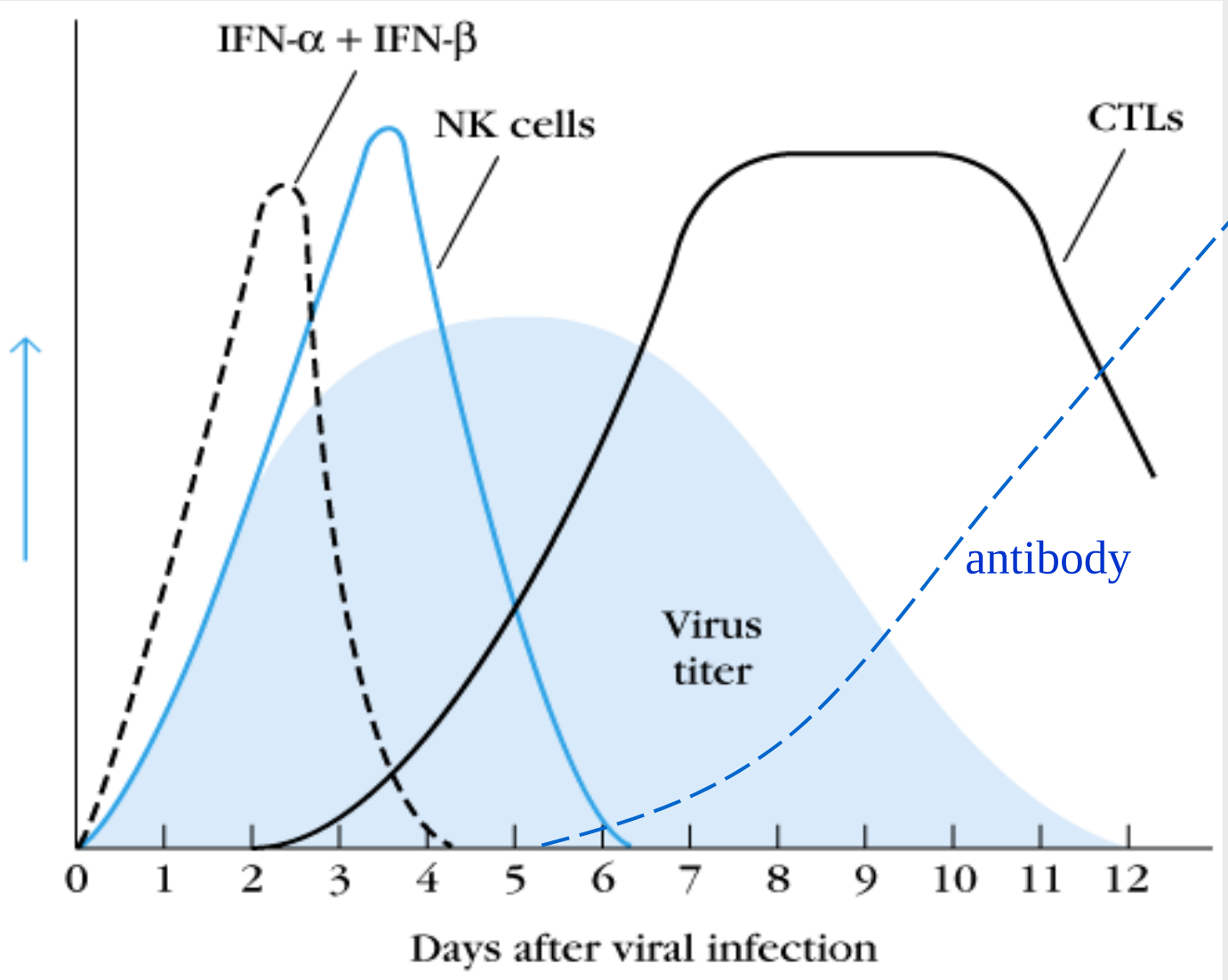
Imperial College London

T cell dynamics and retroviral infection

Charles R M Bangham
Department of Immunology,
Wright-Fleming Institute
Imperial College Faculty of Medicine

In the race between microorganism and immune system, different immune weapons are used at different times



How do we know CTLs are necessary in the immune response to a virus?

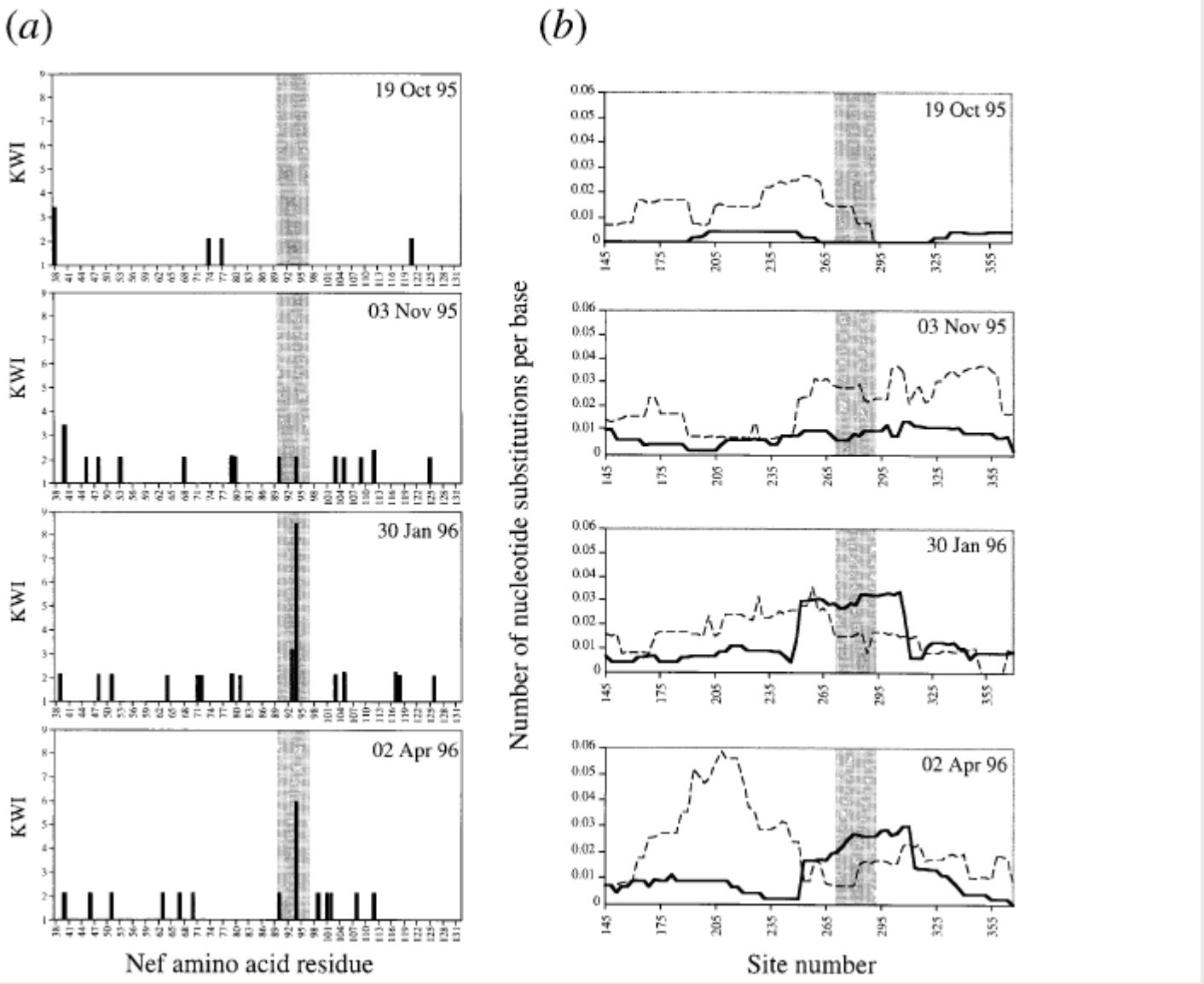
Three types of evidence:

1) deficiency of CTL number:
   depletion of CTLs in mice by anti-CD8 antibody
   CMI defect in humans: experiment of nature

2) deficiency of CTL function: knockout of beta-2 microglobulin gene in mice or perforin gene

3) passive ('adoptive') transfer of CD8+ T cells to a deficient recipient
   - mice
   - humans (HIV, EBV, CMV)

CTLs select amino acid mutations in HIV-1 Nef



D. Price et al 1997 PNAS

Human T-lymphotropic virus type 1 (HTLV-1)

- infects 10-20 million people.
- endemic (1-20% of adults) in South America, Caribbean, Central Africa, southern Japan.
- 5% develop an aggressive T-cell leukaemia/lymphoma
- 1-2% develop a chronic inflammatory disease either of CNS, eyes, muscles, joints, lungs or skin.
- >90% remain healthy carriers of HTLV-1.

HTLV-1 persistence and inflammatory disease

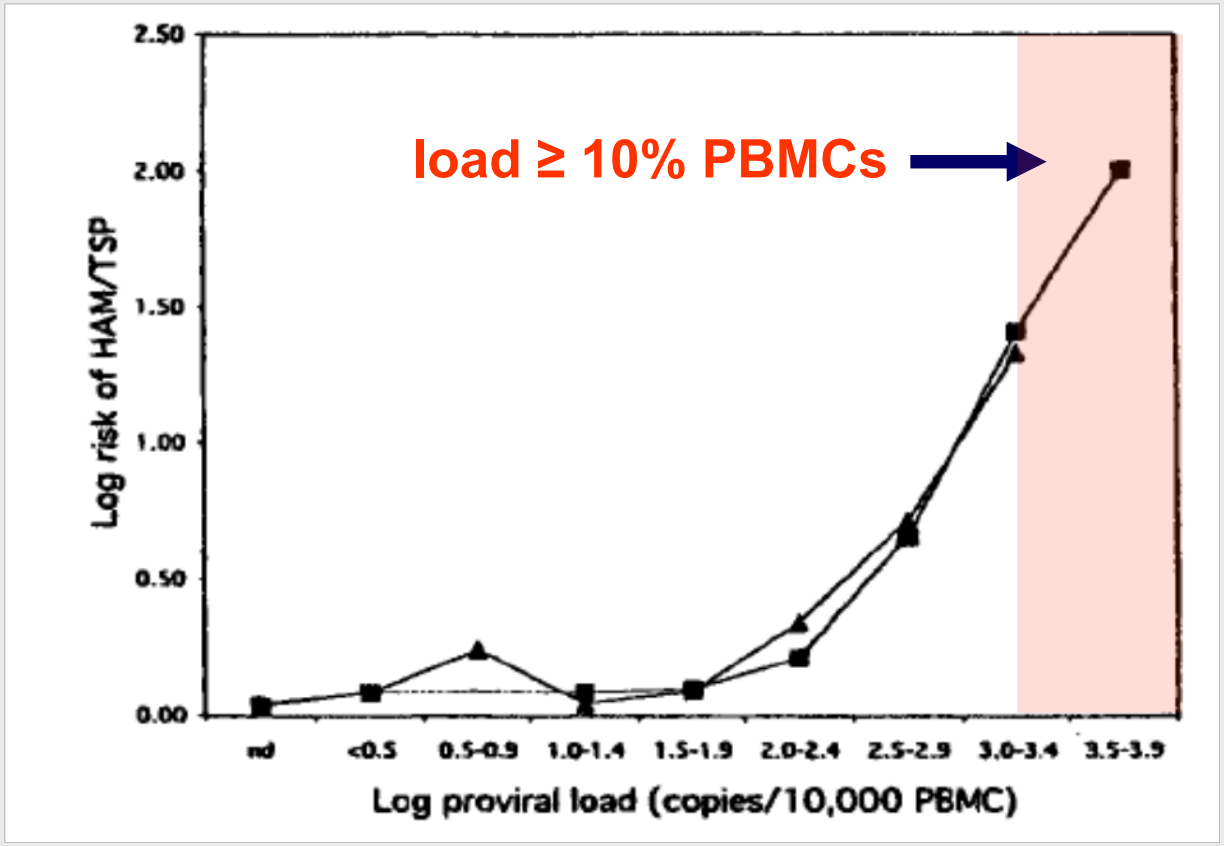
Three main questions:

1. How does HTLV-1 persist?

2. How does it spread?

3. Why do some develop HAM/TSP, whereas most remain healthy carriers?

The proviral load of HTLV-1 correlates with the risk of HAM/TSP



median proviral load

(copies/100 PBMCs)

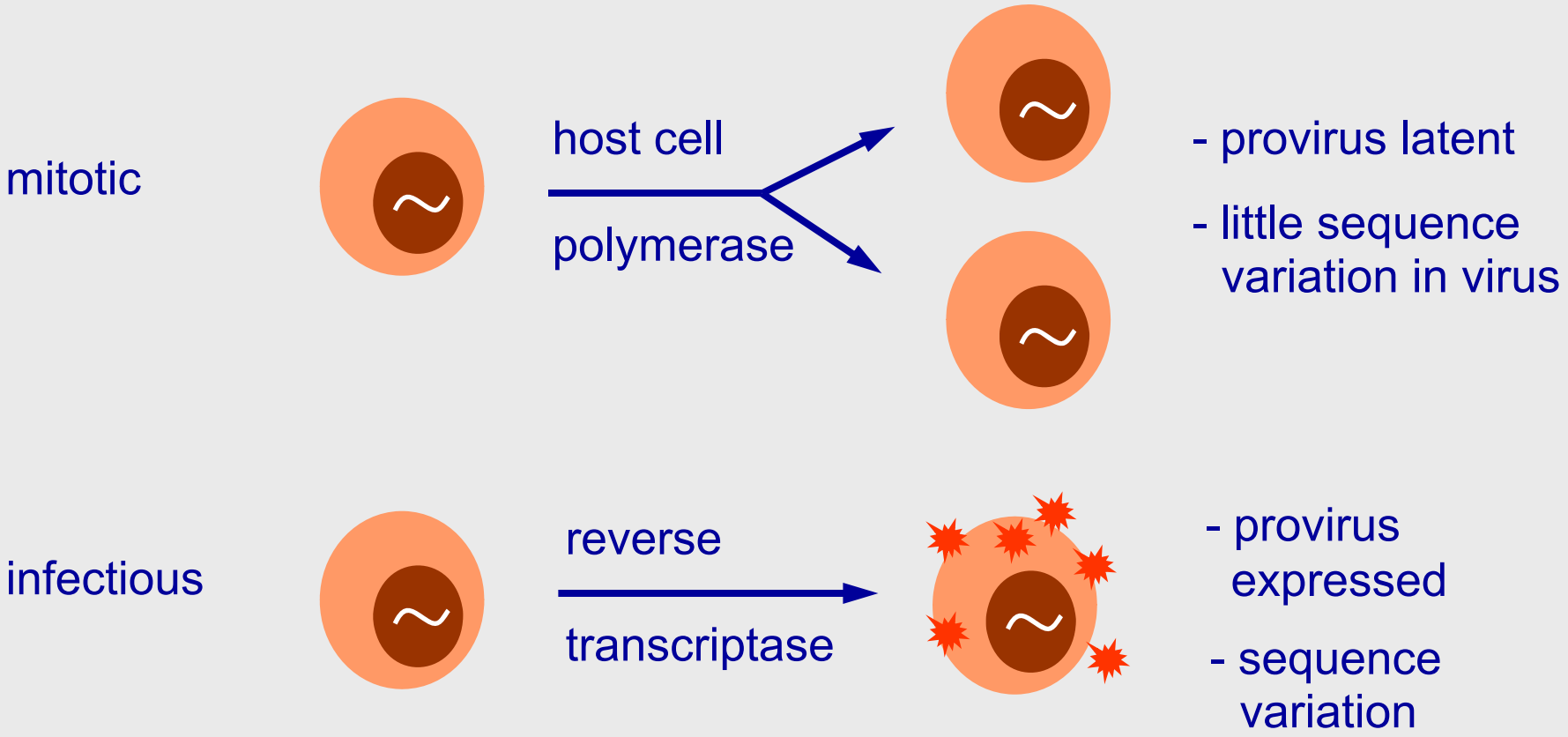
HAM/TSP: 5.4

asymptomatic: 0.34

Jeffery et al: PNAS (1999) **96**, 3848

How is the high proviral load maintained?

Retroviruses replicate by two routes:

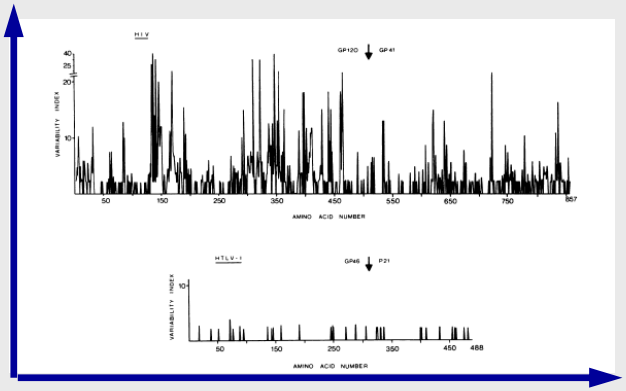


Evidence for latency of HTLV-1

1. HTLV-1 varies little in sequence:

variability (Kabat-Wu)

Env



HIV-1

HTLV-1

amino acid position

Daenke et al. 1990: J. Virol. **64**, 1278

2. HTLV-1 mRNA and proteins are usually undetectable in PBMCs.

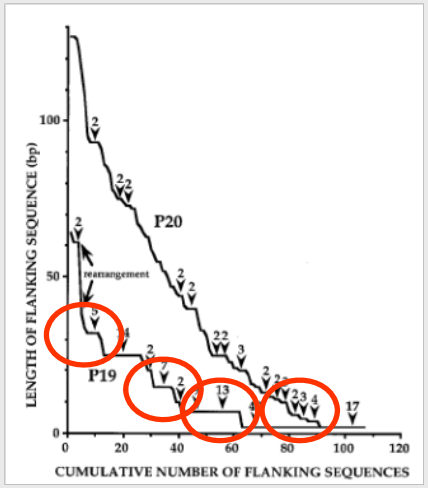
3. Virions are absent and plasma is non-infectious.

'Standard model' of HTLV-1 persistence

HTLV-1 is maintained by passive proliferation of provirus-containing lymphocytes.

A fraction of cells express HTLV-1, but too few to allow the immune response to make an impact on proviral load.

Supported by observation of large clones of HTLV-1$^+$ lymphocytes *in vivo*:



Wattel et al. 1995:
J. Virol.**69**, 2863

What is wrong with the 'standard model'?

There is a persistent, strong immune response to HTLV-1.

- antibody: virus-specific IgM persists in many individuals
- cytotoxic T lymphocytes (CTLs) are chronically activated

- does the CTL response make any impact?

CTLs reduce proviral load and risk of HAM/TSP

- strong anti-Tax response exerts positive selection on *tax* gene[1]
- spontaneous Tax mutants escape CTLs[2]
- granzymes and perforin are more highly expressed in individuals with a low proviral load[3]
- CTLs spontaneously kill autologous HTLV-1$^+$ cells ex vivo[4]
- HLA-A2 and -C8 confer protection in s. Japan[5]
- High CTL avidity correlates with low proviral load and expression[6]

[1] Niewiesk et al. 1994: J. Virol. **68**, 6778; Kubota et al. 2007: J. Immunol. **178**, 5966
[2] Niewiesk et al. 1995: J. Virol. **69**, 2649
[3] Vine, Heaps et al. 2004: J. Immunol. **173**, 5121
[4] Hanon et al. 2000: Blood **95**, 1386 ; Asquith et al. 2005: J. Gen. Virol. **86**, 1515
[5] Jeffery et al. 1999: Proc. Nat. Acad. Sci. USA **96**, 3848
[6] Kattan, Rowan, Macnamara et al. 2009: J. Immunol. **182**, 5723

Protective role of HLA class 1 indicates that CTLs limit HTLV-1 expression in vivo

1. Possession of *either HLA-A*02 or HLA-Cw*08* :

   - reduced proviral load by 3-fold
   - halved the odds of HAM/TSP

HLA-A2 and HLA-Cw8 prevent 36% of potential HAM/TSP cases.

2. HLA class 1 heterozygosity was associated with a lower proviral load.

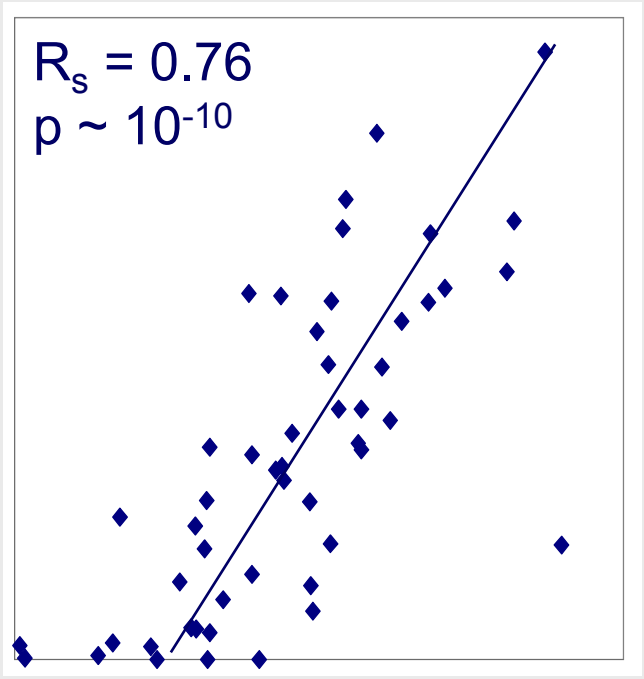
Jeffery et al: PNAS (1999) **96**, 3848; J Immunol (2000) **165**, 7278
Vine et al: J Infect Dis (2002) **186**, 932

How do HLA-A2 and -C8 protect against HTLV-1?

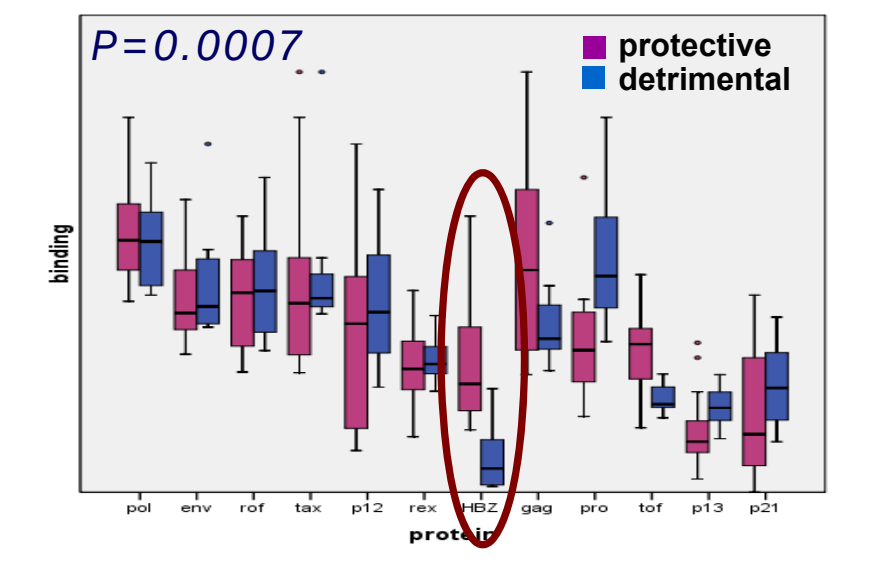
MacNamara, et al. 2010, PLoS Pathogens.

Epitope prediction

200 peptides: 100 Tax, 100 HBZ

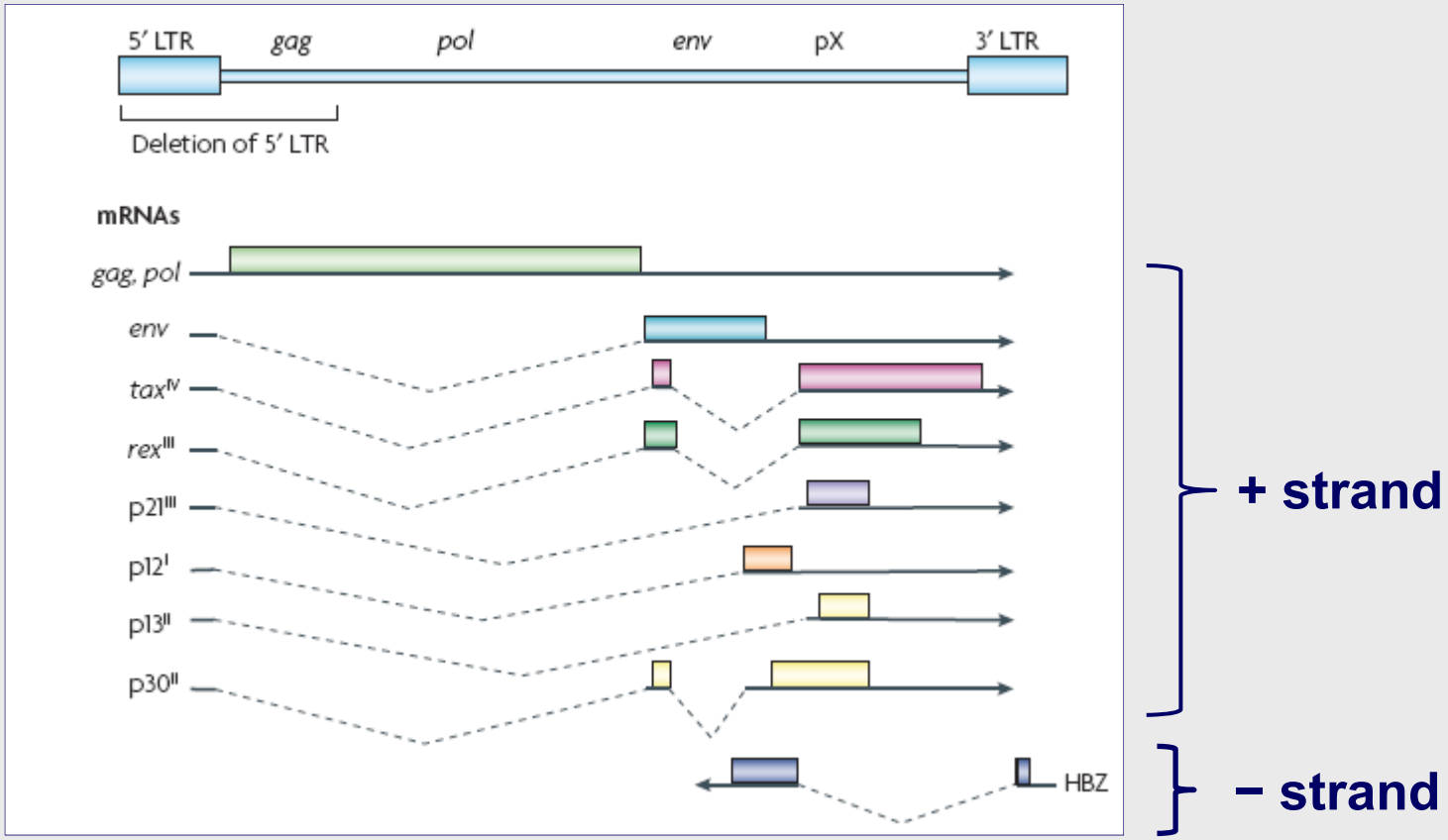


Which HTLV-1 proteins bind best to A2 and Cw8?



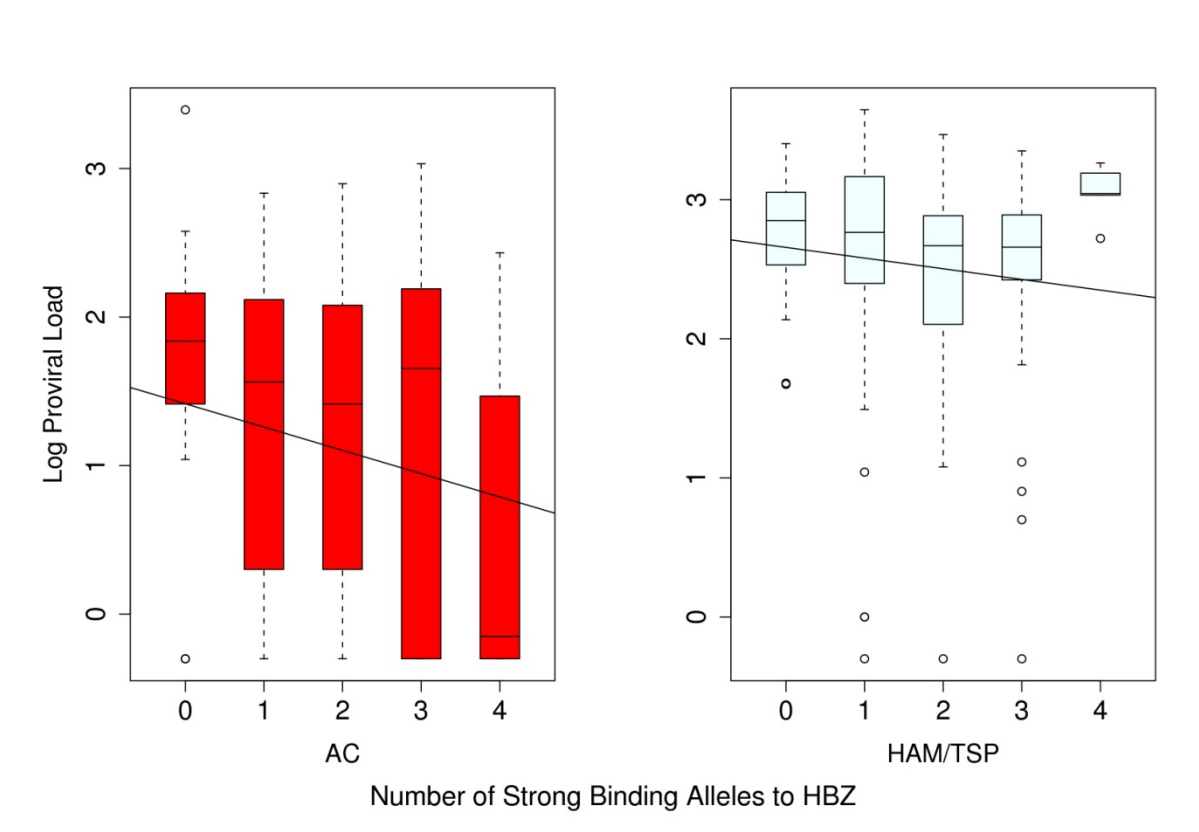
Conclusion: protective alleles (A02 & C08) are those that bind HBZ strongly.

HBZ – the only known transcript from the negative strand of the HTLV-1 provirus



Matsuoka & Jeang 2007, Nat. Rev. Cancer

Strong binding of HBZ peptides correlates with low proviral load



P = 0.016 (Spearman)

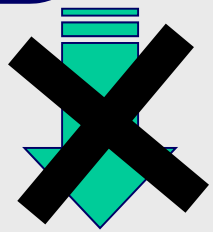
MacNamara, et al. 2010, PLoS Pathogens.

Why is HBZ the critical target?

HBZ inhibits expression of other HTLV-I genes

=> Evade immune response

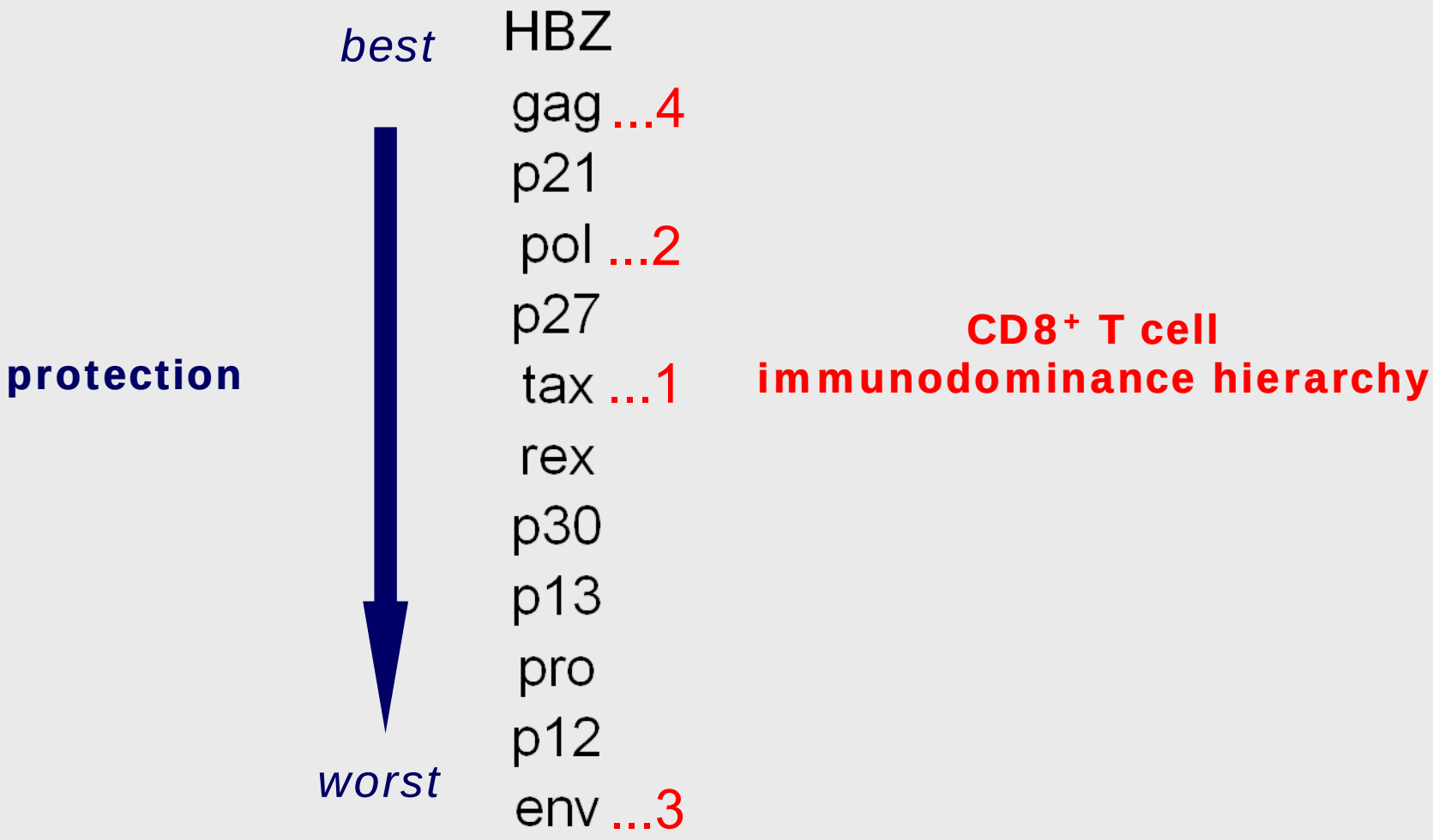
HBZ expression drives infected cell proliferation



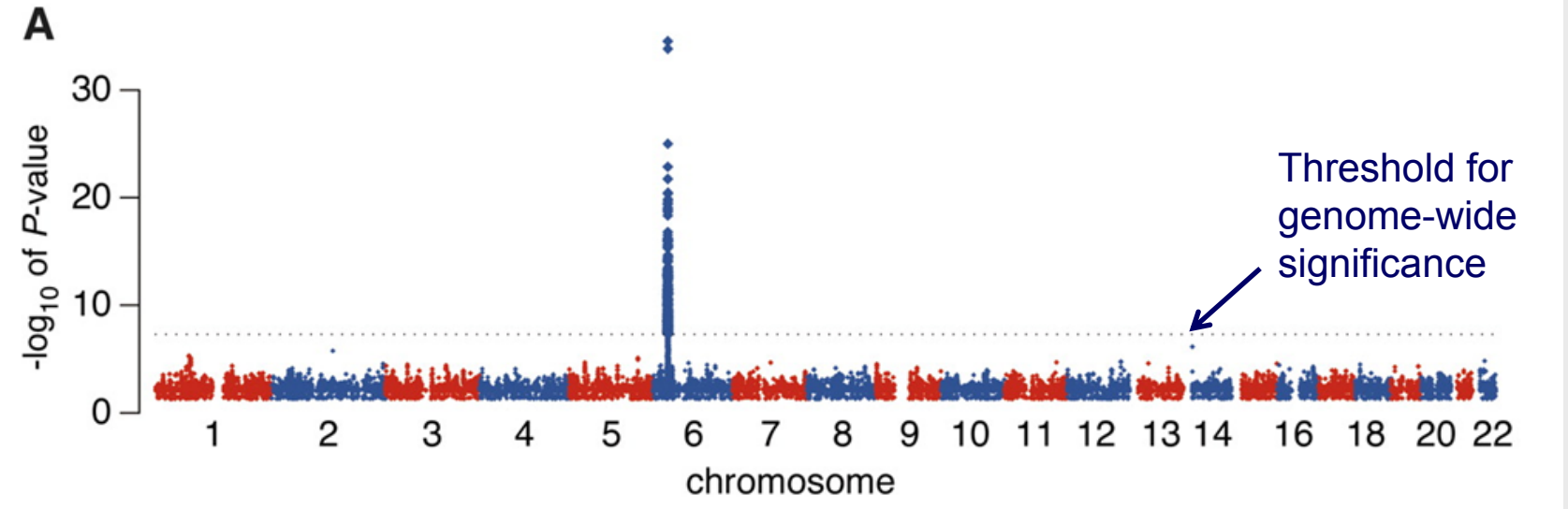
HBZ-specific CTLs

Proviral load increases

CTL protection is unrelated to immunodominance in HTLV-1 infection



HIV-1 is controlled by CTLs



GWAS with 1.3 million autosomal SNPs in 1712 individuals

313 SNPs significantly associated with efficiency control of viral load

Result: all 313 SNPs lie in the MHC class 1 region

GWAS = genome-wide association study
SNP = single nucleotide polymorphism

International HIV Controllers Study 2010
Science **330**, 1551-1557

Conclusions (1): CTL response

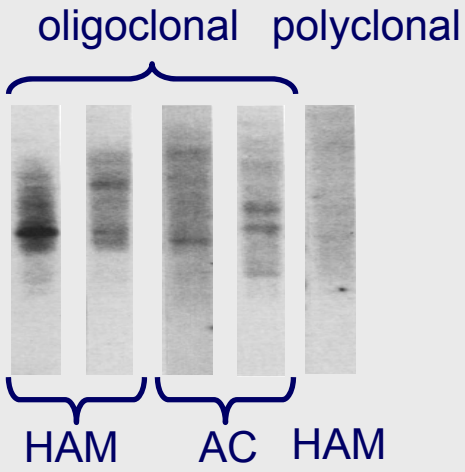
1. CTL response to HBZ determines the outcome of HTLV-1 infection
2. Strength of CTL response to Tax is *consequence, not cause*, of efficient host control of HTLV-1
3. HLA Class 1 protection prevents ~50% of cases of HAM/TSP
4. Frequency, phenotype* and function** of CTLs cannot be used to measure CTL effectiveness in persistent infections

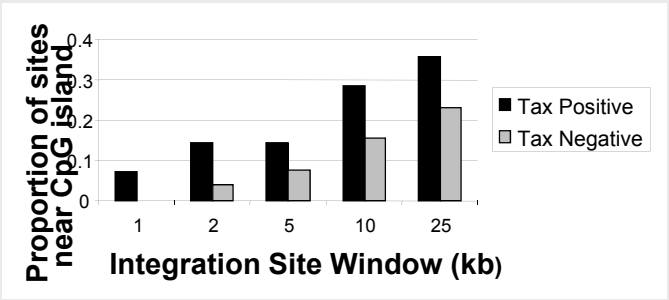
* phenotype: e.g. PD-1 expression

** function: e.g. “polyfunctionality” – ability to carry out >1 effector mechanism (CTL lysis; IFN; IL-2; etc.)

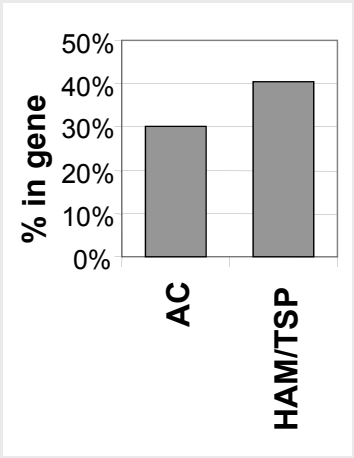
Questions raised by clonal proliferation of HTLV-1⁺ T cells

- how many clones are present in each host?
- what determines the size of a clone?
- how can we define and measure 'clonality'?
- what is the relationship between clonality and:
  - proviral load?
  - disease status – can we predict ATL?
  - immune surveillance?
  - intercurrent infections – Strongyloidiasis; TB; infectious dermatitis?





Meekings et al. 2008:
PLoS Path. **4**, e1000027



Current view of HTLV-1 clonality in vivo

- **number of infected T-cell clones in chronic phase:**

  - unknown with precision, but believed to lie between 10 and 100 on the basis of Southern blot analysis.

- **oligoclonality**

  - appears higher in HAM/TSP than in asymptomatic carriers (Southern blot)

Hypothesis

Genomic integration site determines clonal fitness and pathogenic potential of a HTLV-1^{+} T cell clone

– transcriptional activity of flanking DNA

– identity (‘ontology’) of flanking host genes.

Required: a technique to map proviral integration sites in PBMCs:

- sensitive
- high-throughput
- quantitative

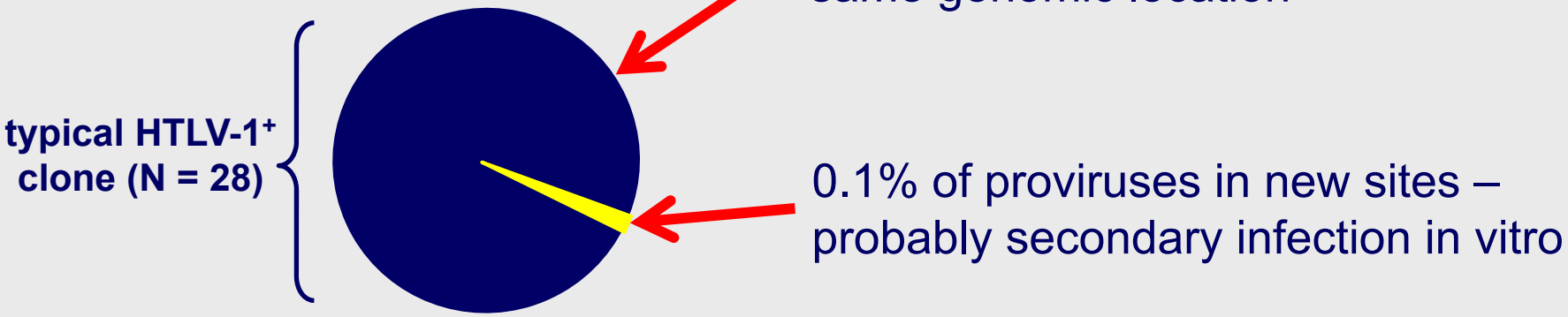
1. How many provirus copies/cell?

Method

CD4$^+$ HTLV-1$^+$ T-cell clones isolated by limiting dilution in presence of HTLV-1 integrase inhibitor (raltegravir) to minimize secondary infection

HTLV-1 proviral integration sites mapped & quantified

Results



Conclusion: HTLV-1-infected cells carry a single provirus in vivo

Cook et al., 2012, *Blood*

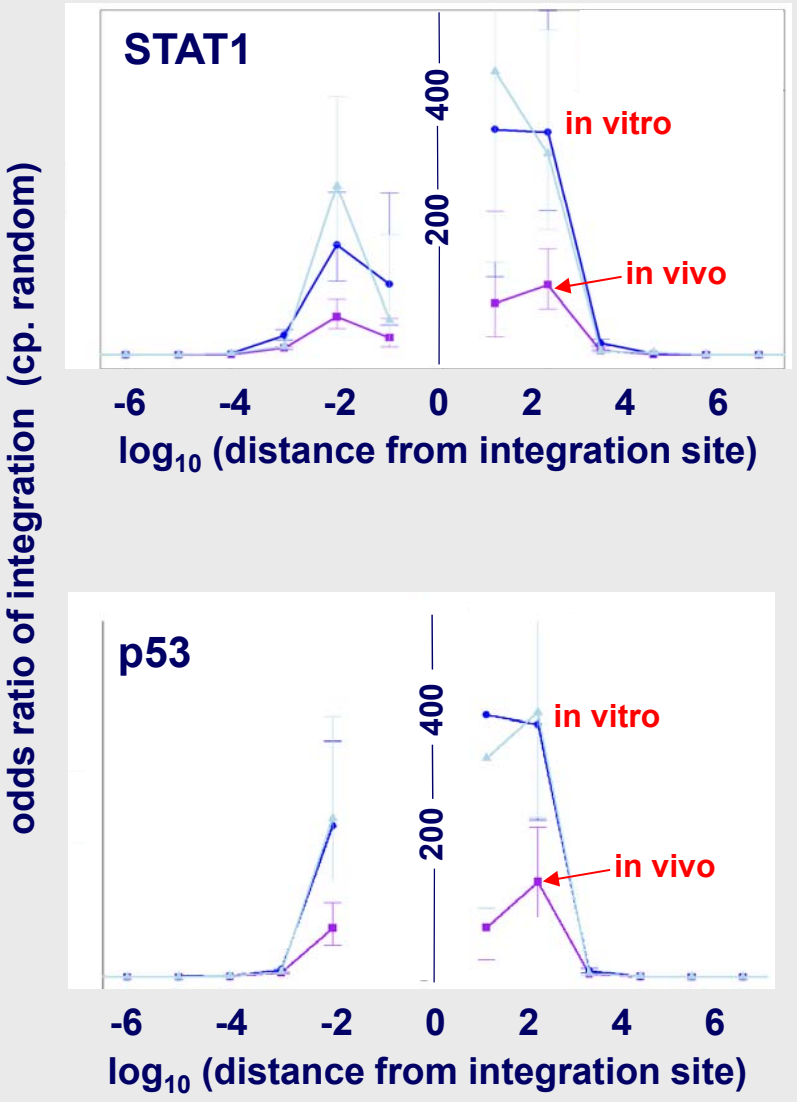
2. Targeting of HTLV-1 integration site

Proviral integration is not random

Genomic attributes tested:

- histone marks
- transcription factor binding sites
- DNAse 1 hypersensitive sites
- CpG islands
- gene density
- proximity to genes
- gene ontology
- nucleosomes
- chromatin remodelling factors

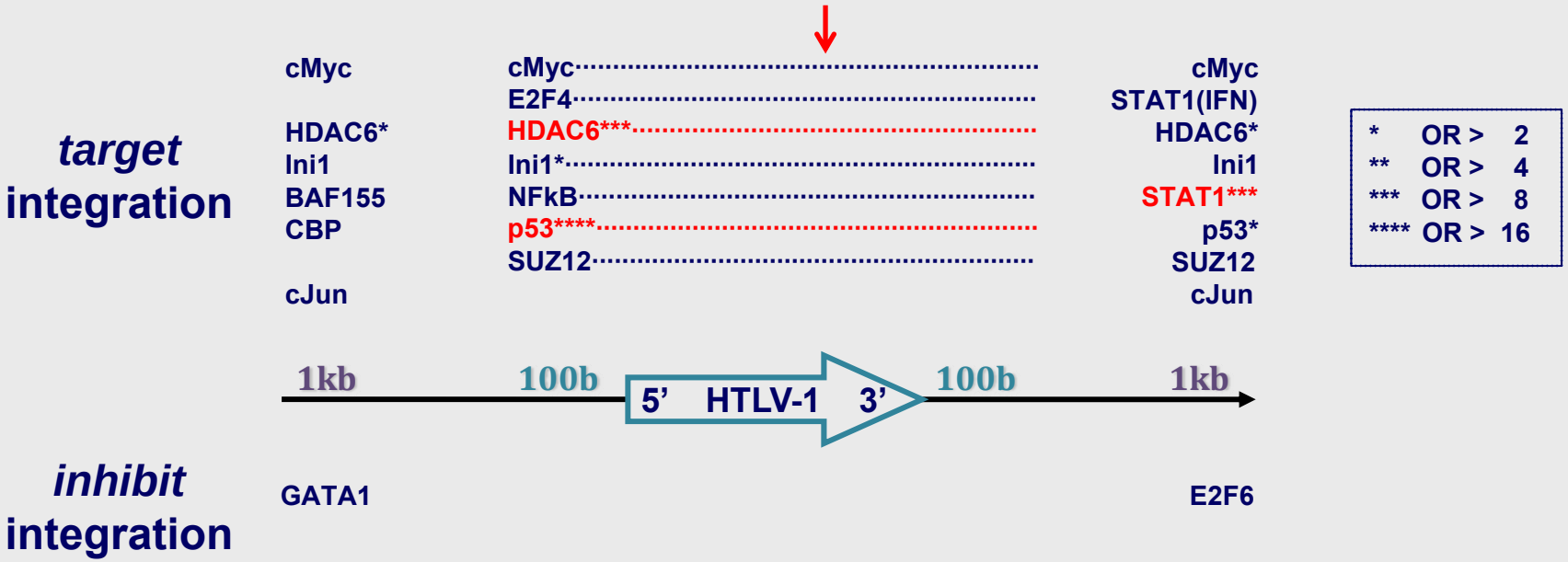
Targeting effects are
- short-range (<1kb)
- typically symmetrical



Anat Melamed, unpublished

HTLV-1 integration targeting to sites of host DNA-binding proteins: summary

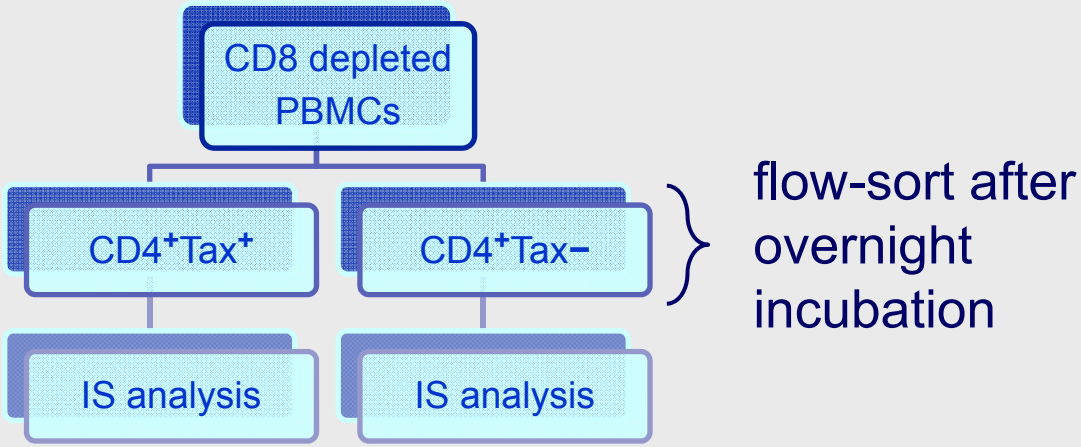
integration is favoured symmetrically, upstream and downstream of sites

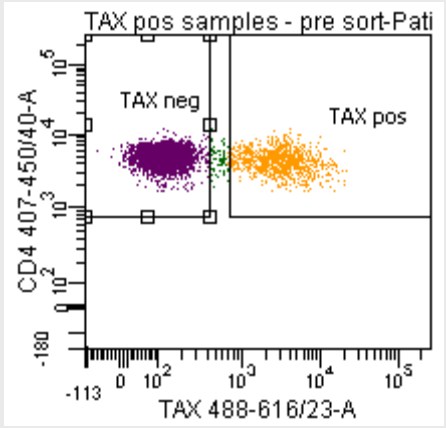


Independent, significant correlates of integration targeting (logistic regression).

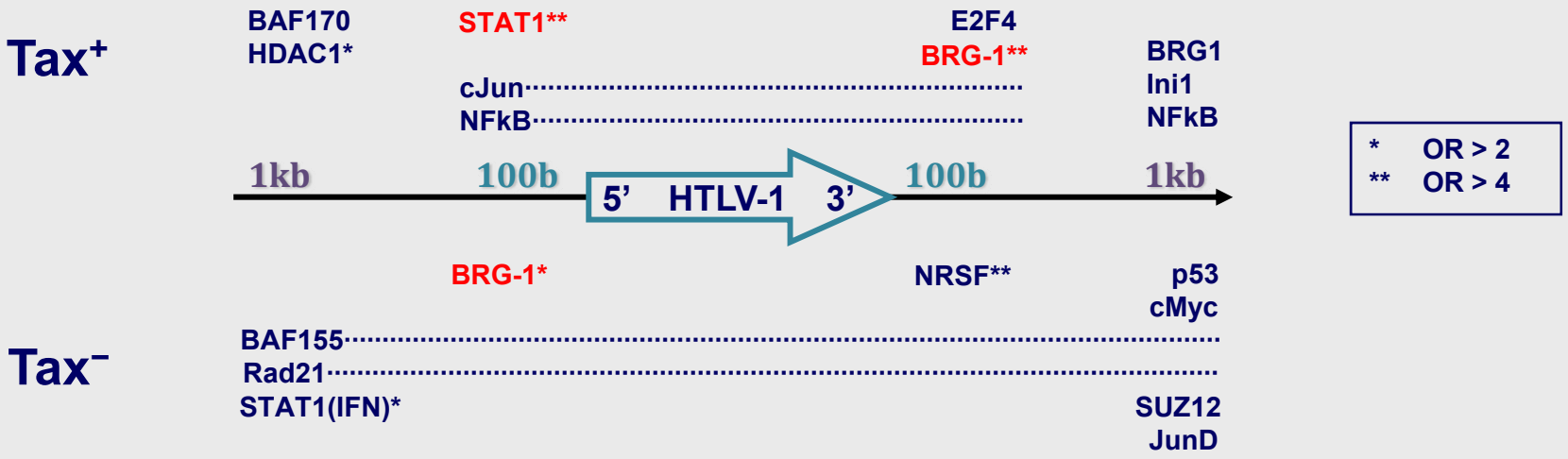
A. Melamed, unpublished

What determines spontaneous proviral expression?





Influence of flanking host genome on Tax expression: summary

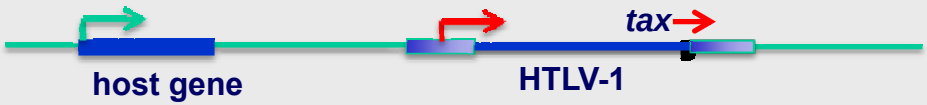


Asymmetry of effects (upstream/downstream) contrasts with integration targeting.

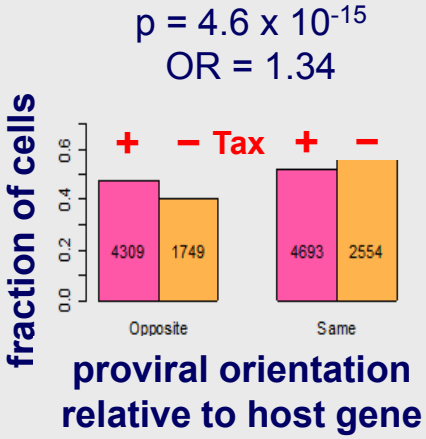
A. Melamed, unpublished

4. Spontaneous Tax expression depends on orientation relative to flanking host gene

Proviral orientation

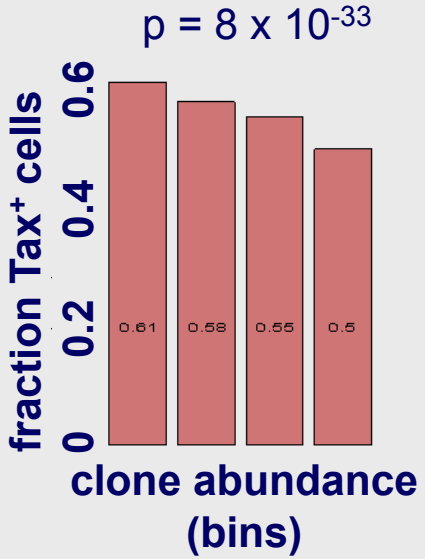


same-sense orientation *suppresses* Tax expression:



Clone abundance

- *inversely* correlated with Tax expression



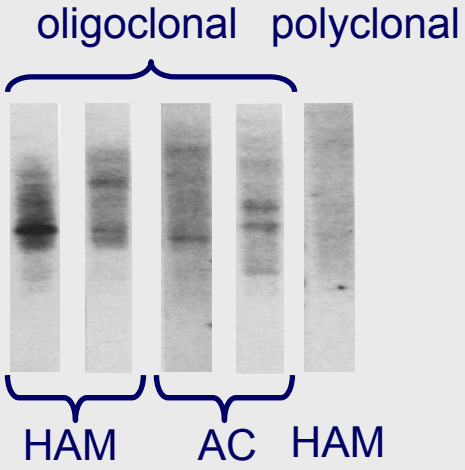
Anat Melamed, unpublished

5. How many HTLV-1^{+} clones in one host?

Previous estimates:

~ 10 to 100 in a typical AC
or HAM patient

~ 1 in a patient with ATLL



Estimation of total number of HTLV-1⁺ clones in one host

Laydon 2012, unpublished

- Fit many models (~70) to all patients' datasets and subsets thereof

•Score models against following criteria:

1) Goodness of fit

2) Accuracy

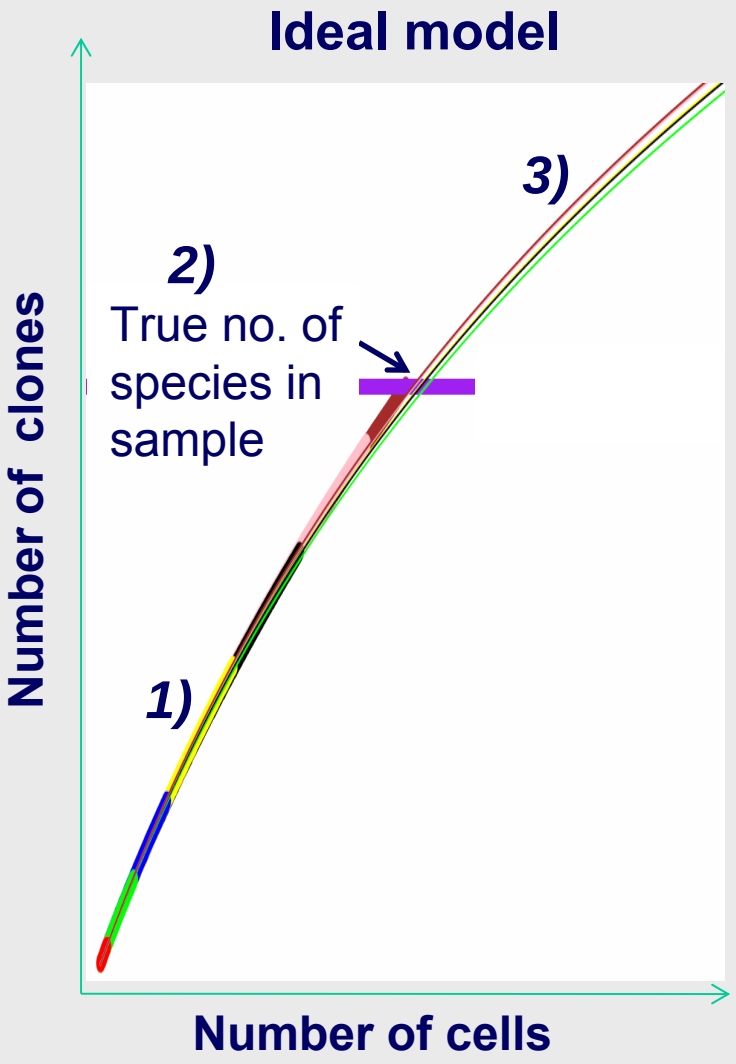
From all subsample sizes, model must "predict" true no. clones in total sample

3) Similarity

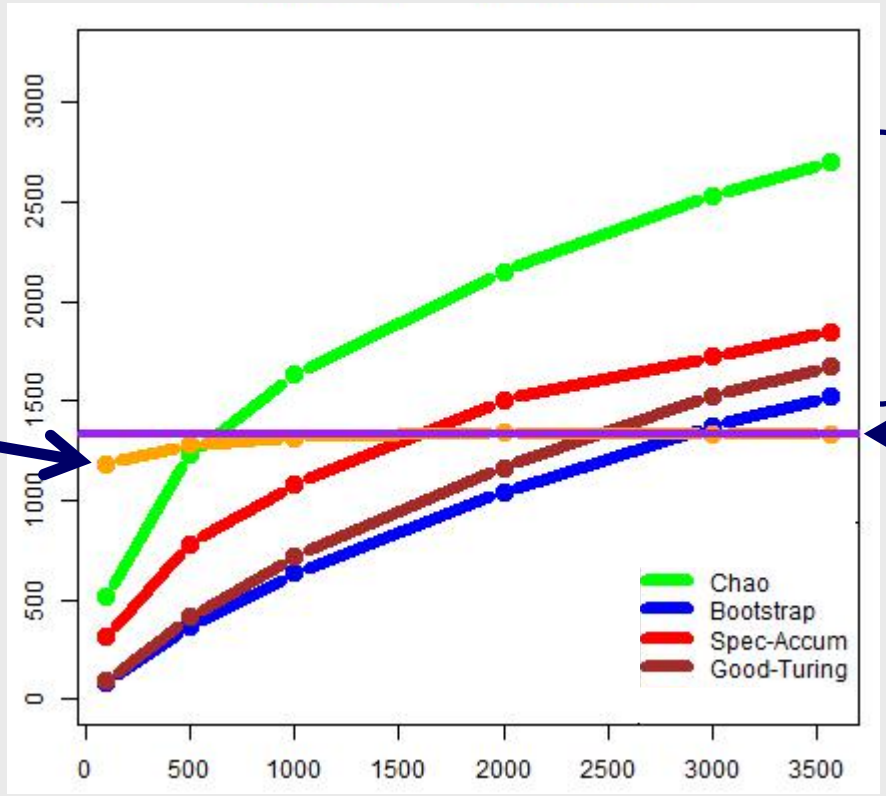
Area between curves extrapolated from all subsamples must be minimal

4) Plausibility

More cells should not result in fewer clones



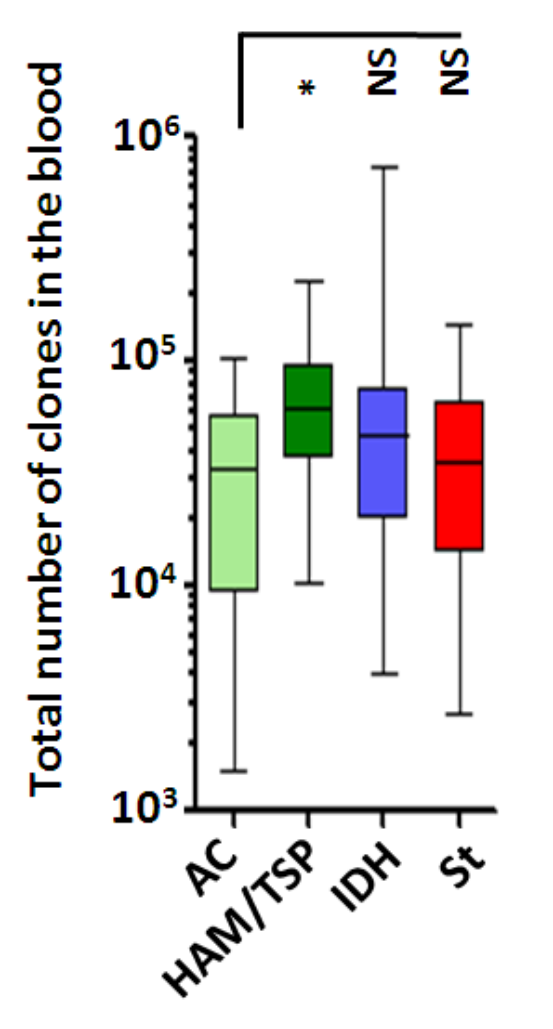
New method surpasses ecological predictors



Data on representative patient with HAM/TSP

Laydon 2012, unpublished

How many HTLV-1^{+} T-cell clones in one host?



Number of clones

observed (in ≤10μg DNA)	200 to 3500
estimated total in circulation (DivE)	10^3 to 10^6 *(mean ~ 60000)*

Gillet et al 2012, unpublished
Laydon 2012, unpublished

Conclusions

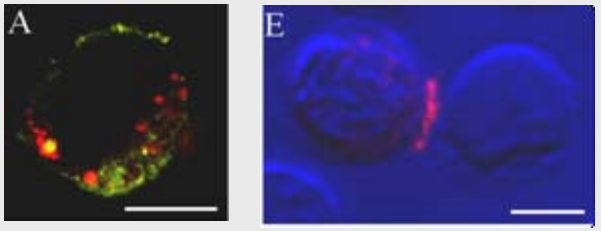
	Previous belief	**New conclusion**
Total clone number	10 to 100	10^3 to 10^6
HAM/TSP	associated with oligoclonal proliferation	associated with greater *number* of clones
Targeting of integration	random	targeted to specific transcription factor binding sites
Proviral orientation	same-sense *favours* Tax	same-sense *suppresses* Tax
Clone abundance	associated with Tax^+	associated with Tax−
Proviral copy number	multiple	one copy/cell

If HTLV-1 is expressed in vivo, where are the virus particles?

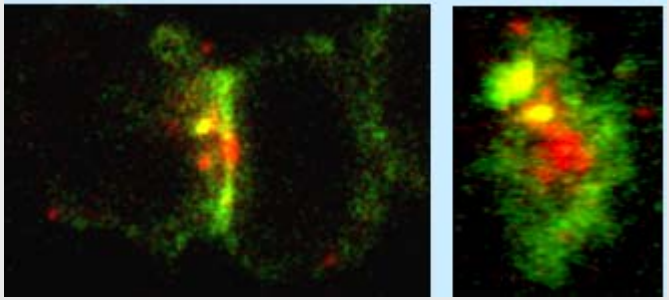
- serum from HTLV-1-infected people is not infectious; HTLV-1 is usually undetectable by EM and by PCR
- only ~1 in 10^5 to 10^6 HTLV-I virions (from a cell line) is infectious
- cell contact is required for efficient spread of HTLV-I, both between individuals (transfer of lymphocytes) and *in vitro*

HTLV-1 is transmitted directly between cells across an organized cell-cell contact – the virological synapse

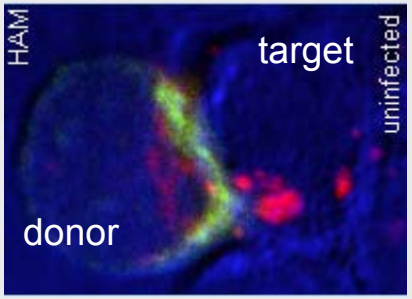
Gag protein complexes (red) polarize to the cell-cell contact area



- which contains organized adhesion domains (green)

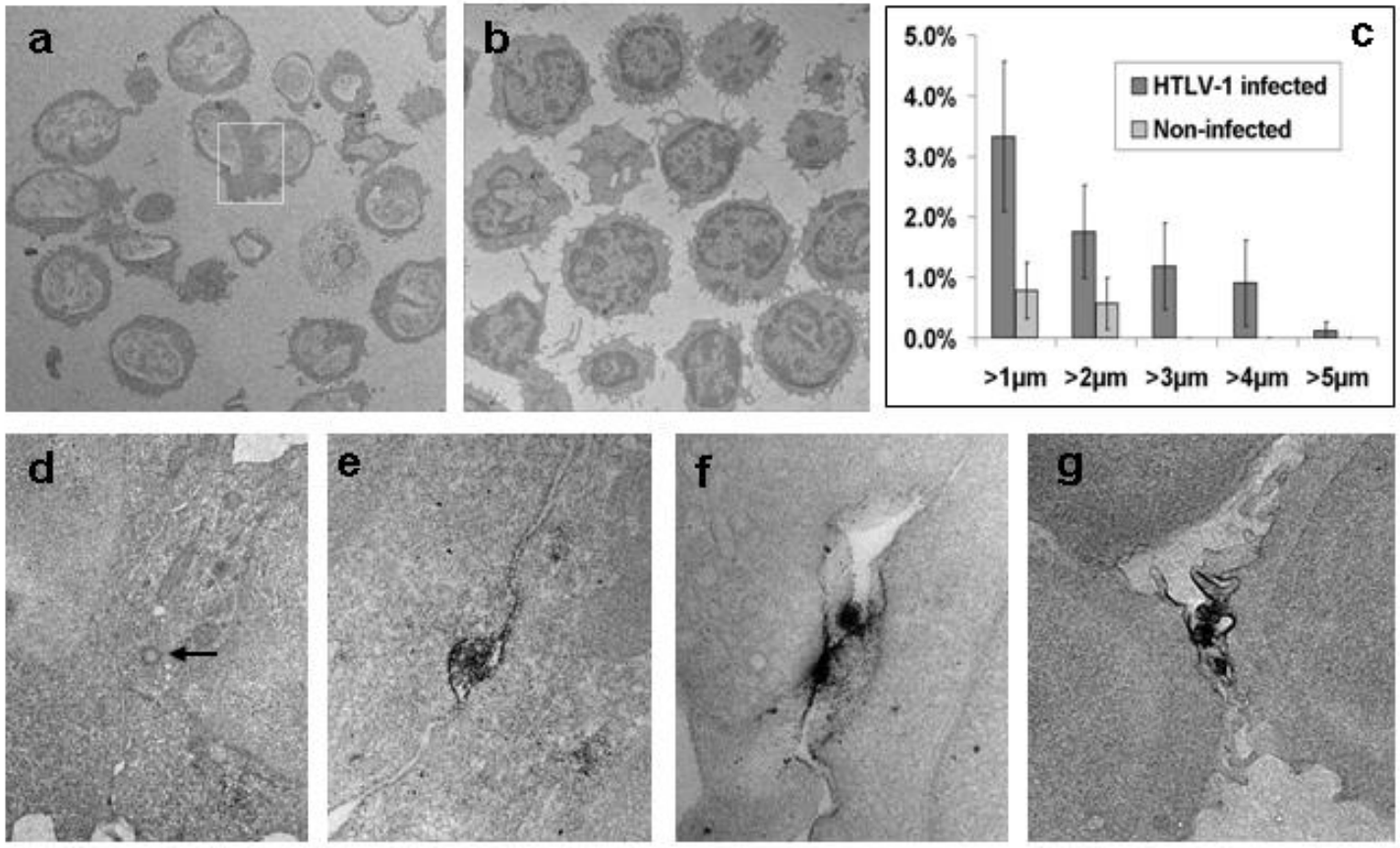


Gag is then transferred with the HTLV-1 genome to the target cell



Igakura et al 2003: Science **299**, 1713-6

HTLV-1 Gag^{+} particles are trapped between membranes at the VS



Majorovits, Nejmeddine et al, 2008

How does HTLV-1 persist and cause disease?