This three day international event will explore new research into TB detection, treatments and vaccination as well as new development in controlling and preventing TB Infection.

This event has CPD accreditation

This abstract book will be finalised two weeks before the event

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Day 1:

Invited Speakers Abstracts

“Dangerous” M. tuberculosis genotypes their transmission and acquisition of drug resistance - implications for treatment and infection control.
Dr Richard Anthony, Royal Tropical Institute, Amsterdam, The Netherlands
Recent insights into the changing distribution of tuberculosis genotypes indicate some clades are spreading more effectively than others although it is too early to be sure if this represents an emerging epidemic of more aggressive strains or random drift. More worryingly is the observation that in many settings drug resistance in Mycobacterium tuberculosis is rapidly increasing in prevalence and is disproportionally concentrated in a very few successful strains. These successful highly resistant strains deserve particular attention as treatment options remain limited. Tools to easily identify these strains and halt their spread are urgently needed.

MDR-TB - excellent treatment outcome in the Netherlands - teamwork with all stakeholders
Dr. Tjip S van der Werf, MD PhD, pulmonologist, Professor of Medicine, Infectious Diseases Service, Dept. of Internal Medicine, and Pulmonary Diseases & Tuberculosis, UMCG, University of Groningen, Groningen, The Netherlands

Acute minimally invasive surgical treatment in Pott's diseases
Dr Stefano Rigotti, Ospedale Sacro Cuore, Negar, Verona, Italy
Pott's disease is a very serious condition for the spine and it is the most frequent secondary localization of pulmonary TB.
In the acute phase of infection there is an abscess that destroys the bone of the vertebral body and discs. The outcome in patients is very serious because severe vertebral deformities resulting (scoliosis and kyphosis), which may also lead to disabling compression on the spinal canal and neurological deficits following. The purpose of our work is to treat patients who have had an early diagnosis and initiate drug therapy; in these patients is practiced early spinal vertebral stabilization, even in the acute phase, adopting the most innovative minimally invasive techniques for going to let a quick recovery in patients who often presents a picture of weakness and comorbilità drug.

Role of surgery in active TB
Professor Khaled M. AlKattan, Professor of Surgery Dean, College of Medicine, Alfaisal University, Saudi Arabia
Surgery plays an important role in the management of TB. Surgical intervention may be required in diagnosis, treatment or as palliative intervention with TB. In thoracic surgery this could be pulmonary or extrapulmonary TB. For the later it include chest wall, pleura, mediastinum and pericardium. It could also be utilized to manage sequelae or complications of TB from infection to lung transplantation. The talk will highlight the role of thoracic surgery in these involvement.

Design, Syntheses and Studies of New Potent and Selective Anti-tuberculosis Agents Based on Imidazopyrine Cores
Dr Marvin J. Miller, George and Winifred Clark Professor of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, IN, USA
Multidrug (MDR) and extensive drug resistant (XDR) forms of TB are becoming common. New treatments are desperately needed. Our early studies focused on syntheses of mycobactins (essential virulence factors for Mtb, that facilitate iron uptake), analogs and drug conjugates. Simultaneous fragment based screening of all synthetic intermediates led to the discovery of new small molecule anti-TB agents including appropriately functionalized imidazopyridines that have remarkably potent anti-TB activity. Subsequent combined studies with the NIH, Eli Lilly & Co, IDRI, UIC and others indicate that imidazo[1,2-a]pyridine-3-carboxamides are an exciting new class of potent, selective anti-TB agents that merit additional development opportunities.

Immunoprophylactic and immunontherapeutic roles of different immunomodulators during the disease process
Professor Subrata Majumdar, MSc., Ph.D. FNAsc, FAsct, Division of Molecular Medicine, Bose Institute, India
CD1 ANTIGENS: STRUCTURE ELUCIDATION AND AVAILABILITY THROUGH CHEMICAL SYNTHESIS
A.J. Minnaard
Stratingh Institute for Chemistry, University of Groningen, Nijenborgh 7, 9747 AG Groningen, The Netherlands
A.J.Minnaard@rug.nl

Around 20 years ago, it was reported that CD1 proteins present glycolipid antigens to T cells. Since that time, a series previously unknown glycolipids have been identified in the lipid envelope of Mycobacterium tuberculosis, that bind specifically to CD1a, CD1b, CD1c and CD1d. Several of these mycobacterial lipid antigens activate human T cells, which expand in number during human tuberculosis infections.

To aid in the discovery and structure elucidation of these mycobacterial glycolipid antigens, and in order to provide sufficient material in pure form to allow immunological research including animal studies, we started a research program a decade ago. In this program we developed chemical synthesis routes to the most important glycolipids found in pathogenic Mycobacterium tuberculosis: including mannosphosphomycoketide, bis-acylsulfglycolipid, phthiocerol dimycoserosate A, the triglycosyl phenolic glycolipid PGL-tb1, mycoside B, and sulfolipid-1. A synthesis route towards mycolic acid is under study. The produced material, in milligram quantities, has been instrumental in molecular immunological research focusing on the binding of these antigens to the CD1 proteins, and their processing.

Currently, several of the developed synthetic routes are further developed to scales > 100 mg to allow animal studies. Key points of this approach are that the compounds are homogeneous, that is, not consisting of mixtures of homologues or closely related compounds. This strongly facilitates a molecular interpretation of the experimental results. In addition, the compounds are chemically pure and not (potentially) contaminated with traces of immunogenic oligosaccharides or peptides. This makes the immunological research more reliable and opens to door to application of these glycolipids in human.

In this presentation, an account will be given on the strategies to produce these mycobacterial glycolipids and their application in immunology, This in order to stimulate the scientific community to apply these, now available, compounds in their research.

The bioinformatic analysis revealed SNPs in type II TA genes specific to different genotypes. SNP in MazE3 gene classifies M. tuberculosis in two large groups: Euro-American and Asian. Using 6 loci (VapC6, HigBA, VapC37, VapC38, Rv2656c and VapC12) the Asian group can be subdivided in EAI, Dheli/Cas and Beijing. EAI-Manila can also be distinguished from EAI, and Beijing genotype can be split in 4 subgroups: Beijing-Modern, Beijing-Ancient, Beijing-like and Russian endemic W/B0, associated with drug resistant and hiper-virulent phenotype.

Using 8 loci (VapC6, VapC30, VapC17, VapC38, VapC10, VapC3, VapC47, MAZE8) one can divide the Euro-American branch in Ural, Haarlem, LAM S and T genotypes, distinguishing LAM1, LAM4/F15/KZN, LAM9 and LAM2 in LAM lineage, and the SMI-049 cluster in the T-type. LAM4/F15/KZN and SMI-049 genotypes, which could be previously detected by RFLP-typing, are also associated with enhanced virulence phenotype like W/B0. In the contrary, the Ural genotype is associated with decreased virulence. Thus using 13 loci one can detect 16 main types of M. tuberculosis, including 3 types with enhanced virulence. This set of loci is minimal, when increasing the number of genes, we can increase the resolution of this method.

Using this method we analysed a collection of 62 clinical isolates from Central Tuberculosis Research Institute (Moscow). We typed forty-three strains as Beijing-Modern (including six W/B0), one Haarlem, two Ural (H4), four LAM9, two LAM, six T strains and 4 strains of unknown genotype. The results were confirmed by spoligotyping and housekeeping genes sequencing. We developed a new genotyping method of M. tuberculosi based on Real-Time PCR of 13 genes of TA systems.

The application for the international patent PCT/ RU2014/000559 24.07.2014

MODULAR CAPACITY BUILDING APPROACH OF DOCTORS AND HEALTH CARE WORKERS INCREASES CASE DETECTION RATE IN CHILD TB: A BANGLADESH MODEL

1. Dr. Shakil Ahmed
2. Prof. M Ruhul Amin
3. Prof. ARM Luthful Kabir
4. Prof. Md. Abid Hossain Mollah
5. Dr. Masuda Mohsena

Dr. Shakil Ahmed, Assistant Professor of Pediatrics, Shaheed Suhrawardy Medical College, Dhaka-1207, Bangladesh. E-mail: shakildr@gmail.com

Abstract: Diagnosing child TB is a challenge in 22 High Burden Countries (HBCs) including Bangladesh. WHO estimates the case detection rate should be 6-10% of total TB cases. Recent publication shows it can be 4-22%, with sporadic reports form HBCs up to 25%.

In Bangladesh, case detection was 3.1% in 2011, 2.85% in 2012 and it was 2.74% in 2013. Moreover, case detection rate in the sub-district hospitals (Upazila Health Complex-UHC) were even lower than the national average. One of the important reasons is lack of clinical skills of doctors and awareness of health care workers on child TB at these community hospitals.

To develop capacity among these groups of stakeholder, between 2012-13 Bangladesh has developed generic interactive training modules, training video, flip chart and other training aids on Child TB. By using these tools, capacity building program for doctors and health care workers was conducted between November 2013-July 2014. This was done with support from USAID under the guidance of National Tuberculosis Control Program (NTP) with Bangladesh Pediatric Association (BPA) as technical & implementing partner. Total 1181 doctors and 8345 health care workers have been trained on child TB in 17 districts and 122 sub-districts of Dhaka Division. Pre- and post-test analysis, of doctors trained, showed statistically significant (p<0.001) improvement of knowledge of the participant irrespective of age, qualification and portfolio. Andragogy modular training
Case detection rate showed a raising trend in the sub-district hospitals of Dhaka division. The number of cases detected in the project area was 752 in 2013, while 992 cases were detected till 30th September 2014 (132% of 2013) sparing one quarter (October to December) of 2014. Year end data will provide further light on increment.

Since no other intervention was undertaken during this period in Dhaka Division, it can be assumed that the increase in the case detection rate was due to this capacity development program of the doctors and health care workers.

**LINEAGE 7 MYCOBACTERIUM TUBERCULOSIS STRAINS ARE ASSOCIATED WITH LONGER PATIENT DELAY IN PULMONARY TB PATIENTS**

SA Yimer, G Norheim, A Namouchi, ED Zegeye, W Kinander, T Tønjum, S Bekele, T Mannsåker, G Bjune, A Aseffa, C Holm-Hansen

*Department of Microbiology, Unit for Genome Dynamics, Oslo University Hospital, PO Box 4950, Nydalen, NO-0424 Oslo, Norway*, Email: yimsalo@yahoo.com

**Background**

This study investigates the genetic diversity of *Mycobacterium tuberculosis* (Mtb) strains among pulmonary TB patients in Amhara Region, Ethiopia, and the association between specific Mtb lineages, sociodemographic and clinical parameters.

**Methods**

DNA was isolated from Mtb-positive sputum specimens (n=240) and analyzed by PCR/24-locus MIRU-VNTR and spoligotyping. Bioinformatics was used to assign Mtb genotypes to global lineages. Associations between patient characteristics and genotype were evaluated using logistic regression analysis.

**Results**

Mtb strains (n=138) were assigned to seven sub-lineages, four of which were not represented in the MIRU-VNTRplus database. The largest sub-lineages (n=60; 26.0%) belonged to Central Asian (CAS), the next (n=36; 15.6%) to lineage 7, and the third (n=35; 15.2%) to Haarlem. The four novel sub-lineages designated NW-ETH3, NW-ETH2, NW-ETH4 included 24 (10.4%), 18 (7.8%), 8 (3.5%) and 5 (2.2%) isolates, respectively. Patients infected with lineage 7 strains were highly likely to delay in seeking medical attention compared to patients infected with CAS strains (AOR=4.7, 95% CI 1.6, 13.5). Cases of Harlem infection (OR= 2.8 95% CI 1.2, 6.6) and NW-ETH3 (OR= 2.8 95% CI 1.0, 7.3) appeared in defined clusters.

**Conclusion**

The study revealed a high diversity of modern and pre-modern Mtb lineages of which approximately 25% were not previously reported. Infection with Mtb lineage 7 strains is associated with longer patient delay that suggests a possible increased duration of illness among these patients. Intensified active case finding and contact tracing activities in the study region are needed to expedite diagnosis and treatment of TB.

**RESISTANCE TO PYRAZINAMIDE IN RUSSIAN MYCOBACTERIUM TUBERCULOSIS ISOLATES**

D.A. Maslov, O.B. Bekker, T.G. Smirnova, Elena E. Larionova, Sofya N. Andreevskaya, L.N. Chernousova, Y. Zhang, V.N. Danilenko

*Vavilov Institute of General Genetics, Russian Academy of Sciences, Moscow, Russia; Central TB Research Institute, Russian Academy of Medical Sciences, Moscow, Russia; Division of Global Health Equity, Brigham and Women’s Hospital, Harvard University, Boston, MA 02115, USA; NPO SRC «BIOAN», Moscow, Russia.*

*Corresponding author - V.N. Danilenko: Vavilov Institute of General Genetics, Gubkin str. 3, 119991, Moscow, GSP-1, Russia; e-mail: valerid@vigg.ru.*

Tuberculosis (TB) is a major global health problem with an estimated of 9.0 million new TB cases and 1.5 million TB deaths in 2013. Pyrazinamide (PZA) is a key frontline drug in TB chemotherapy, which shortens the lengthy anti-TB therapy to 6 months due to its sterilizing ability against persisters. PZA is recommended for treatment of both drug-susceptible and drug-resistant TB cases. The phenotypic testing for PZA susceptibility can show both false-positive and false-negative results (Piersimoni et al., 2006). Up to 40% of false-positive resistance can be obtained on Bactec MGIT 960 (Chedore et al., 2010). Sequencing of *pncA* gene is also recommended (Simons et al., 2012). Mutations in gene *pncA* coding pyrazinamidase (PZase) are the major mechanism of PZA resistance in *M. tuberculosis* (Scorpio and Zhang, 1996; Scorpio et al., 1997; Cheng et al., 2000). Mutations in *rpsA* gene, encoding S1 ribosomal protein, a key element of trans-translation, were found in some PZA-resistant
We analyzed 64 *M. tuberculosis* clinical isolates from patients from the European part of Russia. The isolates were classified in 3 groups: 20 isolates from patients with long and ineffective chemotherapy; 21 isolates from patients with long and effective chemotherapy; a control group of 23 drug-sensitive isolates from new-TB cases. The collection included 6 mono- or poly-drug resistant isolates, 25 MDR and 10 XDR isolates. The genotyping by spoligotyping revealed 70% of the isolates to belong to Beijing lineage. The share of Beijing genotypes varied from 48% in the control group to 95% in the group with long and ineffective treatment.

The isolates were tested for phenotypic PZA resistance in Bactec MGIT 960 system and for PZase activity using Wayne method. The genes controlling PZA resistance (*pncA*, *rpsA* and *panD*) from these isolates were sequenced. Twenty-four isolates were found to be PZA-resistant after the first Bactec MGIT 960 DST, twenty-three of them carrying mutations in *pncA* and showing negative PZase activity; five more phenotypically sensitive to PZA isolates also harbored mutations in *pncA* and had negative PZase activity, three of them confirmed PZA resistance on the second DST. Mutations in *rpsA* and *panD* not leading to PZA resistance in clinical diagnostics concentration (100 mkg/ml) were found, which still may lead to low-level PZA resistance. We have found 1 PZA-resistant isolate with no mutations in known genes, which may harbor a new mechanism of PZA-resistance.

The PZA-resistance may highly impact the clinical outcome in case of MDR-TB treatment. Russia has a significant MDR-TB problem. Although PZA resistance in MDR-TB (ZR-MDR-TB) in some parts of the world has been found to vary between 20-80%, no such information is available in Russian MDR-TB strains. We show that over 73% of MDR-TB isolated in the European part of Russia is PZA-resistant, according to Bactec MGIT 960 testing of 519 MDR-TB isolates, collected.

Thus we show a high burden of PZA resistance in Russian MDR-TB. Ineffective regimen may be a factor of artificial selection of PZA-resistant MDR-TB strains in clinics, most of them, as we show in this study, of Beijing genotype, which is shown to be one of the most difficult-to-cure in Russia and one of the most abundant.

**Poster Presentation Abstracts**

**QUALITY OF TUBERCULOSIS CARE IN PRIVATE HEALTH FACILITIES OF ADDIS ABABA, ETHIOPIA**

Gezahegn Gebrekidan, 1 Gezahegn Tesfaye, 2 Mitiku Teshome Hambisa, 2 and Negussie Deyessa 3

Presenting author: Gezahegn Tesfaye
Email address of the corresponding author: gezahegnkidan@yahoo.com

**Background:** Ensuring provision of good quality tuberculosis (TB) care, especially in private for profit health facilities, is an important component of TB control strategy to reduce poor medical practice which results in multidrug resistant TB (MDR-TB), enhances clients' satisfaction and service utilization. Therefore the aim of this study was to investigate quality of TB care in private health facilities of Addis Ababa.

**Methods:** A facility based cross-sectional study was conducted based on Donabedian’s structure-process-outcome model of health care quality. Quality of care was determined by adherence to National TB Program guidelines, treatment success rate, and client satisfaction. Exit interview was conducted on 292 patients on the intensive phase of treatment and 384 patient records were reviewed in eight private health facilities.

**Results:** All resources recommended by the National TB Program guidelines including trained staff, laboratory facilities and drugs were continuously available, except for a shortage of streptomycin, inconsistent supply of laboratory reagent and unavailability of IEC materials. Initial diagnostic AFB test was done for 95.4% of pulmonary TB patients. Most important components of TB care recommended by national guidelines were delivered for a significant proportion of patients. Majority (75%) of the clients were found to be satisfied with each component of TB care. The treatment success rate was 90.9%.

**Conclusions:** The quality of TB care was fairly good in that the structural and process of components of TB care service almost fulfills the minimum requirements for implementation of TB care in private health facilities compared with the national guideline. Compliance with national guidelines was also found to be satisfactory. However, only three forth of the patients were counseled for HIV testing. Strengthening HIV counseling and testing, tackling shortage of streptomycin and laboratory reagent at private TB clinic is crucial.
Asadbeyk Mahsa 2, Zaeifi Davood 2, Asadi Mahsa 3, Atabaki Yasamin *1
1) Institute of molecular and microbial research of Viravigene
2) Department of Biology, University of Tehran, Tehran, Iran.
3) Department of Microbiology, Islamic Azad University of Karaj, Karaj, Iran

Postal address: nom 51, of Taghavi alley, North-Soehre vardi St, Tehran, Iran
Postal code: 1568639313
Email: y.atabaki@yahoo.com

Abstract:
Prevention and treatment of drug-resistant clones is important in guiding TB control strategies. The simultaneous rapid detection of the type of mutation conferring resistance and the genotype reflect the extent of drug resistant TB transmission within the communities. Mutations conferring resistance to rifampin in rifampin-resistant clinical Mycobacterium tuberculosis isolates occur mostly in the 81 bp rifampin-resistance-determining region (RRDR) of the rpoB gene.

DNAs were extracted and both techniques of RFLP, RAPD-PCR and sequencing of the rpoB gene were performed for 30 rifampin resistant M. tuberculosis isolates from patients. Mutations in the RRDR of the rpoB gene were identified in all of rifampin-resistant isolates. Fifty percent of Central Asian Strain isolates carried a mutation in codon 516, 14.3% of isolates carried a mutation in codon 531 and 36.7% of isolates carried a mutation in codon 526. Overall, there appeared to be a similar accuracy between the genotype and specific mutations conferring resistance to rifampin by RFLP and RAPD-PCR both.

Keyword: rpoB mutations, Rifampin, Mycobacterium tuberculosis

M. smegmatis APHVIII+ TEST-SYSTEM FOR PRIMARY SCREENING OF MYCOBACTERIAL SERINE/THREONINE PROTEIN KINASE(S) INHIBITORS
Bekker O.B.2, Maslov D.A.2, Danilenko V.N.2,3
2Vavilov Institute of General Genetics, Russian Academy of Sciences, Moscow, Russia; 3NPO SRC «BIOAN», Moscow, Russia.
Corresponding author: Danilenko V.N., Vavilov Institute of General Genetics, Russian Academy of Sciences, 119991, Gubkin str., 3, Moscow, Russia, valerid@vigg.ru.

Recently tuberculosis (TB) extends in the MDR and XDR forms (WHO, 2014). Nowadays researchers all over the world are searching for drugs with a new mechanism of action. Mycobacterial serine/threonine protein kinase(s) are proposed as new biotargets for anti-TB drugs (Danilenko V.N. et al, 2011).

M. smegmatis is a fast-growing non-pathogenic bacterium and is thus used as a model organism for M. tuberculosis (Koen A. et al, 2005). We’ve developed the M. smegmatis APHVIII+ test system for primary screening of serine/threonine protein kinase(s) inhibitors from chemical libraries. The mycobacterial cell wall consists of several layers. The outer layers are mostly represented by lipids, most of which are mycolic acids and their derivatives. Their low reactivity provides high chemical stability to the mycobacterial cell wall (Ramón-García et al, 2011). The M. smegmatis APHVIII+ test system consists of the M. smegmatis mc2155 strain, which harbors the plasmid, a derivative of the tetracycline-inducible pMIND shuttle-vector (Blokpoel et al, 2005) that lacks the kanamycin-resistance gene and carries the S. rimosus aphVIII gene (GenBank: DQ007043.1). Therefore, the M. smegmatis APHVIII+ test system can detect mycobacterial protein kinase inhibitors that are able to penetrate through the cell wall of mycobacteria. The M. smegmatis APHVIII+ test system is based on the ability of APHVIII to phosphorylate and inactivate kanamycin. And M. smegmatis become resistant to kanamycin. The activity of APHVIII is dependent on its phosphorylation by serine/threonine protein kinase(s) of M. smegmatis. The M. smegmatis genome contains 13 serine/threonine protein kinase genes that are homologous to the protein kinase genes of M. tuberculosis. In S. lividans one of serine/threonine protein kinase, namely Pk25 (SCO), phosphorylates APHVIII (Bekker et al, 2008; Bekker et al, 2010). The similarity of the amino acid sequences of the substrate-binding sites of the STPKs PK25 from S. lividans and MSMEG_5513 from M. smegmatis suggests substrate identity.

Pk25 S. lividans GSADKSVGSGPE
MSMEG_5513 M. smegmatis GDADKAIGSMS

We classified STPKs based on nine variable amino acids located in the ATP-binding pocket of the catalytic domain of the protein kinases. The signature of nine variable amino acids determines the binding specificity of
The protein kinase inhibitors (Zakharevich et al., 2012). According to this classification, *M. tuberculosis* PknA, and *M. smegmatis* MSMEG_5513 and belong to group 1.

\[
\begin{array}{c|c}
\text{MSMEG_5513} & \text{M. smegmatis} \\
\text{PknA} & \text{M. tuberculosis} \\
\end{array}
\]

I V A A M L V L T
V V A V M Y V L T

Therefore, we can select potential inhibitors of *M. tuberculosis* PknA through the *M. smegmatis* APHVIII+ (MSMEG_5513). PknA from *M. tuberculosis* is essential for cell division of *M. tuberculosis* (Thakur et al, 2006)

References

MODELING WHIB7-DEPENDENT SYSTEM OF INTRINSIC DRUG RESISTANCE OF MYCOBACTERIUM TUBERCULOSIS ON THE MODEL OBJECT - MYCOBACTERIUM SMEGMATIS

Shur K.V.\(^a\), Maslov D.A.\(^b\), Bekker O.B.\(^a\), Danilenko V.N.\(^a\)

\(^a\)Laboratory of Bacterial Genetics, Vavilov Institute of General Genetics, Gubkina str. 3, 119991, Moscow, Russia
Corresponding author - V.N. Danilenko: Vavilov Institute of General Genetics, Gubkina str. 3, 119991, Moscow, GSP-1, Russia; e-mail: valerid@vigg.ru.

In the process of adaptation of *Mycobacterium tuberculosis* to the action of anti-TB agents strains with multiple (MDR) and extensive (XDR) drug-resistance phenotypes emerged (Prozorov et al. 2012). Besides acquired resistance, there are systems of intrinsic antibiotic resistance in mycobacteria, mediated by proteins that activate transcriptional responses to drugs (Zhang et al. 2013). One of the controllers of the intrinsic resistance is *whiB7* gene, encoding a transcriptional regulator. Strains mutant for *whiB7*, are sensitive to low concentrations of some drugs including tetracycline, macrolides, aminoglycosides, lincosamides, etc. WhiB7 transcription factor controls the expression of several genes directly responsible for resistance to antimicrobial agents. The system of genes controlled by WhiB7 is called its regulon; it includes the following genes: *tap*, encoding an efflux pump (provides resistance to aminoglycosides and tetracyclines); *eis* – a predicted acetyltransferase, involved in intracellular survival and related acetyltransferases (resistance to aminoglycosides); *erm(37)* - 235 rRNA methyltransferase (resistance to macrolides); and *rv1473*, encoding a possible macrolide ABC transporter ATP-binding protein (provides resistance to macrolides), and other genes, that don’t affect drug resistance.

The objective of the research was to functionally analyze *M. tuberculosis* intrinsic drug resistance mediated by the WhiB7 regulon on *M. smegmatis*. We selected *M. smegmatis* mc\(^2\) 155 as a model for WhiB7 regulon research because this organism has genes orthologous to the genes of *M. tuberculosis* WhiB7 regulon (Reeves AZ et al., 2013). In addition a non-functional *tap*-gene homolog with a frame-shift mutation was found in *M. smegmatis* mc\(^2\) 155.

The analysis of WhiB7-regulon genes’ sequences in 33 complete and 104 draft genomes of *M. tuberculosis* strains stored in NCBI GenBank revealed polymorphism: we found a six SNPs in *tap* gene (insC581, T1029C, A68T, C191G, insT482; delG524); three SNPs in *rv1473* (C1575A, insC451, insC467); two SNPs in *eis* gene (C65G and delG664). The gene *whiB7* itself had only one polymorphic variant – *whiB7ΔC188* detected in three strains: T17, T92 and T46. We also analyzed SNPs in 70 *M. tuberculosis* clinical isolates from Russia and detected SNPs in *tap* gene - insC581, in *eis* - C855T and in *rv1473* - A289G. The SNPs in *eis* and *rv1473* genes were not previously described.

Two pairs of genes – *whiB7*, *tap* and their mutant variants - *whiB7ΔC188* and *tap* (insC581) were cloned in *M. smegmatis*. The resistance levels to different antibiotics were analyzed by paper disc method. The overexpression of *whiB7* gene increased resistance of *M. smegmatis* to five antibiotics: clarithromycin, amikacin, tetracycline, chloramphenicol and imipenem. The overexpression of the mutant variant did not show significant difference in resistance levels from negative control. The overexpression of the *tap* gene increased resistance levels towards erythromycin, kanamycin, oxytetracycline and tetracycline, while the overexpression of the mutant copy of *tap* gene did not affect resistance levels. These results demonstrate that *tap* gene provides resistance not only for aminoglycosides and tetracyclines, but for macrolides too. The impact of *rv1473* and *erm(37)* on resistance of *M. smegmatis* to different antibiotics will be analyzed in future research.
Salicylanilides demonstrated a wide range of biological activities. Several derivatives, developed by our group, have already proved to be active against various bacteria and fungi, while their cytotoxicity is a major drawback. We have recently discovered that the introduction of nitro-group at the salicylic part of the molecules resulted products with moderate antimycobacterial activity, yet high cytotoxicity.

Herein, the synthesis and the antimycobacterial activity of 2-methoxy-3-nitrobanzamides as derivatives of 3-nitrosalicylanilides is been presented. The newly synthesized compounds exhibited enhanced activity over their corresponding 3-nitrosalicylanilides (2-4 fold decreased MIC values against \( M_t b \). \( H_3\text{R}_{\text{V}} \)) and a notably better cytotoxic profile over HepG2 cells (10-25 fold increased \( IC_{50} \) values). The most active compound of the series proved to be \( N-(3,5\text{-bis(trifluoromethyl)phenyl})-2\text{-methoxy-3-nitrobenzamide} \) (MIC=4\( \mu \)M, \( IC_{50}=79.2\mu M \)) while the less cytotoxic active compound was 2-methoxy-3-nitro-\( N-(4\text{-}(trifluoromethyl)phenyl) \)benzamide (MIC=8\( \mu \)M, \( IC_{50}=208.2\mu M \)). Our results indicate that 2-methoxy-3-nitrobanzamides represent promising candidates for further development.

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3. Submitted manuscript.

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CHILDHOOD TUBERCULOSIS PRESENTING WITH LYMPHADENOPATHY

SC Sozmen, O Anal, S Asilsoy, S Isik, N Uzuner, O Karaman, N Esen
Dept. of Pediatric Allergy and Immunology, Dokuz Eylul University School of Medicine, Inciralti 35340 IZMIR-TURKIYE
Corresponding e-mail: ozden.anal@deu.edu.tr

Tuberculous lymphadenitis (TL) is the most common extrapulmonary manifestation of tuberculosis. Peripheral lymph node enlargement has many causes in children, including infectious and malignant conditions. High index of suspicion is needed for diagnosis of tuberculosis in such cases. Tissue specimens for pathological examination, and for mycobacterial culture and antimycobacterial drug susceptibility is important in timely decision for appropriate treatment. Five pediatric cases are presented here with mycobacterial infection detected in cervical lymph nodes.

Case 1. Nine years old girl with Down syndrome had enlarged posterior cervical, supraclavicular and left axillary lymph nodes. Pathologic examination showed necrotizing granulomatous lymphadenitis. She had pulmonary tuberculosis that was treated with a 12 months course of isoniazide, rifampycin, pyrazinamide and streptomycin.

Case 2. Fifteen years old girl had two months history of abdominal pain and painless swelling in right cervical region. Surgically extirpated supraclavicular lymph node grew \( M_t b \) in culture. The patient who was diagnosed as pulmonary and abdominal tuberculosis, recovered completely following a 12 months course of antituberculous treatment.

Case 3. Fourteen years old girl had nine months history of painless cervical mass. Her sputum culture was positive for \( M_t b \) which was sensitive to streptomycin, ethambutol, rifampicin, isoniazid.

Case 4. Ten years old girl presented with draining purrulant mass under the mandible suggesting scrofula. Culture of lymph node was positive for \( M_t b \). Patient’s lesions were totally regressed following treatment with isoniazide, rifampycin and pyrazinamide.

Case 5. Seventeen years old boy had painful swelling located in left upper gingival mucosa and left cervical adenopathy. Excision of lymph node was performed and pathological findings showed chronic granulomatous
inflammation. Non-tuberculous mycobacteria grew in culture. Patient’s lesions were totally regressed without any drug treatment.

All of the cases were negative for HIV, and immune deficiency states were ruled out during their follow-up period.

Surgical excision and drainage of a suspected superficial lymph node facilitates the diagnosis of mycobacterial infection. In pediatric patients, antituberculous treatment should be individualized according to patient’s clinical and laboratory investigations, in order to decrease the potential drug toxicity.

**RIFAMPICIN-LOADED POLY(AMIDO AMINE) NANOGELS**


*Biomaterials Science & Technology, MIRA Institute, University of Twente, Drienerlolaan 5, 7522 NB, Enschede, The Netherlands*

E-mail: a.e.ekkelenkamp@utwente.nl

Tuberculosis (TB) remains one of the most deadly diseases in the world. Although treatment is available, therapy is long and the drug regime has a high drug load. Furthermore, inadequate treatment increases the risk of generating drug-resistant mycobacterium tuberculosis (Mtb) strains.1,2

The development of new drugs is essential to tackle the problem of drug-resistance.3 However, improving therapeutic efficacy and therefore lowering the risk of drug-resistance is also essential. Therefore, TB therapy needs to become more effective, lowering both the administered drug dose and dosage frequency.4 The challenges faced in TB treatment are similar to the research in the controlled drug delivery field: ensuring targeted delivery of chemotherapeutics and achieving sustained release.5 Improved delivery of anti-TB chemotherapeutics can improve therapy efficacy and lower the administered dosage.4

Polymeric nanoparticles have become one of the cornerstones for the controlled delivery of drugs.5 Polymeric-based delivery strategies have already been reported.6,7 Reported strategies frequently employ polyester-based biomaterials to improve sustained release. Here we present the development of peptidomimetic poly(amine amine)8 nanoparticles (nanogels) with a diameter of 100 nm. The nanogels were loaded with rifampicin as a concept for controlled drug delivery. Furthermore, the nanogels show minimal cell toxicity and contain disulfide bonds, which are cleaved in the cytosol of the cell to deliver antibiotics into Mtb-infected cells. We also have shown that the nanogels were able to enter murine macrophages, showing that poly(amine amine) nanogels are a promising anti-TB delivery vehicle.

Day 2:

Invited Speakers Abstracts

Investments in tuberculosis research 1997-2013 - an analysis of the UK research portfolio
Dr Michael Head, Research Associate, University College London, UK
This talk will outline the R&D investments related to tuberculosis research that have been awarded to UK institutions between 1997 and 2013. Areas of relative research strength and research gaps will be identified, along with a comparison of investments of other major global diseases.

Pulmonary tuberculosis and systemic mycosis co-infection - clinical Implications
Dr Rinaldo Poncio Mendes, M.D., Ph.D.

Immunopathogenesis of tuberculosis in Asian elephants (Elephas maximus)
Jennifer (Jaime) Landolfi, DVM, PhD, Diplomate ACVP, University of Illinois, Zoological Pathology Program, US
Tuberculosis is an important health concern for Asian elephant (Elephas maximus) populations worldwide. Most infections are due to Mycobacterium tuberculosis, though mechanisms underlying tuberculosis susceptibility are unknown. Investigations into elephant tuberculosis immunopathogenesis to date have demonstrated differences in immune cell function between cell culture samples from positive and negative animals. Additional studies have also illustrated patterns of local inflammation reminiscent of both human latent and active pulmonary tuberculosis lesions. The foundation of knowledge established by these findings serves to promote continued investigation of elephant tuberculosis immunopathogenesis for the long-term conservation of this endangered species.

Mycobacterial ParE2: A DNA Gyrase inhibitor and its role in mycobacterial persistence
Mr. Manish Gupta, Teri University and Bsl-3 Lab, School Of Biotechnology, Jawaharlal Nehru University, New Delhi, India
Mycobacterium tuberculosis, the etiological agent of Tuberculosis (TB), elaborates its pathogenicity by virtue of its metabolically inactive and non-replicative persisters, latency associated reactivation and drug resistance. A similar non-replicative, dormant phase is triggered by the activation of Toxin-Antitoxin addiction systems under stress. We propose that specific TA systems may be involved in the process of adaption to environmental cues within the host macrophages. In this context, we have examined the parDE2 TA system which is conserved in M. tuberculosis complex (MTBC) and absent in other non-pathogenic mycobacteria implying their potential role in TB pathogenesis and persistence.

Synthesis and evaluation of potential inhibitors of M.Tb. bearing aminoheterocyclic cores
Dr Michel Baltas, CNRS LSPCMIB UMR 5068, CNRS, France
Tuberculosis caused by Mycobacterium tuberculosis remains a major cause of mortality worldwide with nearly three million people deaths annually. Our own efforts have focused on the development of potential inhibitors of InhA and M. Tuberculosis drugs possessing a central amino heterocyclic core. Three different families were designed and were synthesized. The first family of inhibitors was designed based on GEK structure analogues bearing succinimide and reduced succinimide rings. The second family was based on (di)ketotriazole and triazole core. The third family concerns derivatives of 4-alkoxy cinnamic acid. Synthesis and activities will also be presented.

Oral Presentation Abstracts

ROLE OF TNF-α, IFN-γ, IL-12p70, IL-4 & IL-1β AS EARLY TREATMENT RESPONSE BIOMARKERS FOR THE DETECTION OF MDR-TB
Nazish Fatima*, Mohammad Shameem**, Nabeela*, Haris M Khan*, Indu Shukla*
*Department of Microbiology, Jawaharlal Nehru Medical College, AMU, Aligarh.
**Department of TB & Respiratory Diseases, Jawaharlal Nehru Medical College, AMU, Aligarh.
Address of corresponding author: - Dr. Nazish Fatima, Department of Microbiology, JNMC, AMU, Aligarh, India.
E-mail- nazsham28@gmail.com
Background: The worldwide emergence of multidrug-resistance tuberculosis (MDR-TB i.e., resistance to at least rifampicin &isoniazid) is continuously increasing. The prevalence of MDR TB among new and previously treated cases is increasing all over the world as well as in India.

Resistance to Mtb infection is mediated by macrophages, T-cells and their interaction and is dependent on the interplay of cytokines produced by each cells. The discovery of biomarkers for TB treatment response is therefore important for both clinical practice and clinical trials of new anti-TB drugs. The present study aims to screen TNF-α, IFN-γ, IL-12p70, IL-4 & IL-1β among TB patients and controls.

Methodology: This study was conducted at department of Microbiology, JNMC, AMU, Aligarh. Samples were collected from the department of TB & Respiratory diseases, JNMC. Different cytokines levels were measured in 65 samples of TB patients of whom 30 (46.1%) were new TB cases and 35 (53.8%) were suspected MDR TB cases along with 15 BCG vaccinated healthy controls by ELISA (Diaclone France). A complete clinical, radiological and treatment data was collected. Informed consent was taken from all subjects. Study was approved by Institutional Bioethical Committee. Statistical analysis had been performed by using Sigma plot (10.0).

Results: The concentration of TNF-α, IFN-γ, IL-12p70 & IL-1β were significantly higher in new & MDR TB cases (P<0.001) as compared to control, while IL-4 concentration increased only in MDR TB patients (P<0.001) as compared to control. All these cytokines showed no significant variations according to the site of involvement in pulmonary vs. extra-pulmonary TB cases.

Conclusions: More large sized studies are required to establish the immuno-regulatory and diagnostic roles of these cytokines as early treatment response biomarkers. This concept, if validated, could lead to the development of clinical interventions and accelerate the conduction of TB clinical trials.

TH1 AND TH17 LEVELS IN TUBERCULOSIS PATIENTS WITH DIABETES MELLITUS

Adil Wafi CG, Zuber Ahmed, Mohammad Owais, Mohammad Shameem, Sheelu Shafique, Mazhar Alam, Mohd Arif Alam, Arshad Ejazi

Correspondence: Dr Mohammad Shameem MD;FRCP(Edin.)
Department of Tuberculosis and Chest Diseases; JN Medical College, Aligarh Muslim University; Aligarh UP 202002 India
E Mail: drshameem123@gmail.com

Back ground: The link between diabetes mellitus and tuberculosis has been recognised for centuries. In recent decades, tuberculosis incidence has declined in high-income countries, but incidence remains high in countries that have high rates of infection with HIV, high prevalence of malnutrition and crowded living conditions, or poor tuberculosis control infrastructure. At the same time, diabetes mellitus prevalence is soaring globally. However the role of Th1 and Th17 levels, two important cytokine involved in controlling inflammation has to be studied in Tuberculosis patients with Diabetes Mellitus

Method: This study was carried out in the department of Tuberculosis And Respiratory Diseases ,Jawaharlal Nehru Medical college ,AMU ,Aligarh from October 2012 to October 2014 .Total number of patients were 45,They were divided into two groups. First group study group comprising patients of pulmonary tuberculosis with diabetes mellitus having 21 patients. Second group comprising 24 controls matched for age and sex with study group having pulmonary Tuberculosis. Blood samples of each patient and control were taken and the levels of IFN-γ, (a signature cytokine of Th1 cells) through sandwich ELISA in samples belongs to both groups of patients. We measured the levels of RORγt level(chief master transcription regulator of Th17 type cells); through Real Time quantitative PCR

Results: Th1 and Th17 levels in patients of TB with DM were significantly increased than pulmonary TB without DM ( Th1in study group –M=935.15,Sdx±284pg/ml p value < 0.005, control group 670.69±SD134pg/ml),Th17 were increased in TB with DM group as depicted in the figure 8 (n=24, p value < 0.05 ).Taken together these results suggest that Th1 Th17 levels in TB with DM were significantly increased than those in pulmonary TB cases. Our data support the prediction that diabetes exacerbates tuberculosis severity to a significant degree and therefore provides a rationale for treating latent tuberculosis in the diabetic population in India. We also found that in group of patients having pulmonary TB with DM, IFN gamma levels were higher in age group 15 to 24(mean–1349.18 and SD ±530.7) than other age groups. One possible reason for this phenomenon may be the fully flourished immune system in young age, however further studies are required towards understanding this finding. Our data supporting the effects of an excessive but otherwise intact adaptive immune response to M. Tuberculosis during diabetes provided a rational basis for testing combined antimicrobial and anti inflammatory therapies in diabetic patients with tuberculosis. In addition to this, some type of immune modulator may be use as an adjunct to chemotherapy.
MYCOBACTERIAL ParE2 TOXIN: POTENTIAL ROLE IN PERSISTENCE AND PATHOGENICITY

M Gupta1,2, R Sitaraman2, R Bhatnagar1, N Banerjee1,2
1Molecular and Cell Biology Department, School of Biotechnology, Jawaharlal Nehru University, New Delhi-110067, India and 2Department of Biotechnology, TERI University, New Delhi-110067, India
*Corresponding author (nirupamaban@yahoo.com)

The chronicity of Mycobacterium tuberculosis (Mt b) infection is attributed to metabolically inactive, non-replicative persisters, latency-associated reactivation and drug resistance. A similar non-replicative phase is triggered by the activation of Toxin-Antitoxin (TA) addiction systems under stress. Presence of 88 putative TA modules in the Mt b H37Rv genome and conservation of the same only in the M. tuberculosis complex (MTBC), suggests an important role for these genes in Mt b evolution. In this context, we have examined two transcriptionally active TA modules (parDE1 and parDE2), present in the Mt b H37Rv genome, that are preserved in MTBC but absent in other non-pathogenic mycobacteria. These two putative TA loci are homologous to the archetypal parDE TA module encoded by the RK2 plasmid of E. coli. ParE, the toxin component of this family binds to DNA gyrase and inhibits bacterial replication and transcription. The inhibition is relieved by the ParD antidote that binds to ParE and sequesters it.

We found that overexpression of Mt b ParE2 toxin but not ParE1, in heterologous bacterial host like E. coli and M. smegmatis, resulted in growth and replication arrest, suggesting that the cellular target of ParE2 is conserved in both the organisms. ParE2 inhibited the Mt b DNA gyrase (GyrA₂-GyrB₂ complex) in supercoiling assays by generating a linear form of DNA intermediate, which is a hallmark of stabilization of the gyrase-DNA cleavable complex. Understandably, the effect of ParE2 toxicity is dependent on the growth phase, with early log phase cells exhibiting a greater loss of viability as compared to late log phase cells. The ParD2 antitoxin effectively prevented the inactivation of gyrase by the cognate ParE2 toxin. However, it is unable to reverse ParE2 toxicity in in vitro DNA supercoiling assays. The activity of ParD2 is consistent with its observed role in vivo in preventing ParE2-mediated killing. Surface plasmon resonance and isothermal titration calorimetry studies suggested that mycobacterial ParE2 binds to the GyrB subunit and not GyrA. Moreover, ParE2 had no ill-effects on the ATPase activity of GyrB suggesting that the GyrB-ATPase domain is not involved in ParE2 binding. Majority of E. coli cells expressing ParE2 under the tightly controlled and inducible ara promoter exhibited cell stasis, but not cell death. Microscopic studies of such cells reveal extensive cell filamentation, cell elongation, loss of membrane integrity and intracellular formation of uncharacterized vacuole-like spaces. The Mt b parDE2 operon responded to adverse environmental conditions like oxidative stress and hypoxia, similar to what is encountered by the pathogen inside the host macrophages by decreasing cellular replication and generation of non-culturable phenotype of cells. In the light of these data, it is plausible that the physiological role of this TA system might lie in enabling mycobacteria to adapt within the host leading to persistence. Our results revealed that a functional parDE2 in Mt b H37Rv may contribute to latency and persistence, making it an attractive drug target.

GENDER DIFFERENCES IN THE EPIDEMIOLOGY OF TUBERCULOSIS SUSPECTS AND TUBERCULOSIS PATIENTS IN GUINEA-BISSAU

F Rudolf, AM Mendes, CB Patsche, A Sifna, MS Mendes, V Gomes, C Wejse
Folehaven 133, 2.tv, 2500 Valby, Denmark

Introduction: We assessed gender differences among patients included in an observational cohort study among pulmonary Tuberculosis suspects (PTBS) from June 2010 to June 2014, established at the Bandim Health Projects (BHP) study site in Bissau, Guinea-Bissau.

Methods: We included adult patients with signs and symptoms suggestive of pulmonary Tuberculosis seeking medical care at primary health care centers in the BHPs study area. All included patients were visited at home two weeks after first encounter and patients being symptomatic at the two weeks follow-up were referred to chest x-ray (CXR) and treated with broad spectrum antibiotics for seven days. After finished treatment the patients were reassessed and if still symptomatic, they were referred to take another CXR and seen by a TB-experienced physician. The possible diagnoses after this second examination were Tuberculosis (TB), not TB and maybe TB. If the diagnosis was maybe TB the patient was treated with a second antibiotic for another seven days and another chest x-ray was taken. Final diagnosis, TB or not TB, was given by the same physician evaluating the patients after first course of antibiotics. All patients diagnosed were referred to treatment and invited for inclusion in the Bandim TB cohort.

Results: We included 2019 PTBS, 54.5% female. Females were younger than males (33 (95%CI 32 – 34) vs. 36 (95%CI 35 – 37)) and more often Human immunodeficiency virus (HIV)-infected (20.1% vs 12.0%, p< 0.001), while more male patients had TB previously (13.8% vs. 8.5%, p<0.001) and were diagnosed with TB more often (14.8% vs. 7.3%, p<0.001). Of the diagnosed patients the proportion of smear positive cases was significant
Tuberculosis caused by *Mycobacterium tuberculosis* remains a major cause of mortality worldwide with nearly three million people deaths annually. Antibiotic resistance is a growing problem in multidrug-resistant tuberculosis (MDR-MT) and in extensively drug-resistant tuberculosis (XDR-MT). More than ever, there is an urgent need to develop new potent and fast acting anti-tuberculosis drugs to combat the spread of TB.

Our own efforts have focused on the development of potential inhibitors of InhA and M. Tuberculosis drugs possessing a central amino heterocyclic core. Three different families were designed and were synthesized.

The first family of inhibitors was designed based on GEK structure analogues bearing succinimide and reduced succinimide rings. Synthesis and biological results of these new compounds will be presented. A few of them present good activities against InhA and promising activities against *M. tuberculosis*. The second family was based on ketotriazole (diketotriazole) and triazole core. Synthesis of these new families and their activities will be presented. Among them, 1,4-Diketotriazoles are a new family of inhibitors for *M. tuberculosis* and *M. tuberculosis* resistant-strains but the protein target has to be identified. The third family concerns derivatives of 4-alkoxy cinnamic acid. Among them, 4-isopentenyloxycinnamyl triazolophthalazine derivative was found to be 100-1800 times more active than isoniazid (INH) when tested for its ability to inhibit the growth of INH-resistant *M. tuberculosis* strains. Synthesis and activities will also be presented.

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   Recent advances in the development of cinnamic-like derivatives as antituberculosis agents. *Expert Opin. Ther., 2012*, 155-168 (review).
THE ROLE OF EFFLUX PUMP INHIBITORS ON FIRST- AND SECOND-LINE ANTI-TUBERCULOSIS DRUGS IN RIFAMPICIN MONO-RESISTANT CLINICAL ISOLATES OF MYCOBACTERIUM TUBERCULOSIS

C.M Pule1, G.E Louw2, R.M Warren1, P.D Van Helden1, T.C Victor1

DST/NRF Centre of Excellence in Biomedical Tuberculosis Research / MRC Centre for Molecular and Cellular Biology, Division of Molecular Biology and Human Genetics, Department of Biomedical Sciences, Faculty of Health Sciences, Stellenbosch University, South Africa.2 NIAID/LCID/Tuberculosis Research Section, Bethesda, MD, USA.

Rationale: Recent reports suggest that specific rpoB mutations are associated with altered intrinsic resistance to other first and second-line anti-tuberculosis drugs.

Objective: To determine the effect of efflux pump inhibitors (EPIs) on the susceptibility of rifampicin mono-resistant Mycobacterium tuberculosis clinical isolates to first and second-line anti-tuberculosis drugs.

Methods: Rifampicin mono-resistant clinical isolates (n=8) from different strain families with the same rpoB mutation (Ser531Leu) were selected. The effect of the EPIs (verapamil, CCCP) to first-line (isoniazid, pyrazinamide, ethambutol, streptomycin) and second-line (ciprofloxacin, moxifloxacin, ofloxacin, amikacin, capreomycin and ethionamide) drugs was determined by the MGIT 960 in combination with EpiCenter Technology. The isolates were exposed to the above drugs at MIC in the presence/absence of EPIs. Thereafter, the fractional inhibitory concentration (FIC) was calculated and the interaction values were classified as synergistic when FIC index ≤ 0.5-0.9, indifference/additive when FIC index = 1-1.9 and antagonistic when FIC index ≥ 2.

Findings: The FIC values for first-line drugs in combination with verapamil and CCCP respectively were as follows: isoniazid (1.0;0.4) streptomycin (0.4;1.2), ethambutol (1.4;1.5) and pyrazinamide (0.7;0.2), then for second line drugs amikacin (0.7;1.7), capreomycin (0.8;0.7), ciprofloxacin (0.8;2.6), ofloxacin (1.1;1.8), moxifloxacin (1.0;1.1) and ethionamide (0.2;0.9). Verapamil had an effect on the anti-TB drugs MICs with p-value= 0.0001 and in contrast to CCCP (p= 0.01196) in rifampicin mono-resistant strains with rpoB mutation 531.

Discussion: Our findings demonstrated that EPIs enhanced the susceptibility to certain first- and second-line anti-TB drugs in rifampicin mono-resistant strains. Thus suggests the involvement of efflux pumps activities in defining the level of intrinsic resistance.

TARGETED DELIVERY OF ANTITUBERCULAR DRUGS TO THE BRAIN FOR THE TREATMENT OF TUBERCULOUS MENINGITIS.

S Majeda1, S Sharma3, BD Radotra2, P Singh2, N Sharma2

a: Lab 334, Department of Biochemistry, PGIMER, Chandigarh, -160012 India
b : Department of Histopathology, PGIMER c. Department of Neurology, PGIMER d. Department of Internal Medicine, PGIMER

Correspondence: Professor Sadhna Sharma, Dept of Biochemistry, PGIMER, Chandigarh -160012 (India), Phone : 91-0172-2755180, Fax: 91-0172-2745078, 2744401, Email: sadhnabiochem@gmail.com

Background: Tuberculous meningitis is the most severe form of extra pulmonary tuberculosis. Though it represents roughly 1% of all cases of tuberculosis, it causes mortality or severe disabilities in almost fifty percent of the patients affected. The conventional therapy employs use of Isoniazid, Rifampicin, and Pyrazinamide for a period of 6-24 months depending upon severity of the disease. Serious limitation of the therapy is the limited ability of antitubercular drugs to cross the blood brain barrier and its lengthy treatment schedule causing patient non-compliance. This study was designed to synthesize nanoparticles containing antitubercular drugs for their effective delivery to the brain and to study pharmacokinetic and therapeutic effects in mice.

Methodology: Polybutylcyanoacrylate(PBCA) nanoparticles encapsulating anti-tubercular drugs were synthesized by anionic polymerization method and double coated with PEG+P-80 for oral delivery in mice. Pharmacokinetic effects of drugs in plasma and tissues were evaluated by high performance liquid chromatography. Murine model of tuberculous meningitis was developed by intracranial injection of Mycobacterium tuberculosis infection in mice. Chemotherapy of free or nanoencapsulated drugs was given
orally to infected mice for eight weeks. Free drugs were given daily whereas nanoencapsulated drugs were administered weekly.

**Results:** Nanoparticles obtained were of nanosize range and sterically stable. Pharmacokinetic analysis showed sustained release of drugs in the plasma for about 96h and their retention in the brain for up to six days. Successful generation of murine model was confirmed by increased and stable bacillary loads in mice as well as histopathology of mouse tissues showing meningitis in brain, granuloma in lungs and tuberculous pathology in spleen. Drug loaded PBCA nanoparticles were found to be more effective, 4 or 8 doses of nano-encapsulated formulation were equi-efficacious to 32 or 64 doses of free drugs conventional therapy.

**Conclusion:** Double coated PBCA nanoparticles can be a promising vehicle to deliver anti tubercular drugs to brain and management of tuberculous meningitis. Studies conducted so far have used intravenous route for drug targeting to the brain, our work suggest possibility of using nanoparticle formulation through oral route which is more convenient and patient friendly mode of drug delivery.

**TB CONTROL IN INDIA**

Dr. D. Behera  
Senior Professor & Head, Dept. of Pulmonary Medicine, (WHO Collaborating Centre for Research & Capacity Building in Chronic Respiratory Diseases), Postgraduate Institute of Medical Education & Research, Chandigarh - 160012 (INDIA), And Chairman, National Task Force

India has the highest number of TB cases in the world with 2.2 million new cases every year and 270,000 deaths every year. To counter the problem the country started the DOTs strategy as an integral part of the Revised National tuberculosis Control Program (RNTCP) since 19997. The entire country was covered by the program by March 2006. The goal is to to decrease mortality and morbidity due to TB and cut transmission of infection until TB ceases to be a major public health problem in India and the objectives are to achieve and maintain a case detection of at least 70% of new sputum positive TB patients and to achieve and maintain a cure rate of at least 85% in newly detected smear positive cases. With a population 121, 0,193,422, with Urban area of 31.2% and a total of 36 States and Union Territories, the country has now established 58 Culture-DST Labs, 122 DR-TB Centers to treat MDR-TB cases. There are also 50 Linked DR-TB Centers. The program has 712 Districts Units. In the public sector there are 89 CB-NAAT (Xpert) Sites. Tuberculosis Units (TUs) are 3644 and Designated Microscopy Centers are 13306.

India’s TB control programme is on track as far as reduction in disease burden is concerned. There is 42% reduction in TB mortality rate by 2012 as compared to 1990 level. Similarly there is 51% reduction in TB prevalence rate by 2012 as compared to 1990 level.

The Objectives are modified for the period 2012-2017 to achieve 90% notification rate for all forms to TB cases, to achieve 90% success rate for all new and 85% for re-treatment cases, to significantly improve the successful outcomes of treatment of Drug Resistant TB Cases, to achieve decreased morbidity & mortality of HIV associated TB and to improve outcomes of TB care in the private sector. There is a well defined ACSM (advocacy, communication and social mobilization) strategy based on Communication needs, Target Groups and communication tools/Media options to reach target groups. Roles and responsibilities defined at the central, state and district level. ACSM strategy is modified for addressing newer initiatives like MDR- TB and TB HIV co-infection. Under project Axshaya 374 districts in 23 states are involved. To take care of the Clinically most at risk group like, HIV infected patients, Tribal Action Plan Special provisions for poor and backward areas, TB and diabetes, and bidirectional Screening, there are definite plans. In 2013 there was enhanced outlays for Tribal areas, poor and backward areas. There is an increased trends in the number of registered TB patients with known HIV status, and by now 70% of TB cases know their HIV status. The number of TB-HIV patients receiving
co-trimoxazole prophylaxis is also increasing (over 90% receive the prophylactic therapy). The other strategies include pediatric TB, incorporation of newer diagnostic methods like liquid culture, line probe assays and CB-NAAT are being adopted very quickly by the program. The RNTCP of India is achieving the 70% case detection and more than 88% cure rates.

DIFFERENTIAL MODULATION OF CYTOKINE EXPRESSION BY ERK AND P38 MAP KINASES IN RESPONSE TO MYCOBACTERIUM AVIUM INFECTION IN HUMAN AND CHICKEN CELLS
Mohammed Shukur, Sabine Tötemeyer, Nawzat Issa, Paul Barrow, Michael A. Jones
School of Veterinary Medicine and Science, University of Nottingham, Sutton Bonington LE12 5RD.

Background and Aims
Several cell signaling pathways are involved in the mediation of expression of a number of cytokines induced by mycobacterial infection. The mitogen activated protein kinase (MAPK) pathway plays a crucial role in this process and they are important in the pathogenesis of mycobacterial infection. In this study we aimed to evaluate the role of ERK and p38 MAPK in cellular regulation by Mycobacterium avium infection in human and chicken macrophages-like cells.

Materials and Methods
THP-1 (human monocyte-derived macrophages) and HD11 (chicken macrophage-like cells) were infected with eight clinical isolates of Mycobacterium avium at a multiplicity of infection of 10:1. The cells were pre-treated with highly specific inhibitors of the ERK (PD98059 or U0126) and p38 (SB203580) pathways 30 min prior to infection and the levels of cytokine production at 6 and 24 h post infection were assessed. Pro-inflammatory cytokine production in response to infection was measured in THP-1 cells by ELISA and in HD11 cells by RT-qPCR.

Results
M. avium infection resulted in differential expression of cytokines and chemokines in THP-1 and HD11 cells. Treatment of the cells with PD98059 or U0126 inhibited production of cytokines in THP-1 and completely blocked their expression in HD11 cells. In addition, p38 inhibition differentially modulated cytokine production in THP-1 cells compared to non-inhibited M. avium-infected cells. It inhibited release of IL-6 while the level of IL-1β and TNF-α showed an increase in THP-1 cells in response to M. avium infection following treatment of the cells with SB203580.

Conclusion
The results suggest that signalling events are significantly different in avian and human cells following M. avium infection. Both p38 and ERK are involved in regulation of IL-6 production while p38 negatively regulates IL-1β and TNF-α production in response to M. avium in THP-1 cells. In HD11 cells, it is suggested that multiple signal pathways simultaneously participate in regulation of cytokine signal transduction during M. avium infection.

MOLECULAR CHARACTERIZATION BY SPOLIGOTYPING AND VNTR TYPING OF MYCOBACTERIUM BOVIS ISOLATES FROM CATTLE OF SOUTH KOREA
Jae-Myung Kim, Yunho Jang, Sooyoon Ryoo, Narae Kim, Jin Kyung Kim, Hee Soo Lee
Bacteriology Disease Division, Animal and Plant Quarantine Agency, Anyang, Gyeonggi-do, Korea

Bovine tuberculosis is wide spread and endemic in South Korea despite long attempts to control the disease. The objective of this study was to conduct, molecular characterization of M. bovis strains isolated from slaughter cattle in South Korea. Between August 2012 and June 2013, 1,871 animals were consecutively screened at the abattoirs of Chuangbuk and Gyeongnam. In 242 animals, suspected tuberculosis lesions were detected and put into culture. M. bovis isolated from 7 animals, other bacteria isolated included 7 strains of M. avium complex strains, 31 M. intracellulare strains, 25 non-tuberculous mycobacteria (NTM). The M. bovis strains isolated showed only two spoligotype patterns, SB0140 and SB1040 that were previously observed in strains of European cattle. Spoligotyping combined with VNTR typing allowed us to distinguish 4 distinct types of 7 M. bovis strains. This study provided molecular evidence for the widespread distribution of M. bovis in cattle population in South Korea. Furthermore, this study highlights the importance of both spoligotype and VNTR typing to assist global molecular epidemiological investigations of M. bovis infection.
Background: Tuberculosis (TB) and Diabetes Mellitus (DM) have synergetic relationship. People with diabetes are 2-3 times at higher risk of getting active TB disease. On the other hand TB might cause glucose intolerance. The dual disease of DM & TB is more likely to be associated with atypical disease presentation, higher probability of treatment failure and complications. So there should be a uniform management service for TB-DM co-morbidity.

Objective: Realizing this situation Bangladesh Diabetic Samity (BADAS: A non-profit non-government organization for the management of Diabetes in Bangladesh) with the patronizing of TB CARE II Project, Bangladesh funded by USAID launched a project titled BADAS-USAID TB CARE-II, Bangladesh with the goal of “Integrated approach to increase access to TB services for Diabetic patients”.

Methods: Starting from June 2012 upto December 2014, BADAS-USAID TB CARE-II project has contributed in 3 different areas - 1. TB care service to the patients – TB screening of Diabetic (DM) as well as non-diabetic (non-DM) patients from BIRDEM General Hospital (A tertiary care hospital for diabetic patients in Bangladesh) and other BADAS affiliated centers, investigate the presumptive TB patients with sputum microscopy & GeneX-pert, Chest x-ray and FNAC to confirm the diagnosis and send the patients to DOT (Directly Observed Treatment) center for TB treatment & follow-up. 2. Manpower development – Medical personnel including doctors, nurses, laboratory technicians and others were trained through 3 day & one day long workshop on the management of TB-DM co-morbidity. 3. Develop a national guideline – the project initiated to develop evidence based national guideline, on the basis of existing national TB guideline, with the help of consensus opinion of experts.

Results: 1. During the project period from June 2012 to December 2014, a total of 10,88,303 patients were screened for Tuberculosis. Out of them, 7965 patients [6873 (86.3%) DM, 1092 (13.7%) non-DM] were identified as presumptive TB cases. After investigations 1481 (18.6%) patients were diagnosed as confirmed TB cases who were then referred to DOT for treatment & follow-up.

Among the 6873 presumptive TB cases from DM group 1314 (19.1%) were diagnosed as TB cases. Out of them 1057 (80.4%) were pulmonary TB (PTB) and 257 (19.6%) were extra-pulmonary TB (EPTB). Among the PTB patients in DM group, 745 (70.5%) were smear positive and 312 (29.5%) were smear negative TB. Among the 1092 presumptive cases from non-DM group 167 (15.3%) were diagnosed as TB cases. Out of them 65 (39.0%) were PTB and 102 (61.0%) were EPTB. Among the non-DM PTB patients, 24 (36.9%) were smear positive and 41 (63.1%) were smear negative.

Case detection rate among presumptive TB cases from DM patients: smear positive TB- 10.8%, smear negative TB- 4.6% and extra-pulmonary TB- 3.7 %, from non-DM patients: smear positive TB- 2.2 %, smear negative TB-3.8% and extra-pulmonary TB- 9.3 %.

Sputum for GeneX-pert MTB/RIF was started from November 2013. Upto December 2014 a total of 3029 (100%) patients under went sputum for GeneX-pert microscopy [2324 (76.7%) DM, 705 (23.3%) non-DM] and MTB was detected in 358 (11.8%) patients [303 (13.0%) DM, 55 (7.8%) non-DM]. Out of 358 (100%) cases where GeneX-pert was positive for MTB, Rifampicin was sensitive in 349 (97.5%) cases and Rifampicin was resistant in 9 (2.5%) cases. Rifampicin resistance rate was 2.6% (8) in DM group and 1.8% (1) in non-DM group. Out of 8 cases of Rifampicin resistance in DM group, 3 (0.99%) were new cases of PTB (Primary drug resistance) and 5 (1.65%) were from retreatment group.

2. During the project period period a total of 630 doctors (including 10 master trainers), 216 nurses, 19 laboratory technicians and 333 other staff were trained through 3 day and one day long orientation workshop.

3. A National Guidelines on the Management of TB-DM co-morbidity has been published in November 2014, a pioneer effort in South East Asia.
Mahouts in southern India are a traditional group of animal keepers engaged exclusively in the care and management of captive elephants. In the hands-on and open types of management systems followed for captive elephants in southern India, mahouts are the first line human contacts of these captive wild animals. It is estimated that there are nearly a thousand captive elephants and not less than 3,000 mahouts in the southern Indian states of Kerala, Karnataka and Tamil Nadu. A recent study revealed the seroprevalence of tuberculosis infection in captive elephants in southern India as 15%. Higher seroprevalence of up to 30% was observed in certain groups of captive elephants with greater contact with humans. Twelve different isolates of Mycobacterium tuberculosis were obtained from the post-mortem lung nodules of captive elephants by culture on Lowenstein-Jensen medium and identified by both biochemical and molecular methods. In order to ascertain the zoonotic implications to mahouts of elephants with tuberculosis, a long term study on the morbidity patterns of mahouts in southern India with special reference to tuberculosis was initiated. In a cross sectional study conducted over a period of one year, using 2 TU PPD (SPAN Diagnostics, India), tuberculin skin testing followed by clinical evaluation was performed on 375 randomly selected mahouts attending to elephants in the higher tuberculosis seroprevalence groups. Taking 10 mm sized skin indurations as the cut-off point, the prevalence of latent tuberculosis infection (LTI) among mahouts was estimated to be nearly 53.8%. A contact tracing questionnaire survey was undertaken with nearly 700 mahouts to identify cases of active tuberculosis among mahouts. Fourteen cases of mahouts with active disease with present and past history of anti-tuberculosis treatment were identified. In addition to the already known risk factors for LTI in humans in southern India, history of contact with known tuberculosis positive elephants is examined to check for the risk of zoonosis specifically from infected elephants. A proposed follow-up three year cohort study intends to accurately identify the major risk factors for tuberculin skin test conversions and active disease among the mahouts. This collaborative study intends to suggest evidence based guidelines for the prevention, diagnosis and treatment of tuberculosis in mahouts in the resource poor settings in southern India. The poster gives a pictorial description of the various aspects of the study.

A RAPID SCREENING ASSAY TO DETERMINE THE ANTI-MYCOBACTERIAL PROPERTIES OF METAL/METAL OXIDE NANOPARTICLES
Samantha M K Donnellan*, Karen Stevenson, Vicki Stone
Moredun Research Institute, Heriot-Watt University
*corresponding author smd31@hw.ac.uk

There is an urgent need to develop effective treatments for the disease tuberculosis (TB), caused by the organism Mycobacterium tuberculosis (Mtb). The burden of the disease is enormous, with a third of the world’s population estimated at being infected with latent TB, and it continues to kill up to two million people annually. Mortality rates of multi-drug resistant TB are increasing and there are the continuing problems of treating HIV infected patients. The objective of this project is to create a rapid assay for investigating the anti-mycobacterial activity of different nanomaterials. A related surrogate organism was used, Mycobacterium avium subsp. paratuberculosis (Map) causing paratuberculosis in ruminants, as it could be handled in containment level 2 facilities, making it a relevant and cheaper alternative to Mtb to develop and optimise the assay. Map has a cell wall similar in structure and chemical composition to that of Mtb which hinders the entry of drugs and is resistant to a similar spectrum of drugs. Map has been transformed with a plasmid carrying the gene for Green Fluorescent Protein (GFP) to create a reporter strain (Map-GFP), thus allowing both growth and viability to be tracked by fluorescence. The anti-mycobacterial properties of nanopreparations of silver, copper (II) oxide and zinc oxide have been investigated. Suspensions of pre-weighed nanoparticle (NPs) were prepared in media employing water bath sonication for 16 minutes and serial dilutions of the particulate solutions were made and added to Map-GFP. Growth was monitored over 7 days and a dose response was measured, showing the effects of the different NPs at various concentrations. The efficacy of the NPs can be ranked as ZnO>Cu(II)O>Ag.
Day 3:

Invited Speakers Abstracts

Predictors and prognostic indicators of stroke in CNS tuberculosis
Professor Mohammad Wasay MD, FRCP, FAAN, Aga Khan University, Karachi, Pakistan
Tuberculosis (TB) ranks as one of the leading causes of mortality and morbidity worldwide. In 2010 an estimated 8.8 million incident cases of tuberculosis were recorded globally with approximate 1.45 million deaths. Pakistan currently ranks as the fifth largest contributor to the global TB burden with 0.3% to 0.48 million cases recorded within a population of 187.3 million people.

In CNS tuberculosis, tuberculous meningitis (TBM) accounts for 5-10% of all TB cases and is responsible for more than 40% of the deaths due to TB in developing countries. Tuberculous meningitis is one of the most serious and critical presentations of TB and if undiagnosed and not treated in time, it can advance into complications like space occupying tuberculomas and cerebral infection.

Development of tuberculomas, an obliteratorine vasculitis which causes infarction and inflammatory adhesion exudates can result in obstructive hydrocephalus and multiple cranial nerve palsies.

TBM is characterized by varied clinical manifestation and a myriad of complications. Tuberculoma may be present in 16-40% of patients with central nervous system tuberculosis. Tuberculoma account for 10-30% of intracranial masses in TB endemic areas. Data regarding prognostic value of tuberculoma has been limited with conflicting evidence. Stroke may be present in 15-57% patients with central nervous system TB and has been associated with poor outcome.

A fragment based approach to develop inhibitors of HSAD- an enzyme encoded in a cholesterol catabolism operon in mycobacterium tuberculosis
Dr Elena Polycarpou, Faculty of Science, Engineering and Computing Kingston University London, UK
We have targeted enzymes essential for Mycobacterium tuberculosis survival inside cells. Cholesterol is an intracellular fuel for M. tuberculosis and HsaD is an essential hydrolase cleaving a C-C bond in cholesterol. A fragment based approach identified inhibitors of HsaD. The most effective fragments were co-crystallised with HsaD and fell into two groups binding to overlapping areas of the active site. Structure Activity analyses of a sub-library provided proof of principle that the inhibition of growth of M. tuberculosis on cholesterol as a carbon source, mirrors the enzymatic inhibitory potency, apart from one compound which does not penetrate the mycobacterial cells.

Vaccination of badgers against bovine tuberculosis
Dr Sandrine Lesellier, PhD, MRCVS BAC4, Animal and Plant Health Agency (APHA), New Haw, UK

A fragment based approach identified inhibitors of HsaD. The most effective fragments were co-crystallised with HsaD and fell into two groups binding to overlapping areas of the active site. Structure Activity analyses of a sub-library provided proof of principle that the inhibition of growth of M. tuberculosis on cholesterol as a carbon source, mirrors the enzymatic inhibitory potency, apart from one compound which does not penetrate the mycobacterial cells.

Invited Speakeys Abstracts

Does HIV modify the association between hyperglycaemia and active tuberculosis?
Dr Sarah Lou Bailey, London School of Hygiene and Tropical Medicine, UK
HIV and diabetes mellitus are independently associated with an increased incidence of active tuberculosis (TB). However, the dual effect of HIV and diabetes, or hyperglycaemia, on the risk of developing TB disease is unclear. This case-control study among adults in Lusaka, Zambia aims to determine if HIV modifies the association between hyperglycaemia and active TB. The current analysis suggests that the effect of hyperglycaemia on TB may be greater for individuals with HIV than for those without, but overall there is no evidence that hyperglycaemia is associated with active TB in this population.

The control of Mycobacterium bovis (M. bovis) infection in cattle herds (bovine TB) is one of the current priorities for the government of England and Wales. More than 5% of British herds currently have their TB free status withdrawn and the infection is spreading geographically, costing £90 million/year in testing, compensation, and on diagnosis and vaccine R&D. European badgers (Meles meles) can be chronic carriers and excretors of the bacteria and their role in the transmission of the disease to cattle has been demonstrated, although the exact route of transmission is not fully understood. Vaccination of badgers with injectable BadgerBCG (licensed in the UK in 2010) can significantly reduce the development of visible lesions and excretion...
Background: HIV and diabetes mellitus (DM) are independently associated with an increased incidence of active tuberculosis (TB). However, the dual effect of HIV and DM on the risk of developing TB disease is unclear. This study aims to determine if HIV modifies the association between DM and active TB.

Methods: This is an ongoing unmatched case-control study among adults in Lusaka, Zambia, with diagnosed active TB disease as the outcome and DM as the exposure of interest. Cases with TB were recruited from TB clinics in Lusaka, data for the community controls were taken from a recently conducted population-based cross-sectional study (the ZAMSTAR prevalence survey). Control participants were randomly sampled from within each community using a 2-stage cluster sampling technique. DM is defined as a capillary random blood glucose concentration of ≥11.1mmol/L. HIV status is determined by serological result. Logistic regression was used to explore HIV as a potential effect modifier, adjusting for age, sex, education, body mass index, smoking history and community.

Results: To date 2,375 active TB cases and 6,977 non-TB controls have been recruited. The unadjusted prevalence of DM among cases was 1.7% and among controls was 2.3%. 66% of cases were living with HIV, compared to 18% of controls. The adjusted odds of TB was 9.47 (95% CI 8.05-11.14, p<0.01) times higher in those with DM compared to those without. Among individuals with HIV, the adjusted odds of TB was 4.60 (95% CI 0.97-21.89, p=0.06) times higher in those with DM compared to those without. Among individuals without HIV, the adjusted odds of TB was 1.09 (95% CI 0.52-2.31, p=0.82) times higher in those with DM compared to those without (p-value for interaction = 0.088).


**VPM1002 a new prime vaccine on the horizon**
Dr Leander Grode, *Vakzine Projekt Management GmbH*, Hannover, Germany

VPM1002 is a live vaccine against tuberculosis (TB). As BCG is not sufficiently effective to stop the spread of TB, modifications have been implemented in VPM1002 to improve its immunogenicity. Two Phase I studies and one Phase II using flow cytometry characterized the quality of T cell response following immunization with VPM1002 or BCG. We completed a Phase II clinical trial in neonates in South Africa. Safety and tolerability results from all clinical trials showed no serious adverse reactions after VPM1002 vaccination. VPM1002 induces multifunctional T cell subsets which are thought to play an important role in protection against tuberculosis.

**Oral Presentation Abstracts**

**DOES HIV MODIFY THE ASSOCIATION BETWEEN DIABETES MELLITUS AND ACTIVE TUBERCULOSIS?**
Authors: SL Bailey1,2, S Floyd3, JS Yudkin1, P Godfrey-Faussett2, H Ayles1,2

Affiliation: 1LSHTM TB Centre and Department of Clinical Research, London School of Hygiene and Tropical Medicine, Keppel Street, London, WC1E 7HT, UK; 2Zambart Project, Lusaka, Zambia; 3Division of Medicine, University College London, London, UK.

Presenting and corresponding author email address: slbailey@doctors.org.uk

Background: HIV and diabetes mellitus (DM) are independently associated with an increased incidence of active tuberculosis (TB). However, the dual effect of HIV and DM on the risk of developing TB disease is unclear. This study aims to determine if HIV modifies the association between DM and active TB.

Methods: This is an ongoing unmatched case-control study among adults in Lusaka, Zambia, with diagnosed active TB disease as the outcome and DM as the exposure of interest. Cases with TB were recruited from TB clinics in Lusaka, data for the community controls were taken from a recently conducted population-based cross-sectional study (the ZAMSTAR prevalence survey). Control participants were randomly sampled from within each community using a 2-stage cluster sampling technique. DM is defined as a capillary random blood glucose concentration of ≥11.1mmol/L. HIV status is determined by serological result. Logistic regression was used to explore HIV as a potential effect modifier, adjusting for age, sex, education, body mass index, smoking history and community.

Results: To date 2,375 active TB cases and 6,977 non-TB controls have been recruited. The unadjusted prevalence of DM among cases was 1.7% and among controls was 2.3%. 66% of cases were living with HIV, compared to 18% of controls. The adjusted odds of TB was 9.47 (95% CI 8.05-11.14, p<0.01) times higher in individuals living with HIV compared to HIV negative individuals. Overall, the adjusted odds of TB was 1.47 (95% CI 0.78-2.78, p=0.24) times higher in individuals with DM compared to those without. Among individuals with HIV, the adjusted odds of TB was 4.60 (95% CI 0.97-21.89, p=0.06) times higher in those with DM compared to those without. Among individuals without HIV, the adjusted odds of TB was 1.09 (95% CI 0.52-2.31, p=0.82) times higher in those with DM compared to those without (p-value for interaction = 0.088).
Conclusions: As expected, HIV is a strong risk factor for TB in this population. The current analysis suggests that the effect of DM on TB may be greater for individuals with HIV than for those without, but overall there is no evidence that DM is associated with active TB in this population. Confidence intervals are wide and the study is ongoing so findings will become more definitive later.

A FRAGMENT BASED APPROACH TO DEVELOP INHIBITORS OF HSAD- AN ENZYME ENCODED IN A CHOLESTEROL CATABOLISM OPERON IN MYCOBACTERIUM TUBERCULOSIS

E. Polycarpou¹, N. Lack, D. Evangelopoulos, S. Keany, A. Ciulli, S. Bhakta, T.D McHugh, C. Sieg, O. Eleftheriadou, A. Halman, A. Sinclair, E. Lowe, A. Ryan¹ and E. Sim¹,²

¹ Faculty of Science, Engineering and Computing, Kingston University, Kingston upon Thames, UK
² Department of Pharmacology, University of Oxford, Mansfield Road, Oxford, UK

Background

Exploiting pathways essential for survival of Mycobacterium tuberculosis inside host immune cells has been exploited as a means to identify targets for persistent tuberculosis (TB). Cholesterol is an intracellular fuel for M. tuberculosis and HsaD is a hydrolase responsible for catalysing the cleavage of C-C bonds.¹ The hsad gene has been demonstrated to be part of an operon, the gene products of which catalyse the catabolism of cholesterol. Deletion mutants of genes in the operon affect the ability of M. tuberculosis to grow with cholesterol as the carbon source. The mutants also affect the ability of the organism to survive within macrophages as well as in a guinea pig model of TB. The protein can be expressed in high yields as a recombinant protein and crystallises readily. The active site bears a very wide cleft with a volume of approximately 2100 Å³.¹ An initial study has investigated the effect of mechanism-based inhibitors on the activity of HsaD.²

Methods

We have exploited the large cavity of the enzyme for a fragment based approach to identify inhibitors by the use of differential scanning fluorimetry and NMR as initial screens on a library of 1258 fragments. The seven “hits” obtained, were tested for inhibition of enzymatic activity and from these fragments, two (2 and 6) had IC₅₀ values below 600 µM. Commercially available analogues were subsequently screened for their inhibitory effect. Of these, the three most effective fragments (2, 27 and 32) were co-crystallised with HsaD and the structures of their complexes determined. We made a chemical sub-library and tested for inhibitory activity and also their antibacterial activity against the M. tuberculosis grown on cholesterol as the sole carbon source.

Results and Discussion

The classes of fragments which we identified fell into two groups binding to distinct yet, overlapping areas of the active site of HsaD. One group of fragments which are derivatives of a sulphonamide scaffold, were effective inhibitors and could be readily synthesised to provide more than 50 distinct entities in a series. Of these compounds, we were able to carry out SAR analysis to demonstrate the key features in improving inhibitory potency of enzymatic activity with these small molecules inhibiting at 1 mM. We have also been able to demonstrate that, the growth inhibition of M. tuberculosis on cholesterol as a carbon source, mirrors the enzymatic inhibitory potency, apart from one compound which does not penetrate the mycobacterial cells. We are currently continuing to improve the inhibitory potential of these molecules using the co-crystal structure to guide the synthetic chemistry with the intention to build upon the scaffold which has been demonstrated to be the most potent so far.


Poster Presentation Abstracts

Poster abstracts will be finalised weeks before the event