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

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

Symposium 6 - ATLL (General)

Chairs: Pierluigi Porcu and Lucy Cook

Thomas Jefferson University | Drexel University | Drexel Medicine

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Glucose dynamics predict Post-Transplant Relapse in ATL, revealing broad metabolic reprogramming confirmed by multi-cohort cross-omics analysis

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KU LEUVEN

Conflicts of interest

I have no conflicts of interest

Introduction

- AlloSCT is the only curative modality for people living with Adult T-cell Lymphoma/Leukemia(ATL).
  - However, **relapse remains a primary concern and barrier to long-term survival.**
  - Transplant conditioning alters the systemic microenvironment and nutrient homeostasis.

- **Hypothesis:** ATL cells undergo unique metabolic reprogramming, allowing them to survive conditioning and drive relapse.

Infectious Dermatitis

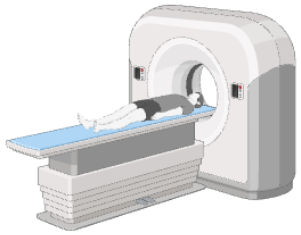
- ID is a strong risk factor for ATL in Afro-Caribbean populations[1-2].
- Flower cells are present in 30% of ID cases[3]
- ID increases clonality[4].

Our framework leverages this as an advantage to study early timeline events.

1.Gonçalves DU, Guedes AC, Carneiro-Proietti ABF, et al. HTLV-1 associated infective dermatitis may be an indolent HTLV-1 associated lymphoma. Braz J Infect Dis. 2000;4:100–102.
2.Farre L, de Oliveira Mde F, Primo J, et al. Early sequential development of infective dermatitis, human T cell lymphotropic virus type 1-associated myelopathy, and adult T cell leukemia/lymphoma. Clin Infect Dis. 2008;46:440–442.
3.Oliveira MFSP, Vieira MG, Primo J, et al. Flower cells in patients with infective dermatitis associated with HTLV-1. J Clin Virol. 2010;48:288–290.
4.Gillet NA, Cook L, Laydon DJ, et al. Strongyloidiasis and infective dermatitis alter human T lymphotropic virus-1 clonality in vivo. PLoS Pathog. 2013;9:e1003263.

Methods

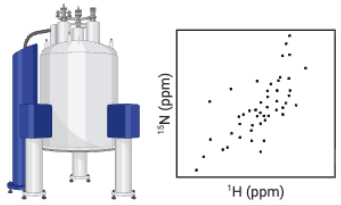
In vivo metabolic demand



FDG PET
n=10
USA

+

Metabolomics



Plasma Samples
^{1}H NMR Spectroscopy
n=64
Brazil

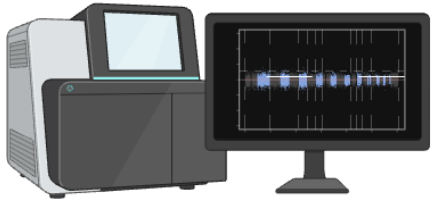
+

Transcriptomics

Multi-cohort
RNAseq and Microarray
n=138
Japan

+

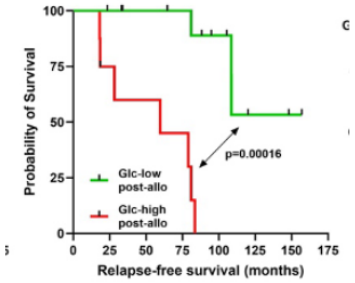
Genomics



Whole Genome Sequencing
(Mutational Burden)
n=168
USA and Japan

+

Clinical Correlation



Transplant Recipients
n=21
USA

=

Integrated Metabolic Model of ATL

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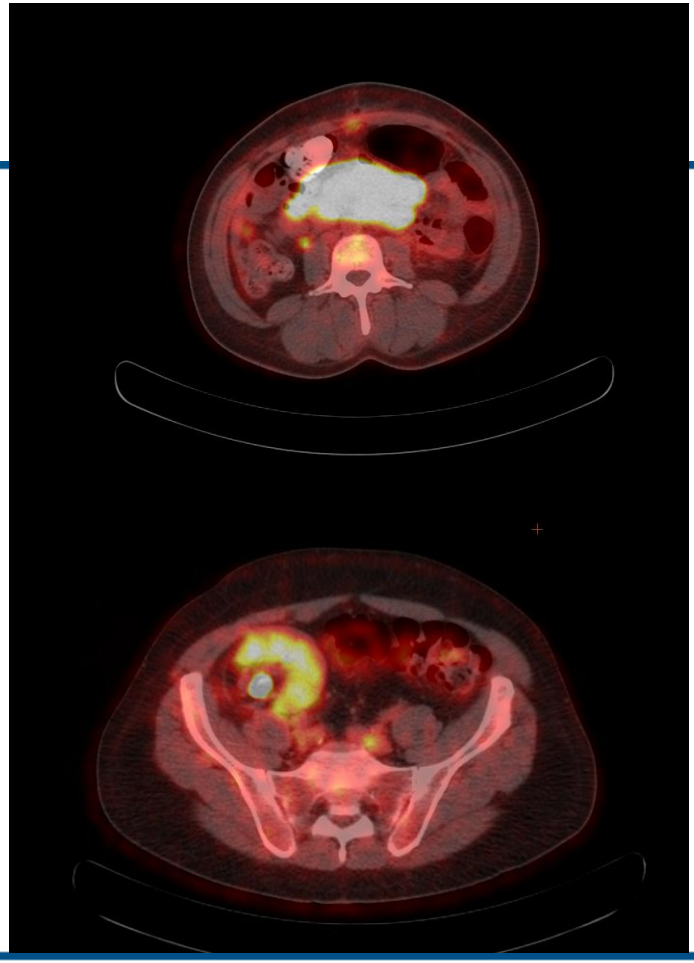
In Vivo: High Glucose Avidity

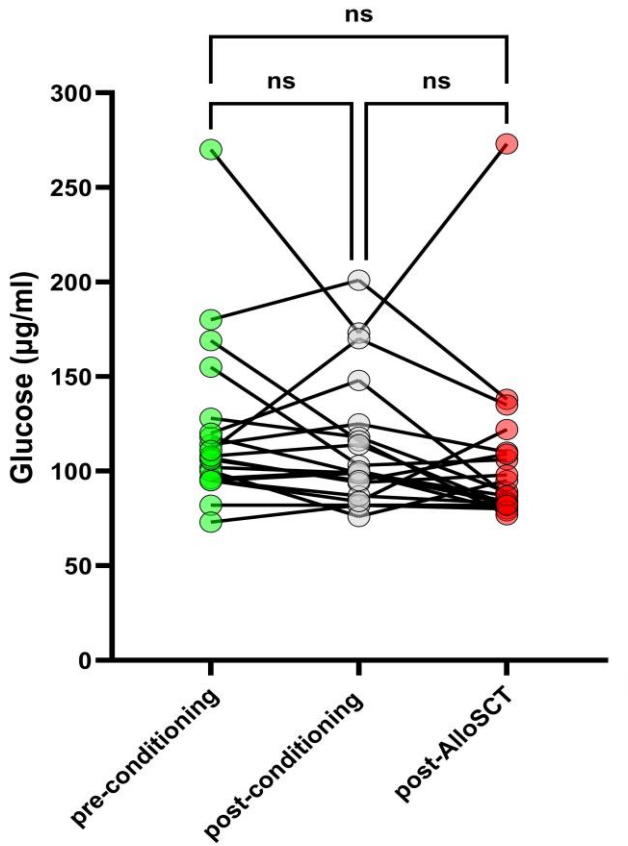
Aggressive ATL is characterized by intense glucose consumption compared to indolent variants.

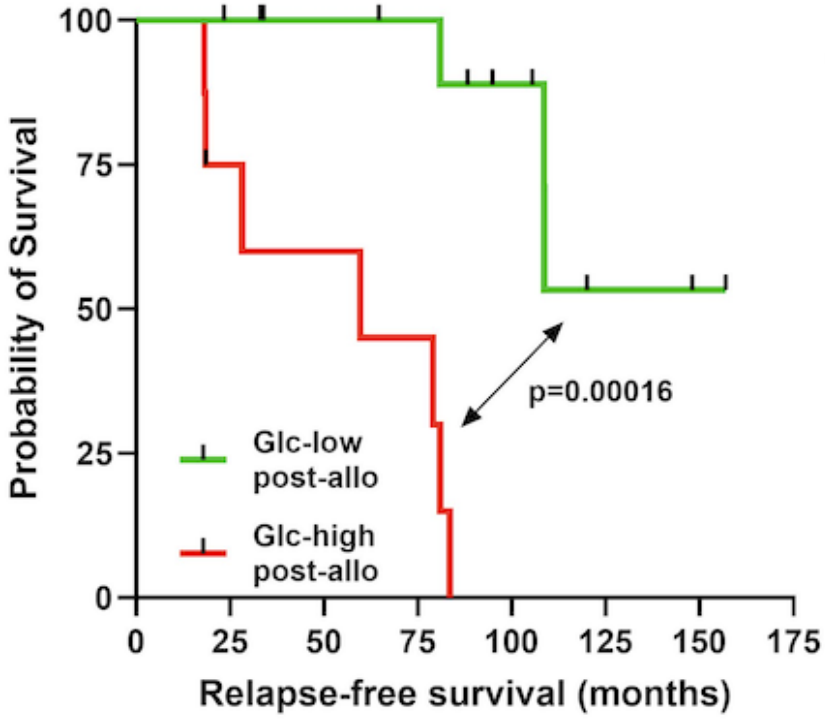
Metric	Aggressive	Indolent
Mean TLR	~5.5	~1.0
SUVmax	Up to 34.0	< 4.0
Status	Hyper-glycolytic	Iso-metabolic

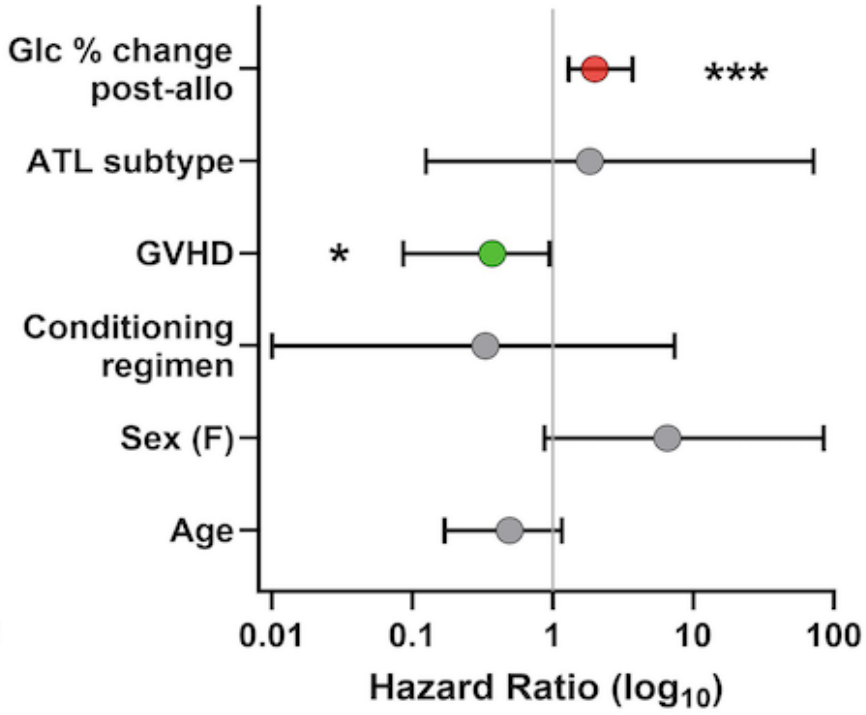


Change in Serum Glucose Predicts Relapse Free Survival in ATL

Hazard Ratio (HR) = 1.035 per unit glucose change
Independent of WBC count, LDH, or pre-existing diabetes status.







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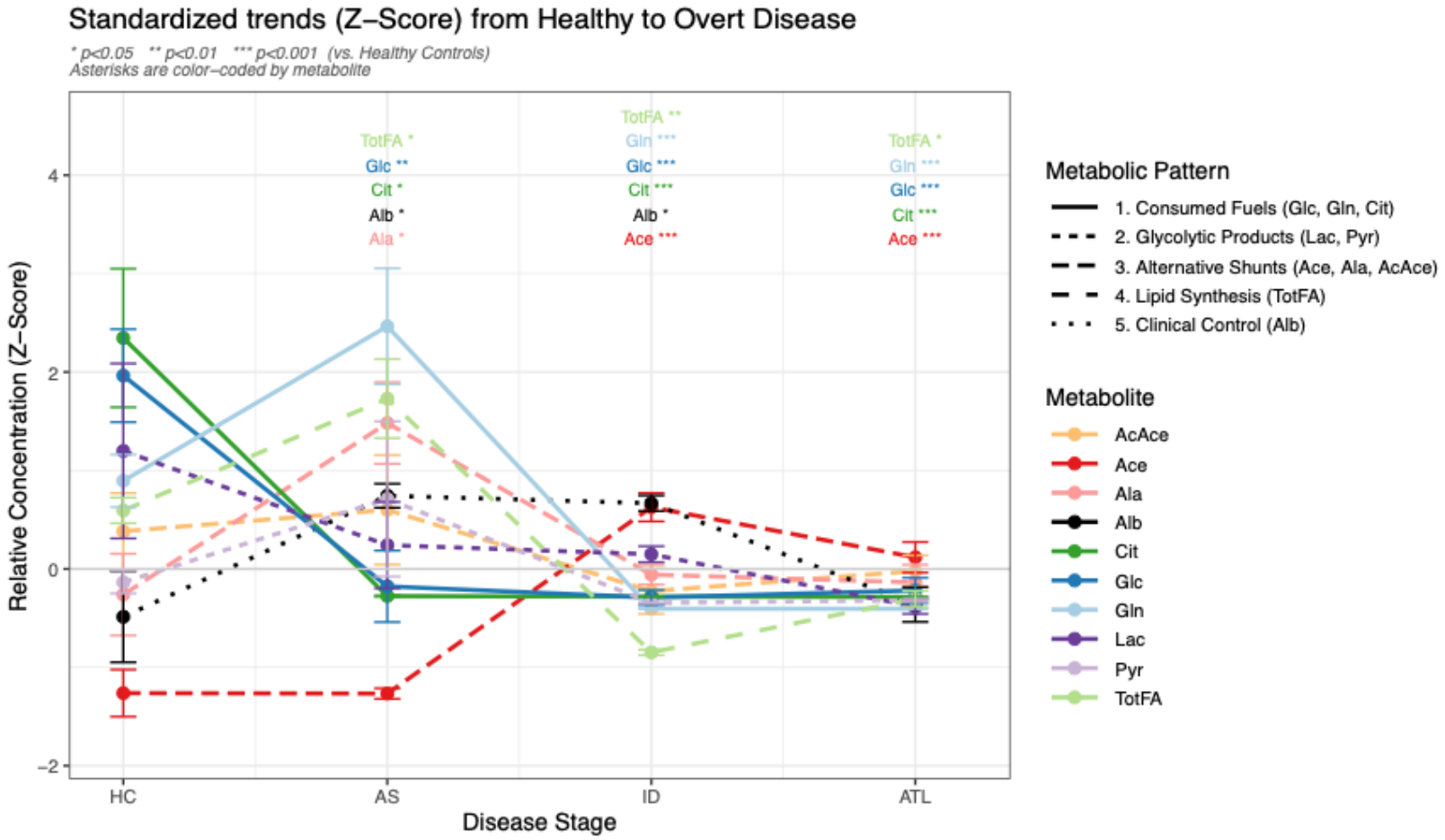
Metabolomics: Timeline of Systemic remodeling



Glucose/glutamine are depleted in ATL while acetate rises in the pre-malignant ID.
Lactate and pyruvate do not accumulate in ATL.

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Transcriptomics: Rewiring Metabolic Flux

Glycolysis

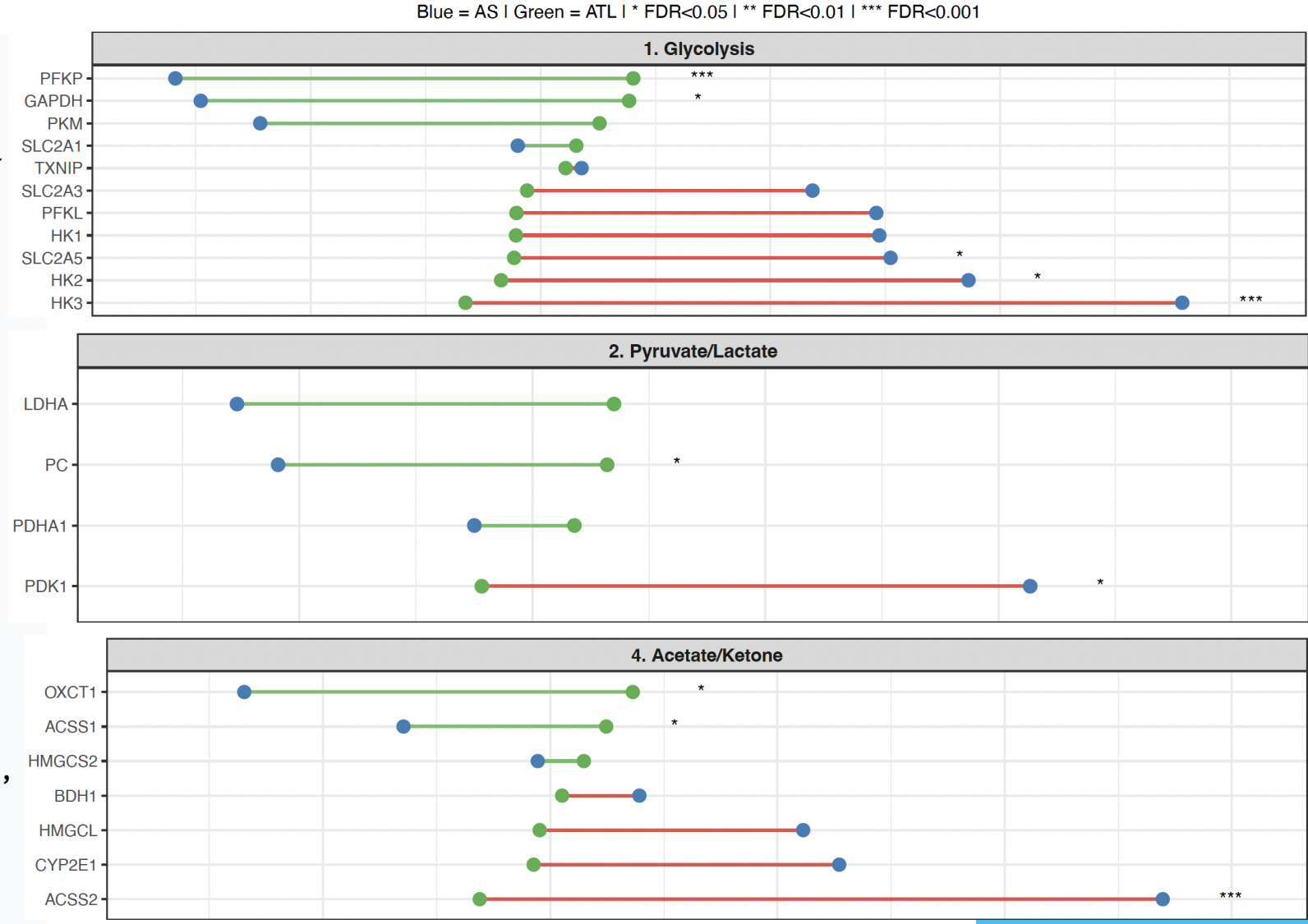
PFKP ↑, GAPDH ↑, SLC2A5 ↓, HK2/3 ↓
transcriptional suppression of glucose transporters and upregulation of select glycolytic flux enzymes

Pyruvate

PC ↑, PDK1 ↓
pyruvate is being shunted toward anaplerosis (oxaloacetate → mitochondria) rather than lactate

Acetate/Ketone

ACSS1 ↑ , OXCT1 ↑, ACSS2 ↓
routing acetate into mitochondria (ACSS1 ↑, OXCT1↑) while *blocking cytosolic lipid synthesis from acetate* (ACSS2 ↓)



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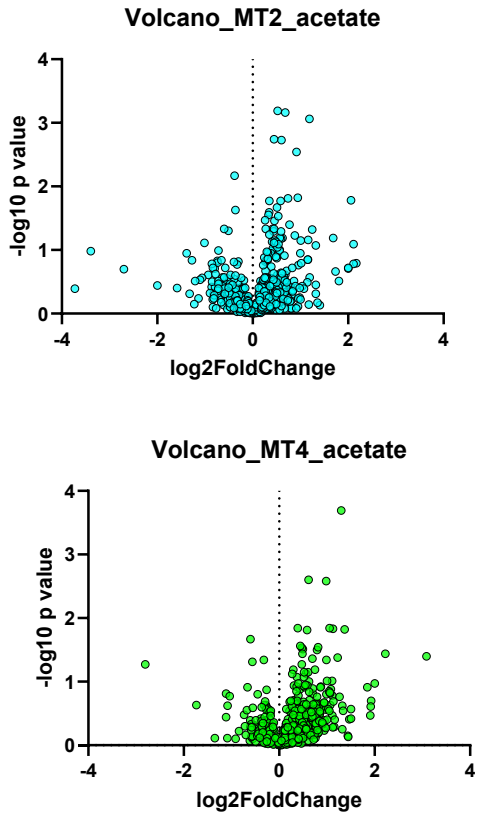
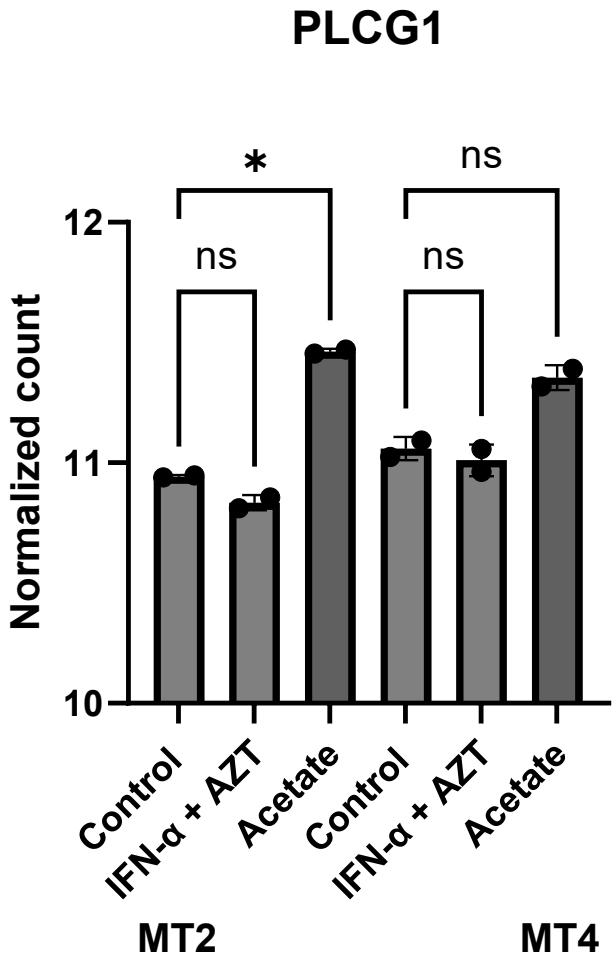
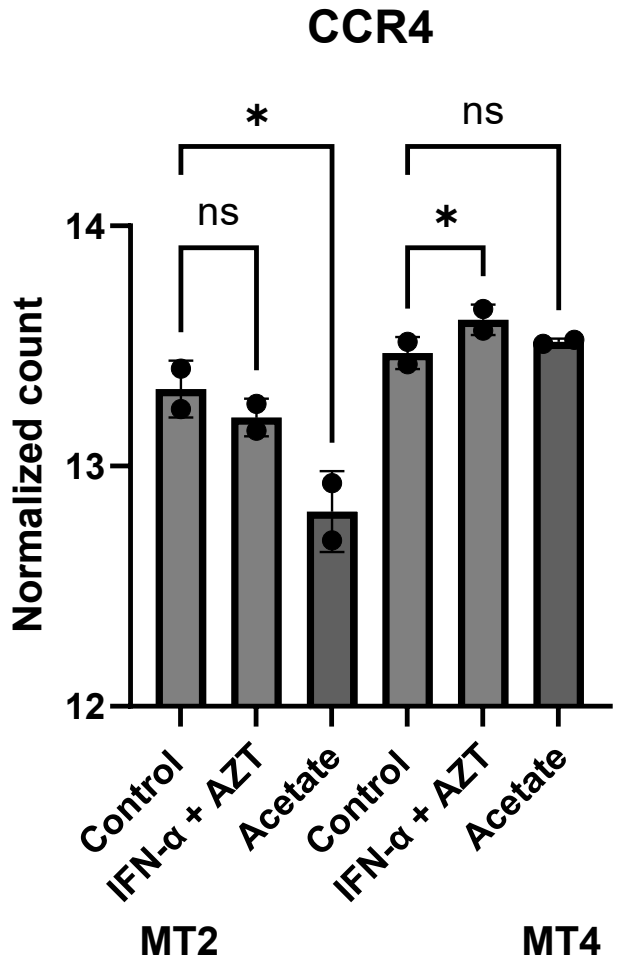
In vitro, acetate increased PLCG1 (most mutated gene) and decreases CCR4 (Moga target)



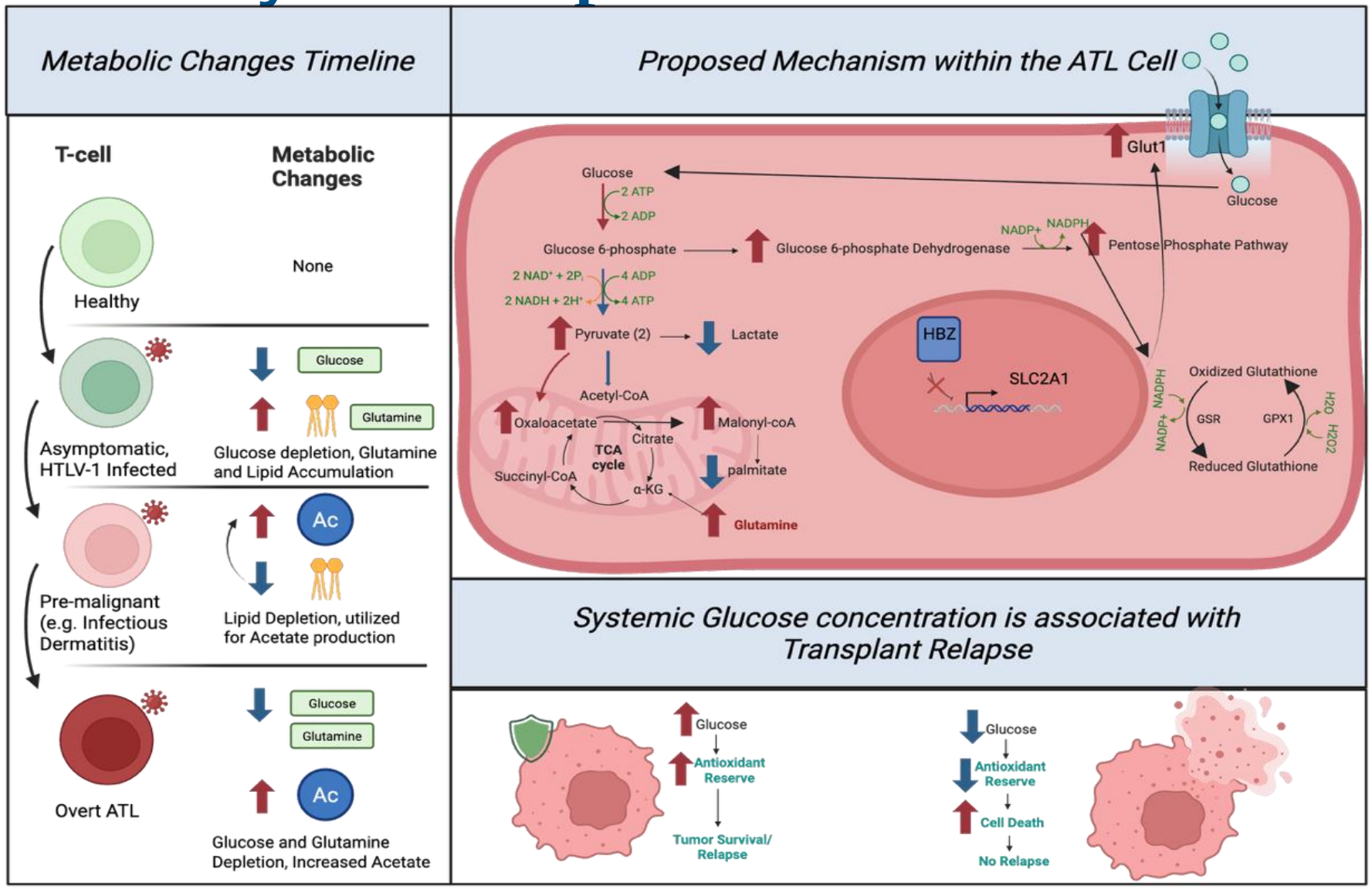




- Acetate increases cell cycle progression

- Link to histone acetylation?

Summary and Proposed Model



ATL cells that have undergone a unique metabolic reprogramming—characterized by glucose/glutamine depletion and elevated acetate levels— are potentially pre-adapted to survive nutrient instability, like that caused by AlloSCT conditioning.

histone acetylation?

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Clinical Implications

Novel Biomarker

Glucose dynamics during conditioning independently predicts post-transplant relapse.

Systemic Insight

ATL involves complex remodeling (lipid scavenging/acetate use).

Targeting Shunts

Targeting metabolic shunts (PPP, Acetate) may sensitize ATL to conditioning and improve RFS.

Thank You

Acknowledgments
Prof. Anne-Mieke Vandamme & Dr. Johan Van Weyenbergh
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Montefiore Medical Center Research Team
Collaborators in Brazil and Japan

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Ethics: UZ Leuven #S57931

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Towards the validation of the Viral Clonality Evenness (VCE) index: a novel prognostic biomarker for adult T-cell leukemia

Anne-Sophie Reuter
GIGA, University of Liège, Belgium

Thomas Jefferson University | Drexel University | Drexel Medicine

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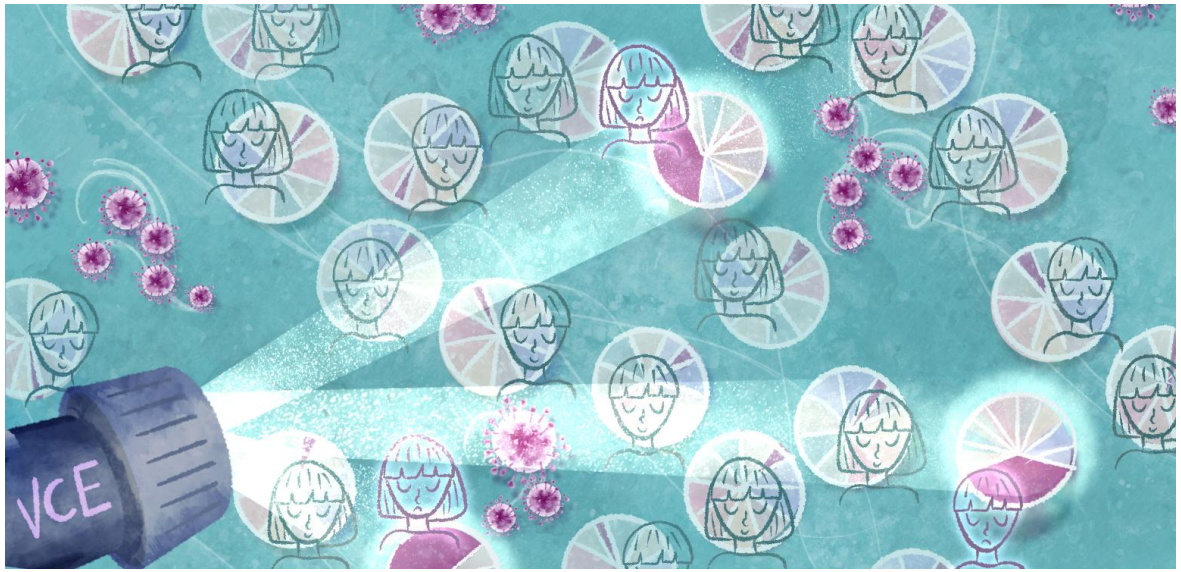


LIÈGE université
GIGA
Medical Genomics

Anne-Sophie Reuter

Unit of Animal Genomics
GIGA Research Institute
University of Liège, Belgium

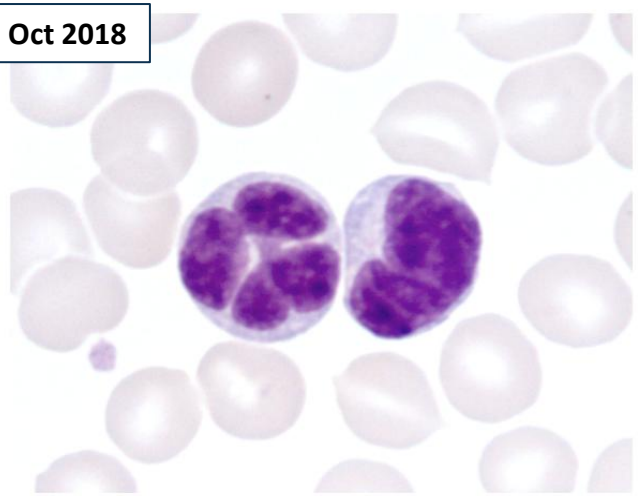
Conflicts of interest

I have no conflicts of interest

HTLV-1: RAISING AWARENESS …..

Nature Medicine Oct 2018

news feature



Adult T cell leukemia cells. Credit: Masao Matsuoka

Back in the spotlight

A long-neglected virus returns to the top of researchers' to-do lists.

Amanda B. Keener

THE LANCET

2018

CORRESPONDENCE | VOLUME 391, ISSUE 10133, P1893-1894, MAY 12, 2018

Time to eradicate HTLV-1: an open letter to WHO

Fabiola Martin • Yutaka Tagaya • Robert Gallo

Published: May 12, 2018 • DOI: https://doi.org/10.1016/S0140-6736(18)30974-7

The truth about HTLV-1: unravelling 30 years of misconceptions

GVN

GLOBAL VIRUS NETWORK

Global Virus Network Issues Open Letter to WHO On Potent Human Carcinogen - Human T Cell Leukemia Virus-1

The Lancet publishes letter from top scientists and advocates from around the world to eradicate HTLV-1, a debilitating and deadly virus

The Guardian 24 Apr 2018

Indigenous investigations

'People are scared': the fight against a deadly virus no one has heard of

WHO announces the development of new recommendations on HTLV-1

Oct 2025

World Health Organization

HTLV-1: RAISING AWARENESS …..

Predictive biomarkers ?

Human T-cell Leukemia Virus 1

~10-20 million infected individuals

Lifetime risk of ATL developement ~ 2-7 %

Asymptomatic infection ~ 20-60 years

↓

Need for risk stratification classifier

news feature

Nature Medicine Oct 2018

Back in the spotlight

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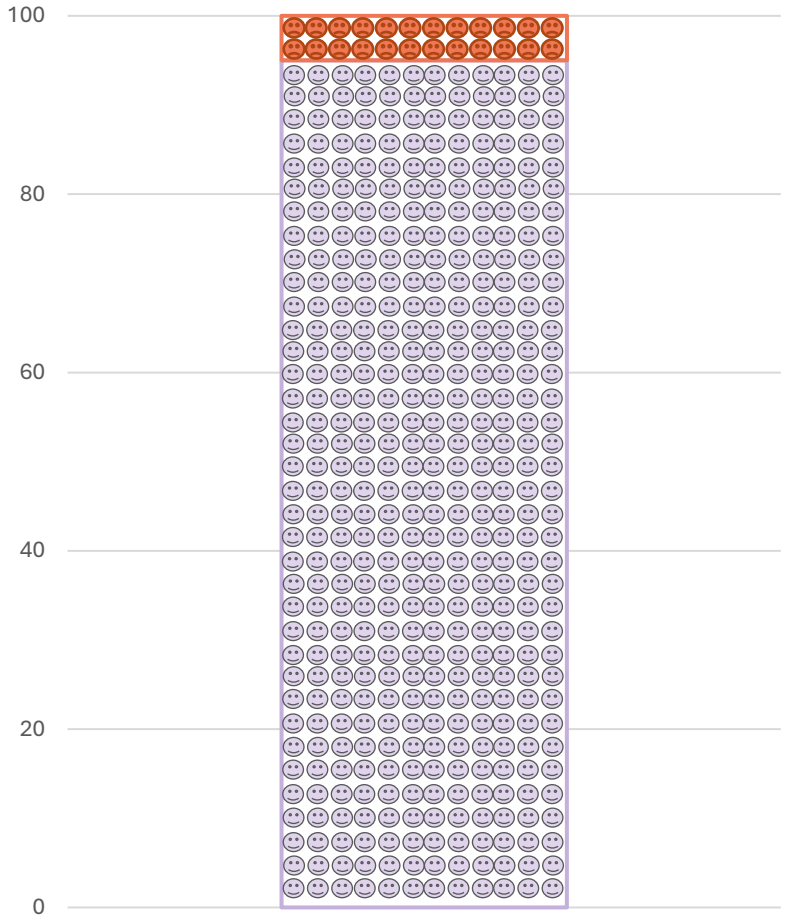
World Health Organization

Predictive biomarker to identify carriers at high risk of progression to ATL ?

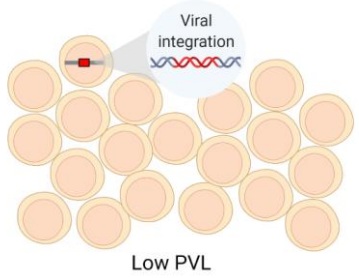
Low risk
High risk
Non-progressor
Progressor

Ideal Biomarker

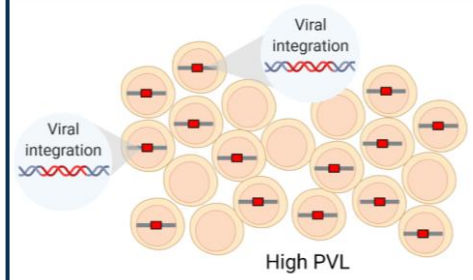


Current biomarker: PVL

= HTLV-1 copies/100 PBMCs

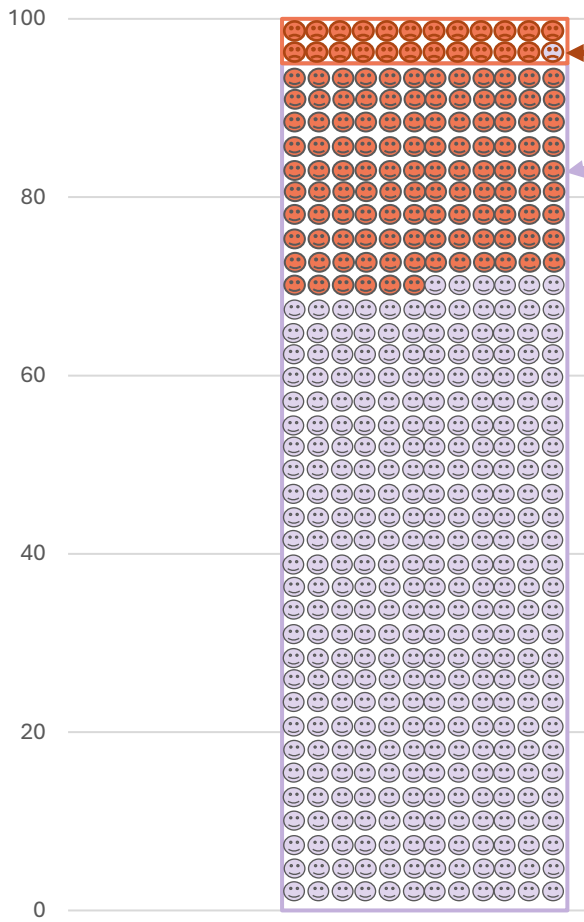


(<4 PVL) = **LR - low risk**



(≥4 PVL) = **HR - high risk**
HR = 25 %

PVL



False negatives
~ 0.1%

False positives

80% high-PVL HR carriers
= 20% all carriers
= 220,000 individuals in Japan!

Unwarranted anxiety

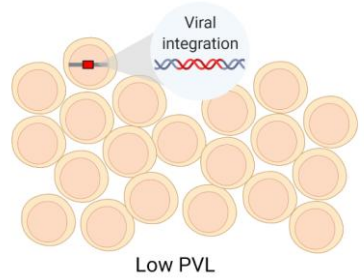
➡ **Need for better predictive biomarker**

Iwanaga, *et al*. *Blood* 2010

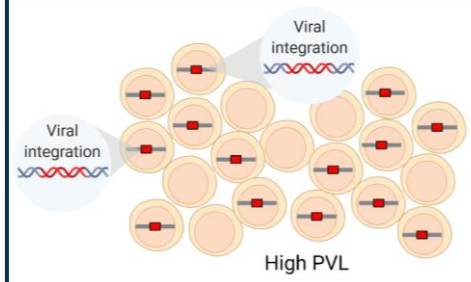
Predictive biomarker beyond PVL

Current biomarker: PVL

= HTLV-1 copies/100 PBMCs



(<4 PVL) = **LR - low risk**



(≥4 PVL) = **HR - high risk**
HR = 25 %

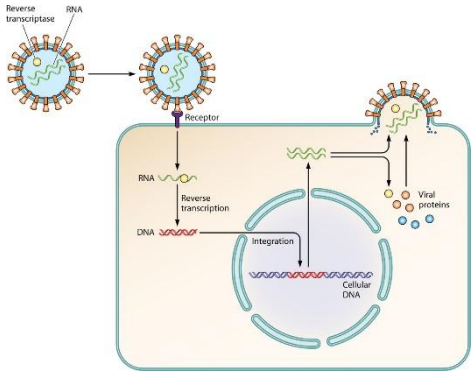
Iwanaga, *et al*. *Blood* 2010

Identification **clonally expanded** cells in the blood of HTLV-1 asymptomatic carriers : **pre-malignant clone**

- Southern blot, low throughput
  Imaizumi et al. Blood 2005
- HTLV-1 Clonality NGS (Linker-mediated PCR) Tag-NGS
  Firouzi et al. Blood Adv 2017
- CD4 $^{+}$ CADM1 $^{+}$ cell percentage (HAS-flow)
  Makiyama et al. Cancer Science 2019
- TCR clonality (OCI-flow)
  Wolf et al. Blood Cancer J 2021
- RAISING - Rapid amplification of IS without contamination of genomic DNA
  Wada et al. Comm Biology, 2022
- Mutational signatures
  Rowan et al. Blood 2020

Method - Proviral integration landscape

Provirus = integrated viral DNA



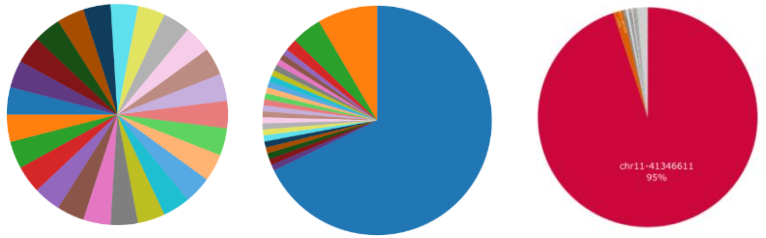
Linker-mediated PCR

NGS

sequencing virus-host junctions
5'/3' Integration Site (IS)

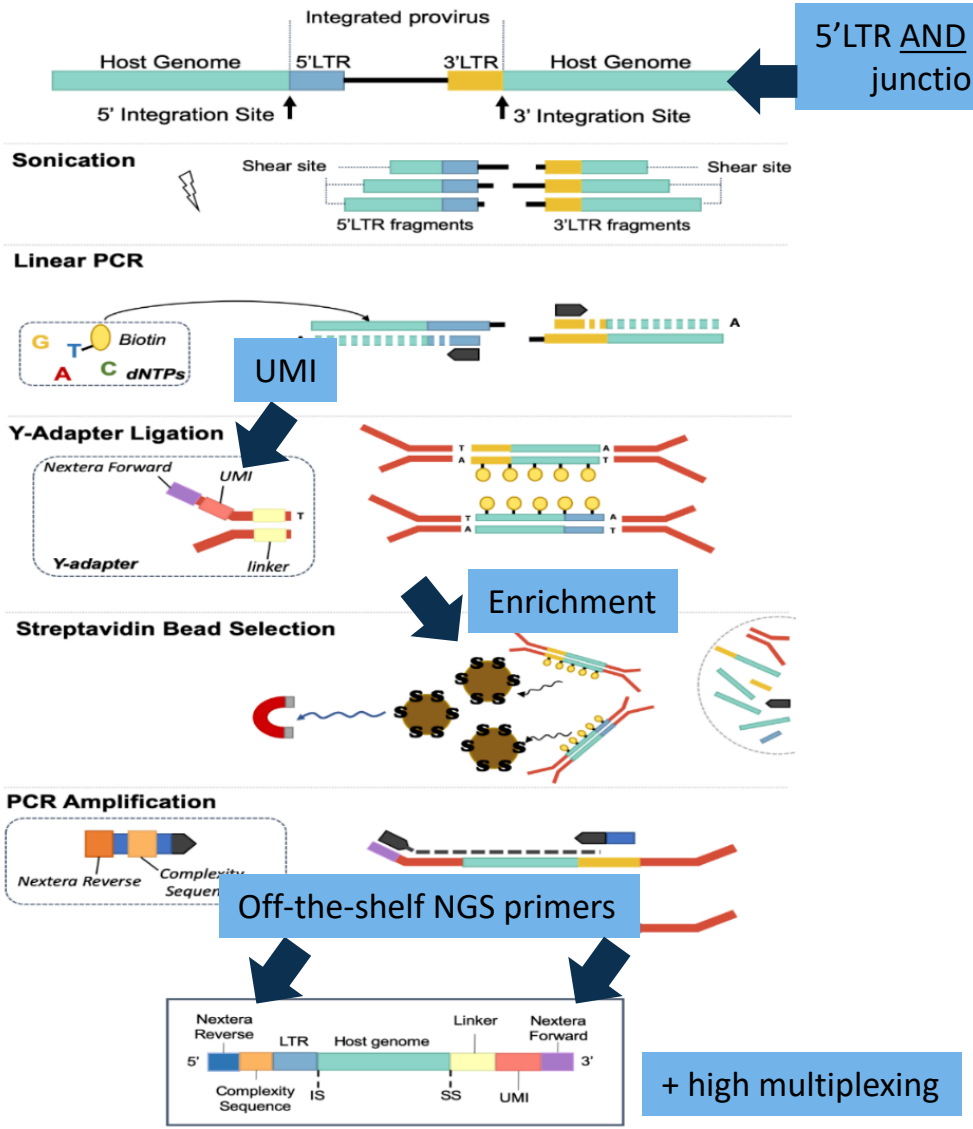
POSITION ← IS + UMI → **ABUNDANCE**

Identifying proviral integration sites genome-wide
Measuring abundance of corresponding clones



Rosewick *et al,* Nat Comm 2017
Artesi *et al,* Leukemia 2017
Rosewick *et al,* Front Microbiol 2020
Marçais *et al,* Leukemia 2021

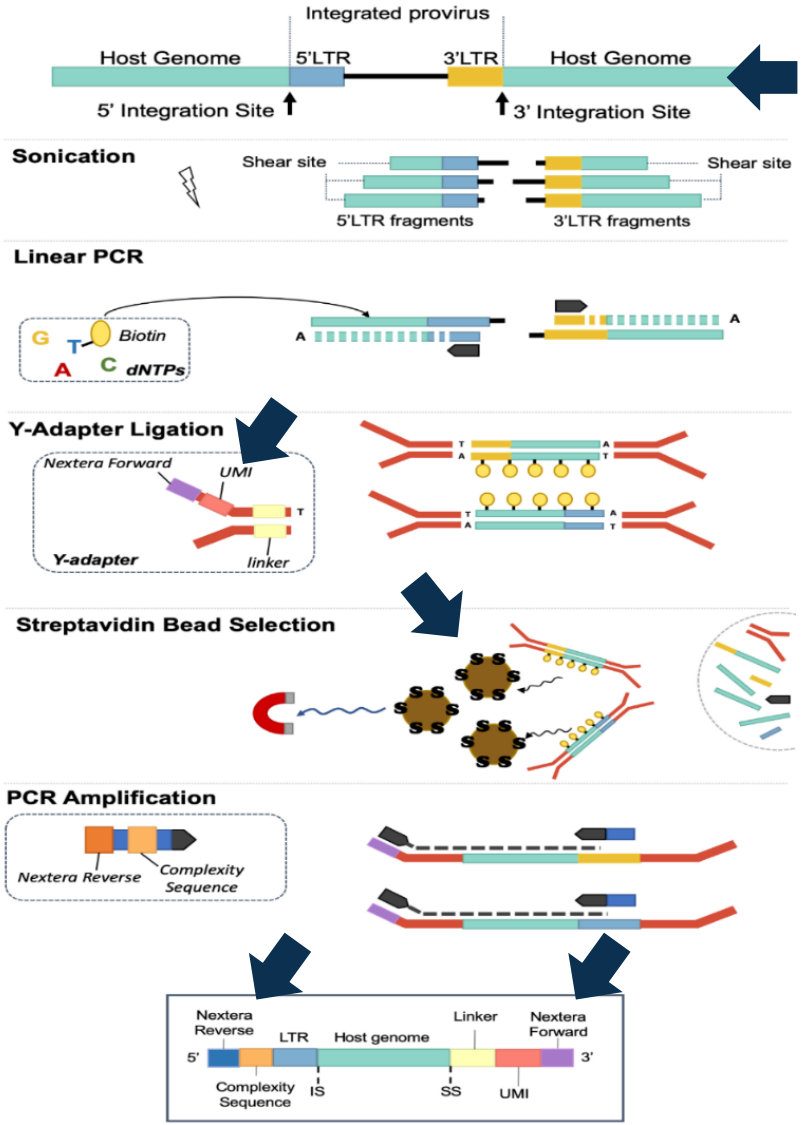
Method - NGS clonality method reveals proviral clonal architecture



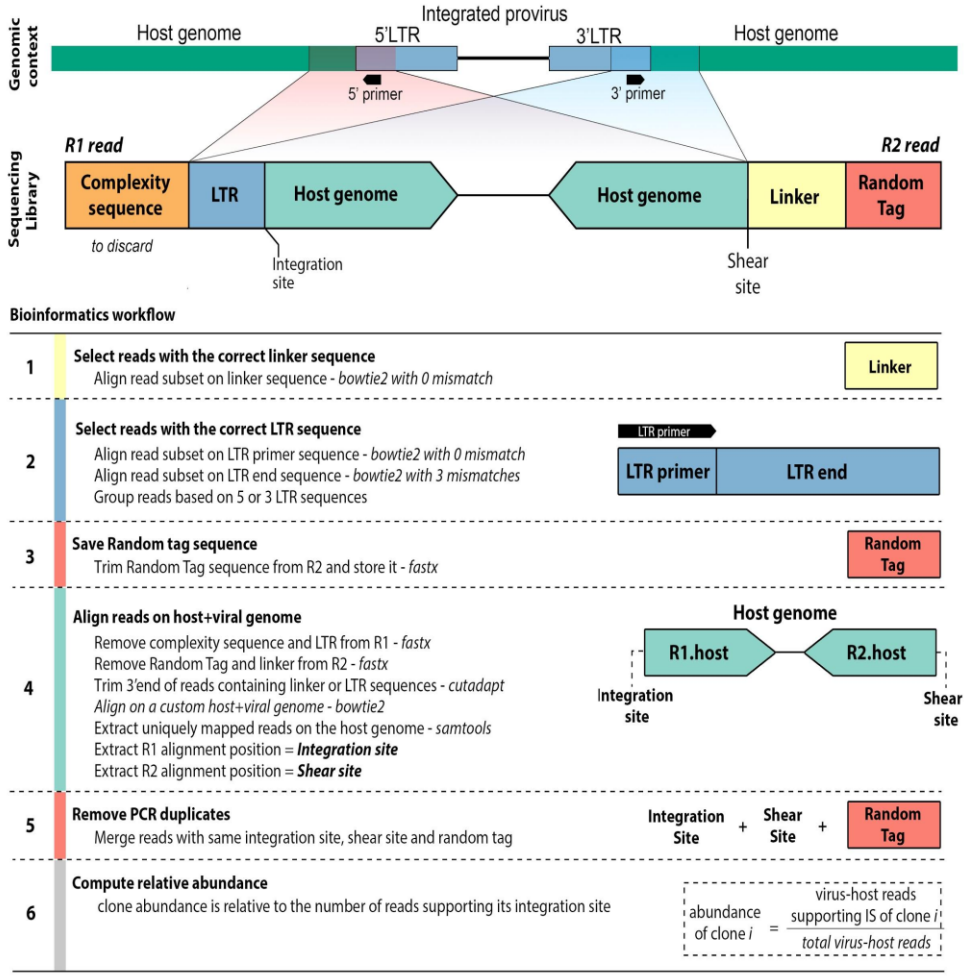
HTLV-1 clonality sequencing method

Sensitivity +++
High-throughput (Illumina)
Multiplexing samples
Cost effective
Only DNA required

Method - NGS clonality method reveals proviral clonal architecture



Optimized HTLV-1 clonality sequencing method



https://github.com/ GIGA-AnimalGenomics-BLV/Public/tree/master/PIC

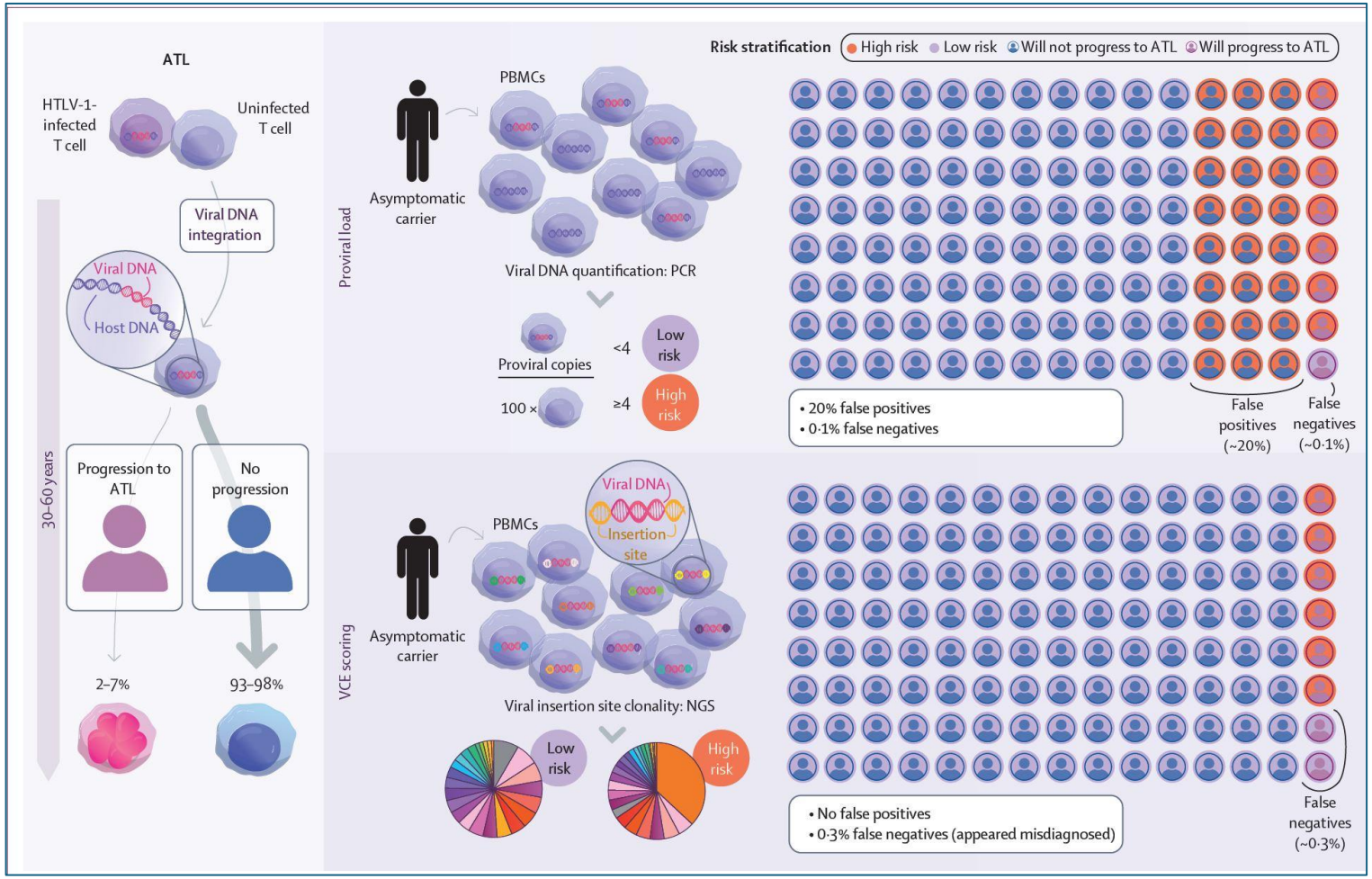
Bioinformatics pipeline

Rosewick *et al*. *Nat Comm* 2017,8:15264
Rosewick *et al*, *Front Microbiol* 2020,11:587306

A viral clonality evenness score to predict progression to adult T-cell leukaemia in asymptomatic carriers of human T-lymphotropic virus type 1 in Japan: a retrospective longitudinal cohort study

Snehal Dilip Karpe, Maria Artesi, Jérôme Wayet, Keith Durkin, Vincent Hahaut, Kaoru Uchimaru, Junya Makiyama, Masako Iwanaga, Atae Utsunomiya, P Martijn Kolijn, Anton W Langerak, Arsène Burny, Ambroise Marçais, Olivier Hermine, Toshiki Watanabe, Michel Georges, Anne Van den Broeke

Karpe et al, *The Lancet Microbe, 2025*



1. HTLV-1 clonality NGS and VCE: a prognostic biomarker for ATL

Discovery cohort - JSPFAD

2. Towards the validation of VCE

- Follow-up study: longitudinal validation in the extended JSPFAD discovery cohort
- Impact of LTR polymorphisms on VCE to generate an “universal” biomarker
- Evaluation of VCE as a therapeutic response marker in an ongoing ATL clinical trial

3. Viral clonality NGS to address fundamental questions

JSPFAD

Joint Study on Predisposing Factors of ATL Development

https://htlv1.jp/jspfad/

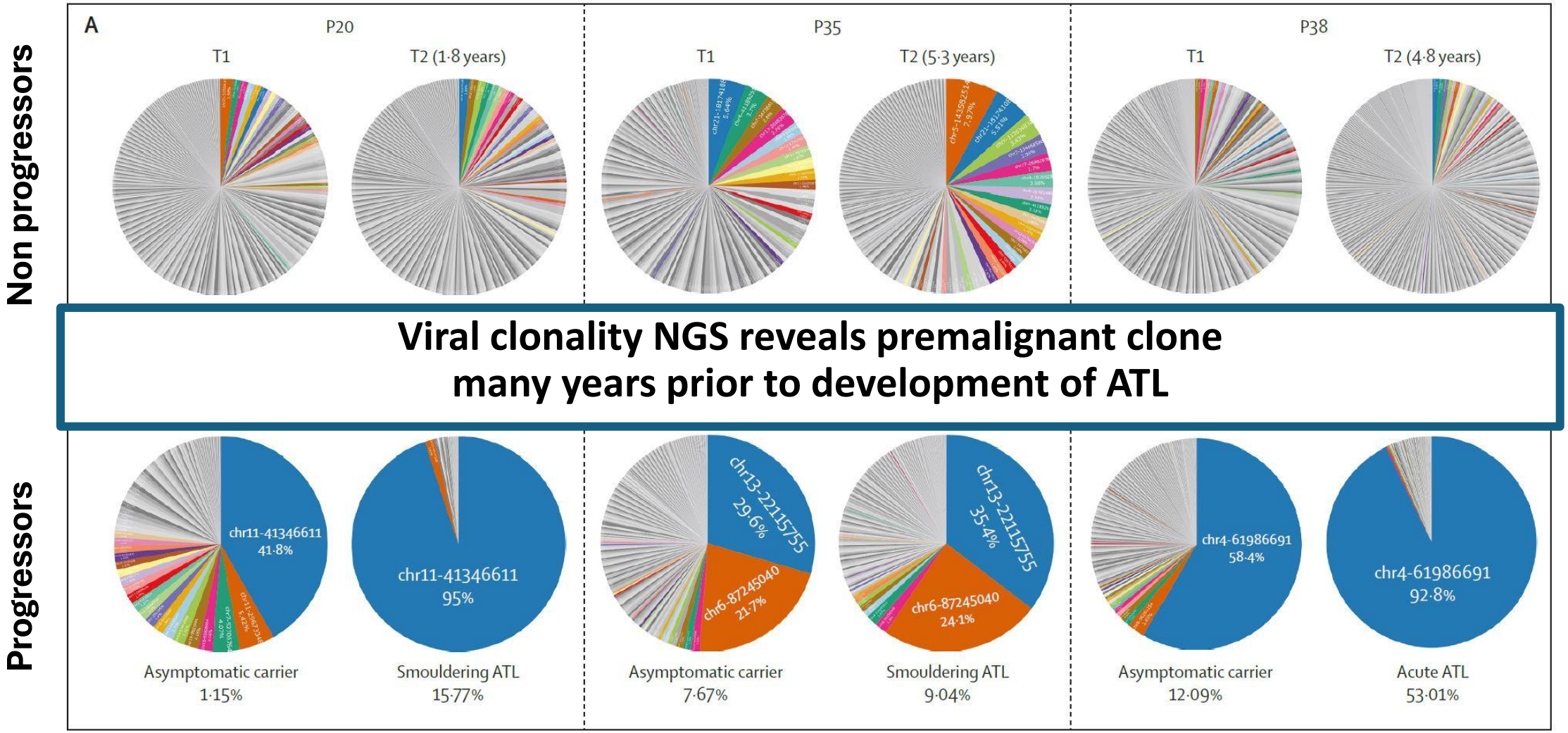
Yoshi Yamano
Toshiki Watanabe
Kaoru Uchimaru
Junya Makyjama
Masako Iwanaga
Atae Utsunomiya

JSPFAD: a unique nation-wide cohort study in Japan

- >4,000 asymptomatic HTLV-1 carriers enrolled
- Since 2002
- Annual medical assessment
- Annual sample collection -> **unique longitudinal biobank**
- ATL + matching retrospective AC samples
- ATL progressors = 1.9 %

Iwanaga *et al, Blood 2010*
Iwanaga et al, Front Microb 2020

Cohort study - HTLV-1 Clonality NGS

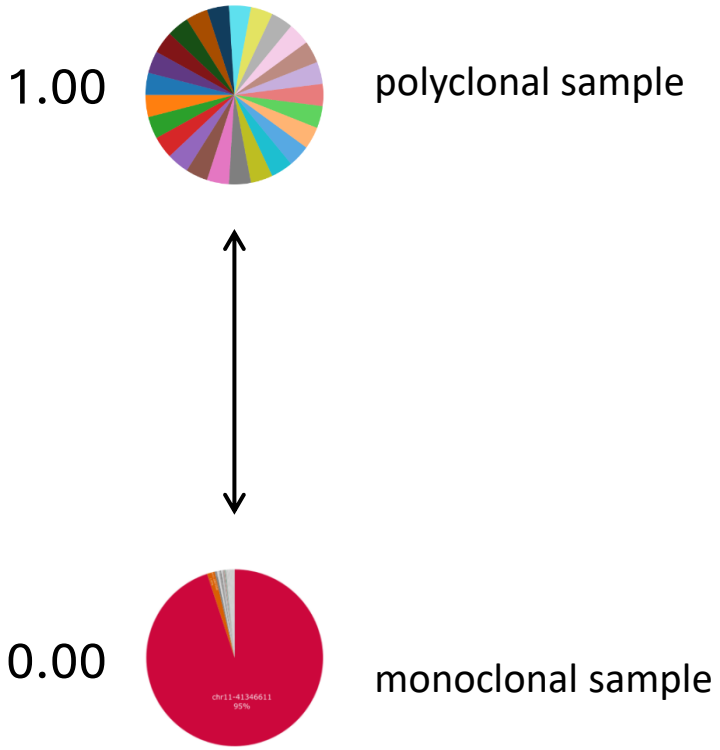


Viral clonality NGS reveals premalignant clone many years prior to development of ATL

Clonality metrics : Viral Clonality Eveness (VCE)

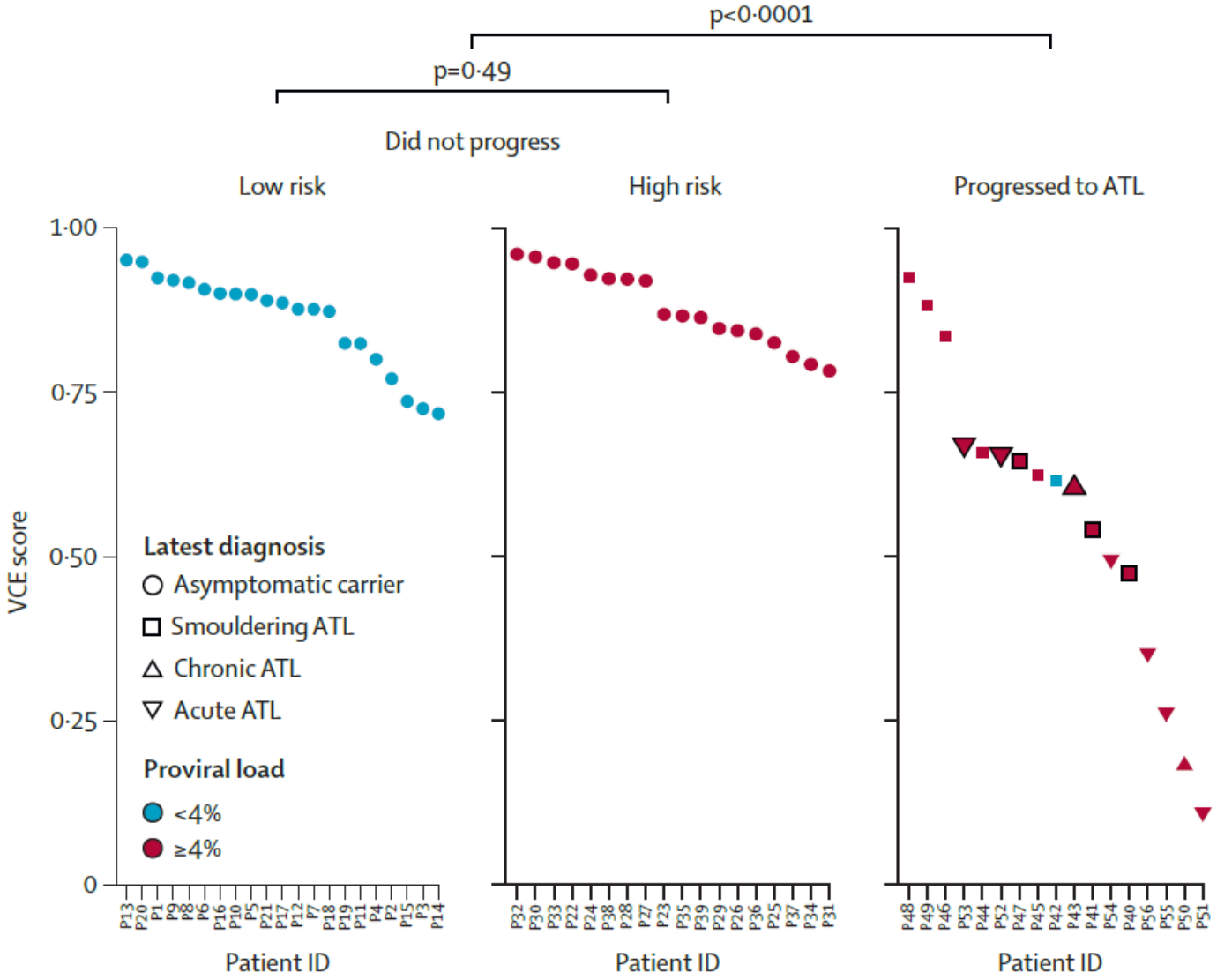
VCE = Shannon Evenness Index (SEI)

VCE closer to 1 = Polyclonal sample
VCE closer to 0 = Monoclonal sample



Clonality metrics : Viral Clonality Eveness (VCE)

VCE at T1 = asymptomatic carrier



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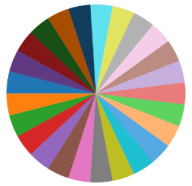
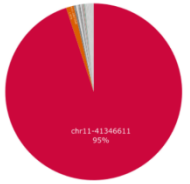


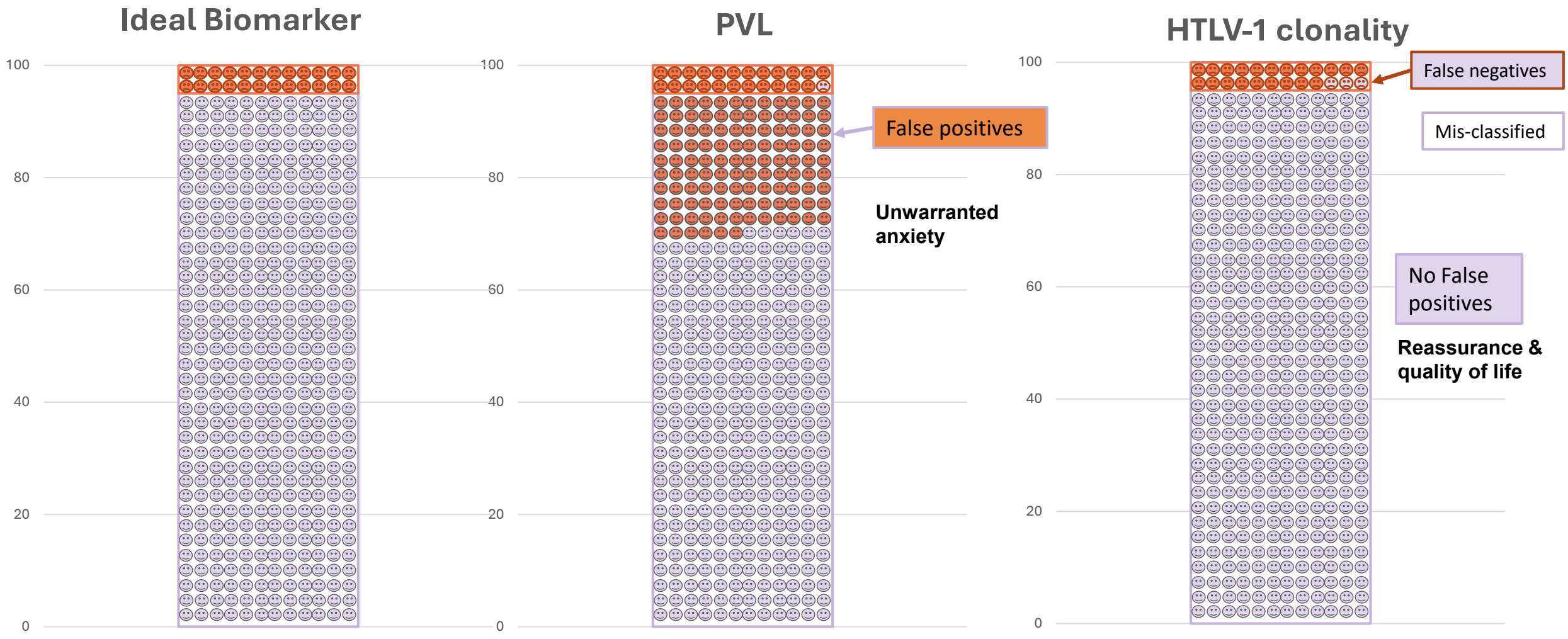
VCE -> 1 = polyclonal sample

0.00



VCE -> 0 = monoclonal sample

Predictive biomarker to identify carriers at high risk of progression to ATL ?



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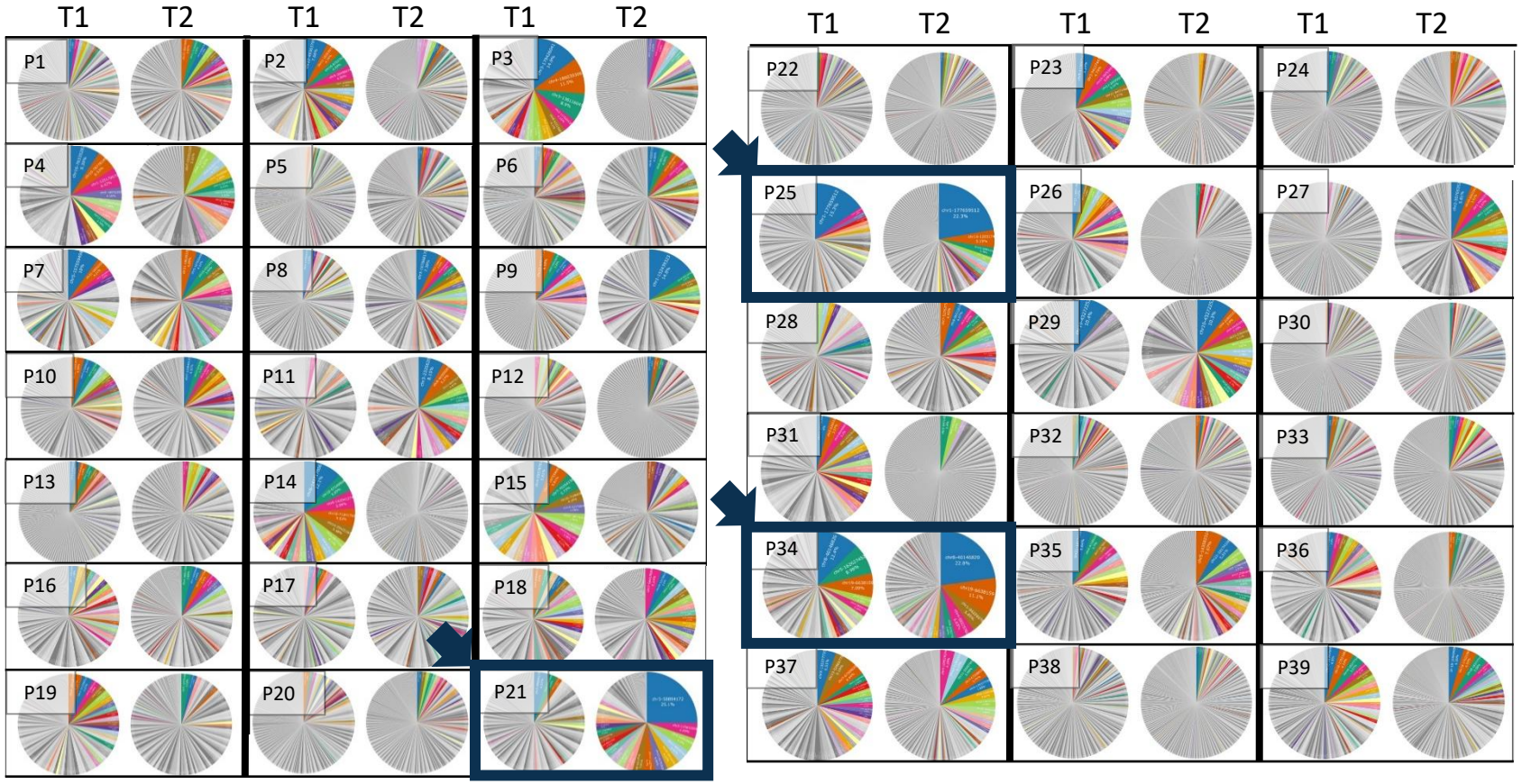
3. Viral clonality NGS to address fundamental questions

HTLV-1 Clonality NGS

Non-progressors



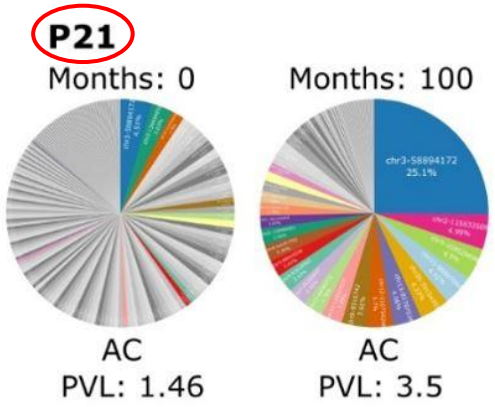
< 4 PVL, Low Risk

≥ 4 PVL, High Risk

VCE as predictive marker

Asymptomatic carriers with VCE < 0.69 at T2 progressed to ATL

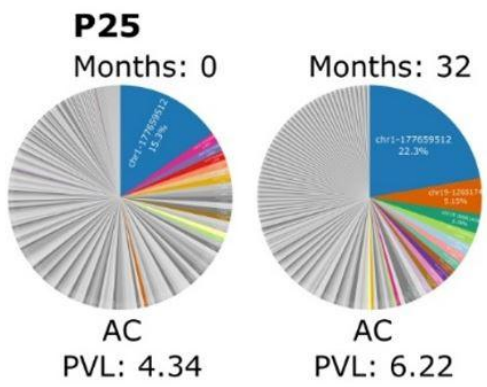
P21



P21	T1-T2
PVL	1.46-3.5
% clone	4.4-21.1
VCE	0.89 - **0.66**

-> **developed chr-ATL**

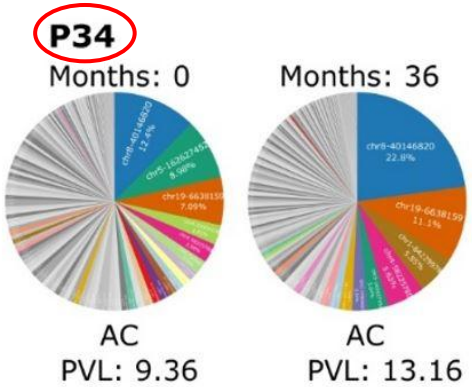
P25



P25	T1-T2
PVL	4.34-6.22
% clone	15.3-22.1
VCE	0.83 - 0.81

-> lost to follow-up

P34

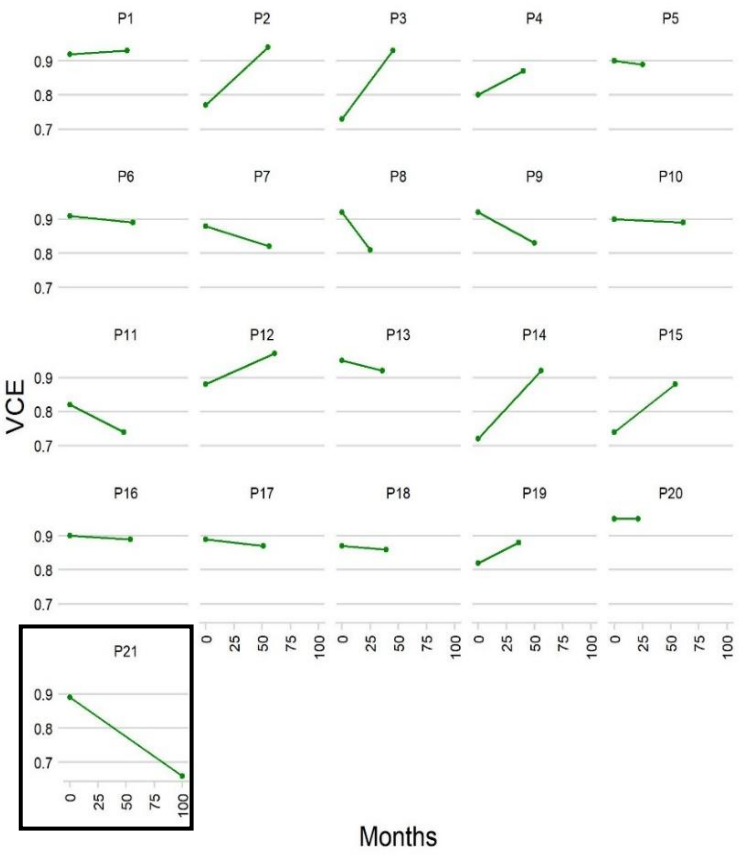


P34	T1-T2
PVL	9.36-13.16
% clone	12.4-22.8
VCE	0.79 - **0.68**

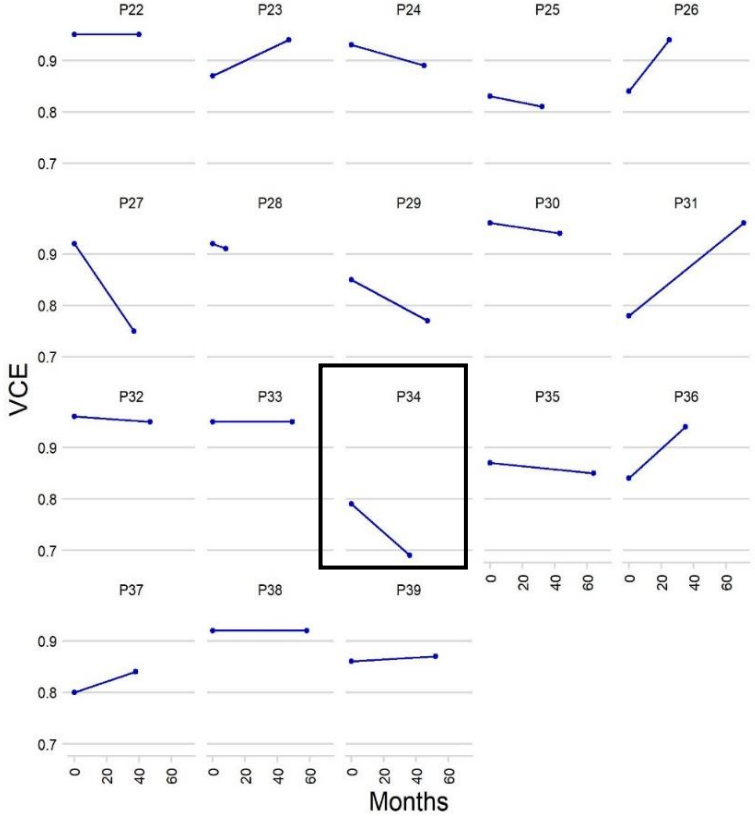
-> **developed SM-ATL -> acute ATL**

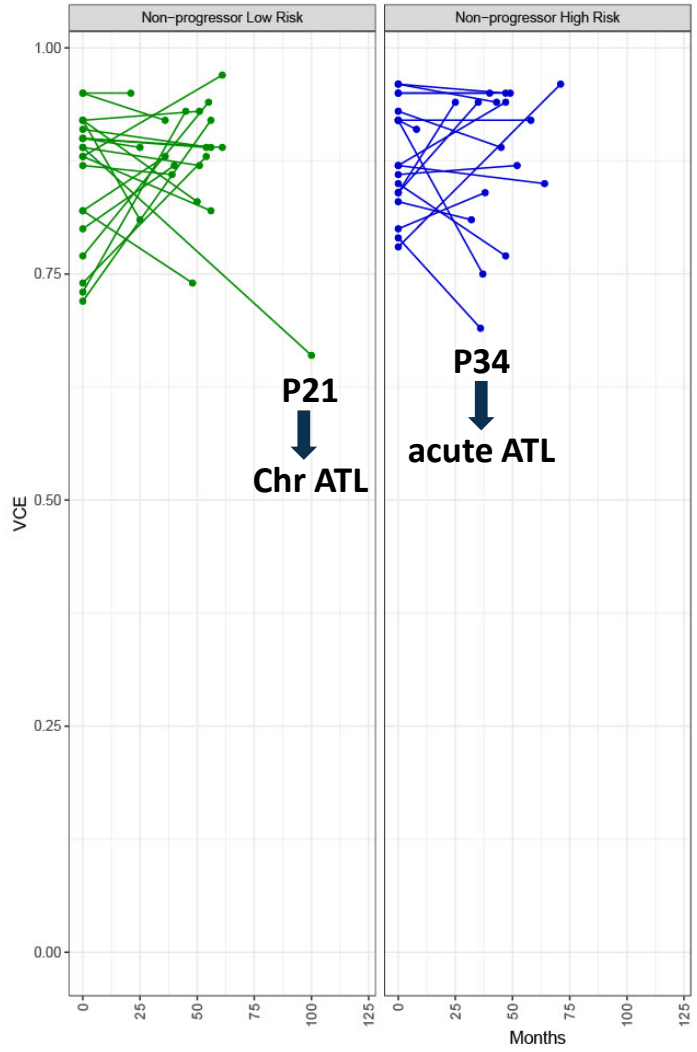
Assess the temporal dynamics of VCE scoring to predict progression

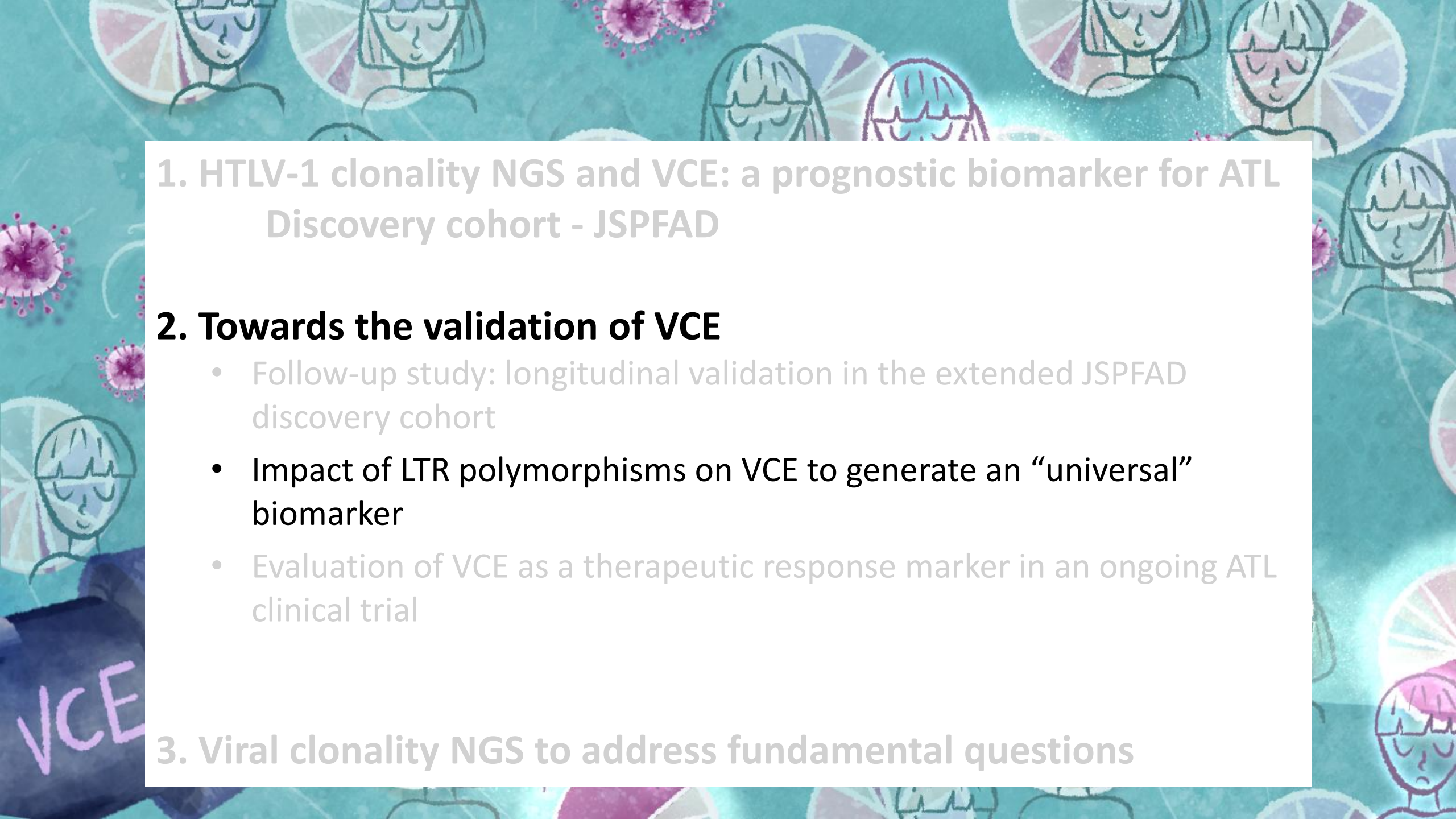
Non progressor Low Risk



Non progressor High Risk







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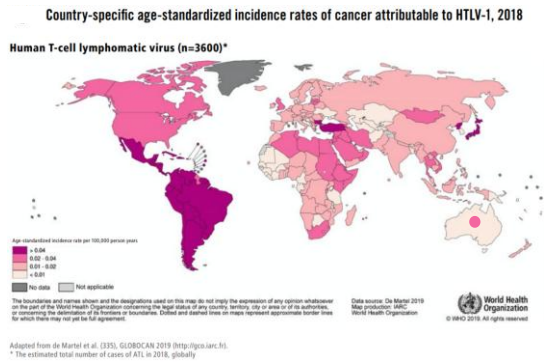
Polymorphisms in LTR sequences



HTLV-1a Japan consensus

TGACAATGACCATGAGCCCCAAATATCCC

HTLV-1a Peru consensus

TGACAATGACCATGAGCCCCAAATATCCC

HTLV-1c consensus

TGATACTGACCATGGGCCCCAAATACCTT

HTLV-1a Japan consensus

GACAGCCCATCCTATAGCACTCT–CAGGAGAGAAATTTAGTACACA

HTLV-1a Peru consensus

GACAGCCCATTCTATAGCACTCTCCAGGAGAGAAACGTAGTACACA

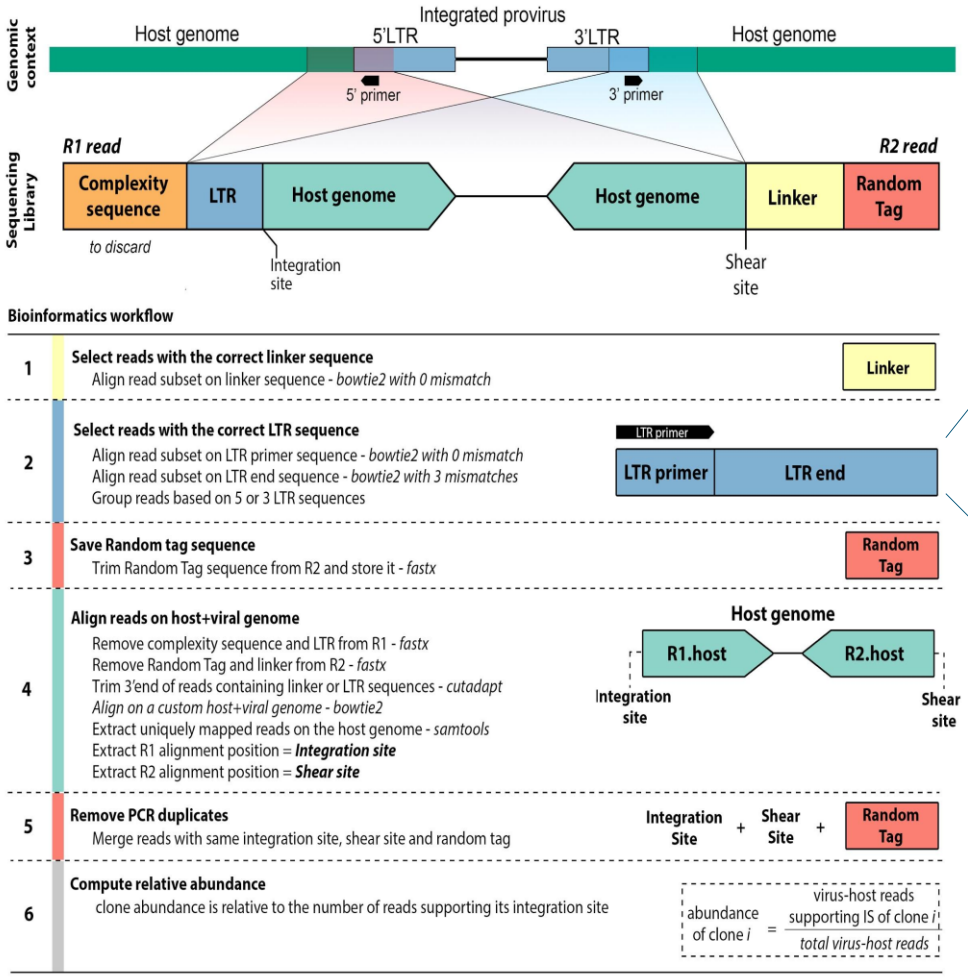
HTLV-1c consensus

GACAGCCCATTCTATACCTCTCTCCAGGAGAGAGACATAGAACACA

Daniel Enriquez-Vera, Kagoshima University, Instituto de Medicina Tropical, Lima
Hirons et al, 2024

Multiple primers

Evaluating the assets of using multiple LTR reference for mapping

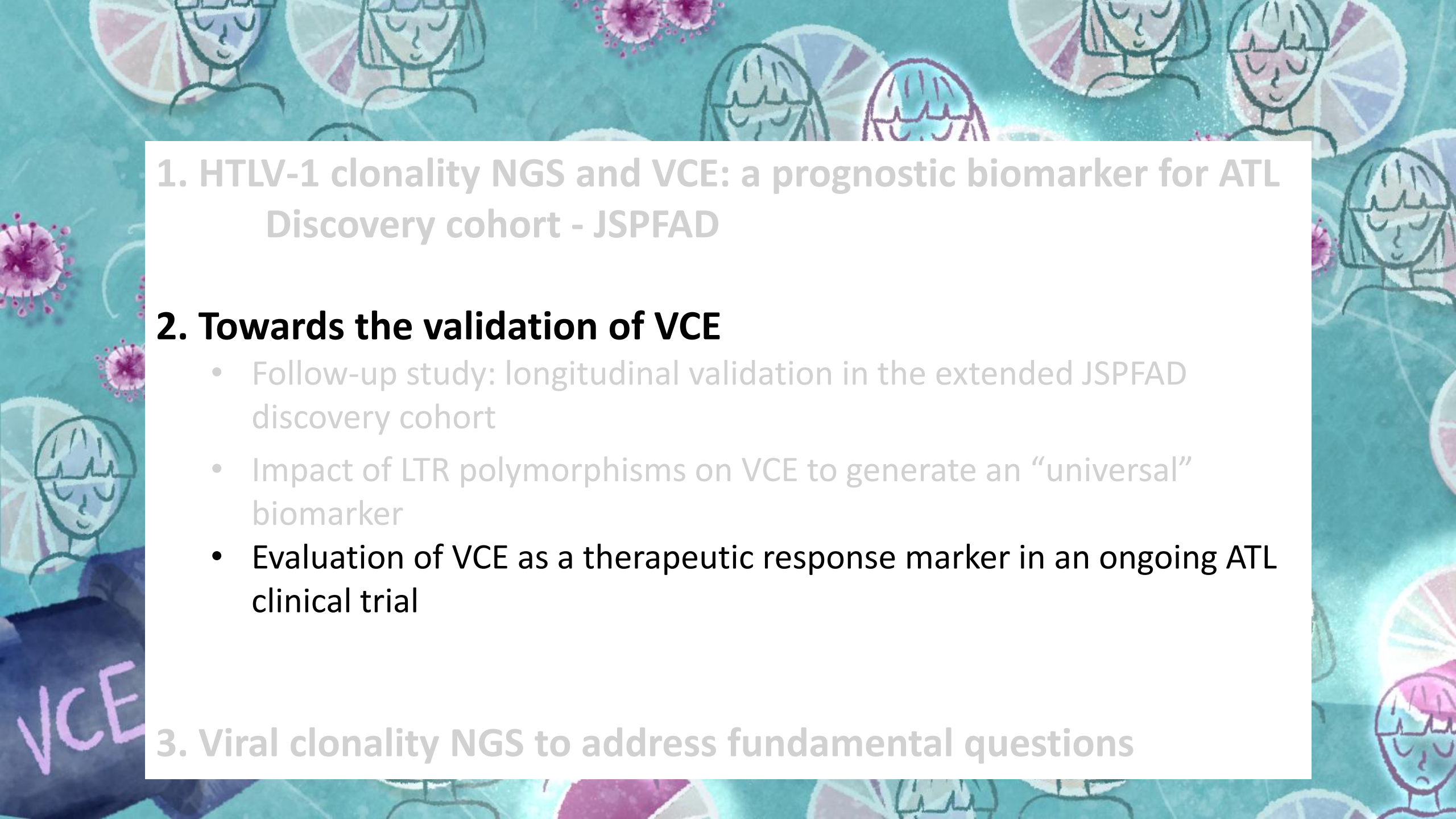


Bioinformatic pipeline
multiple LTR references

>LTR3_HTLV1a_Japan_consensus
GACAGCCCATCCTATAGCACTCTCAGGAGAGAAATTTAGTACACA
>LTR3_HTLV1a_C25
GACAGCCCATCCTATAGCACTCTCCAGGAGAGAAATTTAGTACACA
>LTR3_Peru_consensus
GACAGCCCATTCTATAGCACTCTCCAGGAGAGAAACGTAGTACACA
>LTR3_HTLV1c
GACAGCCCATTCTATACCTCTCTCCAGGAGAGAGACATAGAACACA
>LTR5_HTLV1a_Japan_consensus
TGACAATGACCATGAGCCCCAAATATCCC
>LTR5_HTLV1c
TGATACTGACCATGGGCCCCAAATACCTT

Mapping LTR on multiple variant references
→ Unchanged number of reads and IS detected
→ Unchanged relative abundance of IS

Towards an 'universal' method



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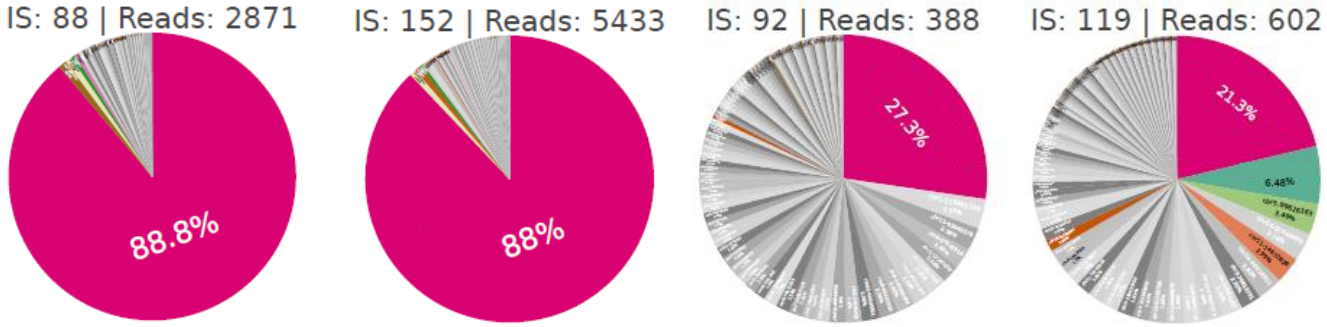
3. Viral clonality NGS to address fundamental questions

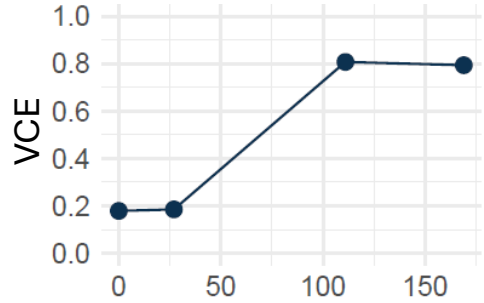
Assessing therapeutic response using VCE

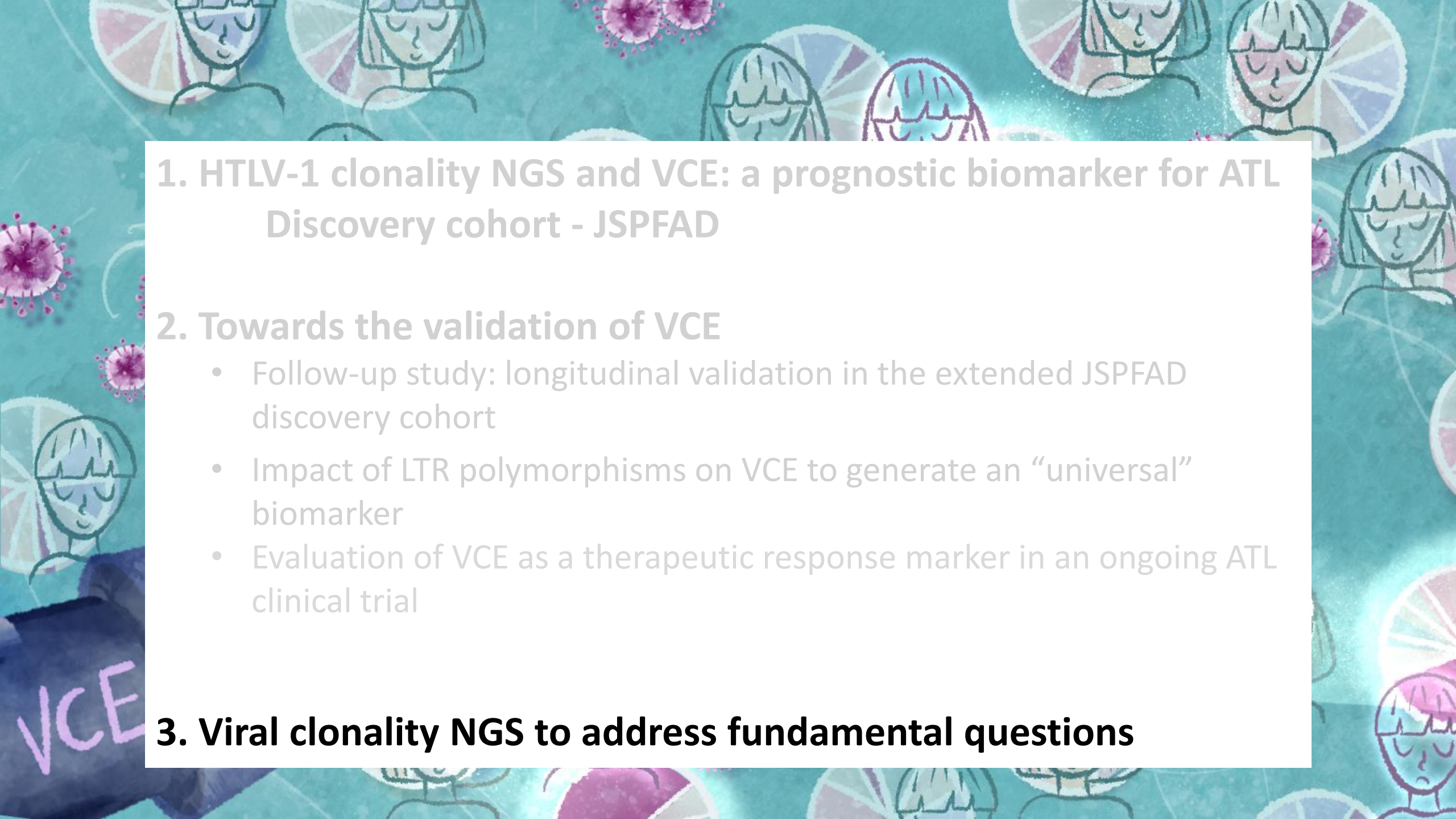
ATL therapeutic clinical trial (*Dr JC Ramos, University of Miami*)
AI-BEL (AZT/Interferon-α in combination with Belinostat) for HTLV-1 related adult T-cell leukemia-lymphoma
PVL and TCR assessment for disease evolution

VCE score as therapeutic response marker
Longitudinal PBMC samples from patients
Tacking evolution and dynamics of malignant dominant clones and VCE score



Time





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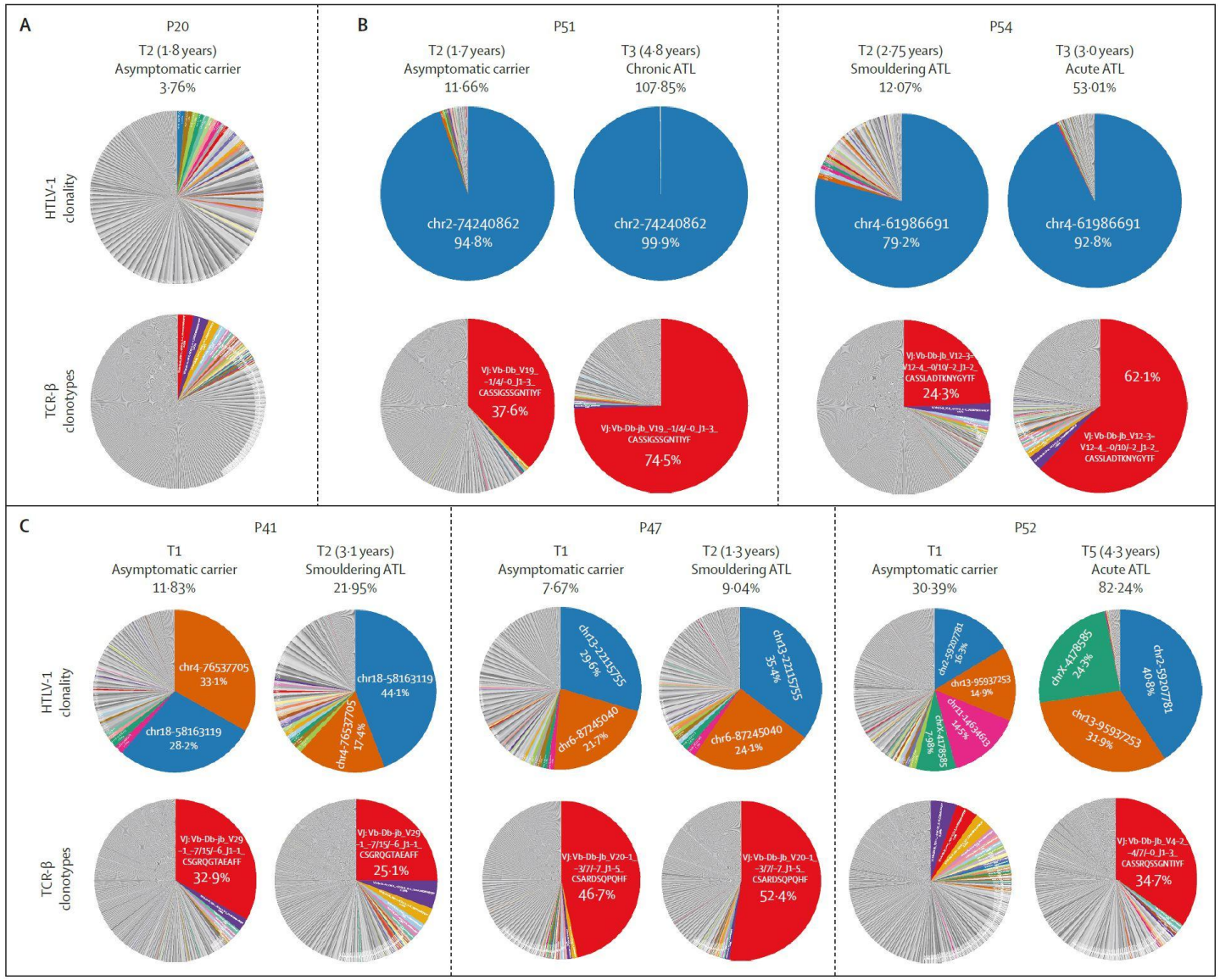
3. Viral clonality NGS to address fundamental questions

ATL cases with multiple dominant integrations in a single T-cell clone



HTLV-1 clonality

TCR-β clonotypes

HTLV-1 clonality

TCR-β clonotypes

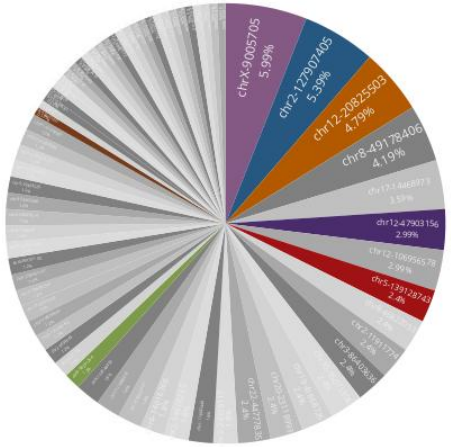
Karpe et al, *The Lancet Microbe, 2025*

VCE is defined by both LTR5 AND LTR3 junctions

P54
ATL-Acute
PVL 59 %

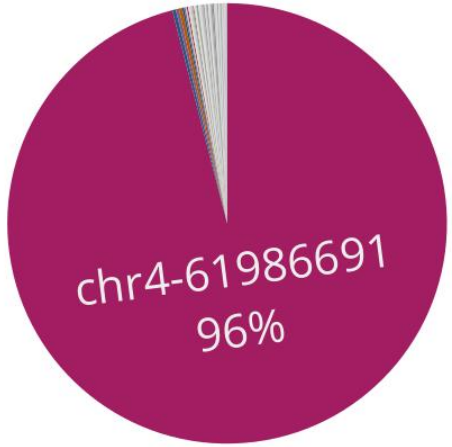
LTR5

IS:68 Reads:167



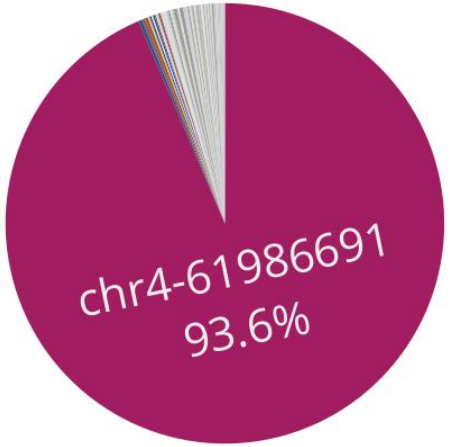
LTR3

IS:68 Reads:4626



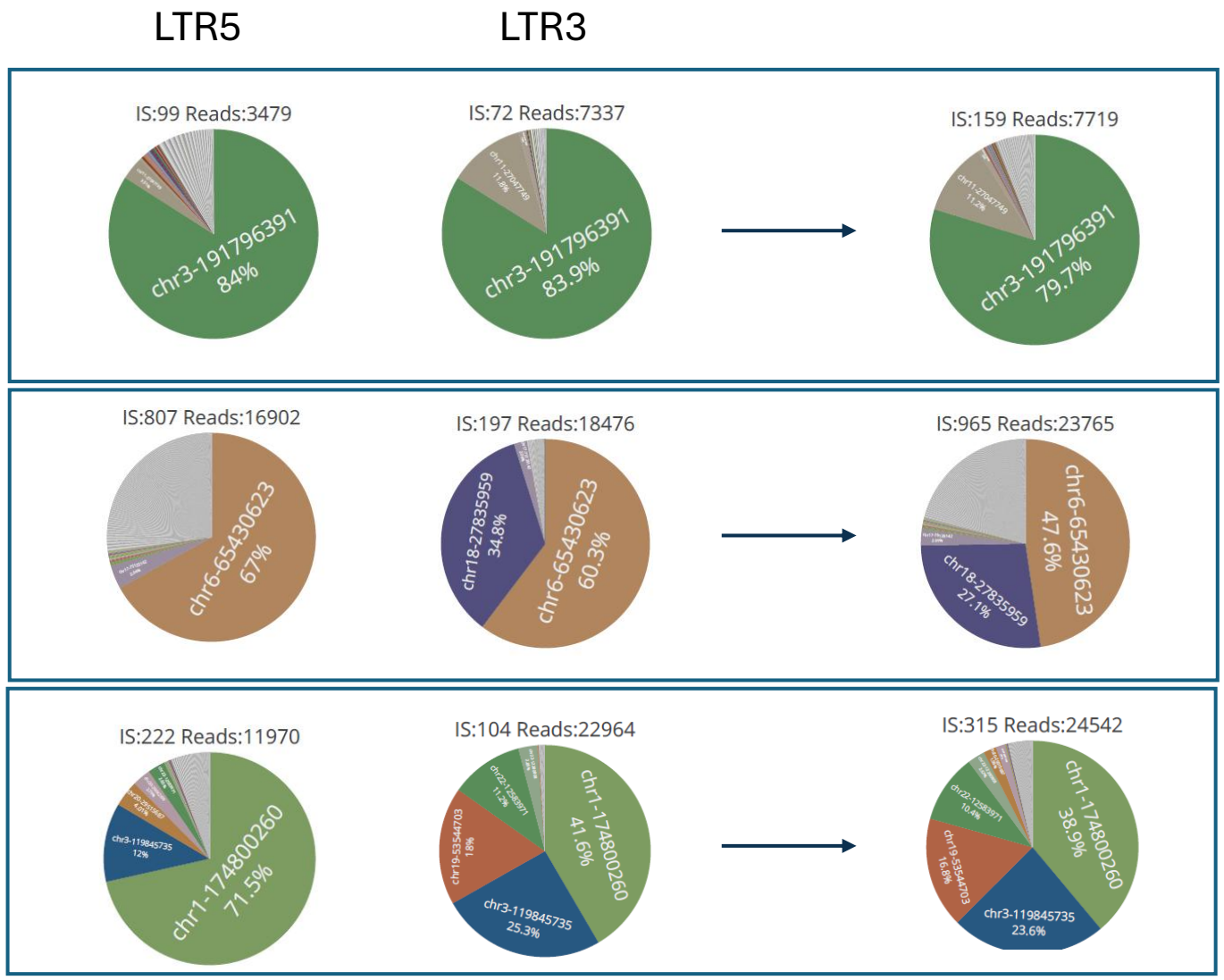
Combined LTR
VCE : 0.111

IS:122 Reads:4746



Clonality assays identify ATL cases with a single dominant LTR5' defective provirus

ATL cases with multiple dominant integrations in a single T-cell clone



VCE = 0.21
Single predominant integration with a 5'LTR-intact provirus

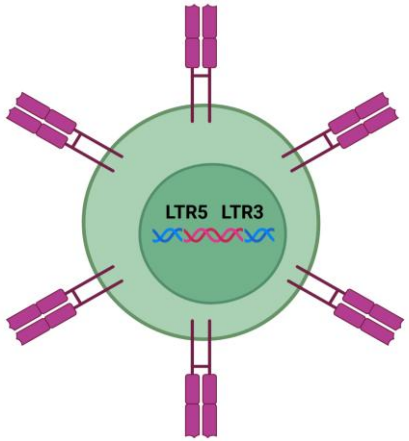
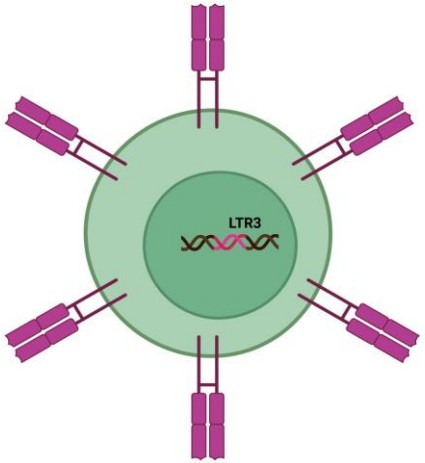
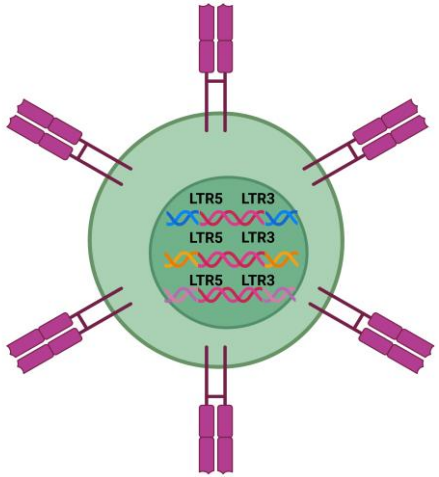
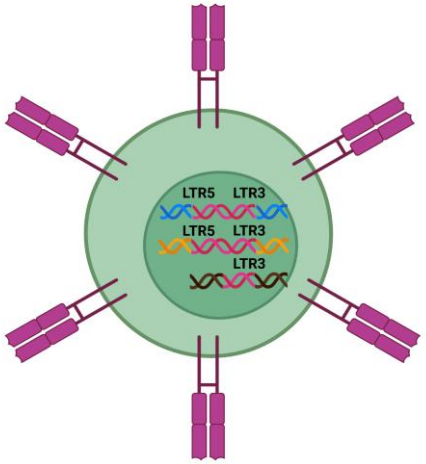
VCE = 0.38
Two predominant integrations: one 5'LTR-intact and one 5'LTR-defective provirus

VCE = 0.32
Three predominant integrations: two 5'LTR-intact and one 5'LTR-defective provirus

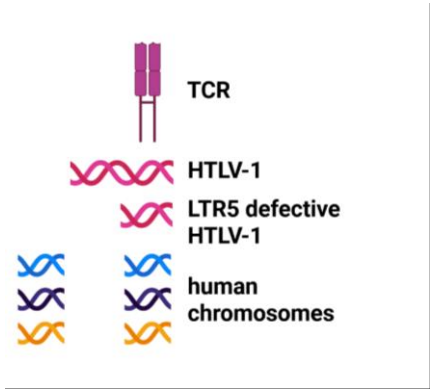
Samples from JSPFAD

In collaboration with A.Bazarbachi

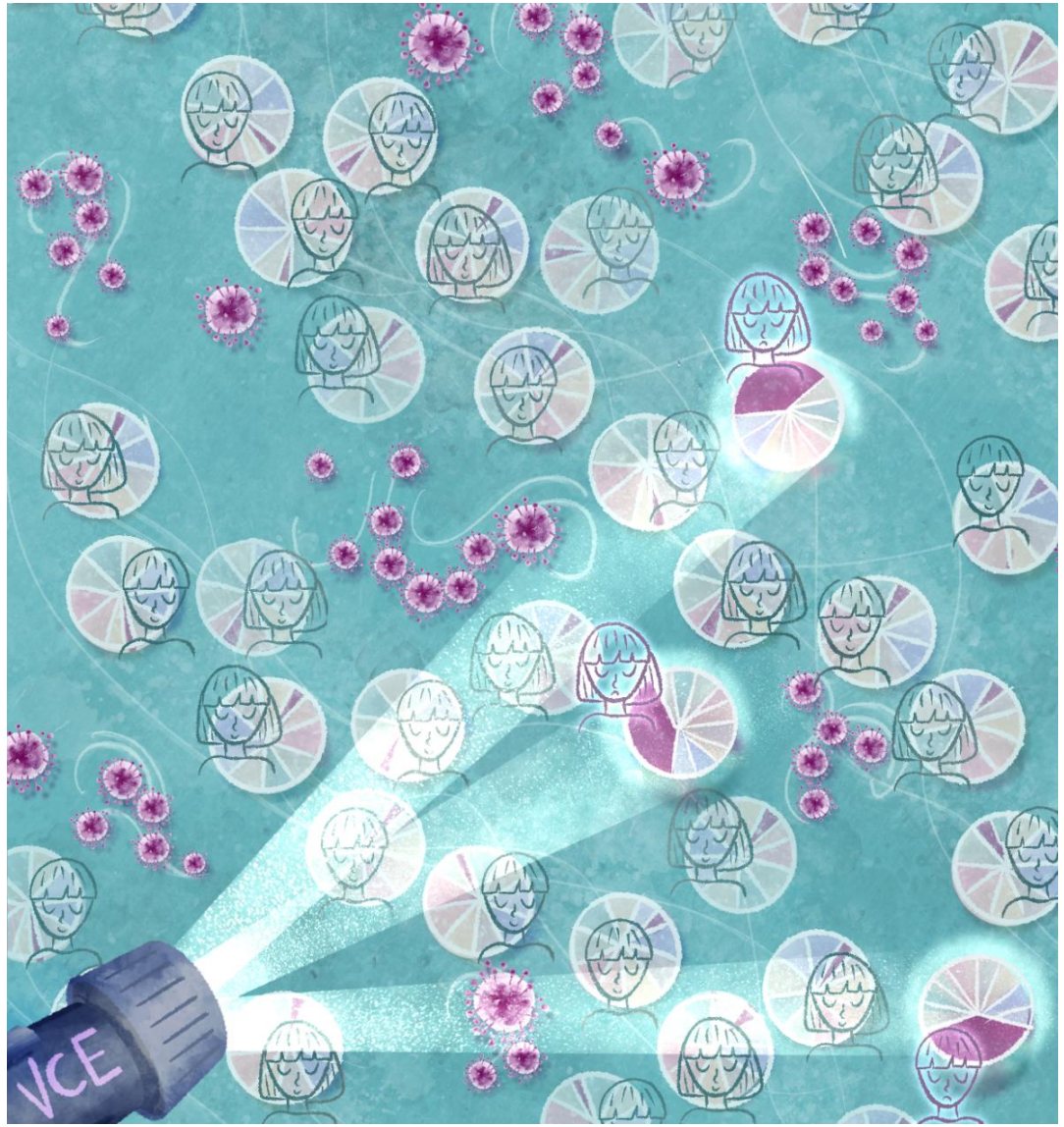
Viral clonality NGS to address fundamental questions

	Intact provirus	Defective provirus
Single provirus		
multiple proviruses in a single transformed T-cell clone		

VCE scoring is robust at identifying individuals at high risk of progression even in the presence of an ATL clone with multiple proviral insertion sites and/or defective clones



Summary and conclusions



Viral clonality and VCE outperform PVL as a prognostic biomarker

- Reassurance for 80% high PVL carriers
- closer follow-up of VCE-low high-risk carriers: candidates for pre-emptive treatment, need for clinical studies (therapeutic response = VCE increase?)

Ongoing validation studies

- Extended discovery cohort, refining threshold for prognostic
- Assessing temporal dynamics
- VCE as biomarker for therapeutic response

VCE as robust universal biomarker

- Despite polymorphisms in LTR
- DNA: implementation clinical practice and easily shipped
- Cost-effective

Need for international collaboration

Jules Bordet Institute & GIGA
Snehal Karpe
Maria Artesi
Keith Durkin
Damien Renette
Vincent Hahaut
Jérôme Wayet
Michel Georges
Anne Van den Broeke

GIGA Genomics platform
Wouter Coppieters, Latifa Karim,
Manon Deckers, Emilie Detry
GIGA Bioinformatics platform
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GIGA Imaging platform
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Raafat Stephan
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VIDO, University Saskatchewan Saskatoon, Canada
Antonio Facciuolo, Philip Griebel

St-Marianna University Kanagawa
Yoshi Yamano, Toshiki Watanabe
The University of Tokyo
Kaoru Uchimaru
Sasebo City General Hospital Nagasaki
Junya Makyjama
Graduate School Medical Sciences Nagasaki
Masako Iwanaga
Imamura General Hospital Kogoshima
Atae Utsunomiya

Hopital Universitaire Necker & Institut Imagine Paris
Olivier Hermine, Ambroise Marçais

Erasmus MC Rotterdam
Anton Langerak, Martijn Kolijn

Instituto de Medicina Tropical, Lima
Eduardo Gotuzzo, Gabriela Garrido, Daniel Enriquez-Vera
Instituto de Medicina Tropical, Lima & MD Anderson, Houston
Luis Malpica

IRVA prognostic working group inter-lab testing partners
Yoshi Yamano, Masumichi Saito, Aileen Rowan, Natasha Jansz, Damian Purcell

University of Miami
Juan-Carlos Ramos

American University of Beirut
Ali Bazarbachi

JSPFAD
Joint Study on Predisposing Factors of ATL Development

HTLV 2026

Utility of Flow Cytometry for Prognostic Prediction in Adult T-cell Leukemia/Lymphoma

Shigeo Fuji[1], Masahito Tokunaga[2], Lucy Cook[3], Atae Utsunomiya[2], Junya Makiyama[4], Youko Suehiro[5], Ki-Ryang Koh[6], Makoto Nakashima[7,8], Yoshihisa Yamano[8], Kaoru Uchimaru[7]

[1]Osaka International Cancer Institute, Japan
[2]Imamura General Hospital, Japan
[3]Imperial College Healthcare NHS Trust, UK
[4]Sasebo City General Hospital [5]NHO Kyushu Cancer Center [6]Osaka General Hospital of West Japan Railway Co.
[7]Graduate School of Frontier Sciences, The University of Tokyo [8]St. Marianna University School of Medicine

Speaker

Shigeo Fuji, M.D., Ph.D.

The authors declare no conflict of interest.

Background

Adult T-cell leukemia/lymphoma (ATL) is a clinically heterogeneous disease caused by HTLV-1 infection.

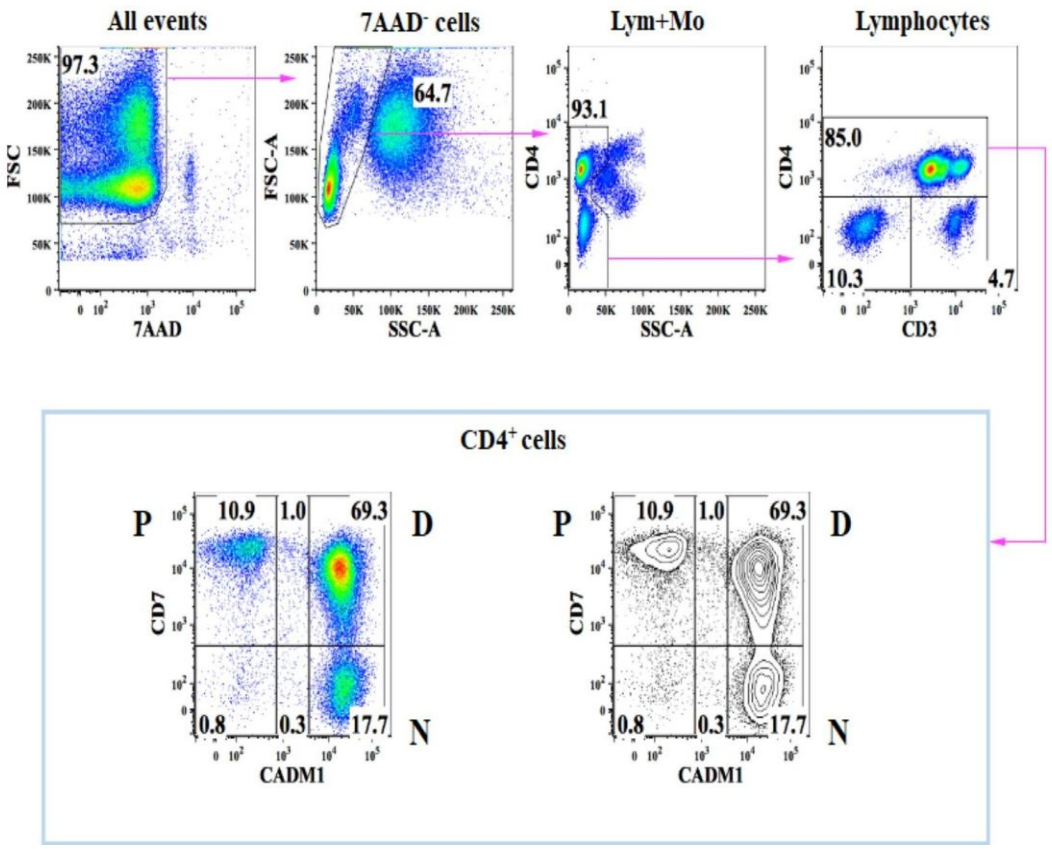
Current classification primarily relies on morphology and routine laboratory findings (Shimoyama classification), which has limited reproducibility and prognostic precision[1,2].

Flow-cytometric assessment could be more accurate to determine the percentage of HTLV-1-infected cells in peripheral blood than morphological assessment, and may provide additional biological information relevant to the risk of disease progression to aggressive ATL.

1. Fuji S, et al. Expert Rev Hematol. 2025, 2. Fuji S, et al. Int J Lab Hematol. 2022

METHOD

HAS-Flow: CADM1 vs. CD7 on CD4⁺ T cells



Representative HAS-Flow gating

Populations

P — CADM1⁻ / CD7⁺

D — CADM1⁺ / CD7⁺~dim

N — CADM1⁺ / CD7⁻

Grouping by D + N (%)

G1	≤ 10 %
G2	10 – 25 %
G3	25 – 50 %
G4	> 50 %

Kobayashi 2014; Makiyama 2019; Jimbo 2024

Kobayashi S, et al. Cancer Sci. 2015
Makiyama J, et al. Cancer Sci 2019

Received: 13 June 2019 | Revised: 2 October 2019 | Accepted: 12 October 2019

DOI: 10.1111/cas.14219

ORIGINAL ARTICLE

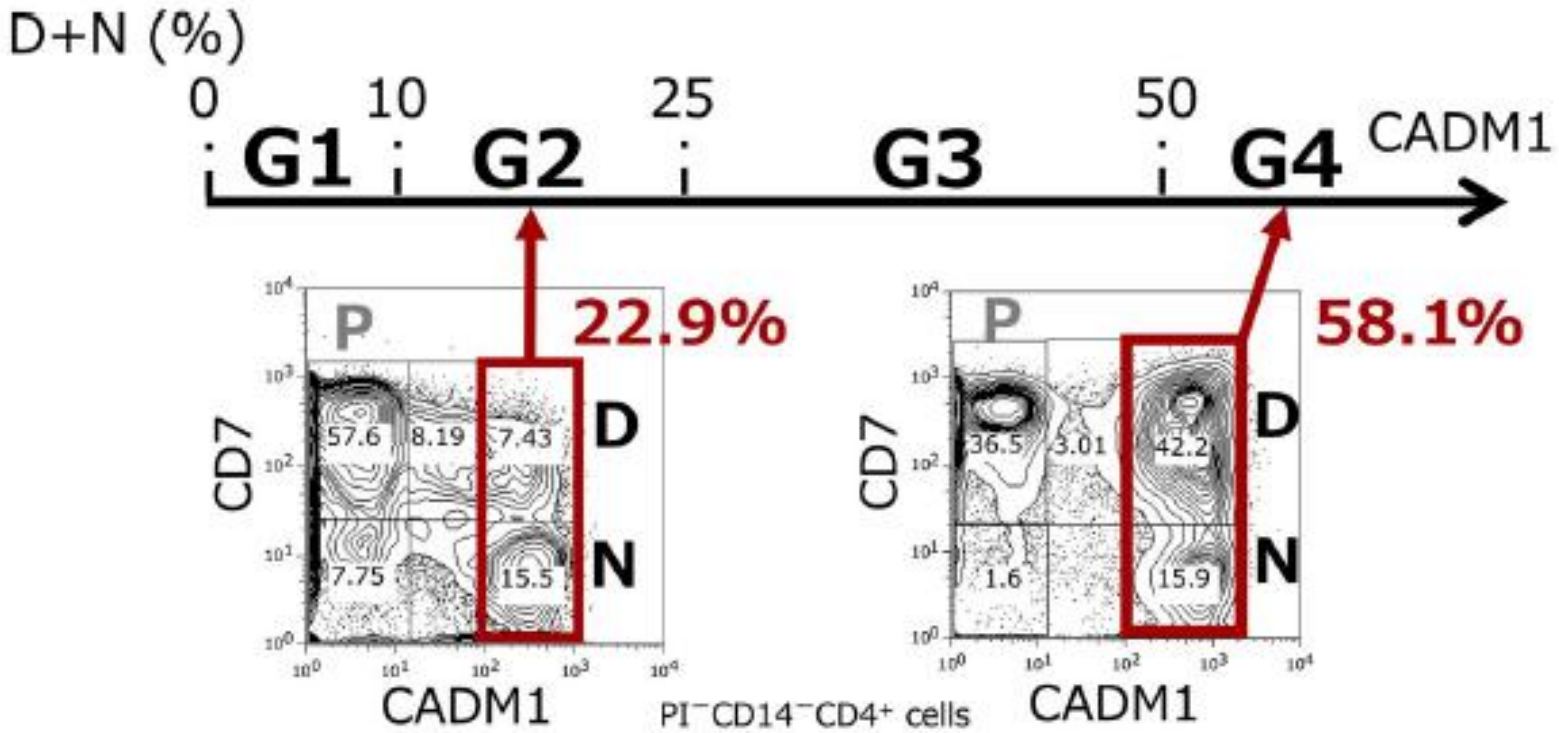
Cancer Science WILEY

$CD4^+CADM1^+$ cell percentage predicts disease progression in HTLV-1 carriers and indolent adult T-cell leukemia/lymphoma

Junya Makiyama[1] | Seiichiro Kobayashi[2] | Eri Watanabe[3] | Tomohiro Ishigaki[4,5] | Toyotaka Kawamata[1] | Makoto Nakashima[6] | Makoto Yamagishi[6] | Kazumi Nakano[6] | Arinobu Tojo[1,2] | Toshiki Watanabe[1,7] | Kaoru Uchimaru[1,6]

Makiyama J, et al. Cancer Sci 2019

HAS-Flow: CADM1 vs. CD7 on CD4⁺ T cells



A

Case 1	AC
Ably	3.0 (%)
PVL	6.9 (copies/100 PBMCs)
sIL-2R	313 (U/mL)

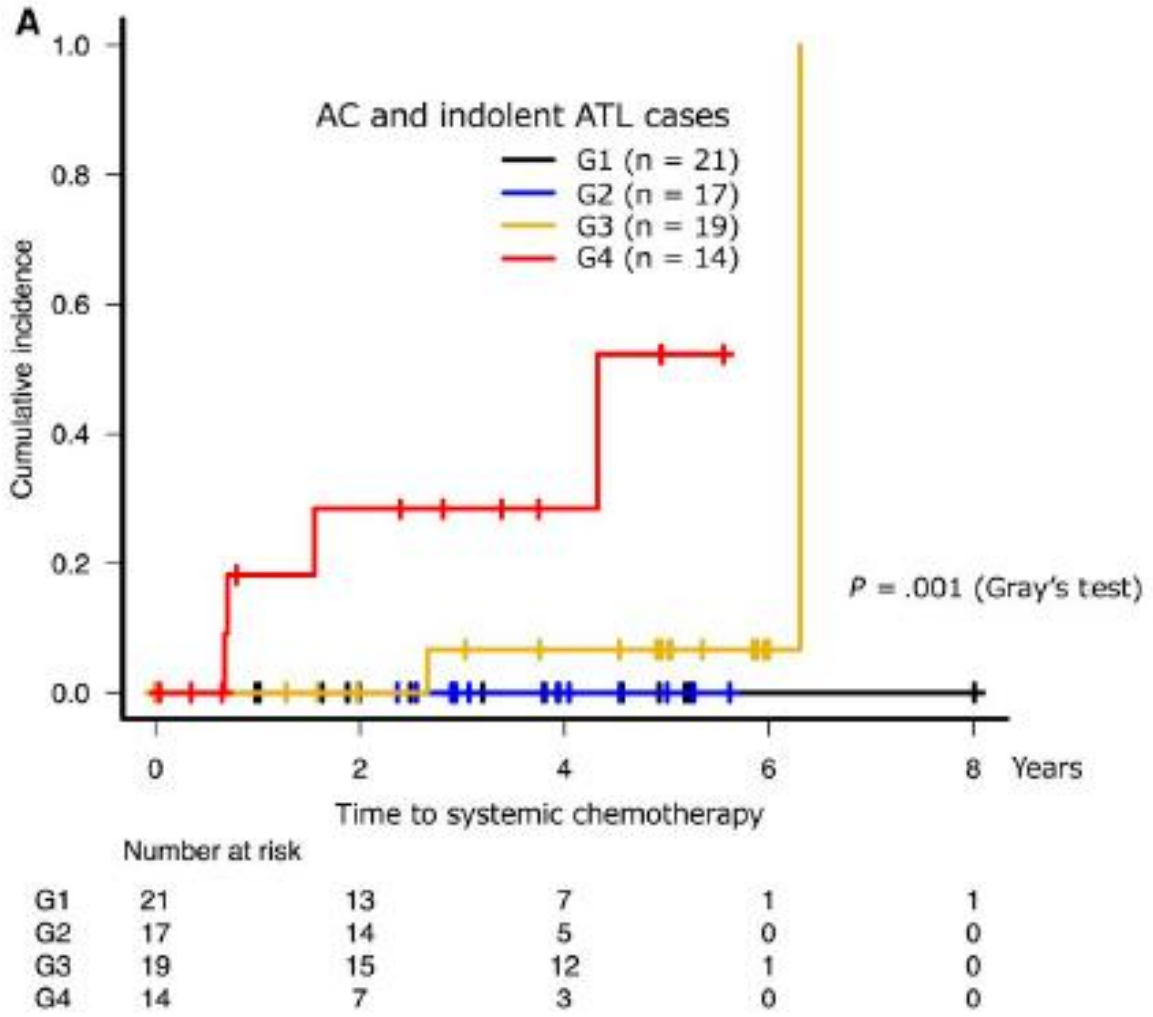
B

Case 2	Chronic type ATL
Ably	24.5 (%)
PVL	36.63 (copies/100 PBMCs)
sIL-2R	1700 (U/mL)

Makiyama J, et al. Cancer Sci 2019

METHOD

HAS-Flow: CADM1 vs. CD7 on CD4^{+} T cells



Makiyama J, et al. Cancer Sci 2019

Aim

To evaluate the prognostic value of flow cytometric analysis in HTLV-1 carriers and indolent ATL

n = 227

Japan — IMSUT (JSPFAD program) & Imamura General Hospital

Median age 63 (29–90); median follow-up 1,639 days (14–5,465).

ENDPOINTS & STATISTICS

PFS — conventional chemotherapy start, progression to acute/lymphoma-type ATL, or death. **ATL-specific PFS** — censoring non-ATL deaths. **OS**. Kaplan–Meier with log-rank; Cox proportional hazards; Spearman correlation. Piecewise linear regression with a CD4 break-point at 1,500 cells/μL (LRT, AICc, 10-fold CV-RMSE).

RESULTS

Baseline characteristics by HAS-Flow & clinical subtype

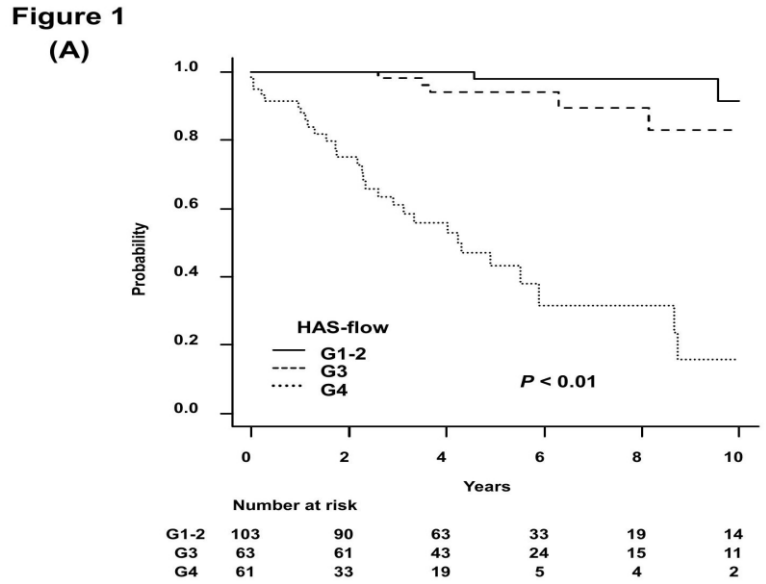
Factor	G1 carrier	G2 carrier	G3 carrier	G3 smold.	G3 chronic	G4 smold.	G4 chronic	P
n	48	55	30	32	1	26	35	—
Age, median	61.5	64	63	61	50	64	64	0.253
Male, %	22.9	32.7	46.7	28.1	100	30.8	34.3	0.305
PVL (copies/100 PBMC)	1.98	6.79	12.9	18.3	18.1	33.2	56.8	<0.001
sIL-2R (U/mL)	333	408	502	555	562	994	1,930	<0.001
Lymphocyte (/µL)	1,509	1,618	1,678	2,113	5,687	2,886	6,469	<0.001
CD4 count (/µL)	645	685	771	795	2,906	1,496	4,055	<0.001
CD4 > 1,500, %	2.1	3.6	3.3	12.5	100	50.0	91.4	<0.001
HAS-Flow D+N, % (median)	4.8	16.0	33.0	37.4	38.2	64.9	87.2	<0.001

Values are medians for continuous variables and percentages for categorical variables. P-values across all groups. PVL, proviral load; sIL-2R, soluble IL-2 receptor; PBMC, peripheral blood mononuclear cells.

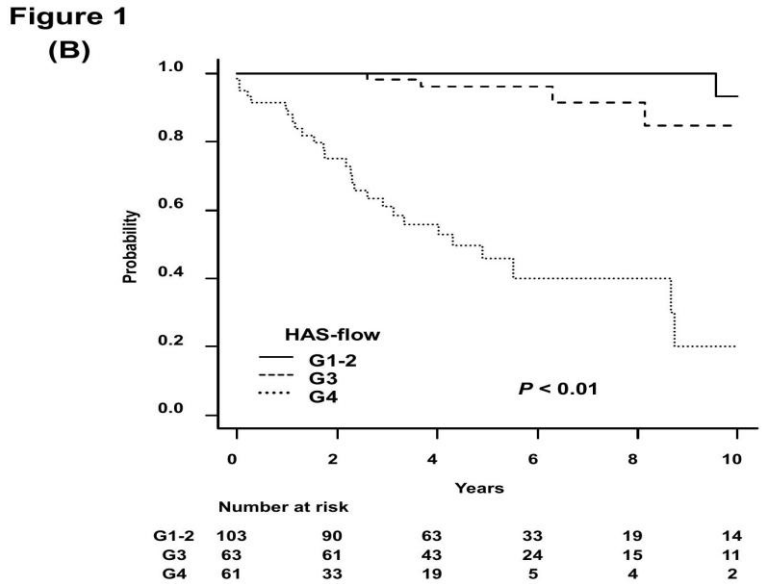
Key observation: PVL, sIL-2R, lymphocyte count, CD4 count, and D+N all rise stepwise from G1 carrier → G4 chronic.

HAS-Flow grouping stratifies progression and survival

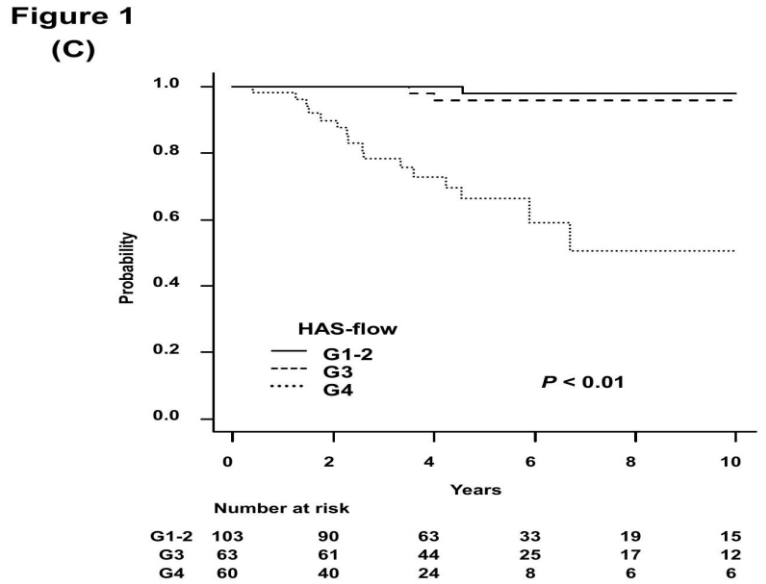
A. PFS



B. ATL-specific PFS



C. Overall survival



G1–G2

4-year rates

PFS	**100 %**
ATL-PFS	**100 %**
OS	**100 %**

All P < 0.01 (log-rank)

G3

4-year rates

PFS	**94.2 %**
ATL-PFS	**96.2 %**
OS	**95.8 %**

All P < 0.01 (log-rank)

G4

4-year rates

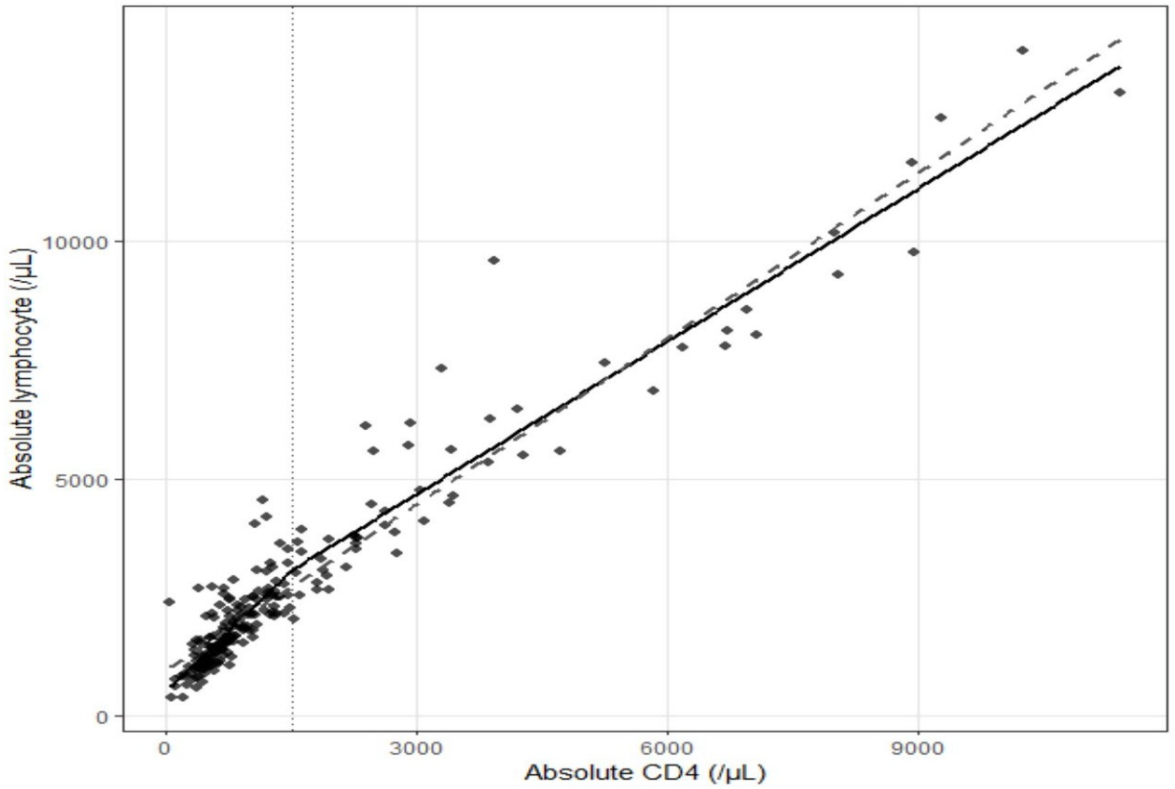
PFS	**55.7 %**
ATL-PFS	**55.7 %**
OS	**72.6 %**

All P < 0.01 (log-rank)

The relationship between absolute CD4 count and total lymphocyte count

CD4–lymphocyte slope changes at 1,500/µL

Absolute CD4 vs. lymphocyte count



LRT P < 0.01
AICc 3,426 vs. 3,446
CV-RMSE 630 vs. 661

Piecewise model with break-point at CD4 = 1,500/µL outperforms a simple linear fit.

When CD4 count exceeds the upper limit of normal at 1,500 cells per microliter, the slope changes, consistent with proliferation of CD4-derived ATL cells dominating the lymphocyte compartment.

RESULTS

Baseline characteristics by HAS-Flow & clinical subtype

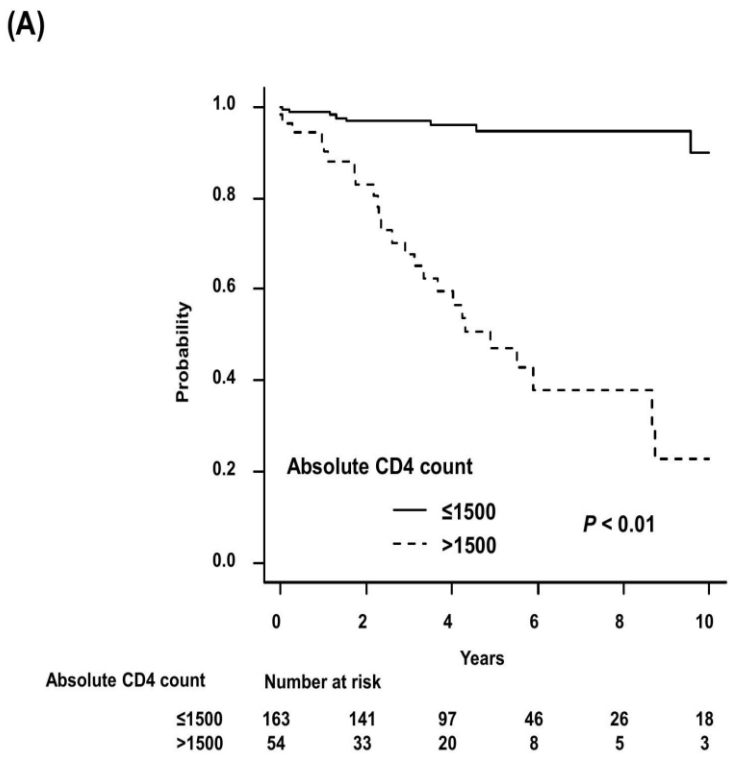
Factor	G1 carrier	G2 carrier	G3 carrier	G3 smold.	G3 chronic	G4 smold.	G4 chronic	P
n	48	55	30	32	1	26	35	—
Age, median	61.5	64	63	61	50	64	64	0.253
Male, %	22.9	32.7	46.7	28.1	100	30.8	34.3	0.305
PVL (copies/100 PBMC)	1.98	6.79	12.9	18.3	18.1	33.2	56.8	<0.001
sIL-2R (U/mL)	333	408	502	555	562	994	1,930	<0.001
Lymphocyte (/µL)	1,509	1,618	1,678	2,113	5,687	2,886	6,469	<0.001
CD4 count (/µL)	645	685	771	795	2,906	1,496	4,055	<0.001
CD4 > 1,500, %	2.1	3.6	3.3	12.5	100	50.0	91.4	<0.001
HAS-Flow D+N, % (median)	4.8	16.0	33.0	37.4	38.2	64.9	87.2	<0.001

Values are medians for continuous variables and percentages for categorical variables. P-values across all groups. PVL, proviral load; sIL-2R, soluble IL-2 receptor; PBMC, peripheral blood mononuclear cells.

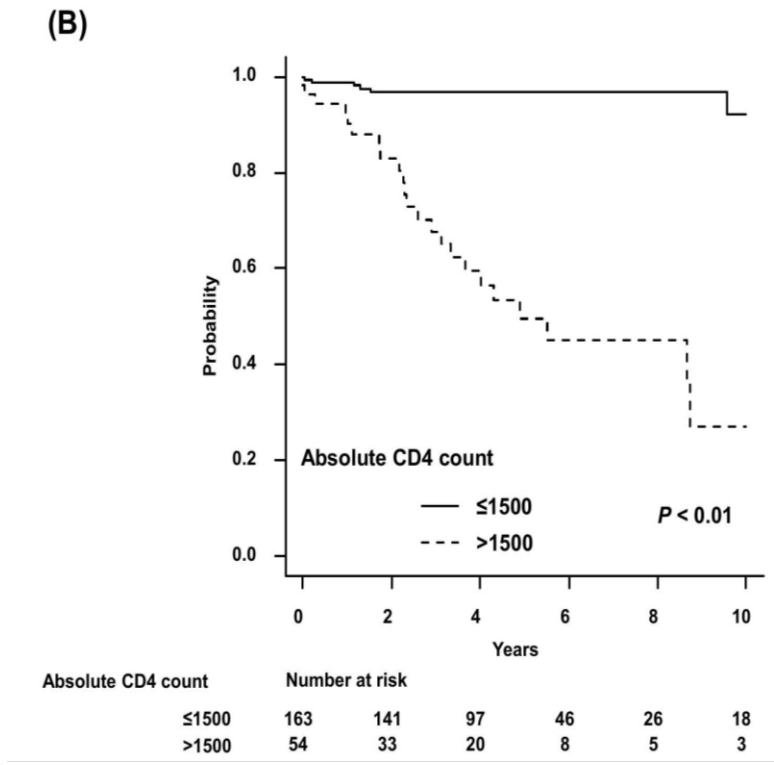
Key observation: PVL, sIL-2R, lymphocyte count, CD4 count, and D+N all rise stepwise from G1 carrier → G4 chronic.

Absolute CD4 > 1,500/µL marks a poor-prognosis group (Japanese cohort)

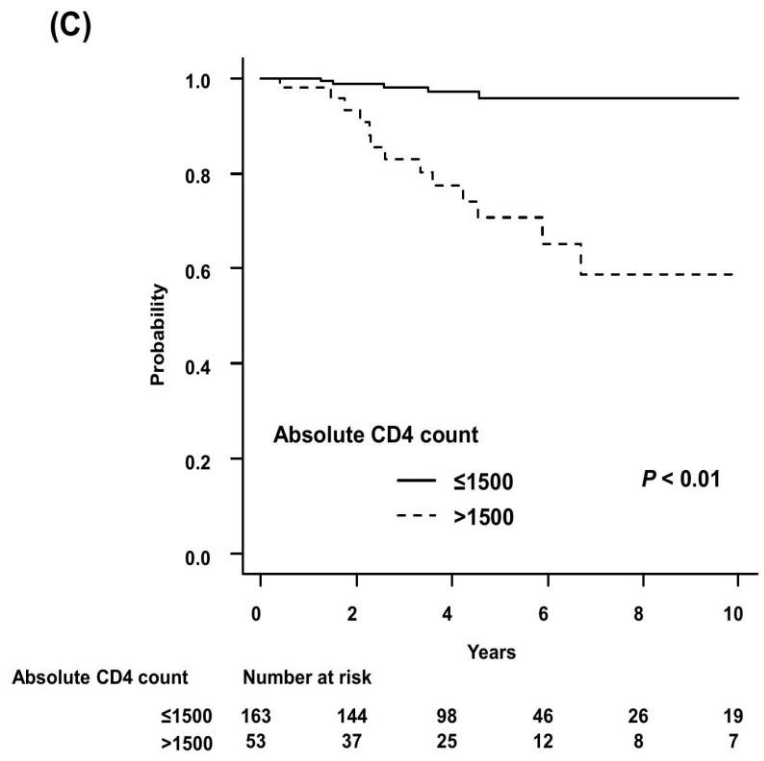
A. PFS



B. ATL-specific PFS



C. Overall survival



CD4 > 1,500/µL shows significantly shorter PFS, ATL-specific PFS, and OS vs. CD4 ≤ 1,500/µL (All P < 0.01).

AIM & METHODS

Two-cohort retrospective study

Aim

To evaluate the prognostic value of flow cytometric analysis in HTLV-1 carriers and indolent ATL

DERIVATION COHORT

n = 227

Japan — IMSUT (JSPFAD program) & Imamura General Hospital

Median age 63 (29–90); median follow-up 1,639 days (14–5,465).

VALIDATION COHORT

n = 81

United Kingdom — Imperial College London

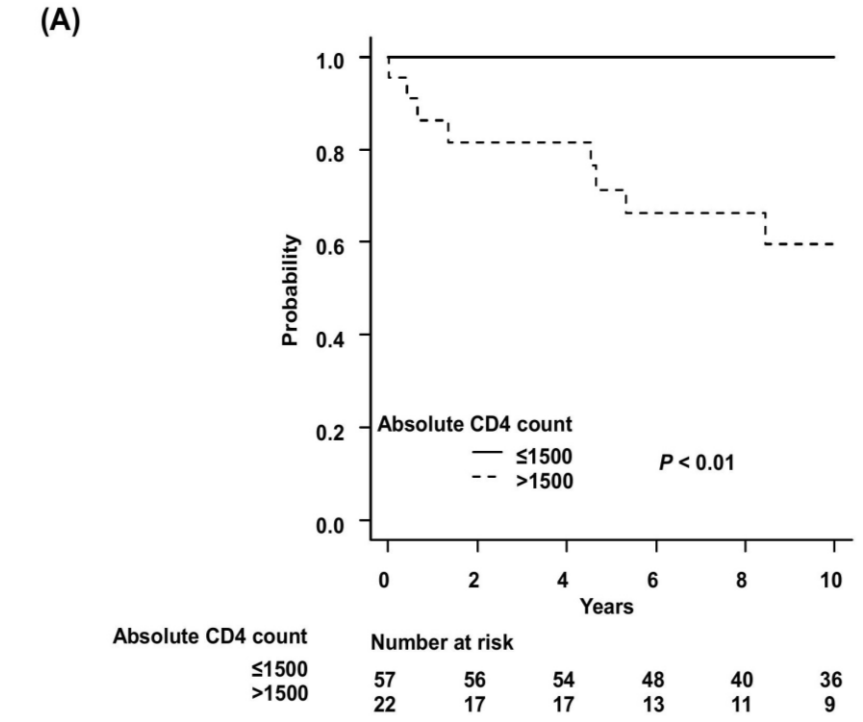
Median age 48 (17–83); carrier 54 / smoldering 10 / chronic 17.

ENDPOINTS & STATISTICS

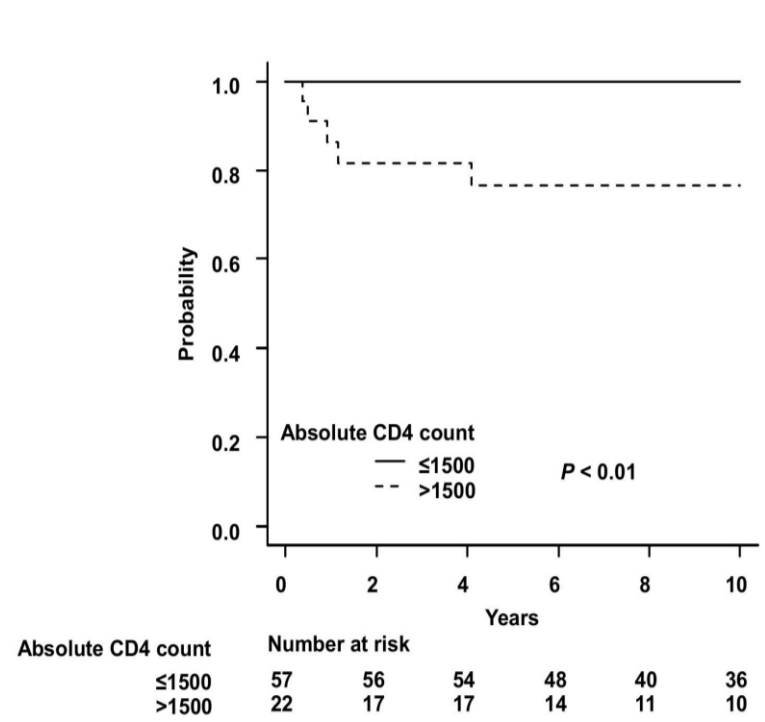
PFS — conventional chemotherapy start, progression to acute/lymphoma-type ATL, or death. **ATL-specific PFS** — censoring non-ATL deaths. **OS**. Kaplan–Meier with log-rank; Cox proportional hazards; Spearman correlation. Piecewise linear regression with a CD4 break-point at 1,500 cells/µL (LRT, AICc, 10-fold CV-RMSE).

External validation in the UK cohort (Imperial College London)

A. PFS



B. Overall survival



The CD4 > 1,500/µL threshold reproduces the prognostic split in an independent UK cohort (P < 0.01 for both PFS and OS).

C O N C L U S I O N

HAS-Flow and absolute CD4 count provide additive prognostic information in HTLV-1-infected individuals.

- **HAS-Flow G4 is the strong independent predictor of outcome.** It carried hazard ratios of approximately 18 to 22 for progression-free survival, ATL-specific PFS, and overall survival.
- **An absolute CD4 count above 1,500/µL marks a poor-prognosis group.** This threshold reproduced the prognostic split in both the Japanese derivation cohort and the independent UK validation cohort, and is a low-cost, globally deployable marker. CD4 count could be an easy and ready-to-use prognostic marker in HTLV-1-infected individuals.
- Incorporating flow cytometric analysis, in addition to morphological determination, would be a meaningful step toward a modern, precise classification of individuals with HTLV-1 infection.

Funding, collaborators, and disclosures

Funding

Japan Agency for Medical Research and Development (AMED Grant JP23fk0108672)

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Dr. Seiichiro Kobayashi (Kanto Rosai Hospital) and Dr. Koji Jimbo (IMSUT Research Hospital) — follow-up data on individuals with HTLV-1 infection.

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

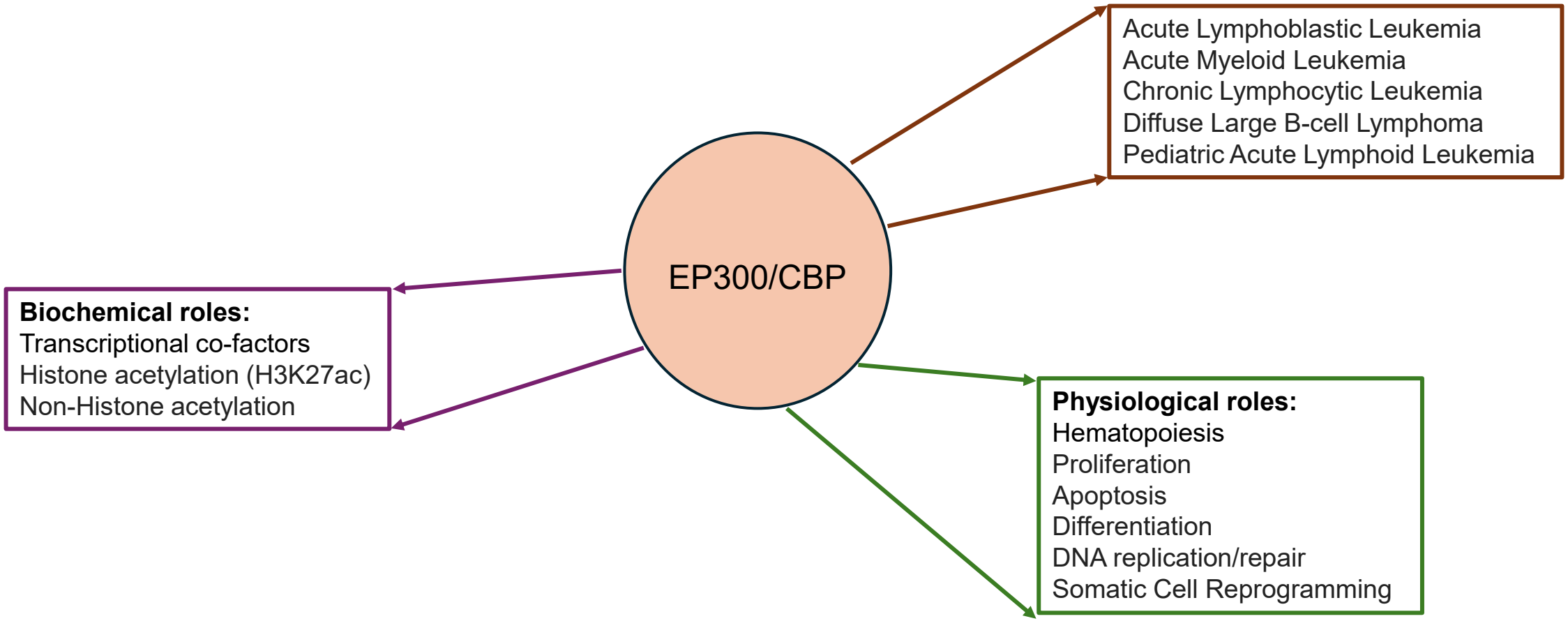
EP300 deficiency leads to chronic replication stress driven aggressive Adult T-cell leukemia/lymphoma

Advaitha Madireddy, Rutgers Cancer Institute

Conflicts of interest

I have no conflicts of interest

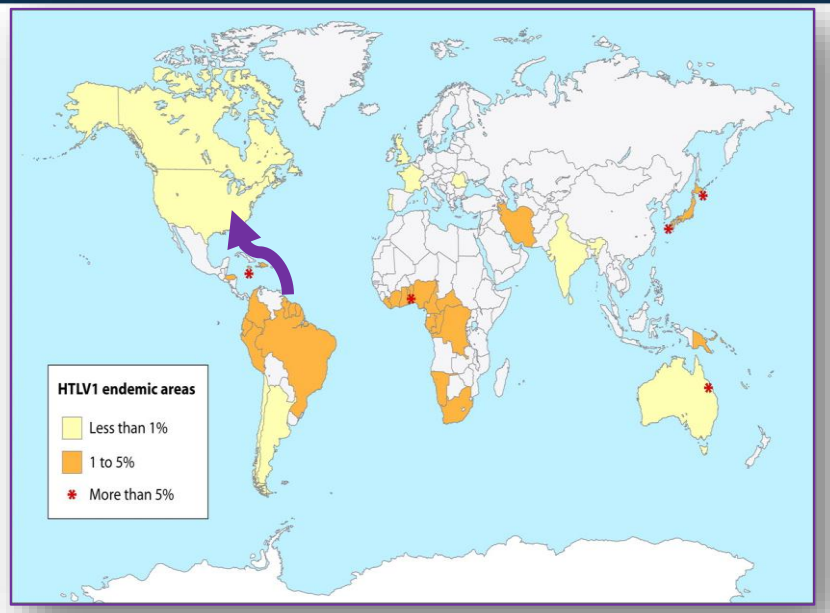
EP300 and CBP are tumor suppressors/promoters



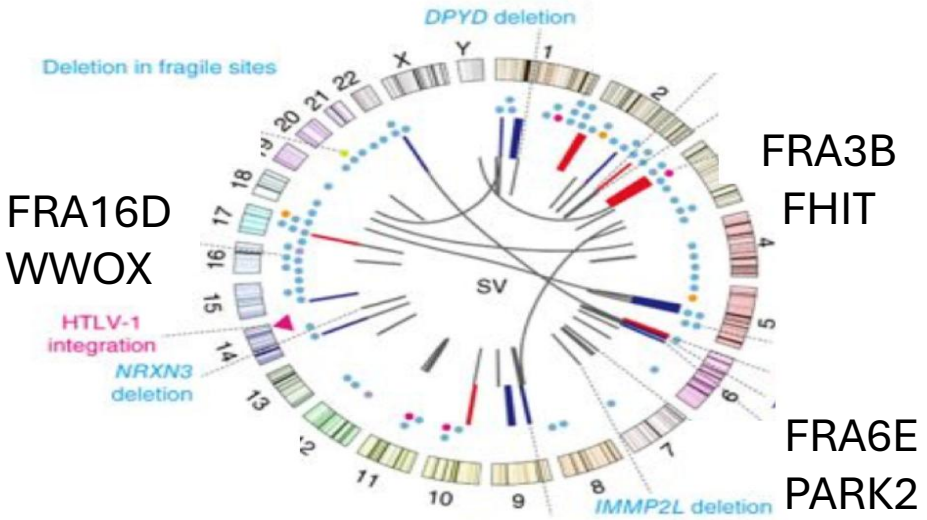
Independent inhibition of EP300 in human cells results in the differential expression of genes involved in cell cycle, DNA replication and DNA repair, unlike CBP

Adult T-cell leukemia/lymphoma (ATLL)

- Rare and aggressive T-cell leukemia/lymphoma
- Linked to Human T-Cell Lymphotropic Virus 1 (HTLV-1) infection
- Endemic to Japan, Middle East, Africa, the Caribbean, Central and South America
- Due to population migration, there is an increase of ATLL patients in North America
- Genomic instability is hallmark of ATLL
  - Cluster at fragile sites
  - However, mechanisms driving genomic instability in ATLL are unclear



Map of HTLV1 endemicity.



North American ATLL patients have aggressive chemo-refractory disease

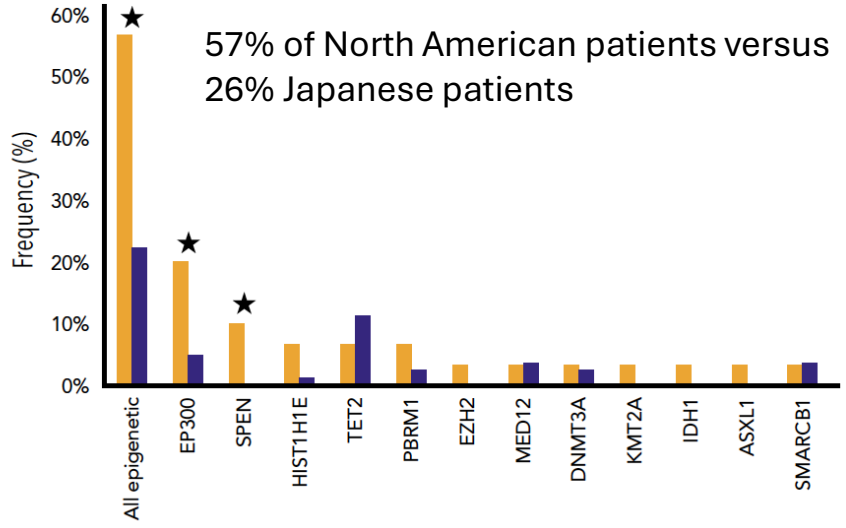
- Prominent difference in the transcriptional and epigenetic landscape between North American and Japanese patients

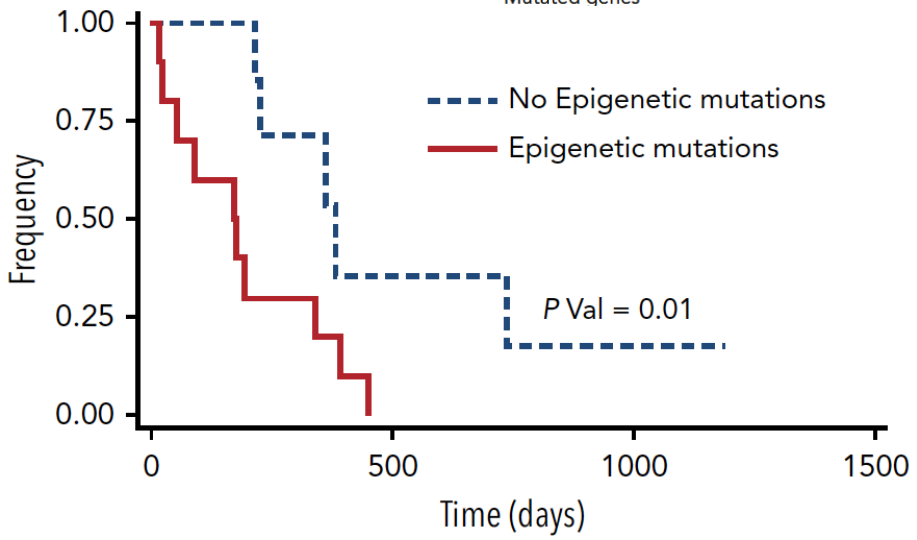
- 57% of NA-ATLL patients present mutations in epigenetic genes
  - 20% of the NA-ATLL patients have EP300 mutations

- Epigenetic mutations correlated with worse prognosis

Epigenetic mutations → ~6 months
No epigenetic mutations → 1 year

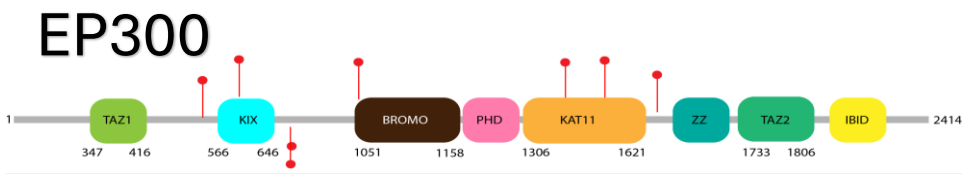
Understanding the mechanisms contributing to severe disease presentation in NA-ATLL is critical to identify new therapeutic targets that could improve quality of life and overall survival for these patients



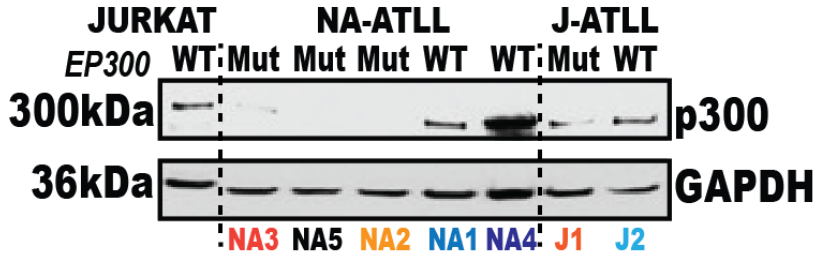


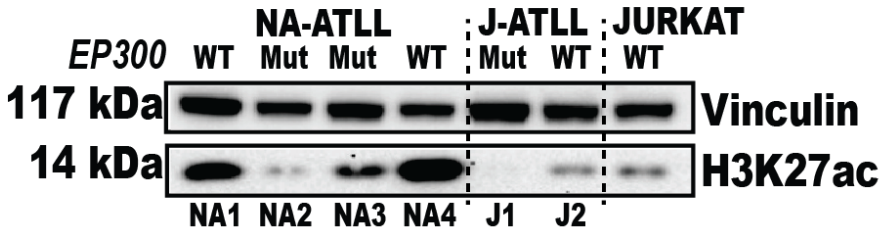
Shah, U, et. Al Blood 2019

Somatic hypermutation in EP300 results in protein downregulation

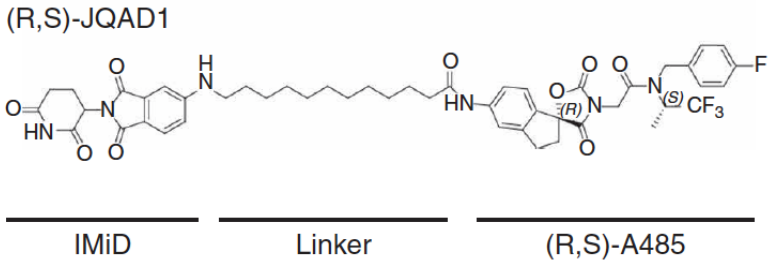


P300 mutation found in ATLL cells
No CBP mutations
missense, splice site, and truncating mutations

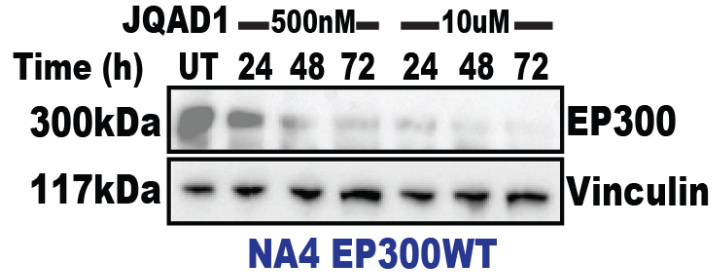


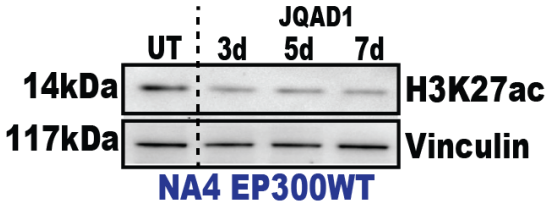


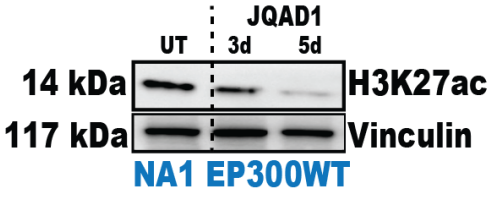
EP300 PROTAC degrader JQAD1 to establish a causal relationship

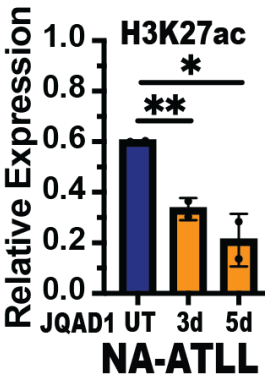


Durbin A, et al. *Cancer Discovery*, 2022

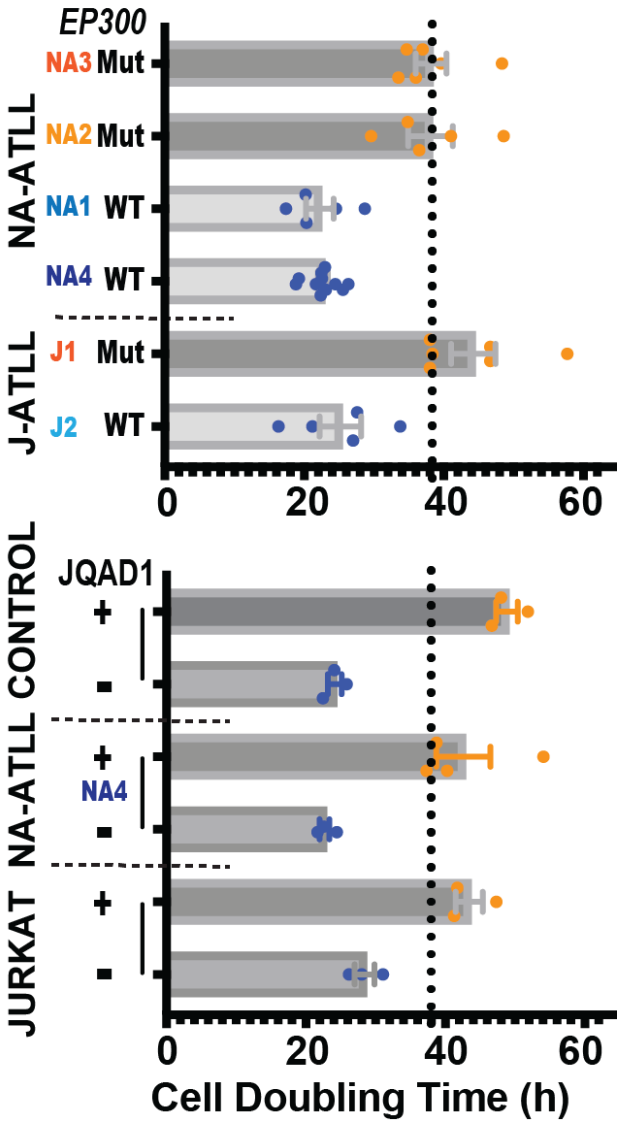


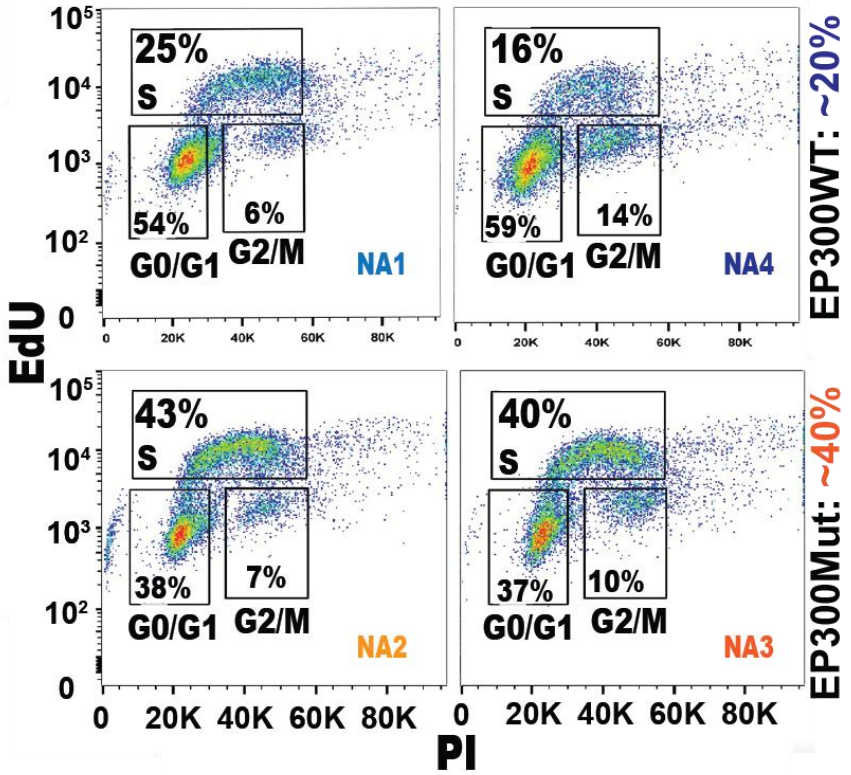


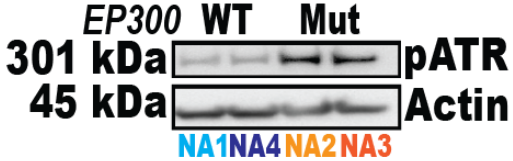


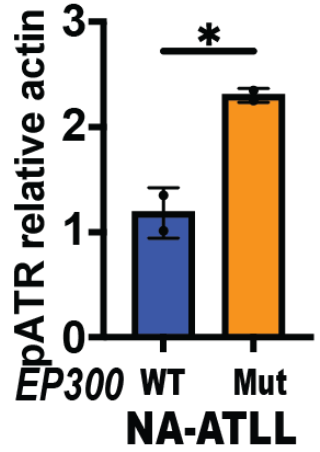


EP300-mutated NA-ATLLs have aberrant cell cycle dynamics, spontaneous DNA damage and replicative checkpoint activation









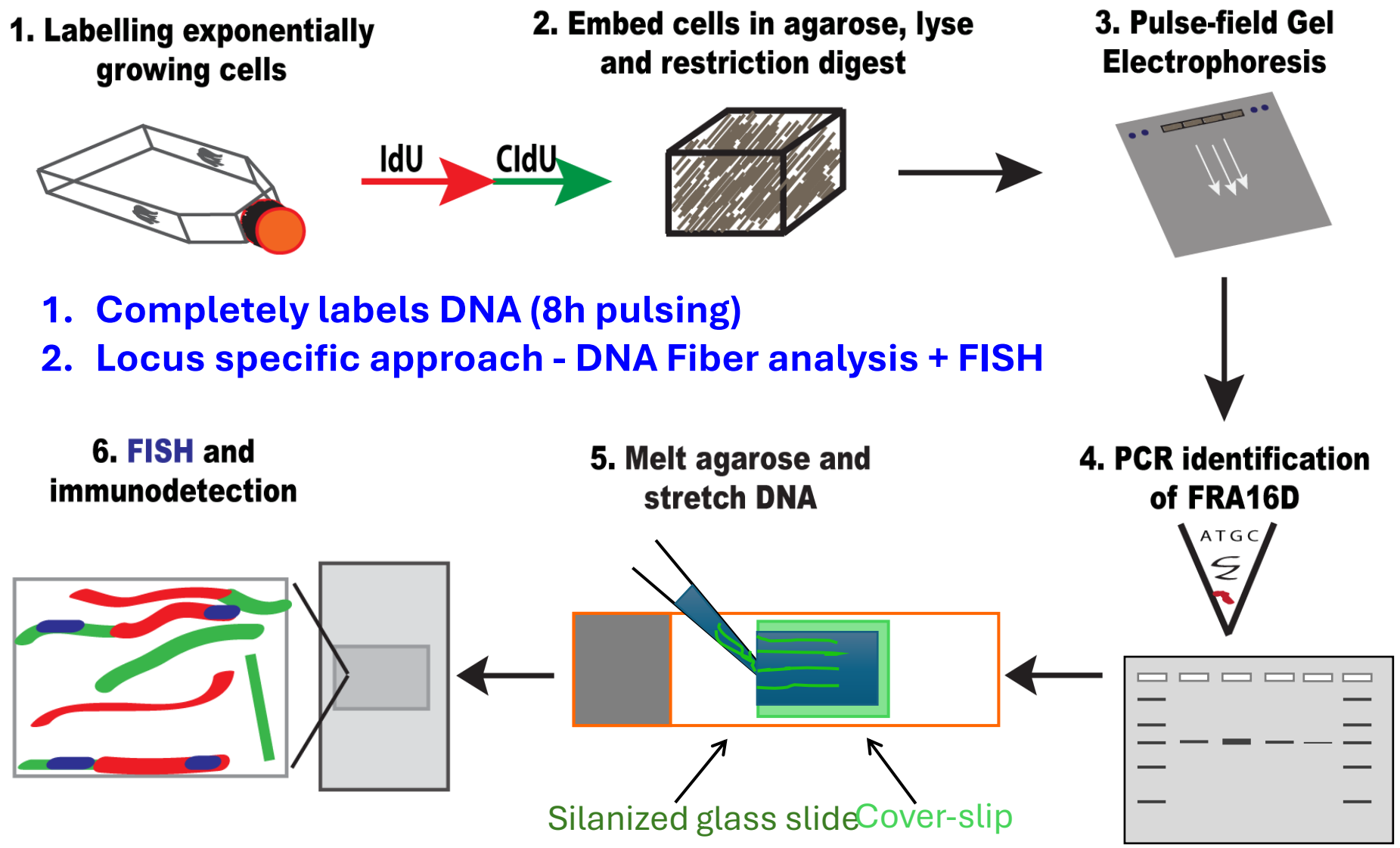
Galvez, AB, Niljikar, M, et. al, Nature Communications, 2025

What's happening during the S-phase when EP300 is deficient?

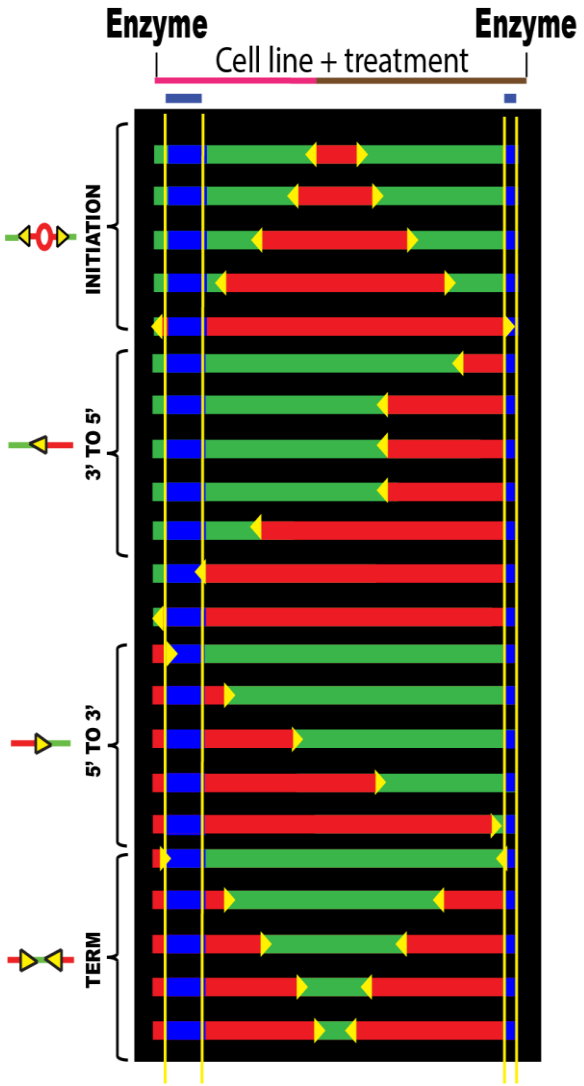
- Locus Specific DNA replication profiling at genomic hotspots of instability in ATLL
  - Single molecule analysis of replicated DNA (SMARD)
  - High-resolution – no need for drugs or inhibitors
  - FRA6E is cytogenetically unstable in NA-ATLL

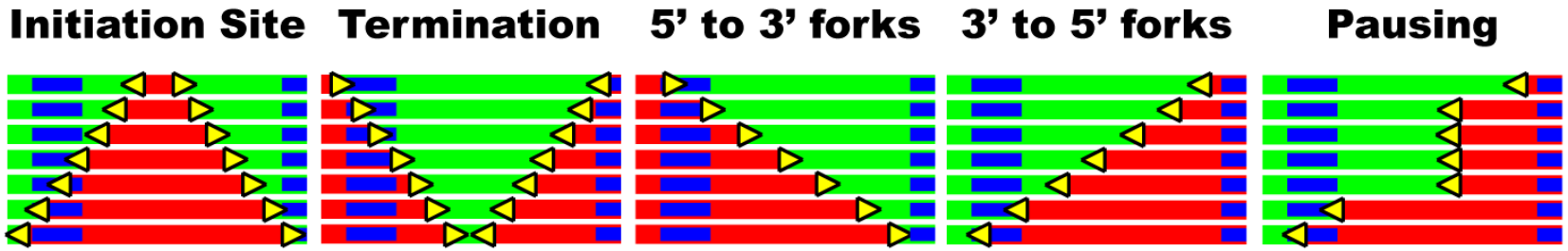
- DNA fiber analysis
  - Genome-wide profiling of DNA replication integrity
  - Need inhibitors

Single Molecule Analysis of the Replicated DNA (SMARD)



SMARD Analysis and Data Interpretation

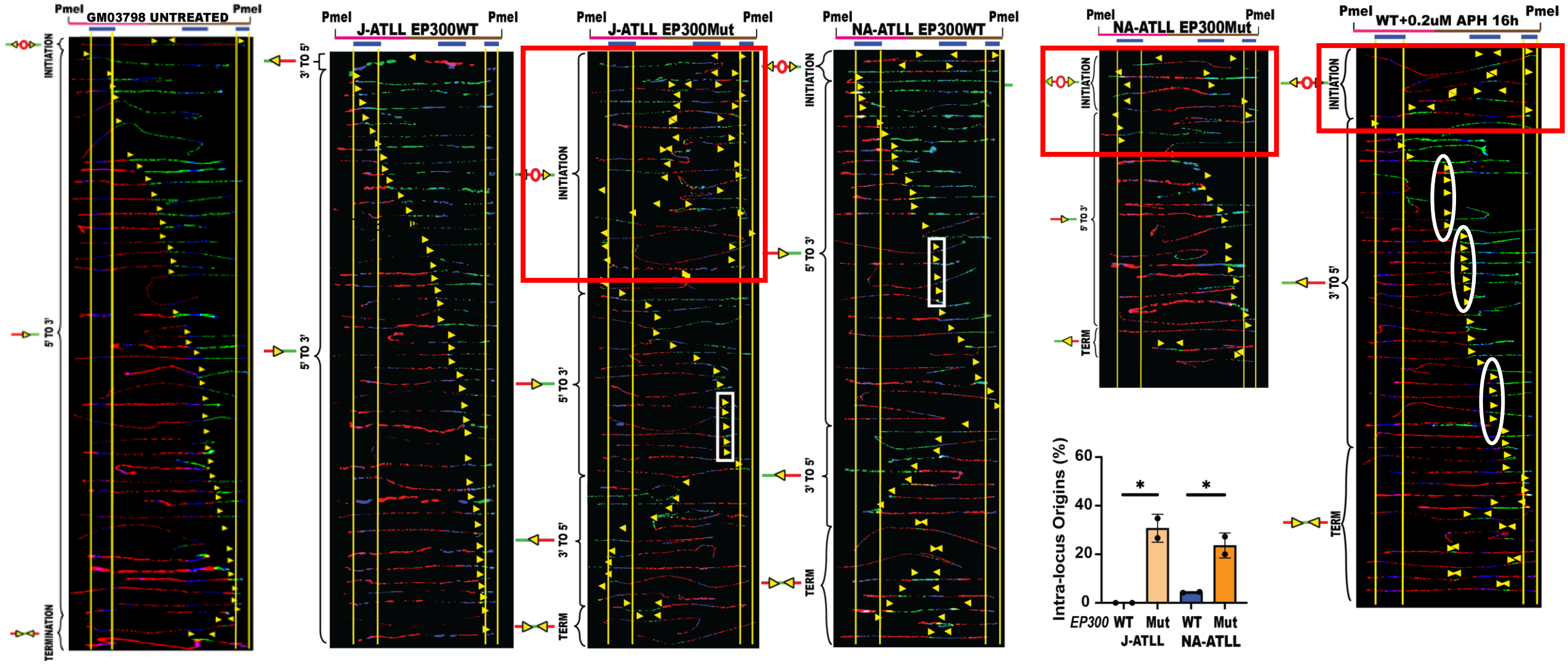




How do we know replication is perturbed?

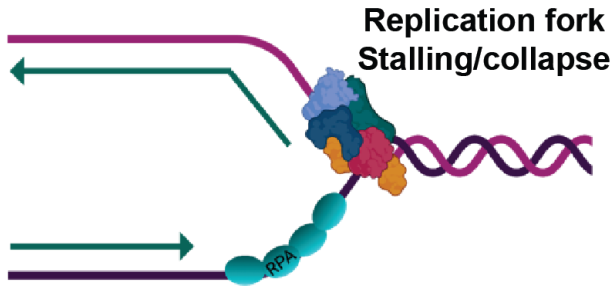
- Replication fork pausing
- Dormant origin activation
- Replication Speed – increase or decrease

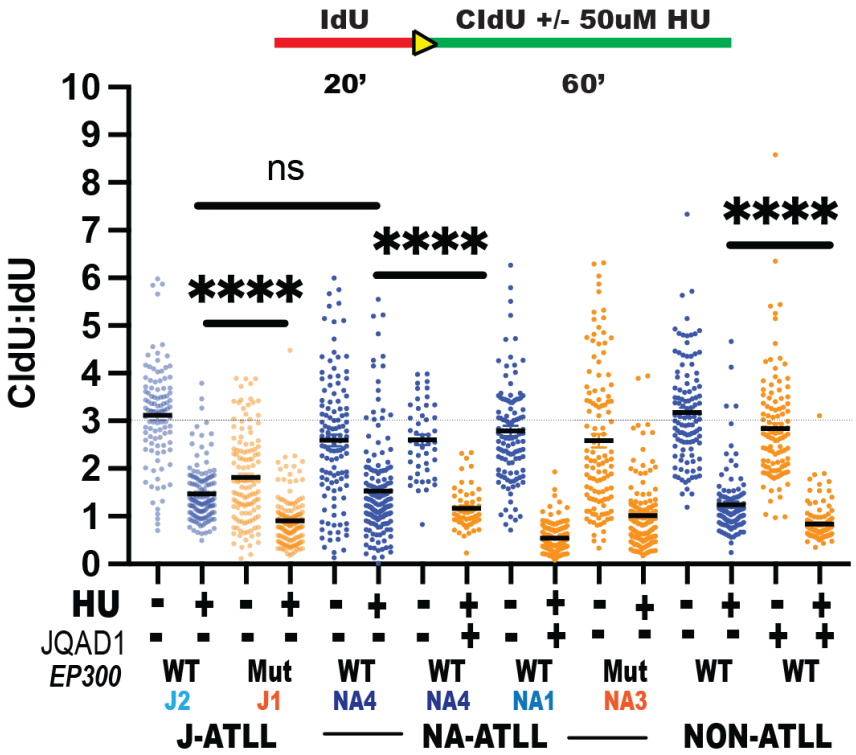
EP300-deficient NA-ATLL cells have aberrant replication dynamics at FRA6E



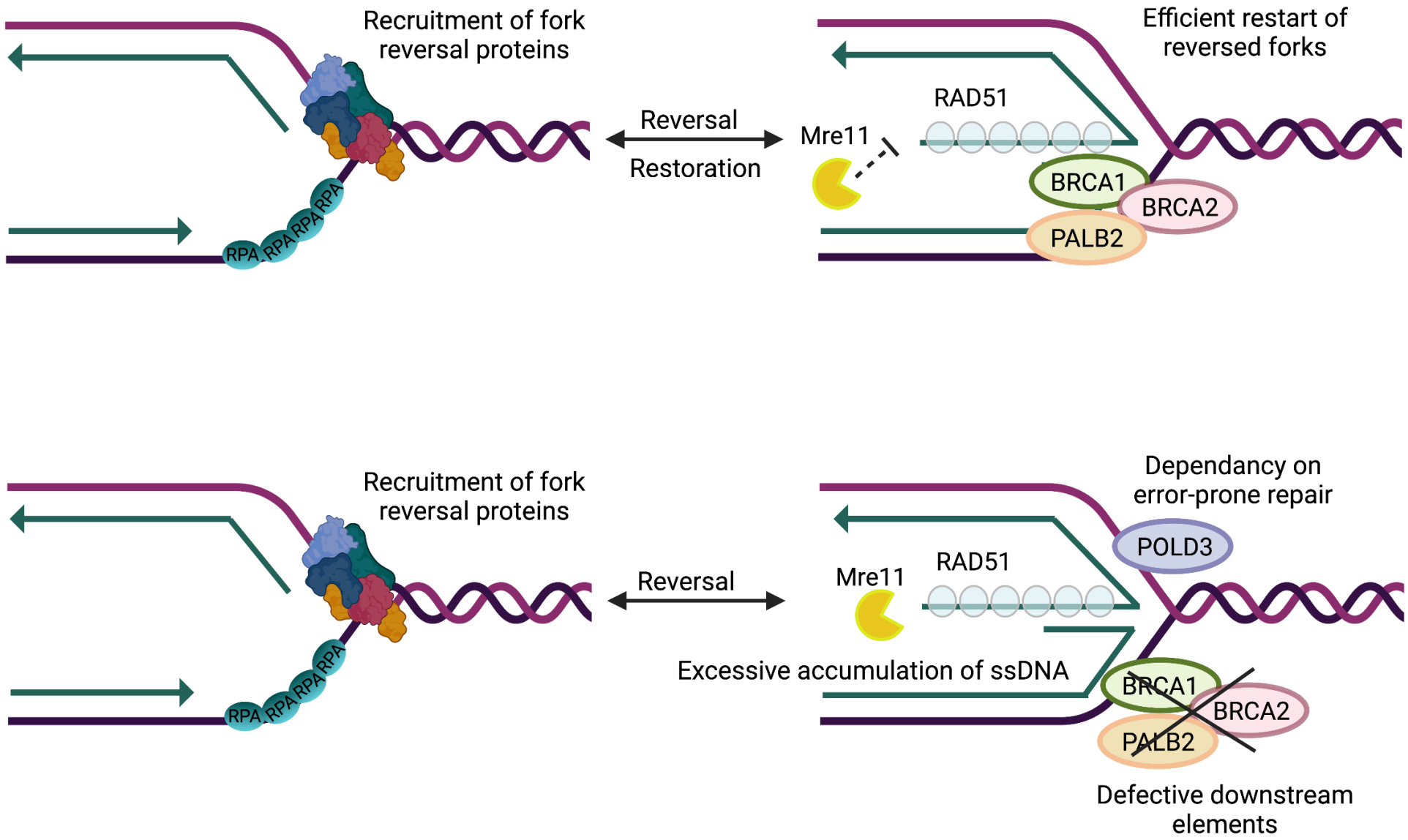
Galvez, AB, Niljikar, M, et. al, Nature Communications, 2025

EP300-deficient NA-ATLL cells display replication fork collapse and nucleolytic degradation

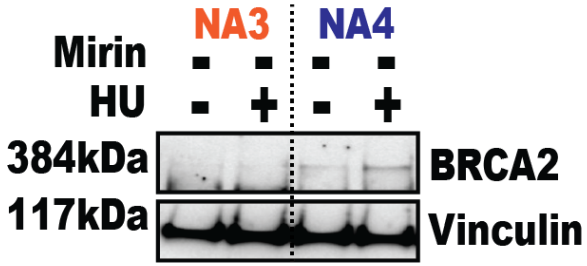


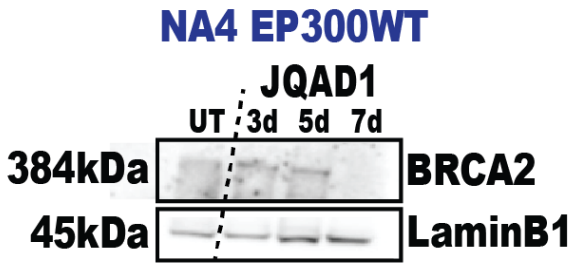


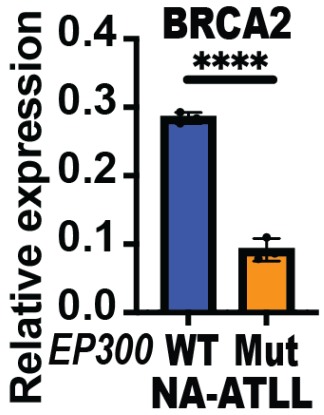
Galvez, AB, Niljikar, M, et. al, Nature Communications, 2025

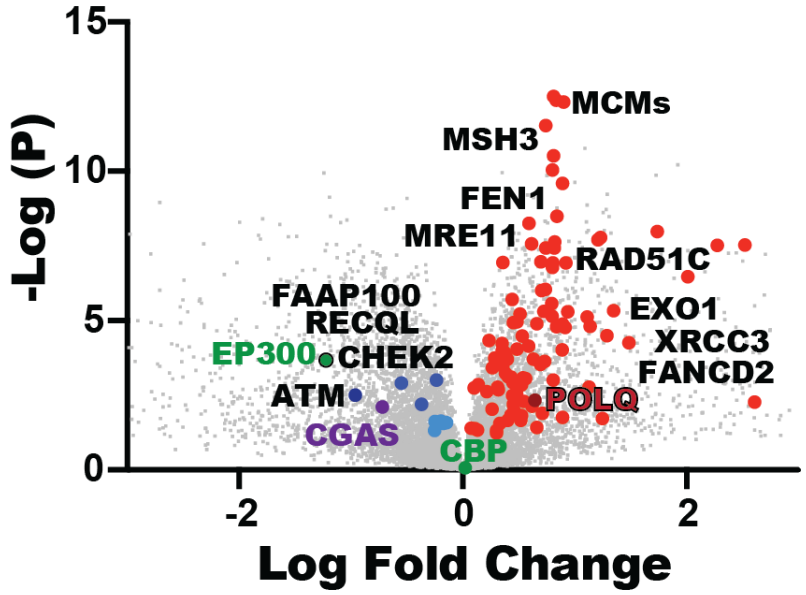

Recruitment of fork reversal proteins
RPA
RPA
RPA
RPA
RPA
Reversal
Restoration
Efficient restart of reversed forks
RAD51
Mre11
BRCA1
BRCA2
PALB2
Recruitment of fork reversal proteins
RPA
RPA
RPA
RPA
RPA
Reversal
Dependancy on error-prone repair
POLD3
Mre11
RAD51
Excessive accumulation of ssDNA
BRCA1
BRCA2
PALB2
Defective downstream elements

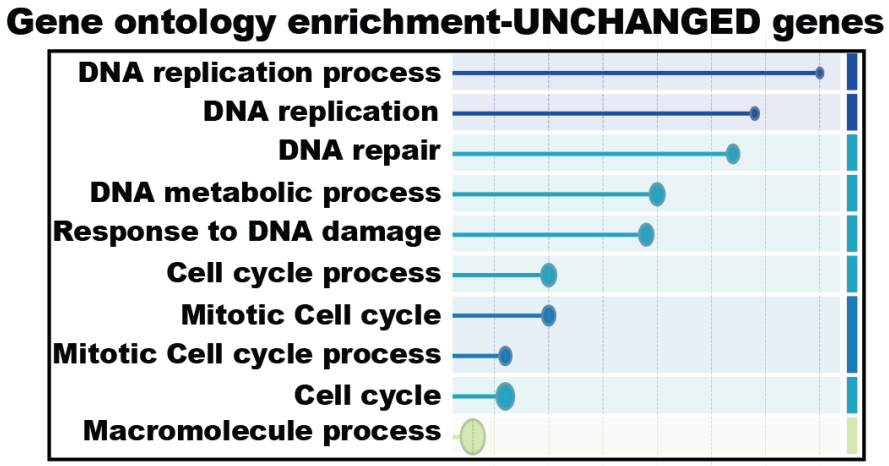
EP300-mutated cells have a BRCA2 deficiency and a downregulation in other HR and fork restart proteins











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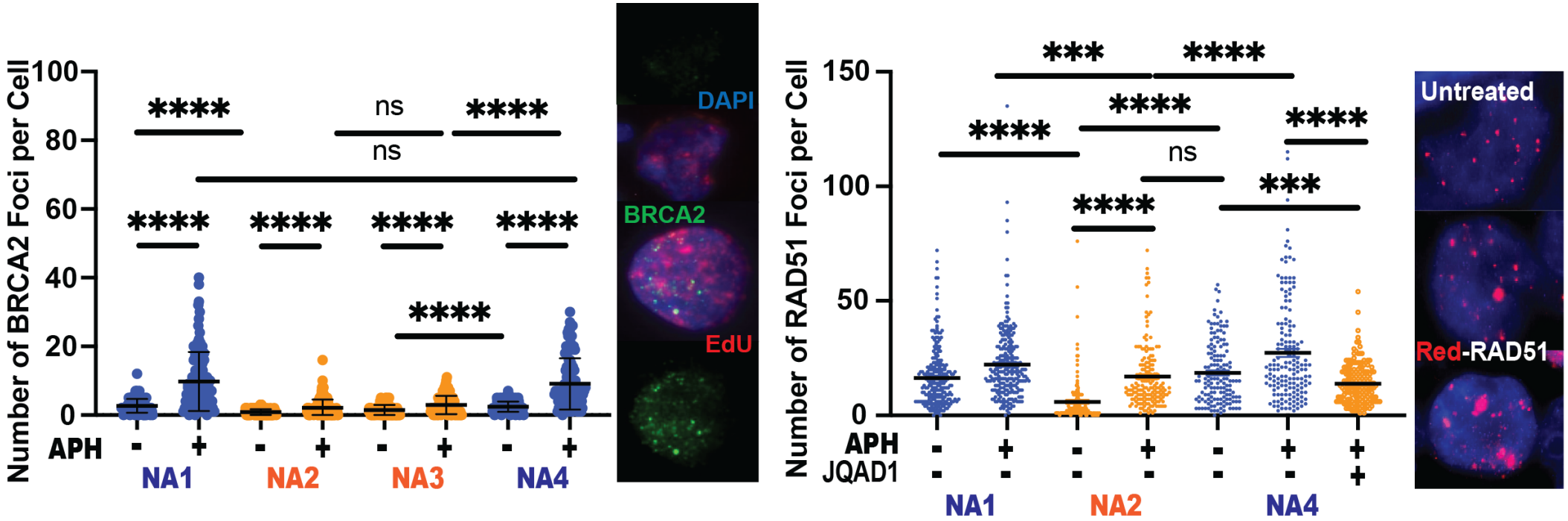
Defect in RAD51 loading is EP300 and BRCA2 associated

BRCA2 protein is a critical mediator of the RPA-RAD51 exchange on single-stranded



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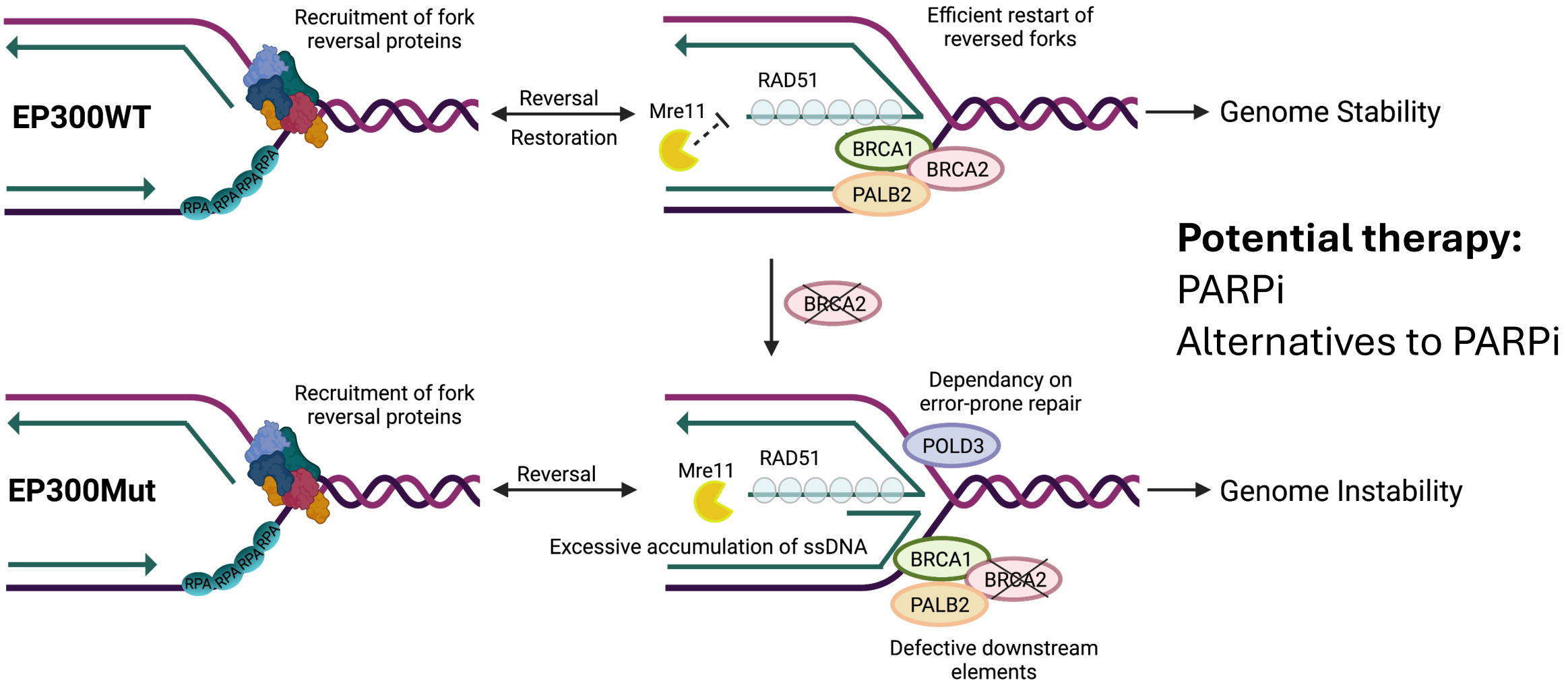
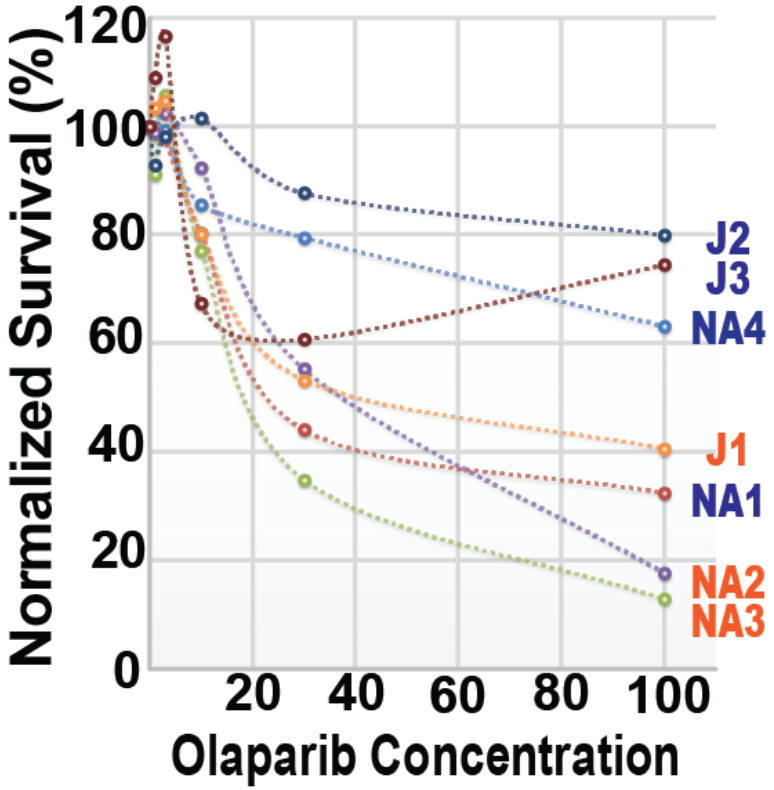
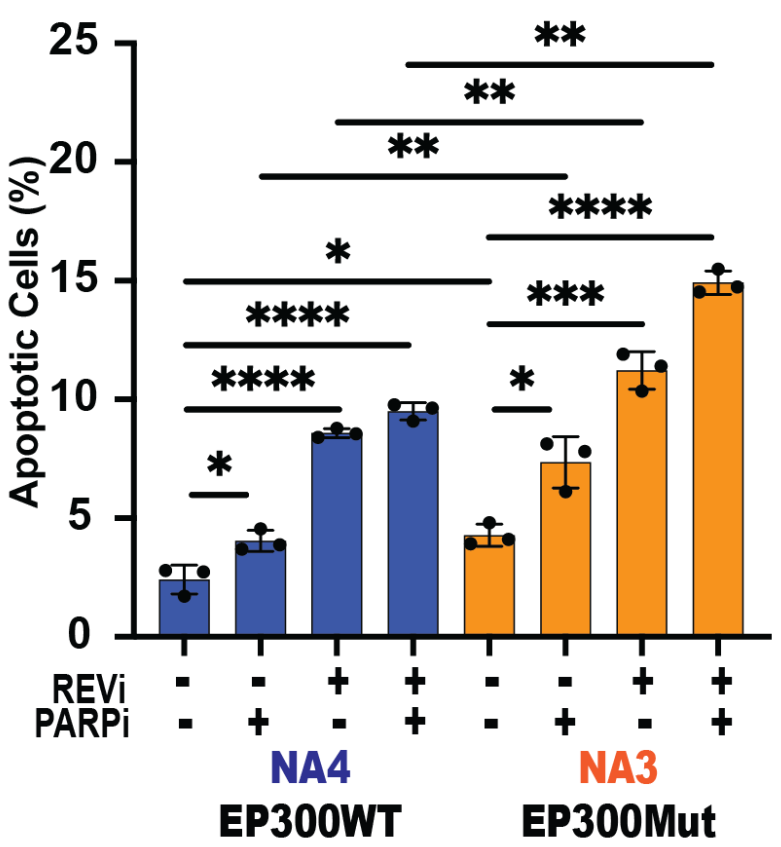
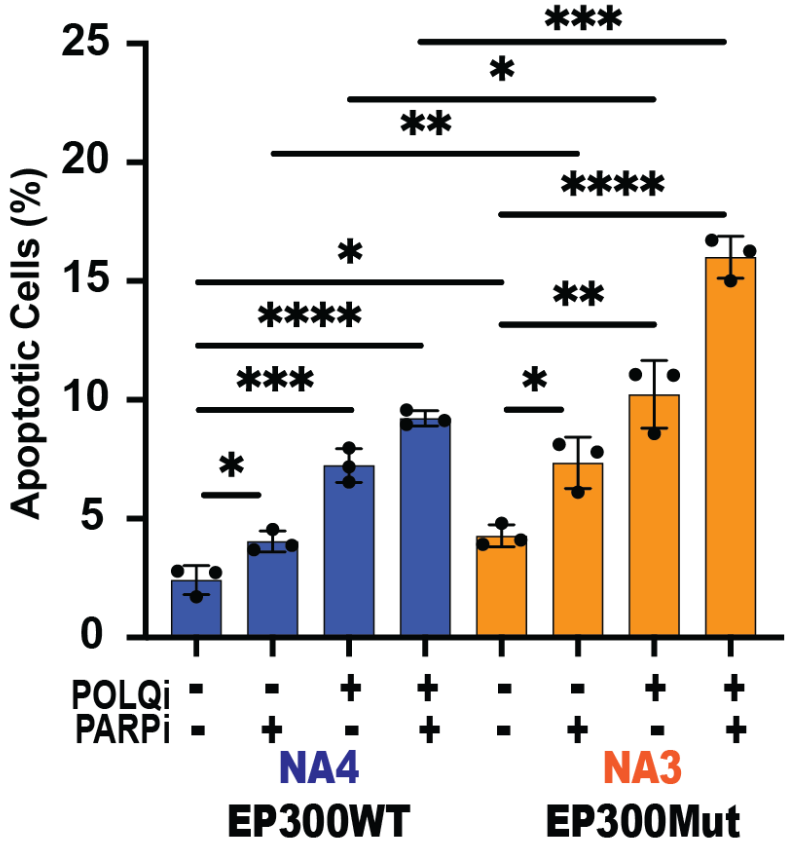
EP300-mutated cells recapitulate features of BRCA-deficient cancers



NA-ATLL cells are sensitive to PARPi (PDX models in progress) and appear to synergize with POLQi and REV1i



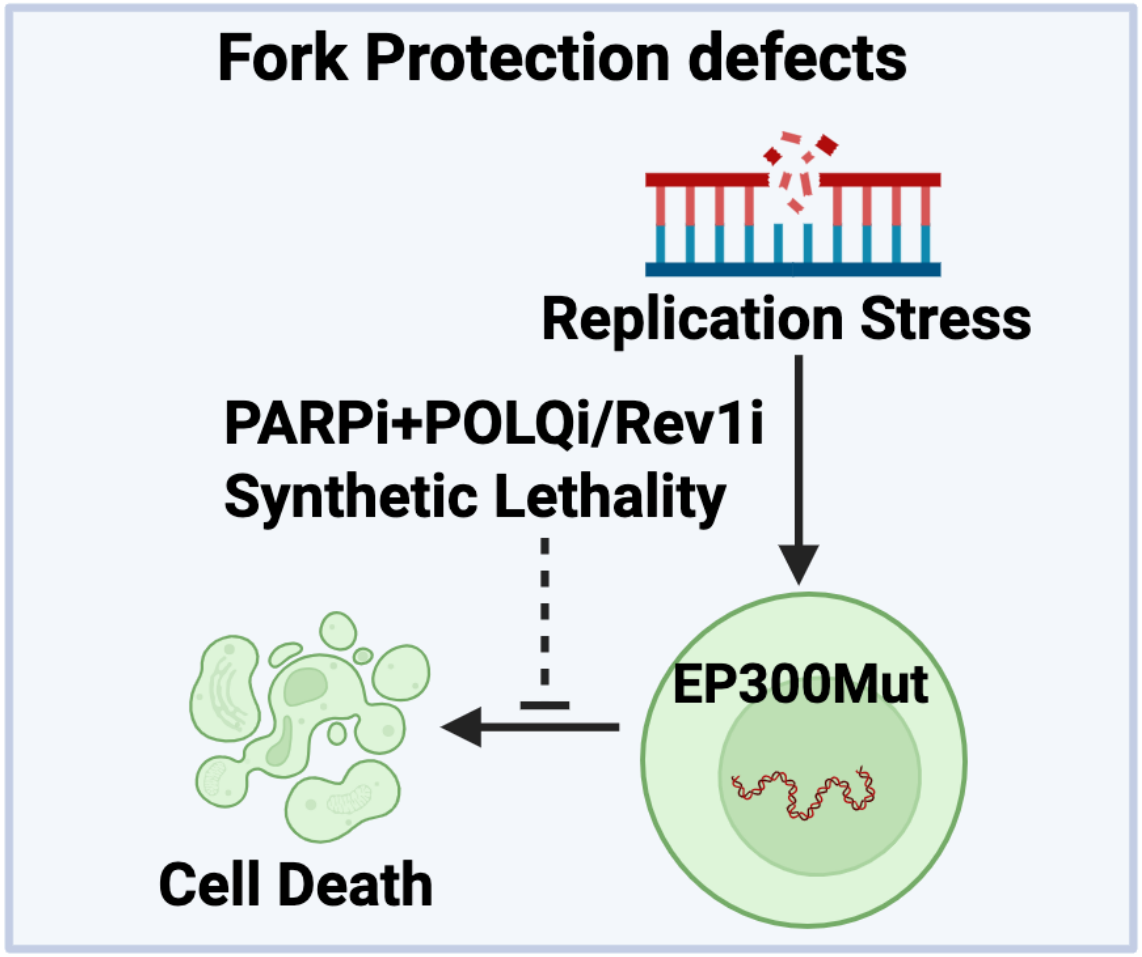




In collaboration with Dr. Hilda Ye

Novel mediators of synthetic lethality in EP300Mut ATLL cells

Summary and working model



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- Dr. Jian Cao (Rutgers CINJ)
- Dr. Cristina Montagna (Rutgers CINJ)
- Dr. Cristina Glytsou (Rutgers Piscataway)

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Center for Pediatric Cancer and Blood Disorders

RUTGERS Cancer Institute of New Jersey

American Cancer Society

NIH National Cancer Institute

Congressionally Directed Medical Research Programs CDMRP Department of Defense

NJCCR New Jersey Commission on Cancer Research

IRF4 drives inflammasome and suppresses pyroptosis in ATLL

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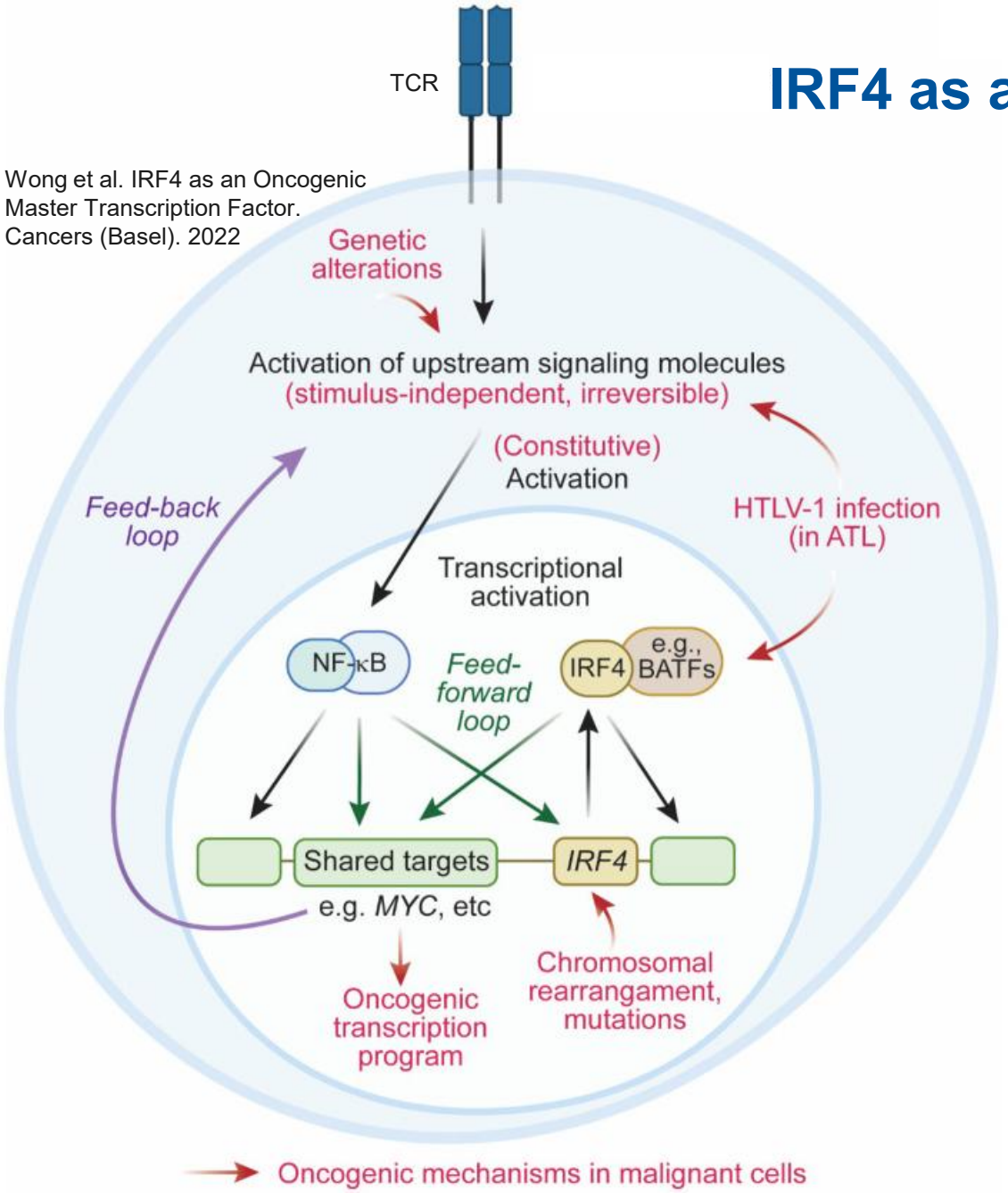
IRF4 as a Prime Driver of ATLL



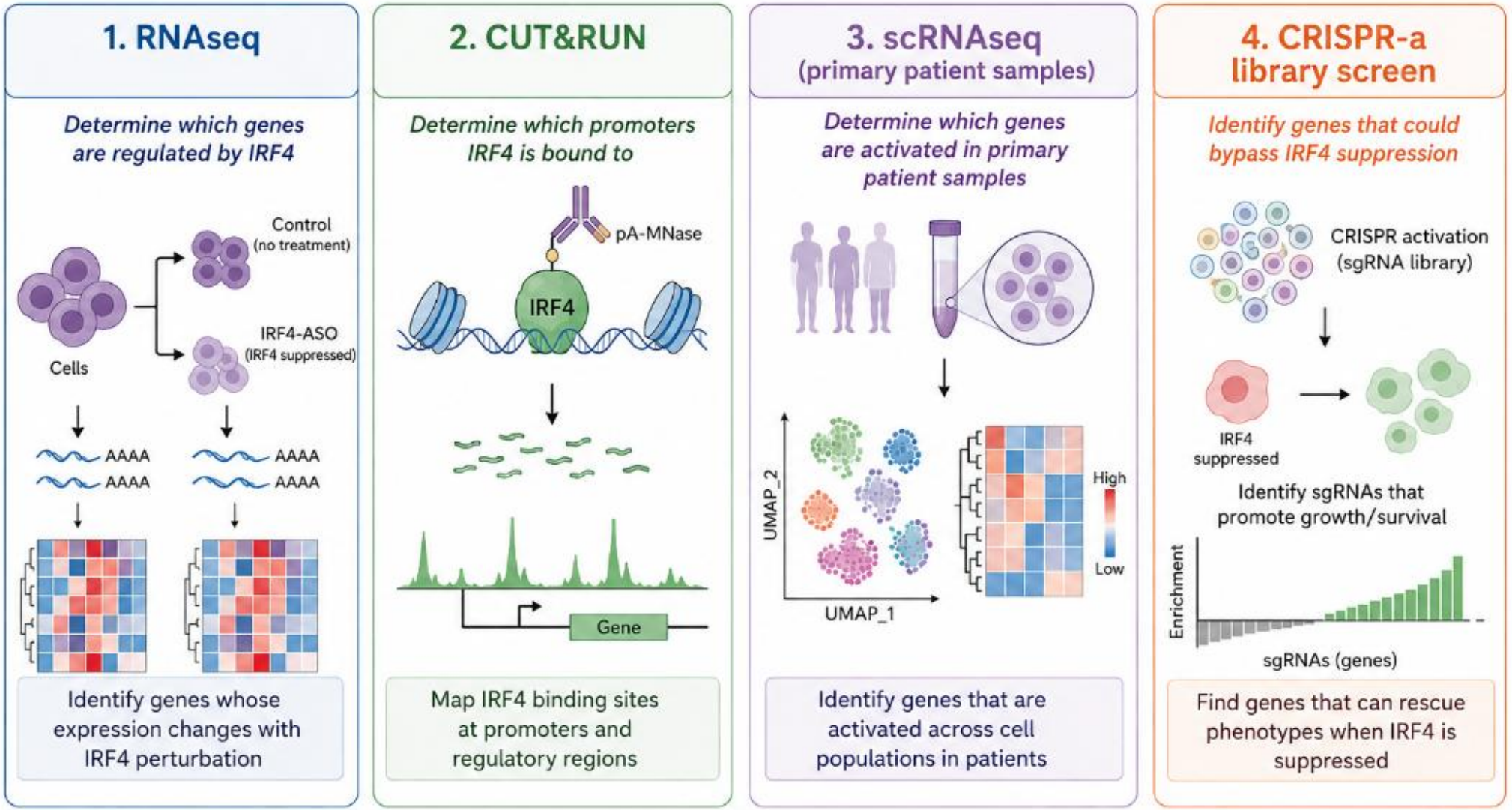
Wong et al. IRF4 as an Oncogenic Master Transcription Factor. Cancers (Basel). 2022

Expressed in ATLL
- frequently amplified / somatic gain of function
- drives constitutive TCR signaling
- feed-forward loop with NFκB
- feed-back loop with MYC

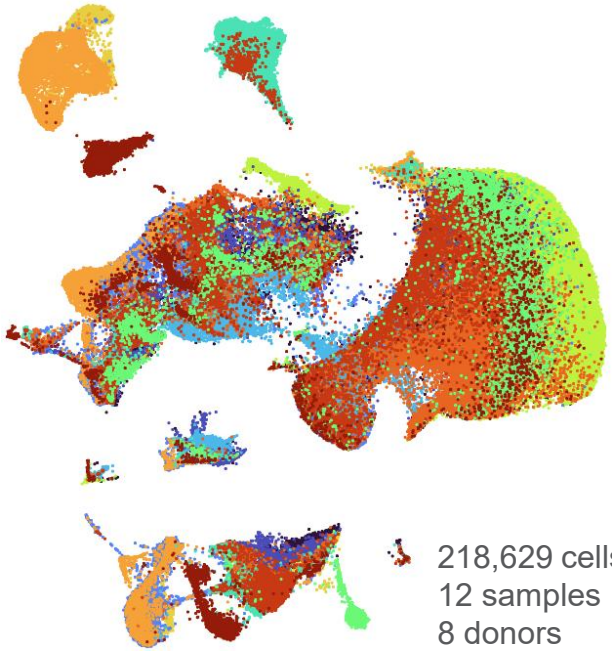
Roles of IRF4
- In T cells
  - Subset differentiation
  - Effector Function
  - Exhaustion, Tolerance
  - Maintenance of memory cells
- Signaling Pathways
  - TCR: Downstream of PLCG1, CARD11, PKC
  - NFκB: Co-occupies with p65 (esp at super enhancers)
  - MYC: Not just in ATLL but also in ALCL, MM
    - Drives aerobic glycolysis and oxidative phosphorylation, proliferation, ribosome biogenesis, survival

IRF4-ASO sensitivity is TAX-dependent

Multi-omic Investigation of IRF4 in TAX^{-} ATLL



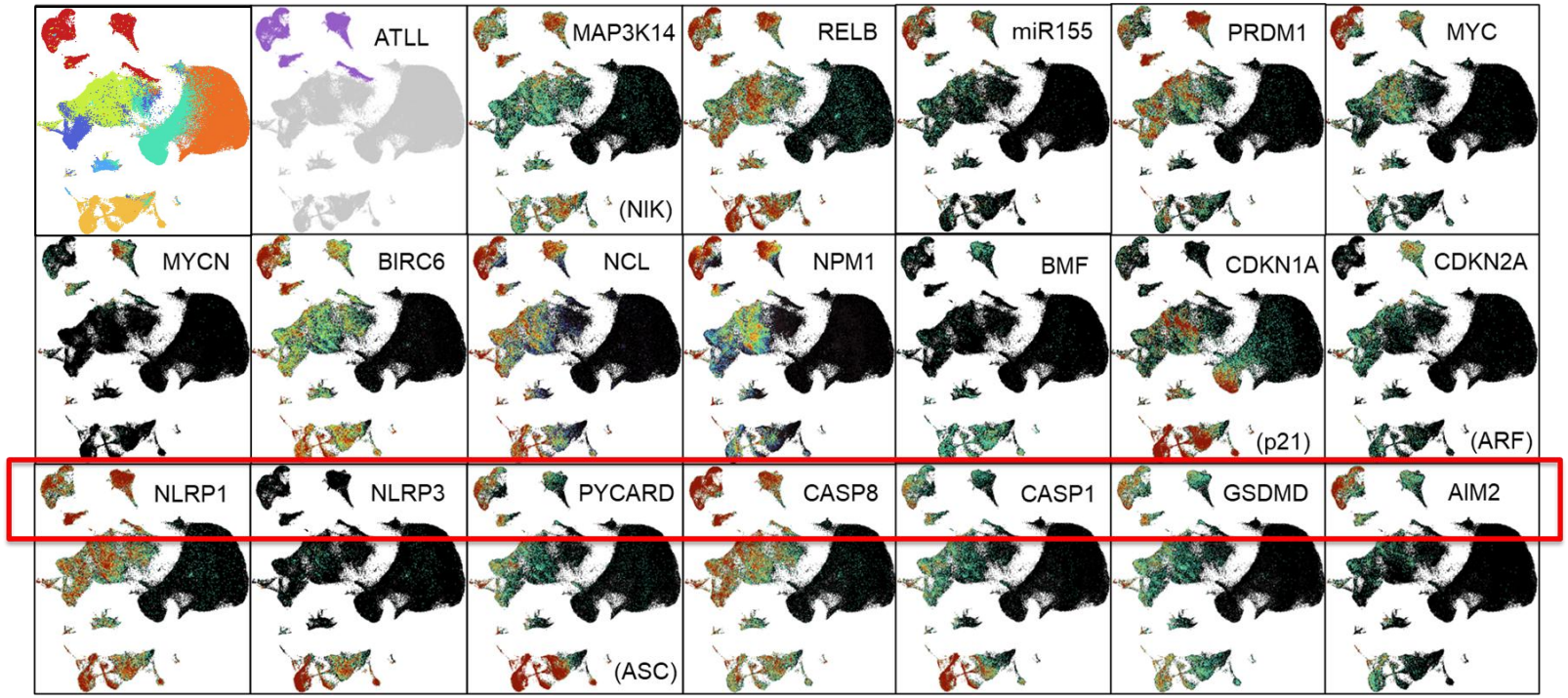
scRNAseq: The Inflammasome is Primed in Acute ATLL Cells

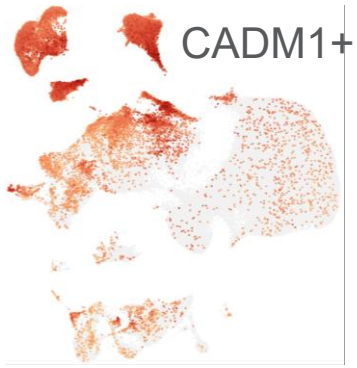


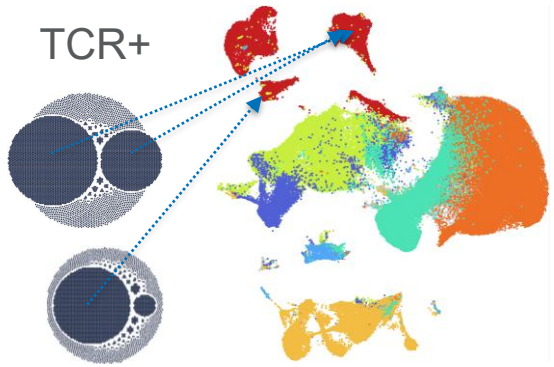
218,629 cells
12 samples
8 donors

ATL01B (40040)
ATL02B (33007)
ATL03B (22972)
ATL04B (6216)
ATL04CR (19679)
ATL04PD (16163)
ATL05B (14581)
ATL06B (26024)
ATL07B (11871)
ATL07CR (12669)
ATL08B (6659)
ATLRBB (8748)

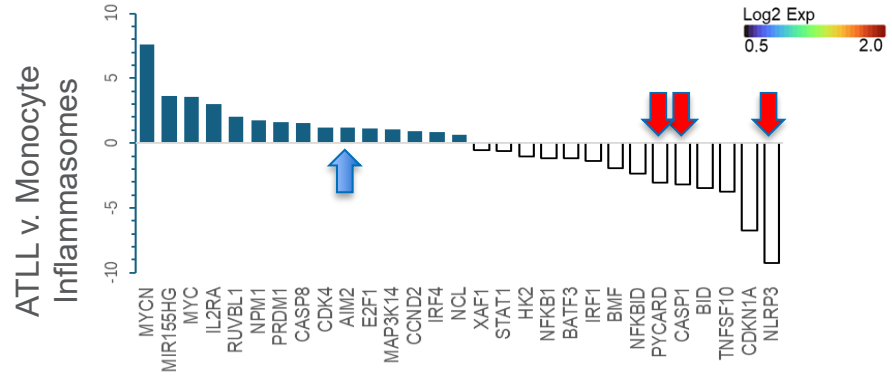
Inflammasome



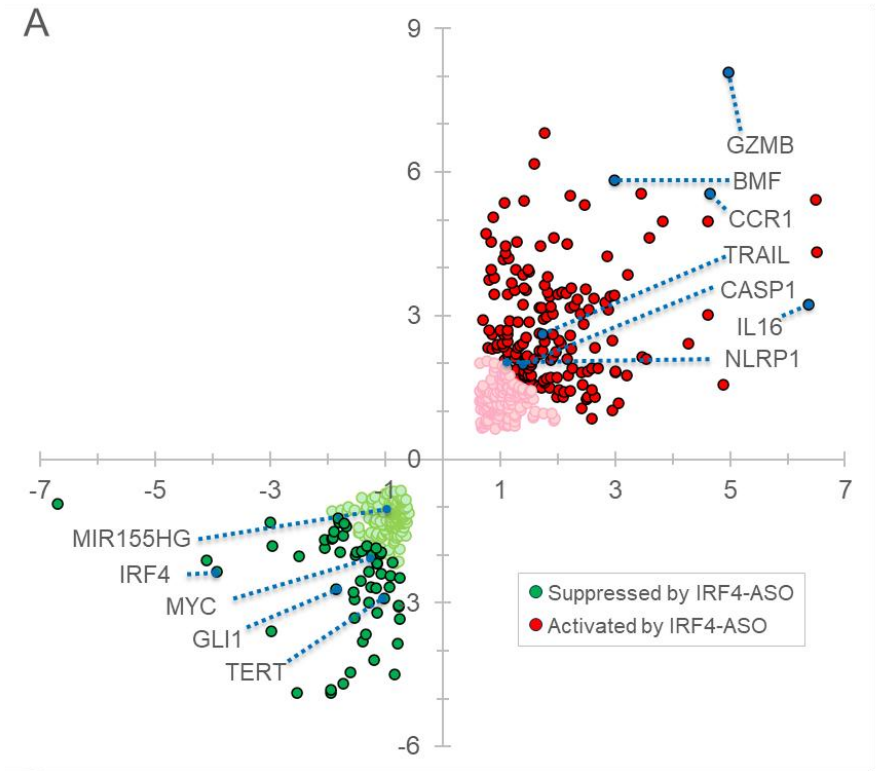


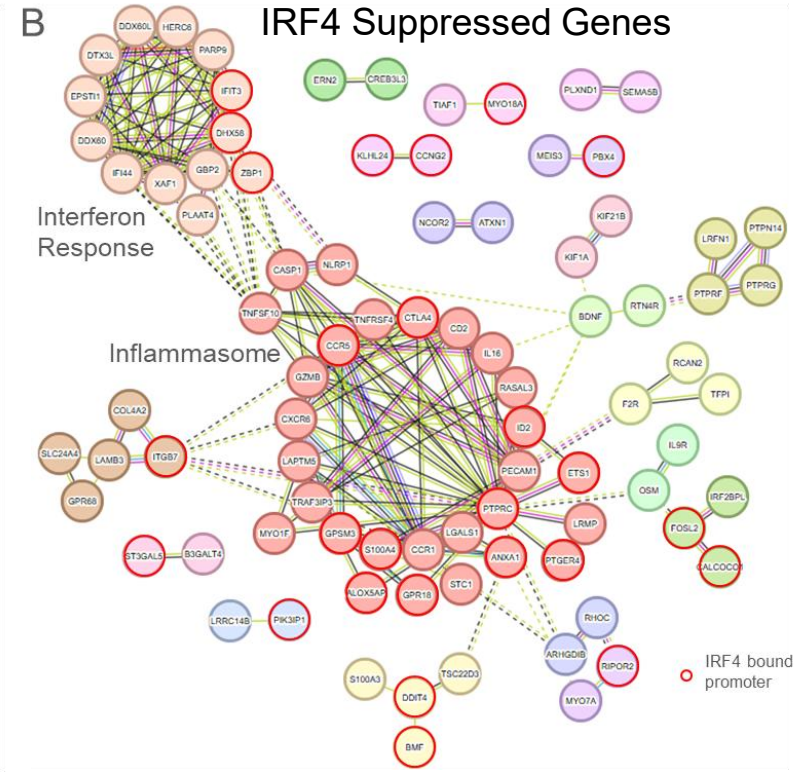


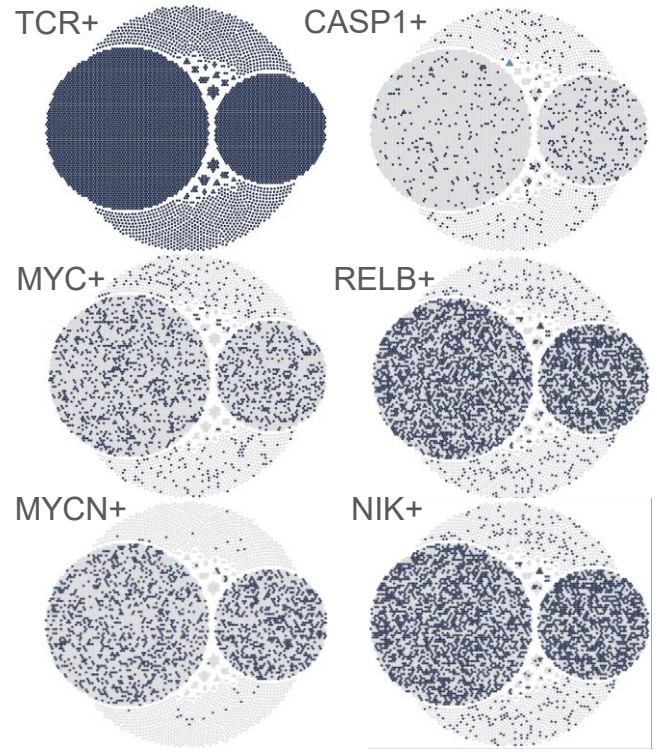
ATLL Clones (26756)
T cells (45181)
NK Cells (9963)
Monocyte (23777)
B cells (3743)
Erythroblast (28958)
Platelets (12856)

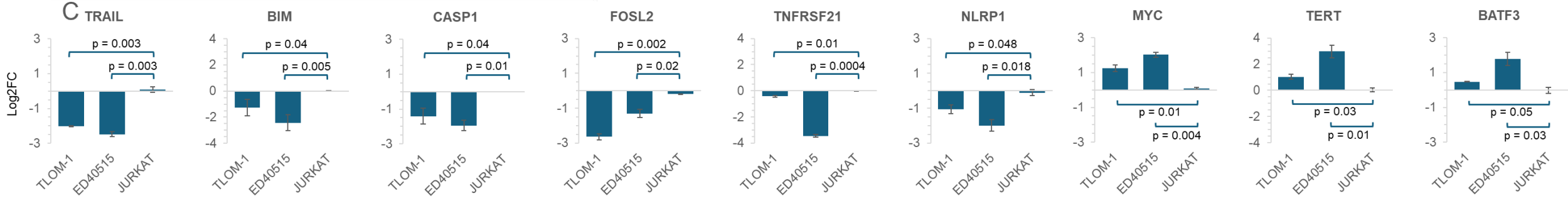


RNAseq: IRF4 Activates NFκB, MYC and. . .Suppresses the Inflammasome

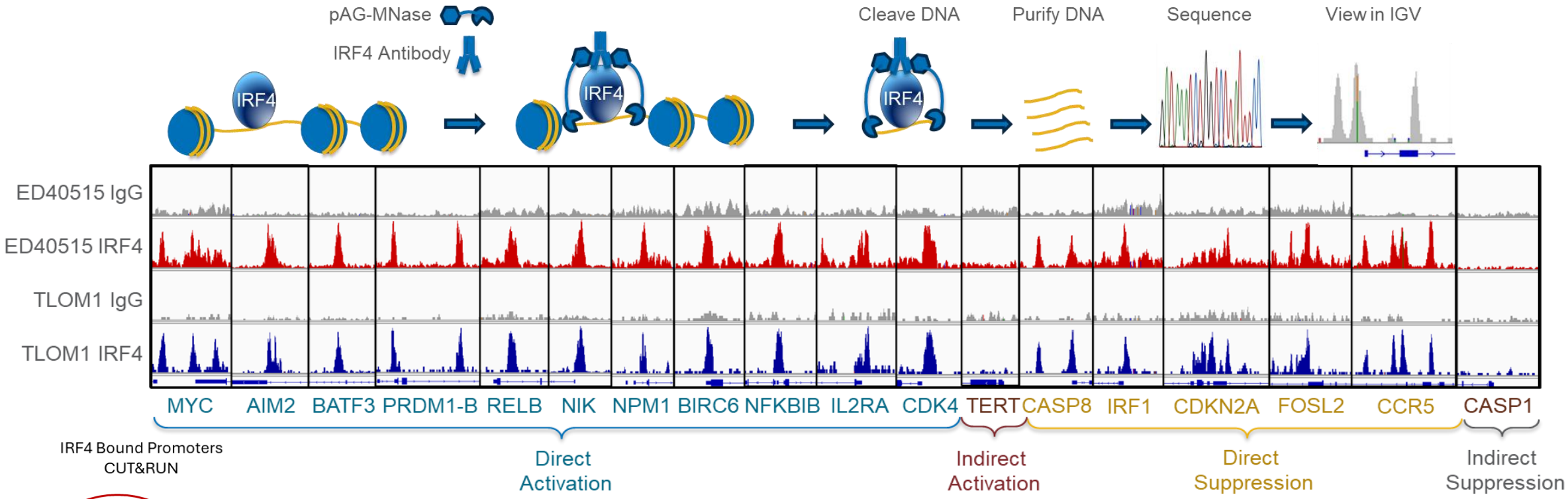




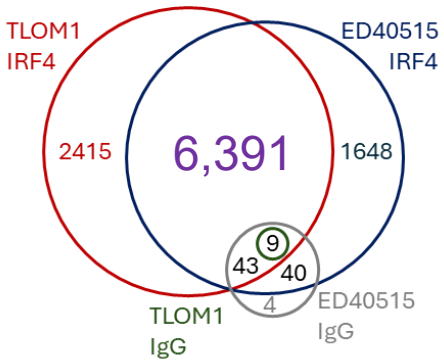




CUT&RUN: IRF4 Directly and Indirectly Regulates ATLL Survival



IRF4 Bound Promoters
CUT&RUN

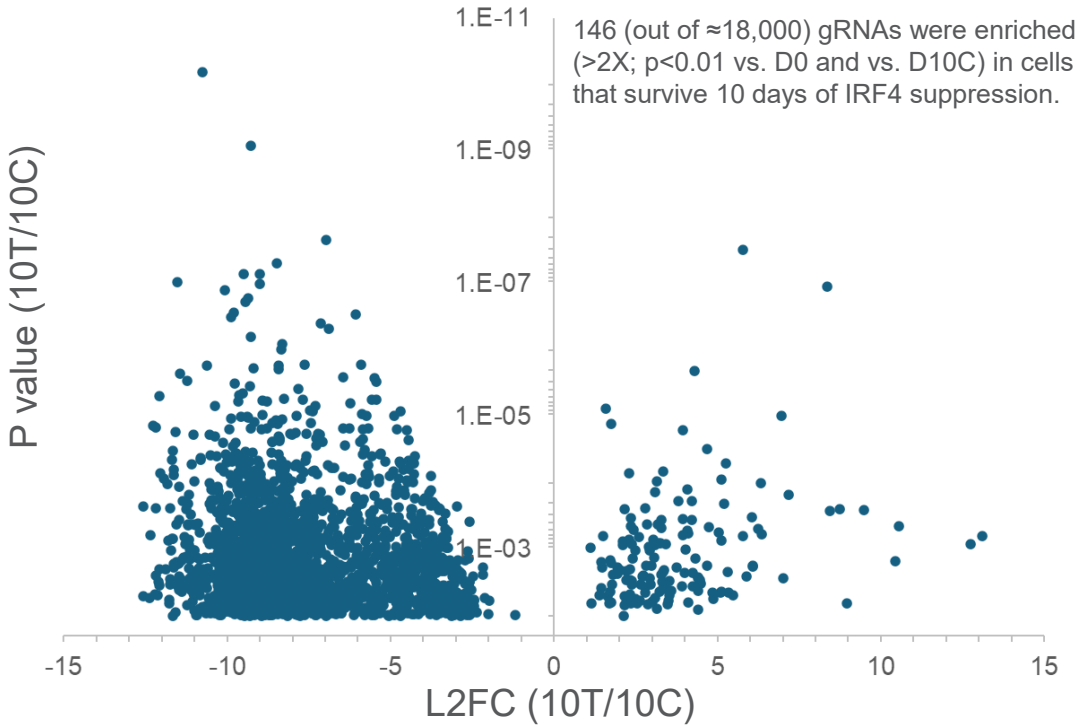


Elements that Form IRF4 heterodimers
AICE: AP1 Family (JUNB, BATF3)
EICE: ETS Family (SPI1, SPIB)
ISRE: IRF Family (IRF1, IRF2, IRF4, IRF8)
ZICE: Zinc Finger Family (IKZF1)
NFKB: NFKB Family (RELA, RELB)
GAS: STAT Family (STAT3, STAT6)

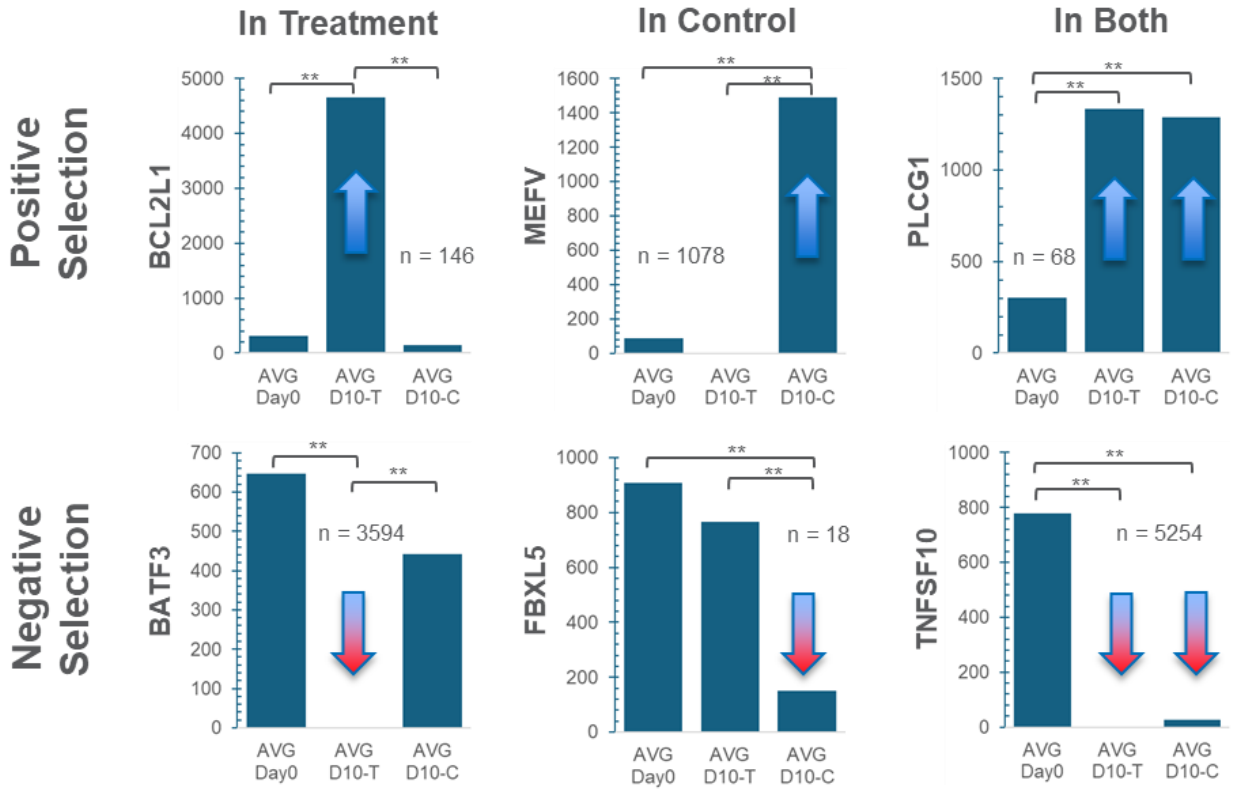
213 IRF4 Activated Genes
(66 with IRF4 at promoter)

320 IRF4 Suppressed Genes
(88 with IRF4 at promoter)

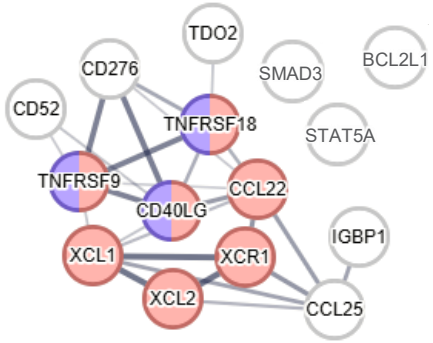
CRISPR-A: IRF4 as an Incoherent Feed-Forward Loop (IFFL)



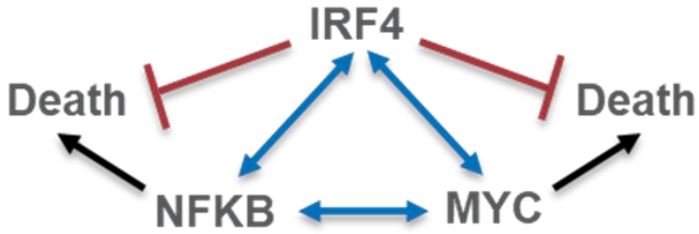
146 (out of ≈18,000) gRNAs were enriched (>2X; p<0.01 vs. D0 and vs. D10C) in cells that survive 10 days of IRF4 suppression.



IRF4 Bypass Cluster

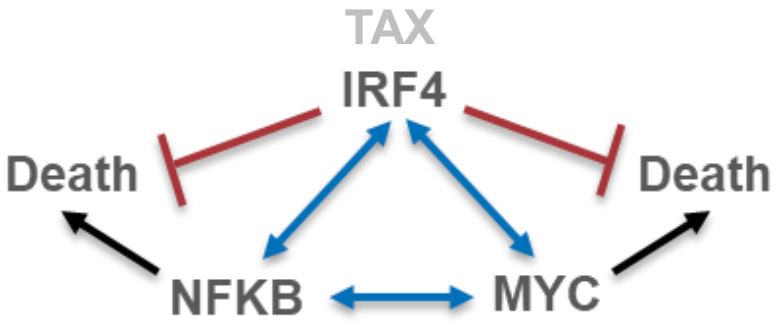


AIM2 Inflammasome
Non-canonical NFκB
BCL-XL Survival



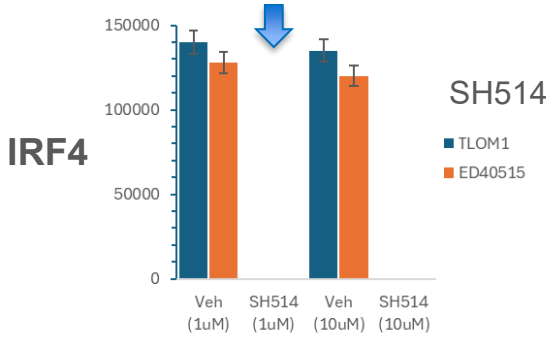
Working Hypothesis: The IRF4 IFFL Replaces TAX IFFLs

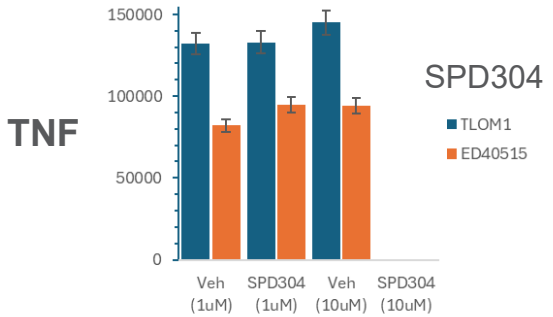
Activation of FAS, FASLG
Activation of TRAIL, TRAILR
Activation of BIM, PUMA
Activation Induced Cell Death (AICD)
TNF, Necroptosis
IRF1,3; Inflammasome; Pyroptosis

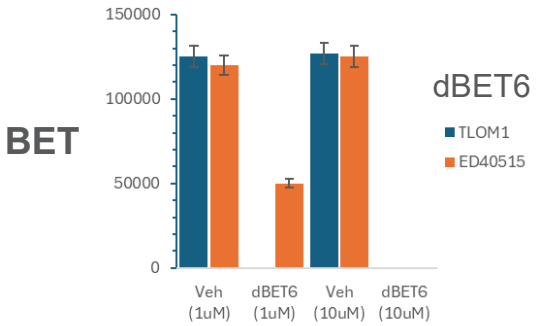


Activation of ARF, TP53
Activation of BIM
Suppression of BCL-XL
Activation of Caspases
Cell Cycle Arrest
Oncogene induced Senescence

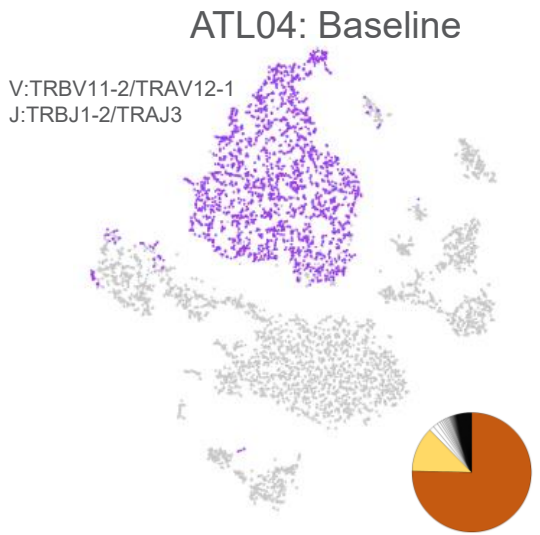
Therapeutics: Targeting the IRF4 Addiction

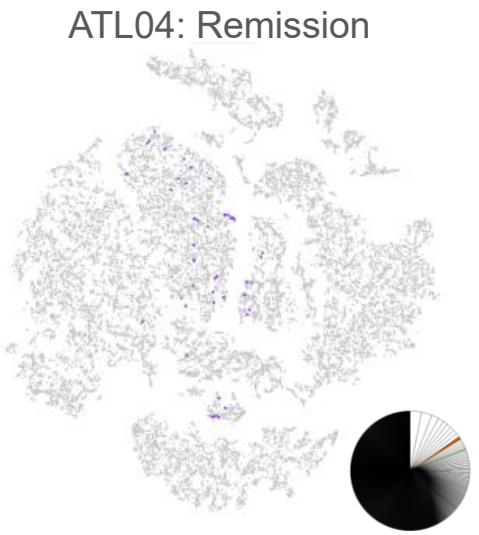


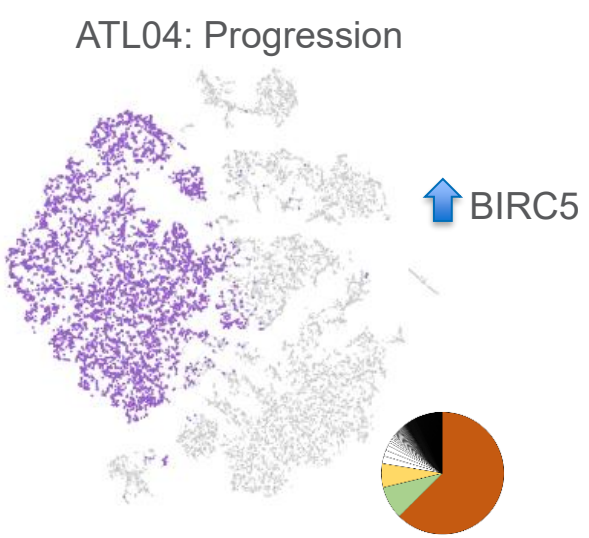


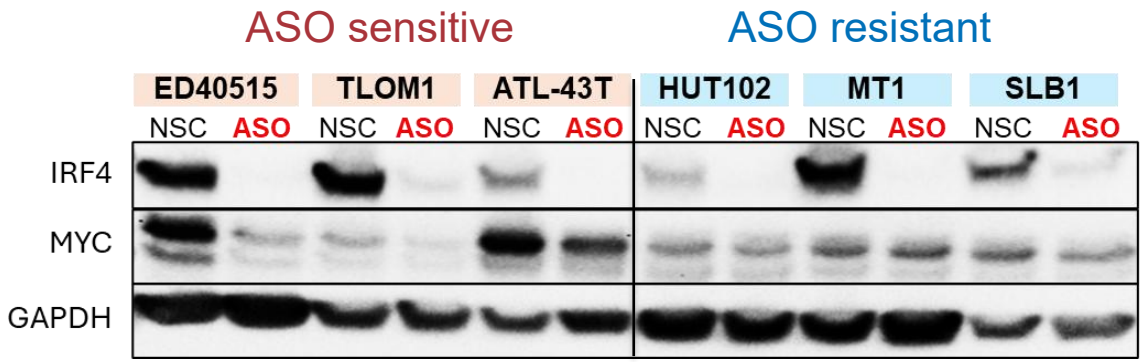


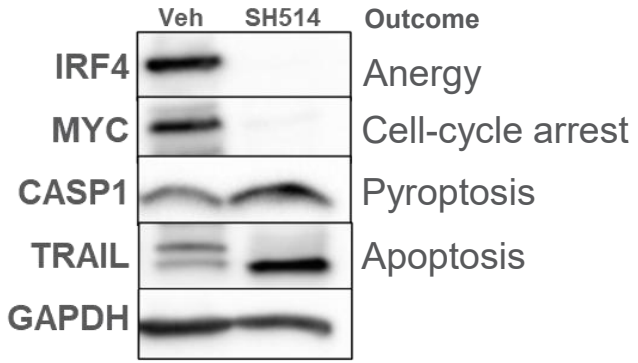
Lenalidomide + EPOCH Clinical Trial (10335)



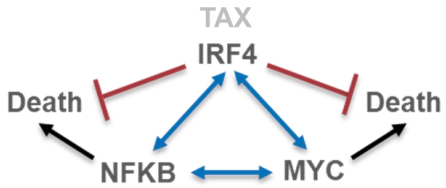








Summary



- The Inflammasome is “primed” in primary acute ATLL
- IRF4 primes the inflammasome and suppresses its activation
- IRF4 activates non-canonical NFKB and MYC
- IRF4 functions as an incoherent feed-forward loop with MYC, NFKB, and downstream death effectors
- IRF4 is part of and may be a substitute for TAX IFFLs
- IRF4 is a critical therapeutic target for ATLL and could cause death by a variety of mechanisms
- Resistance to IRF4 therapy could result from expression of survival factors like BIRC5 or BCL-XL
- In cell lines sensitive to IRF4-ASO, MYC is independent of IRF4. Possibly the result of TAX bypass
- A small molecule IRF4 inhibitor (SH514) is promising in pre-clinical evaluations

Acknowledgments

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