



**BAY AREA
TUBERCULOSIS SCIENCE
SYMPOSIUM**

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SEPTEMBER 7, 2023 • UCSF MISSION BAY
E-BOOK OF ABSTRACTS



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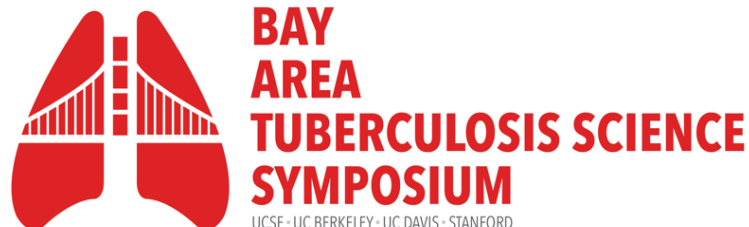
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Symposium program



Thursday, September 7, 2023
9:00am – 6:00pm

Fisher Banquet Room, Mission Bay
 University of California, San Francisco

Hosted by the UCSF Center for Tuberculosis

TIME	PRESENTER(S)	TITLE
8:00-9:00 AM	Registration check-in, poster set-up Continental breakfast & coffee/tea service	
9:00-9:25 AM	Babak Javid , MB BChir, PhD, UCSF Sarah Stanley , PhD, UC Berkeley	Welcome Remarks
9:25-9:30 AM	Payam Nahid , MD, MPH Director, UCSF Center for Tuberculosis	Keynote Introduction: 5min
9:30-10:15 AM	Eric Rubin , MD, PhD Adjunct Professor of Immunology and Infectious Diseases, Harvard University	Keynote talk: “Oxymoronic science - making a safe Mtb strain”
10:15-11:15 AM	Session moderator: Suzanne Dufault , PhD, Assistant Professor, UCSF	Invited talks
10:15-10:35 AM	Priya Shete , MD, MPH Associate Professor, UCSF	“Implementation of Multisectoral Approaches to Ending TB in Uganda: Challenges and Opportunities”
10:35-10:55 AM	Sara Suliman , PhD, MPH Assistant Professor, UCSF	“Host determinants for TB risk”
10:55-11:15 AM	Bennett Penn , MD, PhD Assistant Professor, UC Davis	“Ubiquitin signaling regulates the immune response to <i>M. tuberculosis</i> ”
11:15-11:35 AM	Coffee Break: 20min	
11:35 AM -12:20 PM	Session moderator: Shoshana Zha , PhD, Assistant Professor, UCSF	Oral Abstracts
11:35-11:50 AM	Rebecca Crowder , MPH Research Data Analyst, UCSF	“Head-to-head comparison of the diagnostic accuracy of TB screening tests: Chest-X-ray, Xpert TB host response, and C-reactive protein”
11:50-12:05 PM	Weihao Zheng , PhD Postdoctoral Fellow, UCSF	“Differential responsiveness to interferon gamma in lung mononuclear phagocyte subsets during chronic <i>M. tuberculosis</i> infection”

TIME	PRESENTER(S)	TITLE
12:05-12:20 PM	Colwyn Headley, PhD Postdoctoral Fellow, Stanford University	“Extracellular delivery of functional mitochondria mitigates exhaustion associated surface marker expression on CD4+ T Cells M.tb-infected mice”
12:20-1:50 PM	Lunch and Poster Session: 90min	
1:50-2:50 PM	Bennett Penn, MD, PhD, UC Davis Sarah Hutch, Center Manager, UCSF Center for Tuberculosis	Networking Session: 1hr
2:50-3:10 PM	Coffee Break: 20min	
3:10-4:30 PM	Session moderator: Sarah Stanley, PhD, UC Berkeley	Invited talks
3:10-3:30 PM	Gustavo Velásquez, MD, MPH Assistant Professor, UCSF	Winner, 2023 BATS Rising Star Award (award presented by Payam Nahid) “Towards an all-hands approach to improve treatment for tuberculosis”
3:30-3:50 PM	Pennan Barry, MD Chief, Surveillance and Epidemiology Section, California Department of Public Health TB Branch	“Use of Pretomanid in California”
3:50-4:10 PM	Ashley Wolf, PhD Assistant Professor, UC Berkeley	“The role of the gut microbiome in susceptibility to <i>Mycobacterium tuberculosis</i> infection”
4:10-4:30 PM	Purvesh Khatri, PhD Associate Professor, Stanford University	“Basic and translational research in TB using public data”
4:30-4:35 PM	Babak Javid, MB BChir, PhD, UCSF Sarah Stanley, PhD, UC Berkeley	Poster Awards: 5min
4:35-4:40 PM	Babak Javid, MB BChir, PhD, UCSF	Closing Remarks: 5min
4:45-6:00PM	Wine and cheese reception	

On behalf of the UCSF Center for Tuberculosis and the Bay Area TB Symposium Committee,

Thank You to Our Sponsors



Oral presentations

OP-01

Head-to-head comparison of the diagnostic accuracy of TB screening tests: Chest-X-ray, Xpert TB host response, and C-reactive protein

Rebecca Crowder, UCSF

Email: Rebecca.Crowder@ucsf.edu

Rebecca Crowder¹, Balamugesh Thangakunam², Victoria Dalay³, Welile Dube⁴, Alfred Andama⁵, Dong Van Nguyen⁶, Ankur Gupta Wright^{7,8}, Robert Castro¹, Catherine Cook¹, Tessa Mochizuki¹, Kinari Shah¹, Swati Sudarsan¹, Jason Andrews⁹, Niaz Banaei^{9,10}, Devan Jaganath¹¹, Midori Kato-Maeda¹, John Metcalfe¹, Payam Nahid¹, Patrick Phillips¹, Christina Yoon¹, Claudia M. Denkinger⁸, Adithya Cattamanchi^{1,12}

1. Center for Tuberculosis and Division of Pulmonary and Critical Care Medicine, University of California San Francisco, San Francisco, USA.
2. Christian Medical College, Vellore, India.
3. De la Salle Medical and Health Sciences Institute, Dasmariñas, Philippines.
4. Stellenbosch University, Stellenbosch, South Africa.
5. Makerere University, Kampala, Uganda.
6. Hanoi Lung Hospital, Hanoi, Vietnam.
7. University College London, London, UK.
8. Division of Infectious Disease and Tropical Medicine, University Hospital of Heidelberg, German Center of Infection Research, Heidelberg, Germany.
9. Division of Infectious Diseases and Geographic Medicine, Department of Medicine, Stanford University School of Medicine, Stanford, CA USA.
10. Department of Pathology, Stanford University School of Medicine, Stanford, CA USA.
11. Division of Pediatric Infectious Diseases, University of California, San Francisco, USA.
12. Division of Pulmonary Diseases and Critical Care Medicine, University of California, Irvine, USA.

Background: Accessible, accurate screening tests are necessary to advance tuberculosis (TB) case finding and early detection in high-burden countries.

Methods: We screened adults with ≥ 2 weeks cough presenting to primary health centers in the Philippines, Vietnam, South Africa, Uganda, and India. All participants received chest-X-ray, venous or capillary Cepheid Xpert TB Host Response (HR) testing, and point-of-care C-reactive protein (CRP) testing (Boditech iChroma II). Chest-X-ray images were processed using three computer-aided detection (CAD) algorithms (Delft Imaging CAD4TBv700, Qure.ai qXRv329, Lunit INSIGHTv314111). We assessed diagnostic accuracy against a microbiologic reference standard (MRS) incorporating sputum Xpert Ultra x1 and liquid culture x2. Optimal cut points were chosen for each test to achieve sensitivity $\geq 90\%$ and maximize specificity against the MRS. Two test screening algorithms were considered, defining a positive index test as a positive result on either test.

Results: Between July 2021-August 2022, 1,380 participants were enrolled. 625 (45%) were female, median age was 41 (interquartile range 29-55), 194 (14%) were living with HIV and 303 (22%) had confirmed TB. At 90% sensitivity, all three CAD algorithms had comparable specificity. (CAD4TB: 70.0%, qXR: 71.5%, Lunit: 72.2%). To achieve 90% sensitivity using a single test, $HR \leq 1.3$, $CRP \geq 2.81$, and $Lunit \geq 5.48$ were considered positive. In head-to-head comparisons (with Lunit representing CAD), CAD showed highest specificity (72.2% vs. 65.2% for HR, difference 7.1%, 95% CI 3.3-10.7; 72.2% vs. 49.6% for CRP, difference 22.7%, 95% CI 18.9-26.4). For two-test screening algorithms, at 90% sensitivity, CAD-HR (specificity 79.4%) and CAD-CRP (specificity 74.0%) exceeded WHO target product profile (TPP) minimum accuracy thresholds and had higher accuracy than any test alone. CRP-HR did not achieve TPP targets.

Conclusion: In summary, CAD achieves TPP targets and outperforms HR and CRP. Combining screening tests further increased accuracy. Cost and feasibility of two-test screening algorithms should be explored.

OP-02

Extracellular delivery of functional mitochondria mitigates exhaustion associated surface marker expression on CD4+ T Cells *Mtb*-infected mice

Colwyn Headley, Stanford University

Email: cheadley@stanford.edu

Colwyn A. Headley, Shalini Gautam, Angelica Olmo-Fontanez, Andreu Garcia-Vilanova, Varun Dwivedi, Alyssa Schami, Philip S. Tsao, Jordi B. Torrelles & Joanne Turner.

Tuberculosis (TB) remains a significant global health concern, particularly in populations with weakened immune systems, such as the elderly. CD4+ T cells play a crucial role in the immune response against *Mycobacterium tuberculosis* (*M.tb*) infection. However, chronic TB infection and aging are associated with CD4+ T cell dysfunction, including T cell exhaustion and senescence, which contribute to disease progression and impaired immune control. Understanding the mechanisms underlying CD4+ T cell exhaustion and senescence is essential for developing effective therapeutic strategies to combat TB. Mitochondrial dysfunction has emerged as a critical factor contributing to T cell dysfunction in both aging and chronic infectious diseases. Mitochondria are involved in energy production, calcium signaling, and the regulation of cell survival pathways. Perturbations in mitochondrial function can disrupt cellular metabolism, increase oxidative stress, and impair T cell signaling and effector functions. In the context of CD4+ T cell exhaustion and senescence, mitochondrial dysfunction has been implicated in the dysregulation of cellular metabolism, impaired redox balance, and altered signaling pathways. In this study, we evaluated the impact of mito-transfer on CD4+ T cell differentiation and function using both mouse models and human CD4+ T cells isolated from elderly individuals. Our results demonstrate that mito-transfer in naïve CD4+ T cells from old mice promoted the generation of protective effector and effector memory CD4+ T cells during *M.tb* infection. Furthermore, mito-transfer improved the function of CD4+ T cells from elderly individuals, as evidenced by increased mitochondrial mass, modulation of cytokine production, enhanced T cell activation, and reduced exhaustion and senescence markers. These findings suggest that mito-transfer could serve as a novel strategy for rejuvenating CD4+ T cells, potentially improving immune responses in elderly individuals and patients with chronic *M.tb* infection.

OP-03

Differential responsiveness to interferon gamma in lung mononuclear phagocyte subsets during chronic *M. tuberculosis* infection

Weihao Zheng, UCSF

Email: Weihao.Zheng@ucsf.edu

Weihao Zheng¹, Jason Limberis¹, Joel D. Ernst^{1*}

¹Division of Experimental Medicine, Department of Medicine, University of California, San Francisco, California, USA

*Correspondence: joel.ernst@ucsf.edu

Interferon gamma (IFN- γ) is required for immunity to *Mycobacterium tuberculosis* (Mtb). Although Mtb infects multiple lung mononuclear cell subsets, their distinct responses to IFN- γ remain poorly understood. We have shown that CD11c^{lo} monocyte-derived lung cells termed MNC1 (mononuclear cell subset 1) harbor more live Mtb compared to alveolar macrophages (AM), and CD11c^{hi} MNC2. In this study, we investigated the differential responsiveness to IFN- γ in three lung myeloid cell subsets. By employing RNA sequencing and Gene Ontology analysis of sorted lung myeloid cell subsets, we identified that the biological process 'response to IFN- γ ' is differentially regulated in MNC1 compared to MNC2 and AM. Notably, MNC2 and AM expressed higher levels of IFN- γ response genes, such as *Ifngr2*, *Gbp2*, *Ciita*, and MHCII-encoding genes. Flow cytometry analysis confirmed that MNC1 expressed lower levels of IFNGR2 and MHCII than MNC2 and AM. Furthermore, in vitro IFN- γ stimulation revealed that MNC1 displayed reduced levels of phospho-STAT1 compared to MNC2 and AM, indicating an inferior response to IFN- γ in MNC1. Importantly, the underexpression of MHCII in MNC1 correlated with impaired activation of CD4 T cells. These findings suggest that IFN- γ unresponsiveness contributes to Mtb permissiveness in MNC1, potentially representing a novel evasion mechanism employed by Mtb to establish persistence.

Poster presentations

PP-01

The Role of the Host Gut Microbiome in Susceptibility to *Mycobacterium tuberculosis*

Carolina Agudelo, UC Berkeley

Email: carolinaagudelo@berkeley.edu

Tuberculosis (TB) affects 2 billion people in the world, killing more than 1.7 million people every year. Susceptibility to *Mycobacterium tuberculosis* (*Mtb*) and disease severity are both linked to host genetics, immune system function, and environment. However, no one factor can fully explain the heterogeneity seen in human TB disease. The literature suggests that dysbiosis, or changes in the gut microbiome's composition and function, is linked to alterations in the immune responses to disease manifestations and outcomes in the lungs via the 'gut-lung axis'. Previous studies have demonstrated that antibiotic treatment in a mouse model leads to lower microbial diversity in the gut and a higher susceptibility to *Mtb* infection. We hypothesize that the intestinal microbiome plays a role in determining susceptibility to *Mtb*. To investigate this, we infected conventional, doxycycline-treated and germ-free C57BL/6 mice with the Erdman strain of *Mtb*. Preliminary data suggests a role for certain bacterial taxa in driving host susceptibility to *Mtb*. While we see evidence of gut microbiome-related impacts on *Mtb* infection, the mechanism remains to be discovered. Moving forward, we seek to identify both the bacterial strains and host immunological pathways involved in protection against *Mtb*.

PP-02

Preferences for tuberculosis preventive treatment regimens among persons living with HIV in Uganda – a discrete choice experiment.

Hélène Aschmann, UCSF

Email: Helene.Aschmann@ucsf.edu

Aschmann HE^{1,2,3}, Musinguzi A⁴, Kadota J^{1,2}, Namale C⁵, Kakeeto J⁵, Nakimuli J⁴, Akello L⁴, Welishe F⁴, Nakitende A⁴, Berger C^{1,2}, Dowdy D^{5,6}, Cattamanchi A^{1,2,3,7}, Semitala F^{4,8,9}, Kerkhoff AD^{2,10}

1. Division of Pulmonary Diseases and Critical Care Medicine, University of California San Francisco, San Francisco, CA USA
2. University of California, San Francisco Center for Tuberculosis (CTB), San Francisco, CA USA
3. Department of Epidemiology and Biostatistics, University of California San Francisco, San Francisco, CA USA
4. Infectious Diseases Research Collaboration, Kampala, Uganda
5. Uganda Tuberculosis Implementation Research Consortium, Walimu, Kampala, Uganda
6. Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD USA
7. Division of Pulmonary Diseases and Critical Care Medicine, University of California Irvine, Irvine, CA USA
8. School of Medicine, Makerere University College of Health Sciences, Kampala, Uganda
9. Makerere University Joint AIDS Program, Kampala, Uganda
10. Division of HIV, Infectious Diseases, and Global Medicine, University of California San Francisco, San Francisco, CA USA

Background: Tuberculosis (TB) preventive treatment (TPT) is recommended for persons living with HIV in high TB burden settings. While 6 months of daily isoniazid (6H) is widely available, a short-course regimen consisting of 12 weekly doses of isoniazid and rifapentine (3HP) is being rolled out globally. Other regimens, including 1 month of daily isoniazid and rifapentine (1HP), are considered for future roll-out. However, little is known about individuals' preferences for TPT regimens.

Methods: We administered a discrete choice experiment survey among adults living with HIV at an HIV clinic in Kampala, Uganda. In 9 random choice tasks, participants chose between two regimens based on treatment burden (number of pills, frequency, duration, adjusted HIV antiretroviral dosage) and side effects and indicated its desirability over no treatment. We analyzed preferences using hierarchical Bayesian estimation and simulated predicted TPT choice.

Results: Among 400 participants (median age 44, 72% female, 91% with previous TPT), across tasks, 60% (241/400) accepted all regimens, 39% (157/400) accepted some regimens, and 0.5% (2/400) accepted none. Simulations predicted that if only 6H was available, 13% would prefer no treatment. A 10-pill 3HP regimen was predicted to be equally desirable as 6H (46% 3HP vs. 46% 6H vs. 8% none). However, a 5-pill 3HP fixed dose combination (FDC) regimen was preferred over 6H (90% 3HP vs. 8% 6H vs 2% none). If 1HP was offered in addition, participants preferred a 5-pill 3HP regimen over a 5-pill 1HP regimen, both in scenarios where 1HP required (83% vs. 8%) and did not require antiretroviral dosage adjustment (70% vs. 23%), with few choosing 6H (6%) or no treatment (1%).

Conclusions: Predicted uptake of short-course TPT was high with FDC, and FDC 3HP was highly preferred to 1HP. Development of future, more person-centered TPT regimens should focus on reducing pill burden and non-daily dosing.

PP-03

Frequency of adverse events reported during tuberculosis preventive treatment using 3HP among people living with HIV in Uganda

**Jillian Kadota, UCSF*

Email: Jillian.Kadota@ucsf.edu

**Hélène Aschmann to present Jill Kadota's poster on her behalf*

Jillian L. Kadota¹, Hélène E. Aschmann², Lydia Akello³, Allan Musinguzi³, Fred Welishe³, Jane Nakimuli³, Anne Nakitende³, Joan R. Kasidi³, Devika Patel⁴, Amanda Sammann⁴, Payam Nahid¹, Patrick Phillips¹, Christopher A. Berger¹, David Dowdy^{5,6}, Fred Semitala^{3,7,8}, Adithya Cattamanchi^{5,9}

1. Center for Tuberculosis and Division of Pulmonary and Critical Care Medicine, San Francisco General Hospital, University of California San Francisco, San Francisco CA USA
2. Department of Epidemiology and Biostatistics, University of California San Francisco, San Francisco CA USA
3. Infectious Diseases Research Collaboration, Kampala, Uganda
4. The Better Lab, Department of Surgery, University of California, San Francisco, San Francisco, California, United States of America
5. Uganda Tuberculosis Implementation Research Consortium, Walimu, Kampala, Uganda
6. Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD USA
7. School of Medicine, Makerere University College of Health Sciences, Kampala, Uganda
8. Makerere University Joint AIDS Program, Kampala, Uganda
9. Division of Pulmonary Diseases and Critical Care Medicine, University of California Irvine, Irvine, CA USA

Background: 3HP, a short-course regimen for tuberculosis prevention (12 weekly doses of isoniazid/rifapentine), is being rolled out in high TB-burden countries. Reported adverse event (AE) rates for 3HP may differ between efficacy trials and real-world settings. We aimed to describe the baseline incidence of AEs in a real-world clinical setting among people living with HIV (PLHIV) in Uganda.

Design/Methods: We conducted a pragmatic effectiveness-implementation type 3 study of 3HP TB preventive treatment. We randomized adult PLHIV to facilitated directly observed therapy (DOT), facilitated self-administered therapy (SAT), or choice between DOT and SAT. DOT participants were screened for AEs weekly at the clinic. SAT participants could report AEs by responding to a weekly two-way toll-free interactive voice response phone call or at clinic visits for doses 6 and 12. All participants could report AEs at unscheduled phone calls or clinic visits. We classified AEs according to the Common Terminology Criteria for Adverse Events version 5.0. Clinical staff managed AEs including assessments, evaluations, and treatment decisions.

Results: 272/1655 (16%) participants reported AEs. The most common AEs reported were classified as general disorders (n=117 instances including fever, weakness, oedema, pain, or flu/cold symptoms), nervous system (n=103 including dizziness, peripheral neuropathy, headache, or stroke), musculoskeletal (n=88 including joint, neck, back or chest pain), gastrointestinal (n=83 including nausea, constipation, diarrhea, or change in appetite), or dermatologic (n=56 including rash, itching, or other skin changes). Participants in the DOT arm, women and poor or severely poor participants were more likely to report AEs. Among participants with AEs, 214 (79%) were referred for evaluation by a clinician, 107 (39%) required additional laboratory testing, 18 (7%) had treatment held temporarily and 14 (5%) had treatment discontinued.

Conclusions: 3HP was safe, with >80% of PLHIV feeling well throughout 3HP treatment. Only a few had to stop treatment due to AEs.

PP-04

Investigating mechanisms of stress induced antibiotic tolerance in pathogenic *Mycobacteria*

Nicholas Bates, UC Davis

Email: nabates@ucdavis.edu

Nicholas A. Bates^{1,2,3}, Bennett H. Penn^{2,3}

¹Graduate Group in Immunology, ²Department of Internal Medicine, Division of Infectious Diseases, UC Davis Health, ³Department of Medical Microbiology and Immunology, University of California, Davis

To be effective, antibiotic therapy must navigate numerous biological hurdles. Among these is that a bacterium may exist in one of several, potentially diverse, phenotypic states at any given time. One factor that influences the phenotype of a bacterium is the environment that surrounds it. For a pathogen like *M. tuberculosis*, *in vivo* conditions notably include stressors such as nutrient starvation, hypoxia, low pH, and reactive nitrogen species, which are associated with host immunity in macrophage phagosomes and granulomas. Thus, antibiotics which are tested under standard *in vitro* growth conditions may not display the same activity *in vivo*. To better understand this phenomenon, we searched for examples where host relevant stressors alter the effectiveness of clinically relevant antibiotics used to treat *M. tuberculosis*. As a proof of concept, we began study in the fast-growing organism *M. abscessus*, a highly antibiotic resistant emerging pathogen. In *M. abscessus*, we observed inducible multi-drug tolerance in response to nutrient starvation. Using transposon insertion sequencing, we screened for the genetic requirements for this phenotype. This screen revealed numerous possible regulators of the nutrient stress induced antibiotic tolerance phenotype, including a potential role for cobalamin (vitamin B12) synthesis. We have also identified inducible multi-drug tolerance phenotypes in *M. tuberculosis* and plan to run similar genetic screens in this organism.

PP-05

Anti-PstS1 antibodies trigger NLRP3 activation in *Mycobacterium tuberculosis*-infected macrophages

Rania Bouzeyen, UCSF

Email: rania.bouzeyen@ucsf.edu

Rania Bouzeyen¹, Avia Watson², Tessa Dickson¹, Natalia T. Freund² and Babak Javid¹

¹ Division of Experimental Medicine, University of California, San Francisco, CA, United States

² Department of Clinical Microbiology and Immunology, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv-Yafo, Israel.

The molecular mechanisms by which pathogen-specific antibodies confer protection against intracellular *Mycobacterium tuberculosis* (Mtb) infection are not known. Furthermore, clinical studies looking for markers or correlates of protection against tuberculosis rarely explore antibody functions beyond antigen binding. We previously demonstrated that human monoclonal antibodies induced during active tuberculosis and targeting an Mtb phosphate transporter subunit (PstS1) reduces bacterial burden in an *ex-vivo* human whole blood growth inhibition assay and in Mtb infected mice. Here, we show that anti-PstS1 induces NLRP3 inflammasome activation and caspase1-mediated release of IL-1b in Mtb-infected macrophages. Further, we studied anti-PstS1 mediated-protection efficacy in mice and found that the antibody-mediated protection was accompanied with enhanced recruitment of neutrophils, pro-inflammatory monocytes and antigen specific effector T cells. The relatively facile measurement of antibody-mediated release of IL-1b in an inflammasome-dependent mechanism can readily be included in human and non-human primate studies looking for correlates of antibody-mediated protection. These data advance our understanding of mechanisms by which antibodies may facilitate protection against tuberculosis.

PP-06

A conserved two-gene operon is crucial for virulence and cell membrane function in the pathogen *M. abscessus*

Nick Campell-Kruger, UC Berkeley

Email: nick.ck@berkeley.edu

Mycobacterium abscessus and other mycobacterial pathogens are characterized by the presence of an atypical outer membrane called the mycomembrane. The mycomembrane represents a formidable permeability barrier, giving *M. abscessus* high innate resistance to many classes of antibiotics. Historically, the impermeability of the mycomembrane has been attributed primarily to the α -branched, β -hydroxylated extremely long-chain fatty acids called mycolic acids that make up the inner leaflet and part of the outer leaflet of the mycomembrane. However, recent research has highlighted several genes which, despite their lack of involvement in the metabolism of mycolic acids, are still crucial for mycomembrane function and impermeability. Two such genes, the lipoprotein *lprg* and its operonic partner, a Major Facilitator Superfamily transporter (*mfs*), are widely conserved in mycobacteria but their functions have remained elusive. Recent work in *M. tuberculosis* suggests that this operon may export triacylglycerides (TAGs) to the mycomembrane, but relatively poor sequence conservation between the genes in *M. tuberculosis* and *M. abscessus* brings into question whether this operon plays the same role in these distinct species. To test the contribution of this operon to pathogenesis, mycomembrane function, and lipid transport in *M. abscessus*, we generated a mutant lacking the *lprg-mfs* operon. We found that this mutant is impaired in both macrophage and mouse models of infection. We also found that the mycomembrane's permeability and fluidity is markedly altered in this mutant, and that TAGs are more accessible to solvent extraction. This evidence suggests that *lprg-mfs* is crucial for mycomembrane function in *M. abscessus*, and that the operon either transports a novel lipid species or that the direction of transport is retrograde rather than anterograde.

PP-07

Accuracy of a PCR-based Gene Signature for the Triage of Childhood Pulmonary Tuberculosis

Robert Castro, UCSF

Email: robert.castro@ucsf.edu

Robert Castro, Peter Wambi, Esin Nkereuwem, Moses Nsereko, Beate Kampmann, Eric Wobudeya, Adithya Cattamanchi, Devan Jaganath

Background:

Novel approaches are needed for triage among children with presumptive TB. We evaluated the diagnostic accuracy of a PCR-based three-gene RNA signature (Xpert Host Response, Xpert-HR, Cepheid, USA) among children from Uganda and the Gambia.

Methodology

We prospectively enrolled children 0-9 years old with presumptive TB. All participants completed standard TB evaluation including Xpert MTB/RIF Ultra and mycobacterial culture on respiratory specimens. Venous or capillary blood was collected for Xpert-HR testing using the GeneXpert platform per manufacturer recommendations. A TB score was calculated based on the cycle threshold value of three genes: $(\text{GBP5-DUSP3})/2 - \text{TBP}$. We calculated the area under the receiver operating characteristic curve (AUC) based on a microbiological or composite reference standard (MRS or CRS, respectively), and determined the specificity at a cut-off closest to 90% sensitivity. We compared the accuracy of Xpert-HR to the clinical prediction score from the World Health Organization (WHO) treatment decision algorithm.

Results

We included 128 children (median age 3 years, IQR 1-6), of whom 15 had Confirmed TB, 73 had Unconfirmed TB, and 40 had Unlikely TB. The AUC of Xpert-HR was 0.71 (95% CI 0.55-0.86) using the MRS, and 0.59 (95% CI 0.49-0.70) using the CRS. At a sensitivity near 90%, the specificity of Xpert-HR was 30.1% (95% CI 21.8- 39.4) using the MRS, but 5.0% for the CRS (95% CI 0.61-16.9, Table). Xpert-HR was more specific than the WHO treatment decision algorithm clinical score using the MRS (30.1% vs. 4.4%, $p < 0.001$), but had similarly low specificity using the CRS (5% vs. 10%, $p = 0.41$).

Conclusions

Xpert-HR had only moderate accuracy but greater specificity for microbiologically-confirmed childhood TB than the WHO treatment decision algorithm. Additional genes or complementary tests may be needed to improve specificity, especially for detection of Xpert MTB/RIF Ultra- and culture-negative TB.

PP-08

Context-specific regulation of translation in *Mycobacterium tuberculosis* by kasugamycin

Mohamad Dandan, UCSF

Email: mdandan@fraserlab.com

Mycobacterium tuberculosis (Mtb) is the cause of tuberculosis, resulting in an annual ~1.5 million deaths worldwide. Overuse of antibiotics leads to antibiotic resistance in Mtb. A main target of antibiotics is the ribosome. Recent evidence suggests that antibiotics alter translation in a context specific manner. This is completely unexplored in Mtb. During translation, the ribosome engages with numerous ligands and leads to variations in the properties of the translation complex, both at the codon level and across different genes. We propose the antibiotic kasugamycin (ksg) alters multiple steps of translation via distinct binding sites in a context-specific fashion. The binding site for ksg is unknown in Mtb ribosomes. My preliminary data suggest an occupied ribosome containing tRNA and mRNA in the ksg binding site. To overcome this, *Mycobacterium smegmatis* was grown with ksg followed by visualization of the ribosomal complexes with cryo electron microscopy. In the 30S subunit and 70S complexes, ksg was bound to the P and E site located at h44 of the 16S rRNA and spans between h24 and h28. To determine how ksg alters protein translational activity, an in-vitro translation assay was performed using a green fluorescent protein as a readout. Without ksg, the mean relative fluorescence unit (RFU) was 275 +/- 13.2. Whereas with ksg, the mean RFU was 313 +/- 4 (p=0.0541). Chloramphenicol treatment slightly reduced the mean RFU to 238 +/- 9 but was non-significant as compared to the control (p=0.07). For the next steps, I will evaluate stalled complexes of ribosomal structures bound to ksg with sequence specific transcripts. In all, enhancing our comprehension of these mechanisms will facilitate the development of ksg and other ribosome targeting antibiotics as potential therapeutics for Mtb.

PP-09

Random barcode transposon-site sequencing in *Mycobacterium tuberculosis* to reveal the functions of unknown genes

Kayla Dinshaw, UC Berkeley

Email: kayla_dinshaw@berkeley.edu

Mycobacterium tuberculosis (Mtb) is a human pathogen that often resides in the phagosome of macrophages. During infection, Mtb faces a harsh environment of hypoxia, nutrient starvation, and acid, oxidative, and nitrosative stress. Although the genome of Mtb was sequenced nearly 25 years ago, the function of many individual genes and how they contribute to Mtb survival *in vivo* remains to be discovered. To determine the function of unknown genes, we generated a pooled random barcode transposon-site sequencing (RB-TnSeq) library in Mtb. A unique twentynucleotide barcode in the transposon that is disrupting gene function allows for rapid, high throughput genetic screening without the laborious protocol of standard bacterial TnSeq screens. The Mtb RB-TnSeq library was exposed to a chemical library of antibiotics, stressors, carbon sources, and nitrogen sources. Results from these genetic screens have begun to reveal the function of unknown genes and will expand our knowledge of the genetics behind Mtb pathogenesis.

PP-10

When is a negative MGIT really a negative? Identifying the useful range of time-to-positivity for modeling early treatment response

Suzanne Dufault, UCSF

Email: Suzanne.Dufault@ucsf.edu

BACKGROUND. The BACTEC MGIT machine is the standard approach globally for detecting viable bacilli in a patient's sputum by measuring the decline of oxygen in a liquid culture tube inoculated with the sputum sample. The manufacturers recommend observing samples for no longer than 42 days, at which point the sample is declared "negative" for TB with no evidence for bacterial growth. The time to detection of bacterial growth is referred to as time-to-positivity (TTP) and is of interest as a biomarker wherein change in TTP over time is a measure used for comparing the anti-TB activity of different TB treatments. In practice, very few samples return MGIT TTP values between 30 and 42 days.

MATERIALS AND METHODS. Using data collected weekly to eight weeks post-randomization from the REMoxTB and PanACEA MAMS-TB randomized clinical trials, we investigated whether a lower limit of detection for longitudinal time-to-positivity modeling improves precision and power for detecting differences between treatments.

RESULTS. In the REMoxTB and MAMS-TB studies, only 1.5% and 3.8% of all weekly samples returned TTP measurements between 30 and 42 days, respectively. When the MAMS-TB data are modeled with the 30- rather than the 42-day censoring limit, the precision around the regimen-specific estimated rate of change is either equivalent or improves, resulting in 95% credible interval widths that are reduced by up to 9.4%.

DISCUSSION. While TTP measurements between 30 days and the manufacturer's recommended limit of detection may be important for diagnostic purposes, TTP values captured in this range may not contribute meaningfully to understanding the TTP trajectories for early treatment response. Decreasing the limit of detection used for modeling TTP will result in shorter trial times with improved power for performing regimen selection.

PP-11

Does the innate immune system restrict Mtb?

Marian R. Fairgrieve, UC Berkeley

Email: marianf@berkeley.edu

Marian R. Fairgrieve¹, Ella Brydon^{1,2}, Dmitri I. Kotov¹, Russell E. Vance^{1,2}

¹Division of Immunology and Pathogenesis, Department of Molecular and Cell Biology, Berkeley CA 94720, USA;

²Howard Hughes Medical Institute, University of California, Berkeley, Berkeley CA 94720, USA

Numerous studies have shown that Mtb replication is not controlled until initiation of adaptive immunity, raising the question of why innate immunity is not able to limit the infection. We suspect that Mtb may be intrinsically resistant to innate antimicrobial effectors. To address that possibility, we used an ultra-low dose (ULD) of 1-3 bacilli in a sensitive and rigorous test of whether innate immunity can control Mtb replication. We have demonstrated that even at ULD, Mtb bacterial burdens in the lung are not affected by the absence of key innate immune proteins such as MyD88. This demonstrates that the innate immune response to Mtb is remarkably ineffective in vivo as it is unable to eliminate an infection seeded by as few as 1-3 bacilli. These findings strikingly contrast with in vitro results that suggest that innate immunity alone can restrict Mtb replication and with other bacterial infections in the lung such as *Legionella pneumophila*, where innate immunity can readily clear 10⁶ CFUs. To test the hypothesis that the anti-bacterial immune environment induced by *L. pneumophila* could also control Mtb, we co-infected mice with *L. pneumophila* and Mtb. Interestingly, co-infection with *L. pneumophila* resulted in only a modest reduction in Mtb CFU compared with mice infected with only Mtb. Our preliminary findings indicate that Mtb is resistant to effectors present in a strongly antimicrobial environment stimulated by *L. pneumophila* infection. Together, these findings suggest that Mtb is intrinsically resistant to innate immune effectors.

PP-12

Type I and II IFN crosstalk during *Mycobacterium tuberculosis* infection

Stefan Fattinger, UC Berkeley

Email: sfattinger@berkeley.edu

Stefan A. Fattinger¹, Bianca Parisi¹, Dmitri Kotov^{1,2}, Russell E. Vance^{1,2}

¹Division of Immunology and Molecular Medicine, University of California, Berkeley, CA, USA

²Howard Hughes Medical Institute, University of California, Berkeley, CA, USA

The host immune response against viral and bacterial pathogens relies on cellular communication mediated by cytokines. Interferons (IFNs) are a major class of cytokines which result in the expression of thousands of IFN stimulated genes (ISGs). While type I IFNs signal through IFNAR and promote anti-viral responses, type II IFN signals through IFNGR and has been implicated in responses against bacteria and other intracellular parasites. Despite their rather distinct role in the immune response against pathogens, a potential crosstalk between type I and II IFN signaling has recently been suggested. However, how this crosstalk is mediated remains poorly understood. Here, using unbiased CRISPR screens in mouse and human immune cells, we are addressing how type I IFN signaling suppresses type II IFN response. The CRISPR screen in mouse cells combined with RNAseq analysis of IFN stimulated cells revealed a list of potential candidates mediating this crosstalk. This hit list is currently being validated. Furthermore, we are investigating if this crosstalk might explain the difference in disease outcome for *Mycobacterium tuberculosis* (Mtb) infection associated with type I and II IFNs

PP-13

T cell responses to *Mycobacterium tuberculosis* variable antigens

Zach Howard, UCSF

Email: Zach.Howard@ucsf.edu

A majority of the immunodominant T cell antigens of *Mycobacterium tuberculosis* (Mtb) are hyperconserved. Lack of antigenic variation suggests insufficient selection pressure from T cell responses to infection. We identified 6 variable-sequence Mtb proteins, which we hypothesized represent antigens that vary due to selective pressure from protective CD4+ T cell responses. To investigate the impact of CD4+ T cell responses to these variable-sequence antigens, we vaccinated C57BL/6 and the hypersusceptible SP140-/- mice using a DNA vaccine encoding for a fusion protein of four variable-sequence antigens. We then assayed immune cell populations by flow cytometry, immunopathology by immunofluorescence microscopy and Sytox Green injection, and bacterial burden. Vaccination with variable-sequence antigens caused an increase in Th17/Treg ratios that was associated with a reduced immunopathology in both B6 and SP140-/- mice. Despite reduced immunopathology, vaccination with the variable-sequence antigens did not impact bacterial burden. Antigenicity experiments revealed a unique IL-17 response to the variable-sequence antigen RimJ, and parallel investigations of the response to RimJ in humans also revealed a bias towards a Th17 phenotype in RimJ-responsive T cells. Vaccination of SP140-/- mice with a DNA vaccine encoding for only RimJ demonstrated a similar phenotype to vaccination with the fusion construct, which indicates that RimJ is the primary antigen responsible for the altered immunopathology phenotype. Further investigation is needed to determine the primary mechanism by which RimJ-specific T cells are able to impact immunopathology during Mtb infection without reducing bacterial burden.

PP-14

Health-related quality of life among Ugandan TB survivors

Sophie Huddart, UCSF

Email: Sophie.Huddart@ucsf.edu

Sophie Huddart¹, Alex Kityamuwesi², Shube Bamushaye², Racheal Madaya Nambozo², Vivian Nabacwa², Agnes Sanyu Nakate², Rebecca Crowder¹, Adithya Cattamanchi^{1,3}, Achilles Katamba^{2,4}

1. Center for Tuberculosis and Division of Pulmonary and Critical Care Medicine, San Francisco General Hospital, University of California San Francisco, San Francisco, CA USA
2. Uganda Tuberculosis Implementation Research Consortium, Kampala, Uganda
3. Division of Pulmonary and Critical Care Medicine, University of California Irvine, Irvine, CA USA
4. Clinical Epidemiology and Biostatistics Unit, Department of Medicine, Makerere University College of Health Sciences, Kampala, Uganda

Introduction:

155 million people globally have survived tuberculosis (TB) and many report substantial on-going health issues. There is an urgent need to improve identification of individuals at risk of poor post-TB outcomes. We characterize health-related quality of life (HRQoL) among Ugandan TB survivors a year after treatment completion and identify predictors of poor HRQoL.

Methods:

We surveyed a cohort of Ugandan TB survivors 12-25 months after treatment cessation. HRQoL was assessed with the EQ-5D 5L tool, a 6-item validated generic utility-based instrument that measures capacity in five health domains: mobility, self-care, usual activities, pain and anxiety/depression. We calculated summary index scores using previously validated Uganda specific weights. An index score of one indicates perfect health and zero indicates a health state equivalent to death. We fit a normal linear model to identify demographic and clinical features predictive of the index score.

Results:

We successfully traced 2,110 (87.7%) of 2,406 TB survivors; 138 (5.7%) were deceased. Of the remaining 1,972 TB survivors, 1,923 (97.5%) completed the survey. Participants reported high rates of impairment in most domains. Over a quarter of participants experienced at least some impairment in mobility (26.1%), usual activities (30.6%), and pain/discomfort (35.7%); about half experienced anxiety/depression (48.4%). The median index score was 0.92 (interquartile range: 0.80-1.00). Female sex and older age were associated with lower summary scores (-0.03, 95% confidence interval [CI]: [-0.06, -0.01], and -0.02, 95% CI: [-0.02, -0.02], respectively). Living with HIV was associated with a higher score (0.05, 95% CI: [0.03, 0.08]).

Conclusion:

TB survivors reported challenges in several health domains after TB treatment. Higher HRQoL among people living with HIV suggests that ongoing contact with the healthcare system could ameliorate some of these health issues. TB survivors may benefit from routine health screening and referrals to interventions like pulmonary rehabilitation to promote health post-TB.

PP-15

Elucidating the mechanism and correlates of protection elicited by CDN adjuvanted vaccines for *M. tuberculosis*.

Isabel Lamb-Echegaray, UC Berkeley

Email: isabel.lamb@berkeley.edu

The efficacy of cyclic-di-nucleotide (CDN) adjuvanted vaccines in defending against *M. tuberculosis* (*Mtb*) has been recently discovered, but the mechanism and correlates of protection by which STING activation leads to increased protection remains elusive. While IL-17 and IFN- γ producing CD4 T cells are known to be involved in CDN vaccine protection, the precise phenotypes and localization of Th1 and Th17 cells required for protection are poorly understood. In addition, it is still unclear which of the downstream pathways activated by STING underlie the efficacy of STING activating adjuvants. Here we show that H1/CDN adjuvanted vaccine protection is independent of STAT-6 and IRF3 and that CDNs induce STING-dependent autophagy, distinguishing them from other adjuvants. In addition, we show that although a H1/AS01E adjuvanted vaccine, composed of MPLA and saponin, effectively protects against *Mtb* challenge, while an H1/MPLA adjuvanted vaccine does not. These findings suggest that the terpenoid saponin in AS01E, known to regulate autophagy pathways, might mediate the vaccine's efficacy. Consequently, our results emphasize that the induction of autophagy is an important consideration for the development of new vaccines and that the efficacy of CDN adjuvanted vaccines might be mediated through STING dependent autophagy.

PP-16

Identify IFN-induced key determinants that control *Mycobacterium tuberculosis* in CRISPR library screen

Jingjun Lin, UC Davis

Email: jjnlin@ucdavis.edu

Mycobacterium tuberculosis (*Mtb*) can survive and proliferate in macrophages. Few human immune factors have been identified as potentially important for controlling tuberculosis, which includes interferon gamma (IFN- γ). In addition, IFN- γ knockout mice are highly susceptible to *Mtb* infection. Therefore, identifying IFN- γ associated factors may reveal important *Mtb* pathogenesis mechanisms. Our experiment used an *Mtb* strain that carries an mCherry fluorescent reporter to infect a mouse macrophage cell CRISPR library pre-stimulated with IFN- γ . At the end of the infection, we harvested the macrophage cells and used the fluorescently-activated cell sorting to identify macrophages with high intracellular *Mtb* burden and low intracellular *Mtb* burden. Macrophages were processed for single-guide RNAs (sgRNAs) next-generation sequencing (NGS) to identify significantly enriched sgRNAs. In our first trial, sgRNAs enriched in high *Mtb* burden macrophages did not include the interferon gamma receptor 1 (*Ifngr1*), which was used as the internal positive control. To troubleshoot, we infected the *Ifngr1* knockout (KO) cells and scrambled gRNA-transduced control macrophages (scramble cells) with *Mtb*. Due to the lack of response to IFN- γ , *Ifngr1* KO cells had about 5 to 10-fold higher *Mtb* burden than scramble cells. However, the phenotype largely disappeared when *Ifngr1* KO cells and scramble cells were pooled together and infected with *Mtb*, which resembled the problems that appeared in our first trial. After optimization, the *Ifngr1* KO phenotype was partially restored in pooled cell infections. We then carried out the CRISPR library infection three times. Among the top enriched sgRNAs in the high *Mtb* burden macrophages were *Ifngr1*, *Ifngr2*, *Stat1*, *Stat2*, and *Irf1*. The successful enrichment of interferon-gamma receptors and JAK-STAT pathway genes prompted us to follow up with the screen results. Top-ranking sgRNAs are being validated by constructing gene knockout cells and individually infected with *Mtb* to identify genes essential for IFN- γ dependent restriction of *Mtb*.

PP-17

Impact of a tuberculosis molecular testing strategy on mortality: results of a planned secondary analysis of a cluster-randomized trial in Uganda

Tessa Mochizuki, UCSF

Email: tessa.mochizuki@ucsf.edu

Tessa Mochizuki^{1,2}, Talemwa Nalugwa³, Mariam Nantale³, Denis Oyuku³, Sarah Nabwire³, Diana Babirye³, Stavia Turyahabwe^{3,4}, Moses Joloba⁵, Annet Nakaweesa³, Adithya Cattamanchi^{2,6}, Katherine Fielding⁷, Achilles Katamba^{3,8}

1. University of California, San Francisco, Division of Pulmonary and Critical Care Medicine, San Francisco, United States of America
2. University of California, San Francisco, Center for Tuberculosis, San Francisco, United States of America
3. Makerere University College of Health Sciences, Uganda Tuberculosis Implementation Research Consortium, Kampala, Uganda
4. Uganda Ministry of Health, National Tuberculosis and Leprosy Program, Kampala, Uganda,
5. Makerere University College of Health Sciences, School of Biomedical Sciences, Kampala, Uganda
6. University of California, Irvine, Division of Pulmonary Diseases and Critical Care Medicine, Irvine, United States of America
7. London School of Hygiene and Tropical Medicine, Faculty of Epidemiology and Population Health and the TB Centre, London, United Kingdom of Great Britain and Northern Ireland
8. Makerere University College of Health Sciences, Clinical Epidemiology and Biostatistics Unit, Department of Medicine, Kampala, Uganda

Background: Tuberculosis remains a leading cause of infectious disease death worldwide. Rapid diagnosis of TB is important to improving outcomes and reducing transmission. Xpert MTB/RIF Ultra (Xpert) is a molecular assay that can provide TB results within two hours. Previous studies assessing the impact of Xpert on mortality were inconclusive.

Methods: In this planned secondary analysis of a pragmatic cluster-randomized trial in Uganda, we assessed whether a multi-component intervention strategy decreased mortality among adults evaluated for TB at community health centers. Ten health centers were randomized to the XPEL-TB intervention, which included on-site Xpert testing and implementation supports, and ten to maintain routine TB care, which included on-site smear microscopy and referral-based molecular testing. Vital status was assessed at least six months following TB evaluation through review of TB treatment registers, phone calls, and home visits by community health workers. Our outcome was the mortality rate, censored at 18 months. We performed cluster-level analyses, accounting for stratified randomization and patient-level covariates.

Results: Ascertainment of vital status was high; 9,268 of 10,644 (87%) participants had a known vital status and 325 deaths were observed. When adjusting for sex, age, HIV status, and cluster-level covariates, the mortality rate ratio was 0.77 (95% CI: 0.47—1.28), favoring the intervention. In sub-group analyses, mortality was significantly lower in the intervention arm among people living without HIV (aRR 0.50, 95% CI: 0.26—0.96) and was also lower among women (aRR 0.64, 95% CI: 0.33—1.23).

Conclusion: Due to the low number of deaths among people evaluated for TB at community health centers, we were unable to definitively demonstrate that on-site Xpert testing reduced mortality. Mortality may not be an appropriate outcome for diagnostic trials among non-hospitalized people. Future trials should focus on improvements in TB case detection and treatment initiation.

PP-18

Impact of *Mycobacterium tuberculosis* on monocyte differentiation *in vivo*

Alex Mohapatra, UCSF

Email: alexander.mohapatra@ucsf.edu

A. Mohapatra, W. Zheng, Z. Howard, I. Chang, L. Chen, and J. D. Ernst; Division of Experimental Medicine, University of California, San Francisco

The emergence of multidrug-resistant *Mycobacterium tuberculosis* (Mtb) highlights a critical need to develop new therapies that enhance the immune response to infection. The mechanisms used by Mtb to evade elimination are incompletely understood. In the lungs of Mtb-infected mice, we have shown that monocytes recruited from the blood differentiate into two populations and that one of these populations harbors the majority of viable Mtb. Our goal is to better understand monocyte differentiation in the infected lung to develop therapies that skew differentiation toward cells that restrict, rather than permit, Mtb growth. Emerging evidence suggest that monocyte differentiation may be predetermined by their progenitors before they enter the lung. We enumerated monocytes and their progenitors in the bone marrow (BM), the major source of blood monocytes in homeostasis, and found no difference between Mtb-infected and uninfected mice. Proliferation of BM monocytes and their progenitors, as assessed by incorporation of 5-ethynyl-2'-deoxyuridine, also did not differ between Mtb-infected and uninfected animals. However, these populations all significantly increase in the spleens of Mtb-infected mice. We also found that monocytes from the BM and spleens of Mtb-infected mice have distinct expression of proteins known to be differentially regulated in lung monocyte-derived cells. We hypothesize that pulmonary infection induces systemic changes in the peripheral monocyte pool that skew lung monocyte-derived cells toward a Mtb-permissive state. Further, lung cells derived from splenic monocytes may be predisposed to a permissive phenotype. To begin to test these hypotheses, we are interrogating lung monocyte-derived cells in Mtb-infected mice following adoptive transfer of monocytes and splenectomy. These and future studies will clarify how monocytes from the BM and spleen contribute to Mtb control in the lungs

PP-19

Insights into *Mycobacterium tuberculosis* DNA extraction for targeted deep sequencing using the Deeplex Myc-TB assay: Lessons for improved drug resistance diagnosis

Alina Nalyvayko, UCSF

Email: alina.levin@ucsf.edu

Jason D Limberis^{1*}, Alina Nalyvayko^{1*}, Janré Steyn², Jennifer Williams², Melanie Grobbelaar², Robin M Warren², John Z Metcalfe³⁺

¹Division of Experimental Medicine, University of California, San Francisco, San Francisco, CA, USA

²DSI-NRF Centre of Excellence for Biomedical Tuberculosis Research, SAMRC for Tuberculosis Research, Division of Molecular Biology and Human Genetics, Faculty of Medicine and Health Sciences, Stellenbosch University, Cape Town, South Africa

³Division of Pulmonary and Critical Care Medicine, Zuckerberg San Francisco General Hospital and Trauma Centre, University of California, San Francisco, San Francisco, CA, USA

*Contributed equally

Tuberculosis (TB) is a significant public health concern causing 1.5 million deaths annually. Targeted next generation sequencing (NGS) has revolutionized our understanding of the genetic basis and identification of drug-resistant TB. However, to achieve accurate results reliable enough for clinical implementation, it is essential to extract high-quality *Mycobacterium tuberculosis* DNA from patient-derived samples, often in minimally resourced routine laboratories. We aimed to determine the optimal method for extracting *M. tuberculosis* DNA from both early MGIT culture-positive samples and directly from patient sputum for downstream use in the Deeplex Myc-TB targeted NGS assay, a commercial assay enabling the targeted sequencing of complete genes for drug resistance determination. We performed several representative DNA extraction protocols, including bead-beating and heat lysis, ethanol precipitation and magnetic bead cleanup, and the Deeplex Myc-TB user manual (v5) suggested method. Our findings provide valuable insights into the optimal DNA extraction method for utilization of Deeplex Myc-TB in routine laboratory settings and can inform future experiments evaluating newer generation assays

PP-20

Elucidating the mechanism behind reversible morphology switching in *Mycobacterium avium*

Hannah Nilsson, UC Berkeley

Email: hannah_nilsson@berkeley.edu

Mycobacterium avium (Mav) is an emerging environmental pathogen highly adapted to a wide range of niches, from treated water systems to mammalian tissues. On solid media, Mav forms two distinct colony morphologies, smooth transparent (SmT) and smooth opaque (SmO). These colony morphologies correlate with a broader set of phenotypic states in which SmT cells are more virulent and have greater resistance to antibiotics while SmO cells grow faster than SmT cells in culture. Importantly, Mav interconverts freely between these two metastable morphotypes. The mechanism by which Mav switches between SmT and SmO represents a decades-long conundrum in the field. Here we show that SmT-SmO switching is governed by a reversible transposition event that regulates expression of an enigmatic lipoprotein termed Erp (extracellular repetitive protein). We found that transposition of IS1245, an endogenous insertion sequence, into the erp locus correlated with the SmT-SmO transition, and its precise removal correlated with the switch back to SmT. Genetic studies showed that Erp is required for maintenance of the SmT state and sufficient to drive the switch from SmO to SmT. From a genetic screen we found that the MarP periplasmic protease is required for Erp-mediated switching from SmO to SmT. Thus, we have identified the first three components of an unprecedented regulatory mechanism that controls Mav colony morphology switching, antibiotic resistance and virulence.

PP-21

Distinct *M. tuberculosis* antigens determine human CD4⁺ T cell differentiation

Paul Ogongo, UCSF

Email: Paul.Ogongo@ucsf.edu

Paul Ogongo¹, Anthony Tran¹, Jason Limberis¹, Devin Columbus¹, and Joel D. Ernst

¹Division of Experimental Medicine, University of California San Francisco, 1001 Potrero Avenue, CA 94110

Efforts to identify protective antigens/epitopes against tuberculosis have been hampered by overreliance on studies of a few immunodominant *M. tuberculosis* (*Mtb*) antigens (out of > 4,000 proteins). We stimulated PBMC from recently exposed, QFT⁺ HIV⁻ human adults with distinct *Mtb* antigens; four immunodominant antigens (classical); ESAT-6, PPE18, PPE46, and Espl, and four novel antigens (antigens with T cell epitope sequence variation demonstrating evidence of evolutionary diversifying selection); RimJ, Rv0012, Rv0010c and LldD2, and assessed CD4⁺ T cell differentiation and functionality by intracellular cytokine staining for TNF- α , IFN γ , IL17, and GM-CSF. We discovered that CD4⁺ T cell responses to distinct *Mtb* antigens are highly variable but, unexpectedly, classical antigens induce predominant Th1 while novel antigens elicit predominant Th17 cell responses. Specifically, the novel antigens responses are characterized by IL17 production, expression of ROR γ T, and CCR6, while classical antigens exhibit IFN γ production, expression of T-bet, and CXCR3. Whereas IL17 and IFN γ responses are enriched in novel and classical antigens respectively, TNF- α and GM-CSF responses are prevalent in both classes of antigens. In additional analyses, we uncover that CD4⁺ T cells that respond to *Mtb* antigens by producing IFN γ exhibit a predominantly central memory phenotype, while cells that produce IL17 in response to the same antigens are evenly distributed in effector and central memory phenotypes. This contrasts with Staphylococcus Enterotoxin B (SEB)- IFN γ and IL17 responsive cells that are predominantly effector memory. Studies to determine whether these differences in CD4⁺ T cell responses determine the outcome of *Mtb* infection, progression to active TB disease or non-progression are ongoing.

PP-22

Mtb-human protein-protein interactions modulate bacterial virulence and host immune responses

Abby Ray, UC Davis

Email: abray@ucdavis.edu

Abby Ray¹, Bennett Penn, MD, PhD¹

¹University of California Davis, Department of Internal Medicine

Mtb has the surprising ability to survive and proliferate in the harsh environment of the alveolar macrophage and establish a chronic, often lifelong, infection. Unfortunately, the molecular mechanisms underlying this ability to subvert host defenses remain obscure. The **central hypothesis** of this project is that *Mtb* secretes protein virulence factors into the macrophage to specifically disrupt immune function by selectively targeting mediators of host immunity.

To identify which secreted proteins are involved in virulence, secreted bacterial proteins were tagged and resolved using affinity purification mass spectrometry (AP-MS). The interacting host proteins were also identified, and hundreds of high-specificity interactions were mapped. The goal of this project is to screen through this dataset of 34 biochemically validated bacterial proteins to identify novel virulence factors for subsequent mechanistic studies.

To test the hypothesis that this interactome contains protein virulence factors involved in *Mtb* pathogenesis, wildtype *Mtb* was engineered to delete a single gene of interest, while also inserting a unique barcode. 8 of these barcoded strains were combined into a mixed inoculum at a fixed ratio and this pool was used to infect mice. At multiple timepoints during the 12-week time course, lung and spleens were collected, homogenized, and plated on agar. Bacteria were then harvested from these organ homogenate plates and gDNA was extracted. Then using a barcoded qPCR approach, relative strain proportion was used to identify knockout strains with a fitness disadvantage, thus indicating a possible immunological role for the protein of interest.

Initial experiments with known controls validated that the methodology and approach are sound. The genetic approach to creating knockouts has been optimized and has significantly expedited and improved upon previous techniques. Genes with modest phenotypes (*Rv1075c* and *apa*) are being repeated in subsequent infections to confirm the initial results, while work continues for unscreened genes of interest.

PP-23

Latent tuberculosis infection and hypertension in the US National Health and Nutrition Examination Survey

Argita Salindri, Stanford (not presented)

Email: adsalind@stanford.edu

Argita D. Salindri¹, Sara C. Auld^{2,3,4}, Unjali P. Gujral⁴, Elaine M. Urbina⁵, Jason R. Andrews¹, Moises A. Huaman⁶, Matthew J. Magee^{3,4}

Affiliations:

1. Division of Infectious Diseases and Geographic Medicine, Department of Medicine, Stanford University School of Medicine, Stanford, CA, USA
2. Division of Pulmonary and Critical Care Medicine, Emory University School of Medicine, Atlanta, GA, USA
3. Department of Epidemiology, Emory University Rollins School of Public Health, Atlanta, GA, USA
4. Hubert Department of Global Health, Emory University Rollins School of Public Health, Atlanta, GA, USA
5. GA, USA
6. Department of Pediatrics, College of Medicine, University of Cincinnati, Cincinnati, OH, USA
7. Department of Internal Medicine, College of Medicine, University of Cincinnati, Cincinnati, OH, USA
8. USA

Abstract Summary:

Determining the association between latent tuberculosis infection (LTBI) and hypertension may provide insights into how LTBI increases cardiovascular diseases (CVD) risks. We reported a high prevalence of hypertension (59%) among adults with LTBI in the U.S. Hypertension prevalence was also higher among adults with LTBI and no CVD risk factors.

Background: Latent Tuberculosis infection (LTBI) is marked by dynamic host-pathogen interactions with persistent low-grade inflammation and is associated with increased risk of cardiovascular diseases (CVD) including acute coronary syndrome, myocardial infarction, and stroke. However, few studies assess the relationship between LTBI and hypertension, an intermediate of CVD. We sought to determine the association between LTBI and hypertension using data representative of the adult US population.

Methods: We performed cross-sectional analyses using data from the 2011–2012 US National Health and Nutrition Examination Survey (NHANES). Eligible participants included adults with valid QuantiFERON-TB Gold In-Tube (QFT-GIT) test results who also had blood pressure measures and no history of TB disease. LTBI was defined by a positive QFT-GIT. We defined hypertension by either elevated measured blood pressure levels (i.e., systolic ≥ 130 mmHg or diastolic ≥ 80 mmHg) or known hypertension indications (i.e., self-reported previous diagnosis or use of antihypertensive medications). Analyses were performed using robust quasi-Poisson regressions and accounted for the stratified probability sampling design of NHANES.

Results: The overall prevalence of LTBI was 5.7% (95%CI 4.7–6.7) and hypertension was present among 48.9% (95%CI 45.2–52.7) of participants. The prevalence of hypertension was higher among those with LTBI (58.5%, 95%CI 52.4–64.5) than those without LTBI (48.3%, 95%CI 44.5–52.1) (prevalence ratio [PR]=1.2, 95%CI 1.1–1.3). However, after adjusting for confounders, the prevalence of hypertension was similar for those with and without LTBI (adjusted PR=1.0, 95%CI 0.9–1.1). Among individuals without CVD risk factors of elevated BMI (PR_{normal BMI}=1.6, 95%CI 1.2–2.0), hyperglycemia (PR_{euglycemia}=1.3, 95%CI 1.1–1.5), or cigarette smoking (PR_{non-smokers}=1.2, 95%CI 1.1–1.4), the unadjusted prevalence of hypertension was higher among those with LTBI vs. no LTBI (Figure 1).

Conclusions: More than half of adults with LTBI in the US had hypertension. Importantly, we observed a relationship between LTBI and hypertension among those without established CVD risk factors.

PP-24

A multi-country evaluation of patient preferences for future TB diagnostic tests using a discrete choice experiment

Kinari Shah, UCSF

Email: kinari.shah@ucsf.edu

Kinari Shah¹, Talemwa Nalugwa², Danaida Marcelo³, Robinah Nakawunde⁴, Trang Trinh⁵, Deepa Shankar⁶, Annet Nakaweesa², Ann Schraufnagel¹, Charles Yu³, Maria del Mar Castro Noriega⁷, Robert Castro¹, Catherine Cook¹, Rebecca Crowder¹, Jillian Kadota¹, Tessa Mochizuki¹, Swati Sudarsan¹, Jason Andrews⁸, Niaz Banaei^{8,9}, Devan Jaganath¹⁰, Midori Kato-Maeda¹, John Metcalfe¹, Payam Nahid¹, Patrick Phillips¹, Christina Yoon¹, Andrew D. Kerkhoff¹¹, Adithya Cattamanchi^{1,12}

1. Division of Pulmonary Diseases and Critical Care Medicine, University of California San Francisco, San Francisco, CA USA
2. World Alliance for Lung and Intensive Care Medicines In Uganda (WALIMU), Kampala, Uganda
3. Research Services, De La Salle Medical and Health Sciences Institute, Cavite, Philippines
4. Division of Molecular Biology and Human Genetics, Stellenbosch University, Cape Town South Africa
5. Vietnam National Tuberculosis Program, University of California San Francisco Research Collaboration Unit; Center for Promotion of Advancement of Society, Hanoi, Vietnam
6. Department of Pulmonary Medicine, Christian Medical College, Vellore, Tamil Nadu, India.
7. University of Heidelberg, Heidelberg, Germany.
8. Division of Infectious Diseases and Geographic Medicine, Department of Medicine, Stanford University School of Medicine, Stanford, CA USA.
9. Department of Pathology, Stanford University School of Medicine, Stanford, CA USA.
10. Division of Pediatric Infectious Diseases, University of California, San Francisco, USA.
11. Division of HIV, Infectious Diseases, and Global Medicine, University of California San Francisco, San Francisco, CA USA
12. Division of Pulmonary Diseases and Critical Care Medicine, University of California, Irvine, USA.

Background: The WHO recognizes new and improved diagnostics as a key step to reducing the global TB burden, developing target product profiles (TPPs) to address key priorities. TPPs include the perspectives of providers, product developers, and officials, but not the preferences of people undergoing TB testing. Understanding preferences of people affected by TB is needed to optimize the acceptability and uptake of diagnostic services in high TB burden countries.

Design/methods: We present the preliminary results of an ongoing discrete choice experiment (DCE) among adults with presumptive or microbiologically confirmed TB attending outpatient clinics in 5 countries (Philippines, Vietnam, South Africa, Uganda, and India). The DCE evaluated preferences for 5 attributes related to TB diagnostic test features (sample type, accuracy, cost, location, time to result) with 3-4 levels per attribute. We performed latent class analysis to identify groups with unique preferences and estimated willingness-to-trade for preferred test features.

Results: Among 317 participants (median age 41 years, 53% male, 11% with HIV, 15% with diabetes), 2 groups were identified with distinct preferences for TB diagnostics. Group 1 (66%;n=210) had strong preferences for free, non-sputum-based testing at the health facility, while Group 2 (34%;n=107) strongly preferred sputum-based testing with high test accuracy and testing performed at home or at a community location. Preferences varied by country. Participants in Uganda had stronger preferences for sputum-based testing at a health facility, while participants in Vietnam and the Philippines favored tongue swab or urine-based tests. Willingness-to-trade analyses demonstrated that group 1 would trade lower TB test accuracy for non-sputum-based tests and same-day results.

Conclusions: Persons across 5 countries accessing TB testing services strongly valued same-day test results and a distinct group comprising a majority of individuals valued tongue-swab-based testing, even if this meant the TB test was less accurate. These findings should inform future TPPs and TB diagnostic test development.

PP-25

A prospective multi-country comparison of automated chest x-ray algorithms for the triage of pulmonary tuberculosis

Swati Sudarsan, UCSF

Email: Swati.Sudarsan@ucsf.edu

Swati Sudarsan^{1,2}, William Worodria³, Victoria Dalay⁴, Brigitta Derendinger⁵, Balamugesh Thangakunam⁶, Hanh T T Nguyen⁷, Sandra Vivian Kik⁸, Claudia M. Denkinge^{9,10}, Adithya Cattamanchi^{2,11}, DJ Christopher⁵, Devan Jaganath^{1,2,3}

1. Division of Pulmonary and Critical Care Medicine, San Francisco General Hospital, University of California San Francisco, San Francisco, USA
2. Center for Tuberculosis, University of California San Francisco, San Francisco, USA
3. Walimu and Makerere University College of Health Sciences, Kampala, Uganda
4. De La Salle Medical Health Sciences Institute, Center for Clinical Epidemiology and Biostatistics, Dasmariñas City, Philippines.
5. DSI-NRF Centre of Excellence for Biomedical Tuberculosis Research, SAMRC Centre for Tuberculosis Research, Division of Molecular Biology and Human Genetics, Faculty of Medicine and Health Sciences, Stellenbosch University, Cape Town, South Africa
6. Department of Pulmonary medicine, Christian Medical College Vellore, Tamil Nadu, India
7. Research Collaboration Unit between University of California San Francisco (UCSF) and Vietnam National Tuberculosis Control Program (VNTP)/Center for Promotion of Advancement of Society (CPAS), Hanoi, Vietnam
8. FIND, Geneva 1202, Switzerland
9. Division of Infectious Disease and Tropical Medicine, Heidelberg University Hospital and Faculty of Medicine, Heidelberg University, Heidelberg, Germany
10. German Centre for infection Research (DZIF), Partner site Heidelberg University Hospital, Heidelberg, Germany
11. Division of Pulmonary Diseases and Critical Care Medicine, University of California Irvine, Irvine, USA
12. Division of Pediatric Infectious Diseases, University of California, San Francisco, San Francisco, USA

Abstract Summary: An effective TB triage test could increase TB diagnosis and improve TB-related morbidity and mortality. In a multi-country study of outpatients with presumptive TB, we compared the diagnostic accuracy for pulmonary TB of three computer-aided detection (CAD) algorithms for reading CXRs.

Abstract Body:

Background: Computer-aided detection (CAD) algorithms for chest X-ray (CXR) reading have been endorsed as a TB triage test by the World Health Organization (WHO), but independent, head-to-head comparisons are needed, including among key risk groups.

Design/methods: We enrolled adults with cough ≥ 2 weeks presenting to clinics in Uganda, South Africa, Vietnam, the Philippines, and India. We obtained a CXR and collected sputum for TB testing (Xpert MTB/RIF Ultra [Xpert]; and two liquid cultures if Xpert-negative). We applied three CAD algorithms (Lunit INSIGHT CXR v3 (Lunit), qXR v3, and CAD4TB v7) to CXRs, and selected the optimal cut-point (overall and by country) to maximize specificity at 90% sensitivity in reference to sputum Xpert and culture results.

Results: Among 2219 participants, median age was 41 years (IQR 29-53), 972 (44%) were female, 307 (14%) were living with HIV, 298 (13%) were living with diabetes, and 518 (23%) had confirmed TB. Overall, at 90% sensitivity, Lunit had significantly higher specificity (72.5%, 95% CI 70.2-74.7) than qXR (65.7%, 95% CI 63.3-68.0, $p < 0.001$) and CAD4TB (68.3%, 95% CI 65.9-70.6, $p = 0.001$). Specificity varied by country for each algorithm (Lunit: range 59.5-82.1%; qXR: 55.4-73.2%; CAD4TB: 57.2-84.9%) when using the overall optimal cut-point. However, specificity was $> 70\%$ in South Africa, Vietnam and the Philippines when using country-specific cut-points (Table 1). Lunit had similar or higher specificity than CAD4TB and qXR among people living with HIV and people living with diabetes when using the overall optimal cut-point and when risk group-specific cut-points (Table 1).

Conclusions: CAD algorithms achieved or approached the TB triage test target product profile accuracy overall and in key high-risk groups, but usually only at dedicated cut-points for each group. Lunit performed best, but there was substantial heterogeneity by country and subgroup, highlighting the need for country-led evaluations in different target populations prior to implementation

PP-26

Longitudinal time to positivity as biomarker and surrogate endpoint for predicting unfavorable outcome in drug susceptible tuberculosis treatment

Pieter Van Brantegem, UCSF

Email: pieter.vanbrantegem@ucsf.edu

Pieter Van Brantegem, Vincent Chang, Belén P. Solans, Rada Savic

Department of Bioengineering and Therapeutic Sciences, University of California San Francisco, California, USA

Sputum culture conversion is considered the gold standard biomarker to evaluate the efficacy of tuberculosis treatment (1). However, it has limitations in accurately predicting unfavorable outcome, particularly in patients with comorbidities (2). This study focuses on investigating the utility of longitudinal time-to-positivity (TTP) as a biomarker and surrogate endpoint for predicting unfavorable outcomes in tuberculosis treatment. Data from S31/A5349 (NCT02410772) was used. This is a large phase-3 randomized controlled trial that investigated a 4-month regimen containing rifapentine with (HPZM) or without moxifloxacin (HPZE), compared to the standard 6-month care regimen (HRZE) (3). Non-linear mixed effects models were developed to describe unfavorable outcomes and longitudinal TTP, separately for each treatment arm. A time-to-event model with Weibull hazard function and a linear model described best the time-to-unfavorable outcome and longitudinal TTP data, respectively. GeneXpert cycle threshold (baseline disease burden), impacts both baseline TTP and slope, irrespective of treatment arm. Other variables influencing baseline TTP or slope are presence of cavities, cavity size, African site, race, age and sex, dependent on the treatment arm. The inclusion of the individual predicted TTP slope from its respective model significantly improved the prediction of unfavorable outcome, regardless of treatment arm or other predictors (univariable AUC ROC 0.69, 0.74 and 0.70 for HRZE, HPZE and HPZM, respectively). Interestingly, while the HPZM arm demonstrated non-inferiority to the HRZE arm in terms of primary treatment outcomes, the TTP slopes of patients in the HPZE and HPZM arms are higher than those in the HRZE arm. This suggests a discrepancy between the overall trend of longitudinal TTP and unfavorable outcome. In conclusion, longitudinal TTP contributes to the prediction of unfavorable outcome, but its use is limited and cannot be solely relied upon to forecast unfavorable outcome in a clinical trial.

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PP-27

The role of ESX-1 and PDIM in T cell response to *Mycobacterium tuberculosis* infection

Alex Zilinskas, UC Berkeley

Email: alex_zilinskas@berkeley.edu

Mycobacterium tuberculosis (Mtb) is the causative agent of tuberculosis. Mtb infections generally results in a Th1-mediated response; however, the Stanley lab has previously shown that mice vaccinated with CDN adjuvant and Mtb antigens had enhanced protection mediated by Th17 cells. The ESX-1 (type 7) secretion system, involved in the secretion of many Mtb virulence factors, and lipid virulence factor phthiocerol dimycocerosate (PDIM) are important for Mtb pathogenesis. We show that mice infected with Δ ESX-1 or PDIM-lacking Mtb have lower bacterial burden and a Th17-mediated response compared to WT Mtb infection. We also show that Tbet^{-/-} mice (lacking Th1 response) had a Th17-mediated response to WT Mtb, while maintaining a similar bacterial burden compared to WT mice demonstrating Th17s fully compensated for lack of Th1s. We hypothesize that Mtb directs the host CD4 T cell response, via the ESX-1 secretion system and PDIM, towards a Th1-mediated response for higher bacterial survival, instead of a more protective Th17-mediated response.