Effects of Chemoradiation on Expanded Proviral Clones in an Elite Controller

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BACKGROUND

Clonal expansion of latently infected CD4+ T cells contributes to the latent reservoir. Disruption of this process may be required for viral eradication to be achieved.

Recent studies suggest that intact proviruses integrated in certain Zinc Finger (ZNF) genes have a survival advantage due to reduced viral expression caused by a repressive epigenetic environment. However, direct evidence of this mechanism is lacking.

In this study we analyzed expanded infected-clones in an elite controller before and after he received chemoradiation and immunotherapy for metastatic lung cancer.

METHODS

The study participant is a HLA-B*57+ male elite controller who maintained undetectable HIV RNA without treatment for more than 10 years. In 2019, he was diagnosed with metastatic lung cancer. Details of his clinical history are summarized in Figure 1.

HIV integration sites were characterized by linker-mediated PCR (1, 2). We used digital PCR assays to measure total LTR copies (3), intact proviruses (4) and provirus-specific HIV DNA (5). In order to identify the individual Gag peptides that induced clonal expansion, CD8-depleted PBMCs were stimulated with 11 different pools containing 10-13 peptides each for 10 days.

SUMMARY

- Chemoradiation perturbs CD4+ clones, but its impact is transient.
- By the end of the treatment, two intact proviruses integrated in Zinc Finger genes largely dominated the proviral landscape.
- The intact provirus integrated into *ZNF721* likely had a survival advantage due to lower inducibility upon T cell activation.

Transient decline followed by rebound in reservoir size post chemo-radiation therapy

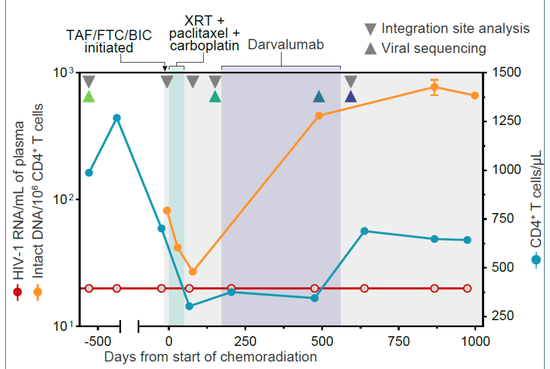


Figure 1. Clinical history of study participant before, during, and after treatment of metastatic lung cancer.
By measuring total and intact HIV DNA longitudinally, we observed an initial decrease in both total and intact DNA, followed by a significant higher rebound for intact DNA. Of note, his plasma HIV RNA remained undetectable (<20 copies/ml) throughout the follow-up.
XRT, chemoradiation therapy, TAF tenofovir alafenamide, FTC emtricitabine, BIC bictegravir, CRT radiotherapy.

Chemoradiation affects expanded clones, including those carrying proviruses

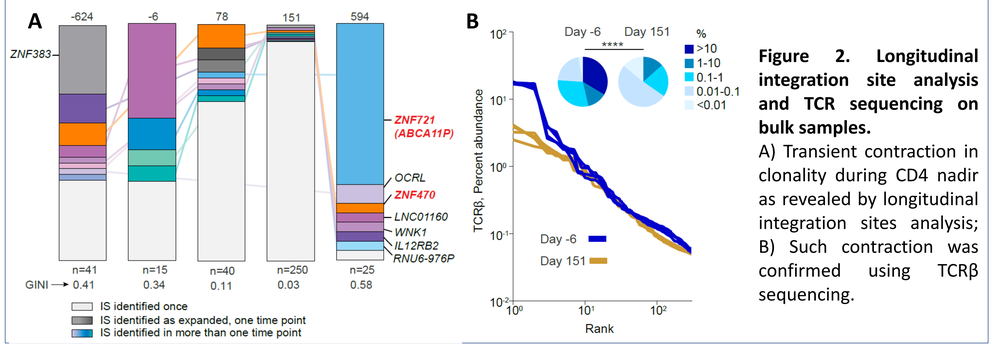


Figure 2. Longitudinal integration site analysis and TCR sequencing on bulk samples.
A) Transient contraction in clonality during CD4 nadir as revealed by longitudinal integration sites analysis; B) Such contraction was confirmed using TCRβ sequencing.

The proviral landscape was largely dominated by two intact proviruses, ZNF470i and ZNF721i

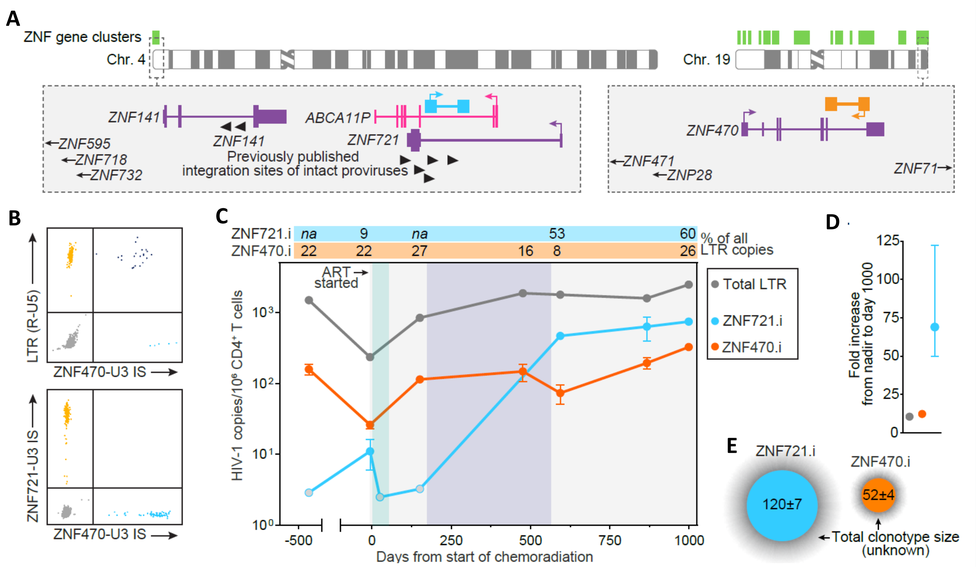


Figure 3. Longitudinal quantification of ZNF721i and ZNF470i before, during and after treatment. We used specific assays to trace the dynamics of the two most abundant clones. ZNF470i was persistent but stable; ZNF721i underwent a 70-fold expansion after chemotherapy was completed.
A) Genomic location and relative orientation of proviruses of interest; B) Representative 2D dPCR plots showing LTR and integration site-specific duplex assays; C) Longitudinal quantification of ZNF721i and ZNF470i proviruses; D) Fold increase of LTR, ZNF470i and ZNF721i from day -6 to day 1000; E) Estimates of total-body clone size.

Stimulation with HIV gag peptides induces proliferation and virus production of ZNF721i and Chr7.d11sc

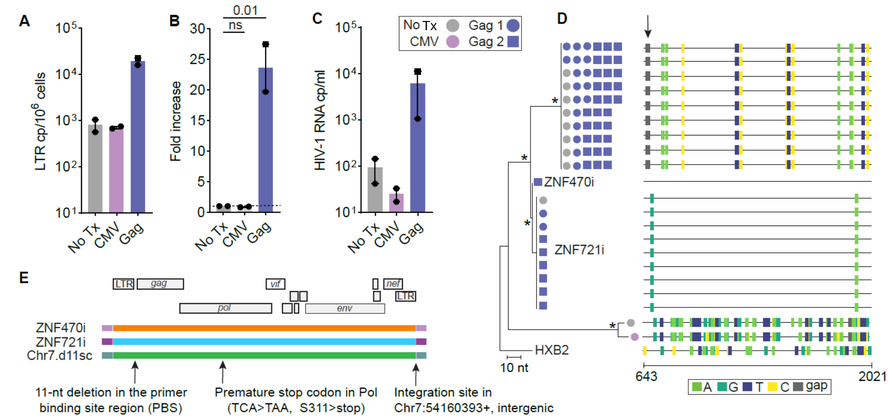


Figure 4. Stimulation with HIV Gag peptides. To investigate the role of antigen-driven selection, we stimulated CD8-depleted PBMCs with CMV and HIV gag antigens. By sequencing of the culture supernatants we detected a new provirus, Chr7.d11sc, highly inducible upon HIV gag stimulation.
A) LTR cp/million cells, B) fold increase compared to no Tx and C) HIV RNA cp/ml following stimulation; D) Maximum likelihood phylogeny analysis of HIV RNA U5-gag single genome sequencing; E) Full genome sequences of ZNF470i, ZNF721i and Chr7.d11sc.

Cells harboring ZNF721i and Chr7.d11sc proviruses are responsive to two HIV gag epitopes

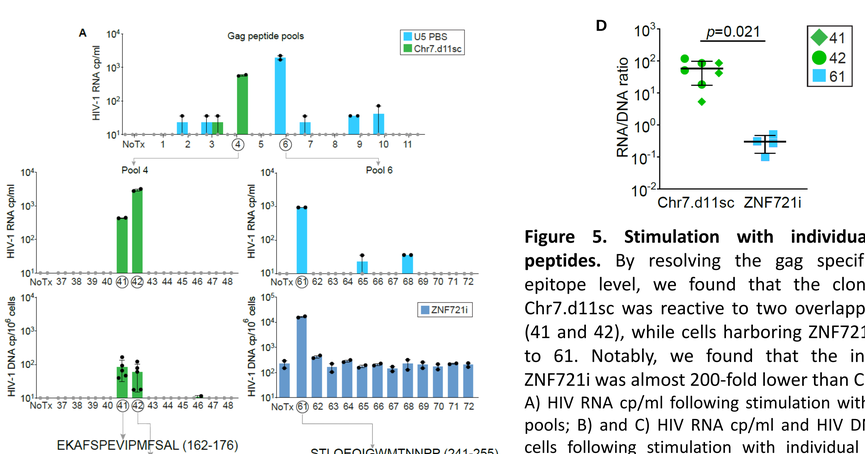


Figure 5. Stimulation with individual HIV Gag peptides. By resolving the gag specificity at the epitope level, we found that the clone harboring Chr7.d11sc was reactive to two overlapping peptides (41 and 42), while cells harboring ZNF721i responded to 61. Notably, we found that the inducibility of ZNF721i was almost 200-fold lower than Chr7.d11sc.
A) HIV RNA cp/ml following stimulation with gag peptide pools; B) and C) HIV RNA cp/ml and HIV DNA cp/million cells following stimulation with individual peptides; D) Differential inducibility between proviruses of interest upon stimulation with cognate Gag peptides.

CONCLUSIONS

Our results suggest that chemoradiation can transiently disrupt clonal expansion and strongly affect the proviral landscape. Following the activation of infected-clones by cognate antigen, the lower inducibility of intact proviruses in certain ZNF genes can play a crucial role in favoring their persistence.

ACKNOWLEDGMENTS

This work was supported by the NIH Early Independence Award (DP5OD031834), the BEAT Martin Delaney Collaboratory (UM1AI164570), the Johns Hopkins University CFAR grant (P30AI094189) and the NIH grants R01AI140789 and R21AI172542

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