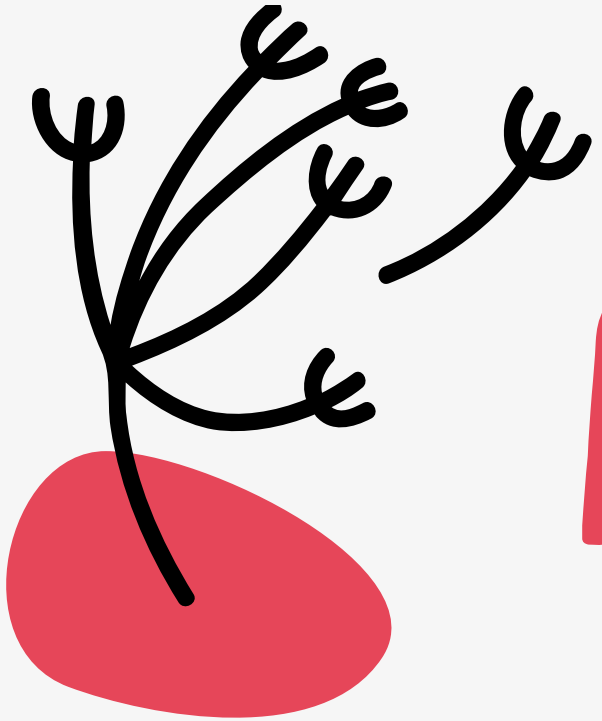

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MISTRAL Investigator meeting

12th September 2023

Agenda

1. Welcome and introduction
2. Update from the wider MISTRAL consortium (Roger Paredes, IrsiCaixa, Spain)
3. Recruitment update + projections
   - Cohort insights
4. Site feedback and discussion
5. Frequently asked questions (FAQs) and data quality/reminders
6. Publication plan and policy
7. Future of MISTRAL
8. General reminders and wrap-up

N.B. This meeting will be recorded

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Background

- Gut microbial dysbiosis has been linked with increased immune activation and various inflammatory markers – and may be a risk factor for serious non-AIDS events
- To date, many studies into the gut microbiome of people with HIV are limited by
  - Sample size
  - A lack of control for key confounders (particularly diet and sexual practice)
  - Lack of association with hard clinical endpoints
  - Cross sectional study designs
- In order to inform future interventional strategies, larger, well characterised cohorts with adequate follow-up are needed

- The EuroSIDA / MISTRAL protocol was therefore designed in order to address this need

Study Design

- Observational study
- Aim is to recruit up to 1,200 participants (to ensure 1000 persons with follow-up) from established EuroSIDA sites
- Participants can be existing EuroSIDA participants or new persons followed at EuroSIDA sites
- Blood and stool collection and MISTRAL questionnaire will occur at baseline and one follow-up visit
- Follow-up clinical data collection will occur during yearly EuroSIDA data collection (Oct-Dec)
- Asides from the additional sample collection and MISTRAL questionnaire, all other data collection and study procedures are the same as EuroSIDA

Study objectives

Primary objective

- 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

Secondary objectives

- To evaluate the associations between the gut microbiome composition and function and pathologic increases in inflammation and coagulation mediators in PLWH

- 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.

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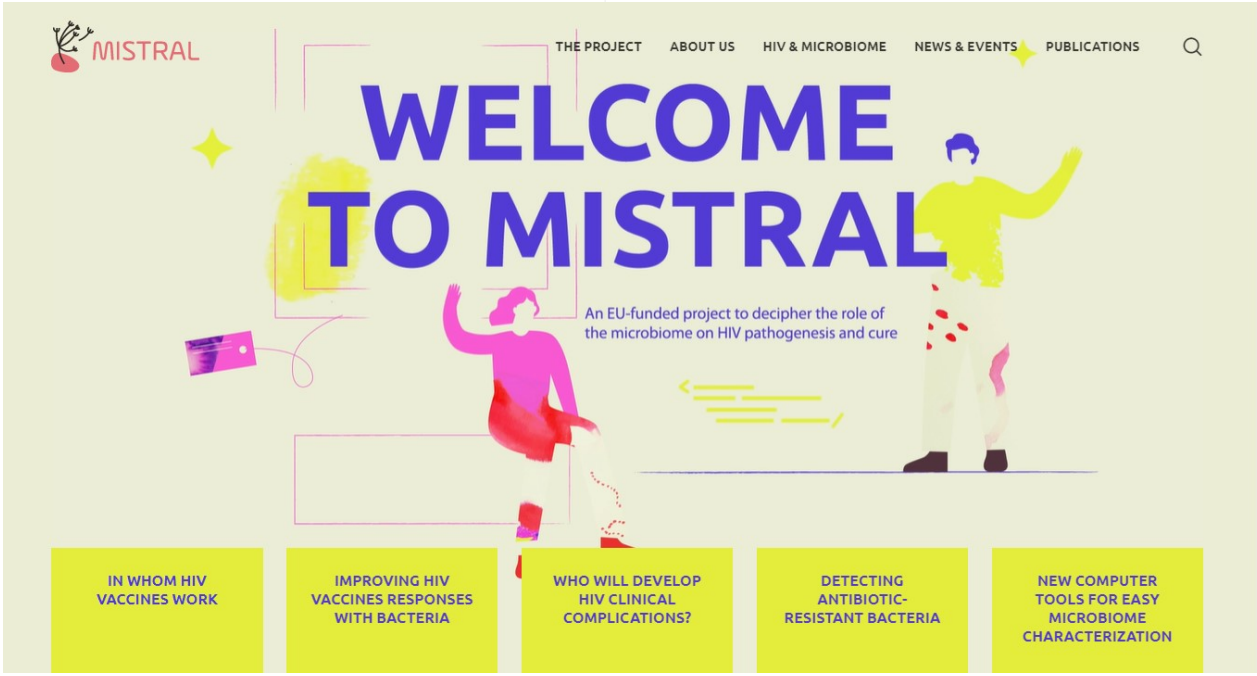
- This protocol is part of a wider EU Horizon2020 funded consortium (*Microbiome-based stratification of individuals at risk of HIV-1 acquisition, chronic clinical complications, antimicrobial drug resistance, and unresponsiveness to therapeutic HIV-1 vaccination*) led by Roger Paredes
- Other work packages address other key questions surrounding HIV and the microbiome as well as data analysis and sharing for these key data (see the MISTRAL website for further details https://www.mistral-hiv.eu/)
- Roger has joined to present some of the highlights today

Roger Paredes

PRINCIPAL INVESTIGATOR



Update from the wider MISTRAL consortium (Roger Paredes, IrsiCaixa, Spain)

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Recruitment update and projections

Current status

Per 8th September 2023

- 21 sites open from 11 countries
- 19 sites have recruited at least one participant
- 405 participants enrolled

- EU Horizon2020 reporting period currently ends June 2025 – an amendment has been submitted to extend until 31st of December 2025

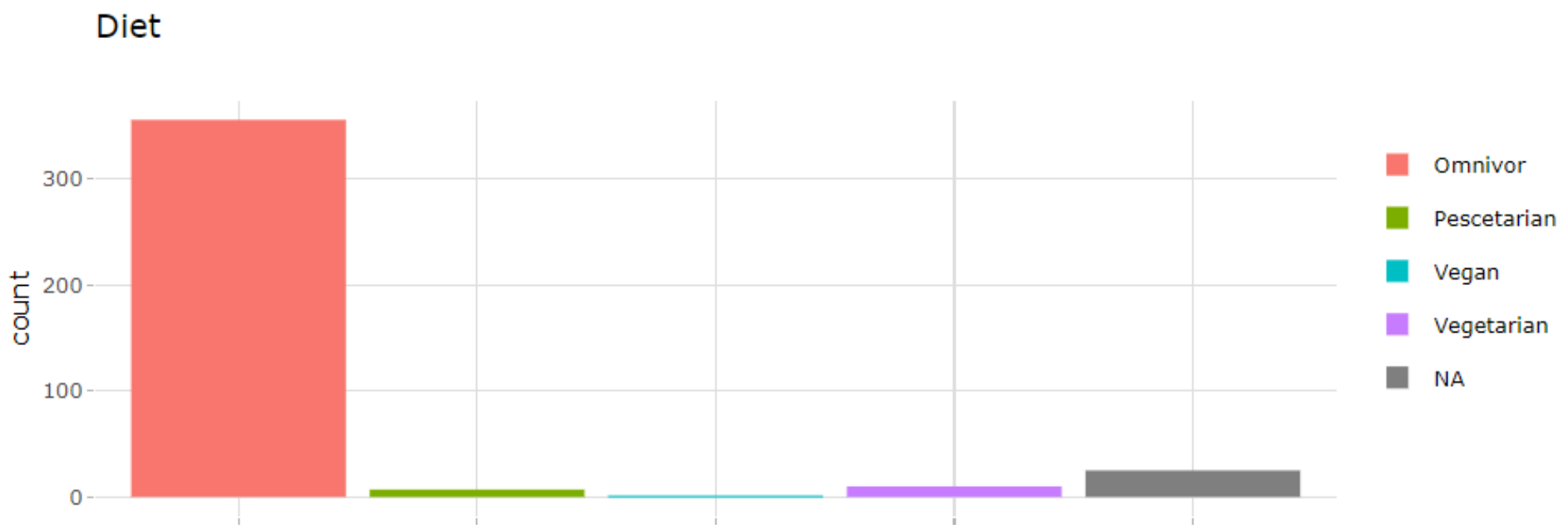
Cohort characteristics

Total participants	405
Existing EuroSIDA, n (%)	132 (33)
Age, median [min, max]	59 [50, 86]
Male, n (%)	321 (85)
Consented to genomics analysis, n (%)	373 (94)
Country of recruitment, n	
Belgium	52
Denmark	58
United Kingdom	90
Germany	116
Luxemburg	19
Spain	18
Czhecia	3
Poland	19
Croatia	24
Ethnicity n*	
White	202
Black	16
Other	5
Unknown	3
Nadir CD4, median [min, max]*	
Cells/µl	289 [3, 1107]
Mode of HIV infection, n (%)**	
MSM	238 (66)
Heterosexual contact	74 (21)
Injecting drug user	9 (2)
Unknown	25 (7)
Other	11(3)

* Only non-EuroSIDA participants
** Not reported for all

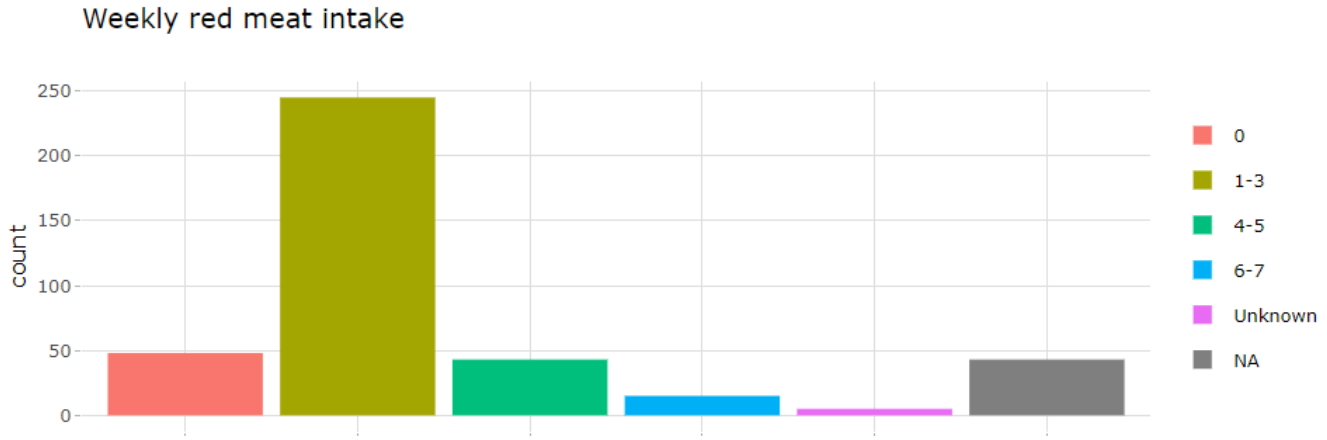
Diet

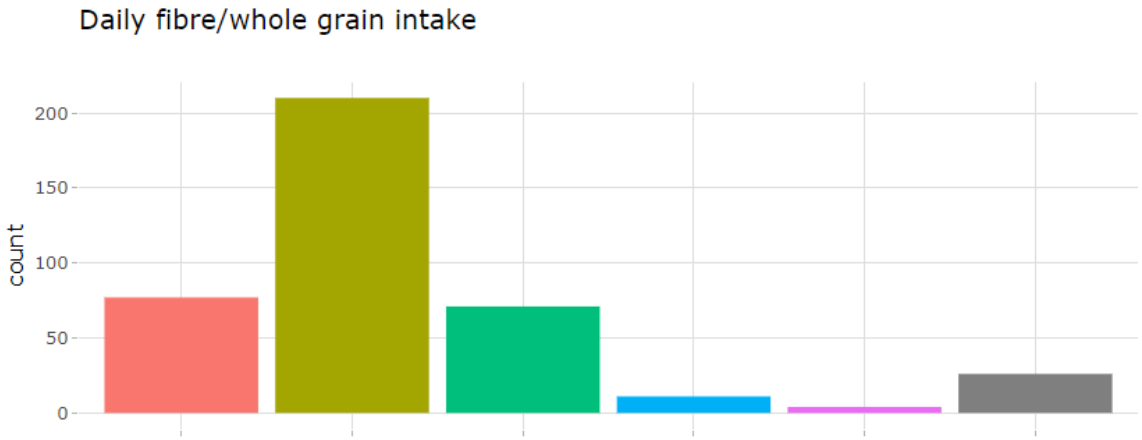
- 95% report an omnivore diet

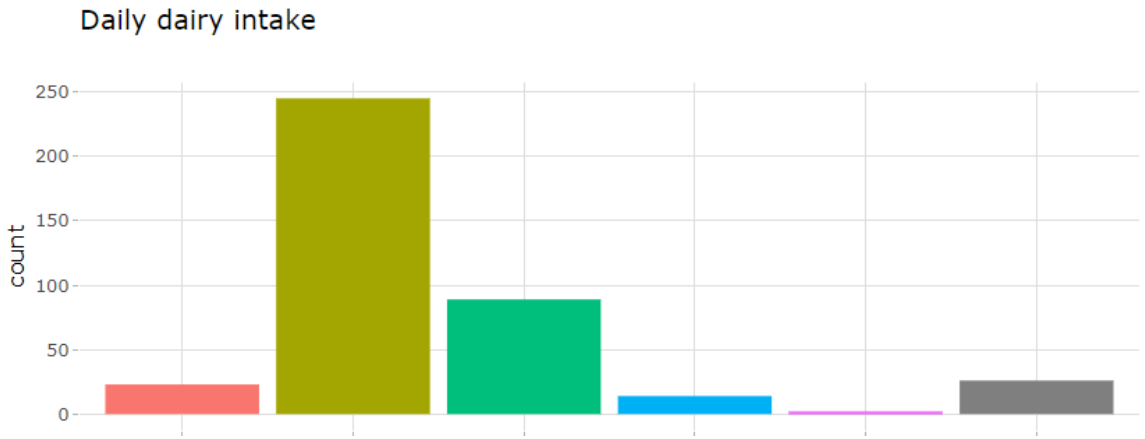


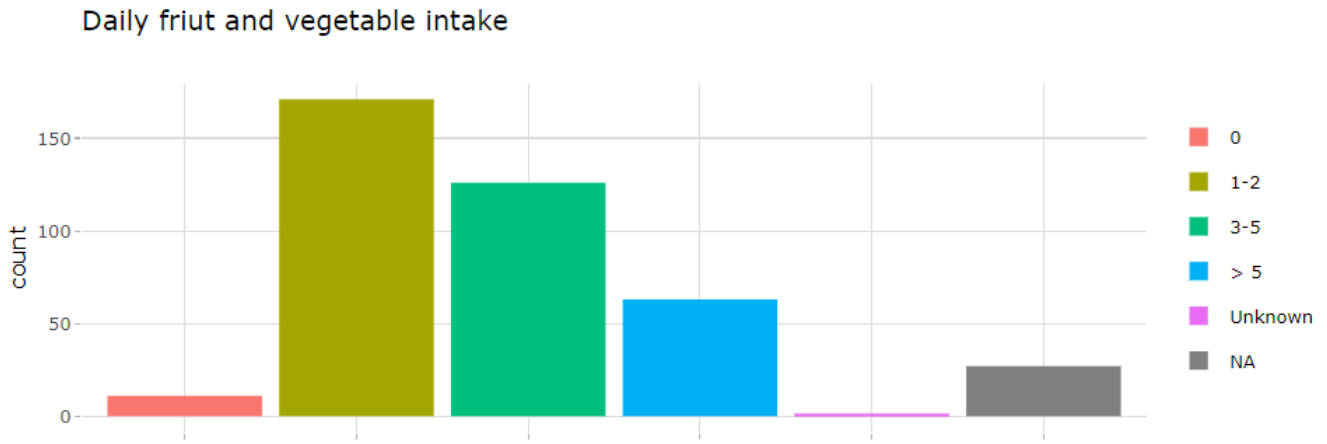
Dietary responses

- 93% of those eating red meat >5 times/week are male
- 71% of participants eat fibres <3 times/week





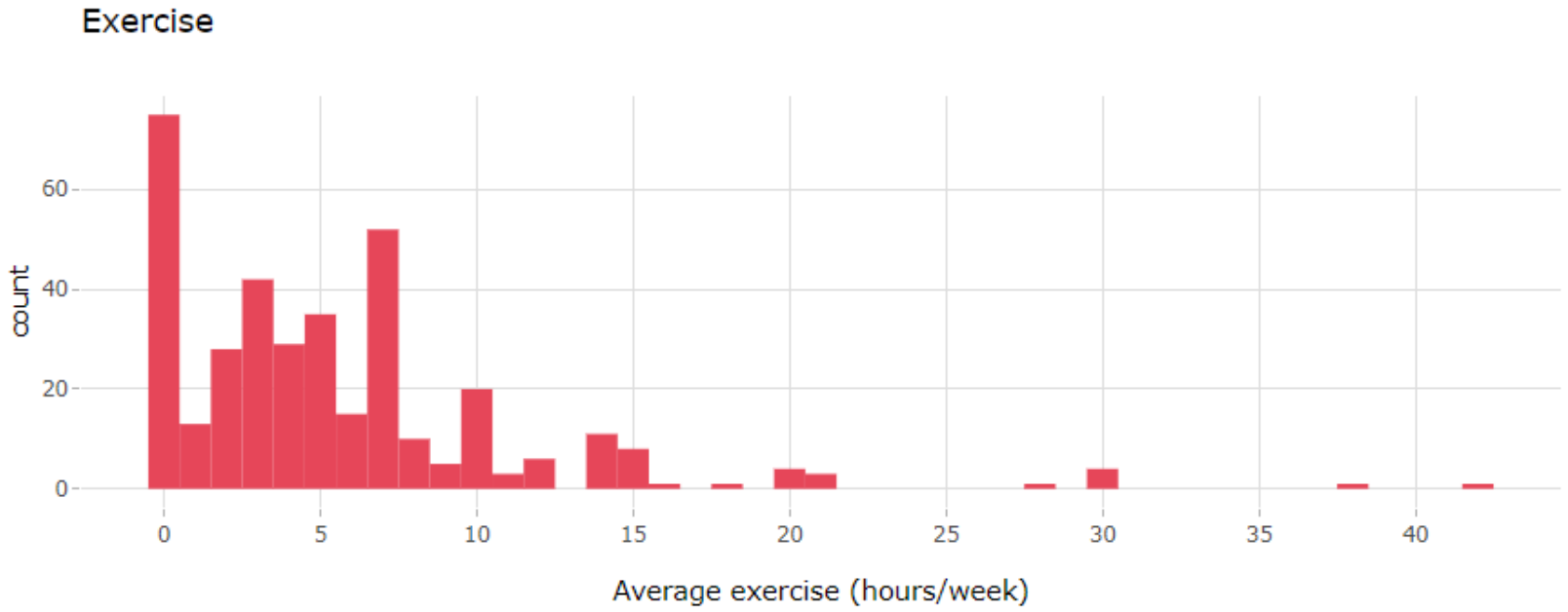


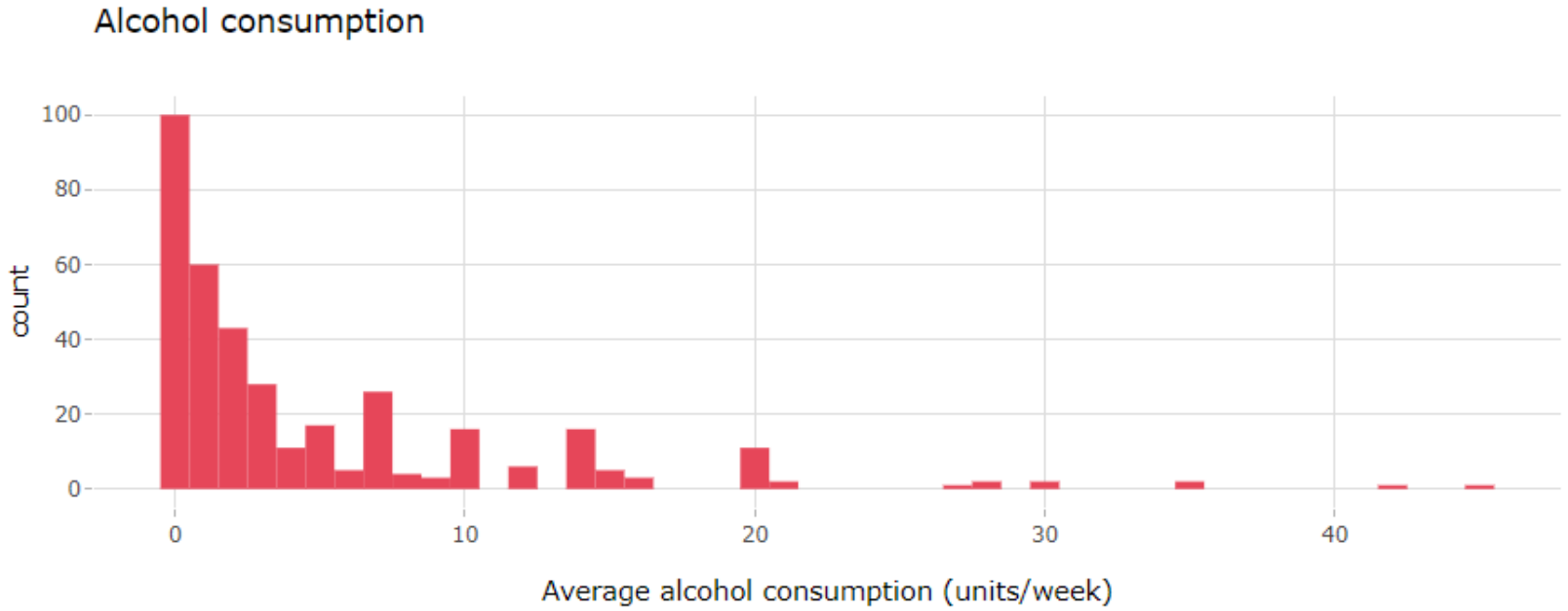


Habits

- 19% report no exercise
- 20% report >7 hours/week

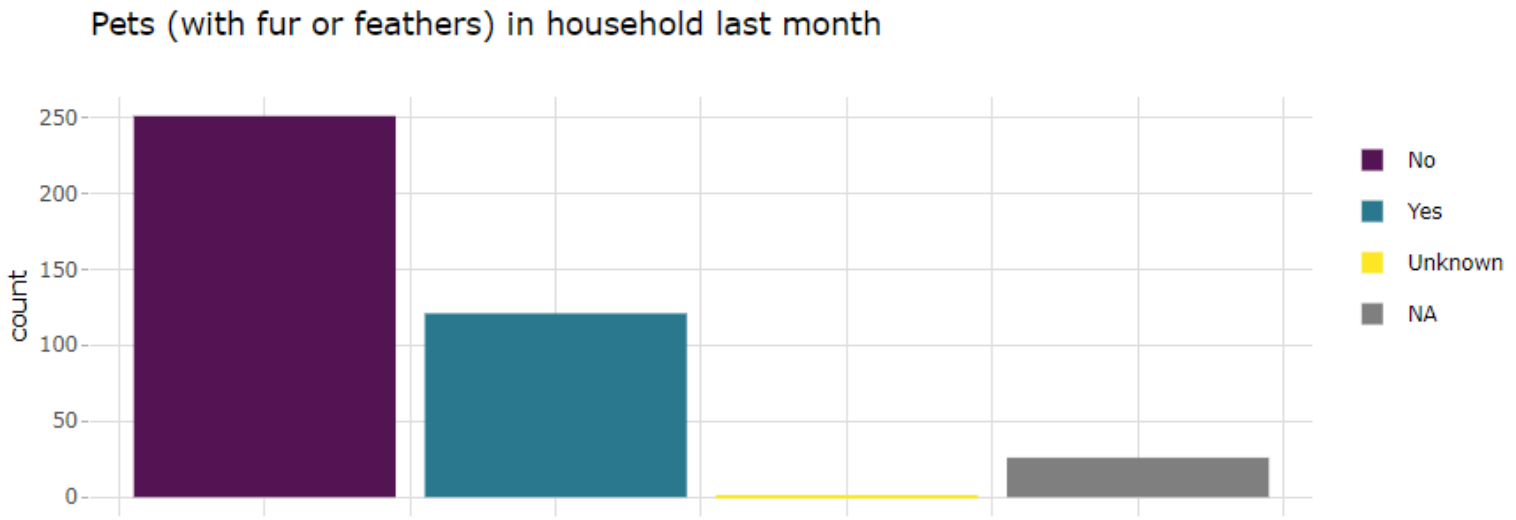
- 25% repost no alcohol consumption
- 92% of those consuming >10 units/week are male
- Only men report >16 units/week

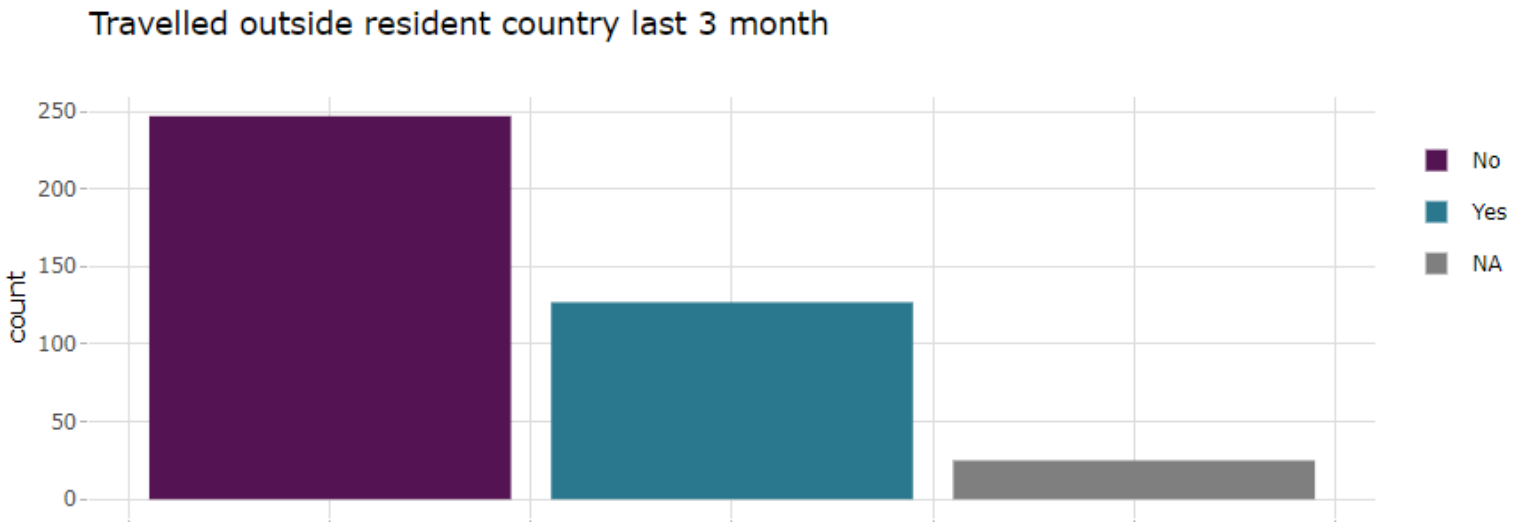




Habits since enrolment

- 43% of females have had pets at home the last month, 30% of males
- 42% of females have travelled outside resident country last 3 months, 36% of males





Summary

- Numbers are still small, but this is an extremely richly characterised cohort in relation to factors that may influence the microbiome
- Preliminary assessments highlight the need to treat these variables carefully given the observed correlations and small numbers of certain subgroups (e.g. women)

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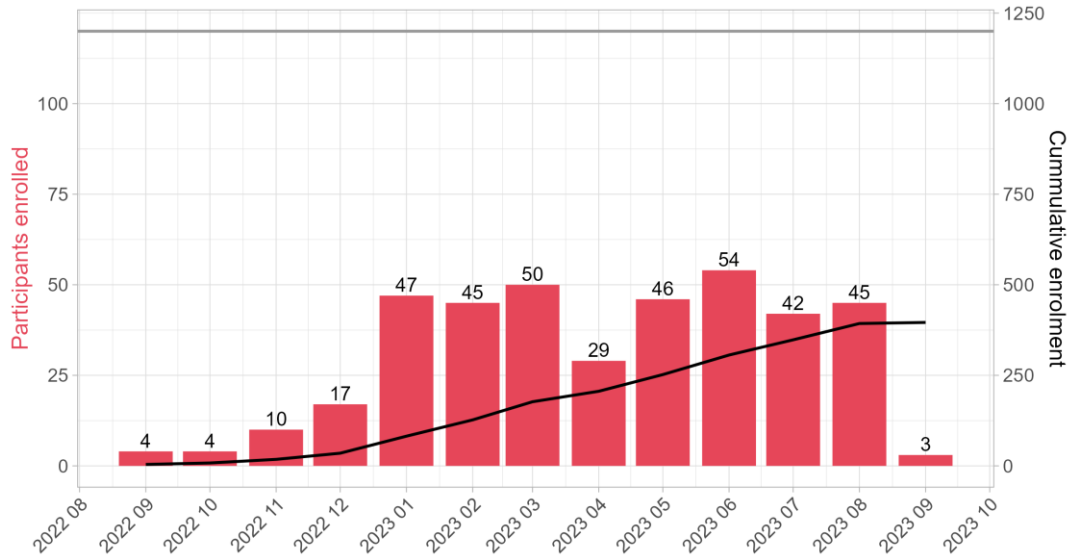
Recruitment projections

- Our original aim was to complete recruitment of 1,200 persons by December of this year in order to ensure adequate time for collection of follow-up sample for 1,000 participants within the EU reporting period
- We would like to complete enrolment by first quarter 2024 – but this requires recruitment to increase to 100 per month
- This new closing date will open the possibility of second samples being collected outside the Horizon2020 reporting period, which we will support, but we will likely close new enrolments by mid 2024
- If we have to close new enrolments prior to full enrolment, this will reduce power for associations with clinical events



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Experiences from sites + Q&A

Your experiences with and questions about..

- Recruitment
- Participant experience and interest
- Data entry/queries
- Monthly reports/updates
- Anything else?

Frequently asked questions (FAQs) and data quality/reminders

Frequently asked questions (FAQs)

- *https://chip.dk/Research/Studies/MISTRAL/Frequently-asked-questions-FAQ*
    - *E.g.:*

Q: If there is no INR recorded, and no mention of end stage liver disease, can the patient be included in the study?

A: If the patient doesn't have ascites and encephalopathy, and INR is missing, calculate the worst case scenario based on the bilirubin and albumin levels alone. Even if both albumin and INR are missing, the CP score cannot be worse than 9 (CP class B) if bilirubin is normal and there is no ascites or encephalopathy.

Q: Is it possible to have some flexibility on the exclusion criteria of 'Child Pugh C end stage liver disease'?
A: The exclusion criteria is written in the protocol so we are limited by that. But you can use the most recent bilirubin, albumin and INR results. If there is no mention of ascites or hepatic encephalopathy or end-stage liver disease (or decompensated liver disease) in the patient chart, there is no need for a clinician to examine the patient for ascites and encephalopathy.

Q: Does the creatinine clearance measurement have to be taken within the last 3 months?
A: Previously, this was a requirement in REDCap, however this has now been changed to the last measurement taken.

Etc.

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Querying process

- Generally, data completeness is excellent
  - Remember to fill in all questions before marking the forms as ‘complete’
- Thank you for adapting to new querying process (via excel sheet)

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Reminder

- Remember to measure key parameters at enrolment and at follow up visits
  - Weight
  - Blood pressure
  - CD4+ T cell count
- These are key variables for clinical management of people with HIV and for the investigation of co-morbidities in HIV observational studies in general and for MISTRAL in particular
- The variables are often not performed annually (as per EACS guidelines) for MISTRAL participants already in EuroSIDA
- In the most recent EuroSIDA follow up dataset, 27/117 MISTRAL participants had no blood pressure reported and for 30/117 the most recent weight was from 2021 or older

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Reimbursement

- Reimbursement is made annually to sites by CHIP in June/July (delayed this year)
- Cutoff date is 1st May for completed and validated forms
  - Forms entered after this date will be paid the following year
- A participant visit is reimbursed 110 Euro:
  i. Visit 1: Baseline form, Questionnaire, Samples and Enrolment form (for non-EuroSIDA participants)
  ii. Visit 2: Questionnaire, Samples
- CoDe and RESPOND Event forms are reimbursed 30 Euro
- EuroSIDA and MISTRAL follow-up forms are reimbursed 20 Euro

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Publication plan and policy

Publications – plans and authorship proposal

- Publications as part of the WP4 activities will include investigators from the sites
- Each recruiting site will have at least one investigator listed as part of the writing group and higher recruiting sites will have more than one (exact number will be decided after full recruitment)
- Wider study group will be listed in the supplemental materials where up to 10 site staff can be acknowledged
- This means that the writing groups will be larger than the usual 22 in EuroSIDA, but we believe this reflects the effort in recruiting to this protocol
- Currently planned publications:
    1. Cohort description and associations between baseline microbiome plus other unique data items in MISTRAL and biomarkers of serious non-AIDS events (IL6, D-dimer and CRP)
    2. Association between microbial factors and cardiovascular disease (primary outcome)
    3. Associations with additional clinical endpoints will depend on number of events collected – as the cohort develops, these may become possible

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Access to data and samples for additional research

- Samples and data will be available for additional research projects from internal (e.g., site investigators or other MISTRAL work packages) and external researchers
- Requests to use MISTRAL samples and data will need to be approved by the EuroSIDA Steering Committee (SC) and MISTRAL data access committee
- MISTRAL data access committee is an oversight committee with representatives from each work package – each work package has right to approve and veto projects requesting data from their own work package –
- EuroSIDA SC will review from a scientific perspective and MISTRAL data access committee, as oversight for the MISTRAL data lake and biobank, will review from an administrative/logistic point of view (e.g., if some funds are needed to facilitate biobank sample picking and shipping)

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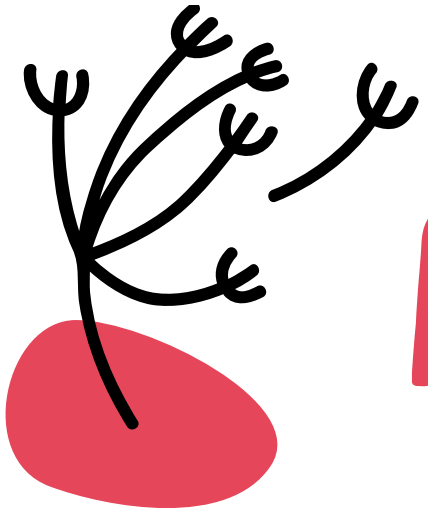
Future of  MISTRAL

Future of MISTRAL

- Horizon2020 reporting period currently ends June 2025 – with an amendment submitted to extend this until 31st of December 2025
- We will continue to support the collection of samples and questionnaires until two visits for 1000 individuals
- Collection of clinical data will then continue through EuroSIDA, ensuring that the cohort becomes even more powerful every year of continued follow-up
- Molecular analyses baseline samples will begin once all baseline samples are collected
- Currently funded analyses include
  - Shotgun metagenomic sequencing of an aliquot of stool (all participants)
  - IL6, CRP and D-dimer ELISA (all participants)
  - Metabolomics on an aliquot of stool (subset of participants only approx. n = 300)
  - Metabolomics and lipidomics on an aliquot of plasma (approx. n=300)
  - Proteomics on an aliquot of plasma (approx. n=300)
- MISTRAL data lake and biorepository will continue beyond the Horizon2020 grant period and hopefully serve as the basis for many more additional projects

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General reminders

- **EuroSIDA and MISTRAL F2F scientific meeting at EACS conference**
  - When: 18th October, 7.30-9.00
  - Location: Hotel Golden Tulip Warsaw Centre

- **MISTRAL 2nd visit**
  - To be completed 10-24 months after patient's 1st visit

- **MISTRAL and EuroSIDA follow-up forms**
  - Available to complete in REDCap from 1st October
  - Both EuroSIDA ('EuroSIDA follow-up') and non-EuroSIDA ('MISTRAL follow-up') participants
    - *MISTRAL follow-up forms only available for complete enrolments prior to July 2024*

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Wrap-up

- Thanks to the site staff and the participants for your continued commitment to this project
- Do not hestiate to contact us at CHIP via mistral.rigshospitalet@regionh.dk or consult the FAQs on our website

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