# MISTRAL Newsletter

*October 2024*

Dear MISTRAL colleagues,

Since our last newsletter in April 2024, we have reached our enrolment goal of 1,000 participants. Thank you all for your hard work and the big push towards the end to reach this huge achievement!

In this fifth MISTRAL study newsletter, we have gathered updates on the study, insights from the data collected during visit 1, and some reminders to your sites. If you have any additional feedback or good ideas to share, feel free to contact us so we can share it with the other sites.

\MISTRAL team at CHIP

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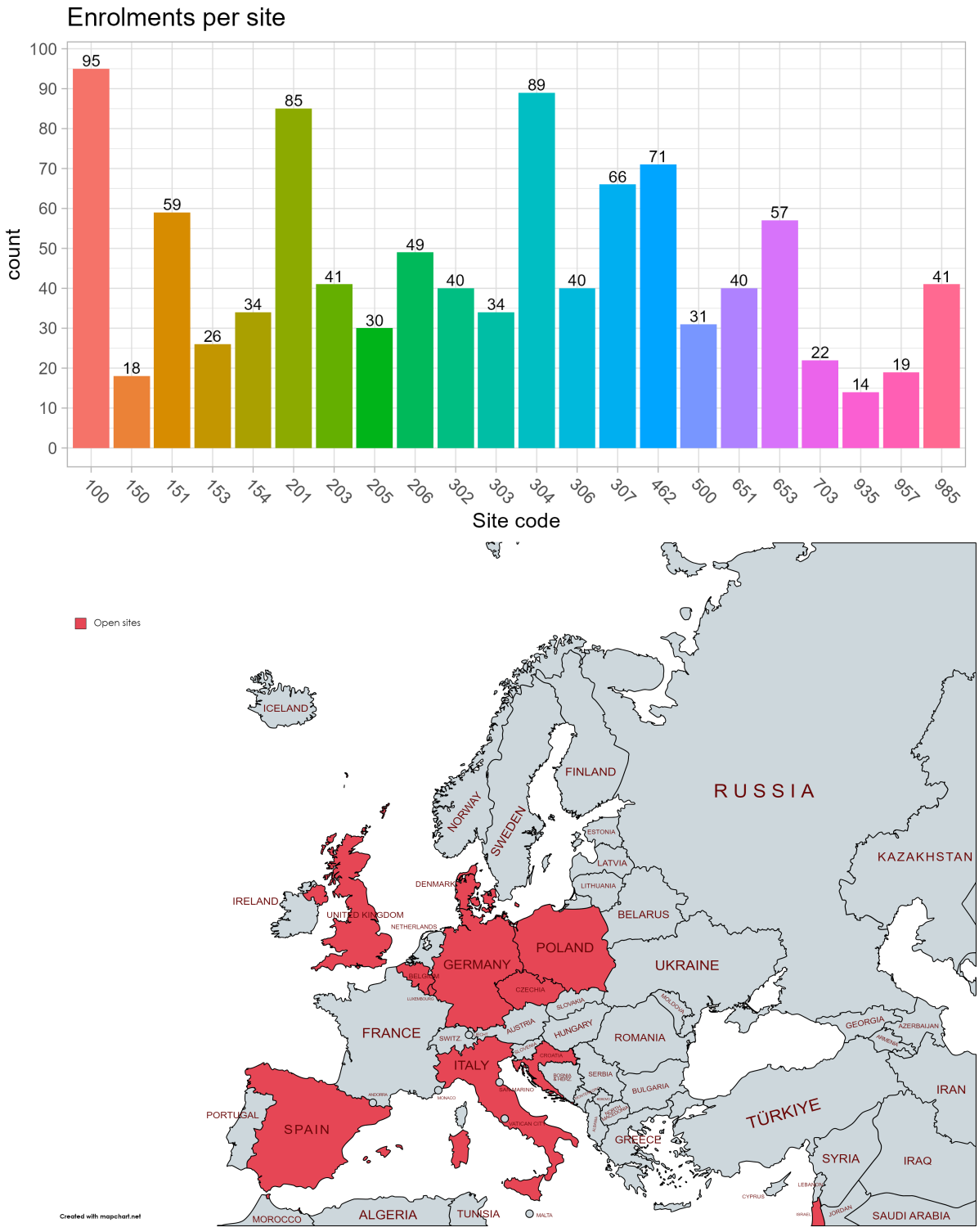
## Site and enrolment status

Twenty-two sites are part of our MISTRAL work package. A total of 1,002 participants completed their first visit, of which 333 were already part of EuroSIDA.




## Cohort characteristics

Now that we have reached full enrolment, we can present a snapshot of some of the cohort characteristics. The group consists predominantly of white men who have sex with men (MSM). Their diet is mainly omnivorous, and the stool samples collected have mostly been of Type 4 of the Bristol Stool Chart.

We have a richly characterised cohort in relation to factors that may influence the microbiome and the risk of serious non-AIDS events. Preliminary assessments also highlight the need to treat these variables carefully given the

observed correlations and small numbers of certain subgroups (e.g., women and non-white ethnicities).

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| **Total participants** | 1002 |
| **Existing EuroSIDA, n (%)** | 333 (33) |
| **Age, median [min, max]** | 59 [50, 86] |
| **Male, n (%)** | 825 (82) |
| **Consented to genomics analysis, n (%)** | 934 (93) |
| **Ethnicity, n** | |
| White | 858 |
| Black | 74 |
| Other | 23 |
| Unknown | 47 |
| **Nadir CD4, median [min, max]** | |
| Cells/µl | 223 [0, 1130] |
| Unknown, n | 111 |
| **Mode of HIV infection, n (%)** | |
| MSM | 621 (62) |
| Heterosexual contact | 226 (23) |
| Injecting drug user | 54 (5) |
| Unknown | 85 (8) |
| Other | 16 (2) |

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## Visit 2 update

As of 29th October 2024, 326 participants have returned to their clinic and completed their second visit.

Please remember to plan the second study visit keeping in mind that the follow-up sample should be collected within 10-24 months after the first MISTRAL study visit.

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## Shipment and analysis of samples

**Shipment of samples**

We have recently shipped almost 10,000 more samples from participants' first visit to IrsiCaixa in Spain, who will be helping us with the analysis of the samples. Thank you so much for the effort you all put in sending your samples to us, we look forward to seeing some first results from the analyses of the first visit!

**Analysis of samples**

As part of our work package, we will be analysing the faecal, plasma and whole blood samples to use in various analyses. In the coming 12 months we will perform faecal shotgun metagenomic sequencing and faecal proteomics as plasma metabolomics, plasma lipidomics and assessment of the inflammatory biomarkers IL-6, C-reactive protein (CRP) and D-dimer. Using bioinformatics

and biostatistical methodologies, these data will be coupled with the clinical data collected through the REDCap questionnaires to elucidate the role of the microbiome in biomarkers of serious AIDS and non-AIDS events in people living with HIV. In the future when we have sufficient power, we will also perform associations between these data and the clinical events themselves.

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## MISTRAL online investigator meeting, 24th September 2024

Thank you for attending our online MISTRAL investigator meeting on 24th September. It was a successful meeting with over 40 investigators participating. For those who were not able to attend you can find the presentation on our website.

As suggested during the meeting, we will work on preparing a patient-friendly update on MISTRAL. Don't hesitate to get in contact with us if you have any other suggestions!

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## Reminders and updates

**Reimbursement 2024**
As we wrote to you by email, we would like to say again here that we apologize for the delays in reimbursement this year. The process is now complete and you should all receive the remuneration for all MISTRAL visits, follow-ups, and events completed before 1st May 2024 shortly. Please do not hesitate to write to us should you have any questions.

**MISTRAL Follow-up 2 dataset (FU2 Autumn 2024) – Open for data entry in REDCap**
As of 24th October 2024, the MISTRAL follow-up dataset (FU2 Autumn 2024) is open for data entry in REDCap. This includes all participants that completed Follow-up 1 last Autumn, as well as participants with enrolment forms checked and locked (validated) in REDCap before 1st May 2024. This is a kind reminder to complete Follow-up 2 by **18th December 2024**. Should you encounter any challenges, please inform us promptly. We are happy to assist you with any support to facilitate the process.

**Finalising forms before May 2025**
The remaining 212 enrolment forms will be downloaded in May 2025. In order

to include enrolled participants for follow-up 3 in the Fall of 2025, you need to finalise their forms before this deadline. This includes responding to pending queries from our side. Do not hesitate to ask if this raises any questions on your end.

**HIV Glasgow Congress 2024**

On Monday, **11th November 2024, 7.30-9am**, in connection with the HIV Glasgow Congress, we will hold a EuroSIDA and MISTRAL update meeting. We look forward to seeing some of you there!

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## Frequently asked questions (FAQ)

**Q: Should the study staff or the patient complete the questionnaire?**
A: The questionnaire should be completed by the study staff.

**Q: A participant consents and takes the stool sample collection kit home with them, when do they have to return?**
A: The participant must return to the clinic with their stool sample within 48 hours of defecation. This should occur as early as possible to the consent, to ensure eligibility criteria are still met at the time of sample collection. However, we allow up to 3 months from date of consent for the participant to collect the stool and return the sample to the clinic.

**Q: Do the sample labels match, in any way, with the participants' PID numbers?**
A: No, you should use one set of labels per participant per visit in the order that you receive the samples. The labels will then be linked to the correct participant and visit when they are scanned into REDCap. You will also need to record which label IDs belong to which patient on the site List of Stored Samples. For more information see slides 43-48 in the Training slides.

All frequently asked questions are gathered on our website. If you have any questions not answered here, other solutions to the problems, or any general tips for participant recruitment/engagement, feel free to contact us at mistral.rigshospitalet@regionh.dk.

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## The MISTRAL consortium

MISTRAL is a large international consortium funded by EU. Research groups

from all over Europe are engaged in the work, which has been split into several work packages each with their own aim.

The primary objective of the work led by CHIP 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 (SNAEs), including cardiovascular events.

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## Interesting research on the gut microbiome

**Kenny et al., "Cholesterol Metabolism by Uncultured Human Gut Bacteria Influences Host Cholesterol Level", *Cell Host Microbe* (2020)**

**Li et al., "Gut microbiome and metabolome profiling in Framingham heart study reveals cholesterol-metabolizing bacteria", *Cell* (2024)**

The gut microbiome plays a role in modulating cholesterol levels of the host. One mechanism is the conversion of cholesterol in the gut to coprostanol - which is poorly absorbed by the host resulting in lower levels of cholesterol in the plasma of the host. This function of gut bacteria has been known since 1930, but the mechanisms were unknown until recently. The first pivotal paper identified the bacterial gene involved in the conversion of cholesterol to coprostanol – the Intestinal sterol metabolising A (ismA) gene (found in 2020 by Kenny et al.). Recently, a study in the large Framingham cohort observed that ismA+ species associated with decreased faecal and serum cholesterol levels, and that these genes were present in Oscillibacter sp. (Li et al., 2024).

These studies highlight the potential for the gut microbiome to modulate the host – in this case, an important risk factor for a variety of clinical complications (namely cholesterol). The bioinformatics methods being developed as part of MISTRAL allow for the characterisation of ismA+ bacterial species, opening the way for studies investigating whether lifestyle factors influence the presence of these bacteria, and whether their presence may impact the cholesterol profiles of PWH and hence the risk of cardiovascular disease, after accounting for other key confounders.

The articles are available here:
Kenny et al. (2020): https://doi.org/10.1016/j.chom.2020.05.013
Li et al. (2024): https://doi.org/10.1016/j.cell.2024.03.014

## Learn more about MISTRAL

You can find all the study documents related to this MISTRAL project at https://chip.dk/Research/Studies/MISTRAL/Study-documents

General information about MISTRAL can be found on this website https://chip.dk/Research/Studies/MISTRAL

Information about all the work packages included in MISTRAL can be found at www.mistral-hiv.eu

Finally, you can follow the MISTRAL consortium on Twitter https://twitter.com/mistralhiv

This was all we had for now. We look forward to sharing more updates with you in half a year.

Sincerely,
*The MISTRAL staff at CHIP*

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