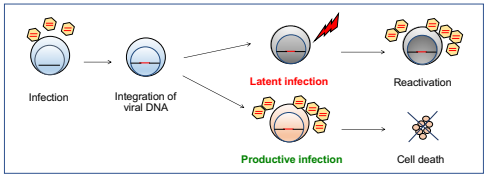
Reporter viruses with protein barcodes to analyze HIV latency establishment

00166

Eun Hye Grace Kim[1,4], Maria C. Bermúdez-González[1], Michael Schotsaert[1,4], Ana Fernández-Sesma[1], Lubbertus C.F. Mulder[1,4], Lara Manganaro[3] and Viviana Simon[1,2,4]
Department of Microbiology[1], Division of Infectious Diseases Department of Medicine[2], Global Health and Emerging Pathogens Institute[4], Icahn School of Medicine at Mount Sinai, New York, NY USA.,
INGM-Istituto Nazionale Genetica Molecolare, Milan, Italy[3]

BACKGROUND

- HIV integration into genome creates a long-lived reservoir
- Combination antiretroviral therapy (cART) suppresses replication **but does not eradicate the virus**
- HIV infection cannot be cured unless the persistent HIV reservoir is purged
- In order to study latency establishment, we need tools to distinguish between latent and productive infection



METHODS

To dissect the molecular mechanisms of latency establishment at the single CD4+ T cell level:

- Infect primary human CD4+ T cells with HIV reporter with protein barcodes
- Generate CyTOF panels suitable to analyze CD4+ T cell subpopulations, infection and cell cycle status
- Compare the frequency of latent and productive HIV infection in different CD4+ T cell subsets

Fig 1. Map of pLAI2 NGFR-V5 HSA-mCherry

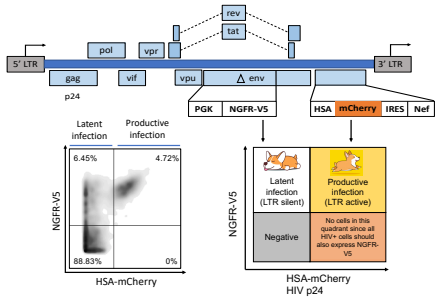
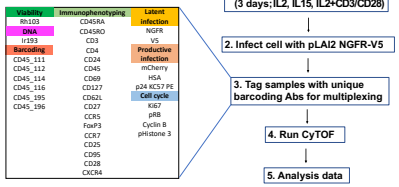


Fig 2. Experimental scheme



TAKE HOME MESSAGE

- Our barcoded HIV (pLAI2 NGFR-V5 HSA-mCherry) identifies both CD4+ T cells that are productively infected or that harbor LTR silent proviruses
- Our virus enables us to distinguish cells with latent and productive infection without promotor interference
- CD4+ T cell activation with IL2+CD3/CD28 alters the distribution of CD4+ T cell subsets
- T regs had the highest level of latent infection
- Our viral toolbox allows us to identify latency establishment and maintenance in primary human CD4+ T cell populations

Fig 3. Frequency of productively infected T cells (p24+, mCherry+, HSA+, NGFR+, V5+)

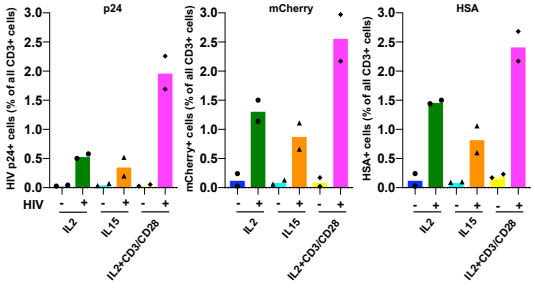


Fig 4. Frequency of latently infected T cells (p24-, mCherry-, HSA-, NGFR+, V5+)

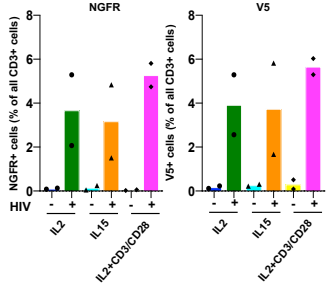


Fig 5. Cell surface markers of the different CD4+ T cell subsets

	CD45RA	CD45RO	CCR7	CD27	CD95	CD127	FoxP3
Naïve	+	-	+	+	-	-	-
TSCM	+	-	+	+	+	-	-
TCM	-	+	+	+	+	-	-
TEM	-	+	-	-	+	-	-
TTM	-	+	-	+	+	-	-
TEMRA	+	-	-	-	-	-	-
T reg	-	-	-	-	-	-	+

Fig 6. Distribution of CD4+ T cell subsets (Mock)

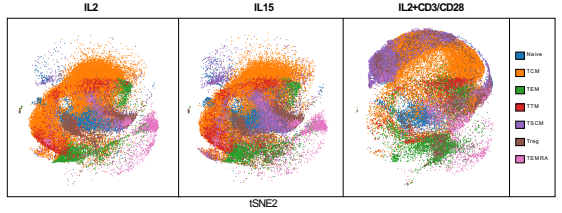


Fig 7. Distribution of CD4+ T cell subsets (Mock)

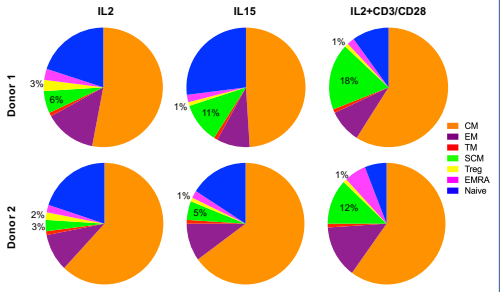
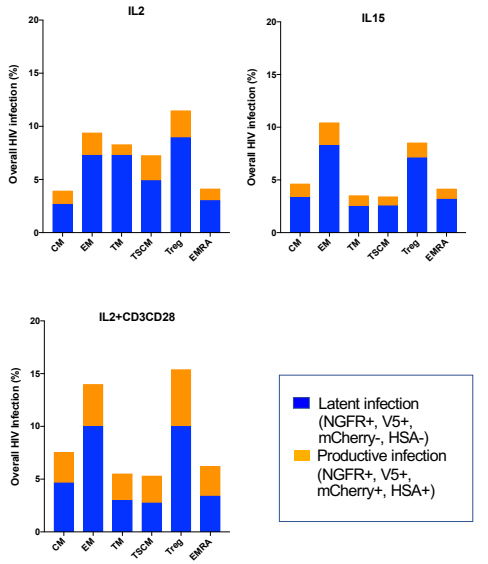


Fig 8. T cells subsets differ in susceptibility to HIV infection



CONCLUSIONS

- We developed a dual-reporter HIV with minimal promoter interference
- Productively infected cells expressed p24, mCherry, HSA. NGFR, and V5
- Latently infected cells expressed only NGFR and V5
- Between 60-70% of infected cells harbor LTR silent proviruses
- T regs had the highest level of latent infection
- Activation alters the distribution of 7 distinct CD4+ T cell subsets from two donors
  - TSCM subsets increased from 6% in IL2 to 18% when treated with IL2+CD3/CD28
  - T reg decreased from 3% in IL2 to 1% in IL2+CD3/CD28
- While IL2 skewed CD4+ T cells towards latent infection, IL2+CD3/CD28 activation resulted in higher frequency of productive infected cells

ADDITIONAL KEY INFORMATION

Author Contact Information: eunhye.kim@mssm.edu or viviana.simon@mssm.edu

Acknowledgements: We thank Dr. Rahman and his team at the Human Immune Monitoring Core (HIMC) of the Icahn School of Medicine at Mount Sinai for CyTOF assistance. We are grateful to Dr. Brown for sharing protein tags and antibodies. This work supported in part by NIH/NIAID R01AI136916 (V.S).