Assay To Measure The Latent Reservoir Of Replication-Competent HIV-1 In Suppressed Patients Based On Ultra Deep Sequencing

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Abstract
Background: Ultra deep sequencing (UDS) is a widely used culture assay to measure the latent HIV-1 reservoir harboring replication-competent HIV-1 in resting CD4+ T cells in patients on HAART. However, the assay is costly, and both labor and resource intensive. To overcome some of these issues with the UDS, we developed an assay using ultra deep sequencing (UDS), which directly analyzes the number of different sequences of the induced viruses to score the number of latently HIV-infected resting CD4+ T cells. In this study, we tested the premise whether the viral sequences derived from different cell types are genetically distinct, since the assay involves a bulk culture.

Hypothesis
Viral sequences induced from different cells are distinct

Most Viral Sequences (env) Induced From Different Cells Derived From Chronic Patients Are Distinct
- Viral Sequences Derived From Acute Patients
- Viral Sequences Derived From Chronic Patients

Primer ID-Based Deep Sequencing Assay Combined With Bulk Culture To Measure Latent HIV-1 Reservoir
- Directly count the number of distinct sequences of the viruses induced to replicate in cultured resting T cells by using deep sequencing analysis.
- Primer ID significantly reduces errors introduced at PCR or sequencing steps.
- Bulk-culture assay could improve work flow with the current gold-standard assay by reducing time, labor, and cost.

Primer ID-Based Deep Sequencing Assay and IUPM calculation Scheme
- 2 million resting T cells in 5 wells and one control well without resting T cells
- 5 replicas (10 million resting T cells total)
- Ultra deep sequencing analysis of individual culture supernatant
- Count distinct viral lineages in each culture supernatant
- Generate phylogenetic tree using all viral lineages
- Count the number of wells containing the same viral lineage
- Determine the type of individual viral lineage based on Poisson distribution
- Add all the lineages from individual viral lineage

Viral Lineage (s) Derived From Individual Culture Well In Outgrowth Assay

Summary
- We were able to directly analyze the number of different sequences of the viruses induced to grow in viral outgrowth assay using ultra deep sequencing (UDS) to score the number of latently HIV-1 infected resting CD4+ T cells.
- Distinct viral sequences were observed when viruses were induced from cells derived from chronic patients, however, viral sequences induced from different cells derived from acute patients were homogeneous suggesting that this assay is not ideal for samples derived from acute patients.
- Positive correlation was observed between the number of viruses observed and the number of resting T cells per well.
- A strong correlation between IUPM-VOA and IUPM-UDS validates Primer-ID based UDS culture assay to measure the latent reservoir of replication-competent HIV-1 in resting CD4+ T cells in suppressed patients.