Role of transcriptionally-active "defective" HIV-1 proviruses in immunological non-responders: a case-control study.

Francesca Scrimieri[1], Estella Bastian[2], Mindy Smith[2], Cathy Rehm[2], Caryn Morse[3], Janaki Kuruppu[3], Mary McLaughlin[2], Joseph Kovacs[3], H. Clifford Lane[2], Hiromi Imamichi[2]

[1]Frederick National Laboratory for Cancer Research, Frederick, MD, United States.
[2]National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD, United States.
[3]Critical Care Medicine Department, NIH Clinical Center, Bethesda, MD, United States.

Francesca Scrimieri, Ph.D.
Email: francesca.scrimieri@nih.gov

IAS 2023 23–26 July

Introduction

Immunological non-responders (INR) exhibit poor CD4⁺ T-cell count recovery despite achieving virologic suppression.

The underlying mechanisms of the immunological non-response are not well-understood.

Objective

We investigated the potential contribution of transcriptionally-competent "defective" HIV-1 proviruses to poor immune recovery observed in INR.

Methods

Fig. 1 Case-control study

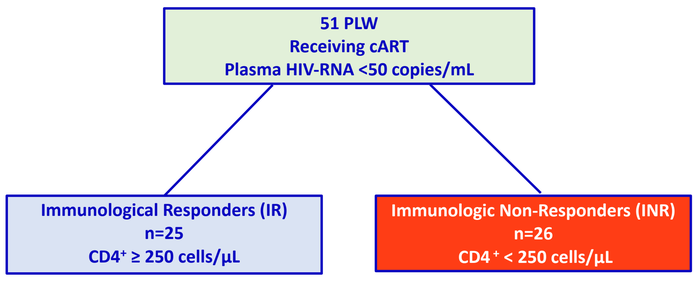
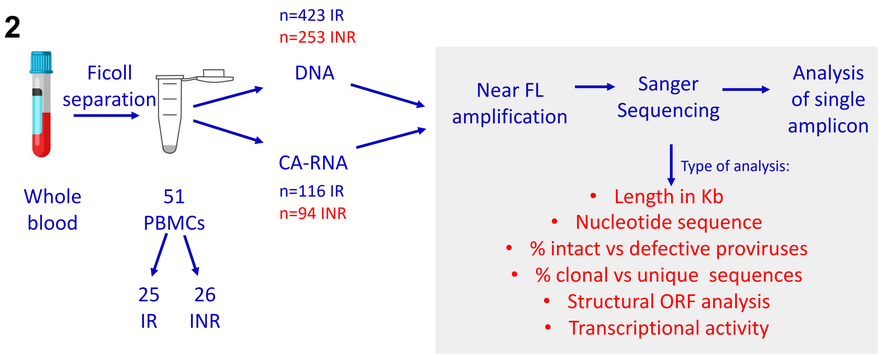
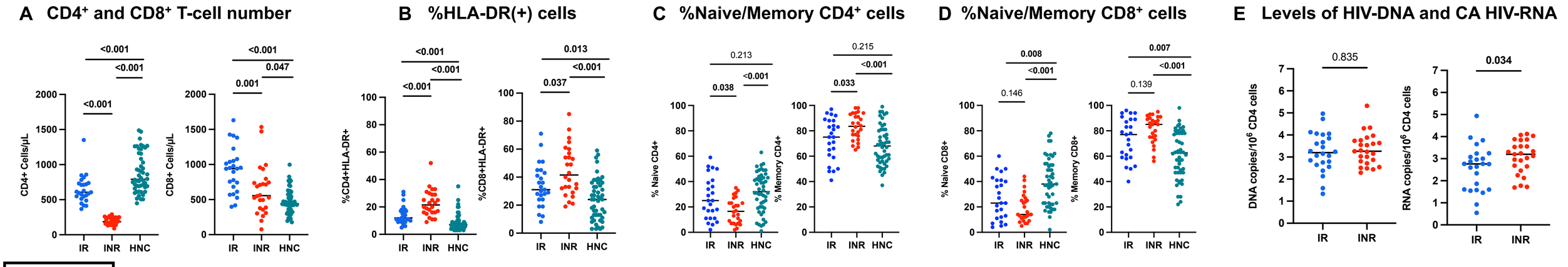


Fig. 2



- **HIV-DNA and cell-associated HIV RNA (CA HIV-RNA) were extracted simultaneously.**
- **423 HIV-DNA & 116 CA HIV-RNA from IR; and 253 HIV-DNA & 94 CA HIV-RNA from INR were generated using 5'LTR-to-3'LTR single-genome amplification and sequenced by Sanger sequencing.**
- **HIV-DNA and CA HIV-RNA viral quasispecies were analyzed with respect of length, quantity, nucleotide sequence and degree of in-vivo clonal expansion.**

Fig. 3. T-cell characteristics and HIV-1 viral load



INR was associated with:

Lower CD4⁺ T-cell count (p<0.001)

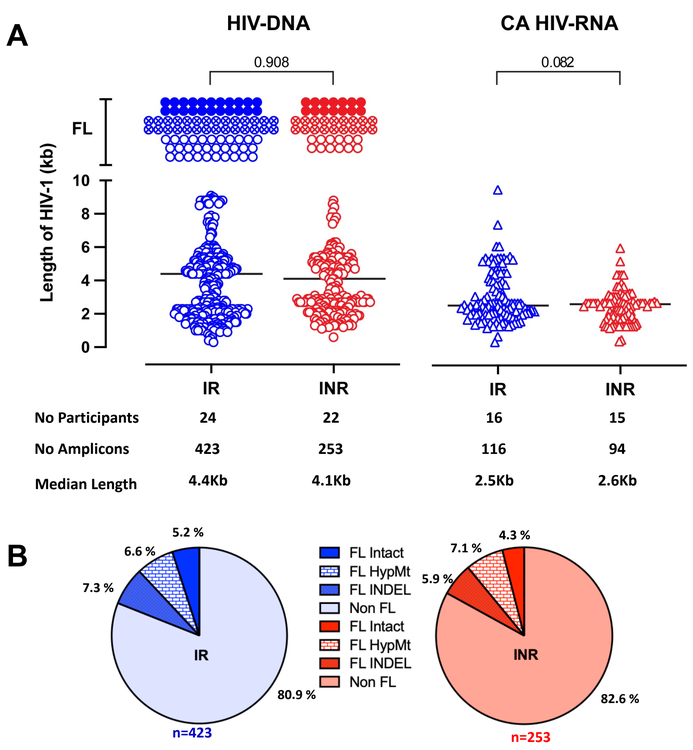
Increase in proportion of activated CD4⁺ and CD8⁺ cells

Skewed toward memory phenotype in CD4⁺

Comparable proportions of Na/Me phenotype in CD8⁺

Higher levels of CA HIV-RNA (p=0.034), while levels of HIV-DNA were similar

Fig. 4. Proportion of near full-length HIV-1



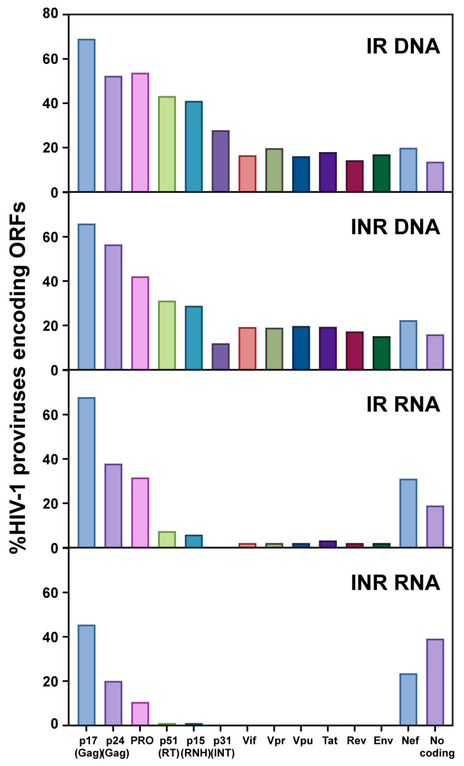
Four types of HIV-DNA structures: full length intact (FLI●), full length hypermutant (FLH⊗), full length with internal insertion (FL INDEL○), and non-FL defective (<10 kb in the length plot).

Similar median length of both HIV-DNA and CA HIV-RNA in IR and INR.

No transcription of full-length provirus in both groups.

Both groups present with similar proportion of FL and defective provirus.

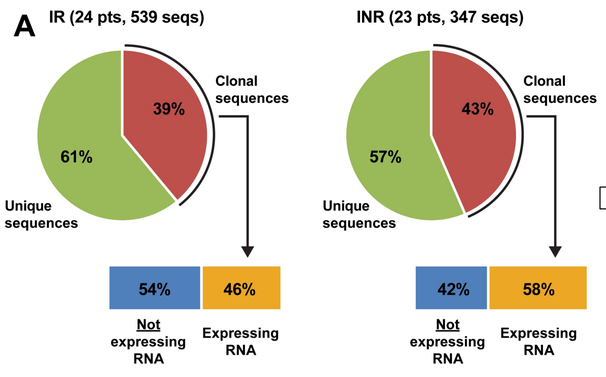
Fig. 5. Number of HIV-1 viral protein-coding regions



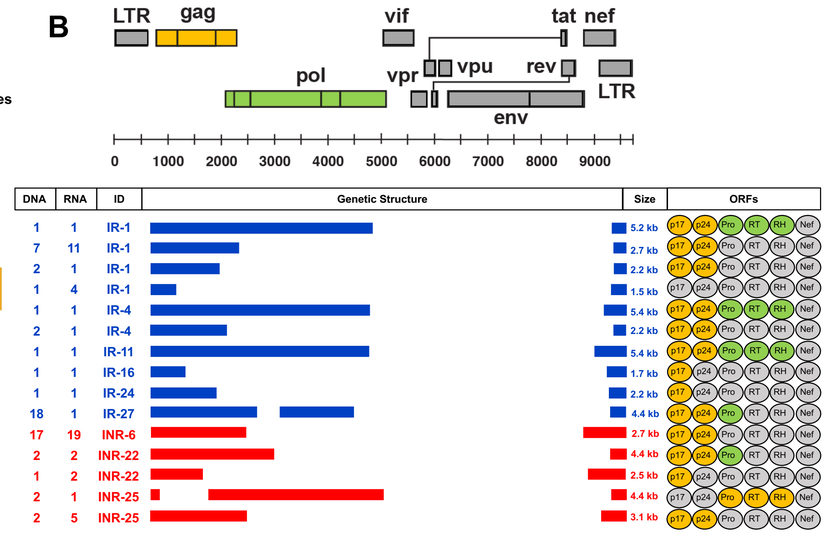
No significant changes in the proportions of HIV-1 proviruses encoding the 13 intact ORFs between IR and INR.

Most preserved viral protein-coding regions were Gag p17 and Gag p24 and Nef.

Fig. 6. Proportion of transcriptionally-competent HIV-1 provirus clones



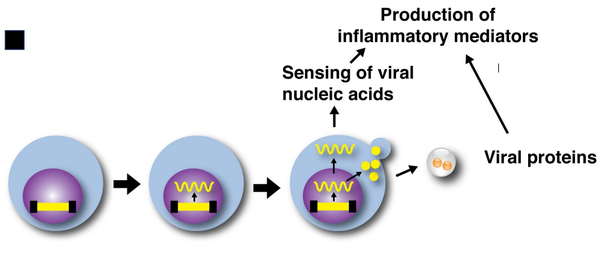
Degree of in-vivo clonal expansion was similar between IR and INR.



10 transcriptionally-active HIV-1 clones in 5 different IRs and 5 transcriptionally-active HIV-1 clones in 3 different INRs were identified.

Gag p17 and p24 coding regions were frequently preserved among the expanded HIV-1 provirus clones expressing CA HIV-RNA

Summary

- **CD4⁺ from INR were lower in number, phenotypically skewed toward memory, were more activated (HLA-DR(+)), and had higher expression of CA HIV-RNA.**
- **Absence of full-length intact CA HIV-RNA suggests that direct cell killing by ongoing HIV-1 replication is an unlikely explanation for poor immune recovery in INR.**
- **Other profiles of HIV-1 quasispecies were similar between IR and INR: Length, levels of HIV-DNA, proportions of FL intact/defective, HIV-1 genome structure, and degree of in-vivo clonal expansion.**
- **Consequences of increased level of RNA transcripts from "defective" HIV-1 proviruses have not been fully elucidated.**
- 

HIV-RNA and/or HIV-1 viral proteins in exosomes may be able to exert pathogenic effect (apoptosis) on infected and uninfected cells.