Mycodays 2022

20-22 June 2022
Amphithéâtre Charles Mérieux
Ecole Nationale Supérieure, Lyon

Abstract book

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This booklet contains the abstracts for the Scientific Session papers as submitted by the authors. Abstracts are in presentation order by day and time.

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PROGRAMME

Monday 20th of June

12h30: Welcome snack

13h45-14h00: Meeting introduction

TB : global disease

14h00-14h30: Lucica Ditiu: Ending TB in the post covid era – can we do it?

14h30-15h00: Jonathan Hoffmann: Implementation of multi-country translational TB research within GABRIEL international network

15h00-15h30: Eamonn Gormley: “Vaccination of badgers with BCG to aid eradication of bovine tuberculosis”

15h30-16h00: coffee break

MTB lipidomics

16h00-16h30: Jérôme Nigou: A *Mycobacterium tuberculosis* fingerprint in human breath allows tuberculosis diagnosis

16h30-17h00: Emilie Layre: Extracellular vesicles released during *Mycobacterium tuberculosis* infection: lipid content and role in host-pathogen interactions

17h00-17h30: Short oral communications session

- Tonia Dargham : Selection of new therapeutic targets in relation with Intrabacterial Lipid Inclusions metabolism in *Mycobacterium abscessus*
- Mélanie Foulon : Acquisition of host derived lipids by intracellular mycobacteria and its impact on pathogenesis

17h30-20h00: Posters and local organic aperitifs

Tuesday 21st of June

New strategies to explore host-pathogen interactions in mycobacterial infections

09h00-09h30: Julien Vaubourgeix : Multiform antimicrobial resistance in mycobacteria

09h30-10h00: Céline Cougoule : Human bronchial organoids unveil druggable pathways against *Mycobacterium abscessus* infection in cystic fibrosis

10h00-10h30: Short oral communications session

- Lucia Grenga : Direct and quick detection of Mycobacterium in cystic fibrosis pulmonary samples, its taxonomical typing, and functional characterization
- Arnaud Machelart : Intrinsic antibacterial activity of beta cyclodextrins potentiates their effect as drug nanocarriers against tuberculosis

10h30-11h00: Coffee break and posters

11h00-12h00: Short oral communications session

- Emilie Doz Deblauwe: Les neutrophiles régulateurs s'accumulent chez la souris formant des granulomes encapsulés
- Jahn Nitschke: A 3R infection model to screen, characterize and decipher the mode of action of novel anti-mycobacterial compounds from a large plant extract
- Audrey Bernut: Pharmacological activation of NRF2 has protective effects during *Mycobacterium abscessus* infection by promoting host defenses and reducing inflammatory damage
- Hamadoun Touré: *Mycobacterium abscessus* resists the innate cytotoxic response by surviving granzyme-mediated cell lysis of infected phagocytes

TB between bench and clinics

12h00-12h30: Maya Hites: Pragmatic management of TB patients in high income countries

12h30-13h30: Lunch and poster

13h30-14h00: Jacinta Bustamante: Mycobacterial diseases in patients with inborn errors of immunity

14h00-14h30: Virginie Rozot: Multidimensional analysis of immune responses in TB

14h30-15h00: Philippe Leissner: Blood transcriptional biomarkers in tuberculosis. Do they meet the needs?

15h00-15h30: Short oral communications session

- Rim Bayaa : Distinct Cellular Signatures Identified by Spectral Flow Cytometry upon Tuberculosis Diagnosis and at Treatment Completion
- Fadel Sayes : Exploring new recombinant BCG Vaccines and different Vaccination Routes for better Tuberculosis Protection

15h30-16h00: Coffee break

Drug discovery for MTB

16h00-16h30: Ruben Hartkoorn A novel potent class of nature inspired anti-tuberculosis molecules: chemical vs genetic plasticity

16h30-17h00: Kamel Djaout : Boosting pretomanid potency: towards reversion of resistance

17h00-17h30: Pierre Santucci: Intracellular localisation of *Mycobacterium tuberculosis* affects efficacy of the antibiotic pyrazinamide

18h00: Visit Lyon and «Fête de la musique»

Wednesday 22nd of June

Pathogenesis of non-tuberculous mycobacteria

9h00-09h30: Estelle Marion: Reconsidering the role of mycolactone in the pathophysiology of Buruli ulcer.

09h30-10h00: Jona Karam: Unraveling the function of tetraspanin CD81 and its cognate adhesins from *Mycobacterium abscessus* during host cell invasion

10h00-10h30: Short oral communications session

- Virginia Pichler : Unpacking the molecular determinants of *Mycobacterium abscessus* infections
- Marion Lagune : Les protéines EsxU et EsxT sécrétées par le système de sécrétion ESX-4 modulent la fitness de *Mycobacterium abscessus*

10h30-11h00: Coffee break

Lessons from Mtb genomics

11h00-11h30: Christophe Guilhot: Emergence of tuberculosis bacilli: the long road to becoming a pathogen

11h30-12h00: Jan Madacki Exploring horizontal gene transfer in predominantly clonal tuberculosis-causing mycobacteria

12h00-12h30: Short oral communications session
Charlotte Genestet : *Mycobacterium tuberculosis* genetic features associated with pulmonary tuberculosis severity
Christophe Sola : Le chaos croissant de la génomique des populations des bacilles de la tuberculose à l'ère des «big data» : il faut trier le bon grain de l'ivraie

12h30-12h45: Meeting conclusions and farewell

Invited speakers

C1. Ending TB in the post covid era – can we do it?

Dr. Lucica Ditiu, Executive Director of the Stop TB Partnership is a Romanian physician, accomplished professional and leader in the global fight against tuberculosis (TB) and other communicable diseases. Dr. Ditiu is driven by the firm belief that we should «leave no one behind» and is one of the strongest advocates within the international community in the fight against tuberculosis. A firm believer in innovation, flexibility, change, breaking the rules and thinking out of the box, Dr. Ditiu is dedicated to driving political commitment and engagement to accelerate the efforts to End TB.

C2. Implementation of multi-country translational TB research within GABRIEL international network

Jonathan Hoffmann, PhD
Tuberculosis research manager
Scientific and Medical Department Fondation Mérieux

Jonathan Hoffmann leads TB research activities in the medical and scientific department of Fondation Mérieux in Lyon, France. Between 2017 and 2020, he coordinated a multi-country clinical evaluation of TB diagnoses for monitoring treatment outcomes in five countries of the international GABRIEL network (HINTT project: Madagascar, Lebanon, Bangladesh, Georgia and Paraguay). Since 2020, he is the co-PI of an operational research program to improve the detection and management of latent TB infection in high-risk groups in Madagascar and Cameroon (APRECIT project). He is also co-supervising a clinical evaluation of innovative diagnostic solutions for pediatric and extra-pulmonary TB in partnership with Icddr,B in Bangladesh (DEDICATE project).
Jonathan Hoffmann joined Fondation Mérieux in 2009 as scientific project manager to oversee the scientific activities of the Rodolphe Mérieux Laboratory in Madagascar. In October 2015, he obtained a PhD in Microbiology/Immunology from the University of Lyon-Ecole Normale Supérieure. Specifically on TB, Fondation Mérieux acts to strengthen diagnostic and research capacities in order to improve screening and care for vulnerable populations/communities affected by this disease in low- and

middle-income countries. Beyond the construction of BSL-3 laboratories, Fondation Mérieux's initiatives include the development and coordination of multi-country operational clinical research programs supporting national TB control programs (NTPs) in the operational implementation of the WHO END-TB strategy.

1- Chedid, C. *et al.* In-Depth Immunophenotyping With Mass Cytometry During TB Treatment Reveals New T-Cell Subsets Associated With Culture Conversion. *Front Immunol* **13**, (2022).
2- Ranaivomanana, P. *et al.* Longitudinal Variations of M. tuberculosis-Induced IFN-γ Responses in HIV-Negative Pregnant Women Exposed to Tuberculosis. *Front Immunol* **12**, (2021).
3- Hoffmann, J. *et al.* Drug-resistant TB prevalence study in 5 health institutions in Haiti. *PLOS ONE* **16**, (2021).
4- Chedid, C. *et al.* Relevance of QuantiFERON-TB Gold Plus and Heparin-Binding Hemagglutinin Interferon-γ Release Assays for Monitoring of Pulmonary Tuberculosis Clearance: A Multicentered Study. *Front. Immunol.* **11**, (2021).
5- Bayaa, R. *et al.* Multi-country evaluation of RISK6, a 6-gene blood transcriptomic signature, for tuberculosis diagnosis and treatment monitoring. *Sci Rep* **11**, (2021).
6- Chedid, C. *et al.* Association of baseline white blood cell counts with tuberculosis treatment outcome: a prospective multicentered cohort study. *International Journal of Infectious Diseases* (2020) doi:10.1016/j.ijid.2020.09.017.

https://www.fondation-merieux.org/en/projects/hintt/
https://www.fondation-merieux.org/en/projects/aprecit/

C3. Vaccination of badgers with BCG to aid eradication of bovine tuberculosis

Eamonn Gormley
School of Veterinary Medicine
University College Dublin (UCD), Dublin, Ireland

Profile

Prof. Eamonn Gormley completed an Honours degree followed by a PhD in Genetics (1988) at Trinity College Dublin. He carried out Post-doctoral work for three years at the Institut Pasteur in Paris and afterwards worked at Massey University, New Zealand researching into the immune responses of cattle vaccinated with BCG (1991-1998). He returned to University College Dublin (UCD) to lead a research programme for the development of a vaccine against tuberculosis for use in wildlife, funded by the Department of Agriculture, Food & the Marine (DAFM). His laboratory also carries out IFNγ blood testing and research on the national herd. He is involved in national and international studies on tuberculosis in cattle and wildlife and has active collaborations with groups in the UK, US, EU, NZ and South Africa. The research conducted in his laboratory provides scientific evidence to support the government strategy to eradicate tuberculosis from the national herd and wildlife.

Abstract : Wildlife species, such as badgers, act as maintenance hosts for *Mycobacterium bovis* and contribute to the spread and persistence of tuberculosis in associated cattle populations. Epidemiological evidence demonstrates a high prevalence of tuberculosis in badgers and controlled studies in Ireland involving comprehensive badger removal have shown that this strategy can serve to significantly reduce cattle reactor rates in the targeted areas. However, as the badger is a protected wildlife species, alternative strategies are required to combat the disease. Targeted vaccination of wildlife species against tuberculosis is an option, which, if successfully employed, could directly facilitate the advancement of bovine tuberculosis eradication in affected areas. Programmes of research into BCG vaccination of badgers are being conducted at UCD and the research team has demonstrated that vaccination of badgers by any number of routes, including oral BCG delivery, generates high levels of protective immunity against challenge with *M. bovis*. The results of a large-scale oral BCG vaccine field trial have also established that the vaccine can induce protective responses that reduces incidence of Tb following natural *M. bovis* challenge. Vaccination of badgers against tuberculosis now forms a key part of the national strategy to eradicate the disease from cattle.

C4. A *Mycobacterium tuberculosis* fingerprint in human breath allows tuberculosis diagnosis

Jérôme Nigou
Institut de Pharmacologie et de Biologie Structurale, Université de Toulouse, CNRS, UPS, France

An estimated one third of tuberculosis (TB) cases go undiagnosed or unreported. Sputum samples, widely used for TB diagnosis, are inefficient at detecting infection in children and paucibacillary (smear negative) patients. Indeed, developing point-of-care biomarker-based diagnostics that are not sputum-based is a major priority for the WHO. Here, we tested exhaled breath condensate (EBC) for *Mycobacterium tuberculosis* (*Mtb*) molecules and assessed whether this approach allows pulmonary TB diagnosis. *Mtb*-specific lipids, lipoarabinomannan lipoglycan, and proteins present in EBCs unambiguously differentiate TB patients from controls. We used EBCs to track the longitudinal effects of antibiotic treatment in *Mtb*-infected children. In addition, *Mtb* lipoarabinomannan and lipid structure in EBC revealed specific metabolic and biochemical states of *Mtb* in the human lung. Our data collectively indicate that EBC analysis can unequivocally diagnose TB across all patient populations and monitor treatment efficacy. This affordable, rapid and non-invasive approach seems superior to sputum assays and can potentially easily be implemented at point-of-care.

Biographical sketch:

- 2011-now: Head of the team “Immunomodulation by Mycobacterial Lipids & Glycoconjugates”, Institut de Pharmacologie et de Biologie Structurale, Toulouse
- 2009: Accreditation to supervise research (HDR), Université de Toulouse
- 2000: PhD in Biochemistry, Université de Toulouse

C5. Extracellular vesicles released during *Mycobacterium tuberculosis* infection: lipid content and role in host-pathogen interactions

Pierre Boyer, Isabelle Vergne, Jérôme Nigou and Emilie Layre.
Speaker: Emilie Layre, PhD, CNRS researcher
Institut de Pharmacologie et de Biologie Structurale, Université de Toulouse, CNRS, Université Paul Sabatier, Toulouse, France

Extracellular vesicles (EV) are tiny objects surrounded by a lipid membrane that allow extracellular release and intercellular exchange of various cargos: proteins, lipids and nucleic acids. They participate to a plethora of biological processes in all kingdoms of life, such as detoxification, homeostatic functions, disease spreading and host-pathogen interaction. During *M. tuberculosis* infection, EVs are released by the infected host cells and by the bacillus itself and in both cases vesicles carry bacterial molecules. Therefore, EV have the potential to participate in host-pathogen interactions and to modulate the microenvironment at the site of infection. For this reason we undertook to characterize the content of these vesicles in bacterial lipids and their immunomodulatory properties. Studying in first instance the vesicles released by mycobacteria of variable virulence, our work first shows that obtaining quality vesicles (devoid of contaminants) requires combining different purification and quality control methods, as often underlined by International Society of Extracellular Vesicles. Purified mycobacteria vesicles were subjected to lipidomic analyses, which provided a detailed characterization of their lipid content. In addition, we have initiated several bioassays to study their ability to interact with and regulate the functions of macrophages *in vitro* (PRR interactions, intracellular traffic, inflammatory mediators secretion and autophagy). Our results highlight EV structural and functional properties including strain-specificities that could have consequences on the immunomodulatory properties of mycobacteria EV *in vivo*.

Fundings: The Fondation pour la Recherche Médicale (DEQ20180339208 and ING20160435108) and the ANR (JCJC ANR-20-CE44-0008)
Acknowledgement: TRI Metatoul and MetaToul-MetaboHUB lipidomic platforms

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C6. Multiform antimicrobial resistance in mycobacteria

Julien Vaubourgeix
MRC-Centre for Molecular Bacteriology and Infection, Imperial College London

Antimicrobial resistance (AMR) is the ability of bacteria to avoid or delay being killed by an antibiotic. AMR can manifest in three ways: resistance, tolerance, or high persistence. Tolerance and persistence complicate treatment of many bacterial infections, including tuberculosis, via contributing to treatment length, treatment failure, disease recurrence, and the emergence of resistance. Therapeutically targeting tolerant and persistent cells could improve outcomes, but the molecular mechanisms underlying tolerance and persistence in mycobacteria are not well understood. To fill this gap, we developed a forward genetic method for the isolation of high survival mutants—which display tolerance and/or high persistence—based on their spatial separation from resisters upon exposure to a drug in vitro. Using this method in the model mycobacterial species *Mycobacterium smegmatis*, we isolated a group of nine mutants that harbored loss-of-function mutations in *argA* or *argD*, two genes in the arginine biosynthesis pathway. We found that perturbation of this essential biosynthetic pathway generated three distinct forms of resistance to diverse antibiotics: tolerance and persistence to aminoglycosides, high survival upon exposure to rifampicin and resistance to macrolides. This extraordinary resilience may help explain how sub-sterilizing exposure to one antibiotic in a multi-drug regimen can induce resistance to others and invites development of drugs targeting mediators of multiple forms of resistance.

Bio: After completing my PhD in the laboratory of Dr. Mamadou Daffé at the Institute of Pharmacology and Structural Biology in 2009 in Toulouse, France, I joined the laboratory of Dr. Carl Nathan at Weill Cornell Medicine in New York, USA, as a postdoctoral fellow (2009-2014), an instructor (2014-2017) and an assistant professor (2017-2018). This experience spurred a continued interest in approaching TB research through multi-disciplinary approaches—spanning microbiology, biochemistry, genetics, structural biology, time-lapse and super-resolution fluorescence photomicroscopy. In 2019, I joined Imperial College London as a Lecturer in Molecular Microbiology in the department of Infectious Disease where my laboratory studies the mechanisms by which mycobacteria, including *Mycobacterium smegmatis* and *Mycobacterium tuberculosis*, survive for a prolonged time upon exposure to otherwise lethal concentration of an antibiotic

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C7. Human bronchial organoids unveil druggable pathways against *Mycobacterium abscessus* infection in cystic fibrosis

Céline Cougoule

Institut de Pharmacologie et de Biologie Structurale (IPBS), Université de Toulouse, CNRS, UPS, Toulouse, France - celine.cougoule@ipbs.fr

INTRODUCTION: *Mycobacterium abscessus* (Mabs) drives life-shortening mortality in cystic fibrosis (CF) patients, primarily because of its resistance to chemotherapeutic agents. Both our knowledge on and models to investigate the host and bacterial determinants that drive Mabs pathology in CF patients remain rudimentary. Here, we evaluated whether the lung organoid technology is appropriate for modelling Mabs infection and whether antioxidant treatment is a candidate therapeutic approach in the context of CF disease.
METHODS: We derived airway organoids (AOs) from lung biopsy and microinjected smooth (S-) or rough (R-)Mabs in the lumen of AOs to evaluate its fitness, responses of AOs to infection, to CF context and treatment efficacy.
RESULTS: We show that S Mabs formed biofilm, R Mabs formed cord serpentines and displayed a higher virulence. While Mabs infection triggers enhanced oxidative stress in AOs, pharmacological activation of antioxidant pathways resulted in better control of Mabs growth. Using CF-patient derived AOs and pharmacological inhibition of the CFTR, we show that S and R Mabs replicated more efficiently and display higher virulence in CF context. Finally, pharmacological activation of antioxidant pathways inhibited Mabs growth and synergized with cefoxitin, a first line antibiotic.
CONCLUSION: we have established AOs as a suitable human system to decipher mechanisms of CF-enhanced respiratory infection by Mabs and confirmed antioxidant approaches as a potential host-directed strategy to improve Mabs infection control.

C8. Pragmatic management of TB patients in high income countries

Maya Hites

The World Health Organisation (WHO) has set the goal of eliminating TB as a public health problem worlwide by 2050. To do so, all active TB cases (but particularly pulmonary TB) must be diagnosed, and treated promptly and adequately, and patients must show good treatment compliance to obtain clinical and microbiological cure. However, TB treatment, even when due to a very susceptible strain of *Mycobacterium tuberculosis*, requires the administration of several drugs for a minimum of 6 months. These treatments may cause secondary effects, and be responsible for drug-drug interactions when patients have co-morbidities requiring other concomitant treatments. Furthermore, patients may have complicated social situations to deal with (e.g : no papers, homeless, etc). Even though most high-income countries

are ones with low TB incidence, significant energy needs to be dedicated to the relatively few TB patients in these countries, to best accompany them throughout their treatment and obtain good outcome results. This talk will discuss how best to accompany and manage these patients.

Biography/ Affiliations :
Maya Hites is an infectious diseases physician who is currently working at Erasme hospital, the academic hospital of the Université Libre de Bruxelles, in Brussels, Belgium. She takes care of patients on a daily basis, and carries out clinical research. Her main research topics are: 1) optimisation of dosage regimens of anti-infectious agents in special patient populations (critically ill, obese, cirrhotic, etc), 2) improving diagnotic techniques for different infections (e.g : community and hospital acquired pneumonia, infections due to *Mycobacteria* spp., *Aspergillosis* spp. and *Pneumocystis jirovicii*), and more recently 3) identifying safe and effective therapeutic options for COVID-19.

C9. Mycobacterial diseases in patients with inborn errors of immunity
Jacinta BUSTAMANTE MD, PhD[1,2,3,4]
1Laboratory of Human Genetics of Infectious Diseases, Necker Branch, INSERM U1163, Necker Hospital for Sick Children, Paris, France, EU
2University of Paris, Imagine Institute, Paris, France, EU
3St. Giles Laboratory of Human Genetics of Infectious Diseases, Rockefeller Branch, The Rockefeller University, New York, NY, USA
4Study Center for Primary Immunodeficiencies, Necker Hospital for Sick Children, AP-HP, Paris, France, EU

Human vulnerability to invasive, disseminated and/or recurrent mycobacterial infections is controlled by a combination of environmental and non-microbial factors, and by genetic and non-genetic host conditions. Tremendous progress has been made towards understanding interindividual clinical variability in susceptibility to non-pathogenic mycobacteria. Clinical disease caused by mycobacteria, including bacillus Calmette-Guérin (BCG) vaccines and environmental mycobacteria, can result from inborn errors of immunity (IEIs). IEIs underlie more than 450 conditions, each associated with an impairment of the development and/or function of hematopoietic and/or non-hematopoietic cells involved in host defense. Only a minority of IEIs confer predisposition to mycobacterial disease. However, all these IEIs share a defining feature: the impairment of immunity mediated by interferon gamma (IFN-γ). More profound IFN-γ deficiency is associated with a greater vulnerability to weakly virulent mycobacteria, whereas more selective IFN-γ deficiency is associated with a more selective predisposition to mycobacterial

disease. Thus, the IFN-γ-mediated immunity is the crucial antimycobacterial circuit, acting as a genetically controlled continuous trait determining the type and outcome of mycobacterial infections.

Biography : Jacinta Bustamante, MD, PhD, "MCU-PH" (Associate Professor with tenure in Université Paris Cité)
Jacinta Bustamante is paediatrician and immunologist by raining. Since 2008, she has led the team working on genetic predisposition to mycobacterial infection in the laboratory of Drs. Abel and Casanova (GHMI-INSERM 1163, Paris branch). She is also responsible for the diagnosis of chronic granulomatous disease and MSMD in patients attending the Center for the Study of Primary Immunodeficiencies (CEDI) at Necker Hospital for Sick Children. She has made substantial contributions to the field of genetic predisposition to infectious disease, including bacterial and mycobacterial infectious diseases, in humans. She has published over 230 papers in peer-reviewed scientific journals and books.

C10. Multidimensional analysis of immune responses in TB
Virginie Rozot

Human clinical research constraints restrict immunological analyses to blood, the most accessible immune compartment, in the context of TB research. Understanding changes occurring in the blood is necessary for the discovery and development of biomarkers that allow tracking of different TB clinical presentations as well as monitoring of treatment success. However, we still do not know the drivers of immune control or pathogenesis, and do not possess correlates of protection that would allow us to design better vaccines and vaccinations strategies. For instance, we know that Th1 cells are necessary but not sufficient for the control of *M. tuberculosis* (Mtb), and, paradoxically, can also mediate pathogenesis. To improve our understanding of immune responses that protect versus those that mediate deleterious functions in the context of TB, blood is likely to be suboptimal. By contrast, studies must focus on tissues affected by Mtb infection and TB disease. Human tissue immunology has been substantially understudied in the field of TB. Human lesions are typically only available from surgical resections where drug therapy has failed, which only represents the extreme profile of a failing immune response.
We propose to use an arsenal of multi-scale imaging and -omics approaches to model the disease trajectory in different anatomic compartments. We will analyse a post-mortem cohort of persons with tuberculosis who are either infected or uninfected with HIV and in control cohorts of Mtb-uninfected and Mtb-infected persons who died of trauma, hypothesized to harbour a successful immune response to Mtb. This study will allow us to study

cell heterogeneity between the different clinical groups and highlight the necessary immune responses for disease control.

Biography : Dr. Rozot possess a non-conventional curriculum as she holds a biotechnology engineering diploma received in 2007 from the renowned Polytech engineering school in Marseille, France. She was next trained in the laboratory of Professor Pantaleo in the CHUV, Lausanne, where she obtained her PhD degree focused on tuberculosis disease and particularly on the study of Mtb-specific CD4 and CD8 T cell responses.
She moved to the South African Tuberculosis Vaccine Initiative (SATVI), where she currently supervises the scientific and operational oversight for the laboratory activities of two vaccine trials with MTBVAC live-attenuated vaccine, one in adults and one in newborns. She will also oversee the MTBVAC phase 3 trial starting in 2022, aiming at assessing efficacy of the vaccine in approximately 7000 newborns. She is also involved in the IMPACT-TB consortium aiming at understanding correlates of (vaccine and natural) protection in multiple cohorts. Identifying immune correlates of risk of TB disease is a major research focus of her group where high dimensional immunological tools are needed. She developed and optimized the first and only Mass Cytometry platform on the African continent.

C11. Blood transcriptional biomarkers in tuberculosis. Do they meet the needs?
Philippe Leissner

Blood transcriptomics have revealed major characteristics of the immune response in Tuberculosis (TB). Whole blood transcriptional biomarkers have been shown to be able to distinguish active TB patients from asymptomatic latently infected individuals and healthy controls and to monitor treatment response of anti-TB therapy. However, consensus has not been achieved regarding the optimal reduced gene sets as diagnostic biomarkers.
Here, we have utilized the information from the entire transcriptome to develop a blood transcriptional signature that discriminates active TB from latently infected and healthy individuals or those with acute viral and bacterial infections. Furthermore, in a unique clinically and temporally well-defined cohort of household contacts and active TB patients, we defined changes in gene expression in incipient TB increasing in subclinical and clinical TB and demonstrated that blood signatures can monitor the treatment response.
Blood transcriptomics thus reveal the evolution and resolution of the immune response in TB, which may help in clinical management of the disease.

BIOGRAPHY. Philippe Leissner has a long-standing interest in the development of biomarkers and diagnostic tests for infectious diseases and oncology. After completing his PhD in Molecular and Cellular Biology at the University Louis Pasteur, Strasbourg, France, he worked at Transgene, a French biotech specialized in gene therapy research activities. In 2001, he joined bioMérieux, a world leader In Vitro Diagnostics company and held several positions in R&D in the field of Oncology, Infectious Diseases, and Personalized Medicine leading different R&D programs and developing strategic collaborations. He has authored over 35 scientific peer-reviewed publications and patent applications and was involved in scientific evaluation of European Health Research Proposals from 2009 to 2012. He joined BIOASTER in 2016 as head of the Diagnostic Program where he has been developing partnerships with industrials, academics and Non-Governmental Organizations in the field of Infectious Diseases for high and low-income countries.

C12. A novel potent class of nature inspired anti-tuberculosis molecules: chemical vs genetic plasticity

Ruben C. Hartkoorn
Centre for Infection & Immunity of Lille (CIIL), Institut Pasteur de Lille, INSERM U1019-CNRS UMR 9017, University of Lille, CHU Lille

Ruben Hartkoorn is a team leader in the CIIL at the Institut Pasteur of Lille. The team focuses it research on innovating in the field of antibiotic drug discovery and vectorisation, including the discovery and development of anti-tuberculosis drugs. Ruben Hartkoorn obtained a BSc in pharmacology from the University of Sheffield, and a PhD in pharmacology and therapeutics from the University of Liverpool, where he was introduced to tuberculosis research. With the aim of continuing research into anti-infective drugs, he then took the opportunity to join the research team of Prof. S. Cole at the EPFL in Lausanne, Switzerland, as a postdoctoral researcher, investigating the mechanism of action of new anti-tuberculosis molecules. With the aid of an ATIP-Avenir, Dr Hartkoorn was then able to setup his own research team in Lille in 2016.
The seminar will present the discovery and development of a new chemical scaffold with potent activity against tuberculosis. Globally this talk will illustrate the R&D process, divulge the mechanism of action, and provide and insight into the *in vitro* and *in vivo* profiling of these very interesting novel antibiotic molecules.

C13. Boosting pretomanid potency: towards reversion of resistance

Kamel Djaout
Société d'Accélération de Transfert Technologique (SATT) Nord, Institut Pasteur de Lille

Bedaquiline-Pretomanid-Linezolid (BPaL) had recently been recommended by the WHO for the treatment of almost all forms of drug-resistant TB. Although Pretomanid has been introduced into TB treatment in 2019, resistance in clinical isolates is already described. All known mutations conferring resistance in clinical isolates occur in the F420-dependent Ddn-mediated activation pathway of this prodrug. High Throughput Screening identified a family of molecules, SMARt-nim, able to restore sensitivity of ddn-mutated M. tuberculosis strains. SMARt-nim elicit an elusive F420-dependant boost of the pretomanid activity without increasing the bioactivation of this prodrug. In this talk, I will present current efforts to understand the mechanism of action of these compounds.

Biography : Dr. Kamel DJAOUT a suivi sa formation initiale à l'Université Pierre et Marie Curie Paris VI « Sorbonne Université », puis il a effectué sa thèse de doctorat au Laboratoire d'Optique et Biosciences de l'Ecole Polytechnique. Après un stage postdoctoral à l'Institut Pasteur de Lille, dans l'équipe d'Alain Baulard, il travaille actuellement à la SATT Nord (Société d'Accélération de Transfert Technologique), détaché à l'institut Pasteur de Lille. Son travail se concentre principalement dans le développement de stratégies innovantes pour lutter contre la tuberculose et l'émergence de souches résistantes aux traitements actuels.

C14. Intracellular localisation of *Mycobacterium tuberculosis* affects efficacy of the antibiotic pyrazinamide

Pierre SANTUCCI[1,2,3]

[1] Host-Pathogen Interactions in Tuberculosis Laboratory, The Francis Crick Institute, London, United-Kingdom

[2] Present address: Aix-Marseille *Univ, CNRS, LISM, IMM FR3479, 13009 Marseille, France*

[3] Correspondence address: *psantucci@imm.cnrs.fr*

Abstract: To be effective, chemotherapy against tuberculosis (TB) must kill the intracellular population of the pathogen, *Mycobacterium tuberculosis*. However, how host cell microenvironments affect antibiotic accumulation and efficacy remains unclear. By combining, high-content fluorescence microscopy with correlative light, electron, and ion microscopy (CLEIM), we investigate how various microenvironments within human macrophages affect the activity of pyrazinamide (PZA), a key antibiotic against TB. We show that PZA accumulates heterogeneously among individual bacteria in multiple host cell environments. We also demonstrate that correlative SEM-NanoSIMS imaging can be used to identify anti-TB drugs distribution and interaction at a subcellular resolution.

Finally, by developing a dual-live imaging approach with pharmacological and genetic perturbations, we show that Mtb can maintain its intracellular pH independently of the surrounding pH in primary human macrophages. We show that unlike bedaquiline (BDQ), isoniazid (INH) or rifampicin (RIF), the front-line drug pyrazinamide (PZA) displays antibacterial efficacy by acting as protonophore which disrupts intrabacterial pH homeostasis *in cellulo*. By using Mtb mutants with different subcellular localisation, we confirmed that intracellular acidification is a prerequisite for PZA efficacy *in cellulo*. Our results may explain the potent *in vivo* efficacy of PZA, compared to its modest *in vitro* activity, and its critical contribution to TB combination chemotherapy.

Keywords: Tuberculosis, Pyrazinamide, Human Macrophages, Subcellular compartments, Mode of action, Endolysosomes, Correlative imaging, Ion microscopy, High-content fluorescence Microscopy, Intracellular Pharmacokinetics

References: Santucci, P., Greenwood, D. J., Fearns, A., Chen, K., Jiang, H., & Gutierrez, M. G. (2021). Intracellular localisation of Mycobacterium tuberculosis affects efficacy of the antibiotic pyrazinamide. Nature communications, 12(1), 3816. https://doi.org/10.1038/s41467-021-24127-3

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C15. Reconsidering the role of mycolactone in the pathophysiology of *Buruli ulcer*

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Mycolactone, a lipid-like toxin, is the major virulence factor of *Mycobacterium ulcerans*, the etiological agent of Buruli ulcer. Its involvement in lesion development has been widely described in early stages of the disease, through its cytotoxic and immunosuppressive activities, but less is known about later stages. Here, we revisit the role of mycolactone in disease outcome and provide the first demonstration of the pro-inflammatory potential of this toxin. We found that the mycolactone-containing mycobacterial extracellular vesicles produced by *M. ulcerans* induced the production of IL-1β, a potent pro-inflammatory cytokine, in a TLR2-dependent manner, targeting NLRP3/1 inflammasomes. We show our data to be relevant in a physiological context. The *in vivo* injection of these mycolactone-containing vesicles induced a strong local inflammatory response and tissue damage, which were prevented by corticosteroids. Finally, several soluble pro-inflammatory factors, including IL-1β, were detected in infected tissues from mice and Buruli ulcer patients.

Our results revisit Buruli ulcer pathophysiology by providing new insight, thus paving the way for the development of new therapeutic strategies taking the pro-inflammatory potential of mycolactone into account.

Mots clés : *M. ulcerans*, extracellular vesicles, mycolactone, inflammation

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C16. Unraveling the function of tetraspanin CD81 and its cognate adhesins from *Mycobacterium abscessus* during host cell invasion

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Mycobacterium abscessus (*Mab*) is a fast-growing mycobacterium that is antibiotic-resistant and causes severe lung infections, particularly in cystic fibrosis patients. In general, inhalation of aerosols containing the infectious bacilli causes *Mycobacterium* infection. The bacilli's adhesion to the surface of macrophages via specific receptors causes phagocytosis and the underlying immunological events. However, the exact mechanisms of *Mab* uptake by host cells remain poorly understood. During my thesis, I investigated the role of CD81's large extracellular loop (LEL), a novel human receptor belonging to the tetraspanin family, and its associated mycobacterial ligands, in *Mab* internalization by macrophages and pneumocytes. My findings showed that CD81LEL deletion, antibodies blocking surface-exposed CD81, and preincubation of bacilli with recombinant GST-CD81LEL protein or synthetic peptides that mimic a small portion of CD81LEL significantly reduced mycobacterial uptake by host cells. Furthermore, pull-down experiments revealed, among other interactant proteins, a mycobacterial ligand from the bacterial antioxidant system, alkylhydroperoxidase C (AhpC), interacting with the LEL of CD81. Interestingly, soluble AhpC saturating macrophages inhibits bacterial internalization, but when overproduced in mycobacteria, AhpC promotes bacilli internalization. Moreover, pre-incubation of macrophages with anti-CD81LEL antibodies completely inhibited phagocytosis of fluorescent beads coated with AhpC, indicating a direct interaction between the CD81LEL receptor and the mycobacterial adhesin AhpC. More research is being conducted to determine the effect of conditional AhpC knockdown on

mycobacteria host cell invasion. In conclusion, my thesis project describes a novel mechanism of pathogenic *Mab* invasion within the host, which may pave the way for future translational applications to reduce or inhibit mycobacterial infections.

Bio-elements to share:

"I am in my third year of PhD studies in the team of Dr. Laurent Kremer at IRIM, in Montpellier, France. My research work focuses on investigating the mycobacterial invasion inside host cells. I am looking forward to pursue my career in the microbiological research field and improve my scientific knowledge. Finally, I am excited to participate in Mycodays and it is with a big pleasure that I present my PhD work among experts in the Mycobacterial field."

C17. Emergence of tuberculosis bacilli: the long road to becoming a pathogen

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Persistence and efficient transmission in humans are the hallmark of *Mycobacterium tuberculosis*, the most frequent tuberculosis bacilli (TB). These features determine the TB pandemics. Pathogenomic evidence suggests that *M. tuberculosis* evolved from an environmental ancestor similar to *Mycobacterium canettii*, a rare human pathogen, which displays reduced transmission in humans and reduced capacity to persist in the mouse model. However, the genetic and phenotypic adaptations responsible for the higher epidemic capacity of *M. tuberculosis* vs *M. canettii* remains poorly characterised. In the lab, we combine hypothesis-driven and genome wide approaches to identify such adaptations. Here, I will present results of experimental evolution of 8 M. canettii populations in mice to select mutants with enhanced persistence in vivo when compared to parental strains, two distant *M. canettii* isolates. Genome sequencing of 140 *M. canettii* mutants, isolated at various steps during the experimental evolution, revealed parallel and convergent evolution of the 8 *M. canettii* populations. Mutations fixed in these populations identified a limited number of targeted pathways corresponding to cAMP metabolism and import/utilization of nutrients. Complementation analysis revealed that mutations in two loci were responsible for the enhanced persistence phenotypes in one *M. canettii* background. Most of the tested mutants were more resistant than their parental strains to several stress encountered *in vivo*, such as nitric oxide, an important effector of immunity against *M. tuberculosis* infection. This resistance was common

to modern *M. tuberculosis* strains but not to *M. canettii* strains. Therefore, our findings demonstrate phenotypic convergence during the experimental evolution of *M. canettii,* which mirrors natural evolution of *M. tuberculosis*. Furthermore, they indicate that the ability to withstand host-induced stresses, such as nitric oxide, was key for the emergence of persistent *M. tuberculosis*.

C18. Exploring horizontal gene transfer in predominantly clonal tuberculosis-causing mycobacteria

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Introduction: Current models of horizontal gene transfer (HGT) in mycobacteria are based on “distributive conjugal transfer” (DCT), an HGT type described in the fast-growing, saprophytic model organism *Mycobacterium smegmatis*, which creates genome mosaicism in resulting strains and depends on ESX-1 and ESX-4 type VII secretion systems. In contrast, only few data on inter-strain DNA transfer are available for tuberculosis-causing mycobacteria, for which chromosomal DNA transfer between two *Mycobacterium canettii* strains was reported, a process which, however, was not observed for *Mycobacterium tuberculosis* strains. Here, we have studied a wide range of human- and animal-adapted members of the *Mycobacterium tuberculosis* complex (MTBC) using an optimized filter-based mating assay together with three selected strains of *M. canettii* that acted as DNA recipients. The role of ESX-secretion systems in this process was also explored.
Material and methods: Mutant strains were prepared using the recombineering system, or the ts-sacB method. For mating assays, donor and recipient bacteria, each carrying a different antibiotic resistance marker, were mixed and incubated on solid medium for 7 days at 37°C and then plated onto double-antibiotic plates. Genomic DNA was isolated from putative recombinants and subjected to Illumina-based whole-genome sequencing. Identifying the donor-derived DNA segments in the recombinant genomes was carried out by analyzing the density of variants between the respective strains.
Results: Unlike in previous approaches, we obtained a high yield of thousands of recombinants containing transferred chromosomal DNA fragments from various MTBC donor strains, as confirmed by whole-genome sequence analysis of 38 randomly selected clones. While the genome organizations of the obtained recombinants showed mosaicisms of donor DNA fragments randomly integrated into a recipient genome backbone, reminiscent of those described as being the result of ESX-1-mediated DCT in *M. smegmatis*, we observed similar

transfer efficiencies when ESX-1-deficient donor and/or recipient mutants were used, arguing that in tubercle bacilli, HGT is an ESX-1-independent process. On the other hand, our preliminary data points to possible ESX-4 involvement in the process. We also show examples of how this form of HGT can affect the phenotype of the recombinant strains.

Discussion: Our results clearly demonstrate the capacity of a wide range of human- and animal-adapted MTBC strains to transfer chromosomal DNA to selected strains of M. canettii. Most interestingly, we found that inter-strain DNA transfer among tubercle bacilli was not dependent on a functional ESX-1 type VII secretion system. These results argue that HGT in tubercle bacilli is organized in a way different from that of the M. smegmatis model, a finding that is also relevant beyond tubercle bacilli, given that many mycobacteria, like, for example, *Mycobacterium avium* or *Mycobacterium abscessus*, are naturally devoid of an ESX-1 secretion system but show recombinogenic, mosaic-like genomic population structures.

Short oral communications

O1. Selection of new therapeutic targets in relation with Intrabacterial Lipid Inclusions metabolism in *Mycobacterium abscessus*

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Introduction: During infection and granuloma formation, Mycobacterium tuberculosis accumulates lipids in the form of Intrabacterial Lipid Inclusions (ILI). These organelles allow the bacilli to escape the host immune system and serve as a carbon source for the persistence of the bacteria within the infected host. Like *M. tuberculosis*, the fast-growing non-tuberculous opportunistic pathogen *Mycobacterium abscessus* can form these lipid structures during its infectious cycle. ILIs are mainly composed of triglycerides surrounded by a phospholipid monolayer and comprising numerous proteins. In addition to their role in the storage of energy, ILI may also provide biosynthetic precursors for the production of complex cell wall lipids. In this context, the identification of the proteins involved in the formation/degradation of ILIs may lead to the discovery of new therapeutic targets to manipulate the persistence of pathogenic mycobacteria.
Materials and methods: Using a peroxidase (APEX) as a bait with known proteins on the surface of ILI, biotinylation of all other unknown proteins located at the vicinity of the bait are expected to lead to their labelling and their subsequent identification at different time points. Indeed, two genes encoding proteins known to be located on the surface of ILIs during their formation have been fused with the apex2 gene. The newly biotinylated proteins were identified by streptavidin-based enrichment and quantitative mass spectrometry analysis. An *in vitro* model mimicking lipid accumulation and consumption developed previously in our laboratory was employed to

identify proteins involved in formation and degradation of ILI.
Results: Using this proximity labelling technique, at 24 and 48 hours, 228 protein candidates were identified at the surface of the ILI and belonging to different families of enzymes (desaturases, synthases, dehydrogenases, oxidoreductases, acyltransferases, hydratases ….). Among them, 123 proteins have been identified at 24 hrs and 143 at 48 hrs. However, only 38 remain present at both time points during the ILI formation stage.
Discussion and conclusions: Based on the presence of orthologous in *M. tuberculosis*, we have selected 18 potential non-essential targets during this ILI synthesis stage. We will next initiate their biochemical characterization and study their physiological role during ILI formation by deleting or overexpressing the corresponding genes in *M. abscessus*. Finally, we expect to discover major enzymes involved in ILI formation that could be useful for the development of new therapeutic strategies. Using the same technology, we also plan to identify the protein candidates participating in ILI degradation. Overall, the complete study will allow to unravel a whole list of protein partners involved in the ILI metabolism.

O2. Acquisition of host-derived lipids by intracellular mycobacteria and its impact on pathogenesis

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Introduction. Intracellular mycobacteria predominantly rely on host-derived lipids such as sterols, phospholipids and fatty acids (FAs) as carbon and energy sources during infection. This has been well described for *Mycobacterium tuberculosis*, but less is known about other pathogenic but non-tuberculous mycobacteria (NTM). Moreover, the mechanisms underlying the exact role of host-derived lipids in the intracellular life of mycobacteria remain to be explored. In this context, our work focus on *Mycobacterium marinum*, an opportunistic human pathogen known to be responsible for a tuberculosis-like infection in fish and amphibians. On the host side, we propose to use the experimental host mode*l Dictyostellium discoideum*, an amoeba well-established to study the interactions between intracellular pathogens and cell-autonomous defense mechanisms.
Material and methods. The capacity of *M. marinum* to grow in minimal media supplemented with defined lipids as single carbon sources was first evaluated. We used GFP-expressing WT and mutant strains defective for fatty acids (FA) and (chole)sterol import systems, tn:mce1a and Δmce4a respectively, as well as mutants defective for lipid utilization (ΔlipY, Δicl1,

Δfacl6), and measured their growth using a fluorescence plate reader. Their capacity to replicate intracellularly in *D. discoideum* was also assessed similarly, and their intracellular localization was investigated by live microscopy and immunofluorescence, using specific markers of the different intracellular compartments.

Results. First, we demonstrated that *M. marinum* WT was able to grow using cholesterol, palmitate or oleate as main carbon sources in a dose-dependent manner. Genetic knock-out of systems involved in lipid import (Mce1/4) and utilization (LipY/Icl1/Facl6) led to growth alterations depending of the lipid and the dose used. These mutations also led to a significant intracellular growth defect in D. discoideum, while their initial capacity to infect cells was not altered, suggesting a limited ability to replicate. To understand this phenomenon, we initiated microscopy experiments aiming at characterizing the subcellular localization of these mutants affected in lipid acquisition. Mutants affected in sterol import (Mce4) and related-detoxification (Icl1) were found more in the cytosol and less in the mycobacteria-containing vacuole (MCV) at early stages of phagocytosis.

Discussion/Conclusion. Host-derived lipid acquisition in mycobacteria is essential for their intracellular life cycle. Loss of function in the systems dedicated to this acquisition impaired the establishment of an MCV, indicating that the use of lipids, particularly sterols, by *M. marinum* might represent a crucial step early in the manipulation of the phagocytosis process leading to MCV genesis. As perspectives, we aim to decipher the dynamics of host lipid availability during infection with *M. marinum* WT and various mutants, from the earliest stages of phagocytosis and intravacuolar growth, to escape to the cytosol and finally until their egress and dissemination.

O3. Direct and quick detection of Mycobacterium in cystic fibrosis pulmonary samples, its taxonomical typing, and functional characterization

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Introduction. Cystic fibrosis (CF) is a polymorphic disease marked by multiple and difficult-to-treat respiratory exacerbations. Like typical CF infections from *Pseudomonas aeruginosa* and *Staphylococcus aureus*, non-tuberculous mycobacteria (NTM) can chronically colonize the airways. The lung microenvironment, including its microbiota, is emerging as potential

driving causes for the worsening of patient symptoms and the variability in the treatment outcome. The dynamic profiles of the respiratory microbiota, including its microbial composition and activities, and the concomitant host response were established. For this, sputum samples collected longitudinally from CF patients chronically colonized by NTM with various antibiotic treatment regimes and disease outcomes were analyzed by metaproteomics.
Methods. A deep metaproteomic analysis was performed on each sample by shotgun tandem mass spectrometry applying our recently developed multi-round search strategy [1]. The taxonomical and functional composition of the microbiota and the host-related functions were obtained. Label-free quantification of taxa and functions was performed. Proteins were KEGG-annotated using the GhostKoala web service. The abundance values for peptides mapped to a KEGG through protein mapping were summed to attribute an abundance value to each functional term. Peptide-to-taxon mapping was performed to obtain taxon-resolved functional quantification [2]. NTM were identified based on their taxon-specific peptide corroborated with their taxon-spectrum matches.
Results. The distribution of the registered mass spectrometry signals as a function of their origin revealed that metaproteomics allows monitoring the dynamics of the CF respiratory microbiome's taxonomical profiles and the host response. Within the microbiota, NTM could be detected and relatively quantified, and their specific functional roles assessed as a function of time, patient health status, and drug treatments. Preliminary results showed differences in the microbiota profiles for samples following different antibiotic treatments, as further confirmed by a peptide-based functional metaproteomics analysis. These analyses revealed mechanisms that potentially confer a competitive advantage to NTM. Similarly, host molecular functions linked to inflammation and immune response were associated with an increased abundance of opportunistic pathogens.
Conclusion. Our innovative metaproteomics pipeline provides a unique approach to identifying Mycobacterium in complex biological samples [3] and studying the CF respiratory microbiome. Applied to a larger number of samples, this approach holds the potential to identify innovative measures for controlling NTM infection and/ or guide future clinical diagnosis.

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O4. Intrinsic antibacterial activity of beta-cyclodextrins potentiates their effect as drug nanocarriers against tuberculosis

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Multi-drug resistant tuberculosis (TB) is a major public health problem concerning about half a million cases each year. Patients hardly adhere to the current strict treatment consisting of more than 10,000 tablets over a 2-year period. There is a clear need for efficient and better formulated medications. We have previously shown that cross-linked poly-β-cyclodextrins (pβCD) are efficient vehicles for pulmonary delivery of powerful combinations of anti-TB drugs. Here, we report that in addition to be efficient drug carriers, pβCD nanoparticles are endowed with intrinsic antibacterial properties.
In mouse infected lung, we observed that pβCD nanoparticles are mainly engulfed by alveolar macrophages and modulate host cells properties impairing *M. tuberculosis* (Mtb) establishment. pβCD hamper colonization of macrophages by Mtb by interfering with lipid rafts, without inducing toxicity. Moreover, pβCD provoke macrophage apoptosis leading to depletion of infected cells, thus creating a lung micro-environment detrimental to Mtb persistence. Taken together, our results suggest that pβCD nanoparticles loaded or not with antibiotics have an antibacterial action by their own and could be used as carrier in drug regimen formulations effective against TB. This activity could fit into the emerging and promising concept of anti-TB approaches by host-directed therapy, which aims to empower host immune properties for the elimination of mycobacteria and/or for the reduction of tissue damage induced by the infection.

O5. Les neutrophiles régulateurs s'accumulent chez la souris formant des granulomes encapsulés.

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La tuberculose (TB) due à l'infection par *Mycobacterium tuberculosis* (Mtb) chez l'homme ou *Mycobacterium bovis* chez le bovin, se caractérise par la formation de granulomes dans le poumon. L'afflux de cellules inflammatoires se structure lors de la phase adaptative de la réponse immunitaire pour faire barrage aux bacilles. Le granulome mature est le lieu d'un équilibre

entre multiplication contrainte des bacilles et inflammation qui peut entrainer la destruction des poumons lors de la TB active, forme contagieuse de la maladie.

Les neutrophiles jouent un rôle clé dans toutes les étapes de la vie du granulome : lors de la TB active, ce sont les cellules les plus représentées dans les lavages bronchoalvéolaires chez l'homme. Ils s'accumulent également dans les lignées de souris sensibles à la TB. Comme les autres types de cellules du système immunitaire inné, les neutrophiles sont présents en plusieurs sous-types. Parmi eux, nous avons décrit récemment une population de neutrophiles régulateurs, proches phénotypiquement des neutrophiles classiques, très inflammatoires, mais différant par leur capacité fonctionnelle à supprimer les lymphocytes T. A ce jour, le rôle des neutrophiles inflammatoires versus régulateurs dans la TB, et notamment dans la maturation et l'évolution du granulome, n'est pas connu. Pour l'étudier nous utilisons la souris C3HeB/FeJ qui, contraitrement à la souris BALB/c ou C57/Bl6 est capable de produire un granulome encapsulé, caractéristique de la TB humaine ou bovine.

Nous avons analysé, chez des souris C3HeB/FeJ infectées par voie intranasale avec Mtb HN878, la charge bactérienne, le recrutement cellulaire dans le poumon et plus précisément le recrutement des neutrophiles inflammatoires (CD11b+/Ly6G+/MHCII-/PDL-1^{lo}) ou régulateurs (CD11b+/Ly6G+/MHCII+/PDL-1^{hi}) ainsi que l'histologie des lésions développées.
Suite à l'infection par Mtb HN878, les animaux se répartissent en deux groupes, selon l'atteinte des points limite (forte perte de poids et signes de morbidité). Ainsi 80% des animaux ont une mortalité précoce (< à 35 jours d'infection) alors que les 20% restants supportent bien l'infection sans déclarer de points limites. Les animaux ayant une survie limitée présentent une forte charge bactérienne dans les poumons. Le recrutement de leucocytes et notamment de neutrophiles dans le poumon est très important. Les neutrophiles majoritaires (90%) ont un phénotype inflammatoire (CD11b+/Ly6G+/MHCII-). Ces animaux présentent également des lésions pulmonaires trés étendues et peu organisées.

Au contraire, les animaux ne succombant pas à l'infection, (20%) ne présentent pas de signes de morbidité ou de perte de poids après 7 semaines d'infection. Ces animaux, ont une charge bactérienne plus faible que ceux de l'autre groupe. Cette plus faible charge est corrélée à un recrutement de leucocytes et de neutrophiles dans le poumon moins important. Dans ce deuxième groupe supportant l'infection les lésions encapsulées dominent et corrèlent avec une charge bactérienne contrôlée. De façon intéressante, les

neutrophiles régulateurs (CD11b+/Ly6G+/MHCII+) recrutés dans le poumon représentent 50% des neutrophiles totaux. Ils expriment fortement PDL-1. De plus le taux de neutrophiles régulateurs corrèle négativement avec la charge bactérienne dans le poumon.

Ainsi, dans le modèle C3HeB/FeJ qui représente bien la physiopathologie de la TB humaine ou bovine avec formation de granulomes matures et encapsulés, nos résultats montrent un afflux important de neutrophiles régulateurs exprimant PDL1. Ils suggèrent que ces neutrophiles régulateurs sont une signature de la formation d'un granulome efficace avec contrôle de l'infection.

O6. A 3R infection model to screen, characterize and decipher the mode of action of novel anti-mycobacterial compounds from a large plant extract library

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Tuberculosis is a high contributor to the global burden of communicable diseases. Low compliance with its treatment, comorbidity with HIV and in particular emerging resistance to antibiotics necessitate innovations in therapeutic intervention. For example, traditional antibiotic therapies might be bolstered by adjuvant therapies with anti-virulence and/or host-targeted approaches.
We exploit an established 3R host-pathogen infection model system, the amoeba and professional phagocyte *Dictyostelium discoideum* (Dd) and *Mycobacterium marinum* (Mm), a genetically close relative to *Mycobacterium tuberculosis*, to screen a large library of plant natural extracts for anti-infective activity.
Infection of Dd with a bioluminescent strain of Mm enables us to capture luminescence as a proxy for intracellular growth of Mm and thus evaluate plant extracts based on their phenotype in this system. To filter out candidates with pure antibiotic activity we counterscreen on bioluminescent Mm in a liquid culture.
We partnered with the French pharmaceutics and cosmetics company Pierre Fabre S.A., which allows us to screen the largest private botanical collection in the world, containing more than 15000 classified samples. This collection is certified with the Nagoya protocol, ensuring fair and equitable sharing of benefits arising out of the utilization of genetic resources.

A cornerstone of this consortium project is a database which is shared between different laboratories from the University of Geneva and ETH Zürich and hosted by the Swiss Institute of Bioinformatics. The Wolfender laboratory, who are experts in phytochemists, selected 1600 plant extracts, profiled them by high resolution MS and mapped them in a molecular network. This makes extracts comparable by chemical and compositional similarity and thus renders the chemical space of the extract library searchable. Different groups investigating biological readouts associated with lipid metabolism, autophagy and anti-infectivity populate this molecular network of chemical structures with biological data. This shared database enables transparent and data-driven decisions in the consortium.
In a proof of concept, a synthetic chemical structure that was previously found to have an anti-infective activity was used as a "seed" to *in silico* browse the molecular network and subsequently to select extracts containing similar structures. This led to the identification of an anti-infective structure, onychin, corroborating previous literature reports about its antibacterial activity.
In addition, information collected on different biological readouts, from collaborating laboratories can be efficiently used to propagate through the screening cascade from the library of natural plant extracts to plant fractions to isolated compounds.
Eventually, we will study and characterize the effect of hit compounds obtained from our phenotypical screen. We will validate target pathways of hit compounds by using a range of cell microbiology tools developed and available in the lab.
Next generation sequencing techniques will be used to characterize the transcriptomic (RNA-seq) and genomic signatures (Tn-seq) during infection. We make use of dual RNA-seq to disentangle host and pathogen signatures and aim at developing an integrative analysis that allows us to study interactions of host and pathogen at a transcriptional level.

O7. Pharmacological activation *of* NRF2 has protective effects during *Mycobacterium abscessus* infection by promoting host defenses and reducing inflammatory damage

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Introduction: Pulmonary disease is the leading cause of morbidity and mortality in cystic fibrosis (CF), a genetic disease caused by biallelic mutations in the CF transmembrane conductance regulator (CFTR), and is characterised by a repetitive circle of persistent infections and chronic inflammatory tissue damage. Among the deleterious bacteria found in CF airway, nontuberculous mycobacteria have emerged as important pathogens of major concern in CF centres worldwide. In particular, infections with the multidrug-resistant *Mycobacterium abscessus* (Mabs) are correlated with accelerated inflammatory lung damage, and are are often refractory to antibiotic therapy. Therefore, we examined whether Curcumin, an activator of the redox-sensitive transcription factor NRF2 that (in other conditions) reduces inflammation while promoting host defence, might provide a beneficial effect against Mabs infection in the context of CF.
Methods: Herein, using CFTR-depleted zebrafish larvae as an innovative vertebrate model of inflammation and infection, combined with human approaches, we sought to determine the effects of curcumin on host immune responses to Mabs infections in CF condition.
Results: Firstly, we show that curcumin exerts anti-inflammatory effects in neutrophilic inflammation induced by Mabs infection and/or injury in CFTR-depleted zebrafish. As a consequence, the reduced number of neutrophils at infected sites prevents tissue damage and abnormal tissue repair. Curcumin reduces overactive neutrophil trafficking and improves tissue repair by restoring NRF2 activity in CF animals. Our findings demonstrate that sufficient modulation of NRF2 function alleviate Mabs-induced inflammatory damage in CF.
Next, while curcumin has no direct antibacterial activity against Mabs, we found that treatment efficiently enhances intracellular bacterial killing of Mabs in THP1 macrophages. Moreover, our results indicated that treatment with curcumin improves the control of Mabs infections in CF zebrafish models, correlating with reduced larval mortality and lower bacterial loads. We show that activation of NRF2-dependent ROS production in professional phagocytes represents a critical host defence against Mabs, and demonstrate that NFR2 is instrumental to efficiently restrict the intracellular growth of Mabs, thereby preventing extracellular mycobacterial spread and more severe infection in CF. These results highlight the crucial role of NRF2 in the

immune control of Mabs by mounting effective oxidative responses.
Discussion-Conclusion: Our findings bring important new understanding of the immune-targeted action of curcumin and show that therapeutic strategies to normalise NRF2 activity might simultaneously enhance bacterial killing and promote inflammation resolution and tissue repair thus prevent infectious and inflammatory lung damage in CF.

O8. *Mycobacterium abscessus* resists the innate cytotoxic response by surviving granzyme-mediated cell lysis of infected phagocytes

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Mycobacterium abscessus, an opportunistic human pathogen, is the most pathogenic species among fast-growing mycobacteria, which are predominantly saprophytic. This virulence is due to its ability to survive within the host, causing severe infections that are difficult to eradicate. How *M. abscessus* colonizes and infects the host and what could explain its pathogenicity remain poorly understood. Using primary murine cells and Drosophila, we demonstrate that intracellular *M. abscessus* is unaffected by the granzyme-mediated cytolysis of infected phagocytic cells by cytotoxic cells. Indeed, we show the existence of such a population in Drosophila, the thanacytes, which induce infected phagocytes apoptosis, depleting the main cell population capable of controlling the infection. *M. abscessus* systemic multiplication is thus favored since the observed antimicrobial peptides production is not required for infection control. These results demonstrate the propensity of *M. abscessus* to resist the host's cytotoxic innate response, reminiscent of that observed with strict pathogenic slow-growing mycobacteria such as *M. tuberculosis.*

O9. Distinct Cellular Signatures Identified by Spectral Flow Cytometry upon Tuberculosis Diagnosis and at Treatment Completion

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Introduction: Tuberculosis (TB) is one of the leading infectious causes of death worldwide. Optimized treatment monitoring methods are needed. The objective of this study was to characterize peripheral immune profiles through spectral cytometry over the course of treatment in patients diagnosed with active TB.
Materials and Methods: This prospective cohort study was based in Tripoli (Lebanon) and Tbilisi (Georgia). Adult, non-immunocompromised patients with culture confirmed drug susceptible TB were followed at treatment initiation (T0), after two months of treatment (T1), and at treatment completion (T2). Whole blood was stimulated with QuantiFERON-TB Gold Plus NIL, TB2 antigenic peptide pool or recombinant methylated *Mycobacterium tuberculosis* (*Mtb*) heparin-binding hemagglutinin produced in *Mycobacterium smegmatis* (rmsHBHA). Cryopreserved white blood cells were analyzed a 30-marker spectral flow cytometry panel. The discriminatory ability of markers evaluated by area under the receiver-operating characteristic curve (ROC AUC).
Results: T-cell phenotypical changes from baseline (T0) to the end of treatment (T2) are detectable in *Mtb*-stimulated samples including a significant increase in terminally differentiated (TEMRA) $CD8^+$ T cell populations and a decrease in effector memory $CD4^+$ T cell populations. Moreover, two subsets of NK cells were identified NK1 and NK2. Following a phenotypic analysis,

we showed that there is a significant increase in NK1 cells between T0 and T2 which had a phenotype consistent with an activated population whereas a significant decrease in NK2 cells were detected between T1 and T2 and which had an inactivated profile. Besides, expression of PD-1 on TEMRA $CD8^+$ T was increased over time and showed a higher accuracy (AUC = 0.9, p = 0.004) to discriminate untreated from successfully treated patients.
Conclusion: Overall, our findings offer crucial insights into the underlying immune factors associated with TB cure, and identify phenotypic signature that may influence treatment outcomes. In sum, these phenotypic changes of *Mtb*-specific T cells and NK cells may represent potential surrogate markers associated with TB control. Among them, abundance of TEMRA $CD8^+$ T cells and their expression of PD-1 are the most promising candidates.

Keywords: *Mycobacterium tuberculosis,* tuberculosis, spectral flow cytometry, treatment monitoring; immunophenotyping; heparin-binding hemagglutinin; unsupervised data analysis

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O10. Exploring new recombinant BCG Vaccines and different Vaccination Routes for better Tuberculosis Protection.

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Introduction: The only currently licensed anti-tuberculosis (TB) vaccine, *Mycobacterium bovis* BCG, provides limited protection against pulmonary TB in adolescents and adults. A feature of this attenuated live vaccine strain is the partial deletion of the genomic locus encoding the ESX-1 type VII secretion system, which in the biosafety level 3 (BSL3) pathogen *Mycobacterium tuberculosis* governs phagosomal rupture and cytosolic pattern recognition, key intracellular phenotypes linked to increased immune signaling.
Methods and Previous Findings: To obtain an improved recombinant BCG Pasteur vaccine strain with increased immune signaling but still low virulence, we have previously integrated the extended esx-1 genomic region of *Mycobacterium marinum,* a BSL2 organism, into a BCG strain of the BCG Pasteur subtype. This recombinant strain named rBCG::ESX-1^{Mmar}, is heterologously expressing ESX-1 functions of *M. marinum* and thereby modulates the host innate immune response via phagosomal rupture-associated induction of type I interferon (IFN) responses and enhanced inflammasome activity. These features result in vaccine-induced higher IL-1β 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, Sayes et al. Cell Reports, 2017*).

Results: In our most recent studies, we have focused on different routes of vaccination, by using parental BCG Pasteur and rBCG Pasteur::ESX-1Mmar. We found that mice vaccinated via the aerosol route with BCG Pasteur or rBCG Pasteur::ESX-1Mmar yielded higher frequencies of IFN-γ-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 Pasteur::ESX-1Mmar via the aerosol route leads to improved TB protection and lower lung pathology compared to subcutaneous vaccination.

Conclusion: The attenuated rBCG::ESX-1Mmar vaccine displayed a superior T-cell immunity and TB protection when mice were vaccinated via aerosol or subcutaneous route compared to parental BCG strain and thereby represents an interesting candidate for defining new promising strategies of vaccination against TB.

O11. Unpacking the molecular determinants of Mycobacterium abscessus infections

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Introduction. *M. abscessus* (*Mab*) resistance mechanisms have been partially characterised, yet a large knowledge gap remains in understanding severe, persistent infections, particularly in the manifestation of phenotypic and genetic changes. The morphological transition from smooth (S) to rough (R), controlled in large part by the glycopeptidolipids (GPL) gene locus, is linked to reduced biosynthesis and/or transport of surface-associated GPLs, intramacrophage trafficking and virulence. In the low-GPL R morphotype, the bacteria forms cords and escape the host's innate immune response, leading to infection persistence. By pairing lipidomics, infection modelling, drug susceptibility testing (DST) and whole genome sequencing (WGS), the link between phenotypic behaviour and genetic determinants will be further elucidated.

Materials and Methods. Clinical *Mab* strains (n=47) were isolated from 20 long-term patients at the St. Paul's Cystic Fibrosis Clinic in Vancouver, Canada. Multiple analytic methods will be used including WGS, DST, lipid analysis, and infection modelling in macrophages. Using microscopy, strains were separated by morphotype. Separated strains underwent lipid extraction and TLC analysis to confirm morphotypes by GPL composition. A subset of serially-collected strains (n=35) belonging to four patients were selected to evaluate bacterial burden (CFU) and cording. Strains were transformed and used for infection in THP-1 macrophages. MICs for amikacin, cefoxitin, clarithromycin, clofazimine, doxycycline, linezolid, meropenem, moxifloxacin and tigecycline were determined. Lastly, strains will undergo WGS to find putative explanatory mutations and indels linking the phenotypes to genotype.
Results. The 47 strains were separated into S and R, yielding a 70 pure strains (38 S, 32 R), with half of the original strains presenting both. Morphotypes were classified as S and R, however many colony features were observed that deviate from the discrete morphologies described in the literature. The GPL profiles matched the assigned morphotypes determined previously, with the exception of four strains, which presented as R yet expressed GPLs. A first round of THP-1 infection with the subset of strains has also been completed with CFU results available after three days of growth. MIC results (µg/mL) ranged as follows: amikacin (16-64); cefoxitin (64-128); clarithromycin (4-64); clofazimine (>128); doxycycline (32- >512); linezolid (16-256); meropenem (32-64); moxifloxacin (4- >16); tigecycline (1-4), with some notable outliers (amikacin 128; meropenem 128) including a 1-4x-fold change in six of the nine antibiotics from a mixed S and R strain to a R-only strain isolated from the same patient.
Discussion-Conclusion. Microscopy is a frequently-used diagnostic method, however, the S and R morphotypes are ill-defined and subject to observer interpretation, which may impact treatment regimens. The presence or absence of GPLs has been a metric for characterising S versus R, however, these results indicate clinical strains present greater variability with what appear to be 'transition' morphologies. Preliminary results link the transition from a mixed S and R population to an exclusively R population with changes in drug tolerance, mostly showing higher resistance. This could indicate that virulence, persistence and resistance may be a consequence of mechanisms beyond the S-to-R transition, to be further evaluated by WGS.

O12. Les protéines EsxU et EsxT sécrétées par le système de sécrétion ESX-4 modulent la fitness de M*ycobacterium abscessus*

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Introduction. Les mycobactéries possèdent des systèmes de sécrétion de type VII (SST7), dénommés ESX. *Mycobacterium abscessus* présente seulement deux loci esx (*esx-3* et *4*) alors que *Mycobacterium tuberculosis* en possède 5 (*esx-1* à *5*). Récemment, notre laboratoire a démontré que le système ESX-4 de *M. abscessus* est un acteur essentiel à sa survie intracellulaire, à l'instar de l'ESX-1 de *M. tuberculosis*. L'absence d'un des gènes codant pour une protéine structurale de l'ESX-4 entraîne une réduction importante de la virulence de *M. abscessus* en l'empêchant de résister à la lyse phagosomale. L'analyse comparative du sécrétome de la souche sauvage et des mutants du système de sécrétion ESX-4 a permis d'identifier plusieurs protéines, dont EsxU et EsxT, codées par deux gènes appartenant au locus *esx-4* de M. abscessus. L'objectif de ce projet est d'étudier le rôle de ces protéines dans la virulence de *M. abscessus.*
Matériels et méthodes. Nous avons purifié les protéines EsxU et EsxT et obtenu un mutant Δ*esxUT*, ce qui nous a permis d'étudier le rôle de ces protéines *in vitro* et *in vivo.*
Résultats. Nous avons d'abord démontré la localisation de ces protéines chez *M. abscessus*. EsxU et EsxT sont associées avec la membrane bactérienne et secrétées dans le milieu extérieur. De plus, elles forment un complexe, ci-après nommé EsxUT, caractéristique des protéines sécrétées par les SST7 des mycobactéries et sont capables *in vitro* d'interagir avec des membranes artificielles pour former des structures semblables à des pores.

Dans les macrophages, leur absence n'influence pas le comportement intracellulaire de la souche mutée Δ*esxUT*, mais empêche la rupture de la membrane phagosomale, maintenant *M. abscessus* Δ*esxUT* dans un phagosome non acidifié. Nous identifions un phénotype hypervirulent associé à Δ*esxUT*, caractérisé par une augmentation de la mortalité et de la croissance bactérienne chez la souris et le poisson zèbre.
Discussion-Conclusion. L'interaction d'EsxUT avec les membranes artificielles et le phénotype du mutant Δ*esxUT* dans les macrophages suggèrent qu'EsxUT sont impliquées dans la rupture de la membrane du phagosome en formant des pores dans les membranes eucaryotes. De plus, EsxUT ne sont pas impliquées dans le blocage de l'acidification du phagosome qui doit faire intervenir d'autres protéines sécrétées par l'ESX-4. L'ensemble de nos résultats *in vivo* suggèrent que l'absence d'EsxUT améliore la survie et la persistance de *M. abscessus* chez les animaux. Ce qui soulève de nombreuses questions du point de vue évolutif quant au rôle de ces protéines chez les autres mycobactéries pour lesquelles la sécrétion d'EsxUT n'a jamais été démontré.

O13. *Mycobacterium tuberculosis* genetic features associated with pulmonary tuberculosis severity

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Background: *Mycobacterium tuberculosis* (Mtb) infections result in a wide spectrum of clinical outcomes, with an array of severity symptoms. Up to now, there are no Mtb proven genetic determinants of these clinical presentations.
Methods: 234 pulmonary tuberculosis (TB) patients diagnosed at the Lyon University Hospital were stratified into mild grade and moderate/severe grade groups according to the Bandim TBscore. Patients' Mtb clinical isolates were explored by whole genome sequencing (WGS) to identify Single Nucleotide Polymorphisms (SNPs). SNPs were classified according to the functional categories of the corresponding loci and heterologous SNP calling was used to detect diversity within Mtb clinical isolates (micro-diversity). Furthermore, we searched for DNA motifs associated with high TB severity scores using genome-wide association study (GWAS) optimised for bacteria. Finally, we performed a structural equation modelling analysis to relate TB severity to various explanatory variables selected from both TB patients' clinical data and aforementioned Mtb genetic features.
Results: Proportions of non-synonymous SNPs were higher in gene categories involved in "cell wall and cell processes" and in "virulence, detoxification, adaptation" for Mtb isolates from the mild grade group and in the "regulatory proteins" gene category for moderate/severe grade group. Besides, GWAS identified an SNP in the promoter of the *espR* gene, a key-regulator of Mtb virulence, associated with the moderate/severe grade group. Model comparisons indicated that TB severity was related to (1) the detection of Mtb micro-diversity within clinical isolates and (2) the GWAS identified SNP.

Conclusions: Taken together, these results provide a new insight to better understand TB pathophysiology. If further confirmed, the detection of Mtb micro-diversity and the GWAS detected espR-related mutation could provide new biomarkers of pulmonary TB severity.

O14. Le chaos croissant de la génomique des populations des bacilles de la tuberculose à l'ère des «big data» : il faut trier le bon grain de l'ivraie !

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Introduction. La bactérie pathogène *Mycobacterium tuberculosis* complex (MTBC) a une structure de population constituée de 9 lignées phylogéographiques. La diversité génomique spécifique d'isolats cliniques au sein de ces 9 lignées est un facteur important de pathogénèse agissant sur la virulence, la transmissibilité, la réponse à l'hôte et l'émergence de la résistance aux antibiotiques. C'est pourquoi il est important de développer des outils de surveillance et de compréhension de l'évolution des génomes.
Matériel et Méthodes. Nous présentons une nouvelle plateforme informatique de biologie computationnelle portant sur *Mycobacterium tuberculosis* complex (MTBC), constituée à partir d'une collection de convenance issue de SRAs (*Sequence Read Archives*) publics ou privés, désignée «*TB-Annnotator*», présentant la description de la structure de la population des MTBC portant sur 16000 génomes représentatifs de 63 pays. Cette plateforme analyse les variants nucléotidiques par rapport à la souche de référence H37Rv (NC_000962.3), la présence ou l'absence des gènes, de régions de différence, et détecte les sites d'insertion des éléments génétiques mobiles. L'objectif de *TB-Annotator* est à la fois de détecter des liens épidémiologiques récents mais aussi de reconstruire des liens spatio-temporels phylogéographiques plus distants entre clones historiques et d'effectuer des études sur le pangénome.
Résultats. Après avoir confronté les étiquetages taxonomiques précédemment développés au cours d'études de référence (Coll *et al*. 2014, Napier *et al.* 2020, Freschi *et al.* 2021, Thawornwattana *et al.* 2021, Coscolla *et al.* 2021) nous construisons un arbre phylogénétique par RaXML et caractérisons un total d'environ 200 lignées ou sous-lignées phylogéographiques, en discutant aussi de l'informativité de certaines SNPs que nous remettons en question ; nous présentons de nouveaux clones phylogéographiques, spécifiques par exemple au sein de la lignée 4.5 ou encore des lignées 5 et 6, et mettant en avant les nouvelles possiblités de recherche qu'offre ce type de plateforme.
Discussion-Conclusion. En nous basant sur des exemples concrets récents, nous montrons que cette plateforme permettra à la fois (1) l'amélioration du suivi épidémiologique des souches circulantes, (2) d'appréhender avec plus de puissance et de finesse, la reconstitution de l'histoire globale et des histoires locales de la tuberculose (3) d'effectuer de nouvelles études sur le pangénome.

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