Cellular, Tissue, and Gene Therapies Advisory Committee Meeting

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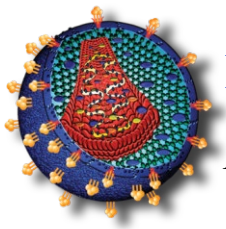
Integration of HIV Proviruses in Oncogenes Can Cause Clonal Expansion of T Cells and Contribute to the Development of T Cell Lymphomas

Stephen Hughes



HIV Dynamics and Replication Program

NCI at Frederick

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Clonal Expansion/Disease Following Lentiviral Vector Treatment in Humans and NHP

Clonal Expansion (Humans)

Cavazzana-Calvo M, et al. Transfusion independence and HMGA2 activation after gene therapy of human β-thalassaemia. Nature. 2010 PMID: 20844535

Fraietta JA, et al. Disruption of TET2 promotes the therapeutic efficacy of CD19-targeted T cells. Nature. 2018 PMID: 29849141

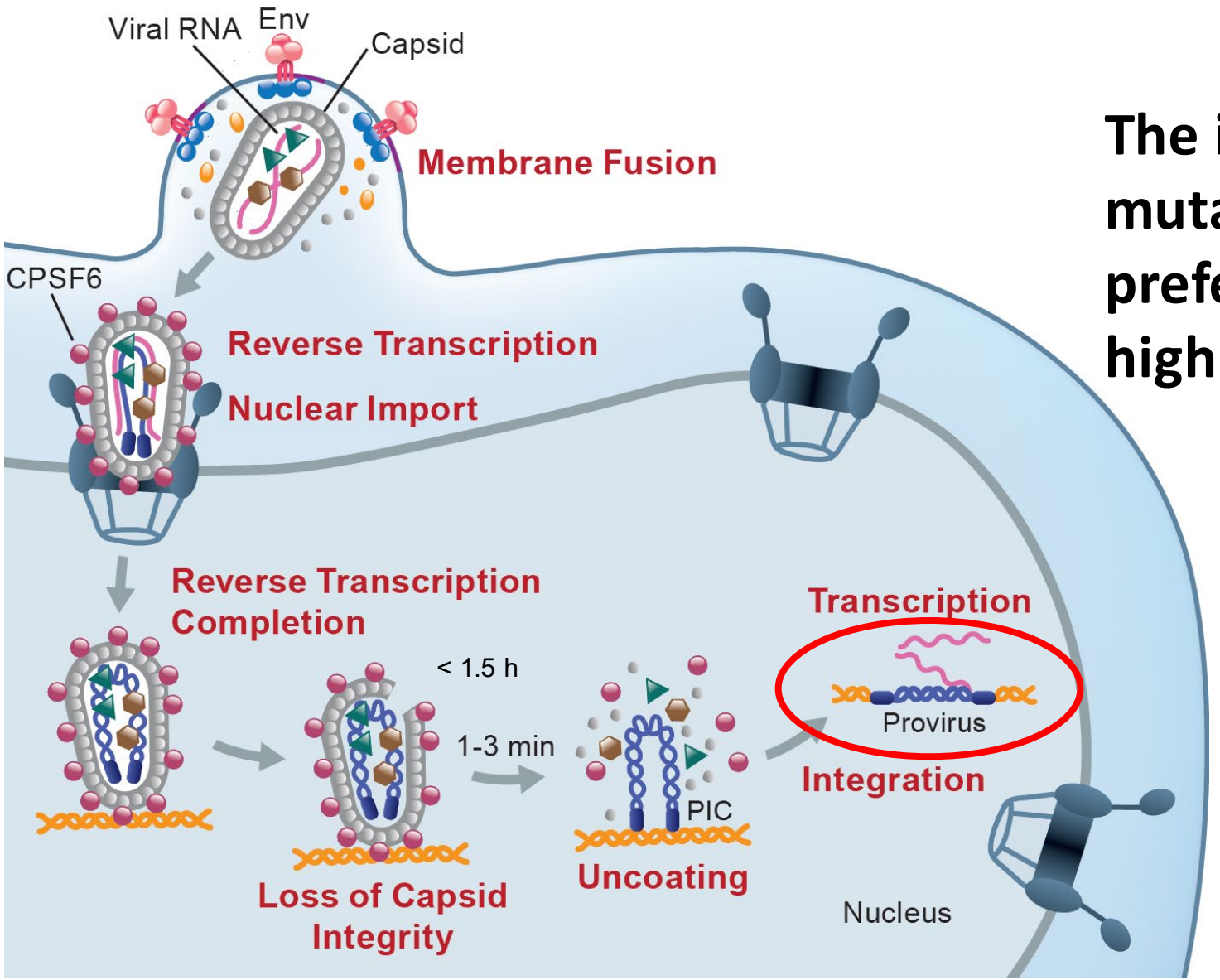
Shah NN, et al. Clonal expansion of CAR T cells harboring lentivector integration in the CBL gene following anti-CD22 CAR T-cell therapy. Blood Adv. 2019 PMID: 31387880

Lethal Disease (NHP)

Espinoza DA, et al. Aberrant Clonal Hematopoiesis following Lentiviral Vector Transduction of HSPCs in a Rhesus Macaque. Mol Ther. 2019 PMID: 31023523

9 proviruses in the problematic cells

Early Stages of HIV-1 Replication



The insertion of a provirus is mutagenic. HIV proviruses preferentially integrate in highly expressed host genes.

HIV Integration and Integration Site Analysis In Vivo

HIV proviruses preferentially integrate into the bodies of highly expressed genes as a result of interactions with two host proteins, CPSF6 and LEDGF.

In vivo, the initial distribution of HIV proviruses/integration sites is affected by both positive and negative selections on the infected cells (and their progeny).

Maldarelli et al. Specific HIV integration sites are linked to clonal expansion and persistence of infected cells. Science. 2014. PMID: 24968937

Wagner TA, et al. HIV latency. Proliferation of cells with HIV integrated into cancer genes contributes to persistent infection. Science. 2014. PMID: 25011556

Sherman E, et al: INSPIIRED: A Pipeline for Quantitative Analysis of Sites of New DNA Integration in Cellular Genomes. Mol Ther Methods Clin Dev. 2016 PMID: 28344990

Wells DW et al. An analytical pipeline for identifying and mapping the integration sites of HIV and other retroviruses. BMC Genomics. 2020 PMID: 32151239; PMCID: PMC7063773.

Coffin JM, Hughes SH. Clonal Expansion of Infected CD4+ T Cells in People Living with HIV. Viruses. 2021 PMID: 34696507

Isolation of Integration Sites and Identification of Clonally Expanded Cells

DNA is fragmented and the host-virus DNA junctions are selectively amplified by Linker-Mediated-PCR.

We amplify both the 5' and 3' LTR/host DNA junctions and both ends of the amplified DNAs are sequenced.

We estimate that we recover ~10% of the proviruses in samples from infected individuals.

All of the cells in an expanded clone are descended from one infected cell; the proviruses in all the cells in a clone are integrated in exactly the same site (this is how we identify clones).

We can monitor the independent isolation of the same host-virus junction in a sample because the sheared ends of the host DNA differ. Repeated isolation of the same host virus junction with different host DNA breakpoints is evidence for clonal expansion.

Why Do HIV Infected T Cells Clonally Expand In Vivo?

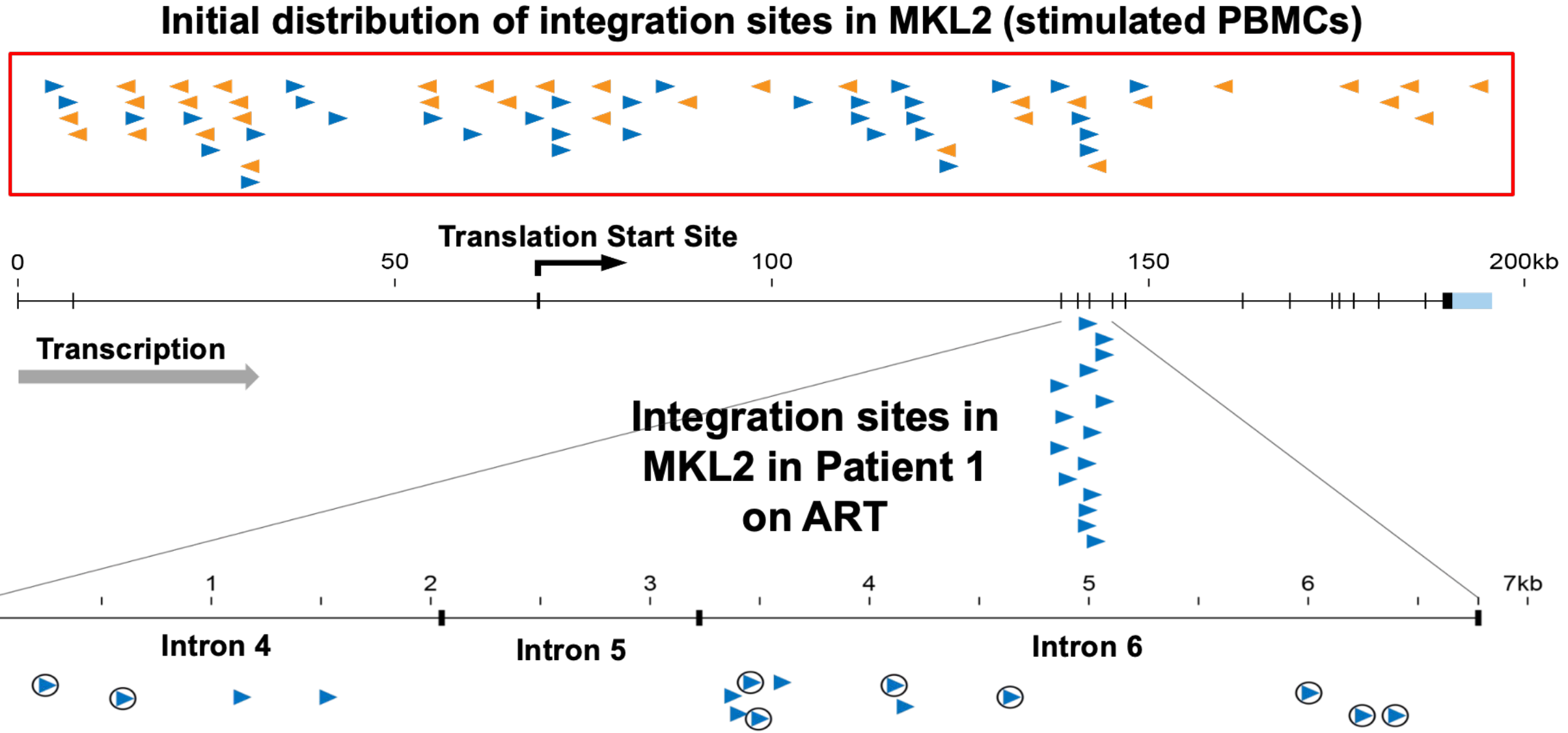
1. **Because the majority of HIV infected cells are T cells, which clonally expand in response to antigens and/or cytokines.**

2. **Because, in the parental T cell, a provirus is integrated in or near an oncogene, altering its expression and promoting the growth of the cells. There are seven genes in which HIV proviruses can cause clonal expansion.**

Recognizing Genes in Which HIV Proviruses Can Contribute to the Growth and Persistence of T Cells

- There is an enrichment for proviruses in the gene in vivo (relative to the starting distribution).
- (HIV) proviruses that cause clonal expansion of T cells in vivo are integrated in a host gene and oriented in the same direction as the gene (neither is always true for other retroviruses).
- (HIV) proviruses that cause clonal expansion in vivo are integrated in specific introns.

There Is a Selection for T Cells with Proviruses in MKL2 in Patient 1 (>10 Years on Therapy)



Genes in Which Proviruses Can Cause Clonal Expansion/Persistence of T Cells

Gene name	Unique IS in genes on-ART	IS in PBMC	PBMC/on ART (Enrichment)	Enrichment probability (Poisson)	Provirus orientation: same as gene/opposite	Orientation probability (Binomial)	Selected IS relative to protein coding exons
All genes	25,731	326,033	12.67 (1.0)		11,476/ 14,255	7.0×10^{-60}	
STAT5B	268	562	2.1 (6.0)	1.4×10^{-114}	197/71	3.5×10^{-15}	Upstream
BACH2	98	132	1.3 (8.7)	9.2×10^{-56}	71/20	3.6×10^{-08}	Upstream
MKL2	49	69	1.4 (8.5)	5.7×10^{-27}	40/6	1.6×10^{-07}	In between
MKL1	85	331	3.9 (3.2)	34.7×10^{-19}	53/30	7.6×10^{-03}	In between
IL2RB	30	68	2.3 (4.8)	1.8×10^{-4}	17/9	9.8×10^{-04}	Upstream
MYB	11	31	3.1 (4.1)	2.3×10^{-05}	10/0	1.5×10^{-01}	In between
POU2F1	15	43	2.9 (4.4)	7.5×10^{-07}	10/5	1.5×10^{-01}	Upstream

The Fraction of the Clones of Expanded T Cells in Which a Provirus Integrated in an Oncogene Causes the Host Cell to Grow and/or Persist is Small (Ca. 2-3%)

In most cases, the clonal expansion of HIV infected T cells is not caused by a provirus integrated in an oncogene, but by the same forces/factors that cause uninfected T cells to clonally expand and persist (antigen stimulation and/or cytokines).

Can HIV Proviruses Directly Contribute to the Development of T Cell Lymphomas?

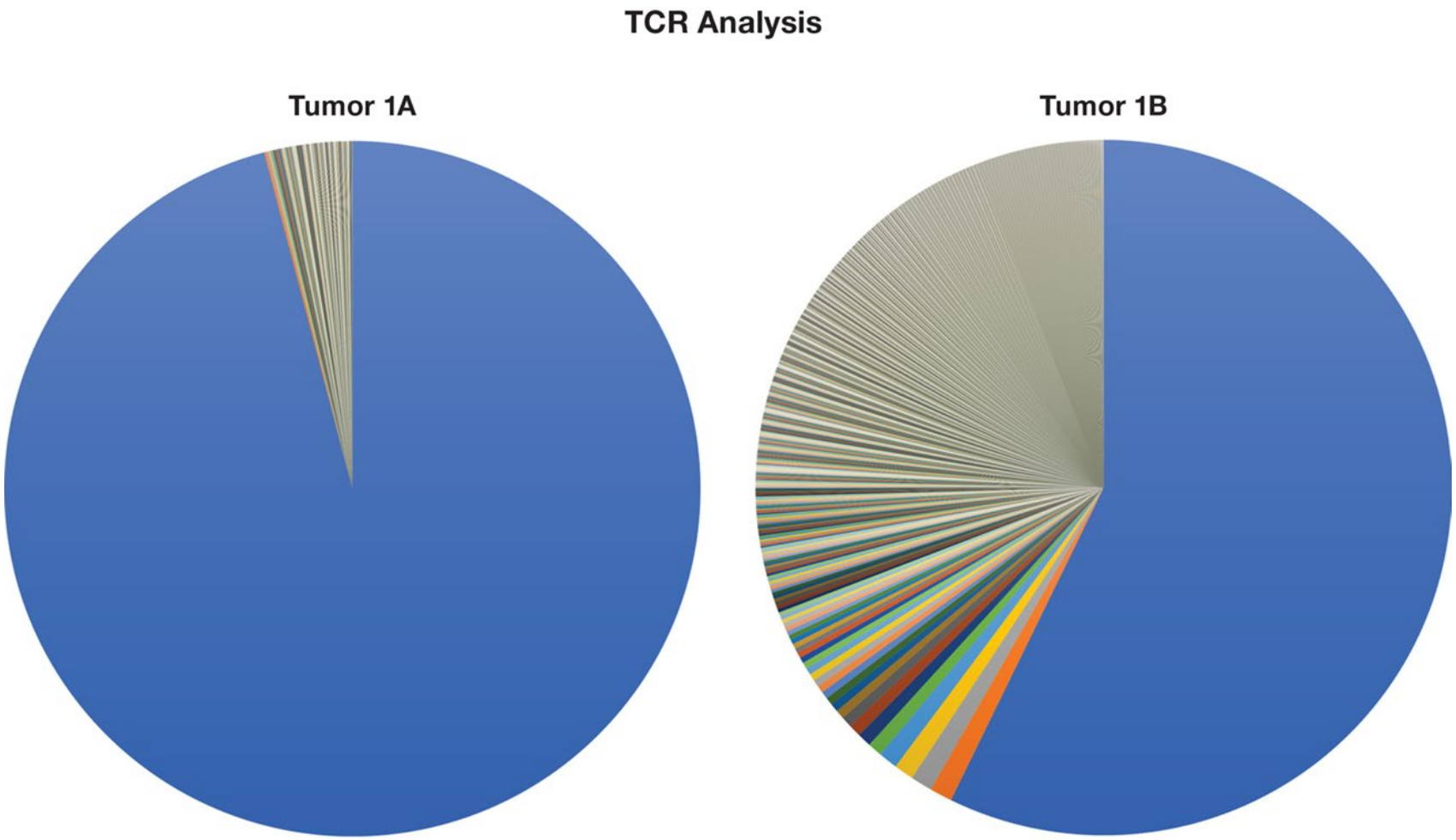
YES

Insertional activation of *STAT3* and *LCK* by HIV-1 proviruses in T cell lymphomas. Mellors JW et al. Sci Adv. 2021 PMID: 34644108

Summary of Specimen Type and Diagnostic Pathology

Donor ID	Specimen Type and Diagnostic Pathology	DNA Ratio (HIV LTR: β-Globin)
1A	Skin, High-Grade T Cell Lymphoma (Frozen Tissue)	2.9
1B	Skin 2nd Site, High-Grade T Cell Lymphoma (Frozen Tissue)	ND
11	Lymph Node, Cutaneous Anaplastic Large Cell Lymphoma (FFPE Tissue)	6.6
12A	Skin, Anaplastic Large Cell Lymphoma (FFPE Tissue)	10.4
12B	Skin 2nd Site, Anaplastic Large Cell Lymphoma (FFPE Tissue)	3.3
10 others	Various T, NK cell, Burkitt's Lymphoma	<.01-.09
	Control Human Heart (FPPE Tissue)	<.01

Lymphomas IA and IB Share a Predominant TCR: Both Tumors Arose from the Same T cell

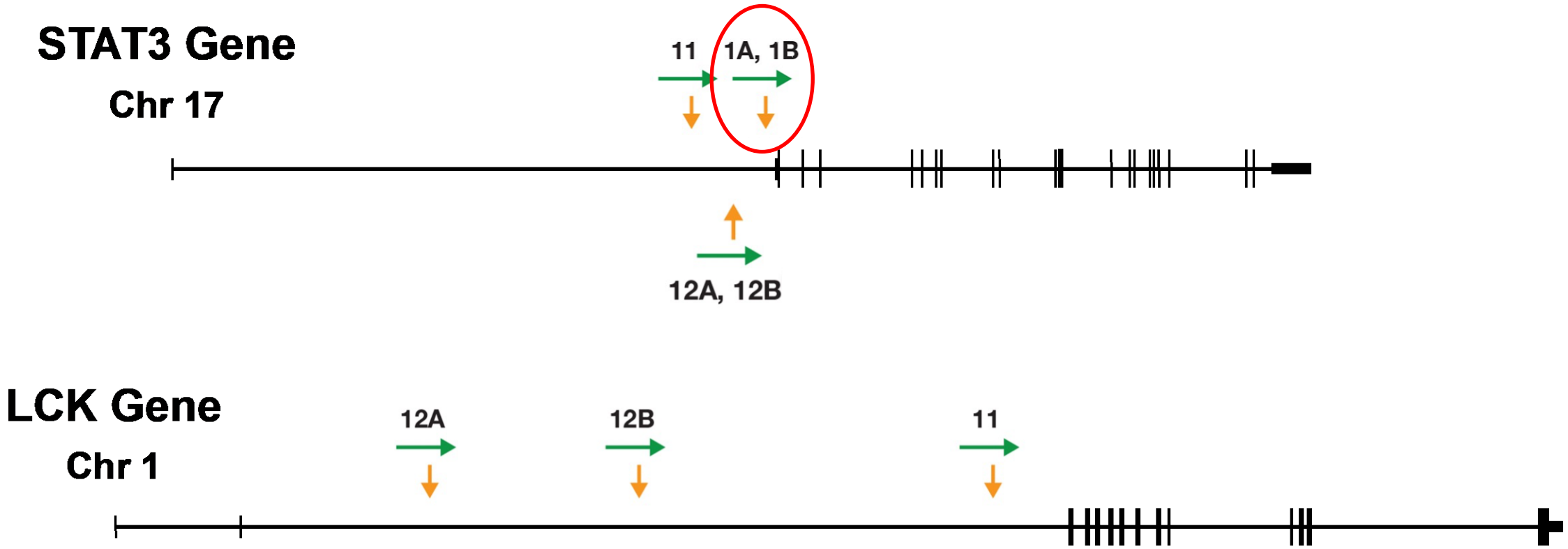


Most cells have the clonotype CASSDGTWNGYTF.

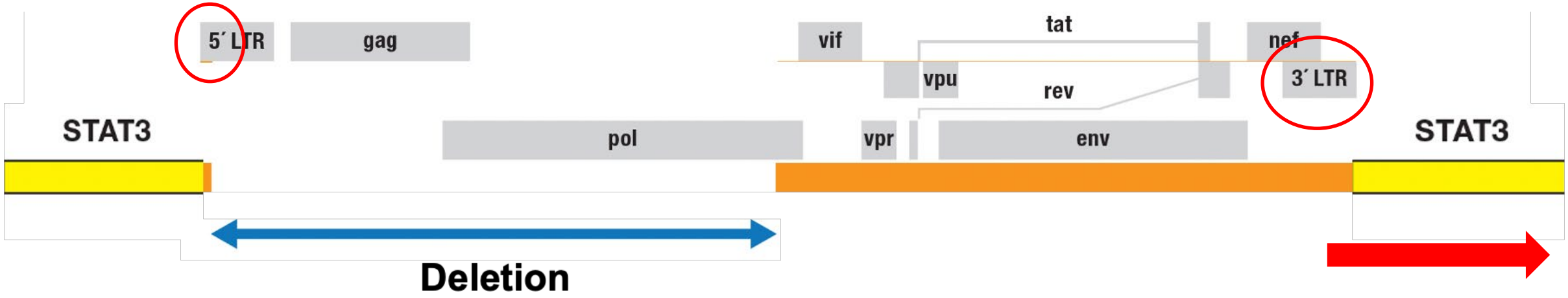
The HIV Positive T Cell Lymphomas Have Proviruses Integrated in STAT3 and LCK; Most Are Heavily Superinfected

Sample	Integration Site, Provirus Orientation in the Host Genome	Provirus Orientation on the Host Gene	Integration Sites Isolated	Integration sites/Breakpoints Identified	
Tumor 1A	chr17, -40,500,566	+STAT3	3LTR/5LTR	~1000	Frozen Tissue
Tumor 1A		All Others		~8000	
Tumor 1B	chr17, -40,500,566	+STAT3	3LTR/5LTR	~500	
Tumor 1B		All Others		~750	
Tumor12A	chr17, -40,502,259	+STAT3	3LTR/5LTR	16	FFPE
Tumor12A	chr1, +32,724,529	+LCK	3LTR/5LTR	10	
Tumor12A		All Others		15	
Tumor12B	chr17, -40,502,259	+STAT3	5LTR	13	
Tumor12B	chr1, +32,729,544	+LCK	5LTR	38	
Tumor12B		All Others		2	
Tumor 11	chr17, -40,506,110	+STAT	3LTR	2	
Tumor 11	chr1, +32,738,734	+LCK	5LTR	2	
Tumor 11	chr3, -45,742,822	+SACM1L	3LTR/5LTR	7	
Tumor 11		All Others		12	

Lymphomas 1A and IB Have a Provirus in Exactly the Same Site in the First Intron of STAT3. The Other Lymphomas Have Proviruses in Both the First Intron of STAT3 and the First Intron of LCK



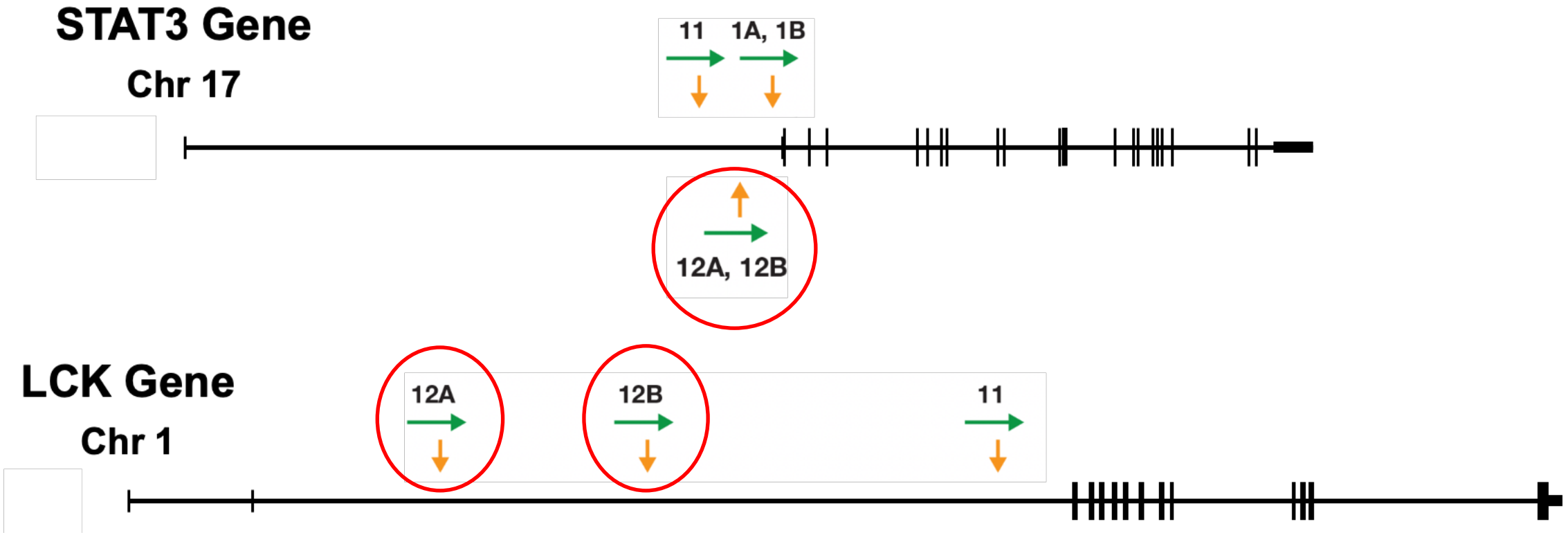
In Lymphomas 1A/1B the Provirus in STAT3 Is Highly Deleted; STAT3 Is Expressed from the 3' LTR



Defective Provirus, 5036 bp

STAT3 is (over)expressed from the 3' LTR promoter

Lymphomas 12A and I2B Have a Provirus in Same Site in the First Intron of STAT3. Both 12A and 12B Have Proviruses the First intron of LCK, but in Different Places (~5 kB Apart)



HIV Proviruses in STAT3 and LCK Can Contribute to the Formation of T Cell Lymphomas

- **HIV proviruses integrated in the STAT3 and LCK can play an important role in the growth and development of T cell tumors.**
- **Integration of a provirus in STAT3 and LCK does not directly cause the clonal expansion of T cells in vivo (STAT3 and LCK are not on the list of 7 genes in which proviruses can cause clonal expansion in vivo).**
- **T cell lymphomas are rare, even in HIV infected individuals. The progression to the lymphomas appears to be a multistep process (we found a somatic mutation in STAT3 in one lymphoma and activation of LCK by an HIV provirus in three others).**
- **For LTR promoter driven expression, Tat would need to be expressed. In the 1A/1B lymphomas, Tat was expressed from the STAT3 promoter.**
- **The HIV infected T cell tumors we analyzed were almost all heavily superinfected late in their development.**

Proviruses of HIV-1 and HIV-1 Vectors Can Affect Host Gene Expression

The LTR promoter has been removed from Self-INactivating (SIN) vectors. Thus, in the SIN vectors, there is no LTR promoter that could drive host gene expression.

The SIN vectors do have an internal promoter.

Deletions and other changes arise frequently in HIV and other retroviruses (and their vectors). Changes in proviruses and can affect the ability of the provirus to alter the expression of host genes.

The primary target cells for HIV infection are CD4+ T cells. T cell tumors are quite rare in both those who are and are not HIV infected.

Animal models based on non-lenti retroviruses suggest that susceptibility to tumorigenesis is both cell type and virus type dependent.

Conversion of a normal cell to a tumor cell is a multi-step process. Having multiple proviruses in infected cells will increase the risk (STAT3 + LCK).

HIV Integration Site Analysis

VDRS: A. Ferris

Leidos: X. Wu, L. Su, A. Guo, D. Demirov, D. Wells

DRP-CRS: F. Maldarelli, F. Simonetti, S. Hill

DRP-TRU: M. Kearney, J. Spindler, Wei-Shao, M. Bale, S. Patro

DRP-VMS: V. Pathak, M. Munshi, R. Burdick

DRP-Tufts: J. Coffin

Pittsburgh: J. Mellors, M. Sobolewski, F. Hong, J. Zerbato, L. Brandt, L. Halvas

Leidos: B. Luke

NIAID: E. Boritz and D. Douek

Special thanks to the patient volunteers

HIV-1 Replication Cycle