Immunological Markers Associated with HIV Persistence During ART Identified by Fuzzy Forests Analysis

Immunological mechanisms of HIV persistence during ART

Latent reservoir
- Cell Proliferation
- Cytokine/Homeostasis
- Antigen/TCR

Active reservoir
- CD4+ T Cell Activation
- Cytokines
- Antigen/TCR

Cell Proliferation
- Virus Spread
- Blunted Immune Clearance (CTL)

Chronic Inflammation

Treg

PD-1

CD8+ T Cell


Courtesy of Steven Deeks
## HIV persistence during ART

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Identifying biomarkers of HIV persistence during ART is of primary importance for the development and the monitoring of curative strategies.
Rational of the study

DARE48 cohort

600 variables

Clinical data
Virological markers
Cell subsets
Chemokines/Chemokine receptors
Proliferation/Activation markers
Imune Checkpoints/IC ligands
IFNs signaling
γ cytokines
IL-6, IL-10 signaling
Inflammation

Gender
Integrated HIV DNA
CD3
CCR5
Ki67
HLA-DR
PD1
pSTAT1
IL-2
pSTAT3
pp38

Ethnicity
Total HIV DNA
CD4
CCR6
CD38
CTLA-4 (CD152)
IL7
sCD14

Age
2-LTR circles
CD8
CCR7
LAG3
IL15

ARVs
CA
US
HIV RNA
CD45RA
CXCR3
TIGIT
CD127

Years on ARVs
CD27
CXCR5
BTLA (CD272)
pSTAT5

CD4 count
CCR7
CCL4
TIM3

CD8 count
CD14
CCL5
CD160

CD4 nadir
CD19
CCL20
2B4

Year of 1st HIV positive test
CD16
CCL19
PDL1

Viral Load
HLA-DR
CCL21
PDL2

CXCL9
Galectin9
CXCL10
CD155
CXCL11
HLA-DR
CXCL12
CD80
CXCL13
CD86

DARE48 cohort
48 subjects on suppressive ART

Important variables associated with HIV persistence during ART

Age, years, median [IQR] 56.5 [50.3-61.75]
Male, n (%) 46 (96%)
Viral load, copies/mL <40
Time since HIV diagnosis, year, median [IQR] 22 [15-26]
Time on ART, year, median [IQR] 8.5 [5.0-12.4]
Self reported lowest CD4 count, cells/uL, median [IQR] 197 [110-285]
CD4 count, cells/uL, median [IQR] 684 [530-862]
CD8 count, cells/uL, median [IQR] 914 [639-1091]
CD4/CD8 ratio

Gating strategy
Fuzzy Forests

1) Create a **network** of variables and identify modules that cluster together. The clusters have high correlation within and low correlation between, suggesting a common pathway or process.

2) Recursive Feature Elimination Random Forests on each module = Filter the best predictors from each module.

3) All "survivors" from the other rounds compete in a **final Recursive Feature Elimination Random Forest** => End up with final list of variables of importance (predictors). This allows one to account for interactions between the modules.

Adapted from Zheng Chen (Washington U, St Louis, USA)
How variables are related between them? 

Network analysis

The network analysis shows that 30% of the variables are clustering in 6 distinct modules that reflect different immunological mechanisms.
Example: Variable of importance for 2-LTR circles

- TEMRA CD8+ PD1+ (%)
- Em CD8+ PD1+ (%)
- CD4+CD8+ PD1+ (MFI)
- CD4+ CD38+ (MFI)
- CD4-CD8- 2B4+ (%)
- CD4+CD8+ PDL2+ (MFI)
- CD16+ Monocytes PDL2+ (%)
- Naive CD4+ CD38+ (%)
- CD4-CD8- 2B4+ (MFI)
- CD4-CD8- PDL2+ (MFI)
Fuzzy Forest analysis identifies distinct predictors for different virological markers of HIV persistence.
The predictors for different virological markers of HIV persistence are enriched for distinct modules.
Fuzzy Forest analysis identifies:

A  Integrated HIV DNA
   IFNs signaling
   Susceptibility to HIV infection

B  Total HIV DNA
   Inflammation
   T cell activation
   IFNs signaling
   Susceptibility to HIV infection

C  2-LTR circles
   CD8 exhaustion

D  US HIV RNA
   IFNs signaling
   Inflammation
   Susceptibility to HIV infection

HIV persists through different mechanisms (latency and replication) that are associated with distinct immunological markers.
Conclusion and Future Steps

- Limitation: Cell-associated DNA/RNA measurements only, replication competent virus was not evaluated
- Fuzzy Forest findings will be validated in an independent cohort
- Fuzzy Forest is a novel, powerful network analytic tool to identify biomarkers of HIV persistence in an unbiased way

The immunological markers of HIV persistence identified by Fuzzy Forest Analysis establish the framework for the development and the monitoring of novel curative immunotherapies
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The study participants
The variables are highly clustered within the module and near independent between the modules.
Variables of importance

**A. Integrated HIV DNA**
- CD4+ CD45RA-CD27-CCR7+ (%)
- Naive CD4+ HLA-DR+CD38+ (%)
- CD4+CD8+ TIGIT+ (%)
- CD8+ pSTAT5+ (%)
- Naive CD4+ pp38+ (%)
- TEMRA CD8+ PD1+ (%)
- Naive CD4+ HLA-DR+ (%)
- Cm CD4+ TIGIT+ (%)
- CD4+ (%)
- CD4+ CXCR3+ (MFI)

**B. Total HIV DNA**
- DCs PDL1+ (MFI)
- Cm CD4+ 2B4+ (%)
- Tm CD8+ pSTAT3+ (MFI)
- Tm CD4+ CCR7+ (MFI)
- CD4+CD8+ PDL1+ (MFI)
- CD8+ pSTAT3 (MFI)
- Naive CD8+ (%)
- Cm CD8+ TIGIT+ (%)
- CD8+ TIGIT (%)
- TEMRA CD8+ pSTAT3+ (MFI)

**C. 2-LTR circles**
- TEMRA CD8+ PD1+ (%)
- Em CD8+ PD1+ (%)
- CD4+CD8+ PD1+ (MFI)
- CD4+ CD38+ (MFI)
- CD4-CD8- 2B4+ (%)
- CD4+CD8+ PDL2+ (MFI)
- CD16+ Monocytes PDL2+ (%)
- Naive CD4+ CD38+ (%)
- CD4-CD8- 2B4+ (MFI)
- CD4-CD8- PDL2+ (MFI)

**D. US HIV RNA**
- pSTAT5+ CD4+ CD45RA-CD27-CCR7+ (%)
- CD4+CD8+ 2B4+ (%)
- Naive CD4+ HLA-DR+CD38+ (%)
- DCs CD86+ (%)
- Naive CD8+ HLA-DR+ (%)
- Td CD4+ pSTAT1+ (MFI)
- CD4+CD8+ pSTAT1+ (MFI)
- Em CD4+ CCR5+ (MFI)
- DCs PDL1+ (MFI)
- Cm CD8+ TIGIT+ (%)

Strength of association