TABLE OF CONTENTS

Plenary Abstracts ......................................................................................................................... 2
   New Horizons for TB Vaccines ............................................................................................. 3
   Retooling the Immune Response to TB ................................................................................ 5
   Issues in Clinical Research and Development ................................................................. 10

Oral Abstracts ............................................................................................................................. 14
   Recognizing the Infected Cell ............................................................................................. 15
   Mucosal Control of Mtb ....................................................................................................... 20
   Correlates of Protection and Disease ................................................................................. 26
   Clinical Research and Epidemiology .................................................................................... 31

Poster Abstracts .......................................................................................................................... 37
Several candidate vaccines have entered, or are about to enter, efficacy trials for prevention of tuberculosis disease, infection or recurrence. These include live attenuated and inactivated mycobacterial, viral-vectored and adjuvanted protein subunit vaccines, often developed for specific target populations defined by age group (infants, children, adolescents and adults), and presence or absence of *Mycobacterium tuberculosis* exposure and co-morbidities such as HIV infection. The path forward for the most advanced candidate vaccines will be reviewed in the context of safety, immunogenicity and potential efficacy in these populations, highlighting current knowledge gaps.

**Funding Sources/Conflicts of Interest**
Institutional grants to University of Cape Town funding several clinical trials of TB vaccine candidates
Tuberculosis remains a major global public health challenge with an urgent need for improved vaccines and treatments. Clinical studies to develop such new approaches can benefit from immunomonitoring that identifies robust correlates of protection and successful treatment responses. We propose the use of standardized whole blood stimulation systems with multi-functional readouts that show more robust results over conventional approaches. As a proof of concept we demonstrated their improved ability to classify active TB disease from latent infection, and are now testing their ability to predict early response to antibiotic treatment. We are also using this strategy in Covid-19 and Influenza vaccination studies to better understand innate immune determinants of vaccine responsiveness. Finally, we aim to integrate results from population based studies that are identifying major determinants of immune variability to support future precision immunology studies.

Funding Sources/Conflicts of Interest
Bill & Melinda Gates Foundation
Agence National de Recherche (ANR)

COI: I have received research support from Rules Based Medicine, Roche Genentech, and Sanofi Pasteur
Targeting immune evasion for effective TB vaccine design

Lin Wang², Peng Cheng¹, Yang Hua², Wang Jie², Liu Haipeng², Wang Fei¹, Baoxue Ge¹,²

¹Tongji University, Shanghai, China
²Shanghai Key Lab of Tuberculosis, Shanghai Pulmonary Hospital, Shanghai, China

Mycobacterium tuberculosis (Mt) is an extremely successful intracellular pathogen that causes tuberculosis (TB), which remains the leading infectious cause of human death. Upon infection, host cells detect Mt through a set of innate immune receptors and launch a range of cellular immune events. However, these defense mechanisms are extensively modulated by Mt to avoid host immune clearance and activation of immune responses. A better understanding of immune evasion mechanisms adopted by Mt will provide new insights into TB pathogenesis and contribute to the development of more effective TB vaccines and therapies.

Funding Sources / Conflicts of Interest
National Key Research and Development Program of China
National Natural Science Foundation of China
Viral vectors, including the potently immunogenic Lentiviral Vectors (LV), are poorly effective at directing antigens to the MHC-II endosomal pathway and eliciting CD4+ T cells. We developed a new generation of LV encoding for antigen-bearing monomers of collectins, substituted at their C-ter domain with CD40 ligand ectodomain to target and activate antigen-presenting cells. Host cells transduced with these optimized LV secreted soluble collectin-antigen polymers with the potential to be endocytosed in vivo and reach the MHC-II pathway. In the murine tuberculosis model, such LV induced efficient MHC-II antigenic presentation and triggered both CD8+ and CD4+ T cells, at the systemic and mucosal levels. They also conferred a significant booster effect, in line with the importance of CD4+ T cells for protection against Mycobacterium tuberculosis. Given the pivotal role of CD4+ T cells in orchestrating innate and adaptive immunity, this strategy can have broad range of applications in the vaccinology field.

**Funding Sources:**
This work was also supported by the Programmes Transversaux de Recherche (PTR # 52-17 from Institut Pasteur to LM and PC), the EU program TBVAC2020 (contract n°643381 to PC), CNRS, University of Toulouse, Agence Nationale de la Recherche/Program d’Investissements d’Avenir (ANR-11-EQUIPEX-0003 to ON), the Fondation pour la Recherche Médicale/Programme d’Investissements d’Avenir (DEQ20160334902 to ON), the Bettencourt Schueller Foundation (Grants Coup d’Élan pour la Recherche Française and Explore-TB to ON).

**Declaration of Interests:**
PC is the founder and CSO of TheraVectys. FA, FM, PA, AN and FN are employees of TheraVectys. FA, JL, FM, CB, PC and LM are inventors of a pending patent directed to the optimized vaccination LV, able to induce CD4+ T-cell responses. Other authors declare no competing interests.
DURTy business: are unconventional lymphocytes important for TB immunity in humans and can we harness them

Al Leslie¹

¹Africa Health Research Institute (AHRI), Durban, South Africa

The cytokines produced by conventional T-cells are essential for protecting against the development of active TB and have been the focus of most TB vaccines to date. However, other T-cell and lymphocyte subsets can produce the same essential cytokines, and there is interest and debate as to whether they can be usefully harnessed by vaccination. An attractive feature of such lymphocytes is that, unlike conventional T-cells, they are not under the control of polymorphic antigen-presenting molecules and can therefore be considered as Donor Unrestricted. To what extent Donor Unrestricted lymphocytes participate in the immune response to TB in humans remains unclear. Using TB infected human lung tissue, we investigated the involvement of Donor Unrestricted T-cells (DURTs) at the site of disease, including MAITs, iNKTs, GEMs, Gamma delta T-cells, CD1C T-cells, and Innate Lymphoid Cells (ILCs). We observed significant expansions of all 3 ILC subsets (producing Th1, Th2, and Th17 cytokines, respectively), suggesting these cell types are engaged in the host-pathogen interaction in the lung. By contrast, we found no evidence of any MAIT subset or iNKTS and GEM T-cell expansion that supported their involvement. However, striking and highly localized accumulations of Delta 1 gamma-delta T-cells and proliferation of CD1C reactive T-cells were observed. The importance of ILC3s is supported by parallel studies in mice and localized production of IL-17 in the lung correlated with less severe disease in humans. Taken together, these data show that certain Donor Unrestricted lymphocytes are involved in the host-pathogen interaction at the site of disease in humans and may be suitable vaccine targets.

Funding Sources / Conflicts of Interest
The Wellcome Trust and the Bill and Melinda Gates Foundation
Collaborative Cross (CC) mice: a new preclinical model for vaccine development

Samuel M. Behar¹

¹UMass Chan Medical School, Worcester, Massachusetts, USA

During the past twenty years, a global effort has developed infrastructure and expertise to clinically evaluate new vaccines against tuberculosis (TB). Testing whether vaccines prevent *Mycobacterium tuberculosis* (Mtb) infection remains complicated. The cost, time, and logistics required for accrual of cases requires large populations followed over years, which limits the capacity to test multiple vaccines concurrently. Therefore, pre-clinical evaluation to identify the best candidates is crucial. Preclinical vaccine development starts in the mouse model and the final gate is the nonhuman primates (NHP). TB pathogenesis in NHP is more like humans, but it is not feasible to test every new vaccine concept in NHP. Therefore, an important scientific and translational goal is to develop better small animal models for vaccine testing. We have leveraged the genetic diversity of Collaborative Cross (CC) strains to improve the ability of the mouse model to evaluate TB vaccines. We have completed a comprehensive analysis of BCG-elicited immunity and protection in 24 diverse CC strains. The CC population produces vaccine responses that are quantitatively and qualitatively different from C57BL/6 mice, and more closely resemble the variable outcomes observed in natural populations. These new data represent a rigorous basis to perform correlative and mechanistic studies to identify pathways associated with vaccine-induced immunity.

Funding Sources / Conflicts of Interest
This work is supported by P01 AI132130 from NIH/NIAID
A new adjuvant induces robust Th1/Th17 memory and increased mucosal recall responses in non-human primates

Joshua Woodworth¹, Gabriel Kristian Pedersen¹, Thomas Lindenstrøm¹, Frank Follmann¹, Vanessa Contreras-Devès², Roger LeGrand², Rasmus Mortensen¹

¹Statens Serum Institut, Copenhagen, Denmark
²Infectious Disease Models and Innovative Therapies, Paris, France

Background: There is a recognized need for diversification of the TB vaccine pipeline and subunit vaccines represent an attractive platform for this purpose. However, the availability of potent adjuvants with distinct immune signatures is limited and despite accumulating evidence for a protective role of Th17 cells, none of the existing clinical candidates induces robust Th17 responses. To address this, we developed a new liposomal adjuvant.

Methods and Results: Comparative data from humans, NHPs and mice demonstrate significant species-dependency on adjuvanticity. We therefore conducted a head-to-head NHP experiment to test this new adjuvant in comparison to two well-characterized adjuvants. In this comparison, the new adjuvant induced significantly increased immunogenicity with strong Th1 responses and sustained Th17 induction. This was associated with a profound recruitment of antigen-specific cells to the lungs of vaccinated animals after mucosal antigen recall (measured by Flow cytometry and PET-CT).

Conclusion: With robust Th17 induction, increased Th1 magnitude and potent mucosal recall responses in NHPs, this new adjuvant holds promise to improve TB immunity and fill a gap in the current TB vaccine development. Based on these results we have initiated GMP manufacturing in preparation for clinical testing with the H107 antigen.

Funding / Conflicts of Interest
NIH is supporting the development of H107 and the new adjuvant. The NHP study was funding by SSI. RM, DEN, GP and JW are co-inventors of an invention covering the new adjuvant. RM and PA are co-inventors of the H107 vaccine.
Many new TB vaccine candidates are in the development pipeline and need to be adequately studied in special populations such as people with HIV, who are at high risk of developing TB infection and disease and tend to develop less robust vaccine induced immune responses. Many questions remain unanswered regarding priority vaccine indications, clinical trial design, measures of safety, immunogenicity, and efficacy considerations that need special considerations for people with HIV. To address these gaps, a roadmap for developing TB vaccines for people with HIV was developed. Consensus statements to strategic questions were developed, which included: 1) What is the use case or rationale for developing TB vaccines for people with HIV?; 2) What is the landscape of TB vaccines and potential risks of administering these to people with HIV?; 3) Which vaccine candidates should be prioritized for study in people with HIV?; 4) What are the TB vaccine trial design considerations in people with HIV?; 5) What is the role of immunological correlates of protection in people with HIV?; and 6) What are the gaps in preclinical models for studying TB vaccines in people with HIV?

Funding Sources/Conflicts of Interest
DAIDS/NIH
Assessing non-specific effects of a new TB vaccine during clinical development

Bernard Fritzell

Independent Consultant, Lyon, France

A recent review of 5 randomized trials and 9 observational studies indicated that BCG vaccination was associated with reduction in all-cause mortality beyond the expected specific effect against tuberculosis. Despite certain limitations, the data suggested that BCG given at or near birth has beneficial effects by reducing mortality and morbidity due to non-tuberculosis infectious diseases in early infancy. Prospective randomized studies comparing BCG administration at birth and BCG delayed at 14 weeks of age would enable assessment whether the reduction in all-cause mortality and severe illness occurs in the first weeks of life, but such studies require a large sample size. A recent much smaller prospective randomized trial in 560 healthy newborns comparing infants given BCG at birth with infants having delayed BCG administration to 6 weeks showed a 25% reduction in the rate of physician-diagnosed non tuberculosis infectious diseases during the first 6 weeks of life. This was the first study to measure and confirm the nonspecific effect of BCG on total infectious disease morbidity in the first weeks of life.

The assessment of beneficial non-specific effects of a new (infant) TB vaccine has relevance from a Public Health perspective. However, separate large prospective randomized studies to measure the non-specific beneficial effects of a new infant TB vaccine cannot be considered in the clinical development before licensure. However, pre-licensure comparative phase 3 efficacy trials that are currently envisioned for new TB vaccines for infant vaccination may provide a framework for the analyses of clinical outcomes in relation to non-specific effects. The randomized design would allow the comparison of endpoints of interest, - all cause death, severe illness requiring hospitalization for reasons other than injury and medically attended infectious diseases between the control BCG and the investigational TB vaccine. Of note, BCG Danish strain was used in most studies reporting beneficial non-specific effects. Such analyses might inform on relevant characteristics of a new infant TB vaccine for Public Health.

Funding / Conflicts of Interest

I am a member of TBVI P&CDT team and as such received honorarium for consultancy on TB vaccines development.
The M72/AS01E tuberculosis vaccine showed 50% (95%CI: 2-74%) efficacy in a phase 2B trial in preventing active pulmonary tuberculosis disease, but potential cost-effectiveness of adolescent immunisation is unknown. We estimated the impact and cost-effectiveness of six scenarios of routine adolescent M72/AS01E-like vaccination in South Africa and India. All scenarios suggested an M72/AS01E-like vaccine would be highly (94-100%) cost-effective in South Africa compared to a cost-effectiveness threshold of 80/disability-adjusted life-year (DALY) averted. For India, a vaccine with efficacy pre- and post-infection was also highly likely (92-100%) cost-effective at a threshold of 4/DALY averted, however a vaccine with only post-infection efficacy had 0-6% probability of cost-effectiveness. In both settings, vaccinating 50% of 18 year-olds was similarly cost-effective to vaccinating 80% of 15 year-olds, and more cost-effective than vaccinating 80% of 10 year-olds. Vaccine trials should include adolescents to ensure vaccines can be delivered to this efficient-to-target population.

Funding Sources/Conflicts of Interest
RCH and GBG report current employment at Sanofi Pasteur, but do not work on TB or TB vaccines in their roles. RCH, GBG, CKW and RW report grants from Bill and Melinda Gates Foundation for the conduct of the study. CKW received funding from UKRI/MRC MR/N013638/1. Funders had no role in study design, data collection, data analysis, data interpretation, or writing of the report. All other authors declare no competing interests.
Engaging communities in TB clinical trials: Lessons learnt from Mbeya, Tanzania

Doreen Pamba¹, Jane Ambindwile¹, Simeon Mwanyonga¹, Lonze Ndelwa¹, Ombeni Chimbe¹, Christina Manyama¹, Lillian Tina Minja¹, Issa Sabi¹, Nyanda Ntinginya¹, Erica Sanga²

¹National Institute for Medical Research (NIMR)-Mbeya Medical Research Center, Mbeya, Tanzania
²National Institute for Medical Research (NIMR)-Mwanza Research Center, Mwanza, Tanzania

Introduction: Community engagement (CE) entails networking with communities and stakeholders for their involvement in TB clinical trials. The National Institute for Medical Research-Mbeya Medical Research Center has been conducting CE activities since 2005 with the objectives of:

- Raising community awareness on TB research
- Establishing and maintaining research trust and mutual understanding between communities, the research team and relevant key stakeholders
- Disseminating trial findings to trial participants and relevant stakeholders including policy makers and communities
- Recruiting and maintaining high participant retention
- Minimizing and managing community rumors and misconceptions about TB research

Engagement strategies: Below are strategies used for engaging participants and communities in collaboration with key TB stakeholders during a trial.

- Community sensitization meetings and participation in World TB Day
- TB screening, diagnosis and HIV testing at community settings
- Community advisory board (CAB) meetings and TB research literacy trainings
- Radio programs to educate communities
- Meetings with relevant community stakeholders (i.e., community leaders, ex-TB members, healthcare providers, traditional and alternative healers)
- Designing and distributing TB education materials
- Biannual participant retention meetings
- Result dissemination to all stakeholders including presentation in scientific meetings

Lessons learnt

- Strong partnership with key stakeholders facilitates smooth trial implementation
- TB research literacy trainings enables CAB members and ex-TB patients to assist in addressing community misconceptions
- Participant retention meetings allows discussions of arising challenges being experienced
- Outreach sessions enhance community awareness of TB research and minimizes misconceptions
- Results dissemination to stakeholders builds research trust and positive attitudes
- Meetings with TB treatment facility staff support trial recruitment activities

Conclusion: Community engagement is crucial to a successful TB clinical trial implementation.

Funding Sources: European and Developing Countries Clinical Partnership (Grant numbers: RIA2016V-1626 POR TB consortium, and RIA2017S-2012 – Simplici-TB)

Conflicts of Interest: None to declare
Characterising the BCG-induced antibody response for M.tb antigen discovery and vaccine development

Rachel Tanner¹, Arlo Randall ², Krista Trappl-Kimmons², Elena Stylianou¹, Deepa Paliwal³, Andrew White⁴, Ian Jones³, Bernardo Villarreal-Ramos⁵,⁶, Angela Yee², Martin Vordermeier⁵,⁶, Sally Sharpe⁴, Helen McShane¹

¹ University of Oxford, Oxford, United Kingdom
² Antigen Discovery Inc., Irvine, United States
³ University of Reading, Reading, United Kingdom
⁴ Public Health England, Salisbury, United Kingdom
⁵ Animal Plant and Health Agency, Addlestone, United Kingdom
⁶ Aberystwyth University, Aberystwyth, United Kingdom

Background: Immunological assessment of BCG vaccination has focussed on the induction of cell-mediated immunity. While a growing body of evidence indicates a role for antibodies in immunity from TB, there is a paucity of literature on the humoral response to BCG vaccination. Improved characterisation of the BCG response in relation to outcomes of mycobacterial challenge may be valuable in determining factors contributing to protection.

Methods: We have taken an unbiased approach to antigen discovery, using whole-protein microarrays spanning the entire proteome of M.tb to identify targets recognised by BCG-induced antibodies in serum from humans, non-human primates (NHP) and cattle. Inclusion of preclinical samples allowed array results to be related to levels of protection from M.tb or M.bovis challenge in matched animals. Proteins of interest were then assessed as protein + adjuvant subunit vaccines in mice, using the ex-vivo mycobacterial growth inhibition assay (MGIA) as a high-throughput surrogate of protective efficacy.

Results: We report top protein targets of BCG-induced IgG across species ranked by fold change in reactivity following vaccination, showing that route of vaccination alters target antigen repertoire and reactivity with superior responses in intravenously BCG vaccinated NHPs. We also demonstrate associations between IgG antigen specificity and improved protection from mycobacterial challenge. Of 7 proteins of interest tested in mice to date, 3 conferred significantly improved control of mycobacterial growth in the MGIA compared with unvaccinated controls.

Conclusion: Such insights into protective BCG-induced immune responses could inform the design of an efficacious new TB vaccine which may benefit from targeting humoral as well as cell-mediated immunity.

Funding Sources / Conflicts of Interest
This work was funded by a Fellowship to RT from VALIDATE (a UKRI-GCRF Network)
Dissecting HLA-E-restricted T-cell responses against Mtb as future targets for vaccination

Linda Voogd¹, Paula Ruibal¹, Kees L.M.C. Franken¹, Ian Derksen², Renate S. Hagedoorn³, Marjolein van Wolffswinkel¹, Krista E. van Meijgaarden¹, Thomas Abeel⁴,⁵, Thomas J. Scriba⁶, Ferenc A Scheeren⁷, Mirjam H. M. Heemskerk³, Tom H. M. Ottenhoff³ and Simone A. Joosten¹.

¹ Department of Infectious Diseases, Leiden University Medical Center, Leiden, The Netherlands; ² Department of Cell and Chemical Biology, Leiden University Medical Center, Leiden, The Netherlands; ³ Department of Hematology, Leiden University Medical Center, Leiden, The Netherlands; ⁴ Broad Institute of M.I.T. and Harvard, Cambridge, MA, USA; ⁵ Delft Bioinformatics Lab, Delft University of Technology, Delft, The Netherlands; ⁶ South African Tuberculosis Vaccine Initiative, Institute of Infectious Disease and Molecular Medicine, Division of Immunology, Department of Pathology, University of Cape Town, South Africa; ⁷ Department of Oncology, Leiden University Medical Center, Leiden, The Netherlands

HLA-E is a non-classical MHC-class-Ib molecule with only two allelic variants, HLA-E*01:01 and HLA-E*01:03, differing in one single amino acid outside the peptide binding groove. HLA-E is enriched in Mycobacterium tuberculosis (Mtb)-phagosomes and is not downregulated by HIV, in contrast to classical HLA-class-Ia molecules, making it an appealing target for presenting conserved Mtb antigens as innovative vaccine. We have previously identified Mtb-specific HLA-E-restricted CD8+T-cells with an unorthodox Th2-like phenotype and capable of inhibiting intracellular Mtb in human macrophages. The mouse Qa-1 homologue to HLA-E further supports that MHC-E presented peptides contribute to protective immunity to TB in vivo.

Through optimized peptide-binding prediction algorithms we identified subtle differences in peptide binding affinity and specificity to HLA-E*01:01, HLA-E*01:03, Qa-1b and the non-human primate homologue Mamu-E and we discovered novel Mtb-derived peptides with HLA-E-binding capacity. We are currently testing TCR and NKG2A/CD94 peptide recognition with optimized HLA-E tetramers. Moreover, ex vivo tetramer staining of PBMCs derived from latent Mtb-infected individuals followed by sorting enabled the selection of HLA-E-restricted Mtb-specific TCR sequences. We transduced these selected TCR sequences into Jurkat cells expressing fluorescent reporter constructs to further investigate the determinants that underly TCR recognition of HLA-E/peptide complexes using in vitro functional assays. With this combined knowledge we can select immunogenic Mtb-derived peptides that can be presented via HLA-E to potentially induce CD8+T-cell responses with the capacity to control Mtb infection.

Altogether, our data will help to further dissect the importance of HLA-E-restricted CD8+T-cell immunity in the context of Mtb and to identify the peptides that represent the best leads for vaccine development.

Funding Sources/Conflicts of Interest
Supported by European Union’s Horizon2020 under the Marie Sklodowska-Curie grant agreements 707404 and 793027 (to PR); The Netherlands Organization for Scientific Research (NWO-TOP Grant Agreement No. 91214038); the National Institute of Allergy and Infectious Diseases of the National Institutes of Health under Award Number R21AI127133 and R01AI141315. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health or any funder. The authors declare no conflict of interest.
Human memory CD4+ T cells that directly recognize macrophages infected with Mycobacterium tuberculosis are a distinct and smaller subset of those that are antigen-specific

Vinicius Suzart1, Daniel Gail1, Weinan Du1, Stephen Carpenter1

1 Case Western Reserve University, Cleveland, United States

Background: A crucial feature of T cell mediated protection is the ability to recognize foreign peptides through their T cell antigen receptor (TCR) in the context of major histocompatibility complexes (MHC). However, evidence from the mouse model of TB and a recent study of memory CD8+ T cells from individuals in the AERAS-402 vaccine study, suggest that not all T cells specific for Mycobacterium tuberculosis (Mtb) antigens recognize cells infected with Mtb.

Methods: Using flow cytometry and co-culture of memory CD4+ T cells from 9 healthy volunteers with latent Mtb infection (LTBI), we determine the frequency of recognition of Mtb-infected monocyte-derived macrophages. We used live cell sorting and single-cell TCR sequencing and transcriptomics to determine the extent to which T cell responses to Mtb-infected macrophages represent a distinct subset of TCR clonotypes.

Results: Significantly fewer memory CD4+ T cells become activated in response to infected macrophages compared to a ~3-fold greater response when either peptides or Mtb lysate were added exogenously. T cells that responded to Mtb-infected cells predominantly expressed a CCR6+ CXCR3+ CCR4- (Th1*) phenotype, whereas when peptides were added, the memory CD4 response contained a larger proportion of CCR6+ CXCR3- CCR4- cells.

Discussion: We posit that targeted immune evasion strategies that redirect T cells such as antigen downregulation or antigen transfer from infected to uninfected cells likely play a major role in Mtb pathogenesis and reactivation from latency.

Conclusion: We favor the emerging view that a goal of TB vaccine design should be to elicit memory T cells that respond specifically to Mtb-infected macrophages.

Funding Sources/Conflicts of Interest
University Hospitals Research Fund and NIH R01 AI124348
Identification of M. tuberculosis antigens that induce dominant IL-17 responses during human infection

Paul Ogongo1,2, Anthony Tran1, Devin Columbus1, Jason Limberis1, Joel D. Ernst1

1University of California, San Francisco, San Francisco, United States
2Institute of Primate Research, Nairobi, Kenya

Studies of CD4 T-cell responses to Mycobacterium tuberculosis (Mtb) have concentrated on a few antigens, mainly ESAT-6 and CFP-10, yet Mtb genome encodes for over 4,000 proteins. We hypothesised that CD4 T-cells with distinct antigen specificity are associated with different functional responses. We stimulated PBMC from recently exposed, QFT+ participants with distinct Mtb antigens: group A: PPE18, PPE46, EsxA, and EspI, and group B: Rv0010c, Rv0012, RimJ and LldD2, and assessed CD4 T-cell responses by intracellular cytokine staining for IFN-γ, TNF-α, IL-17, and GM-CSF. We found that IL-17 responses had the greatest variation by antigen and by participant: CD4 T-cells from 58% of the participants produced IL-17 to group A compared to 79% to group B antigens (p =0.0118). In comparison, 71% of participants produced IFN-γ to group A compared with 58% to group B antigens (p =0.1248). Additionally, we found a higher magnitude of IL-17 response to group B than group A antigens, % of CD4 T-cells expressing detectable IL-17, 0.71±0.53 vs 0.42±0.53 (p =0.007). Analysis of the same participants 6-months later revealed that IL-17 responses to group B antigens were durable while IFN-γ response to group B antigens had waned (p =0.0005). Finally, we observed that CD4+IL-17+ T-cells were less differentiated characterised by dominant central memory phenotype compared to CD4+IFN-γ+ that were predominantly effector memory. Our results provide evidence that distinct CD4 T-cell phenotypes and effector responses are associated with specific antigens, and suggest that inclusion of these antigens in TB vaccines for prevention of disease will broaden the spectrum of T-cell responses and provide enhanced vaccine efficacy.

Funding Sources/Conflicts of Interest
Funding: TBRU-ASTRa: Grant No. U19 AI 111211
Conflict of interest: None
Peripheral blood Mtb-specific T cell frequency and phenotype correlates of TB disease progression

Virginie Rozot1, Miguel Rodo1,3, Carly Young1, Munyrraazi Musvosvi1, Constance Schreuder1, Phu Van3, Greg Finak3, Evan Greene3, Raphael Gottardo3, Holden T Maecker4, Digby Warner5, Valerie Mizrahi5, Cecilia Lindestam-Arlehamn6, Alex Sette6, Willem A Hanekom1,7, Nicole Bilek1, Michelle Fisher1, Francesca Little2, Mark Hatherill1, Tom J Scriba1

1South African Tuberculosis Vaccine Initiative, University of Cape Town, Cape Town, South Africa
2Department of Statistical Sciences, University of Cape Town, Cape Town, South Africa
3Department of Biostatistics and Epidemiology, Fred Hutch Cancer Research Center, Seattle, United States
4Institute for Immunity, Transplantation and Infection, Stanford University, Stanford, United States
5Molecular Mycobacteriology Research Unit, University of Cape Town, Cape Town, South Africa
6La Jolla Institute for Allergy and Immunology, La Jolla, United States
7Africa Health Research Institute, Durban, South Africa

Background: Th1 cells are necessary but not sufficient for control of M. tuberculosis (Mtb), and, paradoxically, can also mediate pathogenesis. The role of antigen-specific T cell magnitude, functional and phenotypic attributes in immune control or TB pathogenesis in humans is poorly understood. We hypothesized that Mtb-specific, Th1/Th17 cytokine-expressing CD4 and Th1 cytokine-expressing CD8 T cells are associated with progression to TB disease and aimed to identify Mtb-specific T cell phenotypic correlates of clinical outcome of Mtb infection.

Methods: We performed a longitudinal study in adolescents with immunological sensitization to Mtb, who remained healthy (controllers, n = 37) or progressed to microbiologically confirmed TB disease (progressors, n = 37). PBMC were stimulated with live Mtb or peptide pools spanning Mtb-antigens or EBV/CMV. T cell responses were characterised by mass cytometry intracellular cytokine staining assay (45 markers). We applied FAUST and an in-house cytokine-gating algorithm to identify cell types automatically, filtered out non-Mtb-specific subsets and assessed associations with TB progression using longitudinal mixed-effects modelling.

Results: Frequencies of Mtb-specific Th1 or Th17 cells and most of their phenotypes did not correlate with clinical outcome. Interestingly, Mtb-specific CD45RA-CCR7-CD27+ IFNg+IL2+TNF+ and IL2+TNF+ CD4 T cells decreased during progression. Progression was also associated with elevated T cell activation, observed as bulk HLA-DR+CD7+ CD4 T cells as well as HLA-DR-expressing Mtb-specific CD4 T cells.

Conclusion: We reveal kinetics of T cell activation and differentiation in human TB progressors that are consistent with elevated in vivo Mtb antigen expression occurring many months prior to TB diagnosis.

Funding Sources/Conflicts of Interest
This work was supported by the Bill and Melinda Gates Foundation (BMGF) Global Health grants OPP1066265, OPP1023483, OPP1065330, and Grand Challenges in Global Health (GC6-74 grant 37772) and the Howard Hughes Medical Institute. The ACS study was also supported by Aeras and BMGF GC12 (grant 37885) for QFT testing. VR was supported by the Swiss National Foundation and EDCTP.

Conflicts of Interest: None
Non-tuberculous mycobacterial mucosal-induced immune response has an enhanced protective effect on BCG’s efficacy against Mycobacterium tuberculosis infection

Taru S. Dutt1, Burton R. Karger1, Amy Fox1, Nathan Youssef1, Rhythm Dhadhwal1, Sarah Cooper1, Elisa Rampacci1, Brendan Podell1, Andres Obregon-Henao1, Marcela Henao-Tamayo1

1Microbiology, Immunology, and Pathology, Colorado State University, Fort Collins, Colorado.

Tuberculosis (TB) continues to increase worldwide despite vigorous attempts to control it. Bacillus Calmette-Guerin (BCG) is the only licensed vaccine currently available for protection against TB. However, its efficacy is highly variable between countries. Some studies have revealed that BCG’s variable protection is due, amongst others, to immunological interference by environmental, non-tuberculous mycobacteria (NTM). However, a definitive mechanism has not been identified so far. Considering the previous, we developed a murine model closely resembling the natural history of human exposure to different mycobacterial species, including 1) BCG vaccination at an early age; 2) exposure to viable NTMs (Mycobacterium avium subsp. avium) via the oral route and 3) maintaining continuous NTM exposure even after TB infection, as occurs in endemic regions. Surprisingly, we found that a low dose of NTM via the oral route enhanced BCG-mediated protection for up to 120 days post-infection, as determined by decreased Mycobacterium tuberculosis (Mtb) burden and in lungs and spleens of infected mice (C57BL/6, C3Heb/Fe and C3H/HeOuJ) and improved pathology scores. This reduction in Mtb is directly correlated to increased numbers of B220+ B2 B-cells and CXCR5+ follicular helper T cells in the lungs. Intestinal Peyer’s Patches likewise had increased numbers of B cells as early as 3 months post NTM exposure, before Mtb aerosol infection. Reduced bacterial counts correlated also with higher numbers of CD8 cytotoxic T cells and NK cells positive for perforin in the lungs of these mice. Immunohistopathology results suggest the presence of tertiary germinal centers in the lungs of mice immunized with BCG taking NTMs in the drinking water. Furthermore, animals receiving NTMs in the drinking water had increased quantities of IgA and IgG against Mtb in bronchoalveolar lavage and serum. Therefore, we hypothesize that B cells are activated by T-dependent B cell activation mechanism and produce antibodies that stimulate the killing of Mtb-infected cells by enhancing antibody-dependent cell-mediated cytotoxicity. These results suggest that chronic live NTM exposure via the oral route elicits a protective humoral mucosal immune response against Mtb. Ongoing experiments are testing the disparity between cytotoxic T cells and antigen-specific B cells from the different groups evaluated. Furthermore, spatial transcriptomics experiments will determine the variances in the granulomatous formation in mice mucosally immunized with NTMs.

Funding Sources/Conflicts of Interest
R01 AI127475 Vaccine induced memory immunity in tuberculosis / no conflicts of interest
Listeria-vectored vaccine expressing multiple Mycobacterium tuberculosis immunoprotective antigens provides potent protective immunity against aerosol challenge with virulent Mycobacterium tuberculosis in mouse and guinea pig models

Qingmei Jia¹, Sasa Maslesa-Galic¹, Marcus Horwitz¹

¹University of California-Los Angeles, Los Angeles, United States

Background: Mycobacterium tuberculosis (Mtb) infects approximately one-third of the world’s population, causing active tuberculosis (TB) in ~10 million people and death in ~1.6 million people annually. The current BCG vaccine provides moderate protection against childhood TB, but poor protection against adult pulmonary TB, the most prevalent form. Hence, improved vaccines against TB are urgently needed.

Methods: We have developed a live attenuated recombinant Listeria monocytogenes (Lm)-vectored TB vaccine that expresses multiple highly immunoprotective antigens of Mtb and evaluated it for immunogenicity and efficacy in mouse and guinea pig models.

Results: In mouse models (BALB/c and C57BL/6), multi-antigenic (5- and 9-antigen) recombinant Lm vaccines (rLm) induce Mtb antigen-specific T cell responses and protect against Mtb aerosol challenge as a standalone vaccine and additionally boost the level of protection afforded by BCG. In the guinea pig model, the rLm vaccines induce Mtb antigen-specific lymphocyte proliferation and provide protective immunity against Mtb aerosol challenge.

Discussion: The rLm vaccine is safe (utilizing an Lm vector with deletions in two major virulence genes) and immunologically potent in mouse and guinea pig models. The rLm vaccine has major advantages over other types of TB vaccines: a) it is cleared quickly in vivo and pre-existing immunity does not affect vaccine efficacy, in contrast to viral-vectored vaccines; b) it expresses multiple key Mtb antigens; c) it is inexpensive to manufacture in broth culture; d) it presents antigens via both MHC class I and II leading to antigen-specific CD4+ and CD8+ T-cells, both important to anti-Mtb immunity; and e) it is generally more potent than viral-vectored and protein/adjuvant vaccines.

Funding Sources / Conflicts of Interest
This work was supported by National Institutes of Health grants AI031338 and AI135631 and Department of Defense PRMRP CDMRP grant W81XWH-12-1-0403.
Mucosal BCG revaccination after intradermal priming prevents TB disease in rhesus macaques

MPM Vierboom¹, KG Haanstra¹, RAW Vervenne¹, SO Hofman¹, CC Sombroek¹, K Dijkman¹, L Meijer¹, MA Stammes¹, J-R Wei², N Howard², F Hopkins², M Chao², AJ Vickers², SM Fortune², FAW Verreck¹

¹Biomedical Primate Research Centre, Rijswijk, the Netherlands
²Dept of Immunology and Infectious Disease, Harvard T. H. Chan School of Public Health, Boston, MA, USA

Previously, we have shown that vaccination via the pulmonary mucosa improves BCG-induced protection in rhesus macaques in association with specific immune signatures primarily in the airways. In the present study we have interrogated the pertinence of these signatures and the protective effect of mucosal BCG after prior standard intradermal BCG.

We found that revaccination by heterologous routing does not impede the typical responses associated with pulmonary mucosal BCG. High frequencies of adaptive polyfunctional Th1/Th17 cells expressing markers of tissue-resident memory and relatively high levels of antigen-specific cytokine secretion, including IL17 and IL10, are apparent in the airways after mucosal BCG boosting as much as after mucosal BCG priming. And these signatures were associated with protection by reduced pathology and mycobacterial tissue burden. As we challenged by repeated limiting dose Mtb exposure, we found that both mucosal priming and boosting, relative to intradermal BCG, induced a significant delay in conversion of a diagnostic IFNg release assay. While we had previously interpreted this finding as a prevention of infection signal, the use of molecularly tagged Mtb stocks per challenge round in this study, allowed to demonstrate that mucosal BCG in this model ultimately confers prevention of disease.

The results of this study shed interesting new light on protective as well as diagnostic immune signatures in relation to TB infection and disease. This warrants further research towards a better understanding of vaccine-induced immune protection. It also provides a rationale for future trials or experimental medicine approaches into the efficacy of mucosal BCG as a revaccination strategy towards impacting the ongoing TB epidemic.

Funding Sources / Conflicts of Interest
None
Maturation of iBALT can be mediated by vaccination and serve as backdoor for T cells to the Mycobacterium tuberculosis (Mtb) infected lung

Thomas Lindenstrøm¹, Mortensen Rasmus¹

¹Statens Serum Institut, Copenhagen, Denmark

Background: One of the biggest barriers for improving protective immunity to TB is for CD4 T cells to get into direct contact with the infected macrophages in the lung. T cell positioning and migration within the Mtb infected lung are therefore critical components of a protective response. Mature induced Bronchus-Associated Lymphoid Tissue (iBALT) express structural features tailored for entry of lymphoid homing cells and could therefore provide a ‘gateway’ for direct lung entry and intralesional positioning of T cells. Vaccines and vaccination strategies that can facilitate and maintain such local immune responses would be a prerequisite to overcome this barrier and to improve protective immunity.

Results and discussion: Using a mouse model of aerosol Mtb infection, we demonstrate that the Th17 inducing vaccine H107/CAF01 promotes the development of fully mature iBALT 4 wk into infection, whereas unimmunized mice show limited signs of iBALT maturation. Hence, only vaccinated mice displayed highly organized iBALTs with clear formation of high endothelial venules (HEVs), aggregated T cell zones, higher levels of CXCL13 as well as increased infiltration of activated B cells. In addition, transition into germinal center B cells was observed. In order to study the significance of iBALT maturation state for lung entry, blockage of HEV-mediated lung entry was performed. In vivo administration of anti-CD62L was found to significantly reduce the lung migration of adoptively transferred CD4 T cells in H107/CAF01 vaccinated, but not unvaccinated, recipients carrying a 4 wk Mtb infection. Our data suggests that iBALT promoting vaccines can facilitate T cell entry and potentially intralesional positioning for improved protection.

Funding Sources/Conflicts of Interest
NIH: 1R01AI134246-01
BALANCE BETWEEN PROTECTION AND PATHOGENIC RESPONSE TO AEROSOL CHALLENGE WITH MYCOBACTERIUM TUBERCULOSIS (MTB) IN MICE VACCINATED WITH TRIFU64, A FUSION CONSISTING OF THREE MTB ANTIGENS

Sadaf Sulman1,2, Benjamin O. Savidge1,3, Kawther Alqaseer1,3,4, Mrinal K. Das1,3, Neda Nezam Abadi1,5, John E. Pearl1,3, Obolbek Turapov1,3, Galina V. Mukamolova1,3, M. Waheed Akhtar2, Andrea May Cooper1,3

1Department Respiratory Sciences, University of Leicester, United Kingdom
2School of Biological Sciences, University of the Punjab, Lahore, Pakistan
3Leicester Tuberculosis Research Group—LTBRG, University of Leicester, Leicester, United Kingdom
4Department of Basic Science, Faculty of Nursing, University of Kufa, Kufa, Najaf Governorate, Najaf, Iraq
5APC Microbiome Ireland, University College Cork, Cork, Ireland

Introduction: Tuberculosis vaccines capable of reducing disease worldwide have proven difficult to develop. BCG is effective in limiting childhood disease, but adult TB is still a major public health issue. Development of new vaccines requires identification of antigens that are both spatially and temporally available throughout infection, and immune responses to which reduce bacterial burden without increasing pathologic outcomes. Subunit vaccines containing antigen require adjuvants to drive appropriate long-lived responses.

Materials and methods: We generated a triple-antigen fusion containing the virulence- associated EsxN (Rv1793), the PPE42 (Rv2608), and the latency associated Rv2628 to investigate the balance between bacterial reduction and weight loss in an animal model of aerosol infection.

Results and discussion: We found that in both a low pattern recognition receptor (PRR) engaging adjuvant and a high PRR- engaging adjuvant (MPL/TDM/DDA) the triple-antigen fusion could reduce the bacterial burden, but also induced weight loss in the mice upon aerosol infection. The weight loss was associated with an imbalance between TNFα and IL-17 transcription in the lung upon challenge. These data indicate the need to assess both protective and pathogenic responses when investigating subunit vaccine activity.

Funding Sources/Conflicts of Interest
This work was supported by MRC-UKRI, grant number MR/P011136/1 and the Royal Society, grant number WM150032 to AMC; a Pakistan Academy of Sciences grant to MWA; a Commonwealth Split-site Scholarship (PKCN-2017-158) to SS; Support from Higher Education and Scientific Research Iraq to KA; a Newton International Fellowship grant number NF160754 to MKD; a UK Biotechnology and Biological Sciences Research Council grant BB/P001513/1 to GVM.

Conflict of Interest: None
Pulmonary BCG induces long-term activation of lung resident macrophages which contribute to protection against tuberculosis

Mata Elena1, Uranga Santiago1, Domínguez-Andrés Jorge3, Peixoto Antonio2, Yuste José4, Martín Carlos1, Nacho Aguilo1

1 University of Zaragoza, Zaragoza, Spain
2 Institut de Pharmacologie et de Biologie Structurale, Toulouse, France
3 Radboud University, Nijmegen, Netherlands
4 Instituto de Salud Carlos III, Madrid, Spain

Background: Bacillus Calmette-Guerin (BCG) is an attenuated bacterial vaccine used to protect against Mycobacterium tuberculosis (Mtb) in regions where infections are highly prevalent. BCG is currently delivered by the intradermal route, but alternative routes of administration are of great interest, including intrapulmonary delivery to more closely mimic respiratory M. tuberculosis infection.

Methods: In this study, mice subjected to pulmonary delivery of GFP-tagged strains of virulent (Mtb) and attenuated (BCG) mycobacteria were studied to better characterize infected lung cellular subsets.

Results: Profound differences in dissemination patterns were detected between Mtb and BCG, with a strong tendency of Mtb to disseminate from alveolar macrophages (AMs) to other myeloid subsets, mainly neutrophils and recruited macrophages. BCG mostly remained in AMs, which promoted their activation. These pre-activated macrophages were highly efficient in containing Mtb bacilli upon challenge and disrupting early bacterial dissemination, which suggests a potential mechanism of protection associated with pulmonary BCG vaccination. Respiratory BCG also protected mice against a lethal Streptococcus pneumoniae challenge, suggesting that BCG-induced innate activation could confer heterologous protection against respiratory pathogens different from Mtb. Importantly, BCG drove long-term activation of AMs, even after vaccine clearance, and these AMs reacted efficiently upon subsequent challenge.

Conclusion: These results suggest the generation of an innate memory-like response in AMs induced by pulmonary BCG vaccination.

Funding Sources / Conflicts of Interest
Supported by Spanish Ministry of “Ciencia, Innovación y Universidades” [grant number RTI2018-097625-B-I00]
Guinea pig PIM-specific, CD1-restricted T cells contribute to granuloma integrity and immune protection against virulent M. tuberculosis

Emmelie Eckhardt1, Jan Schinköthe2, Martine Gilleron3, Max Bastian1

1Friedrich-Loeffler-Institut, Greifswald – Insel Riems, Germany
2Leipzig University, Leipzig, Germany
3Institute of Pharmacology and Structural Biology (IPBS), Toulouse, France

Introduction: Guinea pigs express a functional type I CD1 system and therefore allow studying the role of CD1 restricted, lipid-specific immune responses. In the current study, we investigate the role of Phosphatidylinositol-Mannoside (PIM) reactive, CD1-restricted T cells in immune protection against virulent M.tuberculosis.

Methods/ Results: Guinea pigs were vaccinated with BCG or highly-purified, CAF01-formulated PIM. In-situ hybridization and MALDI-Imaging were used to demonstrate CD1-expression and PIM-synthesis in BCG-infested tissues. After vaccination CFSE dilution was measured by flowcytometry to quantify antigenspecific T cells. Robust CD1-restricted, PIM-specific T cell responses were observed after BCG and to a lesser extent after PIM-CAF01 vaccination. No PIM-specific reactivity was observed in control animals receiving empty CAF01 liposomes. After 80 days all animals were challenged with virulent H37Rv. After four weeks both, BCG and PIM-vaccinated animals showed significantly enhanced pathological scores and reduced CFU counts in the spleen compared to CAF01 controls. Comprehensive histological and transcriptional analyses revealed that the presence of CD1b1 expressing cells in the cortical region of the draining lymphnode was correlated with the abundance of T cells in the tuberculous lesions. Additionally, the number of CFSE-negative, PIM reactive T cells after vaccination was directly correlated with reduced necrotic lesions at the site of infection. Protected animals showed reduced transcript levels of inflammatory cyto- and chemokines in the draining lymphnode.

Conclusion: CD1b-restricted, PIM-reactive T cells contribute to immune protection against virulent M.tuberculosis. Our observations in the guinea pig model suggest that they participate in the spatial orchestration of the local immune defense and help to curtail mycobacteria-induced, hyperinflammatory immunopathology.

Funding Sources / Conflicts of Interest
There are no conflict of interests. The study was funded by the European Commission as part of the TBVAC2020 Consortium (Grant H2020-PHC-643381) and the Deutsche Forschungsgemeinschaft (Grant BA 3885/2-1).
To understand how tuberculosis boosts HIV-1 infection in macrophages: Tunneling nanotubes and Siglec-1 fill the gap!

Maeva Dupont1, Sarah Monard1, Shanti Souriant1, Zoï Vahlas1, Luciana Balboa 2, T. Vu Manh 3, Marcelo Kuroda4, Nuria Izquierdo-Useros5, Isabelle Maridonneau Parini1, Olivier Neyrolles1, Geanncarlo Lugo Villarino1, Christel Verollet1

1 IPBS CNRS UMR 5089, Toulouse, France
2 Institute of Experimental Medicine-CONICET, Buenos Aires, Argentina
3 CIML, Marseille, France
4 University of California, Davis, CA, United States
5 IrsiCaixa AIDS Research Institute, Badalona, Spain

Co-infection with Mycobacterium tuberculosis (Mtbt), the etiological agent of tuberculosis (TB), and HIV-1 is major health issue in the world. The synergistic relationship between HIV-1 and Mtb is known to result in increased pathogen proliferation and associated pathogenesis, worsening diagnosis and treatment. We showed that HIV-1 infection is exacerbated in macrophages exposed to TB-associated microenvironments due to the formation of tunneling nanotubes (TNT). These cellular structures favor HIV-1 spread among macrophages. This mechanism likely participates in enhanced virus load observed in HIV/TB co-infected patients. To identify molecular factors associated with TNT function and function, we performed a transcriptomic analysis in these macrophages, and revealed the up-regulation of the lectin receptor Siglec-1/CD169. We demonstrate Siglec-1 overexpression depends on TB-mediated production of type I interferon. In co-infected non-human primate lungs, Siglec-1 is highly expressed by alveolar macrophages, whose abundance correlates with pathology and activation of the type I interferon/STAT1 pathway. Intriguingly, Siglec-1 expression localizes exclusively on microtubule-containing TNT that are long and carry HIV-1 cargo. Siglec-1 depletion in macrophages decreases TNT length, diminishes HIV-1 capture and cell-to-cell transfer, and finally abrogates TB-driven exacerbation of HIV-1 infection. Altogether, we uncover a deleterious role for Siglec-1 in TB-HIV-1 co-infection, and its localization on TNT opens new avenues to understand the role of Siglec-1+ macrophages in cell-to-cell communication in the lung in TB and co-infection settings.

**Funding Sources/Conflicts of Interest**
ANRS, Sidaction

Tunneling nanotube (TNT) formation in human macrophages (by Scanning Electron Microscopy)
Influenza virus exposure decreases host derived protection against Mycobacterium tuberculosis infection in ferrets

Tuhina Gupta1, Hind Azami1, Kaori Sakamoto1, Stephen Harvey1, Russell Karls1, Frederick Quinn1

1University of Georgia, Athens, United States

Background/Introduction: Influenza virus causes significant morbidity and mortality in humans. Streptococcus pneumoniae and other respiratory bacteria can cause pre-, co-, or post-infections with influenza virus resulting in severe morbidity; however, little is known about influenza virus-Mycobacterium tuberculosis (Mtb) association. Epidemiological records show increased tuberculosis (TB) mortality during the 1918 influenza pandemic suggesting that similar to S. pneumoniae, Mtb infections preceding/ accompanying or following influenza may significantly exacerbate morbidity/ mortality. The ferret model has been used for decades to demonstrate that infections with influenza virus alter the immune response. Thus, we used influenza virus-Mtb infected ferrets to demonstrate a similar profile.

Methods: Six month old female ferrets (n=4/ group) were intranasally exposed to either sterile saline or 106 PFU of influenza strain A/Port Chalmers/1/1973 (H3N2) for 4 weeks followed by intratracheal instillation with 103 CFU of Mtb Erdman. Bacterial viable counts were determined 8 week post Mtb challenge from harvested tissues while splenocytes and whole blood cells were used to determine expression of key cytokines following in vitro stimulation assay with Mtb whole cell lysate.

Results: Splenocytes and blood lymphocytes from Mtb-only infected ferrets released more IFNγ compared to the influenza-Mtb sequentially-infected animals. Interestingly, a higher organ CFU trend was observed in the influenza-Mtb compared to Mtb-alone infected animals. A more thorough analysis of serum and blood lymphocyte cytokine production by ELISA and qRT-PCR is on-going.

Discussion and Conclusion: Ferrets can mimic co-infection dynamics of pathogens such as influenza virus and Mtb and can be used to better understand and identify the factors for successful transmission events and vaccine protective efficacy.

Funding Sources/Conflicts of Interest
No
Efficacy of RUTI immunotherapy against active TB in a mouse model challenges the Koch Phenomenon

Pablo Soldevilla1,3, Anna Buisan4, Sergi Saladrigas1, Lilibeth Arias1, Alexandra Jimenez-Melsió4, Cristina Vilaplana1,3, Mercé Amat4, Pere-Joan Cardona1,2,3

1Unitat de Tuberculosis Experimental (IGTP), Badalona, Spain
2Servei de Microbiologia, LCMN, Hospital Universitari Germans Trias i Pujol, Badalona, Spain
3Centro de Investigación Biomédica en Red de Enfermedades Respiratorias (CIBERES), Madrid, Spain
4Archivel Farma, s.l., Badalona, Spain

Background: Traditionally it has been a lot of concern on therapeutic vaccination on patients with active TB due to its potential toxicity. This precaution is based in the concept summarized in the so called "Koch phenomenon". The main objective of the assay was to test, in a, active TB murine model, whether the immunotherapy with RUTI, alone or combined with chemotherapy at the onset of the disease, would be therapeutic and safe. RUTI comprises liposomed fragments of Mycobacterium tuberculosis (Mtbc).

Methods: C3HeB/FeJ mouse strain was used including both sexes. Animals were infected intravenously with 4.04 E+04 CFU of Mtbc H37Rv. After development of active TB (w5), experimental groups included subcutaneous administration of RUTI combined or not with oral administration of chemotherapy (RHZE) (Figure 1). Treatment was well tolerated, and animals were euthanized to determine the bacillary load, damage in lungs and determinate T-cell response against PPD, ESAT-6, Hsp16-3 and PsTS1 though ELISPOT in splenocytes.

Results: Administration of RUTI alone reduced -1.5 log10 the bacillary load and synergized with chemotherapy (-2.5 log10 reduction) one week after its inoculation, decreasing the lung pathology (Figure 1). The therapeutic effect might be related to the increase of the Th1 response against PsTS1 and HSP16.3 in the combined treatment RUTI plus chemotherapy.

Conclusion: This data encourages the administration of RUTI at the beginning of TB chemotherapy, for a faster reduction of the bacillary load and to explore a shorter drug treatment length. A clinical trial (CONSTAN) recently approved by the Spanish Agency for Medicines and Health Products (AEMPS) will serve to confirm this finding.

Funding Sources / Conflicts of Interest
Archivel Farma, s.l.
Development of a lung-orientated human infection/antigenic challenge model using live BCG and PPD to gain insights into TB immunopathogenesis

Anil Pooran¹, Malika Davids¹, Clemens Hermann², Lynelle Mottay¹, Fawziyah Thompson¹, Jacob Cardenas³, Jinghua Gu⁴, Thearith Koeuth⁴, Jason Limberis¹, Stuart Meier¹, Javan Okendo², Phindile Gina¹, Aliasgar Esmail¹, Jonathan Blackburn², Tawanda Gumbo³,⁴, Keertan Dheda¹,²,⁵

¹Centre for Lung Infection and Immunity, University of Cape Town Lung Institute, Cape Town, South Africa
²University of Cape Town, Cape Town, South Africa
³Praedicare Inc, Dallas, United States
⁴Baylor Scott & White Health Institute for Immunology Research, Dallas, United States
⁵London School of Hygiene and Tropical Medicine, London, United Kingdom

Background: Global elimination of TB is hampered by the lack of an effective vaccine. This is largely due to our incomplete understanding of TB immunopathogenesis and failure to identify reliable correlates of immunity. Despite several candidates in the pipeline, BCG is the only available licensed vaccine, but its efficacy remains suboptimal. Controlled human infection models have facilitated vaccine development for several diseases but safety issues have precluded its use in TB. We have recently developed a lung-orientated mycobacterial controlled human infection/antigenic challenge model using BCG and PPD to study TB immunopathogenesis at the site of disease and potentially identify biosignatures of TB risk.

Methods & Results: We demonstrated that bronchoscopic instillation of live BCG and PPD into the lungs of 74 healthy participants, along a gradient of TB susceptibility (ranging from those with presumed sterilizing immunity to those with multiple recurrent episodes of previous TB), is safe (<10% AEs) and feasible when performed in a TB endemic setting. Furthermore, after 3 days, lung-specific immune responses to pulmonary mucosal administration of BCG and PPD induced several highly localised, lung-specific pro-inflammatory innate (macrophage-mediated, neutrophil-mediated, autophagy) and adaptive (Th1, Th17, IgG responses) immune pathways, which were measured across multiple platforms (cellular, transcriptomic, proteomic). Responses also varied between the different biological compartments (lung, skin and blood).

Conclusion: These data demonstrate that such a model can provide a solid foundation for the advancement of several lines of research including a better understanding of host-pathogen interactions at the site of disease and the subsequent development of better models to evaluate vaccine efficacy and route of administration.

Funding Sources/Conflicts of Interest
South African Medical Research Council, Bill and Melinda Gates Foundation
no conflicts of interest declared
Therapeutic vaccination in tuberculosis: Results from a phase I/II randomized clinical trial of H56:IC31 and adjunctive Cyclooxygenase-2-inhibitor treatment

Synne Jenum¹, Kristian Tonby¹,², Corina S. Rueegg³, Morten Rühwald⁴,⁵, Max P Kristiansen⁶, Peter Bang⁶, Inge C Olsen⁷, Kjersti Seløe¹, Kjerstin Røstad¹, Tehmina Mustafa⁸,⁹, Kjetil Tasken²,¹⁰, Dag Kvale¹,², Rasmus Mortensen⁴, Anne Ma Dyrhol-Riise¹,²

¹ Dept. of Infectious diseases, Oslo University Hospital, Oslo, Norway
² Institute of Clinical Medicine, University of Oslo, Oslo, Norway
³ Oslo Centre for Biostatistics and Epidemiology, Oslo University Hospital, Oslo, Norway
⁴ Center for Vaccine Research, Department of Infectious Disease Immunology, Statens Serum Institut, Copenhagen, Denmark
⁵ Foundation of Innovative New Diagnostics (FIND), the global alliance for diagnostics, Geneva, Switzerland
⁶ Center for Vaccine Research, Vaccine Development, Statens Serum Institut, Copenhagen, Denmark
⁷ Dept. of Research Support for Clinical Trials, Oslo University Hospital, Oslo, Norway
⁸ Centre for International Health, Dept. of Global Public Health and Primary Care, University of Bergen, Bergen, Norway
⁹ Dept. of Thoracic Medicine, Haukeland University Hospital, Bergen, Norway
¹⁰ Dept. of Cancer Immunology, Institute for Cancer Research, Oslo University Hospital, Oslo, Norway

Background: Host-directed-therapy (HDT) strategies are needed to fight tuberculosis (TB). We have studied safety and immunogenicity of H56:IC31 as a therapeutic vaccine, and the immune modulating effects of the adjunctive cyclooxygenase-2-inhibitor (COX-2i) etoricoxib in patients with TB disease.

Methods: In this randomized, open-label, multi-center, safety and explorative phase I/II clinical trial, we enrolled HIV negative adults (aged 18-64) treated for drug-sensitive TB in Norway. Participants were randomized to 120 mg etoricoxib for 140 days, 5 µg H56:IC31 at day 84 and 140, etoricoxib+H56:IC31 combined or standard TB treatment only (controls). Primary outcome was safety up to day 238. Secondary outcomes were antigen-specific T-cells analyzed by fluorospot and intracellular cytokine staining by flow cytometry, and H56 antibodies measured by ELISA. ClinicalTrials.gov number NCT02503839.

Results: Between November 20, 2015 and December 11, 2018 totally 222 TB patients were screened, 51 enrolled and randomized; 13 in the etoricoxib-group, 14 in the H56:IC31-group, 12 in the etoricoxib+H56:IC31-group and 12 controls. Three Severe Adverse Events (SAE) were reported in the etoricoxib-groups; two urticarial rash and one possible disease progression. No SAEs were vaccine related. H56:IC31 induced robust expansion of antigen-specific T-cells. Seroconversion occurred in a higher proportion in the H56:IC31-group compared to controls. Etoricoxib reduced T-cell responses to H56:IC31.

Discussion and Conclusion: We report the first clinical data that therapeutic vaccination with H56:IC31 is safe and immunogenic in TB disease, supporting further studies of H56:IC31 as HDT strategy. Although etoricoxib appears to be safe, our data did not support COX-2i as adjunctive HDT.

Funding Sources / Conflicts of Interest
The Research Council of Norway (RCN, GlobVac no 234493), Oslo University Hospital, Norway, the University of Oslo, Norway and Statens Serum Institut, Denmark. The authors have no conflicts of interests. M.R, M.K, P.B and R.M are employees at SSI that develop the H56:IC31 vaccine. They are not inventors of patents and have no financial interests.
Cytomegalovirus Acquisition in Infancy and the Risk of Tuberculosis Disease in Childhood: A Longitudinal Birth Cohort in Cape Town, South Africa

Leonardo Martinez1, Mark Nicol2,3, Catherine Wedderburn4,5, Attie Stadler4, Maresa Botha4, Lesley Workman4, David le Roux4, Heather Zar4

1Boston University, Boston, United States
2Division of Infection and Immunity, School of Biomedical Sciences, University of Western Australia, Perth, Australia
3Division of Medical Microbiology, University of Cape Town, Cape Town, South Africa
4Department of Paediatrics and Child Health, Red Cross War Memorial Children's Hospital and SA-Medical Research Council Unit on Child and Adolescent Health, Cape Town, South Africa
5Department of Clinical Research, London School of Hygiene & Tropical Medicine, London, United Kingdom

Background: The risk of tuberculosis (TB) after exposure is greatest in the first few years of life, however the mechanisms responsible for this vulnerability are not understood. Acquisition of viral infections, such as cytomegalovirus, may modulate the immune system. We studied the acquisition of cytomegalovirus in infancy and subsequent development of TB throughout childhood.

Methods: We enrolled pregnant women between 20–28 weeks’ gestation attending antenatal care in a peri-urban South African setting in the Drakenstein Child Health Study. Nasopharyngeal swabs for cytomegalovirus detection using qPCR were done at birth, three weeks, six weeks, three months, six months, 12 months, and 24 months. Children were followed prospectively for TB using annual tuberculin skin testing, radiographic examinations with GeneXpert, culture, and smear testing. We compared TB incidence in children with and without cytomegalovirus using Cox regression and hazard ratios (HRs) with 95% confidence intervals (CIs).

Results: Among 963 children tested for cytomegalovirus (Ntests=7,186; median 6 tests/child), 42% had cytomegalovirus by one year. Children who breastfed were at greatest risk (44% versus 14%, P<0.0001). Children were followed for TB for a median of 6.9 years (IQR, 6.0–7.8) and children with cytomegalovirus by one year had an increased hazard of subsequently developing TB (AHR, 3.2; 95% CI, 1.6–6.4) including microbiologically-confirmed disease (AHR, 4.4; 95% CI, 1.2–16.3). Infants with a high cytomegalovirus load were at consistently greatest TB risk.

Discussion and Conclusion: TB prevention in children from high-burden countries may need to include strategies to deter or delay acquisition of cytomegalovirus in the first months of life.

Funding Sources/Conflicts of Interest
This study was funded by the Bill & Melinda Gates Foundation (grant number OPP 1017641), Medical Research Council South Africa, National Research Foundation South Africa, and the National Institutes of Health H3 Africa (grant numbers U54HG009824, U01AI110466).

No conflicts of interest.
Safety and immunogenicity of an adenovirus-vectored tuberculosis vaccine delivered via inhaled aerosol to healthy humans: A dose and route comparison phase 1 study

Mangalakumari Jeyanathan1, Dominik Fritz1, Sam Afkhami1, Emilio Aguirre2, Karen Howie1, Anna Zganiacz1, Anna Anna Dvorkin-Gheva1, Michael Thompson3, Richard Silver4, Ruth Cusack1, Brian Lichty1, Paul O’Byrne1, Martin Kolb1, Maria Fe Medina1, Myrna Dolovich1, Imran Satia1, Gail Gauvreau1, Zhou Xing1, Fiona Smaill2

1Department of Medicine, McMaster University, Hamilton, Canada
2Department of Pathology and Molecular Medicine, McMaster University, Hamilton, Canada
3Department of Chemical Engineering, McMaster University, Hamilton, Canada
4Case Western Reserve University, Cleveland, United States

Background: The objective of this study was to compare the safety and immunogenicity of a human serotype 5 Ad-based tuberculosis (TB) vaccine (AdHu5Ag85A) delivered to healthy humans via inhaled aerosol or intramuscular injection (IM) and characterize the ability of inhaled vaccine to induce respiratory mucosal immunity.

Methods: 31 healthy adults with a history of BCG vaccination were enrolled. AdHu5Ag85A was administered by a single-dose aerosol using the Aeroneb® Solo Nebulizer or by IM injection; 11 in the low dose (LD, 1×10^6 PFU) aerosol group, 11 in the high dose (HD, 2×10^6 PFU) aerosol group and 9 in the IM (1×10^8 PFU) group. Vaccine-related adverse events were collected, and routine laboratory tests and lung function were measured post vaccination. The secondary outcome was comparison of immunogenicity among the different routes and aerosol dose groups. Immunogenicity following vaccination was measured in the peripheral blood and bronchoalveolar lavage samples by Luminex and cell surface and intracellular cytokine immunostaining.

Results: Both LD and HD of AdHu5Ag85A administered by aerosol inhalation and IM injection were safe and well-tolerated. Both aerosol doses but not IM vaccination markedly induced Ag85A-specific airway polyfunctional tissue-resident memory CD4 and CD8 T cells. While as expected, IM vaccination induced Ag85A-specific T cell responses in blood, the LD aerosol vaccination also elicited these T cells in blood. LD aerosol vaccination induced persisting transcriptional changes in alveolar macrophages indicative of trained innate immunity.

Conclusion: Inhaled aerosol delivery of an Ad-vectored TB vaccine is a safe and effective way to elicit respiratory mucosal immunity. These results encourage further development of aerosol vaccine strategies.

Funding Sources/Conflicts of Interest
Funding: The Canadian Institutes for Health Research and the Natural Sciences and Engineering Research Council of Canada
Conflicts of Interest: None
Safety and immunogenicity of the aerosol administered tuberculosis vaccine ChAdOx1-85A

Lerisa Govender¹, Regine Audran¹, Olfa Karoui¹, Aurélie Fayat-Mello¹, Iman Satti², Helen McShane², François Spertini¹

¹Centre Hospitalier Universitaire Vaudois (CHUV), Lausanne, Switzerland
²The Jenner Institute, University of Oxford, Oxford, United Kingdom

Background: The route of M.tb infection is by inhalation of the aerosolised droplets. A boost vaccination via the respiratory mucosa may establish protective immune responses at the primary site of infection. In a phase 1 double-blind clinical trial we compared the safety and immunogenicity of ChAdOx1 85A, administered via aerosol or intramuscular.

Methods: Between January 2019 and March 2020, we enrolled 29 BCG-vaccinated and 10 BCG naïve participants. The first part of the study was a dose escalation of aerosol vaccination performed on 9 BCG-vaccinated participants. In the second, double-blind randomized part of this study, we compared the safety and immunogenicity of the aerosol inhaled versus intramuscular administration routes. A control group of 10 BCG naïve participants received aerosol ChAdOx1 85A. The primary outcome was safety, assessed by the frequency and severity of vaccine-related local and systemic adverse events. The secondary outcome was immunogenicity assessed with laboratory markers of cell- and humoral-mediated immunity in blood and bronchoalveolar lavage samples.

Results: Both administration routes were well tolerated with no serious adverse events (AE). Boosting BCG via aerosol ChAdOx1 85A vaccination induced Ag85A-mucosal and systemic cellular immune responses. Globally, we observed a compartmentalization of the immune responses with aerosol ChAdOx1 85A inducing higher mucosa cellular responses, particularly IFN-γ/IL-17+ CD4+ T cells and ChAdOx1 85A intramuscular inversely inducing higher systemic cellular and humoral responses.

Conclusion: ChAdOx1 85A was safe to be administered via aerosol and promoted lung mucosal and systemic Ag85A-specific T cell responses. These data support further evaluation of the ChAdOx1 85A aerosol route as a BCG boost vaccination strategy.

Funding Sources/Conflicts of Interest
This trial is financed by the H2020 program of the European Commission (TBVAC2020 project) via the Swiss State Secretariat for Education, Research and Innovation (SERI).
A Phase 2a randomized, double-blind, dose-defining trial of MTBVAC compared to BCG in newborns living in a tuberculosis endemic region

Michele Tameris1, Virginie Rozot¹, Claire Imbratta¹, Ingrid Murillo², Hennie Geldenhuys¹, Justin Shenje¹, Angelique Luabeya¹, Simon Mendelsohn¹, Nicolette Tredoux¹, Michelle Fisher¹, Nicole Bilek¹, Simbarashe Mabwe¹, Juana Doce², Nacho Aguilo³, Desislava Marinova³, Eugenia Puentes², Thomas Scriba¹, Carlos Martin³, Mark Hatherill¹

1 SATVI, University of Cape Town, Cape Town, South Africa
2 Biofabri, S.L., Porriño, Spain
3 Department of Microbiology, Faculty of Medicine, University of Zaragoza, Zaragoza, Spain

Background: MTBVAC, a live-attenuated derivative of Mycobacterium tuberculosis (Mtb), was developed to replace newborn BCG vaccination.

Objectives: Evaluate safety and immunogenicity of three escalating doses of MTBVAC vs BCG in newborns in a TB endemic region, Worcester, South Africa, to define the dose of MTBVAC for a phase 3 trial.

Methods: We obtained consent from 228 pregnant women antenatally and enrolled 99 HIV-unexposed, BCG-naive, Mtb-unexposed, healthy newborns, who were randomized 1:3 to receive BCG SSI or MTBVAC at 2.5x10⁴, 2.5x10⁵, or 2.5x10⁶ CFU within 96 hours of birth. QFT -TB Gold Plus was performed at 56, 182 and 365 days after vaccination. Infants with TB contact or QFT conversion at days 182 or 365 were referred for isoniazid preventive therapy.

Results (blinded data): Eighty-three (83.8%) infants across all 3 cohorts had local reactions: 23(69.6%), 29(87.9%) and 31(93.9%) respectively, all rated mild except one grade 2 erythema in cohort 2. Induration (n=3/22/27), swelling (n=6/8/17), erythema (n=5/7/21), and scarring (n=7/21/22) were most common. Systemic AEs were similar across cohorts reflecting mostly childhood illnesses (n=258/265/203) with 47 graded moderate (n=26/11/10) and 1 severe (n=0/1/0). Eleven infants experienced 13 vaccine-unrelated SAEs including an unrelated death due to bronchopneumonia. No related SAEs were recorded. Seven infants have commenced TB treatment (5/1/1) for unconfirmed PTB and 1 (cohort 1) for unconfirmed TBM. Immunogenicity analyses are ongoing.

Conclusion: MTBVAC appeared safe at 3 escalating dose levels in South African newborns compared with BCG. These safety data together with unblinded immunogenicity data will inform dose selection for the Phase 3 trial planned for 2022.

Funding Sources / Conflicts of Interest
Funding: European & Developing Countries Clinical Trials Partnership (EDCTP) Biofabri, SL
Conflict of Interest: IM, JD, EP are employees of Biofabri. CM is co-inventor in a patent application on MTBVAC filed by the University of Zaragoza.
Improving equity in clinical trials using biosurveillance methodology

Wai-Ling Mui2, Falgunee K. Parekh2, Joy Toro1, Taylor Craig1, Maggwa Ndugga1, Ashley Tseng3, Carole Mitnick4, Ghiorghis Belai1

1 FHI Clinical, Durham, United States
2 EpiPointe, Cary, United States
3 Department of Epidemiology, University of Washington, Seattle, United States
4 Center for Global Health Delivery, Harvard Medical School, Cambridge, United States

Background: A highly effective TB vaccine is critical to reducing the global burden of TB. Its development requires identification of sites globally that have both high incidence of TB and capacity to conduct a clinical trial. CROs tend to revisit the same clinical research sites and Key Opinion Leaders (KOLs) that they have experience working with, limiting the global reach. For a phase III TB vaccine trial, a primary focus on epidemiology is critical to site identification to ensure that vaccine efficacy endpoints can be measured.

Methods: We implemented a biosurveillance methodology to conduct global site feasibility for a phase III TB vaccine trial. Based on the subjectivity of available data we utilized an integrated data science approach to identify key criteria relevant to trial conduct, including TB Incidence and Site Capacity to inform site selection. Site-specific epidemiologic and capacity-related data were evaluated in the context of these criteria.

Results: Our methodology to evaluate epidemiologic data enabled a systematic and objective assessment of sites and identification of catchment areas with high levels of TB. Some sites had significant incidence but would need time to build capacity prior to starting the trial. Identifying these needs is critical for trials conducted in LMICs and targeting these sites enhances accessibility to underrepresented populations.

Conclusion: This approach allows for an objective data-driven evaluation of site feasibility, resulting in the identification of sites with high TB burden which may not have been previously considered, to improve equity in clinical trials.

Funding Sources / Conflicts of Interest
None
A tractable and accessible method for generating human Alveolar Macrophage Like (AML) cells in vitro: a new breakthrough in lung biology

Susanta Pahari1, Eusondia Arnett1, Hao Zhang2, Hong Cai2, Yufeng Wang2, Natalie Jarvis1,3, Jan Simper1,3,4, Miranda Lumbreras1, Abul Azad1, Larry S Schlesinger1

1 Host Pathogen Interaction Program, Texas Biomedical Research Institute, San Antonio, United States
2 South Texas Center for Emerging Infectious Diseases, University of Texas at San Antonio, San Antonio, United States
3 UT Health San Antonio, San Antonio, United States
4 Medical Scientist Training Program, UT Health San Antonio, San Antonio, United States

Background: Alveolar macrophages (AM) are unique lung resident myeloid cells and often the first cell type to contact airborne pathogens, e.g., M. tuberculosis. The contribution of human AMs (HAM) to pulmonary diseases remains poorly understood due to the difficulty in accessing them from human donors and their rapid phenotypic change during in vitro culture. There remains an unmet need for cost effective methods for generating human cells with a HAM phenotype, particularly important for translational studies and clinical benefits to humans, including analyses of host cell responses to vaccine candidates.

Methods: We developed cell culture conditions using lung lipids, i.e., Infasurf (synthetic surfactant) and lung-associated cytokines (GM-CSF, TGFβ, and IL10) that facilitate the conversion of human blood-obtained monocytes to an AM-Like (AML) phenotype and function. Analyses include: confocal and transmission electron microscopy, RNA-seq, qRT-PCR, WB, flow cytometry, EPR and Seahorse assay.

Results: These AML cells have: 1) similar morphology to HAM, including the appearance of lipid lamellar bodies, 2) upregulation of key AM transcription factors and PPARγ, TGFβ, and GM-CSF signaling pathways, which are essential for AM development, 3) only 6.2% difference from HAM by RNA-seq, 4) increased oxidative phosphorylation, mitochondrial respiration and reduced glycolysis, similar to what is reported for AMs, and 5) maintained AML phenotype over time in culture.

Conclusion: This study reveals the importance of alveolar space components in the development and maintenance of HAM and provides a readily accessible model to study the impact of HAM on infectious and inflammatory processes and diseases, and impact of therapies and vaccines.

Funding Sources / Conflicts of Interest
R01 AI136831. No conflicts.
An adjuvant system based on a mesoporous silica particle with a mycobacterial surface lipid bilayer

Carlos M. Valdemar-Aguilar, Rufino Nava-Mendoza, Manisekaran Ravichandran, Luz M. López-Marín

1 Universidad Nacional Autónoma de México, Querétaro, Mexico
2 Universidad Autonoma de Queretaro, Querétaro, Mexico

Vaccination has been proven to be one of our greatest weapons against infectious diseases. New generation vaccines using lipid nanoparticles have been approved against COVID-19 having great efficiency. Nanoparticles have been used in biomedical applications due to their easy modulation of properties including chemical composition, surface reactivity, and size. Here we investigated mesoporous silica particles known as SBA-15, decorated with a mycobacterial lipid, phosphatidylinositol mannoside (PIM), through the use of surface lipid bilayers. Since PIMs are known to be TLR-2 agonists, the so-obtained silica-carrier is expected to elicit a strong innate immune response. Mesoporous silica particles have gained interest due to their biocompatibility, absorbance/encapsulation capability, and functionalization properties, while PIMs are part of currently commercialized live vaccines and immunotherapies. We evaluated the biocompatibility and functionality of PIM-silica hybrid particles, and their internalization in THP-1 macrophages using live-cell imaging confocal microscopy, demonstrating that the functional particles provoked a kinetic manifestation in macrophage pseudopods, as well as an increase in size and number of lysosomes. Further analyses are therefore encouraged to better assess the use of PIM-containing particles as a low-cost, immunostimulatory vaccine carrier.

Funding Sources / Conflicts of Interest
Carlos M. Valdemar-Aguilar is a CONACyT fellow (CVU 665176) at the Programa de Doctorado en Ciencias Biomédicas, Universidad Nacional Autónoma de México. This work was supported by Conacyt and DGAPA-UNAM (Mexico) through grants CF2019-53395 and IT200421, respectively.
BCG-primed HEHR/CIA09A boosted vaccination mediated improved protection against hypervirulent Mycobacterium tuberculosis strain M2 in mice by eliciting antigen-specific Th1 and Th17 responses

Kee Woong Kwon¹, Han-Gyu Choi², Hwa-Jung Kim², Sung Jae Shin¹,³

¹Department of Microbiology, Graduate School of Medical Science, Brain Korea 21 Project, Yonsei University College of Medicine, Seoul, Korea (Republic of)
²Department of Microbiology, and Medical Science, College of Medicine, Chungnam National University, Daejeon, Korea (Republic of)
³Institute for Immunology and Immunological Disease, Yonsei University College of Medicine, Seoul, Korea (Republic of)

Background: With BCG-prime boost regimen, tuberculosis (TB) vaccine candidate, Hsp90-ESAT-6, conferred superior protection against the hypervirulent Mycobacterium tuberculosis (Mtb) Beijing clinical strain HN878 compared to BCG-primed ESAT-6 boosted vaccination by demonstrating the essential role of antigen-specific CD4+IFN-γ+IL-17A+ T-cell responses as protective immune determinant.

Methods: A modified version of multiantigenic vaccine Hsp90-ESAT-6-HspX-RipA (HEHR) formulated with CIA09A, TLR4 adjuvant, was evaluated for protective efficacy against hypervirulent Mtb Haarlem clinical strain M2 as BCG-prime boost regimen.

Results: Other than previously reported potential of HspX as TB vaccine candidate, we characterize RipA-induced immunological features by investigating the interaction of RipA with dendritic cells (DCs). RipA-treated DCs displayed increased expression of surface molecules and production of proinflammatory cytokines. Moreover, mice immunized with RipA formulated in GLA-SE, TLR4 adjuvant, displayed remarkable generation of RipA-specific polyfunctional CD4+ T-cells in both lung and spleen. Following challenge with Mtb Beijing clinical strain K, RipA/GLA-SE-immunized mice exhibited similar level of pulmonary inflammation and bacterial loads at 16 weeks post-challenge as BCG did. Thus, we constructed a modified multiantigenic vaccine candidate composed of 4 antigens, HEHR. Notably, given as a BCG-prime boost regimen through intramuscular and subcutaneous route respectively, only HEHR among TB vaccine candidates including ESAT-6 and Hsp90-ESAT6 displayed significance of bacterial reduction against hypervirulent Mtb Haarlem clinical strain M2 infection, compared to BCG. Furthermore, protective immune determinant, represented by antigen-specific CD4+IFN-γ+IL-17A+ T-cell responses, was robustly induced in HEHR boosted mice at 12 weeks post-challenge.

Conclusion: Collectively, these findings will provide the rationale for the continued validation of HEHR vaccine candidate as an effective TB vaccine.

Funding Sources / Conflicts of Interest
Korea Health Industry Development Institute (HI17C0175 and HV20C0139)
Blood RNA signatures of the adjunctive administration of cyclooxygenase-2-inhibitor (Etericoxib) or therapeutic vaccine candidate (H56:IC31) in TB disease

Eleonora Vianello1, Noelia Alonso-Rodriguez2, Suzanne Van Veen1, Synne Jenum2, Kristian Tonby2,3, Rasmus Mortensen4, Tom H.M. Ottenhoff1, Anne Ma Dyrhol-Riise2,3
1Department of Infectious Diseases, Leiden University Medical Center, Leiden, Netherlands
2Department of Infectious Diseases, Oslo University Hospital, Oslo, Norway
3Institute of Clinical Medicine, University of Oslo, Oslo, Norway
4Center for Vaccine Research, Department of Infectious Disease Immunology, Statens Serum Institut, Copenhagen, Denmark

Introduction: Host Direct Therapy pursues the modulation of the immune response functions to improve TB clinical outcome and to increase the success of its treatment. Here we present the preliminary results of the blood RNA signatures of patients who received H56:IC31 vaccine and/or cyclooxygenase-2-inhibitor (COX2i) as adjunctive TB treatments in a randomized open-label phase I/II clinical trial (TBCOX2, NCT02503839).

Material and Methods: Forty TB patients receiving standard TB treatment were included. Experimental groups COX2i, H56:IC31, COX2i +H56:IC31 and Control are detailed in the Table. Blood samples for RNA isolation were collected in PAXgenes on days 1, 84, 98, 140, 154 and 238. Longitudinal transcriptomic responses were identified using a targeted gene expression profiling platform (Fluidigm) on 190 immune- and TB-related genes. Principal Component Analysis (PCA) and differential expressed genes in the course of the treatment were explored.

Results: The most downregulated genes found in the Control-group at day 84 vs day 0 were Complement related genes (C1QA, C1QB, C1QC), IFN signalling genes (CD274, FCGR1A_B_CP, GBP1, GBP5, IFIT2, IFITM1, IFITM3), TB treatment and prognostic related genes (ANKRD22, CATF2, C2, ETV7, SCARF1, SEPTIN4, SERPING1). The most upregulated genes were Myeloid associated genes (CCL22) and Th1 associated genes (IL15, IL2). No significant differences were found in the COX2i-group vs the Control-group. After the second dose of the vaccine, the H56:IC31-group upregulated expression of B cell (CRE, IGHD), NK cell (KLRD1) and T cell markers (PTPRCV1), gene modules associated with an augmentation of immunogenicity.

Conclusion: RNA gene signatures indicate a beneficial response to H56:I31 vaccination in TB patients with active disease.

Funding Sources / Conflicts of Interest
Funding: The Research Council of Norway (RCN, GlobVac no 234493), Oslo University Hospital, Norway, the University of Oslo, Norway and Statens Serum Institut, Denmark. No conflicts of interest.

| Experimental groups and TB clinical parameters |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                | N               | Treatment       | ExtraPulm | Score 2 | Score 1 | Cavities | Fatigue | CRP>10 | ESR>20 |
| COX2i          | 10              | only TB treatment | 3         | 2       | 1       | 1       | 1       | 3     | 3     |
| H56:IC31       | 12              | Etericoxib (Arcoxia®) 120 mg p.o. daily for 140 days since day 0 | 0         | 7       | 1       | 5       | 5       | 6     | 5     |
| H56:IC31       | 12              | 5 μg of H56:IC31 (Statens Serum Institut; SSI, Valneva Austria GmbH) intramuscularly at day 84 and at day 140 | 0         | 9       | 1       | 3       | 2       | 5     | 4     |
| COX2i + H56:IC31 | 8              | Both COX2i and vaccine administration | 3         | 1       | 2       | 0       | 1       | 2     | 4     |

Disease score; 1=1 sympt, 2=2 or more sympt
ESR; Erythrocite Sedimentation Rate
CRP; C-Reactive Protein
Comparison of three methods of Mycobacterium tuberculosis complex spoligotype determination for clinical isolates

Charlotte Genestet¹, Elisabeth Hodille¹, Albin Bernard¹, Guislaire Refrégier³, Maxime Vallée¹, Emilie Westeel², Jean-Luc Berland², Gérard Lina¹, Oana Dumitrescu¹

¹ Centre International de Recherche en Infectiologie (CIRI), Hospices Civils de Lyon, Lyon, France  
² Fondation Mérieux, Lyon, France  
³ Institute for Integrative Biology of the Cell (I2BC), Gif-sur-Yvette, France

Background: To tackle tuberculosis spreading, epidemiological studies are undertaken worldwide to investigate TB transmission chains. Here we compared the spoligotype profiles of Mycobacterium tuberculosis complex (MTBC) clinical isolates by using different methods.

Methods: All MTBC strains isolated from patients during routine practice at the mycobacteria laboratory of Lyon University Hospital, France, between November 2016 and December 2020 were included (n=595) to compare hybridization to membrane-based and WGS (Whole Genome Sequencing)-based spoligotyping. Discrepancies between WGS-based and membrane-based methods were explored thanks to CRISPRBuilder-TB. Among these MTBC strains, 133 were also analysed by hybridization to microbeads-based spoligotyping. Spoligotype profiles were also used for species identification among the MTBC.

Results: Considering the hybridization to membrane-based spoligotyping as the reference, for 86.0% and 80.2% of the clinical strains we obtained the same spoligotype by WGS-based and microbeads-based methods, respectively. Of note, these discrepancies did not impact the species identification. Discrepancies observed between WGS-based and membrane-based methods were due to DR (direct repeat) variants, insertion of IS6110 between the spacer and the DR or within the DR or to truncated spacers. Furthermore, for 28 strains we were not able to conduct identification based on spoligotyping, but WGS allowed to identify 22 M. tuberculosis and 2 M. canettii with SNP-based phylogeny and 4 co-infections.

Conclusions: To conclude, WGS showed very few discrepancies compared to the hybridization-based assay for spoligotyping (including for species identification). In addition to more accurate epidemiological monitoring, WGS provided added value in some cases of species identification. Furthermore, WGS-based spoligotyping allowed a continuous implementation of our database initially generated by membrane-based spoligotyping.

Funding Sources / Conflicts of Interest
This work was supported by the LABEX ECOFECT (ANR-11-LABX-0048) of Université de Lyon, within the program "Investissements d'Avenir" (ANR-11-IDEX-0007) operated by the French national research agency (Agence nationale de la recherche, ANR).
CRISPR/Cas9 approach to generate an auxotrophic BCG for unmarked expression of LTAK63 adjuvant: a new candidate for tuberculosis vaccine

Luana Moraes¹, Monalisa Martins Trentini¹, Dimitrios James Fousteris², Luciana Cezar de Cerqueira Leite¹, Alex Issamu Kanno¹

¹Instituto Butantan, São Paulo, Brazil
²Joint Master Degree Leading International Vaccinology Education, Lyon, France

Introduction: Tuberculosis is one of the deadliest infectious diseases and a huge healthcare burden around the world. New vaccines including recombinant BCG-based candidates are constantly under evaluation in clinical trials. Our group showed that rBCG-LTAK63, a recombinant BCG expressing LTAK63, the genetically detoxified subunit A of LT toxin from Escherichia coli, induces improved protection against Mycobacterium tuberculosis (Mt) in mice models. To remove antibiotic resistance markers, not appropriate for human vaccines, we used CRISPR/Cas9 to generate the unmarked deletion in the lysA gene – the last gene in the biosynthetic operon of lysine and thus obtained a lysine auxotrophic BCG strain.

Method: We have constructed an all-in-one vector containing the Cas9 and sgRNA under control of an inducible promoter, which was used to target the lysA gene. An antibiotic-free mycobacterial vector carrying lysA and ltak63 gene was used to generate a complementation vector and co-express LysA and the LTAK63 antigen in the BCGΔlysA strain.

Results: The new unmarked rBCG-LTAK63 presented stable antigen expression through several passages. Mice immunized with this strain induced superior protection against challenge with pathogenic H37Rv Mt compared to BCG and immunopathology in the lungs were consistently lower.

Conclusion: This work showed the practical application of the CRISPR/Cas9 technology towards the development of new vaccines against tuberculosis suitable to progress into clinical trials.

Funding Sources / Conflicts of Interest
Supported by FAPESP (Projects 2017/24832-6 and 2017/17218-0) and Fundação Butantan.
Deciphering lysosomal enzymatic activities involved in the processing of mycobacterial antigenic glycolipids

Sonia BELKAI1, Christophe CARRAT1, Charlotte DESSAUX1, Alexandre STELLA1, Odile SCHILTZ1, Isabelle VERGNE1, Jérôme NIGOU1, Martine GILLERON1

1Institut de Pharmacologie et Biologie Structurale, IPBS, Université de Toulouse, CNRS, UPS, Toulouse, France

Background/Introduction: The discovery of lipid antigen presentation to T lymphocytes by CD1 proteins has given new insights into the immune system’s capacity to sense and respond to a diverse array of molecules. Even if the main cellular steps of lipid antigen presentation are known, the precise molecular mechanisms and actors involved have yet to be defined. So our work aims at identifying the key actors and mechanisms of lipid antigen processing, focusing on antigens presented by CD1b isoform, which presents the majority of mycobacterial lipid antigens identified to date.

Methods/Results: We have previously discovered that mycobacterial Phosphatidyl-myo-Inositol Mannoside (PIM) are trimmed both in their saccharidic and lipidic parts into a non-conventional acyl-form, through the action of at least three lysosomal enzymes (the α-mannosidase, the pancreatic lipase-related protein 2 (PLRP2) and the lysosomal phospholipase A2 (LPLA2). Here, we developed a global approach to identify within lysosomal preparations the hydrolases capable of trimming lipids. We first isolated lysosomes from antigen presenting cells and characterized their proteome. Next, lysosomal fractions were tested in vitro for their ability to digest PIM. Our preliminary data have revealed lipase degradative activities, among them those previously described.

Discussion and Conclusion: The outcomes of this project will provide a better understanding of the mechanisms of lipid antigens presentation by the CD1b proteins, a prerequisite to the development of a new anti-tuberculosis vaccine.

Funding Sources / Conflicts of Interest
The study was funded by the European Commission as part of the TBVAC2020 Consortium (Grant H2020-PHC-08-2014-643381) and by the Fondation pour la Recherche Médicale (équipe FRM DEQ20180339208). There is no conflict of interest.
Development of Novel Autophagy-Prone BCG Strains as Live Vaccines

Aïcha Bah¹, Claude Gutierrez¹, Denis Hudrisier¹, Jérôme Nigou¹, Olivier Neyrolles¹, Isabelle VERGNE¹

¹IPBS/CNRS-Université Toulouse III, Toulouse, France

Autophagy is an eukaryotic lysosomal degradative process implicated in intracellular pathogen elimination and antigen presentation. Recent studies indicate that autophagy activation plays a major role in vaccine efficacy. Thus, enhancing cellular autophagic response has emerged as a novel and promising strategy to improve BCG vaccine. The challenge in designing such improved vaccine is to limit toxicity associated with autophagy induction and/or to selectively target the autophagy pathway. Thankfully, new autophagy inducers have been recently reported especially from peptide design. Here, we propose an innovative approach to enhance BCG vaccine effectiveness by producing recombinant BCG strains engineered to secrete autophagy-inducing peptides. To allow efficient secretion, the sequence of the pro-autophagic peptide is flanked at its N-terminus by a signal peptide originating from a natural BCG-secreted protein (alpha-antigen). Our preliminary data indicate that these rBCG strains can secrete pro-autophagic peptides in culture media. Furthermore, using both immunoblotting and fluorescence microscopy assays, we found a higher autophagic response in macrophages infected with rBCG as compared to wild-type (WT). Future work will aim to compare the immunogenicity, safety and protection efficacy in mice vaccinated with rBCG to wt BCG.

Funding Sources / Conflicts of Interest
This study was supported by the European Union’s Horizon 2020 Research and Innovation Program (grant 643381TBVAC2020).
Diet and hyperglycaemia affect vaccine immunogenicity and efficacy in mice

Elena Stylianou 1, Rachel Tanner 1, I Cuella-Martin 1, Barbara Kronsteiner-Dobramysl 1, Susanna Dunachie 1, E Haythorne 1, F Ashcroft 1, Helen McShane 1

1University of Oxford, Oxford, United Kingdom

Background: Diabetes is a strong risk factor for developing TB disease. It is estimated that there are 496 million people with diabetes, the majority of which live in low and middle-income countries, where the prevalence of TB is high. Despite this, there is limited information on how diabetes affects TB immune responses, and even less about how it affects vaccine efficacy. Such information is important, as TB vaccines and in particular subunit vaccines will be administered to an increasing diabetic population.

Methods: We established two complementary mouse models of hyperglycaemia that best represent type-2 diabetes. In one model, mice are administered high-fat diets and in the second model, transgenic mice express a conditional mutation where hyperglycaemia develops upon induction. Use of both models, will help distinguish the effect of hyperglycaemia from the secondary side-effects of obesity.

Results: Administration of high-fat diets, resulted in more weight gain and blood glucose levels, compared to control diet mice. Splenocytes from animals on altered diets were able to better control mycobacterial infection using the mycobacterial growth inhibition assay (MGIA). Similarly, BCG vaccination induced stronger immune responses in these animals and had improved efficacy in the MGIA. On-going work is investigating mycobacterial and vaccine immune responses in transgenic mice.

Conclusion: Short to medium-term high-fat diet feeding had a positive impact on mycobacterial and vaccine immune responses. Further work will investigate whether this is also true for long-term high fat diet feeding and the impact of hyperglycaemia, without the presence of obesity. In addition, in-vivo challenge studies will examine the susceptibility of the two mouse models.

Funding Sources / Conflicts of Interest
VALIDATE network
Estimating the potential public health impact of new tuberculosis vaccines in South Africa

Christinah Mukandavire¹, Rebecca A Clark¹, Chathika Weerasuriya¹, Roel Bakker¹, Andrew Iskauskas², Danny Scarponi¹, Arminder Deol¹, Shelly Malhotra³, Allison Portnoy⁴, Matthew Quaife¹, Mark Jit¹, Nicolas A Menzies⁴, Richard G White¹

¹London School of Hygiene and Tropical Medicine, London, United Kingdom
²Durham University, Durham, United Kingdom
³Global Access, IAVI, New York, United States
⁴Harvard T.H. Chan School of Public Health, Boston, United States

Background: New tuberculosis (TB) vaccines will be required to meet the End TB goals. However, there is little research on how these vaccines could be effective in high HIV burden settings. South Africa (SA) has one of the highest TB/HIV rates globally. With promising new TB vaccines in development, we estimated their potential impact on TB burden in SA.

Methods: We developed a dynamic age-structured transmission model of TB/HIV coinfection and calibrated it to SA data. Infant vaccine was implemented as routine neonatal vaccination. The adolescent/adult vaccine included routine vaccination of 9-year-olds and a single mass campaign for ages 10+. Vaccines were introduced in 2028 and assumed to prevent disease with variable duration of protection. We estimated changes in cumulative TB incidence and mortality between 2028 and 2050, comparing vaccination vs no-vaccination scenarios. We examined how impact estimates changed under different assumptions about the populations with respect to their HIV and ART status, for which vaccination was effective.

Results: The model was well calibrated to SA demographic and epidemiological data. Vaccines only effective in HIV-uninfected individuals had the smallest impact on TB. Vaccines also effective in HIV-infected on ART individuals had a larger impact, and vaccines effective in all individuals had the largest impact on TB, and were closest to reaching the End TB goals.

Conclusion: In high HIV burden settings, it is highly advantageous for new TB vaccines to be safe and effective in all individuals. These findings could be used by vaccine developers, and help inform key decision making for future TB vaccination strategies in SA.

Funding Sources / Conflicts of Interest
Bill & Melinda Gates Foundation / None
European commitments to TB R&D investments: Promises made to be broken

Sarah Steingrüber¹, Mike Frick², Elizabeth Lovinger², Annette Gaudino²

¹Treatment Action Group, Berlin, Germany
²Treatment Action Group, New York, United States

Background: The 2018 UN High-Level Meeting on Tuberculosis raised hopes that the promises made at this landmark event would rectify the decades-long neglect for TB research and development (R&D). However, reality has failed to measure up to these commitments. The world remains woefully behind the US billion annual investment needed to make substantive progress on TB R&D, particularly for TB vaccine research. This is particularly true for European funders.

Methods: This presentation will review data on European funding for TB research in relation to political commitments, focusing on TB vaccines. Data on TB R&D funding come from the annual survey conducted by Treatment Action Group (TAG). It will compare European commitments and actual contributions across other relevant infectious diseases, such as COVID-19 and HIV.

Results: The upcoming annual TB R&D Funding Report from TAG has found that in 2020, European investments in TB stood at a mere 31% compared to those made by the United States, and only one of the nine European member states included in the analysis managed to achieve 50% or more of their Fair Share target. Total European investments in TB vaccine R&D in 2020 equated to just 4% of Germany’s public investments in one COVID vaccine candidate (BioNTech; 5 million).

Discussion: There is significant need to increase advocacy across the EU through public awareness raising, coalition building, and direct engagement with national and EU-level stakeholders to mobilize the needed resources for significant advances in TB vaccine development.

Funding Sources / Conflicts of Interest
Stop TB Partnership
Exploring different routes of vaccination against tuberculosis with parental and recombinant BCG vaccines in murine models

Fadel Sayes1, Wafa Frigui1, Alexandre Pawlik1, Jan Madacki1, Magali Tichit1, David Hardy1, Roland Brosch1

1 Institut Pasteur, Paris, France

A feature of the BCG attenuated live anti-tuberculosis vaccine is the partial deletion of the genomic locus encoding the ESX-1 type VII secretion system, which in the pathogen Mycobacterium tuberculosis governs phagosomal rupture and cytosolic pattern recognition, key intracellular phenotypes linked to increased immune signaling. To construct an improved recombinant BCG vaccine strain with increased immune signaling but still low virulence, we have previously constructed a strain that is heterologously expressing the esx-1 region of Mycobacterium marinum. This recombinant strain named rBCG::ESX-1Mmar is able to modulate the host innate immune response via phagosomal rupture-associated induction of type I interferon (IFN) responses and enhanced inflammasome activity, resulting in higher IL-1beta release and higher proportions of CD8+ T cell effectors against mycobacterial antigens and polyfunctional CD4+ Th1 cells specific to ESX-1 antigens. Importantly, rBCG::ESX-1Mmar confers superior protection relative to parental BCG in murine vaccination models (Gröschel et al. Cell Reports, 2017).

In our most recent studies, we have focused on different routes of vaccination, by using parental BCG and rBCG::ESX-1Mmar. We found that mice vaccinated via the aerosol route with BCG or rBCG::ESX-1Mmar yielded higher frequencies of IFN-gamma-producing CD4+ and CD8+ T effectors in the lungs compared to subcutaneous immunized counterparts. Moreover, only aerosol vaccination was able to elicit Th17 and lung resident memory T cells without severe lung pathology. We show that vaccination of mice with BCG Pasteur or rBCG::ESX-1Mmar via the aerosol route leads to improved protection compared to subcutaneous vaccination.

The rBCG Pasteur::ESX-1Mmar vaccine thereby represents an interesting candidate for defining new, promising strategies of vaccination against tuberculosis.

Funding Sources / Conflicts of Interest
European Union’s Horizon 2020 Research and Innovation Program (grant 643381 TBVAC2020)
Extracellular vesicles released during Mycobacterium tuberculosis infection: bacterial lipid content and roles in host-pathogen interactions

Pierre Boyer¹, Jérôme Nigou¹, Emilie Layre¹

¹ Institut de Pharmacologie et de Biologie Structurale, CNRS, Université de Toulouse, Toulouse, France

Mycobacterium tuberculosis (Mtb) remains one of the most successful pathogen in establishing a chronic infection in humans. The lack of efficient tools against tuberculosis infection is mainly due to our incomplete understanding of host-pathogen interactions. As in all other domains of life, extracellular vesicles (EV) are released during Mb infection, by the bacillus itself as well as by infected cells. EV are nanoparticles shuttling various bacterial and host immunomodulatory factors with key role in host-pathogen interactions. Their molecular and functional characterisation is of major interest to further understand how immune responses are regulated at the site of infection.

Mtb produces various lipids acting as Pathogen Associated Molecular Patterns, virulence factors or antigens. However, their roles are mainly conceived at the bacillus envelope. Taking advantage of last generation lipidomic approach we characterized the content of EV in Mtb lipids and glycolipids. In addition, we have undertaken to decipher the immunomodulatory properties of EV released by the bacillus on the functionality of Mtb target cells: the macrophages. More specifically, we describe their interaction with Pattern Recognition Receptors (PRR) such as TLR and lectins, their intracellular trafficking thanks to high resolution microscopy and their capacity to regulate the inflammatory response (production of cytokines and inflammatory mediators and induction of autophagy) though the use of different in vitro bioassays.

Our results provide a detailed description of the lipid content of Mtb EV, highlighting virulent-strain specific lipid. We identified new PRR involved in EV-macrophages interactions. In addition, we highlighted that Mtb EV are internalized by macrophages and regulate autophagy.

Funding Sources / Conflicts of Interest
This work was supported by the Fondation pour la Recherche Médicale (Equipes FRM DEQ20180339208; Aide aux projets innovants: Financement d'un ingénieur ING20160435108).
Host immune correlates of disease persistence in tuberculosis

Shaifali Tyagi¹, Srikanth Sadhu¹, Amit Awasthi¹, Amit Pandey¹
¹THSTI, Faridabad, India

Introduction: Drug-resistant tuberculosis (TB) continues to be a health hazard due to non-compliance to prolonged anti-TB therapy. Persisters; a subset of the drug-tolerant population, likely contributes to this protracted TB regimen. The utilization of host cholesterol by Mycobacterium tuberculosis (Mtbc) is known to be critical for disease persistence. We have identified the role of VapC12 toxin (RNase) in the generation of cholesterol-induced antibiotics and disease persistence in TB. We hypothesize that a comprehensive immune-profiling study with the ΔvapC12 and the wild type (H37Rv) strains will help us identify host immune correlates of disease persistence. The proposed study was designed to identify novel host immune pathways critical for long-term disease persistence.

Methods: Mice infected with different Mtbc strains were sacrificed at different time points (2 and 4 weeks post-infection). Cells were harvested from organs (lungs, spleen and lymph nodes) and immunophenotyping studies were performed using the FACS machine.

Results: Immuno-profiling analysis revealed significant differences in the innate immune response pathways. In comparison to H37Rv, the lungs of mice infected with mutant strain showed an excess of myeloid cell infiltration at both time points. In a survival study performed with high-dose infection, 80% of mice infected with vapC12 mutant strain succumb within 6 weeks post-infection whereas 100% of mice infected with the H37Rv strain survived till 16 weeks.

Discussion & Conclusion: In summary, the mutant displayed a hypervirulent phenotype by modulating host innate immune pathways. Understanding immune correlates of disease persistence would: a) widen our current understanding of TB disease biology b) help design novel host-directed therapeutic approaches against TB.

Funding Sources / Conflicts of Interest
Department of Biotechnology, Govt. of India and Intramural funding by THSTI and Research fellowship from University Grants Commission (UGC).
Identifying immune correlates for BCG-mediated protection in genetically diverse Collaborative Cross

Rocky Lai¹, Travis Williams¹, Abiola Ogunsola¹, Kelly Cavallo¹, Shayla Boyce¹, Yu-Jung Lu¹, Evelyn Chang¹, Daniel Mott¹, Zhou Xing², Roland Brosch³, Chris Sassetti¹, Samuel Behar¹

¹ UMMS, Worcester, United States
² McMaster University, Hamilton, Canada
³ Institut Pasteur, Paris, France

Background: Although the Bacillus Calmette-Guerin (BCG) vaccine has been in use for a century, it is unclear why BCG remains ineffective against pulmonary TB. However, current mouse models of TB infection display a relatively homogenous immune response following BCG vaccination, and fail to replicate the heterogeneous clinical response observed in human populations. Given the lack of genetic diversity in standard laboratory mouse strains, it becomes difficult to identify vaccine-mediated immune correlates of protection that can apply across a genetically diverse population, hindering our efforts to improve global TB vaccination strategies.

Methods: We make use of the recently developed Collaborative Cross (CC) as a platform for identifying immune correlates of protection following subcutaneous BCG vaccination. The CC mice are a panel of recombinant inbred mouse strains that reflects the genetic diversity of an outbred population while retaining the reproducibility of traditional mouse models, and has already been used to identify the genetic determinants behind several human diseases.

Results: Using the CC panel, we are able to identify genetic backgrounds that are protected by BCG and those that are not, and have launched separate efforts to both identify immune correlates within the protected hosts as well as exploring alternative vaccination strategies to protect CC mice that are refractory to BCG vaccination.

Conclusion/Implication: Together, our results demonstrate a two benefits to using the CC panel to study BCG vaccine responses – that as a genetically diverse platform for testing preclinical vaccine formulations as well as for the identification of vaccine-mediated immune correlates that can be applied to a genetically diverse population.

Funding Sources / Conflicts of Interest
None
Influence of aerosol delivered BCG vaccination on immunological and disease parameters following SARS-CoV-2 challenge in rhesus macaques

Andrew White1, Laura Sibley1, Charlotte Sarfas1, Alexandra L. Morrison1, Kevin Bewley1, Susan Fotheringham1, Konstantinos Gkolfinos1, Karen Gooch1, Alastair Handley1, Holly Humphries1, Laura Hunter1, Chelsea Kennard1, Stephanie Longet1, Adam Mabbutt1, Emma Rayner1, Tom Tipton1, Robert Watson1, Yper Hall1, Fergus Gleeson2, Mark Bodman-Smith3, Mike Dennis1, Francisco J. Salguero1, Miles Carroll1, Helen McShane4, William Cookson5, Julian Hopkin6, Sally Sharpe1

1UK Health Security Agency, Porton Down, Salisbury, United Kingdom
2Churchill Hospital, Oxford, United Kingdom
3St. George’s Medical School, United Kingdom
4The Jenner Institute, Oxford, United Kingdom
5Imperial College London, London, United Kingdom
6Swansea University Medical School, Swansea, United Kingdom

The Tuberculosis vaccine, Bacille Calmette Guérin (BCG), can protect against non-tuberculous diseases attributable to heterologous immune mechanisms. Aerosol vaccine delivery can target immune responses toward the site of infection for a respiratory pathogen, such as severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) and therefore, we hypothesised that aerosol delivery of BCG may provide some protection against SARS-CoV-2 infection. Rhesus macaques were given an aerosol BCG vaccination and immune parameters were monitored in vaccinated and unvaccinated animals for 28 days. High-dose SARS-CoV-2 challenge was applied by intranasal and intrabronchial instillation and animals culled six – eight days later. Mycobacteria-specific cell mediated immune responses were detected following aerosol BCG vaccination, but SARS-CoV-2-specific cellular- and antibody-mediated immunity was only measured following challenge. Secretion of cytokine and chemokine markers associated with the innate and adaptive antiviral immune response was detected following SARS-CoV-2 challenge at higher levels in vaccinated animals. Classical CD14+ monocytes and Vδ2 γδ T-cells quantified by whole blood immunophenotyping increased rapidly in vaccinated animals following SARS-CoV-2 challenge, indicating a priming of innate immune cells and non-conventional T-cell populations. However, viral RNA quantified in nasal and pharyngeal swabs, broncho alveolar lavage (BAL) and tissue samples, in-life CT imaging and histopathology scoring applied to pulmonary tissue sections indicated that the disease induced by SARS-CoV-2 challenge was comparable between vaccinated and unvaccinated groups, although an influence of BCG vaccination on the subsequent immune response to SARS-CoV-2 challenge was apparent in immune signatures. However, immune mechanisms primed by BCG vaccination could contribute to the moderation of COVID-19 disease severity in more susceptible species following natural infection.

Funding Sources / Conflicts of Interest
The studies described herein were funded by a grant from the Coalition for Epidemic Preparedness Innovations (CEPI)
MTBVAC vaccination protects rhesus macaques against aerosol challenge with M. tuberculosis and induces immune signatures analogous to those observed in clinical studies

Andrew White1, Laura Sibley1, Charlotte Sarfas1, Alexandra Morrison1, Jennie Gullick1, Simon Clark1, Fergus Gleeson2, Anthony McIntyre2, Cecilia Lindestam Arlehamn3, Alessandro Sette3, Javier Salguero1, Emma Rayner1, Esteban Rodriguez4, Eugenia Puentes4, Dominick Laddy5, Ann Williams1, Mike Dennis1, Carlos Martin6, Sally Sharpe1

1UK Health Security Agency, Salisbury, United Kingdom
2The Churchill Hospital, Oxford, United Kingdom
3La Jolla Institute for Allergy and Immunology, La Jolla, United States
4Biofabri, Ponteverdra, Spain
5Aeras, Rockville, United States
6Universidad de Zaragoza, Zaragoza, Spain

A single intradermal vaccination with MTBVAC given to adult rhesus macaques was well tolerated and conferred a significant improvement in outcome following aerosol exposure to M. tuberculosis compared to that provided by a single BCG vaccination. Vaccination with MTBVAC resulted in significant reduction in M. tuberculosis infection-induced disease pathology measured using in-vivo medical imaging, in gross pathology lesion counts and pathology scores recorded at necropsy, the frequency and severity of pulmonary granulomas, and the frequency of recovery of viable M. tuberculosis from extra-pulmonary tissues following challenge. The immune profiles induced following immunisation with MTBVAC reflect those identified in human clinical trials of MTBVAC. Evaluation of MTBVAC- and TB peptide-pool-specific T-cell cytokine production revealed a predominantly Th1 response from poly- (IFN-γ+TNF-α+IL2+) and multi- (IFN-γ+TNF-α+) functional CD4 T-cells, while only low levels of Th22, Th17 and cytokine-producing CD8 T-cell populations were detected together with low-level, but significant, increases in CFP10-specific IFN-γ secreting cells. In this report we describe concordance between immune profiles measured in clinical trials and a macaque pre-clinical study demonstrating significantly improved outcome after M. tuberculosis challenge as evidence to support the continued development of MTBVAC as an effective prophylactic vaccine for TB vaccination campaigns.

Funding Sources / Conflicts of Interest
This work was supported by the Department of Health, UK and a grant from Aeras. EP, ER, and CM are co-inventors on a patent on MTBVAC held by the University of Zaragoza and Biofabri.
Mucosal vaccination with MTBVAC displays improved induction of trained immunity and broadened local adaptive immune signatures

Michel Vierboom¹, Karin Dijkman¹, Nacho Aguilo², Claudia Sombroek¹, Charel Boot¹, Sam Hofman¹, Richard Vervenne², Krista Haanstra¹, Maarten van der Sande³, Liesbeth van Emst¹, Jorge Dominguez-Andrés³, Simone J.C.F.M. Moorlag³, Jelle Thole⁴, Esteban Rodriguez⁵, Eugenia Puentes⁵, Joost H.A. Martens⁶, Reinout van Crevel³, Mihai G. Netea³, Carlos Martin², Frank Verreck¹

¹BPRC, Rijswijk, Netherlands
²Universidad de Zaragosa, Spain
³RUMC, Nijmegen, Netherlands
⁴TBVI, Lelystad, Netherlands
⁵Biofabri, Pontevedra, Spain
⁶RU, Nijmegen, Netherlands

Introduction: Standard intradermal BCG vaccination fails to curb the ongoing TB pandemic. New vaccine strategies are urgently needed. Exploring alternative routes of vaccination in NHP, we have shown previously that pulmonary mucosal delivery of BCG shows improved protection against TB. This was associated with unique local immune signatures. Alternatively, we explored the use of a new Mtb-derived vaccine candidate MTBVAC. In the present study we have analyzed both adaptive and innate immune response – trained immunity – profiles after mucosal delivery of MTBVAC in comparison with BCG and standard intradermal vaccination.

Methods: Adult rhesus macaques were vaccinated with intradermal (.id) or mucosal (.muc) BCG or MTBVAC. Local and peripheral immune response were analyzed. For trained immunity CD14+ blood and bone marrow derived monocytes were stimulated with lipopolysaccharide (LPS), before and after vaccination.

Results: Mucosal MTBVAC was well tolerated, eliciting PPD specific polyfunctional T helper type 17 cells, interleukin-10, and immunoglobulins in the airway and yielding a broader antigenic profile than BCG in rhesus macaques. In addition, local T cells expressed high levels of mucosal homing and tissue residency markers after pulmonary vaccination. Heterologous stimulation with LPS of unfractionated bronchial lavage cells after mucosal delivery of BCG or MTBVAC did not alter the cytokine production. However, mucosal but not intradermal vaccination, either with BCG or MTBVAC, enhanced innate cytokine production by blood- and bone marrow-derived monocytes after heterologous stimulation. This was associated with a metabolic rewiring signal, typical of trained immunity.

Conclusion: These results provide support to strategies for improving TB vaccination and, more broadly, modulating innate immunity via mucosal surfaces.

Funding Sources / Conflicts of Interest
None
Mycobacterium tuberculosis genetic features associated with pulmonary tuberculosis severity

Charlotte Genestet¹, Elisabeth Hodille¹, Guislaine Refrégier², Alexia Barbry¹, Emilie Westeel³, Jean-Luc Berland³, Gérard Lina¹, Florence Ader⁶, Laurent Jacob⁴, Stéphane Dray⁴, François Massol⁵, Samuel Venner⁴, Oana Dumitrescu¹

¹ Centre International de Recherche en Infectiologie (CIRI), Hospices Civils de Lyon, Lyon, France
² Institute for Integrative Biology of the Cell (I2BC), Gif-sur-Yvette, France
³ Fondation Mérieux, Lyon, France
⁴ Laboratoire de Biométrie et Biologie Évolutive (LBBE), Villeurbanne, France
⁵ Institut Pasteur de Lille, Center for Infection and Immunity of Lille (CIIL), Lille, France
⁶ Hospices Civils de Lyon, Service des Maladies infectieuses et tropicales, Lyon, France

Background: Mycobacterium tuberculosis (Mtb) infections result in a wide spectrum of clinical outcomes, from latent asymptomatic infection to active pulmonary disease, with an array of severity symptoms. But up to now there are no Mtb proven genetic determinants of these clinical presentations.

Methods: 234 pulmonary TB patients diagnosed at the mycobacteria laboratory of Lyon University Hospital were stratified in "mild grade" and "moderate-severe grade" according to the Bandim TBscore, which consider symptoms and clinical findings, and nutritional and immune status of patients were recorded. To investigate the relation between Mtb genetic features and TB severity, we performed whole genome sequencing of the Mtb clinical isolates to explore within-host micro-diversity thought unfixed mutations, classified these mutations according to their functional categories and we also performed genome-wide association study (GWAS).

Results: We showed that Mtb micro-diversity within patient isolates was strongly correlated with TB severity. We found a higher proportion of non-synonymous mutations in regulatory proteins category for Mtb isolates from “moderate-severe grade” TB patients. Moreover, GWAS identified a SNP in the promoter of espR, a regulatory gene highly involved in the regulation of Mtb virulence, associated with TB severity.

Conclusions: Taken together, these results provide a new insight to better understand TB pathophysiology. The effect of the mutations observed on Mtb virulence remain to be explored but could be envisioned as a new biomarkers of TB severity to improve the management of TB patients.

Funding Sources / Conflicts of Interest
This work was supported by the LABEX ECOFECT (ANR-11-LABX-0048) of Université de Lyon, within the program "Investissements d’Avenir" (ANR-11-IDEX-0007) operated by the French national research agency (Agence nationale de la recherche, ANR).
PPM, a potential candidate for RNA vaccine to prevent active TB disease

Chaouki Benabdessalem1,4, Rym Ouni1,5, Kaur Probhjot2, Afifa Jarraya3, Amani Braiek1,5, Amor Baccouche1, Adel Akermi3, Houda Gharsalli1,5, Leila Douik-Elgharbi4,5, Vinay Nandicoory2, Mohamed Ridha Barbouche1,5

1Institut Pasteur de Tunis, Tunis, Tunisia  
2National Institute of Immunology, New Delhi, India  
3Dispensaire de lutte contre la TB, Ariana, Tunisia  
4Abderrahman Mami Hospital, Ariana, Tunisia  
5University Tunis-El Manar, Tunis, Tunisia

Background: Among the eleven ser/thr kinases of Mtb, PknB is an essential kinase and is the only one with an extracellular segment, herein called PPM. PPM acts as a sensor responsible for the transition from slow to rapid replication state. PknB and its PPM have been extensively investigated as a target for drug design. Herein we demonstrate, for the first time, that PPM is a novel immunogen of Mtb that induces correlates of protection.

Material and methods: PBMCs from active TB patients and LTBI (n=75) were collected. We used ELISA to assess IFN-g, TNFa and granzyme B (GrzB) secreted by PBMCs after stimulation with rPPM, PPD, rESAT6 and PHA. We used ICS to study the T cell subset producing GrzB. We evaluated the in vitro growth of H37Rv under hypoxic and normoxic conditions in the presence of anti-rPPM polyclonal antibodies.

Results: PPM induces significantly higher IFNg and GrzB amounts in LTBI when compared to aTB group. CD8 with high GrzB were associated with prevention of TB reactivation in NHPs. ICS experiment showed that CD8 T cells are the source of PPM-specific GrzB. We observed a log fold decrease in CFU of H37Rv under both normoxic and hypoxic conditions upon treatment with 10ug/ml of polyclonal anti-rPPM.

Conclusion: Our data showed that PPM induces correlates of protection and might be target of antibody mediated protection. It has been demonstrated that the addition of rPPM could prevent the in vitro re-growth of Mtb suggesting that a vectorized/RNA vaccine expressing PPM, at the site of the infection, may prevent progression to active disease.

Funding Sources / Conflicts of Interest
Data presented in the abstract are part of a filed patent by Benabdessalem et al. Patent number: TN2020/0192
Pulmonary immunity and durable protection induced by the multiantigenic subunit vaccine candidate composed of Rv0351/Rv3628 against the hypervirulent Mycobacterium tuberculosis strain K

Tae Gun Kang\textsuperscript{1,2}, Kee Woong Kwon\textsuperscript{3}, Ara Lee\textsuperscript{1,2}, Sung Jae Shin\textsuperscript{3,4}, Sang-Jun Ha\textsuperscript{1,2}

\textsuperscript{1}Department of Biochemistry, College of Life Science & Biotechnology, Yonsei University, Seoul, Korea (Republic of)
\textsuperscript{2}Brain Korea 21 (BK21) PLUS Program, Initiative for Biological Functions & Systems, Yonsei University, Seoul, Korea (Republic of)
\textsuperscript{3}Department of Microbiology, Graduate School of Medical Science, Brain Korea 21 Project, Yonsei University College of Medicine, Seoul, Korea (Republic of)
\textsuperscript{4}Institute for Immunology and Immunological Disease, Yonsei University College of Medicine, Seoul, Korea (Republic of)

\textbf{Background:} BCG vaccine is most effective and prevalent vaccine for tuberculosis (TB) prevention. Although TB mortality rate is diminished by BCG vaccine, it has limitations such as less protective effect for adults and hypervirulent Mycobacterium tuberculosis (Mtbc) strains.

\textbf{Methods:} We validated the combination vaccine strategy of BCG and new formulated subunit vaccine candidate composed of selected TB antigen candidates Rv0351/Rv3628, which was expressed in hypervirulent and most prevalent in South Korea clinical Mtbc strain K belonging to the Beijing family.

\textbf{Results:} Mice immunized with BCG, Rv0351/Rv3628 and adjuvants including MPL exhibited more enhanced the Th1-biased immune response and also elevated multifunctional antigen-specific CD4+ T cell responses, co-producing IFN-\(\gamma\), TNF-\(\alpha\), and IL-2 than BCG or Rv0351/Rv3628 only vaccinated group. Furthermore, combination vaccinated mice displayed a significant reduction in bacterial load and more curtailed lung inflammation upon Mtbc K infection state when compared to BCG only vaccinated group. Taken together, our findings demonstrated that boosting BCG vaccination through new subunit vaccine candidate including antigens of hypervirulent Mtbc K confers enhanced protective capacity in elevated Th1 responses along with multifunctional antigen-specific CD4+ T cells, and this protective effect also dampened pulmonary pathology against the hypervirulent Mtbc Beijing infection.

\textbf{Conclusion:} Collectively, these results might be promising vaccine strategy for prevention against worldwide hypervirulent Mtbc strains.

\textbf{Funding Sources / Conflicts of Interest}
Korea Health Industry Development Institute (HV20C0144)


Quantifying the potential health impacts of adolescent / adult vaccination with M72/AS01E and BCG revaccination-like vaccines in India

Rebecca Clark1,2,3, Christinah Mukandavire1,2,3, Chathika Weerasuriya1,2,3, Matthew Quaife1,2,3, Andrew Iskauskas4, Roel Bakker1,2,3, Danny Scarponi1,2,3, Shelly Malhotra5, Rebecca C Harris1,2,3, Nicolas A Menzies6,7, Richard G White1,2,3

1TB Modelling Group and TB Centre, LSHTM, London, United Kingdom
2Department of Infectious Disease Epidemiology, LSHTM, London, United Kingdom
3Centre for the Mathematical Modelling of Infectious Diseases, LSHTM, London, United Kingdom
4Department of Mathematical Sciences, Durham University, Durham, United Kingdom
5Global Access, IAVI, New York, United States
6Center for Health Decision Science, Harvard T.H. Chan School of Public Health, Boston, United States
7Department of Global Health and Population, Harvard T.H. Chan School of Public Health, Boston, United States

Background: India suffers the largest global burden of tuberculosis. Reaching elimination will likely require novel TB vaccines, such as M72/AS01E, and policy changes, including recommending BCG revaccination, both of which have recently completed Phase II trials. To maximize the potential benefit of novel approaches, we must identify optimal vaccine introduction strategies. We evaluated the health impact of these strategies in India while incorporating uncertainties in product and implementation characteristics.

Methods: We developed an age-stratified compartmental dynamic model of TB in India and calibrated it to epidemiologic data. Using this model, we simulated vaccines with clinical trial-derived characteristics over 2028 to 2050, while varying vaccine profiles (efficacy, infection status required for effect, mechanism of effect) and implementation strategies (ages targeted, introduction year, coverage level, number of repeat vaccinations). We estimated rate reductions and cumulative treatments/cases averted for each vaccine scenario compared to the “no new vaccine” baseline.

Results: Higher efficacy and more rapid vaccine introduction led to higher rate reductions and larger cumulative cases/treatments averted. When we compared an M72/AS01E-like vaccine effective in all, and a BCG revaccination-like vaccine effective only in uninfected individuals, the impact of M72/AS01E-like vaccines in India was estimated to be greater due to the larger proportion of the population in which the vaccine was effective.

Discussion: Vaccines with M72/AS01E and BCG revaccination-like characteristics may substantially reduce the burden of TB in India, and vaccine impact may be increased with prompt introduction and scale-up.

Funding Sources / Conflicts of Interest
Bill and Melinda Gates Foundation, WHO
Reducing Delay in Pulmonary Tuberculosis diagnosis by Engaging Informal Healthcare Providers; A Comparative Analysis Between A Semi Rural and an Urban Setting

Sylvie A. Kwedi Nolna¹

¹University of Yaundé, Yaundé, Cameroon

Background/Introduction: Informal Healthcare Providers (IHPs) provide a significant portion of healthcare in many LMICs such as Cameroon. As seeking primary care from IHPs increases the delay in the diagnosis and treatment of PTB, we evaluated the effectiveness of a collaboration between the NTCP and informal healthcare providers in reducing delay TB care.

Methods: IHPs were trained on the identification and referral of TB suspects to the National TB program’s Centers for diagnosis and Treatment (CDTs). For 12 months post-training, the number of bacteriologically confirmed TB cases referred to NTCP for diagnosis was recorded. Descriptive statistics were performed to compare cases diagnosed before and after training and within the healthcare delay systems. Patient delays, the healthcare system delays, and total delays were calculated.

Results: In both cities, of the TB suspects referred, 71.5% were successfully tested in the CDTs within an average time of 3.04 days (95%CI=2.18-3.89) in Douala and 1.68 days (95%CI = 0.96-2.41). A total of 185 (13.36%) were tested positive for TB. The average delay in diagnosis was 2.4 days (95%CI 1.45-3.38) in Douala and 3.6 days (95%CI 2.73-4.49 days) in Ebolowa.

Discussion and Conclusion: IHPs successfully identified and referred presumptive cases allowing for early diagnosis. The health system delay did not vary across settings and over half of referred cases had a diagnostic test done less than a day from referral. Thus, we conclude that integrating IHPs in the NTCP will not likely promote passive detection of cases but will equally enhance the early diagnosis and treatment.

Funding Sources/Conflicts of Interest
This study was funded by the European and Developing Clinical Trial Partnership (EDCTP2) Programme 2 Capacity Building fellowship (Award # TMA2016CDF-1570)
Reduction of chemotherapy by combination with recombinant BCG expressing the LTAK63 adjuvant in the treatment of infection caused by M. tuberculosis

Monalisa Trentini¹, Dunia Rodriguez¹, Alex Issamu Kanno¹, Lazaro Marques-Neto¹, Luciana Cezar de Cerqueira Leite¹

¹Butantan Institute, São Paulo, Brazil

Introduction: Tuberculosis (TB), caused by the bacillus M. tuberculosis, is one of the infectious diseases that causes the greatest number of deaths in the world. Our preliminary results showed that rBCG expressing the LTAK63 adjuvant can reduce Mtb infection in infected mice. Our hypothesis is that the use of vaccines in the treatment of diseases can assist antibiotic chemotherapy, reducing treatment time. The aim of this study was to evaluate the combination of rBCG-LTAK63 vaccine with a reduced antibiotic treatment of Mtb infection in a murine model.

Methods: BALB/c mice were infected with Mtb (500 CFU) intranasally, after four weeks the mice were treated with Isoniazid and Rifampicin via gavage for 15 days (half of the standard treatment). Fifteen days after the end of therapy, the animals were inoculated with rBCG-LTAK63 (106 CFU, single dose) subcutaneously, intranasally or intravenously. The induced immune response and treatment efficacy were evaluated two months after the vaccine dose.

Results: Spleen and lung cells from animals treated with combination therapy using subcutaneous and intravenous rBCG-LTAK63 administration showed a reduction in the inflammatory cytokines (TNF-α and IFN-γ) and an increase in the secretion of IL-6. Furthermore, there was also an increase in IL-10, as well as in regulatory T cells when compared with the untreated groups. Mice treated with half the schedule of chemotherapy combined with the rBCG-LTAK63 vaccine showed reduced bacillary load and immunopathology in the lungs and spleens when compared to the control group.

Conclusion: These results suggest that the immunization with rBCG-LTAK63 vaccine may synergize with reduced chemotherapy in the treatment of TB.

Funding Sources / Conflicts of Interest
Supported by FAPESP (Projets 2017/24832 and 2019/06454-0)
Safety and reactogenicity of MTBVAC and BCG vaccines in South African adults

Angelique Luabeya Kany Kany 1, Claire Imbratta 1, Frances Ratange 1, Virginie Rozot 1, Michele Tameris 1, Justin Shenje 1, Simon Mendelsohn 1, Michelle Fisher 1, Humphrey Mulenga 1, Nicole Bilek 1, Ingrid Murillo 2, Esteban Rodriguez 2, Eugenia Puentes 2, Juana Doce 2, Carlos Martin 2, Dereck Tait 4, Kathryn Rutkowski 4, Cadwill Pillay 4, Thomas Scriba 1, Mark Hatherill 1

1South African Tuberculosis Vaccine Initiative (SATVI), Institute of Infectious Disease and Molecular Medicine, Department of Pathology, University of Cape Town, Cape Town, South Africa
2Biofabri, S.L., Porriño, Pontevedra, Spain
3Department of Microbiology, Faculty of Medicine, University of Zaragoza, Spain, Zaragoza, Spain
4International AIDS Vaccine Initiative (IAVI), Cape Town, South Africa

Background: Tuberculosis remains a global health threat. There is an urgent need of an efficacious vaccine. We evaluated the safety and reactogenicity of a live-attenuated Mycobacterium tuberculosis (Mtb) vaccine, MTBVAC, in a high TB prevalence setting.

Methods: This study was conducted in Worcester, South Africa under clinical trials gov identifier: NCT02933281. We enrolled HIV-negative adults aged 18-50 years, with and without evidence of Mtb exposure assessed by IGRAs tests (QFT) to receive a single dose of MTBVAC or BCG revaccination (BCG Japan; 0.05 mg). MTBVAC was given intradermally at four dose levels: 5 x 103 CFU, 5 x 104 CFU, 5 x 105 CFU, and 5 x 106 CFU. Participants were grouped in 8 cohorts of 18 participants according to dose and Mtb exposure status. Participants were followed up for 12 months.

Results: Out of 485 participants screened, 143 were enrolled. A total of 136/143 (95.1%) vaccinees reported at least one adverse event (AE). Total AEs were 793 with 713/793 (89.9%) classified as mild, 71/793 (8.9%) moderate, and 9/793 (1.1%) severe. Reactogenicity events included pain 16.9% (127/793), redness 5.5% (44/793), swelling 3.9% (31/793) and ulceration at injection site 14.9% (118/793). Cohort 1 and 5 (5 x 103 CFU) had lower AE frequencies 7.7% and 10.9% compared to cohort 4 and 8 (5 x 106 CFU) 18% and 16.4% respectively. Reactogenicity was more pronounced in QFT+ at each dose level. No SAE deemed vaccine related were reported.

Conclusion: Overall, the vaccines were well tolerated among adults with and without Mtb exposure, living in a high TB prevalence setting.

Funding Sources / Conflicts of Interest
Funding: Biofabri, SL, NIH, CDMRP Conflict of Interest IM, JD, EP are employees of Biofabri CM is co-inventor in a patent application on MTBVAC filed by the University of Zaragoza
**Background:** A majority of the immunodominant T cell antigens of Mycobacterium tuberculosis (Mtb) are hyperconserved. Lack of antigenic variation suggests insufficient selection pressure from T cell responses to infection. We identified 6 variable-sequence Mtb proteins, which we hypothesized represent antigens that vary due to selective pressure from protective T cell responses.

**Methods:** To determine if the variable-sequence proteins are immunogenic, we vaccinated C57BL/6 (B6) mice with a DNA vaccine encoding fusions of the variable proteins. We also assayed responses to the variable proteins in Mtb-infected B6 mice, to determine whether they are recognized in infection.

**Results:** Vaccination induced CD4 T cell IFN-γ responses to the variable proteins, confirming that they are immunogenic. We also investigated the CD4 T cell response to these proteins after infection in B6 and CB6F1/J mice. This confirmed that the sequence-variable proteins are antigenic, as reflected by IFN-γ-producing CD4 T cells in the lungs. Interestingly, we observed IL-17 responses in the mediastinal lymph node of CB6F1/J mice to one of the variable antigens RimJ, which also generates IL-17 responses in Mtb-infected humans. Additionally, a naturally occurring epitope variant in RimJ impacted the magnitude of CD4 T cell responses. In an aerosol challenge with Mtb Erdman, vaccination with the variable antigens did not enhance protection in B6 mice, despite enhanced Th1 responses in the lungs.

**Discussion:** Th1 responses to the variable antigens did not reduce lung or spleen bacterial burden in vaccinated C57BL/6 mice after challenge. However, enhanced IL-17 responses to the variable antigen RimJ suggest that mucosal Th17 vaccination has the potential to provide enhanced control.

**Funding Sources / Conflicts of Interest**
None
The DCIR/LRP1 axis in myeloid cells: A new target in immunity to tuberculosis?

Tamara Sneperger¹, Benjamin B A Raymond¹, Alexandre Stella¹, Giulia Trimaglio¹, Corinne Vivès², Franck Fieschi², Odile Burlet-Schiltz¹, Olivier Neyrolles¹, Yoann Rombouts¹

¹Institut de Pharmacologie et de Biologie Structurale, IPBS, Université de Toulouse, CNRS, UPS, Toulouse, France
²Institut de Biologie Structurale, IBS, Université Grenoble Alpes, CNRS, CEA, Grenoble, France

Tuberculosis (TB) is an immunopathology caused by Mycobacterium tuberculosis (Mtbc) and remains one of the top ten causes of death worldwide (WHO’s Global Tuberculosis Report 2020). Therefore, there is an urgent need to improve our understanding of anti-TB immunity in order to develop innovative therapies. We recently discovered that the dendritic cell immunoreceptor (DCIR) negatively modulates anti-TB immunity. In particular, the absence of DCIR in mice results in an improved adaptive immune-mediated pulmonary clearance of Mtbc. However, the absence of a known ligand for DCIR greatly hindered our understanding of the exact functions of this receptor in immunity. To address this issue, we used a combination of mass spectrometry and biochemical approaches to identify the first endogenous ligand for DCIR: the low-density lipoprotein receptor-related protein 1 (LRP1). In addition to carrying LRP1 at their cell surface, myeloid cells strongly express DCIR itself, with macrophages being the cells expressing the most DCIR and LRP1. Using confocal microscopy experiments and proximity ligation assays, we found that DCIR colocalizes with LRP1 at the surface of mouse and human macrophages, supporting the concept of a cis interaction (on the surface of the same cell) between DCIR and LRP1. Of note, DCIR may also interact with LRP1 in trans between cells in close proximity. Importantly, our preliminary experiments showed that DCIR regulates LRP1 functions, including endocytosis of ligands and resulting cytokine production. Collectively, our data provide fundamental insights into the understanding of the role of DCIR in anti-TB immunity and may offer new avenues for the development of host-directed therapy, targeting DCIR/LRP1, to treat TB.

Funding Sources / Conflicts of Interest
Funding Sources: ANR, FRM, UPS
The evaluation of a new recombinant BCG vaccine in cynomolgus macaque model

Natsuko Yamakawa¹, Yasuhiro Yasutomi¹
1 Tsukuba Primate Research Center, National Institutes of Biomedical Innovation, Health and Nutrition, Tsukuba, Japan

Background: Bacillus Calmette-Guerin (BCG) is the only licensed TB vaccine. However, because the BCG vaccine is a weakened live vaccine, its pathogenicity prohibits its use in immunosuppressed people like HIV-positive patients. The mortality rate from TB is about 12%, but increases to about 30% in TB and HIV co-infected patients. Therefore, there is a need to develop a TB vaccine that can be administered to immunosuppressed people.

In the previous study, Mizuno et al tested a new recombinant BCG vaccine (rBCG-SOCS1DN) expressing a mutated suppressor of cytokine signaling 1 (SOCS1) in C57BL/6 mice. SOCS1 is a negative regulator of JAK/STAT signaling. It has been hypothesized that expression of mutant antagonistic SOCS1 activates the host immune response and rapidly eliminates BCG from the host while inducing memory T cells against TB. In fact, rBCG-SOCS1DN prevented TB disease better than control BCG did. Moreover, rBCG-SOCS1DN was not pathogenic in immunodeficient mice (RAG1 knockout mice.)

Methods and Results: The macaque TB model is comparable to human TB patients because it well recapitulates the outcomes of Mycobacterium Tuberculosis (MtB) infection seen in humans. In this study, rBCG-SOCS1DN was evaluated with cynomolgus macaque model. Comparing the number of colony-forming units of MtB in lungs, there was no significant difference between control BCG and rBCG-SOCS1DN. The immune response at the site of administration tended to start earlier and to end earlier with rBCG-SOCS1DN than with control BCG. Moreover, a milder response was observed in macaques administered with rBCG-SOCS1DN. We are currently validating this vaccine using a co-infection model of simian immunodeficiency virus (SIV) and TB.

Funding Sources / Conflicts of Interest
This work was supported by JSPS KAKENHI Grant Number JP21K05995.