Advances in molecular analysis in HTLV infection

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HTLV: Introduction

- First Retrovirus described in humans

- **HTLV-1**, HTLV-2, HTLV-3, HTLV-4

- Transmission
  - IDUs
  - HIV/HTLV

- Prevalence

$> 10^6$

$> 5.10^5$

$> 10^5$

$> 5.10^4$

$> 10^4$

$> 10^3$
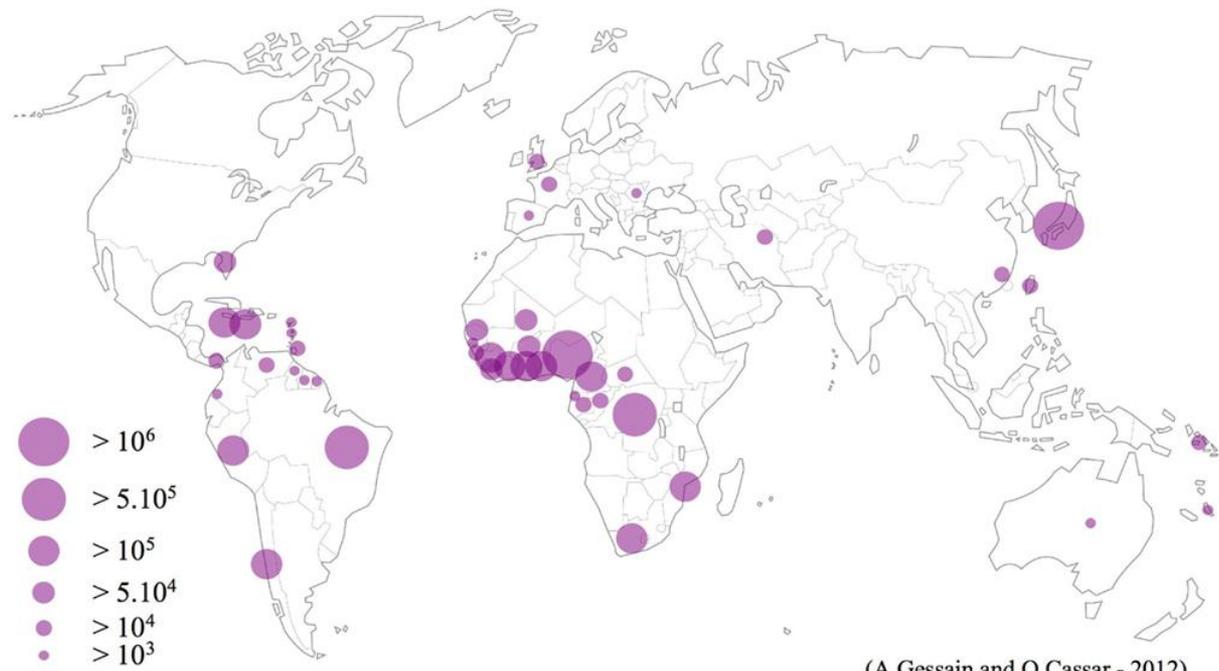
(A.Gessain and O.Cassar - 2012)

HTLV: clinical presentation

- Asymptomatic infection

- 1-5% disease
  - HAM/TSP
  - ATL
  - Other clinical presentation

HAM/TSP
Chronic progressive incapacitating neurologic disease

There is no treatment

HAM/TSP patient

HTLV morphology

Verdonck et al., 2007
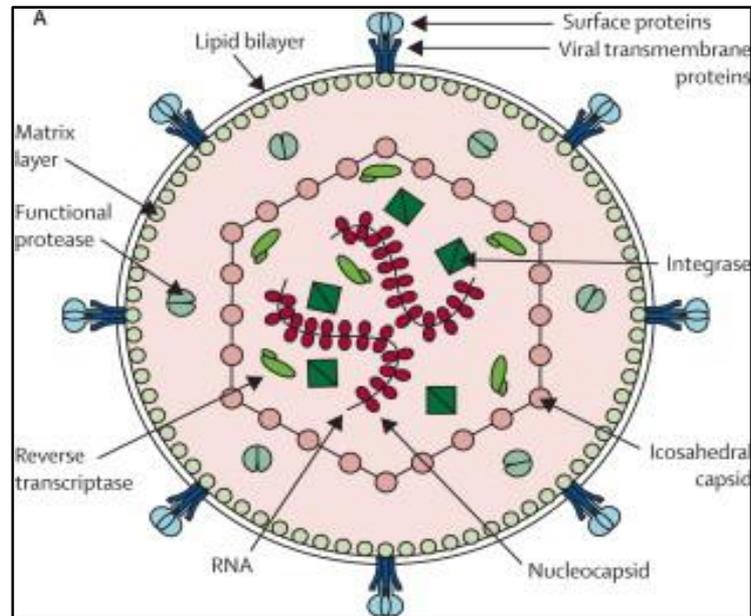
HTLV replication cycle
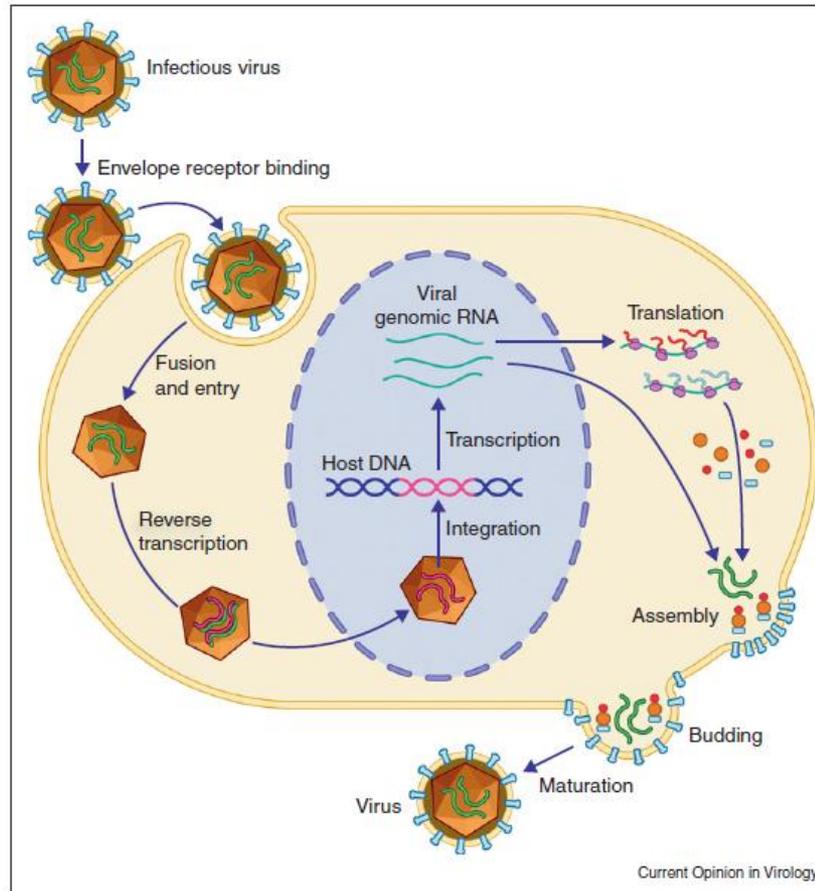
Clonal expansion of Infected T-cell

Lairmore et al., 2012

HTLV proviral genome
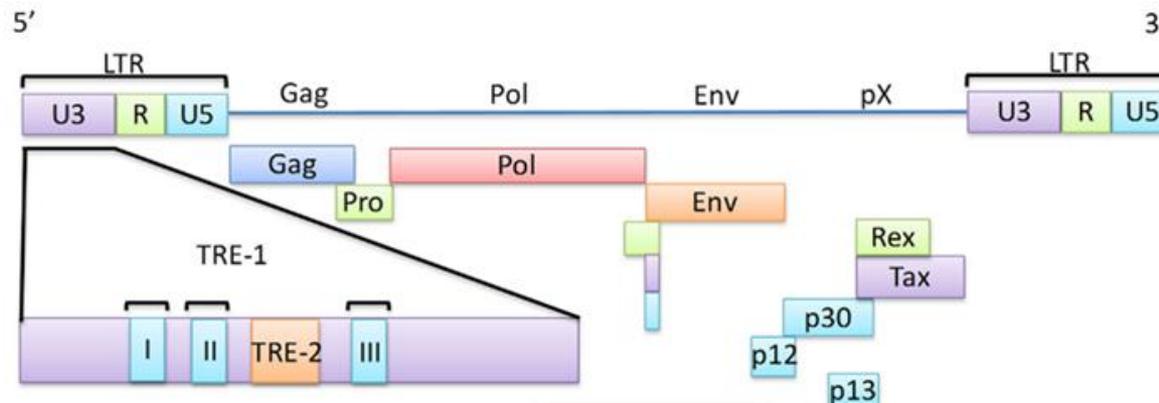
Low mutation rate

Molecular analysis in HTLV infection

➤ Diagnosis

➤ Prognosis

➤ Epidemiology

➤ Pathogenesis

Diagnosis

WHO (OSAME, 1990)	Castro-Costa (2006)
• HAM/TSP Classical symptoms • Abs in blood **and** CSF	• HAM/TSP Classical symptoms • Abs in blood and CSF or PCR positive

Abs detection:
ELISA: screening test
WB: Confirmatory and typing test
Indeterminate WB

Detection of proviral DNA

❖Recent infection

❖Passive transfer of Abs

❖Indeterminate WB

❖Abs in blood and not in CSF in symptomatic patients (HAM/TSP)

❖Identification of HTLV types and subtypes

CASE REPORT

Human T-lymphotropic virus type 2 subtype b in a patient with chronic neurological disorder

Carolina Rosadas · Ana C. P. Vicente · Louise Zanella ·
Mauro J. Cabral-Castro · José M. Peralta ·
Marzia Puccioni-Sohler
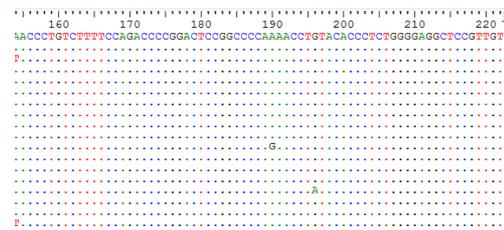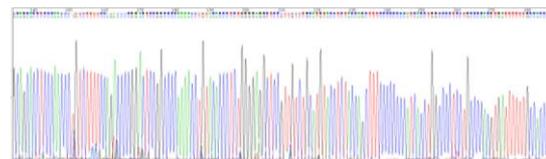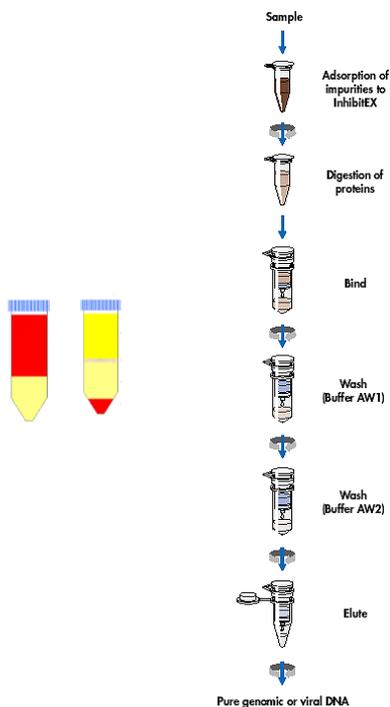
Nucleotide identity

HTLV-2 LTR and Tax region nucleotide identity in percentage between the patient isolate and differents HTLV prototypes

	Prototypes (% similarity)			
Versus patient isolate	NRA (HTLV-2b)	MoT (HTLV-2a)	Kayapo (HTLV-2c)	ATK1 (HTLV-1)
LTR region	99%	93%	95%	81%
Tax gene	99%	96%	97%	77%

LTR (623 nucleotides)
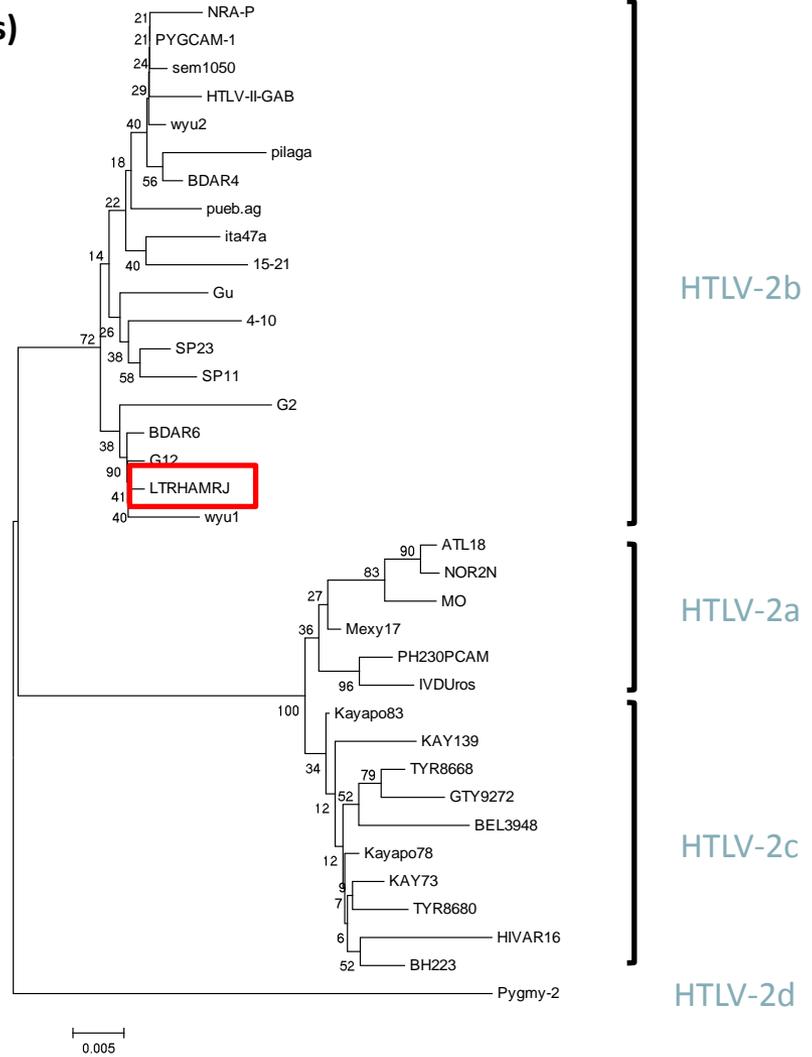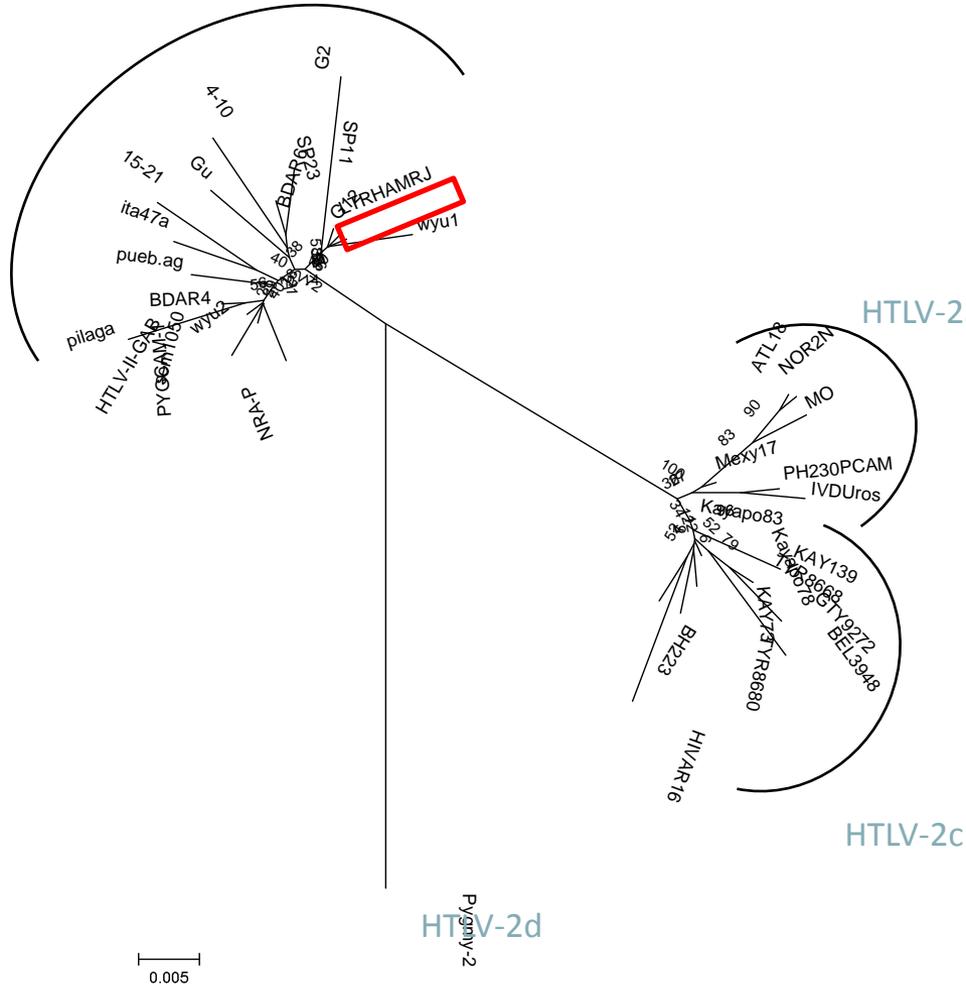
Tax protein

```
TaxHAM-RJ   HFPGFGQSLLYGYPVYVFGDCVQADWCPVSGGLCSTRLHRHALLATCPEHQLTWDPIDGR 60
NRA         HFPGFGQSLLYGYPVYVFGDCVQADWCPVSGGLCSTRLHRHALLATCPEHQLTWDPIDGR 60
Kayapo1     HFPGFGQSLLYGYPVYVFGDCVQADWCPVSGGLCSTRLHRHALLATCPEHQLTWDPIDGR 60
MoT         HFPGFGQSLLYGYPVYVFGDCVQADWCPVSGGLCSTRLHRHALLATCPEHQLTWDPIDGR 60
ATK1        HFPGFGQSLLFGYPVYVFGDCVQGDWCPISGGLCSARLHRHALLATCPEHQITWDPIDGR 60
            **********:************ .****:******:****************:*******

TaxHAM-RJ   VVSSPLQYLIPRLPSFPTQRTSKTLKVLTPPTTPVSPKVPPAFFQSMRKHTPYRNGCLEP 120
NRA         VVSSPLQYLIPRLPSFPTQRTAKTLKVLTPPTTPVSPKVPPAFFQSMRKHTPYRNGCLEP 120
Kayapo1     VVSSPLQYLIPRLPSFPTQRTSRTLKVLTPPTTPISPKVPPAFFQSMRKHTPYRNGCLEP 120
MoT         VVSSPLQYLIPRLPSFPTQRTSRTLKVLTPPTTPVSPKVPPAFFQSMRKHTPYRNGCLEP 120
ATK1        VIGSALQFLIPRLPSFPTQRTSKTLKVLTPPITHTTPNIPPSFLQAMRKYSPFRNGYMEP 120
            *:.*.**:************:.*******  *  :*:::**:*:*:***::*:*** :**

TaxHAM-RJ   TLGDQLPSLAFPEPGLRPQNIYTTWGKTVVCLYLFQLSPPMTWPLIPHVIFCHPRQLGAF 180
NRA         TLGDQLPSLAFPEPGLRPQNIYTTWGKTVVCLYLFQLSPPMTWPLIPHVIFCHPRQLGAF 180
Kayapo1     TLGDQLPSLAFPEPGLRPQNIYTTWGKTVVCLYLYQLSPPMTWPLIPHVIFCHPRQLGAF 180
MoT         TLGDQLPSLAFPEPGLRPQNIYTTWGKTVVCLYLYQLSPPMTWPLIPHVIFCHPRQLGAF 180
ATK1        TLGQHLPTLSFPDPGLRPQNLYTLWGGSVVCMYLYQLSPPITWPLLPHVIFCHPGQLGAF 180
            ***::**:*:*:**:******** :** ** :***:**:*****:****:******* *****

TaxHAM-RJ   LTKVPLKRLEELLYKMFLHTGAVIVLPEDDLPTTMFQPVRAPCIQTAWCTGLLPYHSILT 240
NRA         LTKVPLKRLEELLYKMFLHTGAVIVLPEDDLPTTMFQPVRAPCIQTAWCTGLLPYHSILT 240
Kayapo1     LTKVPLKRLEELLYKMFLHTGAVIVLPEDDLPTTMFQPVRAPCIQTAWCTGLLPYHSILT 240
MoT         LTKVPLKRLEELLYKMFLHTGTVIVLPEDDLPTTMFQPVRAPCIQTAWCTGLLPYHSILT 240
ATK1        LTNVPYKRIEELLYKISLTTGALIILPEDCLPTTLFQPARAPVTLTAWQNGLLPFHSTLT 240
            **:** **:******: * **::*:**** ****:***.***   *** .****:** **

TaxHAM-RJ   TPGLIWTFNDGSPMISGPCPKAGQPSLVVQSSLLIFKKFQTKAFHPSYLLSHQLIQYSSF 300
NRA         TPGLIWTFNDGSPMISGPCPKAGQPSLVVQSSLLIFEKFQTKAFHPSYLLSHQLIQYSSF 300
Kayapo1     TPGLIWTFNDGSPMISGPCPKAGQPSLVVQSSLLIFEKFQTKAFHPSYLLSHQLIQYSSF 300
MoT         TPGLIWTFNDGSPMISGPYPKAGQPSLVVQSSLLIFEKFETKAFHPSYLLSHQLIQYSSF 300
ATK1        TPGLIWTFTDGTPMISGPCPKDGQPSLVLQSSSFIFHKFQTKAYHPSFLLSHGLIQYSSF 300
            ********.**:****** ** *****:*** :*:.**:***:***:**** *******
```

TaxHAM-RJ   HNLHLLFDEYTNIPVSILFNKEEADDN[DQPPE-PAAQGESSTQKVRPSHTNNPK] 54
NRA   HNLHLLFDEYTNIPVSILFNKEEADDN[DQPPE-PAAQGESSTQKVRPSHTNNPK] 54
Kayapo1   HNLHLLFDEYTTIPVSILFNKEEADDN[DQPPE-PATQGESSTQKVRPSHTNNPK] 54
MoT   HNLHLLFDEYTNIPVSILFNKEEADDN[D-------------------------] 29
ATK1   HSLHLLFEEYTNIPISLLFNEKEADDN[HEPQISPGGLEPPSEKHFRETEV----] 51
            *.*****:***.**:*:***::*****

ClustalW2

Prognosis

Determination of proviral load in PBMCs
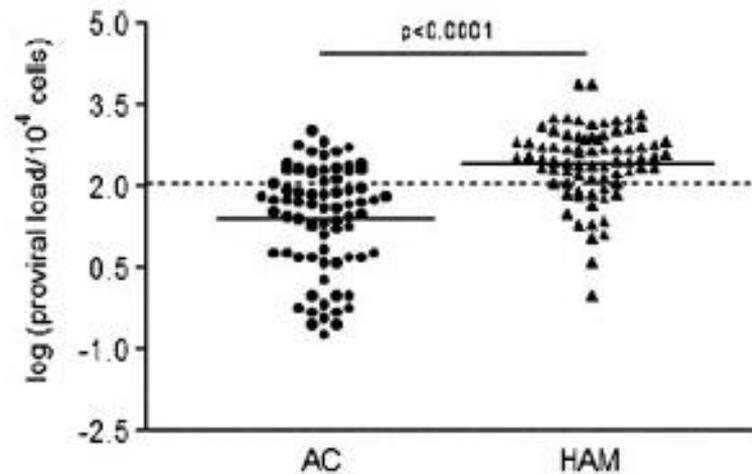
Prognosis

Determination of proviral load in CSF
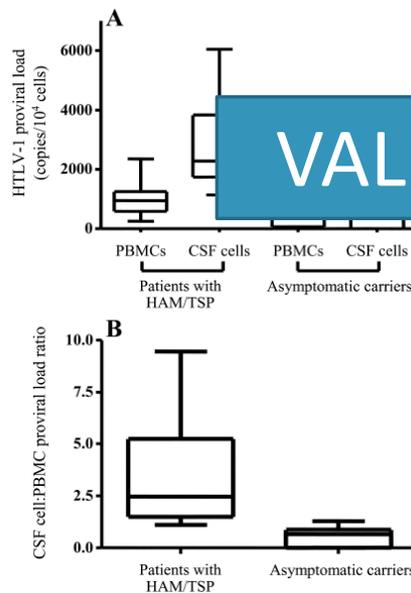
Figure 1. Human T lymphotropic virus type I (HTLV-I) proviral load in peripheral blood mononuclear cells (PBMCs) and cerebrospinal fluid (CSF) cells from patients with HTLV-I–associated myelopathy/tropical spastic paraparesis (HAM/TSP) and from asymptomatic carriers *(A)* and CSF cell: PBMC HTLV-I proviral load ratio *(B). Horizontal bars,* Median and 10th, 25th, 75th, and 90th percentiles.

Table 2 *Mean ± SD of PBMC and CSF of 17 HAM patients (Group I) in comparison with 18 non-HAM patients (Group II)*

	HAM/TSP (Group I), n = 17	Non-HAM/TSP (Group II) n = 18	p Value
PBMC HTLV-I proviral	38 ± 26	9 ± 5	<0.005
		1.9 ± 5	<0.005

PBMC = peripheral blood mononuclear cells: HAM/TSP = human T lymphotropic virus type I (HTLV-I)–associated myelopathy/tropical spastic paraparesis.

VALIDATION IS ESSENCIAL

$r_s = 0.54; p = 0.025; n = 17$
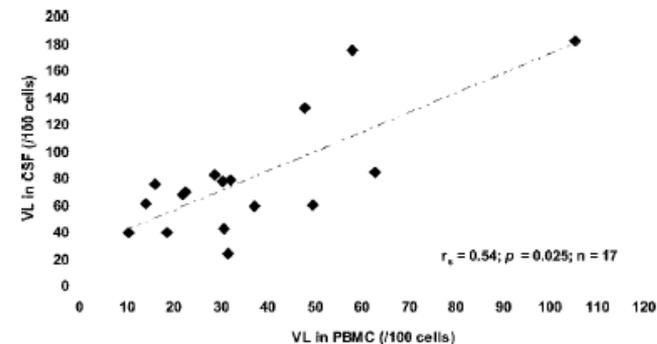
M. Puccioni-Sohler, MD, PhD; Y. Yamano, MD, PhD; M. Rios, PhD; S.M.F. Carvalho, PhD; C.C.F. Vasconcelos, MD; R. Papais-Alvarenga, MD, PhD; and S. Jacobson, PhD

Validation of a quantitative real-time PCR assay for HTLV-1 proviral load in peripheral blood mononuclear cells

CrossMark

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Validation of qPCR for HTLV-1 proviral load in PBMCs

| Actin	
Actin-F	CACATCGTGCCCATCTACGA
Actin-R	CTCAGTGAGGATCTTCATGAGGTAGT
Probe	FAM-ATGCCCTCCCCCATGCCATCCTGCGT-TAMRA
Tax (HTLV-1)	
Tax-F	ACAAAGTTAACCATGCTTATTATCAGC
Tax-R	ACACGTAGACTGGGTATCCGAA
Probe	FAM-TTCCCAGGGTTTGGACAGAGTCTTCT-TAMRA

100ng DNA

Standard curve
PBMC DNA
TARL-2 DNA

Number of HTLV-I (pX) copies/100 cels = (number of copies of pX)/(number of copies of ß-actin/2) x 100 (Nagai et al., 1998)

Validation of qPCR for HTLV-1 proviral load in PBMCs

- Sensibility and specificity
- Limit of detection
- Intra- and inter-assay variability

Validation of qPCR for HTLV-1 proviral load in PBMCs
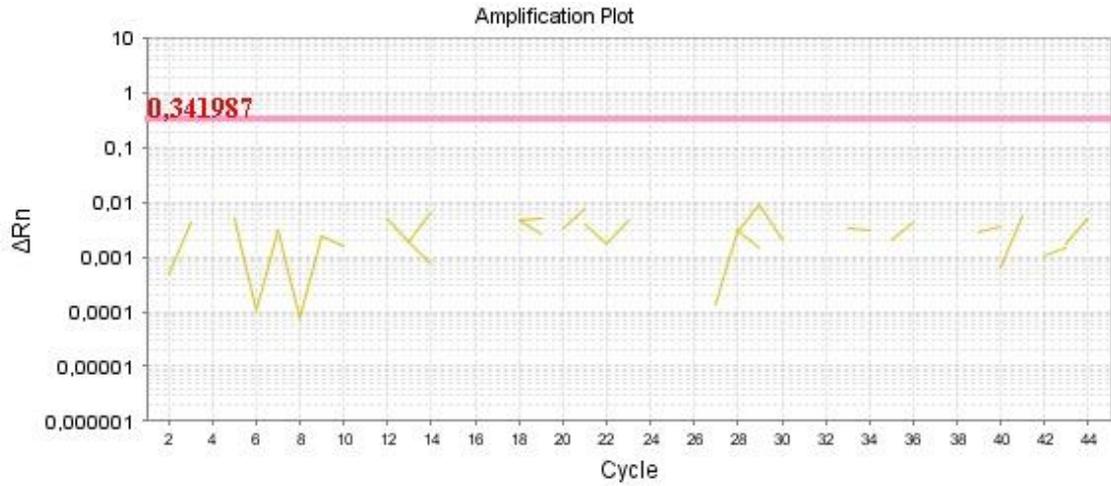
All seropositive samples (ELISA) presented gene amplification (both genes: actin e pX)
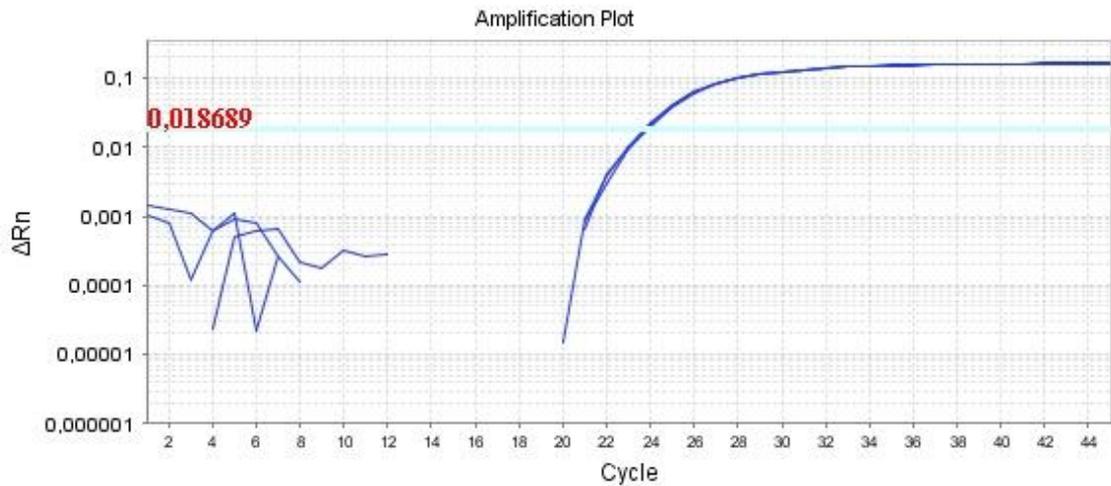
Negative samples (ELISA):
- Not amplify pX gene
- Amplification of actin **Figure**

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Validation of qPCR for HTLV-1 proviral load in PBMCs

◦ Limit of detection: 1 copy/rxn.

◦ qPCR efficiency, slope and r^2: 98,58%, -3,298 e 0,993 respectively.

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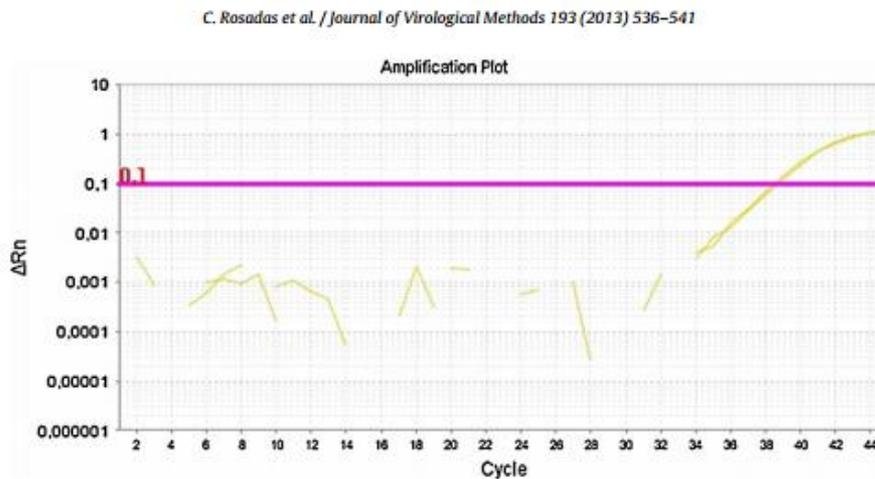
Fig. 4. Amplification plot of the sample containing one copy of pX gene from HTLV-1.

Validation of qPCR for HTLV-1 proviral load in PBMCs

pX

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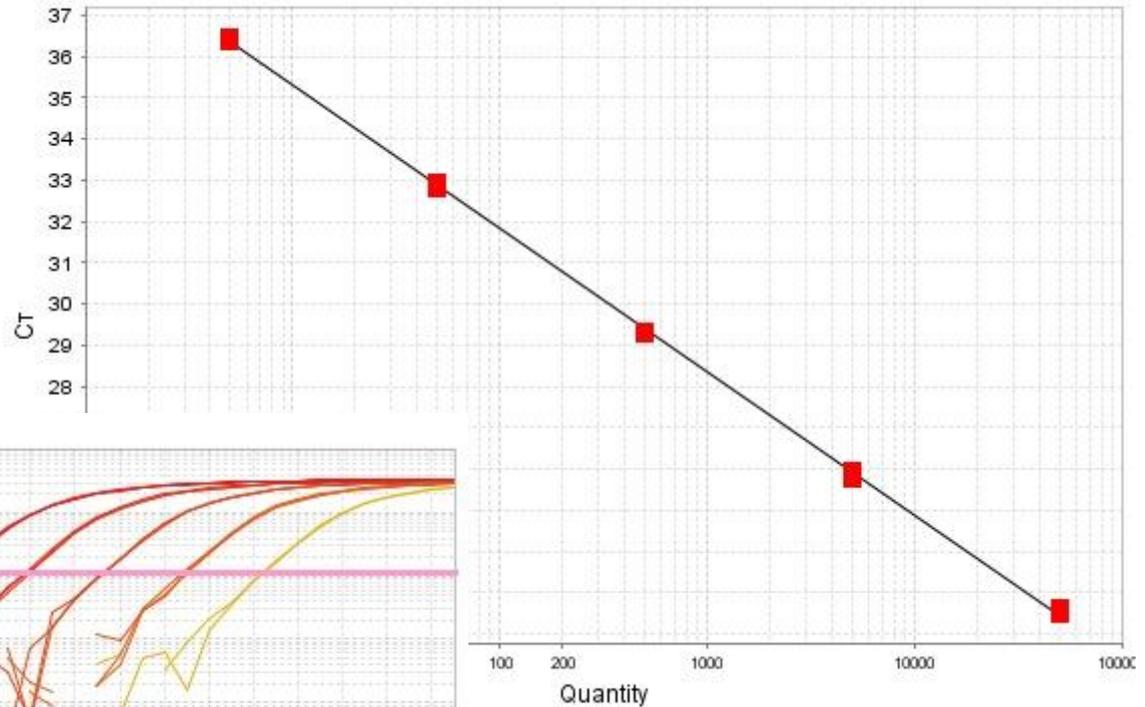
Validation of qPCR for HTLV-1 proviral load in PBMCs

Actin

Standard Curve

Amplification Plot

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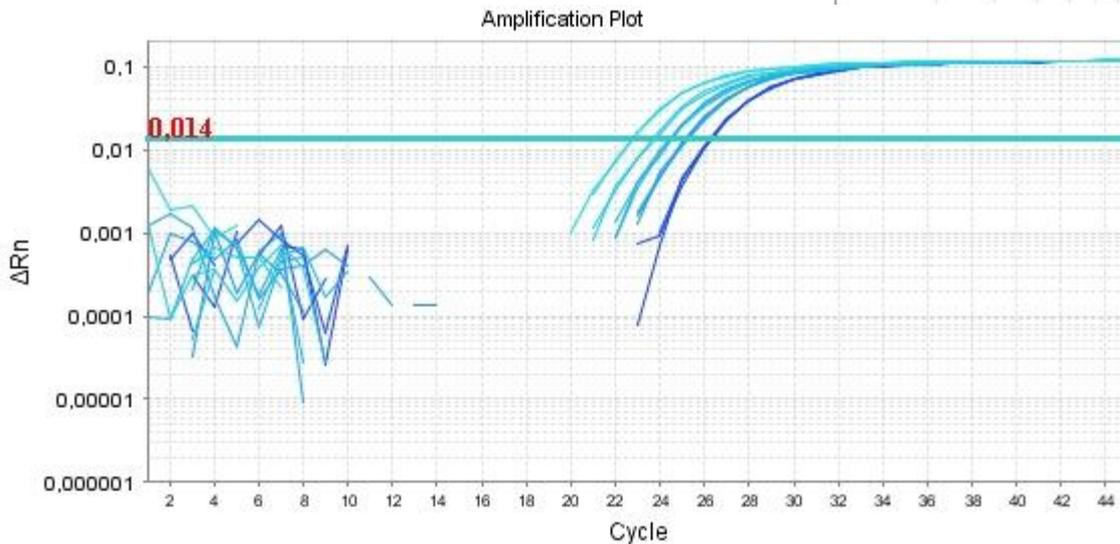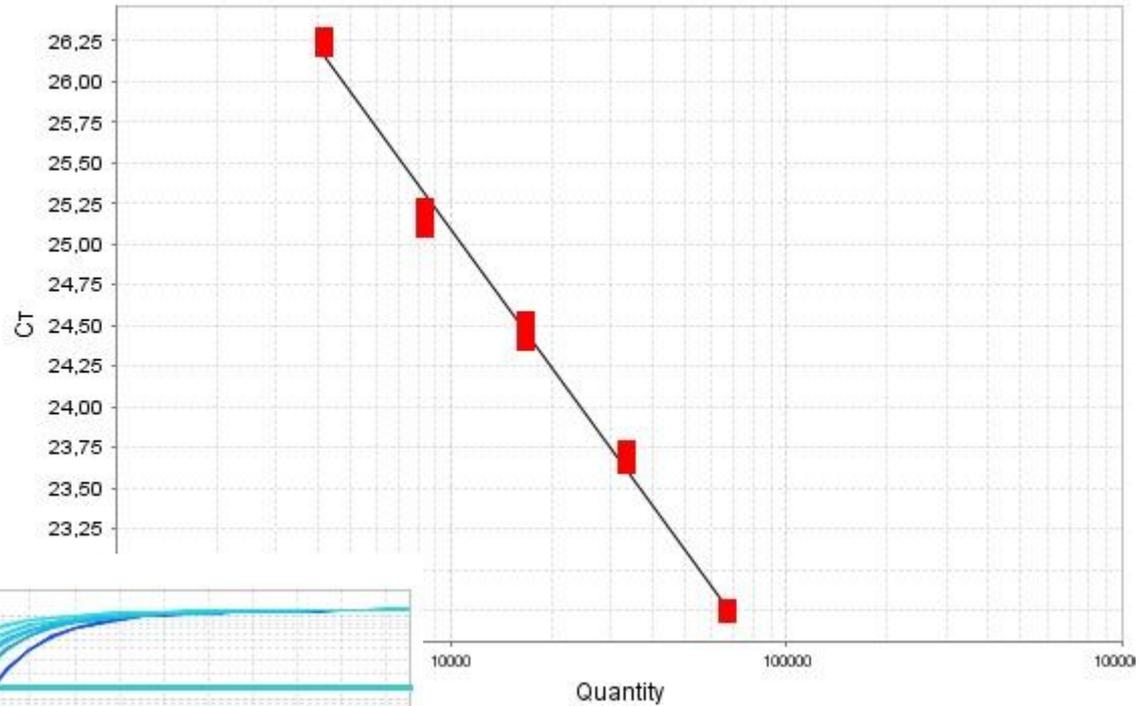
Validation of qPCR for HTLV-1 proviral load in PBMCs

Table 2
Reproducibility experiments: intra- and inter-assay reproducibility of TARL-2 cells DNA standard dilutions analyzed in triplicate on four different assays.

	CT Mean	Intra-assay variability								Inter-assay
		Experiment 1		Experiment 2		Experiment 3		Experiment 4		
		SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	
pX										
50,000	22.7	0.1	0.6	0.1	0.3	0.1	0.3	0	0.1	1.5
50,00	26.0	0	0.2	0.1	0.2	0.1	0.3	0.1	0.4	1.5
500	29.5	0.1	0.4	0.1	0.2	0	0	0.1	0.5	1.2
50	33.0	0.2	0.6	0.2	0.6	0	0	0.2	0.7	0.7
5	36.3	0.6	1.7	0.1	0.3	0.1	0.2	0.8	2.2	2.1
β-actin										
66,600	23.4	0	0.1	0	0.2	0	0	0	0.2	2.2
13,320	24.3	0.1	0.5	0.1	0.5	0.1	0.3	0.1	0.6	2.2
2664	25.0	0.2	0.9	0.2	0.6	0.1	0.4	0.2	0.7	1.9
532	25.6	0.1	0.5	0.1	0.4	0.1	0.2	0.1	0.3	1.8
106.6	26.8	0.1	0.4	0.1	0.3	0.1	0.2	0.1	0.3	2.0

Intra-assay: CV = 0.4%
Inter-assay: CV = 2%

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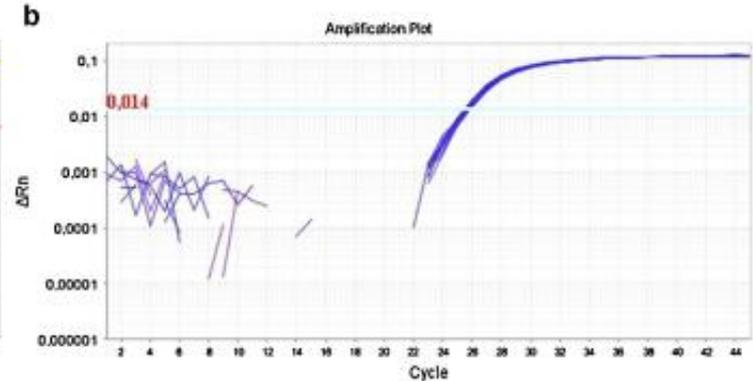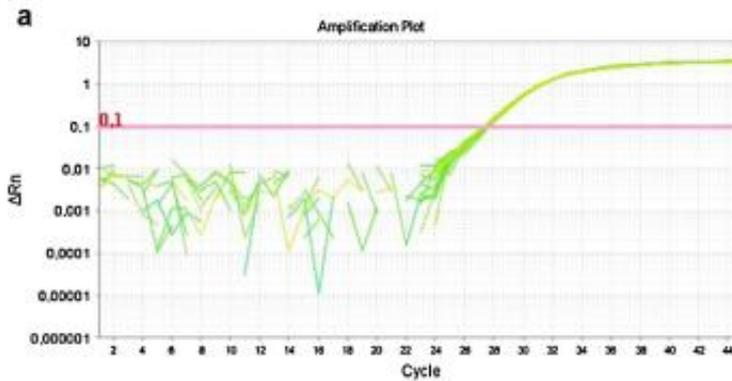
Experiment 1. Same sample 15 times
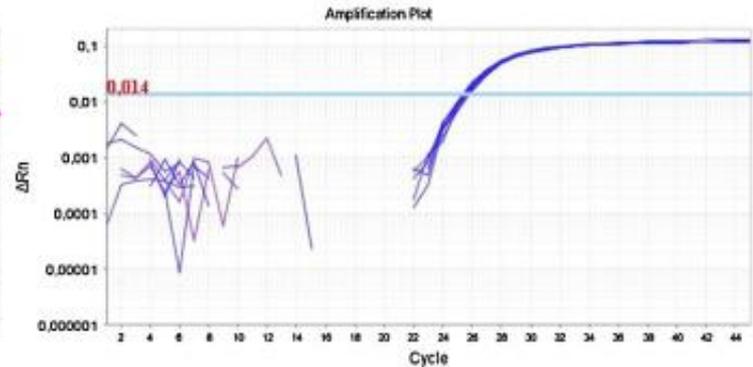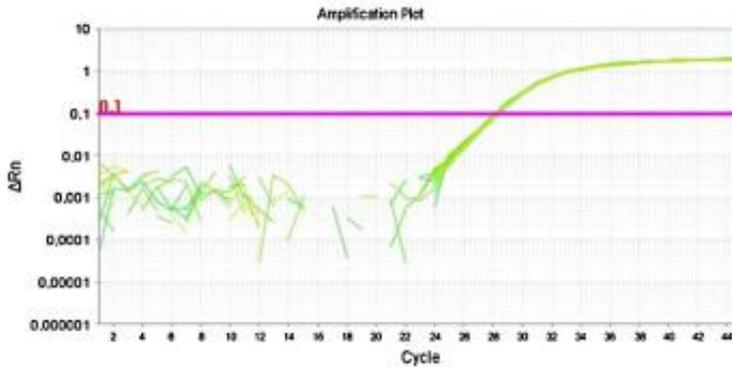
Experiment 2. Same sample 15 times

Validation of qPCR for HTLV-1 proviral load in PBMCs

The assay can reliably quantify HTLV-1 proviral load.

PVL of patients (mean ± SD = 36.3 ± 40.2; SE: 9.5) was higher than in the non-HAM/TSP group (mean ± SD = 6.9 ± 8.8; SE: 2.9; $p < 0.005$).

Epidemiology

Subtypes

Associated with geographic distribution

No association with clinical progression

Gessain & Mahieux, 2012
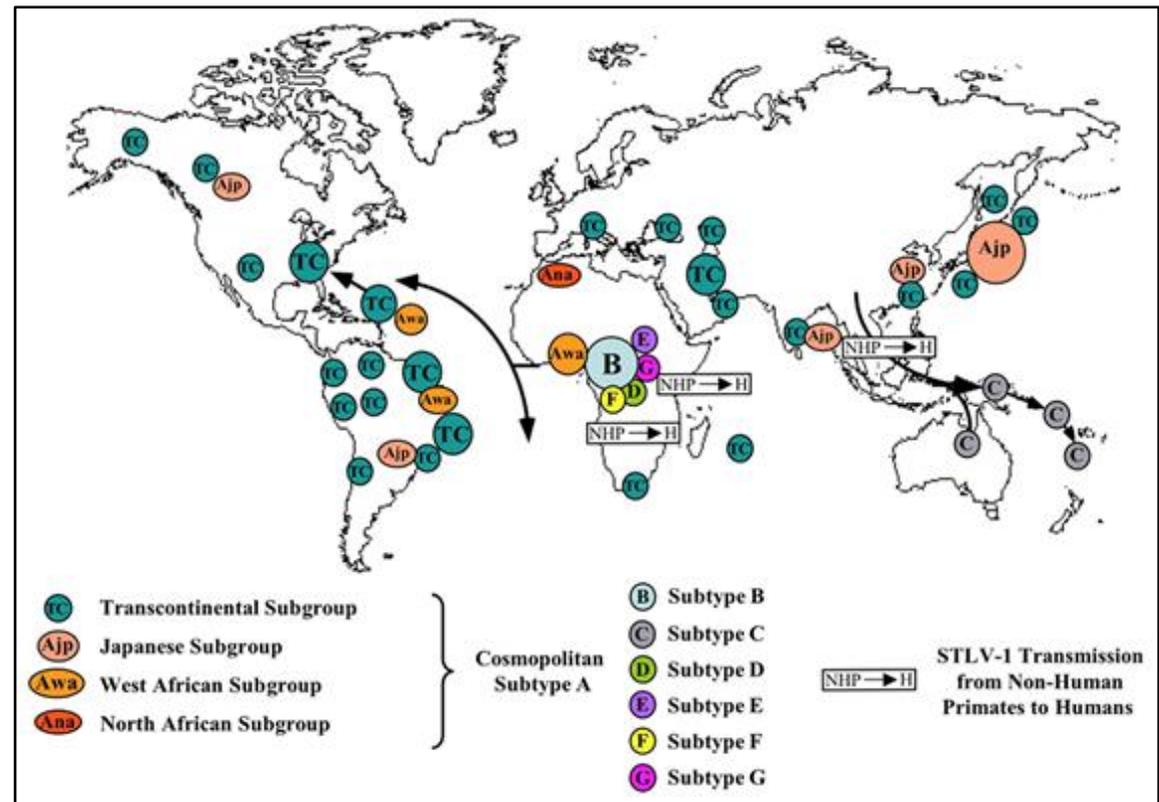
Epidemiology

HTLV-1 subtypes in Rio de Janeiro, Brazil

Pathogenesis

p12
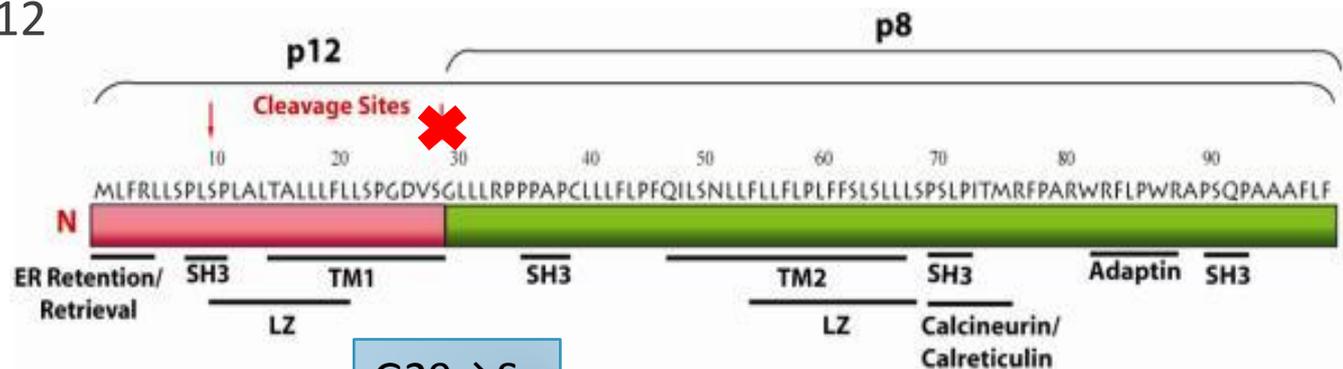
G29→S

↑ p12

Immune evasion of HTLV-1 infected cells
- ↓ MHCI expression
- ICAMI e ICAMII

Infected T-cell proliferation:
- ↑STAT activation,
- ↑ cytoplasmatic calcium→ activation of NFAT

Bind to IL-2 promoter, ↑ IL-2 expression

Important remarks

❖Importance of HTLV-1 screening

❖Molecular assays can be used for different purposes in HTLV infection

❖Prior validation is essential before the implementation in laboratorial routine

Acknowledgments

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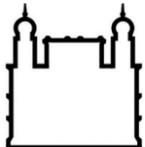
Thank you!

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