1 Publishable summary

Project context and objectives

Tuberculosis (TB) is a disease of poverty affecting primarily young adults in their most productive years. Although the global incidence rate is slowly falling, in 2010 there were 1.4 million deaths related to TB and 8.8 million new TB cases, including 1.1 million cases among people with HIV. TB is among the three greatest causes of death among women aged 15-44[1].

No truly novel compounds for the treatment of TB have been developed in the past 40 years. The present standard of care for the treatment of drug-susceptible TB is unsatisfactory, requiring 6 months of therapy with agents that can produce significant side effects. Moreover, the effectiveness of standard first-line antitubercular chemotherapy in many TB endemic areas is now further compromised by the emergence and spread of multi drug-resistant TB (MDR-TB), which requires the use of second-line drugs (SLDs) that are typically less effective, more toxic, costlier, and some require parenteral administration. Expanded use of SLDs in TB endemic areas and failures in adherence with the treatment regimens have fuelled the emergence of XDR-TB (extremely drug resistant TB) variants that are often beyond chemotherapeutic intervention. M(X)DR-TB represents a significant current threat in global public health and a potential precursor to the emergence of truly pan-resistant TB The imperative for identifying and developing new drugs effective against both drug susceptible and drug-resistant TB has therefore never been greater.

Despite of the number of new TB candidates that over the last ten years have been progressed to studies in humans (clinical trials Phase-I to Phase-III) the armamentarium to fight against TB is still very limited in size and future. The main reasons for this are the extraordinary late attrition rates experimented in anti-infective drug discovery together with the complexity of the infection that requires the use of combination therapies for short treatments and to avoid the spread of resistances. New TB drugs and targets which are rapidly cidal, safe and amenable to combination with other drugs can overcome the spread of resistance to win this fight against TB.

Specifically, the proposed Work Plan of ORCHID consortium is intended to produce the key data that could support the choice of a lead and a backup compound for further preclinical and clinical development.

Early stage Drug Discovery efforts over the last 5 years at GSK Tres Cantos Development Campus have resulted in the identification of a number of promising lead compounds in the fight against TB. These leads need to be further progressed and optimised into candidates for pre-clinical development through the Drug Development progression cascade.

Three compound families are of particular interest:

1) InhA Inhibitors,

2) New Beta-lactam/Beta-lactamase combinations for TB and,

[1] http://www.who.int/tb/publications/2011/factsheet_tb_2011.pdf

3) New potent whole cell anti-tubercular compounds with unknown mode of action,

A preclinical package is already in place for some of them, but further work is necessary for others in order to justify the progression of a single anti-tubercular family to the more resource intensive stages of preclinical and clinical development.

The project encompasses the parallel progression of the three compound families through:

- Lead Optimization Chemistry efforts and MoA studies (Genetic and Proteomic) for whole cell inhibitors,
- *In vitro* and *in vivo* evaluation of a new orally bioavailable Beta-lactam alone or in combination with a Beta-lactamase inhibitor to evaluate the sterilising potential of the new drug/s
- Optimization of an InhA inhibitor for preclinical development.

These efforts will yield candidate molecules for new "information rich" *in vitro* assays of anti-mycobacterial activity (intracellular activity, artificial granuloma, activity against slow/non growing cells and activity against clinical isolates) as well as for *in vivo* safety and efficacy evaluation in different animal models of infection (acute and/or chronic). At this stage a single compound family will be prioritized.

Further studies will be performed assessing the potential for shortening treatment in standalone therapy as well as in combination regimens both *in vitro* and in vivo. All three projects within the mini-portfolio exploit new mode of action paradigms, so no cross-resistance is expected with current drugs or leads in development. At this stage different innovative formulations with new material based on mesoporous silica for drug delivery in oral administration will be evaluated for single compounds and combinations.

Finally a Clinical Development plan will be put in place for the selected candidate molecule.

Work progress and main results achieved so far

All the progress that have been made during this second reporting period can be easily monitored looking at the publication track of the consortium. Many advances have been made in all the key elements that support the choice of compounds for further preclinical and clinical development.

In our way to the candidate selection, the identification of interesting compounds is an essential factor and the paper entitled: “Fueling Open-Source Drug Discovery: 177 Small-Molecule Leads against Tuberculosis” **[1]** illustrates perfectly the effort made to generate new leads. In order to fuel open-source, translational, early-stage drug discovery activities, the results of the recently completed antimycobacterial phenotypic screening campaign against Mycobacterium bovis BCG with hit confirmation in *M. tuberculosis H37Rv* were made publicly accessible. The set of 177 potent non-cytotoxic H37Rv hits identified was made available to maximize the potential impact of the compounds toward a chemical genetics/proteomics exercise, while at the same time providing a plethora of potential starting points for new synthetic lead-generation activities. Another example of the work done by the consortium to produce new interesting hits is showed in the Plos One paper **[2]** where the identification of novel imidazo[1,2-a]pyridine inhibitors targeting M. tuberculosis QcrB was described. Through the use of high throughput whole

cell screening of an extensive compound library a number of compounds were obtained as potent lead molecules active against M. tuberculosis and Mycobacterium bovis BCG.

Once the hits were generated, the consortium dedicated a great amount of work in the Lead optimization as described in the paper published in Plos One in early 2013 **[3]** where the improvement of a class of compounds endowed with high *in vitro* efficacy against *M. tuberculosis* and targeting MmpL3, an essential mycobacterial protein known to be involved in heme uptake, was reported. To improve the physical chemical properties and drug-like parameters of this class of compounds, a medicinal chemistry effort was undertaken. By selecting the optimal substitution patterns, a new series of compounds was produced with lower lipophilicity and improved *in vitro* microsomal stability, two important parameters for the development of antituberculars.

During this second period, the consortium has also been involved in the development of technology that enhances antituberculars discovery. Two articles which illustrated this were published in 2013. The first one: “Encoded Library Technology as a Source of Hits for the Discovery and Lead Optimization of a Potent and Selective Class of Bactericidal Direct Inhibitors of Mycobacterium tuberculosis InhA” **[4]** reported the successful application of a proven novel and robust hit/lead identification platform wherein a large collection of chemotypically diverse DNA-encoded small molecule libraries are screened for affinity toward a desired protein target to the discovery of direct InhA inhibitors. The second article **[5]** described the future of Medecine and more particularly the innocuity assessment of promising nanomaterials (Mesoporous silica particles) for biomedical applications. In a field where there are several major problems associated with the currently available TB treatment, the nanotechnology can be used to improve bioavailability, solubility and drug stability and to protect drugs from the acidic conditions of the stomach, leading to increased drug effectiveness and tolerance.

Finally, in the past 18 months, one of the objectives of the consortium was to decipher the mechanism of action of whole cell inhibitors of the ORCHID project, resulting in the publications of three articles on β-lactams antibacterial activity. The first one **[6]**, explored the basis of the specificity of some β-lactams for L,D-transpeptidase, the main peptidoglycan cross-linking enzymes in wild-type strains of *Mycobacterium tuberculosis*, and identify the features that are critical to efficiently inactivate this target. The second one **[7]**, published in AAC, explored more deeply the interaction of those enzymes with one particular class of β-lactams: the carbapenems. Development of carbapenems for tuberculosis treatment has raised considerable interest within the consortium since these drugs, in association with the β-lactamase inhibitor clavulanic acid, is uniformly active against extensively drug-resistant *M. tuberculosis* and kills both exponentially growing and dormant forms of the bacilli. Finally, the last publication **[8]** investigated one of the major concerns of the consortium: the resistance to antituberculars and more particularly to β-lactams.

Expected final results and their potential impact and use

Scientific expected outcomes:

The compounds that could eventually emerge from this project, after further preclinical and clinical development, are the components of novel regimens of drugs for the treatment of drug-sensitive TB and/or and M(X)DR-TB. Specifically, the proposed Work Plan is intended to produce the key data that could support the choice of a lead and a backup compound for further preclinical and clinical development.

The lead and backup could be either a) compounds that could replace INH in the standard-of-care regimen and circumvent much of the resistance to INH found in circulating M. tuberculosis strains, b) beta-lactams, one of the most effective, safe and well-understood antibacterial classes, for use in drug-sensitive or M(X)DR-TB, or c) a completely new novel anti-tubercular agent for use in combination with the current standard-of-care regimen and/or with the most promising TB regimens that are currently in development to improve and shorten treatment of drug sensitive TB and to address the emerging the MDR problem.

Socio-economic expected impact in the long-term:

Development of new and improved drugs and regimens that shorten and simplify TB treatment, including for drug-sensitive and drug-resistant disease, TB-HIV co-infection and latent infection would have many benefits at the patient and population levels. The ultimate beneficiaries of improved, simplified and shortened TB treatments are TB-affected populations worldwide, but especially in low-income countries where about 90 percent of TB cases and TB-related deaths occur[2]. In addition to saving countless lives of those afflicted with drug-sensitive and drug-resistant TB, improved TB therapy can have a far-reaching development impact by minimizing productivity loss, decreasing the burden on health systems and freeing up valuable resources for use in other critical areas.

In this sense the design and selection of new candidate molecules within ORCHID is including the production costs of the molecules even at the earlier stages. In this way the final candidates would have an affordable access for low income countries. This strategy have been particularly interesting in the case of b-lactams where the compound selected at this stage is orally bioavailable and also an off-patent drug with a significant lower cost that other penems in the same series.

[2] Stop TB Partnership. *TB and Poverty.* Stop TB Partnership Fact Sheet.

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