NONSTRUCTURED TREATMENT INTERRUPTIONS CONTRIBUTE TO LATENT HIV-1 RESERVOIR IN PWID

0322

Gregory Kirk[1,4], Jacqueline Astemborski[1], Shruti Mehta[1], Kristen D. Ritter[2], Janet D. Siliciano[4], Gregory M. Laird[2], Robert F. Siliciano[3,4]

[1]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, [2]Accelevir Diagnostics, Baltimore, MD, [3]Howard Hughes Medical Institute, Baltimore, MD,[4] Johns Hopkins University School of Medicine, Baltimore, MD

BACKGROUND

HIV-1-infected persons who inject drugs (PWID) often struggle to maintain stable viral suppression and experience non-structured treatment interruptions (NTIs). Injecting drugs has been associated with increased inflammation and alterations in T cell homeostasis. However, the long-term effects of NTIs or of injection drug use on the HIV-1 latent reservoir have not been defined. The latent reservoir is the principal barrier to HIV-1 cure, and as cure interventions move forward in clinical trials, it is important to understand how a history of injection drug use may affect the latent reservoir and the potential to achieve a cure among PWID.

METHODS

The latent reservoir in PWID was evaluated with a novel digital droplet PCR assay, the intact proviral DNA assay (IPDA)[1]. This assay distinguishes intact proviruses with the potential to cause viral rebound from the grossly defective proviruses which dominate the population of persistent proviruses in treated patients[2,3]. In this assay, DNA from infected cells is distributed into nanoliter size droplets so that individual proviruses can be probed with multiplex PCRs occurring within the droplets to distinguish intact and defective proviruses (Fig. 1). The number of intact proviruses (those lacking common fatal defects) can be directly enumerated.

Figure 1

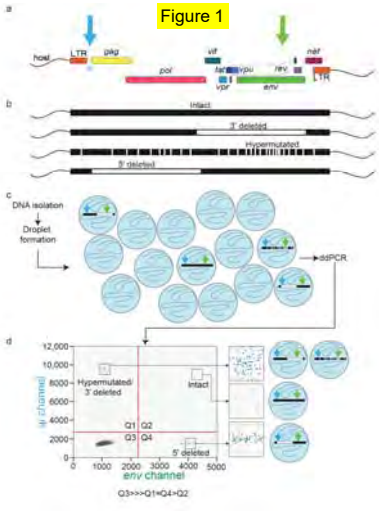


Figure 1. (**a**) Map of the HIV-1 genome indicating positions of the Ψ (blue arrow) and *env* (green arrow) amplicons used to distinguish intact and defective proviruses.

(**b**) Illustration of the common types of fatal defects detected by the IPDA. Proviruses with large deletions and/or APOBEC3G mediated hypermutation fail to give amplification for the Ψ or *env* amplicons

(**c**) Overview of the IPDA: In addition to the HIV-1 proviral discrimination reaction illustrated here, the IPDA includes a parallel ddPCR reaction targeting a host gene to allow determination of input cell number and correction for shearing between Ψ and *env* amplicons.

(**d**) Representative 2-D ddPCR plot from HIV-1 proviral discrimination component reaction of the IPDA. The typical droplet counts follow the pattern: Q3>>>Q1≈Q4>Q2.

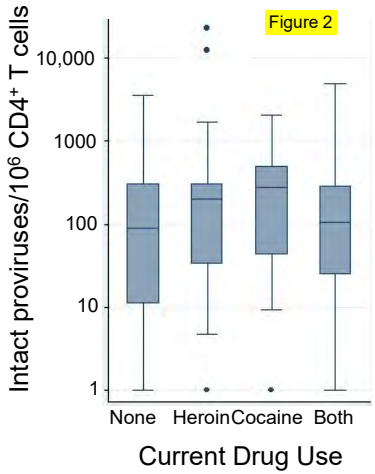
The size of the latent reservoir among drug users is similar to other populations and ***was not greatly influenced by active drug use***.

However, *treatment interruptions and associated viremia* ***were associated*** *with greater levels of intact provirus*.

RESULTS

We performed the IPDA on 108 HIV-1$^+$ adult participants of the AIDS Linked to the IntraVenous Experience (ALIVE) cohort who were on suppressive ART with plasma HIV-1 RNA <50 copies/mL at the time of sampling and at the study visit 6 months prior. A minimum of 5 HIV-1 RNA measurements (2.5 years of observation) was required. Participants were selected based on self-report of current drug use: active heroin use (n=28), active cocaine use (n=23), combined cocaine and heroin use (n=29), and no reported current drug use (n=28). Participants were further selected to include those with a history of prolonged, stable viral suppression (n=36) and those with past periods of viremia due to NTIs (n=72). Participants were 71% male, 96% black, and median age was 53 years.

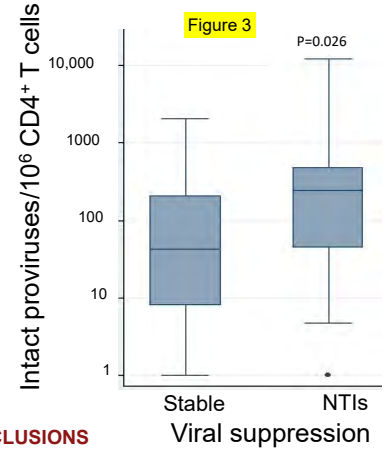
Figure 2



No significant differences were observed by current patterns of drug use, with median frequencies of intact proviruses ranging from 89 to 275 intact proviruses/10^6 CD4$^+$ T cells across groups, values comparable to those seen in other cohorts[1,4] not selected based on illicit drug use (Figure 2).

However, we did observe notably higher intact provirus frequency among persons who had experienced NTIs (Figure 3) compared to those with stable suppression (mean 141 vs. 32 intact proviruses 10^6 CD4+ T cells, respectively; P=0.0011). In multi-variable linear regression adjusting for demographics and drug use, NTIs were strongly associated with higher intact provirus (coef= 0.576; P=0.026).

Figure 3



CONCLUSIONS

We found no apparent long-term effect of injecting drugs on latent reservoir size as measured by IPDA. However, we found a notable increase in reservoir size for those with past periods of viremia due to NTIs compared to those with a history of more $_{\text{stable}}$ viral suppression. Our data have important implications for the field. First, they support the inclusion of PWID with stable suppression in cure studies. Second, they demonstrate that a history of viremia due to NTIs may have lasting effects on the size of the reservoir, and as such, virologic history should be considered when designing or analyzing HIV-1 cure studies.

ACKNOWLEDGEMENTS

This work was supported by NIDA grants R61-129884 & U01-036297

REFERENCES

1) Bruner et al, **Nature** 2019 566:120-125
2) Ho et al, **Cell** 2013
3) Bruner et al, **Nature Med** 2016 22:1043-9
4) Peluso et al, **JCI Insight** 2020 pii: 132997. doi: 10.1172