Mycobacterium Tuberculosis Infection Treatment

Wednesday, 26 March 2014 09:00 - 17:00
Cineworld: The O2, London, SE10 0DX, UK

This event will look at discovering and producing new antibiotics to combat tuberculosis. The conference will discuss target and compound discovery, new potential anti-TB compounds in development, clinical trial design and early trial results. We invite researchers interested in all aspects of drug development and welcome participants from academia, industry, government and non-governmental organizations. We also welcome abstracts for oral and poster presentation. This event has CPD accreditation and is part of the **2014 TB Summit** - www.TBSummit2014.com

Meeting Chair: *Dr. Sanjib Bhakta,* Director of ISMB-Mycobacteria Research Laboratory and University Senior Lecturer, Institute of
Structural and Molecular Biology, Birkbeck, University of London and UCL, UK

The deadline for abstract submissions for oral presentation has now passed. Abstracts for *poster presentation only* can be submitted up to two weeks before the event. You can download the instructions for authors at **www.euroscicon.com/AbstractsForOralAndPosterPresentation.pdf**

Talk times include 5 – 10 minutes for questions

9:30 – 10:15 **Registration**

10:15 - 10:30 **Introduction by the Chair:** *Dr. Sanjib Bhakta,* Director of ISMB-Mycobacteria Research Laboratory and University Senior Lecturer, Institute of Structural and Molecular Biology, Birkbeck, University of London and UCL, UK

10:30 – 11:00 **A study in persistence: overcoming the barriers to shorter treatment for pulmonary tuberculosis**
Dr Derek Sloan, Senior Clinical Academic in Respiratory Medicine, Liverpool School of Tropical Medicine, Liverpool Heart and Chest Hospital, UK
New treatments to cure drug-susceptible pulmonary tuberculosis in less than 6 months are urgently required. Although novel regimens are being assessed in clinical trials, reliable surrogate biomarkers are needed to predict the eventual outcome of therapy from studies of 2 months duration. Pharmacokinetic-pharmacodynamic (PK-PD) modelling of the early bacillary elimination rate may help generate these biomarkers and accelerate drug development. Additionally, fluorescence microscopy can identify sub-populations of M tuberculosis organisms with heterogeneous lipid metabolism; possibly identifying “persister” cells which are difficult to kill and may be associated with treatment failure or relapse.

11:00 – 11:30 **Mycobacterium tuberculosis drug discovery using new targets essential for survival inside macrophages**
Professor Edith Sim, Professor Emeritus of Pharmacology, University of Oxford, UK

The need for new treatment for tuberculosis is evident from the increase in multi drug resistant TB which is compounded by the long duration of existing drug treatments. The need for a pipeline ofpotential new drugs is related to minimising the subsequent development of strains which are resistant in the future. The difficulty in treating tuberculosis apart from the soioeconomic factrs is the life style of the organism which can survive inside cells. Therefore targetting pathways which are essential for intracellular survival is an important strategy. The work presented in this talk will focus on a group of proteins encoded by a gene cluster which is essential for survival inside macrophage and where gene deletion and chemical inhibition have been demonstrated to have similar effects on the survival of mycobacteria.

11:30 – 12:00 **Speakers' photo then mid-morning break and poster exhibition and trade show**
Please try to visit all the exhibition stands during your day at this event. Not only do our sponsors enable Euroscicon to keep the registration fees competitive, but they are also here specifically to talk to you.

12:00 – 12:30 **How to improve the results of the therapy for urogenital tuberculosis**
Professor Ekaterina Kulchavenya, Principal researcher, Head of Urogenital Dpt, Novosibirsk Research TB Institute, Medical University, The Russian Federation
Urogenital Tuberculosis (UGTB) is complicated by bladder tuberculosis (TB) in more than half of cases; late diagnosis and/or absence of pathogenetic therapy leads to the development of shrinked bladder up to full its obliteration. Standard therapy presented poor results: in 57.9% developed complications: posttuberculous cystalgia (36.8%) and microcystis (21.1%). Modified etiopathogenetic therapy, included trospium chloride, increases frequency of recurrence twice and allows avoiding of developing of microcystis at all.

13:30 – 14:00 **Plant-Derived Compounds a Source of Anti-*Mycobacterium Tuberculosis* Agents**
Dr Maria del Rayo Camacho-Corona, Universidad Autónoma de Nuevo León, Mexico

12:30 – 13:30 **Lunch, poster exhibition and trade show**
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14: 00 – 14:30 **Discussion Session**
This discussion session is an informal question and answer session. This is an ideal opportunity to get advice and opinion from experts in this area. This session is not for questions about specific talks, which can be asked after the speakers session, but for discussing either general topics or specific issues. There are three ways you can ask questions:

1. Before the session you can *submit your question to Euroscicon staff* at the registration desk,
2. Before and during the session you can *submit a question or comments, by email*, which will be provided on the day of the event
3. During the session you can *put your hand up* and join in

14:30 – 15:00 **Clinico-radiological response of TB abscesses in children treated with Thalidomide**
Dr Ronald van Toorn, Consultant, Stellenbosch University, South Africa

Tuberculosis abscesses are known to develop or enlarge despite appropriate anti-TB treatment. This phenomenon, the result of immune reconstitution inflammatory syndrome (IRIS), is often more severe in the setting of HIV co-infection and may be life threatening. TB abscesses are notoriously resistant to therapy and require total surgical excision for cure. In our experience, TB abscesses often respond to thalidomide, a potent tumour necrosis alpha-inhibitor. Aim: To describe the clinico-radiological response of TB abscesses in 16 consecutive children treated with thalidomide.

15:00 – 15:30 **Afternoon Tea, last poster session and trade show**

15:30 – 16:00 **The Management of Tuberculosis in children by paediatricians in the private sector in Mumbai, India**

Dr. Carolyn Tauro, Research Head, Co-founder Stop TB India., Tata Institute of Social Sciences, India

Among 1.3 million new tuberculosis (TB) cases in India seven percent were children, many of whom seek care outside of the national TB programme. The wide variety of private providers and their practices poses a problem in TB control. This descriptive study involved sixty-four private paediatricians in Mumbai, who prescribe a variety of investigations and drugs in the management of TB and MDRTB drugs. Challenges included issues with cost, compliance, regimens, unpalatable medicines and child unfriendly drug formulations. Increased public-private partnerships, monitored implementation of standard guidelines and practical solutions to tackle these challenges in India need to be sought.

16:00 – 16:30 **Tuberculosis Therapy under anti-TNF agents**

Dr Tomoshige Matsumoto, Director of Department of Clinical Laboratory Medicine, Osaka Anti-Tuberculosis Association Osaka Hospital, Japan

Although several TNF inhibitors are used for the treatment of moderate-to-severe active rheumatoid arthritis (RA), a substantial number of serious adverse events occur including reactivation of latent tuberculosis (TB) infection. Currently, the American College of Rheumatology recommends that treatment with biologics can be resumed after completion of the anti-TB treatment, while the British Society of Rheumatology suggests that patients on anti-TNF therapy should receive full anti-tuberculosis chemotherapy, but may continue with anti-TNF therapy if clinically indicated. This discrepancy is due to the limited availability of evidence. We previously reported one successful case in which administration of anti-TB medications followed by re-treatment with infliximab could control RA disease activity without exacerbation of TB. Subsequent studies have also reported favorable outcomes for RA patients with TB treated with re-administration of anti-TNF biologics. We will show our experiences concerning anti-TNF inhibitors and TB therapy, including how to prevent paradoxical response from developing.

16:30 - 16>35 **Chairman's summing up and Close of Meeting**

Registration Website: http://www.regonline.co.uk/TBTreat2014

Meeting reports from this event will be published by Virulence and by HONNAO publishing

Keywords: TB, latent, infection, diagnosis, IGRA,Latent TB; M/XDRTB; genome; proteome;

immunome, cell wall, arabinogalactan, drug discovery, benzothiazinone, tuberculosis; treatment; biomarkers; antibiotics, mutation, rpoB, gyrA, gyrB, katG, inhA, Rifampicin resistance, Fluoroquinolone resistance, Isoniazid resistance, Mycobacterium tuberculosis, Thailand, Mangosteen extract, Antimycobacterial activity, air filter, pre-filter, tuberculosis, bladder, complication, urogenital, therapy, TB abscess Thalidomide MRI, TB meningitis; high-dose rifampicin; aspirin; moxifloxacin; eicosanoids, persistence; biomarkers; pharmacokinetics; pharmacodynamics; lipid bodies, anti-TNF agents, biologics, tuberculosis treatment,Pulmonary Tuberculosis, MDRTB, children, private practitioner, India

About the Chair

Sanjib Bhakta is the Director of ISMB-Mycobacteria Research Laboratory at the Institute of Structural and Molecular Biology, Birkbeck, University of London & UCL. His continued research interest (funded by UK-Medical Research Council, Wellcome Trust and EU Research Fund) is focused on developing novel therapeutics to tackle persistence and drug resistance in tuberculosis (XDR-TB, a global health emergency). He has published original research articles for a number of internationally acclaimed journals. Following a BSc (Hons), an MSc and a PhD in Molecular Microbiology & Biochemistry from world class universities and research Institutions in India, Dr Bhakta joined the Oxford University Department of Pharmacology in 2001 as an ISIS innovation Senior Research Scholar and shortly after he was awarded with a Wellcome Trust International Travelling Fellowship. He graduated from The Queen's College, University of Oxford in 2005 completing a second doctoral degree (DPhil) and received a "Sir William Paton Prize" for the best graduate research presentation in Pharmacology. In 2006 he attained his first academic appointment at Birkbeck, University of London as a University Lecturer to lead his TB drug discovery research and research led teaching on antimicrobials. He became a Fellow of the Higher Education Academy, UK after achieving a post graduate certificate in Teaching and Life Long Learning in Higher Education (PGCHE) from the University of London in 2008. He is a core member of Tuberculosis Drug Discovery-UK, the Institute of Structural and Molecular Biology, NIMR/Birkbeck/UCL and an affiliated academic Fellow of the Centre for Infection, Immunity and Disease mechanism, Brunel University. He is a member of a number of international societies and Editorial Board Member of peer reviewed international journals. Sanjib held Visiting Faculty posts at several recognized universities and research institutes in India and actively engaged as an Honorary Consultant for National TB Research at the Indian Development Foundation. He is a STEM-Ambassador on a UK research council funded project and a "Scientist in Residence" at the St Mary's School in Buckinghamshire. He was elected as a Fellow of the Royal Society of Medicine in 2008 and recognised as a Chartered Microbiologist in the UK. He hosted World TB Day events at University of London since 2010 and chaired the EuroSciCon conference "Can we beat…Mycobacterium tuberculosis" in 2013.

About the Speakers

Derek Sloan has worked in high and low income countries, accruing extensive experience in the management of tuberculosis and HIV. After spending time in Kenya, South Africa and Malawi he currently lives in Liverpool. He continues to engage in clinical and academic activities with a global perspective and his research has been funded by the Wellcome Trust. His work focusses on the clinical pharmacology of anti-TB drugs, the metabolic behaviour of TB bacilli under drug pressure and PK-PD modeling of patient responses to anti-TB chemotherapy.

Edith Sim studied Biochemistry in Edinburgh and obtained her doctorate in Oxford. She worked in Grenoble on membrane proteins in bacteria as a Royal Society Research Fellow before returning to Oxford as a junior lecturer where she ran a reserach group mainly funded by the Wellcome Trust. She became Head of the Department of Pharmacology has also served as the Director of the Medical Sciences Division Training Group including post-graduate training programmes. She has published extensively on subjects at the interface between chemistry and protein science in the field of tuberculosis. She has supervised many graduate students and one of her students, Anna Upton, is now Director of Reserach at the TB Global Alliance. Edith Sim is a member of the ACid Fast Club and is currently the Dean of the Faculty of Science Engineering and Computing at Kingston University, Kingston-on-Thames where her reserach team are based. She is a Reserach Fellow at St Peter's College Oxford and an honorary member of the Pharmacology Department, Oxford University.

Ekaterina Kulchavenya has completed her PhD at the age of 28 years from Moscow Research TB Institute; from 2002 she is professor. Now she is a principal researcher of Novosibirsk Research TB Institute and professor of Novosibirsk Medical University. She has published more than 55 papers in reputed journals and 8 monographs and she is an author of chapters on urogenital TB in Russian National Guideline on Urology and in International Consultation on Urological Diseases.She is an author of many new patented techniques, allowed significantly improves the therapy for extrapulmonary tuberculosis as well as prostate and bladder diseases.

Tomoshige Matsumoto is a physician, and the Director of the Department of Clinical Diagnositc Laboratory at Osaka Anti-Tuberculosis Association Osaka Hospital, Japan and co-editor in chief of the Journal of Infectious Diseases and Therapeutics. He is as well Advisor for molecular epidemiology of tuberculosis and especially tuberculosis infection under biologics therapy. His education and career have been made in Osaka, Japan. He at first engaged a molecular cloning and characterization of SOCS-1/SSI-1/CIS, negative feed back regulator of cytokin signaling in cells, under the excellent teaching of Dr. Tetsuji Naka and Dr. Tadamitsu Kishimoto. They proposed the existance of negative feed back regulation system in cells (nature) and characterized the SOCS-1 (pnas). Then, his career has been a clinical application of cytokine signaling for various diseases. He has engaged in developing anti-IL6 receptor therapy, biologic therapy, for castleman disease(blood) and rheumatoid arthritis, which is now available as actemura(R). Then his career has been shifted to the infection therapies especially tuberculosis under the biologics. He has firstly showed a successful anti-TNF treatment for rheumatoid arthritis in a patient with tuberculosis(NEJM). He experienced more than 20 anti-TNF therapies in patients with tuberculosis.

Ronald van Toorn is a South African pediatric neurologist wiith an interest in neuroinfectious diseases, especially TB meningitis. The title of his PHD (near completion) is "Childhood tuberculosis meningitis: challenging current management strategies".

Reinout van Crevel, a Dutch infectious disease specialist, has led a patient-oriented research program on TB in Indonesia, integrating basic sciences, clinical research and public health for more than 10 years. He also set up a program on prevention and care of HIV in the context of injecting drug use in Indonesia. These collaborative research acitivities have a strong aspect of academic capacity building and improving patient services. One of his main topics of interests is TB meningitis

and pharmacokinetic aspects of TB treatment.

Carolyn Kavita Tauro is the research head and co-founder at Stop TB India (www.stoptbindia.org), a non-governmental organization, which works towards research and advocating in the field of TB. She is pursuing Master of Public Health in Social Epidemiology, at Tata Institute of Social Science in Mumbai, India. Her past work experience includes field medical work with Medecines sans Frontieres (MSF) in conflict areas, managing basic health care, Maternal and Child Health programs and tuberculosis. Later, she was part of the MSF national coordination team involved in projects of Mental Health, Tuberculosis, HIV/AIDS, Maternal and Child health and Malnutrition.

POSTER PRESENTATIONS

PLANT-DERIVED COMPOUNDS A SOURCE OF ANTI-*MYCOBACTERIUM TUBERCULOSIS* AGENTS

M. R. Camacho-Corona, E. Garza-González, J.M.J. Favela-Hernández, P.C. Esquivel-Ferriño, N.E. *Sandoval-Montemayor, A.F.* Clemente-Soto, A. García, I. Balderas-Rentería, V. M. Rivas-Galindo, L. Alvarez, M. Y. Ríos

Facultad de Ciencias Químicas, Universidad Autónoma de Nuevo León. Av. Universidad s/n, Ciudad Universitaria, San Nicolás de los Garza, Nuevo León CP 66451. México. Email: maria.camachocn@uanl.edu.mx

Plants have been the source of new drugs since ancient times. Our research group select nine plants used in Mexican traditional medicine to treat tuberculosis and other respiratory diseases. Hexane, chloroform, methanol, and aqueous extracts were prepared from each plant. All extracts were tested against sensitive and resistant strains of *M. tuberculosis* H37Rv. Results showed that hexane extracts of *Citrus aurantifolia*, *Citrus sinensis*, *Foeniculum vulgare* as well as the chloroform extracts of *Nasturtium officinale* and *Larrea tridentata* were the most active extracts. The compounds responsible of the biological activity of above extract were isolated using different chromatographic methods and their chemical structures were determined by spectroscopic techniques. The active compounds from *C. aurantifolia* were 5,8-dimethoxypsoralen, 5-geranyloxypsoralen, palmitic acid, linoleic acid, oleic acid, 4-hexen-3-one, and citral. The active compounds from *C. sinensis* were decanal, caryophyllene oxide, and palmitic acid. The active compounds from *F. vulgare* were linoleic acid, oleic acid, 1,3-benzenediol, undecanal, and 2,4-undecadienal. The active compounds from *L. tridentata* were meso-dihydroguaiaretic acid, 4-epi-larreatricin, 3′-Demethoxy-6-O-demethylisoguaiacin, 5,4′-Dihydroxy-3,7,8,3′-tetramethoxyflavone (4) and 5,4′-dihydroxy-3,7,8-trimethoxyflavone. The above results show that plant-derived compounds are a source of anti-*Mycobacterium tuberculosis* agents. In addition, the mode of action of two active compounds were determined.

THE MANAGEMENT OF TUBERCULOSIS IN CHILDREN BY PAEDIATRICIANS IN THE PRIVATE SECTOR, IN MUMBAI, INDIA

C.K. Tauro, N. Gawde.

Tata Institute of Social Sciences, Mumbai, India *ck.tauro@gmail.com*
Background: Of 8.6 million cases of Tuberculosis (TB) incidence, about 0.5 million were children, according to the Global Tuberculosis Report 2013. The incidence for TB in India, in children was about 7 percent of the reported 1.3 million new TB cases annually. This number is said to be an underestimate of the actual number of those under the age of 15 years having TB. Many TB patients, often those from the poor sector seek and receive care from a large variety of providers outside of the national TB programme. This management if often expensive and of varying quality. Health care providers involved in the provision of Revised National Tuberculosis Control Program amount to more than 3000 Non-governmental organisations, 30,000 private practitioners and 150 corporate health facilities all over India. **Objectives:** To study the management of TB in children in the private sector in the city of Mumbai in India, including the diagnosis, treatment and referral system. **Methods:** This cross sectional study involved 64 private paediatricians from various private health care centers in Mumbai, and data was collected using structured questionnaires that were filled on paper or online, according to the convenience of the doctor. Snow- ball sampling was done, with data entered and analysed. **Results:** A total of 28 different combinations of investigations were advised by the paediatricians, when suspecting TB in a child. Six different combinations of first-line drugs by 96.9% doctors, 13 combinations of second-line drugs by 35.9% doctors, and five combinations of third-line drugs were used to treat TB by 12.5% doctors, while 12 different combinations by 23.4% doctors were used to treat MDRTB. The rest of the participants either referred the case to a specialist or did not choose to answer the question. 68.8% of the doctors knew that TB was a notifiable disease, 17.2% stated that it wasn't and 10.9% were unaware of the status. Apart from finance and compliance issues, difficulties with drug combinations, and unpalatable and drug formulations that were difficult to consume were appeared as challenges faced in the management. **Conclusion:** There is need for increased collaboration of the public and private health sector. Despite the existence of national TB guidelines varied and irrational practices continue. There is need of formulating and implementing separate guidelines for private sector which should also address challenges faced by paediatricians to prevent, detect and treat of TB in children in a high burden country like India.

EVALUATION OF ANTIMYCOBACTERIAL PROPERTIES OF PHYTOCHEMICALS FROM THE PLANT FAMILY ALLIACEAE.

C. Amaning Danquah[1,2], P.N. Mortazavi[2], S. Bhakta[2*] and S. Gibbons[1]

[1]Department of Pharmaceutical and Biological Chemistry, UCL School of Pharmacy, 29-39 Brunswick Square, London WC1N 1AX, UK, [2]Mycobacteria Research Laboratory, Department of Biological Sciences, Institute of Structural and Molecular Biology, Birkbeck, University of London, Malet Street, London WC1E 7HX, UK. [*]Corresponding author: Dr Sanjib Bhakta, s.bhakta@bbk.ac.uk.

Tuberculosis (TB) is confirmed to be a global health emergency as a result of emergence of multi- and extensively-drug-resistant strains of *Mycobacterium tuberculosis* (WHO, 2013). There is a pressing need for lead molecules with novel mechanisms of action to fuel the current anti-TB drug discovery pipeline. This has led to a renewed research interest in natural products, which offer an outstanding source of diverse bioactive chemical scaffolds with the hope of discovering novel anti-mycobacterial compounds. Plants belonging to the family Alliaceae are well-known worldwide for their medicinal properties. Allicin isolated from *Allium sativum* and pyridine-*N*-oxide alkaloids from

Allium stipitatum have been documented to have antimycobacterial activity (O'Donnell *et al*, 2009). This research seeks to use a bioassay-guided approach to isolate and characterize bioactive compounds from different species belonging to the family Alliaceae and to elucidate their structures and their mechanisms of antimycobacterial activity. A whole-cell phenotypic bioassay, which interrogates all of the drug targets simultaneously in a specific physiological context, was used. This is a high throughput agar-based spot culture growth inhibition assay (HT-SPOTi), which allows a rapid but gold-standard determination of minimum inhibitory concentrations (Guzman *et al*., 2013). The results indicated that the family Alliaceae is a promising source of new phytochemicals and antimycobacterial compounds and further studies are ongoing to isolate pure bioactive compounds.
References: 1. WHO (2013) *Global tuberculosis control:WHO annual report*, London: Global Tuberculosis Programme, World Health Organization. 2. Guzman, J. D., Evangelopoulos, D., Gupta, A., Birchall, K., Mwaigwisya, S., Saxty, B., Mchugh, T. D., Gibbons, S., Malkinson, J. & Bhakta, S. (2013). Antitubercular specific activity of ibuprofen and the other 2-arylpropanoic acids using the HT-SPOTi whole-cell phenotypic assay. *British Medical Journal Open,* 3.3. O'Donnell, G., Poeschl, R., Zimhony, O., Gunaratnam, M., Moreira, J. B., Neidle, S., Evangelopoulos, D., Bhakta, S., Malkinson, J. P., Boshoff, H. I., Lenaerts, A. & Gibbons, S. (2009). Bioactive pyridine-N-oxide disulfides from *Allium stipitatum*. *Journal of Natural Products,* 72**,** 360-5

NSAIDs TO TACKLE DRUG RESISTANCE IN MYCOBACTERIUM TUBERCULOSIS - FROM PAIN RELIEF TO TB RELIEF?

A Maitra[1], D Evangelopoulos[1,2], P N Mortazavi[1], A Kovalev[1], J D Guzman[1], N Keep[1],T D McHugh[2], and S Bhakta[1]*

[1]Mycobacteria Research Laboratory, Institute of Structural and Molecular Biology, Department of Biological Sciences, Birkbeck, University of London, Malet Street, London WC1E 7HX. [2]Centre for Clinical Microbiology, Division of Infection and immunity, Royal Free Hospital, Pond St, London NW3 2QG

**Corresponding author: Email: s.bhakta@bbk.ac.uk; sanjib.bhakta@ucl.ac.uk; Tel: +44 (0)20 7631 6355; Fax: +44 (0)20 7631 6246*

Drug resistance in the tuberculosis (TB) causing pathogen, *Mycobacterium tuberculosis*, was observed in 1940s soon after the streptomycin was used as a monotherapy to treat the disease. As of now, there is at least one reported case of an extensively drug-resistant (XDR) strain of the bacterium in 84 countries.[1] A concerted effort to fight TB and develop novel therapeutic regimens to control and reverse the emergence of XDR-TB cases worldwide is on-going. Certain common non-steroidal anti-inflammatory drugs (NSAIDs) have proven to be selectively bactericidal against replicating, non-replicating and multi-drug-resistant clinical isolates of *M. tuberculosis*.[2,3] Our primary focus is to repurpose ibuprofen and carprofen and investigate their novel mechanisms of action in *M. tuberculosis* to help design more potent inhibitors in the future. To this effect we have followed both target-based and whole-cell approaches. We have identified a possible target of the drugs to be translation initiation factor-2 (IF-2). In order to characterise the structure, function and regulation of translational initiation in mycobacteria, we have cloned, heterologously expressed and purified all three translational initiation factors (IF1, 2 and 3) from *M. tuberculosis*. In addition, we have carried out whole cell transcriptomic analyses to assess the effects of the drugs first on a selected set of genes involved in key metabolic pathways and indicated to play essential roles in antimicrobial drug resistance. This revealed the action of NSAIDs on proteins involved in cell wall homeostasis and dormancy mechanisms. Furthermore we have developed an assay to detect efflux modulatory activities in mycobacteria and assayed the synergistic property showed by ibuprofen and carprofen

when used in combination with front-line TB drugs. Finally, trials to generate spontaneous mutants to the drugs are underway to investigate novel mechanisms of action of this group of over-the-counter drugs against *M. tuberculosis*. *REFERENCES:* 1. World Health Organisation. Global tuberculosis report 2013. London (2013).
2. Gold B, Pingle M, et al. (2012) Nonsteroidal anti-inflammatory drug sensitizes Mycobacterium tuberculosis to endogenous and exogenous antimicrobials. PNAS; 109: 16004-11. 3. Guzman JD, Evangelopoulos D, Gupta A, Birchall K, Mwaigwisya S, Saxty B, McHugh T, Gibbons S, Malkinson J, Bhakta S, (2013). Anti-tubercular specific activity of ibuprofen and other 2-arylpropanoic acids using the HT-SPOTi whole-cell phenotypic assay. BMJ Open (3):e002672.

INHIBITION OF FLAVIN DEPENDENT ENZYMES IN DORMANCY

Doris Quay[1], James Torpey[2], Francois-Xavier Chauviac[1], Neil Stoker[3,], Sanjib Bhakta[1], Ambrose Cole[1] and Nicholas Keep[1]
[1]*Department of Biological Sciences, Institute for Structural and Molecular Biology, Birkbeck, University of London, Malet Street, London WC1E 7HX*
[2]*Structural & Molecular Biology, Darwin Building, The Institute for Structural and Molecular Biology , University College London, Gower Street, London WC1E 6BT*
[3]*Department of Pathology and Pathogen Biology, Royal Veterinary College, Royal College Street, London NW1 0TU*

Bacterial dormancy is one of the biggest challenges facing by the world to combat with the recurrence of diseases particularly tuberculosis (TB). *Mycobacterium tuberculosis* dormancy is controlled by DosRS 2CR which upregulates around 50 genes in the infected macrophages. Among them is the *acg* gene, co-regulated with one of the most highly induced protein, Acr1 in dormant cells during hypoxic condition. Acg has been shown to be an unusual FMN-binding protein and indispensable for growth and virulence. Acg does not show the predicted nitroreductase activity; instead it sequesters FMN and so inhibiting flavin dependent enzymes on entry to dormancy. Hence, in this work, we characterised the structure and function of a classical nitroreductase in *M. tuberculosis*. The gene (Rv3368) was cloned and the protein was expressed, purified and crystallised. The yellow crystal was diffracted by X-ray to 1.9Å resolution and the structure was solved by molecular replacement. Rv3368 displays an αβα sandwich fold homodimer, bound with two FMN cofactor molecules, each in the hydrophobic and positively charged pocket. Functional assay shows that Rv3368 is an oxygen-insensitive nitroreductase and the NADPH-reduced enzyme was most active towards nitrofurantoin prodrug. Finally, we were able to show that Acg could inhibit the activity of Rv3368, probably by sequestering FMN out of the protein due to somewhat weaker flavin binding of Rv3368.

This meeting was organised by Euroscicon (www.euroscicon.com), a team of dedicated professionals working for the continuous improvement of technical knowledge transfer to all scientists. Euroscicon believe that they can make a positive difference to the quality of science by providing cutting edge information on new technological advancements to the scientific community. This is provided via our exceptional services to individual scientists, research institutions and industry.

ENDOSCOPY, BACTERIOLOGY OR HISTOLOGY – HOW TO CONFIRM BLADDER TUBERCULOSIS?

Denis Kholtobin, Ekaterina Kulchavenya, Novosibirsk, Russian Federation

Introduction. Bladder tuberculosis (BTB) complicates kidney TB in45.8 – 84.8% and is one of the most severe complications, and leads to the shrinking of bladder and development of terminal renal failure. According to the classification BTB is divided on 4 grades, as well as iatrogenic bladder TB as a complication of the BCG therapy for bladder cancer.

Material and methods. To estimate the value of endoscopy, bacteriology and histology for diagnosis BTB we analyzed 190 patients suspicious on BTB. All underwent X-ray examination, including intravenous urography and multispiral computer tomography, cystoscopy (excluding patients with cystostoma due to extremely low bladder volume), bladder biopsy. Patients with BTB 4 grade underwent cystectomy with iliocystoplastic, bladder tissue was also investigated. Bacteriology included luminescent microscopy of the sediment of urine, microscopy of smear colored by Zhiel-Nelsen technique, culture on 3 mediums, PCR-diagnostic, Bactec and GenExpert.

Results. Among all 190 patients in 18 bladder tuberculosis (BTB) was confirmed, and in 172 – non-specific cystitis (NSC). Decreased volume was revealed in all BTB patients and in 15.7% of NSC patients ($p<0.001$). Trabecules (66.7%), ulcers (11.1%), contact haemorrhages (83.8%), bullous edema (44.4%), deformity of mouth of ureter (94.4%) were found significantly more often in BTB patients. Hyperemia was met in BTB as often as in NSC (accordingly 38.9% and 33.1%).

Histology revealed specific TB inflammation in 11.8% only, in other BTB patients lymphoid and eosinophilic infiltration as well as fibrosis prevailed.

M.tuberculosis was found by culture in 11.1%, by PCR – in 16.7%, none – by microscopy.

Conclusion. There are no any endoscopic findings to confirm BTB, but there are some signs to suspect this disease. Histological and bacteriological confirmation is possible in 11.8% only- mostly due to late examination, after long antibacterial therapy.

ANTITUBERCULAR ACTIVITY OF *ARCTIUM LAPPA* AND *TUSSILAGO FARFARA* EXTRACTS AND CONSTITUENTS

J. Zhao[1], D. Evangelopoulos[2,†], S.Bhakta[2], A.I. Gray[1], V. Seidel [1,*]

[1] Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde,161 Cathedral Street, Glasgow G4 0RE, UK. [2] Mycobacteria Research laboratory, Department of Biological Sciences, Institute of Structural and Molecular Biology, Birkbeck, University of London, Malet Street, London WC1E 7HX, UK.

† Current address: Centre for Clinical Microbiology, Department of Infection, Royal Free Campus, UCL, London,

*UK. *Corresponding author: Dr V. Seidel,* veronique.seidel@strath.ac.uk

Arctium lappa and *Tussilago farfara* (Asteraceae) are two plant species used traditionally as antitubercular remedies. The aim of this study was i) to screen *A. lappa* and *T. farfara* extracts for activity against *Mycobacterium tuberculosis* and ii) to isolate and identify the compound(s) responsible for this reputed anti-TB effect. The activity of extracts and isolated compounds was determined against *Mycobacterium tuberculosis* $H_{37}R_v$ using a high throughput spot culture growth inhibition (HT-SPOTi) assay. The *n*-hexane extracts of both plants, the ethyl acetate extract of *T. farfara* and the dichloromethane phase derived from the methanol extract of *A. lappa* displayed antitubercular activity (MIC 62.5 µg/mL). Further chemical investigation of *A. lappa* led to the isolation of *n*-nonacosane (1), taraxasterol acetate (2), taraxasterol (3), a (1:1) mixture of b sitosterol/stigmasterol (4), isololiolide (5), melitensin (6), *trans*-caffeic acid (7), kaempferol (8), quercetin (9), kaempferol-3-*O*-glucoside (10). Compounds isolated from *T. farfara* were identified as a (1:1) mixture of b sitosterol/stigmasterol (4), *trans*-caffeic acid (7), kaempferol (8), quercetin (9), kaempferol-3-*O*-glucoside (10), loliolide (11), a (4:1) mixture of *p*-coumaric acid/4-hydroxybenzoic acid (12) and *p*-coumaric acid (13). All compounds were identified following analyses of their physicochemical and spectroscopic data (MS, ^{1}H and ^{13}C-NMR) and by comparison with published data. This is the first report of the isolation of *n*-nonacosane (1), isololiolide (5), melitensin (6) and kaempferol-3-*O*-glucoside (10) from *A. lappa*, and of loliolide (11) from *T. farfara*. Amongst the isolated compounds, the best activity was observed for *p*-coumaric acid (13) (MIC 31.3 µg/mL or 190.9 mM) alone and in mixture with 4-hydroxybenzoic acid (12) (MIC 62.5 µg/mL). The above results provide for the first time some scientific evidence to support, to some extent, the ethnomedicinal use of *A. lappa* and *T. farfara* as traditional antitubercular remedies.

SYNTHESIS OF SUBSTRATES AND INHIBITORS OF MUREIN PEPTIDE LIGASE (MPL): A NOVEL STRATEGY TO COMBAT TUBERCULOSIS (TB)

F Scotti[1,2], A Maitra[2], S Nukala[2], T Munshi[2], D Evangelopoulos[2], J P Malkinson[1] and S Bhakta[2*]*

[1] Department of Pharmaceutical and Biological Chemistry, UCL School of Pharmacy, 29-39 Brunswick Square London WC1N 1AX, UK

[2] Mycobacteria Research Laboratory, Institute of Structural and Molecular Biology, Department of Biological Sciences, Birkbeck, University of London, Malet Street, London WC1E 7HX, UK

[*]Corresponding authors: Dr John Malkinson (j.malkinson@ucl.ac.uk) and Dr Sanjib Bhakta (s.bhakta@bbk.ac.uk/ sanjib.bhakta@ucl.ac.uk)

Tuberculosis (TB) is an infectious bacterial disease responsible for the death of millions of people every year. Furthermore the number of multidrug- and extensively drug-resistant cases is increasing alarmingly. It is therefore urgent to find new ways to eradicate this disease (1).

Targeting the cell wall of bacteria is known to be effective in controlling bacterial diseases (2). The cell wall is made up of several components of which the peptidoglycan (PG) layer is essential in maintaining cellular integrity in all bacteria. It has, however, been largely overlooked as a source for anti-tubercular drug targets. Certain bacteria, including *Mycobacterium tuberculosis*, recycle fragments of degraded PG in a bid to conserve energy and resources as well as to enable them to remain hidden from the host's immune surveillance (3). Recycling in these pathogens is therefore able to complement the set of four enzymes (ATP-dependent Mur ligases), the endogenous role of which is to sequentially ligate a number of specific amino acids to the backbone of peptidoglycan (4). Substrate analogues of such recycling enzymes (Murein peptide ligase) have already proved useful in developing novel anti-infective drugs (5). The aim of this project is to characterise the structure

and function of a putative murein peptide ligase (Mpl) from *M. tuberculosis*, in the presence of its natural substrates and their synthetic analogues. Subsequently, these inhibitors will be evaluated for their whole-cell anti-tubercular properties, alone and in the presence of other existing drugs or cell-wall-PG biosynthesis inhibitors. We report here the overexpression and purification of the putative mycobacterial Mpl, as well as the solid-phase synthesis of its substrates and analogues.

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ANTITUBERCULAR ACTIVITY OF *ARCTIUM LAPPA* AND *TUSSILAGO FARFARA* EXTRACTS AND CONSTITUENTS

J. Zhao[1], D. Evangelopoulos[2,†], S.Bhakta[2], A.I. Gray[1], V. Seidel [1,*]

[1] *Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde,161 Cathedral Street, Glasgow G4 0RE, UK.* [2] *Mycobacteria Research laboratory, Department of Biological Sciences, Institute of Structural and Molecular Biology, Birkbeck, University of London, Malet Street, London WC1E 7HX, UK.*

[†] *Current address: Centre for Clinical Microbiology, Department of Infection, Royal Free Campus, UCL, London,*

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p-coumaric acid/4-hydroxybenzoic acid (12) and *p*-coumaric acid (13). All compounds were identified following analyses of their physicochemical and spectroscopic data (MS, ^{1}H and ^{13}C-NMR) and by comparison with published data. This is the first report of the isolation of *n*-nonacosane (1), isololiolide (5), melitensin (6) and kaempferol-3-*O*-glucoside (10) from *A. lappa*, and of loliolide (11) from *T. farfara*. Amongst the isolated compounds, the best activity was observed for *p*-coumaric acid (13) (MIC 31.3 µg/mL or 190.9 mM) alone and in mixture with 4-hydroxybenzoic acid (12) (MIC 62.5 µg/mL). The above results provide for the first time some scientific evidence to support, to some extent, the ethnomedicinal use of *A. lappa* and *T. farfara* as traditional antitubercular remedies.