



A roadmap for drug discovery and its translation to small molecule agents in clinical development for tuberculosis treatment

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Summary

Drug discovery and development, from an initial disease treatment concept to a new drug application (NDA), is a complex, lengthy and expensive process. In this review we discuss the key stages of drug discovery and early development, including target identification and validation, assay development and screening, confirmed hits to leads, lead optimization, and progressing development candidates to an investigational new drug (IND) filing. We also provide particular examples of how this process is beginning to assist in the development of small molecule treatments for tuberculosis, by summarizing the status of the clinical development of several newer classes of drugs. These include the fluoroquinolones, oxazolidinones, diarylquinolines, and nitroimidazooxazoles and -oxazines.

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Introduction

Drug discovery and development is a complex and expensive process, requiring many years and resources from an initial disease treatment concept to a new drug application (NDA).¹ The cost of bringing a new drug to market is estimated to be between \$800 million and \$1 billion.^{2,3} The timeline from initial concept to NDA submission on average ranges from 12–15 years depending on the disease area and the treatment approach (Fig. 1). Currently, there is a huge gap between the number of candidate drug compounds in testing and those that finally get approved. Even for those reaching Phase I clinical trials, less than 10% make it to final

approval, although the success rate for anti-infectives is much higher than in other therapeutic classes. To reduce the cost, time, and failure rate in delivering new medications to the marketplace greater effort is being expended today to build in much better predictors of safety and pharmacokinetic (PK) effects at earlier stages of the discovery and exploratory development process.

In this review we present a high-level view of the fundamental concepts and processes of drug discovery and early stage development, and briefly detail some of the unique features of this process toward the development of drugs to treat tuberculosis (TB). We then provide examples of how this process is beginning to be translated into new small molecule treatments for TB by summarizing the status of the clinical development of several newer classes of drugs. Our aim is to emphasize to researchers in TB some of the steps necessary to translate bench-side findings into bedside applications.

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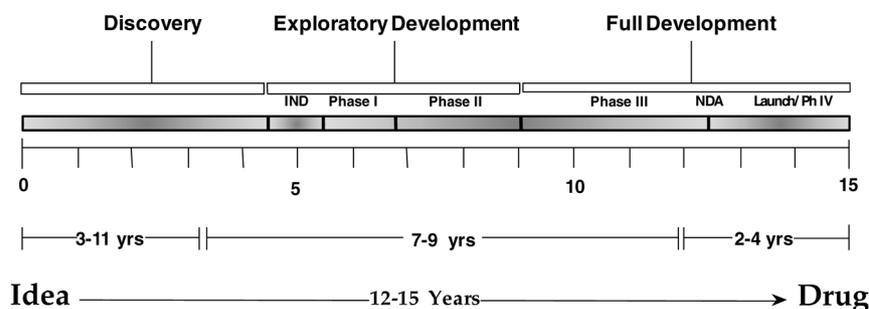


Figure 1 Discovery and Development Process for a New Medicine.

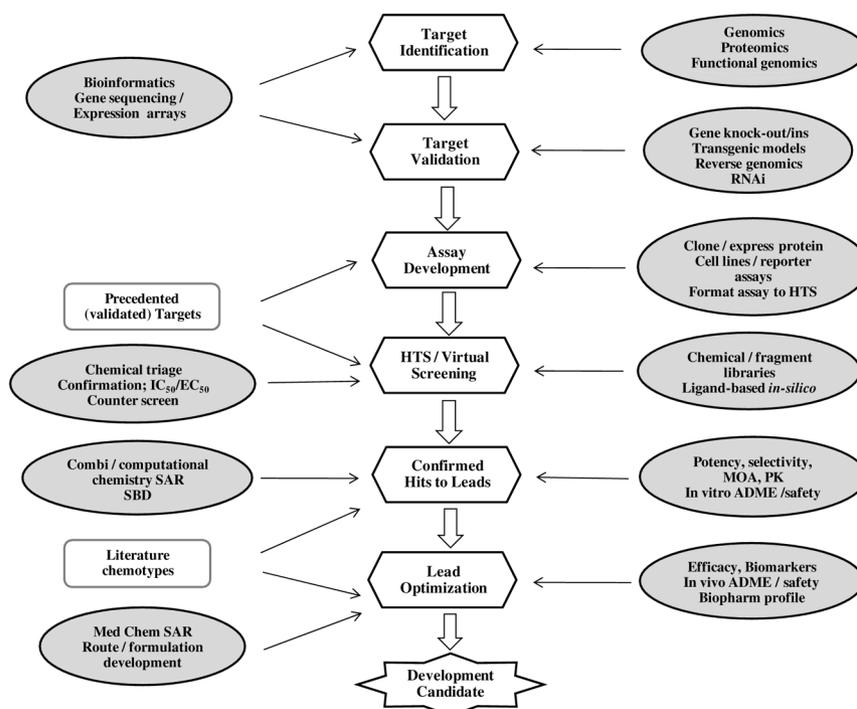


Figure 2 Roadmap to the Discovery of a New Medicine. Hexagons = stages of drug discovery; Ovals = technologies utilized and outcomes; Rectangles = alternate sources of targets and chemotypes.

The drug discovery process

In this section, we detail a roadmap for drug discovery that commences with target identification and culminates in the selection of a development candidate. For the purposes of this review we have arbitrarily divided the process into six major categories (Fig. 2). Within each of these areas, we detail the prominent technologies utilized and summarize the key outcomes. Our objective is to provide the reader with a broad overview of the drug discovery process with extensive citations in the reference section to further details.

Target identification

Most drug discovery today begins with identifying a novel disease target or pursuing a known, highly validated

(precedented) target. The genomics revolution has been the main driver of the target-based approach over the last 10–15 years, providing thousands of human and pathogen genes and their respective gene products. This is inherently a high-risk approach as insight into the normal function of a gene or gene product does not necessarily connect it to a disease. According to a recent review all existing classes of therapeutic drugs together hit 324 different targets.⁴ Another review predicts ~3500 genes in the human genome to be accessible to modulation by high affinity to drug-like small molecules – ~14% of the human genome.⁵

The ability to uncover disease genes has been greatly enhanced by advances in genomics,⁶ proteomics,⁷ and functional genomics. Functional genomics aims to determine disease mechanisms and identify disease genes and markers. This is done by employing the large-scale exploration of gene function, which includes at

the biochemical level the analysis of signaling pathways, regulatory networks, protein-protein interactions, etc. Functional effects are further determined via gene knockouts/gain-of-function studies, and the results of functional complementation of knockouts.⁸ Functional genomics employs high-throughput sequencing and high-density arraying of gene expression and activity of gene products. The information content resulting from these experiments is exceedingly large, which has accelerated the discipline of bioinformatics.

The most common targets for therapeutic intervention can be broadly classified into receptors, proteins and enzymes, DNA, RNA and ribosomal targets. The “druggability” of a given target is defined by the ability of a small molecule drug to fit the biological space and modulate the target. Drug-likeness is defined by a range of molecular properties and descriptors (e.g., permeability, aqueous solubility); those compounds that fit this loose set of criteria must bear some complementarity to a specific biological space or binding site so that high affinity interactions can be effected.⁹ Newer classes of druggable targets, such as G-protein coupled receptors (GPCRs)¹⁰ and protein kinases,¹¹ have now been successfully targeted and a sizable number of recently marketed drugs hit these particular classes.¹² Amongst precedented targets, the bacterial ribosome continues to undergo extensive investigation.¹³

Most existing TB drugs target the processes of cell wall metabolism, DNA replication, or protein synthesis. Many compounds undergoing preclinical and clinical development also target these processes; however, some discovery programs are targeting other essential enzymes (i.e., required for survival) or the bacterial cell membrane.^{14,15} The disclosure¹⁶ of the *Mycobacterium tuberculosis* genome in 1998 and re-annotation thereafter,¹⁷ along with further studies based on transposon mutagenesis¹⁸ and genome-based deletion analysis¹⁹ of clinical strains have influenced the selection of new targets.²⁰ The value of any new target is defined not only in the context of its essentiality for survival in vitro, but also against a variety of properties relevant to the drug discovery process, e.g., selectivity, suitability for structural studies, and ability to monitor inhibition in whole cells. Exploiting the unique cell wall architecture of mycobacteria, because of differences in the lipid content of mammalian and bacterial membrane, presents an attractive approach to target identification. Two reviews outline a number of enzymatic pathways that could form the basis of a drug discovery effort.^{15,21} Another novel multi-disciplinary approach is HTS crystallography that promises to identify and characterize new targets essential for persistent *M. tuberculosis*.²²

Target validation

Target validation requires demonstration that a chosen molecular target is critically involved in a disease process, and that modulation of it is likely to have a therapeutic effect. Validation is usually done first in vitro followed by in vivo experiments in disease-related cell-based models or intact animals. Together these help

to predict a possible therapeutic profile and clinical potential of new drugs in patients.

Target validation is commonly pursued experimentally via the use of knockout,^{23,24} knockin (gain-of-function),²⁵ and transgenic²⁶ models. Knockouts of genes that are essential in development are usually lethal. Over-expression models reactivate gene expression of the target gene, and often ameliorate or even reverse a disease phenotype. Transgenic animals, where a target gene has been knocked out, have become an important approach for the determination of the function of targets (genes) in a whole organism. All these technologies are used to study the effect of modulating or inhibiting the function of a potential drug target. A fundamentally different paradigm today involves using large, diverse chemical libraries in a “reverse genomics” (or “chemical genomics”) approach, to screen for phenotypes generated in cells by exposure to small molecules.²⁷ Such exposure knocks out the function of a gene(s) thus leading to a readable phenotype.

With regard to *M. tuberculosis*, a number of requirements to validate targets prior to initiating a drug discovery program can be highlighted. A matrix system has been presented that ranks targets in terms of confidence in their validity and vulnerability, and may aid in the selection of optimal targets.²⁰

Efficacy in human clinical trials is the ultimate validation of a biological target. Unfortunately, efficacy in animal disease models does not always predict clinical outcomes as the reliability of these models varies widely amongst diseases and must be assessed on a case-by-case basis.

Assay development and high throughput screening (HTS)

The development of an assay that is adaptable to high throughput screening (HTS) is critically important in linking a validated target to druggable chemical matter derived from screening. A reproducible, robust assay must fulfill several criteria, including unequivocal linkage to the target, reliability, practicality, cost, and adaptability to automation (screening of large numbers of compounds in a parallel format). Practically all assays today are against isolated enzymes (lysate or recombinant sources), or engineered cell lines. The latter assays can be quite complex, utilizing reporter gene assays that monitor activation or up-regulation of certain genes or their gene products.^{28,29}

Prior to screening large chemical libraries in an empirical fashion, an assay has to be developed. For isolated proteins, this requires that an expression system be developed or a biological source identified that can provide the quantity of target needed to execute the full HTS. Once a suitable assay with a good signal-to-noise ratio has been developed, it must be formatted for HTS execution. Assays today are commonly run in 1536-well plate format with low μL to high nL volumes per well. To increase speed and efficiency, ultra high-throughput screening (uHTS) utilizing 3456 and higher well formats has been

developed. However, in the last few years a turning point in screening philosophy has occurred with an emphasis on reducing the number of compounds screened in favor of data quality and relevance.³⁰ Hit rates from primary screening of a full compound library typically range between 0.1–5% depending on the cutoff parameters set and the dynamic range of the assay. Most hits have initial potencies of 1–50 μM .

Depending on the quality of the screening library, primary hits may be “triaged” using calculated properties and the input of experienced medicinal chemists to exclude hits likely to have poor ADME (absorption, distribution, metabolism, excretion) properties or which contain structural features that may contribute to toxicity.^{31–33} Steps are then taken to confirm that the primary hits exhibit desired activity. Often hits are retested with the primary assay to eliminate random false positives and in alternative assays to eliminate false positives due to interference with the primary assay signal. Dose-response curves may be generated, where hits are tested at a series of concentrations with results being expressed as IC_{50} or EC_{50} values.

The “confirmed hits” may be further tested in one or more counter screens to garner information on selectivity.³⁴ These assays may include drug targets of the same protein or receptor family, and the results provide valuable information regarding potential for off-target side effects. These secondary screens may also help confirm the mechanism of action (MOA) for a particular active compound. Finally, depending on the source, a hit may not be considered validated until the purity and concentration of the sample is verified; this may involve re-synthesis of the compound or confirmation of structure and integrity of screening material. The ultimate success of screening will depend on the target and assay and the sources, quality and diversity of compound libraries screened.³⁵

Another strategy that is being increasingly utilized to uncover hits, because of lower costs and advances in computing power, is *in silico* screening.³⁶ Powerful beam technologies now permit the elucidation of complex biomacromolecular structures at high resolution. This means that structural information about a given drug target, and the binding conformation of queried structures to that target, are available at earlier stages of drug discovery. *In silico* (virtual) screening can sift through large numbers of compounds based on a user-defined set of selection criteria. More sophisticated algorithms³⁷ provide a three-dimensional description of the interaction of ligands and receptors upon which real or virtual libraries can be computationally docked. Scoring functions are then used to rank compounds that meet selection criteria.³⁸ Thus, *in silico* screening can either reduce the actual number of compounds being screened in a bench-top assay, or enrich a yet-to-be-screened library with compounds that have a better chance of hitting the target.

A recent paper outlines the low effectiveness of HTS screening against enzyme targets in delivering new broad-spectrum clinical antibacterial drugs.³⁹ One of the major challenges is that different bacteria often possess non-homologous genes that code for equivalently

functional proteins. This is less of an issue for agents directed against a single bacterial species, and HTSs vs. *M. tuberculosis* are now commonplace with recent reports of bench-top screens against a number of established^{40,41} and new^{42,43} targets, the discovery of a lead series via virtual HTS,⁴⁴ and the development of whole-cell screens.^{45,46} Goldman et al. also review HTS and other core services available via a consortium that supports preclinical drug discovery and development programs in TB.⁴⁷

Confirmed hits to leads

Once a narrowed list of confirmed hits is in hand, chemical modification is usually required to build in lead-like properties in a “hits-to-leads” campaign. In parallel with the development of high capacity screening technologies, chemists have also developed new approaches involving combinatorial and/or “high-speed” synthesis wherein chemical space is explored around a defined pharmacophore.⁴⁸ Depending on the information available, computational chemistry approaches using the structures of confirmed hits and/or the structure of the target^{49,50} may also be recruited to generate superior leads. In any event, the objective is to generate a structure-activity relationship (SAR) that addresses initial deficiencies of a confirmed hit. These include, but are not limited to, improved potency, aqueous solubility, stability, selectivity vs. relevant related targets. Improved understanding of the kinetics of inhibition may also aid optimization. During this stage, greater evidence of cell-based activity must be demonstrated and some basic safety studies (cardiovascular risk via the dofetilide binding assay⁵¹ and genetic risk via the Ames test⁵²) conducted. These simple *in vitro* assays flag hits at an early stage for two prevalent clinical toxicities. Simple assays on selected confirmed hits (e.g., cassette dosing, stability to liver microsomes, generation of reactive metabolites) to assess potential ADME issues are also conducted.^{53,54} Confirmed hits that pass the above hurdles and are still broadly compliant with the Lipinski “Rule of Five”⁵⁵ are then ready for lead optimization.

Lead optimization

Lead optimization is a complex, non-linear process of refining the chemical structure of a screen lead to improve its drug-like characteristics with the goal of producing a development candidate.⁵⁶ This stage frequently represents the point where a lead falls out in a drug discovery program. The process is highly iterative, using knowledge gained at each prior stage to optimize each new cycle, and is also very chemistry intensive. Through further development of the SAR, medicinal chemists engage all the tools at their disposal to optimize the drug-like properties of a lead series and generate a pharmacological, safety, and biopharmaceutical profile that can lead to choosing one or more candidates for early stage development. The goals of this stage are to:

- a. Further define the pharmacology of the lead series. This includes testing advanced leads in relevant efficacy models that predict for clinical outcomes in humans, characterizing a target or mechanism biomarker that is translatable to humans,⁵⁷ and understanding the in vivo relationship between biomarker response and drug concentration to provide quantitative information for dosing.
- b. Develop a comprehensive ADME package,^{58,59} which includes data on absorption/permeability, mechanisms of clearance, oral bioavailability, a profiling of interaction with cytochrome P-450 isoforms, and plasma protein binding. While many of these studies are conducted in rodents, definitive studies to predict human exposure are usually conducted in higher species such as dogs.
- c. Develop an initial safety profile. This includes the in vitro micronucleus assay,⁶⁰ advanced testing to assess cardiovascular risk (e.g., QT prolongation as assessed by hERG binding⁶¹ and/or in vivo models⁶²), development of safety biomarkers,⁶³ broad ligand profiling,⁶⁴ and an initial in vivo toleration assessment in animal models⁶⁵ to determine appropriate preclinical toxicology species for advanced safety testing.
- d. Develop an initial biopharmaceutical profile⁶⁶ including completing PK and stability studies on a pharmaceutically acceptable formulation that will be compatible with desired delivery options.
- e. Develop a synthetic route that is adaptable to scale-up, lacks safety issues during manufacturing, and is efficient and of low cost.

While the lead optimization process usually begins with a confirmed screen hit, there is an increasing trend toward using a known chemotype with proven drug-like properties as a starting point for SAR.^{67,68} This is due to the high cost of developing lead matter from screening and the statistical probability that a new chemotype will drop out of development due to clinical toxicity.

The lead optimization paradigm for TB drugs is similar to that outlined above. Key to this process is the availability of robust, predictable in vitro assays and in vivo efficacy models. Mouse models have played a key role in developing current tuberculosis agents. However, due to differences in the host immune response to *M. tuberculosis* infection, these models are not expected to reproduce the human disease in all aspects.¹⁴

Early stage development to investigational new drug (IND) filing

Once a lead has been fully optimized for pre-clinical development, additional studies to the point of an investigational new drug (IND) filing are necessary.^{1,69} These entail a significant commitment in terms of money, resources, and time, and include advanced safety and pharmacokinetic/pharmacodynamic (PK/PD) studies toward grooming a compound for Phase I clinical studies in humans. Under FDA requirements, a sponsor must submit data showing that the drug is reasonably safe for use in initial, small-scale clinical studies. Such

data include the toxic and pharmacologic effects of the candidate drug in vitro and in vivo (laboratory animal testing as detailed above in lead optimization). Prior to IND declaration, the FDA will generally ask, at a minimum, that sponsors: (a) develop a pharmacological profile of the drug; (b) determine the acute toxicity of the drug in at least two species of animals, and (c) conduct short-term toxicity studies ranging from two weeks to three months, depending on the proposed duration of use of the drug in the proposed clinical studies. During lead optimization, process chemists start to develop a synthetic route suitable for the manufacture of multi-kilogram quantities of active pharmaceutical ingredient (API) needed for early stage development studies. The FDA requires a Chemistry, Manufacturing and Controls (CMC) documentation package for any drug entering clinical trials.

The culmination of a successful preclinical development program is the filing of an IND application. This generally includes data and information in three broad areas: (a) animal pharmacology and toxicology studies, i.e., preclinical data to demonstrate that the product is reasonably safe for initial testing in humans; (b) manufacturing information, i.e., information pertaining to the composition, manufacture, stability, and controls used for manufacturing the API; and (c) clinical protocols and investigator information, which demonstrate that initial-phase trials will not expose subjects to unnecessary risks.

Issues surrounding the design of clinical protocols for TB drugs have been delineated in two recent reviews.^{14,70} These include identifying a suitable patient population, the duration of trials, the lack of specific diagnostic tools and surrogate markers, regulatory issues, and cost considerations.

Small molecule agents in clinical development as TB drugs

Fluoroquinolones

The use of fluoroquinolones against *M. tuberculosis* began shortly after the discovery of the broad-spectrum antibacterial properties of this class in the early 1980s, when ofloxacin (the racemic form of levofloxacin) was shown to be active in vitro⁷¹ and was evaluated in humans.⁷² To date the newer drug development methods described above have not played a major role in the development of the class, which has advanced largely by more classical chemistry-based methods. A large number of analogs have been prepared and tested experimentally (Table 1), and many have been used clinically in combinations, usually as second-line therapy.⁷³ Levofloxacin, gatifloxacin and moxifloxacin (Fig. 3) are named in the American Thoracic Society guidelines as second-line agents for the treatment of TB, with levofloxacin being the preferred oral agent.⁷⁴ Two excellent recent reviews have covered this subject.^{75,76}

The fluoroquinolones target the bacterial enzymes DNA gyrase (the equivalent of mammalian topoisomerase II) and topoisomerase IV. DNA gyrase is

Table 1 Data for fluoroquinolones against *M. tuberculosis*

Drug	<i>M. tb</i>		<i>M. tb</i> DNA gyrase	
	MIC ($\mu\text{g/mL}$) ^a	Ref	IC ₅₀ ^b ($\mu\text{g/mL}$)	Ref
ofloxacin	0.63–1.25	82	10	86
levofloxacin	0.12–1.0	83	5	74
gatifloxacin	0.03–0.12	83	3	74
moxifloxacin	0.12–0.5	83	4.5	74
sparfloxacin	<0.06–>0.25	84	4.8	86
sitafloxacin	0.06–0.79	85	1.7	86
KRQ-10018	0.05	74		

^aRange of MIC values across sensitive and resistant strains of *M. tuberculosis*.

^bIC₅₀ for DNA supercoiling.

essential for the replication and transcription of bacterial DNA, and exists as a tetramer of two subunits; GyrA and GyrB, together with a C-terminal tail. Gyr A contains the active sites that break and resealed the DNA; the DNA binding domain and the tyrosine residues that temporarily link to the cut DNA phosphates. The Gyr B subunit contains the ATPase active site, and is involved in energy transduction via ATP hydrolysis.⁷⁷ Similarly to the MOA of inhibitors of mammalian topoisomerase II, the fluoroquinolones have little affinity for the DNA gyrase alone,⁷⁸ but form a triple drug/DNA/enzyme complex which inhibits the DNA religation activity of the enzyme, generating a “cleavable complex” that results in lethal DNA double-strand breaks.^{75,79} Unlike most bacteria, *M. tuberculosis* does not possess topoisomerase IV,⁸⁰ leaving DNA gyrase as the sole target of the fluoroquinolones. The *M. tuberculosis* DNA gyrase enzyme has been reviewed in detail recently.⁷⁵ Resistance to fluoroquinolones generally involves mutations in either or both of GyrA and GyrB.^{81,82}

Several fluoroquinolones have been evaluated clinically for TB; this review will focus only on the most recent; the “third-generation” agents levofloxacin, gatifloxacin and sparfloxacin, and the “fourth-generation” agents sitafloxacin, moxifloxacin and the related quinolizinone KRQ-10018 (Table 1, Fig. 3). Major research concerns are the exploration of fluoroquinolones that are active in drug-resistant strains, and in drug combinations that shorten treatment time.

Levofloxacin. The S-enantiomer of ofloxacin, levofloxacin was launched in the Japanese market by Daiichi Pharmaceutical as an antibacterial in 1993, and has been used for second-line treatment of TB. A recent clinical study showed that levofloxacin (1000 mg/day for 7 days) had early bactericidal activity similar to that of isoniazid, and superior to moxifloxacin or gatifloxacin, in pulmonary TB.⁸⁸

Gatifloxacin. This “fourth-generation” fluoroquinolone was launched by Bristol-Myers Squibb in 1999, following its licensing from Kyorin Pharmaceutical. It was withdrawn from use as a general antibacterial agent in the US in 2006, following reports of dysglycemia in some diabetic patients.⁷⁰ However, it is one of the more widely-studied fluoroquinolones for *M. tuberculosis*, and is currently in multicenter clinical trials, being

evaluated as an addition to the ‘standard regimen’ (isoniazid, rifampicin and pyrazinamide), to see if this can shorten the duration of treatment.⁸⁹ A Phase III trial of gatifloxacin in combination with isoniazid, rifampin and pyrazinamide for 4-months, with follow-up treatment with rifampin and isoniazid, is in progress.⁹⁰

Moxifloxacin. This was developed by Bayer AG, and launched in the US in 1999. Multi-center trials are currently evaluating the addition of moxifloxacin instead of isoniazid in combination therapy with rifampicin and pyrazinamide, seeking a shorter dosing regime.⁸⁹ In a mouse model, high-dose (300 mg/kg) gatifloxacin plus rifampin (10 mg/kg) showed excellent activity (only 0.22 cfu/lung after 20 weeks), suggesting that gatifloxacin is superior to earlier analogs such as levofloxacin.⁸⁹ A Phase III trial of moxifloxacin-based therapy is planned to test whether substituting moxifloxacin for either isoniazid or ethambutol over a 4-month period can safely and efficaciously shorten the standard treatment time by 2 months. The study will enroll up to 1,500 patients.⁷⁰

Sparfloxacin. In a comparative study with moxifloxacin and ofloxacin, sparfloxacin was the most potent agent, with an MIC of 0.1 compared to 0.5 $\mu\text{g/mL}$). However, all three compounds showed comparable activity (1–2 $\mu\text{g/mL}$) in a macrophage model. In a murine aerosol infection model, moxifloxacin was the most efficacious (3.0 log CFU/lung reduction), followed by sparfloxacin and ofloxacin (1.5 log CFU/lung reduction). The ratio of the AUC to the MIC was the pharmacodynamic parameter that best described the in vivo efficacy.⁹¹

Sitafloxacin. This is being developed by Daiichi Pharmaceutical, and is one of the more potent fluoroquinolones. In a comparison of sitafloxacin, gatifloxacin and levofloxacin, MICs were determined as 0.06, 1.3 and 0.25 $\mu\text{g/mL}$ respectively.⁹² Activity against *M. tuberculosis* replication in human macrophages correlated with the MICs, and the intracellular uptake of these quinolones by MM6 macrophages and A-549 cells was also in the order sitafloxacin > gatifloxacin > levofloxacin. This suggests that the cellular permeability of these quinolones is an important factor that determines their efficacy to eliminate intracellular *M. tuberculosis* organisms.⁹²

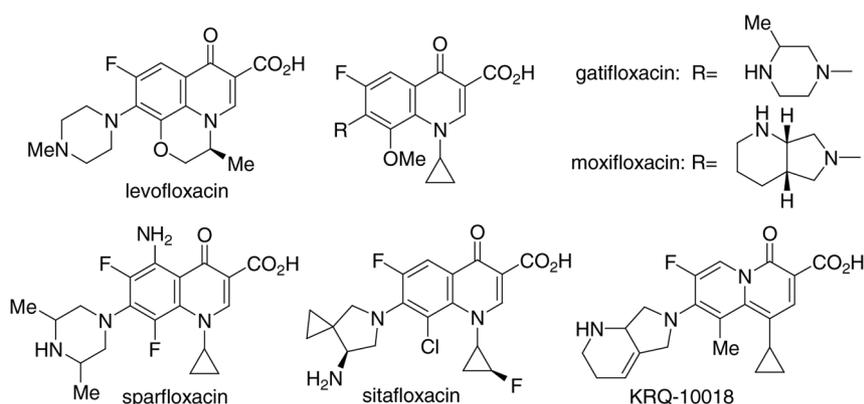


Figure 3 Third- and fourth-generation fluoroquinolones used for tuberculosis.

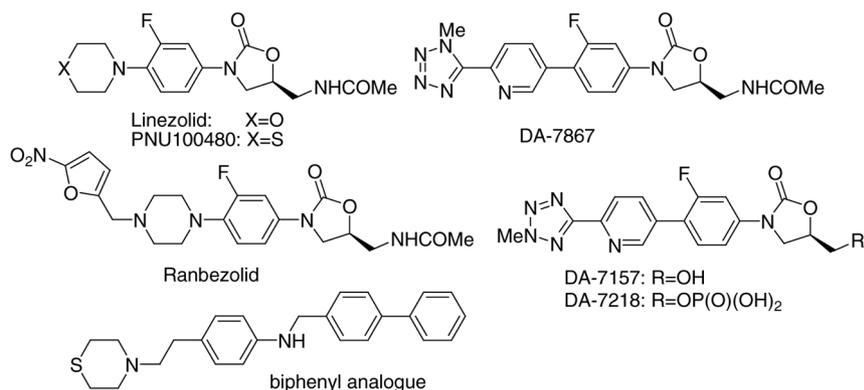


Figure 4 Oxazolidinones investigated for the treatment of tuberculosis.

KRQ-10018. This experimental fluoroquinolizinone is the result of collaboration between the Korea Research Institute of Chemical Technology and the Global Alliance for TB to develop a quinolone that is optimized specifically for TB treatment.⁷⁵ Criteria are long-term safety, ability to shorten therapy duration, and suitability for treating TB/HIV co-infections. Initial studies suggest that KRQ-10018 is more potent than moxifloxacin against replicating *M. tuberculosis* (free and grown intracellularly in macrophages), similar against the non-replicating state, and somewhat less active in an in vivo efficacy mouse model. Other analogs in the class are under study.

Oxazolidinones

Oxazolidinones are a class of broad-spectrum antibacterial agents with a novel MOA, which were originally developed for this utility by classical compound library screening methods, and whose activity extends to mycobacteria.⁹³ These compounds inhibit an early step in the initiation phase of protein synthesis, binding to the 50S subunit of the ribosome.⁹⁴ The lead drug of this class, linezolid (Pfizer), was approved by the FDA in 2000 for use as an antibacterial. Experimental studies show it has good in vitro activity (MICs <1 µg/mL) against a wide

variety of strains of *M. tuberculosis*, including those resistant to most of the widely-used anti-tuberculosis drugs⁹⁵ (Fig. 4). A thiomorpholine analog (PNU-100480) was considerably more potent in vitro against both wild-type (MIC <0.125 µg/mL) and drug-resistant strains (MIC <0.5 µg/mL),⁹⁶ being comparable to that of isoniazid in a mouse model of *M. tuberculosis* infection.⁹⁷ DA-7867 (Dong-A Pharmaceuticals) is one of a series of triaryl oxazolidinones, and shows an MIC of 0.1 µg/mL against resistant strains of *M. tuberculosis*.⁹³ A related compound is Ranbezolid (Ranbaxy), which showed modest in vitro activity against both wild-type (average MIC ~2 µg/mL) and drug-resistant (average MIC 4 µg/mL) isolates.⁹³ This drug is reported as being a clinical candidate.⁹⁸

However, the acetamidomethyloxazolidinone moiety does not seem to be mandatory, as was once thought,⁹⁹ since the biphenyl analog (Fig. 4) is also active against *M. tuberculosis* in vitro (MIC 0.5 µg/mL).¹⁰⁰ More recently, the alcohol DA-7157 was reported to have MICs of ~0.25 and ~0.5 µg/mL respectively against wild-type and drug-resistant clinical isolates of *M. tuberculosis*.¹⁰¹ Since the insolubility of DA-7157 (0.003 µg/mL at pH 7) was a limitation, the much more soluble (150 µg/mL at pH 7) phosphate prodrug DA-7218 was developed. A detailed pharmacokinetic study of DA-7218 showed that it is rapidly converted to DA-7157 by phosphates in rat blood, with complete conversion inside 24 h.¹⁰²

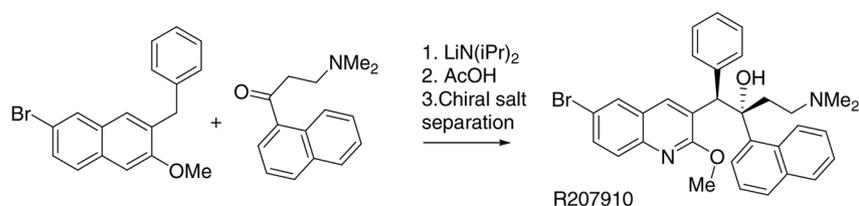


Figure 5 Synthesis of R207910.

Diarylquinolines (R207910 and congeners)

The diarylquinolines, exemplified¹⁰³ by R207910 (Fig. 5), are an exciting new class of compounds with a novel MOA, defined by modern genomic and structural techniques. In vitro studies showed potent activity (MICs from 0.030-0.120 $\mu\text{g}/\text{mL}$) against *M. tuberculosis* H37Rv, including a variety of both antibiotic-susceptible strains, and strains resistant to the standard agents isoniazid, rifampin, streptomycin, ethambutol, pyrazinamide, and moxifloxacin.¹⁰³ Mutant strains of *M. tuberculosis* generated by treatment with R207910 remained sensitive to the major clinical drugs, suggesting a unique target. Genomic analysis of the resistant strains showed consistent mutations in the gene encoding *atpE*, suggesting that R207910 inhibits subunit c (the proton pump) of *M. tuberculosis* ATP synthase,¹⁰³ later confirmed by biochemical and binding assays.¹⁰⁴ Studies¹⁰⁵ on a larger series of mutants found that the most common mutations conferring resistance to R207910 (Ala63Pro and Ile66Met) occur close to Glu61, the carboxyl side chain of which is involved in the proton transfer step required for the creation of ATP. These mutations likely prevent interaction between R207910 and the α -helical c subunits. Studies of the genetic diversity of *atpE* in mycobacterial species showed that the region is highly conserved, except in *M. xenopi* (which is resistant to R207910), where Ala63 is replaced by Met.¹⁰³

An efficient synthesis of R207910 has been reported,¹⁰⁶ involving lithium diisopropylamide-induced condensation of 1-(3-dimethylamino)propionaphthone and 3-benzyl-6-bromo-2-methoxyquinoline, followed by chiral resolution of the correct diastereoisomer by crystallization of a cyclic binaphthyl phosphate salt (Fig. 5).

In studies against established infections in mice (at a bacterial load of 5.94 log units), a single dose of R207910 (25 mg/kg) was at least as active as the triple combination of rifampin, isoniazid and pyrazinamide (Table 2). This result was attributed to the combination of long plasma half-life, efficient tissue penetration, and long tissue half-life (50–60 h).¹⁰³

Evaluation of R207910 in combination with various clinical drugs in mice infected with multidrug-resistant *M. tuberculosis* H37Rv showed that its inclusion provided significantly more active regimens.¹⁰⁷ Thus, CFU counts in spleen and lungs after 2 months were lower when R207910 was added to either of the recommended first-line or second-line therapies (Table 3).

A more recent study¹⁰⁸ assessing 2- and 3-drug combinations of R207910 with isoniazid, rifampin, pyrazinamide or moxifloxacin showed a synergistic interaction

Table 2 Activity of R207910 against *M. tuberculosis* in mice

Dose (mg/kg)	Mean CFU (log10)	
	spleen	lungs
Control (0)	5.77	5.19
12.5	1.91	2.05
25	2.17	1.24
50	1.44	1.07

Data from ref. 103.

Treatment began on day 1 and was administered 5 times weekly for 4 weeks.

Table 3 Comparison of recommended first- and second-line treatment regimens with and without R207910

Regimen	Mean no. of CFU			
	Spleen		Lungs	
	1 mo	2 mo	1 mo	2 mo
Untreated	6.5		5.9	
J	2.6	1.2	2.9	0.2
RHZ	4.5	1.9	3.7	1.0
RHZJ	1.9	0.1	1.8	0
AETMZ	3.2	1.6	2.9	0.1
AETMZJ	1.2	0	0.5	0

Data from ref 107.

J=R207910 (25 mg/kg); M=moxifloxacin (100 mg/kg); R=rifampin (10 mg/kg); H=isoniazid (25 mg/kg); Z=pyrazinamide (150 mg/kg); A=amikacin (150 mg/kg); Et=ethionamide (50 mg/kg).

between R207910 and pyrazinamide (Table 4). The authors concluded that 3-drug combinations containing these two agents have the potential to significantly shorten the treatment duration in patients.

Evaluation of R207910 against several isolates of *M. ulcerans* (the causative agent of the widespread disease Buruli ulcer) showed that, with an MIC₅₀ of 0.006 $\mu\text{g}/\text{mL}$, it was more potent than moxifloxacin, streptomycin, rifampin, amikacin, linezolid, or PA-824, and was active as monotherapy against mouse models of the disease.¹⁰⁹ The drug also showed bactericidal activity against *M. leprae* (in the mouse footpad assay) equivalent to that of rifapentine, rifampin, or moxifloxacin, suggesting that it may also have a role in the treatment of leprosy.¹¹⁰

Table 4 Comparison of three-drug regimens containing R207910

Regimen	Mean lung CFU		
	Day 0	1 mo	2 mo
Untreated	7.2		
J		4.1	2.3
JZ		1.6	0
JZM		1.4	0.03
JZR		2.3	0.07
JZH		1.7	0.18

Data from ref 108.

Abbreviations as for Table 3.

R207910 (as TMC207) is currently in Phase IIA trials for the treatment of active TB, which will compare the effectiveness of the drug in separate combinations with isoniazid, pyrazinamide and rifampin, and together with isoniazid and pyrazinamide.¹¹¹

Nitroimidazo-oxazoles and -oxazines

OPC-67683. While nitroaromatic, and especially nitroimidazoles, have long been of interest as potential antibacterial agents,¹¹² their serious use for tuberculosis began with the initial discovery, by classical compound screening,^{113,114} of the high activity of the 5-nitro-2,3-dihydro[2,1-*b*]imidazooxazoles such as CGI-17341. This compound showed potent in vitro inhibition of *M. tuberculosis* (MIC 0.06 µg/mL), and was active in mouse models of the disease, with an ED₅₀ of 7.7 mg/kg for protection when given on day 12 post-infection with $\sim 4 \times 10^5$ CFU/lung.¹¹⁴ The drug also showed a dose-response for mean survival time, with 20-, 40- and 80 mg/kg doses giving average survival times of 31, 44 and 61 days respectively, compared with 24 days for untreated controls,¹¹⁴ but was mutagenic. Further development of the series showed that larger, lipophilic side chains diminished the mutagenic effects, resulting in development of the series by Otsuka Pharmaceuticals.¹¹⁵ SAR studies showed that the *R*-benzyloxy analog had excellent in vitro potency (MIC 0.05 µg/mL), much better than the corresponding *S*-benzyloxy analog (MIC 3.13 µg/mL) (Fig. 6).

A search for more soluble compounds in the *R*-series led eventually to the piperido analog OPC-67683, which was selected as a clinical candidate.^{115,116} This has been synthesized by the route shown¹¹⁵ in Fig. 7. Coupling of the protected 4-bromophenol and the phenoxy-piperidine under Buchwald conditions, followed by deblocking of the phenol and reaction with the nitroimidazole epoxide resulted in opening of the epoxide and intramolecular cyclization to give OPC-67683 in moderate yield.

OPC-67683 shows extremely potent in vitro inhibition of both wild-type and drug-resistant strains of *M. tuberculosis* (MIC 0.006 µg/mL). Oral dosing (daily for 28 days) in a mouse model infected with 4 log CFU of *M. tuber-*

culosis at 0.5 and 10 mg/kg resulted in 2.5- and >4.4-log reductions in CFU respectively.¹¹⁵ A combination of OPC-67683 (2.5 mg/kg) with rifampin and pyrazinamide was clearly superior to the standard therapy of rifampin/isoniazid/pyrazinamide/ethambutol.¹¹⁶ OPC-67683 was non-mutagenic in the *S. typhimurium* reversion mutation test, and did not affect the activity of liver CYP enzymes. There was little metabolism in both animal and human liver microsomes, but incubation with *M. bovis* BCG resulted in formation of the des-nitro compound. OPC-67683 is in early Phase II clinical trials.¹¹⁶ **PA-824.** Similar development of the 2-nitroimidazo[2,1-*b*]oxazines, where a six-membered rather than five membered ring is fused to the nitroimidazole showed that lipophilic side chains were desirable to lower mutagenicity, but in this case there was a considerable difference between the enantiomers, with the *S* being much more active than the *R*.^{113,117} Data have been reported in the literature for only a limited number of analogs, with much of that being against *M. bovis*, but the broad SAR for the side chain appears to be similar to that for the oxazoles. There was a greater variety of linkers studied, with urea, carbamate, and carbonate linkers all providing compounds of high in vitro potency.¹¹³ The clinical candidate PA-824 (Fig. 8) was selected on the grounds of favorable in vitro, in vivo and mutagenic profiles.¹¹⁸ It proved active against both replicating and static *M. tuberculosis*, suggesting a novel mechanism, and was converted to more polar metabolites only by susceptible strains, suggesting a bioreductive activation step.¹¹⁸

The MOA of PA-824 has yet to be fully defined, but genetic studies suggested the involvement of a protein similar to the F420-dependent glucose-6-phosphate dehydrogenase of *M. smegmatis*. A later paper¹¹⁹ confirmed that while resistance to both CGI-17341 and PA-824 is caused by loss of either a bacterial glucose-6-phosphate dehydrogenase (FGD1) or its deazaflavin cofactor (F420), some bacterial mutants that were wild-type for both FGD1 and F420 were still resistant to PA-824 but not to CGI-17341. Sequencing of these showed changes in an unknown gene product (Rv3547), with complementation with this restoring sensitivity to PA-824. Thus, very subtle changes in drug structure (oxazole vs. oxazine) may have significant effects on the activity profile.

A synthesis of PA-824 has been reported¹¹⁷ (Fig. 8). The product from reaction of 2,4-dinitroimidazole with protected (*S*)-oxiran-2-ylmethanol, followed by protection of the secondary alcohol as a pyranil ether, was cyclized and deprotected to give the nitroimidazo-oxazoline alcohol. This was coupled with 1-(bromomethyl)-4-(trifluoromethoxy)benzene to give PA-824 in good overall yield.

Both pulse radiolysis and scanning electrical microscopy studies of the reduction of the nitro group of PA-824 give comparable values for the dimerization of the subsequent nitro radical anion species; $k_{2,dim}$ of 2.15 and $2.58 \times 10^4 \text{ M}^{-1}\text{sec}^{-1}$, respectively, in an aprotic solvent.¹²⁰

PA-824 showed potent bactericidal activity against both susceptible and multidrug-resistant *M. tuberculosis*, with MICs from 0.015-0.3 µg/mL. Daily oral

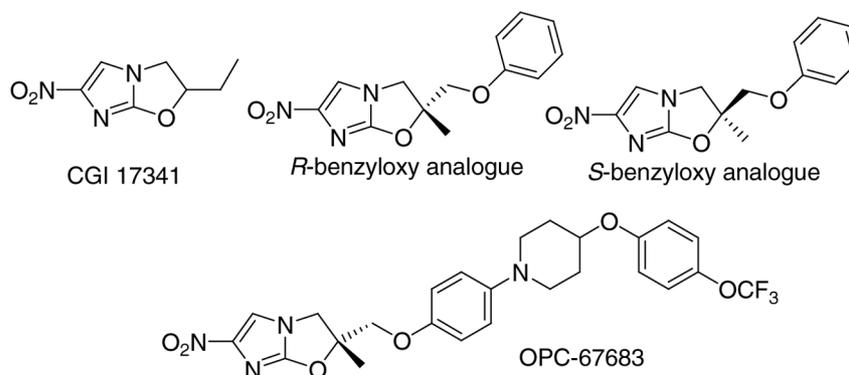


Figure 6 Nitroimidazoles used for the treatment of tuberculosis.

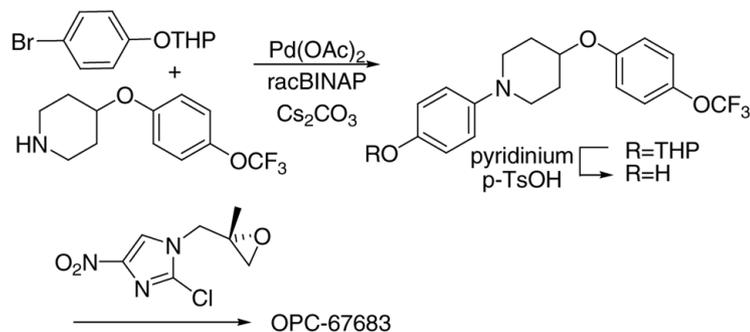


Figure 7 Synthesis of OPC-67683.

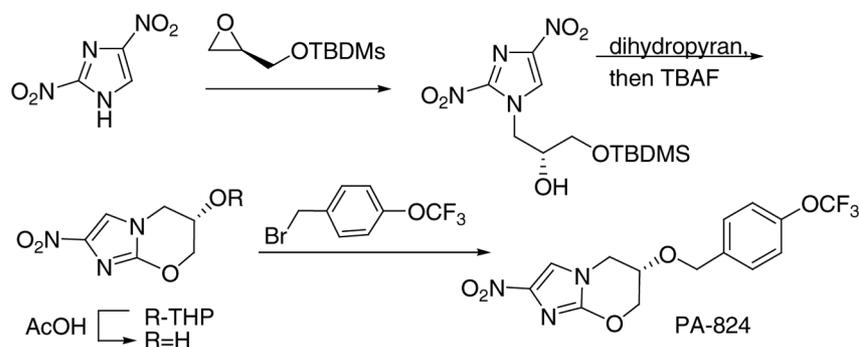


Figure 8 Synthesis of PA-824.

treatment of BALB/c mice infected intravenously with $\sim 10^6$ CFU of *M. tuberculosis* strain H37Rv with PA-824 (50 mg/kg) showed a day 60 CFU reduction of 4.6 logs, compared to a 5.6 log reduction with isoniazid (25 mg/kg).¹¹⁸ In further combination studies in mice,¹²¹ a half-life of 11–12 h was determined, and PA-824 alone was shown to have significant activity. Combination therapy using PA-824, rifampin and pyrazinamide was more effective than the standard regimen of rifampin, isoniazid, and pyrazinamide in lowering CFU counts, but did not significantly change the proportion of mice relapsing after completing 6 months of treatment (Table 5). Substitution of PA-824 for either rifampin or pyrazinamide within the standard regimen was

detrimental, suggesting that PA-824 does not appear to have the sterilizing activity of these agents.

Three Phase I clinical trials of PA-824 have been completed to date; these suggest a drug half-life of about 18 h, and a maximum tolerated dose (in a 7-day multi-dose trial) of about 1000 mg/dose. Other Phase I trials are ongoing.⁷⁰

Concluding remarks

The advancement of compounds from discovery through development is a complex, lengthy, and costly process. In addition to the broad issues normally encountered in

Table 5 Effectiveness of PA-824 in combination regimens

Drug	time	lung CFU (log)	% relapse at 9 mo
control (none)	1 day	3.77	
control (none)	18 day	8.23	
PA-824	2 mo	6.28	
RHZ	2 mo	3.69	
RHZ ^a	6 mo	0	0/46 (0)
PA-824/RHZ	2 mo	3.73	
PA-824/RHZ ^a	6 mo	~ 0	1/19 (5)
PA-824/RZ	2 mo	2.38	
PA-824/RZ ^a	6 mo	~ 0	2/19 (11)
PA-824/HZ ^a	6 mo	~ 2	
PA-824/RH ^a	6 mo		15/19 (79)

Data from ref 121.

^a2 months induction therapy as noted, followed by 4 months maintenance therapy (removal of pyrazinamide).

Dosing: PA-824 (100 mg/kg); R=rifampin (10 mg/kg); H=isoniazid (25 mg/kg); Z=pyrazinamide (150 mg/kg).

other therapeutic areas, researchers seeking improved therapies for TB face a number of daunting challenges related to the discovery stages. These include early screens to uncover agents that will be equally effective for drug-sensitive and drug-resistant strains of TB, as well as addressing persistent forms of *M. tuberculosis* and incorporating assays that flag undesirable safety effects such as drug-drug interactions early on.

To bring into development new TB drugs that offer improvements over current therapies, a broad portfolio of discovery approaches is needed, including an expanded use of modern target-based methods. Exploration of novel targets and MOAs, continued work on well validated targets such as InhA and RNA polymerase, and the utilization of modern drug discovery technologies to uncover both novel and precedented chemotypes will improve the odds of bringing superior small molecule agents into clinical trials. Target-based HTS screening will assist in the latter by allowing much more rapid screening than does organism-based assays.

With respect to new agents currently in trials, the fluoroquinolones represent a well-understood class of compounds acting against a validated target in bacteria (DNA gyrase). They are proving useful against *M. tuberculosis*, but the results do not transfer straightforwardly from other antibacterial indications, emphasizing the marked differences of mycobacteria, and the differences in the host immune response to *M. tuberculosis* infection. The oxazolidinones act on a different bacterial target, binding to the 50S subunit of the ribosome and inhibiting the initiation phase of protein synthesis. Their general antibacterial activity appears to have translated well to *M. tuberculosis*, with compounds likely to be active against a wide variety of resistant strains. The diarylquinolines represent a class aimed at a novel target, the proton pump of *M.*

tuberculosis ATP synthase. While less well-validated as a target, its novelty is exciting and the current trials comparing the effectiveness of TMC207 in combinations with standard TB drugs are being watched with great interest. Finally, the nitroimidazo compounds OPC-67683 and PA-824 represent a new class of drugs of great interest, active against both active and dormant disease and thus with the possibility of significantly shortening treatment, but whose target has not yet been fully elucidated and which may even be different in active and dormant disease. Collectively, this adds up to a promising pipeline of new agents for treating *M. tuberculosis*.

Attaining a robust, steady state to meet the need for novel, optimized drug treatments will require growth in the number of new products in the TB drug development pipeline. However, due to the special challenges of TB drug discovery and development described above, a broad consortium of stakeholders, including pharmaceutical and biotechnology companies, private and government funding agencies, and specialized academic groups will be needed. Only through these partnerships can a meaningful impact be made towards eradicating a large unmet medical need. This now appears achievable.

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