MISTRAL study, an affiliated EuroSIDA study

PROTOCOL v1.0, 7JUL2021 SUMMARY

Full title: MISTRAL study – Gut microbiome correlates of serious AIDS and non-AIDS events
Short title: MISTRAL study

Sponsor: Centre of Excellence for Health, Immunity and Infections (CHIP)

Study coordination: CHIP, Rigshospitalet-University of Copenhagen, Blegdamsvej 9, DK-2100 Copenhagen Ø, Denmark

Background and Rationale: The HIV/AIDS pandemic continues to be one of the major health challenges ever faced by mankind. Far from being resolved, HIV is soaring in Eastern Europe (60% increase in new infections and 27% increase in deaths since 2010) and other regions of the world despite increasing access to antiretroviral treatment[i]. There is emerging evidence that the human microbiome impacts some of the most important clinical aspects of HIV-1 infection, including immune disorders, chronic inflammation and accelerated aging[ii]. Work in other viral diseases and cancer immunotherapy suggest a critical role of the human microbiome also in the outcome of immune therapeutic interventions in HIV-1 infection[iii].

MISTRAL (The Microbiome-based stratification of individuals at risk of HIV-1 acquisition, chronic clinical complications, antimicrobial drug resistance, and unresponsiveness to therapeutic HIV-1 vaccination) is a European Union (EU) Horizon 2020 funded project that aims to explore the impact of the human microbiome on clinical outcomes in people living with HIV (PLWH). The MISTRAL project brings together a team of world-class HIV and microbiome researchers with ideal complementary knowledge and expertise. This team will work to discover and validate novel gut microbiome biomarkers to inform rationally designed, mechanistically-driven interventions on the gut microbiome to mitigate HIV-1 acquisition, systemic inflammation, chronic clinical complications, antimicrobial drug resistance, and boost the efficacy of HIV cure immunotherapies.

Within the MISTRAL project, the MISTRAL study will explore the impact of the microbiome on clinical outcomes in a large clinical cohort of PLWH through the collection and analysis of stool, plasma and blood samples. The MISTRAL study will utilize the long-established network, clinical sites and expertise of the EuroSIDA study, a prospective, observational cohort study of PLWH that has been collecting observational data since 1994.

The stool samples collected in the MISTRAL study will configure a microbiome repository module. The microbiome data/test results will be combined with those of other biomarkers obtainable from testing plasma and blood samples, which could result in increased efficiency and the possibility to use other biomarkers to test the relevance of specific hypothesized pathways. Ultimately, the expected outcome of the MISTRAL study is to have a better basic understanding of the pathophysiological factors of the interplay of HIV infection and the human microbiome.

Study objectives: The primary objective of the MISTRAL study is to strengthen and evaluate the understanding of the association between the gut microbiome composition and the risk of developing serious AIDS and non-AIDS events, including cardiovascular events. The second

objective is to evaluate the associations between the gut microbiome composition and function and pathologic increases in inflammation and coagulation mediators in PLWH. The third objective is to develop a risk score which makes use of information in the gut microbiome as well as other risk factors separately for the different endpoints.

Inclusion criteria: The MISTRAL study plans to enroll 1000 individuals. Participating sites will recruit HIV-1 positive persons age 50 years or older who are prospectively followed in a EuroSIDA site.

Exclusion criteria:

- Creatine Clearance <50
- Child-Pugh C end-stage liver disease
- Any ongoing severe life-threatening disease
- Experiencing any of the following events prior to inclusion: myocardial infarction, stroke, an invasive cardiovascular procedure, AIDS (diagnosed within 5 years of MISTRAL enrolment), and non-AIDS cancers (not including non-melanoma skin cancers)

Study Design: As the MISTRAL study is a non-interventional observational study, data is only collected during participant visits and treatment will not be influenced by the participation. Participants will provide a stool, plasma and whole blood sample at baseline and an additional follow-up sample up to two years after baseline. Participant information about their diet, time and frequency of defecation, medication history and usage and lifestyle data will also be collected at baseline and up to two years after baseline. Clinical data including demographic, laboratory, medical treatment and clinical event history will be collected at enrolment and during annual follow-up. The biological samples will be used to conduct pre-specified analyses into microbiome and immunological related factors, as well as form the basis of an ongoing research biobank for future exploration of the impact on microbiome on clinical outcomes in PLWH. For a subset of the cohort, analyses of host-genetic material will also be performed.

Data Collection: MISTRAL study enrolment, participant information and follow-up data are captured electronically in electronic case reports forms (e-CRFs) using the free secure online browser-based Research Electronic Data Capture tool (REDCap) or RESPOND Electronic Data Submission Tool (REST). Data are collected initially into EuroSIDA servers based at CHIP. At CHIP, the clinical data will undergo quality assurance procedures before being transferred to the MISTRAL data repository for storage. The MISTRAL data repository is contained within Amazon Web Services servers located within the EU and complies with EU data protection. All data is pseudonymised. A de-coding list is held in a safe location by the individual site.

Sample Repository/Research Biobank: Stool, plasma and blood samples will initially be shipped to the coordinating centre at CHIP, Rigshospitalet, Denmark, and stored in secure holding facilities at - 80° Celsius. These samples will then be transferred to the MISTRAL biobank at IrsiCaixa, Hospital Germans Trias I Pujol, Barcelona Spain. Analyses will be performed by members of the MISTRAL Consortium. Samples will not be sent outside the European Union without data transfer agreements. A proportion of the samples collected will be stored for future, yet unspecified research. Samples will be destroyed the latest on 31 December 2045 in accordance with current legal and ethical requirements.

Safety: Participation in the MISTRAL study does not include any risk for participants. The study does not test or investigate any treatments and participation in this study does not interfere with the treatment/care participants may receive at the clinic. There are no direct benefits to the participants. However, the benefit of conducting observational research that includes analysis of biological samples will advance scientific understanding of HIV infection and other co-infections and co-morbidities as well as their complications. This knowledge guides international and European treatment recommendations for the benefit of PLWH and national health systems.

Economy: Sites participating in the MISTRAL study will be reimbursed for enrolment and follow up data collection for each participant, event forms and sending samples.

The MISTRAL study has received funding from the European Commission Directorate-General for Research and Innovation Horizon 2020 Grant Agreement number 847943 (9,994,383.75€).

Participants in the study receive no financial compensation.

Publications: The findings from this study, positive, negative or inconclusive, are intended to be published in peer-reviewed journals and/or presented at medical conferences. Research proposals will be submitted and reviewed under the oversight of the MISTRAL Consortium and EuroSIDA Steering Committee. Final approval of projects will be made by the MISTRAL Consortium and EuroSIDA Steering Committee. Publication will be in accordance with international recognized scientific and ethical standards concerning publications and authorships. Copyrights concerning publication of the sub-study remain with the authors of the publication, regardless of any other provisions regarding intellectual property rights.

Ethical considerations: The Knowledge Center for Data Reviews within the Capital Region of Copenhagen Denmark, RegionH, is data controller for the MISTRAL study. The Knowledge Center for Data Reviews follows General Data protection Regulation (GDPR) in Europe. It is the responsibility of each participating site to ensure that all necessary documents and approvals are obtained according to local and/or national regulations in countries participating in the MISTRAL study as well as other national regulatory approvals as applicable, including the appropriate Competent Authority and/or Data Surveillance Authorities, unless no such requirement applies to observational clinical studies according to national regulations.

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[i] "UNAIDS announces nearly 21 million people living with HIV now on treatment." UNAIDS, 20 Nov. 2017, https://www.unaids.org/en/resources/presscentre/pressreleaseandstatementarchive/2017/november/20171121_righttohealth_report. Press release.

[ii] Li, S, Armstrong, A, et al. Complexities of gut microbiome dysbiosis in the context of HIV infection and antiretroviral therapy, 2016. Clin Pharmacol Ther. 2016; 99(6): 600-611.

[iii] Herrera, S, Martinez-Sanz, J, Serrano-Villar, S. HIV, Cancer, and the Microbiota: Common Pathways Influencing Different Diseases, 2019. Front Immunol. 2019; 10:1466.