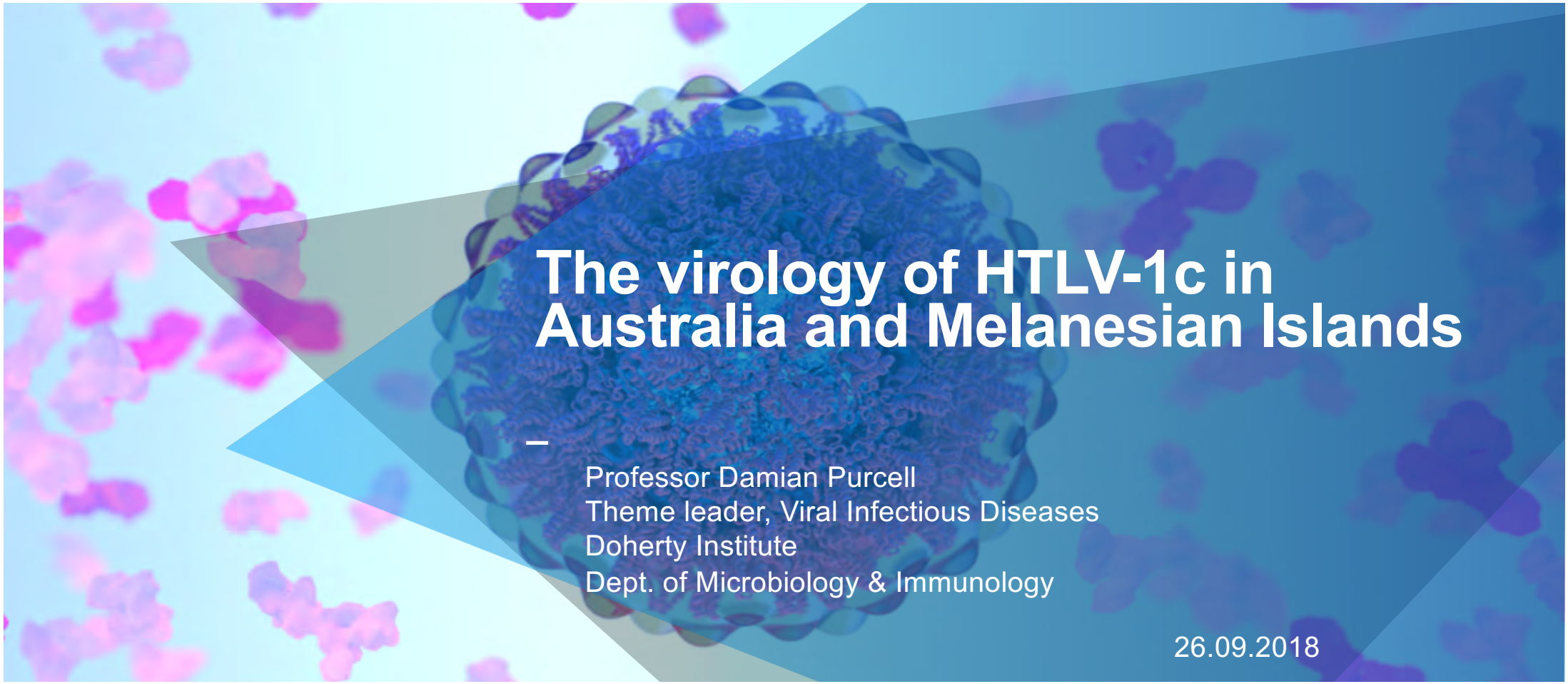


The virology of HTLV-1c in Australia and Melanesian Islands

Professor Damian Purcell
Theme leader, Viral Infectious Diseases
Doherty Institute
Dept. of Microbiology & Immunology

26.09.2018

Doherty Institute

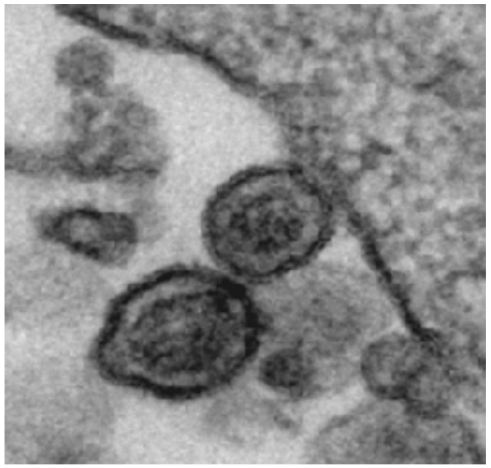
THE UNIVERSITY OF MELBOURNE

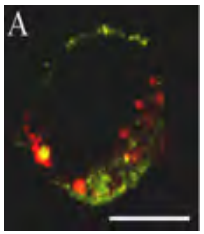
The Royal Melbourne Hospital

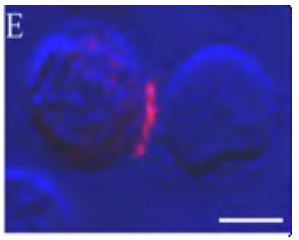
A joint venture between The University of Melbourne and The Royal Melbourne Hospital

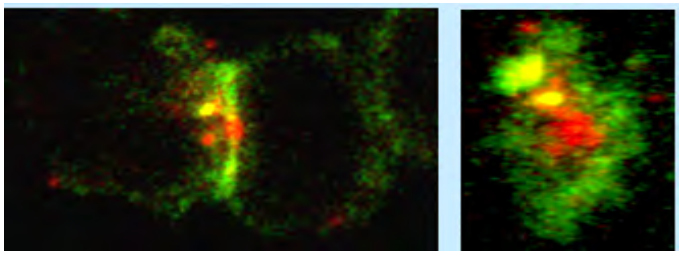
Human T-cell lymphotropic / leukemia virus (HTLV-1)

- 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
- Lifelong infection that invades host DNA
- Virus is found in cells not plasma
- Infects primarily by cell-cell contact.



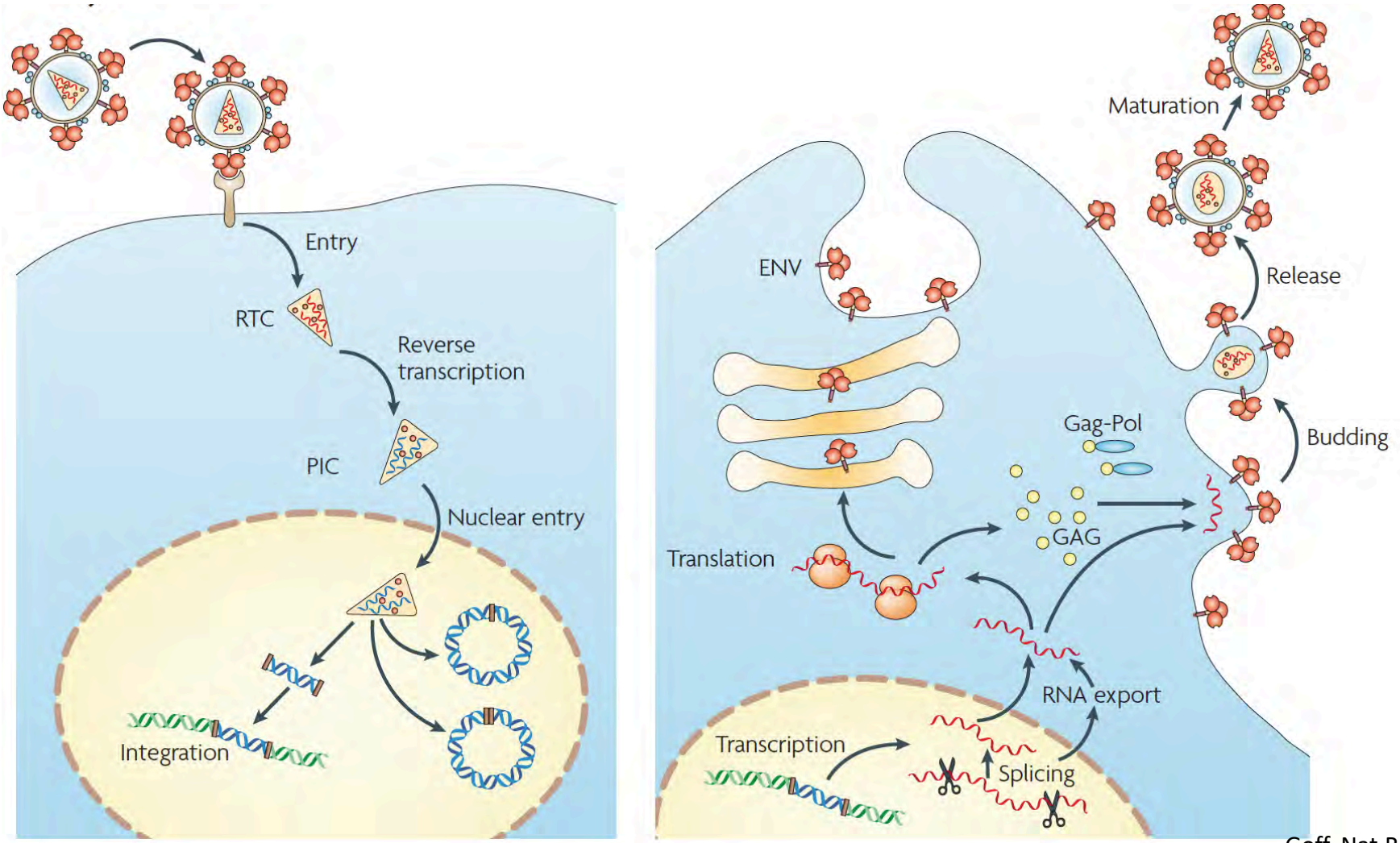






Igakura et al 2003

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



Goff. Nat.Rev.Micro: 2007

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 (+++++)

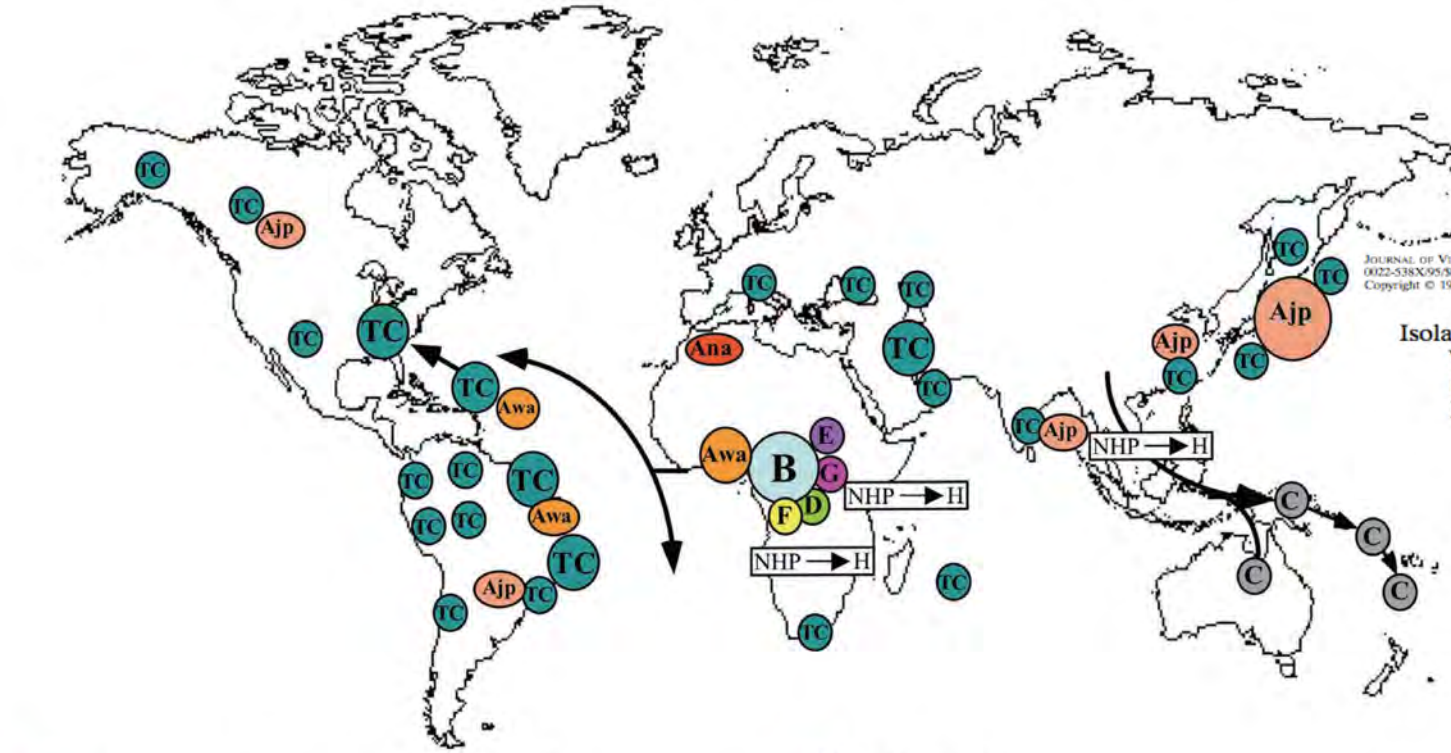
Laboratory diagnosis of HTLV-1

- Antibody serology test (x2) from blood sample (immunoassay)

- Confirmatory serological test:
  - Western blot (~10% indeterminant, slow)
  - Line immunoassay (not ARTG approved)

- PCR proviral load assays - % HTLV-1 positive cells
  - Not ARTG approved
  - qPCR
  - ddPCR - superior but more expensive

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

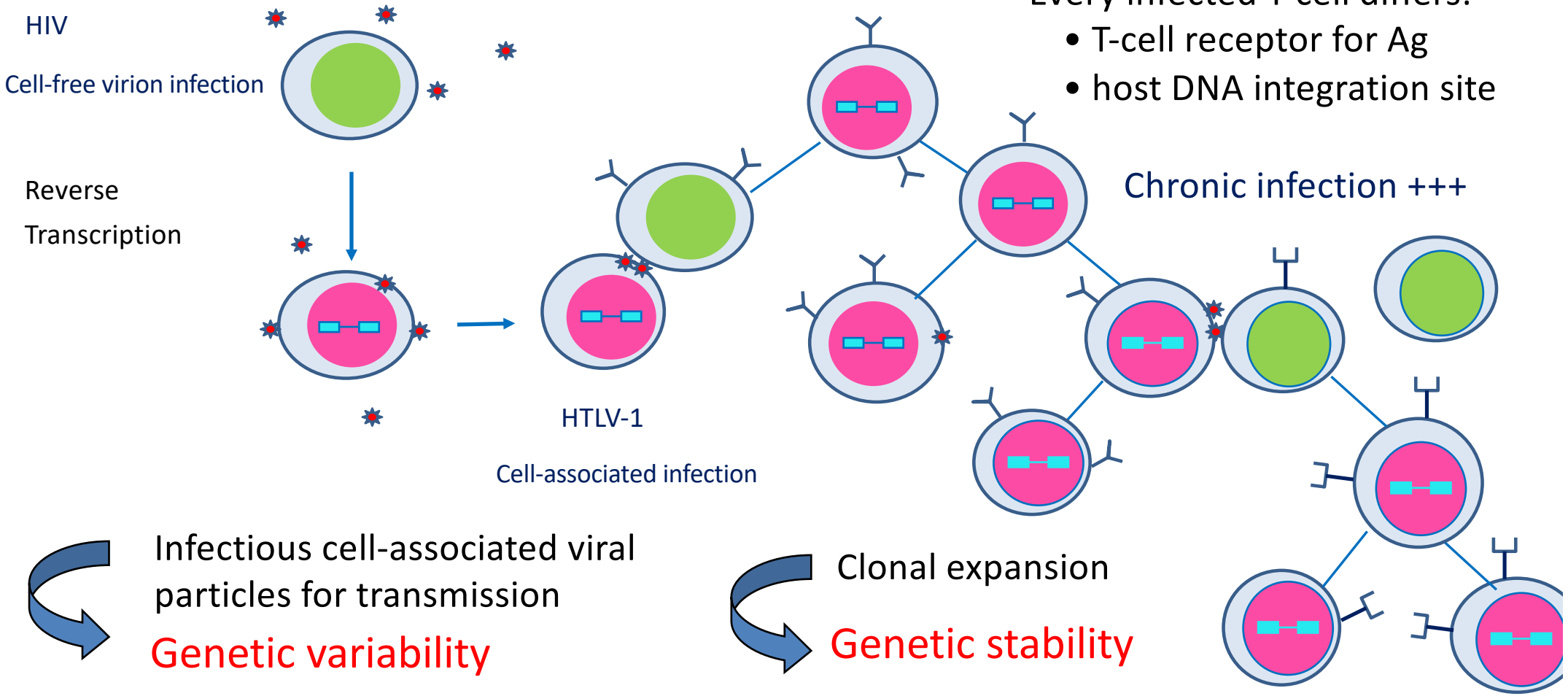
Cosmopolitan Subtype A

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



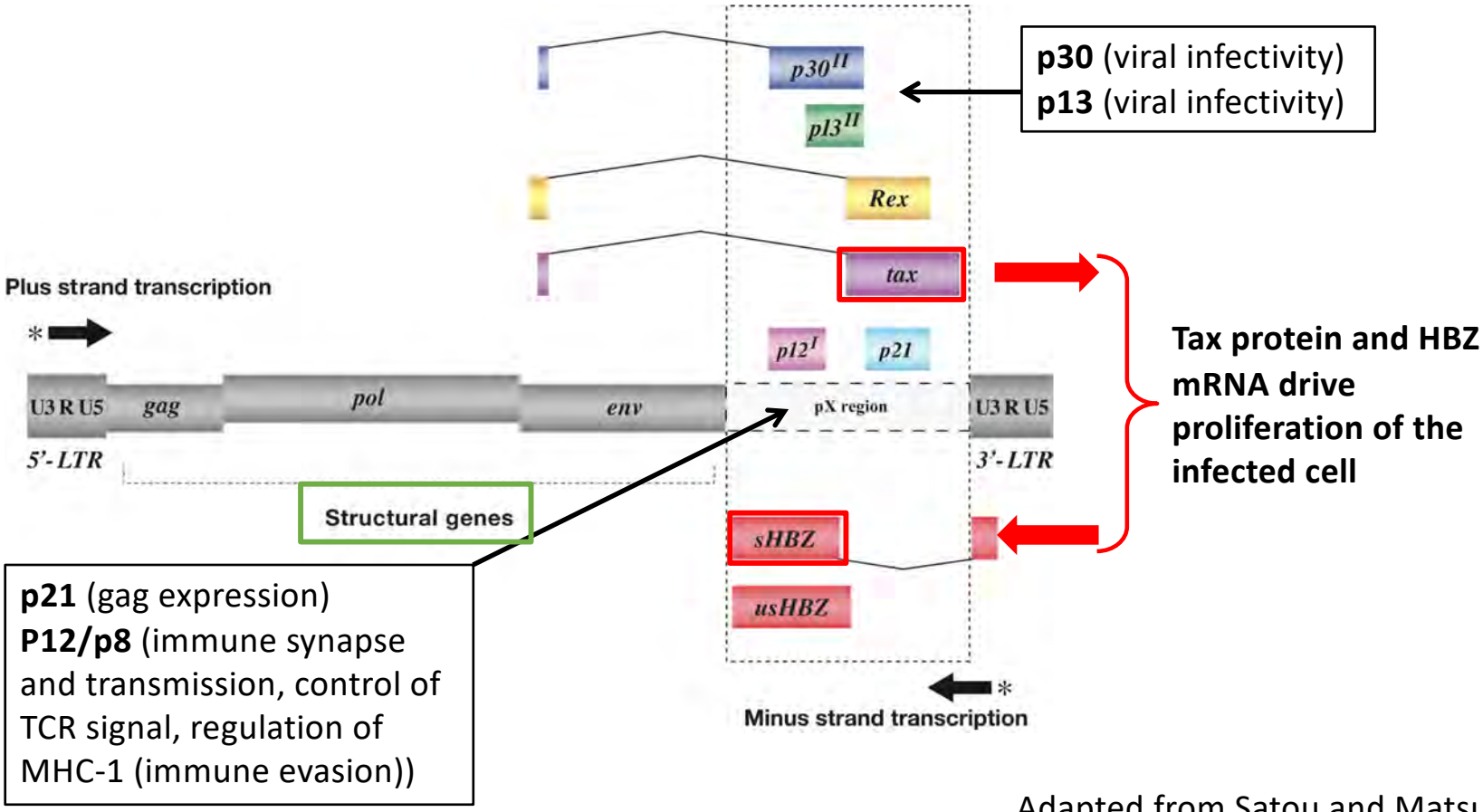
Different disease outcomes in each patient

Depends on:

- Number of years a person has been infected
- The percentage of T-cells infected with the HTLV-1 virus
- Where the HTLV-1 virus inserts into the human host DNA
- What activates an HTLV-1 infected T-cell (such as other pathogens)
- Ability of the infected person to control the number of HTLV-1 infected cells in their body

HTLV-1: genetic structure

- complex retrovirus with many regulatory and accessory genes

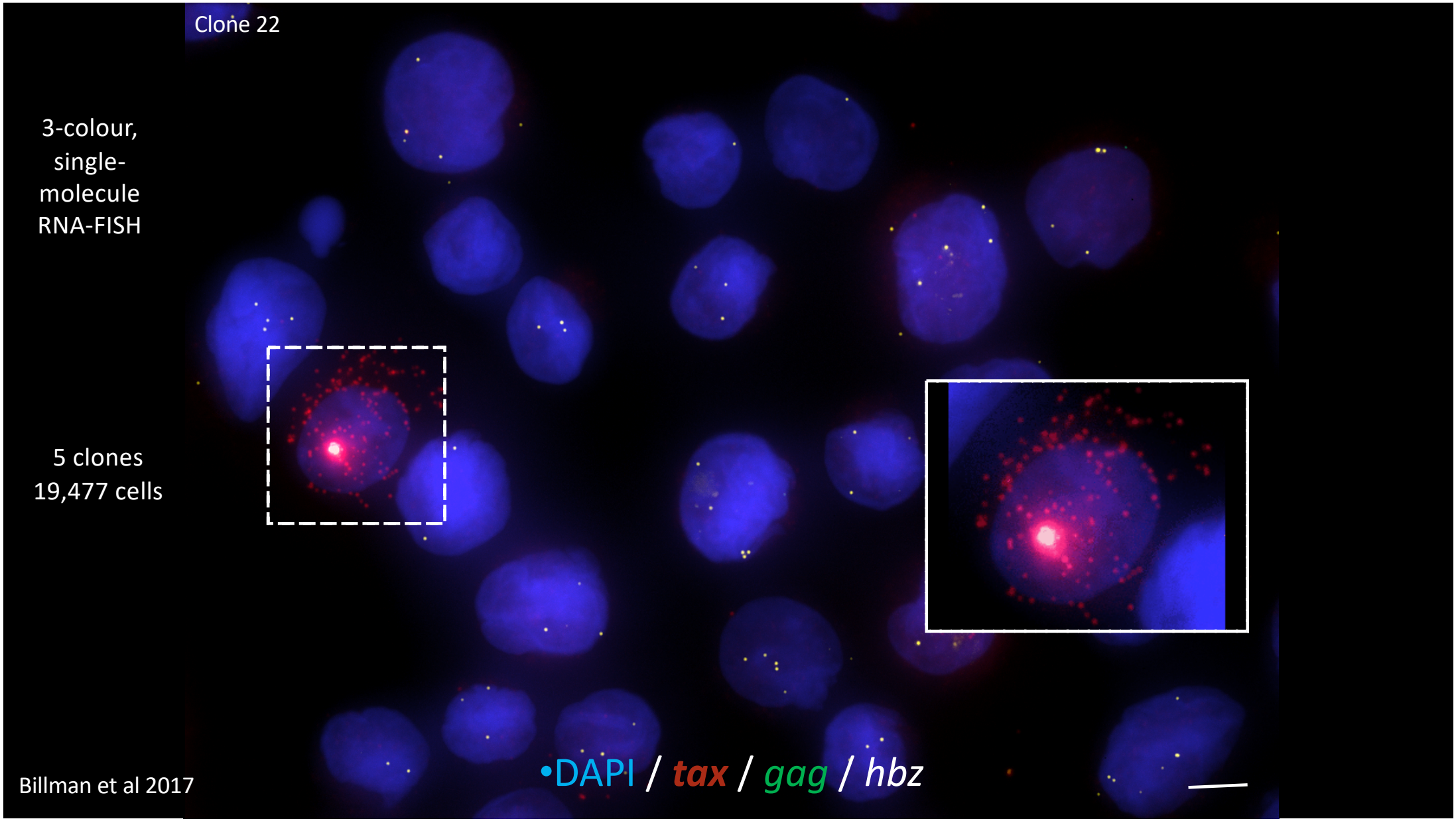


p30 (viral infectivity)
p13 (viral infectivity)

Tax protein and HBZ mRNA drive proliferation of the infected cell

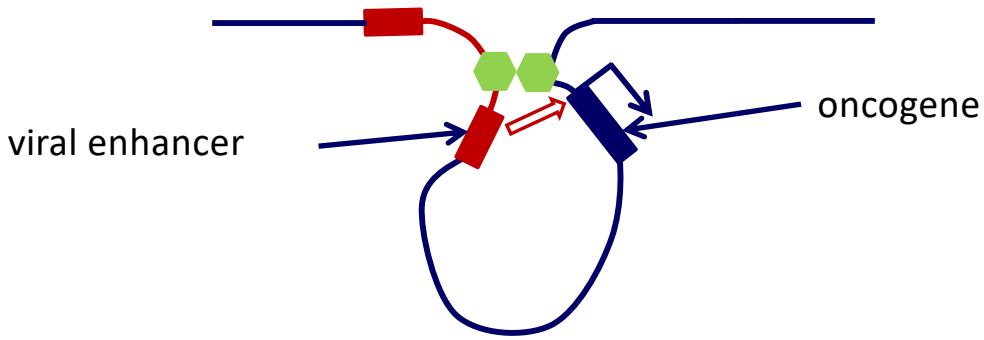
p21 (gag expression)
P12/p8 (immune synapse and transmission, control of TCR signal, regulation of MHC-1 (immune evasion))

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

Leukaemia mechanisms of HTLV-1a

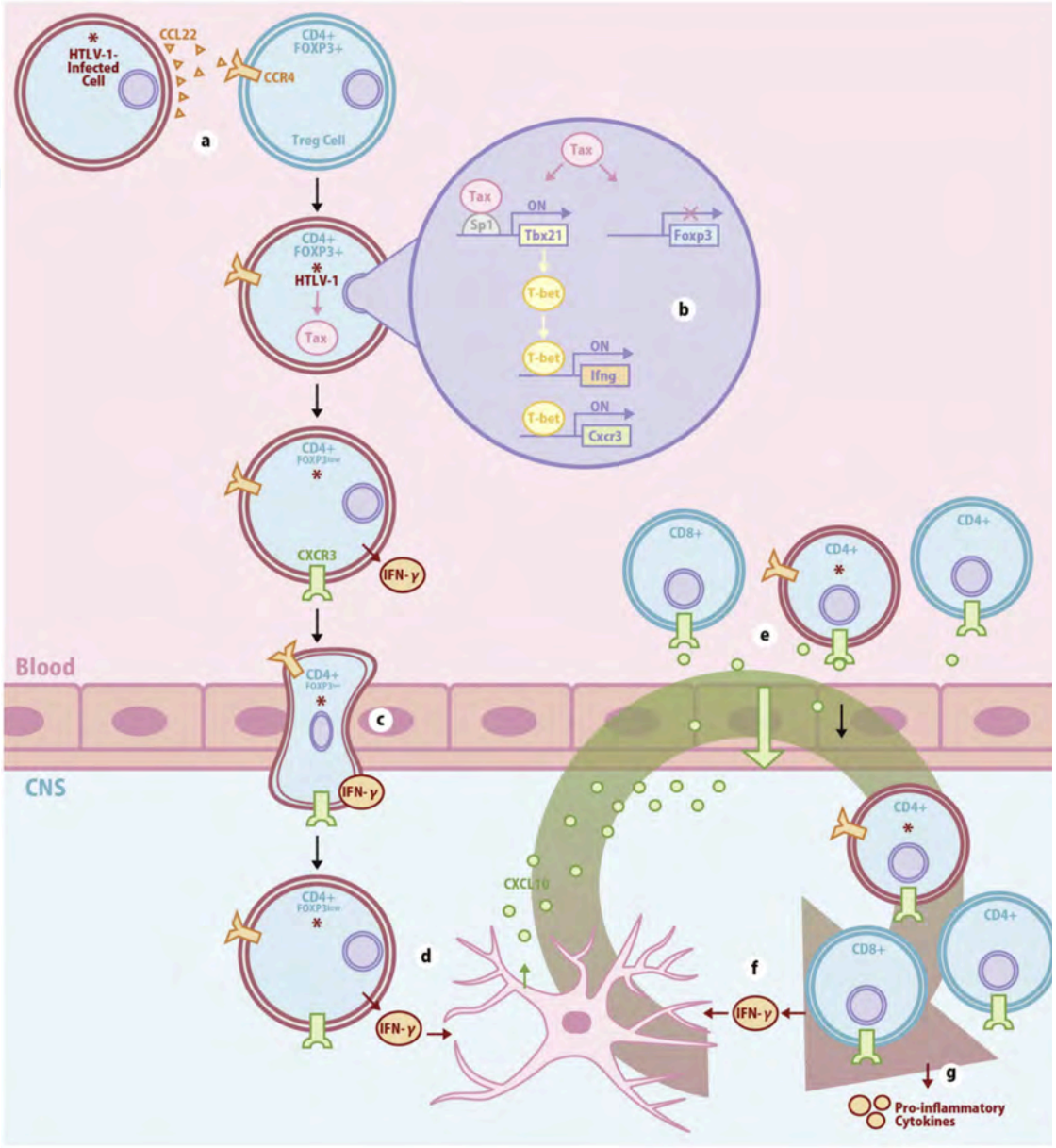
- Ongoing expression of viral RNA & proteins: *hbz*, HBZ, Tax
- Insertional mutation of host DNA
- Long range activation of host oncogenes



Chromatin looping extends the potential for insertional oncogenesis from ~10 kb to ~5 Mb.

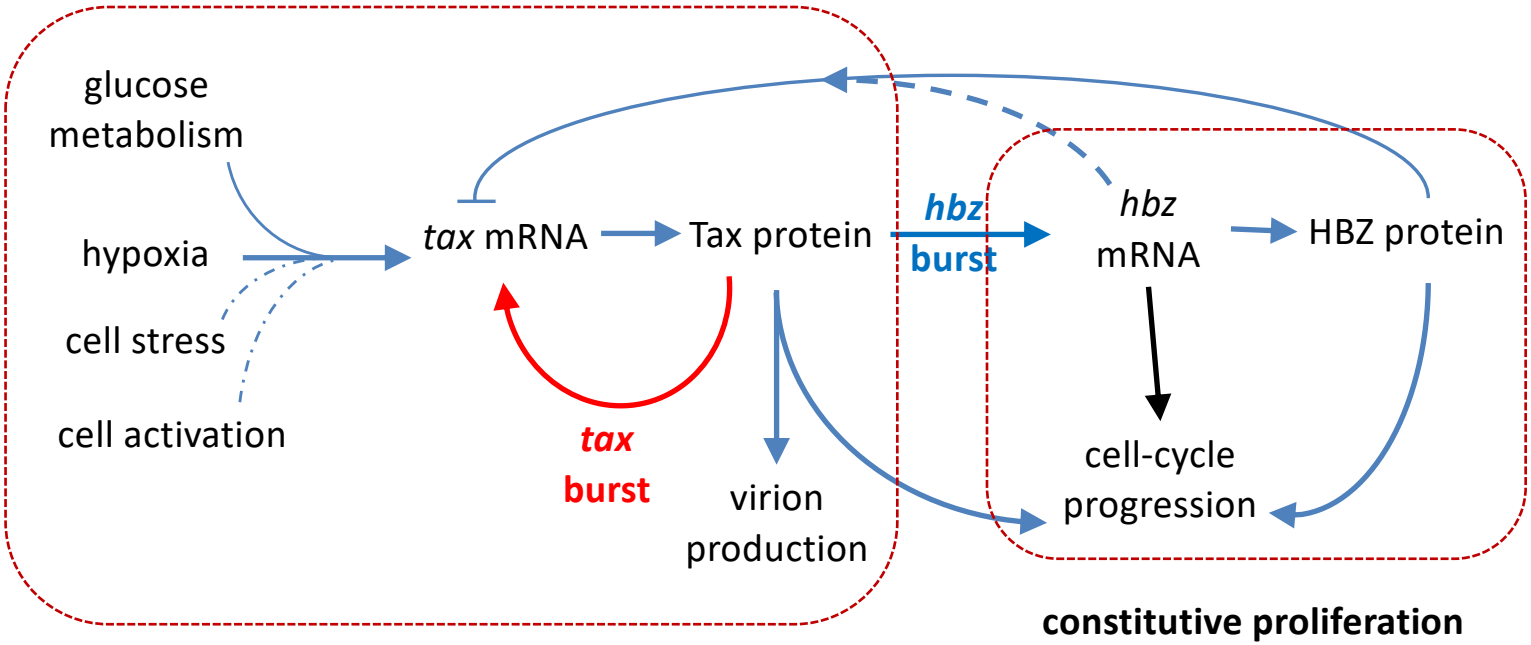
HTLV-1 inflammatory disease through altered cytokine expression

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



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

Regulation of HTLV-1 expression and replication



Kulkarni et al 2017 Cell Chem Biol

Bangham and Ratner 2015

Satou et al 2006; Billman et al 2017

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

Hypothesised that these genetic differences result in novel gene expression in HTLV-1c

- increase inflammation

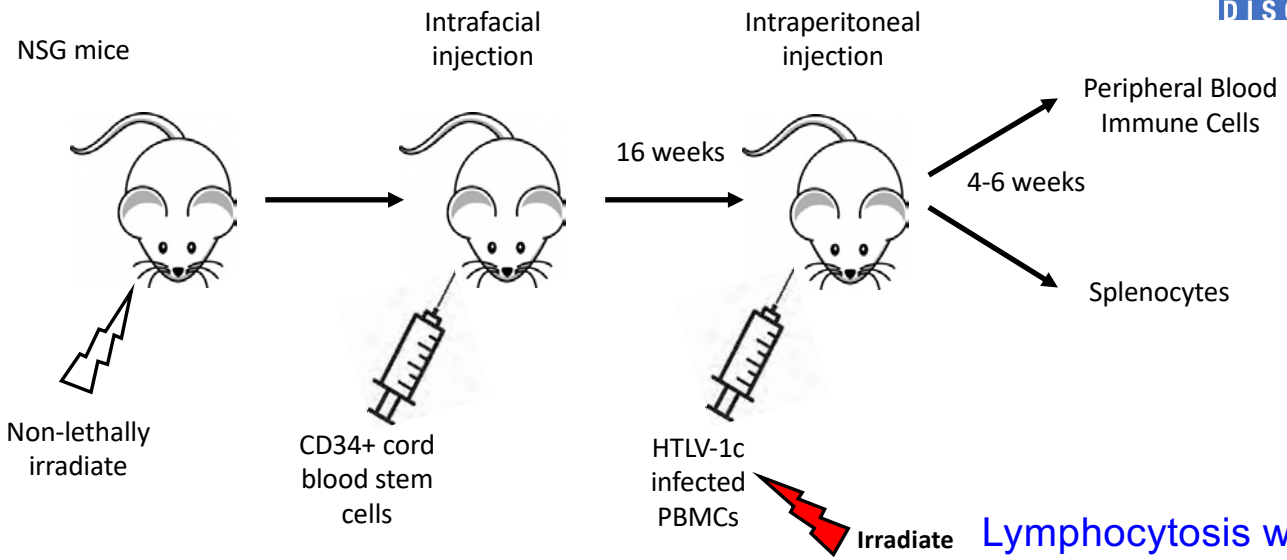
- reduce leukemia induction

Yurick, D. 2017

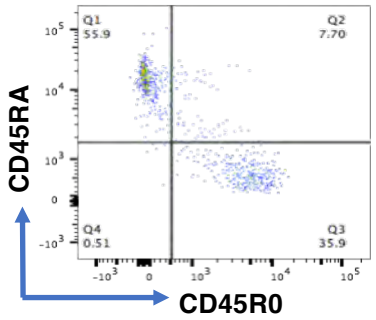
Genomic Region	Nucleotide Divergence %	Amino Acid Divergence %
Rex	5.26	13.23
Env	6.27	3.07
Pol	6.54	3.91
Tax	6.69	7.65
Pro	6.95	8.97
Gag	7.60	3.96
5'LTR	9.14	n/a
3'LTR	9.40	n/a
pX region	9.50	21.95
p30	10.41	15.68
HBZ	12.36	19.12
p27	12.96	22.35
p8	13.33	18.84
p12	19.39	26.80

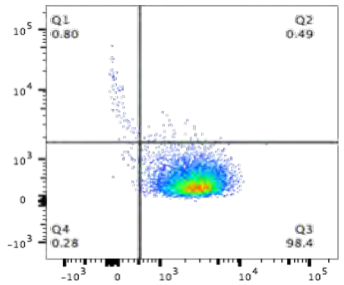
Humanised mouse model to investigate HTLV-1c viral replication

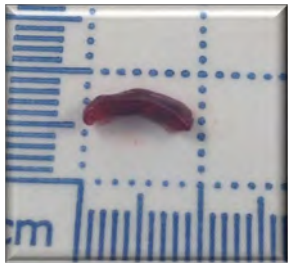
Constructed by the Pellegrini group, WEHI

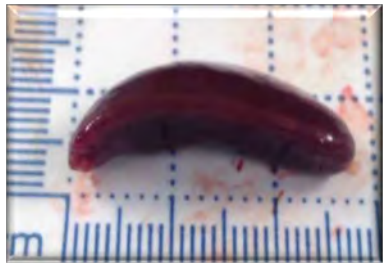


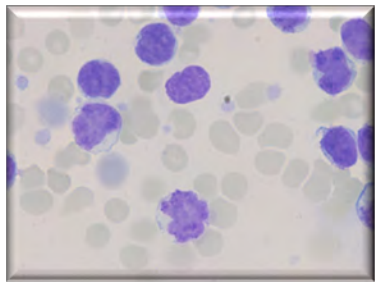
Lymphocytosis with CD4+ memory T-cells



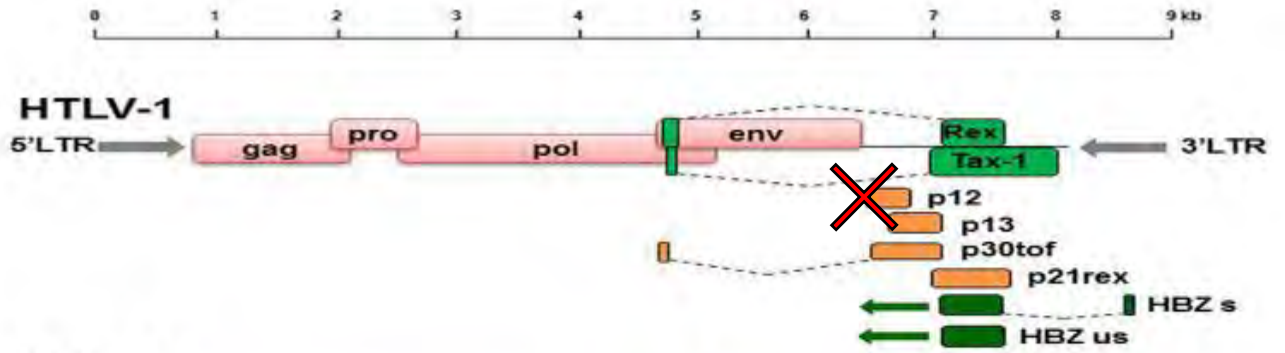




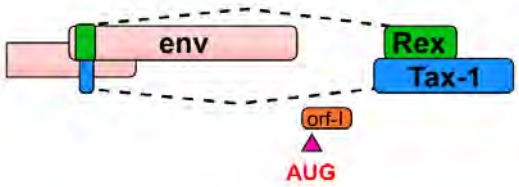




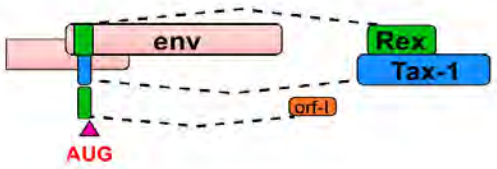
p12 variation between HTLV-1a and -1c



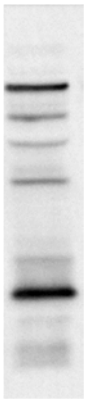
HTLV-1A



HTLV-1C



orf-I **p16**



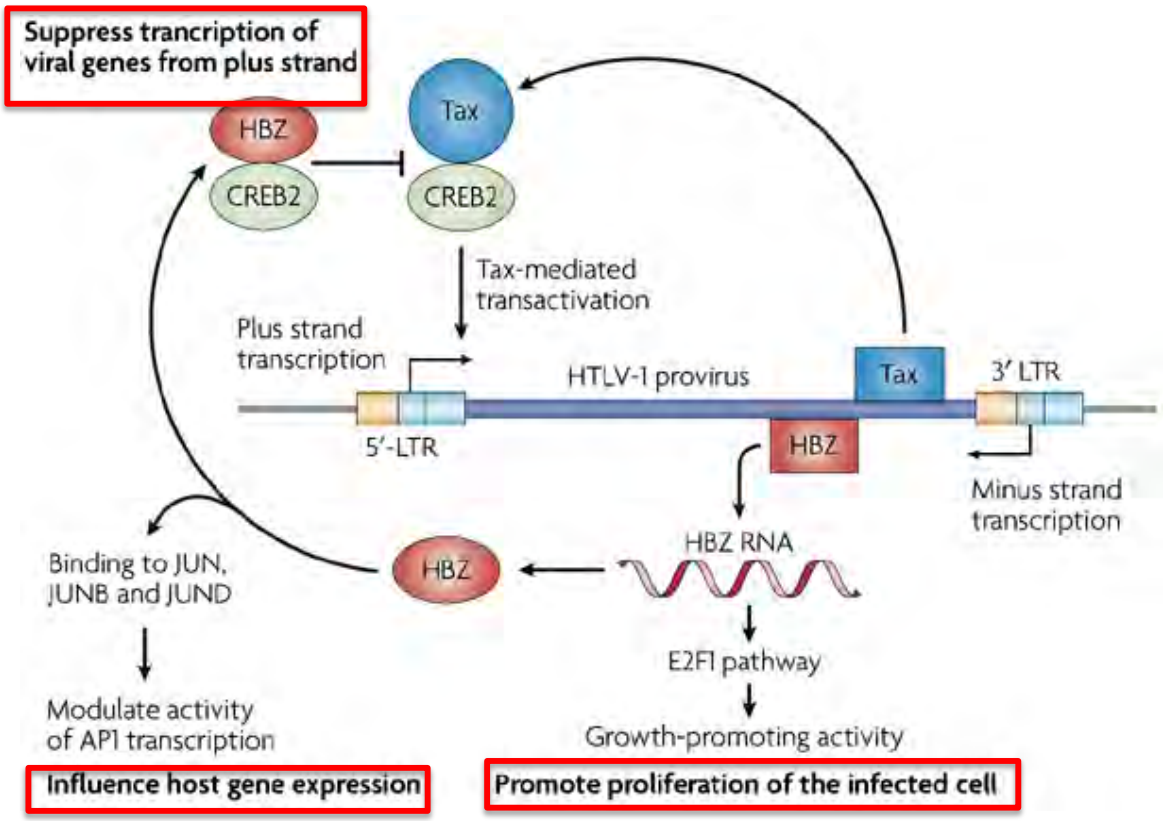
HTLV-1c encodes a p16 variant of the p12 (*orf-I)* using an in frame upstream AUG initiation codon

The p16 variant may contribute to higher inflammatory disease

Galli, Fujikawa, Omsland, Moles, Pise-Masison, Khoury, Yurick, Hirons, Purcell, Franchini (*In Preparation, 2018)*

HTLV-1 basic leucine zipper factor (HBZ)

- Only gene transcribed from the reverse strand
- Expressed constitutively throughout infection
- Low immunogenicity

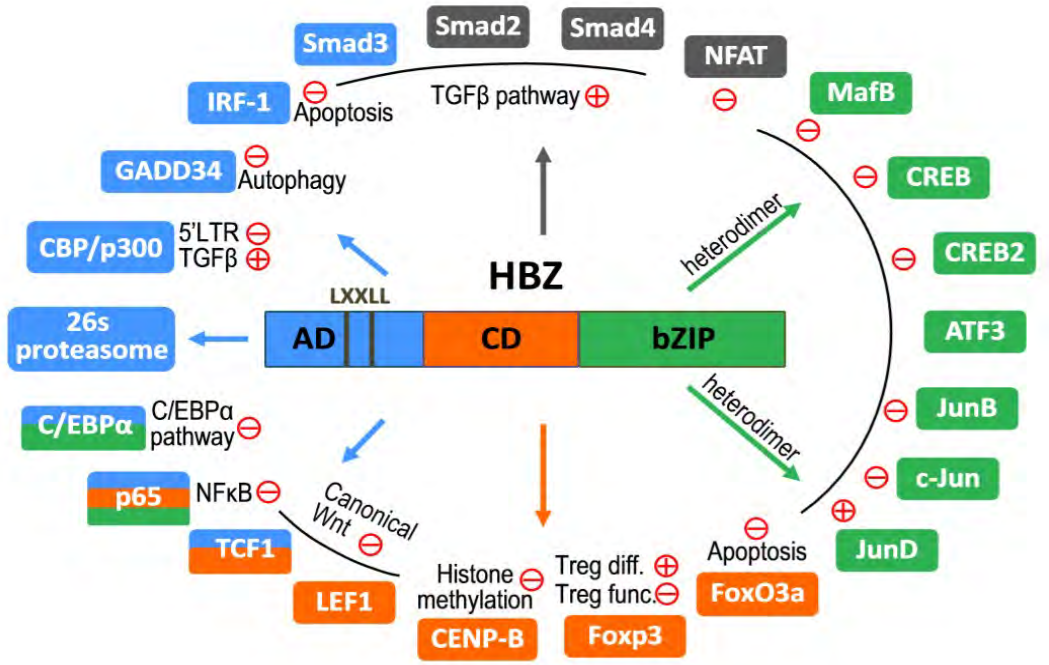


Matsuoka et al. 2007

Deletion in HBZ activation domain deletion that arises by splicing may impact a large range of functions

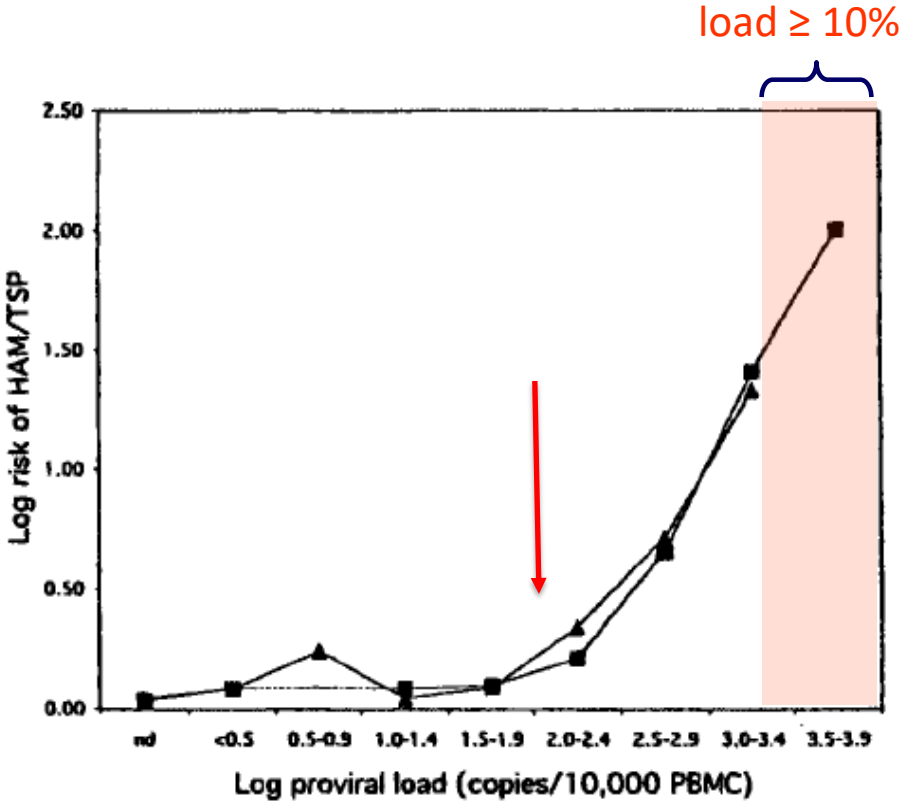
```
HTLV-1a Major HBZ | MAASGLFRCLPVSCPEDLLVEELVDGLLSLEEELKDK-EEEEAVLDGL
HTLV-1c Major HBZ | MAASGPFRCLPVPRPEDLLVEDLVDGLLSLEDDLKDQREEEESVLDGV
HTLV-1c Minor HBZ | MAASGRA-----------------------------------DGV

HTLV-1a Major HBZ | LSLEEESRGRLRRGPPGEKAPPRGETHRDRQRRAEEKRKRKKEREKE
HTLV-1c Major HBZ | LSLEEESR--LRWGLPGEEAPPRGETHRDRRRRAEEKRKRKREREKE
HTLV-1c Minor HBZ | LSLEEESR--LSWGLPGEEAPPRGETHRDRRRRAEEKRKRKREREKE
```



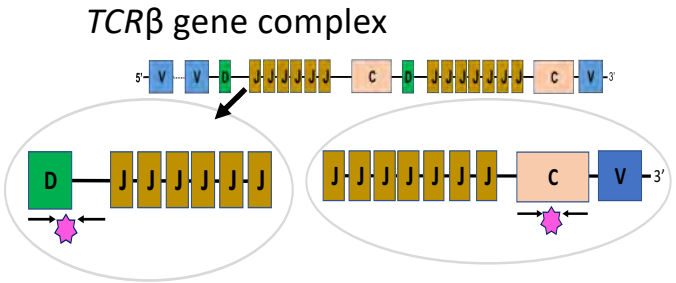
Ma et al., Retrovirology, 2016

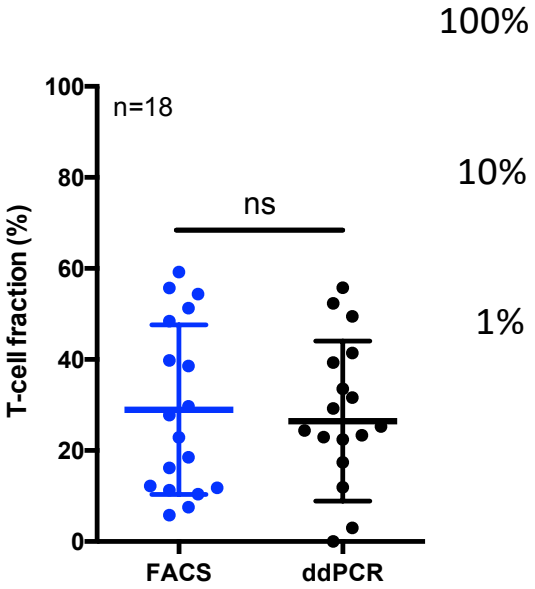
People with high HTLV-1 proviral loads (PVL) have increased risk of leukemia and inflammatory diseases

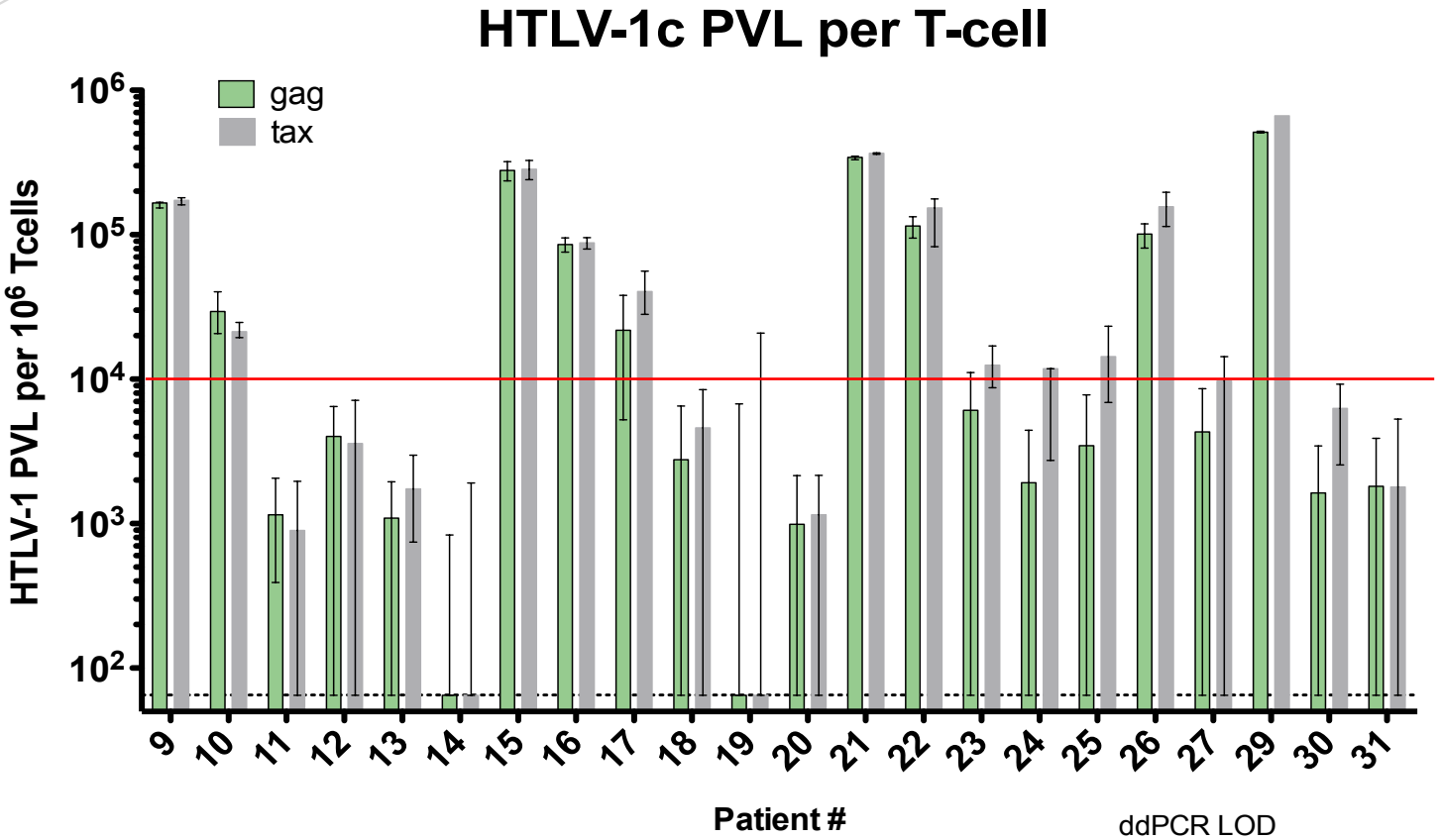


Jeffery et al, 1999, PNAS

An improved ddPCR assay for HTLV-1c proviral load

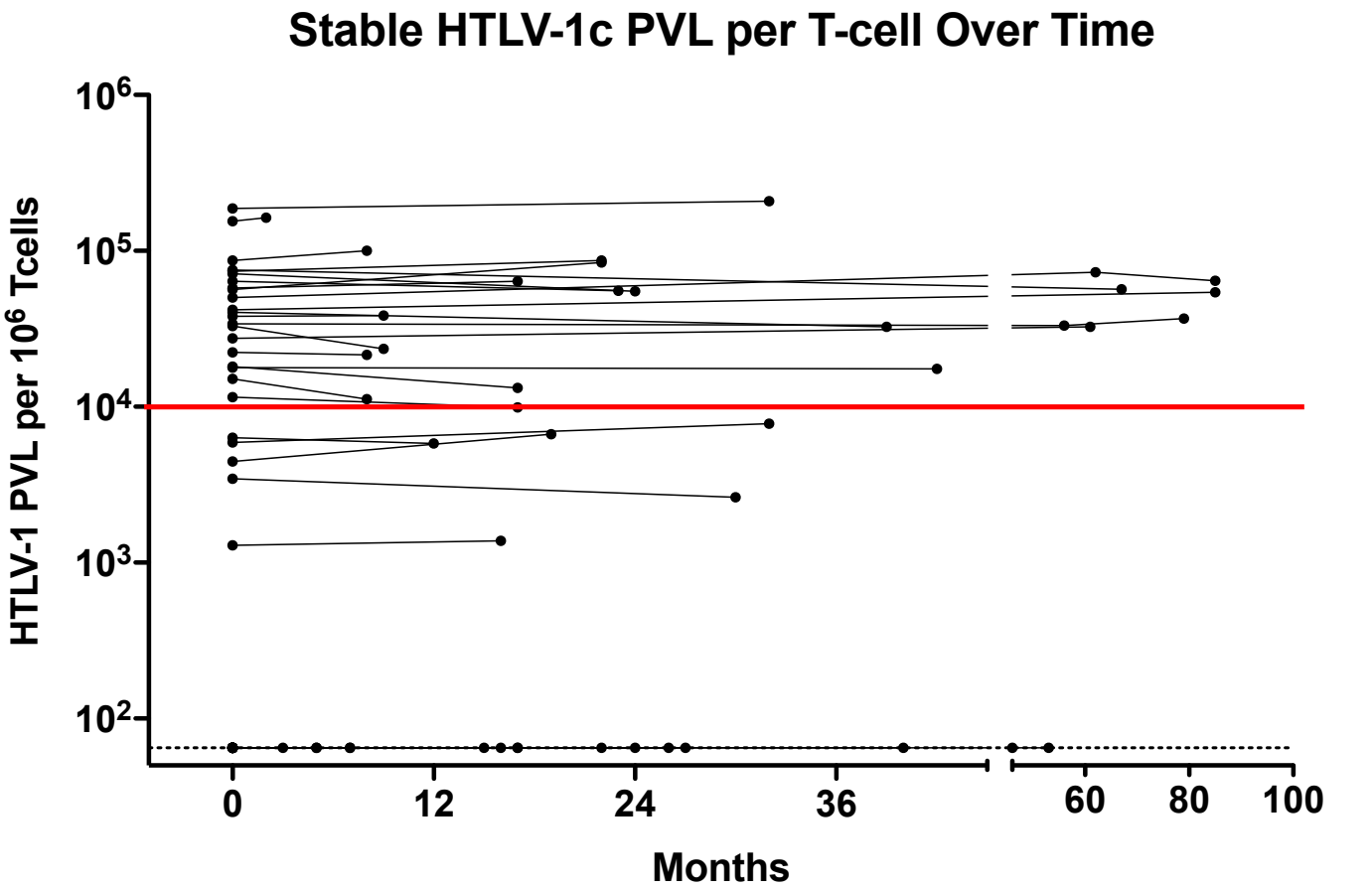






Yurick, D. 2017

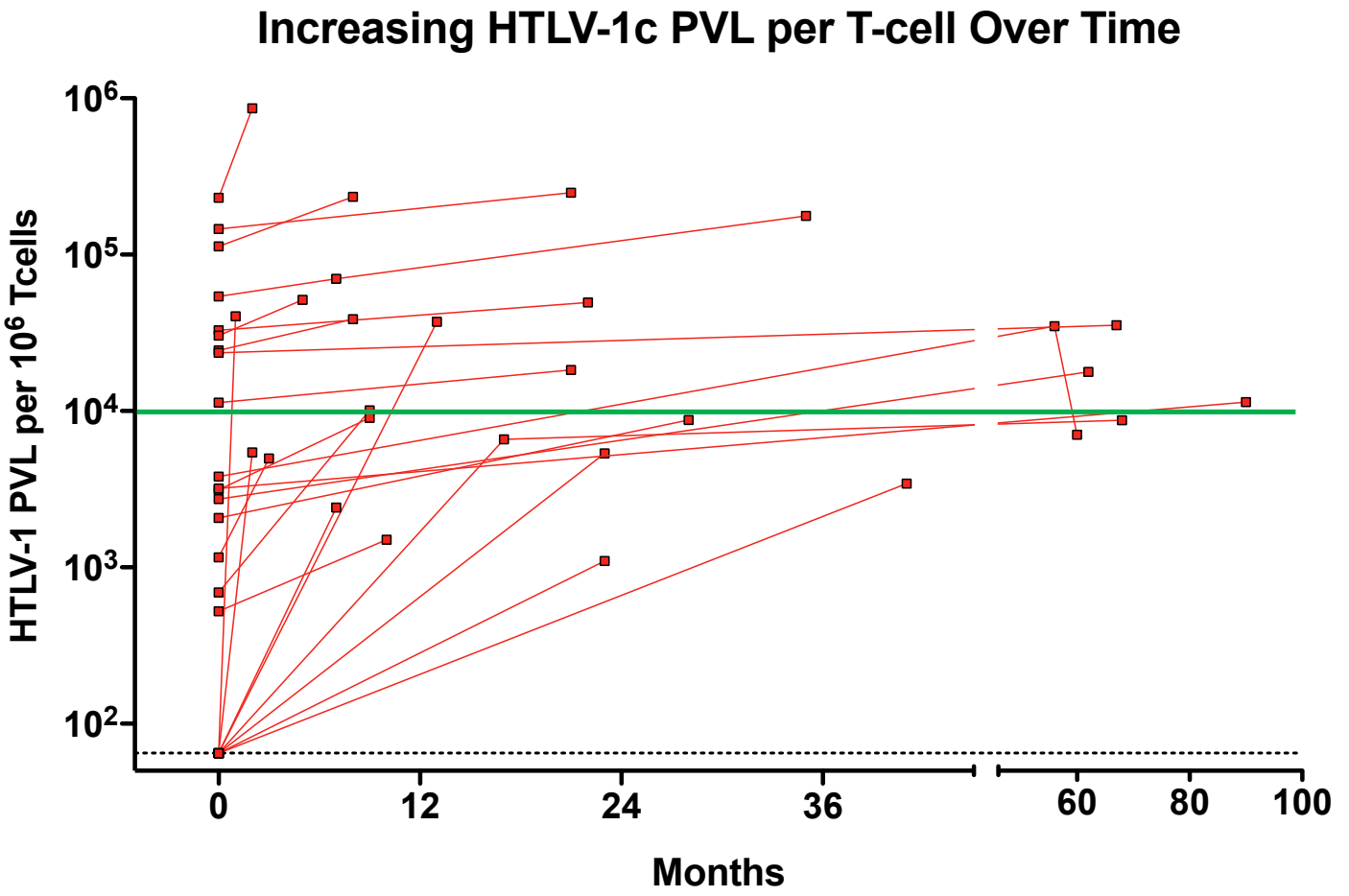
Longitudinal changes in HTLV-1c PVL per T-cell



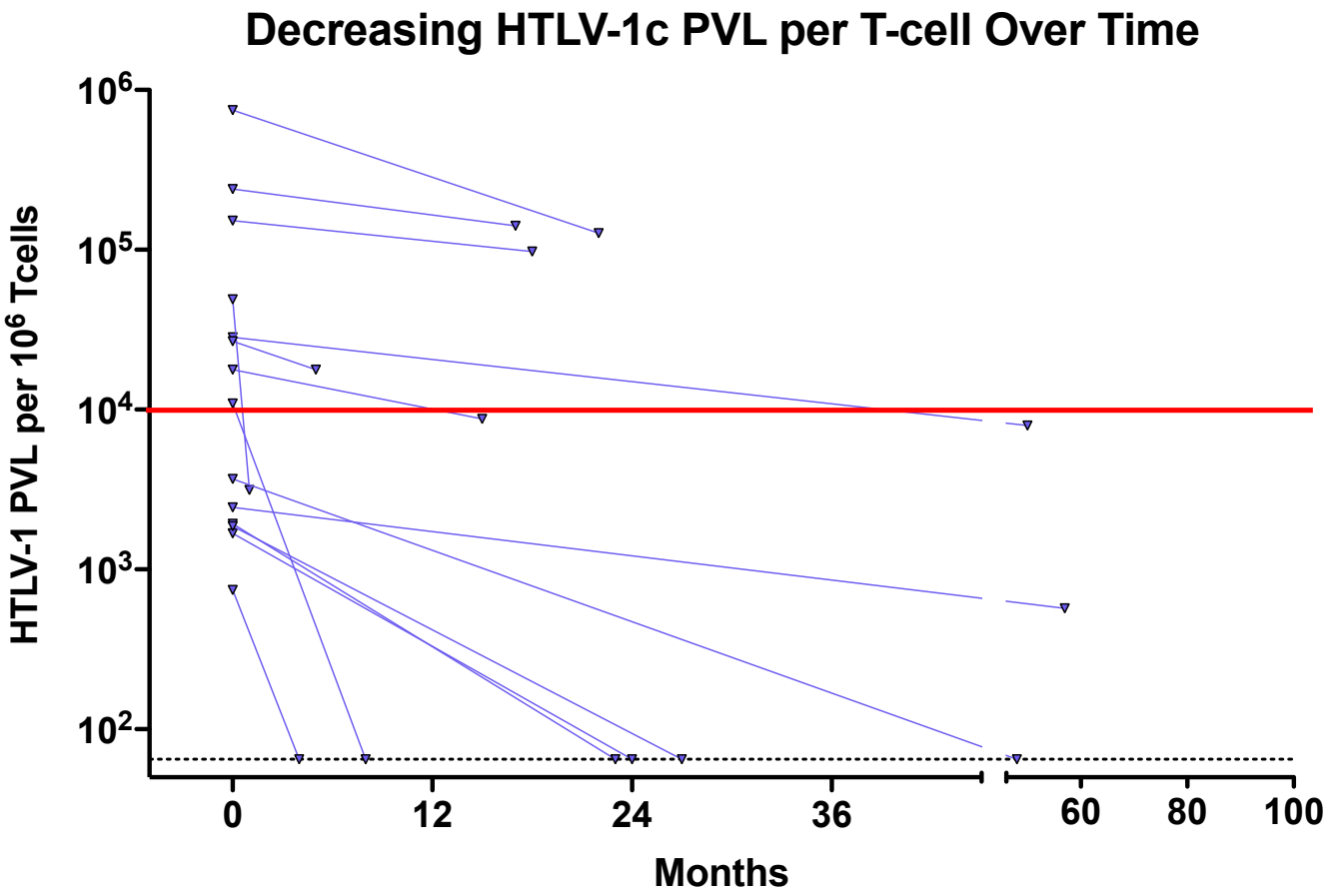
All patients have minimum 2 collection points
Stable conditions: 0.66-fold =< PVL =< 1.5-fold

n = 39 / 83 (47%)

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



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

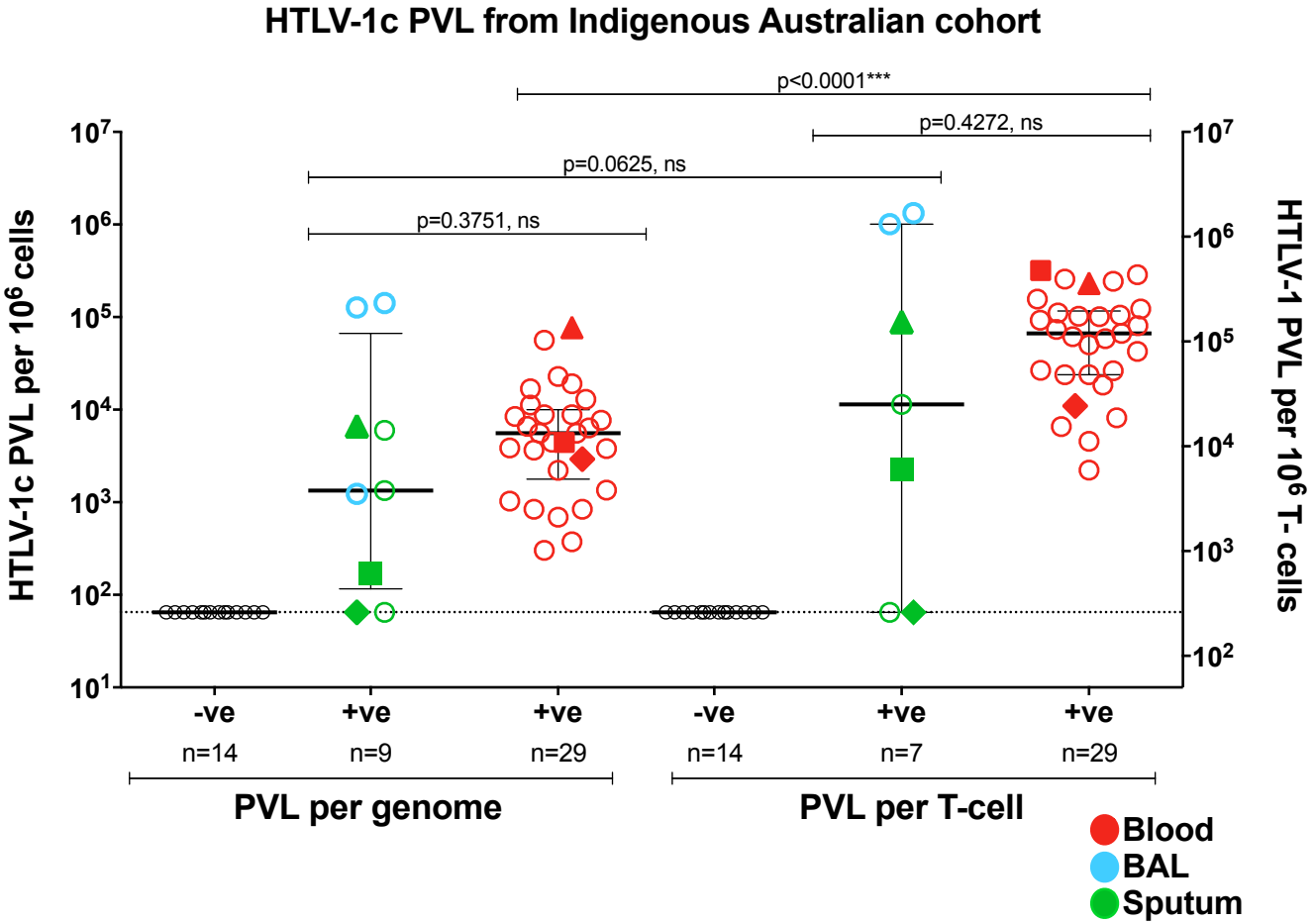


All patients have minimum 2 collection points
Decreasing conditions: PVL =< 0.66-fold

▼ Decreasing PVL per T-cell

n = 14 / 83 (17%)

High HTLV-1c PVL in T-cells from BAL and induced sputum is associated with respiratory diseases such as bronchiectasis



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

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

- p12 / p8 and HBZ

HTLV-1c may be more highly associated with inflammatory disease pathogenesis due to novel functions of diverged viral regulatory proteins

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

Confirmed for HTLV-1c:

- Achieves high proviral loads
  - Promotes transmission and associates with disease
- Causes T-cell proliferation and induction of "Zombie T-cells" in a mouse model
- Different X-region (p16/p8/p30/HBZ) expression profile

Not known with HTLV-1c:

- Pathogenic mechanisms of altered X-region proteins (p16 and HBZ)
  - Increased inflammatory disease?
  - Reduced leukemia induction?
- Assay that accurately predicts 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?

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)

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Con Sonza
Charlene MacKenzie
Chris Gonelli
Michelle Lee
Damian Purcell Lab

Sharon Lewin & Lab - Megan Crane, Jenny Audsley

Patient volunteers! & Community members

Doherty Institute:
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Bridie Clemens
Liyen Loh

NRL Testing:
Kim Wilson

WEHI:
Marc Pellegrini
James Cooney
Cody Allison,

Imperial College UK:
Charles Bangham

NCI, NIH USA:
Genoveffa Franchini