Losartan does not improve lymphatic tissue fibrosis or T-cell recovery in HIV infection

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BACKGROUND AND PREMISE:

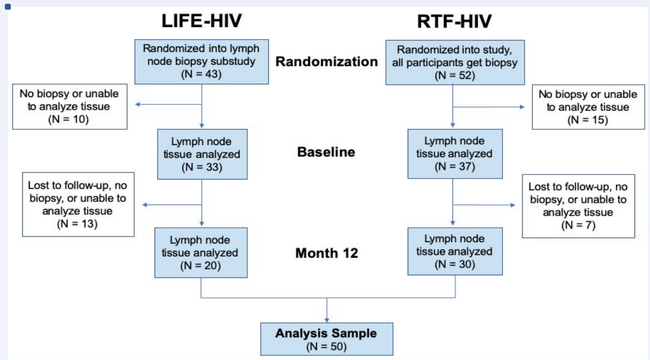
- Incomplete immune recovery among persons with HIV (PHIV) receiving ART is associated with excess clinical risk[1,2]
- Fibrosis (i.e., collagen deposition) within the parafollicular zone of lymphatic nodes (LN) contributes to depletion of naïve and central memory T-cells and impairs immune reconstitution[3-8]
- LN collagen deposition is mediated by transforming growth factor beta (TGF-β) as a consequence of ongoing immune activation[3-8]
- We hypothesized that the angiotensin receptor blocker (ARB) losartan would inhibit fibrosis and improve T-cell recovery, given its established treatment effects in blocking TGF-β signaling

STUDY DESIGN:

We pooled data from two randomized (1:1), double-blind, placebo-controlled trials (RCTs) of losartan (100mg daily):

- **LIFE-HIV**: "Losartan to Reduce Inflammation and Fibrosis Endpoints in HIV Disease"; a subset of n=43 co-enrolled into a lymph node (LN) biopsy substudy. Eligibility Criteria: PHIV age ≥50 years, HIV RNA <200 copies/mL on ART, and CD4+ count in blood of <600 cells/mm3.
- **RTF-HIV**: "Reversing Tissue Fibrosis to Improve Immune Reconstitution in HIV" Eligibility Criteria: PHIV with HIV RNA <200 copies/mL on ART and CD4+ count in blood between 200-650 cells/mm3.

In both studies, participants underwent an inguinal LN biopsy at baseline and at 12 months. **Figure** (below) is flow diagram of participants by study.

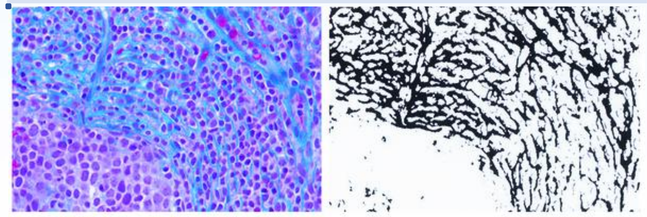


MEASURE METHODS:

Tissue and Blood Analyses: The percent area of collagen and CD4+ T-cells in LN were quantified in the parafollicular T-cell zone, using immunohistochemistry (IHC) followed by quantitative image analysis (QIA). **Figure 2** demonstrates a trichrome stain of collagen (blue) within LN tissue (LEFT), followed by a corresponding image where all collagen is displayed in black and non-collagen tissue removed (RIGHT). The percent area of collagen in tissue is then estimated; corresponding methods were applied to estimate percent area of CD4+ T-cells. ELISA and electrochemi-luminescence assay methods were used to measure biomarkers in blood.

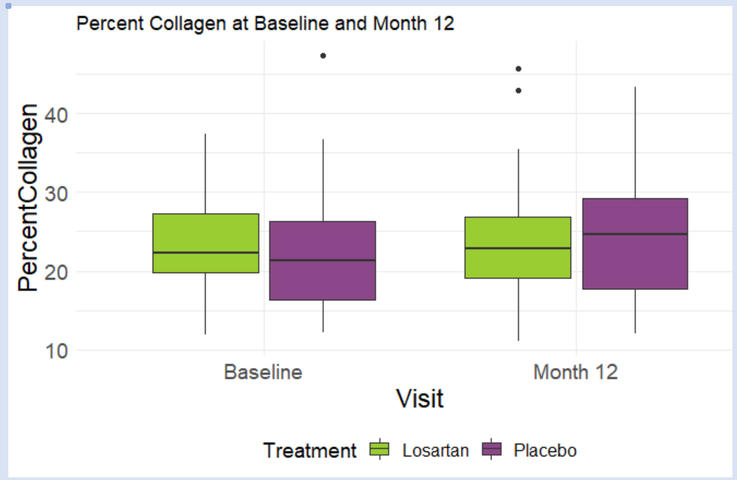
Statistical Methods: Baseline associations were estimated as difference in LN % area associated with a 1-SD difference or presence (vs. absence) of corresponding baseline factor. The main treatment effect over 12 months was defined as change on losartan minus change on placebo, adjusted for baseline level and study.

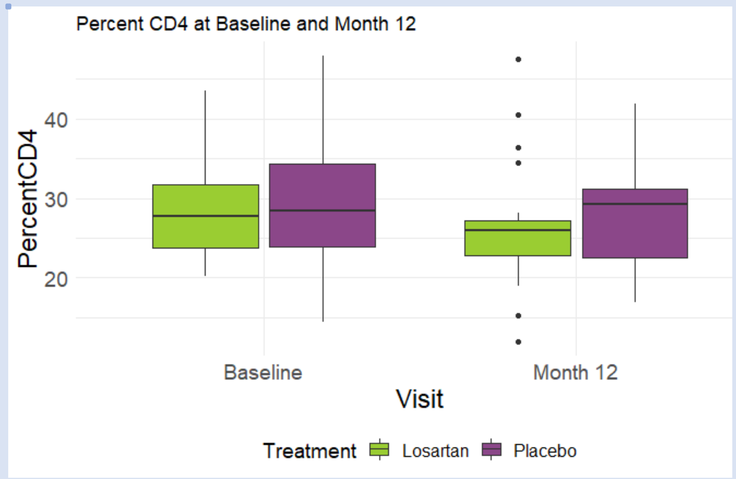
Figure 2: Quantitative Analysis of Collagen in the T-cell Zone in Lymph Node



MAIN FINDING:

Analysis of lymph node (LN) tissue from n=50 with longstanding HIV disease support that LN fibrosis is associated with alterations in T-cell homeostasis, but treatment with losartan (100mg) did not improve LN CD4+ T-cells or collagen when compared to placebo, or within persons, over 12 months.





LN Tissue	Losartan Baseline, Mean (SD)	Placebo Baseline, Mean (SD)	Losartan Month 12, Mean (SD)	Placebo Month 12, Mean (SD)	L vs. P Diff. in Change (95% CI)	L vs. P *p*-value
Collagen, % area	23.6 (6.97)	23.1 (8.72)	24.4 (8.16)	25.4 (8.01)	-1.2 (-5.4, 3.1)	0.58
CD4+, % area	28.8 (6.26)	28.5 (8.19)	26.5 (8.28)	27.1 (5.35)	-0.62 (-5.4, 4.2)	0.79

Table 1: Demographic and Clinical Characteristics

	LIFE-HIV	RTF-Trial	Combined
Sample Size	N=20	N=30	N=50
Demographics	**Mean (SD) or # (%)**		
Age, years	56.1 (3.69)	47.5 (11.7)	50.9 (10.2)
Sex, female	2 (10.0%)	1 (3.3%)	3 (6.0%)
Race/Ethnicity			
White, non-Hispanic	11 (55.0%)	18 (60.0%)	29 (58.0%)
Black, non-Hispanic	8 (40.0%)	8 (26.7%)	16 (32.0%)
Hispanic	0 (0%)	1 (3.3%)	1 (2.0%)
Other	1 (5.0%)	3 (10.0%)	4 (8.0%)
Clinical Factors	**Mean (SD) or # (%)**		
Smoking, current	4 (20.0%)	7 (23.3%)	11 (22.0%)
BMI, kg/m²	26.2 (5.48)	26.6 (4.72)	26.5 (5.01)
Hepatitis C Ab positive	4 (20.0%)	3 (10.0%)	7 (14.0%)
Systolic BP, mm/Hg	126 (11.3)	127 (11.4)	127 (11.3)
Diastolic BP, mm/Hg	81.5 (10.1)	80.0 (7.77)	80.6 (8.70)
HIV Factors	**Mean (SD) or # (%)**		
Duration of HIV diag., yrs	16.5 (7.76)	13.5 (10.1)	14.7 (9.29)
HIV RNA <200 cp/mL	17 (85.0%)	29 (96.7%)	46 (92.0%)
Nadir CD4+ count, cells/μL	173 (137)	179 (125)	176 (129)
CD4+ count, cells/μL	450 (128)	504 (147)	483 (141)
CD8+ count, cells/μL	754 (380)	857 (355)	816 (365)
CD4:CD8	0.79 (0.57)	0.71 (0.44)	0.74 (0.50)
INSTI-based regimen	9 (45.0%)	12 (40.0%)	21 (42.0%)
NNRTI-based regimen	10 (50.0%)	14 (46.7%)	24 (48.0%)
PI-based regimen	4 (20.0%)	9 (30.0%)	13 (26.0%)
Plasma Biomarkers	**Mean (SD)**		
Interleukin-6 (IL-6), pg/mL	1.09 (0.813)	0.91 (0.97)	0.986 (0.904)
TNFR-1, pg/mL	1220 (282)	852 (224)	1000 (306)
sCD163, ng/mL	657 (225)	532 (196)	582 (215)
sCD14, pg/mL	1330 (401)	1020 (233)	1150 (346)
D-dimer, μg/mL	0.37 (0.69)	0.31 (0.55)	0.34 (0.61)
Hyaluronic Acid, ng/mL	41.5 (26.7)	18.7 (15.7)	28.2 (23.7)
Beta-Crosslaps, ng/mL	0.43 (0.21)	0.42 (0.18)	0.42 (0.19)

Table 2: Associations between Lymph Node (LN) Collagen or CD4+ T-cell and Clinical Factors and Blood Biomarkers

	LN Collagen (% area)	LN CD4+ cells (% area)
Clinical Factors	**Estimate (95% CI); p-value**	
Age, years	-0.42 (-2.6, 1.8); 0.71	0.055 (-2.5, 2.6); 0.97
BMI, kg/m²	-0.81 (-3.0, 1.4,); 0.48	-0.5 (-2.8, 1.8); 0.67
Hepatitis C Ab positive	2.9 (-3.3, 9.2); 0.36	-2 (-8.6, 4.6); 0.56
HIV Factors	**Estimate (95% CI); p-value**	
Duration of HIV diag., yrs	2.1 (-0.011, 4.2); 0.057	-1.8 (-4.0, 0.42); 0.12
Nadir CD4+ count, cells/μL	-2.2 (-4.3, 0.003); 0.056	1.8 (-0.43, 4.0); 0.12
CD4+ count, cells/μL	-0.38 (-2.6, 1.8); 0.74	0.38 (-1.9, 2.6); 0.74
CD8+ count, cells/μL	**2.9 (0.86, 5.0); 0.008**	**-4.4 (-6.2, -2.7); <0.001**
CD4:CD8	**-2.8 (-4.9, -0.75); 0.01**	**3.7 (1.8, 5.6); <0.001**
Plasma Biomarkers	**Estimate (95% CI); p-value**	
Interleukin-6 (IL-6), pg/mL	0.51 (-1.7, 2.8); 0.66	-0.9 (-3.5, 1.7); 0.50
TNFR-1, pg/mL	-1.2 (-3.5, 1.0); 0.29	0.97 (-1.4, 3.3); 0.42
sCD163, ng/mL	0.22 (-2.0, 2.4); 0.85	-1.2 (-3.4, 1.0); 0.30
sCD14, pg/mL	-1.3 (-3.5, 0.94); 0.26	2 (-0.45, 4.4); 0.12
D-dimer, μg/mL	-0.46 (-2.7, 1.8); 0.69	-0.077 (-2.3, 2.1); 0.95
Hyaluronic Acid, ng/mL	-2 (-4.2, 0.23); 0.09	1.1 (-1.1, 3.4); 0.33
Beta-Crosslaps, ng/mL	-0.12 (-2.4, 2.1); 0.91	0.048 (-2.3, 2.4); 0.97

**difference in % area associated with a 1-SD difference, or presence (vs. absence), of baseline factor*

SUMMARY OF RESULTS:

- At baseline (**Table 2**), greater LN collagen was associated with less LN CD4+ cells (est: -3.8, p<0.001), and both greater collagen and less CD4+ cells were associated with:
  1. Significantly higher levels of CD8+ T-cells in blood, as well as a lower CD4:CD8 ratio.
  2. Lower nadir CD4+ count and longer duration of HIV diagnosis, but these associations did not reach significance.
- Losartan treatment was not associated with a significant difference in change of LN collagen or LN CD4+ T-cells over 12 months (center **Figure**), nor with biomarkers of fibrosis or blood CD4+ and CD8+ counts (data not shown). Analyses within treatment groups also did not demonstrate significant changes.

IMPLICATIONS:

- Among older persons with longstanding HIV disease, losartan did not alter lymphatic tissue fibrosis or T-cell immune recovery over one year.
- Novel treatments are needed that mitigate lymphatic tissue fibrosis and improve immune reconstitution, given the importance for restoring health among persons living with HIV.

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