BHIVA
British HIV Association

2022 Spring Conference

Wed 20th - Fri 22nd April
Manchester Central, Manchester
Science Session
(Brian Gazzard Lecture)

Chair: Dr Tristan Barber
Co-chair: Dr Matthew Page
BHIVA
British HIV Association

2022 Spring Conference

Wed 20\textsuperscript{th} - Fri 22\textsuperscript{nd} April
Manchester Central, Manchester
HIV pathogenesis & the role of the microbiome

Dr Roger Paredes
Germans Trias i Pujol University Hospital/Irsi Caixa, Spain
HIV pathogenesis & the role of the microbiome

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Department of Infectious Diseases
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Hospital Universitari Germans Trias i Pujol
Badalona, Catalonia, Spain
HIV DAMAGES THE GUT

Is HIV associated with gut dysbiosis?
Aims in HIV

Reduce HIV transmission

Decrease chronic inflammation

Improve vaccine and HIV cure responses

Predict and prevent immune therapy adverse events
Stratification in mAb therapy for COVID-19

a) Seronegative vs seropositive

- Seronegative: Rate ratio, 0.80 (0.70–0.91), P=0.0010 by log-rank test
- Usual care
- REGEN–COV

- Seropositive: Rate ratio, 1.09 (0.95–1.26)
- Usual care
- REGEN–COV

b) All participants

- Rate ratio, 0.94 (0.86–1.03), P=0.17 by log-rank test
- Usual care
- REGEN–COV

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No. at risk, Seronegative
REGEN–COV: 1633  1429  1325  1260  1224
Usual Care: 1520  1308  1173  1088  1059

No. at risk, Seropositive
REGEN–COV: 2636  2452  2322  2252  2201
Usual Care: 2635  2503  2375  2292  2243
MISTRAL
Microbiome-based stratification of individuals at risk of HIV-1 acquisition, chronic clinical complications, antimicrobial drug resistance, and unresponsiveness to therapeutic HIV-1 vaccination

This Project has received funding from the European Union’s Horizon 2020 research and innovation programme under grant agreement No 847943

www.mistral-hiv.eu
MISTRAL-HIV.eu

Data types
Clinical & epidemiological
Virology and reservoir
High-throughput immunology
Host single-cell transcriptomics
HIV-1 Reservoir assays
16S RNA Sequencing
Shotgun sequencing
Fecal proteome
Plasma metabolome
Phenotypic AMR

WP7. Data integration & systems biology
WP8. Microbiome-based patient stratification software

WP1. Correlates of HIV protection and control
WP2. Microbiome-based modulation of HIV immunotherapy (HTI vaccine) in C57/bl6 mice
WP3. HTI vaccine RCT (AELIX-002)
WP4. Microbiome correlates of severe AIDS/non-AIDS events
WP5. Impact of ART in the gut resistome
WP6. RCT to modulate the microbiome in PLWH:
- RCT1: ART initiation in late presenters
- RCT2: Symbiotics in HIV-infected subjects with CD4<500 cells/mm³

WP9. Regulatory procedures
WP10. Management, Exploitation and Communication

This Project has received funding from the European Union’s Horizon 2020 research and innovation programme under grant agreement N0 847943
HIV transmission
Vaginal microbiome affects mucosal homeostasis

*Lactobacillus* dominant

- Low pH
  - Glycogen
  - *Lactobacilli* (crispatus, jensenii, etc.)
  - H$_2$O$_2$
  - Lactic acid
  - Mucins
  - IgG
  - IgA
  - Stratum corneum
  - Stratified squamous epithelia
  - Homeostatic cells and cytokines

Vaginal microbial dysbiosis (Bacterial vaginosis)

- Higher pH
  - *Gardnerella*
  - *Atopobium*
  - *Mobiluncus*
  - *Prevotella*
  - Odour (Putrescine Cadavarine)
  - Short-chain fatty acids
  - Sialidases
  - IgA breakdown
  - Inflammatory cytokines
  - Epithelial disruption
  - Mucins
  - Activated T-cells
  - Inflammatory cells

Adapted from: Burgener et al, Curr. Opinions in Immunology, 2015

- BV associates with a 60% increase in HIV acquisition risk (Atashili *et al*, AIDS, 2008)
Vaginal dysbiosis - TDF metabolism

![Graph showing Lactobacillus dominance and HIV-1 infection rates.](image)

A. Lactobacillus dominant
- Efficacy: 61% (95% CI: 11 to 84%)

B. Non-Lactobacillus dominant
- Efficacy: 18% (95% CI: -77 to 63%)

<table>
<thead>
<tr>
<th>Lactobacillus dominant</th>
<th>Tenofovir</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV-1 infections</td>
<td>9</td>
<td>22</td>
</tr>
<tr>
<td>HIV-1 incidence per 100 person-years</td>
<td>2.7</td>
<td>6.9</td>
</tr>
<tr>
<td>HIV-1 protection effectiveness</td>
<td>61% (95% CI, P-value)</td>
<td>18% (-77, 63), p=0.644</td>
</tr>
</tbody>
</table>

Klatt et al., Science 2017
Vaginal bacterial metabolism associates with HIV acquisition risk

*(Adjustments for age, study arm, contraceptive usage, sex behaviors, and condom usage did not significantly change this observation

Courtesy A. Burgener
Gut microbiome & risk of HIV acquisition & disease progression

- Bacteria candidates to protect from:
  a. HIV-1 acquisition
  b. HIV-1 progression
  c. HIV-1 acquisition and progression

- Preparation of bacterial lysates for testing

- Screening for ability to infect PBMCs with HIV-1 compared with no bacteria

- Confirmation of protection from SIV infection and disease progression in non-human primate models (collaboration with J Brenchley, NIH)

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HIV clinical complications

The microbiome is linked to nadir CD4+ T-cell counts
Gut dysbiosis linked to nadir CD4-T-cells

Richness of human gut microbiome correlates with metabolic markers

Guillen Y et al. Mucosal Immunology 2019
Oxidative stress adaptation
Both sexual practice & HIV infection impact the gut microbiota
Nadir CD4+ T-cell counts linked to dysbiosis
Nadir CD4+ T-cell counts linked to mortality

Mortality According to CD4 Count at Start of Combination Antiretroviral Therapy Among HIV-infected Patients Followed for up to 15 Years After Start of Treatment: Collaborative Cohort Study

Margaret T. May,1 June-Jenesa Vohr-Hicks,1 Adam Trickey,1 Nicole Bohm,1 Peter Salee,15 Fabrice Rouzet,15 Mattea Marie-Krause,4 Natasha Yama2,3,4,5,6,7,8 Matthew Feinberg,9 Michael John Gillis,10 Leah C. Shepherd,11 Ruth M. G當,12 Andrea de Amorim Mendonc,12 Peter A. Dworkin,9 Margaret M. Johnson,13,14 Paul Scholler-Rego,13 Peter Bosch,15 Robert Zangrilli,15 Amy C. Justice,15,16,17,18 Donald R. Giuliano,15 Jana M. Miro,15,19 and Jonathan A. G. Stanford for the Antiretroviral Therapy Cohort Collaborative (ART-CC)
MISTRAL – EuroSIDA microbiome cohort

• To establish a faecal microbiome sample repository for the EuroSIDA clinical cohort of HIV-infected patients for use in metagenomics analyses.

• To evaluate the association between gut microbiome composition and function and the risk of developing cardiovascular disease and severe AIDS and non-AIDS events.
HIV vaccines

Bacteroides / Clostridiales ratio, HIV viremia control and reservoir
Aim of the study

Understanding the potential impact of the gut microbiome in BCN02 vaccine outcome

Vaccine outcome

3 MAP-Controllers
10 MAP-Non-controllers

Mothe et al, Front Immunol., 2020, 11:823
**Bacteroidales/Clostridiales** ratio discriminates between viremic controllers and non-controllers

**Borgognone et al., Microbiome 2022 (in press)**
Gut microbiome-associated signatures confirmed at species level

Borgognone et al., Microbiome 2022 (in press)
Archaea depletion in viremic controllers

Borgognone et al., Microbiome 2022 (in press)
Lower gut microbial diversity and richness in viremic controllers

Borgognone et al., Microbiome 2022 (in press)
B/C ratio negatively correlated with HIV-1 viral reservoir size

HIV-1 DNA

Cell-associated HIV-1 RNA

Borgognone et al., Microbiome 2022 (in press)
Baseline Clostridiales-derived bacterial proteins discriminate controllers and non-controllers

Borgognone et al., Microbiome 2022 (in press)
Host PBMCs transcriptome

- 31 differentially expressed host genes between viremic controllers and non-controllers;
- Upregulated genes in viremic controllers functionally enriched in immune activation and inflammatory response.

Plasma protein inflammation markers

- 7 out of 92 inflammation-related protein differentially expressed between the two groups;
- All differentially expressed proteins were more abundant in viremic controllers.

Borgognone et al., Microbiome 2022 (in press)
Pre-existing Bacteroidales species and immune activation signatures inversely correlated with HIV-1 reservoir size

- Bacteroidales species and immune activation signatures inversely correlates with viral reservoir size (HIV-1 DNA and CA-HIV-1 RNA);

- An opposite trend is observed for Clostridiales species.

Borgognone et al., Microbiome 2022 (in press)
MISTRAL – Vaccine validation studies

DDDMM (N=30) → CCM (N=30) → 24 week Analytical Treatment Interruption (N=45) → Restart Antiretroviral Therapy (N=45)

PPPPP (N=15) → PPP (N=15) → Microbiome Samples → Microbiome Samples → Microbiome Samples → Microbiome Samples

D; DNA-HTI, M; MVA-HTI, C; ChAdOx1-HTI, P; placebo vaccine
MISTRAL – Vaccine validation studies

- Fresh IFNγ ELISPOT in PBMC covering HTI and non-HTI HIV regions (clade B 15mer overlapping peptides - OLP)

![Graphs showing HTI dominance over total HIV and high HTI dominance at ATI start with percentage values]

Mothe B. et al
LB 02669 – vCROI2021
www.mistral-hiv.eu
MISTRAL – Vaccine validation studies

All Cohort (n=41)

Participants Without Beneficial HLA class I alleles (n=32)
MISTRAL – Vaccine validation studies

Participants Without Beneficial HLA class I alleles

* 2 last vaccinees dropped out due to COVID-19 without meeting pre-specified ART resumption criteria
Validation

- RV 306 (CIHR, A Burgener)

Figure 1: RV306 study design

Each RV306 participant received ALVAC-HIV and AIDSVAX B/E either alone or in combination, at the indicated timepoints. Participants were randomised to one of five groups and further randomised within each group to receive either vaccine product or placebo injections at the ratio indicated for each group displayed on the right. Participants were followed for 24 months in total.
## SUMMARY: VAGINAL VS. GUT MICROBIOME

<table>
<thead>
<tr>
<th>Health Status</th>
<th><strong>Vagina</strong></th>
<th><strong>Gut</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy status</td>
<td>Low microbial richness / diversity</td>
<td>High richness/diversity</td>
</tr>
<tr>
<td>Lactobacillus dominance</td>
<td>16S rRNA community types structures 1 &amp; 2</td>
<td>Uncertain Enterotypes not linked to HIV (<em>Prevotella</em> linked to MSM)</td>
</tr>
<tr>
<td>Dysbiosis</td>
<td>High microbial richness</td>
<td>Reduced microbial gene richness</td>
</tr>
<tr>
<td>16S rRNA community types structures 3 &amp; 4</td>
<td>Increase in ROS-resistant bacteria including <em>Proteobacteria</em></td>
<td>Low nadir CD4+ T-cell counts</td>
</tr>
<tr>
<td><em>Prevotella, Gardnerella, Mobiluncus, Atopobium</em></td>
<td>Decrease in methanogens &amp; ROS-sensitive bacteria</td>
<td>Local &amp; systemic inflammation</td>
</tr>
<tr>
<td>Increased pH</td>
<td>Decreased pH</td>
<td><em>Uncertain: HIV acquisition, AIDS progression, ARV metabolism, CV disease, metabolic syndrome, immune reconstitution, HIV persistence</em></td>
</tr>
<tr>
<td>Clinical associations</td>
<td>Local inflammation (CT 3 &amp; 4)</td>
<td>Not yet</td>
</tr>
<tr>
<td>HIV acquisition</td>
<td>HIV acquisition</td>
<td>FMT not effective (small studies, limited engraftment)</td>
</tr>
<tr>
<td>ARV drug metabolism (TDF, dapivirine, but not TAF)</td>
<td>ARV metabolism, CV disease, metabolic syndrome, immune reconstitution, HIV persistence</td>
<td>Probiotics / synbiotics or ART: effect uncertain</td>
</tr>
<tr>
<td>Prospects of effective interventions in the short term</td>
<td>Yes</td>
<td>Engineered Lactobacilli (lactin-V) (post ATB)</td>
</tr>
<tr>
<td>Yes</td>
<td>FMT not effective (small studies, limited engraftment)</td>
<td>Not yet</td>
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<td></td>
</tr>
<tr>
<td>Therapeutic challenges</td>
<td>Preventing recurrences</td>
<td>Understanding the principles for effective, long-term, purpose-driven gut microbiome modulation</td>
</tr>
<tr>
<td>Social / biomedical factors</td>
<td></td>
<td></td>
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</tbody>
</table>

**SUMMARY:** VAGINAL VS. GUT MICROBIOME

Conclusions

• The gut microbiome might be used to stratify HIV patients as to
  • Their risk of HIV acquisition
  • Their risk of developing severe clinical complications
  • Their ability to respond to HIV-directed immunotherapy

• Validation studies in large, independent cohorts and randomized clinical trials are ongoing

• Need for biomarker “simplification” and automated microbiome-based stratification computer tools to facilitate clinical research and interpretation → Ongoing
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