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Dynamics and epigenetic status of regulatory T-cells following antiretroviral therapy (ART) initiation in early HIV infection

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No conflict of interest to declare

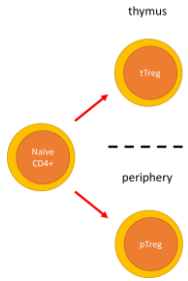
Regulatory T-cells (Tregs):

I. A subset of CD4 T cells with immunosuppressive functions (eg. TGF-β1 production, adenosine production via ectonucleotidase CD39, etc)

II. **Phenotype**: $CD4^{+}CD25^{high}CD127^{low}FoxP3^{+}$

III. Transcription factor **FoxP3** is the master regulator of Tregs functions

IV. **Tregs origin:**



Thymic Tregs (tTregs):

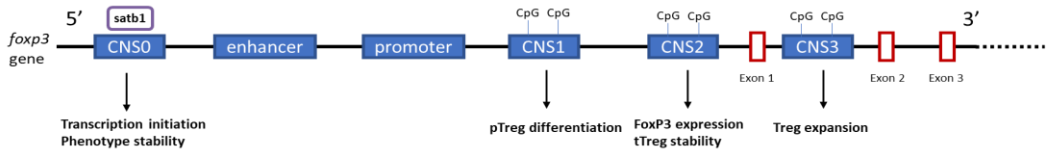
Independent linage that developed from $CD4^{+}CD8^{+}$ thymocytes within the thymus
Helios: Specific marker of Thymic Tregs

Peripheral Tregs (pTregs):

Differentiated from naïve $CD4^{+}$ T cells in peripheral tissues upon antigen stimulation and cytokines: IL-2 and TGF-β1

V. Demethylation of ***foxp3*** locus is required for stable FoxP3 expression and suppressive functions of Tregs.

Conserved non-coding sequences (CNS)



HIV infection:

I. HIV infection is associated with **Increased in Tregs frequencies** which contribute to disease progression via:

a) Inhibition of anti-HIV specific responses and immune dysfunction

b) Tissue fibrosis via **TGF-β1** production

c) HIV Viral persistence

II. $CD39^{+}$ Tregs are linked to disease progression:

Ectonucleotidase CD39 converts inflammatory ATP into ADP and AMP that is finally converted into autoinflammatory adenosine by CD73.

III. Gut-Associated Lymphoid Tissue (GALT) is the main site of SIV/HIV replication, CD4 T-cell depletion and establishment of viral reservoirs during acute infection.

IV. Early antiretroviral (ART) initiation following HIV exposure or diagnosis resulting in:

- Decrease in levels of HIV-integrated DNA => smaller HIV reservoir size
- Decrease in immune-activation
- Decrease in emergence of resistance to treatment
- Decrease in the risk of transmission

Gaps in the knowledge : Treg dynamics and the epigenetics status of *foxp3* gene during (1) acute HIV infection and (2) the impact of early ART initiation remains understudied

METHODOLOGY

Study cohort

123 individuals included in our study

- Non-infected (n=20)
- Chronic ART-Treated (n=21)
- Acute (n=26)
- Elite controllers (EC) (n=18)
- Chronic Non-Treated (n=20)
- Immunological controllers (IC) (n=18)

ART initiation: < 7 months
Median ART: 2 years

20 acute HIV-infected individuals followed for 2 years

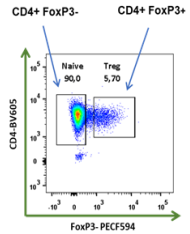
- Acute (n=20)
  - Chronic ART-Treated (n=10)
  - Chronic Non-Treated (n=10)

PBMCs from different cohorts of individuals

Ex vivo phenotypic characterization of human Tregs by multi-parameter flow cytometry

CD4 T cell purification by negative selection with microbeads (STEMCELL)

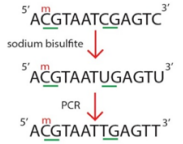
Sort Treg population based on CD4 and FoxP3 expression



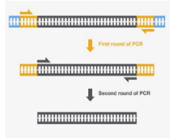
Methylation sequencing (MiSeq)

Bioinformatics analysis of *foxp3* methylation status on around 25000 sequences per region from each individual

Genomic DNA extraction

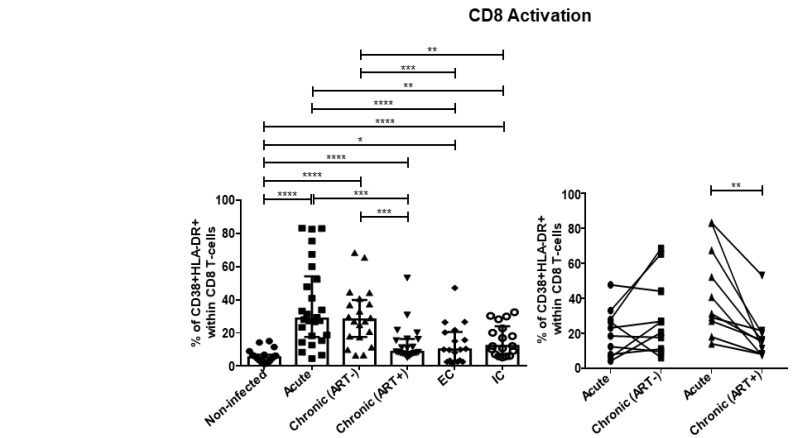


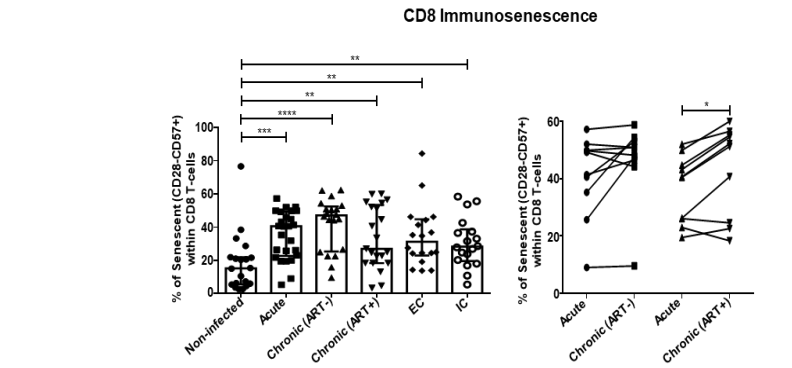
Bisulfite conversion (QIAGEN bisulfite kit)

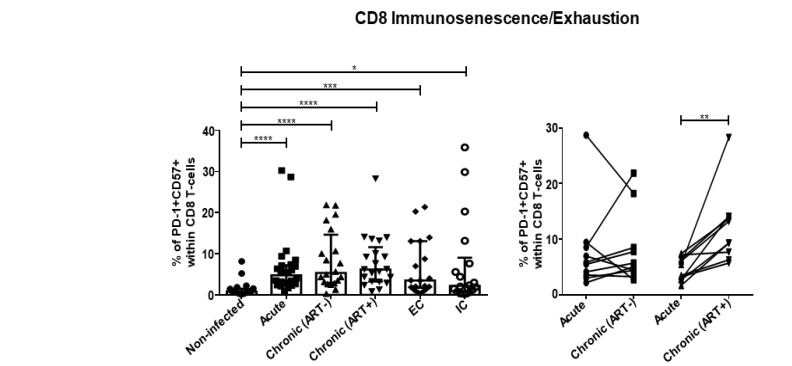


Nested PCRs using specific primers for bisulfited DNA

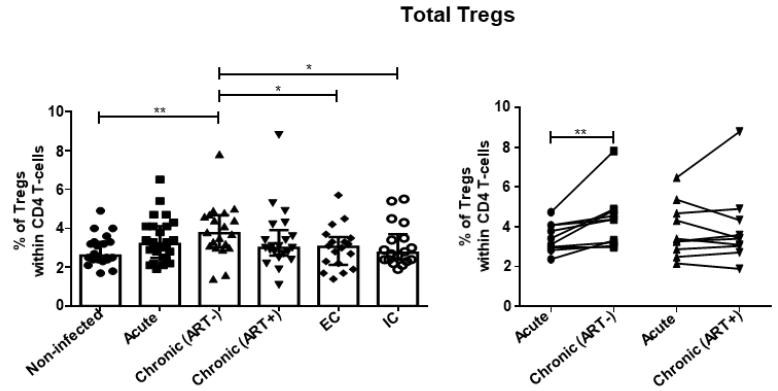
Increased in CD8 T-cell immune activation, senescence and exhaustion overtime during HIV infection, which were reduced, but not normalized by early ART initiation

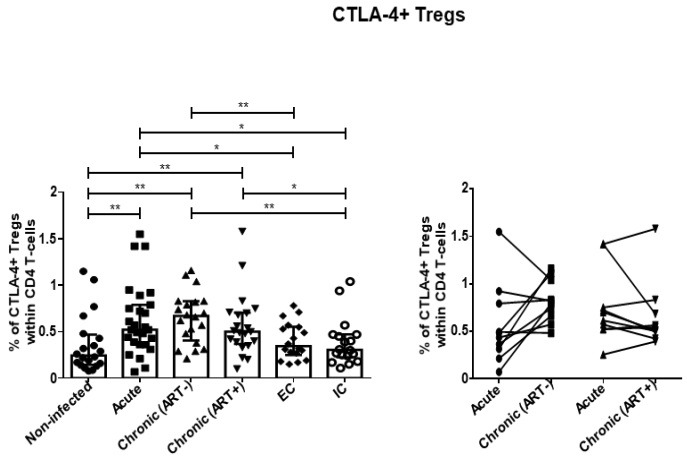


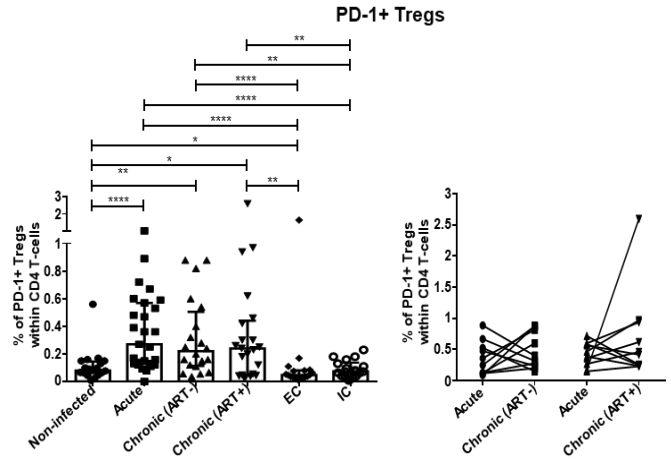


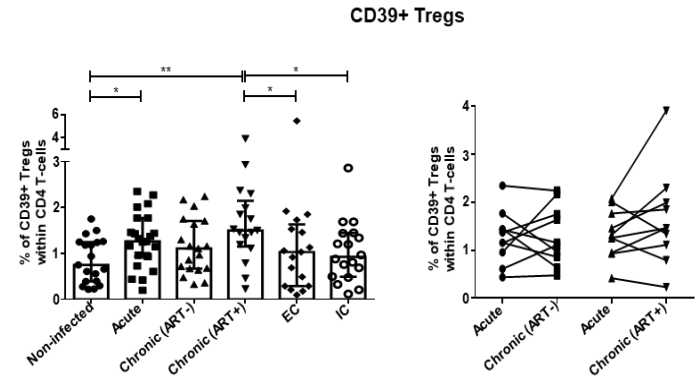


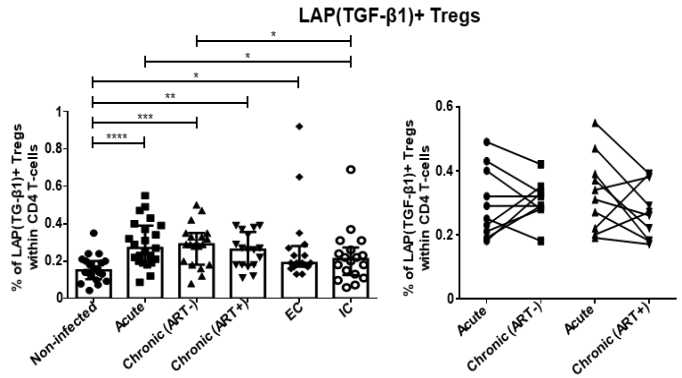
HIV infection increased Treg subsets with highly immunosuppressive capacity, which were not restored following early ART initiation



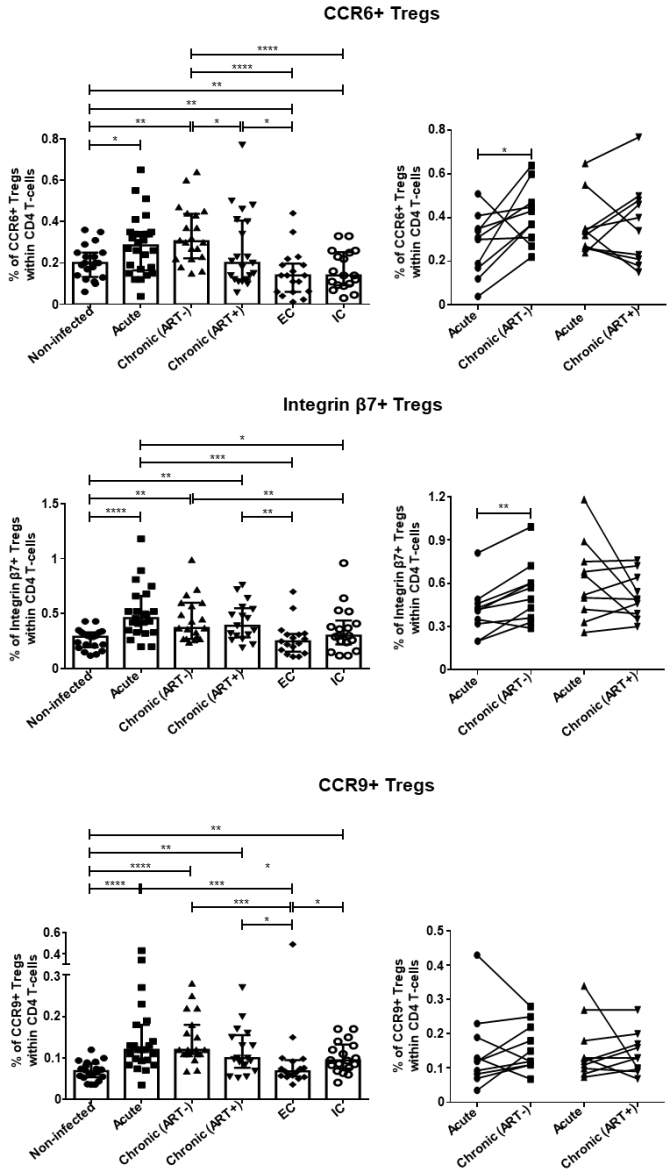




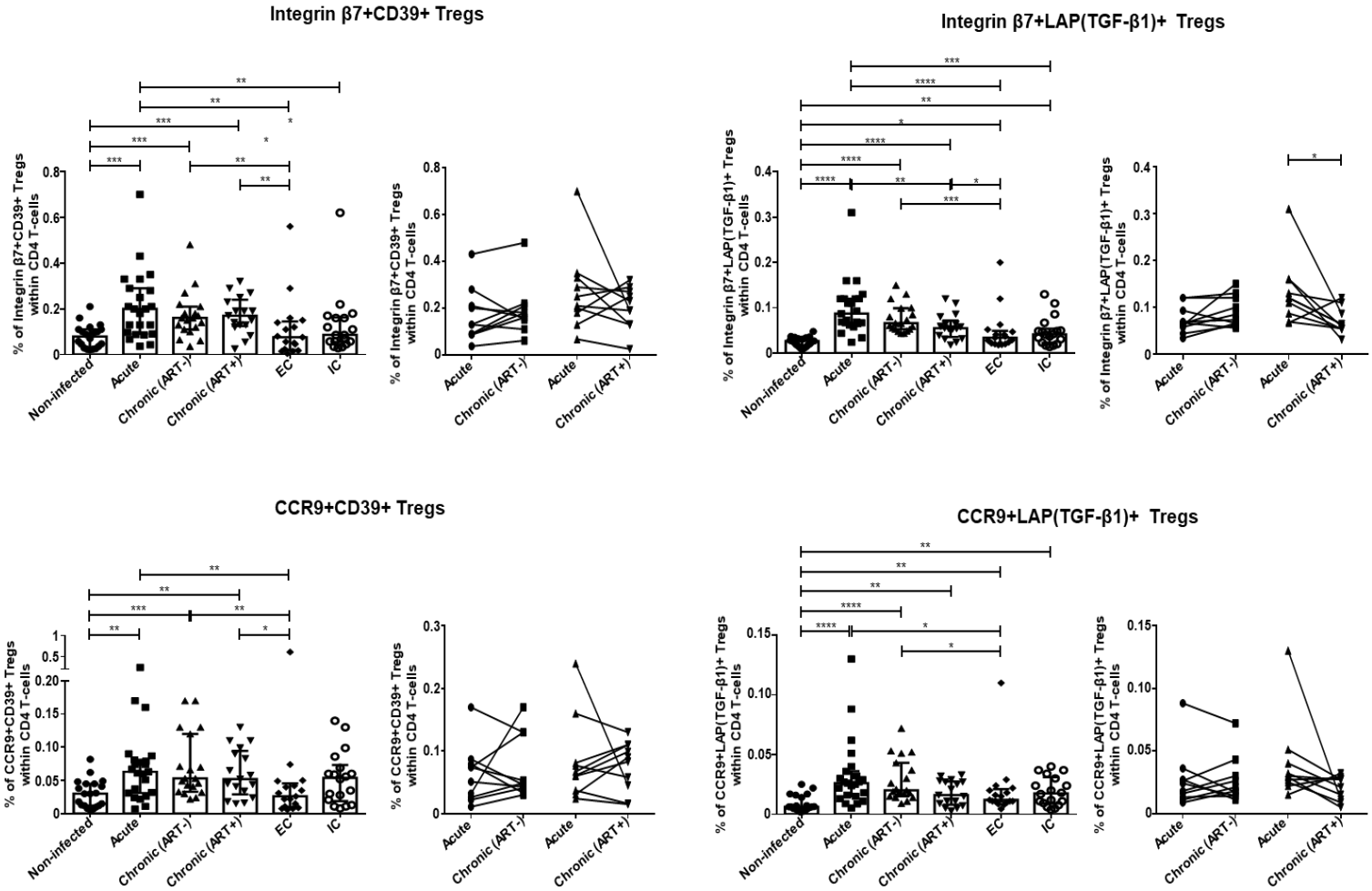




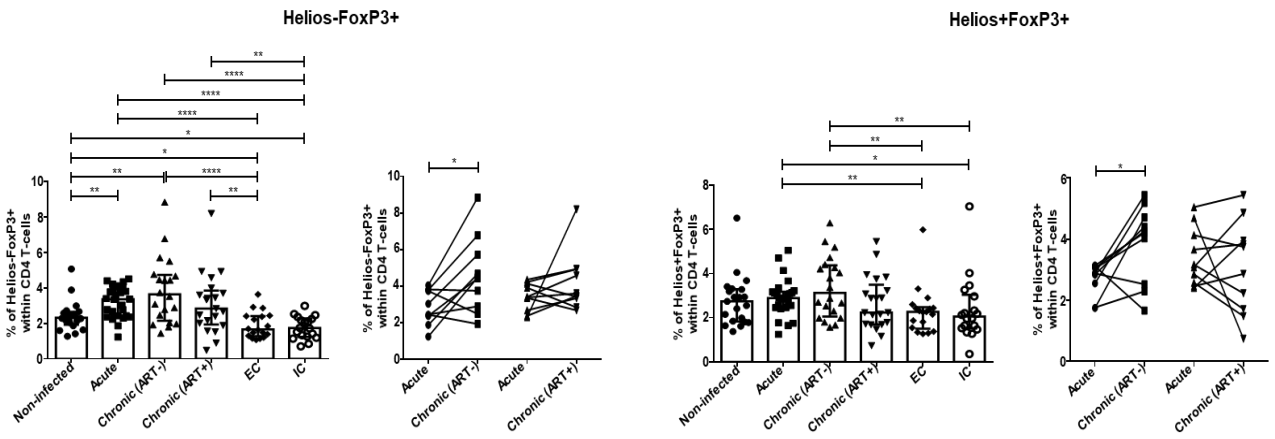
HIV infection was associated with increased expression of gut homing markers by Tregs, while early ART initiation normalized Integrin β7⁺ and CCR9⁺ Tregs



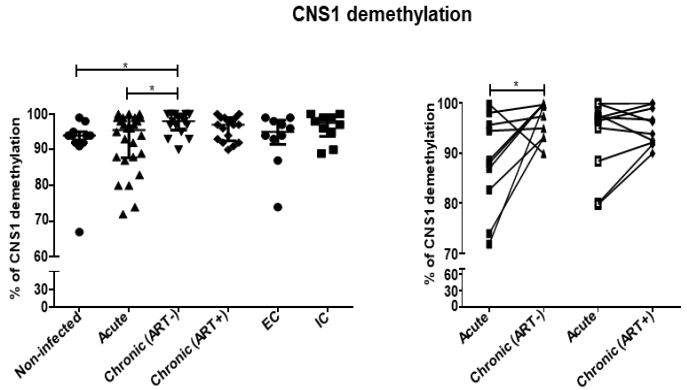
HIV infection increased gut-homing Tregs expressing LAP(TGF-β1) and CD39, which were not normalized following early ART initiation



HIV infection promoted the differentiation of peripheral Tregs (Helios$^-$), which was normalized by early ART initiation



HIV infection increased CNS1 demethylation in the *foxp3* locus, which was reduced following early ART initiation



- CNS1 methylation status is regulated by TGF-β1 (Mohr *et al.* 2018. ClinTransMed)
- CNS1 controls peripheral induction of FoxP3 expression (Sekiya *et al.* 2016. Microbes&Inf)
- Deletion of CNS1 abrogates pTreg differentiation (Mohr *et al.* 2018. ClinTransMed)

CONCLUSIONS:

- Early ART initiation decreased T-cell immune activation, immunosenescence, and exhaustion, as well as total Treg expansion.
- Early ART initiation was unable to control the levels of immunosuppressive Treg subsets and their gut migration potential, which could ultimately contribute to gut tissue fibrosis and disease progression.
- The increase in LAP(TGF-β1)$^+$ Tregs and extra-thymic Helios$^-$FoxP3$^+$ Tregs overtime during HIV infection were consistent with higher demethylation of CNS1 in the *foxp3* gene.
- LAP(TGF-β1)-expressing Tregs in EC and IC were significantly higher than uninfected subjects, while the markers of Treg activation, migration, and function remained similar in these individuals, which is in line of previous reports of mucosal fibrosis in HIV controllers.

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