Adaptation of the HIV transcription profiling assay to measure viral reservoir activity and latency reversal in people with HIV subtypes A1 and D

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Background: In-depth characterization of the HIV latent reservoir, including response to latency reversing agents (LRAs) such as Tat mRNA lipid nanoparticles (LNPs), has primarily focused on assessment of subtype B virus. Here, we adapted the HIV transcription profiling assay to quantify early (LongLTR and Pol), complete (PolyA) and multiply-spliced (Tat-Rev) cell-associated HIV transcripts at baseline and following Tat mRNA-LNP treatment in CD4+ T cells from people with HIV subtypes A1 and D in the Rakai cohort, Uganda.

Methods: Subtype A1 and D primers and probes for each transcript were redesigned using the Los Alamos HIV sequence database and validated using A1 and D consensus standards on the QIAcuity dPCR platform. Baseline HIV RNA expression was assessed in isolated CD4+ T cells from participant samples (n=7 subtype A1, n=10 subtype D). For a subset of participants (n=3 subtype A1, n=2 subtype D), 1-2 million CD4+ T cells were cultured for 72 h with or without Tat mRNA-LNPs, followed by HIV transcription profiling to assess latency reversal.

Results: Adapted A1 and D primer/probe sets demonstrated improved PCR detection of all four transcripts compared to the original subtype B primer/probe sets. Assessment of baseline RNA expression in participant samples demonstrated a step-wise decline in measured transcripts, with highest abundance of LongLTR, followed by Pol, PolyA and Tat-Rev. Ex vivo treatment with Tat-mRNA LNPs led to significant increases in all measured transcripts for both subtype A1 and D participants (mean fold-increase of 10.12 for LongLTR, 17.95 for Pol, 17.94 for PolyA, and 11.04 for Tat-Rev), indicating successful latency reversal.

Conclusion: This work demonstrates the utility of adapting HIV transcription profiling to subtypes prevalent in Africa. Similar blocks to HIV RNA elongation, completion and splicing were observed in subtype A1 and D participant samples. Tat mRNA-LNPs demonstrated cross-subtype ability to reverse latency ex vivo.

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