Development of an In-House Genotyping and Transcription Profiling Pipeline for Investigating Low-Level Viremia in HIV

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Background

Despite the success of antiretroviral therapy (ART), a small subset of people living with HIV (PLWH) experience low-level viremia (LLV), defined as detectable HIV RNA of 50-200 copies/mL in the absence of drug resistance or non-adherence. LLV is associated with a disproportionate burden of adverse health outcomes. While most HIV quantification tools are designed for subtype B, the majority of the global HIV burden comprises non-B subtypes (A, C, D, and circulating recombinant forms). With increasing non-B prevalence in Australia, a robust genotyping tool capable of characterising viral diversity directly from clinical samples is needed to support HIV cure research.

Methods

CD4+ T cells were isolated from peripheral blood mononuclear cells (n=10 participants of our LLV cohort). DNA and RNA were extracted using the QIAamp DNA kit and Zymo Direct-zol RNA kit, respectively. A pipeline was optimised comprising Gag-Pol PCR amplification of a ~3kb fragment, followed by two-primer Sanger sequencing, with subtyping consensus calls generated using three publicly available genotyping algorithms. HIV transcription profiling was performed on RNA from two confirmed subtype B genotyping LLV participants using an in-house validated assay.

Results

Initial Gag-Pol PCR (primers:LongLTR_F/RT-R1) achieved amplification in 5/10 participants. Two additional primer combinations (BLOut_F/BLOut_R and LongLTR_F/RT-R2) yielded amplification in 4/5 previously unresponsive samples, with one failure across these combinations. Subtype calls agreed with validated assays. Transcription profiling revealed expression of key HIV RNA species (LongLTR, Pol, PolyA, and Tat-Rev) in LLV participants, with Tat-Rev levels exceeding those observed in PLWH on suppressive ART.

Conclusion

These preliminary findings support the utility of an in-house genotyping/subtyping pipeline characterising viral diversity in LLV. Further optimisation is required to establish a reliable, comprehensive strategy for virological characterisation of LLV. Notably, our preliminary observation of elevated Tat-Rev expression (a surrogate marker of active viral protein synthesis) suggests ongoing translational activity in LLV.

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