BAY AREA TUBERCULOSIS SCIENCE SYMPOSIUM

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SEPTEMBER 26, 2024 UCSF MISSION BAY

E-BOOK OF ABSTRACTS

Table of contents

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Symposium program

BAY AREA TUBERCULOSIS SCIENCE SYMPOSIUM
UCSF · UC BERKELEY · UC DAVIS · STANFORD

Thursday, September 26, 2024
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 Executive Director, Institute for Global Health Sciences	**Keynote Introduction**
9:30-10:15 AM	**Dr. Megan Murray, MD, ScD** Adjunct Professor of Immunology and Infectious Diseases, Harvard University	**Keynote talk** **"**Genomic Epidemiology of TB in Peru"
10:15-10:55 AM	**Session moderator:** **Sara Suliman, PhD, MPH** Assistant Professor, UCSF	**Invited talks**
	Russell Vance, PhD Professor, Department of Molecular & Cell Biology UC Berkeley, and HHMI Investigator	"The innate immune response during tuberculosis"
	Brenna Henn, PhD Associate Professor, Department of Anthropology, UC Davis	"Illuminating genome-wide host genetic architecture of TB susceptibility"
10:55-11:25 AM	**Coffee Break:** 30min	
11:25 AM-12:10 PM	**Session moderator:** **Bennett Penn, MD, PhD** Assistant Professor, UC Davis	**Oral Abstracts**
	Caitlin Moe, MS, PhD Academic Specialist, UC Irvine/UCSF	"A vibroacoustic signature to classify tuberculosis severity: development and multi-country evaluation"
	Paul Ogongo, PhD Assistant Researcher, Medicine, UCSF	"Increased circulating Th17 cells and differential skewing of CD4 T cell maturation and proliferation in tuberculosis patients with type 2 diabetes"

	Nicholas Bates, BS Graduate Student, UC Davis	"The catalase-peroxidase KatG is a regulator of multidrug antibiotic tolerance in Mycobacterium abscessus"
12:10-1:40 PM	**Lunch and Poster Session**: 90min	
1:40-2:40 PM	**Sarah Hutch**, Associate Director UCSF Center for Tuberculosis	**Networking Session**
2:40-3:10 PM	**Coffee Break:** 30min	
3:10-4:30 PM	**Session moderator:** **Babak Javid, MB BChir, PhD** Professor, Medicine, UCSF	**Invited talks**
	Antonio Pagán, PhD Assistant Professor, Department of Microbiology and Immunology, Stanford University	**Winner, 2024 BATS Rising Star Award (award presented by Babak Javid)** "'Non-canonical' roles of autophagy-related proteins in anti-mycobacterial immunity"
	Amy Tang, MD Director of Immigrant Health North East Medical Services, San Francisco	"Can Primary Care Drive TB Elimination? A Community Health Center's Experience with Scaling Up TB Prevention and Care Services for Non-US Born Populations"
	Jonathan Izudi, MPH, MSc, PhD Honorary Research Fellow, Department of Community Health, Faculty of Medicine, Mbarara University of Science and Technology, Uganda. UC TRAC Global Fellow	"Negative effects of undernutrition on sputum smear conversion and treatment success among retreatment cases in Uganda: a quasi-experimental study"
	Andrew Kerkhoff, MD, PhD, MS Assistant Professor, Medicine, UCSF	"Needs and Wants: Integrating Preferences to Advance People-Centered TB Research and Services"
4:30-4:35 PM	**Devan Jaganath, MD, MPH** Assistant Professor, UCSF	**Poster Awards**: 5min
4:35-4:40 PM	**Babak Javid**, MB BChir, PhD, UCSF	**Closing Remarks**
4:40-6:00PM	**Wine and cheese reception**	

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Oral presentations

OP-01: Nick Bates, UC Davis

The catalase-peroxidase KatG is a regulator of multidrug antibiotic tolerance in Mycobacterium abscessus

Nick Bates, UC Davis

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Nicholas A. Bates[1]; Bennett H. Penn[1]

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In Mycobacteria and beyond, stress conditions leading to a non-replicating cell state are associated with multidrug tolerance—the ability to survive high dose antibiotics for an extended period. Importantly, it is hypothesized that this occurs during in vivo infection, where bacteria encounter many immune system-imposed stressors. In the case of both M. tuberculosis and Nontuberculous Mycobacterial lung disease, antibiotics which kill the vast majority of bacteria within a few days in vitro must be taken for numerous months or even years in vivo. We sought to study this phenomenon by modeling M. abscessus stress induced antibiotic tolerance in vitro and conducting loss of function genetic screens. These screens revealed numerous genes and biological pathways which may contribute to stress induced antibiotic tolerance. One notable result was the catalase-peroxidase gene katG, whose ablation resulted in faster killing with the ribosome targeting antibiotics tigecycline and linezolid. This enhanced killing was observed both in rich media and under nutrient starvation. However, no differential killing phenotype was observed under hypoxia. Additionally, KatG deficient bacteria also displayed enhanced killing phenotypes with the antibiotics levofloxacin and rifabutin, which have distinctive mechanisms of action. Overall, these data indicate that KatG is a regulator of multidrug antibiotic tolerance in in the nontuberculous Mycobacteria species M. abscessus.

OP-02: Caitlin Moe, UCSF

A vibroacoustic signature to classify tuberculosis severity: development and multi-country evaluation

Caitilin Moe, UCSF

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9. De la Salle Medical and Health Sciences Institute- Dasmarinas, Philippines
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Background: TB patients with less severe illness may be eligible for novel shorter treatment regimens, but classification of easier- vs. harder-to-treat phenotypes is currently dependent on chest X-ray (CXR). This hurdle impedes evaluation in many settings and therefore support for trials of stratified treatment approaches. We evaluated the accuracy of a vibroacoustic signature to classify TB severity using a point-of-care (POC) electronic stethoscope.

Methods: Between March 2022-October 2023, we prospectively enrolled people ≥12 years with presumptive TB at health centers in Uganda, South Africa, India, Vietnam, and the Philippines. All participants underwent CXR, which was read by an independent radiologist to identify key features of TB severity. The imPulse UNA electronic stethoscope was used to collect three minutes of audible sounds and vibrations at six chest sites (30 seconds per site) of each participant. We trained a machine learning model to use vibroacoustic data to predict severe disease, defined as lesion(s) involving ≥50% of the thoracic cavity on CXR. A separate test dataset of participants with TB was used to assess the accuracy of the model.

Results: We collected vibroacoustic data for 1,346 participants, with median age 39 (IQR 29-52), 45% female, and 12% living with HIV. The vibroacoustic model achieved an area under the receiver operating characteristic curve of 0.856 (95% CI 0.769-0.934) in the test dataset, with a sensitivity of 84.0% (95% CI 68.0%-96.0%) and specificity of 83.5% (95% CI 75.3%-91.6%). Figure 1 shows visible differences in the vibroacoustic data depending on TB disease severity.

Conclusions: A vibroacoustic signature that utilizes a POC electronic stethoscope shows strong potential to replace CXR in current TB treatment stratification algorithms. With further optimization, this simple tool could provide support for TB drug development clinical trials and implementation of shorter TB regimens.

OP-03: Paul Ogongo, UCSF

Increased circulating Th17 cells and differential skewing of CD4 T cell maturation and proliferation in tuberculosis patients with type 2 diabetes

Paul Ogongo, UCSF

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Although people with diabetes are 3x at greater risk of developing tuberculosis (TB) than those without diabetes, the immunological mechanisms of this increase are not completely defined. Type 2 diabetes (T2DM), the most prevalent form of diabetes, is a result of complex metabolic disorders but obesity with low-grade inflammation and excess free fatty acids which promote insulin resistance exacerbate poor outcomes. CD4 T cells play a critical role in responses essential for controlling initial *Mycobacterium tuberculosis (Mtb)* infection and limiting bacterial growth after the infection is established. Chronic hyperglycemia, a hallmark of T2DM, alters T cell functions including the ability to activate innate cells and enhance their bactericidal activity. Using high-dimensional spectral flow cytometry, we combined expertise in TB immunology and TB-T2DM epidemiology to characterize peripheral CD4 T cell responses in a well-characterized clinical cohort of T2DM participants with and without TB. We discovered that people with TB-T2DM had increased circulating Th17 cell subsets, presented differences in T cell differentiation (e.g. lower naïve T cells and higher central memory T cells), exhibited higher T cell activation, and had increased proliferation of *Mtb*-antigen responsive CD4 T cells. When we used HbA1c levels (%) to group participants into no diabetes (<5.5%), prediabetes (5.7 – 6.4%), and diabetes (>6.5%), we observed a 'dose-response' trend in the increase in circulating Th17 subsets and T cell activation and decrease in the circulating naïve T cells. Finally, we found a significant association between glycemic index (% HbA1c multiplied by the number of years with T2DM) with CD4 T cell activation, circulating naïve T cells, and a trend toward decreased Th17 subsets. Our results reveal that chronic T2DM alters distinct features of CD4 T cells differently which may affect the ability of T cells to effectively control TB. Improved understanding of immunological mechanisms of diabetes-associated risks of TB will inform the development of host-targeted therapies to improve *Mtb* infection outcomes in people with T2DM. Additionally, we hypothesize that discovering immunological mechanisms that increase the risk of TB in T2DM may also guide the discovery of mechanisms that increase TB risk independent of T2DM.

Poster presentations

PP-01: Rachel Abbott, UCSF

Characteristics of incident Tuberculosis (TB) infections among children and adolescents in the SONET study, a longitudinal cohort in rural Southwestern Uganda

Rachel Abbott, UCSF

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Background: An estimated 80%-90% of child TB infections are acquired outside the household, but population-level empirical data in East Africa is limited. In SONET, an ongoing longitudinal cohort of children and adolescents (1-17 years) in 25 villages in rural Uganda, we sought to describe the (1) proportion with incident TB infection, (2) proportion of incident TB infections attributable to a household source and to (2) compare time spent in community-venues between children with and without incident TB infection.

Methods: In SONET, incident TB infection was defined as conversion from negative QuantiFERON PLUS (QFT) at baseline to positive QFT one-year later. TB screening with MTB/RIF GeneXpert Ultra testing was performed for household members aged 7+ living with a child with incident TB infection. Incident TB infections attributable to a known household source were defined as those with self-reported household TB contact in the last year or a household member with a positive Xpert. Participants with incident TB infection and a subsample of the cohort were administered a venue-time questionnaire. We compared median monthly-hours in community-venues (e.g. church) between participants with and without incident TB infection. Caregiver venue-time was used as a proxy for children 1-11 years; adolescents (12-17 years) self-reported venue-time.

Results: Between June 2023-June 2024, 1,754/2,676 (65.5%) baseline QFT-negative participants completed follow-up QFTs, and 50/1,754 (2.9%) had incident TB infection. Among participants with incident TB infection, 54.0% were female, 32.0% ages 1-5 years and 4 (8.0%) were attributable to a known household source: 2 reporting household TB contact and 2 with household members identified by screening. There was a trend toward higher monthly-time spent in community-venues among participants with incident TB (median=78.7 hours/month) than those without (median=61.7 hours/month), p=0.24.

Conclusions: In SONET, few incident TB infections were attributable to a known household source; Xpert screening alone and missingness in outcome ascertainment may underestimate this. There was a trend towards participants with incident TB spending more time in community-venues than those without, suggesting non-household social mixing patterns that may lead to greater exposure. Additional data and novel strategies to interrupt child TB transmission that occurs outside of the home are needed.

PP-02: Shaista Afzal, UCSF

Impact of HIV Acquisition and Timing of Antiretroviral Therapy on Tuberculosis Risk and Inflammatory Pathways: A Longitudinal Study in a Peruvian Cohort

Shaista Afzal, UCSF

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Background: Globally in 2022, tuberculosis (TB), caused by Mycobacterium tuberculosis (Mtb), was responsible for 12.8% of acquired immunodeficiency syndrome (AIDS)-related deaths, which was the leading cause of death in people living with human immunodeficiency virus (HIV). Despite viral suppression with antiretroviral therapy (ART), TB risk remains consistently elevated in people living with HIV and treated, compared to HIV-uninfected people. Studies have developed gene expression signatures to predict risk of TB disease in the presence and absence of HIV infection. It is unclear how HIV infection and time of ART initiation impacts inflammation and TB risk. Therefore, this study aims to determine the risk of TB disease associated with HIV acquisition and time on ART.

Methods: Study cohort: We are leveraging samples from men who have sex with men and transgender women to define how HIV infection and time of ART initiation impact TB risk. Participants were enrolled in Lima, Peru pre-HIV acquisition and evaluated for HIV infection monthly. Upon HIV infection, they were re-enrolled into a secondary study cohort aimed at determining "how early ART should be initiate" and randomized to receive ART soon after HIV diagnosis (early/immediate arm; within 24 weeks) or later (deferred arm), and followed up for a decade. Whole blood samples were collected in RNA later before HIV diagnosis, 3, 6,12, 24 and 48 months after HIV diagnosis. Experimental design: We extracted RNA from blood using the ambion Ampure extraction kit. We determined RNA concentration and quality by Nanodrop and Tapestation, respectively. We generated libraries using the poly (A) kits from New England Biolabs per manufacturer's instructions, and sent samples for next generation sequencing (NGS) at the San Francisco Chan-Zuckerberg Biohub.

Analysis plan: We will filter and align the fastq read files to the human reference genome to determine gene counts. We will determine differentially expressed genes using DESeq2 R package. We will also apply gene set enrichment analysis to define pathways associated with HIV acquisition, ART initiation with participants with and without latent Mtb infection.

Expected results: We expect that TB risk scores will increase after HIV acquisition, decrease with ART, but will remain elevated even after 4 years on ART compared to pre-HIV samples.

Significance: This study design is unique, where the cohort was followed before HIV infection and for a decade after, including samples at the time of ART initiation. With access to this comprehensive dataset, we will explore HIV-associated inflammatory pathways and mechanisms underlying TB risk. Thus, these new findings will inform development of novel therapies to mitigate HIV-associated immune dysfunction and TB risk in people living with HIV.

PP-03: Rania Bouzeyen, UCSF

Antibody-mediated immunity to early M. tuberculosis infection in mice depends on NLRP3 inflammasome formation.

Rania Bouzeyen, UCSF

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The molecular mechanisms by which pathogen-specific antibodies confer protection against intracellular Mycobacterium tuberculosis (Mtb) infection are not known. We previously demonstrated that monoclonal antibodies (mAbs) targeting the Mtb phosphate transporter subunit PstS1, isolated from a patient with active tuberculosis, could reduce bacterial burden in experimental tuberculosis infection including a human whole blood growth inhibition assay and murine aerosol challenge. Many potential antibody-mediated effector functions have been implicated in the protective activity of Mtb-specific antibodies, although mechanistic evidence for which functions mediate protection are lacking. Here, we show that PstS1-specific mAbs induce IL-1β release from Mtb-infected macrophages. Antibody-Mtb immune complexes could trigger ASC speck formation, indicative of inflammasome activation. Through complementary genetic knock-out and pharmacological inhibition approaches, we demonstrated that Mtb-mAb immune complexes specifically trigger NLRP3 inflammasome formation. The role of NLRP3 inflammasomes in immunity to TB in vivo is controversial. Importantly, inhibition of NLRP3 completely abrogated antibody-mediated protection in aerosol challenge of mice by Mtb but had no effect on immunity to Mtb in the absence of Mtb-specific antibodies. These data confirm that antibody-mediated immunity in early infection is a) mediated by NLRP3 inflammasome activation and b) distinct to immunity of naïve animals to TB. Together, these data identify the mechanism by which Mtb-specific antibodies mediate protection against tuberculosis in the early stage of infection and have implications for the rational design of TB vaccines that incorporate antibody-mediated immunity.

PP-04: Nick Campbell-Kruger, UC Berkeley

A conserved two-gene operon is essential for stress-induced lipid remodeling in the pathogen M. abscessus

Nick Campbell-Kruger, UC Berkeley

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Nick Campbell-Kruger[1], Sarah Stanley[1]

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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) or phosphatidylinositol mannosides (PIMs) 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, and that the mycomembrane's permeability and fluidity are markedly altered in this mutant. To determine the lipid cargo of LprG, the M. abscessus and M. tuberculosis proteins were purified and used to isolate lipids from M. abscessus cell lysate. Both proteins bound to an assortment of PIMs, lipids that are essential in mycobacteria. We show that this operon is important for PIM metabolism and remodeling in response to stress.

PP-05: Robert Castro, UCSF

The accuracy of the Uganda national treatment decision algorithm for childhood tuberculosis

Roberto Castro, UCSF

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Background: Diagnosing childhood tuberculosis (TB) is a challenge, and this led the Uganda national tuberculosis and leprosy program (NTLP) to develop a clinical treatment decision algorithm (TDA) for children. However, there is limited data on its accuracy and how it compares to new World Health Organization (WHO) TB TDAs for children.

Objective: To evaluate and compare the performance of the 2017 Uganda NTLP and 2022 WHO TDAs for TB among children in Kampala, Uganda.

Methods: We performed a secondary data analysis of children 0-14 years from Kampala, Uganda who underwent an evaluation for pulmonary TB disease (physical examination, chest x-ray (CXR), tuberculin skin testing, HIV testing, and respiratory specimen collection for Xpert MTB/RIF Ultra testing and culture) between September 2018 and November 2022. Children were classified as living with Confirmed, Unconfirmed, or Unlikely TB per National Institutes of Health (NIH) consensus definitions. We also applied the 2017 NTLP and 2022 WHO algorithms (A with CXR, B without CXR) to diagnose children <10 with and without TB, and calculated accuracy in reference to Confirmed vs. Unlikely TB, as well as a microbiological and composite reference standard. We compared the NTLP algorithm to the WHO treatment decision algorithms if implemented per current Uganda Ministry of Health guidelines.

Results: Overall, 699 children were included in this analysis with 64% under 5 years, 53% were male, 11% (74/669) were HIV positive, and 6% had severe acute malnutrition. Approximately, 57% had a history of TB contact, 38% had abnormal CXR and 12% were Xpert positive. The NTLP algorithm had a sensitivity of 97.9% (95% CI:96.4-99.4) and specificity of 25.9% (95% CI:21.2-30.7). If CXR was considered unavailable, the sensitivity was 97.9% (95% CI:96.4-99.4) and specificity was 28.1% (95% CI:23.2-33.0). In comparison, the WHO TDAs had similar sensitivity, but algorithm A was more specific (32.2%, 95% CI:26.9-37.5) and algorithm B was less specific (15.4%, 95% CI:11.3-19.5).

Conclusion: The Uganda 2017 NTLP and 2022 WHO algorithms perform similarly in settings where Xpert and CXR are available. Both algorithms are likely to lead to over-diagnosis of TB in children 0-14 years due to their low specificity.

PP-06: Javier Cattle, UCSF

The LTBI cascade of care among non-US born pregnant individuals in a community health center setting

Javier Cattle, UCSF

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Background: Tuberculosis (TB) remains a leading cause of death globally among people of reproductive age, with pregnant individuals at increased risk of progression of latent TB infection (LTBI) to active TB and poorer pregnancy related outcomes for mother and child. US guidelines recommend LTBI screening for individuals from places with high TB incidence, including those who are pregnant. However, the quality of LTBI care among pregnant individuals with LTBI in the US is unknown. The aim of this study is to describe the quality of LTBI care among non-US born (non-USB) pregnant individuals at Northeast Medical Services (NEMS), a community health center network serving primarily non-US born Asian patients in the San Francisco Bay Area.

Methods: Individuals receiving care at NEMS with pregnancies during December 01, 2020 – August 15, 2023, were identified using electronic health record data (n = 835) and followed through a cascade of steps in LTBI care using extracted routine clinical data. Individuals who were US born (n =114), had prior history of LTBI or TB (n = 76), and experienced pregnancy loss (n = 16) during the analysis period were excluded from the study.

Results: Among 620 eligible pregnant individuals identified at NEMS, most were non-US born Asian (83.7%), between the ages of 25-34 (69.6%), listed Chinese as their preferred primary language (66.9%), had only one pregnancy during the analysis period (97.7%), and reported being in the US for 10 years or less (58.2%).

A total of 518 individuals were screened for LTBI using recommended tests (83.5%). Of the 505 individuals who completed an LTBI test that was ordered (96.9%), 35 were positive for LTBI (6.9%); with 24 having follow up chest imaging test ordered (68.6%), and 23 having a chest image completed (95.8%). Nine eligible individuals initiated on treatment (25.7%) and 3 completed treatment (33.3%).

Conclusion: Most non-USB pregnant individuals were screened and tested during routine obstetric care at this community health center. Among this group, LTBI prevalence was lower than the general non-US born population. However, of those individuals diagnosed with LTBI, very few were initiated on treatment or completed TB preventive therapy. Rates of treatment initiation and completion were lower than in the general adult non-US born population. Additional research is needed to identify preferences for TB preventive care and barriers to provision of care for non-USB pregnant individuals in community health settings.

PP-07: Lelia Chaisson, UCSF

Sex differences in systematic screening for tuberculosis among antiretroviral therapy naïve people with HIV in Kampala, Uganda

Lelia Chaisson, UCSF

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Background: Systematic tuberculosis (TB) screening is recommended for all people with HIV (PWH) because of its potential to improve TB outcomes through earlier diagnosis and treatment initiation. As such, systematic screening may be particularly important for men, who experience excess TB prevalence and mortality compared to women worldwide. In this study, we aimed to evaluate whether there were sex differences in TB prevalence and severity, diagnostic accuracy of TB screening tools, and TB outcomes among PWH undergoing systematic TB screening in a high TB/HIV burden setting.

Methods: We enrolled and followed adults with HIV (CD4≤350 cells/μL) initiating antiretroviral therapy (ART) at two HIV/AIDS clinics in Kampala, Uganda. All participants underwent TB screening and sputum collection for TB testing (Xpert MTB/RIF [Xpert], culture). We evaluated diagnostic accuracy of four WHO-recommended TB screening strategies (symptom screen; C-reactive protein [CRP]; symptom screen followed by CRP if symptomatic [symptoms+CRP]; Xpert) for culture-positive TB and compared TB prevalence, days-to-treatment initiation, and 3-month mortality by sex.

Results: Of 1,549 participants, 727 (46.9%) were male and 236 (15.2%) had culture-positive TB. Compared to females, males had lower pre-ART CD4 counts (median 139 vs. 183 cells/μL, p<0.001), higher TB prevalence (20.5% vs. 10.6%, p<0.001), and higher mycobacterial load as measured by Xpert semi-quantitative grade (p=0.03). Sensitivity was high (≥89.8%) for all screening strategies except Xpert (Xpert sensitivity 57.2%) and did not differ by sex. Specificity varied widely from 13.9% for symptom screen to 99.2% for Xpert. In addition, specificity was significantly lower for males than females for symptom screen (difference -5%, 95% CI -8.7% to -1.3%), CRP (difference -14.5%, 95% CI -20.0% to -9.3%), and symptoms+CRP (difference -15.0%, 95% CI -20.2% to -9.7%). Among PWH with culture-positive TB, median days-to-treatment initiation (2 vs. 4, p=0.13) and 3-month mortality (9.4% vs. 9.2%, p=0.96) were similar for males and females.

Conclusions: Although ART-naïve males undergoing systematic screening had more advanced HIV and TB than females, days-to-TB treatment initiation and early TB mortality were similar, suggesting that systematic TB screening has the potential to reduce sex-based disparities in TB outcomes.

PP-08: Mohamad Dandan, UCSF

Context-specific inhibition of translation by kasugamycin in Mycobacterium tuberculosis

Mohamad Dandan, UCSF

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Objective: The main goal for this poster presentation is to highlight my recent work of how kasugamycin (ksg) regulates translation in Mycobacterium. Background and significance: Our rationale stems from the following observations that is guided by this central question: How does ksg a) inhibit translation initiation and b) increase translational fidelity in Mtb? 1) Errors in protein synthesis – mistranslation – in Mtb can cause phenotypic resistance to the first-line anti-TB drug rifampicin. Thus, how does ksg alter translational fidelity in Mtb? 2) We also propose ksg alters translation via distinct binding sites in a context-specific fashion in Mtb. Evidence in E. coli and T. thermophilus showed that ksg blocks the path of mRNA in the 30S ribosome. However, ksg's action may also be dependent on the sequence and structure of mRNA. The Gross lab indicated that ksg stalls ribosomal complexes at the translation start site dependent on the mRNA sequence. Thus, how ksg binds the path of mRNA near the decoding center to alter translation initiation in a context specific manner remains unknown in Mtb.

Hypothesis: Our central hypothesis is that ksg impacts translation fidelity and initiation that is dependent upon the mRNA initiation sequence, and the structure of the mRNA binding site in Mtb ribosomes.

Results: Although the structure of the ksg-bound ribosome has been solved for the E. coli and T. thermophilus ribosomes, we have reason to believe that solving the Mtb ksg-bound ribosome is important for the following reasons: a) our preliminary data suggests potentially one ksg binding site in Mycobacterium tuberculosis : 1 primary site in the 30S and in the 70S ribosome, whereas only 1 was observed with E. coli 70S ribosomes, b) our preliminary data suggests ksg occupies the mRNA binding channel that displaces the mRNA, c) in-vitro translation assays showed dose-dependent inhibition of translation by ksg. This suggests translation inhibition, and alteration of the decoding center properties.

Conclusion: Guided by our structural data, we expect to determine the context specific regulation of how ksg modulates translation initiation and fidelity during elongation in Mtb.

PP-09: Fatoumatta Darboe, UCSF

Determining the risk TB disease in persons living with HIV

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Background: Human Immunodeficiency Virus (HIV) acquisition increases the risk of developing tuberculosis (TB) disease up to 10-fold higher than the general population. Antiretroviral therapy (ART) decreases TB risk, but it remains higher than in HIV-uninfected persons, despite viral suppression. We, and others, have previously generated whole blood transcriptional signatures to predict TB disease in the presence and absence of HIV infection. These signatures can be used as markers of TB risk; thus, we aim to determine the risk of TB disease after HIV acquisition and impact of time of ART initiation on TB risk.

Method: We leveraged a unique biorepository of samples from a high-risk group who were followed up from pre-HIV acquisition up to 8 years on ART. Participants were randomized to receive ART within 24 weeks (immediate arm) or after 24 weeks (deferred arm). Whole blood in RNAlater was collected in participants from enrolment till up to 4 years after ART. We selected the following timepoints: baseline sample, the last timepoint before HIV diagnoses, immediately after HIV diagnosis, samples collected at 3,6,12, 24 and 48 months after HIV acquisition. Demographically matched cross-sectional control samples from HIV uninfected persons (n=20) and chronic HIV-infected persons (n=35) were also included. We extracted RNA using the Ribopure RNA purification kit and Illumina sequenced by the Chan Zuckerburg San Francisco Biohub genomics platform. We are aligning the raw data to generate gene counts for analysis of TB gene expression signatures and immune activation pathways. We expect a higher TB risk (as measured by whole blood transcriptomic signatures) in persons who acquire HIV infection, this risk will decrease after ART, but levels will remain elevated than pre-HIV levels. We will also report transcriptional pathways associated with early and late ART.

Conclusion: For the first time we will be able to evaluate how the risk of TB changes in persons after HIV acquisition, timing of ART initiation, and the impact of long-term ART on TB risk. This is a unique cohort and dataset, which will be a resource for understanding TB pathogenesis in individuals co-infected with Mtb and HIV.

PP-10: Suzanne Dufault, UCSF

Finding the optimal duration in TB treatment trials

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The optimal duration of treatment for tuberculosis (TB) must reflect a critical yet delicate balance: it must be long enough to achieve desirable efficacy yet short enough to prevent the development of toxicities, adverse events, and mitigate other arduous aspects related to patient burden. Historically, the approach used to determine duration of treatment for TB has been inefficient. Meeting the WHO's End TB target of developing shorter, more tolerable TB therapeutics will require rigorously re-evaluating best practices in duration-ranging trial designs and statistical methodologies. Many of the challenges in duration-ranging have parallels and proposed solutions in the field of dose-ranging where the literature is substantially more established and where the traditions of qualitative, pairwise comparison studies have been replaced with model-based approaches. Such methods are more efficient and allow for interpolation between the doses observed. Research on efficient study designs and methods for duration-ranging, while similarly attempting to capture a monotonic response relationship, has only just accelerated in earnest over the last two decades. This work examines the utility of cutting-edge dose-finding methods (such as MCP-Mod) for duration-ranging of TB treatments. We compare the operating characteristics of the adapted model-based duration-ranging methodologies against standard qualitative methods for the purposes of estimating optimal duration and describing the duration-response relationship, using a simulation study motivated by a Multi-Arm Multi-Stage Response Over Continuous Intervention (MAMS-ROCI) clinical trial design. We explore three specific targets: 1) power to detect a duration-response relationship, 2) ability to accurately reproduce the duration-response curve, and 3) ability to estimate the optimal duration within an acceptable margin of error. We find that model-based methods outperform standard qualitative comparisons on every target examined, particularly when the sample size is constrained to that of a typical Phase II trial. We conclude that the success of the next era in TB therapeutics duration evaluation trials, and antibiotics duration-ranging more broadly, will meaningfully rely on the ability to simultaneous pair innovative model-based statistical methods with re-imagined study designs such as MAMS-ROCI.

PP-11: Marian Fairgrieve, UC Berkeley

Can innate immunity control M. tuberculosis?

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Numerous studies have shown that Mtb replication is not controlled until initiation of adaptive immunity. Although Mtb infection activates a robust innate immune response, this response nevertheless fails to control infection, raising the question of why innate immunity is unable to limit infection. We hypothesized that Mtb is resistant to innate immune effectors. We investigated if this resistance is due to experimental features of the mouse model or intrinsic resistance mechanisms. First, to demonstrate that innate immunity cannot control Mtb even at low physiological doses, we infected mice lacking genes involved in innate immunity with an ultra-low dose (ULD) of 1-3 bacilli. Even at ULD, Mtb bacterial burdens in the lung were unaffected by the absence of key innate immune proteins such as MyD88. This suggested that the innate immune response to Mtb is remarkably ineffective in vivo and does not contribute to control of doses as low as 1-3 bacilli. Second, we investigated whether the relatively immature immune development of SPF mice contributes to a lack of control. To investigate this, we matured the immune systems of C57BL/6 mice by co-housing with “pet shop” mice. Surprisingly, co-housed C57BL/6 mice were still susceptible to Mtb infection. To take a more targeted approach to induce activation of the innate immune system, we co-infected mice with L. pneumophila and Mtb. Like Mtb, L. pneumophila initially infects alveolar macrophages and elicits a robust inflammatory response. This immune response is sufficient to clear doses of 106 L. pneumophila within days. However, co-infection with L. pneumophila resulted in only a modest reduction in Mtb CFU compared with mice infected with only Mtb, indicating that Mtb is resistant to effectors present in the strongly antimicrobial environment stimulated by L. pneumophila infection. Taken together, our data suggest that Mtb is intrinsically resistant to innate immune clearance mechanisms. Given that adaptive responses ultimately depend on the innate immune response, this intrinsic resistance may also underlie the observed ineffectiveness of the adaptive immune response to provide sterilizing immunity.

PP-12: Stefan Fattinger, UC Berkeley

Type I and II IFN crosstalk during Mycobacterium tuberculosis infection

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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 in which type I IFNs suppress the responsiveness to type II IFN has recently been suggested. However, how this suppression is mediated remains poorly understood. Here, using an unbiased genome-wide CRISPR screen and an ectopic expression approach of ISGs in mouse and human immune cells, we are addressing how type I IFN signaling suppresses type II IFN response. Furthermore, we are investigating if such a crosstalk may explain type I IFN-driven susceptibility to Mycobacterium tuberculosis (Mtb).

PP-13: Sarah Feid, UCSF

Developing strategies to screen for regulators of leaderless mRNA translation in Mycobacteria

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Almost everything we know about the mechanisms of bacterial protein synthesis comes from study of the model organism Escherichia coli, but it's becoming increasingly clear that many pathogens, including Mycobacterium tuberculosis, operate by a different set of rules. Understanding mycobacterial protein synthesis mechanisms offers potential pathways for the development of novel antimicrobials and anti-virulence drugs. One such mechanism is the translation of leaderless mRNAs. Canonical bacterial translation is understood to begin with Shine-Dalgarno assisted formation of the initiation complex. However, the existence of transcripts lacking a clear Shine-Dalgarno or a 5' untranslated region (5' UTR) is well-documented. Very little is known about how translation initiates on 5' UTR-less, so-called leaderless mRNA, and what is known comes primarily from studies in E. coli. E. coli encodes few native leaderless mRNAs, particularly compared to Mycobacteria species, where it is estimated that approximately 20-25% of mRNAs are leaderless. Because leaderless mRNA translation is much more common in mycobacteria than E. coli, M. smegmatis and M. tuberculosis are more useful organisms to model how leaderless translation is initiated, as these organisms seem to incorporate this process into their typical lifestyle. I am developing tools to identify regulators of leaderless translation in mycobacteria through targeted and agnostic CRISPRi approaches. I have designed two leaderless reporters, one utilizing SacB-induced sucrose toxicity and the other a dual-fluorescence reporter. I am characterizing these reporters for their downstream use in screening CRISPRi knockdowns for effects on leaderless mRNA translation.

PP-14: Adam Fillion, UCSF

Identification of small molecule inhibitors of the essential Mycobacterium tuberculosis anti-toxin DarG using a novel macrodomain-targeting small molecule library

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Toxin-antitoxin (TA) modules can be found throughout the genomes of many single-cell microorganisms. Mycobacterium tuberculosis (M. tb) has approximately 80 unique TA modules. In most cases, the deletion of TA loci has little effect on the growth of M. tb under standard culture conditions. However, three TA modules have been shown to impact the viability of M. tb when disrupted. One of these is the DarTG TA module, with the DarG antitoxin being essential. DarT encodes a DNA ADP-ribosyl transferase toxin. DarG is a DNA ADP-ribosyl glycohydrolase that works to reverse the ADP-ribosylation of the M. tb genome. Due to its essentiality, DarG makes for a promising drug target candidate. DarG is a macrodomain (Mac1)-containing enzyme. Mac1 domains are found in proteins across many domains of life, and this class of protein fold has been previously considered undruggable. Until recently, in silico targeted drug design has been difficult. Recent advances in machine learning have been able to overcome many prior roadblocks. Fragmenstein, a chemical structure prediction algorithm, was used to generate a library of promiscuous macrodomain-targeting small molecule fragments, which were predicted to bind and potentially inhibit many Mac1-containing enzymes. We screened 916 small molecules from this Mac1-specific library against M.tb-H37Rv. The initial screen identified 27 hits. The top ten hits were re-screened, and all were confirmed to inhibit M.tb with micromolar-level MICs. This study highlights the potential of leveraging machine-learning mediated in silico small molecule design to target promising drug targets that were previously deemed undruggable. Future efforts will focus on confirming on-target specificity via genetic and structural approaches and optimizing potency via medicinal chemistry.

PP-15: Sydnee T Gould, UC Berkeley

A Novel Mouse Model to Identify Host Immune Factors that Mediate Susceptibility to Tuberculosis Meningitis (TBM)

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Mycobacterium tuberculosis (Mtb) naturally infects via the respiratory route, resulting in pulmonary TB (PTB) disease in roughly 10% of infected individuals. After initial growth in the lung, Mtb can disseminate via the bloodstream and reseed the lungs as well as result in secondary infections of extrapulmonary organs such as the brain. TB meningitis (TBM) is characterized by the presence of Mtb in the meninges and has a higher mortality rate than PTB. However, there is no clear mechanism for Mtb entry into the brain due in large part to a lack of representative animal model of TBM. The two prevailing theories of how Mtb initially passes through the blood-brain barrier (BBB) are that extracellular Mtb in the blood cross through leaky endothelial cell junctions, or that intracellular Mtb within circulating immune cells are ferried across the endothelial barrier via cell trafficking pathways. Although alternative explanations to the route of entry exist, our study focuses primarily on testing these two models. Recently a novel mouse strain, SARB, has been identified to be hypersusceptible to TBM, providing an opportunity to study bacterial trafficking to the brain. We propose to initially determine if dissemination occurs as a result of bacteria traveling through the blood intracellularly, extracellularly, or a combination of both. Our working model is that Mtb is ferried out of the lung within phagocytic cells, and that specific interactions of the infected cells with the capillary endothelium triggers the first step of CNS infection by promoting entry to the brain through the BBB. Using closely-related but non-susceptible mouse lines (SARA, SARC), we will also compare host genetic factors that contribute to the SARB line's hypersusceptibility such as immune cell functionality, differential cell population activation, and recruitment. Lastly, by utilizing clinical TBM strains, we hope to identify novel bacterial factors that drive TBM manifestation as well as test putative TBM Mtb genes in our new mouse model.

PP-16: Jolyn Hoang, UC Berkeley

The role of a conserved membrane transport protein MmpL4 in the virulence of Mycobacterium marinum

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To cause disease, Mycobacterium tuberculosis and related mycobacteria survive within host immune cells by manipulating their intracellular environment. Mycobacterial membrane protein large (MmpL) proteins are resistance-nodulation-cell division (RND) transporters, some of which export lipids required for the virulence of M. tuberculosis. Deletion of mmpL4 in M. tuberculosis induces a severe virulence defect in ex vivo murine macrophage and in vivo infection models. Interestingly, this defect is only observed in the presence of interferon γ (IFNγ), a cytokine important for activating bactericidal mechanisms in macrophages, suggesting that mmpL4 is required specifically in response to host immune activation. While RND family transporters in gram-negative bacteria are known to interact with other proteins as part of a multiprotein efflux system, a similar system for transporting molecules across the unique mycobacterial cell envelope has yet to be found. We have identified three putative helper proteins that may interact with MmpL4 to facilitate substrate transport. However, the mechanism of MmpL4-mediated virulence and the role of the putative helper proteins is unknown. Due to the technical challenges and safety considerations of working with M. tuberculosis, we used M. marinum as a model system. We hypothesize that MMAR_0771, the nearest homolog of mmpL4 in M. marinum, and its putative helper proteins are required for virulence. Here, we created genetic deletion mutants in M. marinum using CRISPR and tested these mutants using an ex vivo murine bone marrow macrophage infection model.

PP-17: Zach Howard, UCSF

A Mycobacterium tuberculosis variable antigen vaccine induces infection tolerance

Zach Howard, UCSF

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More than 95% 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 representing hypothetical 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 hypersusceptible SP140-/- mice using a DNA vaccine encoding a fusion protein of four variable-sequence antigens (Rv0010c, RimJ, Rv2719c, and Rv0990c). We then assayed bacterial burdens, immune cell populations by flow cytometry, and immunopathology by immunofluorescence microscopy and Sytox Green injection.

Vaccination with variable-sequence antigens did not reduce bacterial burdens but caused markedly reduced immunopathology in SP140-/- mice. Vaccination reduced lung necrosis, measured as a reduction in total Sytox Green fluorescence in whole lungs. Vaccination of SP140-/- mice with a DNA vaccine encoding for RimJ alone demonstrated a similar phenotype to vaccination with the fusion construct, which indicates that responses to RimJ are sufficient for the altered immunopathology phenotype.

In other fields of biology, reduction in pathology without reduction in pathogen burden is termed infection tolerance, which describes the effect of our vaccine. Vaccination induced increases in RORγt+ CD4 T cells, but further investigation is needed to determine the primary mechanism by which RimJ-specific vaccine responses induce infection tolerance. Most preclinical studies evaluate candidate vaccines for their ability to reduce bacterial burden, while our results demonstrate a distinct phenotype and endpoint for identifying promising candidate vaccines, since reduction of tissue damage can reduce TB morbidity and potentially reduce TB transmission.

PP-18: Mollie Hudson, UCSF

The impact of social protection interventions on treatment and socioeconomic outcomes of tuberculosis-affected individuals and households: A systematic review and meta-analysis

Mollie Hudson, UCSF

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Background: Social protection interventions, endorsed by World Health Organization (WHO) and included in UN high level meeting commitments, have the potential to curb the TB epidemic by addressing the underlying social and structural determinants of disease. We conducted a systematic review and meta-analysis to quantify the impact of social protection in conjunction with biomedical interventions on TB treatment and socioeconomic outcomes for affected people and households.

Methods: We conducted a comprehensive search across multiple electronic databases for articles published from January 2012 to September 2023, reporting studies that described at least one social protection intervention and focused on treatment and/or socioeconomic outcomes for people with TB or their households using standardized PICOT approach. Random-effects meta-analysis was used to analyze our primary outcome of interest, TB treatment success, across included studies. Risk of bias was assessed using the Newcastle Ottawa Scale and the Cochrane Risk of Bias tool. This review was registered prospectively in the PROSPERO database (registration number CRD42022382181).

Results: Out of 44,404 articles identified in our search, 46 were eligible for inclusion. Thirty-three studies reported TB treatment outcomes, seven studies reported on socioeconomic outcomes, and two studies reported both TB treatment and socioeconomic outcomes. Eight studies described implementation challenges, with the most common reason (n=6) for poor implementation fidelity being administrative related barriers. Random-effects meta-analysis found that individuals who were recipients of social protection interventions in conjunction with standard biomedical treatment had 2.12 times the odds of TB treatment success (95% CI 1.7, 2.6).

Conclusion: Social protection interventions significantly improve rates of TB treatment success. Additional studies that systematically collect data on socioeconomic outcomes, mortality, and implementation are still required. The standardized outcomes and definitions used in this systematic review and meta-analysis have the potential to guide further research, monitoring and evaluation on social protection programs for TB-affected populations.

PP-19: Nalin Abeydeera Kekiriwara Godage, UCSF

Optineurin: A Novel Host Restriction Factor for Mycobacterium tuberculosis Infection

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Background: Mycobacterium tuberculosis (Mtb) is a highly successful human pathogen, causing over 1.5 million deaths annually. The increasing prevalence of drug-resistant Mtb necessitates innovative treatment approaches to combat this global health threat. Autophagy has emerged as a crucial innate immune mechanism for eliminating intracellular pathogens, including Mtb. Enhancing host immune factors such as autophagy presents a viable strategy for combating drug-resistant Mtb.

Methods: We identified several autophagy receptors, including Optineurin, that undergo phosphorylation during Mtb infection in bone marrow-derived macrophages. These phosphorylations occur at six distinct sites within or near the LC3 and ubiquitin-binding domains, indicating Optineurin's significant role in Mtb pathogenesis. To investigate Optineurin's role in Mtb infection, we utilized Cas9+ conditionally immortalized macrophages to create Optineurin-deficient macrophages, alongside control cells expressing scramble sgRNA.

Results: Infection of a single-cell clonal population with 100% editing efficiency resulted in increased bacterial growth compared to the scramble control, indicating that Optineurin restricts Mtb replication. Further, we complemented the knockout using lentiviral transduction with Optineurin expressing synonymous mutations in the sgRNA recognition site and PAM, preventing Cas9 from targeting the complemented allele.

Conclusions: Our results demonstrated restricted Mtb growth in Optineurin-complemented cells compared to Optineurin-deficient macrophages. In vivo assessment using a murine model of aerosol Mtb infection showed a 1-log increase in mean Mtb CFU in the lungs of Optineurin-deficient mice at 21 days post-infection compared to wild-type mice, confirming Optineurin's role in Mtb pathogenesis. Immunofluorescence microscopy revealed a 55% decrease in Mtb colocalization with LC3 in Optineurin-deficient macrophages compared to the scramble control, suggesting that Optineurin specifically directs Mtb toward selective autophagy. Our findings demonstrate that Optineurin is a newly identified host restriction factor. Augmentation of Optineurin function is a potential therapeutic approach for limiting Mtb growth.

PP-20: Jingjun Lin, UC Davis

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

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Mycobacterium tuberculosis (Mtb) can survive and proliferate in macrophages. Few human immune factors have been identified as potentially important for controlling tuberculosis, including interferon gamma (IFN-γ). IFN-γ knockout mice are highly susceptible to Mtb infection. Therefore, identifying IFN-γ associated factors may reveal important Mtb pathogenesis mechanisms. We used an Mtb strain carrying an mCherry fluorescent reporter to infect a mouse macrophage cell CRISPR library pre-stimulated with IFN-γ. Macrophages with high and low intracellular Mtb burdens were sorted and sequenced to identify significantly enriched knockout genes. Unexpectedly, interferon gamma receptor 1 (Ifngr1) mutant cells were not enriched in macrophages with high Mtb burden. After optimizing the infection conditions, we enriched Ifngr1, Stat1, and Irf1 genes in the high Mtb burden macrophages, prompting us to follow up on the screen results. Among the top enriched sgRNAs in the high Mtb burden macrophages, we found that Lrrc8a gene knockout resulted in higher Mtb growth in mouse macrophages and human macrophage THP1 cells. LRRC8A (or SWELL1) has been shown to regulate cell volume and various biological processes, but its involvement in controlling Mtb by macrophages is unknown and is being investigated.

PP-21: Tessa Mochizuki, UCSF

Cepheid Xpert TB/LTBI shows promising accuracy as a triage test for tuberculosis

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Background: Non-sputum triage tests are needed to improve TB detection in high-burden settings. The Xpert TB/LTBI cartridge (research use only, Cepheid, USA) is a novel assay that detects nine mRNA targets from MTB-antigen stimulated blood. We evaluated its diagnostic performance as a TB triage test in comparison to QuantiFERON (QFT)-TB Gold Plus, and assessed whether it met WHO target product profile (TPP) accuracy targets (≥90% sensitivity, ≥70% specificity).

Methods: Between March-September 2023, we enrolled consecutive people ≥12 years at health centers in Uganda and Vietnam with presumptive TB. Participants provided sputum for TB testing (Xpert MTB/RIF Ultra and culture) and venous blood for Xpert TB/LTBI and QFT-Plus. We performed logistic regression to predict TB status using cycle threshold values for Xpert TB/LTBI mRNA targets and receiver operating characteristic analysis to assess accuracy in reference to sputum results.

Results: We included 216 people with valid QFT-Plus results. Median age was 42 years (IQR: 29-56), 123 (56.9%) were male, 20 (9.3%) were living with HIV, 20 (9.3%) had diabetes, 64 (29.6%) had confirmed TB and 129 (59.7%) were QFT-positive. The area under the curve for Xpert TB/LTBI was 0.90 (95% CI 0.86-0.95). The specificity of Xpert TB/LTBI was 77.0% (95% CI 69.5-83.4) at a cut-point that achieved ≥90% sensitivity (90.6%, 95% CI 80.7-96.5), and was higher than for QFT-Plus (67.8% vs. 55.3%, difference=12.5% [95% CI 4.3-20.7, p<0.01]) at a cut-point that achieved the same sensitivity as QFT-Plus (95.3%, 95% CI 86.9-99.0). In people living with HIV, sensitivity was 100.0% (95% CI 39.8-100) and specificity was 81.2% (95% CI 54.4-96.0).

Conclusions: Xpert TB/LTBI exceeded WHO TPP accuracy targets for TB triage tests and outperformed QFT-Plus. Xpert TB/LTBI should be further evaluated as a tool to both screen for prevalent active TB and predict future risk of incident TB with a single test.

PP-22: Alexander Mohapatra, UCSF

A novel mouse model for tuberculosis vaccine antigen discovery

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Vaccination is the most efficient way to limit transmission, lower mortality, and achieve the end goal of eradicating tuberculosis. The discovery that CD4 T cells coordinate elimination of cells infected with M. tuberculosis (Mtb) in humans led to the identification of several T cell antigens that underlie the current vaccine pipeline. However, the antigens that confer the most protective T cell response against Mtb remain unknown.

Three candidate vaccines in clinical trials include the Mtb protein “early secreted antigenic target of 6 kDa” (ESAT-6), based on the high proportion of T cells in Mtb-infected humans and animals that recognize this antigen and modest protection in vaccinated animals. Despite this immunogenicity, ESAT-6 mutations that would limit T cell recognition are exceedingly rare among Mtb clinical isolates and Mtb-exposed humans with an ESAT-6-specific T cell response are not protected from developing tuberculosis.

These results suggest that ESAT-6-specific T cells are more beneficial for the pathogen than for the host. To test the hypothesis that ESAT-6-specific T cells are detrimental to Mtb elimination by masking responses against other Mtb antigens, we developed an ESAT-6-tolerant mouse (termed “6T”). These mice express the CD4 T cell epitope of ESAT-6 recognized by I-Ab in the thymus to delete any developing ESAT-6-specific T cells before they enter the circulating pool. Whereas ~9% of all lung CD4 T cells in Mtb-infected wildtype mice are specific for ESAT-6, none are present in infected 6T mice.

Further, lung mycobacterial burden is unchanged between Mtb-infected 6T mice and littermate controls, suggesting that ESAT-6-specific T cells are dispensable for pathogen control. Mtb-infected lung CD4 T cell responses to a pool of Mtb peptides that includes ESAT-6 were preserved between wildtype and 6T mice, raising the possibility that novel T cell clones expand in the absence of ESAT-6-specific T cells.

In ongoing studies, we will identify these unmasked T cell clones and their cognate antigens via T cell receptor sequencing and peptide pool screens, respectively, to inform development of new tuberculosis vaccine candidates.

PP-23: Hannah Nilsson, UC Berkeley

Elucidating the mechanism behind reversible colony morphology switching in Mycobacterium avium

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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 are representative of 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 gene 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 also identified a mutant in a periplasmic protease, MarP, that is trapped in the SmO state and blocks Erp-mediated switching 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-24: Oshiomah Oyageshio, UC Davis

Integrating single cell multiomics and ancestry to investigate TB susceptibility

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Recent approaches have begun to identify expression quantitative trait loci (eQTLs), genetic variants that regulate gene expression, in response to Mtb infection. However, they are limited by bulk RNA sequencing or analyzing a single cell type. We propose a novel eQTL mapping approach combining single-cell RNA sequencing and ancestry-adjusted analyses on the repertoire of peripheral blood mononuclear cells (PBMCs) from TB cases (positive GeneXpert) and latent (LTBI) controls (positive interferon-gamma release assay and negative GeneXpert) to discover critical variants associated with TB.

TB is endemic in our study population, with an incidence of 643/100,000. We have sampled PBMCs from 75 LTBI controls and 75 cases. Our participants identify as South African Coloured (SAC), a population with high genetic diversity and admixture. Our previous research has shown that the SAC is a 5-way admixed population with majority Khoe-San ancestry. Khoe-San ancestry harbors the highest genetic diversity of all human populations as measured by low linkage disequilibrium levels and high heterozygosity. We will leverage this diverse genetic architecture to optimize variant discovery.

First, we will use CITE-seq for single-cell profiling of the entire PBMC repertoire and its cell surface proteins. This leverages multimodal clustering to integrate gene and protein expression profiles to accurately define cell types and states. Then, we will identify differentially expressed genes (DEGs) in these cell clusters between cases and controls.

Next, we will identify genomic variants (eQTLs) that modulate the TB immune response. To do this we will utilize whole genome sequences (WGS) from our cases and controls and estimate global and local ancestry. Then we will combine WGS data with significant DEGs. Finally, we will use eQTL mapping models that incorporate ancestry proportions to identify novel TB susceptibility variants.

PP-25: Hayley Poore, UC Irvine

Evaluation of tongue swab and sputum-dipped swab molecular testing for tuberculosis on the novel Pluslife platform

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Background: Accessible point-of-care (POC) testing methods are needed to aid TB case finding. Swabs are emerging as promising, easy-to-use sampling tools. The Pluslife platform includes a swab preparation device and a Mycobacterium tuberculosis complex (MTBC) test card that provides results within 30 minutes. We assessed whether Pluslife's MTBC assay meets the draft WHO target product profile (TPP) accuracy thresholds for a near POC, non-sputum diagnostic (≥75% sensitivity and ≥98% specificity).

Methods: Between April-May 2024, we enrolled people ≥12 years with presumed TB at health centers in Kampala, Uganda. Two tongue dorsum swabs and sputum samples were obtained from each participant. Swabs were processed with Pluslife's automated sample prep device or a Biospec bead beater, and tested using Pluslife's MTBC test card. A third swab was dipped into sputum, swirled 10 times, and processed for Pluslife testing. Diagnostic accuracy of swab-based testing was assessed against sputum Xpert Ultra

Results: Of 58 participants enrolled to date, we excluded one with a trace result on sputum Xpert. Median age was 32.5 (IQR 23-41), 34 (59.7%) were male, 17 (29.8%) were living with HIV, 20 (35.1%) had diabetes, 6 (10.5%) had prior TB, and 13 (22.8%) had a positive sputum Xpert result. Both tongue swabs, processed with Pluslife device or Biospec bead beating, demonstrated sensitivity of 92.3% [95% CI 64.0-99.8] and specificity of 100% [95% CI 92.0-100]. Sputum-dipped swabs achieved sensitivity of 100% [95% CI 75.3–100] and specificity of 95.5% [95% CI 84.5 - 99.4].

Conclusions: Our preliminary findings indicate that tongue swabs and sputum-dipped swabs tested on Pluslife's platform are likely to exceed the draft WHO TPP accuracy thresholds for a near POC, non-sputum TB diagnostic. Further analysis with increased sample size and MGIT culture results is forthcoming. Future studies should explore Pluslife's performance in different populations and community screening settings.

PP-26: Dvijen Purohit, UCSF

Impact of Rifampicin Dose on Dexamethasone Pharmacokinetics in Tuberculous Meningitis: Insights from the ALTER Trial

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Background: Tuberculous meningitis (TBM) represents the most severe manifestation of extrapulmonary tuberculosis, characterized by infection of the meninges, the protective membranes enveloping the central nervous system. Treatment of TBM involves a combination of antibiotic chemotherapy and adjunctive corticosteroids. Rifampicin and corticosteroids are both used in the treatment of TBM, however, their co-administration raises concerns about drug-drug interactions, primarily due to their influence on the activity of hepatic CYP3A4 enzymes which may lower concentrations. Our aim was to assess the pharmacokinetics (PK) of dexamethasone in cerebrospinal fluid (CSF) and plasma from the ALTER trial (NCT04021121).

Methods: Participants aged 18 years and older were recruited from Masaka Regional Referral Hospital in Masaka, Uganda, and randomized in a 1:1 ratio to receive either high-dose (35 mg/kg) or standard-dose (10 mg/kg) rifampicin. Additionally, they were further randomized in a 1:1 ratio to receive linezolid 1200 mg daily or no linezolid for the initial 4 weeks, concurrently with isoniazid, pyrazinamide, ethambutol, and steroids. CSF and plasma samples were collected on days 2, 14, and/or 28 for PK analysis. Day 2 included dense sampling with pre-dose trough levels and 2, 4, and 8-hour post-dose measurements along with a single CSF draw at various time points. On days 14 and/or 28, one plasma sample and one CSF sample were obtained at different time points per participant.

Results: Of the 40 participants enrolled in this trial (55% women, median age 37 years, median weight 47.5 kg, 98% HIV+), median dexamethasone concentrations in CSF and plasma were 4.50 mg/L and 33.65 mg/L, respectively. Among all timepoints collected during the sampling visits, median dexamethasone concentrations were consistently lower in the plasma and CSF of those receiving high dose rifampicin versus standard dose. The partition coefficients (ratio of plasma to CSF levels) were comparable between groups, with a median value of 0.36 and 0.31 in the high and standard dose groups respectively.

Conclusions: These preliminary findings suggest that increasing the dose of rifampicin may lower the concentrations of dexamethasone in both CSF and plasma while preserving the partition coefficients. Further population PK analyses will be performed to confirm this relationship.

PP-27: Christopher Rae, UC Berkeley

How Do Lipids Traverse the Mycobacterial Cell Envelope?

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The mycobacterial cell envelope is a poorly understood interface between the pathogen and the host. It is unclear how hydrophobic molecules are effluxed across this uniquely impermeable cell envelope and what proteins facilitate their movement. Mycobacterial membrane protein large (MmpL) proteins are a family of transporters that initiate the export of hydrophobic molecules by extracting them from the inner membrane. Here we focus on MmpL4, a small molecule exporter present across all mycobacterial species and essential for the virulence of M. tuberculosis. We used biochemistry and structural biology, combined with mycobacterial genetics to show that MmpL4 functions as part of a multi-protein efflux apparatus containing an inner membrane multimer of the transporter and periplasmic adaptor components that provides a conduit across the periplasm.

PP-28: Abigail Ray, UC Davis

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

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Despite the potent antimicrobial activity of alveolar macrophages, Mycobacterium tuberculosis (Mtb) has the surprising ability to survive and proliferate in these cells by halting phagosome-lysosome fusion, disrupting antigen presentation, and dysregulating cytokine signaling. Unfortunately, the specific molecular mechanisms underlying this ability to subvert host defenses remain obscure. We hypothesize that Mtb secretes protein virulence factors into the macrophage to specifically disrupt immune function by selectively targeting mediators of host immunity. Previous work used a biochemical approach to identify physical interactions between secreted Mtb proteins and human proteins.

These interactions were mapped within a high-specificity protein-protein interaction network as a means of isolating host-pathogen interactions with putative roles in virulence. Next, we sought to identify functionally relevant results within this interactome. With bacterial genetics we were able to characterize the Mtb components of this dataset in vivo. For each of the top 40 bacterial effectors, a knockout strain of Mtb was engineered by disrupting the open reading frame of the gene and inserting a traceable barcode. Up to eight of these barcoded mutant strains were then pooled together into a mixed inoculum at a fixed ration and used to infect C57/B6 mice via aerosolization.

To capture the dynamics of both the innate and adaptive immune response, lungs and spleens were collected from mice at 0, 10, 21, 42, and 90 days post infection. These samples were homogenized, plated on agar, and subsequently, bacilli were recovered from these organ homogenate plates. Genomic DNA was isolated and then analyzed via barcode qPCR to quantify the relative strain proportion.

This in vivo screening approach has yielded several bacterial effectors with modest fitness defects, thus indicating a possible immunological role for the protein of interest. These candidates are now being validated with additional clones, complementation experiments, and prepared for macrophage infections to confirm their role in human cells before proceeding with subsequent mechanistic studies.

PP-29: Alyssa Sales, UCSF

"That is the reason why I trust": Perceptions and implementation considerations for tuberculosis tongue swab testing in Viet Nam and Zambia

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Background: In high tuberculosis (TB)-burden settings, tongue swabs are expected to facilitate the expansion of molecular TB testing. Thus, understanding tongue swab acceptability and feasibility among people with TB symptoms is critical to uptake.

Methods: From September 2023-February 2024, we conducted qualitative, in-depth interviews with adults undergoing TB evaluation (n=42) at six primary health centers in Viet Nam and Zambia. All participants were offered a health worker-collected tongue swab in addition to routine sputum collection. Interviews explored participants' experiences and perceptions of tongue swabs to diagnose TB. We used thematic analysis to elucidate preferences, usability, and acceptability for tongue swabs.

Results: Among participants in Zambia (n=21; 57% female, 67% who had tongue swab and sputum collected, 24% sputum only, and 10% tongue swab only) and Viet Nam (n=21; 43% female, 100% had tongue swab and sputum collected), tongue swabs were considered feasible and generally easy to collect, with many participants noting them as a welcome complementary or alternative to sputum. Most participants felt the ease of tongue swab sample collection promoted usability, particularly for children, older adults, and those unable to produce sputum. Trust, a multifaceted construct described across interviews, drove the acceptability of tongue swabs. Knowledge of diagnostic accuracy, past swabbing experiences (primarily from COVID-19 and HIV testing), concerns about increased risk of TB transmission through sample collection, instruction and counseling by health workers, and sample source within the body all drove trust in tongue swabs. Some participants agreed to tongue swab collection because they felt it would deliver rapid results. In contrast, others declined swabs because they feared throat pain or were concerned about their dental hygiene. Participant-driven implementation suggestions included standardized instructions or health worker counseling for tongue swabs and home collection to reduce time and increase comfort.

Conclusion: Tongue swabs are highly feasible and easy to use. To increase the acceptability of tongue swabs, future facility, and community-based promotion or collection should target drivers of trust by developing and including standardized information on swab accuracy compared to sputum, time to result, and collection procedures. Context-specific considerations for the physical location of tongue swab collection may minimize discomfort and transmission concerns.

PP-30: Kinari Shah, UCSF (on behalf of Jill Kadota)

Dissaving is common among patients undergoing TB diagnostic evaluation across four high-TB burden countries

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Background: Tuberculosis (TB)-affected households often face dire socioeconomic consequences of disease which are associated with poor TB outcomes. Examples include catastrophic costs, which can be difficult to enumerate, and negative financial coping strategies, such as dissaving, which are simpler to identify. We assessed the prevalence of dissaving among people accessing TB diagnostic services in 4 high-TB burden countries, as well as demographic and clinical factors associated with dissaving.

Methods: From December 2022-March 2024, surveys were conducted among adults with no prior history of TB undergoing TB diagnostic evaluation at health facilities in the Philippines, Viet Nam, Uganda, and India. Surveys collected self-reported health and sociodemographic data, including questions about dissaving and unmet social needs. Dissaving was defined as having: taken out a loan, sold a household asset, reduced household food consumption, withdrawn from long-term savings, or taken a child out of school to cover the costs of TB care. We used multivariate logistic regression to assess for characteristics associated with dissaving.

Results: Among 571 participants (median age 45 years, 50.6% female, 2.6% HIV-positive, 16.5% diabetic), 317 (56.6%) engaged in any type of dissaving, with withdrawal from savings being most common (Table). Multivariate logistic regression revealed that lower education was associated with a greater odds of dissaving (No education: odds ratio [OR]=3.11, 95% confidence interval (CI): 1.20-8.05; Primary/some primary: OR=2.68, 95% CI: 1.25-5.77; Secondary/some secondary: OR=2.02, 95% CI: 1.35-3.01). Additionally, those with HIV (OR=3.58, 95% CI: 2.74-4.69), multidimensionally poor people (OR=1.75, 95% CI: 1.40-2.20), and those with diabetes (OR=1.44, 95% CI: 1.04-1.99) were at greater risk of dissaving.

Conclusions: Dissaving is prevalent even among people undergoing testing for TB. Poverty, less education, and coexistent diagnoses made these individuals significantly more vulnerable to dissaving. Targeting socioeconomic interventions for those with existing health conditions and/or heightened socioeconomic risk should be considered to improve TB outcomes.

PP-31: Kinari Shah, UCSF

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

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Background: Recognizing new and improved diagnostics as a key step to reducing the global tuberculosis (TB) burden, the World Health Organization (WHO) publishes target product profiles (TPPs) to guide development of novel TB diagnostics; however, TPPs have not included the preferences of people undergoing TB testing. Understanding their preferences is crucial for optimizing the acceptability and uptake of TB diagnostic services.

Design/methods: We conducted a discrete choice experiment (DCE) among adults with presumptive or microbiologically confirmed TB in 5 countries (Philippines, Vietnam, South Africa, Uganda, and India). The DCE evaluated preferences for 5 TB diagnostic test attributes (sample type, accuracy, cost, location, time to result) with 3-4 levels per attribute. We estimated mean preference weights for attribute levels using Hierarchical Bayesian models and conducted willingness-to-trade analyses for preferred test features.

Results: Among 1,033 participants (median age 43 years, Interquartile Range: 30-55), 52% were female, 10% had HIV, 14% had diabetes, 36% had current or prior TB), the most strongly valued test features were free cost, high diagnostic accuracy, and rapid (within 15 minutes) result availability. Among less valued attributes, facility-based testing (location) and sputum (sample type) were most preferred. The relative importance of attributes and their levels differed by country. In willingness-to-trade simulations, participants in each country were willing to trade 10-20% lower accuracy for the availability of rapid tests but were unwilling to trade accuracy for alternative sample types (e.g., tongue-swab, urine, blood) or testing locations (e.g., home- or community-based); participants in India and Vietnam were willing to pay $3.20 and $4.00 USD, respectively, for tests with rapid results.

Conclusions: Persons accessing TB services in five high-burden countries universally value free cost, high diagnostic accuracy, and rapid result availability. However, many were willing to accept lower test accuracy and some were willing to incur small out-of-pocket costs to access rapid tests. To align with preferences of TB-affected communities, future TPP updates and new test development should prioritize rapid results while maintaining high accuracy and minimizing costs.

PP-32: Jacob Sussman, The University of Utah

The concentration of tuberculosis within Paraguay's incarcerated and Indigenous populations, 2018-2022

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Background: While incidence of tuberculosis (TB) has decreased globally, in Paraguay, considered a medium-incidence country by the WHO, TB incidence has increased slightly from 42 per 100,000 in 2010 to 46 per 100,000 in 2022.

Methods: We conducted a retrospective study of TB cases notified to the Paraguay National Program for Tuberculosis Control (NPTC) from 2018 to 2022 and quantified trends in specific populations identified as vulnerable.

Results: Of the 13,725 TB cases notified in Paraguay from 2018 to 2022, 2,331 (17%) occurred among incarcerated individuals and 1,743 (12.7%) occurred among self-identified Indigenous individuals. In 2022, the relative risk of TB was 87 and 6.4 among the incarcerated and Indigenous populations, compared with the non-incarcerated and non-Indigenous populations respectively.

Conclusions: We found significant heterogeneity in TB incidence across Paraguay's 17 departments. Our findings highlight the urgency of expanding access to TB diagnosis, treatment, and prevention in populations at heightened risk of TB in Paraguay.

PP-33: Brittney Sweetser, UCSF

The accuracy of a three-gene host response signature to classify tuberculosis severity in children

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Summary: New tools are needed to determine disease severity in children with TB and guide the use of shorter treatment regimens. We assessed the role of the Cepheid Xpert 3-gene host response cartridge to distinguish TB severity in children from The Gambia and Uganda.

Background: Children with non-severe TB are eligible for shorter treatment regimens, but new tools are needed to guide disease classification. We evaluated the role of the blood-based Cepheid 3-gene Xpert Host Response (HR) cartridge to stratify TB disease severity in children.

Methods: We included children under 15 years with microbiologically Confirmed or Unconfirmed TB in Uganda and The Gambia. Disease severity was defined according to WHO guidelines. Less than 1 mL of blood was collected and Xpert-HR was used to measure the cycle threshold for each gene and calculate an HR TB score with the equation of (GBP5-DUSP3)/2 – TBP. We performed receiver operating curve (ROC) analysis to calculate the area under the curve (AUC) and determine the accuracy to classify severe disease at cut-off values closest to 90% sensitivity to minimize undertreatment.

Results: Thirteen (11.0%) children who met the above criteria had an invalid HR result. Of 105 children included, the median age was 4 years (IQR 1-7), 20 (19.1%) had Confirmed TB and 27 (25.7%) had severe TB per WHO criteria. The median HR TB score was significantly lower in the severe group versus the non-severe group (-1.5 versus -1.0, p = 0.003). In the Confirmed TB group, Xpert HR achieved an AUC of 0.71 (95% CI 0.43-0.99) to detect severe TB, with a specificity of 62.5% (95% CI 24.5-91.5) at 91.7% sensitivity (95% CI 61.5-99.8). However, when including the Unconfirmed TB group, the AUC reduced to 0.69 (95% CI 0.58-0.80) with a specificity of 43.6% (95% CI 32.4-55.3) at 88.9% sensitivity. This corresponded to 55.2% overall concordance with WHO criteria, and 52.4% concordance with CXR classification of severe TB.

Conclusions: Xpert-HR had low specificity to detect severe TB in children and could lead to overtreatment. Accuracy was higher among children with Confirmed TB, but child-specific TB gene signatures may be needed to increase accuracy.

PP-34: Nora West, UCSF

A mixed methods evaluation of preferences for tongue swab-based testing among people with presumptive tuberculosis in five high-burden countries

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Background: The use of tongue swabs for tuberculosis (TB) testing could expand the accessibility of testing in high TB-burden settings. However, the perceptions of and preferences for tongue swab-based testing among people undergoing TB evaluation at routine health facilities is unknown.

Methods: We utilized a convergent, parallel, mixed-methods study design to evaluate preferences for tongue swabs for TB testing across five high TB-burden countries. We conducted a cross-sectional survey from July 2023-January 2024 among participants undergoing sputum and tongue swab collection as part of the Rapid Research in Diagnostics Development for TB Network (R2D2 TB Network) in India, the Philippines, South Africa, Uganda, and Viet Nam. A subsample of R2D2 TB Network participants completed qualitative interviews. Quantitative and qualitative data were triangulated and interpreted through comparisons for concurrence/discordance.

Results: Among 861 participants who completed the preference survey (49% male; median age 48, 7% living with HIV), 55% reported an overall preference for tongue swab, 23% for sputum, and 22% no preference. Discomfort during collection was more common for sputum compared to tongue swabs (42% vs.13%, $p<0.001$). Discomfort or difficulty providing a tongue swab did not differ by sex ($p=0.634$). Nearly half of men (n=209, 49%) and the majority (n=269, 62%) of women endorsed tongue swab as their preferred sample type (compared to sputum and no preference) ($p<0.001$). Most participants (n=522, 61%) reported that tongue swab was easiest to provide, followed by sputum (n=176, 20%), and no difference (n=163, 19%). Qualitative interviews (n=40) revealed key influences on preference for tongue swabs as compared to sputum: the importance of where in the body a sample comes (lung vs. tongue), the balance between ease of collection and accuracy of sample testing, the influence of health workers, and differences in discomfort experience among men and women.

Conclusions: Perceptions of tongue swabs among people undergoing TB diagnostic testing are generally favorable. This multi-country mixed methods analysis revealed that preference for sample type is driven by more than ease of collection; it is grounded in a combination of multi-level factors. These findings provide important considerations for recommendations and implementation of tongue swabs as a complementary or alternative TB diagnostic approach.

PP-35: Jillian Kadota, UCSF (presented by Nora West)

Baseline assessment of social assistance programs for people with TB and their households in Uganda: a situation analysis

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Summary: Social protection is vital for supporting people with tuberculosis (TB) and their households, and its widespread implementation aligns with global commitments. We completed a mixed-methods study to evaluate accessibility of social protection for people with TB in Uganda. Interventions addressing specific challenges faced by TB-affected households are needed.

Introduction: Robust social protection systems are crucial for supporting people with TB and align with global TB control targets and commitments from the 2023 UN High-Level meeting. We conducted a participatory action mixed-methods study to understand the social protection landscape for people with TB in Uganda.

Methods: Between October-November 2023 we conducted document reviews and completed surveys and semi-structured interviews with key informants using a WHO-recommended protocol adapted for Uganda. We collected data on social protection program characteristics, coverage, and accessibility, and categorized programs as TB-specific or TB-sensitive, and responsive or inclusive. We summarized qualitative barriers to program access/implementation from the supply and demand-side perspective.

Results: Surveys (n=26) among TB and social protection program representatives, governmental and non-governmental organization employees, community members, and TB survivors revealed a multitude of programs (n=19) managed by diverse entities, including governmental, non-governmental, and community-led initiatives. Among these, the Enablers Program (funder: Global Fund) emerged as the sole TB-specific initiative both inclusive and responsive to the needs of people with multi-drug resistant TB. Key informant interview data (n=18) revealed administrative challenges and limited resource allocation as significant implementation barriers. Prominent demand-side barriers included lack of program awareness among intended beneficiaries and bureaucratic complexities. These results demonstrated insufficient awareness and differing perspectives on program inclusiveness, key population coverage, and ability to address socioeconomic shocks among intended beneficiaries.

Conclusion: TB-specific programs responsive to the needs of people with drug-sensitive TB are lacking in Uganda. TB-sensitive programs fail to benefit people with/at-risk of TB due to supply, policy, and programmatic barriers that hinder access. Lack of unified understanding of program characteristics including their ability to meet the needs of people with TB underscores the need for a more coherent approach to program implementation. Addressing these barriers is imperative to ensure equitable access to social protection for TB-affected households.

PP-36: Ava Xu, UCSF

Pyrazinamide Safety, Efficacy, and Dosing for Treating Drug-Susceptible Pulmonary Tuberculosis

Ava Xu, UCSF

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Rationale: Optimizing pyrazinamide dosing is critical to improve treatment efficacy while minimizing toxicity during tuberculosis treatment. Study 31/ACTG A5349 represents the largest Phase 3 randomized controlled therapeutic trial to date for such investigation.

Objectives: We sought to report pyrazinamide pharmacokinetic parameters, risk factors for lower pyrazinamide exposure, and relationships between pyrazinamide exposure with efficacy and safety outcomes. We aimed to determine pyrazinamide dosing strategies that optimize risks and benefits.

Methods: We analyzed pyrazinamide steady-state pharmacokinetic data using population nonlinear mixed-effects models. We evaluated the contribution of pyrazinamide exposure to long-term efficacy using parametric time-to-event models and safety outcomes using logistic regression. We evaluated optimal dosing with therapeutic windows targeting ≥95% durable cure and safety within the observed proportion of the primary safety outcome.

Measurements and Main Results: Among 2255 participants with 6978 plasma samples, pyrazinamide displayed 7-fold exposure variability (151-1053 mg·h/L). Body weight was not a clinically relevant predictor of drug clearance and thus did not justify the need for weight-banded dosing. Both clinical and safety outcomes were associated with pyrazinamide exposure, resulting in a therapeutic window of 231-355 mg·h/L for the control and 226-349 mg·h/L for the rifapentine-moxifloxacin regimen. Flat dosing of pyrazinamide at 1000 mg would have permitted an additional 13.1% (n=96) participants allocated to the control and 9.2% (n=70) to the rifapentine-moxifloxacin regimen dosed within the therapeutic window, compared to the current weight-banded dosing**.**

Conclusions: Flat dosing of pyrazinamide at 1000 mg daily would be readily implementable and could optimize treatment outcomes in drug-susceptible tuberculosis.

PP-37: Alex Zilinskas, UC Berkeley

Understanding how Mycobacterium tuberculosis alters the T helper response

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Mycobacterium tuberculosis (Mtb) is the causative agent of tuberculosis. Mtb infections generally results in a type 1 T helper (Th1)-mediated response; however, the Stanley lab has previously shown that mice vaccinated with CDN adjuvant and Mtb protein antigens had enhanced protection mediated by type 17 T helper (Th17) cells. Other labs have also indicated that humans and non-human primates are more protected to Mtb infection when the hosts generate a Th1 and Th17 response. The type 7 secretion system ESX-1, involved in the secretion of many Mtb virulence factors, and lipid virulence factor phthiocerol dimycocerosate (PDIM) are important for Mtb pathogenesis. We show that WT C57BL/6J (B6) mice infected with ΔESX-1 or PDIM-lacking Mtb have lower bacterial burden and a Th17-mediated response compared to WT Mtb infection. Additionally, we show that type I interferon signaling does not contribute to the Th1 vs Th17 bias. 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 during early infection with protection being IL17-dependent demonstrating that Th17s fully compensated for the lack of Th1s. We hypothesize that Mtb directs host CD4 T cell response, via the ESX-1 secretion system and PDIM, towards a Th1-biased response for higher bacterial survival instead of the host inducing Th17s to provide enhanced protection.