IL-18 and IL-3 in Extracellular Vesicles: Biomarkers for a Durable Elite Control

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Elite Controllers, an exceptional model of functional cure

✓ HIV-1 *elite controllers (EC)* represent a rare subset of PLWH (≈ 1%) able to spontaneously control HIV-1 replication without antiretroviral therapy (ART), representing an exceptional model of functional cure.

✓ EC are heterogeneous

- Transient controllers (TC): lose HIV-1 control over time
- Persistent controllers (PC): sustain durable HIV-1 control

✓ Only small studies have compared inflammatory profiles in TC and PC

EVs & Transport of cytokines

- ✓ Several studies have found cytokines encapsulated in and embedded on the surface of EVs. *Fitzgerald et al., Sci Rep 2018*

- ✓ EVs is a generic name that describes a heterogeneous collection of membrane-bound vesicles that are natural released from different cell types into different bodily fluids (i.e. blood, urine, saliva, semen or breast milk).

Kusama: Laurence King Publishing

- ✓ EVs have been associated with HIV pathogenesis. We have recently observed higher levels of a specific EV subpopulation (CD9+ microvesicles platelets-derived) during HIV-1 infection which were particularly elevated among those with long-term natural control of HIV-1 replication. *Poveda et al., J Infect Dis 2022*

Objective

✓ To evaluate cytokine profiles in EVs among well-characterized cohorts of PLWH, including EC with durable control (PC) and transient control (TC)

Methods

Study population: 120 donors divided into 5 groups:

- 30 ART-naïve (median 7 days after HIV diagnosis, not acute infections)
- 30 ART-treated with nondetectable viremia (median time on ART 9 years)
- 30 EC (ECRIS) who controlled viremia for a median of 14.4 years:
  - 15 TC (median of 1.4 years [IQR: 0.9-2.8] before the loss of control) & 15 PC.
- 30 HIV-uninfected controls

Objective

✓ To evaluate cytokine profiles in EVs among well-characterized cohorts of PLWH, including EC with durable control (PC) and transient control (TC)

Methods

Cytokine measurements: 39 cytokines were quantified in and on EVs isolated by ExoQuick from stored plasma using a multiplexed bead-based assay.

Statistical analyses: Random forest, PCA, and decision trees were performed to identify specific cytokines as a signature of each study group.

Heat Map of the relative cytokines levels in EVs

Overall, the median levels of cytokines were 1.33 fold higher for PLWH than for the HIV uninfected control group. Within PLWH, EC showed the highest levels of cytokines (1.11 fold higher than for ART-exposed and 1.32 higher than for ART-naïve). Within the EC the levels of cytokines were 1.36 higher for PC than for TC.

Poveda et al., CROI 2022

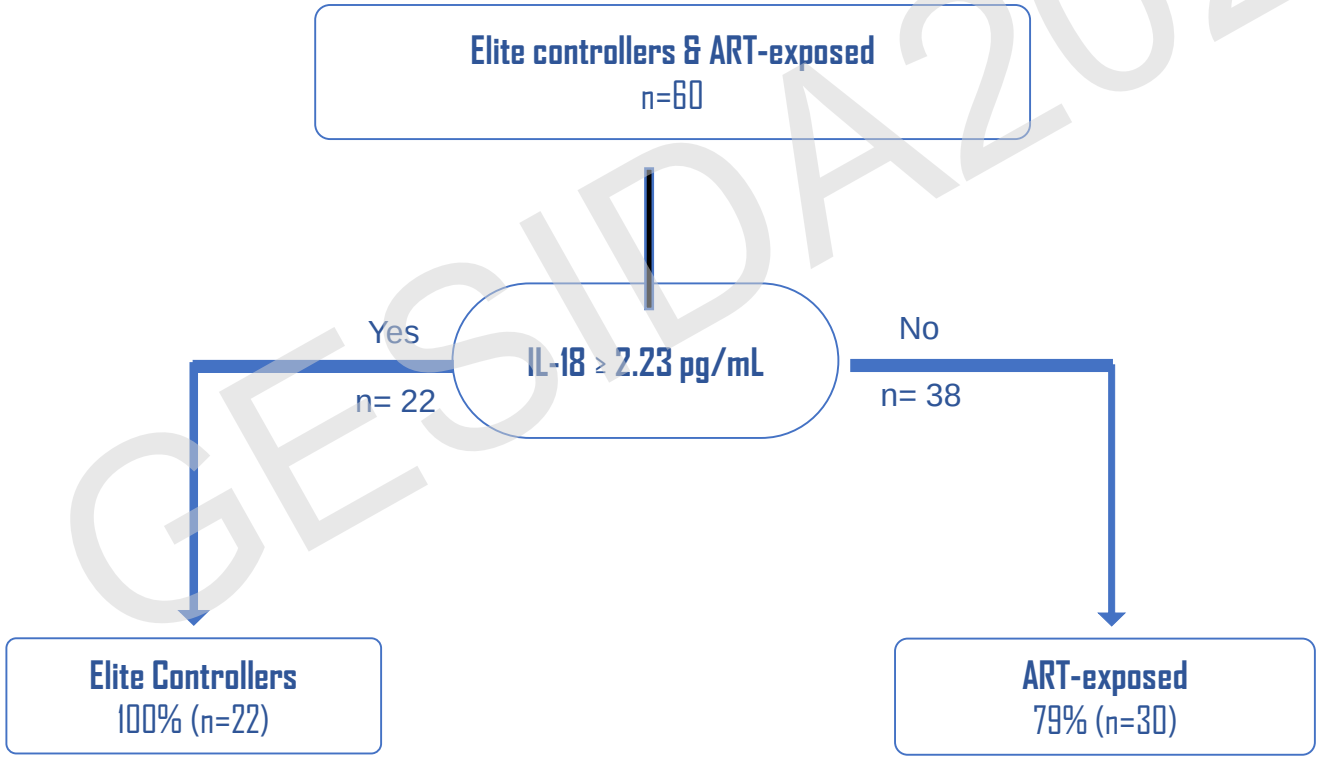
Evaluation of Specific cytokines in EVs as potential biomarkers of PLWH groups.

Groups (median)	Cytokines	AUC	Sen/Spe (%)	Cut-off pg/mL
PLWH vs. HIV- 1.15 vs. 0.26	IL-18	0.741	70/77	0.489
EC (↑) vs. ART-exposed 3.98 vs. 0.15	IL-18	0.942	73.3/100	2.23
EC (↑) vs. ART-naïve 3.98 vs. 1.56 14.08 vs.7.67 3.33 vs. 2.00 0.39 vs. 0.23	IL-18 MIG IL-22 MIP-1β	0.746 0.741 0.705 0.701	73.3/66.7 70.0/76.7 83.3/56.7 73.3/66.7	2.192 10.49 2.23 0.31
TC vs. PC (↑) 2.32 vs. 4.50 3.16 vs. 9.09 80.56 vs. 97.94 10.35 vs. 17.00	IL-3 TRAIL INF-λ MIG	0.824 0.813 0.782 0.707	73.3/86.7 86.7/73.3 66.7/93.3 93.3/60.0	3.97 3.71 81.41 10.87

Poveda et al., CROI 2022

Decision Trees Results in EVs

✓ **EC vs. ART-exposed:** 100% of EC with *IL-18 levels ≥ 2.23 pg/mL* were correctly identified within the group of PLWH and supressed viremia as EC.



Main findings

✓ EC showed higher levels of EV-associated cytokines compared with other PLWH groups.

✓ In the setting of suppressed viremia, EC showed higher levels of IL-18 than did ART-treated individuals in EVs. **IL-18** plasma levels distinguished these two populations with high sensitivity (73.3%) and specificity (100%). Within EC, higher levels of **IL-3** best discriminates between PC and TC (sensitivity-73.3%; specificity-86.7%).

✓ This study identified cytokines as potential biomarkers of EC vs. ART-exposed and within the EC, cytokines to predict sustained virologic control in the absence of treatment.

✓ Protected transport of cytokines in EVs might represent a sophisticated mechanism to deliver targeted signals to host cells selectively maintaining local environments of activation and inflammation that control HIV replication.

Biological functions

✓ **IL-18** is a pleiotropic pro-inflammatory cytokine that is increased in the circulation of PWH. It is activated by NLPR3 inflammasomes and released from cells promoting inflammatory responses such as T cells and NK cells.

✓ **IL-3** is a T-cell derived pluripotent hematopoietic colony-stimulatory factor required for the proliferation, maturation, and survival of a number of immune cells. A role for IL-3 during HIV-1 has not been well defined, its importance may reside in the support of these immune cells, in particularly plasmocytoid DC.

✓ **TRAIL** (TNF-related apoptosis-inducing ligand) is an immunoregulatory protein that can eliminate immune cells including infected cells.

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