HIV-1-driven cancer-related gene expression as a mechanism of integration site-dependent clonal expansion and a therapeutic target

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HTLV-1 → Adult T cell leukemia (ATL) → ?

HIV-1 → ?
HTLV-1 causes adult T cell leukemia in infected CD8+ T cells in ~5% of infected individuals after ~50 years of infection.

Charles Bangham, Phil. Trans. R. Soc. B 2017
HTLV-1 infection leads to clonal expansion of CD8+ T cells

Charles Bangham, Blood 2011
Uncontrolled clonal expansion of HTLV-1 infected cells leads to adult T cell leukemia (ATL)

Charles Bangham, Seminars in Cancer Biology 2014
It takes 50-60 years from HTLV-1 infection to ATL

Charles Bangham, Phil. Trans. R. Soc. B 2017
How does HTLV-1 infection cause cancer?

Accumulation of replicative mutations in HTLV-1-infected T cell clones that persist for decades, typically over 40 years.

Cytotoxic T lymphocytes shape the landscape of HTLV-1 infected clones.

Abnormal chromatin looping in the host genome caused by HTLV-1 CTCF sites can deregulate host gene expression.

Charles Bangham, AACR 2017
Charles Bangham, Seminars in Cancer Biology 2014
HTLV-1

Clonal expansion
Immune evasion
Chromatin looping

Adult T cell leukemia (ATL)

HIV-1

?
>50% of replication competent HIV-1 come from clonally expanded HIV-1-infected cells

Hosmane, Siliciano et al., JEM 2017  Bui, Mellors et al., PLOS Pathogens 2017  Lorenzi, Nussenzweig et al., PNAS 2016
~40% of HIV-1-infected cells are clonally expanded

The frequency of clonally expanded cells increases over time

Maldarelli et al., Science 2014; Wagner et al., Science 2014; Cohn et al., Cell 2014
HIV-1-infected cells undergo clonal expansion through antigen stimulation and homeostatic proliferation

Antigen stimulation

Antigen

IL-7

IL-15

Homeostatic proliferation

Douek et al., Nature 2002; Simonetti et al., PNAS 2016; Chomont et al., Nature Med 2009; Wang et al., PNAS 2018
HIV-1 which are integrated into specific sites in the cancer-related genes underwent clonal expansion

**In vitro** infection

**In vitro** infection

**In vivo:** cells from HIV-1-infected individuals on ART

Maldarelli *et al.*, Science 2014; Wagner *et al.*, Science 2014
An in vivo selection mechanism favoring the persistence of HIV-1 infected cells remains unknown.

Do HIV-1 integration sites matter?

Maldarelli et al., Science 2014; Wagner et al., Science 2014; Cohn et al., Cell 2015
Hypothesis. HIV-1 modulates the host gene expression at the integration site and promotes the survival

**a**

![Pie charts showing gene expression in whole blood and CD8+ CAR+ cells](image)

**b**

*TET2* allele disrupted by lentiviral integration

Fraietta *et al.*, Nature 2018
Studying mechanisms of HIV-1-host interactions ex vivo using clinical samples: challenges

- Only $1 \text{–} 100/10^6 (<0.1\%)$ CD4$^+$ T cells contain infectious HIV-1 in an ART-treated, virally suppressed, HIV-1-infected individual.

- There is no reliable surface marker which can identify HIV-1-infected cells from HIV-1-infected individuals for detailed mechanistic analysis.

HIV-1 SortSeq identifies HIV-1-infected cells from virally suppressed individuals for single-cell RNAseq

Resting CD4\(^+\) T cells from ART-treated, virally suppressed, HIV-1-infected individuals

PMA/ionomycin 16 hours +ART

Activate → Stain → Sort → RNAseq
HIV-1 drives aberrant NFATC3 transcription

HIV-1 major splice donor (MSD)  NFATC3 known Splice acceptor

Position: 154_21  chr16 + 68,085,344  NFATC3  Exon 8/10

68,183,366  98,022  68,218,090  132,747

GGCGGCAGCTGCTCACATTGGCTTTGAAGTTCCTCCATATCATAACCAGCATGCTGCGAGGTGCACT

Sequence read starts from the transcription start site at HIV-1 R

Host transcription orientation  HIV-1 transcription orientation and HIV-1-host splice junction

Host translation start site  HIV-1 integration sites previously reported in vivo
HIV-1 overrides the host promoter and drives aberrant host gene transcription at the integration site.
HIV-1 drives high levels of aberrant host gene expression within the transcription unit at the integration site.

Maldarelli et al.
HIV-1 drives high levels of aberrant host gene transcription downstream but not upstream of the integration site.
HIV-1-driven aberrant splicing turns host introns into exons while HIV-1 transcription remains intact.
HIV-1 driven aberrant host gene transcription leads to aberrant truncated cancer-related protein expression

N-terminal truncated VAV1 increases oncogenesis and induces cancer transformation

*Uncontrollably active GDP/GTP nucleotide exchange factor (GEF)
*Increases uncontrolled growth

Shalom et al., Oncogenesis 2018
CRISPR-mediated HIV-1-specific repression decreases proliferation of HIV-1-infected cells harboring HIV-1 integrated into \textit{VAV1}

*Transduced cells were sorted based on fluorescent protein expression.
*Cell count was measured by flow cytometric counting of viable cells per ml.
CRISPR-mediated HIV-1-specific repression decreases proliferation of HIV-1-infected cells harboring HIV-1 integrated into VAV1

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Uninfected Jurkat

- dCas9-VP64-mCherry (Activating)
- dCas9-Krab-mCherry (Inhibiting)

HIV-1 specific activation

8B10 HIV-1-Jurkat (VAV1)

- dCas9-VP64-mCherry (Activating)
- dCas9-Krab-mCherry (Inhibiting)

HIV-1 specific repression

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HTLV-1

Clonal expansion
Immune evasion
Chromatin looping

Adult T cell leukemia (ATL)

➢ 50-60 years
➢ 5% of infected individuals

HIV-1

Clonal expansion
Immune evasion
Chromatin looping

Treatment?
Antiretroviral therapy (ART) alone does not cure HIV infection. Why not?

ART inhibits the activity of viral enzymes but does not kill the infected cells.

ART does not inhibit HIV promoter activity. An active HIV-1 promoter contributes to immune activation and exhaustion.
Single-cell analysis identified genes required for HIV-1 expression

Heat map shows differential expression level in Z-score, which means the number of standard deviations away from mean. The P value is calculated using a Wald Test (Love et al., 2014). The adjusted P value is calculated by Benjamin-Hochberg corrections.
HIV-1 SortSeq identifies *IMPDH1* which is required for HIV-1 reactivation

De novo purine synthesis

**IMPDH**

**Mycophenolic acid**

The active form of MMF

Depletes guanine nucleotides in lymphocytes
Impact of IMPDH inhibition in vivo is currently being examined:

“MMF for HIV Reservoir Reduction”
Phase II, PI Schiffer/Hladik
ClinicalTrials.gov #NCT03262441
Clonal expansion
Viral cytopathic effect
Immune selection pressure
HIV-1 persistence
Chromatin Looping?
Ongoing (R61)
Antigen stimulation
Homeostatic proliferation
HIV-1-driven proliferation
Ongoing (R01)

Single cell analysis
Cellular environment
HIV-1-driven cancer-gene expression

Resting CD4+ T cells from ART-treated, virally suppressed, HIV-1-infected individuals
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HIV-1 SortSeq
Activate → Stain → Sort → RNAseq

NFATC3

Ongoing (R61)

Immunological
selection
pressure
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Impact of HIV-1-driven aberrant host gene transcription: Construction of a HIV-1 reporter cell line for mechanistic analysis

- Confirmed HIV-1 integration site in \textit{proliferation-related genes associated with in vivo clonal expansion}
- No additional transcription terminator in the cassette
- Contains Tat, TAR, Rev, RRE, and all splice sites
- Single-round infection

\textbf{HIV-1-Jurkat model}

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