

### Cytolytic CD8+ T cells infiltrate lymph node follicles and limit HIV replication in spontaneous controllers

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### Natural control of HIV infection is mediated by cytotoxic CD8 T cells



### Natural control of HIV infection in lymphoid tissues



- HIV persists in lymph node follicles which are immune-privileged sites largely excluding CD8 T cells during homeostasis (Beck et al., Front. Immunol. 2019; Connick et al., J. Immunol. 2007)
- CXCR5<sup>+</sup> CD8 T cells can overcome barriers to follicular access and are enriched in chronic viral infections (Leong et al., Nat. Immunol. 2016)
- *Ex vivo* cytotoxic effector molecule expression is lower in lymph nodes compared to peripheral blood which was lowest in HIV elite controllers (Reuter et al., Cell Rep. 2017; Nguyen et al., Sci. Transl. Med. 2019)
- Lymphoid CD8 T cell responses in elite controller macaques restrict vRNA replication to the follicle (Fukazawa et al., Nat. Med. 2015)

Studying people living with HIV that show a range of ongoing viral replication in the lymph node but durably control plasma viral loads will help clarify the mechanism of active viral control

### Study design and patient characteristics



	EC O	vc 🔵	ART	HIV- 🗱
Number of participants	8	11	17	7
Plasma viral load (RNA copies/mL, median (IQR))	< 20 (0)	72 (201)	< 20 (0)	N/A
Duration of suppression (years, median (IQR))	23 (11)	23 (13)	12 (10)	N/A
Tissue viral burden (HIV gagpol RNA counts/mm², median (IQR))	71 (358)	37 (145)	23 (201)	N/A

## Controllers

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# HIV-specific CD8 T cell frequencies are comparable in peripheral blood and lymph nodes



### Lymphoid HIV-specific CD8 T cells show similar proliferation and cytotoxic potential to blood-derived CD8 T cells



Strong clonotypic overlap between blood and lymph node suggests inflammatory recruitment from circulation



HIV-specific CD8 T cells from circulation show innate activation while lymphoid CD8 T cells have higher T cell activation gene signatures



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# Higher frequencies of cytotoxic CD8 T cells in lymph nodes of spontaneous controllers *ex vivo*

![](_page_8_Figure_1.jpeg)

![](_page_8_Figure_2.jpeg)

# Frequencies of cytotoxic lymphoid CD8 T cells are proportional to tissue viral burden

![](_page_9_Figure_1.jpeg)

![](_page_9_Picture_2.jpeg)

![](_page_9_Figure_3.jpeg)

# Cytotoxic CD8 T cells in lymph node follicles are located in proximity to HIV RNA+ cells in controllers

#### Cell segmentation for intra-GC spatial localization analysis

![](_page_10_Figure_2.jpeg)

![](_page_10_Figure_3.jpeg)

![](_page_10_Figure_4.jpeg)

![](_page_10_Figure_5.jpeg)

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### Summary

- In spontaneous controllers, cytotoxic CD8 T cell responses in the lymph node contribute to the containment of HIV infection inside the follicle
- Follicular CD8 T cells have the potential to express cytotoxic effector molecules in the presence of antigen
- High clonotypic overlap and gene expression signatures suggest inflammatory recruitment of circulating CD8 T cells to infected lymphoid tissues
- Both, the **localization** and **function** of CD8 T cells are important aspects to consider in curative strategies

# Acknowledgements

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### Study participants!!

![](_page_12_Picture_5.jpeg)

![](_page_12_Picture_6.jpeg)

#### FAU Erlangen

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![](_page_12_Picture_9.jpeg)

<u>AHRI</u>

Zaza Ndhlovu

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## Supplement

#### **Patient Characteristics**

		All	EC	VC	ART	HIV-
n		43	8	11	17	7
Age (median, IQR)		53 (13)	59 (11)	56 (15)	53 (9)	47 (18)
Sex	Female	8 (16.3%)	0 (0%)	2 (18.2%)	4 (23.5%)	1 (14.3%)
(n, %)	Male	35 (83.7%)	8 (100%)	9 (81.8%)	13 (76.5%)	6 (85.7%)
Ethnicity (n, %)	African American	14 (32.6%)	1 (12.5%)	6 (54.5%)	7 (41.2%)	0 (0%)
	Caucasian	27 (62.8%)	7 (87.5%)	5 (45.5%)	8 (47.1%)	7 (100%)
	Hispanic/Latino	1 (2.3%)	0 (0%)	0 (0%)	1 (5.9%)	0 (0%)
	Native American	1 (2.3%)	0 (0%)	0 (0%)	1 (5.9%)	0 (0%)
Plasma Viral Load (HIV RNA copies/mL; median, IQR)		<20 (10)	<20 (0)	72 (201)	<20 (0)	N/A
Duration of Control (years; median, IQR; *by ART)		N/A	23 (11)	23 (13)	12* (10)	N/A
CD4⁺ T Cells (cells/µL blood; median, IQR)		753 (539)	836 (445)	912 (478)	554 (503)	N/A
Peak Pre-ART Viral Load (n, %, median HIV RNA copies/mL)		N/A	N/A	N/A	n = 8 (47%) 3.0 x 10 <sup>5</sup>	N/A
Protective HLA-B Alleles (n, %) [ <i>B*14, B*27, B*52, B*57, B*58, B*81</i> ]		N/A	6 (75%)	7 (63.3%)	4 (21.1%)	N/A

## Immunodominant responses

	Controllers	ART treated
0	B*57 Gag TW10	A*02 Pol IV9
0	B*58 Gag TW10	B*51 Pol LI9
	B*53 Nef YY9	B*18 Pol NY10
0	B*57 Gag KF11	C*08 Gag TL9
0	B*57 Gag TW10	B*35 Pol TY9
	B*57 Gag TW10	B*57 Gag TW10
0	B*57 Gag KF11	None Detectable
0	B*08 Gag El8	A*03 Gag KK9
	B*57 Gag IW9	B*44 Gag AW11
0	B*57 Gag KF11	B*08 Gag El8
0	A*24 Gag KW9	B*14 Gag DA9
	B*53 Gag QW9	B*52 Gag RI8
	B*81 Gag TL9	C*04 Env SF9
	B*57 Gag KF11	A*03 Env TK10
	B*57 Gag KF11	B*13 Nef RV9
	B*42 Gag TL9	B*15 Pol VI10
	B*57 Gag KF11	B*40 Pol IL8
	B*57 Gag KF11	
	A*26 Gag EL9	

# Flow cytometric validation of gene expression signatures

![](_page_15_Figure_1.jpeg)

# Local vRNA expression in lymphoid tissue does not correlate with plasma viral load

![](_page_16_Figure_1.jpeg)

# Memory subsets and cytolytic effector molecules single positives

![](_page_17_Figure_1.jpeg)

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### Gating Strategies

![](_page_18_Figure_1.jpeg)

## Supplement

![](_page_19_Figure_1.jpeg)

![](_page_19_Figure_2.jpeg)

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