Abstract # 287

PRE-ART TUBERCULOSIS AND TB-IRIS AFFECT THE HIV-1 RESERVOIR

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STUDY RATIONALE

Activated CD4+ T cells are important HIV-1 reservoirs prior to antiretroviral therapy (ART)[1]. Higher proportions of activated CD4+ T cell subsets are characteristic of active tuberculosis (TB) infections[2]. On initiating ART, rapid expansions of TB-specific CD4+ T cells drive TB associated immune reconstitution inflammatory syndrome (IRIS) – Figure 1. Elevated pro-inflammatory cytokines and increased CD4+ T cell activation during TB and TB-IRIS may facilitate further recruitment of CD4+ T cell targets for HIV entry and replication, potentially seeding the HIV reservoir[3,4].

Activation of CD+ T cells at baseline versus during TB-IRIS event

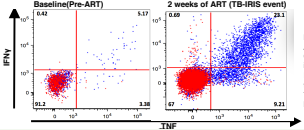


Figure 1. FACS Analysis of TB-specific CD4+ T cells secreting interferon gamma, IFNγ, (y-axis) and tumor necrosis factor, TNF, (x-axis). Baseline = Pre-ART.

STUDY HYPOTHESIS

Baseline TB and TB-IRIS have long-term effects on the HIV-1 reservoir.

STUDY AIMS

1. To determine if starting ART with baseline comorbid TB affects the HIV-1 reservoir differently from those without comorbid TB.
2. To determine how TB-IRIS in persons receiving ART affects the HIV-1 reservoir.

SAMPLING STRATEGY

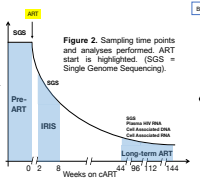
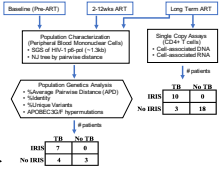


Figure 2. Sampling time points and analyses performed. ART start is highlighted. (SGS = Single Genome Sequencing).

METHODS



RESULTS

Lower levels of cell-associated RNA and lower ratio of RNA:DNA in CD4+ T cells after 96 weeks of ART in TB, No IRIS patients

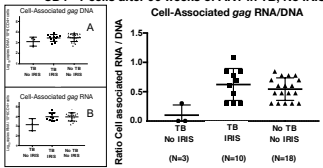


Figure 3. Median and IQR of CD4+ T cell-associated *gag* (A) DNA and (B) RNA were normalized to CCR5. (C) Ratio of CD4+ T cell-associated RNA:DNA.

TB patients had higher proportions of APOBEC3G/F hypermutated clones whose presence were associated with TB infection

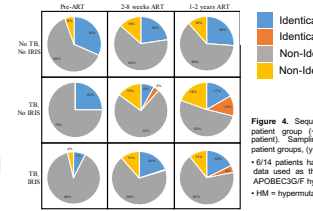


Figure 4. Sequence identity within each patient group (~120 SG sequences per patient). Sampling time points, (x-axis); patient groups, (y-axis).
• 6/14 patients had baseline RNA sequence data used as the reference sequence for APOBEC3G/F hypermutation analysis.
• HM = hypermutants.

TB-IRIS patients had less population identity after long term ART (Mann-Whitney p<0.05)

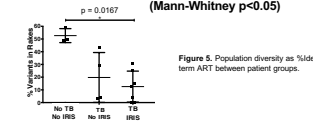


Figure 5. Population diversity as %Identity, after long term ART between patient groups.

TB-IRIS patients had more genetically diverse populations after long term ART (Mann-Whitney p<0.05)

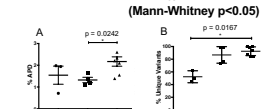


Figure 5. Population diversity as (A) %APD (B) %Unique Variants after long term ART between patient groups.

Baseline variants persist in TB infected patients during long term ART

(A) No TB, No IRIS

Weeks of ART	#Sequences (PBMC DNA)
0	63
8	48
128	65

(B) TB, No IRIS

Weeks of ART	#Sequences (PBMC DNA)
0	20
4	54
64	61

(C) TB, IRIS

Weeks of ART	#Sequences (PBMC DNA)
0	17
4	61
96	44

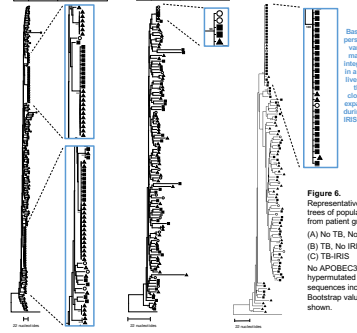


Figure 6. Representative NJ trees of populations from patient groups.
(A) No TB, No IRIS;
(B) TB, No IRIS; and
(C) TB-IRIS
No APOBEC3G/F hypermutated sequences included. Bootstrap values >80 shown.

SUMMARY

Our data suggest:

- a relationship between clonal expansion and baseline TB infections
- a shift to a more diverse proviral population after long term ART in TB-IRIS patients.

IMPLICATIONS

Active TB infections, in addition to IRIS, may drive T cell activation and compartmentalization to have long term effects on HIV-1 persistence.

FUTURE DIRECTIONS

- Expansion of cell-associated DNA and RNA datasets
- SGS: RNA; various T cell subsets
- Population compartmentalization analyses
- Detailed analyses of proviral structures
- Virus production

REFERENCES

1.Cockerham LR, Siliciano JD, Sinclair E et al. PLoS One 2014; 9: e110731.
2.Sullivan ZA, Wong EB, Ndung'u T et al. EBioMedicine 2015; 2: 334– 340.
3.Sereti I, Rodger AJ, French MA. Curr Opin HIV AIDS 2010; 5: 504– 510.
4.Vandergeeten C, Fromentin R, Chomont N. Cytokine Growth Factor Rev 2012; 23: 143–149.

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