Quantitative Systems Pharmacology approaches for Development of Host-Directed Therapies against Mycobacterium tuberculosis infections

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Abstract

Host-directed therapies (HDT) that modulate host-pathogen interaction offer an innovative strategy to combat Mycobacterium tuberculosis (Mtb) infections. When combined with conventional anti-tuberculosis regimens, HDT strategies could contribute to improving treatment outcomes, reducing treatment duration, and preventing resistance development. It is however challenging to evaluate the interplay of host-pathogen interaction events in response to HDT strategies, and to translate experimental findings towards patients. Quantitative understanding of the multi-faceted nature of the host-pathogen interactions is vital to rationally design HDT strategies. Here, we (1) provide an overview of key host-pathogen interactions as basis for HDT strategies, (2) discuss experimental models to characterize host-pathogen interactions relevant for HDTs, and (3) discuss the utility and approaches of quantitative systems pharmacology (QSP) models to inform design of HDT strategies against Mtb infections. QSP models can be used to identify and optimize treatment targets, to facilitate preclinical to humans translation, and to design combination treatment strategies.

Introduction

Mycobacterium tuberculosis (Mtb) infections are associated with approximately 1.5 - 2 million deaths annually worldwide[1]. The current first-line treatments for tuberculosis (TB) disease include a combination of antibiotics (rifampicin, isoniazid, pyrazinamide, and ethambutol) for at least six months[2]. However, the ongoing emergence of multidrug resistant Mtb threatens the effectiveness of the treatment with conventional antibiotics[3]. Host-directed therapy (HDT) strategies targeting the host immune response against Mtb to complement conventional antibiotic treatment strategies have received increasing attention[4–11] to enhance treatment outcomes, shorter treatment durations, and avoidance of resistance development.

HDTs target interactions between the host immune response and the Mtb pathogen. The host immune response to Mtb infection is reliant on the cumulative activities of various defence mechanisms such as macrophage activation, phagocytosis, autophagy, antigen presentation, and cytokine and T-lymphocytes production. Mtb has several mechanisms to modulate the host response enabling evasion of immune system-mediated clearance[1,11]. Pharmacological targeting of specific host-pathogen interaction mechanisms reflects an important approach for HDTs. Understanding the multiscale nature of host-pathogen interactions is essential to identify relevant drug targets for HDTs, and design appropriate combination treatments and dosing schedules.

A major challenge in the discovery and development of HDTs for TB is the prediction of treatment responses associated with specific pharmacological modulation of an immune response-associated target due to complex systems-level host-drug-pathogen interactions[4,6,12]. The translation of systems-level responses to HDT strategies from preclinical models to patients is challenged by inter-species differences in immune responses to Mtb pathogen. Mathematical modelling, and in particular the use of quantitative systems pharmacology (QSP) modelling can serve as a valuable tool to identify relevant HDT targets, and to inform subsequent design of combination drug treatment strategies and dosing schedules[13–18]. The utility of quantitative modelling to design improved treatment strategies for TB have already been demonstrated extensively for conventional antibiotic therapies[17–19]. For design of HDTs, however, QSP approaches remain have not yet been developed.

Here, we discuss the utility of QSP modelling strategies to support discovery and development of HDT strategies. We summarize high-potential host-pathogen interactions of relevance for HDTs. We then provide an overview of several relevant infection models to characterize host-pathogen interactions of Mtb. Based on this, we discuss how QSP models can be developed with a focus on required model components and the integration with experimental and clinical data, for application in target selection, inter-species translation and for clinical study design and treatment optimization.

Host -Pathogen Interactions as basis for Host-Directed Therapy Strategies

Several host-pathogen interactions of Mtb involved in its pathogenesis and immune system evasion offer potential targets for design of HDTs[11] (**Figure 1**), and are of relevance to capture in QSP modelling approaches.

Induction of Autophagy

Autophagy involves the formation of a double-membrane phagophore, elongation of the phagophore, autophagosome maturation, and fusion with lysosomes for degradation of the selected cellular material, and requires a complex interplay between various protein complexes. Autophagy plays an essential role in controlling Mtb infections[20–23] and has been studied extensively as potential HDT strategy for Mtb[4,11,20,24]. Currently, two therapeutic targets, mammalian target of rapamycin (mTOR) and intracellular cholesterol, are being studied to induce autophagy.

mTOR Inhibitors

Mammalian target of rapamycin complex 1 (mTORC1) plays a role in regulation of autophagy by two mechanisms, (1) inhibition of unc-51-like kinase 1 (ULK1) and transcription Factor EB (TFEB) phosphorylation[24] and (2) activation of glycolysis[25]. Mtb activates mTORC1 and thus inhibits autophagy.

Metformin is the most evaluated mTOR inhibitor as potential HDT treatment for Mtb infections. Metformin inhibited the growth of intracellular MDR Mtb strains in vitro[26]. Adjunctive treatment of metformin with isoniazid induced phagosome-lysosome fusion, enhanced the immune response, and reduced intracellular growth of Mtb in mice[26]. Study of transcriptional changes in healthy human volunteers following metformin dosing reported that metformin alters mTOR signalling, induces autophagy, and enhances the host response to Mtb[27]. Multiple reports suggest that metformin adjunctive therapy in diabetic TB patients improved TB therapy success rate and lowered mortality rate[26,28,29].

Everolimus, an mTOR inhibitor, showed significant potential against Mtb as an HDT. In a human granuloma model, everolimus treatment alone or in combination with isoniazid or pyrazinamide showed significant reduction in Mtb load as compared to the controls.[30] Adjunctive everolimus treatment with rifabutin-substituted standard TB therapy improved lung functions as measured by forced expiratory volume (FEV1) when compared to a control group in a randomized clinical trial[31]. A recent study identified that protein kinase inhibitor ibrutinib as a potential HDT against Mtb. Ibrutinib therapy alone significantly promoted auto-lysosome fusion in vitro, inhibited the mTOR pathway in vitro, and reduced Mtb load in mice[32].

Overall, induction of autophagy via mTOR inhibitors, especially in combination with conventional Mtb therapy, holds a potential as an adjunctive HDT strategy for treatment of TB.

HMG-CoA Inhibitors

Autophagy is also dependent on intracellular cholesterol. Key proteins, 1A/1B-light chain 3 (LC3) and lysosomal associated membrane protein 3 (LAMP3), and Ca^{2+} are essential for autophagosome maturation and autophagosome-lysosome fusion. LC3, LAMP3, and Ca^{2+} are inhibited by intracellular cholesterol[5,33], and thus cholesterol inhibits autophagy and promotes Mtb survival.

The HMG-CoA reductase pathway has been associated with intracellular cholesterol reduction, autophagy induction and improved Mtb clearance. Therapy with HMG-CoA inhibitors, such as simvastatin, pravastatin, and fluvastatin, as adjunctive therapy to conventional anti-TB drugs improved bacterial clearance by the host and improved the efficacy of first-line TB drugs by promoting phagosome maturation and autophagy in macrophage cell cultures and in mice models.[7,34–36] In vitro screening and experiments in mice for eight HMG-CoA inhibitors discovered that pravastatin, simvastatin, and fluvastatin had the most favourable anti-TB activity and pravastatin showed the least toxicity and drug-drug interactions when used as an adjunctive to standard anti-TB treatment[7,33]. On the other hand, a population-based cohort analysis of data from newly diagnosed TB patients recognized no statistically significant difference in hazard ratio between patients who were using statins (as a lipid lowering treatment) in addition to standard TB treatment as compared to patients who did not use statins[37]. Several retrospective clinical studies have identified that chronic use of statins reduced the risk of developing TB; however, to our knowledge, no studies have evaluated statins as a treatment in active TB patients alone or in combination with conventional anti-TB therapy[38]. As such, prospective clinical studies assessing the use of statins, especially pravastatin, at different doses as adjunctive to standard TB therapy may be needed.

Regulation of Host Epigenetics

Infection with Mtb alters some host gene functions important for the ensuring immune response. Two key pathways involved in Mtb-induced host epigenetic alterations are histone deacetylases1 (HDAC1) and TLR3-BMP-miR27a pathway both of which can be pharmacologically exploited for HDTs[39–41].

HDAC Inhibitors

Upregulation of HDAC1 was noted in macrophages containing live Mtb and HDAC1 recruitment suppressed the expression of IL-12B that plays a vital role in initiating type 1 T cell immunity to Mtb. HDAC1 is also known to modulate autophagy associated genes[42,43]. Treatment with a broad-spectrum HDAC inhibitor (Trichostatin A) decreased bacterial growth in both M1 and M2 macrophage cell cultures, while selective HDAC inhibitors (TMP195, and TMP269) reduced bacterial growth in M2 macrophage cell cultures. Vorinostat, an HDAC inhibitor, promoted immune response by human macrophage cell cultures[44]. In zebrafish embryos infected with Mm, HDAC inhibition significantly reduced microbial burden[40]. Additionally, HDAC inhibition significantly inhibited Mtb growth in lungs and showed increased production of key cytokines in mice[45].

Abl Tyrosine Kinase Inhibitors

Abl tyrosine kinase is involved in entry and survival of Mtb within macrophages through TLR3-BMP-miR27a pathway. Abl tyrosine kinase also inhibits expression of vATPase pump-relevant genes, and thus inhibits acidification of autolysosomes. Pharmacological inhibition of Abl tyrosine kinase using imatinib improved containment of Mtb within macrophages, induced expressions of iNOS, increased acidification of phagosomes, and decreased bacterial load in human macrophage cell cultures and in mice[11,41]. A clinical study assessing effects of imatinib alone and in combination with conventional anti-TB drugs in drug-resistant- and HIV co-infected- TB patients[46] is currently ongoing.

Modulation of Cytokine Response

The kinetics of the key cytokines, such as interferon gamma (IFN-γ) , tumour necrosis alpha (TNF-α), interleukin (IL)-1β, IL-10, IL-4, IL-12, and IL-2, during the course of Mtb infections have been well studied in vitro and in vivo[47–51]. IFN-γ is one of the most important players to the host immune response and its main role is activation of macrophages. IFN-γ also induces infected macrophage apoptosis via induction of more than 200 pro-apoptotic genes (i.e. Fas/Fas ligand, cathepsin, protein kinase R, etc.)[52,53]. Activated macrophage produce reactive nitrogen intermediates (RNIs) and pro-inflammatory cytokines, TNF-α and IL-1β, that possess microbicidal properties against Mtb. Resident macrophages also produce RNIs, TNF-α, and, IL-1β· however activated macrophage-mediated production is much more efficient[54–56]. Excessive production of pro-inflammatory cytokines, however, can lead to tissue damage[57]. Anti-inflammatory cytokines, IL-10 and IL-4, are also induced upon macrophage phagocytosis and balance pro-inflammatory cytokine levels by macrophage deactivation[57]. However, excessive production of anti-inflammatory cytokines may result in limiting the host immune systems' microbicidal activities[58]. Thus, the fine balance between the pro- and anti-inflammatory cytokines may determine the overall outcome of the Mtb infection.

Adjunctive treatment with IFN-y have been evaluated in various clinical studies; however, different patient conditions, routes of administration (intravenous vs. subcutaneous) and dosing regimen resulted in varying outcomes[59]. Adjunctive treatment with aerosolized IFN-y showed benefits in reducing cavitary lesions and induced negative sputum conversion in TB patients in clinical studies[60,61].

Anti-inflammatory agents such as cyclooxygenase (COX) 1/2 inhibitors, corticosteroids, 5-lipoxygenase inhibitor (Zileuton), phosphodiesterase (PDE) inhibitors, and matrix metalloproteinases (MMP) inhibitors have been shown to reduce Mtb burden in vitro or in preclinical species[4]. However, treatments with corticosteroids and celecoxib (COX1 inhibitor) in combination with conventional anti-TB drugs did not show significant benefits of these additional HDT in human subjects[4,62]. Adjunctive treatments with PDE inhibitors and MMP inhibitors have not been evaluated yet in human subjects to our knowledge. Retrospective analysis of existing data where TB patients took approved anti-inflammatory drugs, especially PDE inhibitors, as concomitant medications for other conditions and their impact on TB outcome can be a valuable approach.

Enhancing T-cell Mediated Host Response

The overall innate immune reaction play an important role in the initiation of adaptive immune response by antigen presentation, cytokines, and costimulatory signals[55]. Two to three weeks after the initial infection, antigen-presenting cells (APCs) that drain into regional lymph nodes initiate adaptive T-lymphocytes mediated immune response. Upon antigen presentation, the APCs, through antigen presentation via major histocompatibility molecules (MHC)-I and II , prime CD8+ T cells (cytotoxic T cells) and CD4+ T cells, respectively to initiate adaptive immune response.[63–65] Both activated CD4+ and CD8+ T cells secrete IFN-γ, IL-2, IL-17A, and IL-10. The production of CD4+ mediated IFN-γ is further stimulated by activated macrophages, whereas the production of CD8+ mediated IFN-γ is driven by concentrations of IL-12 and correlates with bacterial load[50]. Mature dendritic cells secrete IL-12p70 which helps increasing recruitment of additional CD4+ T cells[66,67]. IL-2 play a role in further proliferation of T cells[57]. CD8+ cells have direct microbicidal capabilities through perforin, granzymes, and granulysin or induce apoptosis through Fas/Fas ligand interaction[68].

Adjunctive cytokine supplementation with IL-12 and IL-2 have been evaluated in clinical studies, but did not result in significant benefits[4,59]. However, recombinant human IL-2 supplementation showed significant improvements in negative sputum culture conversion rates and in enhanced X-ray resolution in MDR TB patients[69]. Therefore, the use of recombinant IL-2 supplementation as HDT strategy for TB should be further evaluated.

Experimental approaches to inform design of Host-Directed Therapies

Several experimental approaches experimentally modelling (parts of) Mtb infection are of relevance to inform design of HDTs. Such experiments can provide quantitative understanding about components of drug-host-pathogen interactions (**Figure 2**), which can be combined and integrated through the use of QSP modelling. Key aspects include the immune modulatory effect of therapeutic agents on immune cells that in turn lead to changes in Mtb inhibition-dynamics of immune cells, immune system evasion strategies induced by Mtb, and direct pharmacodynamic effects of antimicrobial agents used in combination with HDTs. Parametrization of QSP models requires quantitative data of both the time course of effects (i.e., rates) and the concentration-effect relationships for therapeutic agents studied; as well as the ability to perform time course measurements. Data to be measured during such experiments both include endpoints such as Mtb disease burden, cell counts of lymphocytes, and regulatory proteins and biomarkers that can explain observed treatment response[70]. Here, we discuss key in vitro and in vivo preclinical models that can be of specific relevance for characterization of HDTs using a QSP modelling approach.

In Vitro Macrophage Infection Models and Advanced Cell Culture Systems

Human-derived macrophage and peripheral blood mononuclear cell (PBMC) cultures are extensively used to screen for the activity of antimicrobials but also identify potential compounds with HDT potential[27,71–73]. The in vitro setting allows conducting experiments in high-throughput setting including the use of reporter cell lines to screen for specific effects at the molecular level, e.g., autophagy induction. This approach thus readily allows characterization of the time course and dose response relationship of compounds.

Several advanced cell culture systems have been increasingly used to study Mtb host-pathogen interactions and for screening of compounds including HDTs, such as based on 3D cell cultures and organoids[71,74], and the development of a lung-on-chip system[75]. Similar to simpler cell culture systems, the longitudinal measurement of cytokines, chemokines, and bacterial load is possible, and has been used to study HDT interventions[74]. The use of lung organoids and lung-on-chip and their overall use in drug discovery and development is yet to be advanced further.

The in vitro hollow fibre infection model (HFIM) is commonly used to study the direct effects of antimicrobial agents on Mtb, but it also readily allows to include co-cultures with macrophages to better reflect aforementioned in vitro intracellular infection systems. In the HFIM, Mtb is cultured in a closed chemostat system with continuous flow of medium, while it allows simulation of concentration-time profiles that occur in patients. The corresponding changes in bacterial load over time can be quantified, allowing characterization of underlying PK/PD relationships of antibiotic and/or HDTs in combination with PK/PD modelling[76].

Whilst all in vitro cell culture-based approaches are attractive for purposes of screening and quantitative characterization of key mechanisms and phenotypic response profiles, these systems remain a simplified model system that does not include all aspects related to the host immune response. The use of QSP modelling could facilitate translation of such in vitro responses based on human host cells towards expected in vivo response.

Zebrafish Infection Models

Adult zebrafish *Mycobacterium marinum* (Mm) infection models have gained increasing attention as a preclinical Mtb infection model[77–79]. Zebrafish embryos and larvae are of interest due to their optical transparency and thus allowing the use of advanced imaging methods. Zebrafish possess an innate immune system that is highly similar to that of mammals; therefore, it has been used in many studies for the analysis of cellular and systemic responses to infection[78,80,81]. Because infection with various mycobacteria, including Mtb leads rapidly to the formation of granuloma structures that are highly similar to those observed in

human tuberculosis patients, it has been a successful model to study the progression of tuberculosis and the effects of drug treatment[82,83]. It also enables pharmacological screening of drugs, to treat mycobacterial infection at a high throughput level with an emphasis on the measurement of drug uptake characteristics[84]. Knockdown and overexpression experiments in zebrafish combined with QSP modelling would especially provide insights into contribution of certain component to overall immune response and anti-TB effects. A recent study provided the proof of concept that use of zebrafish larvae combined with translational PK/PD modelling can accurately predict effects of anti-TB drugs in humans[85]. Thus, zebrafish is a promising experimental TB model that can be used to generate the data required for QSP models to evaluate HDT strategies.

Vertebrate Infection Models

Rodent infection models using mice, rabbits, and guinea pigs are commonly used as infection model for Mtb[77]. Mice have been used in TB research from a long time due to the small size, availability of humanized and genetically modified strains, and cost-benefits over other vertebrate in vivo models, such as rabbits, guinea pigs, and NHPs. Even though rodent infection models such as mice incorporate a full immune system, differences between the human immune response remain[86] and lead to translational challenges[71]. QSP models could help address some of those translational challenges. Mice infected with ultra-low dose aerosol Mtb showed heterogeneous disease progression and granuloma formation similar to humans. Analysis of the transcriptomics data obtained from the ultra-low dose Mtb infected mice and the controls enabled predictions of risk of progression to active TB disease following Mtb infection in humans.[87] QSP models can incorporate various doses of Mtb inoculum and findings from the transcriptomics data analysis, and can enable translational predictions of treatment outcome[88–90].

NHPs have been widely used in immunology research and TB vaccine studies. NHP-Mtb infection models are of interest to generate HDT-relevant data due to their similarities to humans in basic physiology, immunology, and disease pathology. The use of these models has been however limited in TB treatment research due to the requirements of scientific and financial resources as well as safety issues due to highly infectious and contagious nature of Mtb.[91] QSP models can fill in the gaps between in vitro, zebrafish, smaller vertebrates such as mice, and humans to overcome the limitations of NHP models. In general, QSP models can link results from various experimental infection models to enable predictions in humans.

Design of Host-Directed Therapies using Quantitative Systems Pharmacology Modelling

The overall outcome of Mtb disease and treatment is reliant on the integrated results of the molecular and cellular events, and their reflection at tissue, organ, and host level dynamics occurring at different time scales. As such, it can be challenging to predict patient responses to different HDT strategies. Species differences in immune response characteristics make it more challenging to translate the results from preclinical studies to clinical scenarios. In addition, determination of the effects of treatments and disease progression in specific patient populations, can be challenging, i.e., in patients with weakened immune response and/or other conditions, patients with specific genotype known to affect certain pharmacology. QSP modelling can address these hurdles through quantitative integration of Mtb host-pathogen interaction mechanisms with PK and PD aspects of HDTs, making it a relevant tool to guide drug discovery and development of HDTs for Mtb. Here we discuss three main components of the QSP framework to evaluate HDTs for Mtb infection, (1) drug PK models, (2) host immune response models, and (3) pathogen dynamic models. The considerations for identification of drug- and system specific parameters to facilitate scaling, and the incorporation of variability are also discussed. Lastly, we discuss applications of the QSP models to evaluate HDTs. An overview of the QSP framework components and applications is provided in**Figure 2** and **Figure 3** , respectively.

Pharmacokinetics

Pharmacokinetics describes the concentration-time profile of drugs and is determined by absorption, distribution, metabolism. and elimination processes, which may differ between organisms. Consideration of concentration-effect relationships, and therefore the PK, is of essential value for design of HDT strategies. Mathematical PK models quantitatively characterize PK based on parameters accounting for the underlying processes.

Physiologically based PK (PBPK) models describe the concentration profiles in specific tissues of interest and are informed by both drug- and system-specific parameters. PBPK models are of relevance to scale PK between preclinical species and towards humans in a mechanistic-fashion. For Mtb infection, PBPK models describing lung exposure are of specific relevance. In addition, their mechanism-based approach allows for incorporation of drug-drug interactions, which often occur for Mtb combination therapies[92]. In the clinical phase, quantifying inter-patient variability in PK is important. Here, population PK (PopPK) models are of relevance, which capture inter-individual variation in underlying PK parameters that can be explained by specific patient-specific covariates[93]. It is furthermore helpful that because many HDTs involve approved drugs, often PK models are available already to characterize their PK[94,95].

Immune Responses

Models describing the key immune response components, such as dynamics of macrophage counts, cytokines, and CD4+ and CD8+ T lymphocytes are essential for QSP models to study HDTs. Systems biology models describing the host-Mtb interactions within the site of infection (lungs)[56] have been previously developed, and later linked with lymphatics[50] and blood circulations[96]. The states included in these models were resting-, activated-, and critically infected-macrophages, cytokines, such as IFN-γ, IL-10, and IL-12, immature- and mature- dendritic cells, CD4+ lymphocytes, and intra- and extra-cellular Mtb populations. The key feature of this model was contributions of various immune components on intra- and extra-cellular Mtb. The above-developed model was later expanded to include CD8+ cells dynamics in lungs and lymph[49,97]. The parameters in these models were identified from published human-derived or non-human primate (NHP) experimental results or model fitting to in vitro or in vivo (mice) data. These models can be expanded to include key drug targets involved in Mtb HDTs and their downstream effects on functional immune response changes and the quantitative interaction with Mtb bacteria.

To the best of our knowledge, there are currently no mathematical models available in literature describing HDT-relevant pathways, such as autophagy in Mtb infections; however, components and parameter estimates from single cell systems biology models[98–103] can be adapted and extended using experimental in vitro and in vivo data. For example, a HDT model containing key biological features of autophagy[98] including HDAC1-related components may be developed. The model parameters can be informed using prior knowledge available in literature[98] and data from in vitro experiments[40]. The model may describe dynamics of the phagocytic cells and zebrafish infection with Mm bacterial load overtime in HDAC1 inhibitors exposed macrophage cell cultures as compared to controls, and this would allow estimation of parameters relevant to HDAC1 effect. The simulations from the models may be compared with the experimental outcomes, preferably from different experimental conditions than the original experiments used for parameter estimation. This allows validation of the model structure and parameter estimates. In the above example, the simulations from the QSP model including autophagy components may be validated against data from zebrafish exposed to HDAC1 inhibitors (at various HDAC1 levels) experiments[40].

Pathogen Dynamics

Models for the population dynamics of pathogens include the effect of antimicrobial drug on the growth and inhibition-dynamics of Mtb bacteria and emergence of treatment resistance. In vitro and in vivo kill dynamic studies have enabled our understanding of parameters of Mtb growth rates[18], bactericidal and bacteriostatic effects of conventional anti-TB drugs[76], and resistance development rates of bacteria[104,105]. Through

the use of PK/PD modelling, dosing strategies can be designed that optimize dosing schedules for maximal bacterial control and reduced risk of resistance development. The incorporation of immune cell effects on pathogen killing is a key required step to study the effects of HDTs on Mtb treatment. Published host-Mtb interaction models[50] can be updated to include contributions of key HDT components on pathogen killing, as well as pathogen evasion mechanisms. For example, an autophagy model may contain quantitative relationship between bacterial load, mTOR, and autophagy. This will allow evaluations and predictions of various mTOR inhibitors on Mtb clearance by autophagy.

Implementation and Applications of the QSP Modelling Framework

QSP modelling have successfully influenced various decision making processes at different stages starting from discovery to late phase development in various therapeutic areas[16] and offer potential for the challenges faced in translation and design of HDT (combination) treatments in Mtb infections. A QSP framework to translate and optimize optimal HDTs should contain a combination of aforementioned model components for PK of one or more (investigational) drugs, immune/host response and pathogen dynamics, including their interactions. Depending on the type of HDT drug studied, QSP models may be parametrized and/or adapted in specific ways, e.g., to capture the drug-specific parameters for PK, pathogen kill and immune system effects, and induction of specific immune system effects. Various considerations and applications of the HDT QSP modelling framework are discussed below.

Target Identification and Drug Discovery

QSP models integrate various host-pathogen interactions and drug PK/PD components; therefore, they can readily provide assessment of target engagement upon stimulation or inhibition of certain target molecules at various doses and affinities and its impact on overall treatment outcome. This allows evaluations of the iterative process of hypotheses generation, designing new experiments, hypotheses validation and/or generation of new hypotheses. This approach can be applied to evaluate known HDT targets and HDT candidate molecules, to discover new HDT targets, or to discover and evaluate new HDT molecules. With advances in technologies, applications of combining QSP modelling and machine learning approaches to screen virtual drug compounds to enable discovery of drugs with optimal PK/PD characteristics are being evaluated[106].

Translational Predictions

With increased complexity and innovation in design of new drugs within the last two decades, mechanistic QSP models are increasingly being applied to inform translation of the results across different experimental conditions and species[107,108]. The systematic incorporation of system-specific parameters not only for various species, such as zebrafish, mice and humans, but also incorporation of differences between in vitro systems and in vivo models, is crucial to enable translation towards clinical HDT treatment designs[77,86]. In some cases, i.e. for scaling from in vitro HFIM to humans, such scaling is already well studied[76], whilst further studies are needed for the host's immune response components[109]. Consolidating immune-relevant differences between preclinical models and humans[109] may be challenging and resource intensive, as there are varying strains of models used across different experiments depending on the objectives of the experiments. On the other hand, the shown evolutionary conservation of the metabolic responses to mycobacterial infection in human patients and mice and zebrafish animal models show that basic disease symptoms such as wasting syndrome are not depending on species or varying strains[110]. Gene expression analysis data across species may be used to inform parameters of expressions of genes responsible for certain immune functions[111]. Such expression data studies can be used to predict metabolism in a whole-genome metabolic network theoretical modelling approach in various model organisms such as zebrafish[112]. Factors such as state or severity of infection, intensity of resistance, and sensitivity of drugs to bacterial strains (for example between Mtb and Mm) may also be applied within the QSP framework.

Variability and Precision Medicine

The presentation and severity of TB is variable amongst patients, and thus treatment responses, especially to HDTs, are variable. Many factors such as age, sex, genotypes, co-morbid conditions (HIV, type 2 diabetes) play role in determining the outcome of the disease and treatment. Thus, considering these factors in the QSP framework is very important. For example, known differences in PK and immune-response components for HIV co-infected TB patients may be incorporated in the framework, and extrapolate results from studies in TB patients to HIV-TB co-infected patients[113]. Many PopPK models have evaluated these factors' impact on variability in PK of conventional anti-TB drugs[114], and thus can be included in QSP simulations framework. In addition to external factors, considering immune-response relevant endotypes is also important[115,116]. Technological advances within the last century enabled generation of large-scale data, including omics data. The large-scale omics data may enable us to better understand the inter-individual variations associated with the parameters of the QSP models[117,118]. For example, parameters, together with inter-individual variations in them, describing the expression of baseline state of immune response components within lymph nodes and blood were estimated using data from a flow cytometry analysis of blood leukocytes and genome-wide DNA genotyping from 1000 healthy humans[117]. In addition, parameters, together with inter-individual variations in them, describing fractions of various lymphocytes within tumour microenvironment were informed using transcriptomics data from cancer patients.[117] Gene expression analysis of omics datasets from total of 443 TB patients enabled stratification of the patients into two groups. One of the two groups was characterized by increased gene activity score for inflammatory response and decreased gene activity score for metabolism-relevant pathways, and patients in this group showed slower time to negative TB culture conversion and poor clinical outcome[115,116]. Similarly, gene expression data can be used to include variability in the QSP models and inform outcome of certain HDT treatment.

Selection of Optimal Dosing Regimens and Combination Therapies

QSP models are also suitable to evaluate various combination therapies with optimal dosing regimen efficiently and can be especially valuable for difficult to treat diseases, such as TB. A QSP model enabled simulations of multiple combination therapies and identified the most effective dual-drug combination for the treatment of advanced castration-resistant prostate cancer where effectiveness of immunotherapy was previously insufficient [119]. In the TB disease space, QSP modelling has recently been applied to predict patient outcome with intensive dosing regimen and to explore shorter treatment duration scenarios for conventional anti-TB drug therapy[18]. Overall, the use of QSP modelling can serve as a valuable tool to efficiently design and develop HDTs for treatment of TB.

Conclusions

HDTs offer a unique treatment strategy to combat Mtb infections, but are challenged by complex and multiscale interactions between drug, host and pathogen. Several key mechanisms are of interest to be exploited as HDTs but are facing challenges in translation towards clinically effective treatment strategies. The combined use of multiple in vitro and in vivo experimental infection models can offer a more complete, quantitative and predictive understanding of drug-host-pathogen interactions. This should be combined with QSP modelling strategies that integrate data to enable translation towards patients and to help designing optimal clinical treatment strategies for HDTs in combination with classical antibiotics.

Figures

Figure 1. The Host-Pathogen Interactions as Basis for Host-Directed Therapy Strategies for the Treatment of Mtb Infections.

Initiation of the host innate immune response occurs shortly after inhalation of aerosols containing Mtb bacteria and Mtb implantation in macrophages. Both resident and activated macrophages stimulates the release of pro-inflammatory cytokines, such as TNF-α and IL-1β, following phagocytosis and autophagy. Antigen presenting cells (macrophages and dendritic cells) that drain into local lymph nodes activate CD4+ and CD8+ T-cell mediated adaptive immune responses. Antigen presenting cells also stimulate the release of IL-12, which helps recruit additional CD4+ T-cells. CD4+ T-cells secrete IFN-γ that stimulates macrophage activation, IL-2, TNF-α, and also IL-10 that helps balance the pro-inflammatory response by deactivation of macrophages. CD8+ cells have cytotoxic activities. CD4+ T-cell secreted IL-2 drives further proliferation of CD4+ as well as CD8+ T-cells. Autophagic pathways start with parting of a section from endoplasmic reticulum, the phagophore, followed by the elongation of phagophore with engulfment of Mtb, autophagosome formation and maturation, and fusion of the autophagosome with lysosomes. Mtb activates mTORC1 and thus inhibits autophagy, while mTORC1 activates aerobic glycolysis. Intracellular cholesterol inhibits LC3, Ca2+, and LAMP3, and thus inhibits autophagy mediated Mtb killing. Mtb activates HDAC pathway and thus downregulates various genes responsible for innate and adaptive immune response.

Potential host-directed therapy strategies are presented in green text.

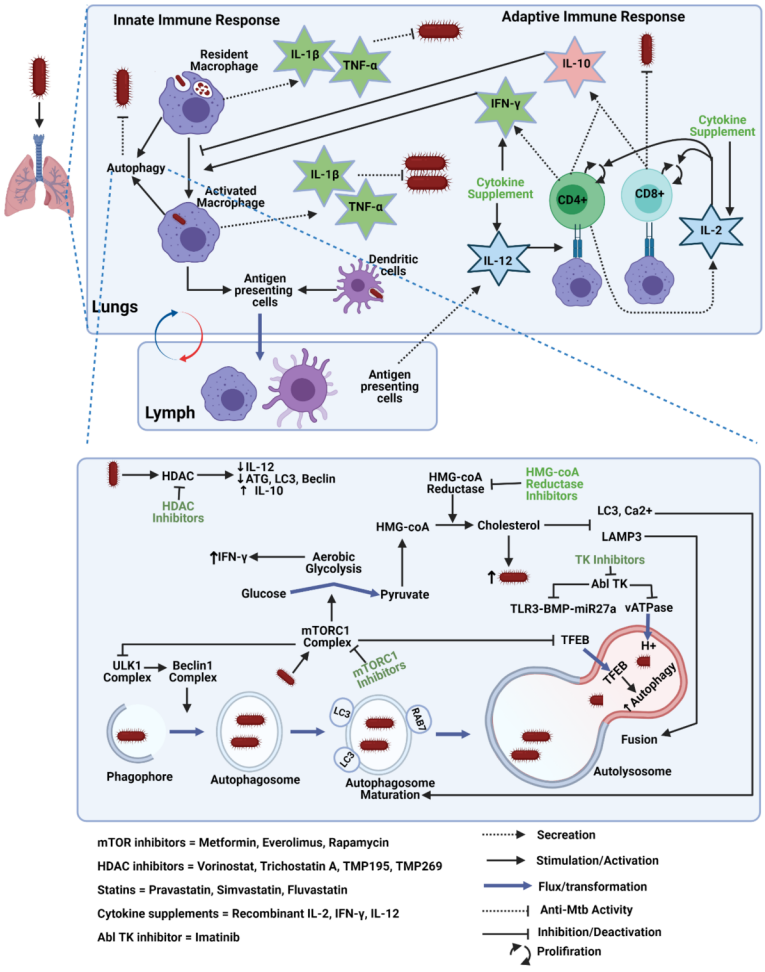


Figure 2. Components of the Conceptual Quantitative Systems Pharmacology (QSP) Framework to Assess HDTs for Treatment of TB

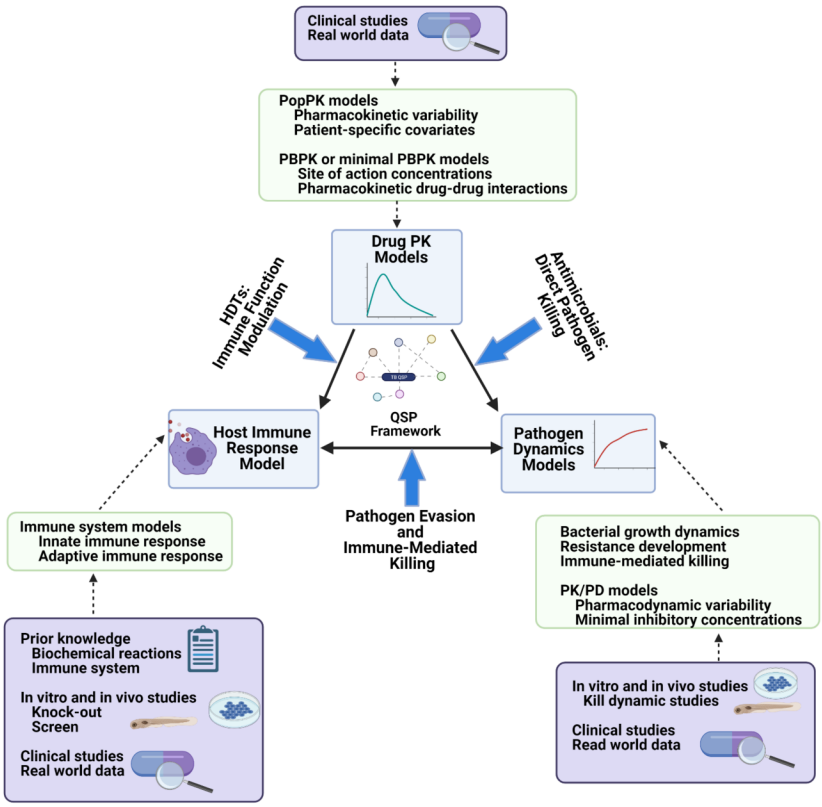
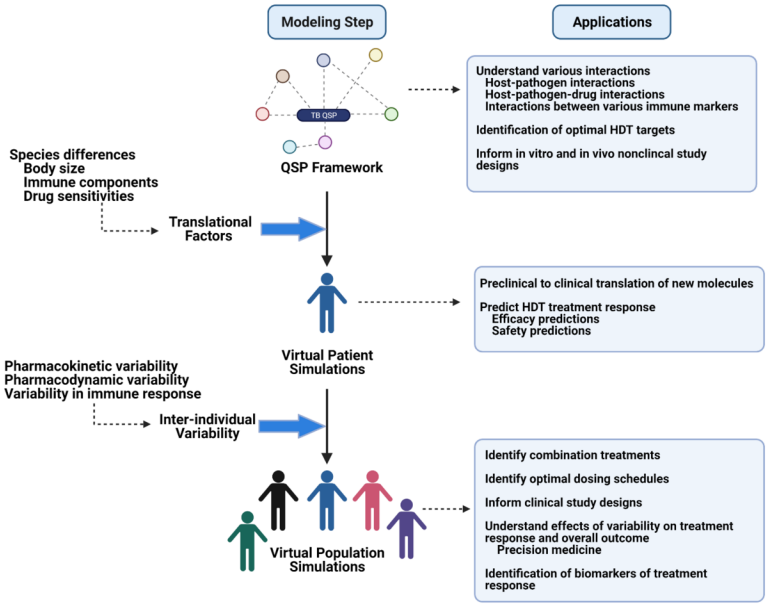


Figure 3. Applications of the Conceptual Quantitative Systems Pharmacology (QSP) Framework to Assess Host-Directed Therapies for Treatment of Tuberculosis



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