Host-directed therapy for the treatment of tuberculosis: rewiring the host to recover control

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Appendices

English Summary

Tuberculosis (TB), caused by Mycobacterium tuberculosis (Mtb), remains one of the world's leading causes of infectious disease-related deaths. The emergence of multidrug-resistant (MDR), extensively drug-resistant (XDR), and totally drug-resistant (TDR) strains, increasingly undermine TB control efforts. *Mtb*'s ability to establish latent, metabolically dormant infections within host cells further complicates treatment, as conventional antibiotics primarily target actively replicating bacilli, leaving dormant populations unaffected. Standard treatment regimens require six months or longer of multidrug therapy, causing significant toxicity and poor adherence, which drive further antibiotic resistance. The only licensed vaccine, Bacille Calmette-Guérin (BCG), offers only partial and inconsistent protection in adults.

Mtb persists by manipulating host immune pathways. It hijacks macrophage signalling, delays adaptive immune responses, and promotes granuloma formation to evade clearance. These host-pathogen interactions demand therapies that not only kill the pathogen, but also reprogram host responses to improve bacterial clearance.

Host-directed therapy (HDT) strengthens the host's own antimicrobial mechanisms by targeting pathways *Mtb* exploits. Unlike antibiotics, HDTs act on host cells, making them less likely to be rendered ineffective through bacterial resistance. They also target dormant *Mtb*, work synergistically with antibiotics, and may shorten treatment duration. Because many host pathways are conserved across pathogens, HDTs hold cross-pathogen potential, such as against nontuberculous mycobacteria (NTM) and *Salmonella enterica* (*Stm*). Lastly, the repurposing of existing clinically approved drugs with established safety profiles can fast-track their development as HDT agent for the treatment of TB. Mechanistically, HDTs may enhance the intrinsic cellular defence mechanisms, modulate immune responses to enhance pathogen clearance while minimizing tissue damage, regulate host cell death pathways, and reprogram epigenetic states to induce bactericidal macrophage phenotypes. Despite all advantages, effective HDTs for TB remain limited, which requires systematic discovery and validation.

To this end, this thesis describes new tools to accelerate HDT discovery (**Chapter 2**). A medium-throughput, flow cytometry-based screening platform was developed to rapidly quantify intracellular bacterial burden in infected cells. By combining fluorescently tagged bacteria with automated cytometry, analysis time was reduced from weeks to days compared to traditional colony-forming unit (CFU) assays. This

platform was complemented with a machine learning model trained on screening data and integrated with public bioactivity databases. This computational model predicted active HDT candidates by identifying compounds likely to modulate host pathways essential for intracellular bacterial survival. Together, these approaches enable the systematic discovery of multiple HDT candidates.

The initial screening of 1,260 pharmacologically active compounds revealed several hits with HDT activity, with receptor tyrosine kinase (RTK) inhibitors emerging as a major druggable pathway (**Chapter 2**). The machine learning model successfully predicted additional effective RTK inhibitors, with greatly increased success rate compared to the initial screen. An unbiased human kinome-wide siRNA screen confirmed the essential role of RTK signalling in regulating intracellular *Mtb* survival. These findings collectively established RTK signalling as a central regulators in intracellular *Mtb* survival and positioned RTK inhibitors as strong HDT candidates. Notable compounds include dovitinib, AT9283, and ENMD-2076, which significantly reduce intracellular *Mtb* burden, including MDR strains.

Next existing HDT candidates were optimized to improve efficacy. The AKT1/PKB inhibitor H-89, previously identified as an HDT candidate, only shows moderate activity against *Mtb*. To find more effective analogues, a library of 76 structural analogues of H-89 was screened (**Chapter 3**). This led to the discovery of 97i, which demonstrated strong activity against drug-sensitive and MDR-*Mtb* strains in primary human macrophages and showed synergy with suboptimal concentrations of Rifampicin. 97i activity was confirmed cross-species in non-human primate alveolar macrophages, and *in vivo* in a zebrafish embryo model. Mechanistically, 97i promotes autophagy and lysosomal activity, accelerating intracellular bacterial degradation. Unlike H-89, 97i also inhibits PCTAIRE family kinases, CAMK1, and PFKL, broadening its host-targeting profile. This work highlights structure-guided optimization as a strategy to refine HDT efficacy.

Autophagy plays a central role in host defence against *Mtb*. To exploit this, a library of 96 autophagy-modulating compounds was screened which led to the identification of six potent HDT candidates (**Chapter 4**). Two of these, the structural analogues Pimozide and Fluspirilene, were investigated in detail. Both drugs enhance lysosomal biogenesis and autophagy through TFEB nuclear translocation, a master regulator of lysosomal and autophagic gene expression. Pimozide additionally modulates immune signalling by inhibiting STAT5 phosphorylation, reducing CISH accumulation on *Mtb* phagosomes, which promotes phagosomal acidification. It also uniquely induces reactive oxygen and nitrogen species (ROS/RNS), known contributors to bacterial

killing. Importantly, both drugs are effective against MDR-*Mtb* and *Stm*, reinforcing autophagy and lysosomal activation as key host mechanisms.

The autophagy screen also identified Tamoxifen, an anti-cancer drug and selective estrogen receptor modulator (SERM). Despite its established role as a SERM, mechanistic studies, including ER knockout mutants and transcriptomics, confirmed that Tamoxifen's HDT activity is independent of estrogen receptor signalling (**Chapter 5**). Instead, Tamoxifen activates TFEB, enhances lysosomal biogenesis and increases *Mtb* localization to the lysosome. Tamoxifen effectively reduced MDR-*Mtb* in human macrophages and in zebrafish embryos, emphasizing its potential for rapid translation through drug repurposing.

While HDT agents that directly stimulate cellular defence processes are obvious candidates, reprogramming of the macrophage through epigenetic modulation might hold promise as well. First was shown that *Mtb* infection alters histone deacetylase (HDAC) expression, implicating HDACs in intracellular *Mtb* infection (**Chapter 6**). Subsequently, both pan-HDAC inhibitors, such as Trichostatin A (TSA), and selective class IIa HDAC inhibitors, TMP195 and TMP269, were found to significantly reduce intracellular *Mtb* burden. HDAC inhibition polarizes the macrophages toward a bactericidal phenotype and suppresses pro-inflammatory cytokine production, improving pathogen clearance. The HDT activity of HDAC inhibition was validated in an *in vivo* zebrafish embryo model, thus identifying HDACs as promising HDT targets.

Despite their diverse chemical structures and primary targets, multiple HDT candidates, 97i, Pimozide, Fluspirilene, and Tamoxifen, converged on a shared mechanism, that is TFEB-mediated lysosomal activation and autophagy induction. This highlights their role as a central host pathway for controlling intracellular pathogens. The cross-pathogen efficacy of these compounds against *Stm* and the validation of HDT candidates in primary human macrophages, bronchoalveolar lavage (BAL) cells from non-human primates, and zebrafish models, demonstrate their translational potential. Their activity on MDR-*Mtb* and synergy observed with a conventional antibiotic further supports the integration of HDTs into current TB treatment regimens.

This thesis provides strong preclinical evidence that HDT can transform TB treatment. By targeting host pathways involved in autophagy, lysosomal activation, RTK signalling, and epigenetic regulation, HDTs enhance bacterial clearance, combat antibiotic-resistant strains, and may shorten treatment duration. The combination of phenotypic screening, predictive machine learning, and mechanistic validation yielded several

promising HDT candidates, some of which, such as Pimozide, Fluspirilene and Tamoxifen, are already clinically approved, enabling accelerated translation.

By training the host immune system to fight more effectively, HDTs represent a paradigm shift in TB therapy and hold promise for TB as well as other intracellular infections.