Novel aspects of the Australian HTLV-1c stains.

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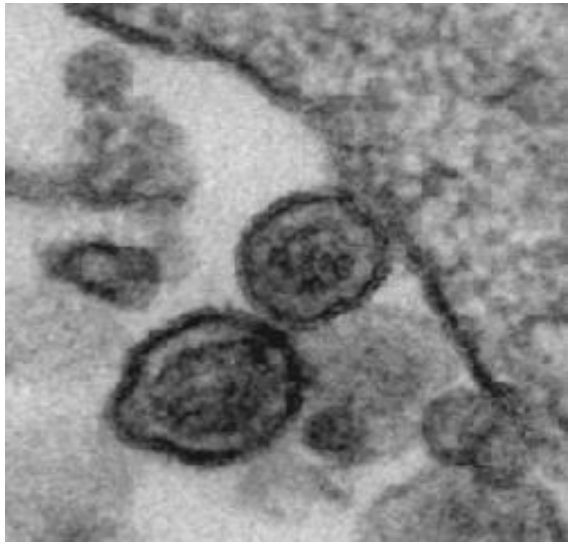
17. 09. 2019

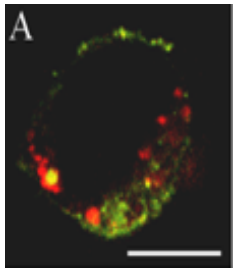
Doherty Institute
THE UNIVERSITY OF MELBOURNE
The Royal Melbourne Hospital

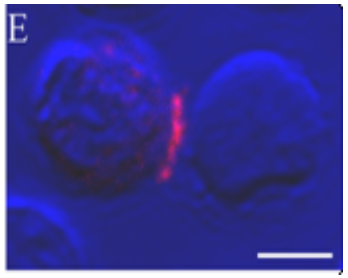
A joint venture between The University of Melbourne and The Royal Melbourne Hospital

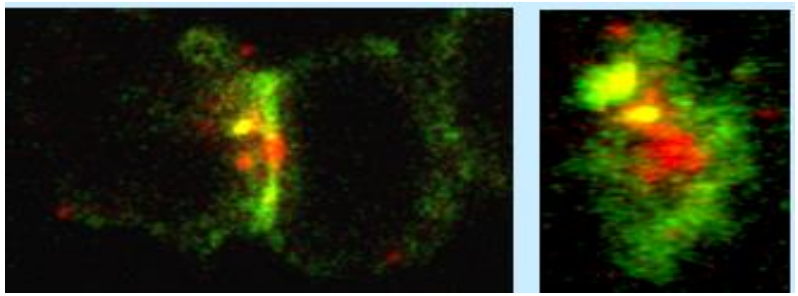
Human T-cell lymphotropic / leukemia virus (HTLV-1)

- First described Retrovirus of humans (1980)
- 10 – 15 Million infections globally
- Primarily targets T-cells ($CD4^+$ and CD8+)
  - Can infect other cells
  - B-cells, monocytes, DCs, myeloid cells, endothelial cells
- Transmission: blood, sexual, breastmilk
- Infects primarily by cell-cell contact
- Lifelong infection that invades host DNA



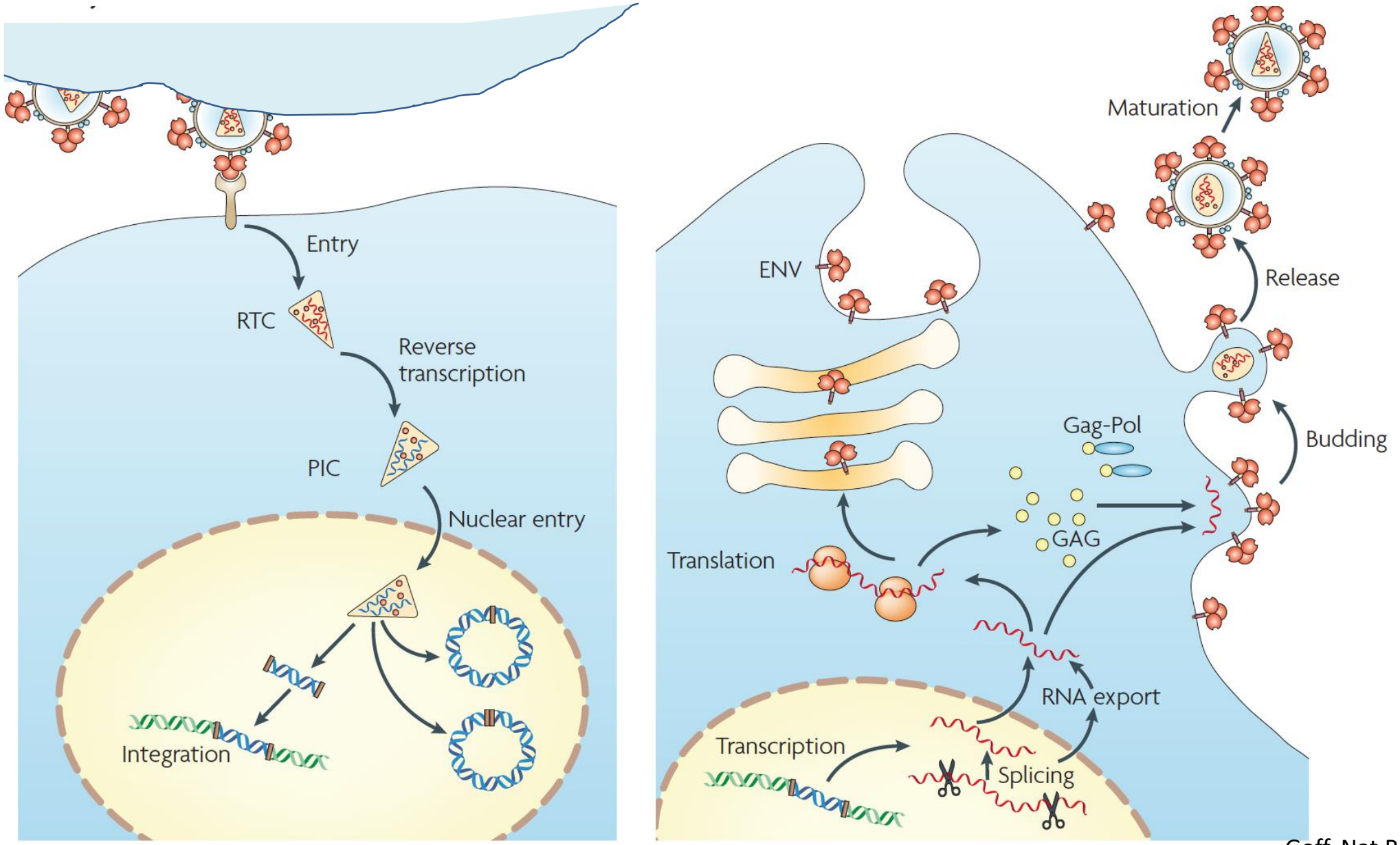






Igakura et al 2003

HTLV-1: an RNA virus that mostly exists in cellular DNA



Goff. Nat.Rev.Micro: 2007

HTLV-1 associated diseases

ATL

Adult T-cell leukemia / lymphoma,

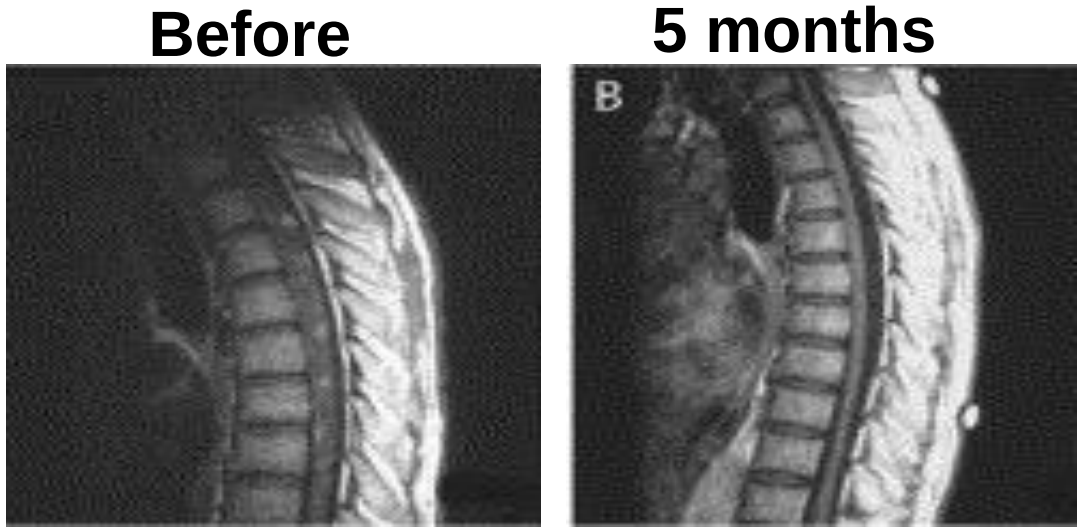
- median survival < 1year
- 5% develop ATL
- resistant to chemotherapy

HAM

HTLV-1-associated myelopathy

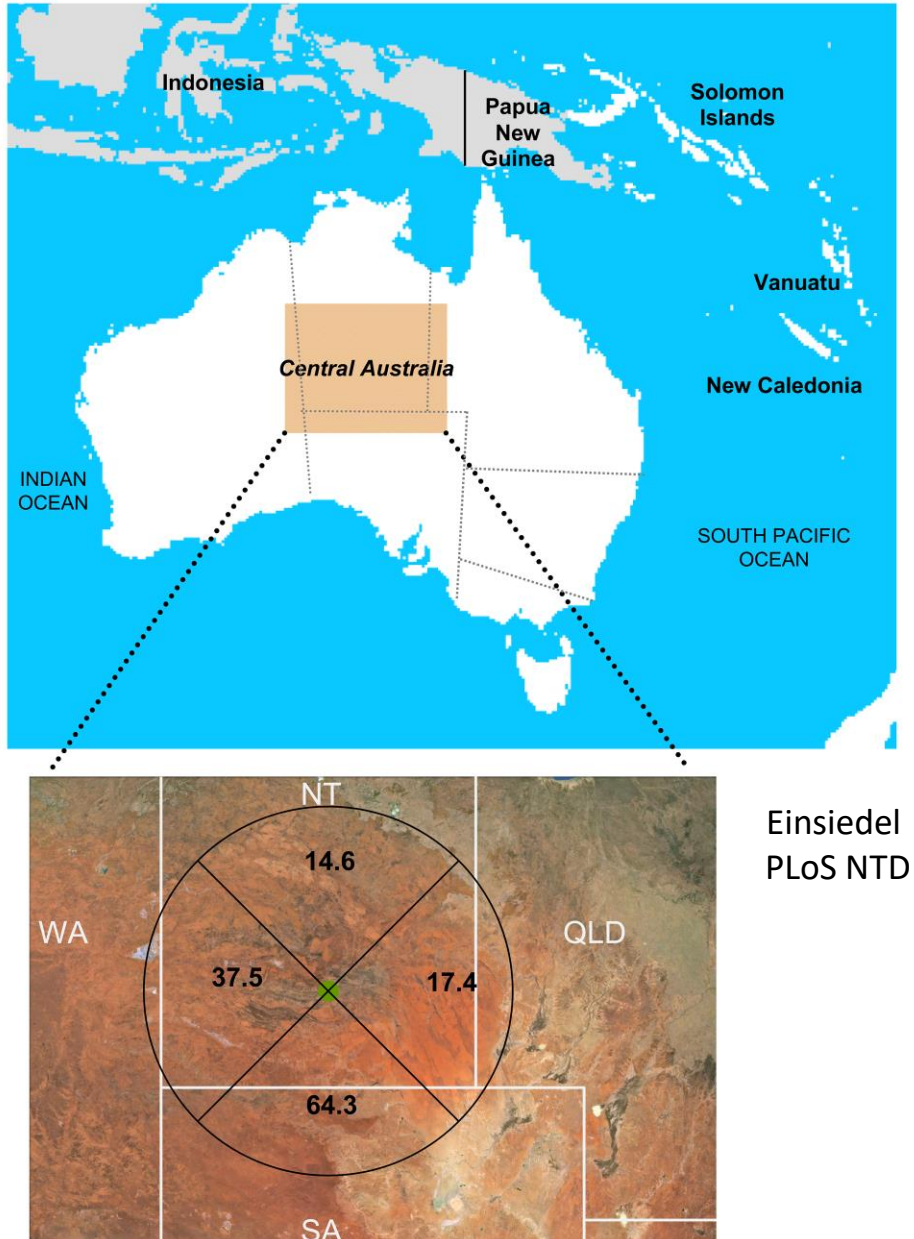
- 4% develop HAM / TSP
- no effective therapy
- Spasticity / paralysis of lower extremities
- bladder dysfunction
- constipation
- impotence

Kao et al., 2015 Derm Sinica



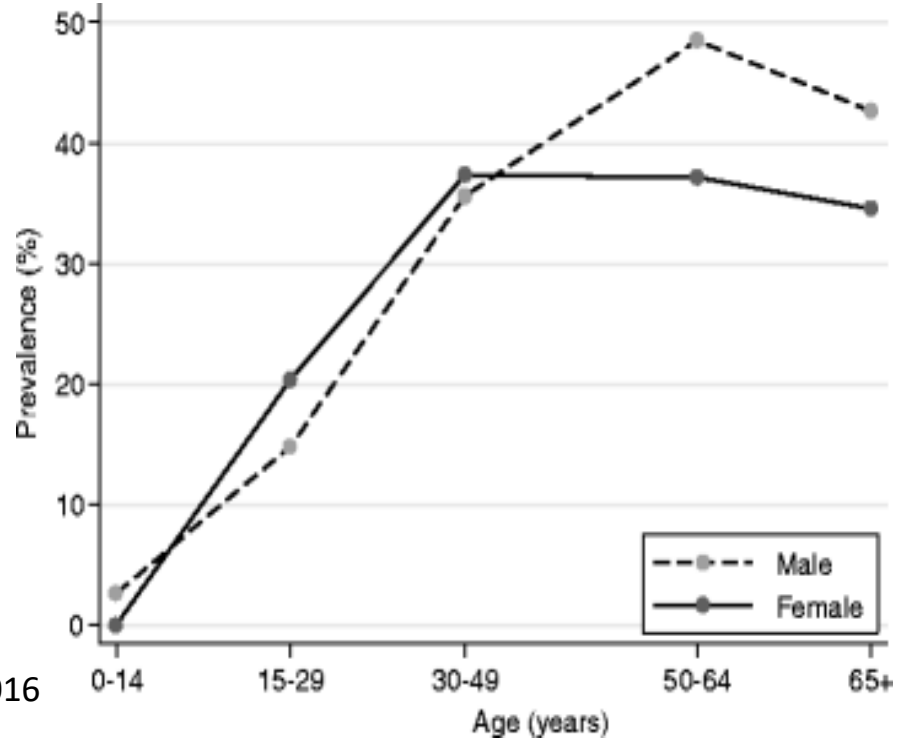
Araujo and Silva, 2006, Lancet Neurology

HTLV-1c: High prevalence in remote central AUS



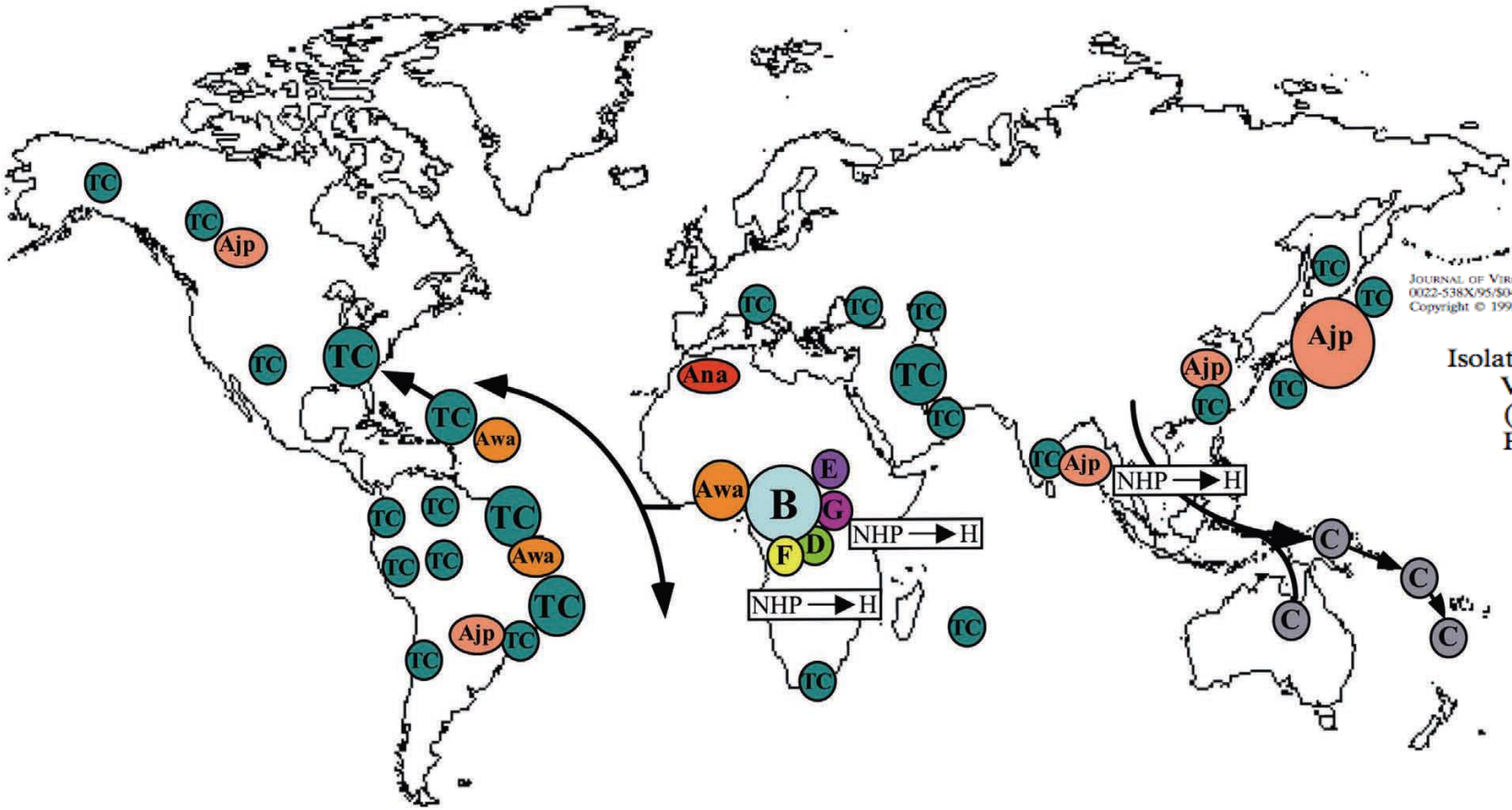
Einsiedel et al
PLoS NTD 2014

- 43% prevalence in Alice Springs Hospital survey *Einsiedel and Woodman, MJA 2010*
  Einsiedel et al., PLoS NTD 2014
- 40% prevalence in a prospective community based survey *(Einsiedel et al. MJA 2016)*
  > 60% in some communities *(Einsiedel, PLOS NTD 2018)*



Einsiedel L., et al.,
BMC Public Health, 2016

Origin: ancient primate transmission & movement of infected persons.



JOURNAL OF VIROLOGY, Nov. 1995, p. 6980–6993
0022-538X/95/$04.00+0
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Vol. 69, No. 11

Isolation and Characterization of a New Simian T-Cell Leukemia Virus Type 1 from Naturally Infected Celebes Macaques (*Macaca tonkeana*): Complete Nucleotide Sequence and Phylogenetic Relationship with the Australo-Melanesian Human T-Cell Leukemia Virus Type 1

FERA IBRAHIM, GUY DE THÉ, AND ANTOINE GESSAIN*
Unité d'Epidémiologie des Virus Oncogènes, Institut Pasteur, 75728 Paris Cedex 15, France

TC Transcontinental Subgroup
Ajp Japanese Subgroup
Awa West African Subgroup
Ana North African Subgroup

Cosmopolitan Subtype A

B Subtype B
C Subtype C
D Subtype D
E Subtype E
F Subtype F
G Subtype G

NHP → H STLV-1 Transmission from Non-Human Primates to Humans

Gessain and Cassar: Frontiers in Microbiology, 2012

HTLV-1 is similar to HIV-1, but subtly different.

Property	HIV	HTLV-1
Main immune cell targets	$CD4^+$ T-cells	$CD4^+$ and $CD8^+$ T-cells
Duration of infection	Lifelong	Lifelong
Infectious transmission	Virus particles	Virus infected cells
Effect of infected T-cells	Killed by virus	Proliferation from expressed viral products
Effect on immune function	Immune-deficiency from lack of "$CD4^+$ T-cell help"	Over-active inflammation from "Zombie T-cells"
Chronic immune activation	+++	++++
Tumour induction	Indirect (+)	Direct (+++++)

HTLV-1 undergoes cell-associated transmission and causes expansion of defective immune T-cells

HIV >> HTLV-1

Cell-free virion infection

Reverse Transcription

RT

HTLV-1 > HIV

Cell-associated infection

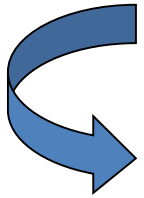
Every infected T-cell differs:

- T-cell receptor for Ag
- Host DNA integration site

Chronic infection +++

Infectious cell-associated viral particles for transmission

Genetic variability



Clonal expansion

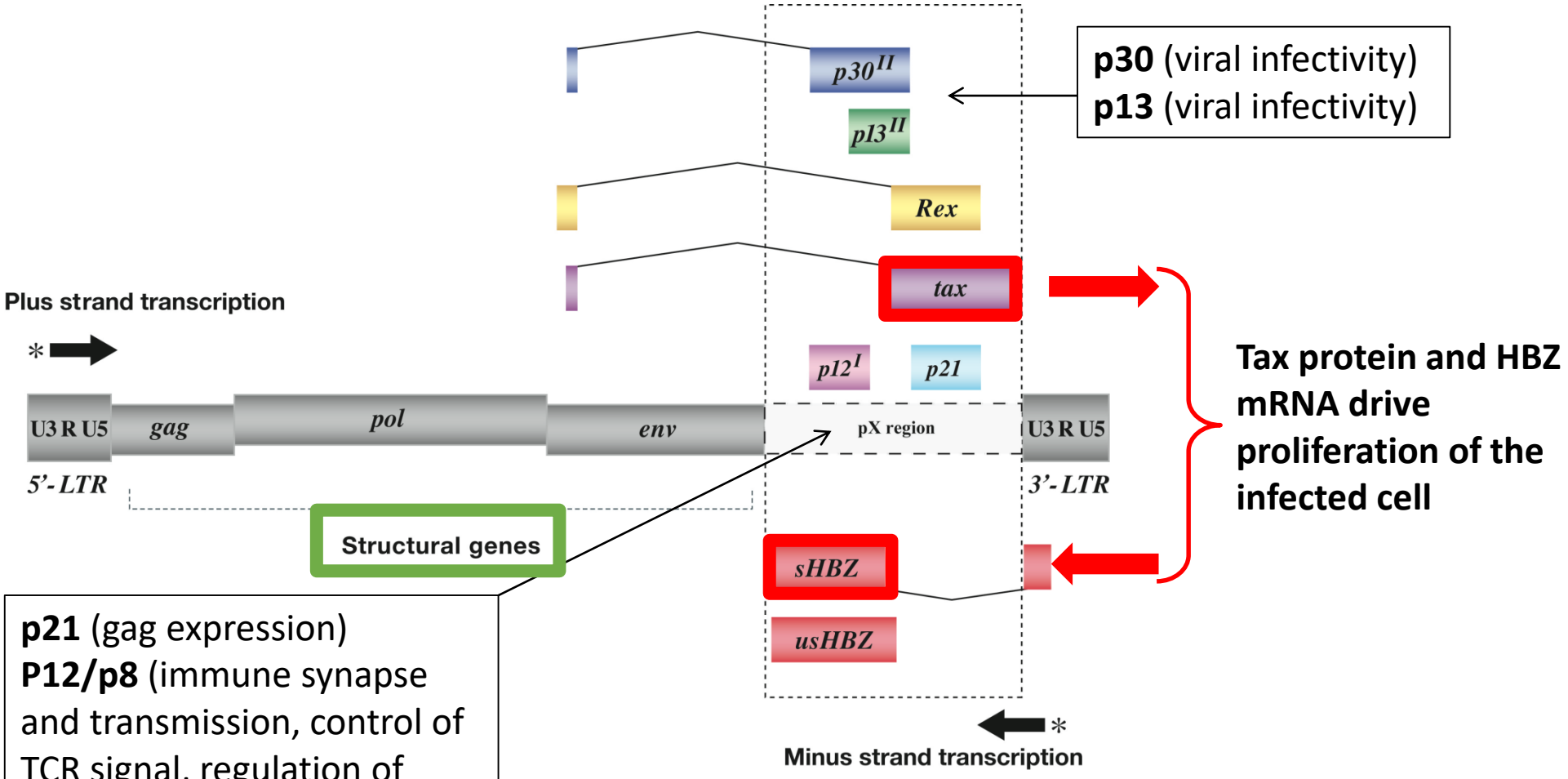
Genetic stability

Different disease outcome from HTLV-1 in each patient

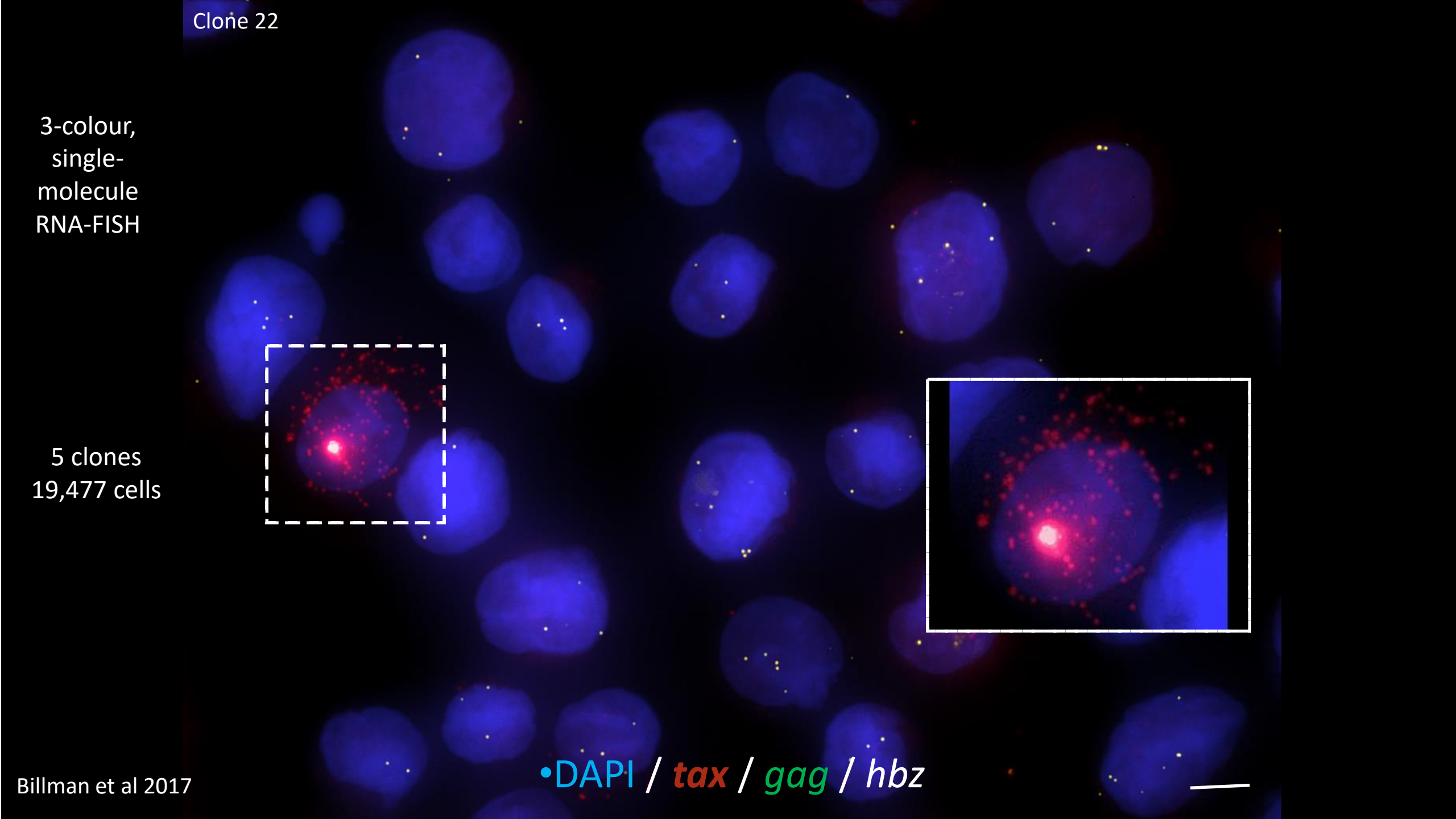
- Number of years of infection (disease in older age)
- The proviral load (% HTLV-1 infected T-cells)
- Proviral genome structure and expression
- HTLV-1 integration site in host DNA
- Antigen receptor of HTLV-1 infected-cells (eg other pathogens)
- Immune control of HTLV-1 infected cells

HTLV-1a: genetic structure

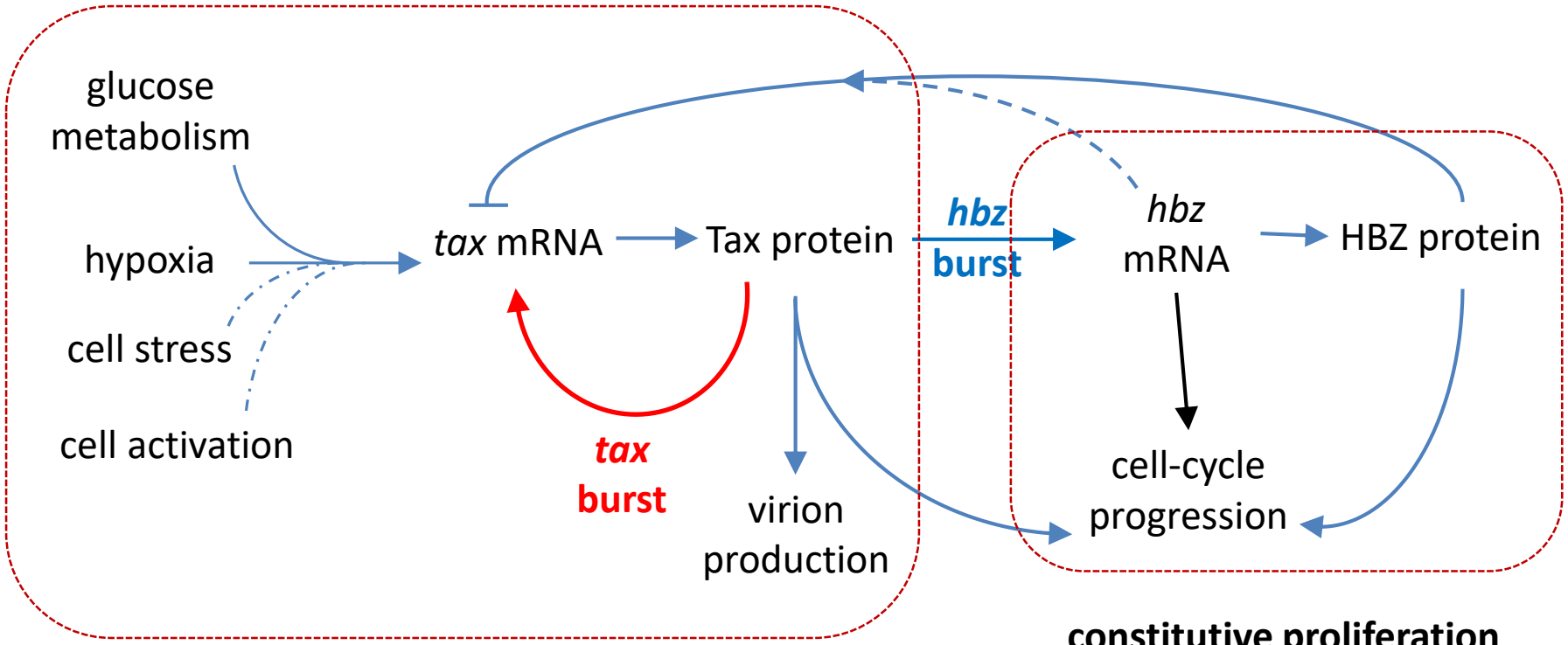
- a complex retrovirus with many regulatory and accessory genes



Adapted from Satou and Matsuoka, 2013


Clone 22
3-colour, single-molecule RNA-FISH
5 clones
19,477 cells
•DAPI / tax / gag / hbz
Billman et al 2017

Regulation of HTLV-1a expression and replication



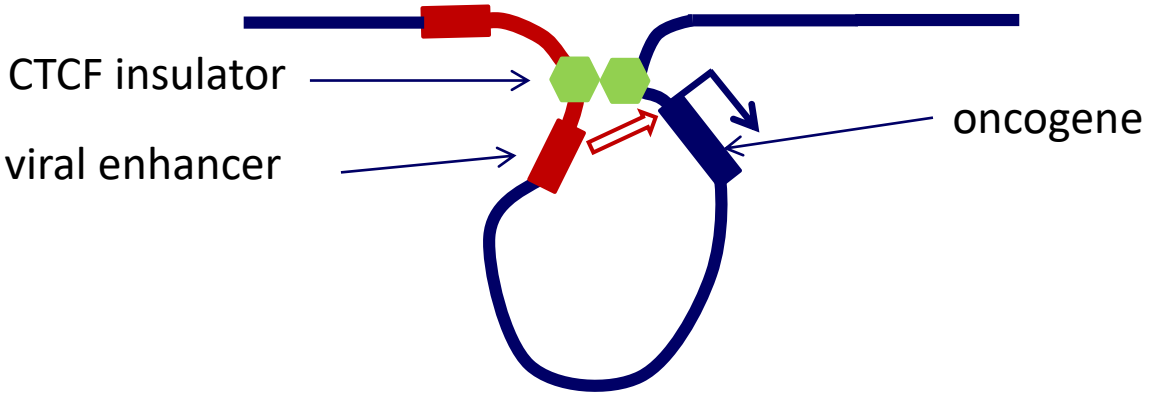
Kulkarni et al 2017
Cell Chem Biol

Bangham and Ratner 2015

Satou et al 2006; Billman et al 2017

Leukaemia mechanisms of HTLV-1a

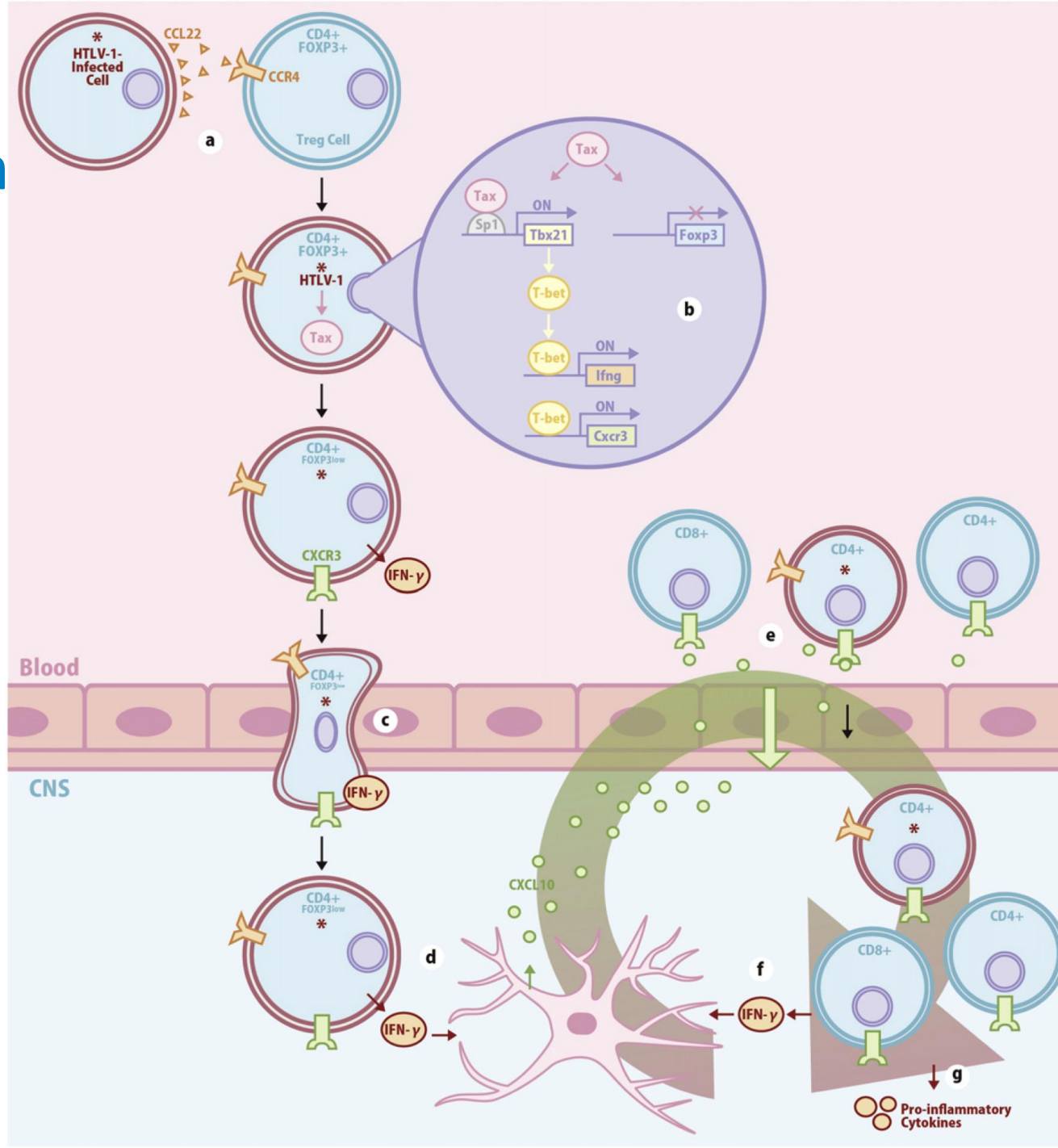
- Ongoing expression of viral RNA & proteins: *hbz*, HBZ, Tax
- Insertional mutation of host DNA
- Long range activation of host oncogenes



Chromatin looping due to CTCF insulator extends insertional oncogenesis from ~10 kb to ~5 Mb.

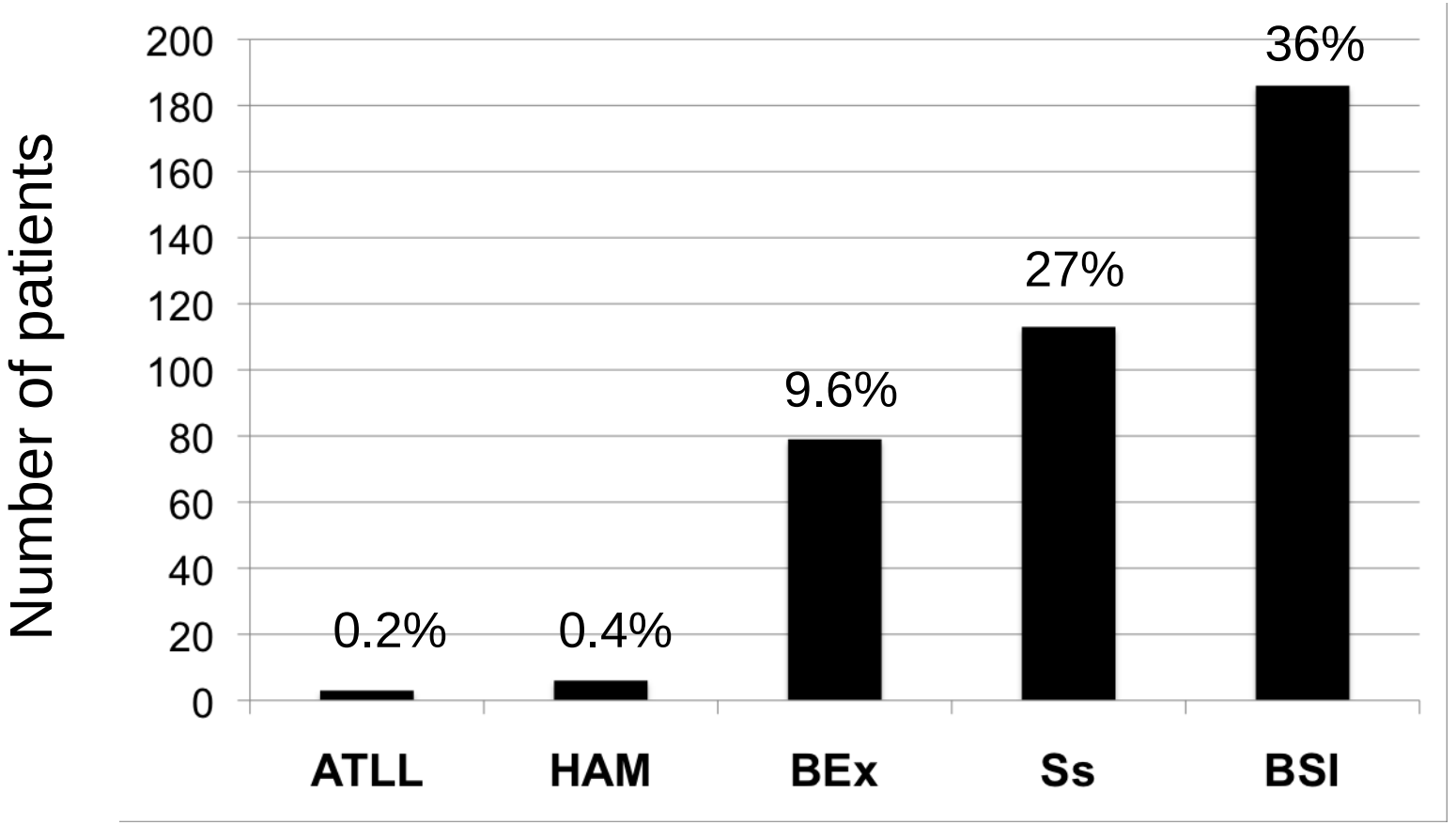
HTLV-1 inflammatory disease through altered cytokine expression

- HTLV-1$^+$ cells mostly CD4$^+$/CCR4$^+$ (T-Reg) cells
- Infected T-cells express HTLV-1 Tax:
  - Reprogramed into “Zombie T-cells”
  - Secrete pro-inflammatory cytokines (IFNγ)
- Bystander support cells in organs become activated → further cytokine expression
- Influx and activation of more dys-regulated T-cells
- Out of control positive inflammatory feedback loop



Yamano and Coler-Reilly (2017), J Neuroimmunol 304; 51

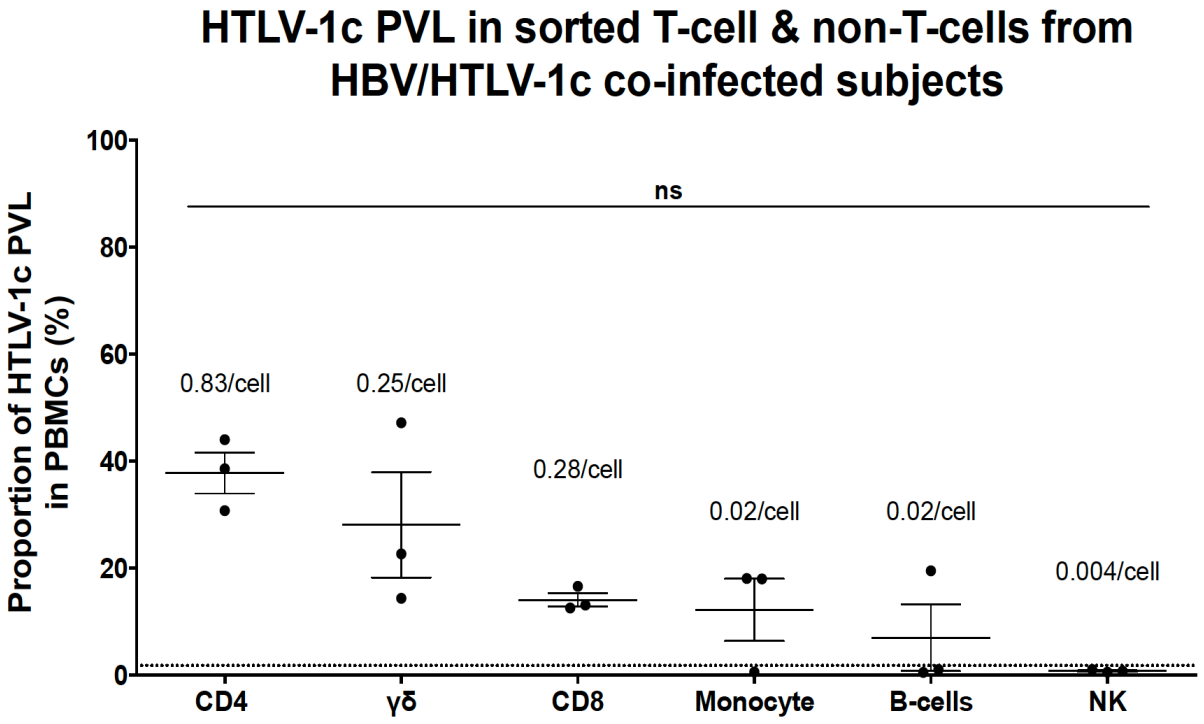
The relative burden of HTLV-1c associated conditions in central Australia

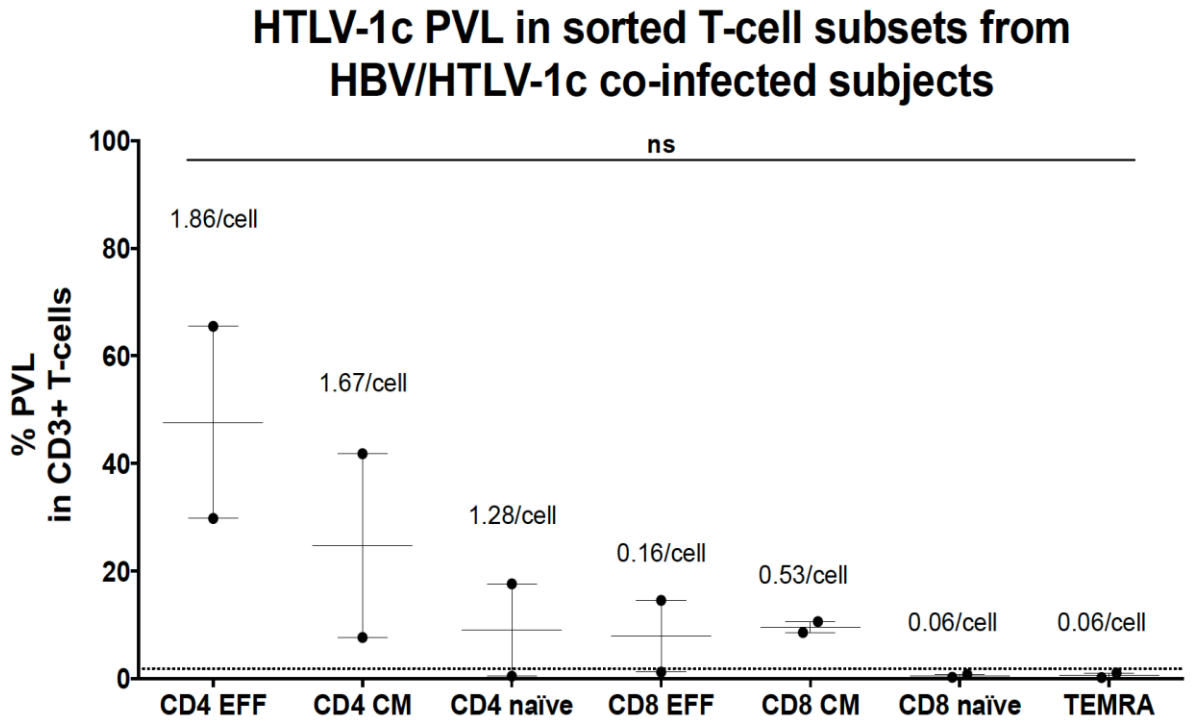


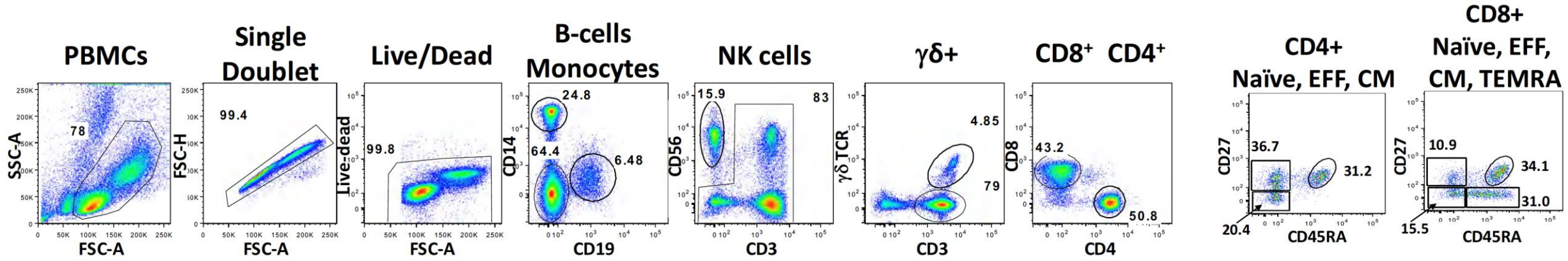
507 HTLV-1 WB+ adult patients

Einsiedel et al., *PLoS Negl Trop Dis,* 2014

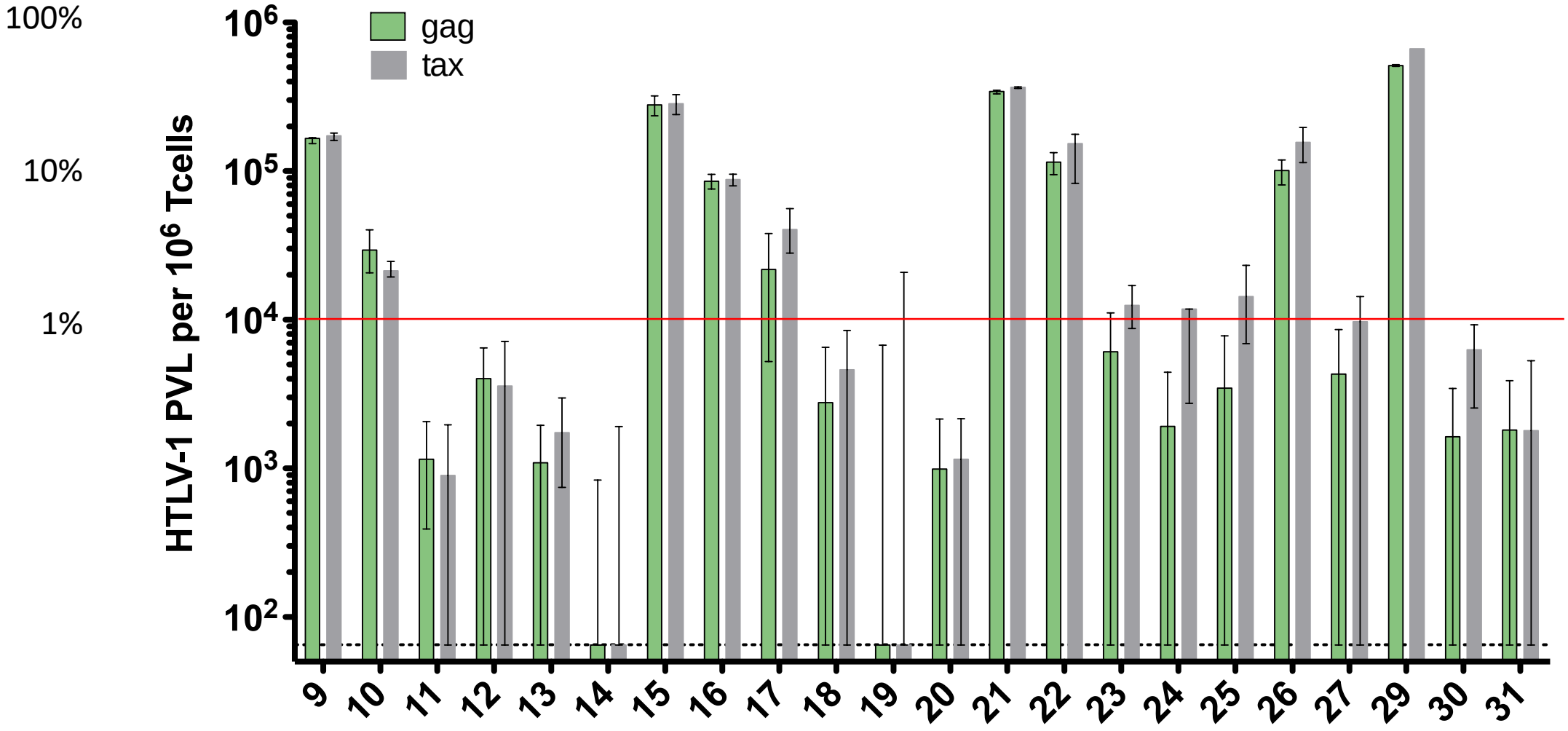
HTLV-1c provirus found predominantly in T-cells of the effector memory phenotype in some HTLV/HBV co-infected patients (n=4).







HTLV-1c PVL per T-cell

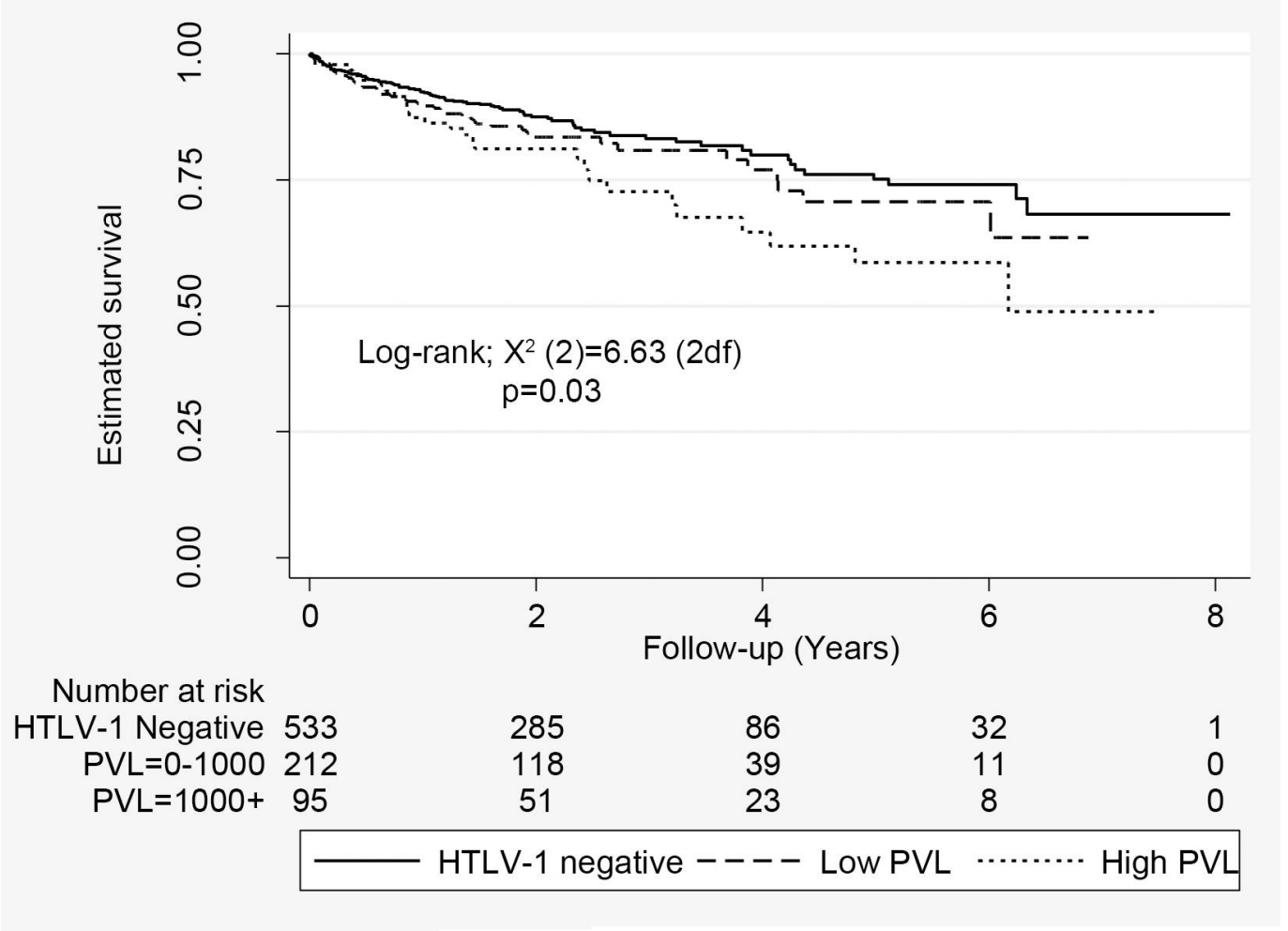


Yurick et al, 2019, J. Clin. Micro

ddPCR LOD
65 copies per
10^6 Tcells

Significant increase in all cause mortality

The Alice Springs Prospective Community Cohort (n=843)



PLOS | NEGLECTED TROPICAL DISEASES

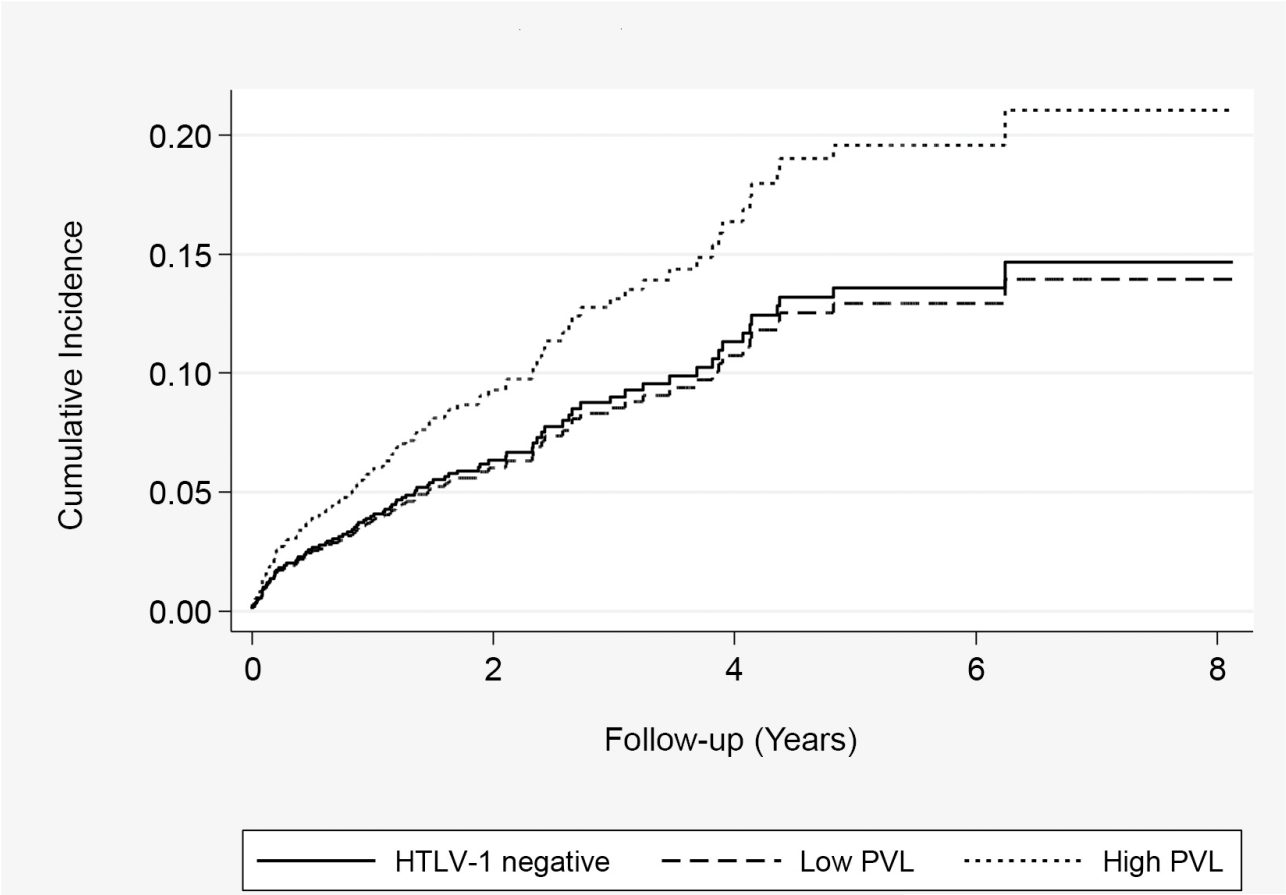
PLOS Neglected Tropical Diseases | https://doi.org/10.1371/journal.pntd.0006281 March 12, 2018

RESEARCH ARTICLE

Human T-Lymphotropic Virus type 1c subtype proviral loads, chronic lung disease and survival in a prospective cohort of Indigenous Australians

Lloyd Einsiedel[1]*, Hai Pham[1], Kim Wilson[2], Rebecca Walley[3], Jocelyn Turpin[4], Charles Bangham[4], Antoine Gessain[5], Richard J. Woodman[6]

High HTLV-1c PVL >1% increases risk of BEx related death



Sugisaki et al.,
Am. J.Trop Med. Hyg.
1998

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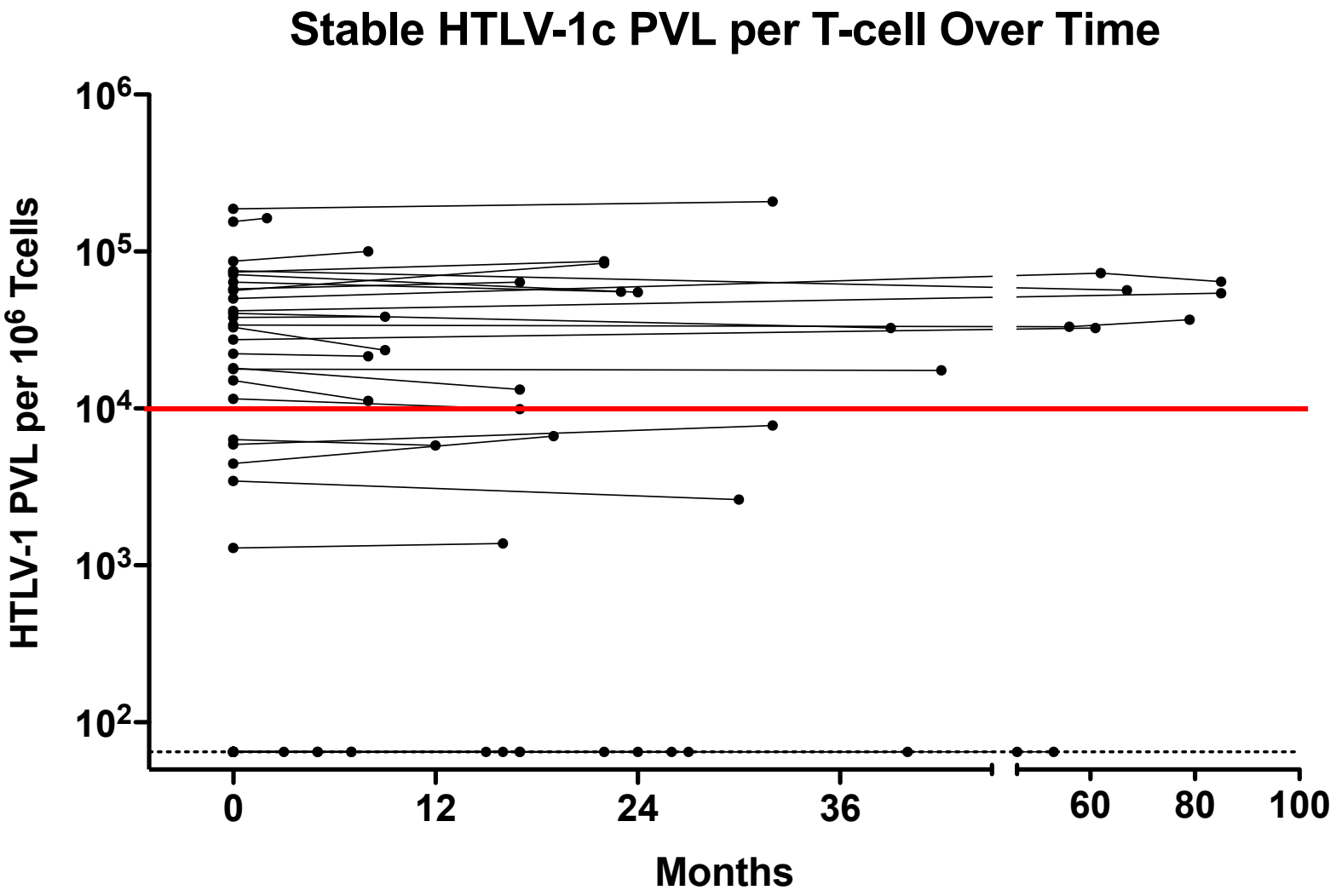
Longitudinal changes in HTLV-1c PVL per T-cell

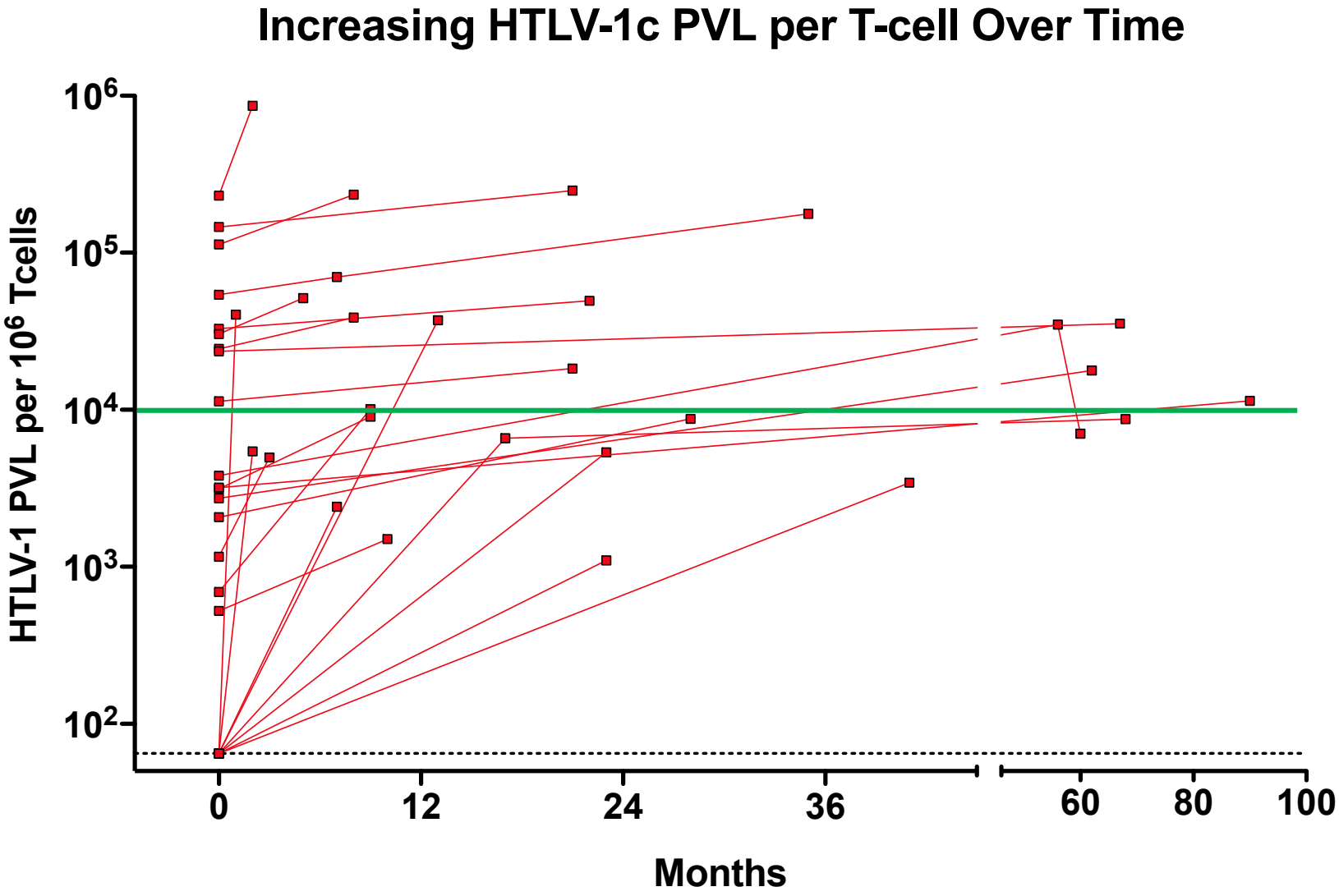


All patients have minimum 2 collection points
Stable conditions: 0.66-fold =< PVL =< 1.5-fold

● Stable PVL per T-cell

n = 39 / 83 (47%)

Longitudinal changes in HTLV-1c PVL per T-cell

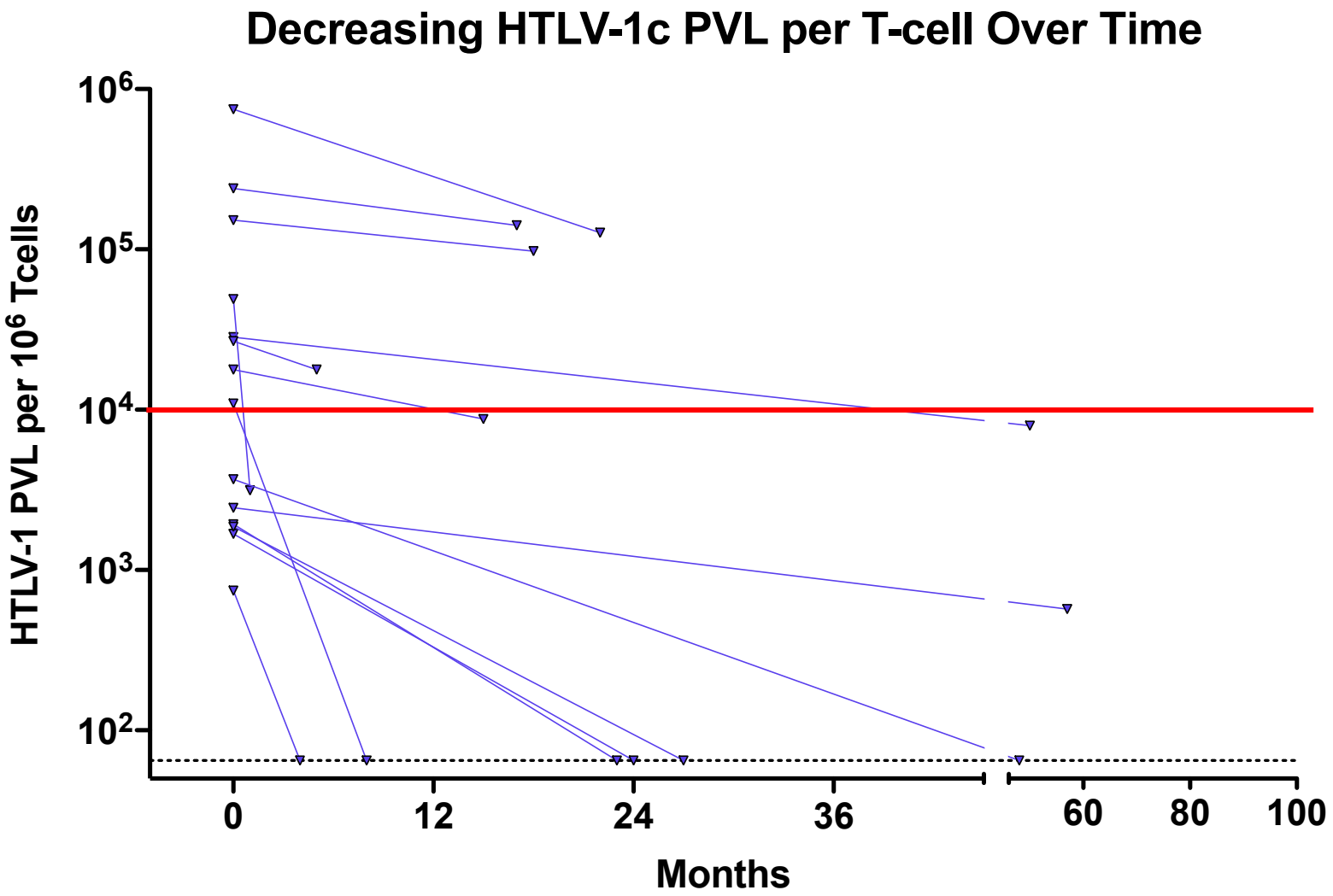


All patients have minimum 2 collection points
Increasing conditions: PVL >= 1.5-fold

Increasing PVL per T-cell

n = 25 / 83 (30%)

Longitudinal changes in HTLV-1c PVL per T-cell



All patients have minimum 2 collection points
Decreasing conditions: PVL =< 0.66-fold

▼ Decreasing PVL per T-cell

n = 14 / 83 (17%)

Significant genomic differences between HTLV-1a and -1c

HTLV-1c genomic consensus sequence generated from 22 patients from Alice Springs Hospital

Significant divergence found towards 3' end

- Impacts pX region and reverse transcripts

Hypothesis: Genetic differences result in novel gene expression in HTLV-1c

- increase inflammation

- reduce leukemia induction

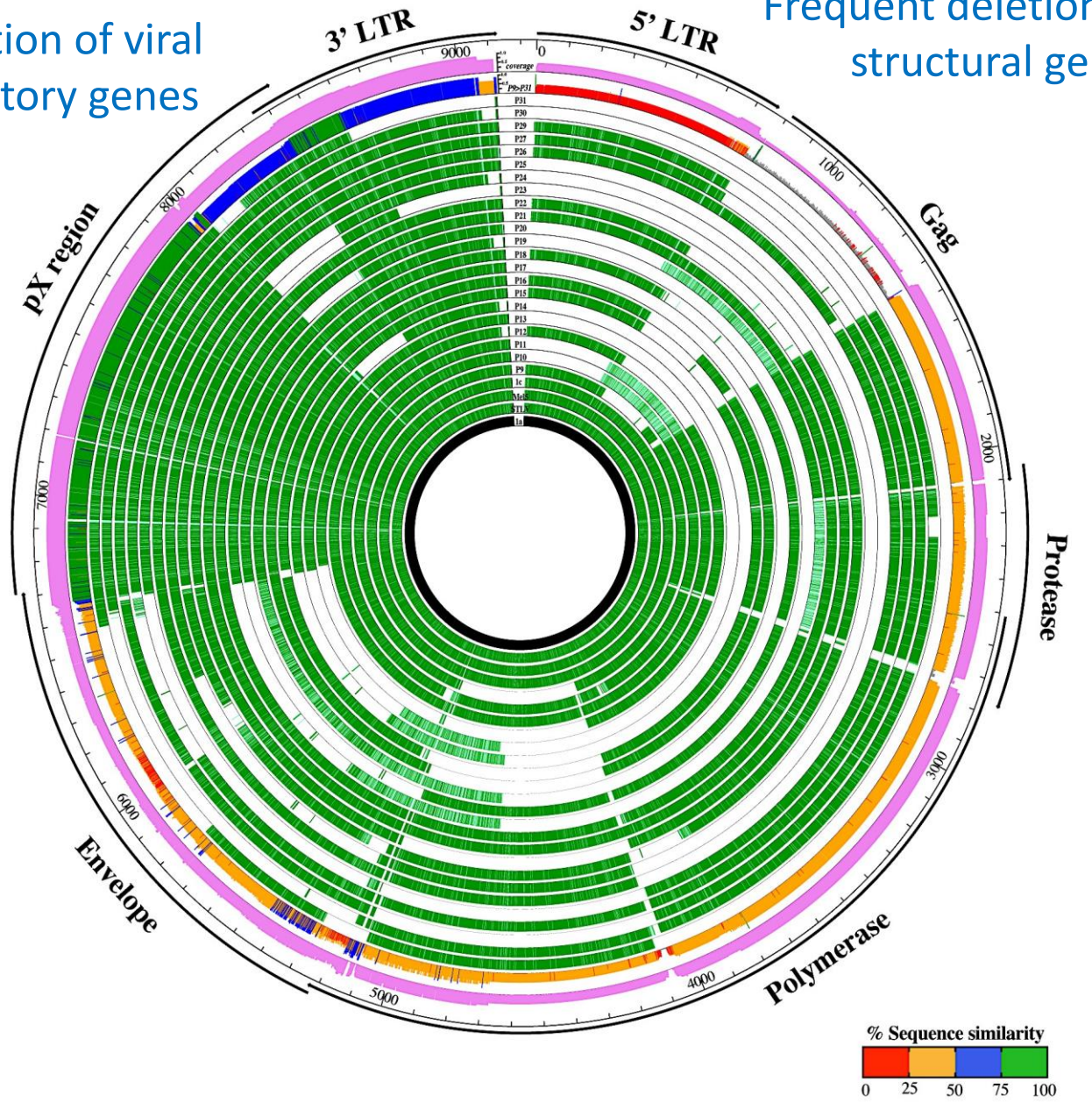
Yurick, D. 2017

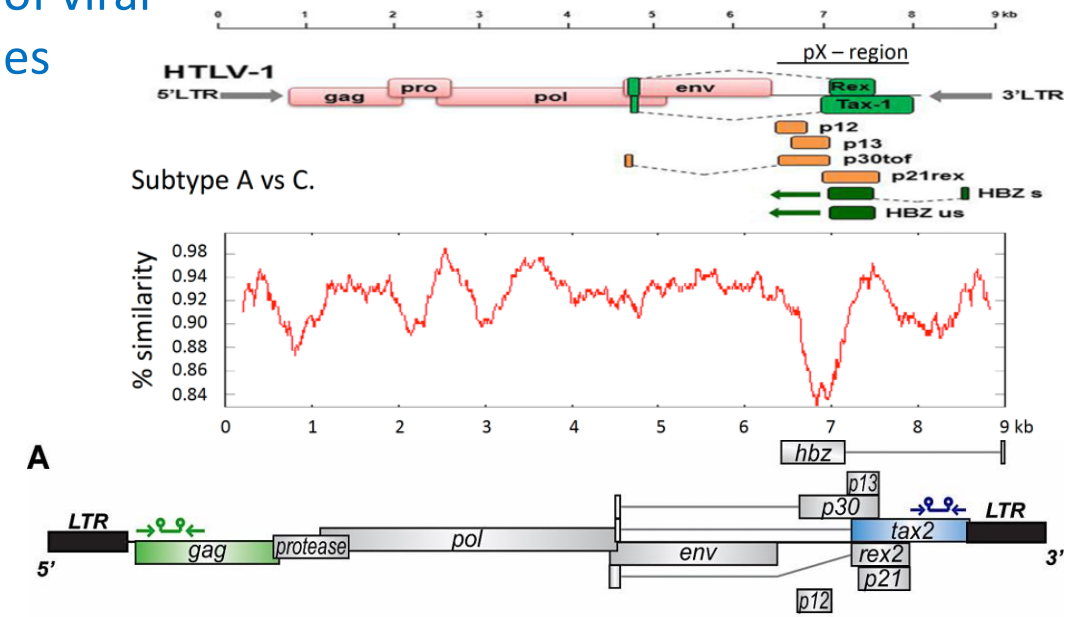
Genomic Region	Nucleotide Divergence %	Amino Acid Divergence %
Rex	5.26	13.23
Env	6.27	3.07
Pol	6.54	3.91
Tax	6.69	7.65
Pro	6.95	8.97
Gag	7.60	3.96
5'LTR	9.14	n/a
3'LTR	9.40	n/a
pX region	9.50	21.95
p30	10.41	15.68
HBZ	12.36	19.12
p27	12.96	22.35
p8	13.33	18.84
p12	19.39	26.80

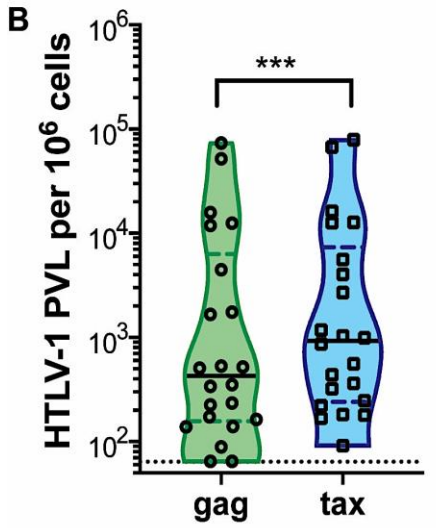
Genomic differences between HTLV-1a and -1c

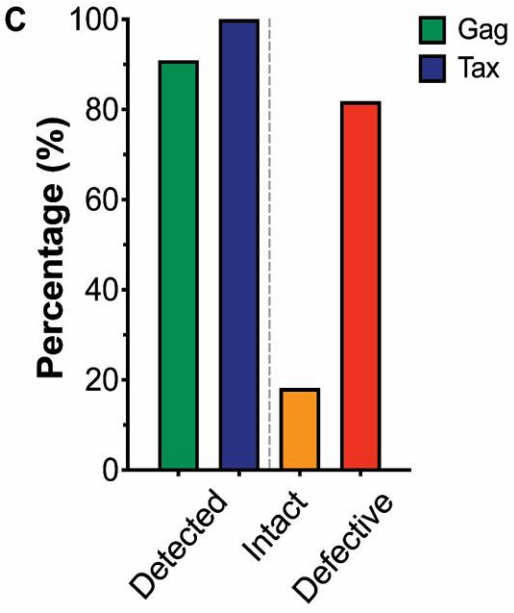
Retention of viral regulatory genes

Frequent deletion of viral structural genes







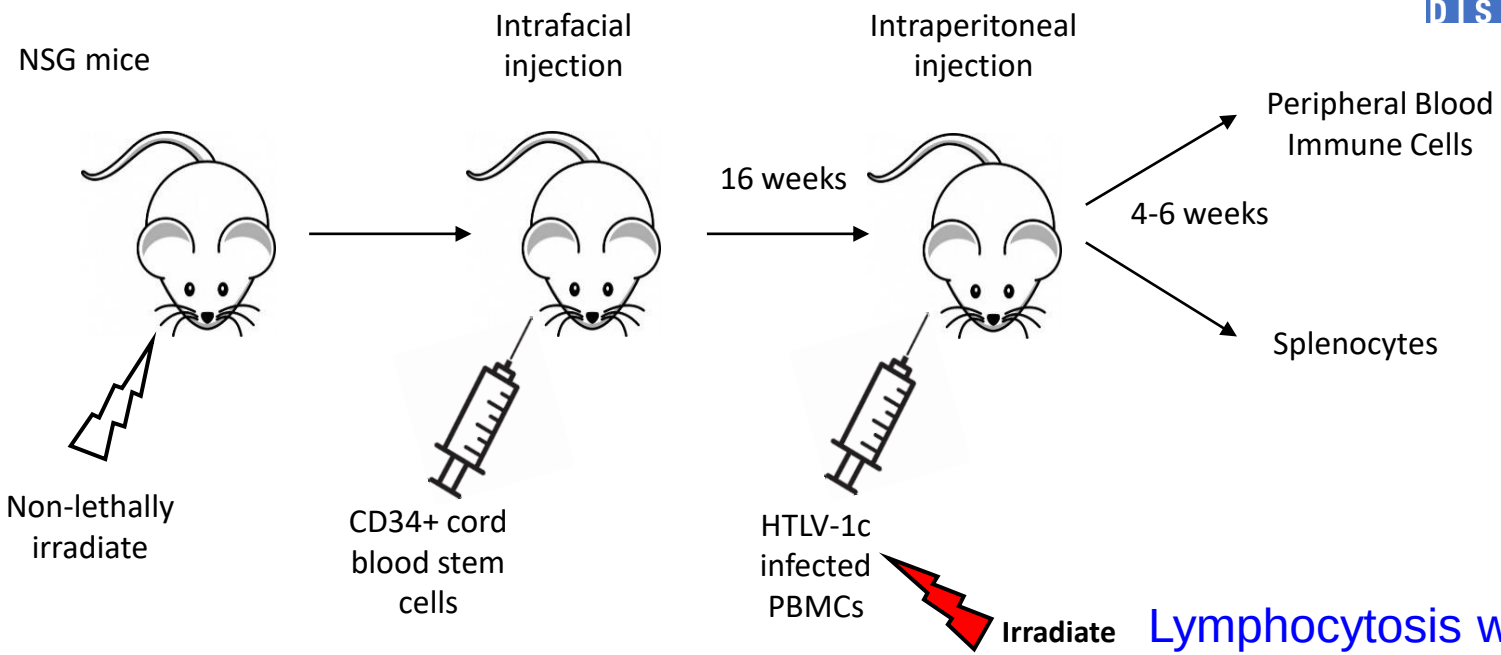


Two selective pressures at work:

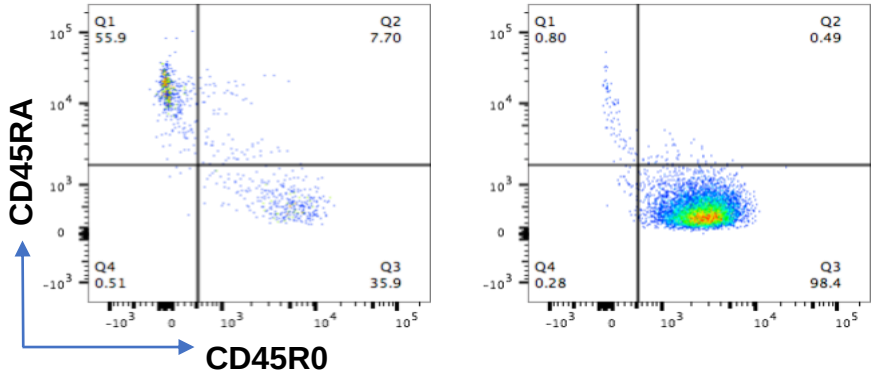
- Specific deletion of structural genes (gag)
    - Immune mechanisms – CTL?
    - Genetic mechanism – piRNA?
- Retention of the X-region
    - Cell proliferation
    - Immune evasion

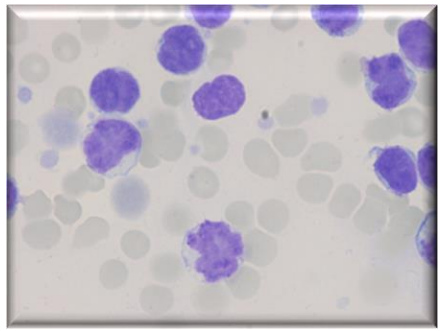
Humanised mouse model to investigate HTLV-1c viral replication

Pellegrini group, WEHI



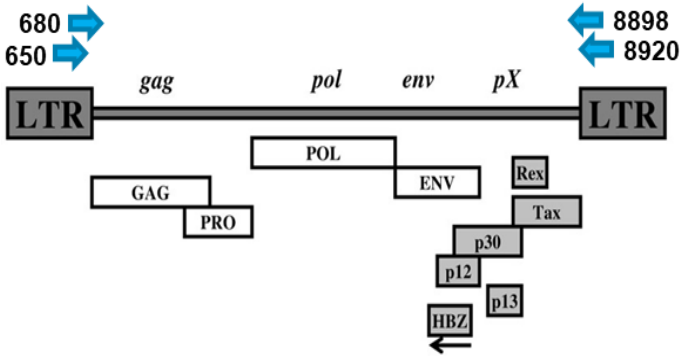
Lymphocytosis with CD4+ memory T-cells



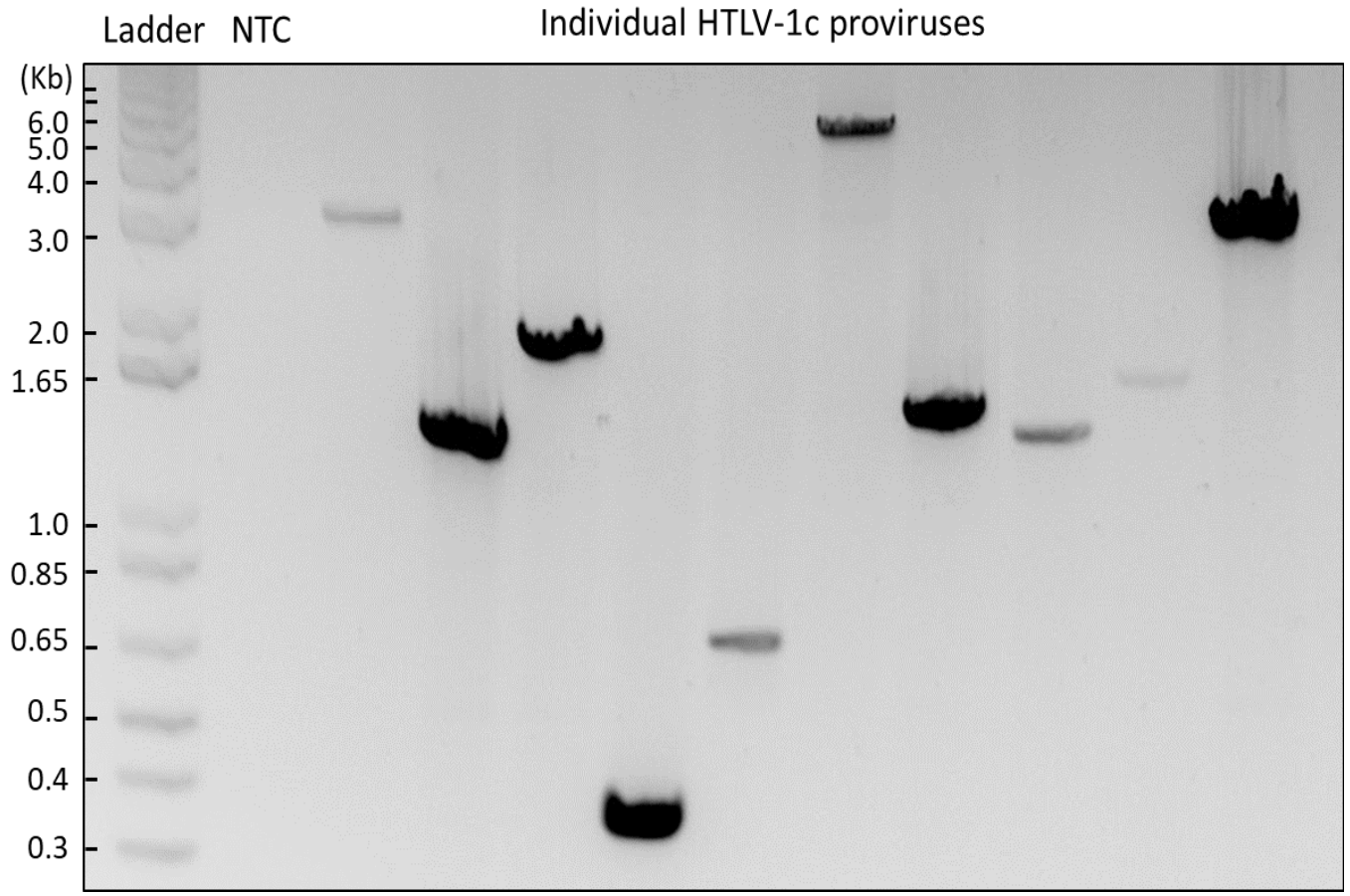


Humanized-mice lacking functional immunity internally delete HTLV-1 proviral DNA.

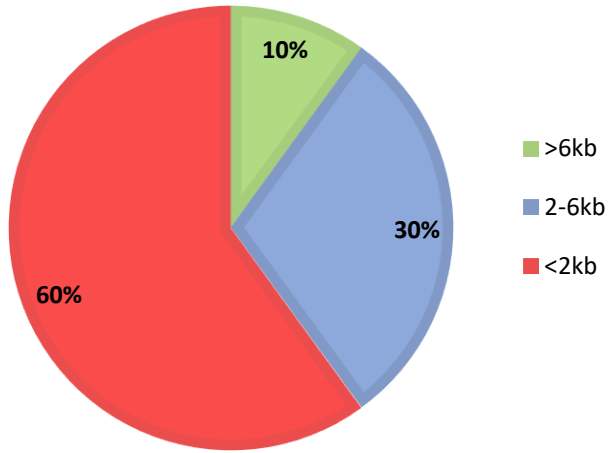
A



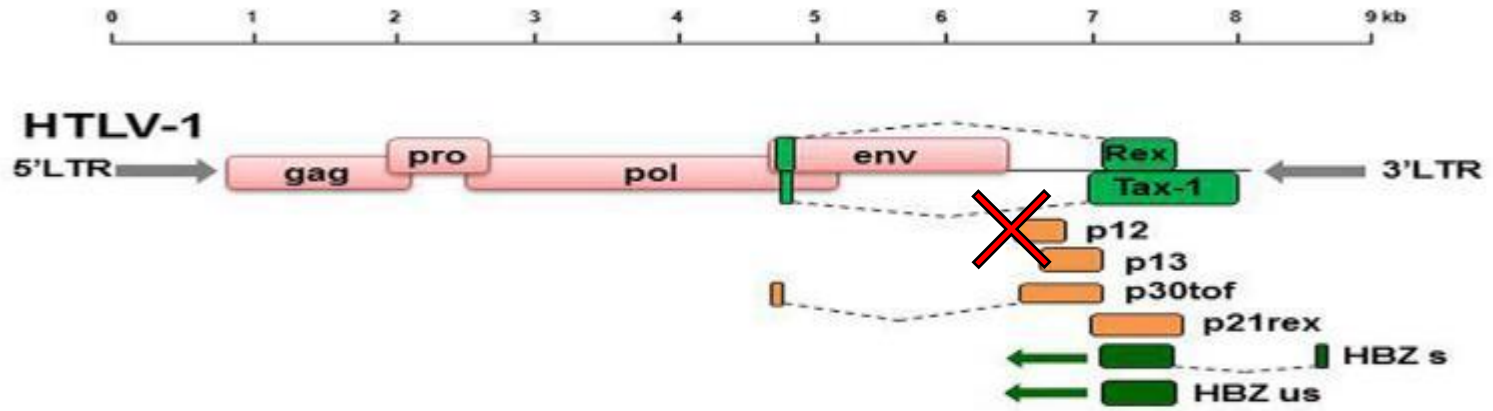
B



C

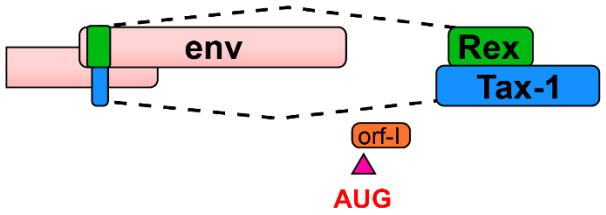


p12 (orf-1) variation between HTLV-1a and -1c

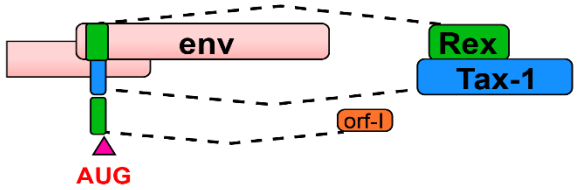


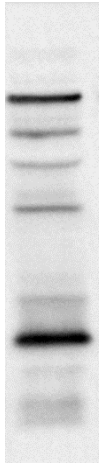
orf-I **p16**

HTLV-1A



HTLV-1C



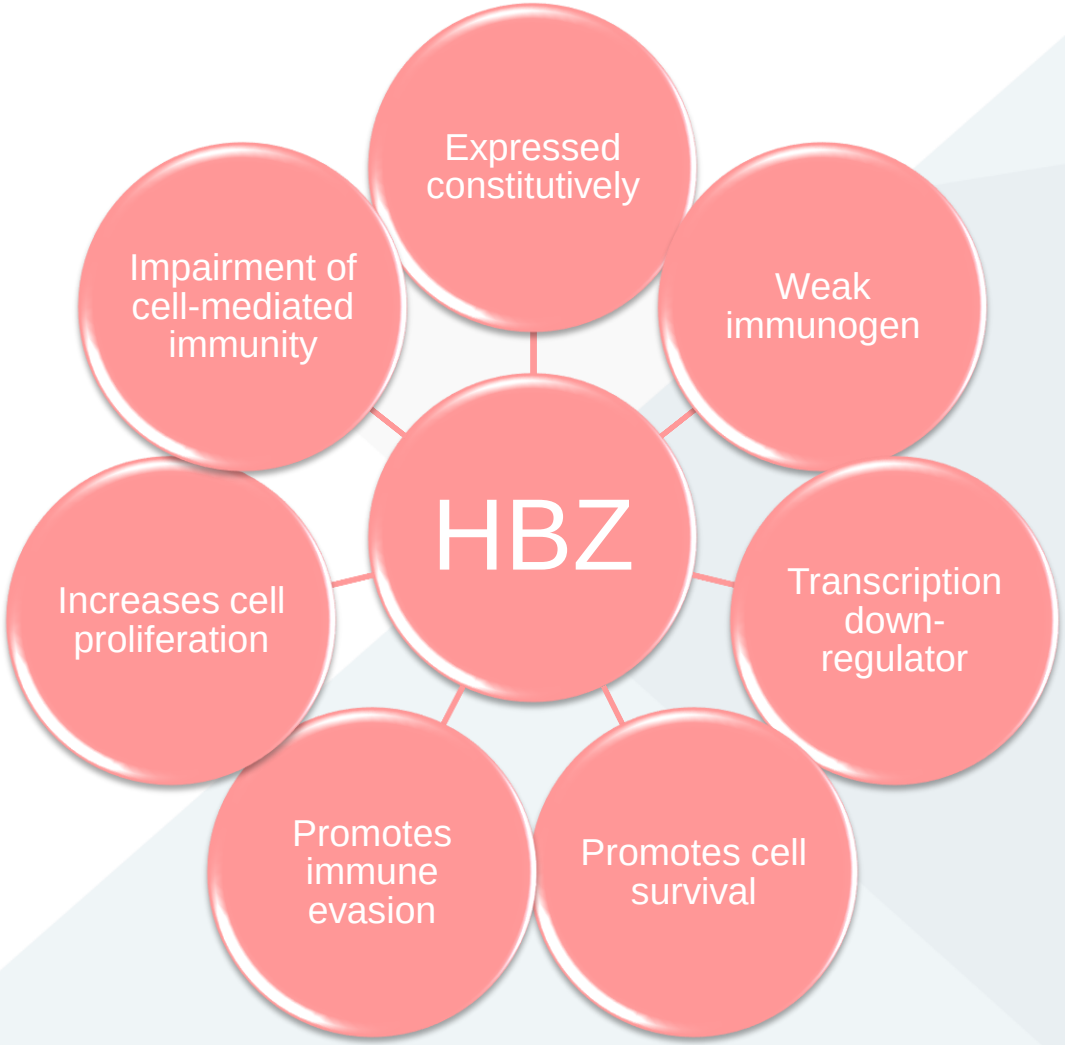


HTLV-1c encodes a p16 variant of the p12 (*orf-I)* using an in-frame upstream AUG initiation codon

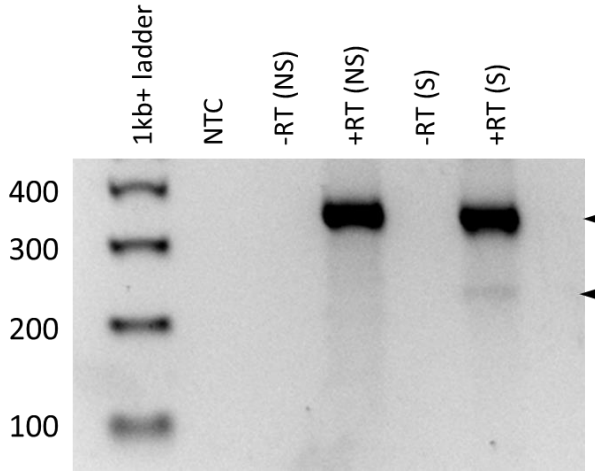
The p16 variant may contribute to higher inflammatory disease

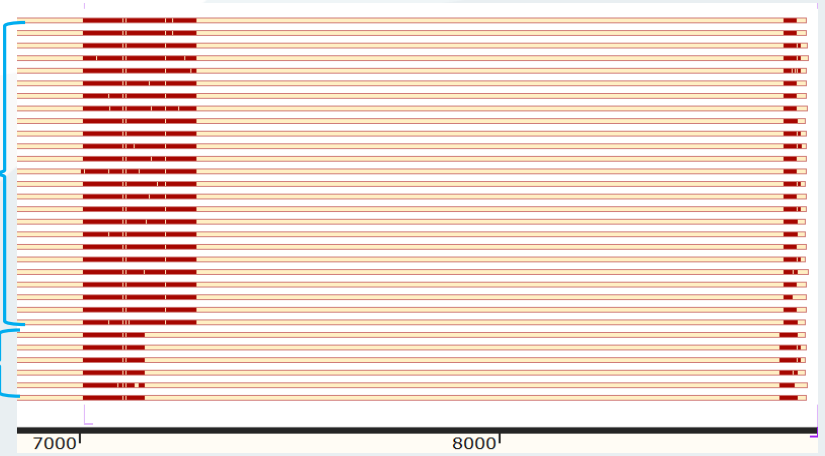
Sarkis, Galli, Fujikawa, Omsland, Moles, Khoury, Yurick, Purcell, Franchini, Pise-Masison (*In Review)*

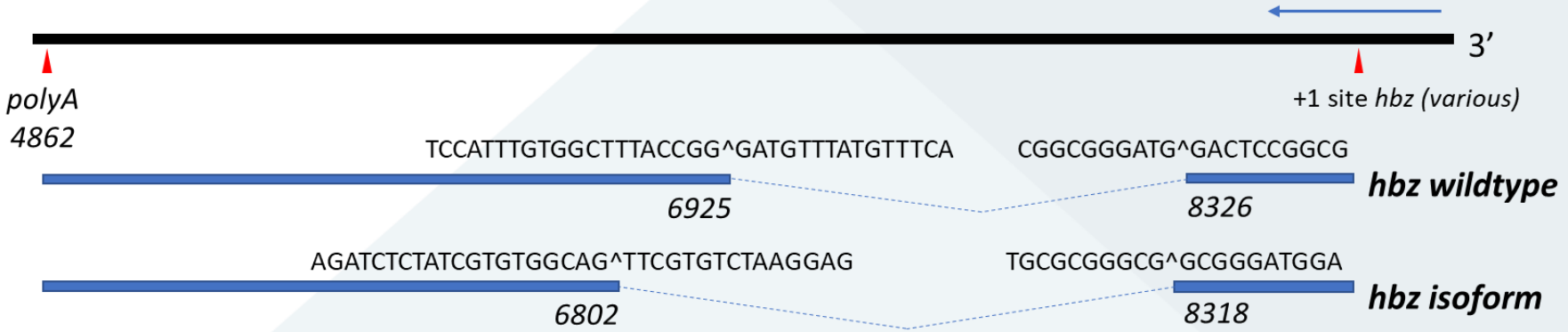
HTLV-1a basic leucine zipper factor (HBZ)



Novel minor HTLV-1c *hbz* mRNA isoform carries a deletion of 114 nucleotides



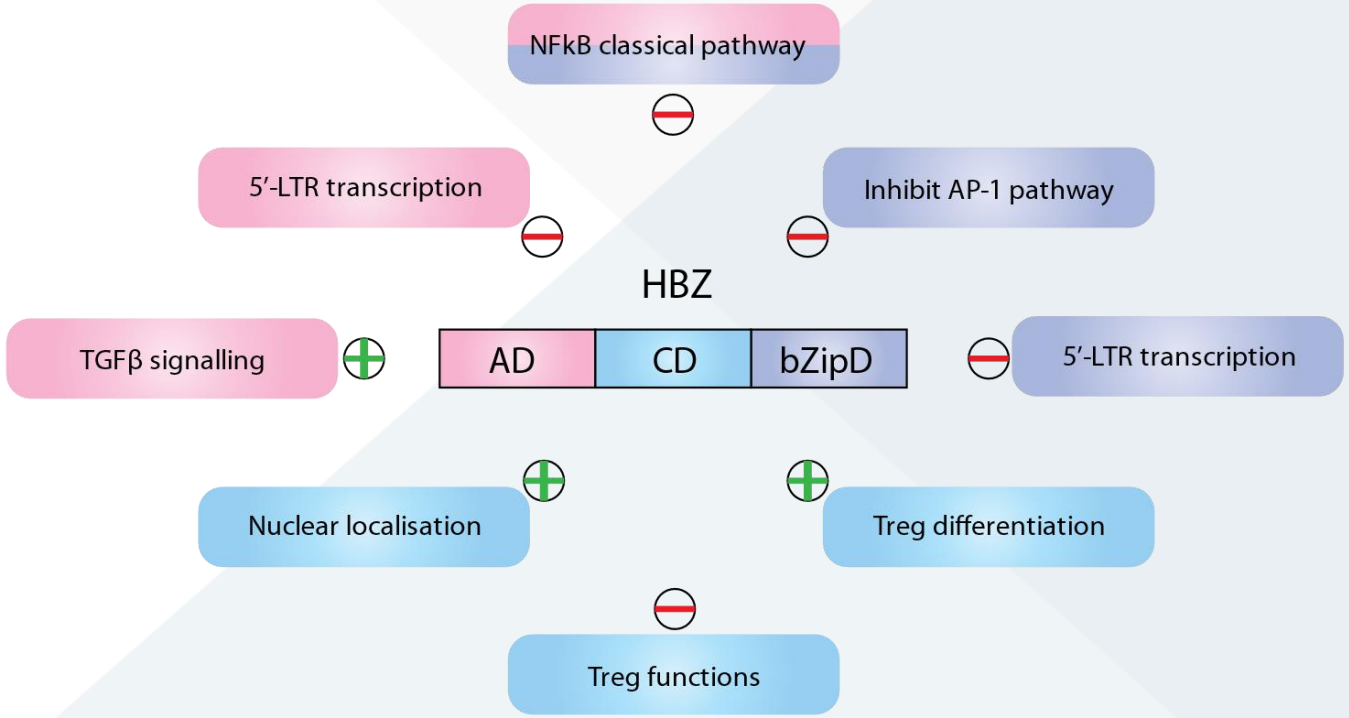




*Numbering relative to fwd +1

Novel HBZ isoform truncated in activation domain may impact its regulatory functions

```
HTLV-1a HBZsp   | MAASGLFRCLPVSCPEDLLVEELVDGLLSLEEELKDK-EEEEAVLDGLLSLEEESRGRLRRGPPGE
HTLV-1c HBZsp   | MAASGPFRCLPVPRPEDLLVEDLVDGLLSLEDDLKDQREEEESVLDGVLSLEEESR--LRWGLPGE
HTLV-1c HBZiso  | MAASGRA-------------------------------------DGVLSLEEESR--LSWGLPGE
```



HTLV-1c p30 - mutations in each of the functional domains

The p30A of HTLV-1a is essential for *in vivo* infectivity and promotes ATL

- Alters cellular gene expression and immune response
- Impedes DNA repair and alters cell-cycle progression

The p30C of HTLV-1c has **aa mutations in all known functional domains**:

- TRE transcription repression site
- TIP60 binding domain
- Rex binding domain,
- nucleolus retention sequence (NoRS)
- nuclear localization sequence (NLS)

HTLV-1c infections in Australia and our region

Genetically distinct HTLV-1c in indigenous communities in Australia

- Highly prevalent in remote central Australian Aboriginal communities
- Prevalence in other parts of Australia unknown
- Same strains present in PNG and Melanesia
  - prevalence unknown
- High levels of defective provirus retaining X-region

HTLV-1c subtype diverges in genes associated with leukaemia (ATL) and HAM

- p12 / p8, p30 and HBZ
- Relatively high conservation in Env and structural genes

HTLV-1c Env trimers offer a possible vaccine and pathway to monoclonal NAb

Australian HTLV-1c: What's known, what's unknown

Not known with HTLV-1c:

- Pathogenic mechanisms of altered X-region proteins (p16, p30 and HBZ)
    - Increased inflammatory disease?
    - Reduced leukemia induction?
- Assay to predict the onset of inflammatory disease / leukemia?
- Expanded HTLV-1c invasion into & pathogenesis of myeloid cells?
- Drugs or vaccines that prevent viral replication, or eliminate cells with provirus?
- Antibody protection from transmission?
    - Passive protection from mAb during pregnancy and breast-feeding?
    - Prophylactic vaccine?

HTLV-1 pathway forward – lessons from HIV

- HIV
  - Testing ✓
  - Treatments ✓
  - Preventives ✓
  - Education ✓
  - Confront stigma ✓
- HTLV-1
  - Testing 🔄
  - Treatments ✗
  - Preventives ✗
  - Education ✗
  - Confront stigma ✗

National (ACH2 / NCHECR / NCSR) and International coordination (WHO)

HTLV-1 pathway forward – lessons from HIV

- HIV
  - Testing ✓
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  - Education ✓
  - Confront stigma ✓
- HTLV-1
  - Testing 🔄
  - Treatments ✗
  - Preventives ✗
  - Education ✗
  - Confront stigma ✗

National (ACH2 / NCHECR / NCSR) and International coordination (WHO)

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Charlene MacKenzie

Damian Purcell Lab

Sharon Lewin & Lab –

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& Community members

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Doherty Institute
A joint venture between the University of Melbourne and Melbourne Health

Baker IDI
HEART & DIABETES INSTITUTE

THE UNIVERSITY OF MELBOURNE

SAVE THE DATE

20th International Congress on HTLV 2021

23-26 May 2021 | Melbourne, Australia

Melbourne Convention and Exhibition Centre (MCEC)

Global experts will present on HTLV Virology – Cytopathology and Immune modulation, Epidemiology - Transmission, Public Health, Pathogenesis of associated diseases (HAM/TSP, ATL, Other conditions), Biomedical Prevention – Vaccines and Antivirals, Diagnostics and treatment - Longitudinal monitoring and Disease prognosis. This is an excellent opportunity to provide awareness about the situation of these infectious diseases both here in Australia and globally.

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