Discovery and development of SQ109: a new antitubercular drug with a novel mechanism of action

Katherine A Sacksteder[1], **Marina Protopopova**[1], **Clifton E Barry 3rd**[2], **Koen Andries**[3], and **Carol A Nacy**[1,*]

[1]Sequella, Inc., Rockville, MD, USA

[2]Tuberculosis Research Section, National Institute of Allergy & Infectious Diseases, National Institutes of Health, Bethesda, MD, USA

[3]Antimicrobial Research, Janssen Infectious Diseases, Beerse, Belgium

Abstract

Existing drugs have limited efficacy against the rising threat of drug-resistant TB, have significant side effects, and must be given in combinations of four to six drugs for at least 6 months for drug-sensitive TB and up to 24 months for drug-resistant TB. The long treatment duration has led to increased patient noncompliance with therapy. This, in turn, drives the development of additional drug resistance in a spiral that has resulted in some forms of TB being currently untreatable by existing drugs. New antitubercular drugs in development, particularly those with mechanisms of action that are different from existing first- and second-line TB drugs, are anticipated to be effective against both drug-sensitive and drug-resistant TB. SQ109 is a new TB drug candidate with a novel mechanism of action that was safe and well tolerated in Phase I and early Phase II clinical trials. We describe herein the identification, development and characterization of SQ109 as a promising new antitubercular drug.

Keywords

antitubercular agent; clinical trial; drug discovery; drug resistance; MDR-TB; pharmacology; tuberculosis; XDR-TB

TB is a global health crisis

TB is a systemic disease with typical pulmonary manifestations caused by exposure to *Mycobacterium tuberculosis* (Mtb), which is transmitted from person to person by airborne bacteria. Approximately a third of the world's population is currently infected with Mtb (Figure 1). Only 10% of people infected with the bacterium will develop active pulmonary disease in their lifetime, although people coinfected with HIV or suffering from diabetes, rheumatoid arthritis or other pathologies treated with certain drugs [1,2] have a much higher incidence of disease than the general population. TB is the cause of the largest number of

human deaths attributable to a single etiologic agent: of approximately 8.8 million incident cases each year, nearly 1.5 million people die [101]. Figure 1 shows the distribution of TB around the world: no country is exempt, but the highest incidence rates are in Asia, parts of South America and much of Africa as a result of the burgeoning HIV epidemic.

Treatment of TB is a logistical nightmare with existing drugs: the recommended therapeutic regimen for drug-sensitive (DS)-TB involves four different first-line drugs: isoniazid (INH), rifampicin (RIF), pyrazinamide (PZA) and ethambutol (EMB). The complete four-drug regimen is administered for 2 months, and then both INH and RIF are administered for an additional 4 (WHO) to 6 (CDC) months. These drugs were all discovered more than 40 years ago and have significant safety concerns, including liver, ocular, skin and peripheral nerve toxicities, influenza-like symptoms, rash, headaches and gastrointestinal and neurological complications. These side effects contribute to poor compliance with the standard drug regimens, and outside of clinical trials, such compliance rates are poor, generally between 60 and 80% [3], although some studies demonstrated compliance rates as high as 90% [4,5].

Patients who take the medications erratically can continue to transmit their infection to others for extended periods [6–8]. More importantly, noncompliance with TB treatments can influence development of multidrug-resistant (MDR)-TB, TB that is resistant to INH and RIF [9]. The WHO currently estimates that there are 650,000 prevalent cases of MDR-TB [101]. MDR-TB is even more difficult to treat than DS-TB, with regimens lasting up to 24 months. Even with this long treatment with multiple drugs, a recent systematic review suggested that, worldwide, only 62% of patients treated for MDR-TB were cured or completed treatment [10]. There are now many reports of extensively drug-resistant (XDR)-TB, TB that is resistant not only to INH and RIF, but also to the fluoroquinolones and at least one of the second-line injectable agents, capreomycin (CM), kanamycin (KAN) or amikacin (AMK) [11]. XDR-TB is significantly more expensive to treat and has even poorer outcomes than MDR-TB [12,13]. HIV coinfection worsens these already dismal outcomes [14–16], underscoring the need for a new treatment regimen that could be used in both HIV-negative and HIV-positive patients with DS- and drug-resistant (DR)-TB.

Several new TB drug candidates are in clinical development

After a long hiatus during which no new TB drugs were discovered, pharmaceutical companies restarted programs to develop drugs for both DS-TB and MDR-TB in the 1990s. There are now promising novel, second-generation and repurposed TB drug candidates in clinical trials (see recent reviews [17–21]). Table 1 outlines the TB drug candidates currently in clinical development.

SQ109 (Sequella) and bedaquiline (TMC207; Janssen Infectious Diseases/TB Alliance) are two drug candidates with new and distinct mechanisms of action (MOA). Bedaquiline is a diarylquinoline that targets ATP synthase. It has been tested in a 7-day early bactericidal activity (EBA) study [22] and a Phase II clinical trial for the treatment of MDR-TB [23]. SQ109 is a 1,2-ethylenediamine that targets MmpL3 in Mtb [24,25]. MmpL3 is a mycolic acid transporter required for incorporation of mycolic acid into the Mtb cell wall. SQ109 is in Phase II clinical trials for DS-TB and is the subject of this review.

Repurposed TB drug candidates include linezolid, an oxazolidinone antibiotic for treating a variety of serious Gram-positive infections [26] and used off-label to treat MDR- and XDR-TB with some success [27–30]. An EBA study showed minimal killing activity [31], but additional clinical trials to investigate its potential in XDR-TB are ongoing [102]. Metronidazole is a nitroimidazole that has been used to treat certain anaerobic infections for more than 45 years [32]. As Mtb are found in low-oxygen granulomas, investigators thought

it might be useful in the eradication of dormant Mtb [33,34]. It is not effective in mouse models of latent TB [33,35–37], but mice do not form granulomas. The National Institute of Allergy and Infectious Diseases (NIAID) at the NIH is sponsoring a Phase II clinical trial to investigate the efficacy of metronidazole to treat MDR-TB [103]. Finally, the fluoroquinolones ofloxacin and levofloxacin are approved for severe bacterial infections, and are also commonly used to treat MDR-TB [17]. The newer fluoroquinolones, gatifloxacin and moxifloxacin (MXF), have activity comparable to INH in mouse models [38], and both are in clinical evaluation for the treatment of TB.

Four TB drug candidates are from known drug classes, but have novel structures optimized for activity against Mtb. Delamanid (Otsuka) and PA-824 (TB Alliance) are novel nitroimidazoles. Delamanid showed activity in an EBA study [39] and is currently being evaluated in a Phase III clinical trial for the treatment of MDR-TB. PA-824 also showed activity in an EBA study [40] and is being evaluated in a Phase II study in combination with MXF and PZA for the treatment of DS- and MDR-TB. Sutezolid (PNU-100480: Pfizer) and AZD5847 (AstraZeneca) are both oxazolidinones, with MOA similar to linezolid. Sutezolid was identified contemporaneously with linezolid, and was noted to have better antimycobacterial activity both *in vitro* and in a mouse model of TB [41–43]. Sutezolid has an improved safety profile compared with linezolid [44] and shows better time-dependent killing in an *ex vivo* whole-blood culture test [45]. A Phase II EBA study is ongoing. AstraZeneca completed Phase I clinical trials for AZD5847; a Phase II EBA study is planned but is not yet recruiting.

SQ109 identified by a multistep filtering process

In January 1999, Sequella and the Laboratory of Host Defenses, NIAID, NIH, signed a Collaborative Research and Development Agreement to begin a combinatorial chemistry program to discover a new TB drug. We synthesized and screened a 63,238-member chemical library designed around the active 1,2-ethylenediamine pharmacophore found in EMB, one of the first-line anti-TB drugs. We focused on EMB for several reasons: the lack of combinatorial chemistry and high-throughput screens (HTS) in the 1960s prevented the developers of EMB from a complete evaluation of the structure–activity relationship (SAR) around the pharmacophore [46]; the early clinical trials of EMB at 25 mg/kg showed clear and convincing signs of therapeutic efficacy, even when delivered as monotherapy in patients with severe disease [47,48], but ultimately this dose had to be reduced due to ocular toxicity; and the EMB MIC on Mtb is moderate, and it is difficult to achieve blood levels much higher than the MIC, thereby restricting its efficacy in humans [49]. We hoped that by revisiting this series, we could identify an EMB-like drug candidate with an improved MIC and decreased toxicity.

The library was synthesized with a primary focus on modification at the amine substituents, leaving the ethylenediamine core unaltered. Approximately 300 commercially available amine monomers, NH_2R_1 and NHR_2R_3, were used to combinatorially create the ethylenediamine library. Aliphatic, aromatic, open-chain and strained bicyclic amines, amino alcohols and amino acids were selected for significant molecular diversity in size, shape, polarity and charge [46]. The compounds were then filtered through microbiology studies and multiple standard *in vitro* interspecies absorption, distribution, metabolism, elimination and toxicology (ADMET) assays designed to narrow the number of candidates based on antimicrobial activity, low toxicity and other desirable characteristics for a new TB drug (Figure 2).

Compounds were synthesized in groups of ten and evaluated in two *in vitro* HTS: a broth microdilution assay to determine MIC against Mtb and a bioluminescence HTS in which

recombinant Mtb produces light in response to cell wall inhibition [50]. The use of dual assays was important: the microdilution assay allowed us to identify compounds that penetrated the bacterial cell wall to access their target, while the luminescence assay provided MOA specificity to minimize selection of generally toxic compounds. Each active ten-compound pool was resynthesized to enumerate each of the ten discrete compounds to identify the active structures, and our chemists evaluated the SAR of the series. In total, we identified 2796 ten-compound mixtures that were active and 170 individual compounds that had activity in both HTS and MICs in the potentially interesting (<6 μg/ml) range.

The majority of active compounds were lipophilic. The most frequently occurring fragments in the active compounds were: highly α-branched aliphatic moieties, 2,2-diphenylethyl and 3,3-diphenylpropyl fragments, tricyclic skeletons derived from adamantane-containing amine monomers, myrtanylamine and isopinocamphylamine and isoprenoid structures. We used this SAR to create a new 30,000+ diamine library to further develop the structural diversity of our library by modification of the diamine linker, which led to the discovery of novel scaffolds with activity against Mtb [51–53].

The top 27 1,2-ethylenediamine active compounds were evaluated in additional *in vitro* assays that included an *in vitro* cytotoxicity assay to determine IC_{50} on HepG2 cells and a calculation of the selectivity index (SI; IC_{50}/MIC). The SI estimates the therapeutic window of a compound, which is the concentration range at which the compound should be both safe and efficacious. SQ109 had the lowest MIC (0.78 μg/ml) and the highest SI (16.7) of this group. We also determined the minimal bactericidal concentration (MBC), calculated the lipophilicity of each compound (logP) and evaluated the efficacy of the best 13 compounds in Mtb-infected macrophages, since Mtb is a facultatively intracellular organism. The best 11 compounds were advanced to *in vivo* efficacy studies in mice, and the most promising three compounds from those studies were evaluated in maximum tolerated dose (MTD) and pharmacokinetic (PK) studies in mice.

SQ109 had both the best antibacterial activity and the most promising toxicity, MTD and PK parameters of all of the active drug compounds [54,55], and had the same MIC against DS and MDR Mtb strains (Table 2). Moreover, it was efficacious in both a screening and a chronic model of TB in mice [56] at 10 mg/kg. SQ109 (Figure 2) was selected as the lead TB drug candidate from the library [54] and underwent extensive *in vitro* and *in vivo* studies and pharmacological and toxicology (14-, 28- and 90-day) evaluation in rats, dogs and monkeys prior to starting its clinical evaluation.

Since our selection of SQ109 to move forward, other research groups synthesized and evaluated SQ109 analogs by modifying the adamantane and geranyl fragments [57–59]. However, none of these analogs demonstrated notable improvement over SQ109 MIC or *in vivo* efficacy, supporting selection of SQ109 as best in class.

It is important to note that, although SQ109 was identified from a focused combinatorial library based on EMB, the chemical structures of EMB and SQ109 are very different (Figure 2) and the potency and activities of both drugs are quite distinct, as are their MOA (see below). We started these studies looking for a better and safer EMB, but we ended up with a substantially better new drug with an entirely new MOA and important new properties.

SQ109 has significant *in vitro* activity against *Mycobacteria*

SQ109 is active on all Mtb, including MDR- and XDR-TB clinical strains (Table 2), as well as against other *Mycobacteria* in the Mtb family (*Mycobacteria bovis* and *M. bovis* BCG) and *Mycobacteria fortuitum*. It has lower activity against other pathogenic *Mycobacteria*, including *Mycobacteria avium*, *marinum*, *chelonae* and *abscessus*, with MIC in the range of

4–16 µg/ml. In addition, SQ109 kills Mtb inside macrophages at a level equivalent to INH and superior to EMB [60]. At its MIC, it reduces intracellular Mtb by 99% [54]. Intracellular activity is important because Mtb are facultative intracellular pathogens: they are phagocytosed by pulmonary macrophages, but halt the maturation of the phagosome to create a protected cellular niche for their replication [61].

We also determined MBC to assess whether SQ109 is bacteriostatic or bactericidal. In our tests, SQ109 was bactericidal at a concentration of 0.64 µg/ml, approximately its MIC (range 0.2–0.78 µg/ml). The Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF) also identified SQ109 as bactericidal at its MIC (0.78 µg/ml). By contrast, EMB is bacteriostatic at its MIC and at doses that can be used to treat adult pulmonary TB [62,63].

Importantly, Mtb has a very low spontaneous mutation rate (2.55×10^{-11}) for SQ109 resistance [Reddy V, Unpublished Data], a rate 100–1000-times better than other antitubercular drugs or drug candidates. If this translates to a low rate of resistance development *in vivo*, it would be a significant new attribute of this drug.

SQ109 has a novel MOA

Although SQ109 was originally identified by creating analogs of EMB, our data clearly demonstrate that it is not active by the same mechanism as EMB. For example, SQ109 is active against a number of EMB-resistant strains (Table 2) [54]. Boshoff *et al.* described the transcriptional response of Mtb after exposure to various antibiotics [64], which confirmed that SQ109, similar to other well-characterized mycobacterial cell wall inhibitors, induces the transcription of the *iniBAC* operon. Consistent with this observation, the overall transcriptional profile of SQ109-treated cells closely resembles other cell wall-active agents such as INH, ethionamide (ETA), thiolactomycin and EMB. However, two genes upregulated by EMB were not upregulated by SQ109, and two genes downregulated by SQ109 were not downregulated by EMB.

In a study conducted by the National Cancer Institute (NCI), NIH, researchers examined postantibiotic protein changes in Mtb after exposure to SQ109, EMB and INH [65]. As with the transcriptional response studies, SQ109 had a signature profile similar to other cell wall inhibitors. However, the profiles of EMB and SQ109 were sufficiently different to suggest a distinct target.

Recently, the molecular target in Mtb of SQ109 was identified by two independent groups, further substantiating the differences in MOA between this diamine and EMB [24,25]. Treatment of Mtb with SQ109 decreased the incorporation of mycolic acids into the cell wall. However, this effect was localized to incorporation into trehalose dimycolate and mycolates attached covalently to the arabinogalactan polymer. Incorporation into trehalose monomyclate (TMM) actually increased, suggesting that SQ109 did not target mycolate synthesis, but rather transport and processing. Although the authors were unable to create spontaneous SQ109-resistant mutants, they did create mutants resistant to similar ethylenediamine compounds that had some cross-resistance to SQ109. The sequencing of these mutants revealed mutations in *MmpL3*, the putative transporter of TMM, and the precursor of both trehalose dimycolate and cell wall mycolates (Figure 3) [24]. Work by the second group demonstrated that other adamantyl-containing compounds also target MmpL3 and prevent translocation of TMM across the membrane, further supporting the notion that this transporter is likely the target of SQ109 [25].

By contrast, EMB inhibits the synthesis of arabinogalactan and lipoarabinomannan [66,67] and is assumed to act via inhibition of three arabinosyltransferases: EmbA, EmbB and

EmbC. Table 3 summarizes the characteristics of SQ109 and EMB and demonstrates that the two drugs have distinct actions and MOA.

SQ109 activity on other pathogens

SQ109 also has activity against organisms with relevance to human health, besides Mtb:

- It is bactericidal for *Helicobacter pylori:* all *H. pylori* clinical isolates are susceptible to SQ109, with a MIC range of 2.5–15 μg/ml. In addition, SQ109 in time–kill assays is better than amoxicillin and metronidazole, two of the first-line drugs commonly used to treat *H. pylori* infection [Mokobongo M, Einck L, Merrell D. *In vitro* characterization of the anti-bacterial activity of SQ109 against *Helicobacter pylori*. (2012), Manuscript in Preparation]. Sequella filed an SQ109 Investigational New Drug (IND) application for this indication; a Phase II clinical trial is ongoing (2012).

- Sequella collaborates on an NIH R01 grant with the Division of Infectious Disease and Global Health, University of Virginia (VA, USA), to investigate SQ109 and SQ109-related compounds for activity to kill *Clostridium difficile.* SQ109 MIC for this organism is 8–16 μg/ml.

- Interestingly, SQ109 also has activity against eukaryotic microorganisms, both filamentous and yeast fungi such as *Aspergillus fumigatus* (MIC of 8 μg/ml), *Candida* spp. (MIC of 1–8 μg/ml) and *Cryptococcus neoformans* (MIC of 0.25–4 μg/ml).

- Additional organisms against which SQ109 has *in vitro* activity include *Enterococci* spp. (MIC of 8–32 μg/ml), *Haemophilus influenzae* (MIC of 1–32 μg/ml), *Neisseria gonorrhoeae* (MIC and MBC of 0.038 μg/ml) and *Streptococcus pneumoniae* (MIC of 2–4 μg/ml).

It is interesting to note that MmpL3, the identified target of SQ109 in Mtb, only has homologs in other *Mycobacteria*, and does not have an obvious functional homolog in any of these other susceptible bacteria or fungi. It is possible that SQ109 has a unique target in these organisms that has not yet been identified, or that the inability to generate SQ109-resistant Mtb mutants directly [24] may indicate an as-yet unidentified second (or other) target present in both Mtb and the other susceptible microorganisms.

SQ109 enhances activity of existing & investigational TB drugs

We investigated *in vitro* interactions of SQ109 with currently used first-line drugs [68]. SQ109 demonstrated synergistic interactions with INH and RIF, and additive effects when combined with EMB or streptomycin. The strongest synergistic combination was SQ109 and RIF, and this synergy worked both ways: <MIC quantities of SQ109 synergistically improved RIF activity eightfold, and <MIC RIF synergistically improved SQ109 activity fourfold. Interestingly, *in vitro* studies demonstrate that SQ109 can reduce the MIC of RIF in RIF-resistant Mtb strains [68].

Importantly, no *in vivo* adverse drug–drug interactions or PK changes were found in research and formal pairwise studies of SQ109 with INH, RIF or streptomycin in mice or rats, supporting the value of the incorporation of SQ109 into a first-line treatment regimen.

Because SQ109 has activity against all DR-Mtb (Table 2), it could also be a part of regimens to treat MDR- or XDR-TB. Therefore, we assessed SQ109 in combination with each of the second-line TB drugs *in vitro* and *in vivo* to determine any interactions [Reddy V & Nikonenko B, Unpublished Data]. In two-drug combinations *in vitro* with AMK, ETA,

MXF, K AN, cycloserine (CS), para-aminosalicylic acid or CM, SQ109 showed *in vitro* synergy with some drugs (CM, MXF and AMK) and additive effects with others (ETA, KAN, CS and para-aminosalicylic acid). No antagonistic effects were observed in any of the combinations. In PK evaluations in mice, pharmacology profiles of SQ109 and MXF were not affected by coadministration. In the presence of CS and KAN, exposure of mice to SQ109 was slightly decreased.

Clofazimine (CFZ) is a group 5 drug on the WHO list of drugs to be used in MDR-TB. Group 5 drugs have unclear efficacy or an unclear role in MDR-TB treatment. While there are toxicity concerns with CFZ, new data suggest that it may be a useful drug for combination treatment of TB [69,70]. SQ109 showed synergy with CFZ, both *in vitro* (Table 4) and in a macrophage intracellular killing assay [Reddy V, Unpublished Data]. Studies in mice are ongoing.

SQ109 has interesting activity when combined with investigational TB drug candidates in development by other companies:

- SQ109 has profound synergy with all the rifamycins, including rifapentine (Sanofi) and rifabutin (Pfizer), which are drugs in the same class as RIF that have a longer half-life (rifapentine) or a reduced activation of CYP3A4, a human P450 enzyme that metabolizes many drugs, including drugs to treat HIV (rifabutin). RIF and rifapentine have identical synergy results with SQ109 *in vitro*. Rifapentine is in clinical trials to evaluate whether it can shorten TB treatment: SQ109 has a similar half-life to rifapentine and, based on mouse studies, could be paired with this drug to both improve efficacy and shorten treatment time [71].

- SQ109 and sutezolid have additive effects *in vitro*, and SQ109 improved synergistically the time to kill intracellular Mtb in an infected macrophage assay [72]. SQ109 and bedaquiline are apparently the two best drugs to pair with sutezolid in a new TB regimen for all forms of DS- and DR-TB [73].

- SQ109 and bedaquiline were also synergistic *in vitro*: small amounts of SQ109 (0.5 MIC) improved the MIC of bedaquiline four- to eight-times over its MIC and also improved the rate of Mtb killing when the two drugs were evaluated in combination. Small amounts of bedaquiline (0.5 MIC) also improved SQ109 activity fourfold. In addition, SQ109 increased the postantibiotic effect of bedaquiline from 9 to 14 h [74].

SQ109 is active in mouse models of TB

PK studies based on drug serum concentrations in mice suggest that SQ109 has low oral bio-availability, but tissue distribution studies show that SQ109 achieves concentrations well above its MIC, at least 45-fold, in TB murine target organs (lung and spleen) compared with plasma (Figure 4) [60].

Meng *et al.* investigated esterase-sensitive carbamate prodrugs of SQ109 with the goal of improving bioavailability [75]. They identified a prodrug that improved bioavailability of SQ109 from 21.4 to 91.4% in rats, with a high tissue distribution to the lung and spleen. However, MIC of the carbamate compound was higher than SQ109 *in vitro*, and the compound was unstable in rodent and human serum, and also in phosphate-buffered saline. In mice, we were unable to reproduce the increases in plasma concentrations that were observed in rats.

In a rapid, high-dose model of TB infection developed for initial screening of drug candidates [56], SQ109 (10 mg/kg) significantly reduced weight loss in treated infected

mice compared with untreated infected mice. Similarly, 10 mg/kg SQ109 administered to mice for 6–8 weeks in a model of chronic TB showed better activity than EMB at 100 mg/kg [76]. In dose-ranging experiments, 25 mg/kg SQ109 showed improvement over the 10 mg/kg dose in the spleen, but no difference in lungs [54]; higher concentrations were similar to 25 mg/kg in both lungs and spleens. These efficacious doses are well below the MTD of orally delivered SQ109 in mice (600 mg/kg [54]).

Interestingly, the effects of SQ109 combined with INH and RIF are most evident with >2 weeks of treatment (Table 5): activity is significantly better than EMB at 3 and 4 weeks [76]. This may indicate that SQ109 drug action occurs not on the rapidly replicating bacteria in tissues, but those sequestered in macrophages or in a dormant state.

SQ109 improves overall efficacy of the standard four-drug treatment regimen (INH, RIF, PZA and EMB) when it replaces EMB for 8 weeks in a mouse model of TB [76]. In fact, no bacteria remained in lungs of half the mice in the SQ109-containing regimen, and an average 18 CFU remained in the rest; by contrast, mice treated with INH + RIF + PZA + EMB had 32-fold more CFU (average 568 CFU) than in the SQ109-containing regimen (Figure 5). These data suggest that SQ109 works effectively within the existing first-line treatment regimen to replace EMB: it may shorten treatment time and have significant therapeutic benefits over EMB.

We recently evaluated SQ109 interactions with bedaquiline *in vivo* in a mouse model of TB: by 3 months of combination drug treatment, the regimen SQ109 + bedaquiline + PZA induced a durable cure in Mtb-infected mice (Figure 6), eliminating lung and spleen CFU better than standard of care [Nikonenko B, Unpublished Data] with a 50% reduction in time (3 vs 6 months). Although such models must be interpreted carefully as predictive ability in humans is, as yet, uncertain, these results support further clinical evaluation of the combination.

These new *in vitro* and *in vivo* drug combination data are particularly exciting because of the toxicity and poor efficacy of current second-line TB drugs. One can now envision an entirely new treatment regimen for MDR-TB composed of several of the current investigational drugs, and this new combination may also prove effective in DS-TB. Sequella has collaborative research agreements with pharmaceutical developers of rifapentine, sutezolid and bedaquiline, and will build on these exciting data to underpin future clinical evaluations of new drug combinations.

IND-directed preclinical development of SQ109

The ADMET profile of SQ109 was determined [77]. Tissue distribution in rats showed the highest concentrations of SQ109 in liver and, similar to the distribution in mice, also high concentrations in the lung and spleen, the target organs for TB treatment. When radioactive SQ109 was orally administered to rats, the highest level of the drug was in liver, suggesting that the drug may undergo a first-pass metabolic step there.

Metabolic profiling of SQ109 in liver microsomes of various animal species, including humans, revealed potential metabolites suggestive of oxidation, epoxidation and N-dealkylation of the drug. CYP450 profile studies of SQ109 on human CYP450 enzymes established the formation of four major metabolites produced primarily by CYP450 2D6 and 2C19 enzymes [77]. The CYP450 enzyme of concern in drug–drug interactions, 3A4, is not activated by SQ109, nor is it metabolized by the enzyme.

Table 6 shows *in vitro* and *in vivo* safety and toxicity tests that have been performed on SQ109 to date and the results of these assays.

Extensive IND-directed preclinical safety, toxicology and pharmacology studies were performed on SQ109, many supported by the NCI Developmental Therapeutics Program. SQ109 was evaluated in IND-directed dose-ranging 14- and 28-day and definitive 14- and 90-day studies in dogs, and definitive 90-day studies in rats and nonhuman primates. At 40 mg/kg/day, there were no cardiovascular, hepatic, hematologic, neurological or ocular toxicities, or any adverse events (AEs) at all, except emesis in dogs, in the three animal species. The drug did cause phospholipidosis, similar to virtually all lipophilic drugs and antibiotics. The 'no observed AEs level' (NOAEL), depending on species, was from 30 to 40 mg/kg/day. The efficacious dose of SQ109 for treatment of TB in the murine model of acute infection is 10 mg/kg/day (~1 mg/kg/day human-equivalent dose). Based on the toxicology NOAEL, we anticipated that a dose several-fold greater than the human-equivalent dose could be explored in clinical trials to maximize clinical efficacy.

SQ109 safety in humans

Fast-track designation for SQ109 was granted by the US FDA in January 2007 under IND #104,669, and Orphan Drug Status was also granted by the EMA and FDA in 2007.

Safety of SQ109 was evaluated in three Phase I studies (80 healthy humans; Table 7) and one Phase IIa study in TB patients (75 adult patients with sputum-positive, DS pulmonary TB).

Phase Ia (study conducted by Sequella)

There were no serious AEs (SAEs), and any AEs were not dose-related. All AEs were self-limited and none required intervention. The mean maximum concentration for 10- and 300-mg oral doses of SQ109 administered to fasting human subjects were 0.729 and 20.2 ng/ml, respectively. Both the maximum concentration and area under the curve increased in a dose-dependent manner. Increase in half-life was nonlinear, with substantially larger values for the 200- and 300-mg doses (61.1 h for the 300-mg dose cohort), which is consistent with a large volume of distribution also measured for these doses. Administration of SQ109 with a high-fat, high-calorie meal resulted in a decrease in the rate of absorption with minimal effect on the extent of absorption, resulting in a decreased maximum serum concentration and increased half-life.

Phase Ib (study conducted by NIAID, NIH)

There were no SAEs, and AEs were not dose-related and did not require intervention. SQ109, whether administered as a 75-mg dose daily for 14 days or as a 150-mg dose daily for 5 days followed by single 150-mg doses on days 9 and 14, was well tolerated.

Phase Ic (study conducted by NIAID, NIH)

There were no SAEs, although one subject withdrew due to treatment-emergent AEs of nausea, vomiting and abdominal discomfort. Vital sign, ECG, neurological, mini-mental assessment and ophthalmological data did not reveal any clinically meaningful trends or changes throughout the study.

Steady-state conditions were achieved by day 10 with daily administration of 300 mg SQ109. Based on the preclinical relationship observed between plasma and lung concentrations, steady-state plasma concentrations associated with once-daily dosing of 300 mg SQ109 correlate with lung concentrations approximately 5.8-fold greater than the SQ109 MIC. In addition, based on the plasma concentration–time profile, lung concentrations are expected to be above the MIC for Mtb 12 h after dosing in approximately 50% of subjects.

Phase IIa (study conducted in collaboration with the European Developing Countries Clinical Trial Partnership)

We recently completed enrollment to evaluate safety, tolerance, EBA and PK of 14 days of daily doses of SQ109 at 75, 150 or 300 mg monotherapy, or SQ109 150 or 300 mg plus standard-dose RIF. Standard-dose RIF, the positive control, was administered to one cohort of subjects. The study was conducted at three sites in Cape Town, South Africa. Preliminary safety analysis indicates that all doses of SQ109, with or without concomitant RIF, were safe and well-tolerated. The only AEs were gastric and gastrointestinal complaints, which for all but one patient resolved spontaneously; one patient who was administered SQ109 withdrew because of pain from pre-existing, but undiagnosed, esophageal candidiasis. PK and efficacy data are being evaluated and should be available in mid-2012.

Future perspective

Recent years have shown a rise in interest in the application of modern drug-discovery techniques to the field of TB, leading to an unprecedented number of new TB drug candidates in clinical trials. On the downside, recent years also saw the application of many prejudices of modern drug development to anti-infective and antitubercular drugs. SQ109 does not fit the mold of traditional pharmaceuticals, and its development has been slowed by concern that its basic structure does not fit the norms of other nonantibiotic drug classes. These prejudices slowed new antitubercular clinical evaluation, which has not kept pace with the rapidly expanding worldwide need for new TB drugs. It is important to continue to explore new ideas and new chemical space to combat public health threats such as TB.

The goal of the SQ109 clinical program is to investigate the utility of this agent in both DS and DR strains of Mtb by replacing EMB in the current standard-of-care regimen to treat disease caused by DS strains and adding SQ109 to second-line regimens that are tailored to the specific MDR-TB disease. The low bacterial mutation rate (2.55×10^{-11}) observed *in vitro* combined with the extensive tissue penetration observed *in vivo* suggest that the drug has significant potential clinical utility to treat pulmonary TB and to decrease relapses/recurrences due to the emergence of antibiotic resistance. Synergy between SQ109 and RIF or INH may also shorten treatment times for uncomplicated TB and could reverse the phenotypic resistance of some MDR-TB strains so that the first-line TB drugs can also be used in certain MDR-TB cases.

While initial SQ109 clinical studies are focused on replacing a single drug or being added to an existing drug regimen, a tantalizing scenario is clinical evaluation of a novel treatment regimen containing multiple TB drug candidates. Because of limited resources, diagnosis of DR-TB often takes place only after treatment failure. This approach leads to an increase in morbidity and mortality, as well as the development of further drug resistance that is difficult or impossible to treat. The FDA recently released draft guidance for the codevelopment of two unmarketed drug candidates, signaling their willingness to consider registration of new drug combinations prior to individual drug registration [104]. This raises the possibility that a completely novel drug regimen could eventually be used to treat both DS and DR-TB. The long half-life of SQ109 may facilitate combination of SQ109 with other TB drug candidates such as bedaquiline (which also has a long half-life) or sutezolid, with which it interacts well *in vitro*, thereby enabling treatment regimens with less frequent dosing and improved patient compliance.

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Executive summary

TB is a global health crisis

- A third of the world's population is infected with *Mycobacterium tuberculosis*.

- There are 8.8 million incident TB cases each year and more than 1.5 million deaths.

- Existing treatment regimens leave significant room for improvement because of toxicity and long durations.

- Drug-resistant TB, multidrug-resistant TB and extensively drug-resistant TB threaten our control of this deadly disease.

Several new TB drug candidates are in clinical development

- The field of TB drug development has seen a recent resurgence.

- Several drugs and drug candidates for TB are in clinical trials.

SQ109 is a novel TB drug candidate & has promising characteristics

- SQ109 has the following promising characteristics:

    – Significant in vitro activity against Mycobacterium tuberculosis;

    – Novel mechanism of action;

    – Pulmonary accumulation of the drug in preclinical animal studies;

    – Activity on nontubercular pathogens;

    – Enhances activity of existing and investigational TB drugs;

    – Active in mouse models of TB;

    – Shows promise in Investigational New Drug-directed preclinical studies;

    – Found to be safe and well tolerated in Phase I and II clinical studies at doses up to 300 mg.
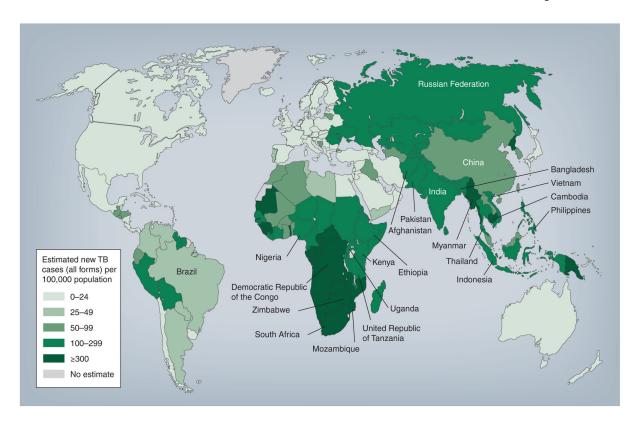
Figure 1. WHO estimated TB incidence rates, 2011
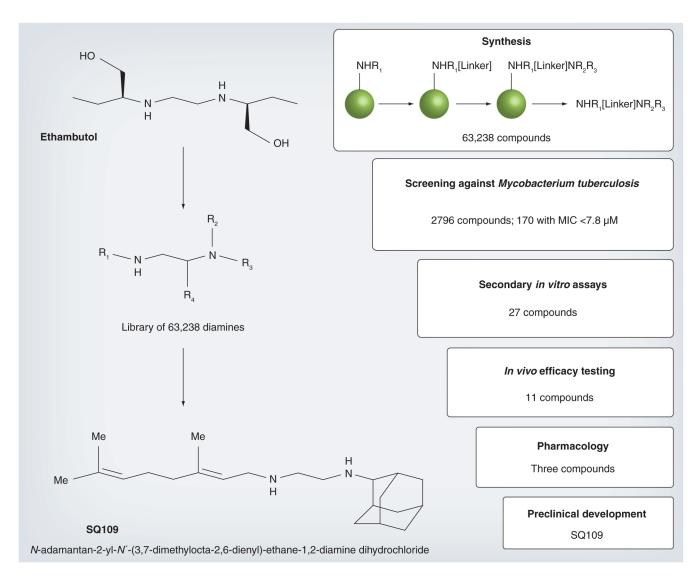Reproduced with permission from [101].

Figure 2. Process to identify new drug candidate, SQ109, from a focused combinatorial library of 1,2-ethylenediamine small-molecule compounds
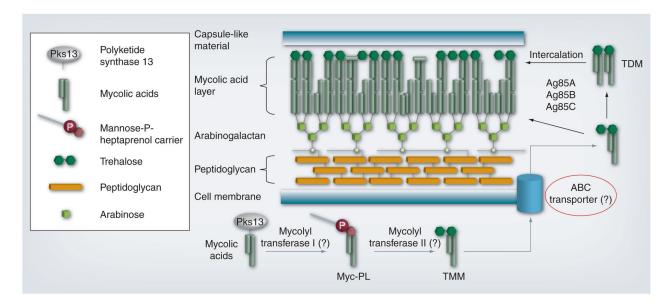Data taken from [46,54].

Figure 3. SQ109 mechanism of action
The figure depicts stylized components of the cell wall, while primarily focusing on TMM and TDM. Hypothetical or proposed steps are indicated by gray lines, and question marks in parentheses represent hypothetical proteins/enzymes not yet identified. The inset legend represents the shapes used to depict the components shown. Note that the diagram is not to scale and does not include detailed structures or all components of the mycobacterial cell wall. MmpL3, the target of SQ109, is proposed to be the ABC transporter (red circle). TDM: Trehalose dimycolate; TMM: Trehalose monomycolate.
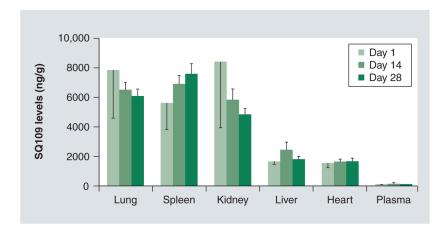Adapted with permission from [24].

Figure 4. SQ109 tissue levels after oral administration to mice for 28 days
SQ109 at 10 mg/kg; drug levels were measured 1 h after the last administration of the drug.
Reproduced with permission from [60].

INH + RIF + SQ109 + PZA   INH + RIF + EMB + PZA
(standard of care)
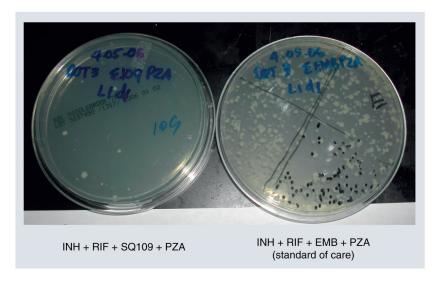
Figure 5. SQ109 activity compared with ethambutol in the standard drug regimen
Estimation of CFUs (white colonies of *Mycobacterium tuberculosis* on plates of 7H10 agar incubated at 37°C, 5% CO_2 in a humidified incubator for 3 weeks) in undiluted lung homogenates of *Mycobacterium tuberculosis*-infected mice treated for 6 weeks with standard of care TB drugs (right plate: ~568 CFU) or a combination containing SQ109 in place of EMB (left plate: ~18 CFU).
EMB: Ethambutol; INH: Isoniazid; PZA: Pyrazinamide; RIF: Rifampicin.
Data taken from [76].

Figure 6. Treatment of mice with bedaquiline alone or incombination with SQ109 and/or pyrazinamide

After 3 weeks of infection with *Mycobacterium tuberculosis*, female C57BL/6 mice were either treated with nothing (infected control, ten mice) for 3 months or with one 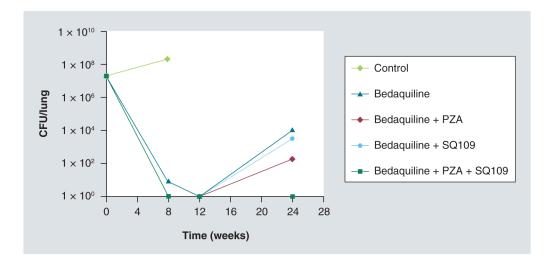of the described regimens in groups of 30 mice/regimen. Drug concentrations were: bedaquiline, 25 mg/kg; SQ109, 25 mg/kg; PZA, 150 mg/kg. Mice were evaluated for CFU/lung at 2 and 3 months. After 3 months of drug therapy, all control mice were dead, and lungs of ten mice in all groups were harvested and plated to evaluate CFU. Lung tissues from all drug-treated groups were culture-negative (no CFU). After therapy termination, 20 additional mice of each drug-treated group were rested (no treatment) for 3 months. Lungs were extracted, homogenized and plated undiluted on 7H10 agar for 3 weeks. CFU/lung 1×10^0 means no detected CFU (limit of detection = 1 bacterium/ml of undiluted lung homogenate). PZA: Pyrazinamide.

Table 1

Clinical-stage TB drugs and drug candidates.

Drug candidate	Class	Intellectual property	Clinical phase
Delamanid (OPC-67683)	Nitroimidazole	Otsuka	III
Moxifloxicin	Fluoroquinolone	Bayer AG/TB Alliance	III
Gatifloxacin	Fluoroquinolone	WHO/Bristol-Myers Squibb	III
Bedaquiline (TMC207)	Diarylquinoline	Janssen Infectious Diseases/TB Alliance	II
SQ109	Ethylenediamine	Sequella	II
PA-824	Nitroimidazole	TB Alliance	II
Sutezolid (PNU-100480)	Oxazolidinone	Pfizer	II
Linezolid	Oxazolidinone	Pfizer	II
Metronidazole	Nitroimidazole	Generic	II
AZD5847	Oxazolidinone	AstraZeneca	I

Table 2

In vitro activity of SQ109 against *Mycobacterium tuberculosis*.

Susceptibility profile	Identification number	Assay	MIC (µg/ml)
H37Rv (pan-susceptible)	ATCC 27294	BACTEC	0.2
H37Rv (pan-susceptible)	ATCC 27294	Alamar	0.39[†]
Erdman (pan-susceptible)	ATCC 35801	Alamar	0.39[†]
EMB-resistant	Not reported	Alamar	0.78[†]
INH-resistant	ATCC 35822	Alamar	0.78[†]
RIF-resistant	ATCC 35838	Alamar	0.39[†]
CIP-resistant	Not reported	Alamar	0.39[†]
KAN-resistant	Not reported	Alamar	0.78[†]
XDR, also EMB-resistant	576W1[‡]	Microbroth	0.20
XDR, also EMB-resistant	587W1[§]	Microbroth	0.20

[†]Work performed by the Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF).

[‡]Strain from the laboratory of C Barry at the National Institute of Allergy and Infectious Diseases (NIAID): resistant to streptomycin, INH, RIF, EMB, ethionamide, KAN, CIP, capuramycin and cycloserine.

[§]Strain from the laboratory of C Barry at NIAID: resistant to streptomycin, INH, RIF, EMB, ethionamide and KAN; sensitive to CIP, capuramycin and cycloserine.

CIP: Ciprofloxacin; EMB: Ethambutol; INH: Isoniazid; KAN: Kanamycin; RIF: Rifampicin; XDR: Extensively drug-resistant.

Table 3

Distinct characteristics of ethambutol and SQ109.

Characteristic	EMB	SQ109	Ref.
Molecular weight	204.31 g/mol	330.55 g/mol	[46]
MIC on Mtb	5–7 µg/ml	0.12–0.36 µg/ml	[46,54]
MIC on EMB-resistant Mtb	184 µg/ml	0.12–0.78 µg/ml	[54]
Induces Mtb P_{0341} promoter, which is activated with cell wall disruption	Yes	Yes	[46]
Kills nonreplicating Mtb	No	Yes	[Reddy V, Unpublished data]
Activity on Mtb gene clusters *GC82* and *GC120*	Upregulates	Downregulates	[64]
Effect on acid-fastness of Mtb	Decreases	No effect	[64]
Synergy with RIF	No	Yes	[68]
Synergy with INH	No	Yes	[68]
Activity against *Candida*, *Helicobacter pylori*, *Haemophilus influenzae*, *Streptococcus pneumoniae* and *Aspergillus fumigatus*	No	Yes	[Sequella, Unpublished data]
Location of mutations in resistant Mtb	*embA*, *embB* and *embC*	*mmpL3*	[24,78,79]
Target	Inhibition of AG and LAM biosynthesis	Inhibition of TMM transport	[24,80]

AG: Arabinogalactan; EMB: Ethambutol; INH: Isoniazid; LAM: Lipoarabinomannan; Mtb: Mycobacterium tuberculosis; RIF: Rifampicin; TMM: Trehalose monomyclate.

Table 4

Checkerboard titration of SQ109 and clofazimine.

Experiment	Drug combination	MIC (µg/ml)		ΣFIC
		Alone	Combination	
1	SQ109	0.5	0.125	0.37
	CFZ	0.5	0.062	
2	SQ109	0.5	0.125	0.37
	CFZ	0.5	0.062	

Drug interactions were determined by checkerboard titration of SQ109 and CFZ by a 96-well microdilution assay described previously using Mycobacterium tuberculosis H37Rv [68]. From the MIC values, the FIC of each drug was calculated as (MIC of drug in combination)/(MIC of individual drug). The ΣFIC is the sum of the FICs of the two drugs. ΣFIC 0.5 is synergistic; 4.0 is antagonistic and in between is additive or indifferent.

CFZ: Clofazimine; ΣFIC: Fractional inhibitory concentration index.

Table 5

SQ109 antitubercular activity during intensive-phase drug treatment.

Drug regimen	Log_{10} CFU in lung	Log decrease
2 weeks		
Untreated	6.16 ± 0.02	–
INH + RIF + EMB	4.64 ± 0.23	1.52
INH + RIF + SQ109	4.46 ± 0.12	1.70
3 weeks		
Untreated	6.34 ± 0.34	–
INH + RIF + EMB	4.38 ± 0.05	1.96
INH + RIF + SQ109	3.80 ± 0.10	2.54
4 weeks		
Untreated	6.42 ± 0.76	–
INH + RIF + EMB	3.86 ± 0.14	2.56
INH + RIF + SQ109	3.26 ± 0.12	3.16

C57BL/6 mice were inoculated intravenously with 10^4 Mycobacterium tuberculosis H37Rv and infection was allowed to progress for 21 days. Chemotherapy started on day 21 and continued for 4 weeks; mice were treated with the indicated combinations. Lung CFUs were assessed at 2, 3 and 4 weeks of treatment. Drugs were administered at the following concentrations: INH, 25 mg/kg; RIF, 20 mg/kg; SQ109, 10 mg/kg. At each time-point, one group of mice for each drug combination (six mice per group) was sacrificed; lung homogenates were plated in tenfold serial dilutions on 7H10 agar dishes. CFUs were calculated after 3 weeks of growth, and are expressed ± standard deviation.

EMB: Ethambutol; INH: Isoniazid; RIF: Rifampicin.

Data taken from [76].

Table 6

In vitro and *in vivo* safety studies of SQ109.

Assay	Outcome
In vitro *mutagenicity studies*	
Salmonella–Escherichia coli/microsome plate incorporation assay	Negative
Mouse lymphoma mutation assay	Negative
Micronucleus assay in rats	Negative
Cardiovascular safety, beagle dogs (single oral dosing at 75, 140 and 300 mg/m^2)	
Clinical observations	No deaths
Body weights	No change
Clinical pathology: hematology or clinical chemistry	No change
Blood pressure: at 75 and 140 mg/m^2	No change
Heart rate/ECG data	No change
ECG waveforms (rhythms and morphology)	No change
Neurological safety assessment, rats (90-day oral dosing at 30, 120 and 240 mg/m^2/day)	
Functional observational battery parameters	No change
Neurological safety assessment, beagle dogs (90-day oral dosing at 100, 300 and 1000 mg/m^2/day)	
Functional observational battery parameters	No change
Pulmonary safety testing, beagle dogs (single oral dosing at 75, 140 and 300 mg/m^2)	
Respiratory rate	No change
Tidal volume	No change
Minute volume	No change
Reproductive toxicology studies	
Segment 1: effects on male and female gamete maturation, copulatory performance, fertilization, zygote development and embryo implantation	No impact
Segment 2: effects on pregnant female and development of embryo and fetus from implantation	No impact

Table 7

SQ109 clinical studies.

Study	Cohorts	Dose (mg)	Dosing schedule[†]	Subjects (n)	
				SQ109	**Placebo**
Phase Ia, escalating single dose	Fasting	5, 10, 20, 50, 100, 200, 300	Single oral dose after overnight fast	42	14
	Food effect	300	Single oral dose after high-calorie, high-fat meal	6	0
Phase Ib, multiple daily doses	1B-1	75	Days 1–14	8	2
	1B-2	150	Days 1–5, 9 and 14	8	2
	1B-3	150	Days 1–14	8	2
Phase Ic, multiple daily 300 mg doses	1C-1	300	Days 1–14	8	2
Phase IIa, safety and EBA[†]	2A-1	75	Days 1–14	15	0
	2A-2	150	Days 1–14	15	0
	2A-3	300	Days 1–14	15	0
	2A-4	75 + RIF	Days 1–14	15	0
	2A-5	150 + RIF	Days 1–14	15	0
	2A-6	300 + RIF	Days 1–14	15	0
	2A-7	RIF	Days 1–14	15	0

[1] SQ109 administered in morning after overnight fast for all studies, except Phase Ia – 'Food effect' cohort.

EBA: Early bactericidal activity; RIF: Rifampicin 10 mg/kg.