in, for example, clinical trials. Further, the basic discoveries uncovered through the screening campaigns described here, can form unique starting points and feedback into basic research to develop new compounds to improve potency. Overall, drug repurposing holds strong promise in complementing traditional drug discovery. With strategic intellectual property planning and increasing funding initiatives and partnerships, common hurdles of the process could be mitigated. With advances in screening platforms, and the advent of data repositories and associated analytical methods, coupled with the increased access to libraries of FDA-approved drugs and innovative partnerships, there is hope that lead discovery based on drug repurposing could deliver on the next generation of antimicrobial agents. Repurposing drugs could provide breakthrough therapies for antibiotic-resistant pathogens, especially as de novo drug discovery and development is in decline. Ultimately, when it comes to the challenge of treating drug-resistant infections, failure is not an acceptable option, and all reasonable prospects for success should be explored.

Received: 28 September 2017; Accepted: 3 January 2019;
Published online: 4 March 2019

References

1. O'Neill, J. *Review on Antimicrobial Resistance: tackling a crisis for the health and wealth of nations* (HM Government, 2014).
2. Rossolini, G. M., Arena, F., Pecile, P. & Pollini, S. Update on the antibiotic resistance crisis. *Curr. Opin. Pharmacol.* **18**, 56–60 (2014).
3. Tanwar, J., Das, S., Fatima, Z. & Hameed, S. Multidrug resistance: an emerging crisis. *Interdiscip. Perspect. Infect. Dis.* **2014**, 541340 (2014).
4. Ariey, F. et al. A molecular marker of artemisinin-resistant *Plasmodium falciparum* malaria. *Nature* **505**, 50–55 (2014).
5. Kauffman, C. A., Pappas, P. G. & Patterson, T. F. Fungal infections associated with contaminated methylprednisolone injections. *New Engl. J. Med.* **368**, 2495–2500 (2013).
6. McCarthy, M. Hospital transmitted *Candida auris* infections confirmed in the US. *BMJ* **355**, i5978 (2016).
7. Carroll, M. W. et al. Temporal and spatial analysis of the 2014–2015 Ebola virus outbreak in West Africa. *Nature* **524**, 97–101 (2015).
8. Kreuels, B. et al. A case of severe Ebola virus infection complicated by gram-negative septicemia. *New Engl. J. Med.* **371**, 2394–2401 (2014).
9. Snitkin, E. S. et al. Tracking a hospital outbreak of carbapenem-resistant *Klebsiella pneumoniae* with whole-genome sequencing. *Sci. Transl. Med.* **4**, 148ra116 (2012).
10. Liu, Y. Y. et al. Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. *Lancet Infect. Dis.* **16**, 161–168 (2016).
11. Hughes, D. & Karlen, A. Discovery and preclinical development of new antibiotics. *Upsala J. Med. Sci.* **119**, 162–169 (2014).
12. Payne, D. J., Gwynn, M. N., Holmes, D. J. & Pompliano, D. L. Drugs for bad bugs: confronting the challenges of antibacterial discovery. *Nat. Rev. Drug Discov.* **6**, 29–40 (2007).
13. Waring, M. J. et al. An analysis of the attrition of drug candidates from four major pharmaceutical companies. *Nat. Rev. Drug Discov.* **14**, 475–486 (2015).
14. Langedijk, J., Mantel-Teeuwisse, A. K., Slijkerman, D. S. & Schutjens, M. H. Drug repositioning and repurposing: terminology and definitions in literature. *Drug Discov. Today* **20**, 1027–1034 (2015).
15. Paolini, G. V., Shapland, R. H., van Hoorn, W. P., Mason, J. S. & Hopkins, A. L. Global mapping of pharmacological space. *Nat. Biotechnol.* **24**, 805–815 (2006).
16. Glicksberg, B. S. et al. An integrative pipeline for multi-modal discovery of disease relationships. *Pac. Symp. Biocomput.* **20**, 407–418 (2015).

17. Moroney, J. et al. Phase I study of the antiangiogenic antibody bevacizumab and the mTOR/hypoxia-inducible factor inhibitor temsirolimus combined with liposomal doxorubicin: tolerance and biological activity. *Clin. Cancer Res.* **18**, 5796–5805 (2012).
18. Moroney, J. W. et al. A phase I trial of liposomal doxorubicin, bevacizumab, and temsirolimus in patients with advanced gynecologic and breast malignancies. *Clin. Cancer Res.* **17**, 6840–6846 (2011).
19. Ashburn, T. T. & Thor, K. B. Drug repositioning: identifying and developing new uses for existing drugs. *Nat. Rev. Drug Discov.* **3**, 673–683 (2004).
20. Chong, C. R. & Sullivan, D. J. Jr. New uses for old drugs. *Nature* **448**, 645–646 (2007).
21. Norrby, S. R. et al. Lack of development of new antimicrobial drugs: a potential serious threat to public health. *Lancet Infect. Dis.* **5**, 115–119 (2005).
22. Kepplinger, E. E. FDA's expedited approval mechanisms for new drug products. *Biotechnol. Law Rep.* **34**, 15–37 (2015).
23. Hernandez, J. J. et al. Giving drugs a second chance: overcoming regulatory and financial hurdles in repurposing approved drugs as cancer therapeutics. *Front. Oncol.* **7**, 273 (2017).
24. Oprea, T. I. et al. Drug repurposing from an academic perspective. *Drug Discov. Today Ther. Strateg.* **8**, 61–69 (2011).
25. Paul, S. M. et al. How to improve R&D productivity: the pharmaceutical industry's grand challenge. *Nat. Rev. Drug Discov.* **9**, 203–214 (2010).
26. Oprea, T. I. & Overington, J. P. Computational and practical aspects of drug repositioning. *Assay Drug Dev. Techn.* **13**, 299–306 (2015).
27. Sun, W., Sanderson, P. E. & Zheng, W. Drug combination therapy increases successful drug repositioning. *Drug Discov. Today* **21**, 1189–1195 (2016).
28. Zeitlinger, M. A. et al. Impact of plasma protein binding on antimicrobial activity using time-killing curves. *J. Antimicrob. Chemoth.* **54**, 876–880 (2004).
29. Burian, A. et al. Plasma protein binding may reduce antimicrobial activity by preventing intra-bacterial uptake of antibiotics, for example clindamycin. *J. Antimicrob. Chemoth.* **66**, 134–137 (2011).
30. Schulz, M., Iwersen-Bergmann, S., Andresen, H. & Schmoldt, A. Therapeutic and toxic blood concentrations of nearly 1,000 drugs and other xenobiotics. *Crit. Care* **16**, R136 (2012).
31. Schulz, M. & Schmoldt, A. Therapeutic and toxic blood concentrations of more than 800 drugs and other xenobiotics. *Pharmazie* **58**, 447–474 (2003).
32. Mullard, A. 2013 FDA drug approvals. *Nat. Rev. Drug Discov.* **13**, 85–89 (2014).
33. Lin, F. & Wang, S. J. Identification of the factors that result in obviousness rulings for biotech patents: an updated analysis of the US Federal Circuit decisions after KSR. *Hum. Vacc. Immunother.* **9**, 2490–2495 (2013).
34. Wittich, C. M., Burkle, C. M. & Lanier, W. L. Ten common questions (and their answers) about off-label drug use. *Mayo Clin. Proc.* **87**, 982–990 (2012).
35. Morello, L. More cuts loom for US science. *Nature* **501**, 147–148 (2013).
36. Pantziarka, P. et al. The repurposing drugs in oncology (ReDO) project. *eCancermedicalscience* **8**, 442 (2014).
37. Issa, N. T., Kruger, J., Byers, S. W. & Dakshanamurthy, S. Drug repurposing a reality: from computers to the clinic. *Expert Rev. Clin. Pharm.* **6**, 95–97 (2013).
38. Bessoff, K., Sateriale, A., Lee, K. K. & Huston, C. D. Drug repurposing screen reveals FDA-approved inhibitors of human HMG-CoA reductase and isoprenoid synthesis that block *Cryptosporidium parvum* growth. *Antimicrob. Agents Ch.* **57**, 1804–1814 (2013).
39. Debnath, A. et al. A high-throughput drug screen for *Entamoeba histolytica* identifies a new lead and target. *Nat. Med.* **18**, 956–960 (2012).
40. Lucumi, E. et al. Discovery of potent small-molecule inhibitors of multidrug-resistant *Plasmodium falciparum* using a novel miniaturized high-throughput luciferase-based assay. *Antimicrob. Agents Ch.* **54**, 3597–3604 (2010).
41. da Cruz, F. P. et al. Drug screen targeted at *Plasmodium* liver stages identifies a potent multistage antimalarial drug. *J. Infect. Dis.* **205**, 1278–1286 (2012).
42. Chong, C. R., Chen, X., Shi, L., Liu, J. O. & Sullivan, D. J. Jr. A clinical drug library screen identifies astemizole as an antimalarial agent. *Nat. Chem. Biol.* **2**, 415–416 (2006).
43. Chen, C. Z. et al. High-throughput *Giardia lamblia* viability assay using bioluminescent ATP content measurements. *Antimicrob. Agents Ch.* **55**, 667–675 (2011).
44. Chockalingam, K., Simeon, R. L., Rice, C. M. & Chen, Z. A cell protection screen reveals potent inhibitors of multiple stages of the hepatitis C virus life cycle. *Proc. Natl Acad. Sci. USA* **107**, 3764–3769 (2010).
45. Dyall, J. et al. Repurposing of clinically developed drugs for treatment of Middle East respiratory syndrome coronavirus infection. *Antimicrob. Agents Ch.* **58**, 4885–4893 (2014).
46. Gastaminza, P., Whitten-Bauer, C. & Chisari, F. V. Unbiased probing of the entire hepatitis C virus life cycle identifies clinical compounds that target multiple aspects of the infection. *Proc. Natl Acad. Sci. USA* **107**, 291–296 (2010).
47. He, S. et al. Repurposing of the antihistamine chlorcyclizine and related compounds for treatment of hepatitis C virus infection. *Sci. Transl Med.* **7**, 282ra249 (2015).
48. Madrid, P. B. et al. Evaluation of Ebola virus inhibitors for drug repurposing. *ACS Infect. Dis.* **1**, 317–326 (2015).
49. Johansen, L. M. et al. A screen of approved drugs and molecular probes identifies therapeutics with anti-Ebola virus activity. *Sci. Transl Med.* **7**, 290ra289 (2015).
50. Kouznetsova, J. et al. Identification of 53 compounds that block Ebola virus-like particle entry via a repurposing screen of approved drugs. *Emerg. Microbes Infect.* **3**, e84 (2014).
51. Barrows, N. J. et al. A screen of FDA-approved drugs for inhibitors of Zika virus infection. *Cell Host Microbe* **20**, 259–270 (2016).
52. Butts, A. et al. A repurposing approach identifies off-patent drugs with fungicidal cryptococcal activity, a common structural chemotype, and pharmacological properties relevant to the treatment of cryptococcosis. *Eukaryot. Cell* **12**, 278–287 (2013).
53. Krysan, D. J. & Didone, L. A high-throughput screening assay for small molecules that disrupt yeast cell integrity. *J. Biomol. Screen.* **13**, 657–664 (2008).
54. Siles, S. A., Srinivasan, A., Pierce, C. G., Lopez-Ribot, J. L. & Ramasubramanian, A. K. High-throughput screening of a collection of known pharmacologically active small compounds for identification of *Candida albicans* biofilm inhibitors. *Antimicrob. Agents Ch.* **57**, 3681–3687 (2013).
55. Zhai, B. et al. Polymyxin B, in combination with fluconazole, exerts a potent fungicidal effect. *J. Antimicrob. Chemoth.* **65**, 931–938 (2010).
56. Chopra, S. et al. Repurposing FDA-approved drugs to combat drug-resistant *Acinetobacter baumannii*. *J. Antimicrob. Chemoth* **65**, 2598–2601 (2010).
57. Jacobs, A. C. et al. Adenylate kinase release as a high-throughput-screening-compatible reporter of bacterial lysis for identification of antibacterial agents. *Antimicrob. Agents Ch.* **57**, 26–36 (2013).
58. Pothineni, V. R. et al. Identification of new drug candidates against *Borrelia burgdorferi* using high-throughput screening. *Drug Des. Dev. Ther.* **10**, 1307–1322 (2016).
59. Sun, W. et al. Rapid antimicrobial susceptibility test for identification of new therapeutics and drug combinations against multidrug-resistant bacteria. *Emerg. Microbes Infec.* **5**, e116 (2016).
60. Younis, W., Thangamani, S. & Seleem, M. N. Repurposing non-antimicrobial drugs and clinical molecules to treat bacterial infections. *Curr. Pharm. Design* **21**, 4106–4111 (2015).
61. Schenone, M., Dancik, V., Wagner, B. K. & Clemons, P. A. Target identification and mechanism of action in chemical biology and drug discovery. *Nat. Chem. Biol.* **9**, 232–240 (2013).
62. Zheng, W., Thorne, N. & McKew, J. C. Phenotypic screens as a renewed approach for drug discovery. *Drug Discov. Today* **18**, 1067–1073 (2013).
63. Gregori-Puigjane, E. et al. Identifying mechanism-of-action targets for drugs and probes. *Proc. Natl Acad. Sci. USA* **109**, 11178–11183 (2012).
64. Farha, M. A. & Brown, E. D. Unconventional screening approaches for antibiotic discovery. *Ann. NY Acad. Sci.* **1354**, 54–66 (2015).
65. Farha, M. A. et al. Antagonism screen for inhibitors of bacterial cell wall biogenesis uncovers an inhibitor of undecaprenyl diphosphate synthase. *Proc. Natl Acad. Sci. USA* **112**, 11048–11053 (2015).
66. Stokes, J. M., Davis, J. H., Mangat, C. S., Williamson, J. R. & Brown, E. D. Discovery of a small molecule that inhibits bacterial ribosome biogenesis. *eLife* **3**, e03574 (2014).
67. Xu, M. et al. Identification of small-molecule inhibitors of Zika virus infection and induced neural cell death via a drug repurposing screen. *Nat. Med.* **22**, 1101–1107 (2016).
68. Imperi, F. et al. Repurposing the antimycotic drug flucytosine for suppression of *Pseudomonas aeruginosa* pathogenicity. *Proc. Natl Acad. Sci. USA* **110**, 7458–7463 (2013).
69. Imperi, F. et al. New life for an old drug: the anthelmintic drug niclosamide inhibits *Pseudomonas aeruginosa* quorum sensing. *Antimicrob. Agents Ch.* **57**, 996–1005 (2013).
70. Engel, J. C. et al. Image-based high-throughput drug screening targeting the intracellular stage of *Trypanosoma cruzi*, the agent of Chagas' disease. *Antimicrob. Agents Ch.* **54**, 3326–3334 (2010).
71. Breger, J. et al. Antifungal chemical compounds identified using a *C. elegans* pathogenicity assay. *PLoS Pathog.* **3**, e18 (2007).
72. Rajamuthiah, R. et al. Whole animal automated platform for drug discovery against multi-drug resistant *Staphylococcus aureus*. *PLoS ONE* **9**, e89189 (2014).
73. Brown, S. A., Palmer, K. L. & Whiteley, M. Revisiting the host as a growth medium. *Nat. Rev. Microbiol.* **6**, 657–666 (2008).
74. Colquhoun, J. M., Wozniak, R. A. & Dunman, P. M. Clinically relevant growth conditions alter *Acinetobacter baumannii* antibiotic susceptibility and promote identification of novel antibacterial agents. *PLoS ONE* **10**, e0143033 (2015).

75. Andersson, J. A. et al. New role for FDA-approved drugs in combating antibiotic-resistant bacteria. *Antimicrob. Agents Ch.* **60**, 3717–3729 (2016).
76. Czyz, D. M. et al. Host-directed antimicrobial drugs with broad-spectrum efficacy against intracellular bacterial pathogens. *mBio.* **5**, e01534–01514 (2014).
77. Singh, S. B. Confronting the challenges of discovery of novel antibacterial agents. *Bioorg. Med. Chem. Lett.* **24**, 3683–3689 (2014).
78. Ejim, L. et al. Combinations of antibiotics and nonantibiotic drugs enhance antimicrobial efficacy. *Nat. Chem. Biol.* **7**, 348–350 (2011).
79. Spitzer, M. et al. Cross-species discovery of syncretic drug combinations that potentiate the antifungal fluconazole. *Mol. Syst. Biol.* **7**, 499 (2011).
80. Planer, J. D. et al. Synergy testing of FDA-approved drugs identifies potent drug combinations against *Trypanosoma cruzi*. *PLoS Neglect. Trop. D.* **8**, e2977 (2014).
81. Sun, W. et al. Synergistic drug combination effectively blocks Ebola virus infection. *Antivir. Res.* **137**, 165–172 (2017).
82. Farha, M. A. et al. Inhibition of WTA synthesis blocks the cooperative action of PBPs and sensitizes MRSA to beta-lactams. *ACS Chem. Biol.* **8**, 226–233 (2013).
83. Aeschlimann, J. R., Dresser, L. D., Kaatz, G. W. & Rybak, M. J. Effects of NorA inhibitors on in vitro antibacterial activities and postantibiotic effects of levofloxacin, ciprofloxacin, and norfloxacin in genetically related strains of *Staphylococcus aureus*. *Antimicrob. Agents Ch.* **43**, 335–340 (1999).
84. Van den Driessche, F., Brackman, G., Swimberghe, R., Rigole, P. & Coenye, T. Screening a repurposing library for potentiators of antibiotics against *Staphylococcus aureus* biofilms. *Int. J. Antimicrob. Ag.* **49**, 315–320 (2017).
85. Delattin, N. et al. Repurposing as a means to increase the activity of amphotericin B and caspofungin against *Candida albicans* biofilms. *J. Antimicrob. Chemoth.* **69**, 1035–1044 (2014).
86. Stokes, J. M. et al. Pentamidine sensitizes Gram-negative pathogens to antibiotics and overcomes acquired colistin resistance. *Nat. Microbiol.* **2**, 17028 (2017).
87. Lehar, J. et al. Synergistic drug combinations tend to improve therapeutically relevant selectivity. *Nat. Biotechnol.* **27**, 659–666 (2009).
88. Delcour, A. H. Outer membrane permeability and antibiotic resistance. *Biochim. Biophys. Acta.* **1794**, 808–816 (2009).
89. MacNair, C. R. et al. Overcoming mcr-1 mediated colistin resistance with colistin in combination with other antibiotics. *Nat. Commun.* **9**, 458 (2018).
90. Gygli, S. M., Borrell, S., Trauner, A. & Gagneux, S. Antimicrobial resistance in *Mycobacterium tuberculosis*: mechanistic and evolutionary perspectives. *FEMS Microbiol. Rev.* **41**, 354–373 (2017).
91. Bollenbach, T. Antimicrobial interactions: mechanisms and implications for drug discovery and resistance evolution. *Curr. Opin. Microbiol.* **27**, 1–9 (2015).
92. Eliopoulos, G. M. & Moellering, R. C. Jr. Antibiotic synergism and antimicrobial combinations in clinical infections. *Rev. Infect. Dis.* **4**, 282–293 (1982).
93. Bonhoeffer, S., Lipsitch, M. & Levin, B. R. Evaluating treatment protocols to prevent antibiotic resistance. *Proc. Natl Acad. Sci. USA* **94**, 12106–12111 (1997).
94. Mouton, J. W. Combination therapy as a tool to prevent emergence of bacterial resistance. *Infection* **27**, S24–S28 (1999).
95. Torella, J. P., Chait, R. & Kishony, R. Optimal drug synergy in antimicrobial treatments. *PLoS Comput. Biol.* **6**, e1000796 (2010).
96. Worthington, R. J. & Melander, C. Combination approaches to combat multidrug-resistant bacteria. *Trends Biotechnol.* **31**, 177–184 (2013).
97. Kinnings, S. L. et al. Drug discovery using chemical systems biology: repositioning the safe medicine Comtan to treat multi-drug and extensively drug resistant tuberculosis. *PLoS Comput. Biol.* **5**, e1000423 (2009).
98. Forsberg, M. et al. Pharmacokinetics and pharmacodynamics of entacapone and tolcapone after acute and repeated administration: a comparative study in the rat. *J. Pharmacol. Exp. Ther.* **304**, 498–506 (2003).
99. Khodaverdian, V. et al. Discovery of antivirulence agents against methicillin-resistant *Staphylococcus aureus*. *Antimicrob. Agents Ch.* **57**, 3645–3652 (2013).
100. Astolfi, A. et al. Pharmacophore-based repositioning of approved drugs as novel *Staphylococcus aureus* NorA efflux pump inhibitors. *J. Med. Chem.* **60**, 1598–1604 (2017).
101. Carlson-Banning, K. M. et al. Toward repurposing ciclopirox as an antibiotic against drug-resistant *Acinetobacter baumannii*, *Escherichia coli*, and *Klebsiella pneumoniae*. *PLoS ONE* **8**, e69646 (2013).
102. Deng, L., Sundriyal, S., Rubio, V., Shi, Z. Z. & Song, Y. Coordination chemistry based approach to lipophilic inhibitors of 1-deoxy-D-xylulose-5-phosphate reductoisomerase. *J Med. Chem.* **52**, 6539–6542 (2009).
103. Ekins, S., Mestres, J. & Testa, B. *In silico* pharmacology for drug discovery: methods for virtual ligand screening and profiling. *Brit. J. Pharmacol.* **152**, 9–20 (2007).
104. Wang, Y. et al. PubChem BioAssay: 2014 update. *Nucleic Acids Res.* **42**, D1075–D1082 (2014).
105. Wishart, D. S. et al. DrugBank: a knowledgebase for drugs, drug actions and drug targets. *Nucleic Acids Res.* **36**, D901–D906 (2008).
106. Bento, A. P. et al. The ChEMBL bioactivity database: an update. *Nucleic Acids Res.* **42**, D1083–D1090 (2014).
107. Brown, A. S. & Patel, C. J. A standard database for drug repositioning. *Sci. Data* **4**, 170029 (2017).
108. Shameer, K. et al. Systematic analyses of drugs and disease indications in RepurposeDB reveal pharmacological, biological and epidemiological factors influencing drug repositioning. *Brief Bioinform.* **19**, 656–678 (2017).
109. Sateriale, A., Bessoff, K., Sarkar, I. N. & Huston, C. D. Drug repurposing: mining protozoan proteomes for targets of known bioactive compounds. *J. Am. Med. Inform. Assn.* **21**, 238–244 (2014).
110. Chavali, A. K. et al. Metabolic network analysis predicts efficacy of FDA-approved drugs targeting the causative agent of a neglected tropical disease. *BMC Syst. Biol.* **6**, 27 (2012).
111. Lamb, J. The Connectivity Map: a new tool for biomedical research. *Nat. Rev. Cancer* **7**, 54–60 (2007).
112. Josset, L. et al. Gene expression signature-based screening identifies new broadly effective influenza a antivirals. *PLoS ONE* **5**, e13169 (2010).
113. Coelho, E. D., Arrais, J. P. & Oliveira, J. L. Computational discovery of putative leads for drug repositioning through drug-target interaction prediction. *PLoS Comput. Biol.* **12**, e1005219 (2016).
114. Berenstein, A. J., Magarinos, M. P., Chernomoretz, A. & Aguero, F. A multilayer network approach for guiding drug repositioning in neglected diseases. *PLoS Negl. Trop. D.* **10**, e0004300 (2016).
115. Iwata, H., Sawada, R., Mizutani, S. & Yamanishi, Y. Systematic drug repositioning for a wide range of diseases with integrative analyses of phenotypic and molecular data. *J. Chem. Inf. Model.* **55**, 446–459 (2015).
116. Malo, N., Hanley, J. A., Cerquozzi, S., Pelletier, J. & Nadon, R. Statistical practice in high-throughput screening data analysis. *Nat. Biotechnol.* **24**, 167–175 (2006).
117. Li, Y. Y. & Jones, S. J. Drug repositioning for personalized medicine. *Genome Med.* **4**, 27 (2012).
118. March-Vila, E. et al. On the integration of in silico drug design methods for drug repurposing. *Front. Pharmacol.* **8**, 298 (2017).
119. Meyerhoff, A. U. S. Food and Drug Administration approval of AmBisome (liposomal amphotericin B) for treatment of visceral leishmaniasis. *Clin. Infect. Dis.* **28**, 42–48 (1999). discussion 49–51.
120. Cohen, H. G. & Reynolds, T. B. Comparison of metronidazole and chloroquine for the treatment of amoebic liver abscess. A controlled trial. *Gastroenterology* **69**, 35–41 (1975).
121. Katlama, C., De Wit, S., O'Doherty, E., Van Glabeke, M. & Clumeck, N. Pyrimethamine-clindamycin vs. pyrimethamine-sulfadiazine as acute and long-term therapy for toxoplasmic encephalitis in patients with AIDS. *Clin. Infect. Dis.* **22**, 268–275 (1996).
122. Tan, K. R. et al. Doxycycline for malaria chemoprophylaxis and treatment: report from the CDC expert meeting on malaria chemoprophylaxis. *Am. J. Trop. Med. Hyg.* **84**, 517–531 (2011).
123. Ben Salah, A. et al. Topical paromomycin with or without gentamicin for cutaneous leishmaniasis. *New Engl. J. Med.* **368**, 524–532 (2013).
124. Robert-Gangneux, F. & Darde, M. L. Epidemiology of and diagnostic strategies for toxoplasmosis. *Clin. Microbiol. Rev.* **25**, 264–296 (2012).
125. Medina-Franco, J. L., Giulianotti, M. A., Welmaker, G. S. & Houghten, R. A. Shifting from the single to the multitarget paradigm in drug discovery. *Drug Discov. Today* **18**, 495–501 (2013).
126. Fairlamb, A. H., Gow, N. A., Matthews, K. R. & Waters, A. P. Drug resistance in eukaryotic microorganisms. *Nat. Microbiol.* **1**, 16092 (2016).
127. Weisman, J. L. et al. Searching for new antimalarial therapeutics amongst known drugs. *Chem. Biol. Drug Des.* **67**, 409–416 (2006).
128. *World malaria report 2017* (WHO, 2017).
129. Attaran, A. Where did it all go wrong? *Nature* **430**, 932–933 (2004).
130. Gottlieb, S. Antihistamine drug withdrawn by manufacturer. *BMJ* **319**, 7 (1999).
131. Derbyshire, E. R., Prudencio, M., Mota, M. M. & Clardy, J. Liver-stage malaria parasites vulnerable to diverse chemical scaffolds. *Proc. Natl Acad. Sci. USA* **109**, 8511–8516 (2012).
132. Roman, G., Crandall, I. E. & Szarek, W. A. Synthesis and anti-Plasmodium activity of benzimidazole analogues structurally related to astemizole. *ChemMedChem* **8**, 1795–1804 (2013).
133. Gunther, J., Shafir, S., Bristow, B. & Sorvillo, F. Short report: Amebiasis-related mortality among United States residents, 1990–2007. *Am. J. Trop. Med. Hyg.* **85**, 1038–1040 (2011).
134. Andrade, R. M. & Reed, S. L. New drug target in protozoan parasites: the role of thioredoxin reductase. *Front. Microbiol.* **6**, 975 (2015).
135. Roder, C. & Thomson, M. J. Auranofin: repurposing an old drug for a golden new age. *Drugs R.D.* **15**, 13–20 (2015).
136. Tejman-Yarden, N. et al. A reprofiled drug, auranofin, is effective against metronidazole-resistant *Giardia lamblia*. *Antimicrob. Agents Ch.* **57**, 2029–2035 (2013).

137. Berglof, F. E., Berglof, K. & Walz, D. T. Auranofin: an oral chrysotherapeutic agent for the treatment of rheumatoid arthritis. *J. Rheumatol.* **5**, 68–74 (1978).
138. Hill, A. & Cooke, G. Medicine. *Hepatitis C can be cured globally, but at what cost? Science* **345**, 141–142 (2014).
139. Huang, R. et al. The NCGC pharmaceutical collection: a comprehensive resource of clinically approved drugs enabling repurposing and chemical genomics. *Sci. Transl Med.* **3**, 80ps16 (2011).
140. Chen, L. H. & Hamer, D. H. Zika virus: rapid spread in the Western hemisphere. *Ann. Intern. Med.* **164**, 613–615 (2016).
141. Tang, H. et al. Zika virus infects human cortical neural progenitors and attenuates their growth. *Cell Stem Cell* **18**, 587–590 (2016).
142. Khanim, F. L. et al. Redeployment-based drug screening identifies the anti-helminthic niclosamide as anti-myeloma therapy that also reduces free light chain production. *Blood Cancer J.* **1**, e39 (2011).
143. Jordan, V. C. Tamoxifen: the herald of a new era of preventive therapeutics. *J. Natl Cancer I.* **89**, 747–749 (1997).
144. Friedman, Z. Y. Recent advances in understanding the molecular mechanisms of tamoxifen action. *Cancer Invest.* **16**, 391–396 (1998).
145. Dolan, K. et al. Antifungal activity of tamoxifen: in vitro and in vivo activities and mechanistic characterization. *Antimicrob. Agents Ch.* **53**, 3337–3346 (2009).
146. Wiseman, H., Cannon, M. & Arnstein, H. R. Observation and significance of growth inhibition of Saccharomyces cerevisiae (A224A) by the anti-oestrogen drug tamoxifen. *Biochem. Soc. T.* **17**, 1038–1039 (1989).
147. Wiseman, H., Cannon, M., Arnstein, H. R. & Halliwell, B. Enhancement by tamoxifen of the membrane antioxidant action of the yeast membrane sterol ergosterol: relevance to the antiyeast and anticancer action of tamoxifen. *Biochim. Biophys. Acta* **1181**, 201–206 (1993).
148. Beggs, W. H. Anti-Candida activity of the anti-cancer drug tamoxifen. *Res. Commun. Chem. Path.* **80**, 125–128 (1993).
149. Edlind, T., Smith, L., Henry, K., Katiyar, S. & Nickels, J. Antifungal activity in *Saccharomyces cerevisiae* is modulated by calcium signalling. *Mol. Microbiol.* **46**, 257–268 (2002).
150. Parsons, A. B. et al. Exploring the mode-of-action of bioactive compounds by chemical-genetic profiling in yeast. *Cell* **126**, 611–625 (2006).
151. Antimisiaris, D., Bae, K. G., Morton, L. & Gully, Z. Tamoxifen pharmacovigilance: implications for safe use in the future. *Consult. Pharm.* **32**, 535–546 (2017).
152. Lass-Florl, C., Dierich, M. P., Fuchs, D., Semenitz, E. & Ledochowski, M. Antifungal activity against *Candida* species of the selective serotonin-reuptake inhibitor, sertraline. *Clin. Infect. Dis.* **33**, E135–E136 (2001).
153. Lass-Florl, C. et al. Antifungal properties of selective serotonin reuptake inhibitors against *Aspergillus* species in vitro. *J. Antimicrob. Chemoth.* **48**, 775–779 (2001).
154. Zhai, B., Wu, C., Wang, L., Sachs, M. S. & Lin, X. The antidepressant sertraline provides a promising therapeutic option for neurotropic cryptococcal infections. *Antimicrob. Agents Ch.* **56**, 3758–3766 (2012).
155. Rhein, J. et al. Efficacy of adjunctive sertraline for the treatment of HIV-associated cryptococcal meningitis: an open-label dose-ranging study. *Lancet Infect. Dis.* **16**, 809–818 (2016).
156. Villanueva-Lozano, H. et al. Clinical evaluation of the antifungal effect of sertraline in the treatment of cryptococcal meningitis in HIV patients: a single Mexican center experience. *Infection* **46**, 25–30 (2018).
157. Andrews, P., Thyssen, J. & Lorke, D. The biology and toxicology of molluscicides, Bayluscide. *Pharmacol. Therapeut.* **19**, 245–295 (1982).
158. Mook, R. A. Jr et al. Structure-activity studies of Wnt/beta-catenin inhibition in the Niclosamide chemotype: identification of derivatives with improved drug exposure. *Bioorgan. Med. Chem.* **23**, 5829–5838 (2015).
159. Ye, Y., Zhang, X., Zhang, T., Wang, H. & Wu, B. Design and evaluation of injectable niclosamide nanocrystals prepared by wet media milling technique. *Drug Dev. Ind. Pharm.* **41**, 1416–1424 (2015).
160. Lu, W. et al. Niclosamide suppresses cancer cell growth by inducing Wnt co-receptor LRP6 degradation and inhibiting the Wnt/beta-catenin pathway. *PLoS ONE* **6**, e29290 (2011).
161. Li, R. et al. Niclosamide overcomes acquired resistance to erlotinib through suppression of STAT3 in non-small cell lung cancer. *Mol. Cancer Ther.* **12**, 2200–2212 (2013).
162. Liang, L. et al. Inhibitory effects of niclosamide on inflammation and migration of fibroblast-like synoviocytes from patients with rheumatoid arthritis. *Inflamm. Res.* **64**, 225–233 (2015).
163. Jurgeit, A. et al. Niclosamide is a proton carrier and targets acidic endosomes with broad antiviral effects. *PLoS Pathog.* **8**, e1002976 (2012).
164. Amos, H. & Vollmayer, E. Effect of pentamidine on the growth of *Escherichia coli*. *J. Bacteriol.* **73**, 172–177 (1957).
165. Wien, R., Harrison, J. & Freeman, W. A. Diamidines as antibacterial compounds. *Brit. J. Pharm. Chemoth.* **3**, 211–218 (1948).
166. Wien, R., Harrison, J. & Freeman, W. A. New antibacterial diamidines. *Lancet* **1**, 711 (1948).
167. Libman, M. D., Miller, M. A. & Richards, G. K. Antistaphylococcal activity of pentamidine. *Antimicrob. Agents Ch.* **34**, 1795–1796 (1990).
168. Fox, K. R., Sansom, C. E. & Stevens, M. F. Footprinting studies on the sequence-selective binding of pentamidine to DNA. *FEBS Lett.* **266**, 150–154 (1990).
169. Minnick, M. F., Hicks, L. D., Battisti, J. M. & Raghavan, R. Pentamidine inhibits *Coxiella burnetii* growth and 23S rRNA intron splicing in vitro. *Int. J. Antimicrob. Ag.* **36**, 380–382 (2010).
170. Sun, T. & Zhang, Y. Pentamidine binds to tRNA through non-specific hydrophobic interactions and inhibits aminoacylation and translation. *Nucleic Acids Res.* **36**, 1654–1664 (2008).
171. Mullard, A. Drug repurposing programmes get lift off. *Nat. Rev. Drug Discov.* **11**, 505–506 (2012).
172. Corsello, S. M. et al. The Drug Repurposing Hub: a next-generation drug library and information resource. *Nat. Med.* **23**, 405–408 (2017).
173. Weir, S. J., DeGennaro, L. J. & Austin, C. P. Repurposing approved and abandoned drugs for the treatment and prevention of cancer through public-private partnership. *Cancer Res.* **72**, 1055–1058 (2012).
174. Kwok, A. K. & Koenigbauer, F. M. Incentives to repurpose existing drugs for orphan indications. *ACS Med. Chem. Lett.* **6**, 828–830 (2015).
175. Simarro, P. P., Franco, J., Diarra, A., Postigo, J. A. & Jannin, J. Update on field use of the available drugs for the chemotherapy of human African trypanosomiasis. *Parasitology* **139**, 842–846 (2012).
176. Smorenburg, C. H. et al. Phase II study of miltefosine 6% solution as topical treatment of skin metastases in breast cancer patients. *Colloq. Inse.* **11**, 825–828 (2000).
177. Yarchoan, R. et al. Administration of 3'-azido-3'-deoxythymidine, an inhibitor of HTLV-III/LAV replication, to patients with AIDS or AIDS-related complex. *Lancet* **1**, 575–580 (1986).
178. Simpson, P. B. & Reichman, M. Opening the lead generation toolbox. *Nat. Rev. Drug Discov.* **13**, 3–4 (2014).
179. Nilsson, N. & Felding, J. Open innovation platforms to boost pharmaceutical collaborations: evaluating external compounds for desired biological activity. *Future Med. Chem.* **7**, 1853–1859 (2015).
180. Lipinski, C. A., Lombardo, F., Dominy, B. W. & Feeney, P. J. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv. Drug Deliver. Rev.* **46**, 3–26 (2001).
181. Magarinos, M. P. et al. TDR Targets: a chemogenomics resource for neglected diseases. *Nucleic Acids Res.* **40**, D1118–D1127 (2012).
182. von Eichborn, J. et al. PROMISCUOUS: a database for network-based drug-repositioning. *Nucleic Acids Res.* **39**, D1060–D1066 (2011).
183. Kuhn, M., Letunic, I., Jensen, L. J. & Bork, P. The SIDER database of drugs and side effects. *Nucleic Acids Res.* **44**, D1075–D1079 (2016).
184. Siramshetty, V. B. et al. SuperDRUG2: a one stop resource for approved/marketed drugs. *Nucleic Acids Res.* **46**, D1137–D1143 (2018).

Acknowledgements

This work was supported by a Foundation grant from the Canadian Institutes of Health Research (FRN-143215) and a Tier I Canada Research Chair award to E.D.B.

Author contributions

Both authors researched data for the article, substantially contributed to discussion of content, wrote the article, and reviewed and edited the manuscript before submission.

Competing interests

The authors declare no competing interests.

Additional information

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