2023

Novel therapeutics in the treatment of drug-resistant tuberculosis: a review

BOSTON UNIVERSITY

ARAM V. CHOBANIAN & EDWARD AVEDISIAN SCHOOL OF MEDICINE

Thesis

NOVEL THERAPEUTICS IN THE TREATMENT OF DRUG-RESISTANT TUBERCULOSIS: A REVIEW

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B.A., University of Colorado Boulder, 2016

Submitted in partial fulfillment of the

requirements for the degree of

Master of Science

2023

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DEDICATION

I would like to dedicate this work to my patient spouse Beca.

ACKNOWLEDGMENTS

I would like to thank the readers of this thesis who have instilled in me a passion for science as well as perseverance. Thank you to my program director who has always made time to answer short messages or phone calls at any time of day. Lastly, I would like to acknowledge my classmates who were always present and would respond, no matter the question.

NOVEL THERAPEUTICS IN THE TREATMENT

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ABSTRACT

Mycobacterium tuberculosis is the causative agent of Tuberculosis infection (TB). TB is still endemic throughout the World and is a significant cause of morbidity and mortality, killing over 4,000 people every day. Although a number of antibiotics have been available for the treatment of TB since the late 1940s, treatment duration, drug-resistance, and barriers to effective health delivery have thwarted efforts for elimination (and eventually, eradication). Here is a compendium of several novel therapies that are being considered to address this global pandemic.

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LIST OF ABBREVIATIONS

AIC	Autophagy-Inducing Compound
ARDS	Acute Respiratory Distress Syndrome
BDQ	Bedaquiline
CFU	Colony Forming Units
CLR	C-type Lectin Receptor
CML	Chronic Myelogenous Leukemia
COPD	Chronic Obstructive Pulmonary Disorder
DC	Dendritic Cell
DR-TB	Drug-Resistant Tuberculosis
EMA	European Medicines Agency
FDA	U.S. Food and Drug Administration
HDT	Host-Directed Therapy
HIV	Human-Immunodeficiency Virus
MDR-TB	Multi-Drug Resistant Tuberculosis
MET	Metformin
MIC	Minimum-Inhibitory Concentration
Mm	Mycobacterium Marinum
MOMP	Mitochondrial Outer Membrane Permeabilization

MPT	Mitochondrial Permeability Transition
MR	Mannose Receptor
MSC	Mesenchymal Stromal Cells
Mtb	Mycobacterium Tuberculosis
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
NET	Neutrophil Extracellular Trap
NK	Natural Killer Cell
Pa	Pretomanid
PAMP	Pathogen-Associated Molecular Patterns
PBA	4-Phenylbutyrate
PRR	Pattern Recognition Receptors
SSRI	Selective Serotonin Reuptake Inhibitor
TB	Tuberculosis
TDM	Trehalose-6,6-Dimycolate
TK	Tyrosine Kinase
TLR	Toll-Like Receptor
WHO	World Health Organization
XDR-TB	Extensively Drug-Resistant Tuberculosis

INTRODUCTION

Mycobacterium tuberculosis is the responsible agent for tuberculosis (TB), an infectious disease that has co-evolved with mankind for several million years and has evaded current approaches for eradication.[1] The World Health Organization (WHO) estimates that up to a quarter of the global population, 1.8 billion people, are infected with TB; those within two years of infection have a roughly 10 percent risk of progressing to active disease.[2] The majority of TB cases are relegated to the global South, with Southeast Asia, Africa, and the Western Pacific bearing the majority of the global burden of TB. Thirty countries are defined by the WHO as being the highest-burden TB disease countries spanning the entire globe, with incidence rates being highest in Lesotho, South Africa, and the Philippines.[3] Despite the development of effective therapeutic regimens, tuberculosis still kills more people every year than any other infectious disease, second only to COVID-19, and kills more people annually than HIV and malaria combined. If left untreated, TB infection has a mortality rate of over 50%.[4] Up to 35% of HIV-related deaths can be directly attributable to co-infection with mycobacterium tuberculosis, the causative agent for TB.[5] An estimated one million children get TB disease each year, of whom roughly 25% die. The global number of new individuals developing active tuberculosis has declined by

approximately 1.6% per year since 2000—a glacial pace—and there are still an estimated 440,000 new cases of multidrug-resistant tuberculosis developing every year, creating a substantial challenge to public health globally.[6]

Part of the difficulty in treating tuberculosis is the burdensome treatment regimen in curing TB. The standard chemotherapeutic regimen for drug-sensitive tuberculosis consists of four antibiotics (rifampin, isoniazid, pyrazinamide, and ethambutol) taken daily for two months, then two antibiotics (rifampin and isoniazid) taken daily for four months.[7] However, if drug-resistance develops, treatment duration can increase to anywhere from six to 24-months–sometimes with dozens of pills being taken every day along with toxic injectable antibiotics being administered several times a week.[8] The development of drug-resistance is widely variable from patient to patient and even fully adherent patients can develop drug-resistance.[9–15] An alarming proportion of individuals will acquire or develop TB that is resistant to two of the most potent first-line antibiotics, rifampin and isoniazid, termed multi-drug resistant TB or MDR-TB **[Figure 1]**. TB resistant to isoniazid, rifampicin, any fluoroquinolone, and either bedaquiline or linezolid is termed extensively drug resistant TB or XDR-TB.[16] Treatment for drug-resistant forms of TB is extremely difficult and has a wide range of side-effects including ototoxicity, hyperpigmentation,

neuropsychiatric side-effects, hepatotoxicity, and bone marrow toxicity. The global burden of MDR-TB is increasing at an annual rate of more than 20% every year and it is estimated that DR-TB (drug-resistant TB) will kill 75 million people and cost the global economy almost $17 trillion in the next 35 years.[17,18]

Historically, bacterial infections have been a major cause of human mortality since time immemorial, with the oldest documented cases being recorded over 10,000 years ago.[19–24] Antimicrobial agents were first developed in the early 20th century with the advent of arsphenamine (salvarsan), to treat bacterial syphilis and the parasitic infection, African trypanosomiasis; both major infectious scourges to humankind. Since then, hundreds of antimicrobial agents, or "antibiotics," have been developed to be utilized to treat microbial infections. The term antibiotic was first utilized by a naval officer in 1860 to describe an opposition to the belief in life beyond Earth. The term was then co-opted by Professor Selman Waksman to describe streptomycin and its activity against mycobacterium tuberculosis–the first treatment ever developed for the disease. However, it has been a recurring theme throughout the antibiotic era for bacterial infections, mycobacterium tuberculosis being a notable example, to develop resistance to new therapeutics — sometimes even while the new drugs are still undergoing clinical trials. While most of the drug-resistance for TB is

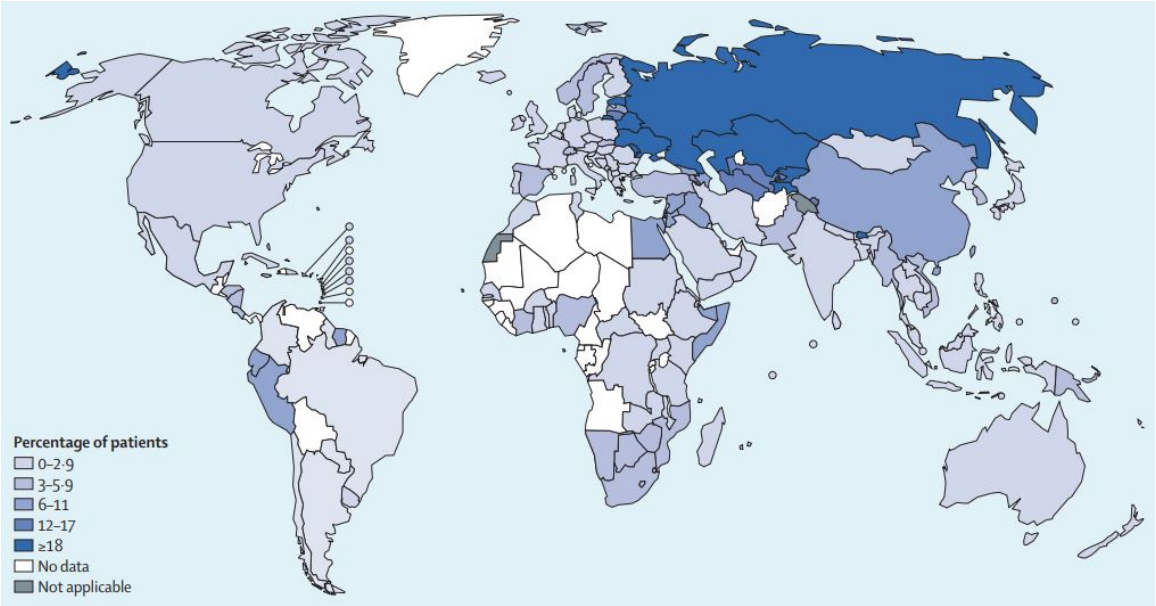

Figure 1 – Percentages of patients with multidrug-resistant tuberculosis globally. Figures are based on the most recent year for which data have been reported to WHO, which varies among countries (2002-2018). Figure taken from Lange 2019.[25]

linked to transmission of resistant strains, the situation is exacerbated by programs that do not invest sufficiently in supports for patient adherence, underfunded and under-supported health systems, and long, difficult therapeutic regimens that challenges even the most disciplined healthcare practitioners and steadfast patients.

Despite the early view of antibiotics as solely agents utilized to kill surrounding microbes, it has been theorized that antibiotics may instead play a role as signaling molecules that modulate the activity of neighboring bacteria to

change their transcriptional profile. Antimicrobial activity has since been understood to be a phenomenon which occurs when those same antibiotics are present in high enough concentrations to kill neighboring bacteria.[26] This lends to the idea that antibiotics, given in insufficient quantities for a pathogenic bacterial infection, may instead serve to modulate a pathogen's transcriptional profile to develop into one of antimicrobial resistance. This is, indeed, what is observed in the clinical setting as insufficient or inconsistent doses of antibiotics, whether due to the administration or adherence by the patient, augments the development of a drug-resistant pathogen, which includes drug-resistant TB. This has been sufficiently documented in a number of clinical settings, including in Uganda where TB in a cohort of individuals treated with antibiotics were found to have, first, a rapid decline in the pathogen itself and, shortly thereafter, a small subpopulation of TB with an altered transcriptional profile suggesting that drug-tolerant pathogens are in a non-growing to slow-growing but metabolically active state termed "quiescence."

Lifecycle of Mycobacterium Tuberculosis

Mycobacterium tuberculosis is an acid-fast bacillus, which features both gram-positive and gram-negative components in its cell wall.[27] It is the causative

agent for tuberculosis, an airborne infectious disease that primarily infects the lungs as pulmonary TB, but can disseminate throughout the body via hematogenous spread (extra-pulmonary TB). The most common sites of extrapulmonary TB include the lymph nodes, pleura, and osteoarticular areas, although any site in the body can be involved.[28] TB is projected from an infected individual into the air, where it is suspended in airborne droplets and aerosols and then inhaled by individuals in the immediate vicinity **[Figure 2]**. Once inhaled, the mycobacterium travels to the lungs where it is often recognized by the host's immune system. More specifically, the innate immune system is able to recognize Mtb (Mycobacterium tuberculosis) through toll-like receptors (TLRs), C-type lectin receptors (CLRs), Nod-like receptors (NLRs), Dectin-1, Mannose receptor (MR), and CD-SIGN–all located on the surface of innate immune cells of macrophages, dendritic cells, neutrophils, and natural killer cells, among others.[29] Most importantly, TLR-2, TLR-4, and TLR-9 along with their adaptor protein MyD88 facilitate the recognition of Mtb.[30] Alveolar macrophages have the principle role to ingest any foreign particles in the alveoli and deactivate them if recognized as being foreign. TB is no exception and is swiftly phagocytosed by macrophages through the recognition of pathogen-associated molecular patterns (PAMPs) on the surface of the bacterium utilizing pattern

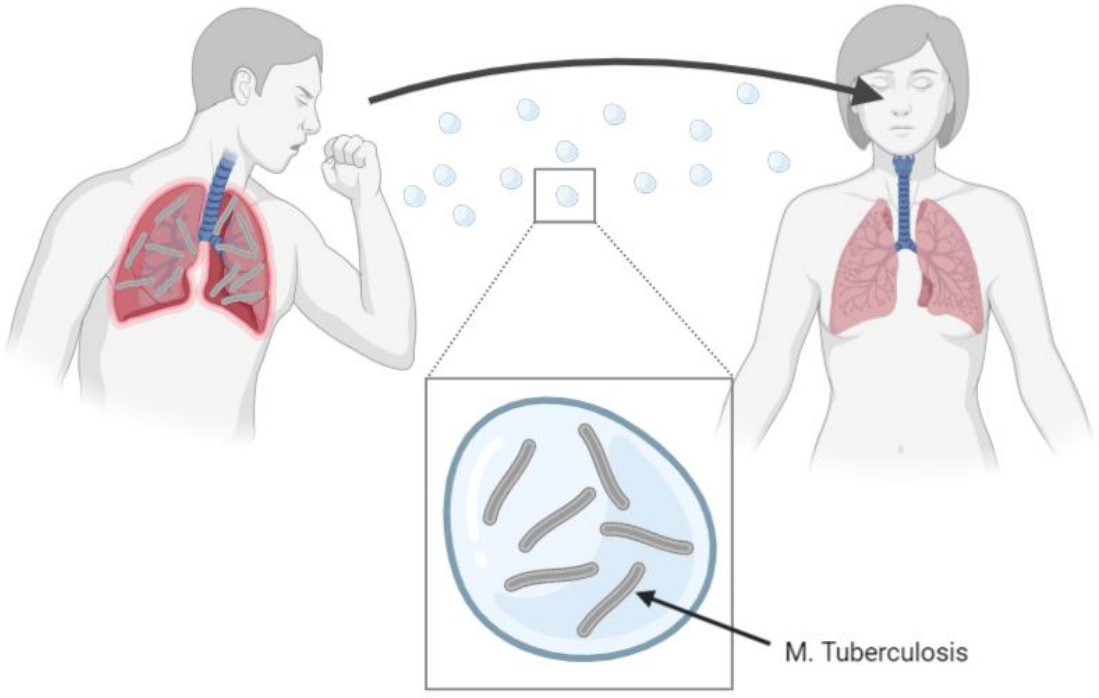

Figure 2 – Airborne Transmission of TB.

recognition receptors (PRRs)–such as those listed above–on the immune cell surface. Concurrently, anti-inflammatory cytokines are released by Mtb-infected macrophages, mediated by the recognition of mannose expressed on the surface of the Mtb through a mannose receptor belonging to a family of C-type lectin receptors, as stated previously.

Once ingested, the macrophage will attempt to fuse the phagocytosed TB with intracellular lysosomes containing oxidative free radicals in an attempt to neutralize and kill the bacterium **[Figure 3]**. Autophagy is a key first-line defense

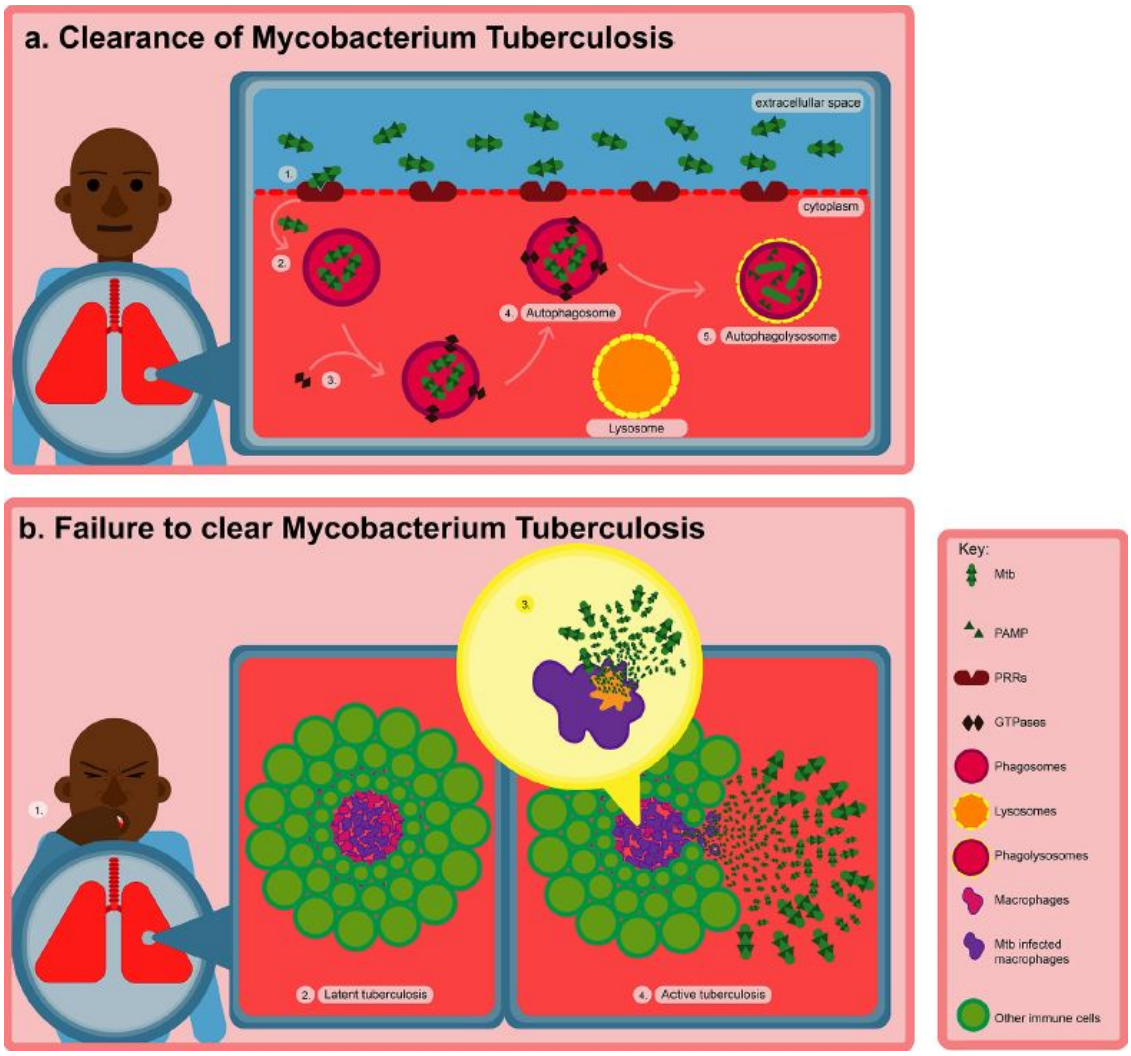

Figure 3 – Transmission and pathogenesis of TB after Mtb infection. (a) Macrophages utilize PRRs on their surface to (1) identify Mtb PAMPs, (2) phagocytose Mtb into intracellular phagosomes, (3) convert phagosomes into mature autophagosomes utilizing Rab GTPases, (4) fuse with intracellular lysosomes, and (5) create the final autophagolysosomes. (b) Failure to eradicate after infection results in either (2) latent tuberculosis or (3) necrosis of infected macrophages and (4) the development of active tuberculosis. Taken from Maphasa et al. 2021.

mechanism of the innate immune response utilized in the update and degradation of an array of invading intracellular pathogens. Once taken up into macrophages through phagocytosis, the now intracellular TB-filled vesicle is meant to be fused with acidic lysosomes containing the reactive oxygen species created and superoxide; both suitable to directly destroy the bacterium or any other invading pathogens. Sometimes, this key defense step is subverted by the invading TB as it prevents the fusion of lysosomes with the Mtb-containing phagosome. However, as we will see, this mechanism can potentially be enhanced to induce a host-directed response through autophagy inducing compounds.

Although the Mtb can halt the fusion of the intracellular lysosomes with the Mtb-containing phagocytic vesicles and is additionally able to persist inside the macrophages, the host's immune response is still viable and able to recognize an invading pathogen. The macrophage inducible Ca^{2+}-dependent lectin receptor (Mincle) expressed on the surface of macrophages is able to recognize and bind to a mycobacterial cord factor, Trehalose-6,6-dimycolate (TDM), one of the most common glycolipids on the surface of the Mtb bacterium.[31,32] Recognition of TDM by the Mincle receptor has been shown to increase the recruitment of Th1/Th17 innate immune cells, pro-inflammatory cytokine production, and granuloma

formation. Another PRR, Clecsf8, interacts with the TDM of Mtb to trigger multiple intracellular reactions including NF-κB activation, pro-inflammatory cytokine production, phagocytosis, and respiratory burst.[32]

Neutrophils are known to be recruited en masse to areas of Mtb-infection in response to systemic chemokines. Neutrophils play a vital role in the induction of autophagy inside the Mtb-infected macrophages as well as directly killing the Mtb utilizing antimicrobial peptides, including cathelicidin LL-37, defensins, lactoferrin, and lysozyme. Interestingly, neutrophils have also been implicated in assisting macrophages through direct recruitment of a ROS-creating enzyme, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase–a critical component of cellular metabolism–to the surface of phagosomes, which will go on to fuse with lysosomes culminating in the Mtb-killing autophagolysosomes. Neutrophils additionally "activate" macrophages through a number of cytokines such as azurophil granule proteins, heat shock proteins, and neutrophil extracellular traps (NETs). However, the release of NETs–which is comprised of nucleosome, elastase, and histones–into the host's circulation has recently been implicated in the pathogenic host response to TB infection.[33] Elastase serves as an important component in bacterial degradation through the degradation of chromatin de-condensation as well as increasing

macrophage capacity to kill intracellular pathogens and further release pro-inflammatory cytokines. Dendritic cells (DCs) have also been shown to be a key first line component of the innate immune system as they can phagocytose the Mtb bacterium and present antigen to T-cells in the adjacent draining lymph nodes. However, DCs have additionally been implicated in providing a niche replicating environment allowing TB to replicate and persist in the host.[34] Mtb is able to pair with DCs utilizing a cell-surface receptor Hip1, which additionally serves as a serine hydrolase.[35] An additional and critical component of the innate immune system is the natural killer (NK) cells, which play a number of vital roles in combating TB infection. Primary among these functions is their cytotoxic function exerted through perforins, granzymes, and granulysins. One of the key cytokines in the recruitment and activation of macrophages is IFN-γ, secreted primarily by NK cells in the innate immune response to TB infection.

If the infection progresses to a certain irreparable level, the infected macrophage will destroy itself in one of two methods: apoptosis or necrosis. Apoptosis is a process in which the cell will destroy all intracellular contents, forming membrane-bound "blebs." Necrosis is the process in which the cell contents are similarly destroyed, however, the key difference is that the cell bursts open, spilling the intracellular contents into the surrounding environment.

Ideally, apoptosis is the preferred method of cellular self-destruction as the infected cell, the Mtb-infected macrophage, contains the intracellular Mtb in encapsulated blebs that will not be allowed to infect other neighboring cells. Apoptosis is facilitated by an intrinsic pathway, which perforates and releases the contents of mitochondria, inducing apoptosis.[36] One method of releasing the contents of mitochondria is through mitochondrial outer membrane permeabilization (MOMP), releasing apoptosis inducing factor, Smac-DIABLO, and cytochrome c among others, which activates caspase-3/7, inducing apoptosis. Apoptosis can also be triggered independently through the pro-apoptotic Bcl-2 family of proteins BAX and BAK, which facilitate the penetration of the mitochondrial outer membrane and subsequent activation of caspase-9, caspase-3, and finally apoptosis. Conversely, Mtb has been shown to induce necroptosis through the pathogenic opening of the inner mitochondrial membrane pore instead of the outer membrane as mentioned previously, releasing another set of factors including mitochondrial permeability transition (MPT).[37] MPT has been shown to puncture the inner mitochondrial membrane, allowing water to enter the inner mitochondrial matrix causing it to swell and burst, ultimately inducing necrosis.[36]

Tuberculosis is able to persist in a dormant state due to the inhibition of apoptosis in Mtb-infected macrophages. Although actively replicating TB is able to be killed by a slew of currently available antibiotics, dormant bacilli are intrinsically resistant to many antibiotics.[17] How they are able to change to a virulent form is through the further inhibition of apoptosis in addition to actively stimulating necroptosis in Mtb-infected macrophages. In this way, they are able to disseminate Mtb throughout the host by actively utilizing the innate immune system while avoiding innate immune mechanisms of destroying the infection. One of the key chemicals implicated in the induction of apoptosis in Mtb-infected macrophages is TNFα, but remarkably, the Mtb has found a mechanism to secret soluble TNFα-receptors to sequester the necessary TNFα through an intermediary IL-10 dependent mechanism.

However, if the macrophages are not strong enough, or if the number of bacteria overwhelms the macrophages, then the TB will be allowed to replicate inside of the macrophages themselves and infect neighboring macrophages, allowing the number of bacteria to increase exponentially. However, in most healthy adults, the number of immune cells recruited to the site of infection is equal to the number of macrophages replicating–eventually reaching a steady state of infection that does not expand, leaving the host asymptomatic. Nine out

of 10 individuals infected with TB enter this stage of senescence where the immune cells are able to contain the infection within a centralized area of caseous necrosis and prevent further growth or spread. Neighboring lymph nodes may be infected as well. The immune cells that have swallowed the TB bacilli are then contained in a focused area of the lungs termed a Ghon complex or granuloma. The TB bacteria may continue to exist in the infected macrophages but are incapable of infecting neighboring alveolar cells. In this "latent" form of TB infection, macrophages, lymphocytes, and dendritic cells are recruited to the area to contain the Mtb in a centralized granuloma. The granuloma is composed of centrally contained, necrotic, Mtb-infected macrophages. This core structure is surrounded by innate lymphocytes which stimulate the secretion of key immune cytokines.[38–40] The centrally located, necrotic macrophages are constantly replenished by newly recruited phagocytes as well as vascular and tissue remodeling; all of which are crucial to maintaining a latent form of the infection.[38] This latent form of TB can persist in individuals for years, often up to the lifetime of the infected individual.

In a select number of individuals (approximately 5-10%) who are infected by TB who were able to suppress their initial infection, their infection will subsequently become active, often 1-2 years after initial exposure.[41] This

"reactivation" of the bacteria is more likely to happen when the host has a number of predisposing factors such as HIV infection, malnutrition, smoking, alcohol intake, diabetes, or vitamin D deficiency, to name a few.[42] The rate of necrosis of Mtb-infected macrophages and replenishment of phagocytes becomes untenable, and the TB can then break through the immune complex and is then allowed to infect neighboring cells. Often this phase of infection is isolated to the neighboring alveolar cells where the TB creates a cavity that the immune system cannot access to stave off the replicating infection. At this stage the infected individual starts to exhibit signs of active infection by loss of appetite, fever, night sweats, and coughing, thus spreading the highly contagious bacteria in airborne droplets to infect other potential future hosts in the immediate vicinity.[43]

Standard Treatment and Development of Drug-Resistance

The current recommended course of treatment for TB consists of a four-antibiotic regimen that must be strictly adhered to for a minimum of six months, which begins as an intensive two-month regimen of four antibiotics (rifampin, isoniazid, pyrazinamide, and ethambutol), followed by an additional four-months of two of the first line antibiotics, rifampin and isoniazid. Given the magnitude of global cases, the difficulty of the treatment duration, the need for

consistent supply chains, poor adherence due to the overwhelming number of pills and debilitating drug toxicity results in poor cure rates globally, which subsequently fuels the development of drug resistance in non-compliant patients.[43]

To make matters worse, a recent study has found that two of the first-line chemotherapeutics, isoniazid and rifampin, are chemically incompatible at physiologic conditions–effectively reducing their bioavailability and efficacy.[44] Other adverse interactions have been elucidated for critical first-line anti-TB drugs.[44]

As a result, the WHO reported that in 2018, there were approximately 500,000 new cases of rifampin-resistant tuberculosis, of which, 78% had resistance to multiple chemotherapeutic agents. The development of drug-resistance to isoniazid, rifampicin, one of the fluoroquinolones, and bedaquiline or linezolid is known as extensively-drug resistant tuberculosis; an affliction that accounted for an average 6.2% of all TB cases in 128 countries WHO member states over the last 15 years.[45]

The first barrier preventing the treatment of macrophage infected tuberculosis is the difficulty of the antibiotics being in sufficient concentration in the lungs, penetrating the cell membrane of the host-macrophage, and to be

taken up by the intracellular mycobacteria.[46] However, findings suggest that most TB drugs are malabsorbed, especially so for those with diarrhea, HIV infection, or who have eaten recently as it is recommended to take these drugs on an empty stomach.[47] Even in concentrations sufficient to penetrate into the alveolar macrophages, the tubercle membrane itself has been found to have reduced penetrance which contributes to drug-tolerance–even in dormant forms of the bacteria. Intracellular accumulation of antibiotics has also been shown to be significantly reduced in nutrient-deprived, non-replicating mycobacterium tuberculosis.[48]

One key reason tuberculosis has evaded all major curative efforts is its ability to evade chemotherapeutic treatment paradigms utilizing a number of peculiar mechanisms. One key mechanism is the avoidance of innate and adaptive immunological defenses. In particular, mycobacteria have the ability to hamper autophagocytic processes of white cells that have endocytosed the bacterium into lytic vesicles. In addition to halting the innate processes of phagocytosis, it has recently been shown that Mtb is in fact capable of thickening its cell wall in response to antibiotic stress, even in the absence of drug resistance-conferring mutations.[49] What contributes to a substantial amount of

treatment failure is the difficult regimen that patients must adhere to so as to ensure adequate uptake and penetrance of antibiotics at the site of infection.

The surge in drug-resistance calls for a new and diverse arsenal of therapeutics and treatment methodologies to aid front-line tuberculosis providers. There are a number of therapies in existence which can be repurposed to combat TB, or novel therapies have recently been developed that can target either the components responsible for the development of drug resistance or the mycobacterium itself. In this compendium, we outline novel therapeutics currently being developed which could be utilized in the treatment of mycobacterium tuberculosis as well as the mechanism of action of each therapy.

METHODS

This review was first initiated by searching the National Center for Biotechnology Information's database using search terms "novel" "therapy" "tuberculosis." The search criteria were limited to the past ten years or from 2012 to 2022. From the search results, we selected review articles that were broad in scope to get an idea of the various possible therapeutic modalities and interventions. Each intervention was then explored in-depth through review of published literature and further exploration of the cited literature in these

reviews. Only therapies that have initiated human clinical trials or had extensive in vivo testing were included in this review.

NOVEL THERAPEUTICS

Antibiotics

Although drug-resistance to new line antibiotics is well known, there are a number of antibiotics being developed which can be utilized in the fight against tuberculosis. Antibiotics are traditionally classified based on their mode of action or the particular cellular component they inhibit: whether that be on DNA synthesis, RNA synthesis, cell wall synthesis, and the like.[50] With the rise of drug-resistance in TB, clinicians have tried to utilize new combinations of low-sterilizing activity antibiotics to increase their overall antimicrobial activity, but these new combinations came with a concurrent increase in toxicity. Although there are a number of new antibiotics developed that seem like promising new treatments, their side-effect profiles are prohibitively toxic which deters their use in a clinical setting.

Bedaquiline

Bedaquiline (BDQ) was approved for clinical use by the United States Food and Drug Administration in 2012 and the European Medicines Agency the following year for use against MDR-TB–the first drug approved to treat tuberculosis in over 40 years.[51] BDQ is classified as a diarylquinoline and has a critical heterocyclic nucleus with tertiary alcohol and tertiary amine groups at its pharmacologically active site responsible for its antimycobacterial activity. BDQ specifically inhibits the ATP synthase critical for metabolism in actively and non-actively replicating TB,[52,53] making it a potent bactericidal antibiotic **[Figure 4]**. The ability of BDQ to actively target a key component of TB metabolism makes it an ideal candidate for both drug-sensitive and drug-resistant TB and it has been found to be highly effective at low-doses of administration.[54] Clinical trials have found that the inclusion of BDQ among a cocktail of antitubercular drugs overall improved outcomes for individuals infected with MDR-TB. However, isolating and assessing BDQ's standalone efficacy against TB has yet to be determined in phase III clinical trials. In combination with other anti-tubercular drugs, BDQ has been shown to be effective in shortening treatment regimens, increasing both overall adherence and cost-efficacy.[55–60] There are some cardiac concerns–mainly QT-prolongation–in individuals taking BDQ, especially considering it is often in

combination with other drugs found to also have QT-prolongation effects, so it is recommended that individuals undergoing treatment have monthly check-ins for electrocardiography monitoring.[52,61] Liver function needs to additionally be monitored closely based on side-effects found in initial trials.[52]

Despite its prospect as a powerful bactericidal therapy, studies have already found BDQ-resistance strains of TB. These high-level mutations were found in genes encoding the ATP-synthase, the site of action for BDQ. However, BDQ administration in combination with other drugs that the TB-strain is still susceptible to solves this issue. Further studies are required to elucidate treatment-resistance, utilization in special settings (e.g. HIV infection, malnutrition, pregnancy, etc.), and its implications on treatment regimens in the setting of MDR- and XDR-TB.

Delamanid

Delamanid was first discovered through extensive laboratory development and was then approved by the European Medicines Agency (EMA) for use against adult pulmonary MDR-TB in 2014.[62] Delamanid has been found to inhibit the synthesis of mycolic acids, disrupting cell-wall synthesis to facilitate

drug penetration into mycobacteria **[Figure 4]**. However, the exact mechanism is unknown as trial data of TB-strains resistant to Delamanid is insufficient.

Delamanid is well tolerated and is distributed throughout the body with 50% of orally administered Delamanid being readily bioavailable.[63,64] Studies on rats have found that ^{14}C-labeled Delamanid that is orally administered was then subsequently found in therapeutic concentrations in the central nervous system, eyes, bone, placenta, and fetus of pregnant rats.[65] This finding suggests that orally administered Delamanid may be a suitable therapeutic drug for extrapulmonary tuberculosis, however, additional clinical data will be required to confirm this. Additional studies have found that Delamanid can be administered in far lower doses for clinical efficacy. One study found that 0.625 mg/kg of Delamanid resulted in a 95% reduction in TB CFU (colony forming units), compared with 3.5 mg/kg of rifampin and 5 mg/kg for isoniazid to achieve the same results.[66] A study by Chen et al. utilizing a TB-infected guinea pig model (which is more comparable to humans than traditional Balb/c mice as they form necrotic caseous lesions while mouse-models do not) found that solo administration of Delamanid resulted in a complete sterilization of the lungs within 8 weeks of monotherapy, comparable to the commonly accepted MDR-TB regimen. This study finding was especially enticing considering Delamanid was

capable of destroying bacilli in hypoxic regions of the guinea pig lung granulomas and subsequently eliminating hypoxic lesions, lending evidence that Delamanid would be an effective agent in previously difficult to reach hypoxic regions of the lungs.[67]

It has been proposed to use Delamanid in combination with BDQ to create a potent new combination regimen in the treatment of MDR- and XDR-TB. However, the side effect profile significantly increases with this combination, including cardiac complications such as QT-prolongation, but this was found to be of little concern and the combination was deemed safe after several subsequent studies.[68–70] Delamanid also has the pragmatic clinical benefit of being absorbed readily after a meal, almost a 2-fold increased absorption as compared to a fasted state as well as being minimally excreted in the urine.[64] Early clinical trials demonstrated a 5% increase in certain adverse events such as nausea, vomiting, headache, and QT-prolongation in comparison to the placebo arm– giving it an overall favorable safety profile relative to other second line anti-TB drugs.[64] Additional trials have found Delamanid to be well tolerated in pediatric populations as well.[71]

Pretomanid

Pretomanid (Pa) has been allowed to be used in the treatment of rifampin-resistant tuberculosis since 2019 under operational research conditions.[61,72] Pa is classified as an oral nitroimidazole with activity against Mtb–both in vitro and in vivo.[73–75] Like Delamanid, Pa kills Mtb through the active suppression of mycolic acid biosynthesis, a critical component of the Mtb cell wall **[Figure 4]**. As stated previously, this is advantageous in a clinical setting as this allows Pa to have anti-tubercular activity in hypoxic conditions in non-replicating Mtb.[76,77] Pa uptake additionally has clinical appeal as its uptake and bioavailability is highest after intake of a high-calorie meal, unlike most current TB therapeutics which require the patient to be in a fasted state.[78,79] Pa is additionally only required to be taken once a day with a half-life of 16-20 hours.[80]

Early clinical trials have found that administration of Pa results in no significant adverse events or side-effects.[81,82] However, a meta-analysis of several trials displayed an increase in hepatotoxicity among trial participants receiving Pa-containing regimens.[83–86] Trial data showed a 7% (1/15) withdrawal on each experimental regimen in trials in South Africa in 2012 and 2015. 13% of trial participants in a clinical trial arm with Pa experienced treatment interruptions in a trial in South Africa and Tanzania. Finally, anywhere between 3-8% of trial

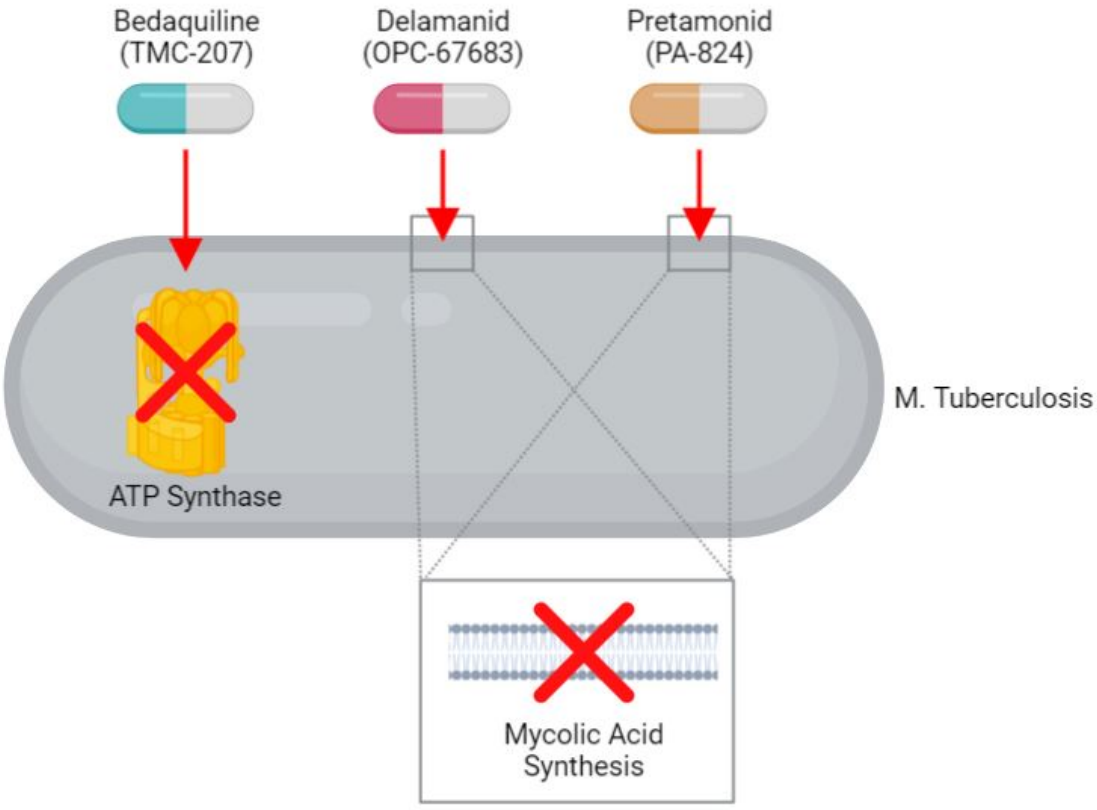

Figure 4 - Mechanism of action of several novel antibiotics.

participants in various arms containing Pa in a clinical trial in several high-burden TB countries were withdrawn from trial participation due to liver-enzyme elevations. Early clinical trial data showed only minimal increase in the minimum-inhibitory concentration (MIC) for Pa against Mtb after treatment completion including a 6% increase MIC for Pa in a study of 65 individuals in South Africa. The same study showed only a 0.5% increase in MIC of 206

individuals who completed treatment in South Africa and Tanzania. Despite this, a number of studies did not report acquired resistance to Pa.[87] Alarmingly, three hepatotoxic lethal adverse events were identified in one trial, but this is believed to have been attributed to delayed recognition of medication- induced liver failure.[88] Cardiac complications arose in a number of trials containing Pa, which resulted in participant withdrawal including one patient experiencing QT-prolongation, atrioventricular block, and cardiac arrhythmia.[83,85,88,89]

Host-Directed Therapies

A novel approach to combating tuberculosis is the utilization of compounds that focus on host-pathogen interactions and enhance the infected individual's immune response to the pathogen. Host-directed therapy, or HDT, utilizes small molecules (with or without accompanying antibodies) to enhance a burgeoning field of study with a number of possible therapeutics being developed to enhance the host's immune system in mitigating TB infection or disease. Targets of HDT include several diverse molecules involved in the pathogenesis of disease including cytokines, immune cells, and enzyme activity, among many others. Current HDTs for TB have one of two primary aims; to enhance the host's immune system in response to pathogenic infection or to

control excess inflammation to prevent permanent lung tissue damage. However, HDTs can be potentially adapted to facilitate treatment in numerous different ways, including signal transduction-mediating cytokines, antimicrobial processes, immune cell regulation, and epigenetic modulation, among many others.

One distinct advantage in the utilization of HDTs is that this therapy is not dependent on the invading pathogen as the compounds do not directly interact with the mycobacterium and instead modulate and enhance the host-response to infection. As a result, there is little to no risk of developing drug-resistance to the compounds themselves.[90,91] HDT can even serve as a possible modality in the treatment of MDR- and XDR-TB as they directly modulate pathways in the host. Despite HDT's promise as a novel therapeutic in the treatment of tuberculosis infection, this modality, along with other therapeutic approaches, is still limited by the therapy's ability to penetrate to the infected tissue as well as having unwanted side-effects on non-infected cells throughout the host's body.[91] Several HDTs will be explored further in this review, including autophagy inducing compounds, nanoparticles, metformin, monoclonal antibodies, and stem cell therapy.

Autophagy Inducing Compounds

As highlighted earlier, autophagy is an innate feature of all cells that the immune system uses for intracellular lysosomal degradation of invading pathogens. Autophagy of the infected host cells that contain TB can be enhanced through the utilization of autophagy-inducing compounds (AICs). In general, with an infected individual, the autophagy of the host macrophages containing tuberculosis can be halted by the bacterium, allowing the infection to persist in the host by remaining protected inside the "disabled" macrophages. AICs can override this protective mechanism and allow the host's own immune system to stymie and eliminate the TB. This is especially important as AICs hold promise as a treatment strategy against drug-resistant strains with or without adjunctive therapy.[91–93]

AICs are preferable to standard regimens as they are less toxic, costly, and pragmatically less complicated than antibiotic regimens. A number of compounds of interest are currently being studied as possible therapeutics to aid in the autophagy. Nortriptyline was found to be successful in inducing autophagy in Mtb-infected macrophages, significantly reducing their intracellular survival.[94] Trehalose was additionally found to be capable of inducing autophagy and xenophagy–a more selective type of autophagy that

targets and eradicates bacteria and damaged organelles–in Mtb-infected macrophages.[95] The added advantage of trehalose is that it is effective in treating both TB and HIV infection as it can overcome the HIV-induced blocked of autophagy.[95] A number of studies of saracatinib (AZD0530) could act as an AIC as it is capable of inducing autophagy and lysosomal maturation.[96] Fluoxetine, an selective serotonin reuptake inhibitor (SSRI), has been shown to increase TNFα, facilitating a subsequent increase in autophagy of Mtb-infected macrophages.[97] Paik et al. showed that baicalin acts as a powerful antibacterial agent through the activation of autophagy pathway, PI3K/Akt/mTOR pathway while simultaneously inhibiting the PI3K/Akt/NFκB pathway.[91] Zymosan, a TLR2 agonist derived from the yeast cell wall, that holds promising immunomodulatory effects, including the upregulation of TNFα, an increased recruitment of LC3 autophagy protein to phagosomes, the activation of NOX2 NADPH oxidase–all shown to promote the maturation of phagosomes and increase autophagy.[98,99]

Vitamin D in particular holds significant promise as an AIC to be utilized in the treatment of TB. The presence of vitamin D has been shown to greatly increase the efficacy of innate autophagy-based mechanisms in vitro and in vivo in TB infected individuals.[100] Vitamin D3's mechanism of action is to upregulate

the expression of the antimicrobial peptide cathelicidin, Atg5, Beclin-1, CAMP, DEFB4, as well as reactive oxygen and nitrogen intermediates–all critical components of autophagy.[100,101] Vitamin D3 given in combination with 4-phenylbutyrate (PBA) has been shown to overcome the inhibition of cathelicidin expression to enhance anti-mycobacterial activity.[102,103] Notably, one study found that Vitamin-D3 supplementation in Vitamin-D3 deficient individuals was capable of recovering phagosome-lysosome fusion and autophagy in macrophages, highlighting how critical Vitamin-D3 is in maintaining innate immune functionality.[104] Further studies are necessary in order to fully elucidate the role that Vitamin-D3 plays in immune modulation and its role in protecting from TB.

Metformin

Another host-directed therapy considered to be utilized as a novel therapeutic for TB infection is the drug metformin (MET), primarily utilized to lower blood-glucose levels in diabetics but recently proposed to be repurposed as a new host-directed therapy to combat Mtb infection.[105,106] MET was first approved for broad public use by the U.S. Food and Drug Administration (FDA)

in 1994 and is currently on the list of essential medicines by the WHO.[107,108] In vitro studies have found that MET is capable of enhancing the macrophage fusion of intracellular phagosomes containing Mtb with intracellular lysosomes.[109] MET has additionally been shown in vitro to reduce chronic inflammation in the lungs, enhance immune response by bolstering CD8+ and CD4+ T cells in the lungs, increase mitochondrial reactive oxygen species production, and actually serve to augment the antimicrobial activity of standard TB drugs; although, there was a study that conflicted with the above mentioned effects, showing almost no effect on Mtb-infected mice treated with MET alongside first-line TB drugs.[110]

Preliminary data from clinical trials utilizing MET concomitantly with standard TB therapeutics have shown promising results. One trial studying the effect of TB therapy on diabetic patients found that patients taking MET and anti-TB drugs showed a reduced likelihood of being culture positive at two months in addition to a decrease in overall mortality.[106] The same study found an increased expression of genes responsible for reactive-oxygen species–findings corroborated by in vitro studies as well.

How MET mitigates and improves TB infection may be due to a number of host-related factors, either through direct or indirect pathways.[111,112] First and

foremost, glycemic control has been shown to provide a protective effect against TB infection. In effect, the decreased blood-glucose in TB exposed and infected individuals through MET may explain its protective capabilities. MET has incidentally also been shown to increase the levels of circulating immune cells, including neutrophils, monocytes (pre-cursors to macrophages). Other studies of MET have shown a significant increase in macrophage autophagy activity, control of localized inflammatory processes as indicated by an increase in anti-inflammatory markers such as TNFα, interleukin-β, and interferon- γ, and an increase in innate and adaptive immunological pathways.

Imatinib

Imatinib, otherwise known by its brand name Gleevec, is a tyrosine kinase (TK) inhibitor has traditionally been used as an effective therapy against particular cancers, primarily in ABL1-mutated chronic myelogenous leukemia (CML). Imatinib was only recently explored as a possible therapeutic in the treatment of Mtb infection. Imatinib exerts its effect by inhibiting ABL1/2 receptors and related tyrosine kinases, all critical in the development of CML and other cancers.[113] Although the exact mechanism is unclear, a number of bacterial

infectious diseases utilize ABL receptors as part of their normal pathogenesis, however, some studies have discovered the importance of ABL and TKs in motility, cellular trafficking, release from infected cells, and autophagy.[114–118]

Napier et al. utilized cell lines lacking ABL and TKs to show that Mtb infection is dependent on this family of receptors for proliferation and growth.[119] In their studies, they found that imatinib reduces bacterial load synergistically with common first-line anti-TB therapeutics in mice infected with both Mtb or its close relative Mm (Mycobacterium marinum). Imatinib's ability to modulate ABL1/2 and related tyrosine-sensitive kinases presents a possible modality to broaden the arsenal of therapeutics utilized in the fight against Mtb infection. The authors additionally found that administration of imatinib helped facilitate a 5.8-fold increase in macrophage phagosome-lysosome fusion and intracellular trafficking of mycobacteria. Most strikingly, the authors found that administration of imatinib as well as a first-line TB therapeutic in Mtb-infected mice resulted in a 185-fold decrease in median-cfu as compared to Mtb-infected controls. It is additionally important to note that the authors found that imatinib was slightly more efficacious at lower concentrations, lending evidence that the drug may have a concentration-dependent effect.

Although imatinib is a possible adjuvant therapeutic in additional to standard chemotherapeutic regimens, it is possible that there are other therapies that can be utilized which would inhibit ABL or related tyrosine-kinases that are necessary for Mtb entry into macrophages, intracellular trafficking, lysosomal fusion, and phagolysosomal degradation. Considering its low toxicity, imatinib is an appealing therapeutic to Mtb infection, although some recent studies have found side-effects after long-term administration.[120,121] However, utilization in conjunction with standard therapeutics could hopefully shorten the duration of treatment, mitigating the risk of any long-term side effects. Additionally, the lack of cold-chain requirements provides an added advantage, although its shelf-life is limited to only two years.[122] Conversely, imatinib's high annual cost makes it prohibitive for utilization in low- to middle-income countries, which bear the highest burden of TB.

Stem Cell Therapy

Stem cell transplantation has recently been considered as a viable option in the treatment of infectious diseases, including drug-resistant tuberculosis. The central idea of stem cell therapy revolves around the utilization of bone-marrow

derived mesenchymal stromal cells (MSCs) to hijack the host's diffuse inflammatory response to be directed to anti-pathogen responses. MSCs have been shown to support stem cells in the bone marrow and broncho-alveolar stem cells, thus it may facilitate the reorganization of alveolar tissue and temper chronic inflammation; both necessary in the healing of Mtb infection. MSCs achieve these desired outcomes through a targeted change in dendritic cells which regulate the host T-cells. MSCs can additionally curb inflammatory processes and reduce epithelial damage by mitigating oxidative stress, increasing the efficacy of phagocytic processes which can help magnify microbial clearance, and help to battle infection through direct antimicrobial activity.[123]

MSCs can be isolated from patients even though they comprise ~0.001% of bone marrow mononuclear cells. Once isolated, they can be expanded ex-vivo and then re-infused into patients. Interestingly, the re-infused MSCs are able to migrate to sites of injury and inflammation to promote tissue repair. MSCs can restore lung epithelium and increase the proliferation of broncho-alveolar stem cells. In addition, the MSCs themselves can differentiate into the various cell types of the lungs including Type I and Type II pneumocytes, bronchoalveolar epithelium.[124]

Skrahin et al. have utilized autologous stem cell transplantation of MSCs in multi-drug and extensively drug-resistant tuberculosis in an open-label phase 1 safety trial and subsequent phase 2 efficacy trial.[125,126] Their published findings, although with a small cohort of individuals in Belarus, have found a remarkable 81% of subjects treated with autologous stem cell transplantation had a successful outcome, with 75% of subjects being completely cured compared to just 22% of controls given the standard treatment regimen.[126] Kaufmann et al. have been studying immune markers and immunologic anti-TB responses in patients infused with autologous MSC therapy in South African patients with MDR/XDR-TB.

MSCs have been shown to serve additional functions in the host's inherent immune functions and restorative processes. Trials are currently underway to study the clinical efficacy of utilizing MSCs in patients with acute respiratory distress syndrome (ARDS) and chronic obstructive pulmonary disorder (COPD), with initial phase I trials showing that autologous MSC therapy is well tolerated in subjects.

Nanoparticles

Although autophagy inducing compounds are an appealing therapeutic, there is still the limitation in delivery of the drugs to target sites within the Mtb-infected macrophages. With oral administration, there are considerations for the drugs to penetrate the gastrointestinal wall, transport through the blood system, penetrate the endothelium and epithelium of the lungs, infiltrate complex lung lesions, penetrate the cell membrane of macrophages and, finally, for the drugs to be taken up by the intracellular Mtb and accumulate in sufficient quantities to exert its antibacterial effects.[46] Although not explicitly a novel therapeutic per se, nanoparticles offer an exciting therapeutic modality to deliver drugs to be delivered in parts of the body which were previously difficult to access–namely inside the Mtb-containing caseous granuloma inside the lungs.

Nanoparticles can be utilized to improve the activity of small-molecules (namely AICs) to enhance their absorption and delivery to target sites.[127,128] Nanoparticles are able to have a sustained drug-release at the target site, which has the distinct advantage of not only having greater penetrance, but additionally requires a decreased dosing frequency and decreased drug toxicity. In particular, inhaled nanoparticles hold special promise as a drug-delivery model as they have the advantage of delivering drugs to the area predominantly

affected by TB, the lungs. Nanoparticles can be readily taken up by Mtb-infected alveolar macrophages in the lungs as inhaled particles smaller than 0.1 microns.[129] The nanoparticles can additionally be localized to the site of infection through the utilization of surface-bound ligands to target Mtb-infected alveolar macrophages **[Figure 5]**.[130] Inhaled nanoparticles have been found to additionally show better localization and uptake than nanoparticles that were orally or intravenously administered.[131,132] Inhalation of nanoparticles has the added benefit of being quickly absorbed as the lungs have a large surface area, a thin blood-air barrier, they can bypass the liver thereby increasing bioavailability, and lastly increases patient compliance as this method of administration is non-invasive.[131,133,134] One in vitro study of Mtb-infected cells found that the utilization of microparticles containing silver nanoparticles and zinc oxide nanoparticles along with a standard first-line antituberculosis drug increased the efficacy of drug delivery by 76%.[135] Another study of direct administration of ethambutol-loaded solid lipid nanoparticles converted to a dry inhalable powder did not cause significant cytotoxicity unlike its common clinical counterpart, ethambutol hydrochloride when administered orally. Another study of aerosol delivery of several first-line antituberculosis drugs in a guinea pig model comparing administration of drugs orally, intravenously, or via drug-loaded PLG-

nanoparticle spray. The study found that a nanoparticle spray every 10-days was able to elicit the same therapeutic as a 46-day daily oral regimen, measured in disappearance of colony-forming units.[136,137] The most pragmatic limitation in this delivery method is that the nanoparticle suspensions must be kept stabled. Considering early trial results and the prospect of easing the patient of burdensome daily oral regimens, nanoparticle-bound drug delivery systems show significant promise as a drug delivery modality and should be explored further in clinical settings.

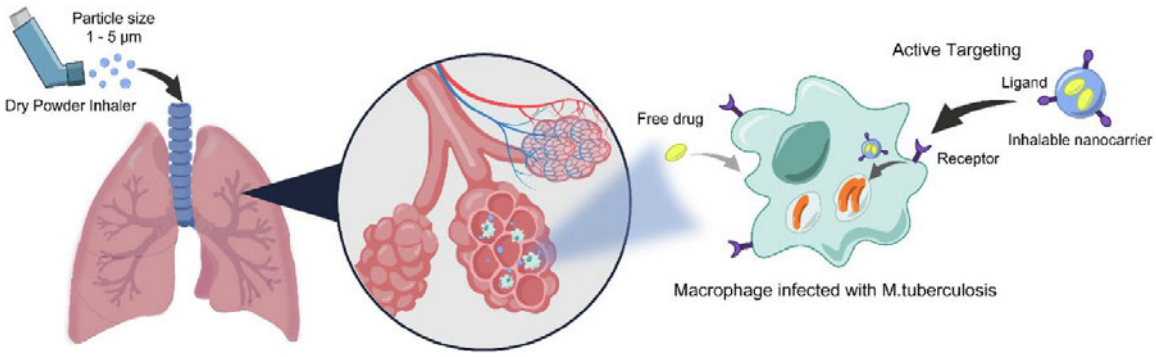

Figure 5 – Route of administration of inhalable nanoparticles in the treatment of M. tuberculosis. Taken from Chae et al. 2021.[130]

Phage Therapy

Although not a novel therapy, bacteriophage therapy has received renewed interest as a possible treatment of TB. In the early 20th century — concurrently with the development of the first antibiotics — there was an equal, if not greater, interest in the development of phage therapeutics for bacterial infections. Bacteriophages were named so due to a plate of shigella bacteria, which had clear spots when cultured with filtered fecal samples from dysentery patients. French microbiologist Felix d'Herelle theorized that there was some substance that was consuming the bacteria, hence, the term bacteriophage.[138,139] Bacteriophages came to be commercially produced and widely distributed in some countries in the 1920s through the 1940s for the prophylaxis or treatment of a number of infectious diseases at the time.[140–142] However, the efficacy and safety of phage therapy was not well understood and with the rise of penicillin and the antibiotic era, phage therapy was soon abandoned in most Western countries.

What is most interesting about mycobacteriophages is their remarkable ability to efficiently lyse mycobacteria, including mycobacteria tuberculosis **[Figure 6A]**. All mycobacteria encode lysins that are effective in penetrating, cutting, and thereby lysing the mycobacterial cell wall **[Figure 6B]**.[143] Mycobacteriophages encode a number of critical genes that encode proteins

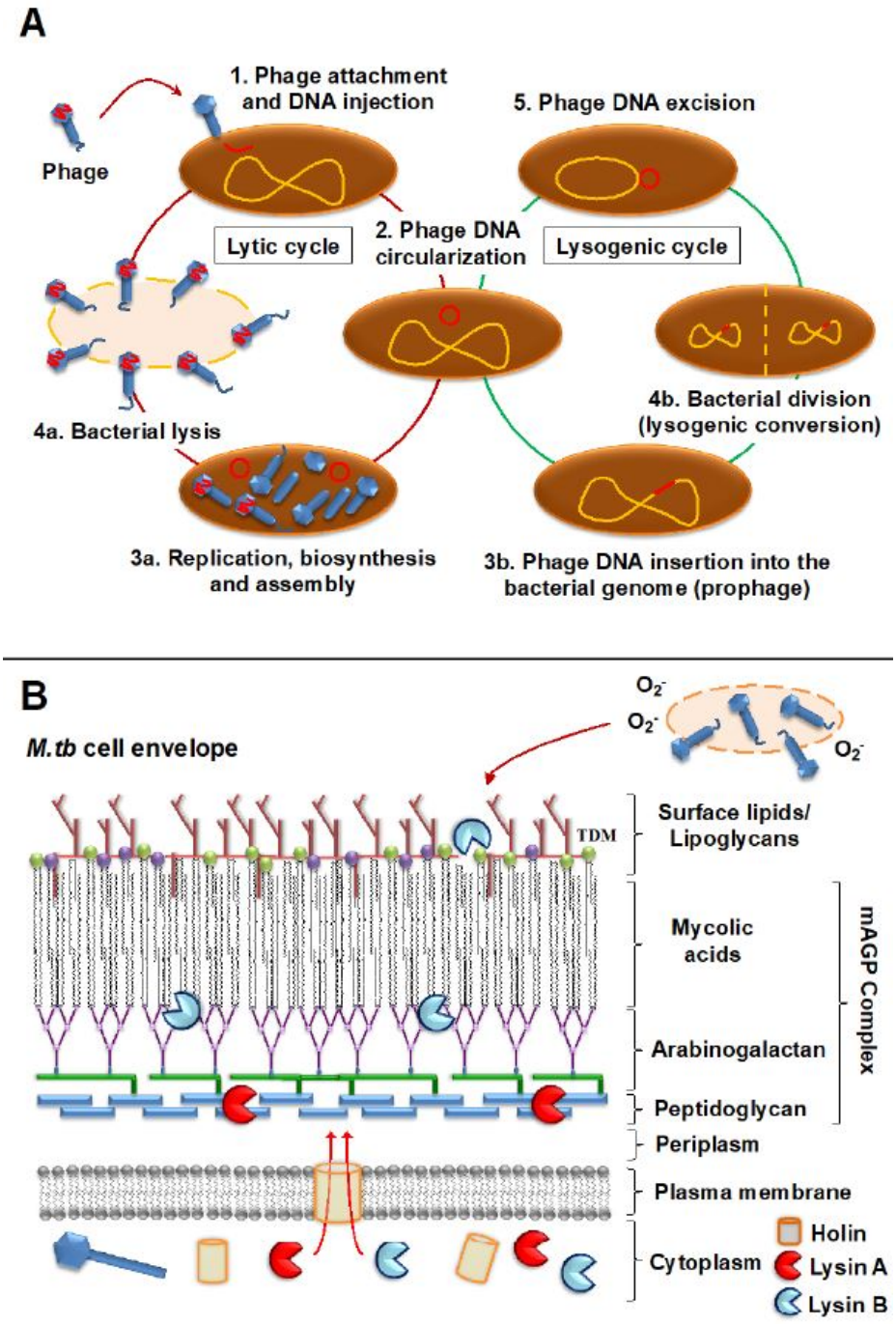

Figure 6 – Infection of M. tuberculosis by mycobacteriophages. Figure 6A – Steps during mycobacteriophage infection. Figure 6B – Action of mycobacteriophage proteins. Taken from Allue-Guardia et al. 2021.[144]

essential for the functionality against mycobacterium: namely, 1) endolysins that target the integrity of the Mtb cell envelope; 2) holins, which are membrane proteins that aid in the translocation of lysins to their target destination past the cell membrane; and, 3) Lysin B, an esterase that is capable of cleaving the ester bonds which are critical to the Mtb cell envelope **[Figure 6B]**.[144–146] Although the preferred method of cell death for mycobacteriophages is lysis of the cell wall, two in vivo studies using a guinea pig model showed significant promise in treating TB with mycobacteriophages,[147,148] although more in vivo studies are they also are capable of releasing superoxide (O_2^-) radicals **[Figure 6B]**.[144] So far, for the treatment of TB, only a number of phages have been studied,[149] however, critically needed.

There are advantages of utilizing bacteriophages in the treatment of TB infection. First, mycobacteriophages are only capable of replicating inside of their target cells, the Mtb bacillus, while their neighboring eukaryotic cells are left unharmed.[150] Compared to antibiotics, this has the distinct advantage of a targeted approach to the mycobacteria infection, leaving the host's microbiota intact.[151] Lastly, bacteriophages are one of the most abundant organisms on the planet, while with traditional antibiotics it is difficult and costly to isolate and study antibiotics and then bring them to market.[152] On the other hand, there are

several limitations to the use of mycobacteriophages; the first being the difficulty in delivering the phages to the site of infection, considering the immune response walling off the bacterial infection, precluding the penetrance of the therapy where it is needed. One proposed solution is the utilization of a liposomal delivery system of the phages, but even this proposed solution is still limited by the impenetrable wall of the granulomata. Additionally, it is still not clearly understood how mycobacteriophages are going to interact with the host cells and immune system, thus, extensive safety trials to see if they are safe for human use. Lastly, it is going to be critical to understand how the host's immune system is going to create phage-neutralizing antibodies and whether this is going to have a significant effect on the dose, route of administration, and the host's immune status. Clearly, there is an urgent need to study phage therapy as a potential therapeutic strategy for use against TB infection and more clinical trial data is warranted.

DISCUSSION

Mycobacterium tuberculosis is an infectious disease that is still widely endemic throughout the World. Although there is a plethora of treatments widely available, supply chain, costs, toxicity, and difficult treatment regimens

impede successful treatment and eradication of this ancient disease. However, there has been a growing interest in exploring new therapeutics in the treatment treatment of mycobacterium tuberculosis. First, a number of new antibiotics used of TB. Here is outlined a compendium of potential new therapeutics in the in the treatment of TB infection were outlined. Antibiotics have been the predominant, preferred, and often the only method of treatment for TB infection. Although there has been a significant lag in the development of new antibiotics for TB treatment, a number of new drugs have been developed to be utilized in instances of drug-resistant tuberculosis including bedaquiline, delamanid, and pretomanid. Although these new drugs hold promise in the treatment of drug-resistant TB, like older antibiotics in wide-spread use, there is concern for drug toxicity as well as the development of drug-resistance, which has already been well documented in several clinical trials.

A number of new therapeutic modalities were then explored including a number of host-directed therapies including autophagy inducing compounds, metformin, imatinib, vitamin D and stem cell therapy. These therapies rely on amplifying or augmenting the host's innate immune functions to facilitate the eradication of disease. Notably, there has also been the development of a new modality to deliver and target drugs to deliver medications to the TB-containing

granuloma through the use of nanomedicines. Aerosolized nanomedicines hold particular promise as they are inhalable therapies that can be targeted directly to TB in the granuloma in the lungs. Several trials have preliminary data to support this delivery method, but more extensive testing is needed before aerosolized nanomedicines can be brought to wide clinical use. Lastly, we explored the history and mechanism of the utilization of bacteriophages, specifically, mycobacteriophages which are live viruses that can target Mtb, replicate inside, and lyse the Mtb to efficiently and methodically eliminate Mtb from the infected host, although, more safety and efficacy data is needed. With this compendium, it is hoped that new therapeutics and treatment modalities will be shared to facilitate further research to help work towards eradicating this prevalent and disastrous epidemic once and for all.

BIBLIOGRAPHY

1. Hirsh AE, Tsolaki AG, DeRiemer K, Feldman MW, Small PM. Stable association between strains of Mycobacterium tuberculosis and their human host populations. *Proceedings of the National Academy of Sciences*. 2004;101(14):4871-4876. doi:10.1073/pnas.0305627101

2. World Health Organization. *Tuberculosis: Key Facts*. World Health Organization; 2021. Accessed June 27, 2022. https://www.who.int/news-room/fact-sheets/detail/tuberculosis

3. World Health Organization. *WHO Global Lists of High Burden Countries for Tuberculosis (TB), TB/HIV and Multidrug/Rifampicin-Resistant TB (MDR/RR-TB), 2021-2025*. World Health Organization; 2021. Accessed June 28, 2022. https://cdn.who.int/media/docs/default-source/hq-tuberculosis/who_globalhbcliststb_2021-2025_backgrounddocument.pdf?sfvrsn=f6b854c2_9

4. Khan FN, Zaidi KU, Thawani V. Stem cell therapy: an adjunct in the treatment of mdr tuberculosis. *Current Stem Cell Research and Therapy*. 2017;3(3). doi:10.15406/jsrt.2017.03.00099

5. Kim YR, Yang CS. Host-Directed Therapeutics as a Novel Approach for Tuberculosis Treatment. *Journal of Microbiology and Biotechnology*. 2017;27(9):1549-1558. doi:10.4014/jmb.1705.05032

6. *Facing the Reality of Drug-Resistant Tuberculosis in India: Challenges and Potential Solutions: Summary of a Joint Workshop by the Institute of Medicine, the Indian National Science Academy, and the Indian Council of Medical Research*. National Academies Press (US); 2012. doi:10.17226/13243

7. Grace AG, Mittal A, Jain S, et al. Shortened treatment regimens versus the standard regimen for drug-sensitive pulmonary tuberculosis. *The Cochrane Database of Systematic Reviews*. 2019;12(12):CD012918. doi:10.1002/14651858.CD012918.pub2

8. *The PIH Guide to the Medical Management of Multidrug-Resistant Tuberculosis*. 2nd Edition. United States Agency for International Development TB CARE II; 2013.

9. Wallis Robert S., Patil Shripad, Cheon Seon-Hee, et al. Drug Tolerance in Mycobacterium tuberculosis. *Antimicrobial Agents and Chemotherapy*. 1999;43(11):2600-2606. doi:10.1128/AAC.43.11.2600

10. Afzal A, Rathore R, Butt NF, Randhawa FA. Efficacy of Vitamin D supplementation in achieving an early Sputum Conversion in Smear positive Pulmonary Tuberculosis. *Pakistan Journal of Medical Sciences*. 2018;34(4):849-854. doi:10.12669/pjms.344.14397

11. Visser ME, Stead MC, Walzl G, et al. Baseline Predictors of Sputum Culture Conversion in Pulmonary Tuberculosis: Importance of Cavities, Smoking, Time to Detection and W-Beijing Genotype. *Public Library of Science One*. 2012;7(1):e29588. doi:10.1371/journal.pone.0029588

12. Musteikienė G, Miliauskas S, Zaveckienė J, Žemaitis M, Vitkauskienė A. Factors associated with sputum culture conversion in patients with pulmonary tuberculosis. *Medicina*. 2017;53(6):386-393. doi:10.1016/j.medici.2018.01.005

13. Unsematham S, Kateruttanakul P. Factors predicting sputum smear conversion and treatment outcomes in new smear-positive pulmonary tuberculosis. *Journal of the Medical Association of Thailand*. 2013;96(6):644-649.

14. Aliyu MH, Salihu HM, Ratard R. HIV infection and sputum-culture conversion in patients diagnosed with Mycobacterium tuberculosis: A population-based study. *Wiener Klinische Wochenschrift*. 2003;115(10):340-346. doi:10.1007/BF03041486

15. Olaru ID, Heyckendorf J, Grossmann S, Lange C. Time to Culture Positivity and Sputum Smear Microscopy during Tuberculosis Therapy. *Public Library of Science One*. 2014;9(8):e106075. doi:10.1371/journal.pone.0106075

16. World Health Organization. *Global Tuberculosis Report*. World Health Organization; 2017.

17. Dheda K, Gumbo T, Maartens G, et al. The epidemiology, pathogenesis, transmission, diagnosis, and management of multidrug-resistant, extensively drug-resistant, and incurable tuberculosis. *The Lancet Respiratory*

Medicine. Published online March 15, 2017:S2213-2600(17)30079-6. doi:10.1016/S2213-2600(17)30079-6

18. World Health Organization. *WHO Treatment Guidelines for Drug-Resistant Tuberculosis, 2016 Update*. WHO Team Global Tuberculosis Programme; 2016.

19. Hershkovitz I, Donoghue HD, Minnikin DE, et al. Detection and Molecular Characterization of 9000-Year-Old Mycobacterium tuberculosis from a Neolithic Settlement in the Eastern Mediterranean. *Public Library of Science One*. 2008;3(10):e3426. doi:10.1371/journal.pone.0003426

20. Holloway KL, Henneberg RJ, de Barros Lopes M, Henneberg M. Evolution of human tuberculosis: A systematic review and meta-analysis of paleopathological evidence. *HOMO: Journal of Comparative Human Biology*. 2011;62(6):402-458. doi:10.1016/j.jchb.2011.10.001

21. Baker O, Lee OYC, Wu HHT, et al. Human tuberculosis predates domestication in ancient Syria. *Tuberculosis*. 2015;95:S4-S12. doi:10.1016/j.tube.2015.02.001

22. Masson M, Bereczki Z, Molnár E, et al. 7000 year-old tuberculosis cases from Hungary – Osteological and biomolecular evidence. *Tuberculosis*. 2015;95:S13-S17. doi:10.1016/j.tube.2015.02.007

23. Pálfi G, Dutour O, Perrin P, Sola C, Zink A. Tuberculosis in evolution. *Tuberculosis*. 2015;95:S1-S3. doi:10.1016/j.tube.2015.04.007

24. Piombino-Mascali D, Jankauskas R, Tamošiūnas A, et al. Evidence of probable tuberculosis in Lithuanian mummies. *HOMO: Journal of Comparative Human Biology*. 2015;66(5):420-431. doi:10.1016/j.jchb.2015.01.004

25. Lange C, Dheda K, Chesov D, Mandalakas AM, Udwadia Z, Horsburgh CRJ. Management of drug-resistant tuberculosis. *Lancet*. 2019;394(10202):953-966. doi:10.1016/S0140-6736(19)31882-3

26. Clardy J, Fischbach MA, Currie CR. The natural history of antibiotics. *Current Biology*. 2009;19(11):R437-R441. doi:10.1016/j.cub.2009.04.001

27. Fu LM, Fu-Liu CS. Is Mycobacterium tuberculosis a closer relative to Gram-positive or Gram-negative bacterial pathogens? *Tuberculosis*. 2002;82(2-3):85-90. doi:10.1054/tube.2002.0328

28. Golden MP, Vikram HR. Extrapulmonary tuberculosis: an overview. *American Family Physician*. 2005;72(9):1761-1768.

29. Liu CH, Liu H, Ge B. Innate immunity in tuberculosis: host defense vs pathogen evasion. *Cellular and Molecular Immunology*. 2017;14(12):963-975. doi:10.1038/cmi.2017.88

30. Kleinnijenhuis J, Oosting M, Joosten LAB, Netea MG, Van Crevel R. Innate immune recognition of Mycobacterium tuberculosis. *Clinical and Developmental Immunology*. 2011;2011:405310. doi:10.1155/2011/405310

31. Ishikawa E, Ishikawa T, Morita YS, et al. Direct recognition of the mycobacterial glycolipid, trehalose dimycolate, by C-type lectin Mincle. *Journal of Experimental Medicine*. 2009;206(13):2879-2888. doi:10.1084/jem.20091750

32. Marakalala MJ, Ndlovu H. Signaling C-type lectin receptors in antimycobacterial immunity. *Public Library of Science Pathogens*. 2017;13(6):e1006333. doi:10.1371/journal.ppat.1006333

33. van der Meer AJ, Zeerleder S, Blok DC, et al. Neutrophil extracellular traps in patients with pulmonary tuberculosis. *Respiratory Research*. 2017;18(1):181. doi:10.1186/s12931-017-0663-1

34. Ahmed S, Raqib R, Guðmundsson GH, Bergman P, Agerberth B, Rekha RS. Host-Directed Therapy as a Novel Treatment Strategy to Overcome Tuberculosis: Targeting Immune Modulation. *Antibiotics (Basel)*. 2020;9(1). doi:10.3390/antibiotics9010021

35. Madan-Lala R, Sia JK, King R, et al. Mycobacterium tuberculosis Impairs Dendritic Cell Functions through the Serine Hydrolase Hip1. *The Journal of Immunology*. 2014;192(9):4263. doi:10.4049/jimmunol.1303185

36. Green Douglas R., Kroemer Guido. The Pathophysiology of Mitochondrial Cell Death. *Science*. 2004;305(5684):626-629. doi:10.1126/science.1099320

37. Behar SM, Martin CJ, Nunes-Alves C, Divangahi M, Remold HG. Lipids, apoptosis, and cross-presentation: links in the chain of host defense against Mycobacterium tuberculosis. *Microbes and Infection*. 2011;13(8):749-756. doi:10.1016/j.micinf.2011.03.002

38. Ehlers S, Schaible U. The Granuloma in Tuberculosis: Dynamics of a Host–Pathogen Collusion. *Frontiers in Immunology*. 2013;3. https://www.frontiersin.org/article/10.3389/fimmu.2012.00411

39. Hajipour MJ, Fromm KM, Akbar Ashkarran A, et al. Antibacterial properties of nanoparticles. *Trends in Biotechnology*. 2012;30(10):499-511. doi:10.1016/j.tibtech.2012.06.004

40. Nasiruddin M, Neyaz MK, Das S. Nanotechnology-Based Approach in Tuberculosis Treatment. *Tuberculosis Research and Treatment*. 2017;2017:4920209. doi:10.1155/2017/4920209

41. World Health Organization. *Global Tuberculosis Report 2018*. World Health Organization; 2018.

42. Patterson B, Smith D, Telford A, et al. Vitamin D deficiency predicts latent TB reactivation independent of preventive therapy: a longitudinal study. *The International Journal of Tuberculosis and Lung Disease*. 2020;24(9):916-921. doi:10.5588/ijtld.19.0605

43. Mueller P, Pieters J. Modulation of macrophage antimicrobial mechanisms by pathogenic mycobacteria. *Immunobiology*. 2006;211(6):549-556. doi:10.1016/j.imbio.2006.06.004

44. Arbex MA, Varella M de CL, Siqueira HR de, Mello FAF de. Antituberculosis drugs: drug interactions, adverse effects, and use in special situations. Part 1: first-line drugs. *Jornal Brasileiro de Pneumologia*. 2010;36(5):626-640. doi:10.1590/s1806-37132010000500016

45. World Health Organization. *Global Tuberculosis Report 2019*. World Health Organization; 2019.

46. Dartois V. The path of anti-tuberculosis drugs: from blood to lesions to mycobacterial cells. *Nature Reviews Microbiology*. 2014;12(3):159-167. doi:10.1038/nrmicro3200

47. Gurumurthy Prema, Ramachandran Geetha, Hemanth Kumar A. K., et al. Decreased Bioavailability of Rifampin and Other Antituberculosis Drugs in Patients with Advanced Human Immunodeficiency Virus Disease. *Antimicrobial Agents and Chemotherapy*. 2004;48(11):4473-4475. doi:10.1128/AAC.48.11.4473-4475.2004

48. Sarathy JP, Lee E, Dartois V. Polyamines Inhibit Porin-Mediated Fluoroquinolone Uptake in Mycobacteria. *Public Library of Science One*. 2013;8(6):1-10. doi:10.1371/journal.pone.0065806

49. Goossens SN, Sampson SL, Van Rie A. Mechanisms of Drug-Induced Tolerance in Mycobacterium tuberculosis. *Clinical Microbiology Reviews*. 2020;34(1). doi:10.1128/CMR.00141-20

50. Kohanski MA, Dwyer DJ, Collins JJ. How antibiotics kill bacteria: from targets to networks. *Nature Reviews Microbiology*. 2010;8(6):423-435. doi:10.1038/nrmicro2333

51. Mahajan R. Bedaquiline: First FDA-approved tuberculosis drug in 40 years. *International Journal of Applied and Basic Medical Research*. 2013;3(1):1-2. doi:10.4103/2229-516X.112228

52. European Medicines Agency. *Sirturo: European Public Assessment Report - Product Information*.; 2019. Accessed June 28, 2022. https://www.ema.europa.eu/en/documents/product-information/sirturo-epar-product-information_en.pdf

53. Koul A, Vranckx L, Dendouga N, et al. Diarylquinolines are bactericidal for dormant mycobacteria as a result of disturbed ATP homeostasis. *The Journal of Biological Chemistry*. 2008;283(37):25273-25280. doi:10.1074/jbc.M803899200

54. Patel H, Pawara R, Pawara K, Ahmed F, Shirkhedkar A, Surana S. A structural insight of bedaquiline for the cardiotoxicity and hepatotoxicity. *Tuberculosis*. 2019;117:79-84. doi:10.1016/j.tube.2019.06.005

55. Gao M, Gao J, Xie L, et al. Early outcome and safety of bedaquiline-containing regimens for treatment of MDR- and XDR-TB in China: a multicentre study. *Clinical Microbiology and Infection*. 2021;27(4):597-602. doi:10.1016/j.cmi.2020.06.004

56. Salhotra VS, Sachdeva KS, Kshirsagar N, et al. Effectiveness and safety of bedaquiline under conditional access program for treatment of drug-resistant tuberculosis in India: An interim analysis. *The Indian Journal of Tuberculosis*. 2020;67(1):29-37. doi:10.1016/j.ijtb.2019.10.002

57. Olayanju O, Esmail A, Limberis J, Dheda K. A regimen containing bedaquiline and delamanid compared to bedaquiline in patients with drug-resistant tuberculosis. *The European Respiratory Journal*. 2020;55(1):1901181. doi:10.1183/13993003.01181-2019

58. Mpobela Agnarson A, Williams A, Kambili C, Mattson G, Metz L. The cost-effectiveness of a bedaquiline-containing short-course regimen for the treatment of multidrug-resistant tuberculosis in South Africa. *Expert Review of Anti-Infective Therapy*. 2020;18(5):475-483. doi:10.1080/14787210.2020.1742109

59. Kim JH, Kwon OJ, Kim YS, Park MS, Hwang S, Shim TS. Bedaquiline in multidrug-resistant tuberculosis treatment: Safety and efficacy in a Korean subpopulation. *Respiratory Investigation*. 2020;58(1):45-51. doi:10.1016/j.resinv.2019.08.004

60. Kang H, Jo KW, Jeon D, Yim JJ, Shim TS. Interim treatment outcomes in multidrug-resistant tuberculosis using bedaquiline and/or delamanid in South Korea. *Respiratory Medicine*. 2020;167:105956. doi:10.1016/j.rmed.2020.105956

61. World Health Organization. *WHO Consolidated Guidelines on Drug-Resistant Tuberculosis Treatment*. World Health Organization; 2019. Accessed June 29, 2022. https://www.ncbi.nlm.nih.gov/books/NBK539513/

62. Liu Y, Matsumoto M, Ishida H, et al. Delamanid: From discovery to its use for pulmonary multidrug-resistant tuberculosis (MDR-TB). *Tuberculosis*. 2018;111:20-30. doi:10.1016/j.tube.2018.04.008

63. Sasahara K, Shimokawa Y, Hirao Y, et al. Pharmacokinetics and Metabolism of Delamanid, a Novel Anti-Tuberculosis Drug, in Animals and Humans: Importance of Albumin Metabolism In Vivo. *Drug Metabolism and Disposition*. 2015;43(8):1267-1276. doi:10.1124/dmd.115.064527

64. European Medicines Agency. *Assessment Report: Deltyba*. Committee for Medicinal Products for Human Use (CHMP); 2013. Accessed June 29, 2022. https://www.ema.europa.eu/en/documents/assessment-report/deltyba-epar-public-assessment-report_en.pdf

65. Shibata M, Shimokawa Y, Sasahara K, et al. Absorption, distribution and excretion of the anti-tuberculosis drug delamanid in rats: Extensive tissue distribution suggests potential therapeutic value for extrapulmonary tuberculosis. *Biopharmaceutics and Drug Disposition*. 2017;38(4):301-312. doi:10.1002/bdd.2064

66. Matsumoto M, Hashizume H, Tomishige T, et al. OPC-67683, a nitro-dihydro-imidazooxazole derivative with promising action against tuberculosis in vitro and in mice. *Public Library of Science Medicine*. 2006;3(11):e466. doi:10.1371/journal.pmed.0030466

67. Chen X, Hashizume H, Tomishige T, et al. Delamanid Kills Dormant Mycobacteria In Vitro and in a Guinea Pig Model of Tuberculosis. *Antimicrobial Agents and Chemotherapy*. 2017;61(6). doi:10.1128/AAC.02402-16

68. Kwon YS, Jeon D, Kang H, Yim JJ, Shim TS. Concurrent use of bedaquiline and delamanid for the treatment of fluoroquinolone-resistant multidrug-resistant tuberculosis: a nationwide cohort study in South Korea. *The European Respiratory Journal*. 2021;57(3):2003026. doi:10.1183/13993003.03026-2020

69. Pontali E, Sotgiu G, Tiberi S, et al. Combined treatment of drug-resistant tuberculosis with bedaquiline and delamanid: a systematic review. *The European Respiratory Journal*. 2018;52(1):1800934. doi:10.1183/13993003.00934-2018

70. Vambe D, Kay AW, Furin J, et al. Bedaquiline and delamanid result in low rates of unfavourable outcomes among TB patients in Eswatini. *The*

International Journal of Tuberculosis and Lung Disease. 2020;24(10):1095-1102. doi:10.5588/ijtld.20.0082

71. Hafkin J, Frias M, Hesseling A, Gler M, Hittel N. *Pharmacokinetics and safety of delamanid in children ages 6-17 years with MDR-TB.* Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC), Poster A-960, San Diego, 2015.

72. World Health Organization. *Rapid Communication: Key Changes to the Treatment of Drug-Resistant Tuberculosis*. World Health Organization Accessed June 29, 2022. https://www.who.int/publications/i/item/WHO-UCN-TB-2022-2

73. Singh Ramandeep, Manjunatha Ujjini, Boshoff Helena I. M., et al. PA-824 Kills Nonreplicating Mycobacterium tuberculosis by Intracellular NO Release. *Science*. 2008;322(5906):1392-1395. doi:10.1126/science.1164571

74. Stover CK, Warrener P, VanDevanter DR, et al. A small-molecule nitroimidazopyran drug candidate for the treatment of tuberculosis. *Nature*. 2000;405(6789):962-966. doi:10.1038/35016103

75. Tyagi S, Nuermberger E, Yoshimatsu T, et al. Bactericidal activity of the nitroimidazopyran PA-824 in a murine model of tuberculosis. *Antimicrobial Agents and Chemotherapy*. 2005;49(6):2289-2293. doi:10.1128/AAC.49.6.2289-2293.2005

76. Manjunatha U, Boshoff HI, Barry CE. The mechanism of action of PA-824: Novel insights from transcriptional profiling. *Communicative and Integrative Biology*. 2009;2(3):215-218. doi:10.4161/cib.2.3.7926

77. Shirley M. Pretomanid in drug-resistant tuberculosis: a profile of its use. *Drugs and Therapy Perspectives*. Published online 2020. doi:10.6084/m9.figshare.12278645.v1

78. Ginsberg AM, Laurenzi MW, Rouse DJ, Whitney KD, Spigelman MK. Safety, tolerability, and pharmacokinetics of PA-824 in healthy subjects. *Antimicrobial Agents and Chemotherapy*. 2009;53(9):3720-3725. doi:10.1128/AAC.00106-09

79. Winter H, Ginsberg A, Egizi E, et al. Effect of a high-calorie, high-fat meal on the bioavailability and pharmacokinetics of PA-824 in healthy adult subjects. *Antimicrobial Agents and Chemotherapy*. 2013;57(11):5516-5520. doi:10.1128/AAC.00798-13

80. Keam SJ. Pretomanid: First Approval. *Drugs*. 2019;79(16):1797-1803. doi:10.1007/s40265-019-01207-9

81. Ginsberg AM, Laurenzi MW, Rouse DJ, Whitney KD, Spigelman MK. Assessment of the effects of the nitroimidazo-oxazine PA-824 on renal function in healthy subjects. *Antimicrobial Agents and Chemotherapy*. 2009;53(9):3726-3733. doi:10.1128/AAC.00112-09

82. Winter H, Egizi E, Erondu N, et al. Evaluation of pharmacokinetic interaction between PA-824 and midazolam in healthy adult subjects. *Antimicrobial Agents and Chemotherapy*. 2013;57(8):3699-3703. doi:10.1128/AAC.02632-12

83. Diacon AH, Dawson R, von Groote-Bidlingmaier F, et al. 14-day bactericidal activity of PA-824, bedaquiline, pyrazinamide, and moxifloxacin combinations: a randomised trial. *Lancet*. 2012;380(9846):986-993. doi:10.1016/S0140-6736(12)61080-0

84. Diacon AH, Dawson R, von Groote-Bidlingmaier F, et al. Bactericidal activity of pyrazinamide and clofazimine alone and in combinations with pretomanid and bedaquiline. *American Journal of Respiratory and Critical Care Medicine*. 2015;191(8):943-953. doi:10.1164/rccm.201410-1801OC

85. Dawson R, Diacon AH, Everitt D, et al. Efficiency and safety of the combination of moxifloxacin, pretomanid (PA-824), and pyrazinamide during the first 8 weeks of antituberculosis treatment: a phase 2b, open-label, partly randomised trial in patients with drug-susceptible or drug-resistant pulmonary tuberculosis. *Lancet*. 2015;385(9979):1738-1747. doi:10.1016/S0140-6736(14)62002-X

86. Tweed CD, Dawson R, Burger DA, et al. Bedaquiline, moxifloxacin, pretomanid, and pyrazinamide during the first 8 weeks of treatment of patients with drug-susceptible or drug-resistant pulmonary tuberculosis: a

multicentre, open-label, partially randomised, phase 2b trial. *The Lancet Respiratory Medicine*. 2019;7(12):1048-1058. doi:10.1016/S2213-2600(19)30366-2

87. Gils T, Lynen L, de Jong BC, Van Deun A, Decroo T. Pretomanid for tuberculosis: a systematic review. *Clinical Microbiology and Infection*. 2022;28(1):31-42. doi:10.1016/j.cmi.2021.08.007

88. Diacon AH, Dawson R, du Bois J, et al. Phase II dose-ranging trial of the early bactericidal activity of PA-824. *Antimicrobial Agents and Chemotherapy*. 2012;56(6):3027-3031. doi:10.1128/AAC.06125-11

89. Diacon AH, Dawson R, Hanekom M, et al. Early bactericidal activity and pharmacokinetics of PA-824 in smear-positive tuberculosis patients. *Antimicrobial Agents and Chemotherapy*. 2010;54(8):3402-3407. doi:10.1128/AAC.01354-09

90. Kolloli A, Subbian S. Host-Directed Therapeutic Strategies for Tuberculosis. *Frontiers in Medicine*. 2017;4. doi:10.3389/fmed.2017.00171

91. Paik S, Kim JK, Chung C, Jo EK. Autophagy: A new strategy for host-directed therapy of tuberculosis. *Virulence*. 2019;10(1):448-459. doi:10.1080/21505594.2018.1536598

92. Gupta A, Misra A, Deretic V. Targeted pulmonary delivery of inducers of host macrophage autophagy as a potential host-directed chemotherapy of tuberculosis. *Advanced Drug Delivery Reviews*. 2016;102:10-20. doi:10.1016/j.addr.2016.01.016

93. Kimmey JM, Stallings CL. Bacterial Pathogens versus Autophagy: Implications for Therapeutic Interventions. *Trends in Molecular Medicine*. 2016;22(12):1060-1076. doi:10.1016/j.molmed.2016.10.008

94. Sundaramurthy V, Barsacchi R, Samusik N, et al. Integration of chemical and RNAi multiparametric profiles identifies triggers of intracellular mycobacterial killing. *Cell Host and Microbe*. 2013;13(2):129-142. doi:10.1016/j.chom.2013.01.008

95. Sharma V, Makhdoomi M, Kumar P, et al. Induction of autophagy by trehalose limits opportunistic mycobacterial infections in HIV-infected

macrophages. *bioRxiv*. Published online January 1, 2017:202697. doi:10.1101/202697

96. Chandra Pallavi, Rajmani R. S., Verma Garima, Bhavesh Neel Sarovar, Kumar Dhiraj, Stallings Christina L. Targeting Drug-Sensitive and -Resistant Strains of Mycobacterium tuberculosis by Inhibition of Src Family Kinases Lowers Disease Burden and Pathology. *mSphere*. 1(2):e00043-15. doi:10.1128/mSphere.00043-15

97. Stanley SA, Barczak AK, Silvis MR, et al. Identification of Host-Targeted Small Molecules That Restrict Intracellular Mycobacterium tuberculosis Growth. *Public Library of Science Pathogens*. 2014;10(2):e1003946. doi:10.1371/journal.ppat.1003946

98. Huang J, Canadien V, Lam GY, et al. Activation of antibacterial autophagy by NADPH oxidases. *Proceedings of the National Academy of Sciences of the United States of America*. 2009;106(15):6226-6231. doi:10.1073/pnas.0811045106

99. Yang CS, Lee JS, Rodgers M, et al. Autophagy protein Rubicon mediates phagocytic NADPH oxidase activation in response to microbial infection or TLR stimulation. *Cell Host and Microbe*. 2012;11(3):264-276. doi:10.1016/j.chom.2012.01.018

100. Campbell GR, Spector SA. Autophagy induction by vitamin D inhibits both Mycobacterium tuberculosis and human immunodeficiency virus type 1. *Autophagy*. 2012;8(10):1523-1525. doi:10.4161/auto.21154

101. Yuk JM, Shin DM, Lee HM, et al. Vitamin D3 induces autophagy in human monocytes/macrophages via cathelicidin. *Cell Host and Microbe*. 2009;6(3):231-243. doi:10.1016/j.chom.2009.08.004

102. Rekha RS, Rao Muvva SSVJ, Wan M, et al. Phenylbutyrate induces LL-37-dependent autophagy and intracellular killing of Mycobacterium tuberculosis in human macrophages. *Autophagy*. 2015;11(9):1688-1699. doi:10.1080/15548627.2015.1075110

103. Mily A, Rekha RS, Kamal SMM, et al. Oral intake of phenylbutyrate with or without vitamin D3 upregulates the cathelicidin LL-37 in human

macrophages: a dose finding study for treatment of tuberculosis. *Biomed Central Pulmonary Medicine*. 2013;13:23. doi:10.1186/1471-2466-13-23

104. Fabri M, Stenger S, Shin DM, et al. Vitamin D is required for IFN-gamma-mediated antimicrobial activity of human macrophages. *Science Translational Medicine*. 2011;3(104):104ra102. doi:10.1126/scitranslmed.3003045

105. Singhal A, Jie L, Kumar P, et al. Metformin as adjunct antituberculosis therapy. *Science Translational Medicine*. 2014;6(263):263ra159. doi:10.1126/scitranslmed.3009885

106. Degner NR, Wang JY, Golub JE, Karakousis PC. Metformin Use Reverses the Increased Mortality Associated With Diabetes Mellitus During Tuberculosis Treatment. *Clinical Infectious Diseases*. 2018;66(2):198-205. doi:10.1093/cid/cix819

107. Corcoran C, Jacobs TF. Metformin. In: *StatPearls*. StatPearls Publishing; 2022.

108. World Health Organization. *WHO Model List of Essential Medicines - 22nd List, 2021*. World Health Organization; 2021. Accessed June 29, 2022. https://www.who.int/publications/i/item/WHO-MHP-HPS-EML-2021.02

109. Lee YJ, Han SK, Park JH, et al. The effect of metformin on culture conversion in tuberculosis patients with diabetes mellitus. *The Korean Journal of Internal Medicine*. 2018;33(5):933-940. doi:10.3904/kjim.2017.249

110. Dutta NK, Pinn ML, Karakousis PC. Metformin Adjunctive Therapy Does Not Improve the Sterilizing Activity of the First-Line Antitubercular Regimen in Mice. *Antimicrobial Agents and Chemotherapy*. 2017;61(8). doi:10.1128/AAC.00652-17

111. Arai M, Uchiba M, Komura H, Mizuochi Y, Harada N, Okajima K. Metformin, an antidiabetic agent, suppresses the production of tumor necrosis factor and tissue factor by inhibiting early growth response factor-1 expression in human monocytes in vitro. *The Journal of Pharmacology and Experimental Therapeutics*. 2010;334(1):206-213. doi:10.1124/jpet.109.164970

112. Lachmandas E, Eckold C, Böhme J, et al. Metformin Alters Human Host Responses to Mycobacterium tuberculosis in Healthy Subjects. *The Journal of Infectious Diseases*. 2019;220(1):139-150. doi:10.1093/infdis/jiz064

113. Druker BJ, O'Brien SG, Cortes J, Radich J. Chronic myelogenous leukemia. *Hematology, The American Society of Hematology Education Program*. Published online 2002:111-135. doi:10.1182/asheducation-2002.1.111

114. Yogalingam G, Pendergast AM. Abl kinases regulate autophagy by promoting the trafficking and function of lysosomal components. *The Journal of Biological Chemistry*. 2008;283(51):35941-35953. doi:10.1074/jbc.M804543200

115. Burton EA, Plattner R, Pendergast AM. Abl tyrosine kinases are required for infection by Shigella flexneri. *The European Molecular Biology Journal*. 2003;22(20):5471-5479. doi:10.1093/emboj/cdg512

116. Reeves PM, Bommarius B, Lebeis S, et al. Disabling poxvirus pathogenesis by inhibition of Abl-family tyrosine kinases. *Nature Medicine*. 2005;11(7):731-739. doi:10.1038/nm1265

117. Reeves PM, Smith SK, Olson VA, et al. Variola and monkeypox viruses utilize conserved mechanisms of virion motility and release that depend on abl and SRC family tyrosine kinases. *Journal of Virology*. 2011;85(1):21-31. doi:10.1128/JVI.01814-10

118. Swimm A, Bommarius B, Li Y, et al. Enteropathogenic Escherichia coli use redundant tyrosine kinases to form actin pedestals. *Molecular Biology of the Cell*. 2004;15(8):3520-3529. doi:10.1091/mbc.e04-02-0093

119. Napier RJ, Rafi W, Cheruvu M, et al. Imatinib-sensitive tyrosine kinases regulate mycobacterial pathogenesis and represent therapeutic targets against tuberculosis. *Cell Host and Microbe*. 2011;10(5):475-485. doi:10.1016/j.chom.2011.09.010

120. Kerkelä R, Grazette L, Yacobi R, et al. Cardiotoxicity of the cancer therapeutic agent imatinib mesylate. *Nature Medicine*. 2006;12(8):908-916. doi:10.1038/nm1446

121. Mumprecht S, Matter M, Pavelic V, Ochsenbein AF. Imatinib mesylate selectively impairs expansion of memory cytotoxic T cells without affecting the control of primary viral infections. *Blood*. 2006;108(10):3406-3413. doi:10.1182/blood-2006-04-018705

122. European Medicines Agency. *Glivec*. European Medicines Agency; 2009. Accessed June 29, 2022. https://www.ema.europa.eu/en/medicines/human/EPAR/glivec

123. Krasnodembskaya A, Song Y, Fang X, et al. Antibacterial Effect of Human Mesenchymal Stem Cells Is Mediated in Part from Secretion of the Antimicrobial Peptide LL-37. *Stem Cells*. 2010;28(12):2229-2238. doi:10.1002/stem.544

124. Tropea KA, Leder E, Aslam M, et al. Bronchioalveolar stem cells increase after mesenchymal stromal cell treatment in a mouse model of bronchopulmonary dysplasia. *American Journal of Physiology Lung Cellular and Molecular Physiology*. 2012;302(9):L829-837. doi:10.1152/ajplung.00347.2011

125. Skrahin A, Ahmed RK, Ferrara G, et al. Autologous mesenchymal stromal cell infusion as adjunct treatment in patients with multidrug and extensively drug-resistant tuberculosis: an open-label phase 1 safety trial. *The Lancet Respiratory Medicine*. 2014;2(2):108-122. doi:10.1016/S2213-2600(13)70234-0

126. Skrahin A, Jenkins HE, Hurevich H, et al. Effectiveness of a novel cellular therapy to treat multidrug-resistant tuberculosis. *International Journal of Mycobacteriology*. 2016;5 Suppl 1:S23. doi:10.1016/j.ijmyco.2016.11.018

127. Dube A, Lemmer Y, Hayeshi R, et al. State of the art and future directions in nanomedicine for tuberculosis. *Expert Opinion on Drug Delivery*. 2013;10(12):1725-1734. doi:10.1517/17425247.2014.846905

128. Tukulula M, Gouveia L, Paixao P, Hayeshi R, Naicker B, Dube A. Functionalization of PLGA Nanoparticles with 1,3-β-glucan Enhances the Intracellular Pharmacokinetics of Rifampicin in Macrophages. *Pharmaceutical Research*. 2018;35(6):111. doi:10.1007/s11095-018-2391-8

129. Detampel P, Ganguly A, Tehranian S, et al. In vivo clearance of nanoparticles by transcytosis across alveolar epithelial cells. *Public Library of Science One*. 2019;14(9):e0223339. doi:10.1371/journal.pone.0223339

130. Chae J, Choi Y, Tanaka M, Choi J. Inhalable nanoparticles delivery targeting alveolar macrophages for the treatment of pulmonary tuberculosis. *Journal of Bioscience and Bioengineering*. 2021;132(6):543-551. doi:10.1016/j.jbiosc.2021.08.009

131. Azarmi S, Roa WH, Löbenberg R. Targeted delivery of nanoparticles for the treatment of lung diseases. *Advanced Drug Delivery Reviews*. 2008;60(8):863-875. doi:10.1016/j.addr.2007.11.006

132. Liu WT. Nanoparticles and their biological and environmental applications. *Journal of Bioscience and Bioengineering*. 2006;102(1):1-7. doi:10.1263/jbb.102.1

133. Abdelaziz HM, Gaber M, Abd-Elwakil MM, et al. Inhalable particulate drug delivery systems for lung cancer therapy: Nanoparticles, microparticles, nanocomposites and nanoaggregates. *Journal of Controlled Release*. 2018;269:374-392. doi:10.1016/j.jconrel.2017.11.036

134. Thorley AJ, Ruenraroengsak P, Potter TE, Tetley TD. Critical Determinants of Uptake and Translocation of Nanoparticles by the Human Pulmonary Alveolar Epithelium. *American Chemical Society Nano*. 2014;8(11):11778-11789. doi:10.1021/nn505399e

135. Ellis T, Chiappi M, García-Trenco A, et al. Multimetallic Microparticles Increase the Potency of Rifampicin against Intracellular Mycobacterium tuberculosis. *American Chemical Society Nano*. 2018;12(6):5228-5240. doi:10.1021/acsnano.7b08264

136. Pandey R, Sharma A, Zahoor A, Sharma S, Khuller GK, Prasad B. Poly (DL-lactide-co-glycolide) nanoparticle-based inhalable sustained drug delivery system for experimental tuberculosis. *The Journal of Antimicrobial Chemotherapy*. 2003;52(6):981-986. doi:10.1093/jac/dkg477

137. Pandey R, Khuller GK. Solid lipid particle-based inhalable sustained drug delivery system against experimental tuberculosis. *Tuberculosis*. 2005;85(4):227-234. doi:10.1016/j.tube.2004.11.003

138. Wilkinson L. Félix d'Herelle and the origins of molecular biology. *Medical History and Bioethics*. 2001;45(2):294-295. doi:PMC1044364

139. Chanishvili N. Phage therapy--history from Twort and d'Herelle through Soviet experience to current approaches. *Advances in Virus Research*. 2012;83:3-40. doi:10.1016/B978-0-12-394438-2.00001-3

140. Sulakvelidze A, Alavidze Z, Morris JGJ. Bacteriophage therapy. *Antimicrobial Agents and Chemotherapy*. 2001;45(3):649-659. doi:10.1128/AAC.45.3.649-659.2001

141. Dublanchet A, Bourne S. The epic of phage therapy. *The Canadian Journal of Infectious Diseases and Medical Microbiology*. 2007;18(1):15-18. doi:10.1155/2007/365761

142. Kokin G. *Phage Therapy and Phage Prophylaxis of Gas Gangrene.* Vol Volume 3. Medgiz; 1946.

143. Saadhali SA, Hassan S, Hanna LE, Ranganathan UD, Kumar V. Homology modeling, substrate docking, and molecular simulation studies of mycobacteriophage Che12 lysin A. *Journal of Molecular Modeling*. 2016;22(8):180. doi:10.1007/s00894-016-3056-3

144. Allué-Guardia A, Saranathan R, Chan J, Torrelles JB. Mycobacteriophages as Potential Therapeutic Agents against Drug-Resistant Tuberculosis. *International Journal of Molecular Sciences*. 2021;22(2). doi:10.3390/ijms22020735

145. Gigante AM, Hampton CM, Dillard RS, et al. The Ms6 Mycolyl-Arabinogalactan Esterase LysB is Essential for an Efficient Mycobacteriophage-Induced Lysis. *Viruses*. 2017;9(11). doi:10.3390/v9110343

146. Payne K, Sun Q, Sacchettini J, Hatfull GF. Mycobacteriophage Lysin B is a novel mycolylarabinogalactan esterase. *Molecular Microbiology*. 2009;73(3):367-381. doi:10.1111/j.1365-2958.2009.06775.x

147. Sula L, Sulová J, Stolcpartová M. Therapy of experimental tuberculosis in guinea pigs with mycobacterial phages DS-6A, GR-21 T, My-327. *Czechoslovak Medicine*. 1981;4(4):209-214.

148. Peng L, Luo Y, Chen B, et al. Therapeutic effect of bacteriophage D29 in the treatment for guinea pigs infected with sensitive strain of Mycobacterium tuberculosis. *Chinese Journal of Zoonoses*. 2009;25(08):733.

149. Azimi T, Mosadegh M, Nasiri MJ, Sabour S, Karimaei S, Nasser A. Phage therapy as a renewed therapeutic approach to mycobacterial infections: a comprehensive review. *Infection and Drug Resistance*. 2019;12:2943-2959. doi:10.2147/IDR.S218638

150. Ooi ML, Drilling AJ, Morales S, et al. Safety and Tolerability of Bacteriophage Therapy for Chronic Rhinosinusitis Due to Staphylococcus aureus. *Journal of the American Medical Association Otolaryngology - Head and Neck Surgery*. 2019;145(8):723-729. doi:10.1001/jamaoto.2019.1191

151. Bogovazova GG, Voroshilova NN, Bondarenko VM. [The efficacy of Klebsiella pneumoniae bacteriophage in the therapy of experimental Klebsiella infection]. *Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii*. 1991;(4):5-8.

152. Principi N, Silvestri E, Esposito S. Advantages and Limitations of Bacteriophages for the Treatment of Bacterial Infections. *Frontiers in Pharmacology*. 2019;10. doi:10.3389/fphar.2019.00513

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