Final Report Summary - MM4TB (More Medicines for Tuberculosis)

Executive Summary:
Tuberculosis (TB), a colossal public health problem, is one of the major obstacles to economic growth and social stability in the world. TB is currently the leading cause of death due to an infectious disease and has overtaken HIV as the number one killer. One reason for the resurgence of TB in Europe and elsewhere is the lengthy duration of treatment, that uses drugs developed 60 years ago, and the emergence of strains of Mycobacterium tuberculosis (M. tb) that display extensive resistance to these drugs.

The principal aims of the More Medicines for Tuberculosis consortium (MM4TB) were to stimulate and catalyse TB drug development by discovering and validating pharmacologically new drug targets and by identifying new chemical entities that could serve as candidates for novel TB drugs. The project employed four work-packages (WP) to achieve this objective. WP1 generated hits from phenotypic screens; WP2 used the hits to find the targets; WP3 used structural biology to progress from target to drug while WP4 provided the enabling chemistry for drug synthesis. In WP1, we employed an algorithm of novel whole cell-based approaches with M. tb to find molecules with antibacterial activity known as “hits” in libraries of natural products or synthetic organic compounds and then in WP4 we used medicinal chemistry to generate optimized hits and leads for candidate drugs (CD). The targets of the hits were found in WP2 using whole genome sequencing of resistant M. tb mutants and by subsequent genetic and biochemical validation. In parallel in WP3, a prioritized set of pre-validated and newly discovered targets was investigated extensively and used to underpin a rational drug-design approach involving structure determination and fragment-based screens (WP4). The latter approach was also used to progress new lead compounds. In addition, an extensive range of new tools and strains for TB drug discovery were established and disseminated.

At the outset of MM4TB our explicit objectives were to validate at least five new drug targets pharmacologically and to discover at least one family of CD. These objectives were successfully met, since, firstly, 10 new targets for drugs that met our progression criteria were identified and, secondly, PBTZ169, the consortium’s flagship CD, completed preclinical development and entered clinical trials. In addition, the leads from two series of compounds targeting an enzyme involved in central metabolism and another enzyme required for cell wall synthesis are approaching optimized lead/CD status.

Other notable products and achievements during the lifetime of MM4TB were the publication of 125 scientific articles, the filing of five provisional patents and the creation of a not-for-profit foundation for TB drug development. As a direct result of their training and research in MM4TB, several young scientists achieved independent status as group leaders in leading European institutions.

Project Context and Objectives:
TB is a chronic and complex disease resulting from infection with the slow-growing pathogen, M. tb, which may be present in intra- and extracellular forms; these display widely different metabolic states, ranging from exponential growth to latency, in different lesions within the same patient. TB is arguably the most important disease in the history of humanity and decimated European populations during the 18th-19th centuries. Today, TB accounts for the annual loss of ~1.9 million lives as a result of poverty, homelessness, synergy with the HIV/AIDS pandemic and the emergence of multidrug-resistant (MDR) and extensively-drug resistant (XDR) strains of M. tb. While most of the 9.6 million new cases that arose in 2015 occurred in the developing world the industrialized nations, especially in Europe, are also at increasing risk due to the inexorable spread of...
drug-resistant disease, global travel and immigration. A new concern is both active and latent TB among refugees from the Middle East and North Africa.

TB treatment is compounded by the relative inefficacy, by today's standards, of the drugs available and the variety of physiological states in which M. tb can exist. In particular, non-replicating or latent bacilli constitute a challenge to therapy because of their phenotypic drug resistance. Any anti-TB compounds in development must cope with this reservoir of bacilli in order to eliminate the disease and this is a tall order given that a third of the world's population is latently infected with M. tb. Furthermore, since many TB patients are also co-infected with HIV, new TB drugs must be compatible with antiretroviral therapy. An additional concern is overlap with the growing type 2 diabetes epidemic as diabetic patients respond poorly to current TB medication.

In the decade before MM4TB began, the pharmaceutical industry favoured a target-based approach to drug discovery in all therapeutic areas. Here, an essential function, generally an enzyme, was used in high throughput screening (HTS) to identify inhibitors present in vast chemical libraries of synthetic compounds. Medicinal chemistry was then employed to progress hits, meeting predefined criteria, through to leads and ultimately to candidate drug (CD) status. After passing preclinical toxicological evaluation, the CD entered clinical trials. While successful in some cases, the HTS approach has failed miserably in the antibacterial discovery area as documented candidly by Dr. Payne and colleagues from GlaxoSmithKline and many other experts. In our own experience, target-based screens generate hits but these usually fail to show useful minimum inhibitory concentrations (MIC) against M. tb. The reasons for this collective failure remain unclear but may include the inability of many synthetic compounds to enter mycobacteria and find their target, highly active efflux systems or other innate resistance mechanisms. It is also conceivable that the physico-chemical properties of the kinds of compounds developed for cancer or metabolic diseases, and which make up a large part of current screening libraries of pharmaceutical companies, are more suited to eukaryotic than to prokaryotic cells. In addition, the use of genetic validation as the main criterion for defining target essentiality has been misleading but has highlighted important metabolic differences between bacteria growing in vitro and during infection.

Overall, this has led to the realization that pharmacologically validated targets are of far greater value than genetically validated ones but, in the case of M. tb, and excluding the ribosome, only a handful of these were known prior to the MM4TB project, namely, RNA polymerase (RNAP), DNA gyrase, folate pathway enzymes like thymidylate synthase (TS), InhA (NADH-dependent enoyl-[Acyl-Carrier-Protein] Reductase), decaprenyl-phosphoribose epimerase (DprE1) and ATP synthase. With the exception of fluoroquinolones, all of the TB drug candidates that were in development (SQ109, BTZ043) or in clinical trials (TMC207, PA-824, OPC67683) when MM4TB began were discovered on the basis of their whole cell activity.

This concise survey led to the inescapable conclusion that the most direct and effective route for hit-generation and target discovery would be to screen synthetic compounds or natural products for whole cell activity against M. tb. In the past this was not possible in Europe owing to the lack of dedicated screening facilities in biosafety level 3 conditions but such infrastructure was an important asset of the MM4TB consortium. It should also be remembered here that many TB drugs are prodrugs and that compounds requiring intracellular activation are only detectable in whole cell screens.

While the MM4TB project was underway a number of notable setbacks to TB drug development were reported. These included the failure of fluoroquinolone drugs to reduce treatment duration in three separate clinical trials, clinical trials involving pretomanid (PA-824) being placed on hold on several occasions due to unexplained side effects, and more recently, to deaths in the so-called STAND trial, to the failure of TBA354, the pretomanid back-up, in phase I trials and to the lack of anti-TB activity of SQ109 in phase II trials directed by the Panacea consortium. These failures underline the many difficulties that confront TB drug development.

To underpin TB drug discovery in the MM4TB project we chose to use four work-packages (WP). Using phenotypic screens of natural product and heterocyclic compound libraries WP1 generated hits that showed MIC <4 μM. These hits were also tested
for activity against intracellular and latent M. tb using novel models available to the consortium. In WP2 the targets of the hits were found using state-of-the-art technologies, particularly, whole genome sequencing of M. tb mutants resistant to the hits. The targets thus identified were validated using orthogonal assays such as gene replacement, conditional knock-down mutants, transcriptomics, proteomics and enzymology. WP2 enabled target-based studies to be initiated and facilitated the transfer of well-validated targets to WP3, which used structural biology to understand the interaction of the hits and leads with their targets. This enabled rational, structure-guided approaches to be used for lead optimization. The latter task was performed as part of WP4, which provided the enabling chemistry for drug synthesis. Another component of WP4 was the use of animal models of TB to test targets for their essentiality during infection and lead compounds for activity against M. tb during disease. These models were used to test extensively PBTZ169, MM4TB's candidate drug (CD), and to design new regimens through the use of systematic studies of TB drug combinations with PBTZ169.

The objectives of the MM4TB consortium were to support TB drug discovery by finding and validating pharmacologically five targets for new drugs and to progress at least one series of compounds to CD status. Both these objectives were accomplished thanks to the effective and productive partnerships fostered by MM4TB. Ten new targets for TB drugs were discovered, our CD, PBTZ169, completed preclinical development and entered clinical trials, and two series of compounds targeting GuaB2, an enzyme involved in central metabolism, and InhA, an enzyme required for cell wall synthesis, are at the LO stage and likely to achieve CD status. Thus, despite the rather gloomy situation concerning TB drug development worldwide, the MM4TB consortium achieved its goals and provided new impetus and resources to this field that is of critical importance for global health.

**Project Results:**

MM4TB used four interlinked work-packages (WP) to meet its TB drug delivery goals and each WP had a set of precise objectives. These objectives will be stated in the appropriate section of the WP below and precede the main results obtained.

**WP 1: Hit generation from phenotypic screens**

**Objectives**

The goal of this WP was to use innovative whole cell models to screen widely diverse libraries of natural products or synthetic molecules for bactericidal compounds that kill M. tb under three different growth conditions. The desired properties of such bactericidal compounds were:

- Confirmed minimal inhibitory concentration (MIC) <4 μM on actively growing bacteria
- MIC against replicating, non-replicating, and intracellular bacteria
- Minimal Bactericidal Concentration (MBC) < 4X MIC
- Solubility >10 μM
- Antimycobacterial activity confirmed following re-synthesis
- Structure activity relationship (SAR) emerging

**Results**

The overall success of MM4TB depended to a large extent on the timely completion of tasks in WP1 as the hits discovered there progressed to WPs 2 and 4 for further exploitation. WP1 used innovative whole cell screens of widely diverse libraries of synthetic compounds and natural products to find molecules that kill M. tb under three different physiological conditions: active growth, latency and intracellular growth. Whole cell screening was performed with different chemical libraries: 1 (1,113 known hits), 2 (~320,000 compounds), 11 (~18,000 compounds) and 20 (~1,200 compounds), and with 16,000 purified natural products, library 24. In Phase I, these compounds were first screened for whole cell activity at a concentration of 20 μM. Hits displaying >80 % inhibition were retained for further characterization. Phase II involved determination of their MIC, MBC and cytotoxicity for human cells. Compounds with an MIC <4 μM with no or limited cytotoxicity were selected for further testing. Screening of library 1 was completed at month 6 and the eight compounds, which met the progression criteria, transferred to
WP2. Libraries 2, 11, 20 and 24 were screened using the same algorithm and screening was completed during year 2. These screens yielded 357, 9, 3 and 54 hits, respectively, and after hit expansion and nearest neighbour testing, >400,000 compounds were tested in total. All hits were tested for intracellular activity and cytotoxicity to help further prioritization. Very few non-cytotoxic compounds were active against M. tb under all three conditions with the most interesting hits stemming from thienopyrimidine libraries or the natural product collection. In summary, 20 hit series, some containing several compounds, advanced to WP2 and WP4. When possible, all compounds showing acceptable MICs were tested for IC50 against purified enzymatic targets; some hits against RNA polymerase from library 2 were identified and pursued in 2013. A newly available library, the NCCR Chemical Biology library, was screened in year 4, in order to generate additional hits for WP2 since this WP had completed its allotted tasks with the compounds from libraries 2, 11, 20 and 24. Screening the NCCR library generated 90 new hits, representing >15 different scaffolds. 68 of these hits were reconfirmed after testing a new batch of the compound and distributed to other partners for hit to target activity.

To facilitate compound progression we also used time-lapse microfluidic-microscopy for real-time single-cell analysis. This new analytical method contributed to understanding the mechanism of action (MOA) as well as the reasons for failure of antibiotics as exemplified by the problem of bacterial persistence. Microfluidics was used to study the uptake and activity of several different DprE1 inhibitors (BTZ, aminooquinolines, Ty3Re, etc.), cell division and protein synthesis inhibitors, and to monitor the response of conditional knockdown mutants under non-permissive conditions.

Phenotypic screening was performed with thyx and wag31 mutant strains whose gene expression can be conditionally modulated and this has been facilitated by the development of a series of promoters of different strengths so that the expression level obtained with conditional knock-down mutants is close to the physiological level of the selected M. tb genes. Work was successfully undertaken with thyA and thyX, which encode two different thymidylate synthases, and with the upp and pyrR genes, also from the pyrimidine biosynthesis pathway.

The outstanding progress made in WP1 provided a very solid foundation for a successful outcome for MM4TB.

WP 2: Hit to target

Objectives

WP2 aimed to find the molecular targets of prioritized molecules, already available, as well as natural products and synthetic compounds (hits) that were discovered within WP1 and proven to be effective in models of active or latent TB. Several integrated methodological approaches were harnessed to achieve our objectives. These included:

• selection of resistant mutants;
• whole-genome shotgun sequencing (WGS)/Bioinformatic analysis of drug resistant mutants;
• target fishing;
• target confirmation;
• production of antibodies or recombinant proteins.

Results

An ideal TB drug target should be essential for bacterial growth and persistence, restricted to the pathogen, accessible to the drug, and unaffected by permeability barriers. The simplest, and by far the most successful, way to find targets with these properties is to use an active compound for target finding. WP2 identified the targets of prioritized molecules, namely the hits from WP1 and compounds already available from NM4TB such as the benzoquinolines. In addition, we investigated the old antibiotic, pyridomycin, as it kills M. tb effectively, and showed that pyridomycin inhibits the well-validated TB drug target, InhA, but that it binds in a new pocket of the active site.

Resistant mutants were isolated to prioritized hits from library 1 and their genomes sequenced in an attempt to find the targets. Resistance to nitrophenes was due to loss of activity of the Ddn nitroreductase that activates the TB drug, PA-824, so in light of this knowledge, work on the thiophene was discontinued. Four other compounds were shown to be activated by the mono-oxygenase, EthA, and the thienopyrimidines by Rv2466c, a DSBA oxidoreductase. One of the metabolites produced by
EthA inhibits the essential enzyme, PyrG (CTP synthase), and other likely targets for hits from library 1 included HrcA and PanK, for which validation was completed.

Using genetics and biochemistry the target for the aminoquinolone scaffold from library 2 was shown to be the flavoprotein, DprE1, the well-understood BTZ-target, whereas the oxadiazole-piperidine scaffold from the same library targets MmpL3. The targets for two hits from library 11 were shown to be the essential cell division protein Wag31 (DivIVA) and GuaB2 (inosine-5′-monophosphate dehydrogenase). The target of Ty38c from library 20 was shown to be DprE1 once the detoxifying resistance mechanism mediated by the decarboxylase Rv3406 had been inactivated. Characterization of 5-FU-resistant mutants has uncovered lesions in uracil phosphoribosyltransferase, Upp and the pyrimidine biosynthesis regulator, PyrR. Investigation of the resistance mechanisms to three natural products from library 24 identified the undecaprenyl-phosphate alpha-N-acetylglosaminyltransferase (WecA), the ribosomal protein, RplK, and the tRNA synthetase, TrpS, as the respective targets. Despite being discovered at a relatively late stage of MM4TB the targets or resistance mechanisms for many of the hits from the NCCR library were also identified. The targets included the promiscuous target MmpL3, involved in trehalose monomycolate export; Rv0338c, an enzyme involved in iron-sulphur centre production; and, lctd2, an isocitrate dehydrogenase. Mutations conferring resistance to some compounds were found in mmr, encoding the multidrug resistance transporter, which plays a role in drug efflux as evidenced using mmr knockout mutants. Finally, as part of the hit to target cascade WP2 showed that two series of nitro-aromatic compounds were activated by an F420-dependent nitroreductase. The active species appears to target NadD (nicotinate mononucleotide adenylyltransferase).

In order to reduce the time required to isolate mutants that are resistant to hit compounds and thus speed up target validation, the faster-growing tubercle bacillus, Mycobacterium canettii was investigated. Selected INH, RIF, STR and BTZ-resistant strains of M. canettii were screened for characteristic mutations in katG, rpoB, rpsL, rrs and dprE1 genes; these were essentially the same as those reported previously in M. tb thus validating the utility of this approach which is nearly three times faster. The CDD database was heavily used for storing, managing and analysing screening data and an in silico platform for metabolic pathway reconstruction in M. tb was also established with priority given to the pyrimidine and decaprenyl-phosphoribose (DPA) pathways so that the effects of genetic and chemical knockdowns of targets in these pathways could be compared.

Target validation was undertaken using inducible/repressible expression systems in vitro, ex vivo and in vivo and technological improvements made to the system. The essentiality and vulnerability of the following genes and their functions was completed: guaB2, nadD, nrdE/F2, rv2361c, rv3807c, rv3806c, garA and pimA. Conditional knock-down mutants were made for every gene in the DPA pathway thereby demonstrating that, with one exception (rv3807c), all genes were essential. The most vulnerable functions were DprE1, UbiA and PrsA with the latter enzyme, phosphoribosyl-pyrophosphate synthetase, appearing to be a particularly attractive new target for drug discovery. Various target proteins were expressed and antibodies generated.

Excellent progress was achieved in WP2 and seven targets were transferred to WP3. The objective of finding five new pharmacologically validated targets was met twelve months ahead of schedule and 10 new targets were uncovered in total.

WP 3: Target-to-Drug

Objectives

WP3 progressed compounds using a target-based IC50 approach where possible, but otherwise used MIC as a driver e.g. for a membrane-protein with no readily measurable activity. The target-based approach involved biochemical characterization, crystallographic structure determination, fragment-based lead generation, virtual screening and rational drug design. Lead generation approaches included HTS against random and focused libraries.

Results
Enzymes and functions in cell wall biosynthesis such as DprE1 and PimA make excellent drug targets. Great progress was made in the D-arabinose pathway (DPA) with biochemical and structural studies of DprE1 advancing spectacularly. Structures of recombinant M. tb and M. smegmatis DprE1 were solved at 1.9 and 2.1Å resolution, respectively. More importantly crystal structures have also been obtained of DprE1 in complex with BTZ043, PBTZ169, as well as other benzothiazinones, and with Ty38c and seven other carboxyquinoxalines, at resolutions ranging from 1.8 - 2.5 Å. These are rare but invaluable examples of a TB drug target in complex with its cognate inhibitors, and this represents a major achievement that will enhance rational drug design. Another key enzyme in the DPA pathway, PRPP synthetase or PrsA, was investigated intensively. Mycobacterial PrsA and its human orthologue have been biochemically characterized and two assays suitable for HTS developed. The crystal structure of M. smegmatis PrsA was determined at 2.4Å resolution and revealed differences in the active site with respect to the human enzyme. Medium throughput screening for PrsA inhibitors was carried out and 24 compounds identified with IC50 values ranging from 17 - 48 µM, with some displaying MIC values ranging from 0.49 - 21 µM.

As outlined above, PimA - phosphatidylinositol mannosyltransferase – appears to be a high value target as it is very vulnerable in vivo. Despite successful structural and biochemical characterization of PimA work on this target was terminated due to its chemical intractability and lack of suitable lead compounds.

NAD metabolism is another priority area for TB drug discovery. Soluble truncated forms of type II NADH:menaquinone oxidoreductase (Ndh2), a membrane-bound enzyme central to NAD metabolism, were suitable for biochemical and biophysical studies, and provided support for the mode of action of known inhibitors. However, these did not reflect the true properties of this monotypic membrane protein. Expression of the full-length M. tb Ndh2 in E. coli provided only low amounts of protein so expression in M. smegmatis and Pichia pastoris was undertaken. Additionally, inhibitors were tested against a yeast homologue, Ndi, for which expression and structural work have been successful elsewhere but again to no avail. Active NAD synthetase (NadE) was produced in recombinant form and an enzymatic assay amenable for screening developed. However, owing to lack of chemical leads this target has been abandoned. As outlined above, several nitro-aromatic hits appear to target NadD and enzymological confirmation for this will continue after termination of MM4TB.

Enzyme systems essential for nucleic acid synthesis, such as the classical target RNA polymerase (RNAP), were investigated. Successful expression of RNAP was achieved and crystallization experiments undertaken together with inhibition studies with lipiarmycin and ripostatin but these compounds proved untractable. The thymidylate synthases, ThyA/ThyX, produce important precursors for nucleic acid synthesis, are druggable and were successfully expressed. However, since crystals of M. tb ThyX protein did not diffract at low enough resolution a surrogate enzyme, TM_ThyX, was used for fragment-based ligand design. Crystals of the TM_ThyX with inhibitors were generated and their structures solved.

Expression and biochemical characterisation of the M. tb Topoisomerase (Topo I) and DNA gyrase advanced well and these enzymes were tested for inhibition by the hits found in the whole cell screens but no potent inhibitors were found. The structure of the ATPase domain of M. tb DNA gyrase was solved and a preliminary diffraction dataset of a complex with diospyrin was generated. Topo I crystals were obtained but these diffracted poorly.

Other WP3 targets that have emerged from WP2, or been selected using other MM4TB criteria, include Wag31, GuaB2, PrsA, PyrG, Rv2466c, TrpS and the pyridomycin target, InhA. Three new structures of PyrrG in complex with substrates (AMP-PCP, 6-diazo-5-oxo-L-norleucine, UTP) were obtained at resolutions between 2.0 and 3.7Å. AMP-PCP binds in the expected ATP-binding pocket, in close proximity to the site of resistance to our lead compound. The structure of Rv2466c, the activator of thienopyrimidines such as TP053, was obtained at 1.51Å and attempts at co-crystallization undertaken. The redox (bio)chemistry of this enzyme has been intensively studied and the active form of TP053 appears to target the protein Rv0579. The structure of M. tb tryptophanyl tRNA synthetase, TrpS, was solved at a resolution of 2.6Å and also in complex with ATP and chaungxinmycin, a natural product inhibitor. The 2.3Å resolution crystal structure of InhA, complexed with pyridomycin, revealed the antibiotic to occlude both the NADH- and the substrate-binding sites, a highly unusual property. This information was of immense value for rational design of better pyridomycin derivatives and this approach was also pursued.
intensively with InhA in conjunction with fragment-based screens. Finally, high resolution structures of GuaB2 from M. thermoresistibile alone and in complex with inhibitors were obtained and used to instruct structure-based drug design with the M. tb enzyme and to model interactions with the lead compound VCC234718.

WP 4: Enabling chemistry

Objectives

WP4 provided enabling chemistry to pharmacologically validate at least five new drug targets and to develop at least one family of candidate drugs. This involved resynthesis of whole cell screen (WCS) and HTS hits, lead identification (LI) and lead optimisation (LO) chemistry following either MIC, IC50 driven SAR or using structure-based or fragment based approaches when the structures of the target proteins were known. As part of WP4 animal models of TB were used to measure the activity of lead compounds and to assess the essentiality of genes using conditional knock-down technology.

Results

The BTZ series kill M. tb by inhibiting DprE1 through the formation of a covalent bond between a nitro group on the compound and the active site cysteine residue. Non-covalent BTZ inhibitors were synthesized and these compounds retain activity albeit much weaker. The aminoquinolone series also contains non-covalent inhibitors of DprE1, and these were progressed through LO and structure-assisted design programs. Unfortunately, despite major improvements in IC50/MIC and host cell penetration, the best candidates failed to show efficacy in rodent models of TB due to poor PK. Work on aminoquinolines was thus discontinued but replaced by the carboxyquinoxaline series, for which eight DprE1 complexes were available to support structure-guided approaches.

LO activity was also undertaken on derivatives of the synthetic compounds cyclic hydroxamic acid, thieno[2,3-d]pyrimidines, pyrano[3,2-b]indoles and spiroindenes, and with the natural products chuangxinmycin, ripostatin, amicetin, and pyridomycin. A range of pyridomycin analogs was chemically synthesized and several of these inhibited InhA activity and the growth of M. tb but to date none was shown to be more active than pyridomycin itself. Understanding these effects and designing better derivatives were both greatly facilitated by the availability of the InhA-pyridomycin co-crystal structure from WP3. To date, pyridomycin failed to show activity in murine models due to liver metabolism and this issue is still being addressed.

Considerable effort was devoted to understanding the SAR of Amicetin in an attempt to improve its potency and PK, this led to the finding that the sugar moiety plays a major role inactivity against M. tb. The total synthesis of derivatives of ripostatin was also achieved but since these showed neither anti-mycobacterial activity nor RNAP inhibition the project was halted.

Fragment-based drug design is still on-going with InhA, ThyX and TrpS. Work was initiated with a new dithiocarbamate series since the lead compound met the MIC progression criterion and also appeared to inhibit the essential carbonic anhydrase enzymes.

Using mouse models and conditional knock-down mutants of M. tb we demonstrated the essentiality of the dprE1, guaB2 and pimA genes. The activity of many lead compounds and PBTZ169, our CD, was determined using the murine model of chronic TB. By this means we established that PBTZ169 in combination with bedaquiline and pyrazinamide was more effective than the standard TB treatment. The combination of PBTZ169 with clofazimine, a leprosy drug, was also highly active. Importantly, these results underlined the potential of PBTZ169 for the treatment of TB in humans and we are pleased to report that the preclinical development of PBTZ169 was completed during the last year of MM4TB. Finally, two approaches were undertaken to obtain potent inhibitors with CD status from the optimised leads against GuaB2. The first of these was a fragment-based approach whereas the second employed traditional SAR and medicinal chemistry to progress the lead VCC234718. Owing to lack of time, these series have not yet completed testing in murine models of TB.

Potential Impact:

The need for new drugs to combat TB is arguably greater than for any other therapeutic area of human medicine, including cancer, owing to the truly exceptional global dimension associated with those chronic, highly transmissible disease. We are
confident that the MM4TB consortium, which took a highly competitive yet totally realistic route to TB drug discovery, has helped to meet this unmet medical need. It was our initial goal to use a combination of novel whole cell screening approaches and whole genome sequencing to discover five new drug targets that were chemically validated and pharmacologically tractable. In fact, we discovered ten such targets and these were exploited both within the consortium but also made available to others as a result of publication of the results once the appropriate steps had been taken to protect any intellectual property that arose. The reason for this open target policy was to maximize the chances of a new TB drug being found since there is such a dearth of well-validated targets in Mycobacterium tuberculosis (M. tb).

It was our ambition to generate at least one family of candidate drugs by which we mean one or more structurally related chemical entities, built around the same or a different pharmacophore, that kill M. tb by inhibiting the same cellular function. MMTB’s flagship compound, PBTZ169, successfully completed preclinical development thereby achieving CD status and has entered clinical trials in Russia. In addition a not-for-profit Foundation, Innovative Medicines for Tuberculosis (iM4TB), was created, in order to exploit the results obtained in both NM4TB (FP6) and MM4TB. The main mission of iM4TB is to pursue the clinical development of PBTZ169, which will shortly enter clinical trials in Switzerland. It is conceivable that the other optimized leads from MM4TB, that are approaching CD status (inhibitors of central metabolism and cell wall synthesis), may later be exploited by iM4TB, another MM4TB partner or a pharmaceutical company.

The success of MM4TB was only possible due to the pooling of resources on a European-wide basis and the ability to include key participants from ICPC. When the complexity of the drug discovery process is taken into consideration it is obvious that this could only have been achieved by selecting the strongest academic scientists and industrial partners in order to align the panoply of ideas and technologies required. We set ourselves ambitious yet attainable goals which, as described above, took a CD through late preclinical development and into phase I clinical trials. The CD will then progress to phase Ila trials involving TB patients and this could be done for instance in the framework of EDCTP. The delivery of a CD by this consortium thus represents a valuable contribution to the Millennium goals concerning TB and may help alleviate a major poverty-related disease.

Dissemination, communication and promotion of the results, achievements and products of MM4TB are a cornerstone of our activities. The main findings have been published in a series of high-profile papers in the scientific literature, after taking due care of the protection of intellectual property rights, and the results were also presented at national and international meetings and symposia. Five provisional patent applications were filed by MM4TB participants. We have ensured whenever possible that the participants in MM4TB, in so far as they have acquired privileged access rights, had the possibility to obtain exploitation rights either free of charge or for privileged licensing fees. The relationships between the participants with respect to these aspects had been regulated in the Consortium Agreement.

Electronic means of communication have been routinely used for both internal and external purposes. A domain for a public MM4TB website (http://www.MM4TB.org) was purchased in order to engage with the general public. We also engaged with the general public through the website and in face-to-face meetings in order to convey the significance of the MM4TB mission. Video-taped interviews with MM4TB members were made available on the website so that they could explain in layman’s terms the reasons for undertaking this project and how the public will benefit from its conduct. It is of vital importance to explain the necessity to pursue research on infectious diseases like TB at a time when the situation is particularly concerning given the steady advance of drug-resistant diseases, both community- and hospital-acquired, and the emergence of new pathogens. The website was continually developed and maintained throughout the project.

Several videos and a DVD publicizing MM4TB were also produced although it is believed that the main means of attracting attention to the project was through the scientific literature and press releases, as well as through the website. The website provided the public and potential backers or investors with information about the overall objectives of the project, details of the participants, non-confidential progress reports and a list of publications. When necessary, for instance following major publications, patents or the discovery of a new “lead”, press conferences were held. Newly developed technologies were
made available to the wider scientific community via publication in peer-reviewed journals and presentation at international conferences. Nowadays, most scientific projects are web-managed thereby allowing instant and efficient reporting and querying procedures. A private website served as an important tool for sharing protocols, technologies, results and information among consortium members. It allowed communication and dissemination of information between the participants, including information concerning project meetings, presentations, minutes of Executive Board Meetings, General Assembly meetings, reports from the Scientific Advisory Board etc. Data from target validation, screening assays and compound production were stored there and news of technology development was made available to the consortium on a routine basis. Regular electronic-meetings on technology development also enabled the rapid dissemination of new methods to all MM4TB participants and allowed for quicker assimilation of new targets into the multiple screening and discovery platforms.

List of Websites:
www.mm4tb.org

Related information

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