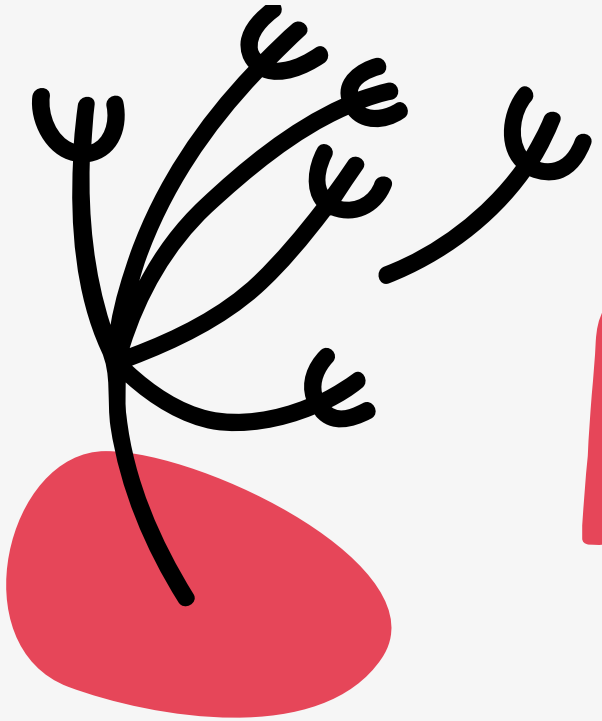

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MISTRAL Investigator meeting

24th September 2024

Agenda

1. Welcome and introduction
2. Recruitment and visit update
3. Update from the wider MISTRAL consortium (Alessandra Borgognone, IrsiCaixa, Spain)
4. Analysis of data
5. Publication plan and policy
   - Bioinformatics QA tool
6. 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

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Study Design

- Observational study
- Aim is to recruit over 1000 participants 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

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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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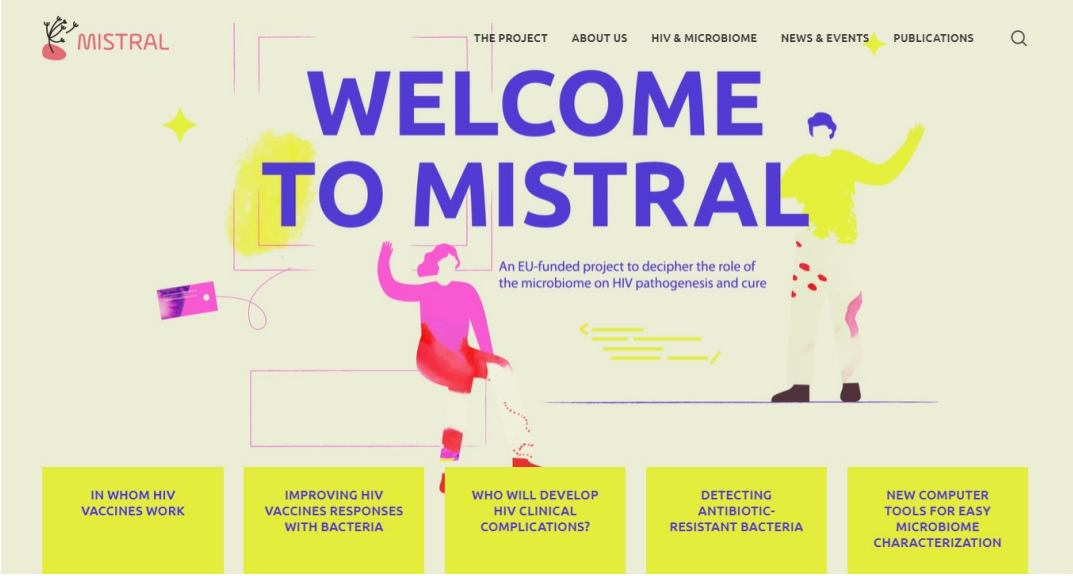
- This protocol is part of a wider EU Horizon2020 funded consortium (*Microbiome-based stratification of individuals at risk of HIV-1 acquisition, chronic clinical complications, antimicrobial drug resistance, and unresponsiveness to therapeutic HIV-1 vaccination*) led by Roger Paredes

- 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/)

- Alessandra from IrsiCaixa has joined to present some of the highlights today

Roger Paredes

PRINCIPAL INVESTIGATOR



Recruitment and visit update

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Current status

Per 24th September 2024

- 22 sites from 11 countries have recruited
- 1,002 participants enrolled
- 273 participants have completed follow-up visit

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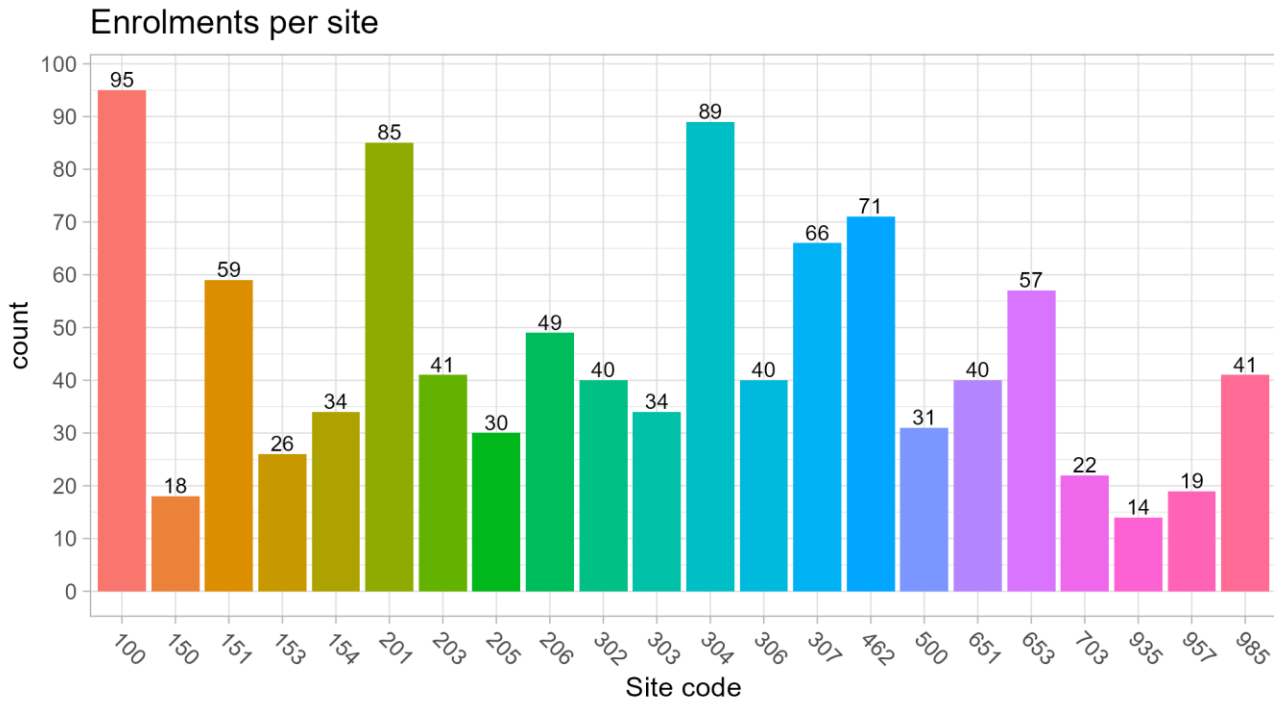
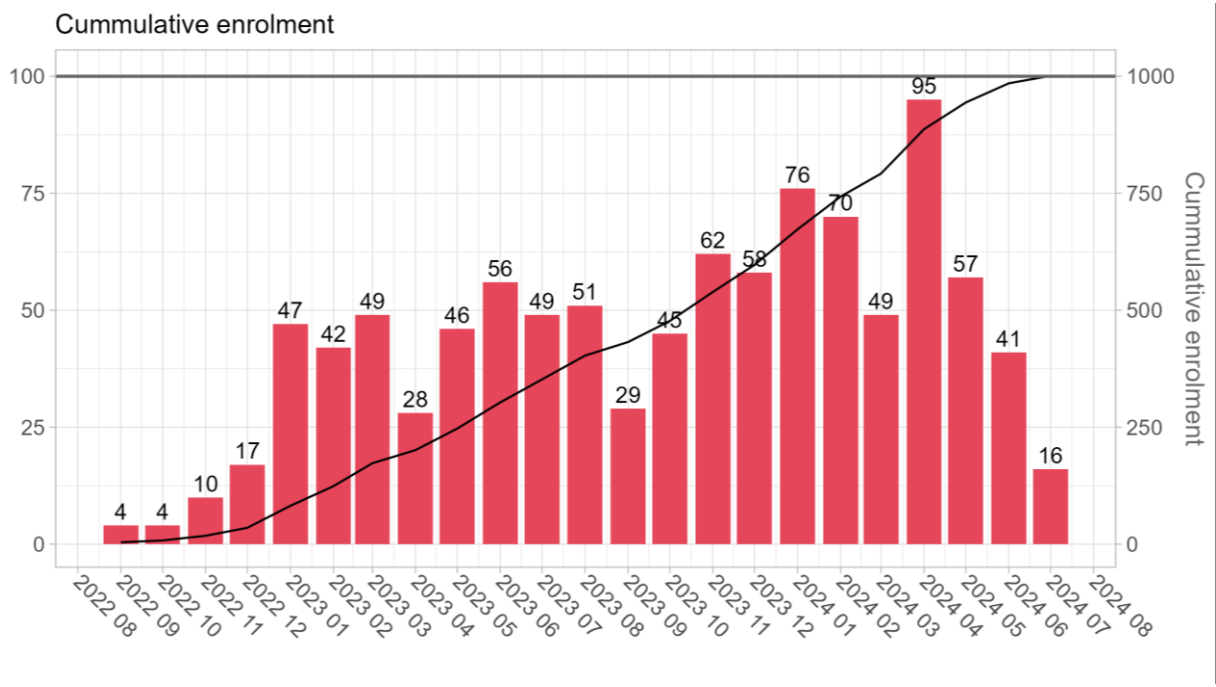
Current status
Per 24th September 2024





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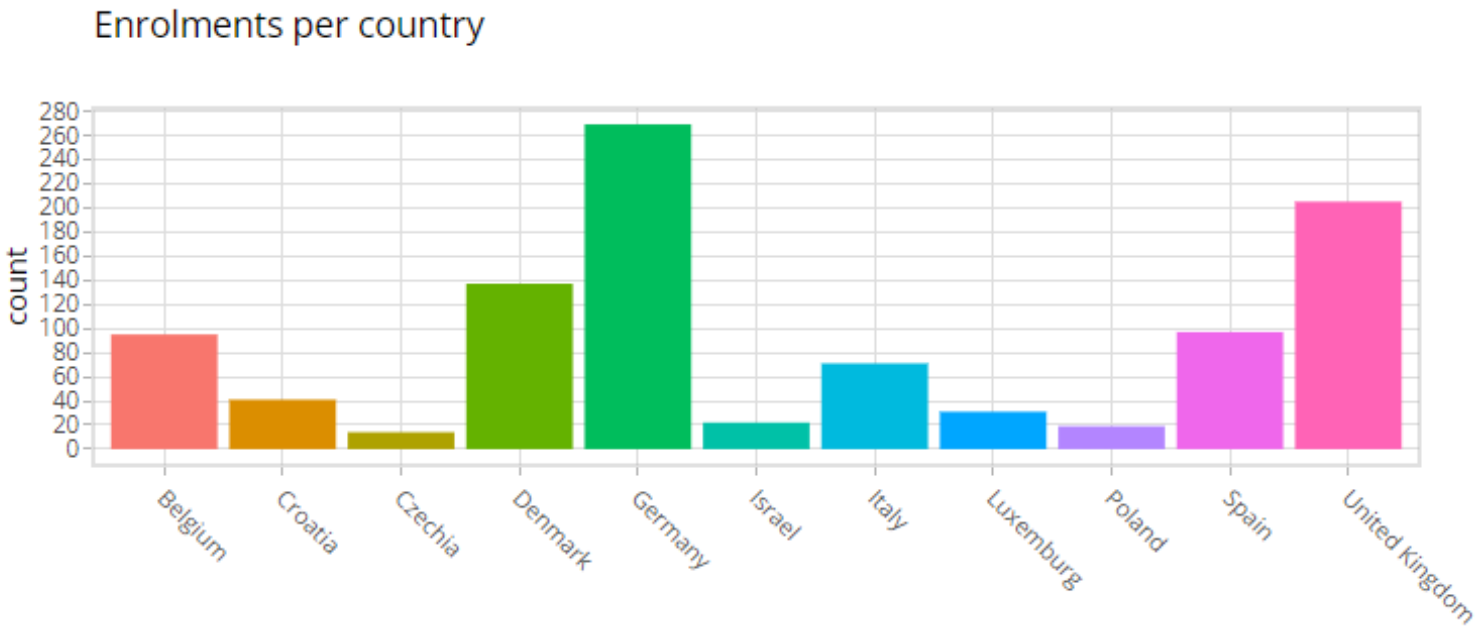
Celebration at CHIP

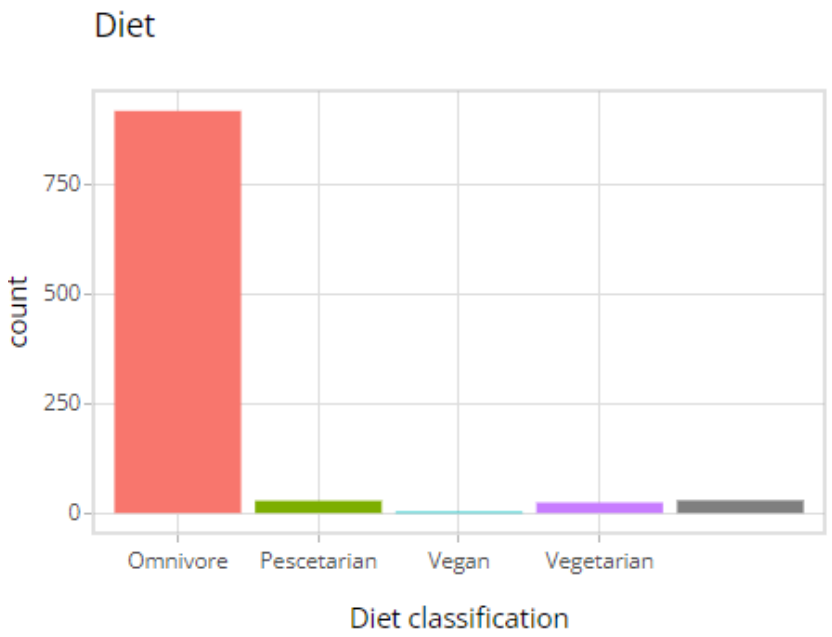
Cohort characteristics

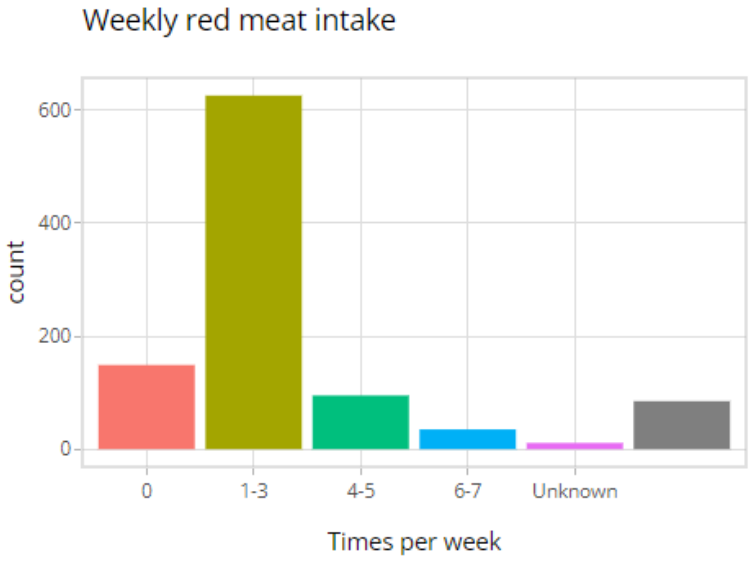
Total participants	1002
Existing EuroSIDA, n (%)	333 (33)
Age, median [min, max]	59 [50, 86]
Male, n (%)	825 (82)
Consented to genomics analysis, n (%)	934 (93)
Ethnicity, n	
White	858
Black	74
Other	23
Unknown	47
Nadir CD4, median [min, max]	
Cells/µl	223 [0, 1130]
Unknown, n	111
Mode of HIV infection,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

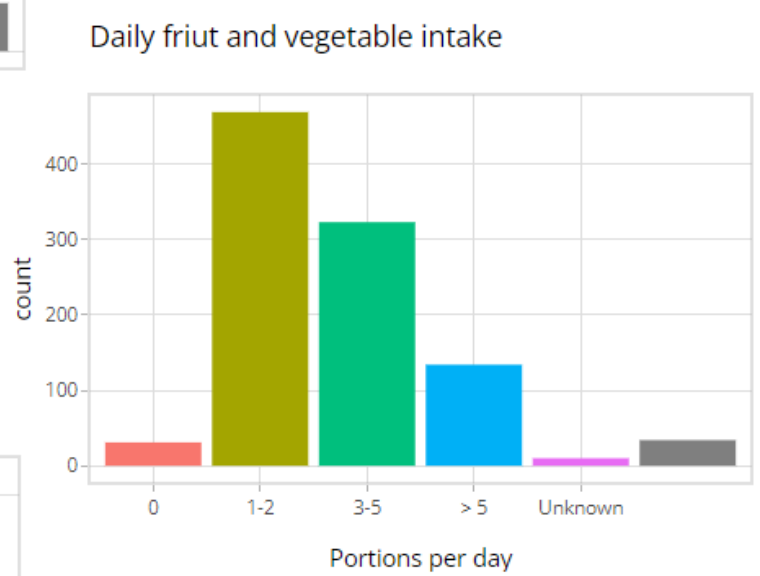


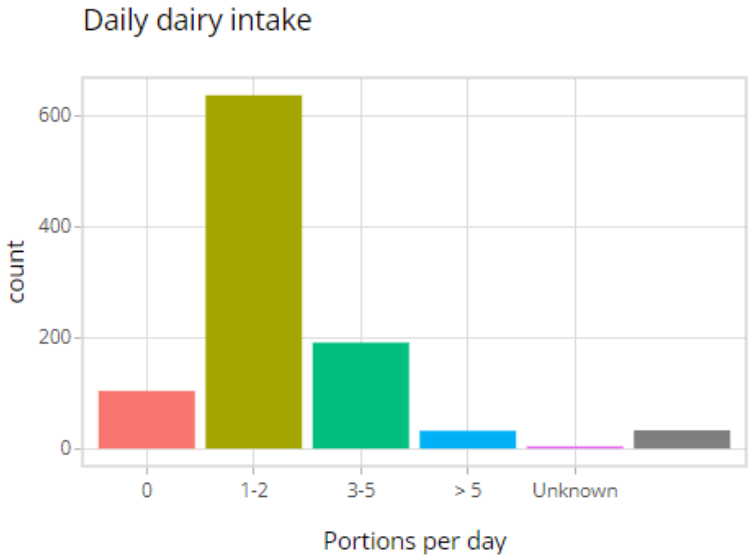
Diet

- 95% report an omnivore diet
- 62% eats red meat 1-3 times/week
- At least 50% eat less fruit and vegetables than recommended (≥5)



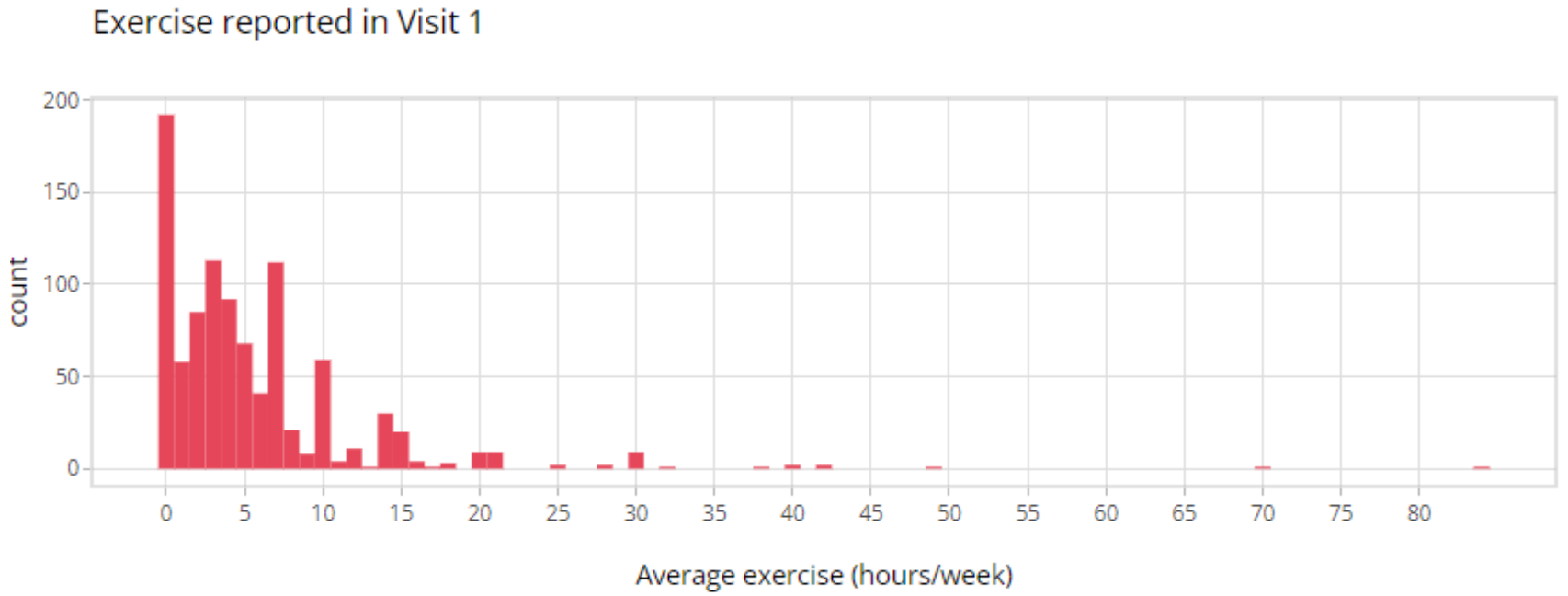


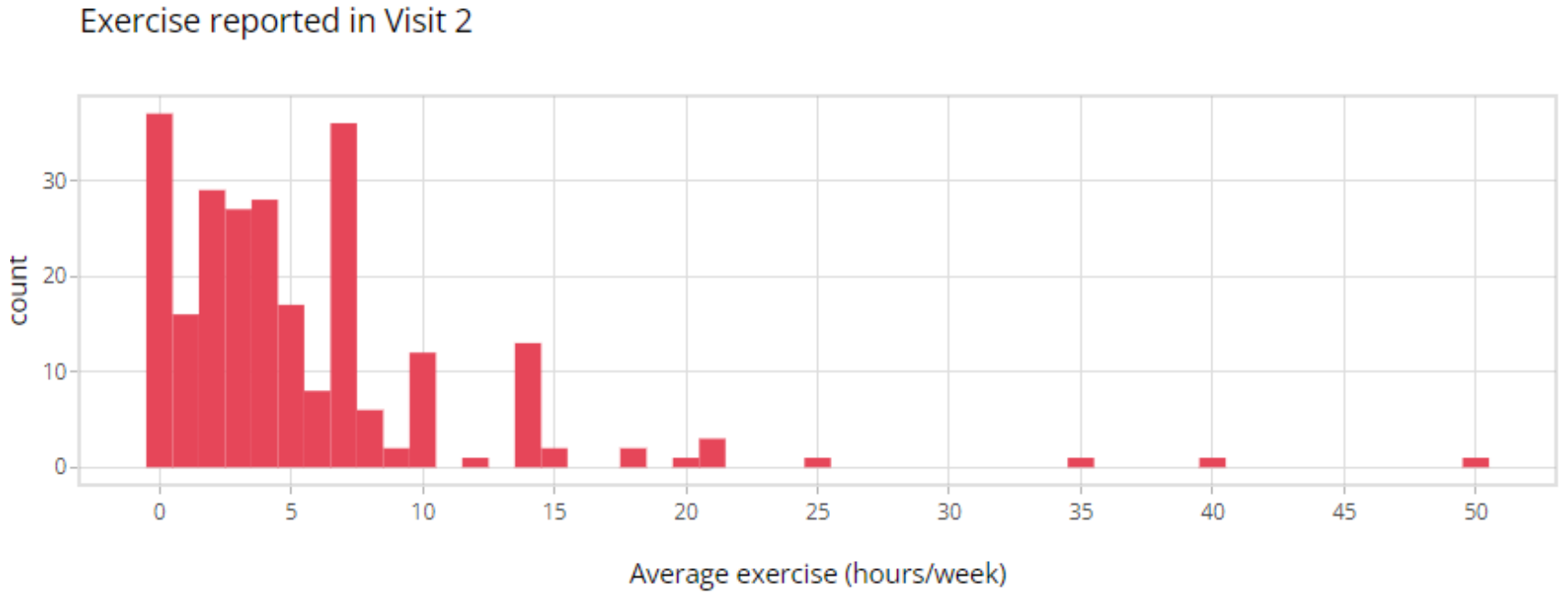




Habits - exercise

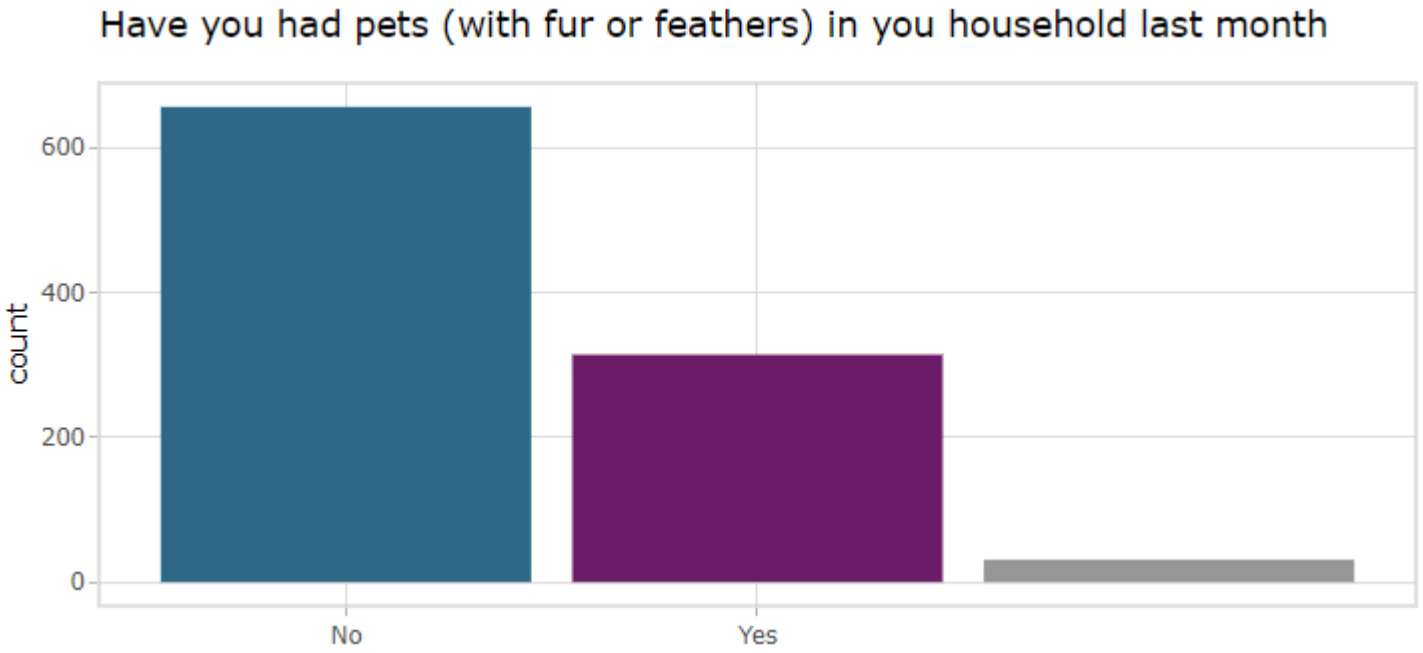
- 14-19% report no exercise
- 80% of those reporting no exercise are male
- 17-20% report >7 hours/week

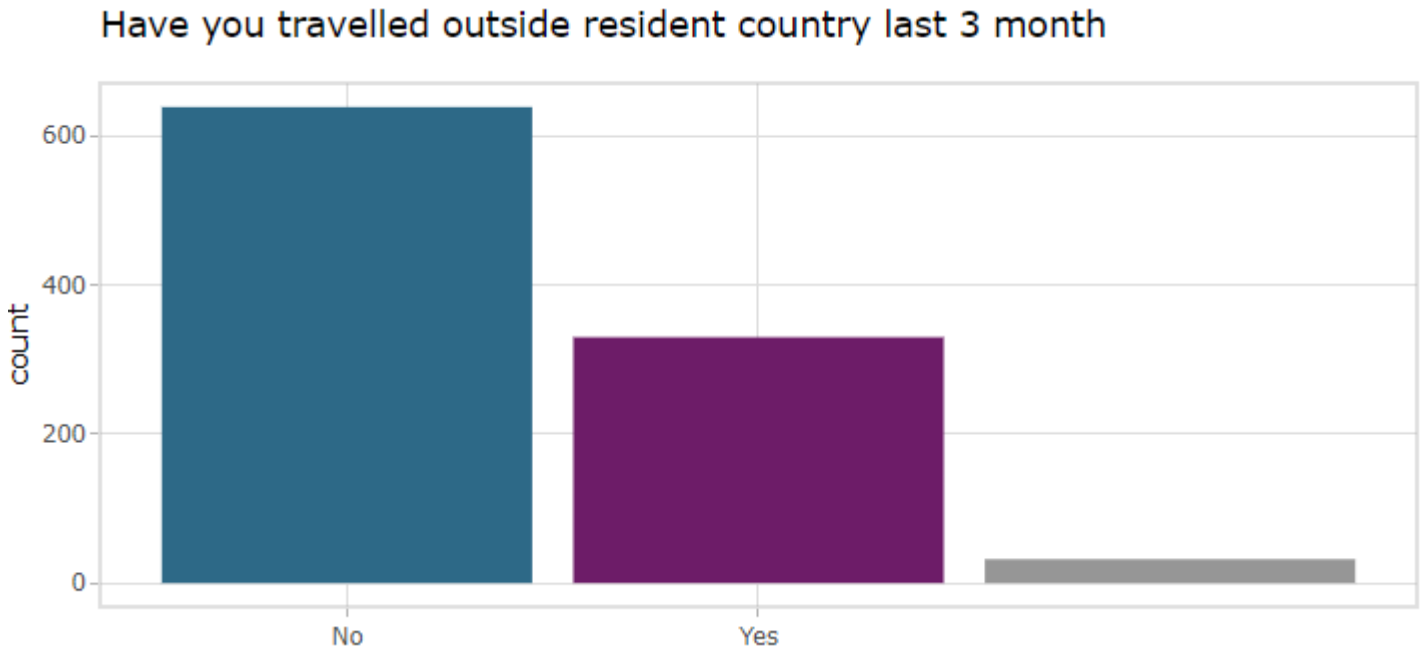




Habits since enrolment

- 41% of females and 30% of males have had pets at home the last month
- 20% of females and 36% of males have travelled outside their resident country last 3 months





Summary

- This is an extremely richly characterised cohort in relation to factors that may influence the microbiome and/or clinical events
- Questionnaire data contains crucial confounding factors that need to be considered for downstream analyses (or entire studies in their own right)
- Preliminary assessments highlight the need to treat these variables carefully given the observed correlations and small numbers of certain subgroups (e.g. women)
- Multiple testing also a concern when exploring the huge numbers of datapoints collected in this study

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Update from the wider MISTRAL consortium (Alessandra Borgognone, IrsiCaixa, Spain)

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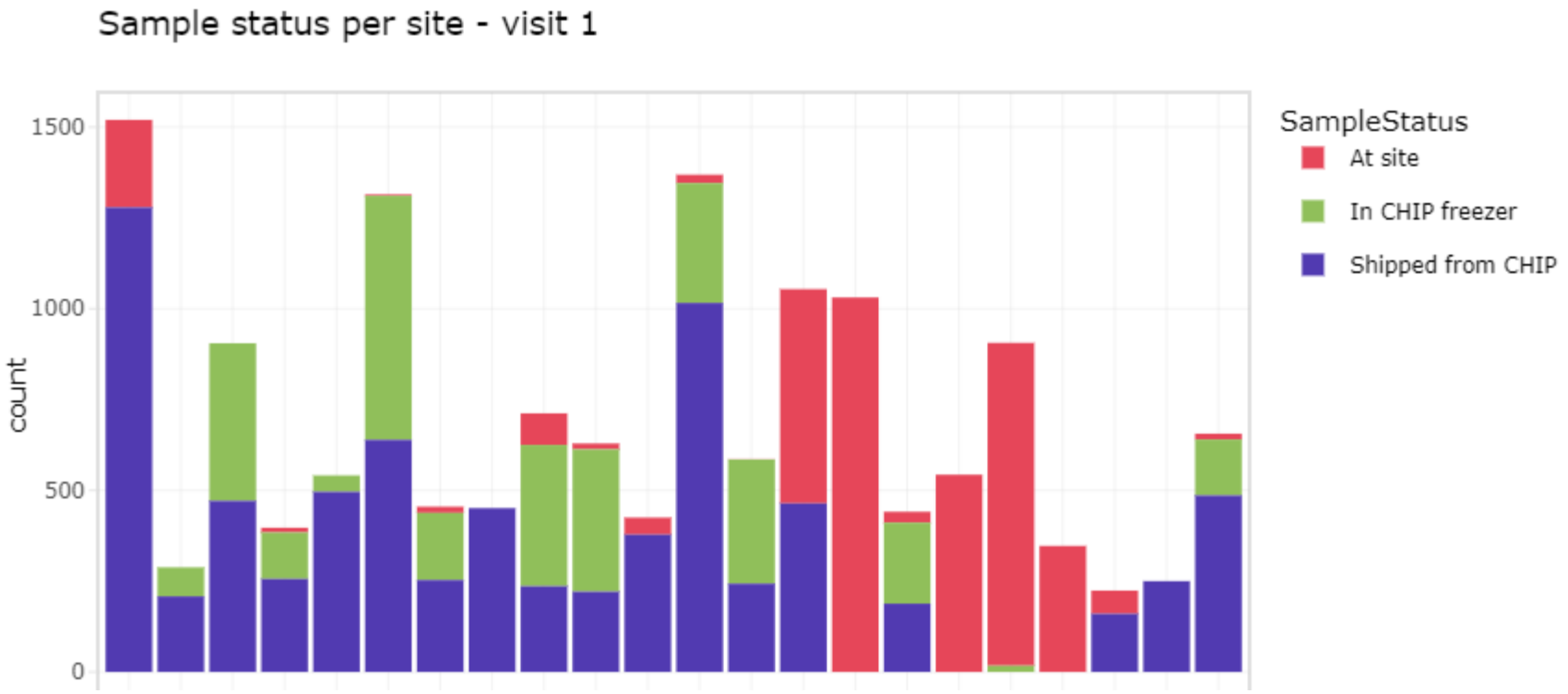
Analysis of data

Sample collection and shipping



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Molecular analyses underway

- Shotgun metagenomics of baseline stool samples
  - DNA sequencing of all microbiota (bacteria, fungi and DNA viruses)
- Proteomics of baseline stool samples
  - Measurement of proteins in stool bacterial pellet (i.e. the proteins inside the bacterial cell wall)
- Metabolomics of baseline plasma samples
  - Unbiased relative quantification of all metabolites in plasma taken at the same time as stool
- Metagenomics and metabolomics to be complete end of 2024 and proteomics in approx. 12 months

- N.B. Orginal plan and budget was to analyse a subset (n=300) of baseline samples – but the molecular labs and MISTRAL leadership were so impressed by the high quality phenotypic data we decided to prioritise EuroSIDA over other projects

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Why shotgun metagenome, fecal proteome and plasma metabolome

Shotgun metagenomics
assesses bacterial DNA
- genes, species, functional potential

Plasma metabolomics
measures the metabolic output of all reactions
- may be **directly** (e.g. secondary bile acids, cholesterol)
- or **indirectly** (e.g. inflammatory processes) influenced by the gut microbiome

Fecal proteomics
(measured from the bacteria)
assesses what is being translated
- real functional output not just potential

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Publication plan and policy

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Future of MISTRAL

- Horizon2020 reporting period has been extended 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 (at least until 2030)
- 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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Publications – plans and authorship proposal for consortium publications

- Major Publications as outlined in the protocols aims will be done as a consortium (i.e. The MISTRAL/EuroSIDA Study Group)
- Each recruiting site will have at least one investigator listed as part of the writing group and sites that recruited over 50 participants will have two representatives
- 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
- We expect multiple consortium publications and authorships can be rotated within a site at the PIs discretion

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Publications – plans and authorship proposal for consortium publications

- **Currently planned analyses (not necessarily individual publications):**
    1. Cohort description
    2. Clinical and lifestyle factors associated with alternations in the gut microbiome
    3. Associations between baseline microbiome and biomarkers of serious non-AIDS events (IL6, D-dimer and CRP)
    4. Association between microbial factors and cardiovascular disease (primary outcome)
    5. 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
- We will nominate 8 MISTRAL/EuroSIDA investigators for proposals based solely on MISTRAL data and 4 investigators for proposals that aim to combine MISTRAL data with other project data – nominations will be rotated across the network
- Projects requesting MISTRAL data/samples
    - Investigating the gut resistome in patients with poor and good immune reconstitution (Jordi Villa and Jose Miro, Hospital Clinic Barcelona)
- If you have ideas or collaborative projects/grants that may benefit from MISTRAL samples, just reach out

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Publication in the making

Real time monitoring of REDCap data using R Markdown to increase data quality and create efficient quality assurance processes

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Background

- REDCap is an excellent and free service for collecting clinical data, but has some limitations
  - Linkage of data and identification of data errors **across different REDCap forms** or different protoocls (e.g. EuroSIDA REDCap vs MISTRAL) or different data collection systems (e.g. REST vs REDCap) is difficult
  - **Visualisation** and **report making** across forms in REDCap is time consuming

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Background

- Bioinformatics is a scientific speciality that uses computational methods to analyse, interpret and visualise complex biological data (e.g. genetics, metabolomics, microbiome etc.)
- We reasoned that these methods could also be used to improve data quality coming in and visualisation of these data for reporting/presentations/monitoring

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Methods

- Querying, data quality and visualisation needs were outlined by the cohort coordination team at CHIP and this was transformed into code by a bioinformatician integrated within the project
- The system was developed in R (a free software for statistical computing and data visualisation)
- Reports and figures were made using R Markdown and presented via interactive HTML files

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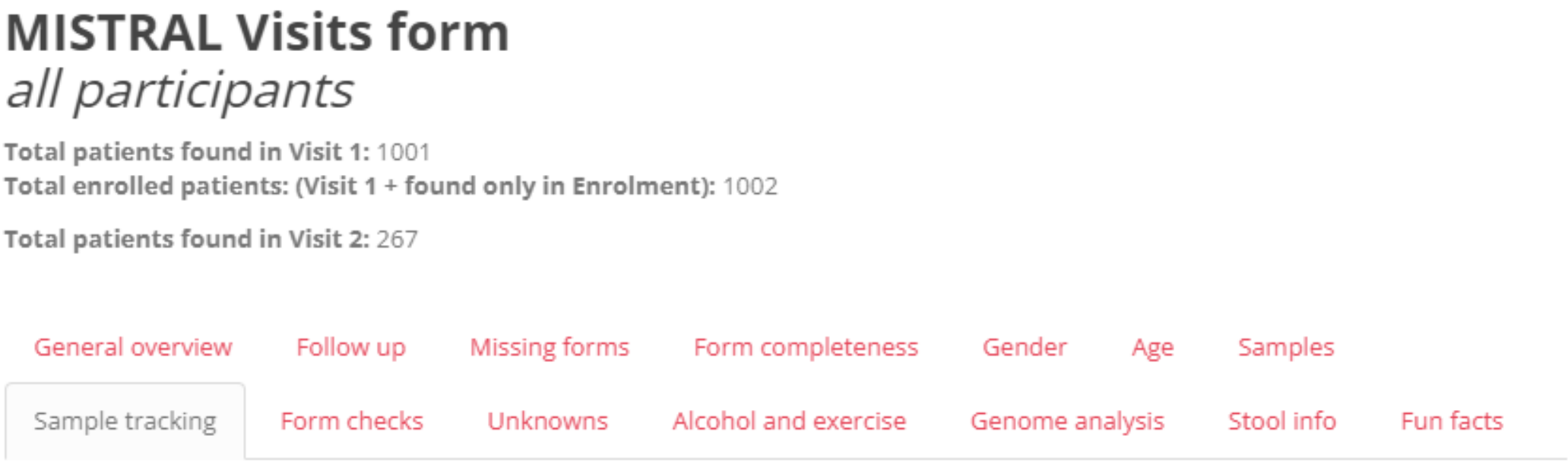
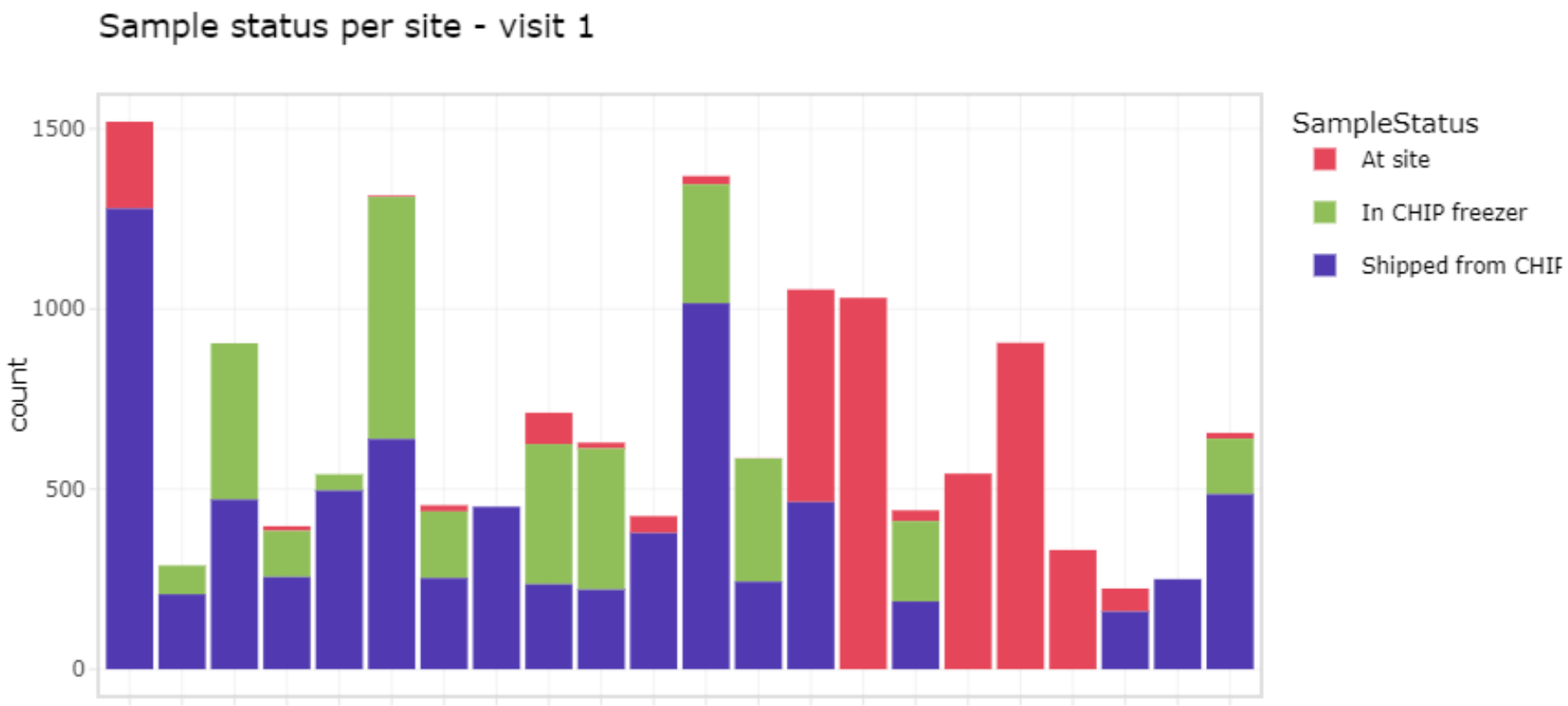
User friendly design for coordination meetings and presentation of interim results



Sample tracking



Visualisation and report generation of potential data errors

Birthdates that does not match with **enrolment**

Patient ID	Enrolment b-day	Visit1 b-day
192943	1969	1946
387410	1970	1970
387414	1971	1971
636534	1953	1954

```r
Enrolment_subset <- Enrolment[(Enrolment$bas_patient %in% Visit1$bas_patient),]
Visit1_subset <- Visit1[(Visit1$bas_patient %in% Enrolment_subset$bas_patient),]

Birthd_subset <- data.frame("bas_patient" = Enrolment_subset$bas_patient,
                            "enrol_bd" = Enrolment_subset$bas_birth_d,
                            "visit_bd" = Visit1_subset$es_bas_birth_d) %>%
  mutate(Error = ifelse(enrol_bd==visit_bd, 0,1))

subset(Birthd_subset,Error==1) %>%
  select(.,-c("Error")) %>%
  mutate(bas_patient = floor(bas_patient*runif(1))) %>%
  # Remove errors that have been fixed in the download
  .[!(.$"bas_patient" %in% c("1518007","3048003","3048032")),] %>%
  datatable(., rownames = F, colnames = c("Patient ID","Enrolment b-day","Visit1 b-day"), #caption = "Birthdate",
            options = list(dom = 't', columnDefs = list(list(className = 'dt-center', targets = "_all"))), class = table_style)
```

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Next steps

- Currently being implemented in EuroSIDA and other CHIP projects
- Abstract being prepared for IWHOD and publication planned with code to be made publicly available

General reminders

- **EuroSIDA and MISTRAL F2F scientific meeting at HIV Glasgow Congress**
  - When: Monday 11th November 2024, 7:30-9:00am
  - Location: Conference venue, Room M4
  - Please let us know if you will be participating

- **MISTRAL and EuroSIDA follow-up forms**
  - Planned to be 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*

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

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Reimbursement

- Reimbursement has been delayed but we are working on getting it to you ASAP
- 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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Wrap-up

- Thanks to the site staff and the participants for your continued commitment to this project
- Do not hesitate to contact us at CHIP via mistral.rigshospitalet@regionh.dk or consult the FAQs on our website
- Look out for our next **newsletter** (November)

MISTRAL

MISTRAL

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EuroSIDA