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Advances in Drug Discovery and Development for Pediatric Tuberculosis

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Abstract

Pediatric tuberculosis is an underappreciated global epidemic estimated to afflict around half a million children worldwide. This problem has historically been overlooked, due in part to their low social status and the difficulty in diagnosis of tuberculosis in children. Children are more susceptible to tuberculosis infection and disease progression, including rapid dissemination into extra-pulmonary infection sites. Treatment of pediatric tuberculosis infections has been traditionally built around agents used to treat the adult disease, but the disease pathology, drug pharmacokinetics and the safety window in children differs from the adult disease. This produces additional concerns for drug discovery and development of new agents. This review examines: (i) the safety concerns for current front and second line agents used to treat complex drug resistant infections and how this knowledge can be used to identify, prioritize and dose agents that may be better tolerated in pediatric populations; (ii) the chemistry and suitability of new drugs in the clinical development pipeline for tuberculosis for the treatment of pediatric infections indicating several new agents may offer significant improvements for the treatment of multi-drug resistant tuberculosis in children.

Keywords

Tuberculosis; pediatric tuberculosis; tuberculosis drug discovery

1. THE GLOBAL TUBERCULOSIS EPIDEMIC: A FOCUS ON PEDIATRICS

Tuberculosis (TB) remains a grave threat to global health, despite being a known and treatable illness for decades. In 2013, 9.0 million people globally fell ill to TB including over half a million pediatric cases, resulting in over a million deaths overall and 75,000 deaths in children. However, estimation of childhood TB cases can be difficult due to its high prevalence in low-income countries and resulting complications in healthcare reporting [1]. The WHO Global Tuberculosis Report 2014 showed 80% of reported TB incidents occurred in 22 countries with new occurrences primarily appearing in East Asia and sub-

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Saharan Africa [2]. TB is caused by bacteria (*Mycobacterium tuberculosis* (M.tb)) and is primarily a lung-residing pathogen that can enter a dormant, non-replicating state which is much more difficult to clear due to a lack of cellular activity [3–6]. It is estimated that approximately one-third of the world has this latent form of TB which, in healthy adults, has a 10% life-time chance of progressing to the active disease state. This percentage is greatly increased in pediatrics, with instances of up to 50% disease progression being observed [1] and rapid dissemination into extra pulmonary infections. Children typically are infected via pulmonary exposure to the M.tb from household contact with a parent or caretaker. Several other factors can affect the spread of TB in pediatric populations; generally the older a child is the more likely they would be to be in contact with a greater number of unique persons and thus increasing their likelihood of exposure closer to adult levels. Other factors such as population density, weather, housing conditions and community associations can also affect the way the disease is spread [1]. After initial exposure to the pathogen, there are factors that influence the host becoming infected. Infection can be correlated directly to the duration of exposure, intensity (confined space, poor ventilation, etc.), virulence of the infecting organism and the immunology of the child [1, 7–9]. Cases of immunosuppression greatly increase this risk, such as age [1, 10–12], malnutrition [13, 14], diabetes, tobacco and alcohol use, or human immunodeficiency virus (HIV)[15]. As stated before, TB remains a very real threat primarily in third-world countries and is in part due to these risk enhancers. In 2012 TB was the leading cause of death in HIV-positive persons and an estimated 1.1 million new cases of TB-HIV co-infection occurred 75% of which were in Africa and east Asia. People co-infected with HIV and TB are 30 times more likely to develop active TB disease than those without [2]. This is exacerbated by the fact that the mainstay of frontline TB therapy, Rifampicin, is a strong inducer of human metabolic enzymes causing antagonism to many Anti-retroviral drugs [16–19].

The current course of therapy for TB takes 6–9 months in optimal conditions [20] and once drug resistance is discovered or developed the treatment time becomes much longer and complicated with the introduction of less efficacious second-line agents [3]. The normal regimen for drug-susceptible infections (known or assumed) consists of four powerful antibiotics: Isoniazid (INH), Rifampicin (RIF), Pyrazinamide (PZA), and Ethambutol (EMB) (Figure 1) taken in combination for two months (intensive phase) followed by four months of INH and RIF (continuation phase). Such a long duration of therapy with agents exhibiting marked adverse events [21], while highly effective, is one reason for poor patient compliance in completing therapy. Other common causes of poor patient compliance includes interruptions in the drug supply or an unwillingness/inability for the patient to regularly return for the entire duration of therapy which may be imparted by a lack of transport options to clinic requiring patients to walk for long periods of time in rural areas. This poor patient compliance ushered the World Health Organization to implement Directly Observed Treatment, Short Course (DOTS) in order to maximize the efficiency of treatment worldwide and to minimize incomplete therapies, the leading cause of drug resistance. DOTS implements five main elements to increase survival and decrease transmittance for most developing countries including: government commitment to treatment, sputum smear microscopy for all cases to assess drug susceptibility, standardized treatment regimen with direct observation by a trained healthcare professional, an uninterrupted drug supply of

primary care agents and a standardized reporting system to maximize data interpretation straight from human cases. Even with these added precautions, cases of resistance continue to emerge across the world. Multidrug-resistant tuberculosis (MDR-TB) is resistant to at least Rifampicin and Isoniazid, the two primary frontline drugs. Further resistance to fluoroquinolones and at least one of the three injectable second-line drugs (i.e. amikacin, kanamycin, and capreomycin) is a subset of MDR-TB known as extensively drug resistant (XDR)[22]. Agents typically reserved for MDR or XDR infections are grouped into four classes (in addition to the first line class) based on efficacy, potency, drug class and experience of use [20, 23] (Figure 2). The first group of second-line agents includes injectable aminoglycosides and polypeptides. The next group is the fluoroquinolones, in both oral and injectable forms, from which generally only one agent is used. The final group of second-line agents are also oral and of various drug classes. A fifth group exists as a third-line of anti-TB drugs which include several repurposed compounds such as Clofazimine (leprosy), Linezolid (respiratory infections), Amoxicillin plus Clavulanate (broad spectrum use), Imipenem plus cilastatin (broad spectrum), and Clarithromycin (respiratory infections). Figure 2 depicts a common progression method to select treatment combinations between these groups of second-line agents as resistance is confirmed. Typically physicians will attempt to always be using at least four drugs that the isolate is susceptible to in order to prevent further resistance from emerging.

1.1. TB in Children

There are certain issues that are especially problematic in diagnosis and treatment of children afflicted by this disease, whom are often a lower priority than adults due to perceptions of a lack of infectiousness, difficulty in obtaining diagnosis using sputum samples and rapid progression to a more serious disease state[24]. Globally children under the age of 15 accounted for half a million infections in 2011 with 64,000 deaths in HIV negative patients. Children under the age of five are several times more likely than adults to progress to more serious extra pulmonary dissemination involved in miliary TB and TB meningitis [1, 10, 24, 25] and most will succumb to infection within a year of initial exposure [26]. With this rapid progression from infection to disease state, children can be quite indicative of an active epidemic at a local level and are useful in distinguishing between latent and infectious populations. Neonates have the highest progression rate to the disease state and in fact can be useful in diagnosis of previously unknown TB in the mother [27]. As a much understudied population, there is little clinical or even pre-clinical data behind the dosing regimens for pediatrics. There is a significant difference in drug delivery, both in their pharmacokinetics and pharmacodynamics [28–32] when considering a child taking the same medication as an adult. Children tend to have a much more sensitive therapeutic index [21, 23, 33], with noted adverse effects to TB therapies worse than adults, especially for second-line agents used for MDR-TB. We will discuss this more in depth later in this review, but for now it should be noted that there is a dearth of knowledge for this patient subpopulation that is just now starting to be addressed. Before 2012, the WHO did not distinguish between pediatric and adult infections when issuing their annual estimate of epidemiology. In addition, the DOTS strategy largely neglects children, as the primary means of detection is sputum smear-positive, which pediatrics often don't display [10, 14, 24]. Other factors contribute to the delicate disease state of pediatric TB infections including

altered immune response [11, 12], difficulties in proper dosing (both amounts and formulation)[34], and the rapid progression to severe disease state. We will discuss the future of small molecule M.tb therapeutics, specifically how it pertains to the pediatric population, as well as alterations in screening and target validation strategies that are proving helpful in the identification of lead candidates to be advanced to the clinic.

1.2. Adverse effects of frontline agents

As previously stated, TB primarily affects impoverished portions of the world and therefore the majority of children being treated for TB live in very resource-scarce areas. This not only makes obtaining the necessary agents required for first or second-line therapy difficult; it also blurs the line for monitoring adverse effects from therapy. Reporting an adverse reaction is very tricky, as many in this patient population are extremely malnourished and co-infected with one or more serious diseases. However, as of late, there has been a more concerted effort in keeping track of toxicological events as it pertains to pediatric tuberculosis treatment, as doses must be safe and well tolerated in order to maintain a high level of patient compliance [35–37]. This is something as a field that needs to be considered going forward as new agents and regimens are developed with pediatric populations in mind.

We will first start with side effects associated with front-line therapies. The front-line treatment for drug sensitive pulmonary TB in children is similar to the adult therapy, with the dosages having recently been raised by the WHO. Two months of RIF (10–20 mg/kg, max dose 600 mg/day), PZA (30–40 mg/kg), INH (10–15 mg/kg, max dose 300 mg/day) and sometimes EMB (15–25 mg/kg, in areas of high HIV or INH resistance prevalence) followed by four months of RIF and INH at the above doses. Despite the recent increase in dosage, it has been discussed by Hiruy et al. that these doses might be inadequate to reach optimal therapeutic plasma concentrations [34], a subject that definitely merits more investigation. However, in this review, we will focus on noted adverse effects for current or lower doses of front and second-line agents.

INH has two major reactions, neurologic and hepatic, both of which are rare in children. INH competes with vitamin B6's (pyridoxine) action as a cofactor in synaptic neurotransmitter biosynthesis [38]. Dose-dependent neurologic toxicity is less common in children than in adults, which results in peripheral neuropathy, clonic seizures and paresthesia [39]. These effects are magnified in children co-infected with HIV or who have vitamin deficiencies. Pyridoxine supplementation is recommended in children whom are severely malnourished or co-infected with HIV [33]. Dosage of INH is dependent on a specific genotype (N-acetyltransferase 2) of a patient population, as it pertains to their rate of acetylation [28]. Slow acetylators are associated with more toxicity issues [36, 40] than intermediate or fast acetylators and genotype differs amongst ethnic groups. Hepatitis is the most severe reaction to INH, with rare reports occurring in children receiving low doses [41, 42]. However, INH is commonly given prophylactically to help clear latent infections. Systemic hepatotoxicity is rare in children and shown in rather large patient studies to have no effect on discontinuation of therapy [43]. Cases that do devolve into liver dysfunction can result in organ failure and require transplantation [44, 45].

RIF is notorious for wreaking havoc on other drugs co-administered with it if they are subject to hepatic metabolism. RIF is a potent ligand for the nuclear hormone receptor PXR, an inducer of Cytochrome p450s (notably 2C9 and 3A4), P-glycoprotein (Pgp or MDR1), ATP binding cassette transporters, lung resistance-related proteins and phase II conjugation enzymes [16, 46–50]. The most troublesome effect is the induction of CYP3A4, which is responsible for the metabolism of many anti-retroviral protease inhibitors, making co-administration of TB and HIV drugs problematic in many parts of the world where therapeutic options for both conditions are limited. There is very limited evidence of RIF having adverse hepatic effects when administered alone in children. A common, albeit benign, side effect of RIF is the discoloration of bodily fluid to an orange-red color that may impart some social stigma. Other rifamycins are being developed with similar potency as RIF but with fewer pharmacokinetic disturbances and greater plasma exposure that are discussed later in this review.

An integral part of the intensive treatment phase of pediatric and adult drug susceptible TB infections is PZA. PZA is a unique drug that barely exhibits any anti-tubercular activity *in vitro*. However, its introduction to front-line therapy reduced therapy times by three months. PZA requires acidic conditions such as inside a macrophage and in granulomas to obtain optimum activity, the exact mechanism of action of pyrazinamide remains a subject of conjecture [51], but it has been hypothesized that bioactivation by pyrazinamidase to pyrazinoic acid within the M.tb cell causes disruption in membrane potential and energy production [52]. The commonly associated complications with PZA therapy are GI distress, hepatotoxicity and joint discomfort. In adults, hepatotoxicity is dose and duration of therapy dependent [36]. Hepatotoxicity from PZA has been shown to be relatively rare in children with little alteration in hepatic enzyme function [30, 53]. Joint problems stem from non-gouty polyarthralgia and gout-induced arthritis via decreasing renal excretion of uric acid. It has not been studied in children, but in adults there have been slight increases of systemic uric acid levels leading to arthritis symptoms.

EMB was developed as a replacement for Thioacetazone in front-line therapy and serves a very similar role in the combination. EMB is only moderately active against M.tb but plays an important role in suppression of resistance emergence in the drug cocktail. Thioacetazone is a thiosemicarbazone that can cause extreme skin reactions, such as lethal Stevens-Johnson reactions, in HIV co-infected patients. Its use is now limited to the most drug-resistant MDR/XDR infections. EMB is not without toxicity concerns, being contraindicated in use for pediatrics up until several reviews of pharmacokinetics, efficacy and toxicity by the WHO and others [54–56]. The biggest concern with EMB use is the development of optic neuritis, an inflammation of the optic nerve [57]. In the case of EMB-induced neuritis, the inflammation occurs at the retrobulbar (posterior) of the nerve and thus is difficult to detect via normal ophthalmoscopic examination. Disease-state diagnosis is very dependent on patient observation and communication of a loss of visual acuity and color vision for which there is difficulty in tracking such an adverse effect in young children (under the age of five) due to their inability to either comprehend changes in their vision or articulate them to a healthcare provider [55, 57]. Retrobulbar neuritis is reversible, if detected early, and therefore early signs of the disease-state progression are imperative. Recent advances in detection could allow for rapid detection of nerve damage and thus discontinuation of

treatment. Specifically the recording visual-evoked potentials (VEPs) in children under the age of five has been tested to see if early detection is possible without being reliant on patient communication of distress [58]. Ocular toxicity with EMB is dose and duration of therapy dependent, with 40% of adults developing symptoms at doses greater than 50 mg/kg and 0–3% at 15 mg/kg [57].

1.3. Second-line agents: Adverse events associated with drug-resistant infection therapy

Second-line therapeutics for treating drug-resistant TB have their own unique, and often worse, adverse events especially in children with toxicity occurring in up to 40% of cases [59]. Any treatment for multi or extensively drug-resistant pathogens will always have a slightly larger toleration to adverse events just because the disease state is much more life threatening. The use of one injectable antibiotic is standard in second-line therapy for drug-resistant infections. Second-line therapy is now often supplemented with broad spectrum antibiotics such as fluoroquinolones and Linezolid which have a better efficacy and safety profile than historic second-line agents such as Ethionamide [60–66].

Important recent studies have begun to focus on developing optimal regimens for the pediatric MDR-TB population. These studies include an examination of pharmacokinetics specific to pediatrics. There are studies of aminoglycosides given short course through IV, but little data on the kinetic profile of those dosed long-term, as in the case with TB therapy, which is generally given intramuscularly (IM) [67, 68]. Injectable agents commonly include aminoglycosides such as Amikacin and Kanamycin and the cyclic polypeptide Capreomycin. Typically the aminoglycosides are given first with favor going towards Amikacin, due to its more potent MIC, with Capreomycin withheld for XDR infections [23, 69]. With a high degree of cross-resistance noted between the aminoglycosides, if resistance develops to one agent, it is generally recommended to progress to Capreomycin or another class. The injectable antibiotics are associated with extensive ototoxicity. As discussed before, when dealing with pediatrics, especially infants and very young children, diagnosis of these adverse effects are quite difficult. The next class for MDR infections are the fluoroquinolones, most common being Moxifloxacin and Levofloxacin. Fluoroquinolones have excellent orally bioavailability and can be administered PO daily, are highly active and display little cross resistance to other TB therapeutics [70]. The fluoroquinolone class of antibiotics has well documented toxicity issues from their wide ranging use, most notably peripheral neuropathy, weakening and inflammation of tendons, dysglycemia and prolongation of QT intervals with associated cardiotoxicity. Other agents used for the treatment of MDR and XDR-TB are listed with their noted adverse events in Figure 3. The need to develop new therapeutics to treat drug-resistant TB without these negative events is imperative if we want to not only cure children afflicted by MDR-TB, but also increase their subsequent quality of life after beating their infection.

1.4. Disease progression in pediatrics

The TB disease progresses differently in pediatrics than it does in an adult population. The majority of children will progress rapidly (inside a year) from exposure to the development of the disease and many develop extra pulmonary disease infections. There are correlations in the age range of children and disease progression rates [1, 24]. The most staggering age

group is infants with an estimated 50% progression from either neonatal or early exposure, five-fold higher percentage than the average adult progression. Young people are much more likely to develop more severe cases of extra-pulmonary TB most commonly in the superficial lymph nodes and the central nervous system (CNS) including TB meningitis [25]. Other conditions common to pediatric populations have a serious effect on the disease burden, including malnourishment, other pulmonary disorders (pneumonia, asthma, etc.), diabetes and HIV co-infection[10].

The difference in disease progression is due to many complicating factors but can most easily be attributed to the relative immune system immaturity in children versus adults [11, 25, 71, 72]. Mycobacterium recognition by macrophages and dendritic cells is impaired in pediatrics which disrupts the link between innate and adaptive immunity. Dendritic cells are responsible for sensitizing naïve T cells to the mycobacterial antigens resulting from innate response. Both cell types recognize mycobacterial surface peptides via pathogen recognition receptors (PRRs) that stimulate the biosynthesis of several cytokines and chemokines [73]. It has been shown there is a significant decrease in macrophage phagocytosis in infants than in adults with similar levels of intracellular killing [74], lending credence to the lack of recognition by the macrophage. Children with active TB fail to mount effective type 1, type 2 and type 17 cytokine responses [12]. CD4+ T lymphocytes associated with type 1 cytokines have been shown to be critically important in protective immunity from the TB disease state [72, 75]. In addition, interleukin 12 (IL-12), gamma interferon (IFN- γ), tumor necrosis factor alpha (TNF- α) and natural killer (NK) cells play roles in induction and maintenance of adaptive immune response [71, 72, 76] and have been shown to be suppressed in pediatrics with TB. Children with TB meningitis are characterized with suppressed IFN- γ and IL-17 production [75], which is evidence supporting that lack of immune response leads to quicker and more frequent disease progression to more serious conditions.

Malnutrition is rampant in areas most affected by TB with children often taking the brunt of both epidemics [77]. There is high correlation with growing evidence that genes related to vitamin metabolism contribute to TB susceptibility [14, 78, 79]. Vitamin D receptors (VDRs) are soluble nuclear receptors found in many immune cells and are an example of this nutrient regulation of immune response to TB. VDRs play a role in cytokine secretion, development of dendritic cells, and the function of effector and regulatory T-cells [78]. Several polymorphisms have been shown to have effects on TB risk and disease outcome [79]. Activation of the Toll-like receptor (TLR) 2/1 by mycobacterium antigen results in expression of VDR and 1- α vitamin D hydroxylase. This enzyme activates the monohydroxyl vitamin D to its active 1,25 dihydroxyl vitamin D, the ligand for VDR which then forms a heterodimer with retinoid X receptor (RXR). This complex is responsible for the formation of LL-37, a cathelicidin protein with known antimicrobial activity while also recruiting other immune cells to the site of the infection [78]. Innate immunity has also been shown to be age-dependent with newborns being dependent on maternal antibodies to compensate for immature antigen-presenting cells, neutrophils, and TLRs [80, 81]. Breast milk has important immune function, containing important immune factors such as: lysozyme, defensins, lactoferrin, soluble CD14, cytokines, antiviral lipids and triglycerides that can be partially digested to free fatty acids that are toxic to many pathogens [82]. Also

breast milk is important in commensal bacterial colony formation, something that precludes other pathogens from residing in the mucus layers of the respiratory tract. Studies have shown that limited non-exclusive breast feeding increases the risk of respiratory tract infections [14, 83].

1.5. Pharmacokinetics and Pharmacodynamics of pediatrics

The differences in pharmacokinetics between children and adults are well-noted and well-studied across many drug classes and disease states [30–32, 67, 84–88]. Children tend to metabolize drugs quicker, have altered drug absorption, different tissue distributions, higher unbound drug concentrations and have differences in total body water volume when compared to adults. These differences can generally be tracked across five different age categories established by the International Conference on Harmonisation of technical requirements for registration of pharmaceuticals for human use (ICH) in 2000; preterm -, term - newborns, infants and toddlers, children and adolescents. In general, drug absorption is altered in neonates and infants more than other ages, primarily due to gastric acid excretion and gastric emptying differences [86, 89, 90]. Acid labile drugs such as beta-lactams are more readily absorbed in neonates and infants via oral administration compared to adults due to relative neutrality of stomach pH until a child reaches adult-like levels around the age of 3 [91, 92]. Gastric emptying is slower in neonates and infants as well, resulting in a slower rate of drug removal from the stomach [90, 92]. This will not only result in a lower peak concentration of drug, but also a delay in the time required to achieve maximum concentration. Although this phenomena rarely affects the area under the concentration curve of total drug exposure, when dealing with antibiotics, maintaining a concentration above the MIC is often required for complete eradication which could be affected without proper dose adjustments.

Drug distribution of therapeutics varies drastically throughout all stages of life, due to alterations of tissue concentrations with respect to age, changes in membrane permeability and the affect that plays on CNS concentrations, alteration in plasma protein binding affinity and changes in extracellular fluid levels compared to total body water [85, 93]. Membrane permeability is high in immature neonates, as the blood brain barrier has not yet fully developed. This is important in drug regimen planning in neonates with pulmonary and extra pulmonary TB infections, especially meningitis as the distribution of therapeutic agents will be different in the CNS than adult PK estimations would predict. Plasma protein binding is a contentious issue in the drug development world with leaders having very different opinions in the importance of plasma-bound drug concentrations [94, 95]. In general, acidic drugs will bind to albumin where basic drugs are a little more promiscuous in binding to globulin, α -acid glycoprotein (AAG) and lipoproteins. Levels of plasma protein fluctuate with age, with unbound fractions generally higher in all pediatrics compared to adults [96]. Neonates express lower levels of serum albumin levels compared to adults, levels that approach adult levels within a year and fetal albumin displays lower binding affinities for many drugs [97]. Pure plasma concentrations are not indicative in predicting tissue perfusion, especially to specific and difficult-to-reach tissue. Similar to solid tumors, necrotic pulmonary lesions are poorly vascularized and make drug penetration difficult. However, advanced *in vivo* modeling of cell-specific concentrations can be very helpful in fleshing out where drugs are

getting to and in what concentrations [98–102]. It was shown using non-compartmentalized analysis of rabbit tuberculosis that a phase III agent, Moxifloxacin, exhibited several-fold higher concentrations in the lesion than predicted by a mixed-effect PK model system [98]. While Moxifloxacin accumulates in granulomas, front-line drugs such as RIF exhibited a fraction of their plasma concentrations. Coupling this *in vivo* model in rabbits with advances in mass spectroscopy (MALDI-MSI) and drug distribution imaging [103–105], it is much easier to quantify the relevant concentrations of anti-TB drugs at sites specific to various states of TB infection. This could be especially important in developing regimens specific for pediatric populations, as their disease state rapidly disseminates and is found extra pulmonary in a myriad of tissues. To be able to quantify and optimize a therapy for a specific state of TB infection would be a powerful tool in more customizable treatments. Another factor that affects drug disposition is the amount of water in the body relative to body weight. Neonates exhibit a very high percentage of water (80%) compared to an adult who decreases to around 60%. This will result in a higher volume of distribution in childhood than in an adult, also affected by the relative abundance of adipose tissue at various stages of life. This is particularly important in the TB drug discovery paradigm, as many active compounds coming down the discovery pipeline are highly lipophilic.

Metabolic profiles vary greatly with respect to age, most pronounced again in neonates and infants. There has been a great surge in knowledge for the expression profiles of the most important drug metabolizing enzymes [106], including the cytochrome p450's that play a critical role in TB drug metabolism. Perhaps the most important enzyme for all TB populations is the CYP3A4 enzyme, induced by RIF and responsible for the metabolism of several clinically relevant agents. This enzyme is expressed at very low levels at birth, with rapid progression to 30–60% of adult levels within a week and full expression at one year [107]. Drug metabolism only gets more complicated with co-infection with HIV, an unfortunately common occurrence. The need for a front-line agent with fewer pharmacokinetic interactions than the ones exhibited by RIF is clear.

1.6. Formulations and FDCs

One of the easiest ways to improve patient compliance in all populations is to reduce the pill burden one has to receive [108]. This can be achieved in fixed dose combinations (FDCs) in which combinations of therapeutics are compounded into one pill for a scheduled dose. However, most FDCs are designed around adult dosing ranges and often must be crudely cut into an estimated proportion to match dosing parameters of a child. The WHO did release guidance about dosing with currently available FDCs [109]. With more knowledge emerging about the differences in pediatric pharmacokinetics and drug exposure levels at various tissues, fixed dose regimens can and should be created with pediatrics in mind. This will undoubtedly place a burden on the manufacturing practices in place currently, but the ramifications of easy-to-dispense, tailor-made combinations could be immeasurable. The ability to deliver the needed medications in the correct proportions to the places that need them most could lead to faster and more complete sterilization of endemic infections and slow disease progression.

Another way to ease the burden on children is improved formulations. Many of the second-line agents are injectable and highly unpleasant, making treating MDR and XDR infections difficult. Even the oral agents can be difficult to take, mainly due to the large size and number of pills. Emulsions and powder food additives are viable options in increasing patient compliance in fulfilling a full duration of therapy providing there are no taste issues such as bitterness associated with the drugs.

2. NEW DRUGS

The discovery and development of new anti-TB agents has seen a relative uptick in activity in recent years. This increased activity can be attributed to increased awareness of the global disease burden and the rise of MDR and XDR strains lending credence to the fear of a potential new global epidemic comprised of drug-resistant strains. However, simply identifying the most potent new compound *in vitro* is not good enough, as is the case in many drug development areas the issue has more nuance. In order for a new compound to be adopted into clinical use, it should have a profile containing most of the following metrics: unique mechanism of action to belay cross resistance, oral bioavailability with once-a-day dosing pharmacokinetic properties, ability to shorten the current duration of therapy, a high safety profile and compatibility with other partner anti-tubercular and anti-HIV agents and low cost of production. Each of these conditions stems from unique dilemmas associated with treating TB; from the long duration of treatment and M.tb latency to the low affluence of the majority affected and prevalence of co-morbidities including HIV co-infection. This section of the review of the new drugs has been broken down to three categories: newly approved agents in the clinic, repurposed antibiotics historically used to treat other infections and new agents in the preclinical pipeline.

2.1. New agents approved for adult TB indications already in the Clinic

Bedaquiline (Figure 4) is the first novel class of TB drug approved in over 40 years. Bedaquiline (Sirturo or TMC207) is a member of the Diarylquinoline class of drugs and acts as an ATP synthase inhibitor by binding to the c subunit [110, 111] resulting in ATP depletion and pH imbalances inside the organism [112]. The c subunit in mycobacterium ATP Synthase differs by three amino acid residues from its human counterpart forming a unique binding site for Bedaquiline garnering its selectivity for the mycobacterium [113]. Discovered via phenotypic screening [112] by Janssen Pharmaceutica and manufactured by Johnson and Johnson, it was approved on December 28th, 2012 by the FDA to treat MDR-TB [114].

It was potent and selective pre-clinically in both drug sensitive (MIC 0.03 mg/mL) and drug resistant (MIC 0.12 mg/mL) isolates in both acute and chronic infections [112]. Murine *in vivo* models showed a robust reduction in treatment time compared to background combination therapy [64, 115, 116], thus Bedaquiline was pushed into combination optimization clinical trials for pulmonary MDR-TB in adults which lead to its approval. Bedaquiline is a large lipophilic drug which is subject to CYP metabolism, and not surprisingly, it has been noted to have pharmacokinetic interactions with Rifampacin. Bedaquiline use is associated with cardiac liability of QT prolongation and increased

mortality which is the primary reason why, for now, Bedaquiline use is limited to MDR patients [114]. There is limited data available in regards to treatment of pediatric TB cases with Bedaquiline to date; however studies in the future are looking to include pediatric as well as geriatric populations. The International Maternal Pediatric Adolescent AIDS Clinical Trials Network (IMPAACT), a division of the NIH, is developing a protocol to study Bedaquiline in children with MDR-TB with and without HIV (study P1108, www.impaactnetwork.org/studies/).

Delamanid (Figure 5) was also recently approved for the treatment of MDR-TB by the European Union and has shown promising early bactericidal activity (EBA) with a significantly higher sputum culture conversion when added to the first-line regimen [117]. An important point to make here, that holds true for most drugs discussed here in this review, is that most current clinical trials are run through adult patients. There has, however, recently been a case study on compassionate Delamanid use on an Italian 12-year-old that displayed a positive outcome [118], but few long-term conclusions can be drawn from this isolated example.

This lack of available data makes dosing parameters for children difficult to predict and safety profiles are very different from adult to children. Delamanid is in the Nitroimidazole class, a class that also has TBA-354 and Pretomanid (PA-824), the latter being in late stage clinical trials for MDR-TB [63, 119, 120] as a part of novel optimized treatment regimens (OTRs). It was shown by Singh et. Al. in 2008 that these nitroimidazoles killed non-replicating TB via an intracellular release of toxic nitric oxide by a mycobacterium specific enzyme, F₄₂₀-deazaflavin-dependent nitroreductase and disruption of mycolic acid biosynthesis [121].

Both Pretomanid and Delamanid have potent early bactericidal activity (EBA) in humans and have been shown in several *in vivo* models and human trials to clear lungs of bacteria faster than the current front-line drug course when compared to a placebo plus background MDR regimen [122]. Delamanid like Bedaquiline is also a large lipophilic, highly protein-bound drug and has also demonstrated a QT prolongation liability.

2.2. Repurposing antibiotics to treat pediatric TB

Perhaps the most practical approach in developing new agents for pediatric TB is repurposing and optimizing classes of approved antibiotics traditionally used to treat other infections that have a proven safety record in children. These compounds have already undergone significant amounts of clinical safety tests and are generally tolerated in multiple populations. An advantage from the perspective of this review is that some of these compounds will have pediatric PK and dosing studies, something lacking in new TB drug regimen development. This data will not wholly be indicative for childhood TB clearance and safety profiles and could miss drug-drug interactions associated toxicities with other TB drugs. However, it is at least a starting point for future studies to begin from. The existence of clinical data from other antimicrobial applications also is attractive to bringing pharmaceutical companies back into the TB development field, as the cost of running some of the safety studies has already been removed and such application may be suitable for

compassionate use programs from which the company may benefit from positive public relations. The two most advanced examples are Linezolid and Moxifloxacin.

The oxazolidinone Linezolid was originally developed to treat resistant strains of Gram-positive bacterial infections and is notable for its excellent pharmacokinetic properties and ability to penetrate the lung. Linezolid has been found to have efficacy against highly resistant strains of TB. However, its use is limited in duration due to issues of myelotoxicity resulting from inhibition of mitochondrial protein synthesis. It should be noted that typical *in vitro* and *in vivo* pre-clinical testing in mice may have deprioritized Linezolid, as it is only moderately efficacious against drug-susceptible TB compared to other emerging anti-tubercular agents [123]. However, Linezolid has recently showed promising activity treating adult MDR patients even as a mono-therapy [124]. There also have been important recent reports of Linezolid use for the treatment of MDR and XDR-TB infections in children, summarized by Garcia-Prats et al. [125]. In this important study, there is a detailed breakdown of the pharmacokinetic properties of Linezolid in infected children, as well as some noted adverse events and recommendations for use. As known previously, Linezolid is excellently orally bioavailable. It is noted with adverse GI tract complications, hematologic events (myelosuppression) and cases of peripheral and optic neuropathy with long course therapy. In the pooled 18 children, at least one adverse event occurred in nine patients with five requiring a dose reduction and two discontinued treatment. It was this group's suggestion that due to the cost and noted adverse events associated with Linezolid in children, that it should be reserved for XDR infections or pre-XDR (MDR plus resistance to fluoroquinolone OR injectable second line). An interesting note for this review is they also recommend this therapy additive in the cases of disseminated disease, especially in MDR TB-meningitis, due to Linezolid's high cerebral spinal fluid penetration.

Moxifloxacin (Figure 6) is a fluoroquinolone in Phase III efficacy trials for drug-sensitive and drug-resistant pulmonary infections in adults, as part of new combination therapy as well as currently being used as a second-line agent in standard MDR treatment. Fluoroquinolones exhibit their efficacy via interference with bacterial DNA replication, transcription and repair by inhibition of DNA gyrase and Topoisomerase IV [126–128]. Discovered in a drug repurposing study, Moxifloxacin has previously been used to treat respiratory tract infections. Gatifloxacin is another drug in this class under repurposing investigation, but has a black box indication due to dysglycemia and all later generation fluoroquinolones that carry a risk of QT interval prolongation.

Other fluoroquinolones are used in MDR-TB infections as second-line agents. However, Moxifloxacin has the potential to drastically reduce therapy time for drug-susceptible infections, when substituted into the current front line therapy for Isoniazid in mice [116]. The TB Alliance has sponsored a study called the Shortening Treatment by Advancing Novel Drugs (STAND), the purpose being to assess the combination of Moxifloxacin, Pretomanid and Pyrazinamide compared to the current front-line. If proven to be equally or more efficacious to standard care, it could stand as a huge breakthrough for HIV/TB co-infected patients, as RIF would not be involved in the therapy to interact with anti-retrovirals common to HIV treatments. There is little data for the treatment of drug-susceptible TB with fluoroquinolones in pediatrics, but there is a recent review of the treatment of MDR-TB in

pediatrics [128] with an example regimen largely containing Ofloxacin (96%) for 13 months that achieved a cure in 137 of 149 children [129] and several others that achieved similar positive outcomes using Levofloxacin and Moxifloxacin. This recent influx of pediatric data for these repurposed drugs holds great promise as these classes are currently undergoing optimization for their anti-TB efficacy.

2.3. New TB Agents in clinical trials

In addition to these new clinically approved drugs, a host of novel chemical entities are advancing down the gauntlet of late-phase clinical trials and we will discuss their discovery and potential applications in childhood TB. Lastly, we will focus on subsets of novel classes of agents in pre-clinical studies and some aspects of the biology behind their potential in being translational.

2.3.1. 2nd Generation oxazolidinones—Recent clinical trials have shown Linezolid has promising efficacy in treatment of MDR-TB infections including in children. These results have encouraged further examination of the oxazolidinones class of antibiotics for the treatment of TB. [124, 125, 130–132]. Only two agents, Linezolid and Terazolid, are clinically approved for the treatment of gram positive infections. This is a well-explored medicinal chemistry class and a number of second-generation preclinical leads have shown promising anti-tubercular activity and are being repurposed for this indication.

The primary driver is to select agents with a larger therapeutic window, i.e. great TB potency and less relative inhibition of mitochondrial protein biosynthesis.

This approach has led to the advancement of two agents, Sutezolid and AZD5847 (Figure 8) into late-stage clinical trials. Sutezolid (PNU-100480) possess greater tubercularicidal activity than Linezolid and has been found to have additive effects with Bedaquiline and SQ109, but not with nitroimidazoles [133]. With Linezolid shown to be extremely helpful for DR-TB in pediatric populations [125], the improvements in efficacy and mycobacterium selectively that Sutezolid offers is a promising step towards developing better and novel combinations.

2.3.2. Rifapentine/High dose Rifampicin—An analog of Rifampicin, Rifapentine (Figure 9) is in both Phase II and Phase III studies for the treatment of drug-susceptible TB in both its active and latent forms.

All Rifamycins target the beta sub-unit of RNA polymerase and in doing so prevent translation from occurring. Rifapentine is a semisynthetic analog of Rifampicin that exhibits a much longer half-life which allows for a reduced-dosing frequency. [134, 135]. However, Rifapentine does exhibit the same robust induction of many metabolizing enzymes as Rifampicin, so its concurrent use in HIV/TB co-therapy would be mitigated as well as it would be expected to have a deleterious effect with Bedaquiline and other CYP substrates. In addition to the development of new Rifamycins, studies have shown that higher doses of Rifampicin could lead to higher EBA and a potential to shorten the duration of therapy for drug susceptible infections [136] though the safety ramifications, especially for children of such treatment, is not yet known.

2.3.3. Ethylenediamines and MmpL3 Inhibitors—The Ethylenediamine SQ109 (Figure 10) reached phase II clinical trials though its current development status is unclear. The ethylenediamines are thought to target mycolic acid biosynthesis via inhibition of the transport protein MmpL3 [137–140]. MmpL3 is required for the export of mycolic acids to the outer portion of the cell wall as trehalose monomycolates, a vitally important and unique aspect of the mycobacterium's innate durability.

MmpL3 inhibitors are frequently found in phenotypic screening; however some of these compounds (including SQ109) have been shown to have activity against bacteria and fungi that do not produce mycolic acids [141, 142], as well as possessing activity against nonreplicating cells [143, 144]. This has led many to believe that MmpL3 inhibition may only be partially responsible for SQ109 and several others in the class' activity; it has been shown that SQ109 also inhibits menaquinone synthesis, cellular respiration and ATP synthesis [144]. Perhaps the most important method of cellular disruption occurs via dissipation of the transmembrane proton concentration gradient which can interrupt membrane potential [142] causing a deleterious effect on MmpL proteins. This might help explain the synergy that SQ109 exhibits with most anti-TB drugs *in vivo*, as many MmpL proteins have been associated with efflux mediated by a proton antiport [145–147]. SQ109 was designed in a combinatorial chemistry effort around the 1,2-ethylenediamine pharmacophore of EMB. EMB has long been considered the weakest aspect of front-line therapy and the prevailing wisdom is that replacement with a stronger antimicrobial agent would drastically reduce therapy times [138, 139]. SQ109 shows excellent tissue distribution in the lungs and excellent *in vitro* potency, as well as being found safe and well-tolerated in phase I and II clinical trials. The now common place approach of direct MIC screening of vast libraries followed by resistant mutant sequencing efforts to identify the mechanism of action of the phenotypic hits often finds potential MmpL3 inhibitors [148]. This abundance in chemical activity has helped identify many diverse MmpL3 inhibitors, some with better physicochemical properties than SQ109 for which optimization and preclinical testing is ongoing by multiple groups [149, 150].

2.4. New early-stage antitubercular chemotypes with demonstrated *in vivo* efficacy

To conclude our examination of future trends in anti-TB drugs, it is important to note that there are many promising new chemical classes of anti-TB agents that populate an expanding list of advanced preclinical drug candidates (Figure 11). The field as a whole has a promising outlook with broad chemical diversity being displayed in newly identified lead compounds. Phenotypic screening is by and large the most successful tool in this renaissance of discovery [112, 151, 152], empowered greatly by advances in genomic and structural knowledge of druggable targets [153]. The most common targets identified in TB via typical phenotypic whole-cell screening are MmpL3, DprE1 and QcrB [150, 154, 155] and it comes as no surprise that many of the advanced drugs under review and undergoing optimization hit these molecular targets. However, there are a host of targets missed by this approach as it tends to focus on extracellular and membrane bound targets [148]. In order to properly address the emerging threat of drug-resistant mycobacterium, no viable targets should be ignored and thus steps have been taken to advance screening techniques [156, 157]. Screens

can be designed to exploit the most sensitive drug targets, target persisting or latent TB [158] and encompass knowledge of common influx porins and efflux pumps [158–161].

This wealth of novel anti-TB drugs includes inhibitors of Decaprenylphosphoryl-b-D-ribose 2'-epimerase 1 (DprE1) which was first identified as the target of 1,3-benzothiazin-4-ones, which are extremely potent antimicrobials that kill *M.tb* *in vitro*, *ex vivo* and in mouse models of TB with low nanomolar activity [162]. PBTZ169, the lead molecule in this series, is an irreversible inhibitor that requires activation of the nitro group for anti-tubercular activity. An alternative class of DprE1 inhibitors, 1,4-Azaindoles (Figure 11) was identified via a scaffold-morphing approach by AstraZeneca[163]. This compound class inhibits DprE1 via a non-covalent interaction and exhibits efficacy in both 'acute' and 'chronic' TB mouse models. This class also shows good oral bioavailability with AUCs ranging from 200 to 700 $\mu\text{M}/\text{h}$ and free plasma concentrations maintaining above the MIC for 10–24 h (%f T > MIC of 25–100). With its *in vivo* efficacy and drug-like properties, azaindoles are under lead-optimization process for potential against both drug-sensitive and drug resistant TB [163].

InhA enzyme, the fourth enzyme of the type II fatty acid synthase system (FAS II), is one of the key enzymes involved in the elongation cycle of fatty acids in TB. The front-line drug, INH is a pro-drug, which is activated within the mycobacterial cell by the KatG catalase to form an INH-NAD (H) adduct. This adduct inhibits mycolic acid biosynthesis via targeting InhA [164, 165]. The majority of INH-resistant clinical strains result from the emergence of KatG mutants that block the formation of the INH-NAD adduct [166]. The design and development of direct acting InhA inhibitors that bypass KatG activation are promising candidates to combat MDR TB, especially given the historic tractability of other FabI discovery programs. GlaxoSmithKline discovered a series of thiadiazole-based compounds as InhA inhibitors by a high-throughput phenotypic screening [167, 168]. The thiadiazoles (GSK InhA Inhibitor, Figure 11) show low nanomolar InhA inhibition activity (the best compound with an IC_{50} of 43 nM, *M.tb* MIC of 1 μM) and good artificial membrane permeability. The optimization of this potent and selective class of InhA inhibitors provided compounds orally efficacious in *M.tb* infections in mouse models which are undergoing further development.

The third commonly discovered molecular target in HTS phenotypic screening is the cytochrome *bc₁* subunit (qcrB), an essential component of mycobacterial respiration. QcrB catalyzes the transfer of electrons from ubiquinol to cytochrome *c*, a process proven essential for ATP synthesis in *M.tb* and one that if targeted could provide an excellent therapeutic option for latent TB infections. One of the most advanced compounds in this class is Q203, an optimized lead from a phenotypic screen in infected macrophages [169]. Q203 exhibits robust activity in susceptible isolates as well as MDR and XDR clinical strains. It has also been shown to interfere with ATP homeostasis in latent TB colonies and displayed intracellular reduction of ATP levels significantly greater than Bedaquiline. With a clean CYP and PGP profile, Q203 seems to be a prime combination candidate going forward. Another advantage is the ease of synthesis and cost of material [170] making this class an affordable series for treatment of such a third-world affliction.

A promising new class of compounds that could be of specific interest to the pediatric field are the oxaborole-based leucyl-tRNA synthetase (LeuRS) inhibitors being pursued by Anacor [171]. Other tRNA synthetases have been the subject of antimicrobial investigation [172] and have displayed promise as a novel class of antitubercular agents. Similar oxaboroles have been shown to have activity against late-stage central nervous system *African Trypanosomiasis* in mice, which is indicative of good brain penetration and could be relevant in the development of TB meningitis treatment [173].

Spectinomycin is an inhibitor of bacterial protein translation with a distinct mechanism of action from other protein synthesis inhibitors. Unfortunately, it does not display good anti-tubercular activity because it is actively pumped out of the M.tb cell by the Rv1258c efflux pump. Recent efforts have identified semi-synthetic spectinamide derivatives such as 1599 (Figure 11) that overcomes the native efflux and show potent bacterial ribosomal inhibition [161] no cross resistance to other ribosomal targeted drugs used to treat TB and robust efficacy in chronic infection models of TB infection. Most importantly, these agents do not inhibit mitochondrial protein biosynthesis and are likely to lack the ototoxicity side effects of other aminoglycosides. Strong evidence suggests that the Rv1258c pump is up-regulated in MDR-TB strains and during infection, thus making a class of anti-TB drugs that avoid this mechanism a candidate for treatment of chronic infections

3. Summary and discussion

TB remains a grave mortal threat to the children of endemic countries, despite decades of effort dedicated to eliminating this disease. This troubling situation is being compounded by the rise of MDR and XDR-TB strains and HIV co-infection. Recent, increased awareness of this problem led by groups such as the Global TB Alliance, Stop TB Partnership and WHO combined with a plethora of government institutions and input from leaders in the pharmaceutical industry has started to provide this significant problem with the appropriate attention it deserves. The recent implementation of pediatric specific clinical trials, not only with new agents but for existing and repurposed drugs in pharmacokinetically optimized combination studies, increases the likelihood that better pediatric regimens will be developed and implemented soon. The growing pipeline of new anti-tuberculosis agents, that meet modern (higher) safety standards, also offers significant future potential to improve clinical safety and patient outcomes, especially for pediatric TB patients with drug-resistant infections that are currently treated with poorly efficacious and toxic second-line agents.

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Figure 1.
The structure of current front line TB drugs.

Initial Drug Resistance Diagnosis

Figure 2.

General progression of agent addition to MDR-TB infection regimen. Always add one agent until four active agents are being used. *Streptomycin used only if resistance to Amikacin, Kanamycin, and Capreomycin is noted.

Group	Group name	Drugs	Adverse Effects
2	Injectable Agents	Amikacin	Vestibular toxicity, nephrotoxicity, auditory toxicity
		Kanamycin	Vestibular toxicity, nephrotoxicity, auditory toxicity
		Capreomycin	Vestibular toxicity, nephrotoxicity, auditory toxicity
		Streptomycin	Vestibular toxicity, nephrotoxicity, auditory toxicity
3	Fluoroquinolones	Moxifloxacin	Sleep disturbance, GI distress, arthritis, peripheral neuropathy, prolonged QT
		Levofloxacin	Sleep disturbance, GI distress, arthritis, peripheral neuropathy
		Ofloxacin	Sleep disturbance, GI distress, arthritis, peripheral neuropathy
4	Bacteriostatic Second-line (Oral)	Ethionamide	GI distress, hepatotoxicity, hypersensitivity, metallic taste
		Cycloserine	Neurological distress, psychosis, convulsions, rash, drug interactions
		Para-aminosalicylic acid	GI distress, hypersensitivity, hepatotoxicity, sodium load

5		Clofazimine		GI distress, skin discoloration, organ damage due to crystal deposition
		Linezolid		GI distress, headache, myelosuppression, neurotoxicity, lactic acidosis, pancreatitis
		High dose INH		Hepatitis, peripheral neuropathy
	Unclear M.o.A or significant adverse events	β-lactam/ enzyme inhibitor		GI intolerance, hypersensitivity, seizures, liver, renal dysfunction
		Amoxicillin Meropenem Imipenem		Stevens Johnson Syndrome w/HIV co-infection, GI intolerance, hepatitis
		Thiacetazone		GI intolerance, rash, hepatitis, prolonged QT, ventricular arrhythmias
		Clarithromycin		

Figure 3. Groups of commonly administered agents for MDR and XDR infections and their noted adverse events in children.

Figure 4.

Bedaquiline, a new class of anti-TB drug recently approved for MDR-TB infections.

Figure 5.
The nitroimidazoles currently in the clinic or in clinical trials.

Figure 6.
Moxifloxacin, a representative fluoroquinolone.

Figure 7. Schematic of the discovery horizon of novel anti-TB compounds to be discussed. Adapted from the Global TB Alliance drug discovery pipeline. (www.newtbdrugs.org)

Figure 8.
Second generation oxzolidinones, designed for lower myelosuppression that also possess greater mycobacterial efficacy.

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Figure 10.

The lead compound from the MmpL3 inhibitors, the ethylenediamine SQ109.

Figure 11.
New anti-tubercular chemotypes demonstrating broad structural diversity and in vivo efficacy.