Metabolomic Profile Associated with Loss of Spontaneous HIV1 Elite Control
The aim of this work was to perform a metabolomic approach to identify the underlying mechanistic pathways and potential predictive biomarkers associated with the loss of virological control in HIV-1 Elite controllers.
Transient Controllers (n=8)

Persistent Controllers (n=8)
Transient Controllers (n=8)

Persistent Controllers (n=8)
Sample collection/processing & metabolite measurement

- Blood, urine, tissues, cells.
- Tissue homogenisation.
- Metabolite extraction/derivatisation.
- Extracted/derivatised metabolites.
- Metabolite quantification using LC-MS, GC-MS, NMR, by targeted/untargeted approaches.

Metabolomic data processing & analyses

- XIC of +MRM (316 pairs): 454.3/18...
- Raw data.
- Data extraction, metabolite identification, normalisation and scaling.

- Heatmaps, metabolic markers, predictive models, pathway analysis, biological interpretation.

Application of bioinformatic tools (univariate/multivariate statistics, identification of metabolite signatures and integration of information with existing knowledge/data).

Data matrix.
1. **Determination of metabolites of energy metabolism by GC-(EI)qTOF/MS** → **70 metabolites**

2. **Untargeted Lipidomic analysis by LC-qTOF/MS** → **334 lipids.**
Determination of polifunctionality of HIV-specific CD8-T cell response by the intracellular production of IL-2, TNF-α and IFN-γ after Gag stimulation by multiparametric flow cytometry
**Valine**
**Iminodiacetic acid**
**2-ketoisocaproic acid**
**Pyruvic acid**
**Alpha tocopherol**
**Succinic acid**
**Glycolic acid**
**Hexanoic acid**
**Lactic acid**
**Glutamic acid**
**Aspartic acid**
**Isoleucine**
**Leucine**
**Oxaloacetic acid**
**Glycine**
**Phosphoenolpyruvate**
**alpha-Ketoglutaric acid**
**Cholesterol**
**Galacturonic acid 2**
**Malic acid**
**Heptadecanoic acid**
**Glycerol**
**Citric acid**
**Phosphoric acid**
**Limonene**
**Fumaric acid**
**Phosphoglycolic acid**
**trans-Aconitric acid**
**2-hydroxybutyric acid**
**trans-4-hydroxy-L-proline**
**3-Phosphoglyceric acid**
**glycerol-1-phosphate**
**3-Hydroxybutyric acid**
**Aconitic acid**
**Stearic acid**
**Creatinine**
**Pelargonic acid**

OOB estimate of error rate = 12.5%

Confusion Matrix

<table>
<thead>
<tr>
<th></th>
<th>PC</th>
<th>TC</th>
<th>Class. error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Valine</td>
<td>0</td>
<td>8</td>
<td>0.00</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>AUC</th>
<th>p-value</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpha tocopherol</td>
<td>0.891</td>
<td>0.001</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Succinic acid</td>
<td>0.864</td>
<td>0.003</td>
<td>87.5</td>
<td>87.5</td>
</tr>
<tr>
<td>Pyruvic acid</td>
<td>0.891</td>
<td>0.009</td>
<td>87.5</td>
<td>87.5</td>
</tr>
<tr>
<td>hexanoic acid</td>
<td>0.813</td>
<td>0.036</td>
<td>87.5</td>
<td>87.5</td>
</tr>
<tr>
<td>iminodiacetic acid</td>
<td>0.969</td>
<td>0.002</td>
<td>87.5</td>
<td>87.5</td>
</tr>
<tr>
<td>alpha-Ketoglutaric acid</td>
<td>0.844</td>
<td>0.021</td>
<td>87.5</td>
<td>87.5</td>
</tr>
<tr>
<td>2-ketoisocaproic acid</td>
<td>0.953</td>
<td>0.002</td>
<td>87.5</td>
<td>87.5</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>0.797</td>
<td>0.046</td>
<td>87.5</td>
<td>87.5</td>
</tr>
<tr>
<td>Leucine</td>
<td>0.797</td>
<td>0.046</td>
<td>87.5</td>
<td>87.5</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>0.813</td>
<td>0.036</td>
<td>87.5</td>
<td>87.5</td>
</tr>
<tr>
<td>Valine</td>
<td>0.844</td>
<td>0.021</td>
<td>87.5</td>
<td>87.5</td>
</tr>
<tr>
<td>Lactic acid</td>
<td>0.891</td>
<td>0.009</td>
<td>87.5</td>
<td>87.5</td>
</tr>
</tbody>
</table>
alpha tocopherol

pyruvic acid

alpha-ketoglutaric acid

2-ketoisocaproic acid

CD8 T-cell pINDEX 3 functions

r = -0.82
p = 0.001

r = -0.78
p = 0.003

r = -0.61
p = 0.035

r = -0.618
p = 0.032

r = 0.69
p = 0.012
Phospholipids and Mitochondrial dysfunction
There is a specific circulating metabolomic profile before the loss of control associated with the loss of spontaneous HIV-1 elite control.

Valine stands out as the main factor differentiating both studied groups.

All the observed metabolomic differences should be considered not only as potential biomarkers for a rapid screening of future loss of virological control but also as plausible therapeutic targets in HIV infection.
Infection and Immunity Research Group
Francesc Vidal Marsal, MD, PhD
Joaquim Peraire Forner, MD, PhD
Consuelo Viladés Laborda, MD, PhD
Sergi Veloso Esteban, MD, PhD
Miguel López-Dupla, MD, PhD
Montserrat Vargas Laguna
Alfonso Javier Castellano Guerrero
Verónica Alba Elvira
Anna Rull Aixa, PhD
Esther Rodríguez Gallego, PhD

HIV Infection and the Pharmacokinetics of Antiviral Drugs, IBIS
Ezequiel Ruiz-Mateos, PhD
Laura Tarancón-Diez

On behalf of ECRIS integrated in the Spanish AIDS Research Network