Targeting Persisters for Tuberculosis Control

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Mycobacterial persisters, the survivors from antibiotic exposure, necessitate the lengthy treatment of tuberculosis (TB) and pose a significant challenge for our control of the disease. We suggest that persisters in TB are heterogeneous in nature and comprise various proportions of the population depending on the circumstances; the mechanisms of their formation are complex and may be related to those required for persistence in chronic infection. Results from recent studies implicate multiple pathways for persister formation, including energy production, the stringent response, global regulators, the trans-translation pathway, proteasomal protein degradation, toxin-antitoxin modules, and transporter or efflux mechanisms. A combination of specifically persister-targeted approaches, such as catching them when active and susceptible either by stimulating them to “wake up” or by intermittent drug dosing, the development of new drugs, the use of appropriate drug combinations, and combined chemotherapy and immunotherapy, may be needed for more effective elimination of persisters and better treatment of TB. Variations in levels of persister formation and in host genetics can play a role in the outcome of clinical treatment, and thus, these may entail personalized treatment regimens.

"When one realizes that, even though the bacilli vanish and there is . . . truly latent infection, the bacilli are, nevertheless, still there . . . drug susceptible, I think you will agree with me that it shows that ‘you can’t win.’"—Walsh McDermott (50)

A key problem facing effective tuberculosis (TB) control is the requirement for prolonged treatment with multiple drugs for a period of at least 6 months, which makes patient adherence to therapy difficult. Poor adherence would in turn lead to an increased risk of emergence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) TB. The prolonged treatment necessary to cure TB is thought to be due to “persistor” bacteria that are refractory to antibiotic treatment. There is an increasing interest recently in understanding the biology of persisters and in developing new drugs or treatment regimens that are effective not only for drug-resistant TB but also for mycobacterial persisters in order to shorten the lengthy treatment of TB and to target latent infection due to Mycobacterium tuberculosis.

WHAT ARE PERSISTERS?

Persisters are the subpopulation of bacteria which survive the cidal action of antibiotics. They are genetically identical to susceptible bacteria and appear to be nonreplicating or slowly growing. They have noninheritable phenotypic resistance or tolerance to antibiotics, but their progeny remain susceptible to antibiotics upon regrowth (8, 33, 49). We use the term “persistor” here specifically to denote bacteria surviving or with the potential to survive antibiotic (or stress) exposure, while “persistence” is used to denote actual or potential bacterial survival in the face of any stress from immune pressure from the host.

Persisters were first described by Hobby, Meyer, and Chaffee in 1942 when they found that the highly bactericidal antibiotic penicillin only killed 99% of a streptococcal culture, leaving 1% of the bacterial population intact (33). This phenomenon was subsequently more carefully defined by Bigger in 1944 when he termed the 1% surviving bacteria not killed by penicillin as persisters (8). Persistor formation can be viewed as a bacterial adaptation to adverse environments or stress conditions in vivo or in vitro such that, probably as a result of a quiescent state, they are not killed by antibiotics or stresses (49). Persistor formation can be promoted through epigenetic factors in a stochastic manner (77) or in a deterministic manner (24). Persister cells may have morphologies indistinguishable from those of susceptible cells or be distinct in some way, e.g., coccoid shape in old cultures and biofilms or cell wall-defective L-forms (85). The concept of persisters has evolved from the presumed homogeneous persister population (8) to much more heterogeneous and diverse populations (40, 43, 44, 83) that are defined by specific conditions. Persisters are likely to comprise different subpopulations (40, 44, 83) and generally form a small and relatively predictable proportion of a culture. This proportion depends on conditions such as the age of a culture, the length of antibiotic exposure, and the type and concentration of antibiotics used in the model system (40, 44). Persisters can be divided into those that can and those that cannot be propagated under standard culture conditions; the latter can be resuscitated and start growing again when conditions are conducive (53, 85, 88).

In view of the association of persisters with a nonreplicating state, there is overlap with the phenomenon of bacterial dormancy and the terms are sometimes used interchangeably. Dormancy involves a reversible metabolic shutdown (4) and is applicable to some but not all described persister phenomena. Although it is plausible that bacilli with the same phenotypes as persisters underpin latent infection, there is scant direct evidence to support this. Clinically latent infection may or may not be attributable to dormant bacteria (3, 85). Moreover, persister phenomena should...
The yin-yang model depicts a dynamic bacterial population consisting of growing and nongrowing subpopulations in various metabolic states in a continuum (reproduced with permission from reference 83). In the growing bacterial subpopulation (yang), there is a small proportion of nongrowing or slowly growing persisters (yin). As the bacteria enter stationary phase, more persisters form, with a small number of growing bacteria. The persister subpopulation is heterogeneous and consists of a continuum of various subpopulations as a result of stochastic or induced expression of persister genes. The yin-yang model is used to explain why, after a 2-month intensive-phase treatment with isoniazid (INH), rifampin (RIF), pyrazinamide (PZA), and ethambutol (EMB), the remaining persister tubercle bacilli can revert to growing form which can still be killed by INH and RIF in the subsequent 4-month continuation phase of treatment. This model also explains why INH can be used for prophylactic treatment of latent TB infection, where tubercle bacilli revert from nongrowing form to growing form and become susceptible to INH. Furthermore, the yin-yang model can be used to indicate how phenotypic resistance (yin) in persisters can revert to genetic resistance (yang) in growing bacteria and vice versa.

Persisters pose significant challenges to the clinical control of various bacterial infections, as their presence appears to underlie chronic and recurrent infections, biofilm infections, the requirement for lengthy therapy of certain bacterial infections as in TB, and posttreatment bacterial persistence and relapse (39, 49, 85). In this review, we address the noninheritable mycobacterial factors that lead to bacilli surviving chemotherapy as distinct from heritable drug resistance and host factors leading to inadequate drug exposure.

**THE PROBLEM OF PERSISTERS IN TB**

In the mycobacterial persister phenomenon, tubercle bacilli that are drug susceptible in vitro are nonetheless capable of surviving in the body despite intensive therapy with the appropriate antituberculosis drug(s) (49). McDermott proposed that the inability of drugs to completely kill apparently in vitro drug-susceptible *M. tuberculosis* in the body was due to persisters (49) and, with colleagues, he elegantly demonstrated this in a series of mouse model experiments (45–48), while others have made comparable observations in clinical settings (16, 26, 36). While it is clear that mycobacteria persist during TB chemotherapy in both human and mouse infections, exactly where the *M. tuberculosis* cells in question reside in patients is unclear. However, true persisters have been found in lesions, and candidate persisters have been demonstrated in adipose tissue (55) and sputum (29). It is possible that persisters can reside in different locations intracellularly and extracellularly and in different cell types besides macrophages.

During the disease process, tubercle bacilli reside in different microenvironmental conditions that include high oxygen (in cavities) or low oxygen (in host macrophages or in granulomatous lesions) content, nutrient starvation, oxidative stress, and acidic pH, all of which affect their metabolic statuses. Such varied conditions constitute the basis for producing heterogeneous bacterial populations, including nonreplicating persisters and growing bacteria with different capacities for persister formation (83), and therefore, various susceptibilities to antituberculosis drugs. Some bacilli or persisters from patients may or may not have acid-fast staining and adopt different morphologies (85) or may not grow in normal culture media or only grow upon extended incubation for even 6 to 9 months (32) or with resuscitation-promoting factors (Rpfs) (53). A yin-yang model applicable to both patients and experimental systems was proposed to emphasize the heterogeneity, dynamic nature, and interconversion of the diverse bacterial subpopulations present and to provide potential explanations for various degrees of layers of persistence (Fig. 1) (83). The yin-yang model postulates that an overall bacterial population consists of growing, slow-growing, and nongrowing subpopulations with different metabolic statuses in a continuum which can interconvert at the level of the bacteria. The same yin-yang model provides a possible explanation for how latent infection (yin) and active disease (yang) can behave as a continuum and interconvert at the level of the host. Moreover, the model provides a rationale for the current practice of two-phase TB therapy lasting 6 months and why isoniazid can be used for chemoprophylaxis or treatment of latent TB infection (LTBI) (Fig. 2). LTBI, including that induced by host immunity or drug pressure, can involve a relatively small number of nongrowing or slowly growing persisters that can convert to actively growing bacilli and become susceptible to isoniazid treatment upon regrowth. The need for extended courses in treat-
ing latent infection favors this persister-based explanation for latency as opposed to the alternative explanation based on a balance between bacterial growth and immune elimination.

Host genetic, nutritional, neurohumoral, and immune factors play important roles in controlling the persisters and affect the level and outcome of LTBI (Fig. 2) (85). Appropriate immune intervention may help to reduce the reactivation of LTBI to active disease or enhance the activity of chemotherapy for improved control of TB. This review will primarily focus on the bacterial mechanisms of persister formation in TB and leave aside the host immune control of persistence, which has been reviewed previously (76, 85).

**MECHANISMS OF PERSISTER FORMATION IN M. TUBERCULOSIS**

Different *in vitro* models of *M. tuberculosis* survival in the face of potentially lethal stresses have been developed, including starvation in buffer (7), the hypoxic Wayne model (81), and a combination of hypoxia and acid pH (11, 13, 20). The formation of persisters in these models has been assessed in antibiotic tolerance assays, while two models, growing cultures treated with cycloserine (37) and aged cultures treated with rifampin or pyrazinamide, respectively, have been used in drug screen and gene expression studies. While two models, growing cultures treated with cycloserine (37) and aged cultures treated with rifampin or pyrazinamide, have been used in drug screen and gene expression studies, many toxin-antitoxin (TA) modules, universal stress protein (UspA), SigF, anti-sigma factor RsbW, and DnaE2 (37), with 5 genes being common to the different persister models, including Acr2 α-crystallin heat shock protein, GntR transcriptional regulator family protein (Rv1152), PdhA pyruvate dehydrogenase component, Rv3290c (Lat) encoding an l-lysine-episol-aminotransferase, and Rv2517c encoding a hypothetical protein (37). Functional studies will be required before the significance of these genes for persister formation can be determined.

An *in vivo* gene expression study of persister bacilli in chronic TB in mice suggested that the persisters grow slowly and share some similarity to *in vitro* persisters (66). Transposon mutagenesis has identified genes that are important for infection or virulence in the mouse model of TB (12, 65), and these may overlap with genes involved in TB persister formation. It is of interest to note that genes required for persister formation *in vitro* may be different from those required *in vivo*, as demonstrated in the case of reLE, overexpression of which contributes to persister formation *in vitro* but not *in vivo* (69), and *cydC*, which is required for isoniazid persistence in mice but not for *in vitro* persister formation (21). Thus, the genes involved in persister formation *in vitro* versus those required for *in vivo* persistence are likely to vary according to the specific models being used in the study despite some commonality (37, 66). It is likely that multiple mechanisms of varying hierarchy and importance are involved in persister formation in different models.

Several mechanisms or pathways have been identified that may be involved in *M. tuberculosis* persister formation or maintenance, and these have mainly derived from single-gene mutation studies. These are listed in Table 1, where we distinguish between genes that have been associated with persister formation related to antibiotic exposure and those that have been recognized in persistence studies involving chronic *in vivo* infections or macrophage survival. The functional areas involved include the following genes or pathways. (i) Energy-related pathways identified as involved in persister formation include *sucB* (dihydrolipoamide acyltransferase, a subunit of the pyruvate dehydrogenase complex) (DlaT) (9), *menA*, involved in menaquinone biosynthesis and metabolism pathways and ribosomal proteins (37), as found in the starvation model (7) and the Wayne model (78). This study identified 56 upregulated persister-specific genes, including

### TABLE 1 Genes that may be involved in persister formation or survival in *M. tuberculosis*

<table>
<thead>
<tr>
<th>Gene</th>
<th>Physiological function (reference)</th>
<th>Evidence for persister/persistence/nonreplication (reference)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>psaA</em></td>
<td>Cording and mycolic acid cyclopropane ring synthesis (30)</td>
<td>Required for persistent infection and lethality in mouse model</td>
</tr>
<tr>
<td><em>sucB</em> (DlaT)</td>
<td>TCA cycle/glycolysis (9)</td>
<td>Identified as a drug target in nonreplicating (acid- and NO-treated and NRP2) bacilli</td>
</tr>
<tr>
<td><em>menA</em></td>
<td>Menaquinone synthesis (22)</td>
<td>Inhibitors of MenA reduce recovery of bacilli from NRP2</td>
</tr>
<tr>
<td><em>tgs1</em></td>
<td>Triacylglycerol synthase 1 (member of DosR regulon) (20)</td>
<td>Deletion reduces development of antibiotic tolerance (persisters) after multiple stresses</td>
</tr>
<tr>
<td><em>icl1</em></td>
<td>Isocitrate lyase (51)</td>
<td><em>icl1</em> deletion produces loss of persistence in mice</td>
</tr>
<tr>
<td><em>mce4</em></td>
<td>Cholesterol transport (59)</td>
<td>Required for persistence in chronically infected mice</td>
</tr>
<tr>
<td><em>relA</em></td>
<td>Stringent response, ppGpp synthesis (17)</td>
<td>Deletion reduces persistence in chronic infection of mice</td>
</tr>
<tr>
<td><em>carD</em></td>
<td>Regulator of rRNA transcription (70)</td>
<td>Knockdown reduces stress resistance and persistence in mouse infection</td>
</tr>
<tr>
<td><em>prcBA</em></td>
<td>Proteasome core subunits (27, 28)</td>
<td>Required for persistence in chronic infection in mice and long-term survival <em>in vitro</em></td>
</tr>
<tr>
<td><em>cydC</em></td>
<td>Cytochrome bd assembly (21)</td>
<td>Required for persistence in isoniazid-treated mice</td>
</tr>
<tr>
<td><em>phoY2</em></td>
<td>Global cellular metabolism regulator PhoU (68)</td>
<td>Deletion reduces persister formation <em>in vitro</em> and persistence in mouse infection</td>
</tr>
<tr>
<td><em>rpsA</em></td>
<td>S1 ribosomal protein involved in both translation and trans-translation</td>
<td><em>Trans</em>-translation required for survival under stress conditions (67)</td>
</tr>
</tbody>
</table>

*a The presentation of these genes is mainly based on mutant or inhibitor studies that have shown reduced persistence in *M. tuberculosis*. For more genes that are upregulated in persistence models but whose roles are not yet confirmed by mutant or persister studies, see references 7, 37, and 78.*
required for ATP production (22), codC, encoding a transporter for cytochrome bld assembly required for energy production during hypoxia and in vivo infection (21), tgs1 (triacylglycerol synthase 1), which stores fatty acids in triglycerides (20), and icl1, encoding one of two isocitrate lyases, which, distinct from icl2, appears essential for persistence in mouse infection (51). (ii) The stringent response regulator RelA (ppGpp synthase) (17) is important for persistence in M. tuberculosis, since its mutation caused significant defects in long-term survival in vitro and in mice. (iii) The PhoU homolog PhoY2, a phosphate and cellular metabolism regulator, is involved in persistence, since its inactivation caused a defect in the persister phenotype as shown by reduced persister numbers upon rifampin (RIF) and pyrazinamide (PZA) exposure in vitro, as well as a defect in persistence in mice (68). (iv) The target of the critical persister drug PZA has recently been shown to be RpsA (S1 protein), involved in translational-trans (67), which is required for persister survival under stress conditions (74). (v) The protein degradation pathway mediated by proteasome PrcBA (28) has been identified as a persistence mechanism. (vi) Elevated transporter activity, such as Mce4, is involved in cholesterol uptake which enriches persisters (59); in addition, a recent study suggested that M. tuberculosis in macrophages had elevated activity of the efflux pump TAP as a potential mechanism for antibiotic tolerance induced by the intracellular environment (1). (vii) Mutation of the cell wall mycolic acid cydC clopanto synthase PcaA led to decreased cording, persistence, and virulence in mice (30). (viii) Toxin-antitoxin (TA) modules (69) had effects on bacterial antibiotic tolerance. TA modules are the most-studied persister-related mechanism in other bacteria, such as Escherichia coli. It is interesting to note that over 30 TA modules were identified in the genome of M. tuberculosis (60), many of which were induced in cycloserine-tolerant persisters (37). Ectopic overexpression of toxin RelE was shown to induce persisters in M. tuberculosis (69), but the role of other TA modules in persister formation remains to be evaluated. Thus, the mechanisms of persister formation are complex and multiple, and the relative importance of the mechanisms involved may depend on the particular model being used. The verified persister pathways, including those discussed above, could serve as potential targets for developing new drugs active against the persisters for improved treatment of TB.

STRATEGIES TO ERADICATE PERSISTERS FOR IMPROVED TREATMENT

Intermittent drug dosing, which allows persisters to revert to growing forms when antibiotics are removed so that the bacteria become susceptible again, was suggested as a strategy to eliminate persisters by Bigger in 1944 (8). This strategy seems to work for in vitro cultures (8, 14). However, the time period of antibiotic withdrawal is critical for the outcome of the antibiotic to eliminate persisters, as an antibiotic withdrawal period that is too long or too short does not work well (14). This is a potentially interesting strategy to eliminate persisters but has not been rigorously evaluated in clinical settings. This is presumably because the conditions are more complex in patients, where bacteria may grow more slowly and persisters may have been already formed before antibiotics are administered, or “deep” persisters induced by or surviving antibiotics at the end of treatment can take a long time to revive, so that this approach may not work as well in vivo as in vitro. In addition, withdrawal of antibiotics in vivo, unlike in vitro manipulation, may be associated with a more gradual decline of drug concentration, depending on the pharmacokinetics of the drug in question. Besides, the emergence of drug resistance can be a potential risk with intermittent dosing, especially when the bacterial load is high. The use of the hollow-fiber model (25), which can conveniently monitor the increase and decrease of drug concentrations in vivo, could help to address this. Nevertheless, it may be worthy of careful evaluation, since this would be a simple and economic approach to eliminate persisters without resort to persister-targeting new drugs.

The development of new drugs specifically targeting persisters is critically important to achieve the goal of eradicating them and, thus, result in the potential shortening of TB therapy (5, 84). This is exemplified in the remarkable and unique drug pyrazinamide (PZA), whose inclusion in the therapy is critical for shortening the treatment (86), based on a large number of well-conducted clinical trials (26, 52) and animal studies (72, 73). PZA, which is primarily active against persisters, has little activity for growing tubercle bacilli (86). PZA is a prototype persister-targeting drug that depletes membrane energy (87) and inhibits translation in M. tuberculosis (67); both processes are thought to be vital for persister survival under stress conditions. PZA plays a critical and irreplaceable role in shortening TB therapy, since all new drug candidates have to be used with PZA, as removal of PZA from any antituberculosis drug regimen always leads to an inferior therapeutic outcome, especially in terms of disease relapse (35, 72, 73). Almost all current antibiotics are developed based on their activity against growing bacteria, and thus, they have limited activity against nongrowing persisters. However, some antibiotics, such as rifamycins and fluoroquinolones, while primarily having activity against growing tubercle bacilli also have some limited activity against nongrowing persisters. However, since antibiotics, such as rifamycins and fluoroquinolones, while primarily having activity against growing tubercle bacilli also have some limited activity against nongrowing persisters bacilli, and their inclusion in multidrug regimens is associated with a potential shortening of the TB therapy (56).

Rifapentine, when used in enhanced dosing on a daily basis, can cure murine TB in 3 months (61). Currently, a number of clinical trials of the intensification of dosages and/or the frequency of administration of this long-acting rifamycin are ongoing to evaluate whether such an approach can enhance its therapeutic efficacy (38, 82). In the murine model of TB, moxifloxacin-containing regimens demonstrated greatly reduced time to culture conversion (57), and a short duration of such a regimen could produce a stable cure without relapse (58). Based on these findings, the significant sterilizing activity of moxifloxacin, possibly by acting on the bacillary persisters in TB, might enable a shortening of the duration of therapy for drug-susceptible disease. In the early 2000s, a report from India has suggested the potential utility of ofloxacin to shorten the treatment duration for drug-susceptible TB (75). In a study initiated by the Centers for Disease Control and Prevention Tuberculosis Trials Consortium (CDC TBTC), the addition of moxifloxacin to isoniazid, rifampin, and pyrazinamide in the treatment of drug-susceptible TB did not affect the 2-month sputum culture status but did show benefit at earlier time points (10). In a similarly designed study (63), using serial sputum colony counting by nonlinear mixed-effects modeling, the substitution of moxifloxacin for ethambutol appeared superior during the early phase of a biexponential fall in colony counts, but significant and similar rates of acceleration of bacillary elimination during the late phase occurred with both moxifloxacin and gatifloxacin. In another study undertaken in Brazil (15), at 8
weeks, culture conversion to negative had occurred in 80% of patients in the moxifloxacin group, compared with 63% of patients in the ethambutol group (difference of 17.2%; 95% confidence interval, 2.8 to 31.7). The data from these three studies on substituting moxifloxacin for ethambutol allude to the likely sterilizing activity of this fluoroquinolone. A follow-up study by the CDC TBTC (23), however, only showed a nonsignificant increase in sputum culture conversion at week 8 when moxifloxacin was substituted for isoniazid in the standard short-course regimen. This clinical exploration was prompted by the earlier murine TB studies which suggested possible antagonism of the activities of isoniazid and moxifloxacin when given together (57, 58). Recently, a smaller study has also shown that by adding moxifloxacin to the four standard first-line antituberculosis drugs, the time to culture conversion was shortened, and the culture conversion rate after 6 weeks of treatment rose from 61% to 82% (80). A randomized controlled trial known as REMoxTB is now under way to see whether the substitution of moxifloxacin for isoniazid or ethambutol can lead to shortening of the current chemotherapy of drug-susceptible TB to 4 months (http://clinicaltrials.gov/ct2/show/NCT00864383). The OFLOTUB Consortium is also similarly investigating a 4-month regimen based on gatifloxacin.

Among the new antituberculosis drug candidates in clinical studies, the diarylquinoline TMC207, as well as the nitroimidazoles PA-824 and OPC68673, have some activity against persisters (Table 2) and are associated with shortening of the duration of required therapy in the mouse TB model (72). Besides the well-known strong synergy between PZA and TMC207 (2, 35), a recent study has shown that adding clofazimine could further enhance the synergy of PZA and TMC207 in mice (72). Several new early-stage compounds that have activity against TB persisters in vitro are also listed in Table 2. Some of these persister-active compounds have significant problems with bioavailability and toxicity and may only serve as leads for further optimization and mechanism-of-action studies rather than as promising drug candidates themselves.

Various in vitro models have been used for whole-cell-based drug screens to identify promising drug candidates (84). These include the high-throughput-screening-compatible low-oxygen-recovery assay (LORA) system (13), the low-pH/old-bacilli persister screen (11), the low pH/NO screen that identified nitazoxanide (19), and the streptomycin-dependent system (64). However, there are limitations to the in vitro models based on aging, hypoxic, and/or acidic conditions in terms of relevance to in vivo persisters, as shown in recent studies (21, 69), since one model cannot possibly capture all persister populations in vivo (Fig. 1). However, the presence of persisters in sputum presents the opportunity to test this population directly and to determine the conditions necessary to replicate the sputum phenotype in vitro (21, 69). Another approach is to develop inhibitors targeting persister mechanisms (Table 1), such as RelA, PhoY2, and PrcBA (e.g., proteasome inhibitor oxathiazol-2-one (41), energy production (e.g., DlaT inhibitor rhodamine thioxathiazolides (9), and the trans-translation pathway (67). Given the usefulness of the above-described approaches, a potential hurdle in developing new drugs that only kill persisters is the lack of robust animal models to evaluate such activity (31).

While developing persister drugs is important, utilizing and enhancing host immune defense through immunomodulating agents, nutrition, and vaccines (latency therapeutic vaccines) that stimulate innate and adaptive immunity may also play a role in eliminating persisters. The combined use of antituberculosis agents, especially including persister drugs, and the above-described immunotherapeutic approaches could be more effective for eradication of persister bacteria. For example, immunotherapy with the M. tuberculosis Hsp60 DNA vaccine used in combination with antituberculosis chemotherapy has been shown to reduce the incidence of relapse and shorten TB treatment in mice (42). In addition, orally formulated heat-killed Mycobacterium vaccae (V7) is being evaluated in a phase 2 study to improve the efficacy of chemotherapy (http://www.newtbdrugs.org/pipeline.php). More studies are needed to evaluate the immune-based strategy.

Finally, it is possible to “wake up” persisters by altering the environment where they reside, such as by providing certain nutrients or resuscitation factors that trick persisters to revert and start growing so they become susceptible to antibiotics (85). Further studies are needed to assess the different aforementioned strategies for more effective eradication of persisters.

### TABLE 2 Drugs, drug candidates, and compounds that have activity against TB persisters

<table>
<thead>
<tr>
<th>Drug(s) or compound</th>
<th>Target(s)</th>
<th>Activity against persisters</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyrazinamide</td>
<td>Energy production and trans-translation</td>
<td>+++</td>
<td></td>
</tr>
<tr>
<td>Rifampin-rifapentine</td>
<td>RNA polymerase/transcription</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td>Fluoroquinolones</td>
<td>DNA gyrase/DNA synthesis</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>TMC207</td>
<td>FIF0 ATPase/ATP synthesis</td>
<td>+ ++</td>
<td></td>
</tr>
<tr>
<td>Nitroimidazoles</td>
<td>Reactive nitrogen/DNA damage</td>
<td>++/+</td>
<td></td>
</tr>
<tr>
<td>Dithiocarbamates</td>
<td>Unclear/antioxidant and metal chelator</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Niclosamide</td>
<td>Uncoupler/energy production</td>
<td>71</td>
<td></td>
</tr>
<tr>
<td>Hydroxyquinoline</td>
<td>Unclear/antioxidant and metal chelator</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>Oxathiazol-2-one</td>
<td>Proteasome/protein degradation</td>
<td>41</td>
<td></td>
</tr>
<tr>
<td>Rhodanine thioxathiazolines</td>
<td>DlaT/energy production</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Nitzoxanide</td>
<td>Pyruvate-ferrodoxin oxidoreductase/energy production</td>
<td>19</td>
<td></td>
</tr>
</tbody>
</table>

*The activity for killing persisters is expressed by the number of plus signs, with “+++” being the strongest, “++” being moderate, and “+” low or weak activity, based on in vivo activity.*

### FUTURE PERSPECTIVES

Continued study of the mechanisms of mycobacterial persistence is clearly important for developing more effective strategies to combat M. tuberculosis persisters. It is very likely that antituberculosis drugs that inhibit only one persister target...
may have a limited effect on persistence. Developing drugs that inhibit multiple persister targets or pathways/networks using a systems biology approach or synthetic lethality screens may be a more effective way to eliminate persisters. Novel screens targeting persisters, including those in L-forms, in different models may be important to cover the diverse persister populations, as illustrated in Fig. 1. While developing persister drugs is important, antibiotics alone may have a limited effect on eradicating persisters. Utilizing or harnessing host defense mechanisms, including ways to stimulate the immune response to antigens associated with persistence and decrease immune-suppressive mechanisms, and their combined use with antibiotics should be explored.

There may be variations in the levels of persister formation among different bacterial strains (79). For example, it is possible that certain strains of *M. tuberculosis* develop persisters more readily than others and make TB in some clinical cases more difficult to cure or more prone to develop into MDR/XDR TB? Similarly, there may be variations in host genetic susceptibility and immune responses to control persistent infections such that certain individuals may allow persistent or latent infections to develop into active disease and relapse after treatment more readily than others. Identifying such bacterial strains and individuals may help to provide better disease control. Moreover, the recent recognition of lipid body-positive cells and Rpf-dependent cells as potential biomarkers for the extent of the persister population in different patients raises new possibilities to study these host-pathogen interactions further and, possibly, to deploy them to guide therapy (29). Variations in immunity, nutrition, socioeconomic factors, and coinfections such as HIV may also have an impact on the outcome of persistent or latent infections and treatment. Thus, the variability in mycobacterial persistence and host susceptibility and other predisposing factors may lead to heterogeneity in disease expression and severity and to relapse. These factors should be taken into consideration in devising personalized treatment with antibactericidal drugs and immunotherapy tailored for specific individuals in the future.

Can we eradicate persisters? Walsh McDermott’s frustration about persisters that it “shows you can’t win” is still valid today. However, through new systems approaches applied at different levels, including the persister level, host level, and societal and disease control levels, the development and use of new persister drugs and combined use of a repertoire of antibactericidal drugs (i.e., that in the Critical Path to New TB Drug Regimens [CPTR] initiative advocated by TB Alliance, consisting of PZA plus TMC207 or PA-824 as backbone) with other, additional agents that act on different bacterial populations and immunotherapy, as well as novel treatment strategies against persisters, we may be able to better control persisters and shorten the duration of the current 6-month therapy in the years to come.

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We apologize to our colleagues whose work could not be cited due to space limitations.

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