Drexel UNIVERSITY | Drexel MEDICINE

22nd International Conference on Human Retrovirology:
HTLV and Related Retroviruses
HTLV2026

June 3-6, 2026 | Philadelphia, Pennsylvania, USA

# Workshop 2
# **Molecular Virology and Immunopathogenesis**

Moderators: *Edward Harhaj & B. Hilda Ye*

22nd International Conference on Human Retrovirology:
HTLV and Related Retroviruses
HTLV2026

June 3-6, 2026 | Philadelphia, Pennsylvania, USA

# Genetic landscape and clonal evolution of ATLL

Aileen Rowan
Imperial College London, UK

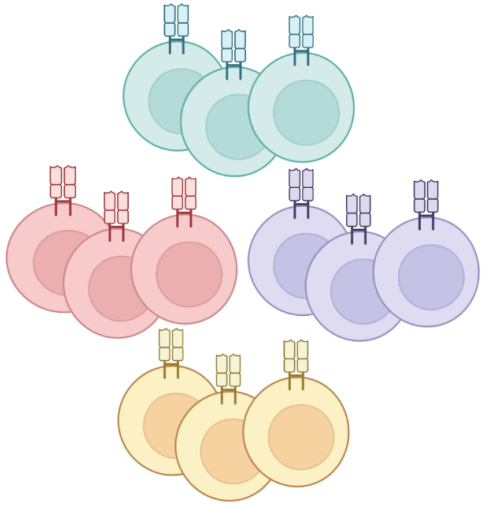
# Genetic landscape and clonal evolution of Adult T cell Leukaemia/Lymphoma

Dr Aileen Rowan
Assistant Professor
Department of Infectious Disease

IMPERIAL

# What happens during Adult T cell leukaemia/lymphoma (ATL) development?

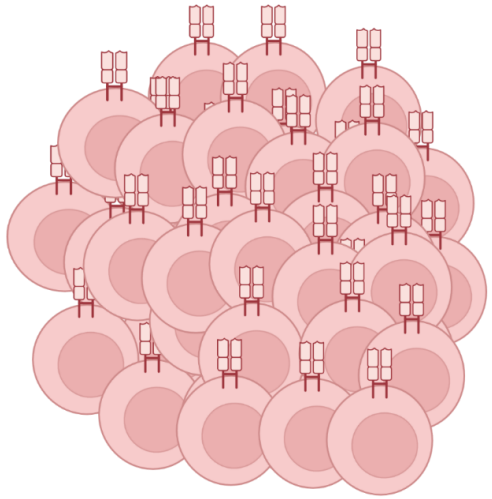
Healthy HTLV-1 carriers



>10,000 clones, each with a distinct proviral integration site and T cell receptor (TCR)

Decades

Adult T cell Leukaemia/Lymphoma

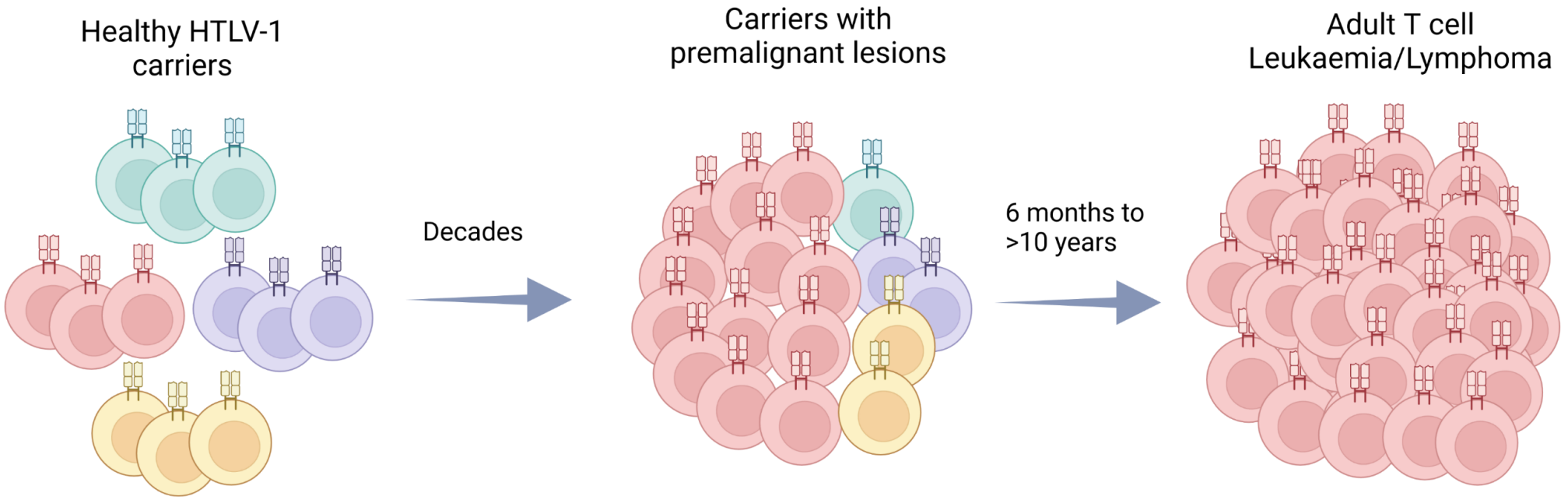


One clone comprises 50-100% of peripheral T cells/lymph node T cells

High proviral load
Family history of ATL
Infection early in life

Yoshida et al, PNAS 1984
Tsukasaki et al, Blood 1997
Cook et al, Blood 2014

# What happens during Adult T cell leukaemia/lymphoma (ATL) development?




**‘ATL-like clones’**

Rowan et al, Blood 2020

n=6, retrospective study

Carrying mutations in cellular genome

# Questions to address:

1. What proportion of carriers with ATL-like clones subsequently develop ATL?

2. What factors favour the emergence of ATL-like clones?

   (in addition to high PVL, age, family history)

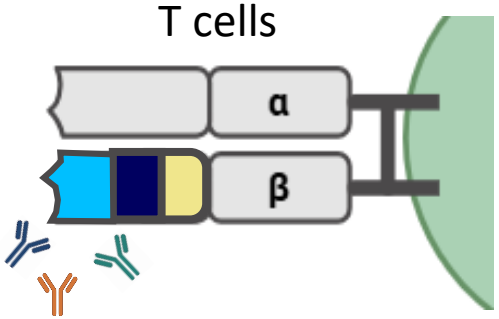
# Q1: Incidence of ATL, prospective study:

## Flow cytometric quantification of ATL-like clones

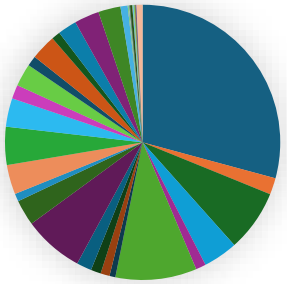
*Wolf et al, Blood Cancer Journal, 2021*

Sonia Wolf
Patricia Watber,
Momoko Usui

Quantify TCRVβ subunits expressed by CCR4CD6- T cells



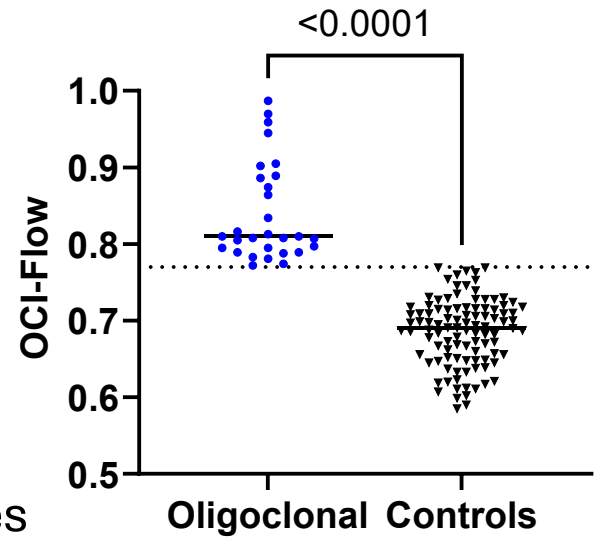

Assign clonality score:

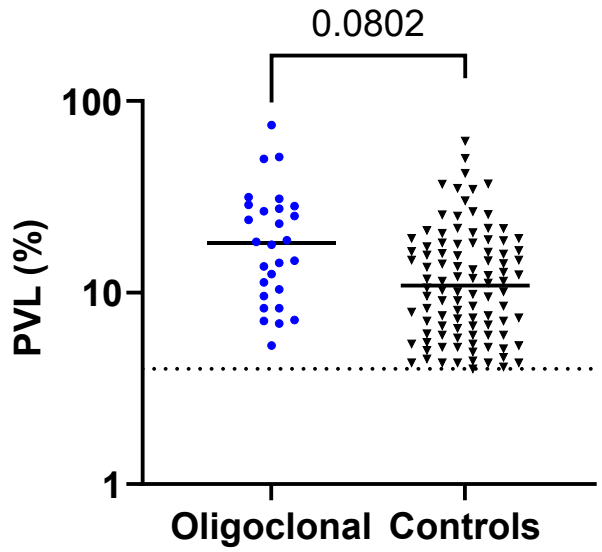


**Approach**

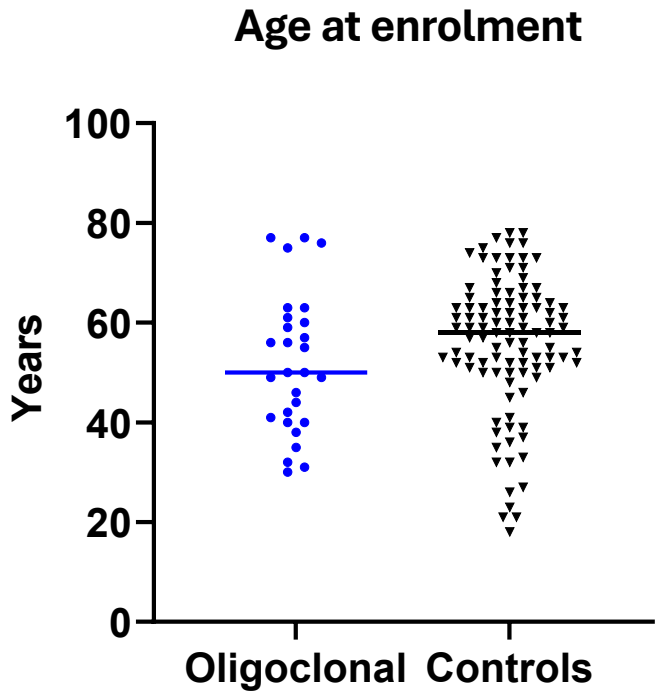
- HTLV -1 infection with high PVL (>4%)
- Stratified by clonality score
- Exclude anyone with a diagnosis of ATL
- Include carriers with any other HTLV-related diseases

| Clone detected OCI>0.770 PVL≥4% | Control OCI≤0.770 PVL≥4% |
|---|---|



| | Clone detected | Control |
|---|---|---|
| N= | **29** | **102** |
| Mean age at start of F/U (range) | 49 (26-77) | 56(18-78) |
| Female | 16 (55%) | 83 (81%) |
| Asymptomatic | 17 (59%) | 36 (35%) |
| HAM | 5 (17%) | 56 (55%) |
| Other (HIV coinfection, Neurological, Polymyositis, Strongyloides, Arthritis) | 7 (24%) | 10 (10%) |
| Total F/U (median, range) | **154 years** (5.9, 1-12.5) | **842 years** (6.3, 0-24) |




996 years all PVL>4% carriers

Expected rate = 2-10/1000 patient-years in PVL>4% carriers

....Expect 2-10 cases

# Incidence of ATL in each group

| | Clone detected | Control |
|---|---|---|
| N= | 29 | 102 |
| Total F/U | 154 years | 842 years |
| ATL cases | | |
| Cases/1000 patient-years (95% CI) | | |

# Incidence of ATL in each group

| | Clone detected | Control |
|---|---|---|
| N= | 29 | 102 |
| Total F/U | 154 years | 842 years |
| ATL cases | 9* | 0 |
| Cases/1000 patient-years (95% CI) | **58.5** (30-112) | 0 |

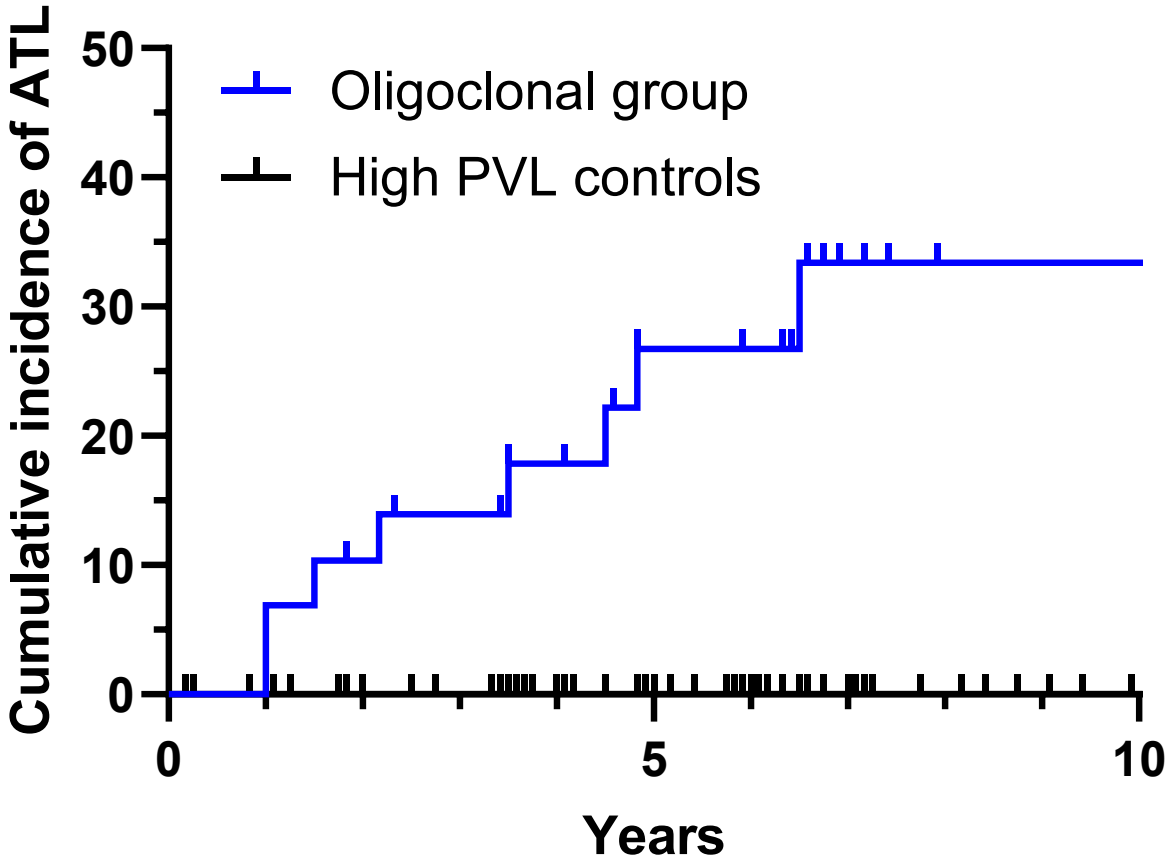
Highly significant:
*Fisher's exact test p<0.0001
vs Control High PVL group, two tailed

9 cases of ATL observed/1000 patient years
95% CI 4.7-17 cases/1000 patient-years

- compatible with predicted rate

HTLV-1 carriers with detectable ATL-like clones are at high risk of developing ATL – **'High-risk' carriers**

# Incidence of ATL over time




**~30% chance of ATL in following 5 years**

p<0.0001

Can variation in rate be explained?

# Test for somatic mutations in host genes

Patricia Watber and
Anat Melamed

Enrich genes of interest from sample- subject to deep sequencing

| Driver gene | Sum % SNV, SV, CNV | Target |
|---|---|---|
| CARD11 | 42.7 | Exon + Intron |
| PLCG1 | 37.3 | Exon |
| CCR4 | 35.3 | Exon |
| CIC | 35.3 | Exon |
| PRKCB | 31.3 | Exon |
| TBL1XR1 | 36.7 | Exon |
| ARID2 | 30.0 | Exon |
| CDKN2A | 29.3 | Exon |
| TP53 | 33.3 | Exon |
| STAT3 | 26.7 | Exon |
| GATA3 | 23.3 | Exon |
| ATXN1 | 26.0 | Exon |
| IRF4 | 22.0 | Exon |
| CD58 | 29.3 | Exon |
| VAV1 | 20.0 | Exon |
| HLA-A | 16.0 | Exon |
| NOTCH1 | 18.7 | Exon |
| POT1 | 15.3 | Exon |
| CBLB | 20.7 | Exon |
| FAS | 13.3 | Exon |
| HLA-B | 14.0 | Exon |

| Driver gene | Sum % SNV, SV, CNV | Target |
|---|---|---|
| CD28 | 15.3 | Exon |
| GPR183 | 17.3 | Exon |
| CCR7 | 12.7 | Exon |
| EP300 | 12.7 | Exon |
| REL | 12.7 | Exon + Intron |
| RHOA | 12.7 | Exon |
| CD274 | 14.0 | Exon + Intron |
| CSNK2B | 9.3 | Exon |
| TET2 | 9.3 | Exon |
| ITGB1 | 8.0 | Exon |
| XPO1 | 8.7 | Exon |
| EEF1A1 | 8.0 | Exon |
| IRF2BP2 | 8.0 | Exon |
| ZNF292 | 8.7 | Exon |
| KLF2 | 6.7 | Exon |
| PIK3CD | 5.3 | Exon |
| ZFP36L2 | 6.7 | Exon |
| PRDM1 | 5.3 | Exon |
| SMARCB1 | 6.0 | Exon |
| CSNK2A1 | 5.3 | Exon |
| FYN | 4.7 | Exon |

151 Kbp host genes
9 Kbp HTLV-1 proviral genome

**Genomic DNA-** PBMC, LN biopsies, dried blood spots

Design based on published datasets-
Kataoka Nat Genetics 2015,
Kogure Blood 2022

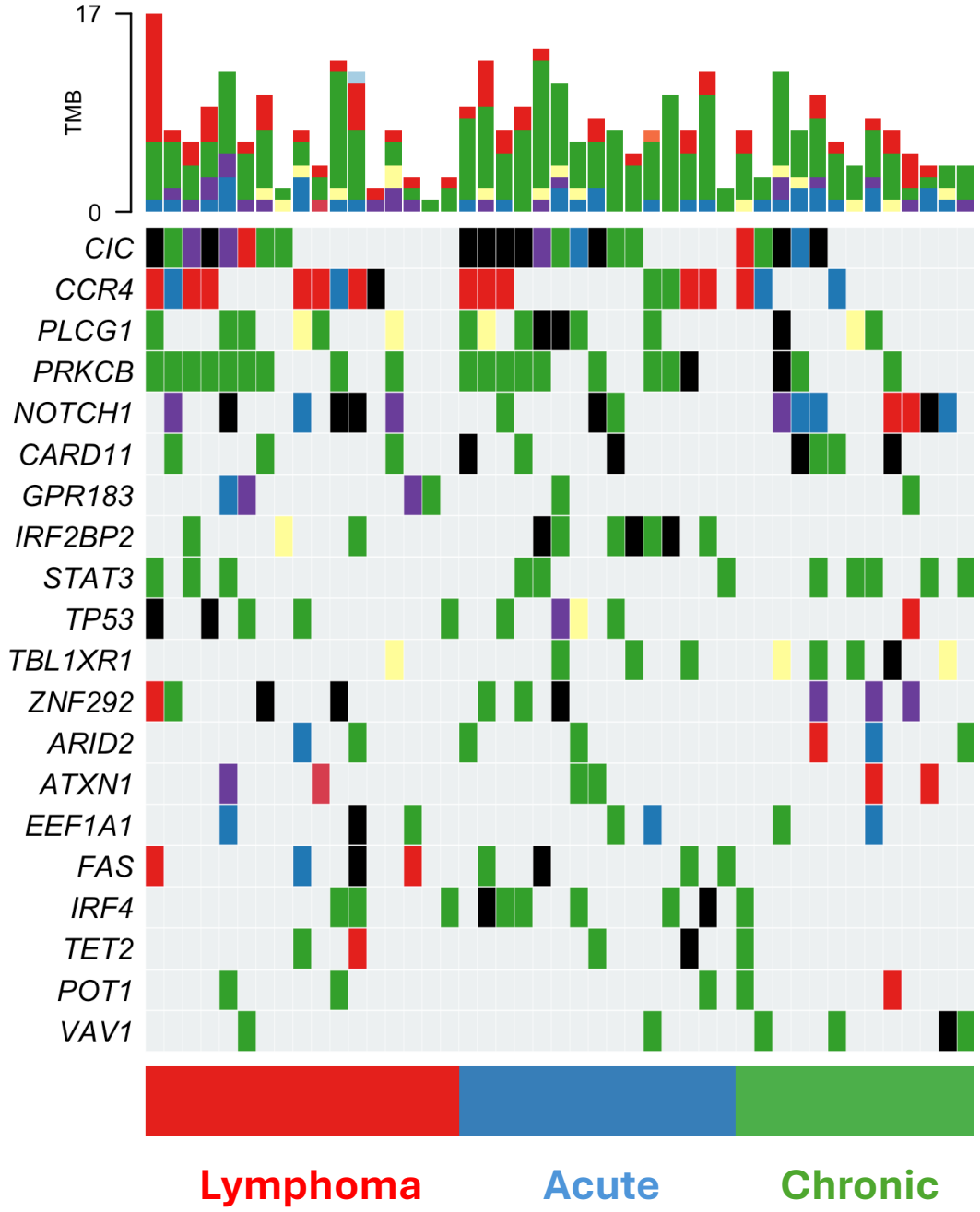
# Study design : Cross sectional

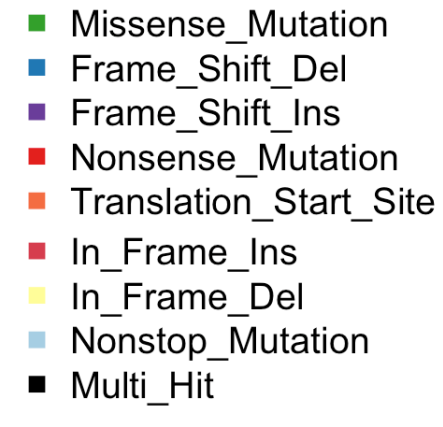
PBMC DNA
ATL: pre-treatment diagnostic
10,000X deep sequencing
+ variant calling

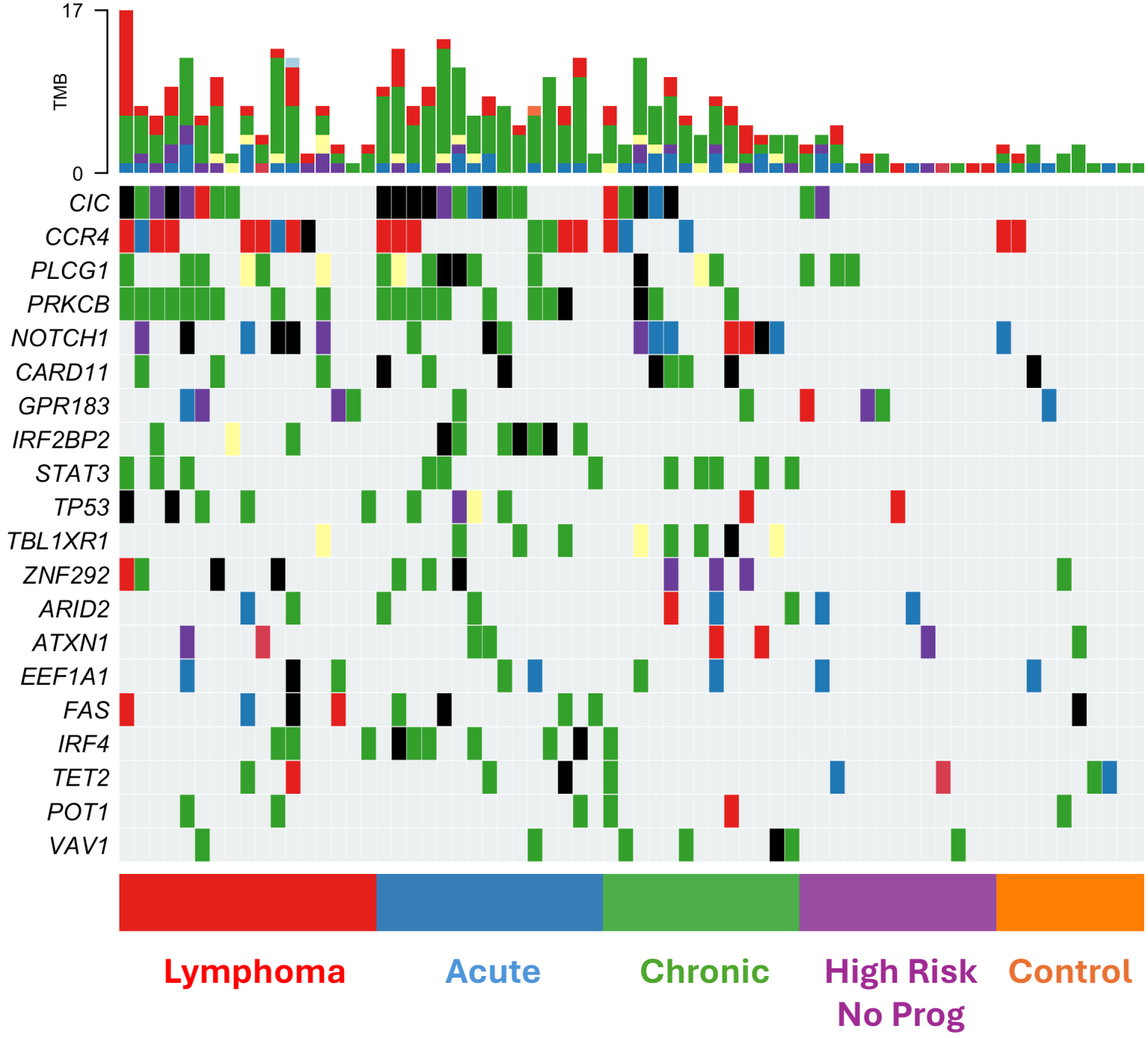
| Asymptomatic | Indolent | Aggressive |
|---|---|---|
| Controls n= 20 | Smoldering n=5 | Acute n=15 |
| High risk n=29* | Chronic n=13 | Lymphoma n=17 |

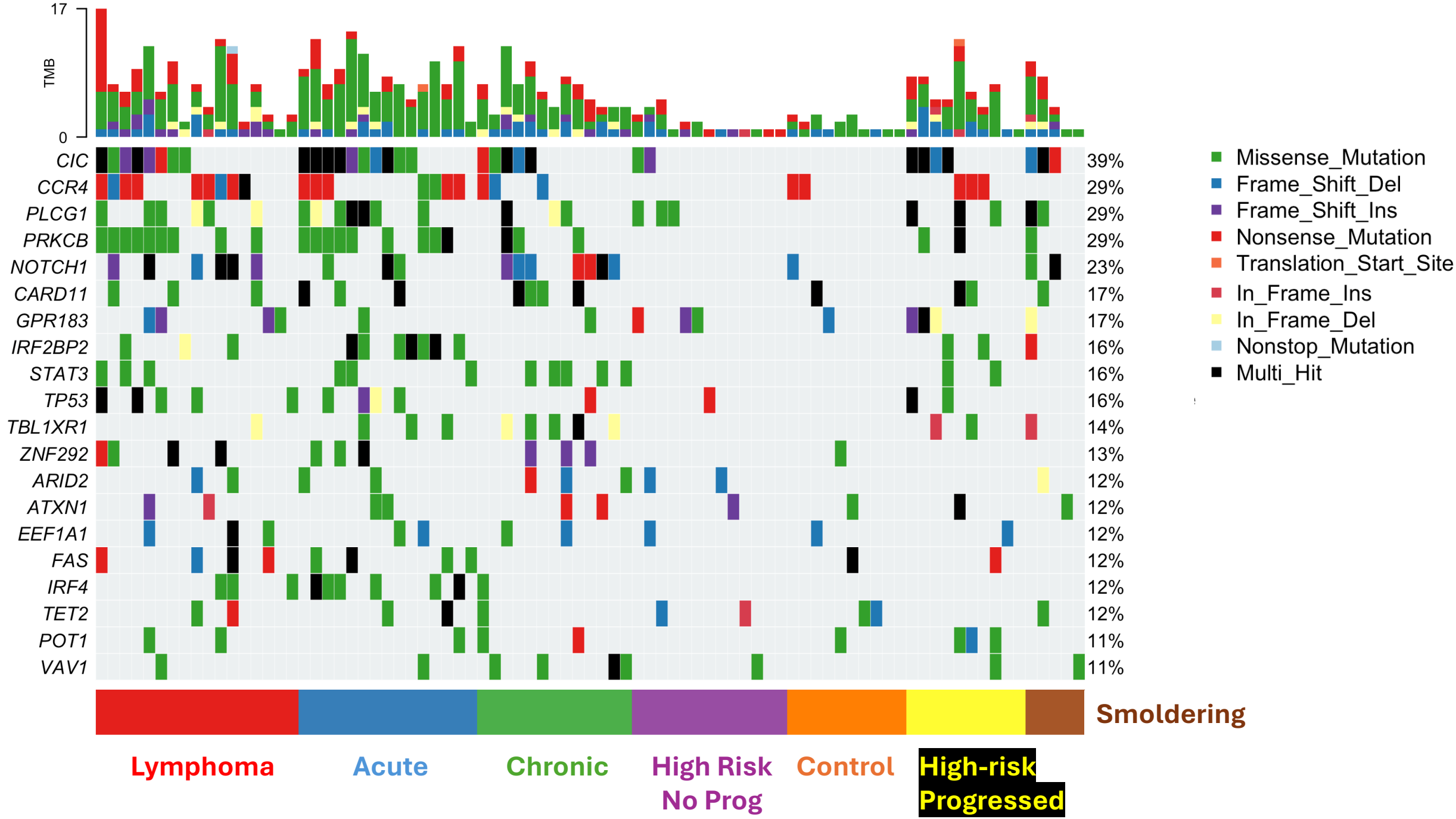
* High-risk group split into two sub-groups

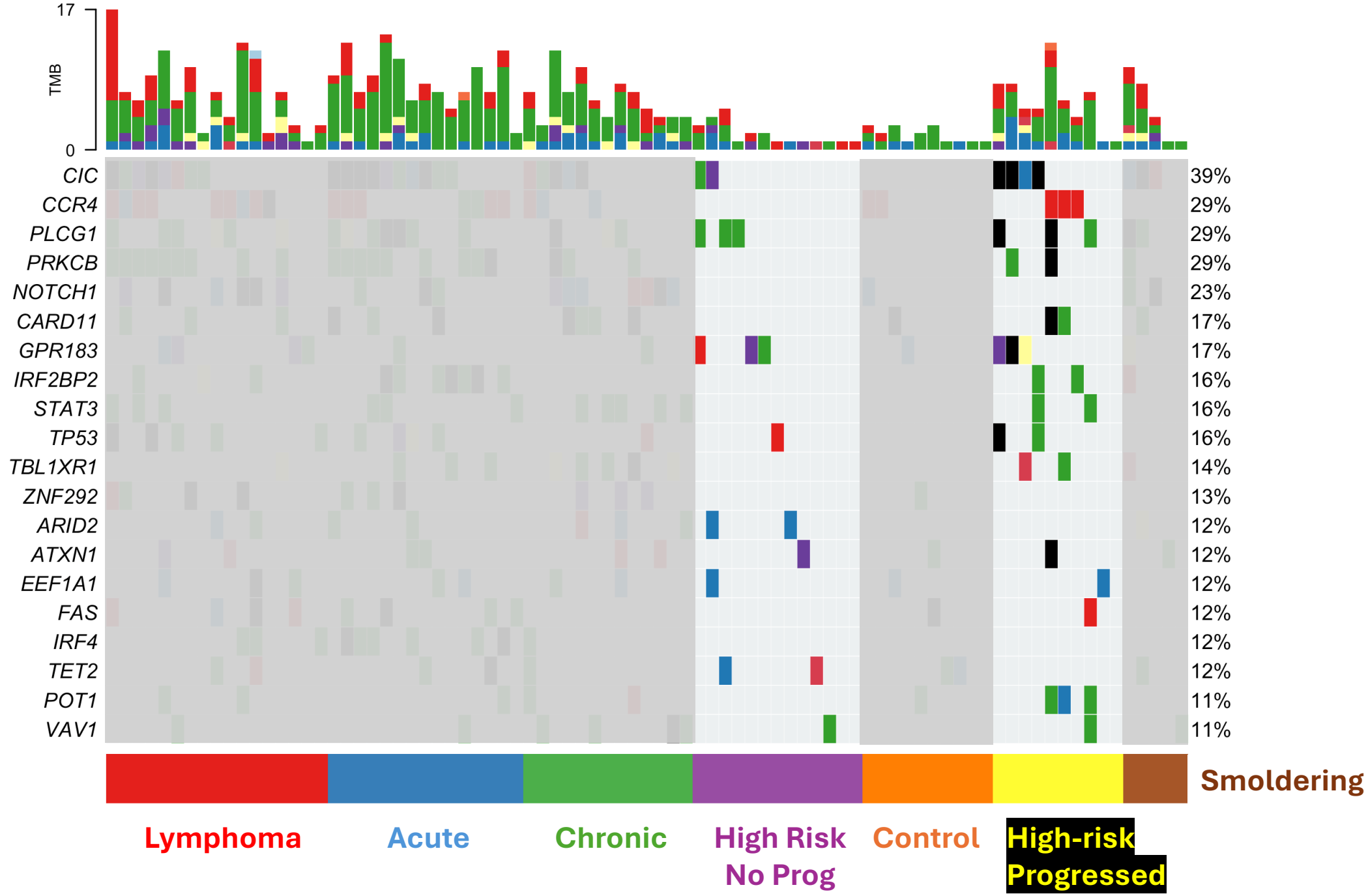
Progressed n=13 ATL in 2+ years
No Progression n= 16 still asymptomatic

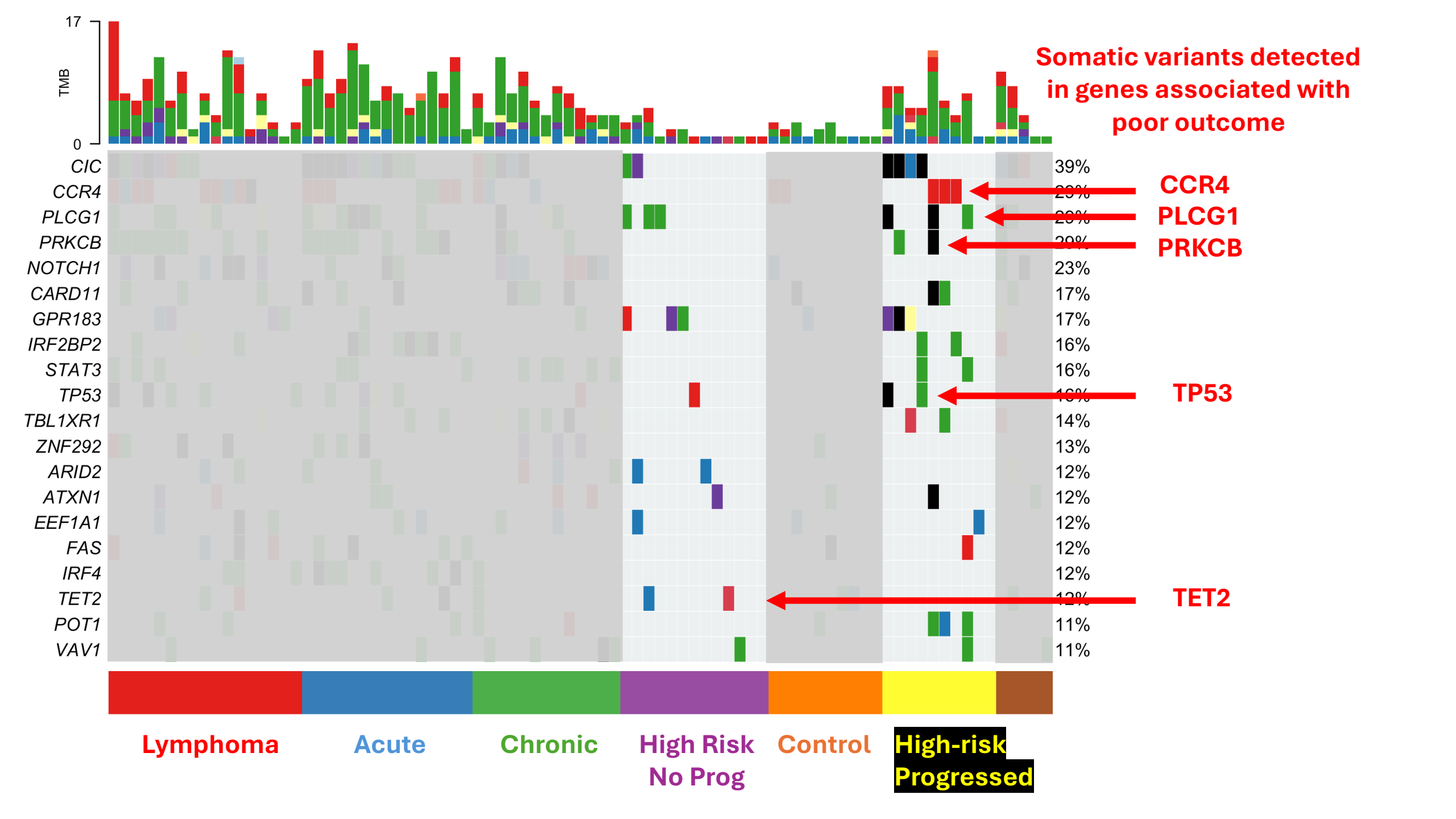

17
TMB
0
CIC
CCR4
PLCG1
PRKCB
NOTCH1
CARD11
GPR183
IRF2BP2
STAT3
TP53
TBL1XR1
ZNF292
ARID2
ATXN1
EEF1A1
FAS
IRF4
TET2
POT1
VAV1
Lymphoma
Acute
Chronic


Missense_Mutation
Frame_Shift_Del
Frame_Shift_Ins
Nonsense_Mutation
Translation_Start_Site
In_Frame_Ins
In_Frame_Del
Nonstop_Mutation
Multi_Hit


17
TMB
0
CIC
CCR4
PLCG1
PRKCB
NOTCH1
CARD11
GPR183
IRF2BP2
STAT3
TP53
TBL1XR1
ZNF292
ARID2
ATXN1
EEF1A1
FAS
IRF4
TET2
POT1
VAV1
Lymphoma
Acute
Chronic
High Risk
No Prog
Control
Missense_Mutation
Frame_Shift_Del
Frame_Shift_Ins
Nonsense_Mutation
Translation_Start_Site
In_Frame_Ins
In_Frame_Del
Nonstop_Mutation
Multi_Hit

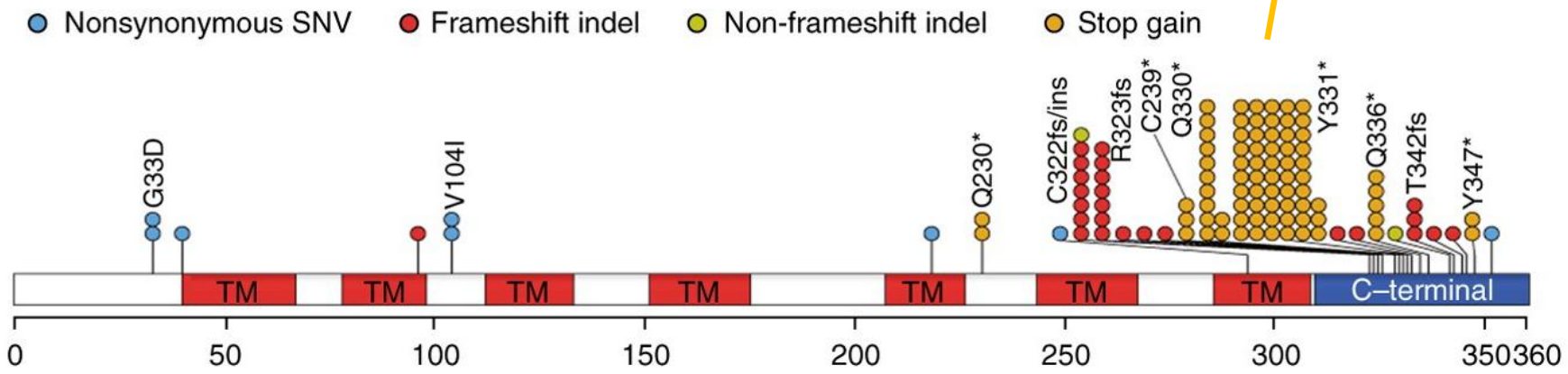

17
TMB
0
CIC 39%
CCR4 29%
PLCG1 29%
PRKCB 29%
NOTCH1 23%
CARD11 17%
GPR183 17%
IRF2BP2 16%
STAT3 16%
TP53 16%
TBL1XR1 14%
ZNF292 13%
ARID2 12%
ATXN1 12%
EEF1A1 12%
FAS 12%
IRF4 12%
TET2 12%
POT1 11%
VAV1 11%
Missense_Mutation
Frame_Shift_Del
Frame_Shift_Ins
Nonsense_Mutation
Translation_Start_Site
In_Frame_Ins
In_Frame_Del
Nonstop_Mutation
Multi_Hit
Smoldering
Lymphoma
Acute
Chronic
High Risk
No Prog
Control
High-risk
Progressed


17
TMB
0
CIC 39%
CCR4 29%
PLCG1 29%
PRKCB 29%
NOTCH1 23%
CARD11 17%
GPR183 17%
IRF2BP2 16%
STAT3 16%
TP53 16%
TBL1XR1 14%
ZNF292 13%
ARID2 12%
ATXN1 12%
EEF1A1 12%
FAS 12%
IRF4 12%
TET2 12%
POT1 11%
VAV1 11%
Smoldering
Lymphoma
Acute
Chronic
High Risk No Prog
Control
High-risk Progressed


17
TMB
0
Somatic variants detected in genes associated with poor outcome
CIC 39%
CCR4
PLCG1
PRKCB
NOTCH1 23%
CARD11 17%
GPR183 17%
IRF2BP2 16%
STAT3 16%
TP53
TBL1XR1 14%
ZNF292 13%
ARID2 12%
ATXN1 12%
EEF1A1 12%
FAS 12%
IRF4 12%
TET2
POT1 11%
VAV1 11%
Lymphoma
Acute
Chronic
High Risk No Prog
Control
High-risk Progressed

# Challenge: Novel variant discovery

Y331X truncates C terminal trapping receptor on cell surface, enhancing signalling




**CCR4 360aa**

*Kataoka et al, Nat Genetics 2015*

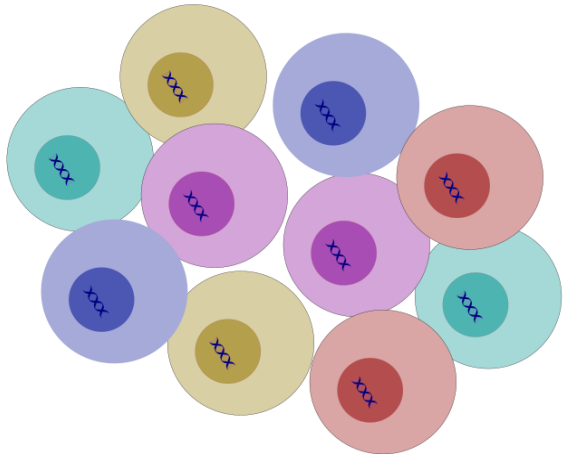
**Our work:**

| Variant | Count | Variant | Count |
|---|---|---|---|
| S24N | 1 | S346Lfs*9 | 1 |
| G33D | 1 | Y331X/Sfs | 13 |
| N300* | 1 | Q330lfs*11 | 1 |
| L328Pfs | 1 | C329X | 3 |
| S345X | 1 | Y347Lfs*9 | 1 |

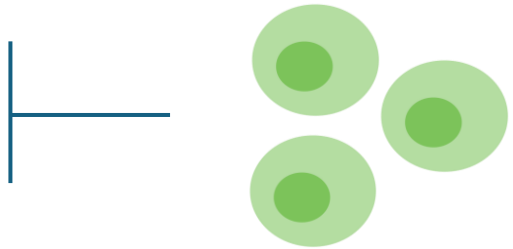
How should we interpret the clinical significance of these previously unseen variants?

# Q2: What factors favour the emergence of ATL-like clones?

**Healthy virus-carriers**

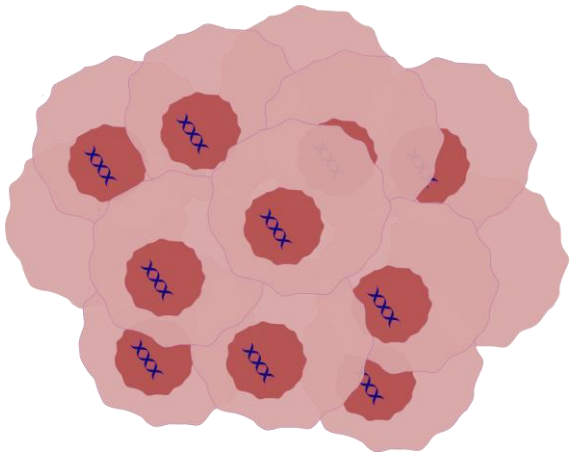


**Polyclonal virus-infected cells**



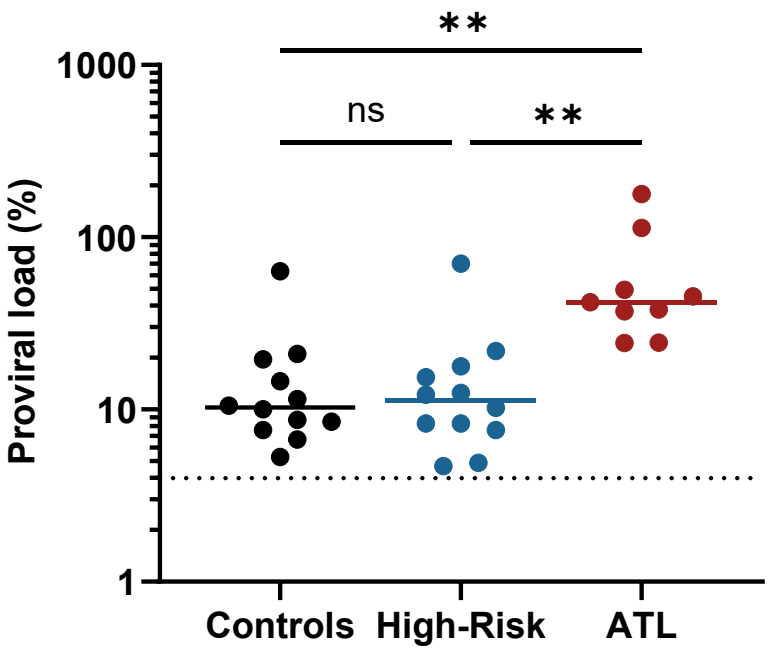
**HTLV-specific CD8+ T cells**

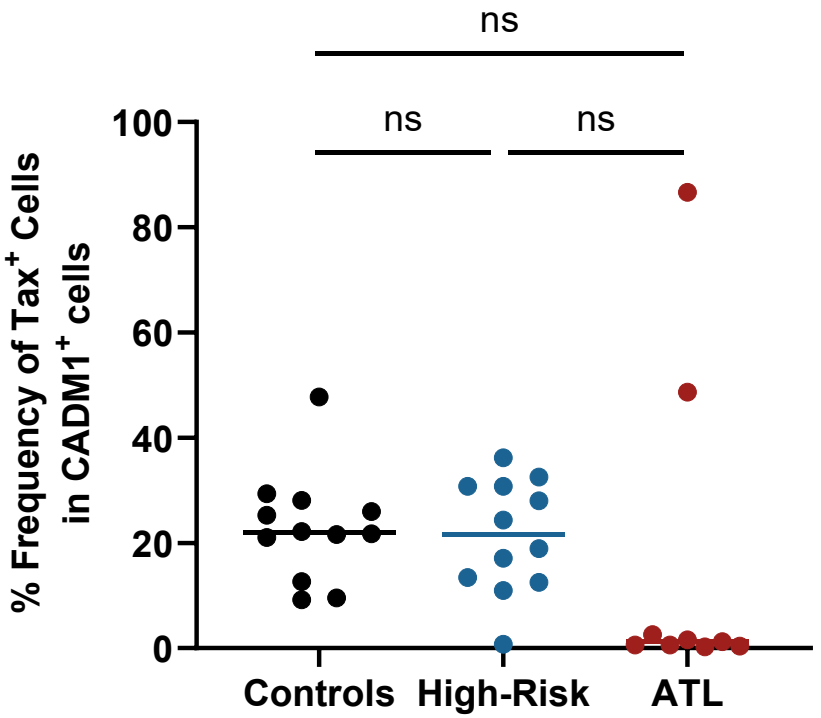
X

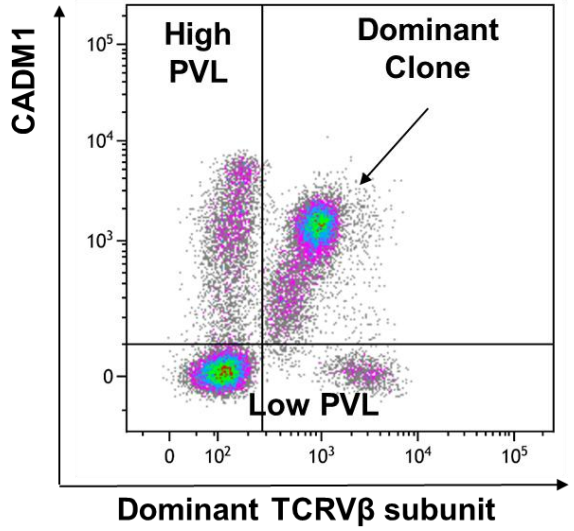


# Cross-sectional study, control donors matched for PVL and antigen burden

Devon Weterings

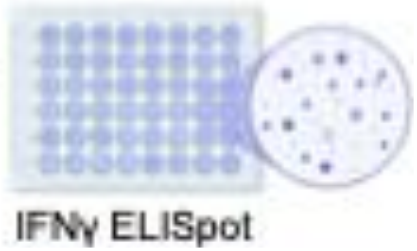


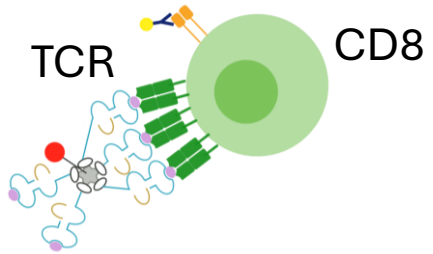
# Very low frequencies of Tax-specific CD8+ T cells in high-risk carriers

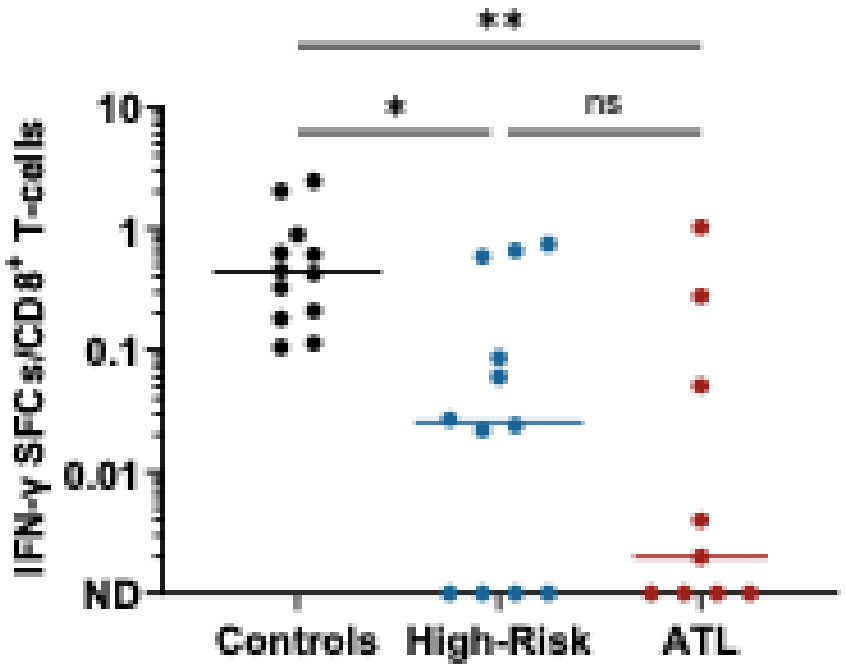
**Functional assay**
Culture PBMC with
Tax/ HBZ
overlapping peptides

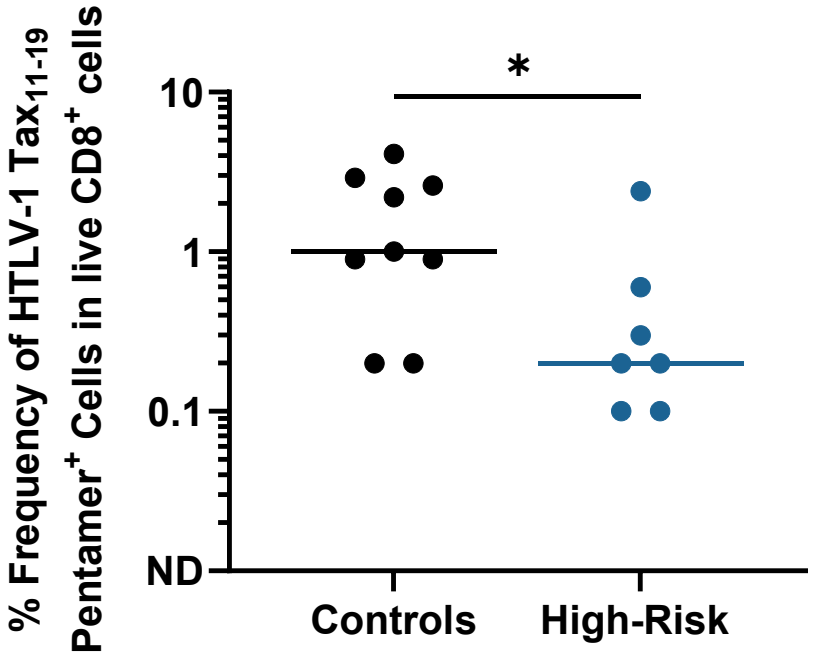



**Absolute quantification**

Tax 11-19/ HLA A*0201
Peptide-MHC Pentamer:

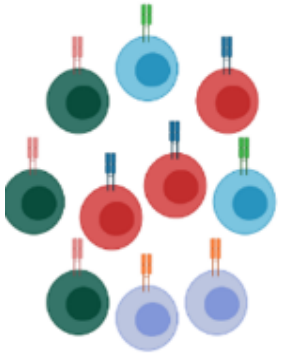


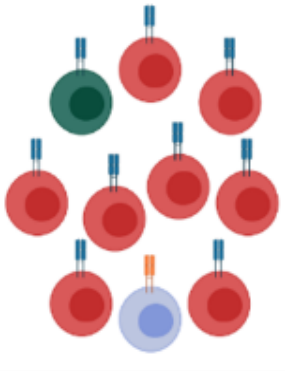



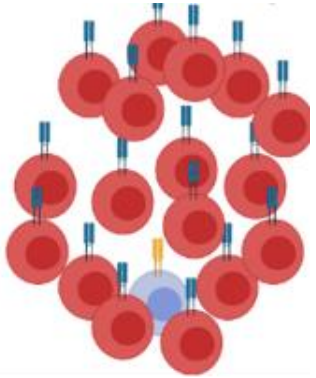




*Weterings et al, in press Blood Neoplasia*

# Emerging picture of immune dysregulation:







| | Controls | High-risk carriers | ATL |
|---|---|---|---|
| Expression of Tax by clone | n/a | Downregulated/lost in 50%, Upregulated in 22% | Downregulated/lost in 78%, Upregulated in 22% |
| Efficiency of autologous CTL in ex vivo assay | Control group | Significantly less than control | Significantly less than control |
| Tax-specific IFN-g by CD8+ T cells | Detected in all low risk carriers | 33% no detectable response | 66% no detectable response |

**Poster A29**
**Devon Weterings**

# Take home messages

1. What proportion of carriers with ATL-like clones subsequently develop ATL?
   - ~30% chance of symptomatic disease within 5 years
   - Associated with burden of somatic variants, some of which have been associated with poor outcome/aggressive disease.
   - Variant detection and interpretation remains a challenge

2. What factors favour the emergence of ATL-like clones?

   Age, PVL, Family history of ATL, diminished CTL surveillance?

**Single cell evolution**
**Poster A60**
**Anat Melamed**

**Clinical implementation**
**Poster A02**
**Prognostic working group**

**TRBC clonality**
**Poster A64**
**Momoko Usui**

# Acknowledgements:

*Thanks to blood donors & clinic staff*


*Section of Virology*

Rowan Group
**Anat Melamed**
**Momoko Usui**
**Patricia Watber**
**Devon Weterings**
**Sonia Wolf**

Taylor group
**Graham Taylor**
**Carol Rosadas**
Huseini Kagdi

Molecular Diagnostic Unit
**Claire Greiller**
Anjna Badhan
Maria Antonietta Demontis
Simon Dustan
Phil Hepplestone
Rachael Quinlan

*National Centre for Human Retrovirology*

**Lucy Cook**
**Divya Dhasmana**
**Adine Adonis**
**Jana Haddow**
**Jerico Baylon**

*Guy's and St Thomas Hospital*
*Kings College*

Richard Dillon
Paul Fields

*Section of Immunology of Infection*

**Charles Bangham**
Aviva Witkover
Jocelyn Turpin
Mun Jun Liew

LEUKEMIA & LYMPHOMA SOCIETY®

UKRI Medical Research Council

NHS National Institute for Health Research

Lymph&Co

22nd International Conference on Human Retrovirology:
HTLV and Related Retroviruses
HTLV2026
June 3-6, 2026 | Philadelphia, Pennsylvania, USA

Thomas Jefferson University | Drexel University | Drexel Medicine

Kumamoto University

3rd June 2026, WORKSHOPS, HTLV 2026

# How Does Persistent HTLV-1 Infection Rewire T-Cell Function and Drives Disease?

Yorifumi Satou

Hokkaido University, Japan

formerly Kumamoto University

# HTLV-1 establishes latent/persistent infection and drives pathogenesis

**The retrovirus HTLV-1 inserts an ectopic CTCF-binding site into the human genome**

Yorifumi Satou[a,b,c,d,e,1], Paola Miyazato[b,c,d], Ko Ishihara[c,e], Hiroko Yaguchi[a], Anat Melamed[a], Michi Miura[a], Asami Fukuda[b,c,d], Kisato Nosaka[f], Takehisa Watanabe[g,2], Aileen G. Rowan[a], Mitsuyoshi Nakao[e,g], and Charles R. M. Bangham[a,1]

[a]Section of Virology, Division of Infectious Diseases, Imperial College, London W2 1PG, United Kingdom; [b]Centre for AIDS Research, Kumamoto University, Kumamoto 860-0811, Japan; [c]Priority Organization for Innovation and Excellence, Kumamoto University, Kumamoto 860-0811, Japan; [d]International Research Centre for Medical Science, Kumamoto University, Kumamoto 860-0811, Japan; [e]Core Research for Evolutionary Science and Technology, Japan Science and Technology Agency, Saitama 332-0012, Japan; [f]Cancer Centre, Kumamoto University Hospital, Kumamoto University, Kumamoto 860-0811, Japan; and [g]Department of Medical Cell Biology, Institute of Molecular Embryology and Genetics, Kumamoto University, Kumamoto 860-0811, Japan

nature COMMUNICATIONS

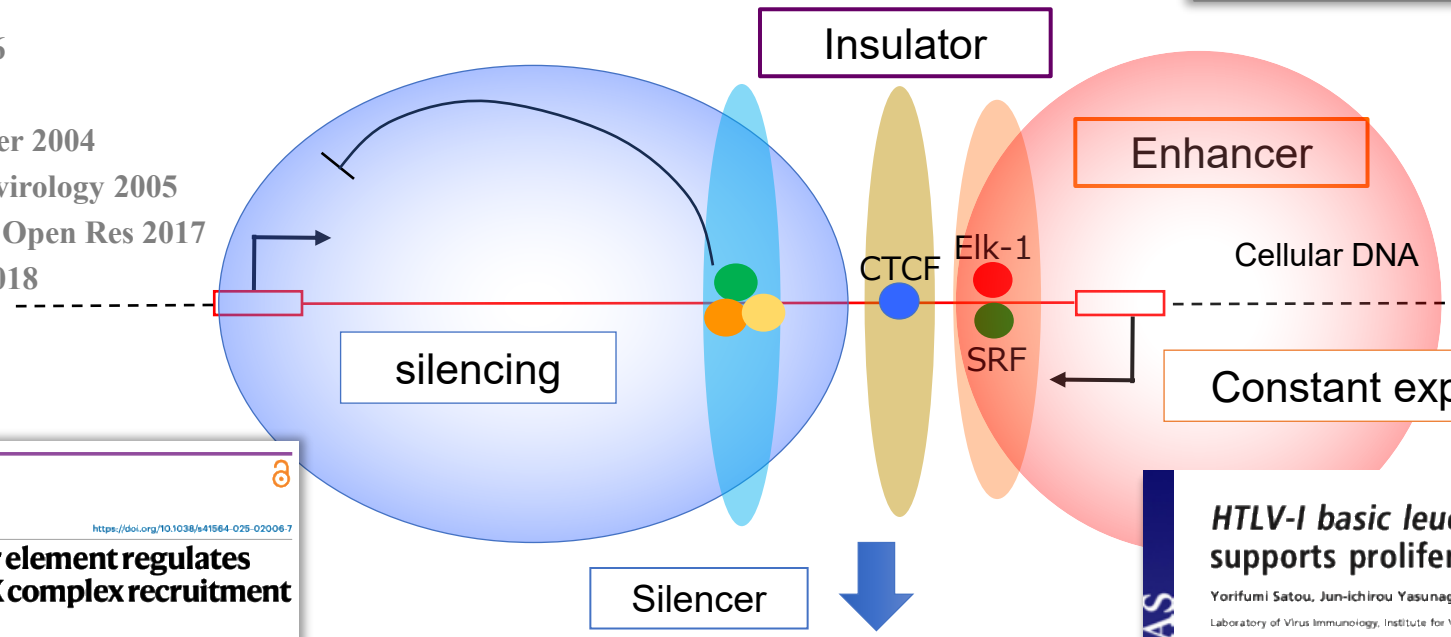
ARTICLE

https://doi.org/10.1038/s41467-022-30029-9 OPEN

**Identification and characterization of a novel enhancer in the HTLV-1 proviral genome**

Misaki Matsuo[1,2], Takaharu Ueno[3,12], Kazuaki Monde[4,12], Kenji Sugata[1,12], Benjy Jek Yang Tan[1,2], Akhinur Rahman[1], Paola Miyazato[1,2], Kyosuke Uchiyama[1,2], Saiful Islam[1,2,11], Hiroo Katsuya[1,5], Shinsuke Nakajima[3], Masahito Tokunaga[6], Kisato Nosaka[7,8], Hiroyuki Hata[9], Atae Utsunomiya[6,10], Jun-ichi Fujisawa[3] & Yorifumi Satou[1,2]

Tamiya et al, Blood 1996
Koiwa et al, JV 2002
Takeda et al, Int J Cancer 2004
Taniguchi Y et al, Retrovirology 2005
Billman et al, Wellcome Open Res 2017
Mahgoub et al, PNAS 2018




Usui et al, Retrovirology 2008
Saito et al, Retrovirology 2009

nature microbiology

Article

https://doi.org/10.1038/s41564-025-02006-7

**Intragenic viral silencer element regulates HTLV-1 latency via RUNX complex recruitment**

Received: 20 April 2024

Accepted: 7 April 2025

Published online: 13 May 2025

Check for updates

Kenji Sugata[1,15], Akhinur Rahman[1,15], Koki Niimura[1,2], Kazuaki Monde[3], Takaharu Ueno[4], Samiul Alam Rajib[1], Mitsuyoshi Takatori[1], Wajihah Sakhor[1], Md Belal Hossain[1], Sharmin Nahar Sithi[1], M. Ishrat Jahan[1], Kouki Matsuda[5], Mitsuharu Ueda[5], Yoshihisa Yamano[14], Terumasa Ikeda[6], Takamasa Ueno[10], Kiyoto Tsuchiya[11], Yuetsu Tanaka[12], Masahito Tokunaga[13], Kenji Maeda[5], Atae Utsunomiya[13], Kazu Okuma[4], Masahiro Ono[14] & Yorifumi Satou[1]

***HTLV-I basic leucine zipper factor* gene mRNA supports proliferation of adult T cell leukemia cells**

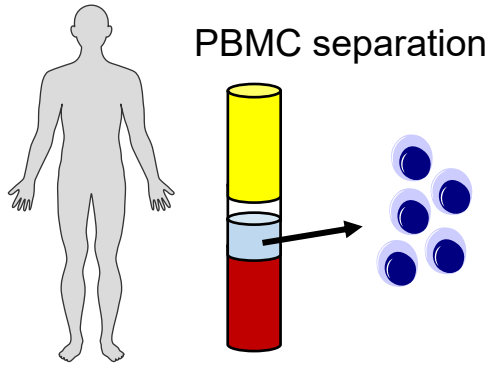
Yorifumi Satou, Jun-ichirou Yasunaga, Mika Yoshida, and Masao Matsuoka*

Laboratory of Virus Immunology, Institute for Virus Research, Kyoto University, 53 Shogoin Kawahara-cho, Sakyo-ku, Kyoto 606-8507, Japan

Edited by Malcolm A. Martin, National Institutes of Health, Bethesda, MD, and approved November 18, 2005 (received for review September 1, 2005)

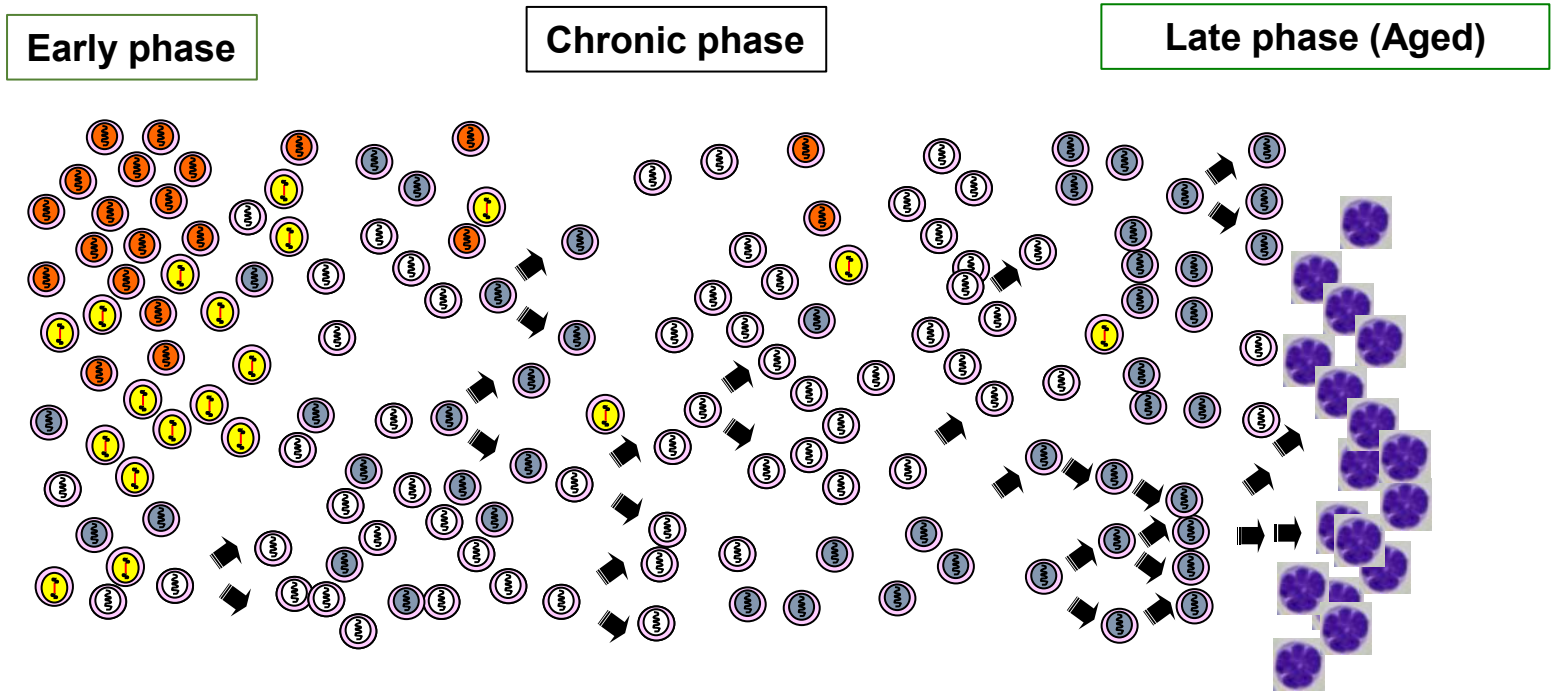
Enabling persistent infection via Immune evasion

# ATL clones emerge during long-term latency




**Various clones with various viral gene expression**

- Tax +++ HBZ+
- Tax ++ HBZ+
- Tax +HBZ+
- Tax - HBZ+



# HTLV-1 infects various cell types but transforms CD4+ T cells almost exclusively

ELSEVIER

Virology

Volume 196, Issue 1, September 1993, Pages 25-33


Regular Article

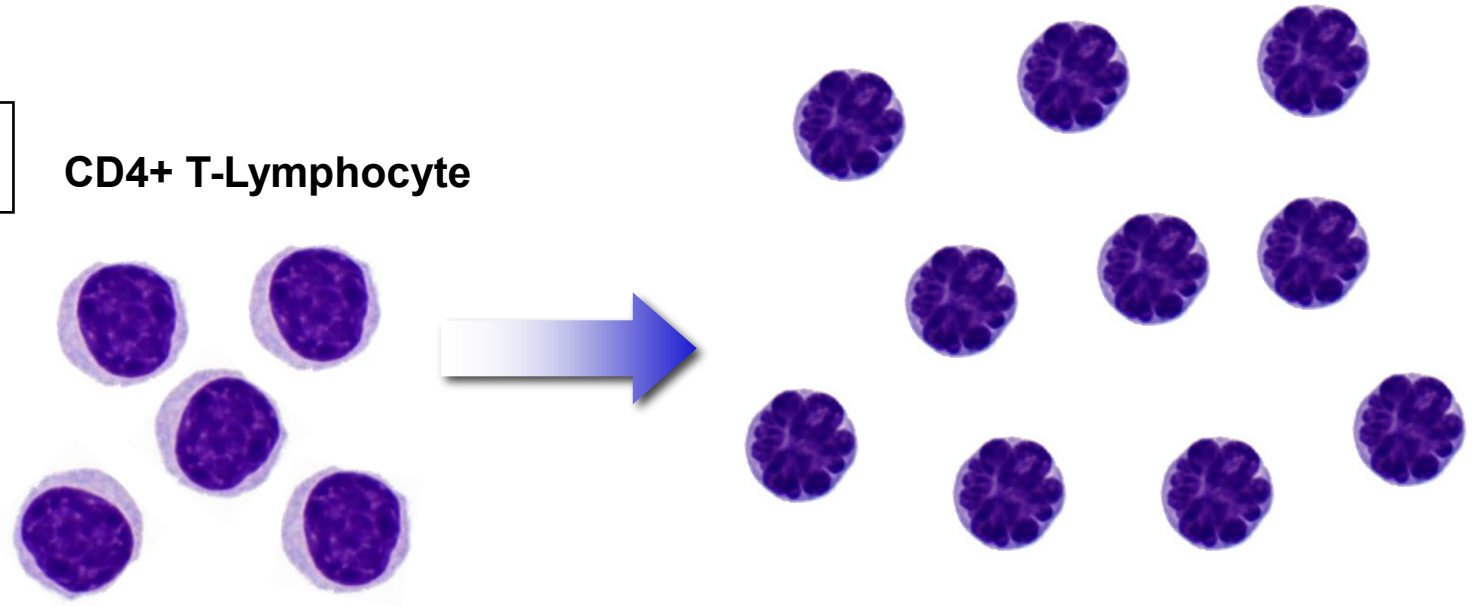
## *In Vivo* Infection of Human T-Cell Leukemia Virus Type I in Non-T Cells

Yoshio Koyanagi, Yasuto Itoyama, Naomi Nakamura, Keita Takamatsu, Jun-Ichi Kira, Teruo Iwamasa, Ikuo Goto, Naoki Yamamoto

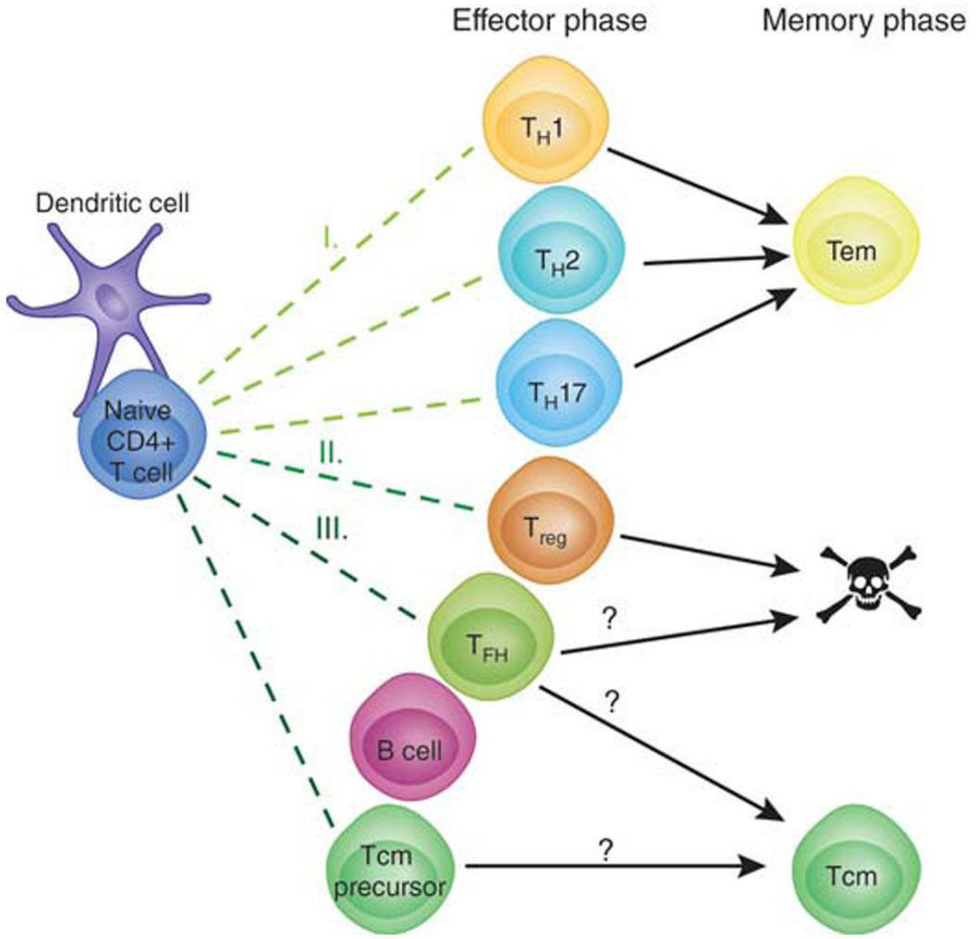
**HTLV-1**

**CD4+ T-Lymphocyte**

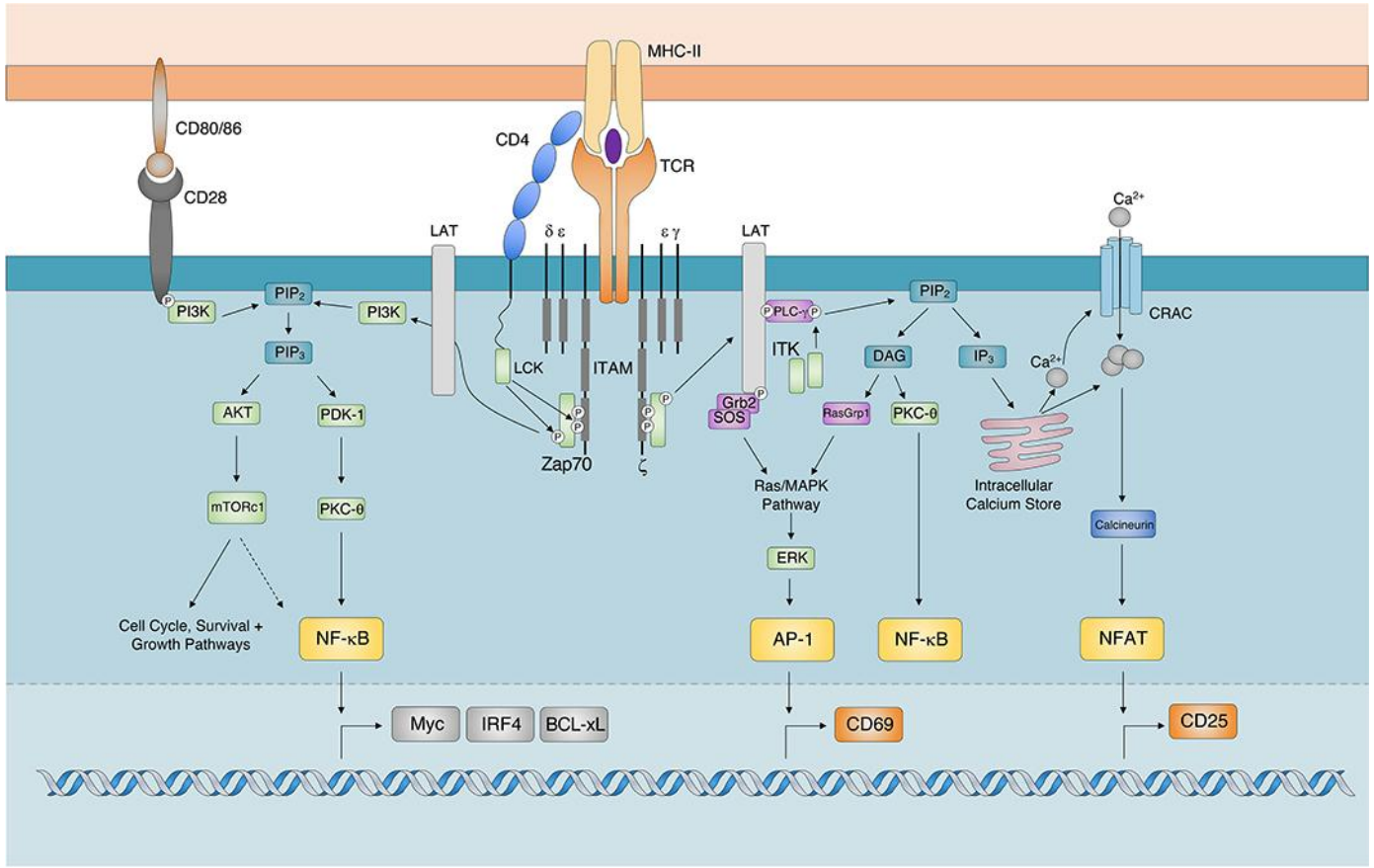
**Proliferation of infected cells – Leukemia**



# CD4⁺ T cells are activated, differentiate, proliferate upon antigen recognition via T-cell receptor



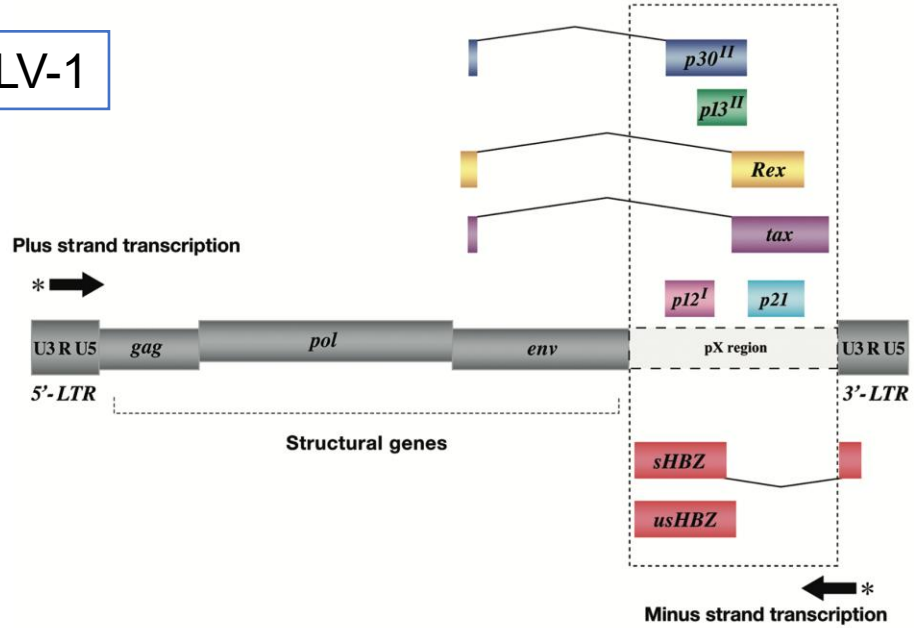

*Pepper M et al 2011 Nat Immun.*

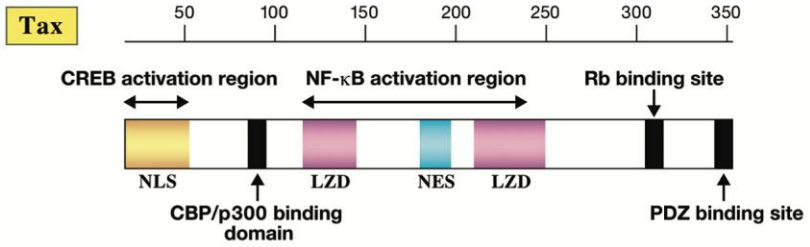


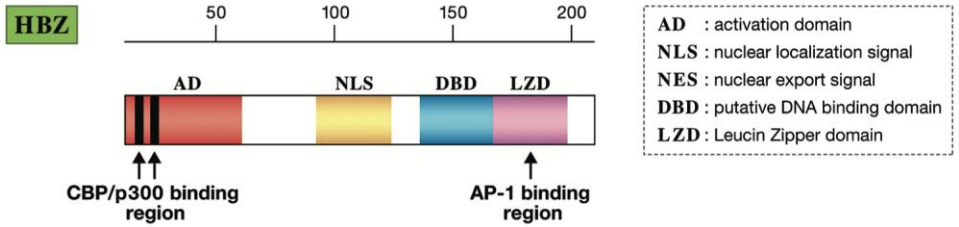

*Bhattacharyya N et al, Front. Immunol.,2020*

# HTLV-1 infection induces abnormalities in TCR signaling pathway

HTLV-1



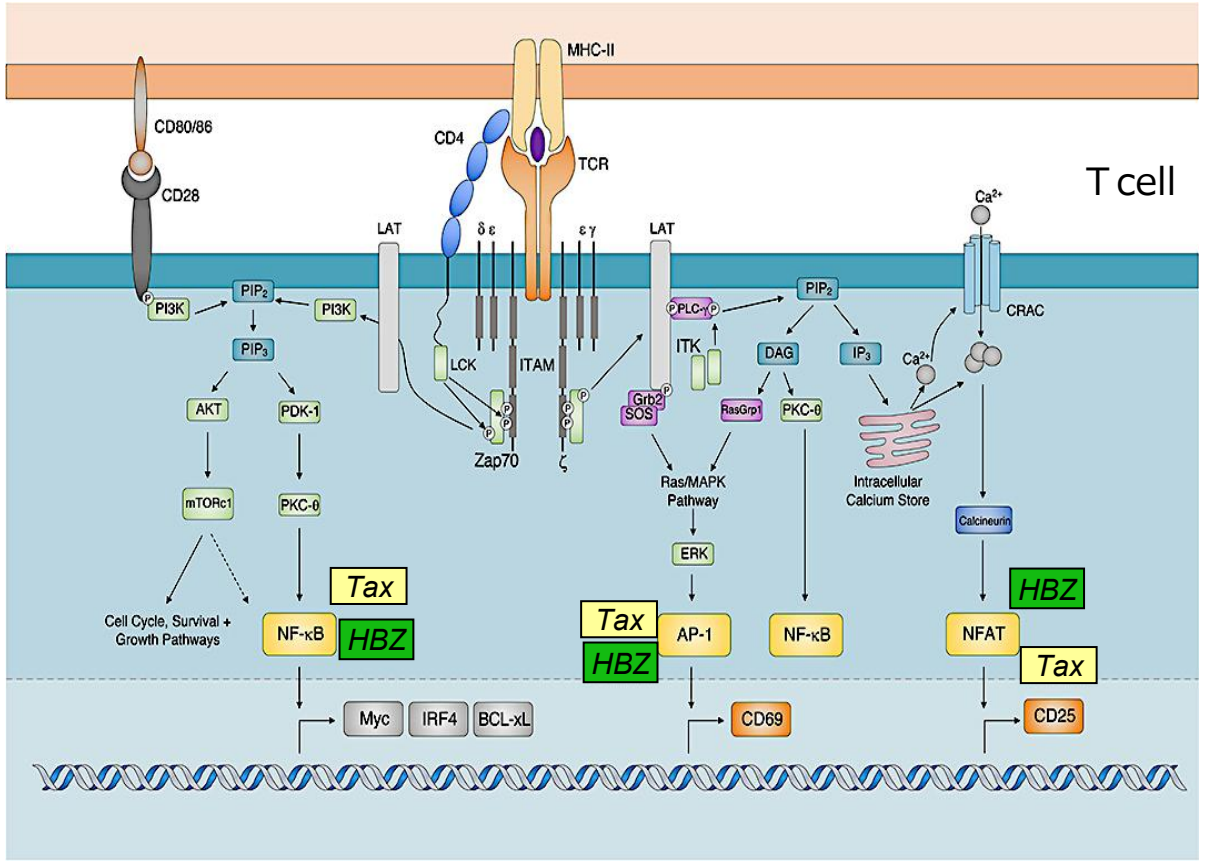



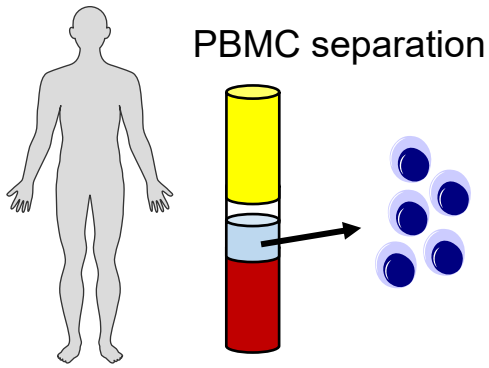

T-cell receptor

APC

T cell

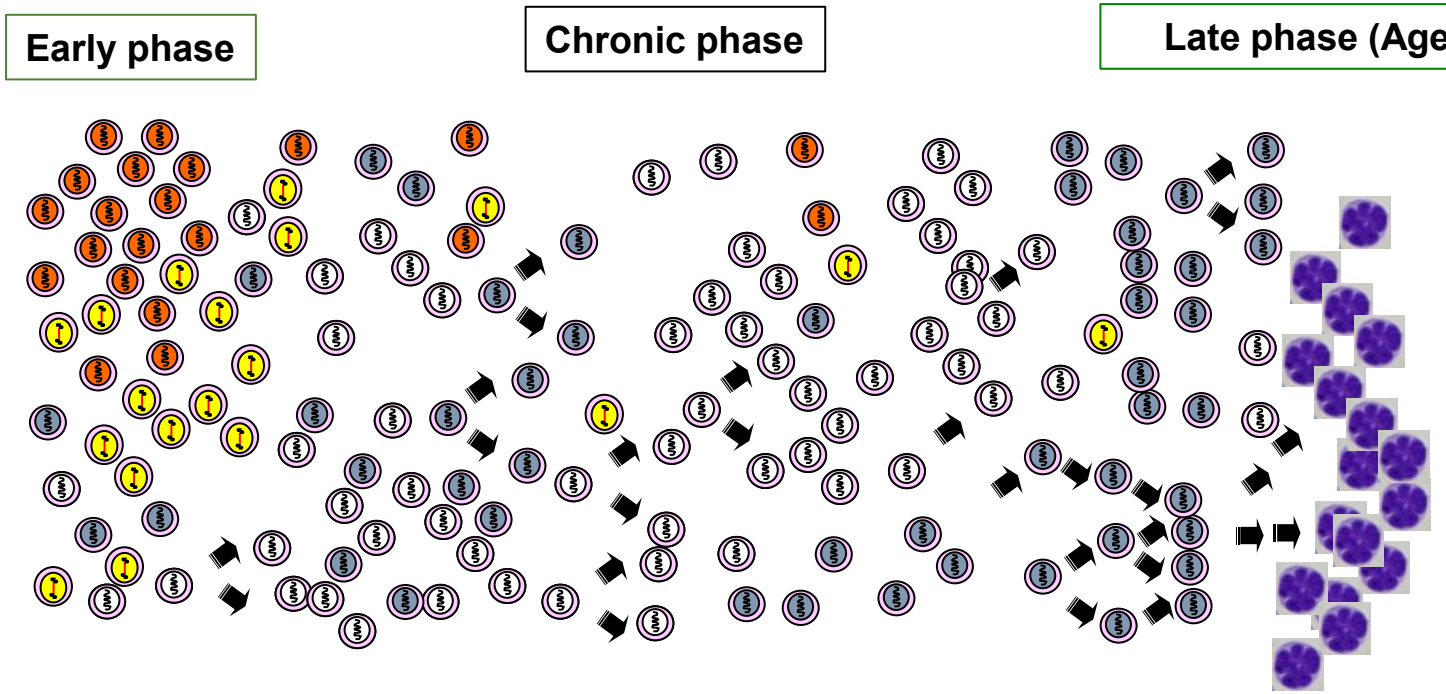


# How does persistent HTLV-1 alter T-cell homeostasis and drives related diseases?



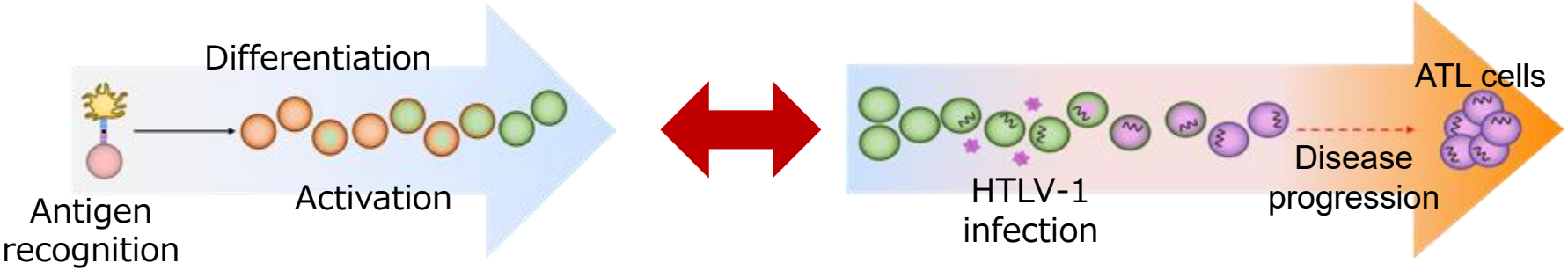

**Various clones with various viral gene expression**

- Tax +++ HBZ+
- Tax ++ HBZ+
- Tax +HBZ+
- Tax - HBZ+

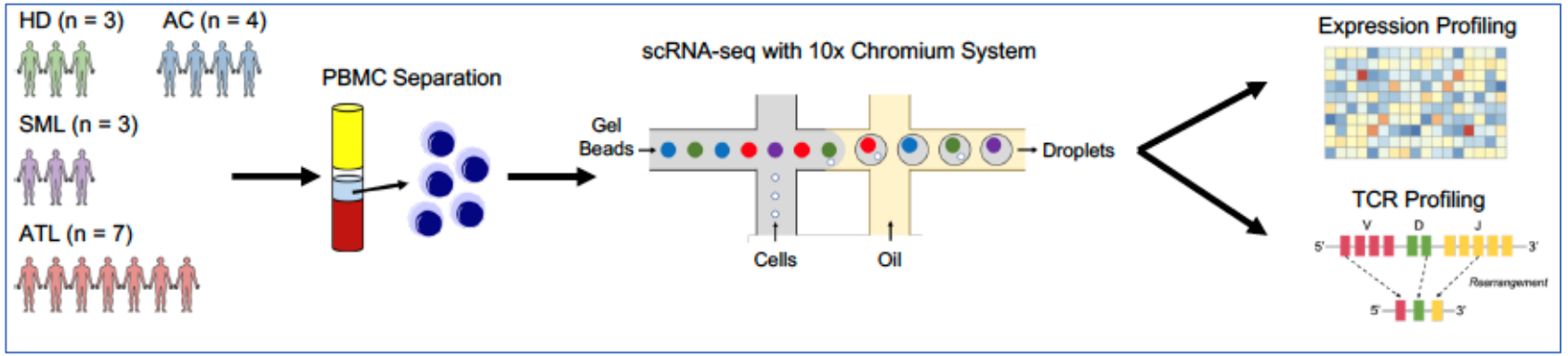


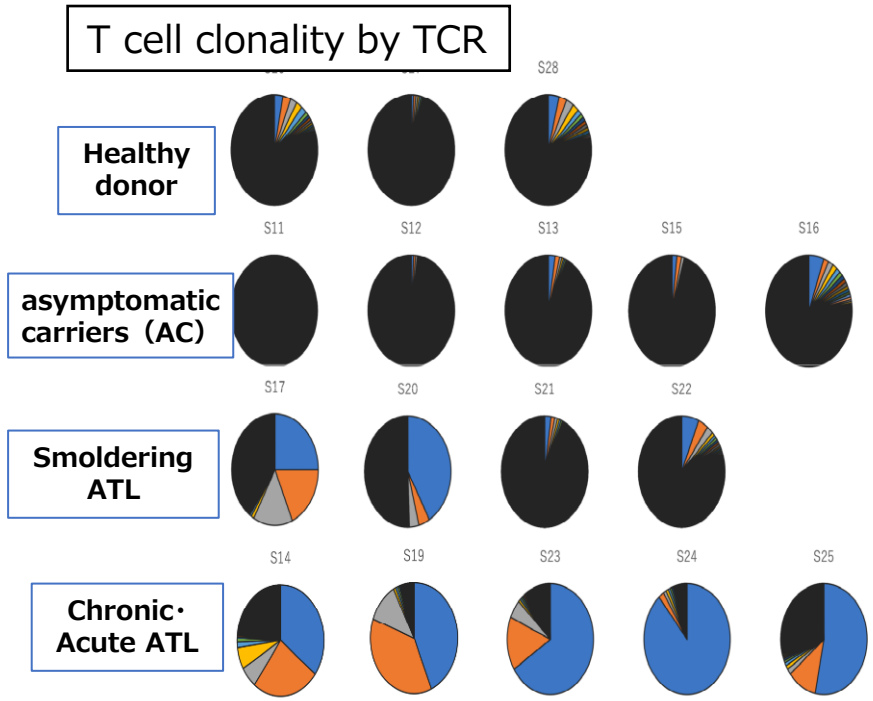

normal CD4⁺ T-cell act. and diff.

HTLV-1-induced transformation



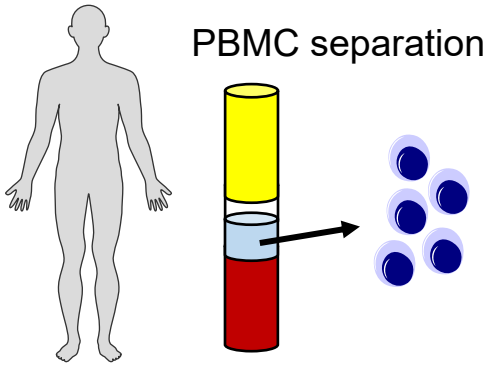
# Transcriptome and TCR information is available at single cell resolution



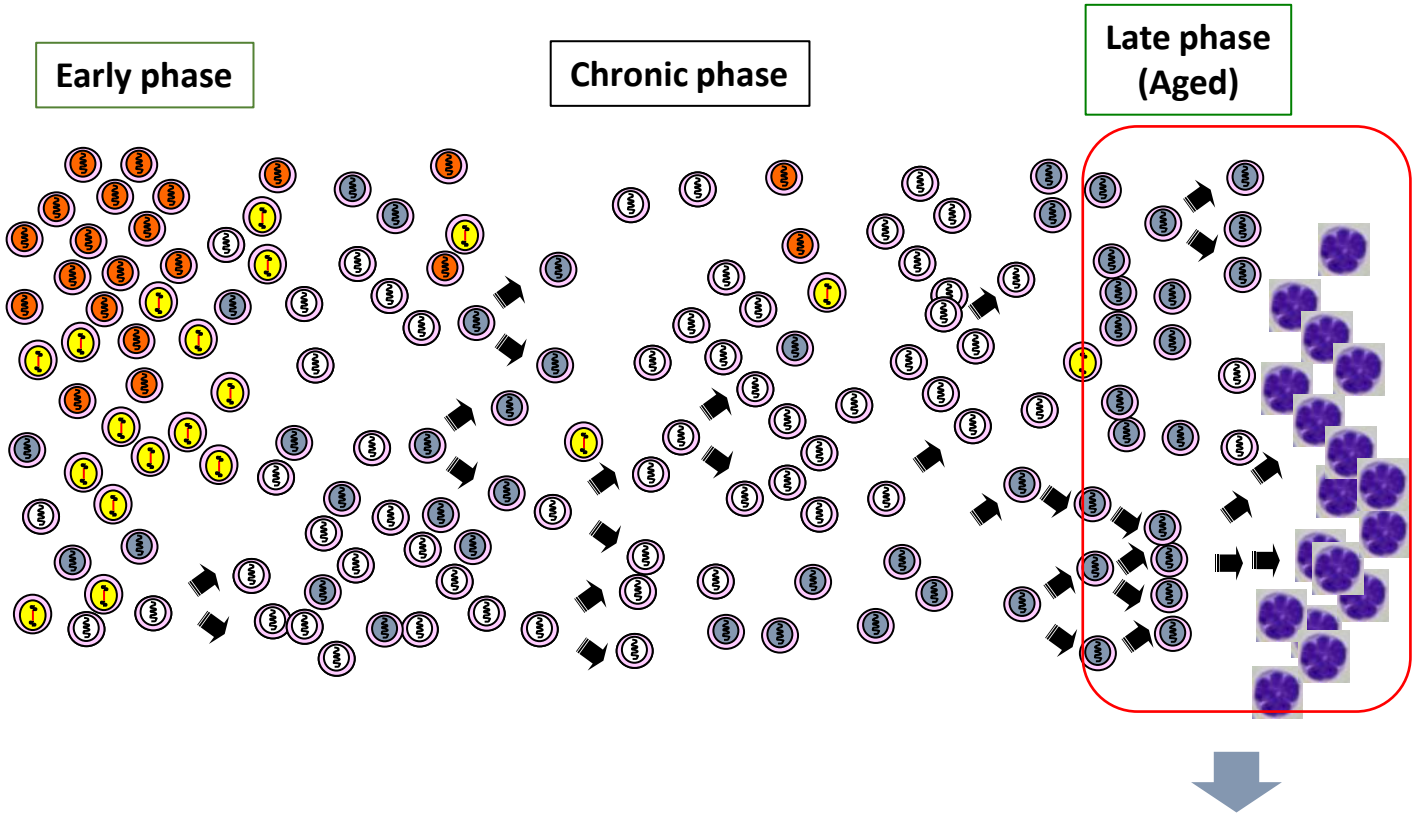




Single cell TCR information should be useful to identify ATL clones

# ATL clones emerge during long-term latency



PBMC separation

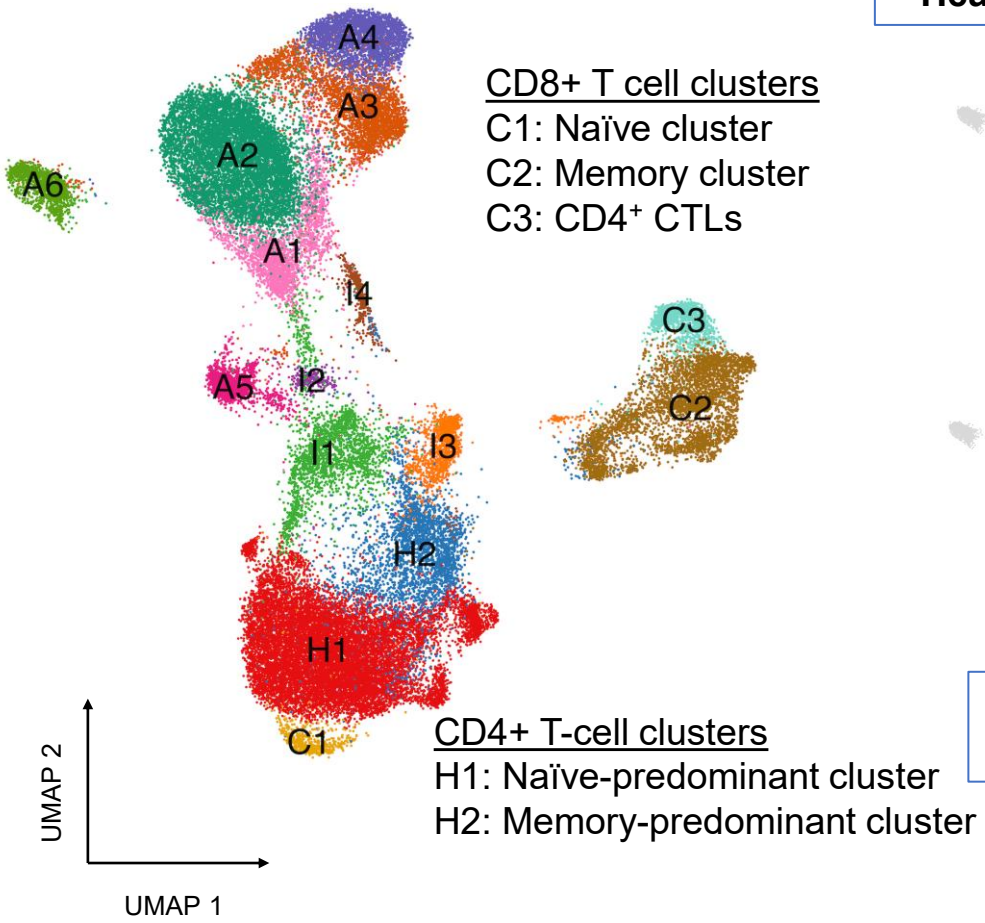



Each T cell clone can be identified by TCR

We aim to analyze the process of ATL development

# Cell clustering (CD4⁺ T cells)

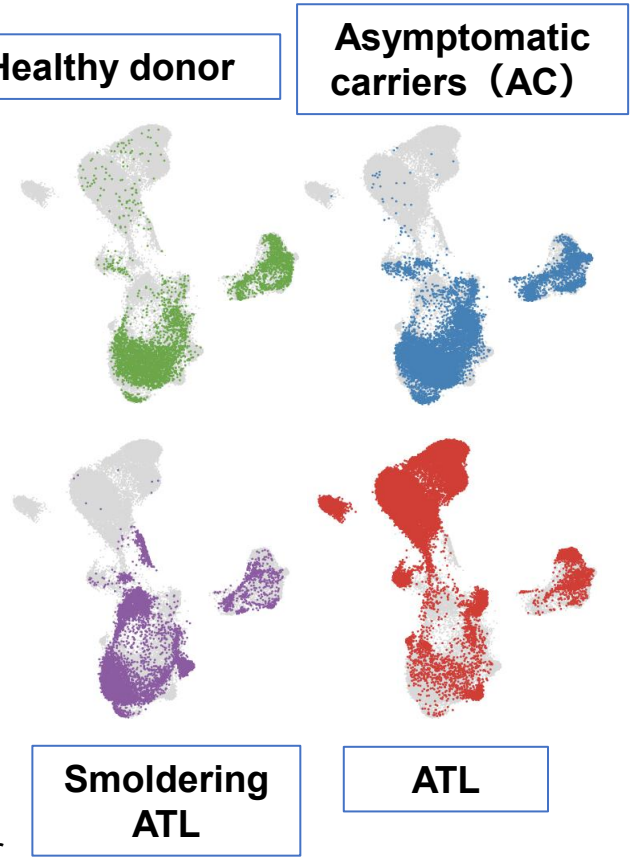
## T-cell clusters: 46,078 cells



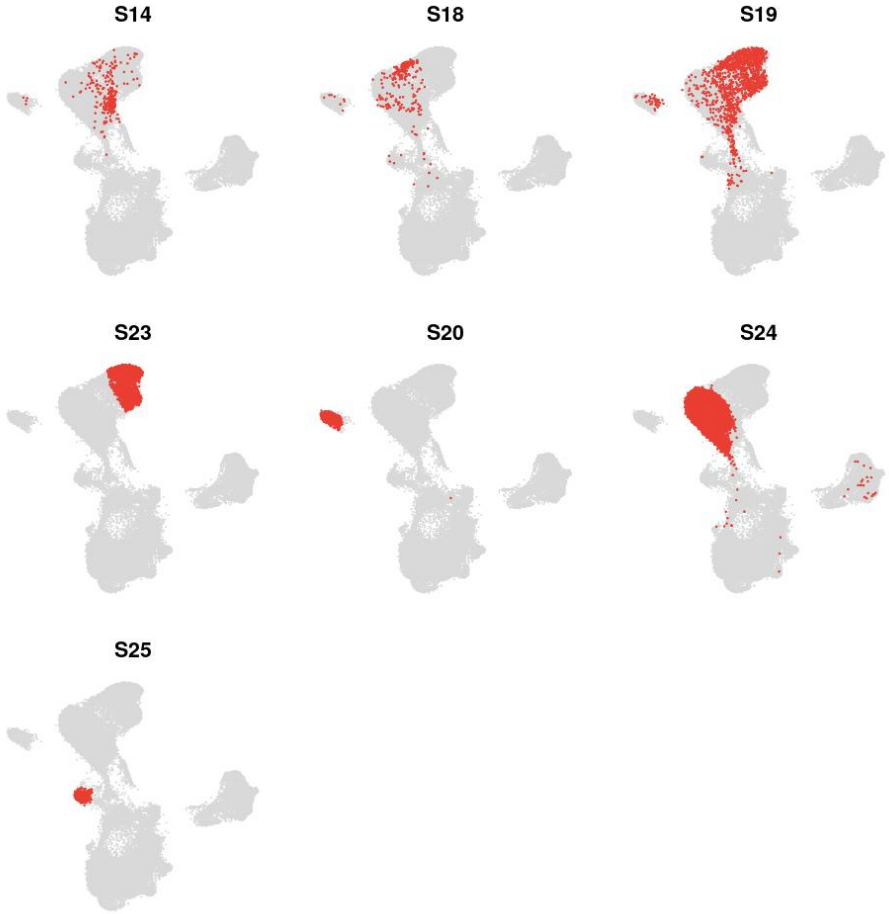

CD8+ T cell clusters
C1: Naïve cluster
C2: Memory cluster
C3: CD4⁺ CTLs

CD4+ T-cell clusters
H1: Naïve-predominant cluster
H2: Memory-predominant cluster

## Clinical status



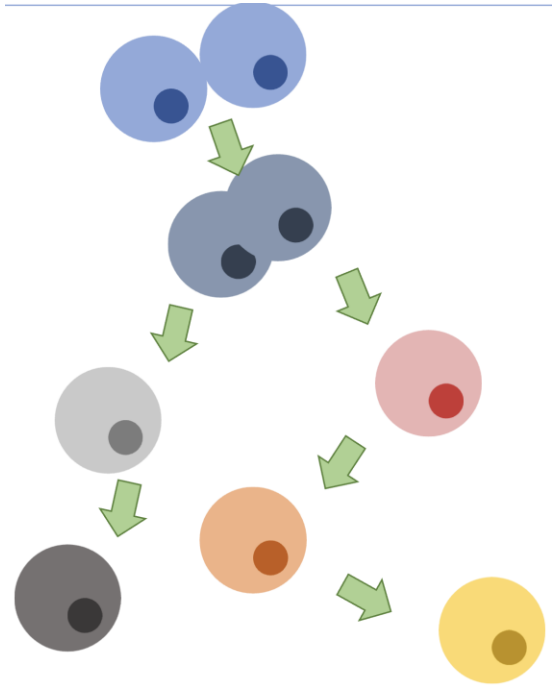

## Most expanded clones

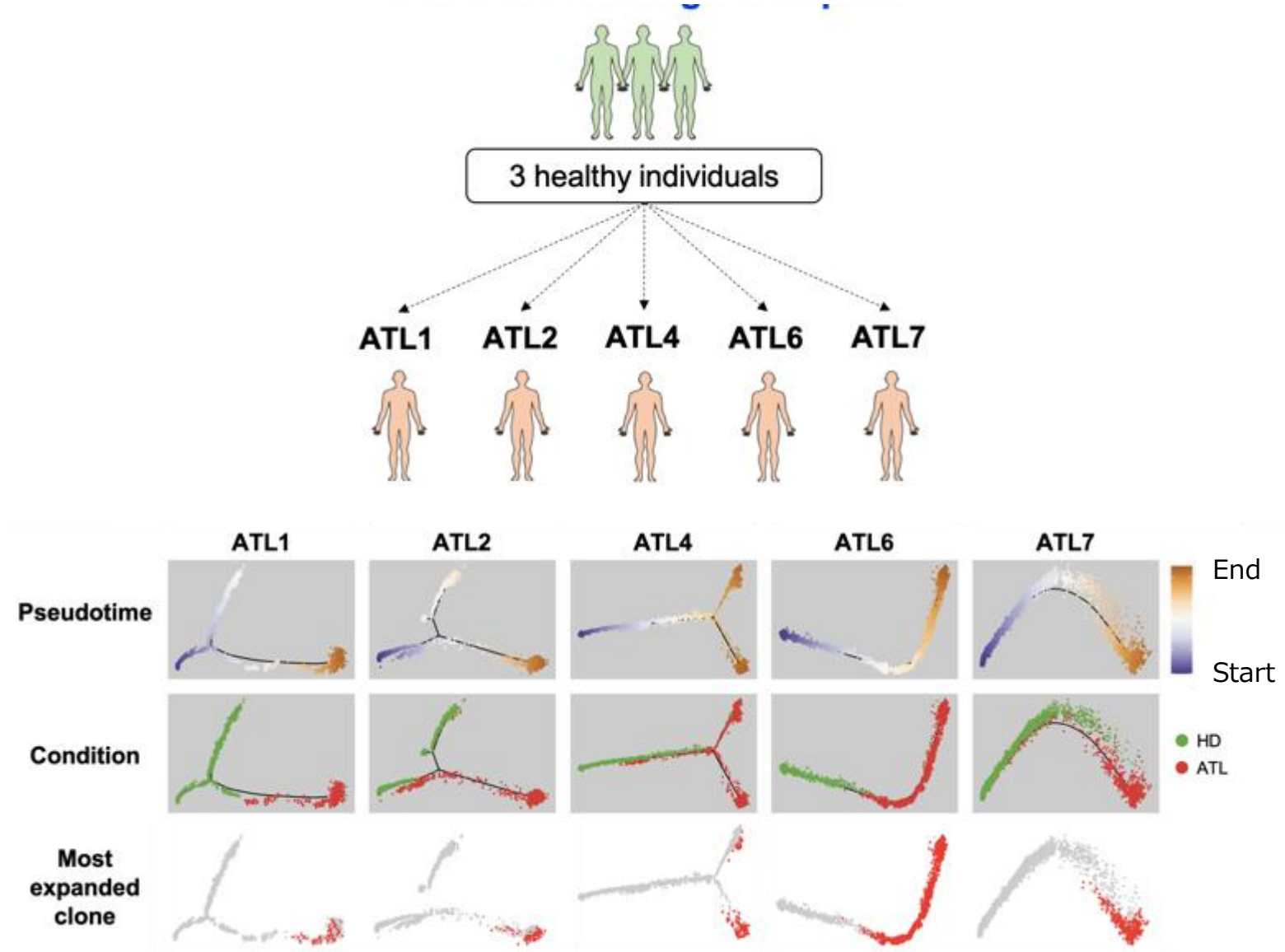



This heterogeneity provides a framework to analyze the process of oncogenesis

# Pseudotime analysis of CD4$^+$ T cells

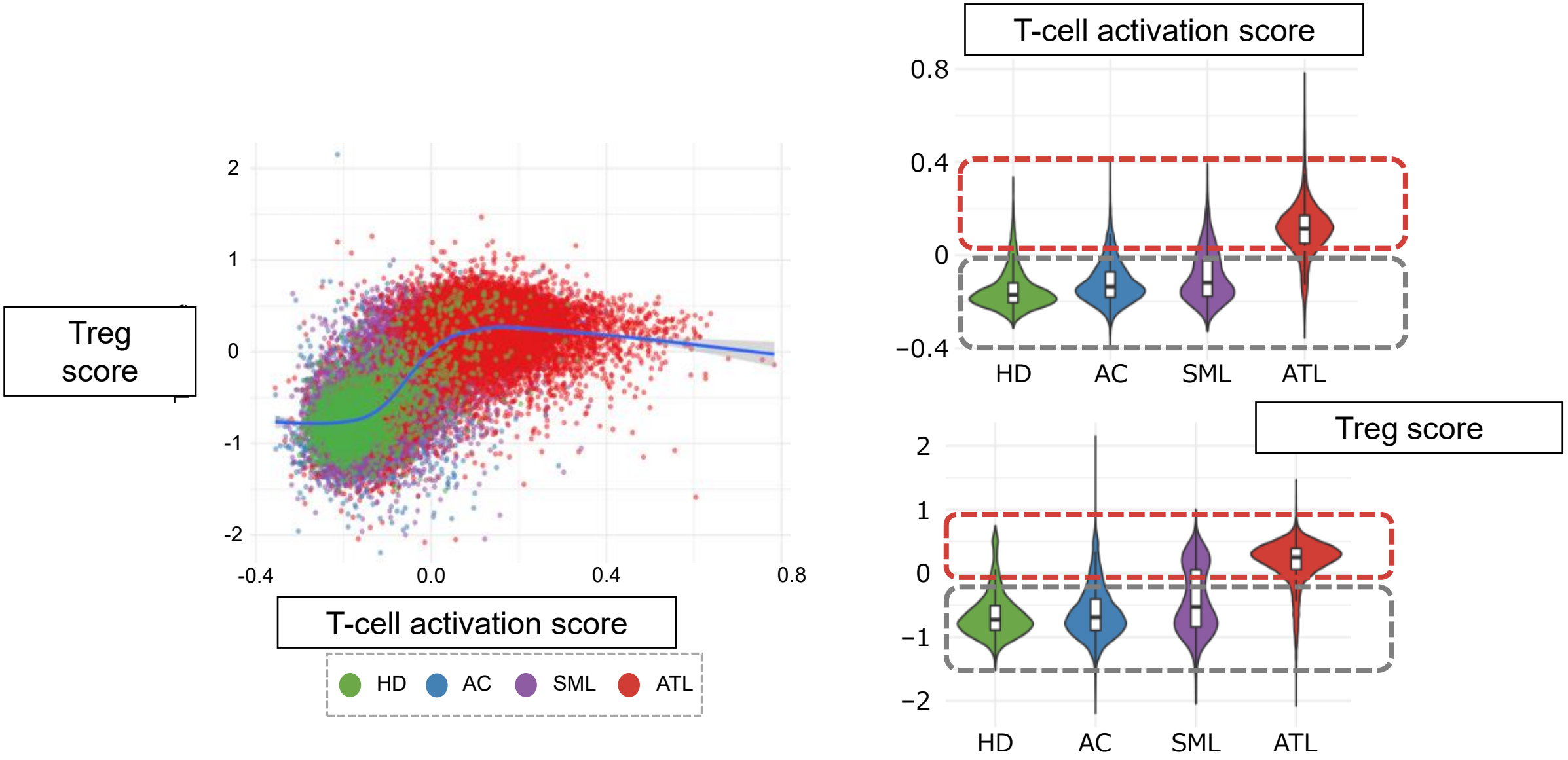
**Pseudotime analysis**





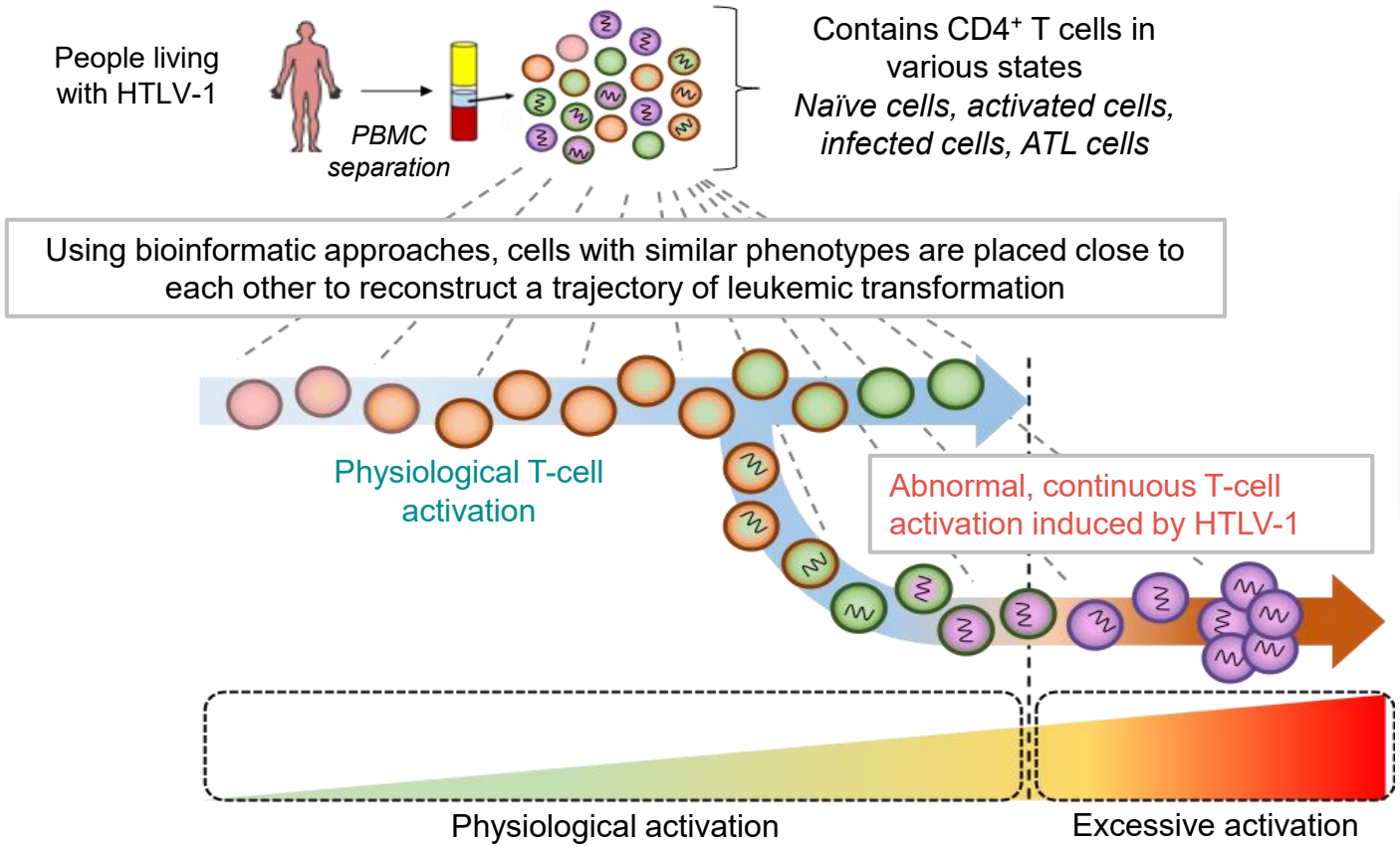

Pseudotime (PT) analysis seems to be reasonable.

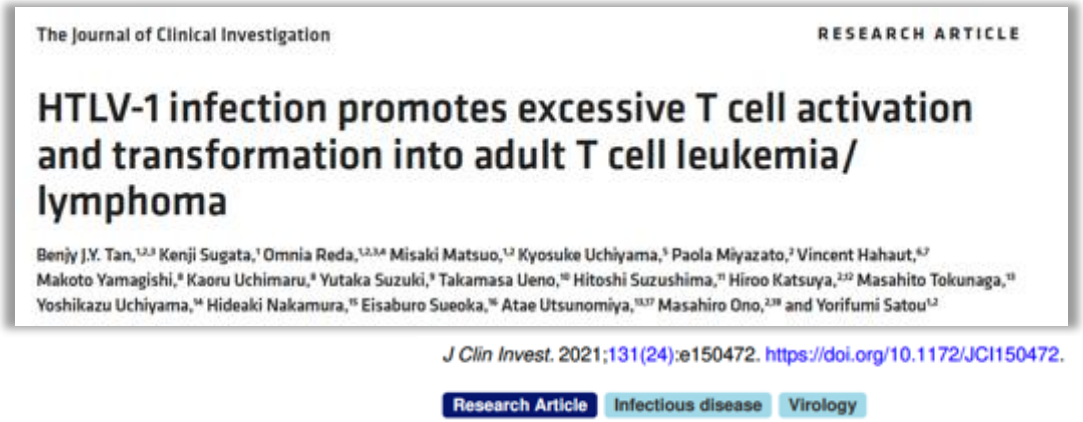
# Distribution of Treg and Tact scores across clinical settings




ATL cells showed an excessive Tact phenotype.

# Summary (1)





The Journal of Clinical Investigation RESEARCH ARTICLE

HTLV-1 infection promotes excessive T cell activation and transformation into adult T cell leukemia/lymphoma

Benjy J.Y. Tan, Kenji Sugata, Omnia Reda, Misaki Matsuo, Kyosuke Uchiyama, Paola Miyazato, Vincent Hahaut, Makoto Yamagishi, Kaoru Uchimaru, Yutaka Suzuki, Takamasa Ueno, Hitoshi Suzushima, Hiroo Katsuya, Masahito Tokunaga, Yoshikazu Uchiyama, Hideaki Nakamura, Eisaburo Sueoka, Atae Utsunomiya, Masahiro Ono, and Yorifumi Satou

J Clin Invest. 2021;131(24):e150472. https://doi.org/10.1172/JCI150472.

Research Article | Infectious disease | Virology

HTLV-1 drives abnormal, continuous T-cell activation

22nd International Conference on Human Retrovirology:
HTLV and Related Retroviruses
HTLV2026

June 3-6, 2026 | Philadelphia, Pennsylvania, USA

# The role of epigenetic regulation in the life cycle of HTLV-1 and BLV

Luc Willems
University of Liege, Belgium

Thomas Jefferson University | Drexel University | Drexel Medicine

22nd International Conference on Human Retrovirology:
HTLV and Related Retroviruses
HTLV2026

June 3-6, 2026 | Philadelphia, Pennsylvania, USA

# Understanding bovine leukemia virus oncogenesis fosters the development of therapeutic and preventive strategies

Luc Willems

June 3, 2026

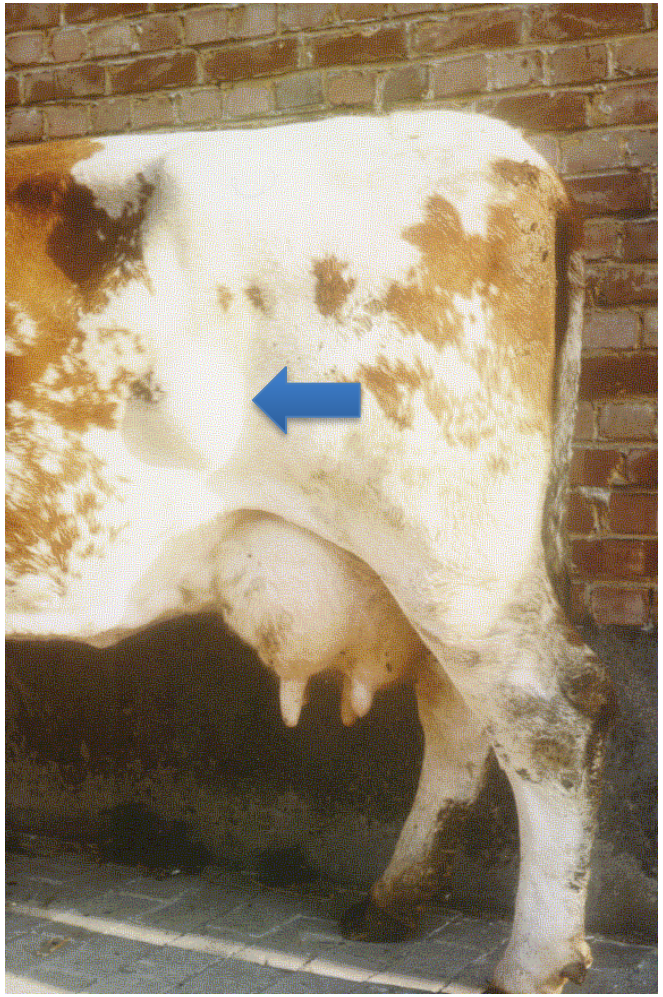
LIÈGE université GIGA institute

LIÈGE université Gembloux Agro-Bio Tech

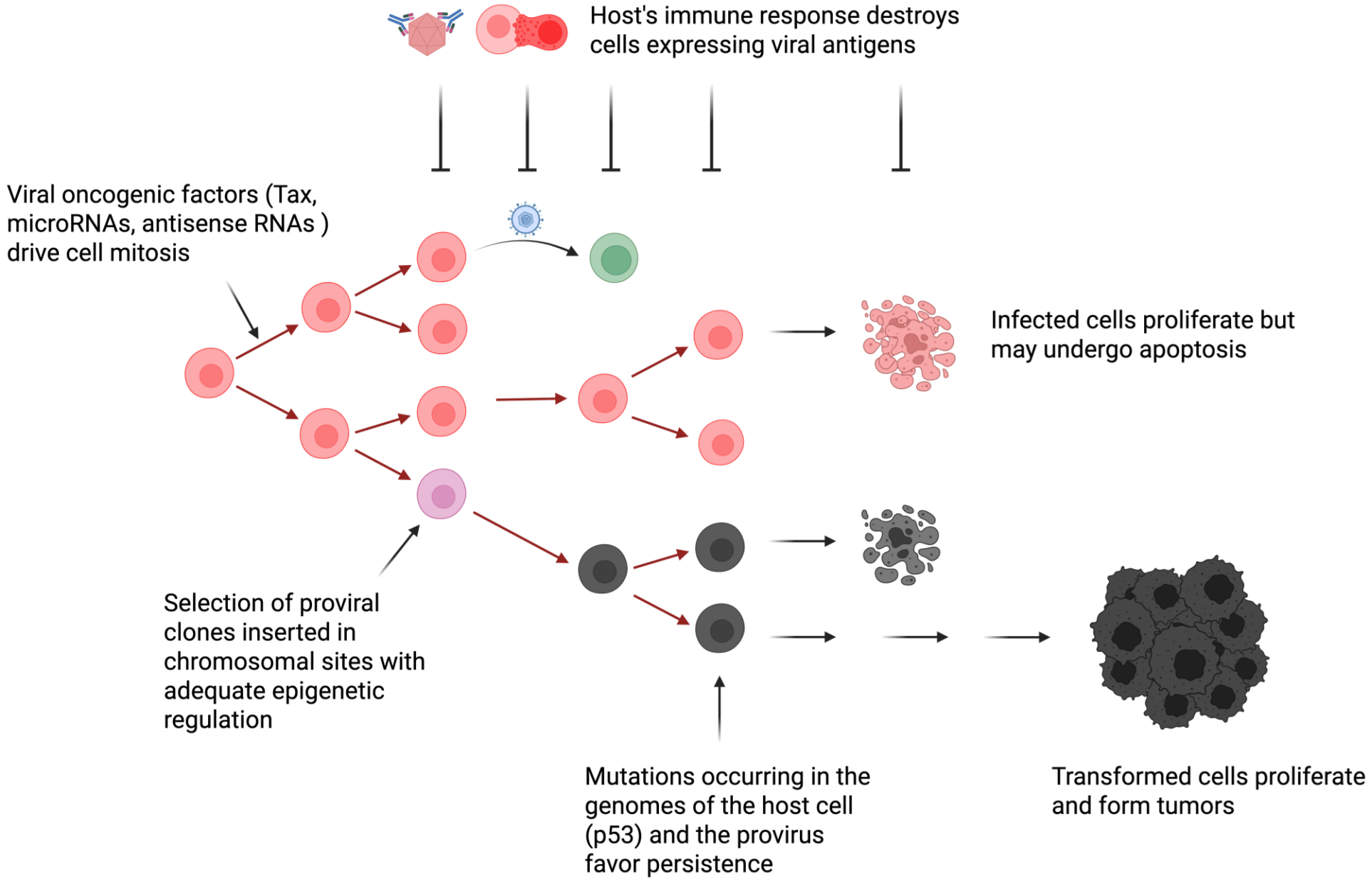
fnrs LA LIBERTÉ DE CHERCHER

# BLV

- naturally infects the bovine species
- can be experimentally transmitted to sheep
- Present in wild-life: llama, yak, bison, water bufalo, zebu, alpaca (Vicugna pacos)
- one third of cows develop a persistent lymphocytosis
- up to 10 % of acute B-cell leukemia or lymphoma
- weakens immune response (mastitis)
- poor effectiveness of vaccination against other pathogens (BHV-1, rotavirus, coronavirus, L Pomona)
- economic loss: death, reduction in milk production, custom restrictions, first cause of carcass condemnation
- a vaccine is available



Gillet et al Retrovirology **4**: 18; Doucet et al Viruses **17**: 10.3390




Gillet et al Retrovirology **4**: 18; Doucet et al Viruses **17**: 10.3390

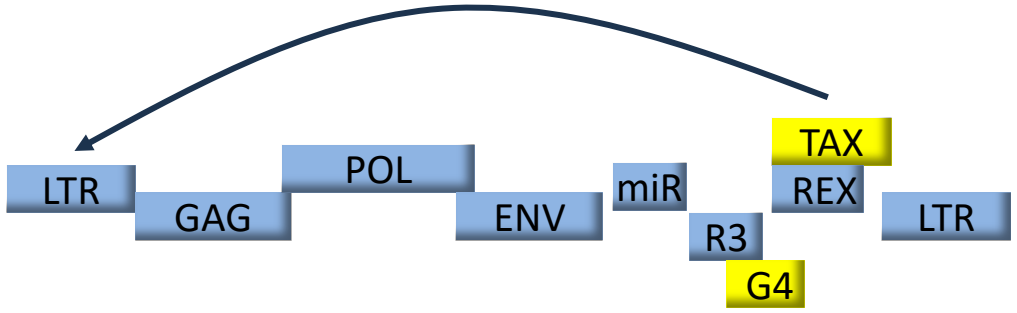


Biorender

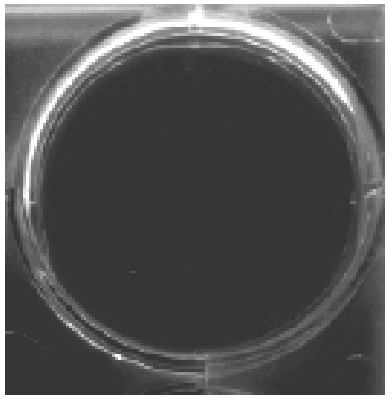
# Understanding BLV oncogenesis

- Viral oncogenes induce cell proliferation and transformation in cell cultures

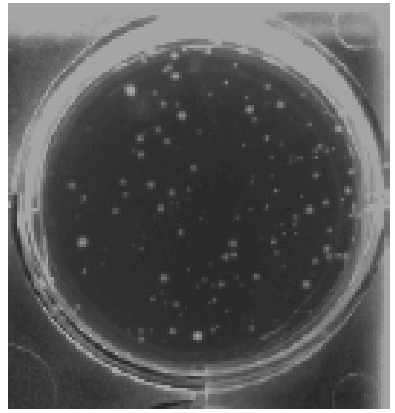
Activation of transcription




Transformation



mock



Tax

Willems et al EMBO J. **9**: 1577; Willems et al Proc. Natl. Acad. Sci. U.S.A. **89**: 3810; Kerkhofs et al Journal of Virology **72**: 2554; Twizere et al The Journal of the National Cancer Institute **95**: 1846

# Understanding BLV/HTLV oncogenesis

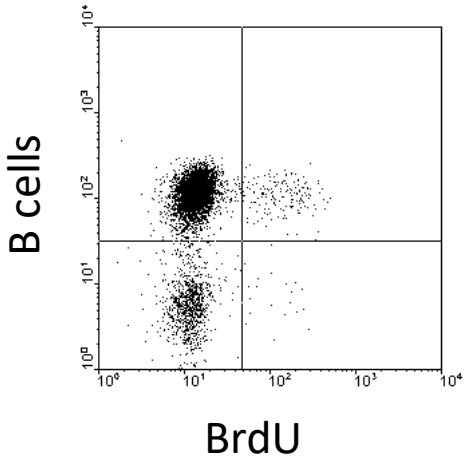
- Viral oncogenes induce cell proliferation and transformation in cell cultures

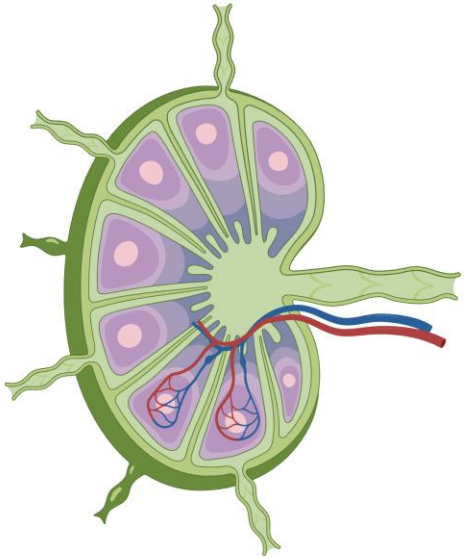
> in infected animals ?

“…BLV infection is latent…”

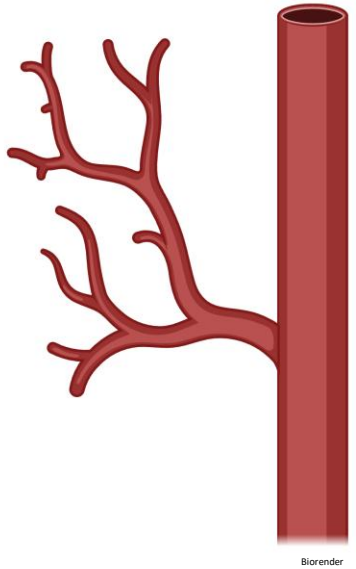
# High turnover of BLV-infected cells

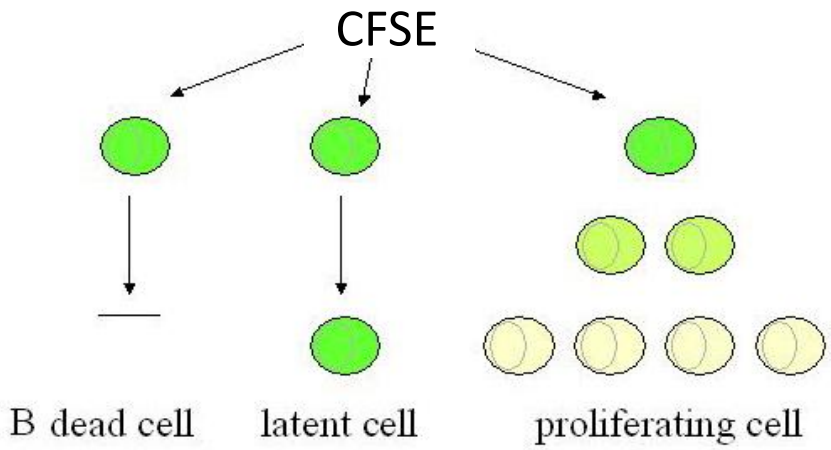
Increased proliferation in lymphoid tissues



Increased death in peripheral blood




Proliferation (% population/day) 2.0 % (BLV) 1.1 % (control) **

Death (% population/day) 11.9 % (BLV) 5.2 % (control) **



Debacq et al Proc. Natl. Acad. Sci. U.S.A. **99**: 10048; Debacq et al Journal of Virology **80**: 9710; Florins et al Journal of Virology **80**: 11998

# Understanding BLV oncogenesis

- Viral oncogenes induce cell proliferation and transformation in cell cultures

- Accelerated cell turnover in BLV-infected animals

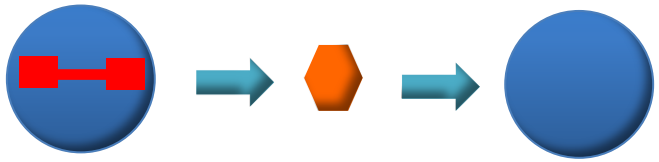
> BLV is not latent during chronic infection

# Understanding BLV oncogenesis

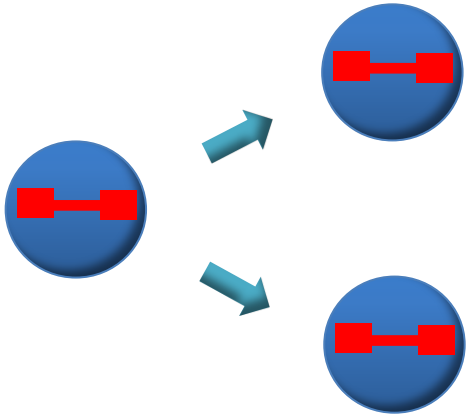
- Viral oncogenes induce cell proliferation and transformation in cell cultures

- Accelerated cell turnover in BLV-infected animals: BLV is not latent during chronic infection

> what is the mode of viral replication ?



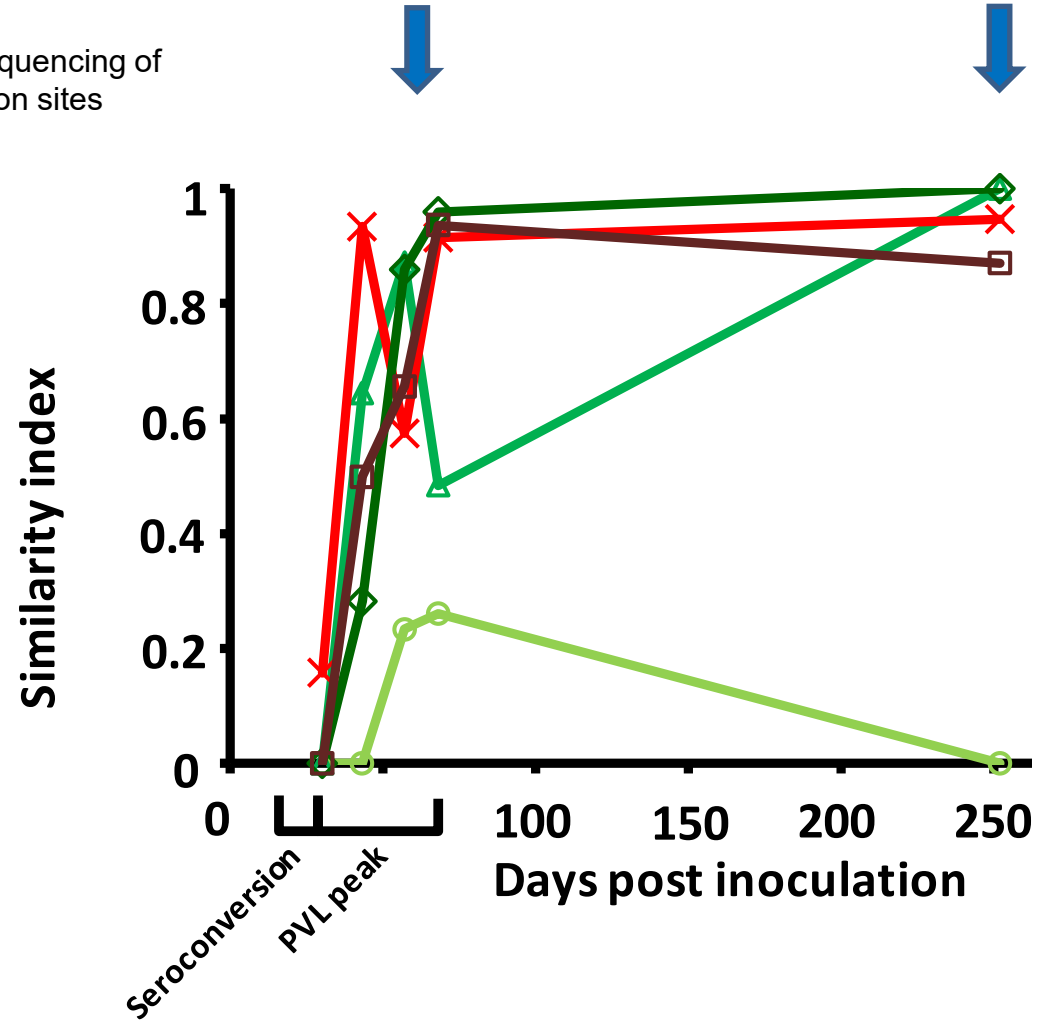
Infectious cycle



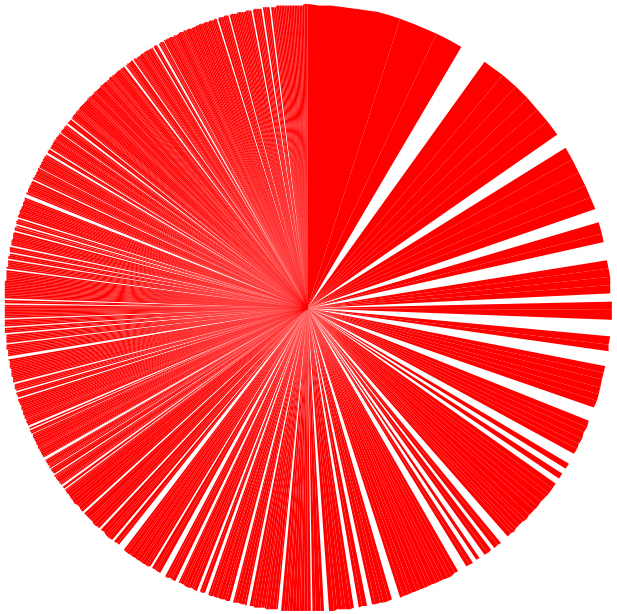
Clonal expansion

# Massive depletion of clones

High throughput sequencing of
BLV proviral insertion sites



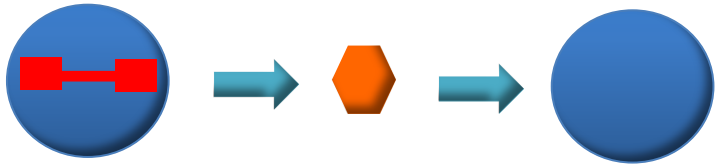

**clones present at seronconversion and no longer detected in the long term**



Gillet et al PLoS Pathogens **9**:e1003687

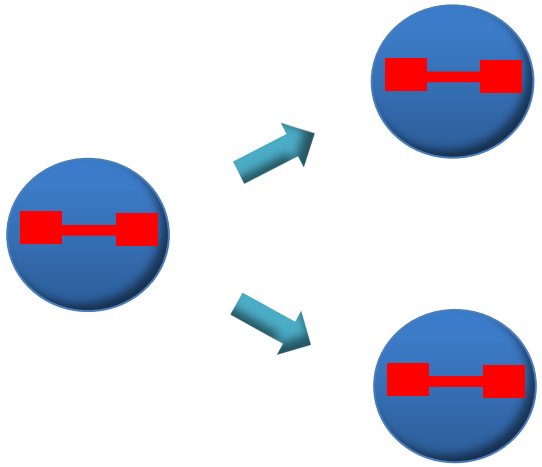
# 2 modes of viral replication

Infectious cycle



Mostly before immune response

Clonal expansion



Mostly after immune response

# Understanding BLV oncogenesis

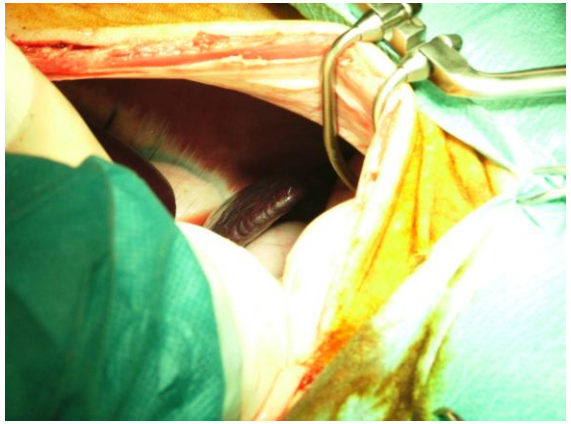
- Viral oncogenes induce cell proliferation and transformation in cell cultures

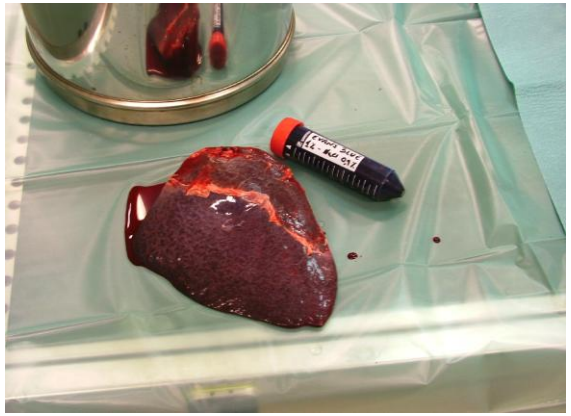
- Accelerated cell turnover in BLV-infected animals: BLV is not latent during chronic infection

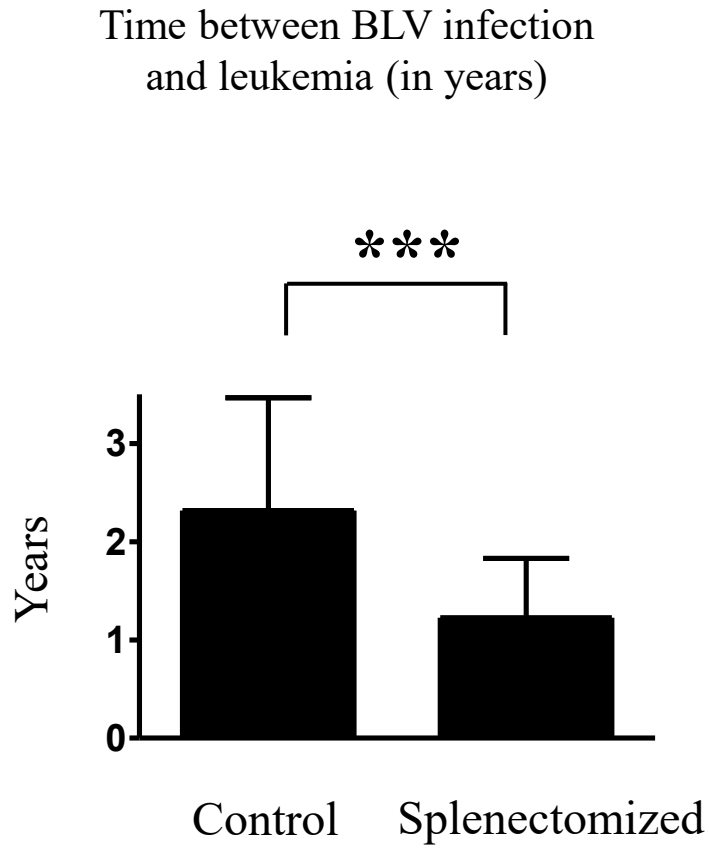
- Massive depletion of infected cell clones after seroconversion

> role of lymphoid organs in immune control ?

# Earlier onset of leukemia after splenectomy










Florins et al PLoS One **4**: e6943

# Understanding BLV oncogenesis

- Viral oncogenes induce cell proliferation and transformation in cell cultures

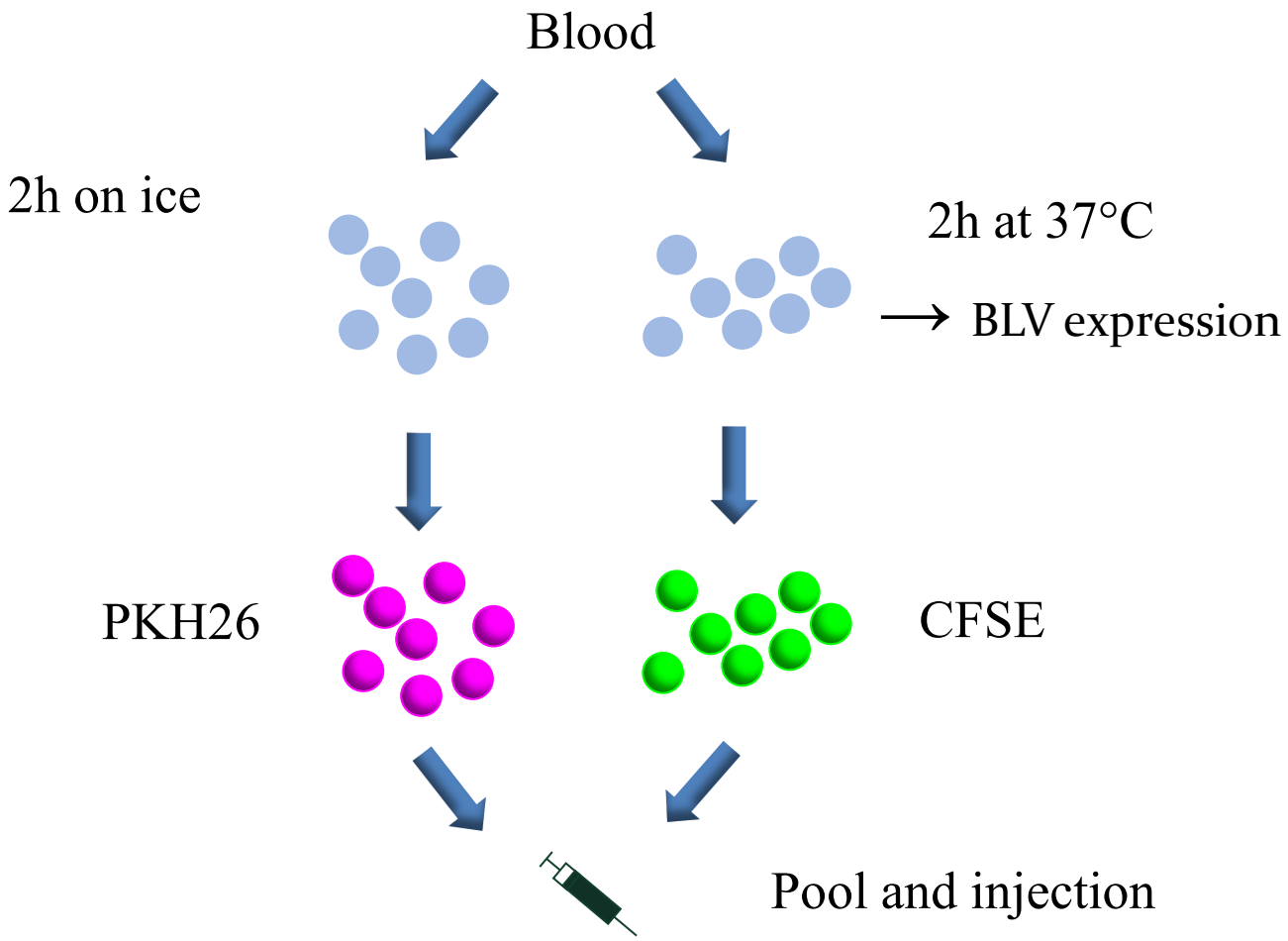
- Accelerated cell turnover in BLV-infected animals: BLV is not latent during chronic infection

- Massive depletion of infected cell clones after seroconversion

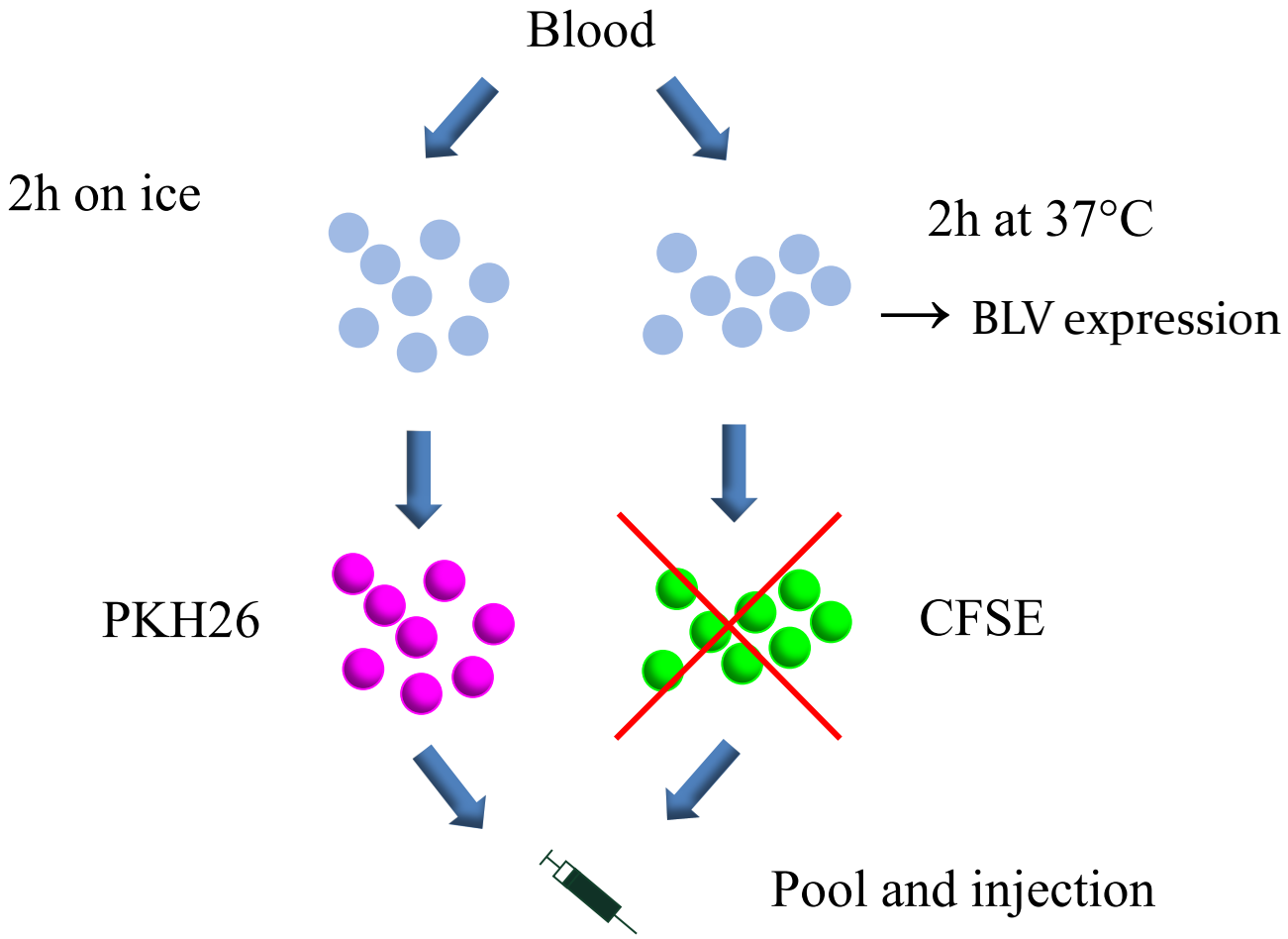
- Role of lymphoid organs in immune control

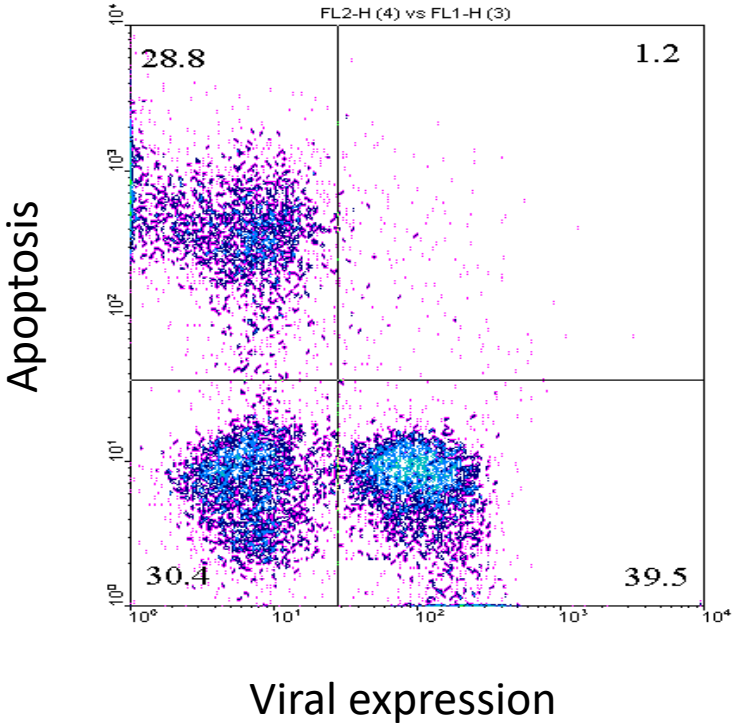
> Role of viral expression ?

# Lifespan of BLV-positive B cells



# Viral expression shortens persistence of B cells






Dequiedt et al Journal of Virology, **71**: 630; Dequiedt et al Journal of Virology, **73**: 1127; Florins et al, Journal of Virology **86**:621

# Understanding BLV oncogenesis

- Viral oncogenes induce cell proliferation and transformation in cell cultures

- Accelerated cell turnover in BLV-infected animals: BLV is not latent during chronic infection

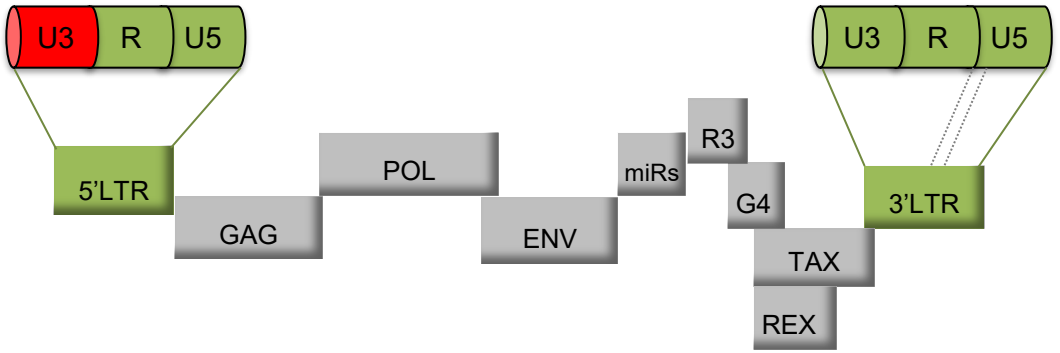
- Massive depletion of infected cell clones after seroconversion

- Role of lymphoid organs in immune control

- Viral expression disfavors persistence of infected cells

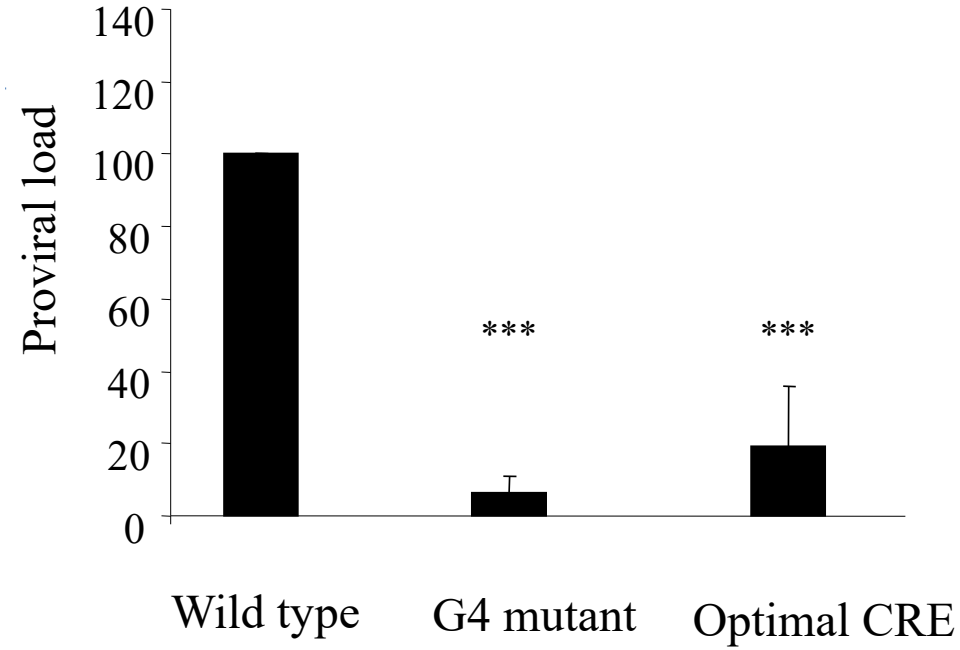
> promoting viral expression ?

# A strong promoter affects viral replication




AGACGTCA
TGACGGCA
TGACCTCA

**TGACGTCA**
Cyclic-AMP response element




Merezak et al Journal of Virology, **75**: 6977

# Understanding BLV oncogenesis

- Viral oncogenes induce cell proliferation and transformation in cell cultures

- Accelerated cell turnover in BLV-infected animals: BLV is not latent during chronic infection

- Massive depletion of infected cell clones after seroconversion

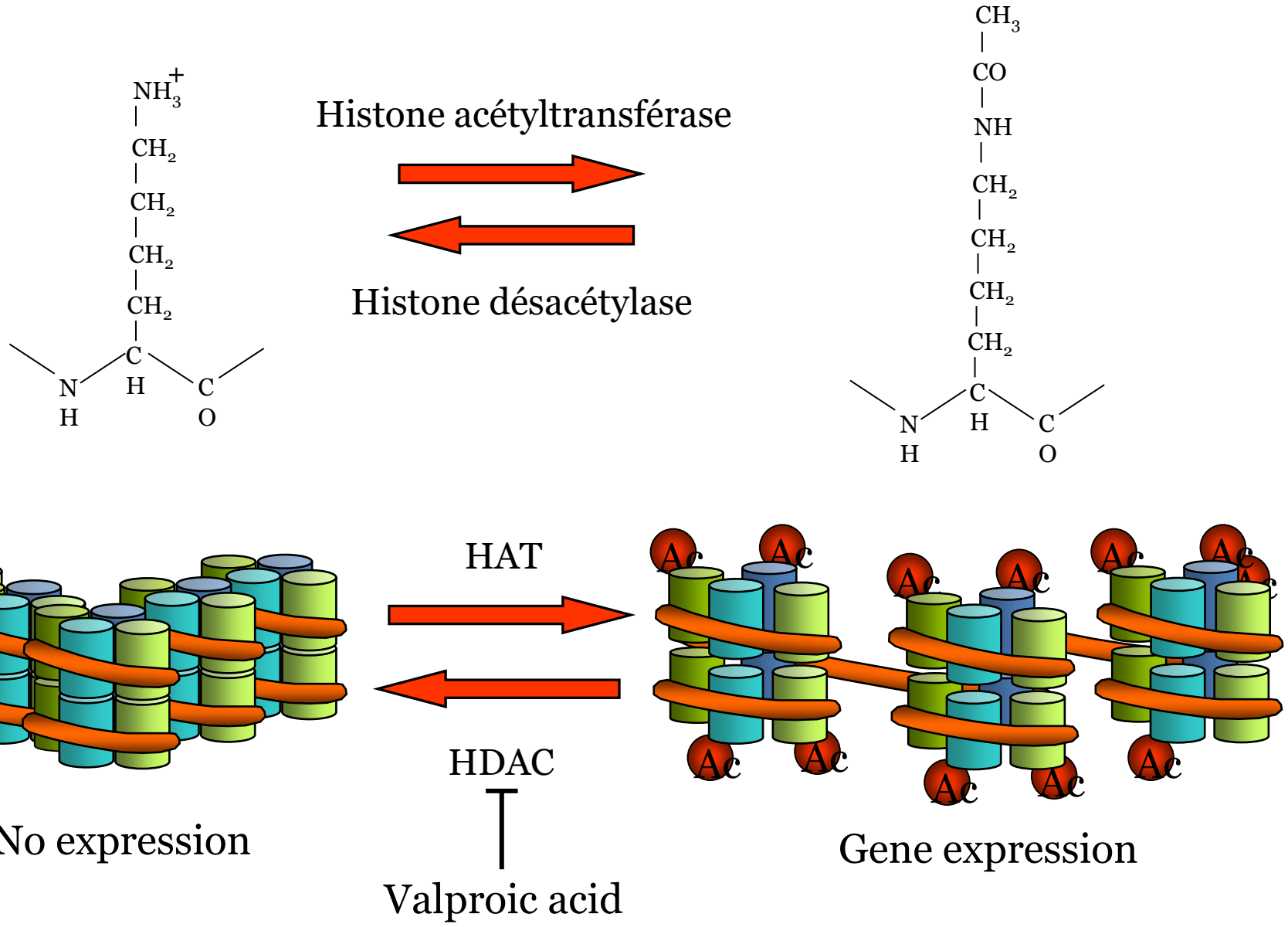
- Role of lymphoid organs in immune control

- Viral expression disfavors persistence of infected cells

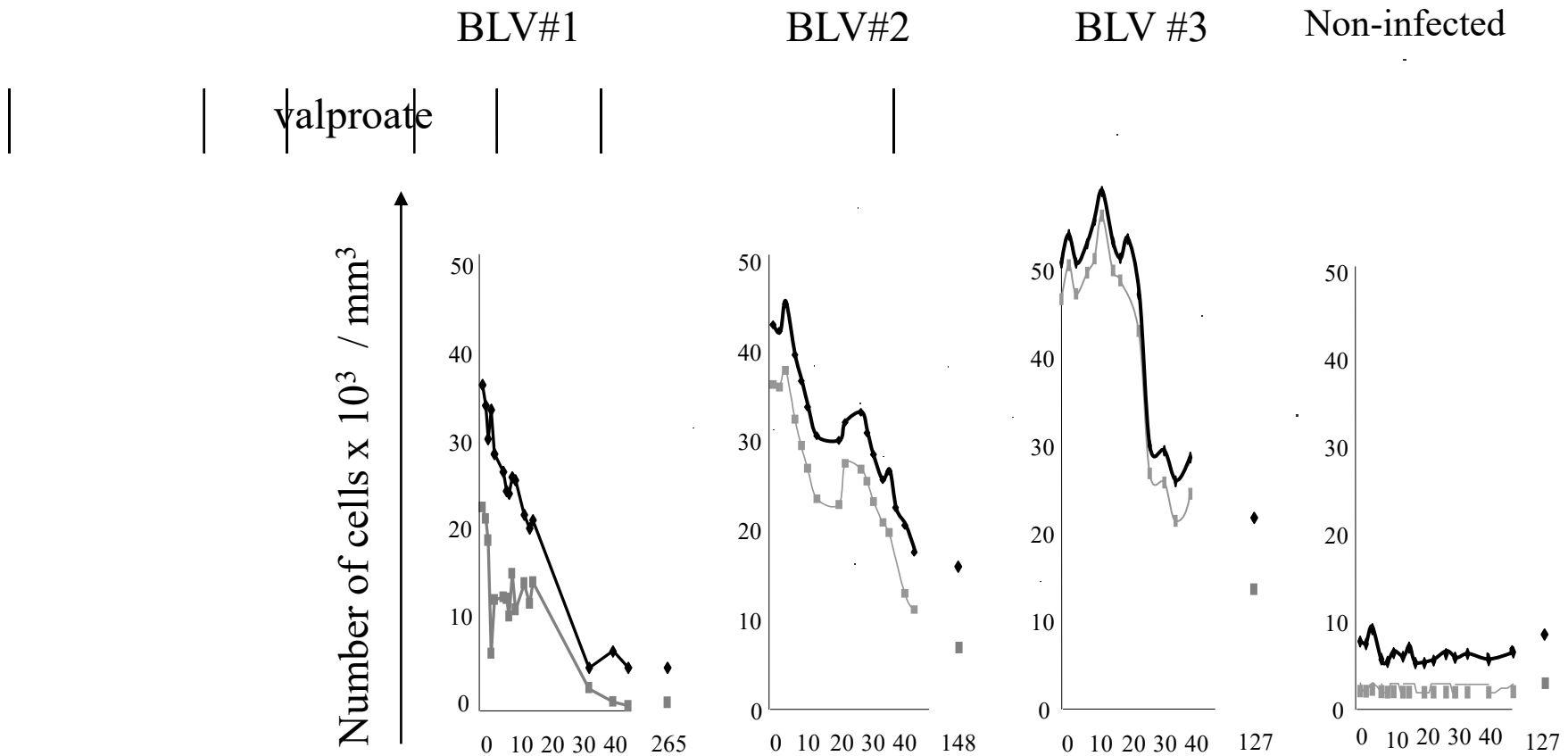
- A strong promoter reduces viral replication

> stimulating viral expression ?

# Epigenetic regulation of gene expression



# Valproate treatment reduces proviral loads






Achachi et al (2005) Proc. Natl. Acad. Sci. U.S.A., 102: 10309

# Understanding BLV oncogenesis

- Viral oncogenes induce cell proliferation and transformation in cell cultures

- Accelerated cell turnover in BLV-infected animals: BLV is not latent during chronic infection

- Massive depletion of infected cell clones after seroconversion

- Role of lymphoid organs in immune control

- Viral expression disfavors persistence of infected cells

- A strong promoter reduces viral replication

- Therapy by activation of viral expression with an HDAC inhibitor

# Understanding BLV oncogenesis

- Viral oncogenes induce cell proliferation and transformation in cell cultures

- Accelerated cell turnover in BLV-infected animals: BLV is not latent during chronic infection

- Massive depletion of infected cell clones after seroconversion

- Role of lymphoid organs in immune control

- Viral expression disfavors persistence of infected cells

- A strong promoter reduces viral replication

- Therapy by activation of viral expression with an HDAC inhibitor

> Epigenetic therapy of HTLV-1 ?

## Cell cultures


Mosley AJ, Meekings KN, McCarthy C, Shepherd D, Cerundolo V, Mazitschek R, Tanaka Y, Taylor GP, Bangham CR (2006) Histone deacetylase inhibitors increase virus gene expression but decrease CD8+ cell antiviral function in HTLV-1 infection. Blood 108:3801

Gazon H, Belrose G, Terol M, Meniane JC, Mesnard JM, Césaire R, Peloponese JM (2016) Impaired expression of DICER and some microRNAs in HBZ expressing cells from acute adult T-cell leukemia patients. Oncotarget. 7:30258

Schnell AP, Kohrt S, Aristodemou A, Taylor GP, Bangham CRM, Thoma-Kress AK (2022) HDAC inhibitors Panobinostat and Romidepsin enhance *tax* transcription in HTLV-1-infected cell lines and freshly isolated patients' T-cells. Front Immunol. 202213:978800

Mourouvin C, Tram J, Marty L, Marie-Delcasse A, Belrose G, Pluta A, Césaire R, Hélias P, Baccini V, Peloponese JM (2026) Valproate reactivates HTLV-1 tax and reduces ABCB1/MDR1 expression in PBMCs derived from ATLL patients. Front Oncol. 16:1721313


## Animal models


Afonso PV, Mekaouche M, Mortreux F, Toulza F, Moriceau A, Wattel E, Gessain A, Bangham CR, Dubreuil G, Plumelle Y, Hermine O, Estaquier J, Mahieux R. (2010) Highly active antiretroviral treatment against STLV-1 infection combining reverse transcriptase and HDAC inhibitors. Blood 116:3802

Zimmerman B, Sargeant A, Landes K, Fernandez SA, Chen CS, Lairmore M (2011) Efficacy of novel histone deacetylase inhibitor, AR42, in a mouse model of human T-lymphotropic virus type 1 adult T cell lymphoma. Leuk Res. 35:1491


## Clinical trials


Lezin A, Gillet N, Olindo S, Signaté A, Grandvaux N, Verlaeten O, Belrose G, de Carvalho Bittencourt M, Hiscott J, Asquith B, Burny A, Smadja D, Césaire R, Willems L. (2007) Histone deacetylase mediated transcriptional activation reduces proviral loads in HTLV-1 associated myelopathy/tropical spastic paraparesis patients. Blood 110:3722

Olindo S, Belrose G, Gillet N, Rodriguez S, Boxus M, Verlaeten O, Asquith B, Bangham C, Signaté A, Smadja D, Lezin A, Césaire R, Willems L. (2011) Safety of long-term treatment of HAM/TSP patients with valproic acid. Blood 118:6306

Isildinha Reis, Michele Manrique, Lisa Cabral, Luis Diaz, Ngoc Toomey, Phillip Ruiz, Juan Carlos Ramos (2012) The Combination of AZT/Interferon-α Therapy with the HDAC Inhibitor Valproic Acid for the Treatment of Adult T-Cell Leukemia/Lymphoma. Blood 120: 4877

Boostani R, Vakili R, Hosseiny SS, Shoeibi A, Fazeli B, Etemadi MM, Sabet F, Valizade N, Rezaee SA (2015) Triple Therapy with Prednisolone, Pegylated Interferon and Sodium Valproate Improves Clinical Outcome and Reduces Human T-Cell Leukemia Virus Type 1 (HTLV-1) Proviral Load, Tax and HBZ mRNA Expression in Patients with HTLV-1-Associated Myelopathy/Tropical Spastic Paraparesis. Neurotherapeutics 12:887

ECM GIGA
(Liege University, Belgium)

Luc Willems

Malik Hamaidia

Alexis Fontaine

Thomas Jouant

Xavier Saintmard

Roxane Terres

Florine Doucet

Manon Zwaenepoel

Songkang Qin

Chen Tan

Dandan Ye

Jean-Rock Jaques

Former members

Thomas Joris

Hélène Gazon

Mégane Willems

Hélène Brossel

Nicolas Gillet

Clotilde Hoyos

Rugi Safari

Hematology
(University Kumamoto, Japan):

Masao Matsuoka

Jun-Ichirou Yasunaga

Virology
(Imperial College, UK):

Graham Taylor

Aileen Rowan

Hematology Lab
(Liege University, Belgium)

Gregory Ehx

Charline Faville

Instituto Nacional de Tecnología Agropecuaria (Buenos Aires, Argentina)

Karina Trono

Bioinformatics platform
(Liege University, Belgium)

Arnaud Lavergne

David Stern

Animal Genomics Lab
(Liege University, Belgium)

Anne Van den Broeke

Anne-Sophie Reuter

Damien Renette

Institut de recherche en infectiologie (Montpellier University, France)

Antoine Gross

Jean-Marie Peloponese

National Veterinary Research Institute (Pulawy, Poland)

Michal Reichert

Jacek Kuzmak

Martinique (Fort-de-France)

Stéphane Olindo

Agnès Lezin

Raymond Césaire

Drexel UNIVERSITY | Drexel MEDICINE

22nd International Conference on Human Retrovirology:
HTLV and Related Retroviruses
HTLV2026

June 3-6, 2026 | Philadelphia, Pennsylvania, USA

# Pathogenesis of HAM/TSP

Christopher Itoh
NINDS/NIH, USA

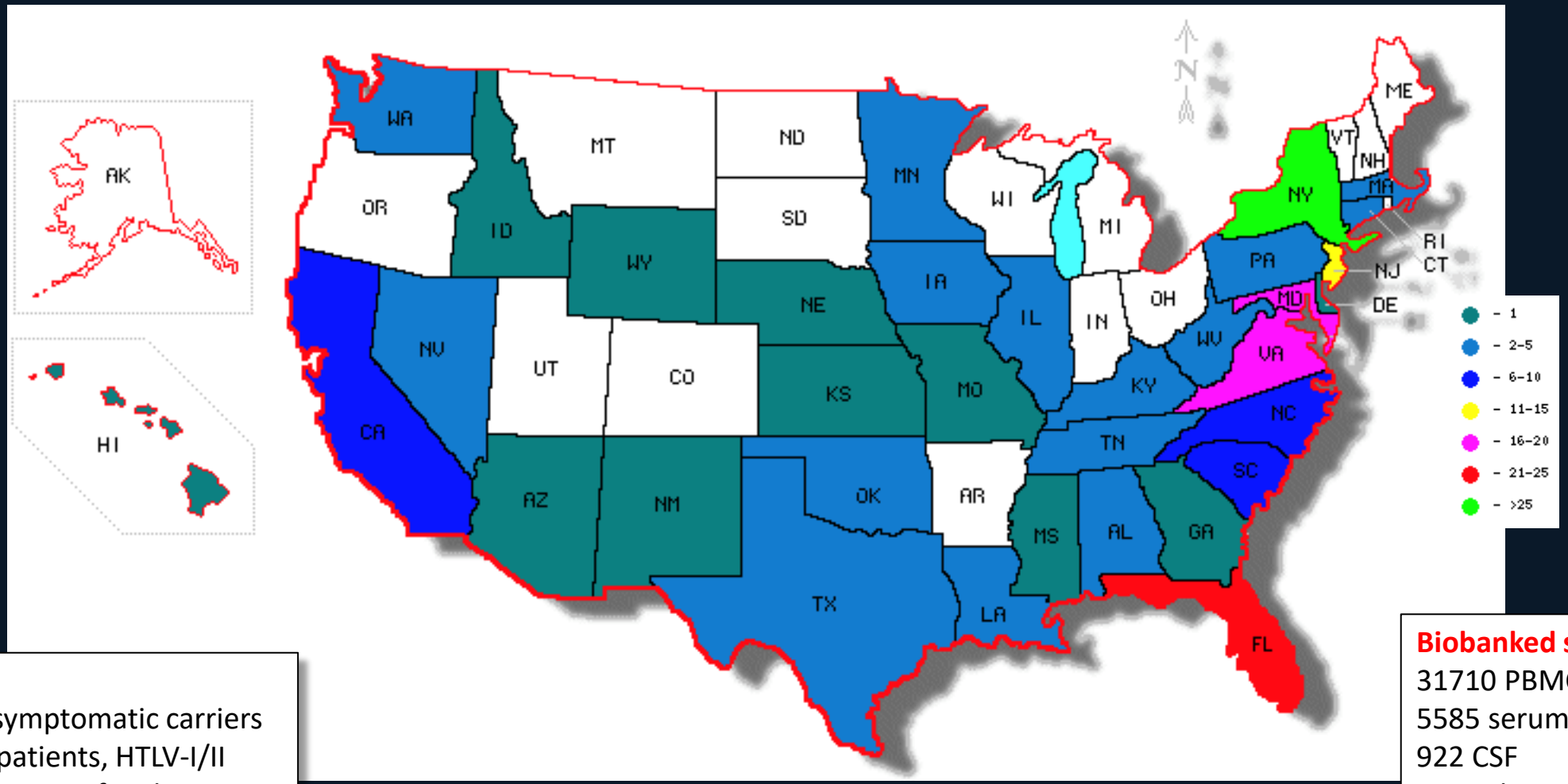
HTLV 2026

# HAM/TSP Pathophysiology

*Bystander damage, transmigration, and the next questions*

Chris Itoh, MD MHS | NINDS/NIH | June 3rd, 2026

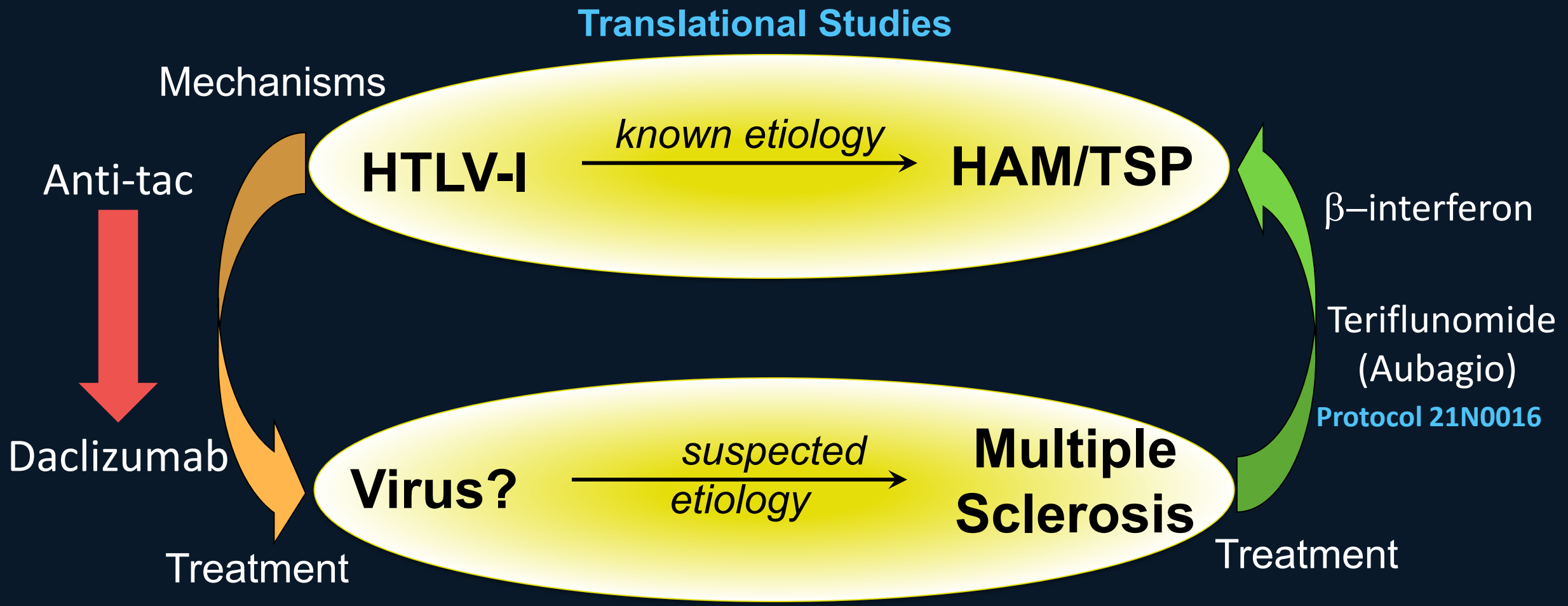
# Viral Immunology Section – Clinical Cohort (US)
# Immuno-Virological Evaluation of HAM/TSP
## Protocol # 98N-0047




**425 patients**
(HAM/TSP, Asymptomatic carriers (AC), HTLV-2 patients, HTLV-I/II seroindeterminates, family members)

**Biobanked samples**
31710 PBMCs
5585 serum
922 CSF
102 saliva
250 plasma

# Association of Viruses and Chronic Progressive Neurologic Disease



# Brief HAM/TSP Summary

## Clinical Symptoms

- Chronic, Progressive, Myelopathy
- Spinal Cord Atrophy
- Heterogeneous rates of progression

## Host Factors

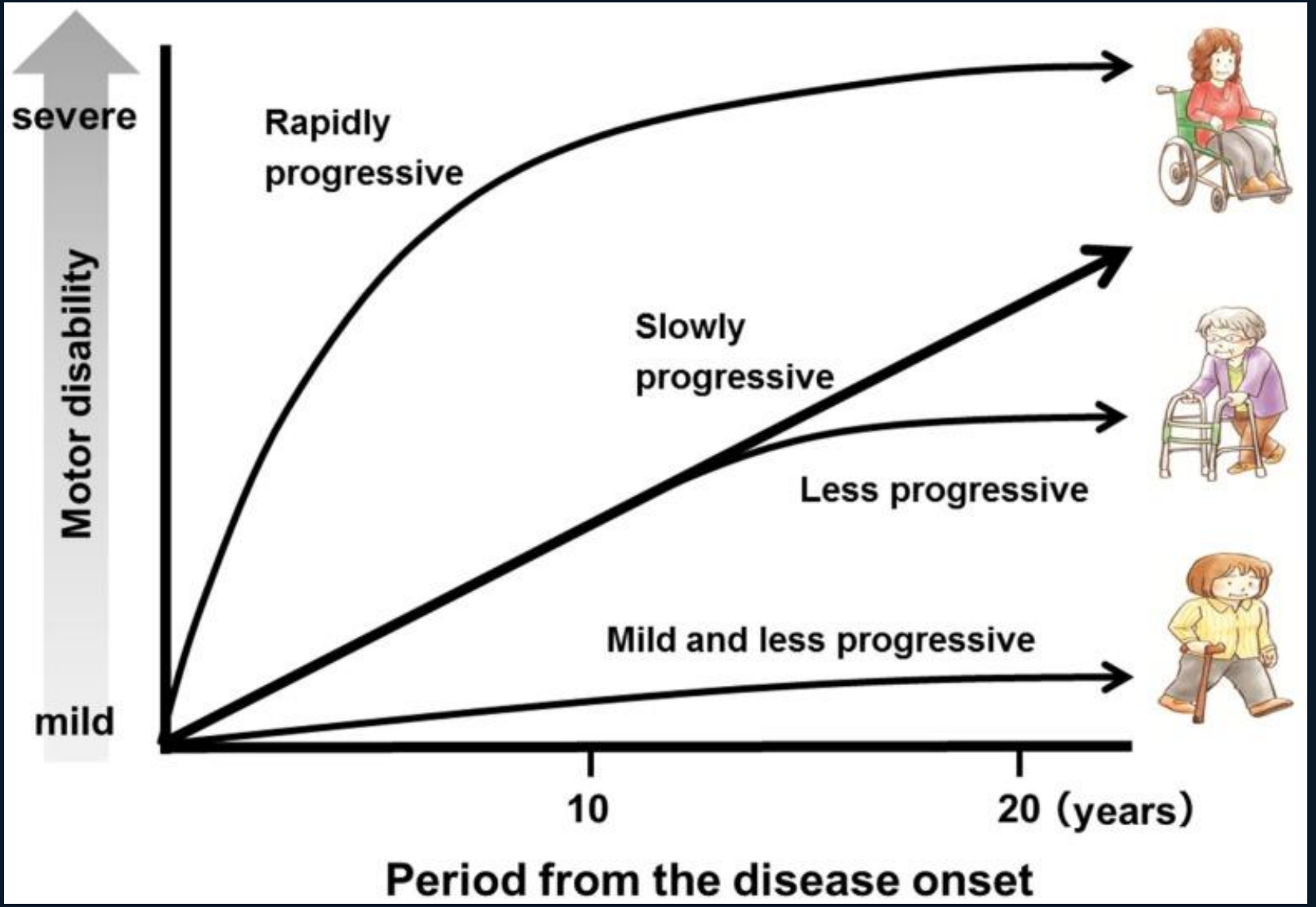
- Age 40-50 at onset
- Female predominance (3:1)
- 0.25-3.8% lifetime risk
- HLA susceptibility / protective

## Viral

- High PVL
- CSF>PBMC PVL
- Various Transmission Routes

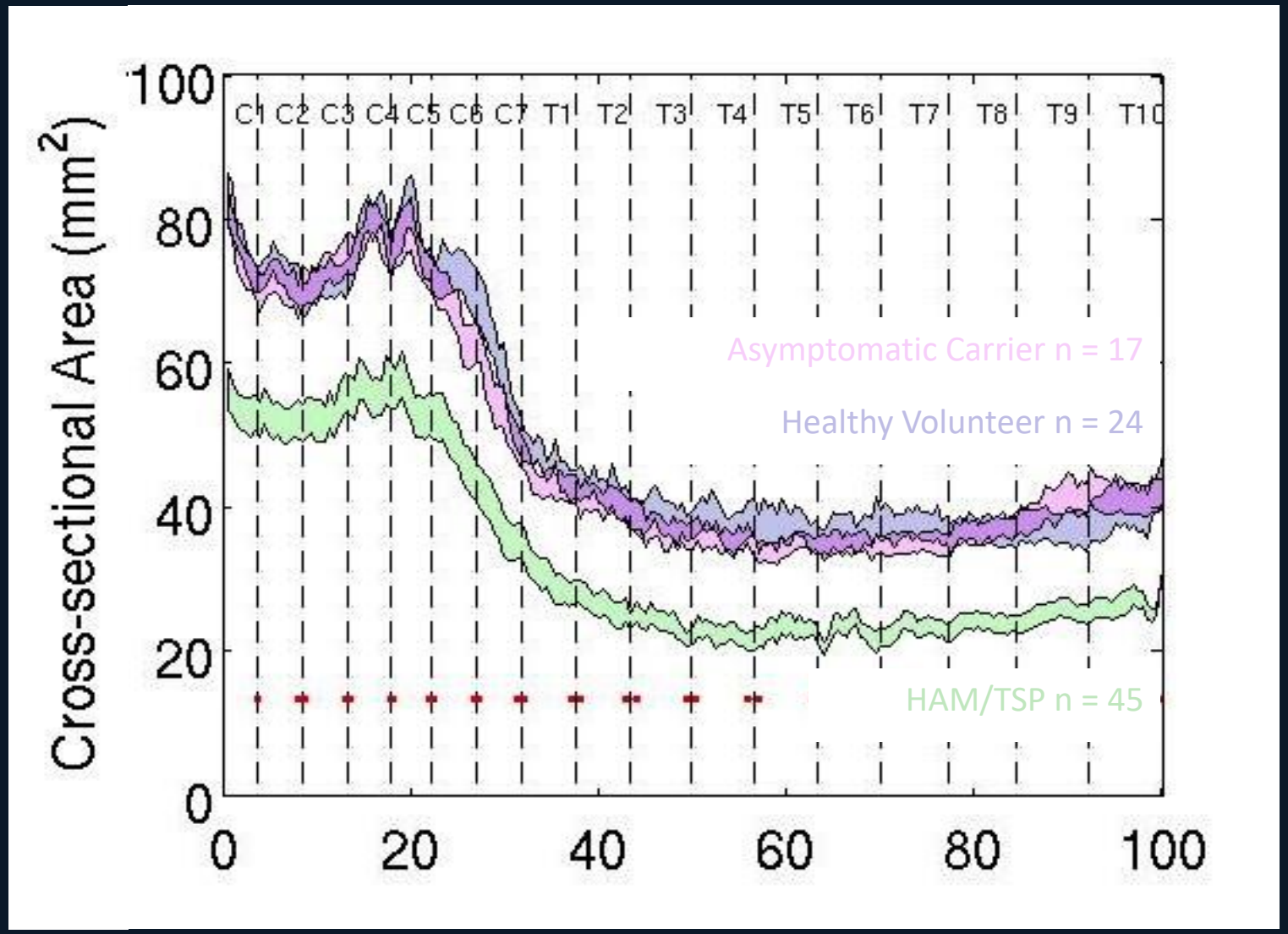
*Nozuma & Jacobson, Front Microbiol 2019*

# Heterogeneous Progression




*Yamano and Sato, Front Microbiol 2012*

# Spinal Cord Atrophy is Associated with Worse Disability, Higher PVL, and Increased CD8+ T cells in CSF




Annals of NEUROLOGY
An Official Journal of the American Neurological Association and the Child Neurology Society
Research Article | Full Access

**Imaging spinal cord atrophy in progressive myelopathies: HTLV-I-associated neurological disease (HAM/TSP) and multiple sclerosis (MS)**

Shila Azodi MD, Govind Nair PhD, Yoshimi Enose-Akahata PhD, Emily Charlip, Ashley Vellucci BS, Irene Cortese MD, Jenifer Dwyer BS, B. Jeanne Billioux MD, Chevaz Thomas BS, Joan Ohayon MSN, Daniel S. Reich MD, PhD, Steven Jacobson PhD ... See fewer authors

First published: 10 October 2017 | https://doi.org/10.1002/ana.25072 | Citations: 21

NeuroImage: Clinical 30 (2021) 102680
Contents lists available at ScienceDirect
NeuroImage: Clinical
journal homepage: www.elsevier.com/locate/ynicl

**Cervical and thoracic cord atrophy in multiple sclerosis phenotypes: Quantification and correlation with clinical disability**

Yair Mina[a,b,1], Shila Azodi[a,c,1], Tsemacha Dubuche[a], Frances Andrada[c], Ikesinachi Osuorah[c], Joan Ohayon[c], Irene Cortese[c], Tianxia Wu[d], Kory R. Johnson[e], Daniel S. Reich[f], Govind Nair[c,g], Steven Jacobson[a,*]

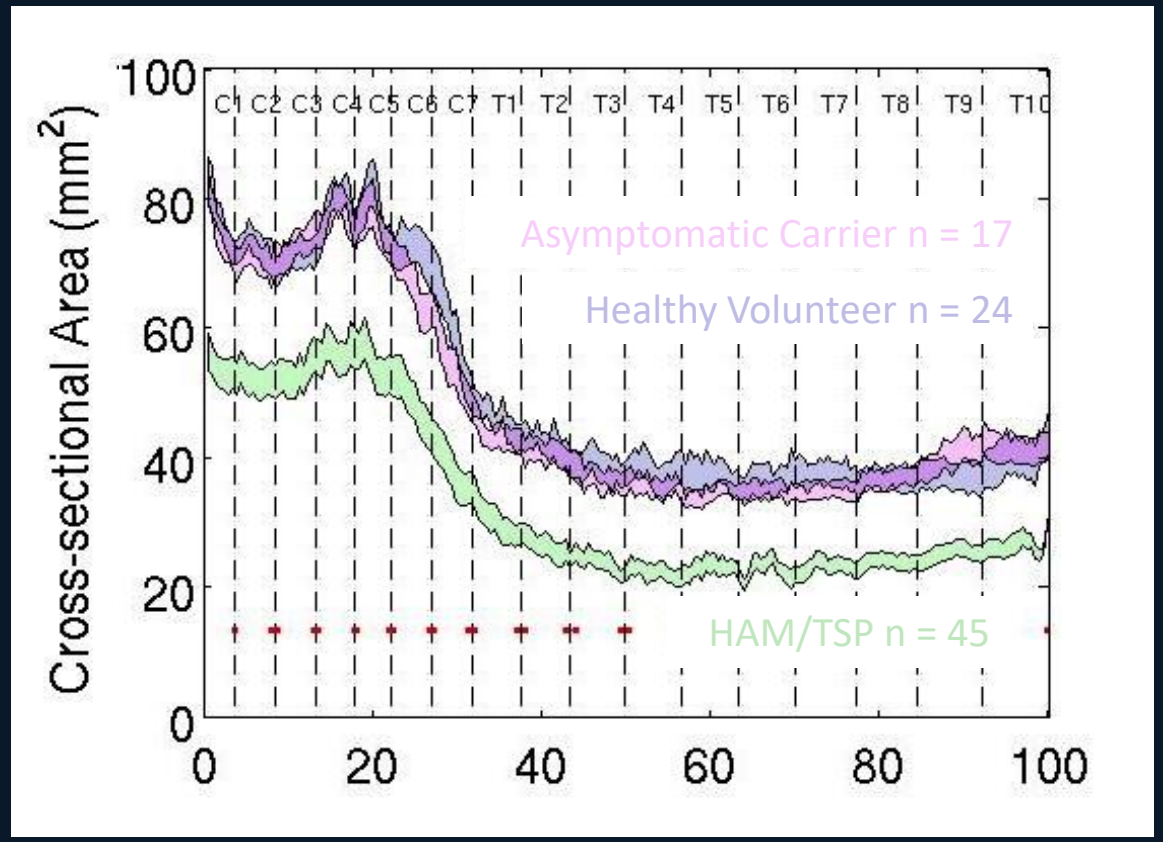
Annals of NEUROLOGY
An Official Journal of the American Neurological Association and the Child Neurology Society
Research Article | Full Access

**In vivo imaging of spinal cord atrophy in neuroinflammatory diseases**

Winston Liu, Govind Nair PhD, Luisa Vuolo MD, Anshika Bakshi BS, Raya Massoud MD, Daniel S. Reich PhD, Steven Jacobson PhD

First published: 04 July 2014 | https://doi.org/10.1002/ana.24213 | Citations: 29

# Spinal Cord Atrophy is Associated with Worse Disability, Higher PVL, and Increased CD8+ T cells in CSF



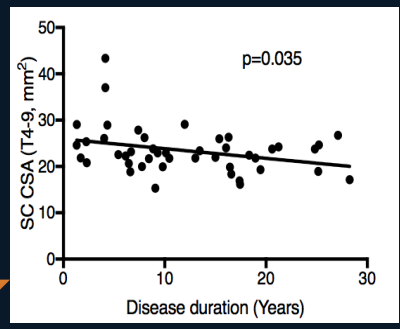

Functional disability scales

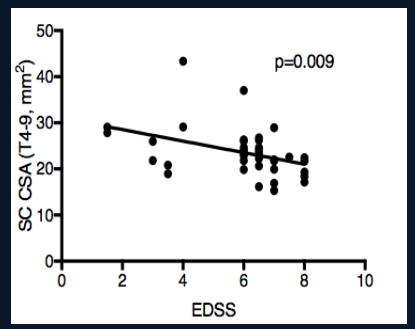
Disease Duration

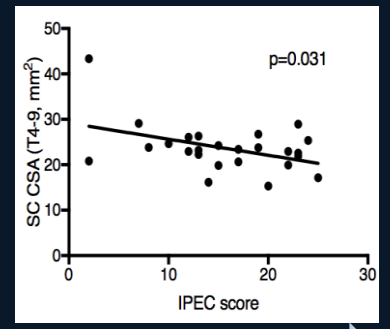
EDSS

IPEC

Thinner



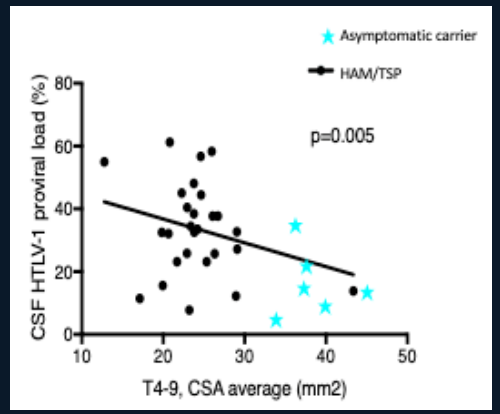



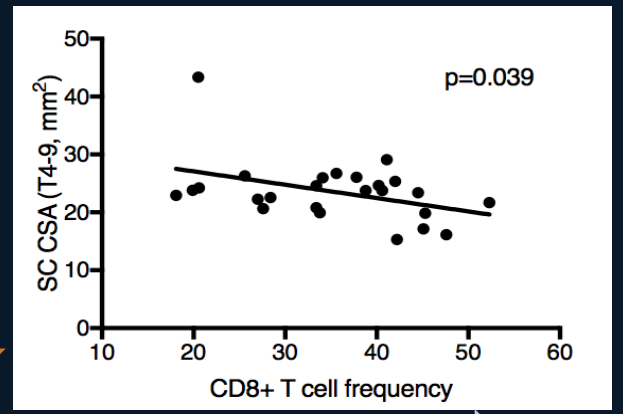

Longer disease duration

Increasing Disability

Higher Proviral Load



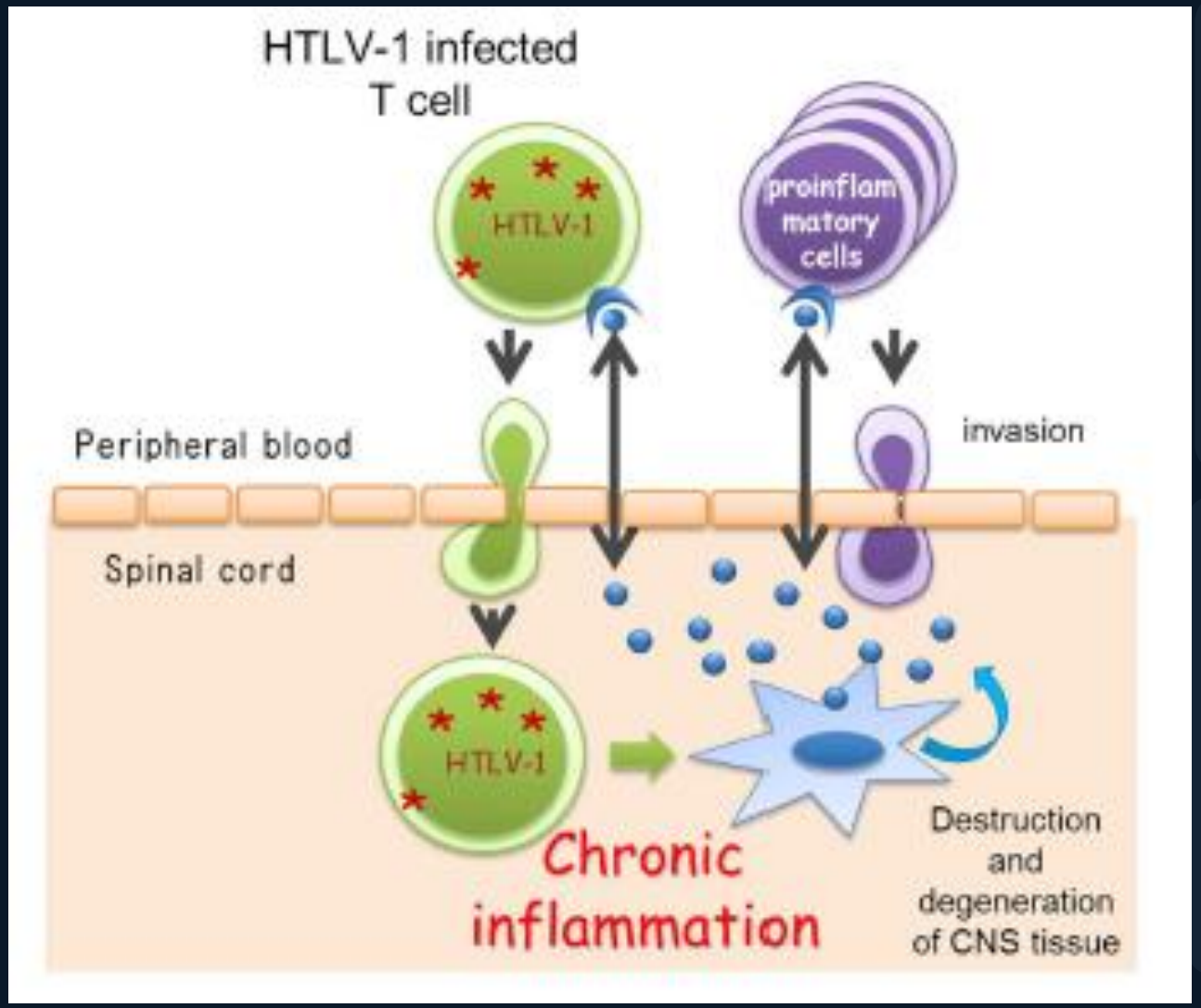

Thinner




Thinner

CD8+ T Cell Frequency

# Pathophysiology: Bystander Model




**1: Traffic in**

**2: Antigen burst**

**3: Amplify**

**4: Bystander damage**

*Yamano & Sato. Front Microbiol 2012*

# What we still cannot fully explain

## Why specifically the thoracic cord?

Low blood flow + low shear stress upregulates VCAM-1?

## Why only minority of carriers develop disease?

Multiple hits: HLA (permissive/protective) + cytokine SNPS + Environmental factors

## What triggers progression after decades of infection?

Environmental/Stress + Genetics

*Levin et al. Nat Med 2002 | Nozuma & Jacobson. Front Microbiol 2019*

# Alternative and complementary hypotheses

## BYSTANDER DAMAGE (dominant)

Chronic CNS inflammation damages uninfected neurons & myelin.

## CTL-MEDIATED

CD8+ CTLs targeting Tax-expressing cells cause direct collateral tissue damage.

## MOLECULAR MIMICRY

Anti-Tax antibodies cross-react with neuronal hnRNP-A1; inhibit neuronal firing ex vivo, however disease specificity and causal importance remain uncertain

## DAMAGE : HEALING IMBALANCE

Disease emerges when viral damage outpaces glial repair. Reframes 'why so few' as a tipping-point problem.

*Levin et al. Nat Med 2002 | Nozuma & Jacobson. Front Microbiol 2019*

# What therapy tells us about pathophysiology

## Antiretrovirals inconclusive

PVL maintained by infected clonal proliferation

## Target the infected cell

Mogamulizumab (anti-CCR4) depletes the reservoir. Phase 3: 65% PVL reduction.

## Target the cytokine loop

Hu-Mikb1, a humanized monoclonal antibody against IL-2/IL-15 receptor b-chain, lead to decreased spontaneous CD8 T cell spontaneous lymphoproliferation, degranulation, and IFN-g expression

## Inhibit Transmission

Raltegravir, integrase inhibitor, showed a subset of patients with mild decrease in PVL

## Disrupt Viral Latency

HDAC inhibitors relax chromatin structure, force infected cells to transcribe Tax, so HTLV1 specific CD8 cells target , leading to decrease in PVL

## Limit downstream damage

Steroids offer transient clinical improvement

*Sato et al. NEJM 2018 / Enose-Akahata et al. Ann Clin Transl Neurol 2019 | Nakamura. Pathogens 2023*

# Summary

1. Understanding pathophysiology of HAM/TSP may offer understanding of other chronic neuroinflammtory diseases

2. Increased VCAM1, low blood flow may contribute to thoracic predominance

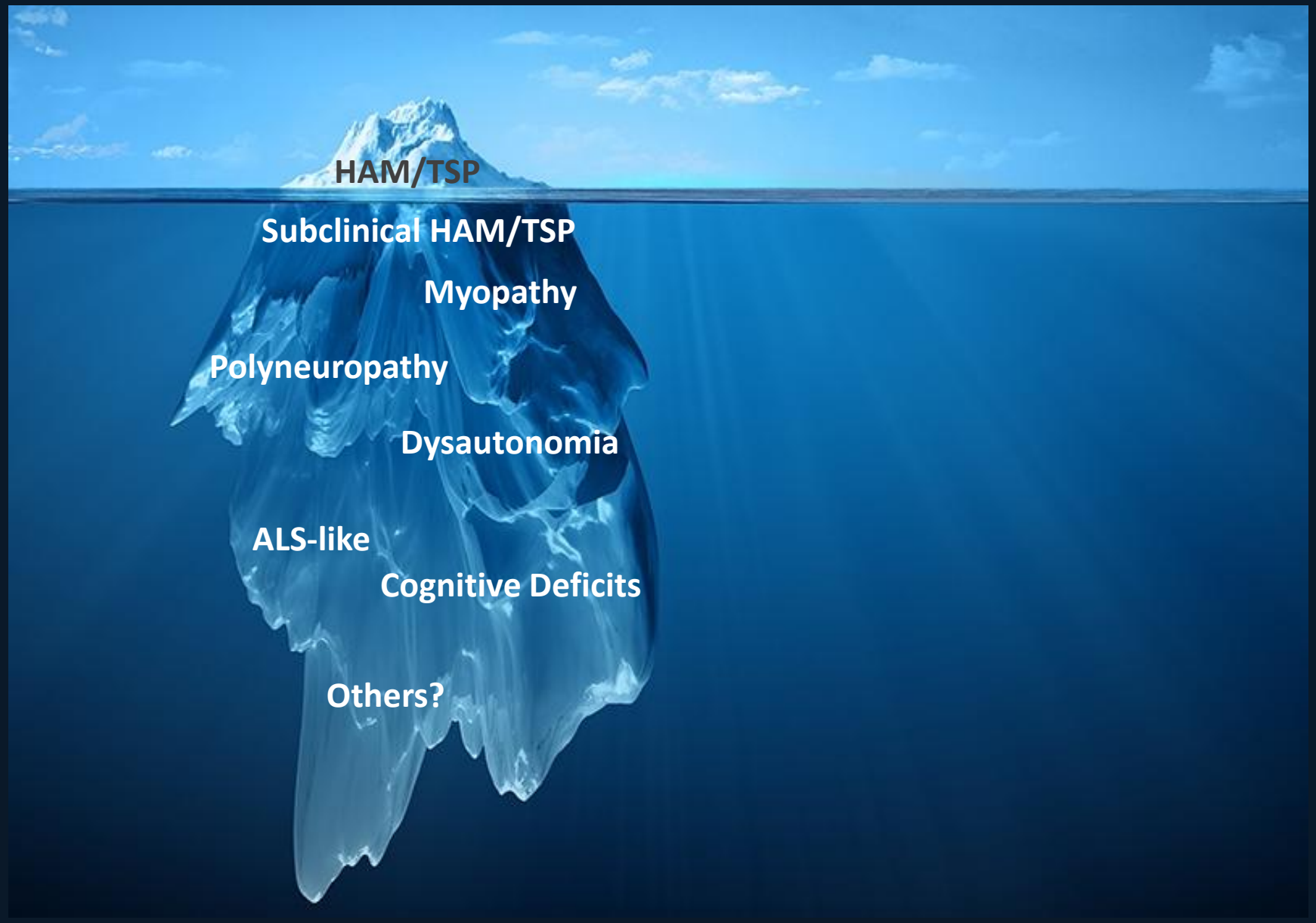
3. PVL is maintained by clonal proliferation, not new infection. Previous therapies may help elucidate pathophysiology

4. Multiple hypotheses (bystander, CTL, damage:healing) likely all contribute

# References


- Araujo, A., Bangham, C. R. M., Casseb, J., Gotuzzo, E., Jacobson, S., Martin, F., Penalva de Oliveira, A., Puccioni-Sohler, M., Taylor, G. P., & Yamano, Y. (2021). Management of HAM/TSP: Systematic Review and Consensus-based Recommendations 2019. *Neurol Clin Pract*, *11*(1), 49-56. https://doi.org/10.1212/CPJ.0000000000000832
- Araujo, A. Q. C., & Silva, M. T. T. (2026). Expanding the neurological spectrum of HTLV-1 beyond HAM/TSP: a contemporary perspective. *Lancet Reg Health Am*, *55*, 101347. https://doi.org/10.1016/j.lana.2025.101347
- Da Silva, S. J., Cabral-Castro, M. J., Faria, L. C., Rosadas, C., de Araujo, M. F. L., Dutra, A. C. S., Yamano, Y., Taylor, G., & Puccioni-Sohler, M. (2025). CXCL-10 in Cerebrospinal Fluid Detects Neuroinflammation in HTLV-1-Associated Myelopathy with High Accuracy. *Viruses*, *17*(1). https://doi.org/10.3390/v17010089
- Enose-Akahata, Y., Oh, U., Ohayon, J., Billioux, B., Massoud, R., Bryant, B., Vellucci, A., Ngouth, N., Cortese, I., Waldmann, T., & Jacobson, S. (2019). Clinical trial of a humanized anti-IL-2/IL-15 receptor β chain in HAM/TSP. *Annals of Clinical and Translational Neurology*, *6*. https://doi.org/10.1002/acn3.50820
- Nozuma, S., & Jacobson, S. (2019). Neuroimmunology of Human T-Lymphotropic Virus Type 1-Associated Myelopathy/Tropical Spastic Paraparesis. *Front Microbiol*, *10*, 885. https://doi.org/10.3389/fmicb.2019.00885
- Sato, T., Coler-Reilly, A. L. G., Yagishita, N., Araya, N., Inoue, E., Furuta, R., Watanabe, T., Uchimaru, K., Matsuoka, M., Matsumoto, N., Hasegawa, Y., & Yamano, Y. (2018). Mogamulizumab (Anti-CCR4) in HTLV-1–Associated Myelopathy. *New England Journal of Medicine*, *378*(6), 529-538. https://doi.org/doi:10.1056/NEJMoa1704827
- Yamano, Y., & Sato, T. (2012). Clinical Pathophysiology of Human T-Lymphotropic Virus-Type 1-Associated Myelopathy/Tropical Spastic Paraparesis [Review]. *Frontiers in Microbiology, Volume 3 - 2012*. https://doi.org/10.3389/fmicb.2012.00389

# Neurologic spectrum of HTLV-1



22nd International Conference on Human Retrovirology:
HTLV and Related Retroviruses
HTLV2026

June 3-6, 2026 | Philadelphia, Pennsylvania, USA

# Molecular Virology of HTLV

Benoit Barbeau
Universite du Quebec a Montreal, Canada

# Cellular localization of HBZ and its functions

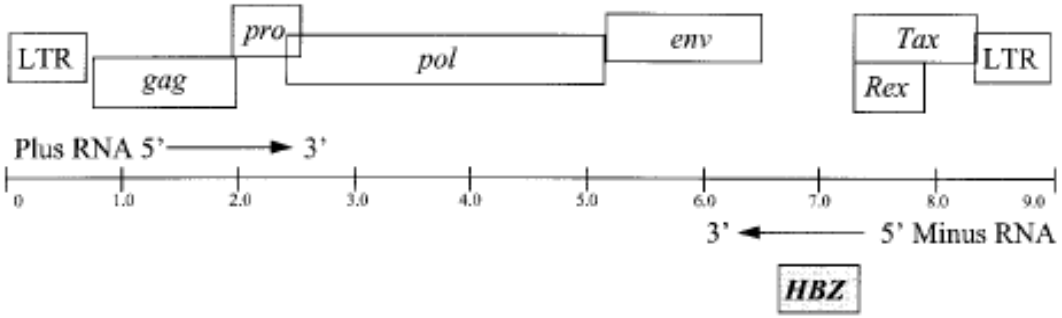
**Benoit Barbeau**

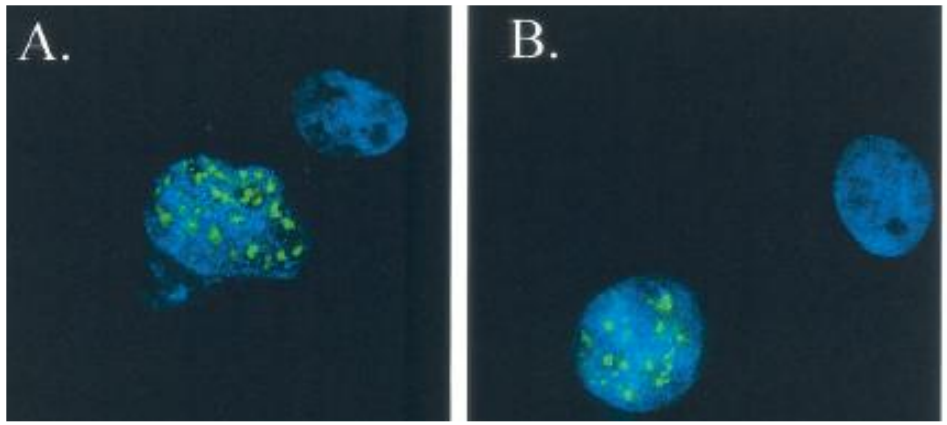
**Département des sciences biologiques**
**Université du Québec à Montréal**

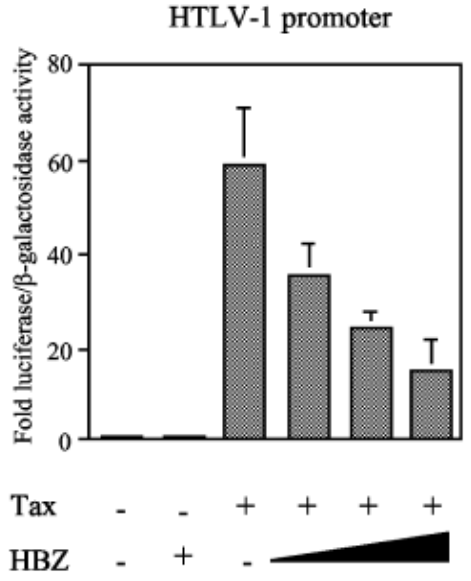
22nd International Conference on Human Retrovirology:
HTLV and Related Retroviruses
HTLV2026

June 3-6, 2026 | Philadelphia, Pennsylvania, USA

# Discovery of HBZ

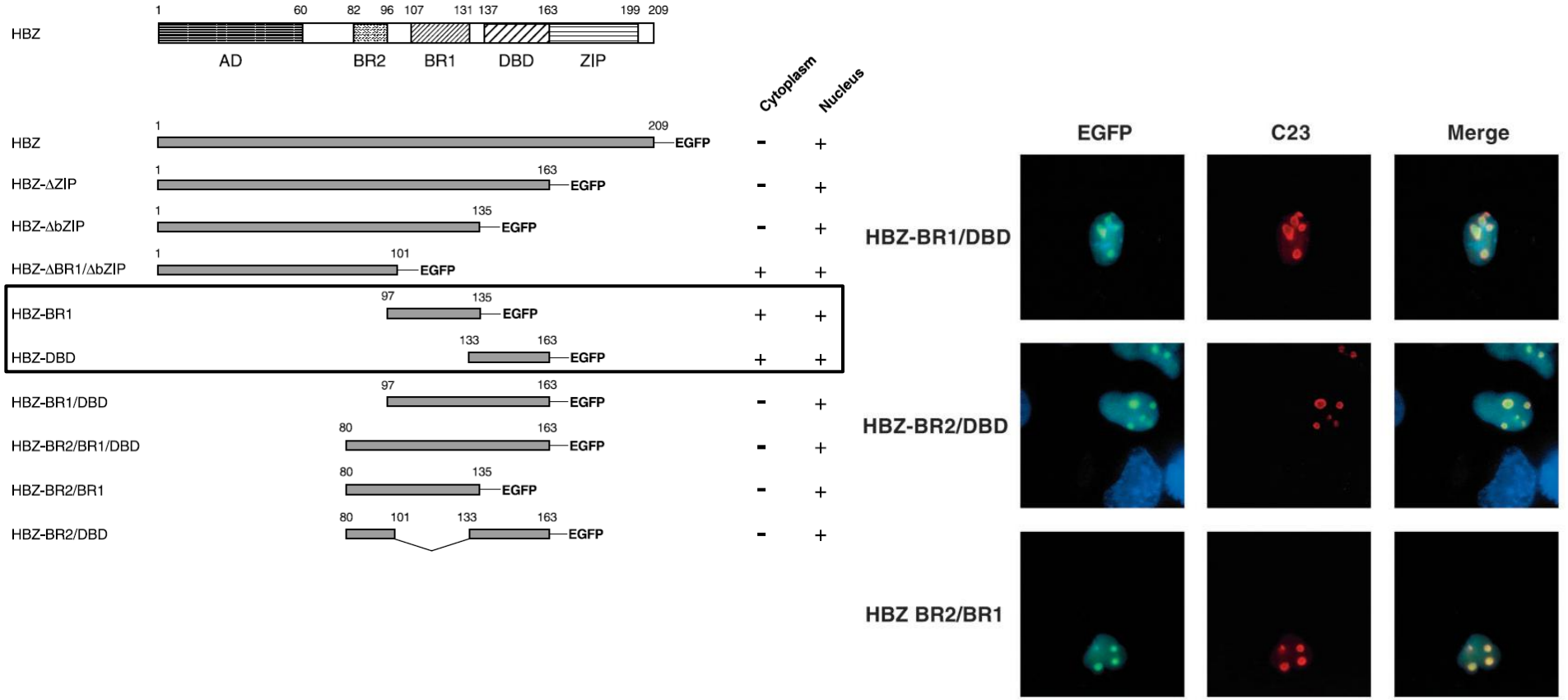


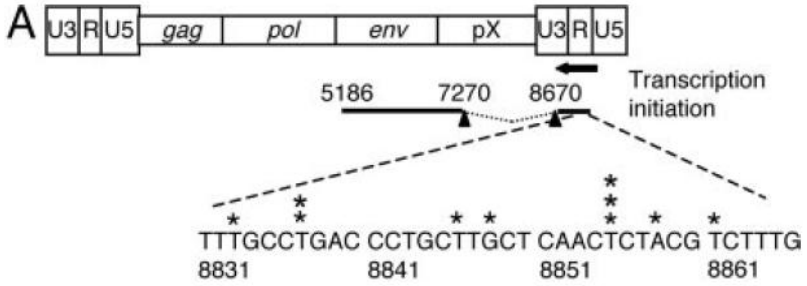

Gaudray et al., 2002

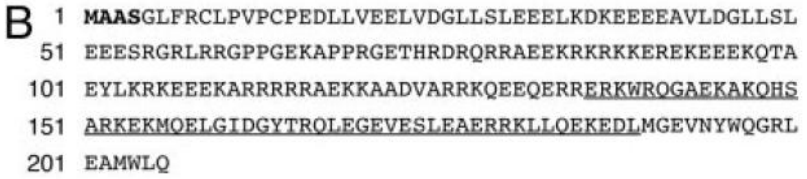
# HBZ, NLS and mutants

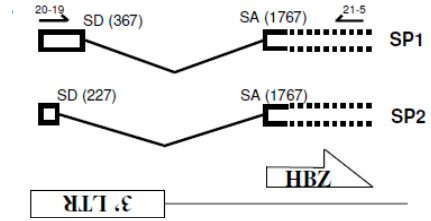


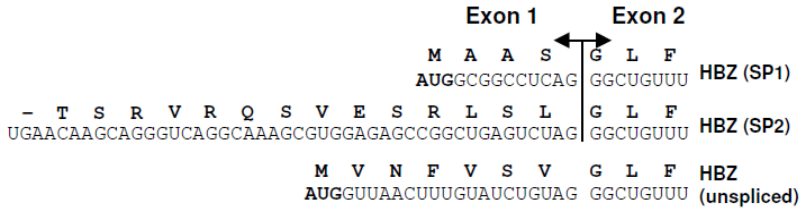

Hivin et al., 2005

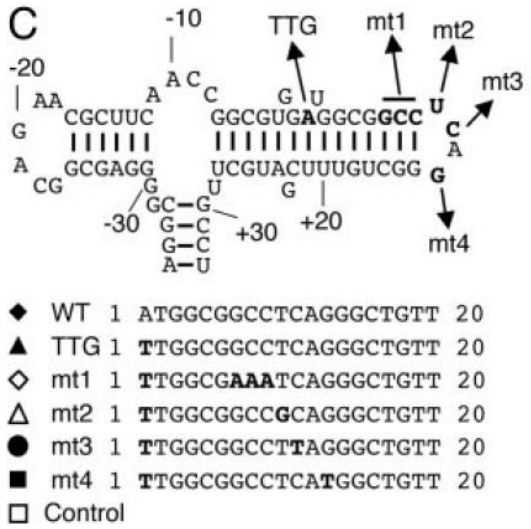
# Early findings related to HBZ isoforms and splicing

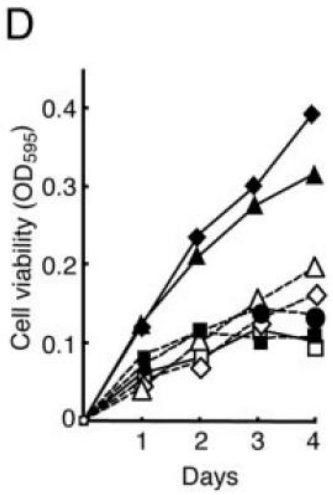


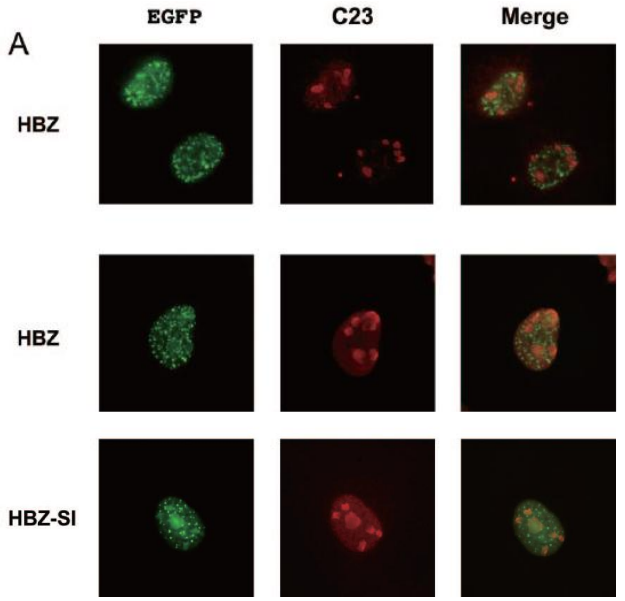



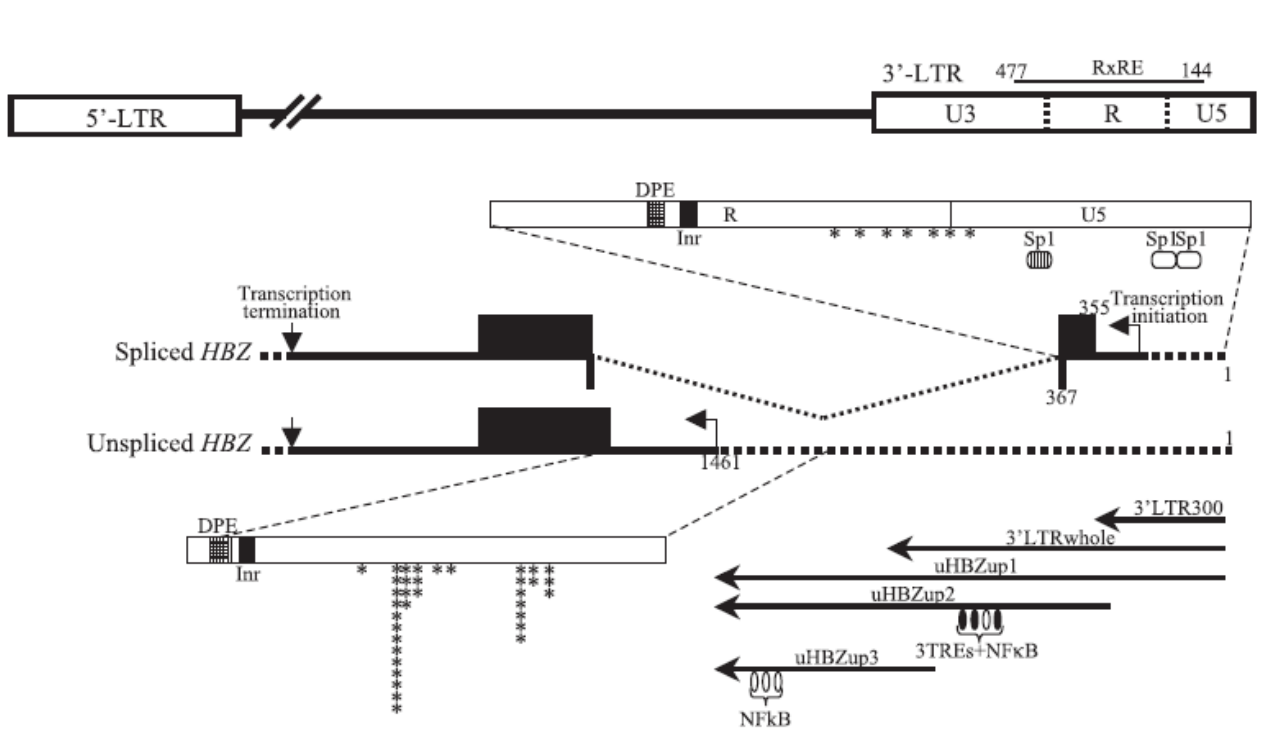



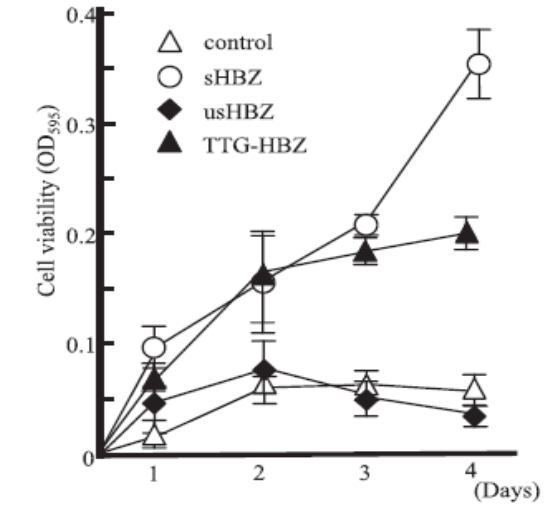



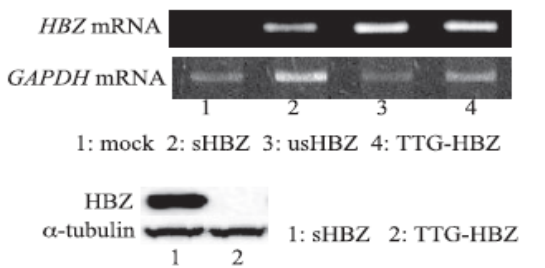




Satou et al. 2006; Cavanagh et al., 2026; Murata et ., 2006

# The unspliced HBZ isoform is expressed from a different mRNA

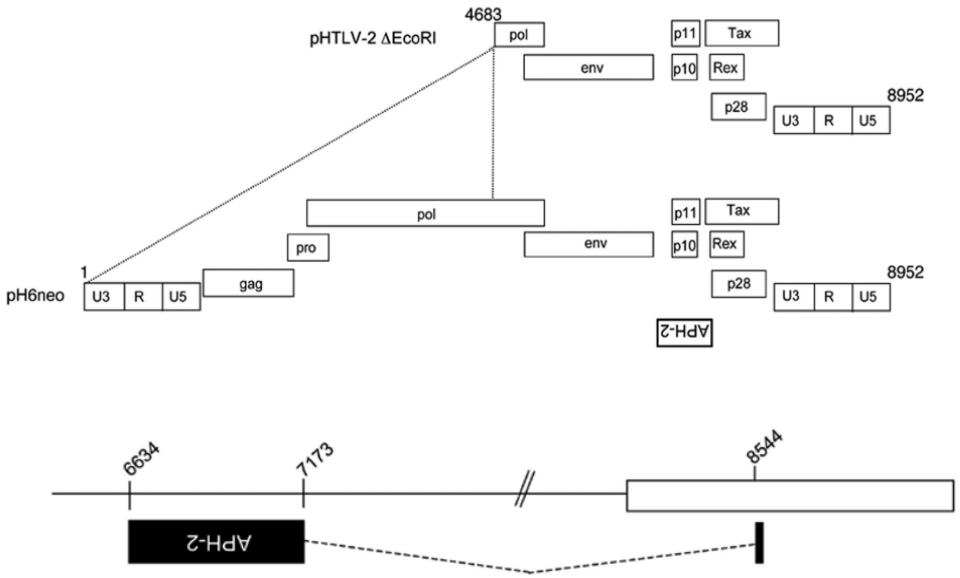


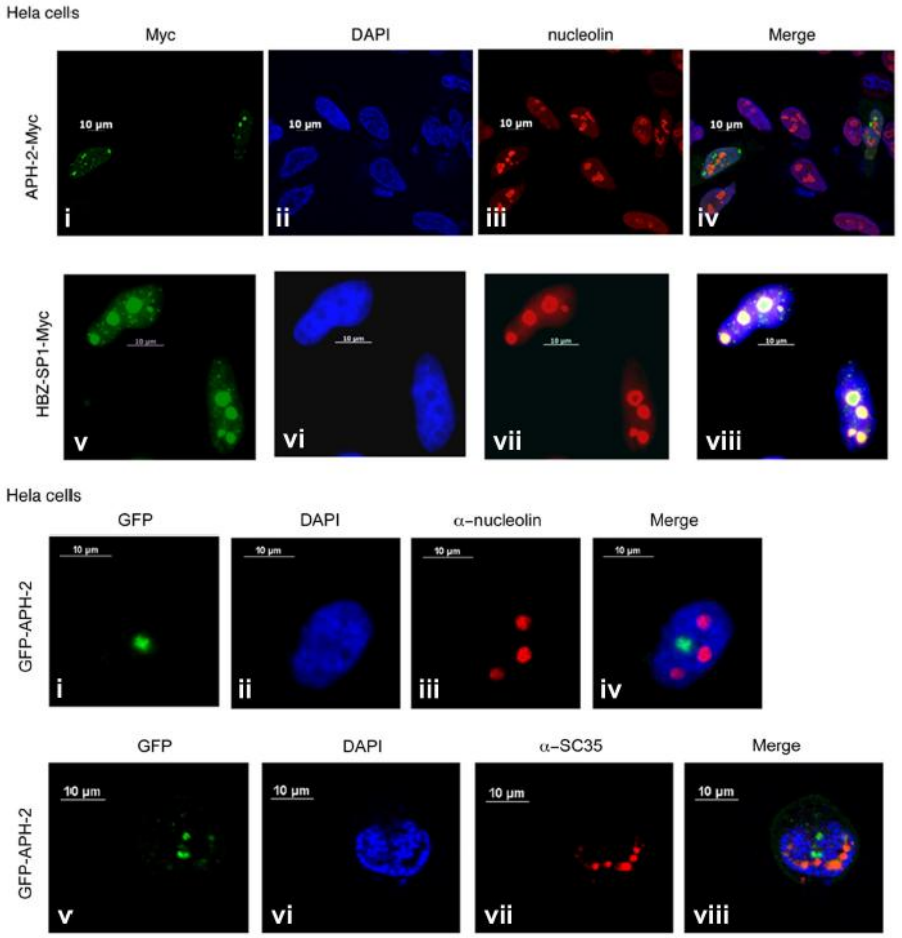




Yoshida et al., 2008

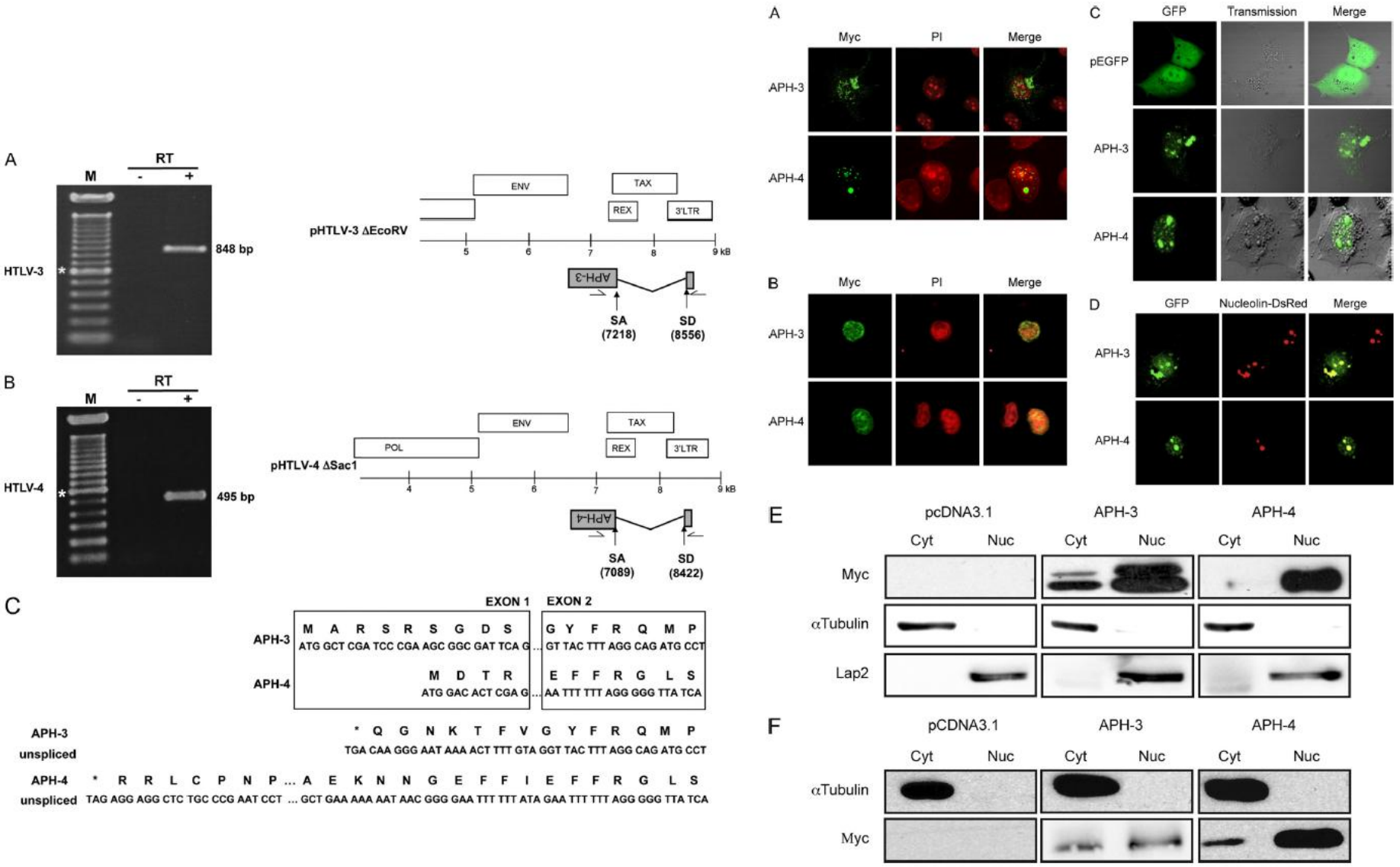
# Comparison between sHBZ and APH-2



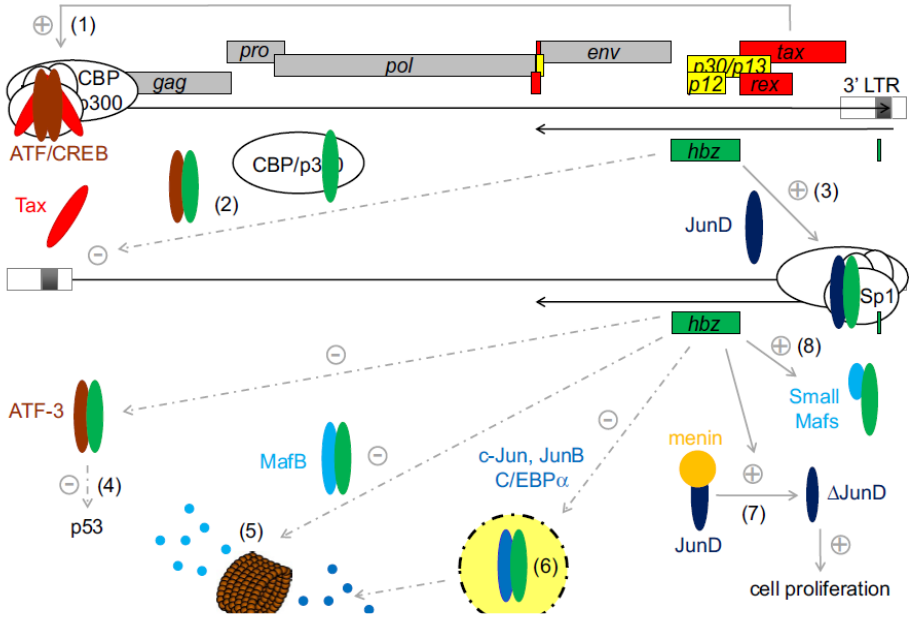

Halin et al., 2009

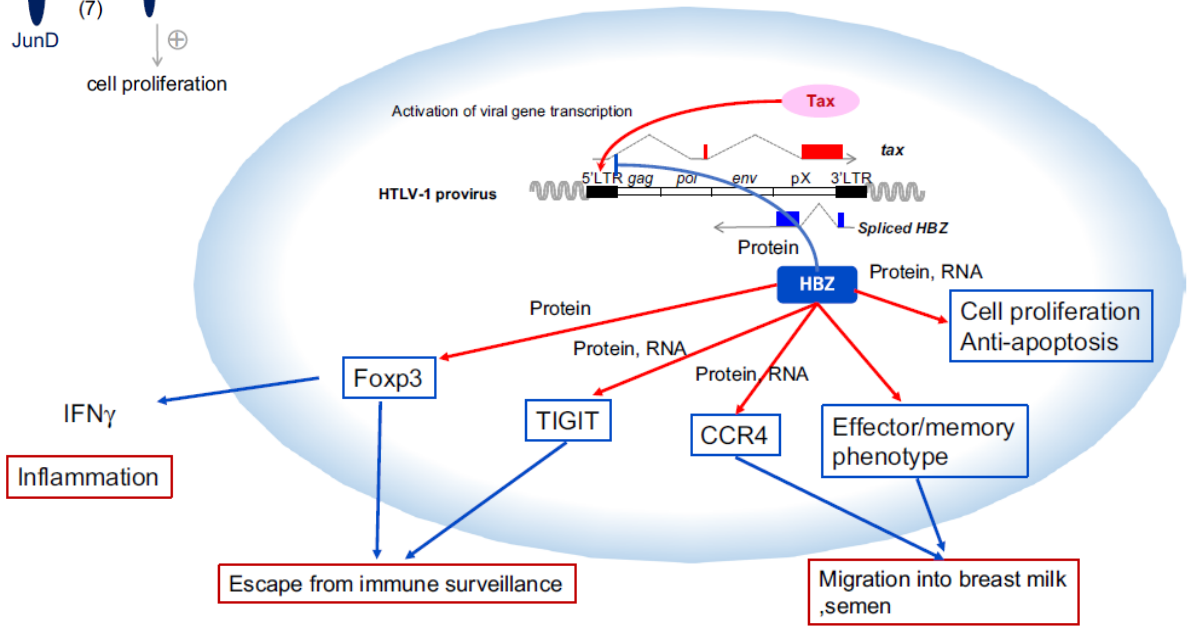
# HTLV-3 and HTLV-4 antisense proteins




Larocque et al., 2011

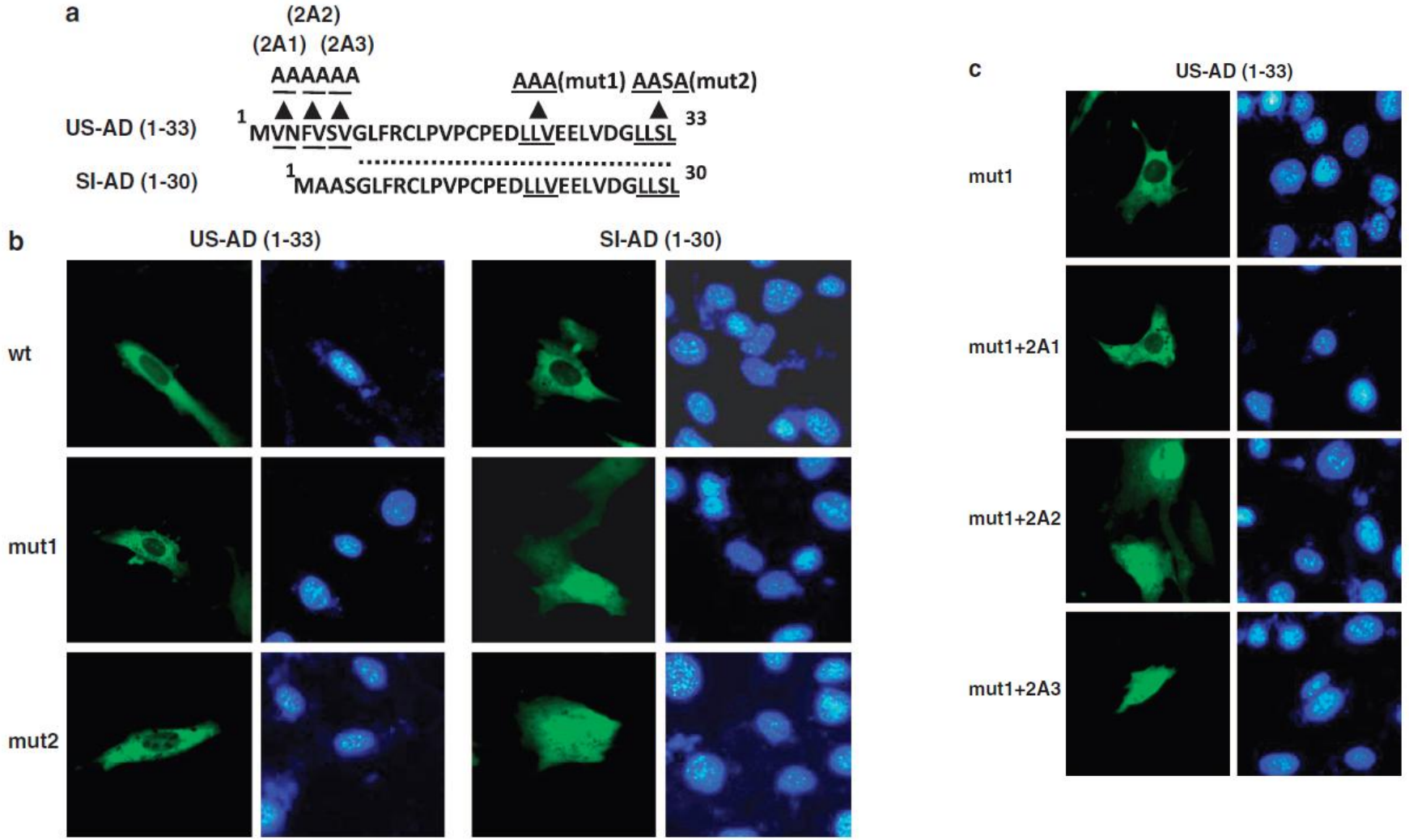
# Functions associated to HBZ



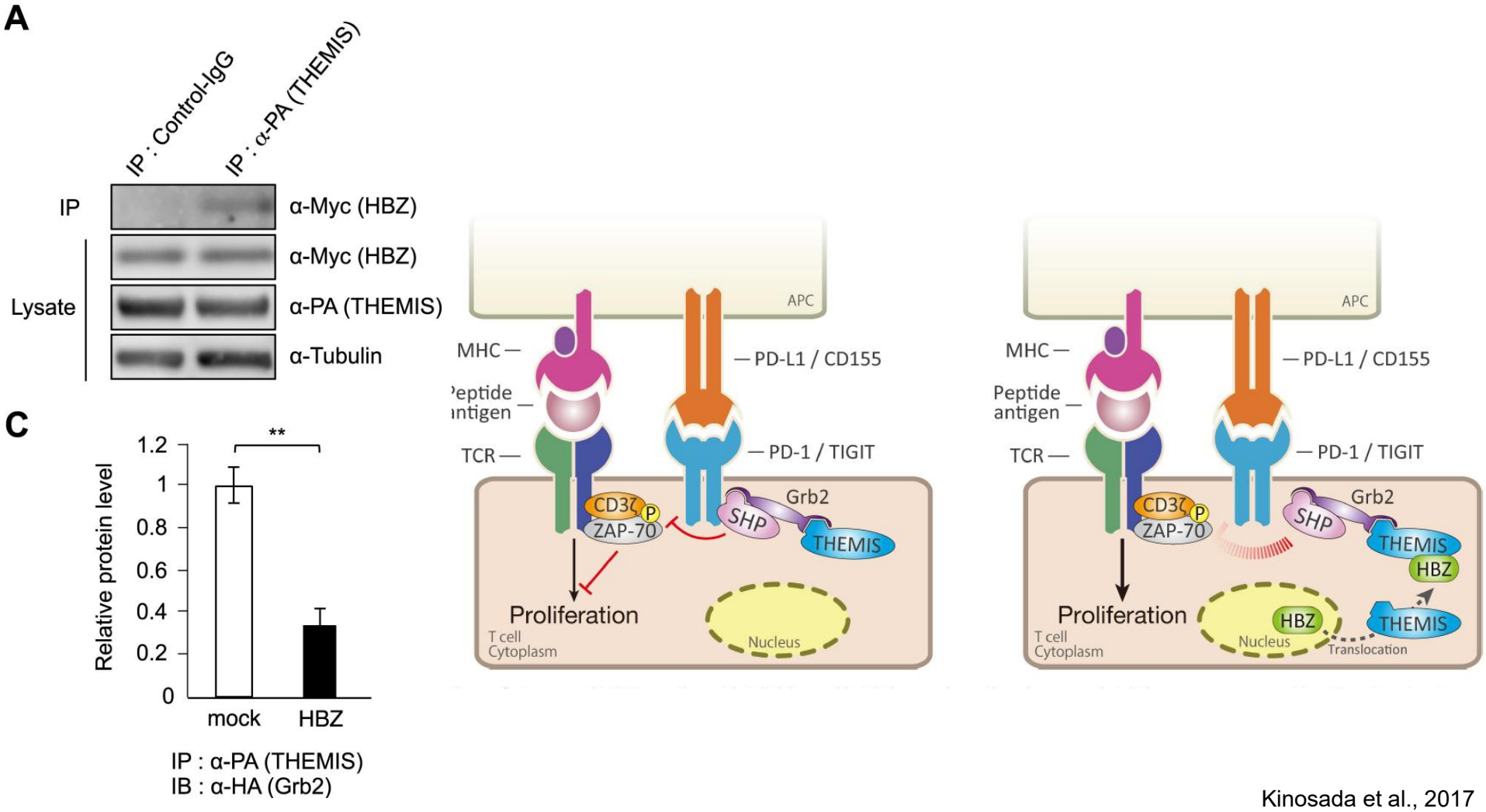

Matsuoka and Mesnard, 2020

# Discovery of NES sequences in HBZ



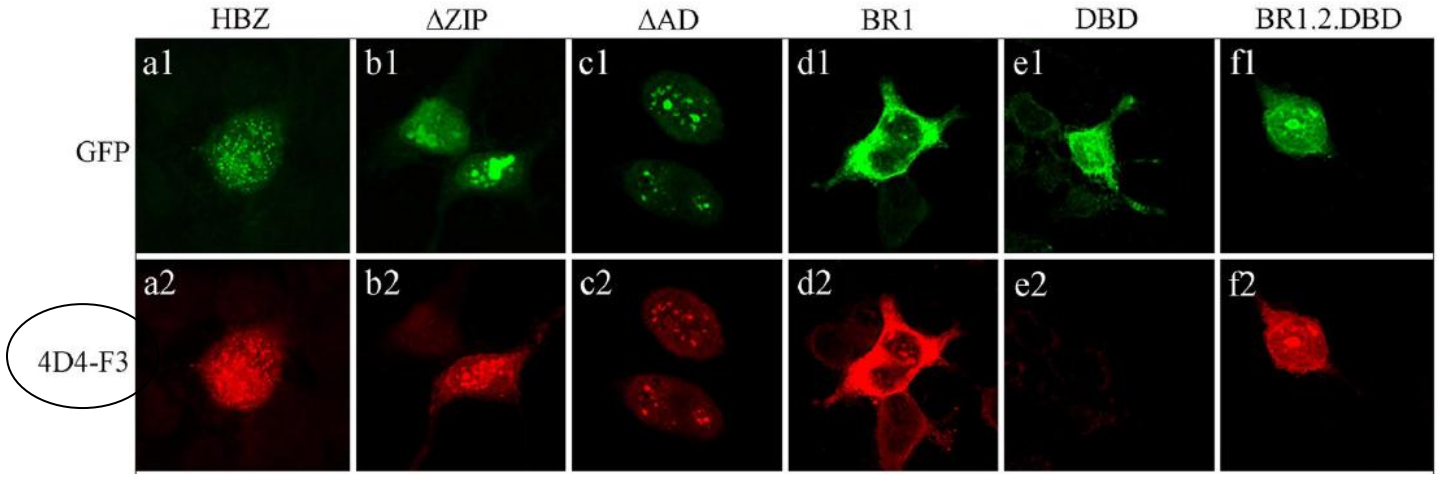

Mukai and Ohshima, 2013

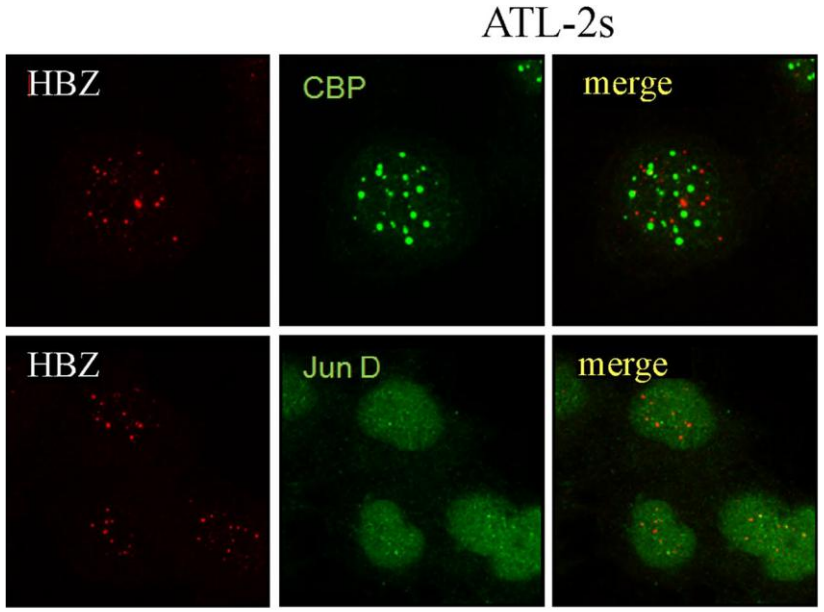
# Functional attributes to cytoplasmic HBZ via potential interaction partner

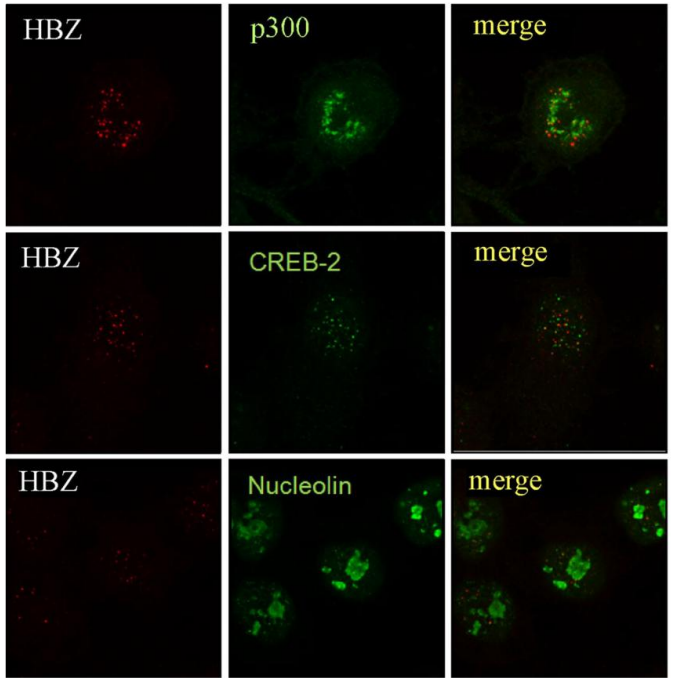



Kinosada et al., 2017

# Reassessment of HBZ localization

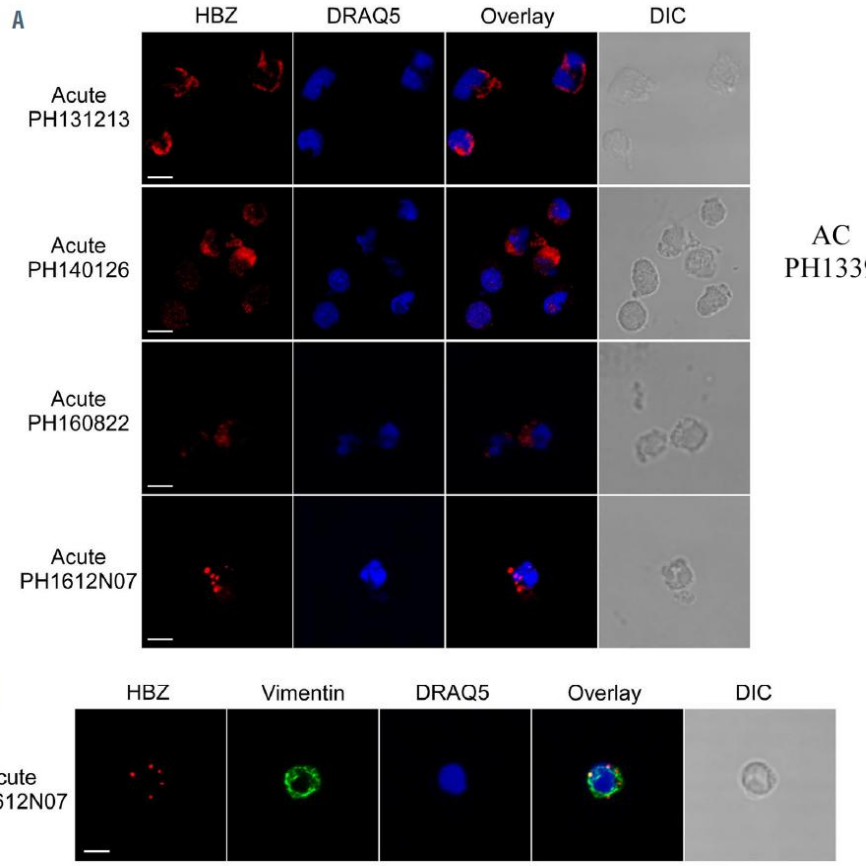


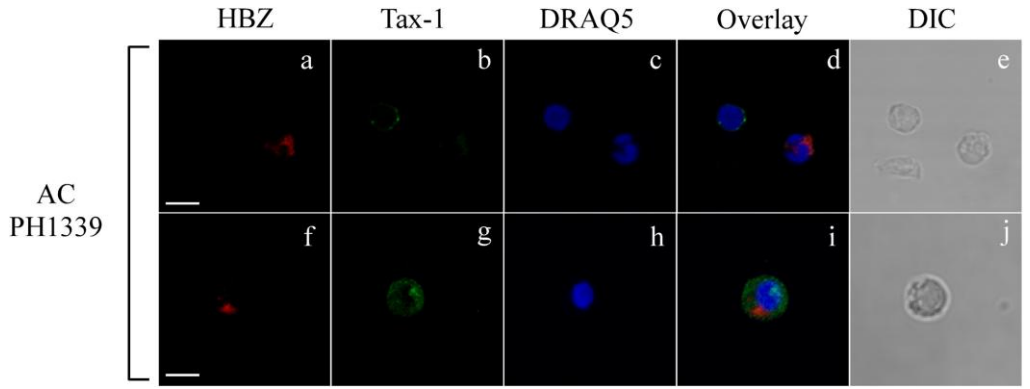




Raval et al., 2015

# HBZ localization in PBMCs from patients

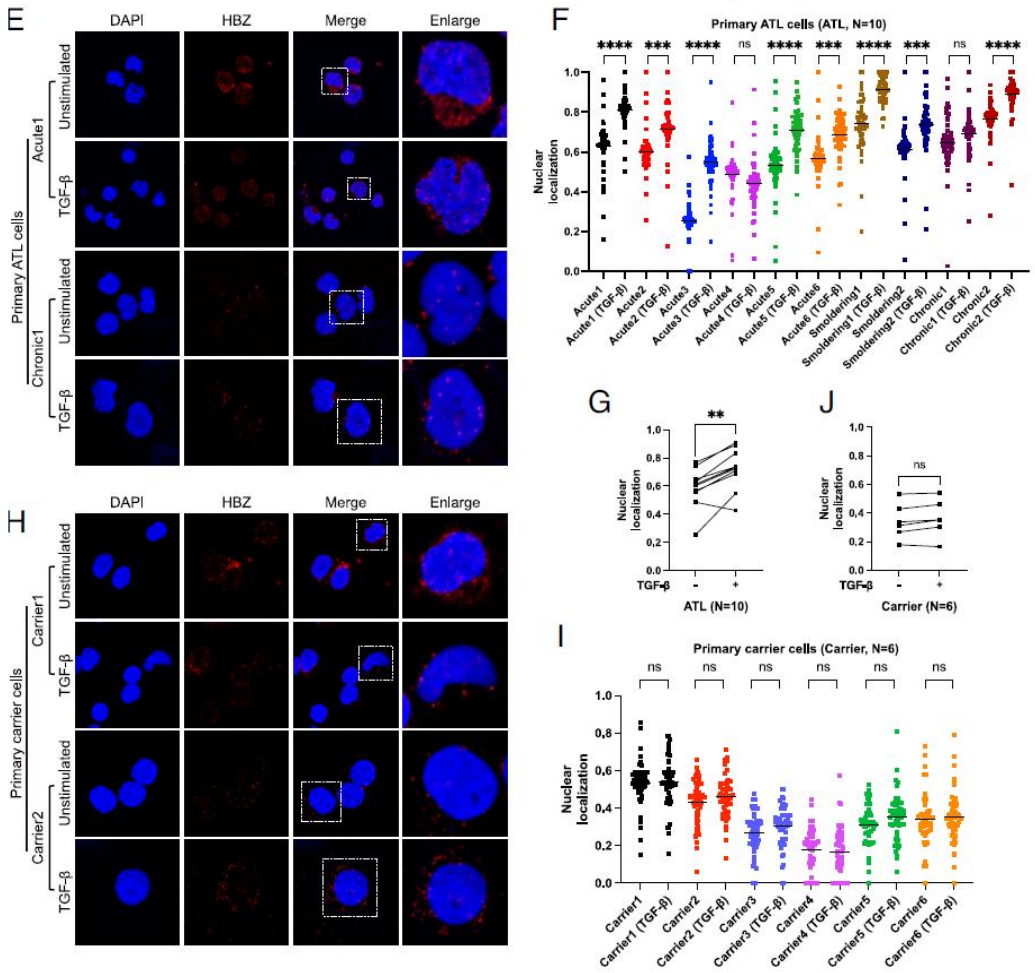


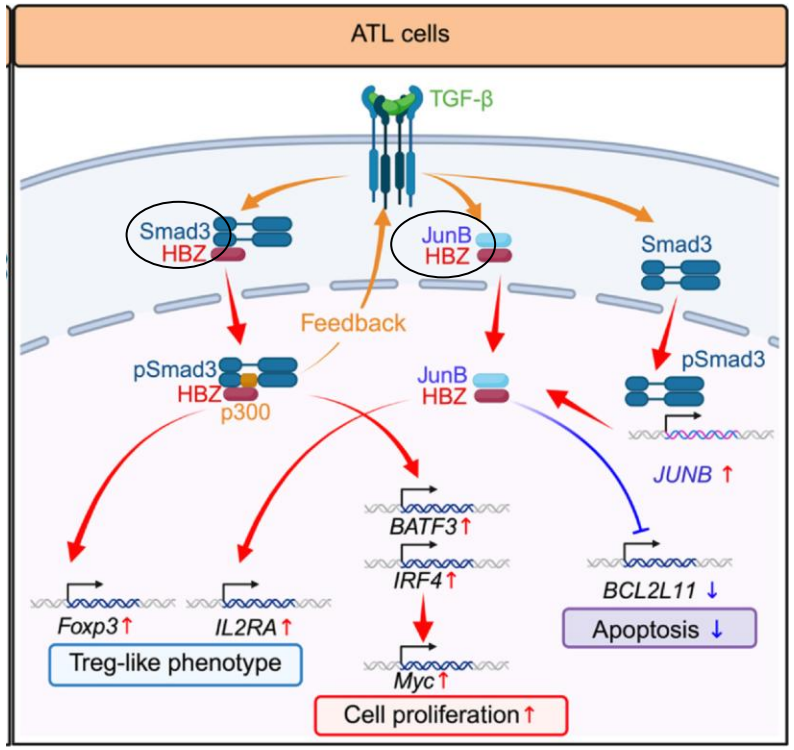




-HBZ-Tax coexpression is rare.
-AC and HAM/TSP PBMCs show rare and exclusive cytoplasmic HBZ detection.
-ATL PBMCs show weak nuclear HBZ signals in a minority of cells.

Baratella et al., 2017; Forlani et al., 2019; Forlani et al., 2021

# TGF-β and HBZ translocation to the nucleus in ATL cells



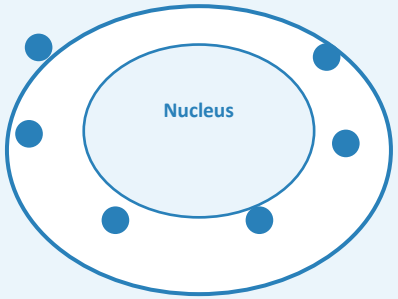




**Proximity ligation assay**

Shichijo and Yasunaga, 2025; Zhang et al., 2025

# HBZ localization *in vivo*

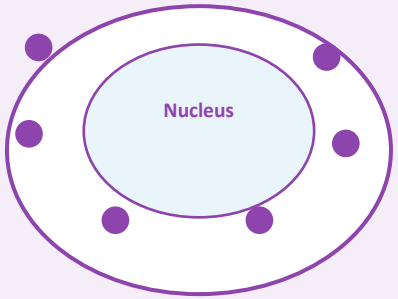
## Asymptomatic Carriers (AC)




**CYTOPLASM only**

- HBZ protein exclusively cytoplasmic
- Lower nuclear HBZ levels

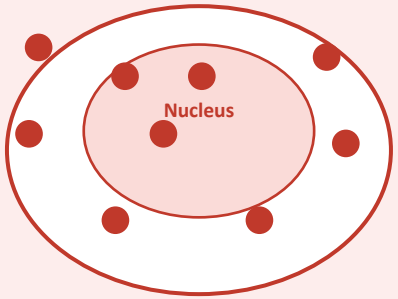
## HAM/TSP Patients




**CYTOPLASM only**

- HBZ remains cytoplasmic
- Similar localization pattern to AC

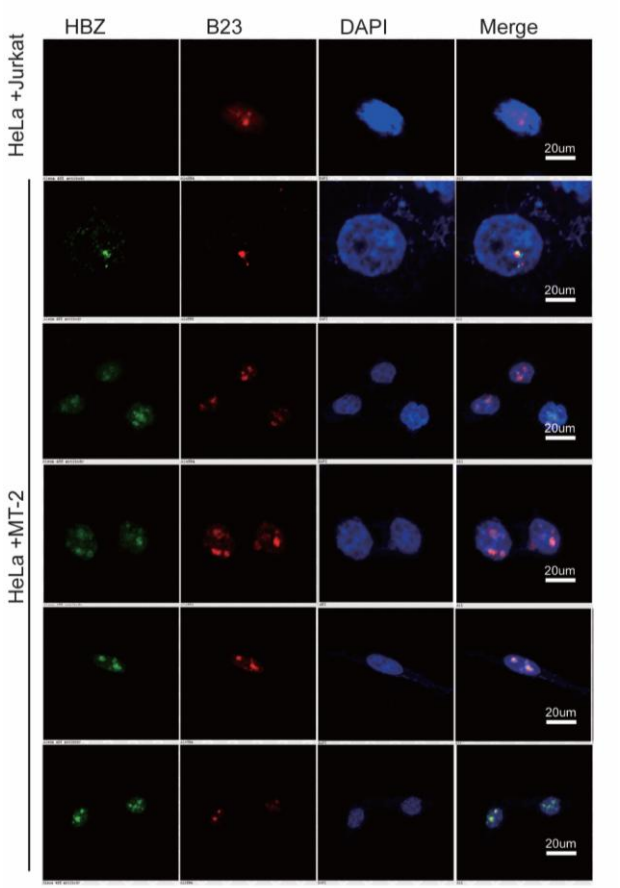
## ATL Patients (Leukemia)

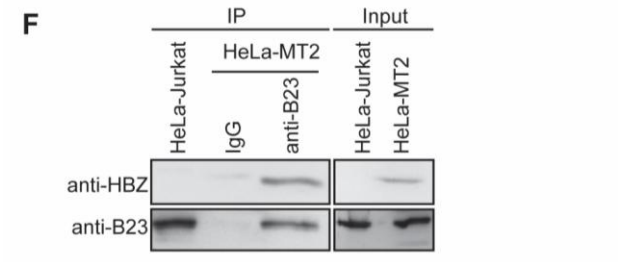


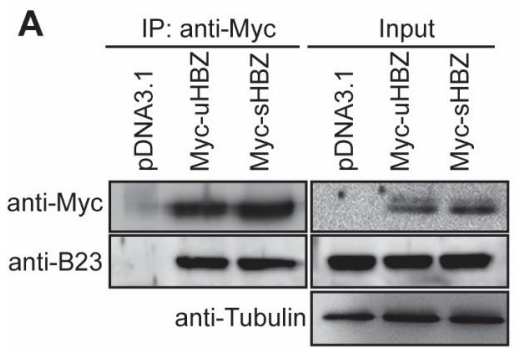

**NUCLEUS + CYTOPLASM**

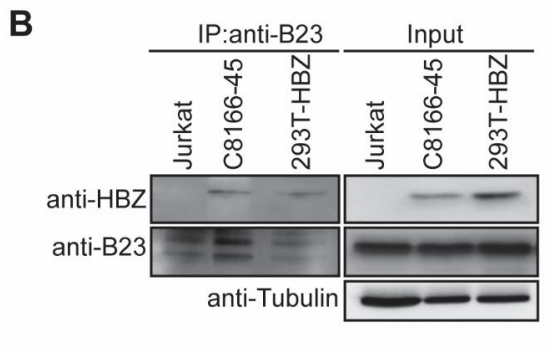
- Nuclear HBZ higher vs. carriers
- TGF-β triggers nuclear translocation

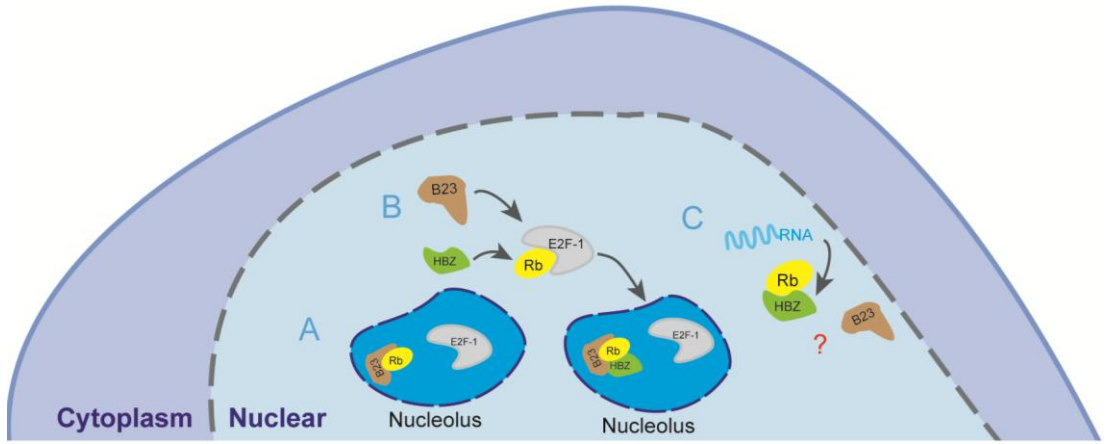
# Association of HBZ with B23 in HBZ-expressing cells




Liu et al., 2022

# Future directions

- What are the mechanisms behind the translocation of HBZ to the nucleus in ATL cells?
- How does abundance of spliced *vs.* unspliced transcript-derived isoforms compare in between clinical conditions?
- Is nucleolar localisation relevant and detectable *in vivo*?
- Implication of HBZ PTM in subcellular localisation?