Cell-associated HIV-1 RNA in people with early acute HIV infection

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> HIV Persistence Workshop December 14, 2022



Walter Reed Army Institute of Research



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No conflicts of interest to declare



Background

Early events during HIV-1 infection are thought to influence subsequent virologic and immunologic outcomes, such as reservoir size, adaptive immune responses, and persistent inflammation.

Robust CD4 T cell infection and inflammatory responses are hallmarks of acute HIV-1 infection, yet detailed characterization of these early events remains poor.

-Large burden of HIV DNA+ cells (Leyre et al., 2020)

-Intense cytokine cascade in acute HIV (Biglino et al., 1996; Stacey et al. 2009)

-HIV DNA levels associated with inflammatory markers (Tiegler et al., 2018)

Key Questions:

- 1) What is burden of HIV RNA+ CD4 T cells in acute HIV?
- 2) Are HIV RNA+ cells replication-active?
- 3) Relationship between vRNA+ cells and inflammation
- 4) Profile of vRNA+ CD4 T cells in early AHI



M. Montero A. Tokarev

Defining cellular infection events by vRNA content



Assay design to detect, define HIV RNA+ cells



- HIV RNA assays target abundant, alternatively spliced vRNA species (Ocwieja et al., Nuc. Acids Res., 2012)
- Extensive viral sequencing data for CRF01_AE in Thailand enabled donor cross-reactive assays (Tovanabutra, Rolland)
- Mirrors assays developed for SIV-infected cell detection in NHP (Bolton et al. PLOS Pathogens 2017)

SEARCH clinic at IHRI, Bangkok, Thailand







RV254/SEARCH010 Acute HIV Infection Cohort

Objectives

- 1. Describe clinical, immunological, and virological characteristics of acute HIV infection
- 2. Identify participants for HIV cure studies

Real-time screening of **459,495** samples in Thailand

Acute HIV infection (n=708 enrolled/921 detected)

> Immediate ART (n=703)

Data as of 30 Sep 2022

Demographics

97% male, 93% MSM 26 years old, 76% CRF01_AE 19 days of infection 98 Fiebig I 164 Fiebig II 324 Fiebig III 86 Fiebig IV 32 Fiebig V

N of optional procedures 254 Sigmoid biopsy 490 Leukapheresis 495 Lumbar puncture 230 Inguinal LN biopsy 872 Brain MRI /MRS 1160 Genital Secretion



AFRIMS laboratories, Bangkok, Thailand

RV254 Fiebig I-V PBMC (N=24) LNMC (N=6)

Participant Symbol	Risk factor	Subtype	Fiebig stage	Plasma VL (RNA copies)	CD4 count
	MSM	CRF01_AE	Ш	30,811,000	621
	MSM	CRF01_AE	III	4,939,160	352
	MSM	CRF01_AE	V	1,329,100	231
	MSM	CRF01_AE	III	4,112,500	206
	MSM	CRF01_AE	III	25,063,500	338
	MSM	CRF01_AE/AG	III	1,642,350	298
	MSM	CRF01_AE/B	III	14,240,700	198
	MSM	CRF01_AE	III	13,922,100	359
	MSM	CRF01_AE	III	2,202,820	532
	MSM	CRF01_AE	III	17,811,300	292
	MSM	CRF01_AE	II	7,263,860	213
	MSM	CRF01_AE	III	2,656,900	350
	MSM	CRF01_AE	Ш	2,412,840	289
	MSM	В	II	1,170,480	265
	MSM/MSF	CRF01_AE	III	47,887,300	392
	MSM	CRF01_AE		30,551,900	295
	MSM	CRF01_AE	III	8,001,600	210
	MSM	CRF01_AE		7,234,700	224
	MSM	CRF01_AE	Ш	3,669,800	384
	MSM	CRF01_AE	III	22,516,400	182
	MSM	CRF01_AE	V	277,506	625
	MSM	CRF01_AE	1	146,194	457
	MSM	CRF01_AE	Ш	2,024,617	158
	MSM	CRF01_AE	III	31650200	184

Participant demographics (RV254)



Method for enumerating, phenotyping vRNA+ T cells



Burden of HIV-1 RNA+ cells in blood during acute HIV-1 infection





- Cells with elongated transcripts (LTR.U3) equally abundant as 5' unspliced RNA+ cells.
- Transcriptionally active cells represent ~10% of "infected" all vRNA+ cells.

9 Andrey Tokarev

RV254

PBMC

N=22

Fiebig II-V



Similar frequency of HIV-1 RNA+ cells in blood and lymph node



• vRNA+ cell burden similar between peripheral blood and lymph nodes during AHI.





HIV-1 RNA+ CD4 T cell burden in CSF during AHI

• Replication-active CD4 T cell infection in CSF during AHI – likely the source of CSF viremia

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• Similar frequency of HIV RNA+ T cells in peripheral blood and CSF

HIV-1 RNA+ cell frequency: correlation across vRNA assays



- LTR and gag vRNA+ cell frequency correlate well with each other
- Spliced vRNA+ assays env/vpu/nef and tat/rev/nef strongly correlate
- Reasonable correlations across all assays



HIV-1 RNA+ cell frequency: correlation with plasma viremia



• Strongest correlation between spliced vRNA+ assay tat/rev/nef and plasma viremia

Consistent with *tat/rev/nef*+ cells supporting replication and producing virions

Spearman p

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HIV RNA+ cell correlations with viremia and DNA

RV254 Fiebig I-V PBMC N=24



14 Nicolas Chomont, Andrey Tokarev



HIV-1 RNA+ cell correlation with serum inflammatory factors



- gag RNA+ cells correlate with IL-12p70, inducer of IFNγ production, Th1 differentiation, CTLs
- APC secretion of IL-12 may be induced by interaction with gag+ CD4 T cells

15 Bonnie Slike, Ningbo Jian, Shelly Krebs, Andrey Tokarev

Single-cell profile, phenotype of vRNA+ CD4 T cells during AHI



• Variable MHC class I downregulation, activation marker expression

Poster PP 8.6: "Single cell transcriptomics identifies PTMA as a host gene that inhibits HIV during acute infection in vivo" A. Geretz

16 Andrey Tokarev; Sharma et al., PLoS Path 2021

Summary

- HIV-1 RNA+ cells represent a median 4% of all memory CD4 T cells at peak AHI.
 - > Most vRNA+ cells lack evidence of viral transcription.
 - > ~0.1% of memory CD4 T cells (<10% of vRNA+ cells) are replication-active.
 - Similar levels in blood, LN, CSF
- vRNA species vary in relationship to virologic and immunologic events.
 - Plasma VL is best predicted by cells expressing spliced vRNA
 - ► Elongated vRNA \cong unspliced 5' vRNA distinct from setting of suppressive ART (Yukl *et al.*, 2018)
 - vRNA+ cells correlate with plasma IL-12p70, suggesting virus-cell interactions may drive inflammatory processes that shape development of adaptive Th1 T cell responses
- vRNA+ cells are heterogeneous for MHC class I downregulation, activation, Tfh



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COMMUNITY SUMMARY

• Key question: What is HIV RNA+ cell burden at peak viremia?

Key finding(s):

EDITION



- Large vRNA+ CD4 T cell burden during acute infection (up to 10-30%), but most do not produce virus
- vRNA+ cells correlate with inflammatory cytokine IL-12
- Next steps: Identify host factors governing latent vs. productive infection

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Acknowledgements

Animal Models & Viral Persistence Section

Hannah King Kathryn McGuckin Wuertz Caroline Subra Emily Tourtellott-Fogt Truong Luu *Maria Montero Andrey Tokarev* Viviana Cobos Jimenez Vishakha Sharma

Université de Montréal Nicolas Chomont



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Institute of HIV Research and Innovation Nittaya Phanuphak

Thai GPO, ViiV Healthcare, Merck, Gilead Sciences

IHRI

Nittaya Phanuphak Praphan Phanuphak Carlo Sacdalan Somchai Sriplienchan Nitiva Chomchev Jintana Intasan Ponpen Tantivitavakul Phillip Chan Ratchapong Kanaprach Eugene Kroon Rapee Trichavaroj Suthat Chottanapund Nipat Teeratakulpisarn Thai Red Cross **AIDS Research Centre** Yuwaree Pichitchok **Tippawan Pankam** Supanit Pattanachaiwit Somsong Teeratakulpisarn Sasiwimol Ubolyam Patcharin Eamyoung **Chulalongkorn University** Kiat Ruxrungtham Supranee Buranapraditkun Sunee Sirivichavakul Rungsun Rerknimitr Netsiri Dumrongpisutikul Phandee Watanaboonyongcharoen Sopark Manasnayakor WRAIR CIDR Nelson Michael Sheila Peel (DCB)

MHRP COL Julie Ake Sandhva Vasan Denise Hsu Merlin Robb Donn Colby **Trevor Crowell** Suteeraporn Pinvakorn Mark de Souza **Diane Bolton** Shelly Krebs **Bonnie Slike** Leigh Ann Eller Morgane Rolland Rasmi Thomas Dominic Paguin Proulx Eric Sanders-Buell Ellen Turk Oratai Butterworth Lisa Reilly Jamie Livengood CO0 DCAC Jintanat Ananworanich* Sodsai Tovanabutra* Michael Eller* Linda Jagodzinski* **AFRIMS** COL Jessica Cowden Michele Spring Alexandra Schuetz Siriwat Akapirat Bessara Nantapinit Pornsuk Visudhiphan COG

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OHSU Jacob Estes Lvdie Trautmann Hiroshi Takata Julie Mitchell U Hawaii Napapon Sailasuta **U** Pittsburgh John Mellors Sharon Riddler BIDMC Dan Barouch Ragon Galit Alter Boris Juelg Janssen Hanneke Schuitemaker Maria Pau Frank Tomaka Michal Sarnecki Daniel Stieh UNC Gail Henderson **RTI International** Holly Peay **U** Minnesota **Timothy Schacker** Drexel Elias Haddad **Case Western** Rafick Sékalv Slim Fourati Adam Burgener **U** Melbourne Sharon Lewin

U Montréal Nicolas Chomont Ghent Linos Vandekerckhove **U** Manitoba Lyle McKinnon **U** Gothenburg Henrik Zetterberg Los Alamos Bette Korber William Fischer Alan Perelson **Johns Hopkins** Robert Siliciano Joel Blankson UCSD **Doug Richman INM RAS Gennady Bocharov U** Nebraska **Courtney Fletcher** Emory Eric Hunter IrsiCaixa Javier Martinez-Picado **Fred Hutchinson** Josh Schiffer IHV Hongshuo Song Duke University Guido Ferrari

And many more!

Acknowledgements

RV254 & substudy participants

Community Advisory Board





Institute of Research





Funding

Division of AIDS, NIAID, NIH **Cooperative Agreement** between HJF and the DoD

Thai GPO ViiV Healthcare Merck **Gilead Sciences**

*Former MHRP

Relative abundance of HIV-1 spliced messages

Ocwieja, Bushman et al., 2012 Nucleic Acid Res

>40 different spliced vRNA species for HIV-1

Aimed to develop (RT-)qPCR assays that:

- Identify HIV-1 transcription (i.e. spliced mRNA)
- 2) Detect abundant HIV mRNA species to maximize sensitivity



Sensitivity, linearity of HIV-1 RT-qPCR assays targeting CRF01_AE



• HIV RT-qPCR assays perform with linear amplification and single-cell sensitivity.





HIV-1 RNA+ cell frequency: correlation with HIV DNA



- LTR and gag vRNA+ cell frequency correlate with total and integrated DNA
- Weaker correlation between spliced vRNA+ cells and HIV DNA
 - · Possible differential regulation of HIV-1 transcription across participants
 - · Or technical artifact due to poor cross-reactivity of spliced vRNA assays with participant sequence