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DNA Methylation Profiling can Classify HIV-associated Lymphomas

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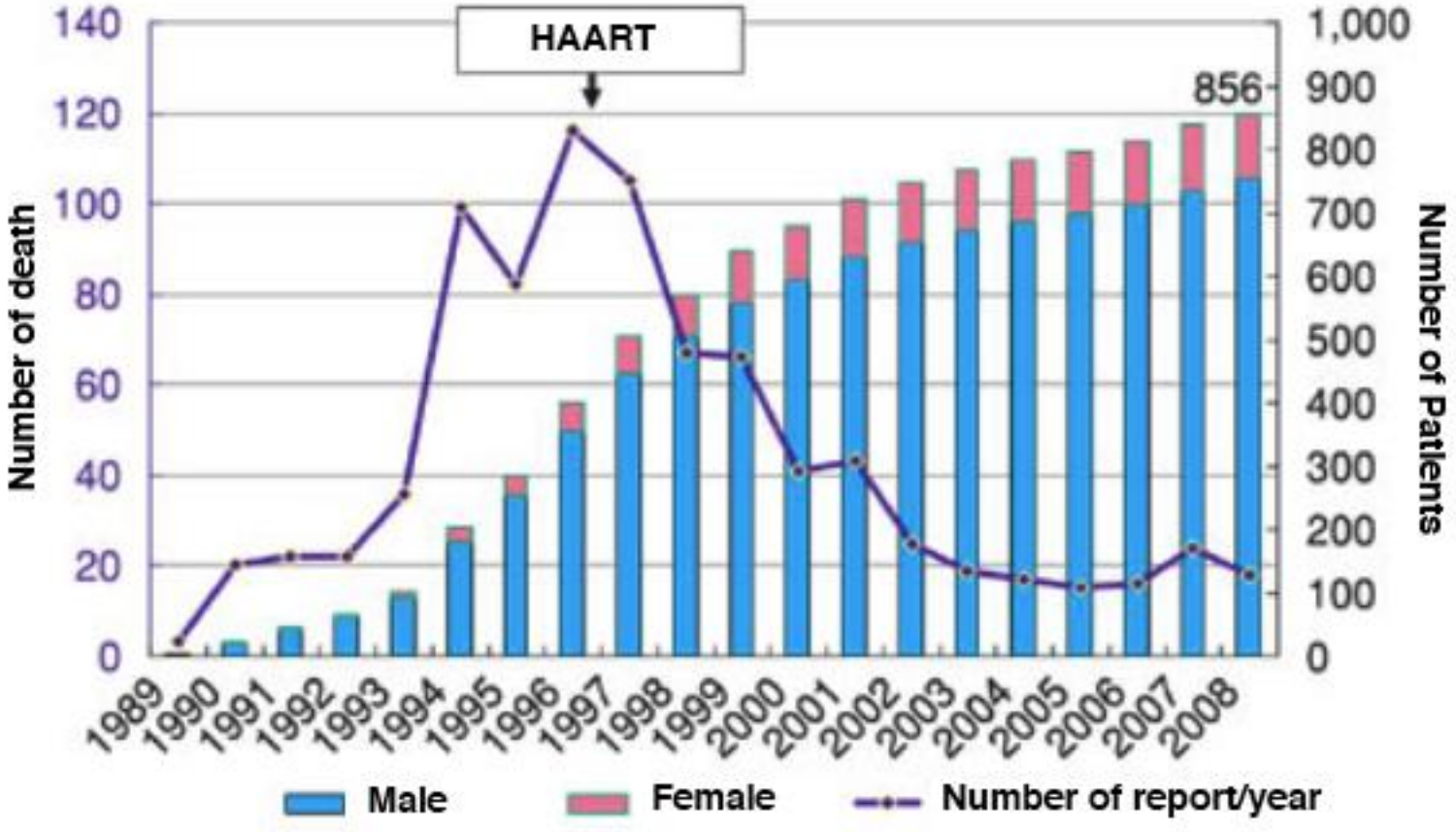
・In 1980s, Hemophilia patients in Japan were infected with HIV-1, which was contaminated in blood products. (Medical malpractice!)

・Approximately 1800 individuals were infected, and 1200 persons are still suffering from the infection.

・Although immunological status is well controlled, persistent infection causes chronic clinical symptoms that include tumorigenesis, cardiovascular diseases and metabolic disorders.

・ Our mission is taking care of these patients and keeping better QOL.

Patients' prognosis is dramatically improved after ART



HAART (Highly Active Anti Retroviral Therapy)

AIDS Committee of Japan , 2010

Malignancy is now the major cause of death

• AIDS-defining diseases	36%
(Non-Hodgkin lymphoma	10%)
• Other tumors (lung, stomach, colon, etc.）	17%
• Hepatitis	15%
• Myocardiac infarction	8%
• Suicide	5%
• Others	4%

Int J Epidemiol 34 , 2005

HIV-positive patients are at high risk of malignancy

Tumors	Incidence rate
Non-Hodgkin lymphoma	
DLBCL	X 21
BL	X 115
PCNSL	X 74
Cervical cancer	X 6

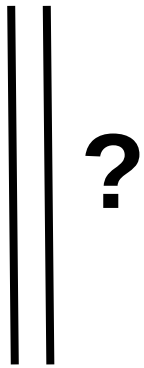
DLBCL: Diffuse large B cell lymphoma
BL: Burkitt lymphoma
PCNSL: Primary central nervous system lymphoma

JAMA 305, 2011

Etiology of malignancy remained unknown.

Question 1

HIV-1 associated lymphomas



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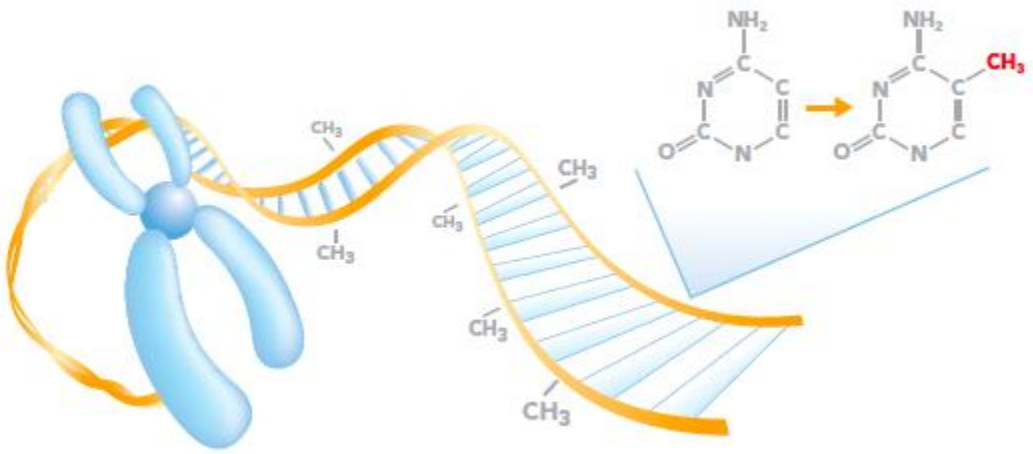
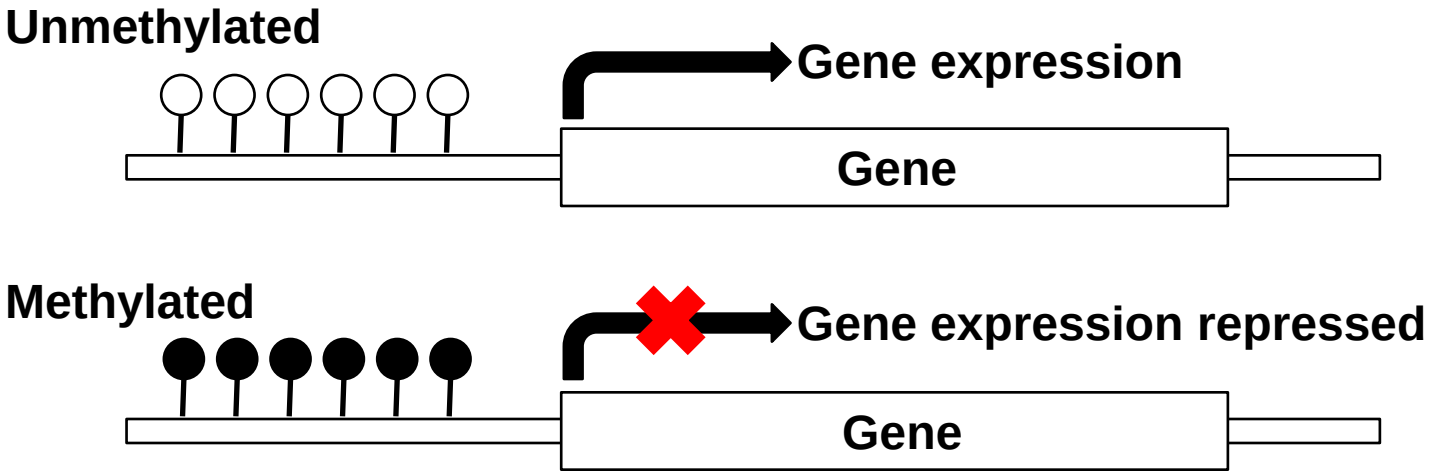
Non-HIV-1 lymphomas

DNA-methylation pattern can characterize tumors



- **The 5’-carbon of cytosine in CpG dinucleotide is epigenetically modified by a methyl residue.**

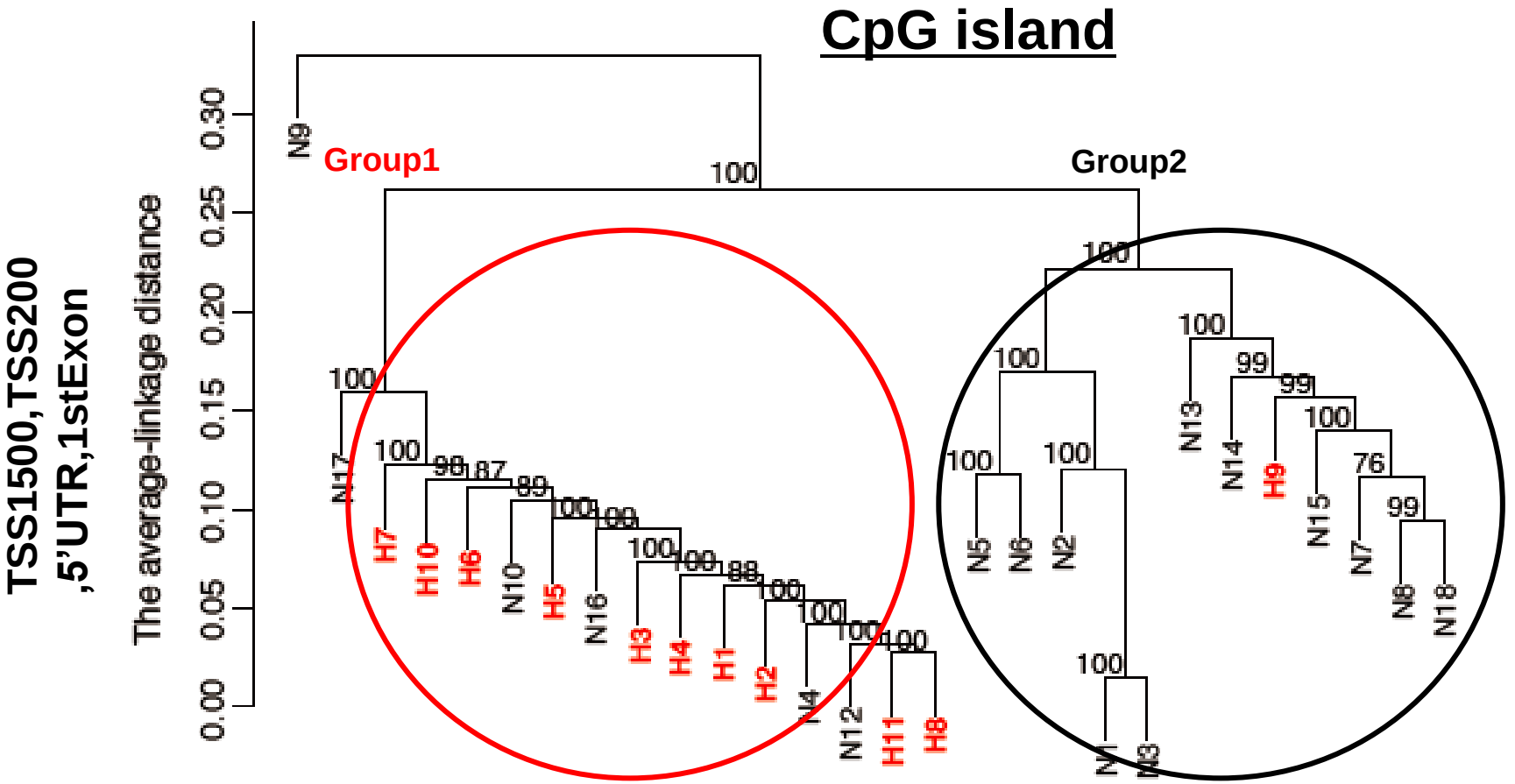


Clinico-pathological features of cohort I

HIV-1 positive lymphomas: 11 cases
Non-HIV lymphomas : 20 cases

Items examined		HIV	non-HIV	*p*-value (HIV vs non-HIV)
Gender	Female	0	10	
	Male	11	10	0.0049*
Age	Mean	45.27	64.35	
	SD**	16.92	10.60	0.018*
Histology	BL	2	3	
	DLBCL	8	17	
	HD	1	0	0.57
Stage	I&II	3	5	
	III&IV	8	12	
	ND***	0	3	0.63
EBV	+	3	7	
	-	8	9	
	ND***	0	4	0.22

HIV-associated lymphomas have unique DNA methylation patterns

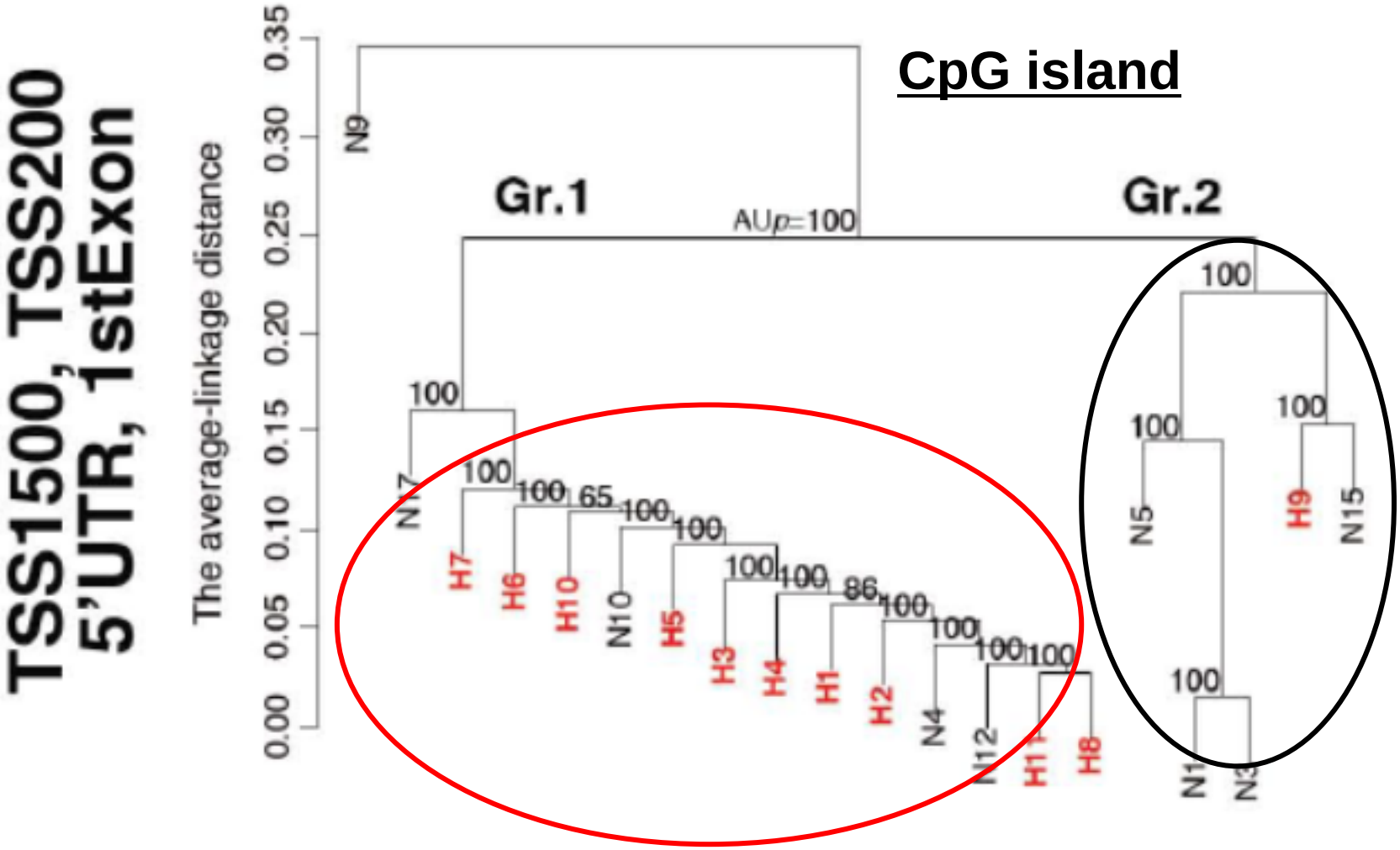


Hierarchical clustering

Red: HIV-positive

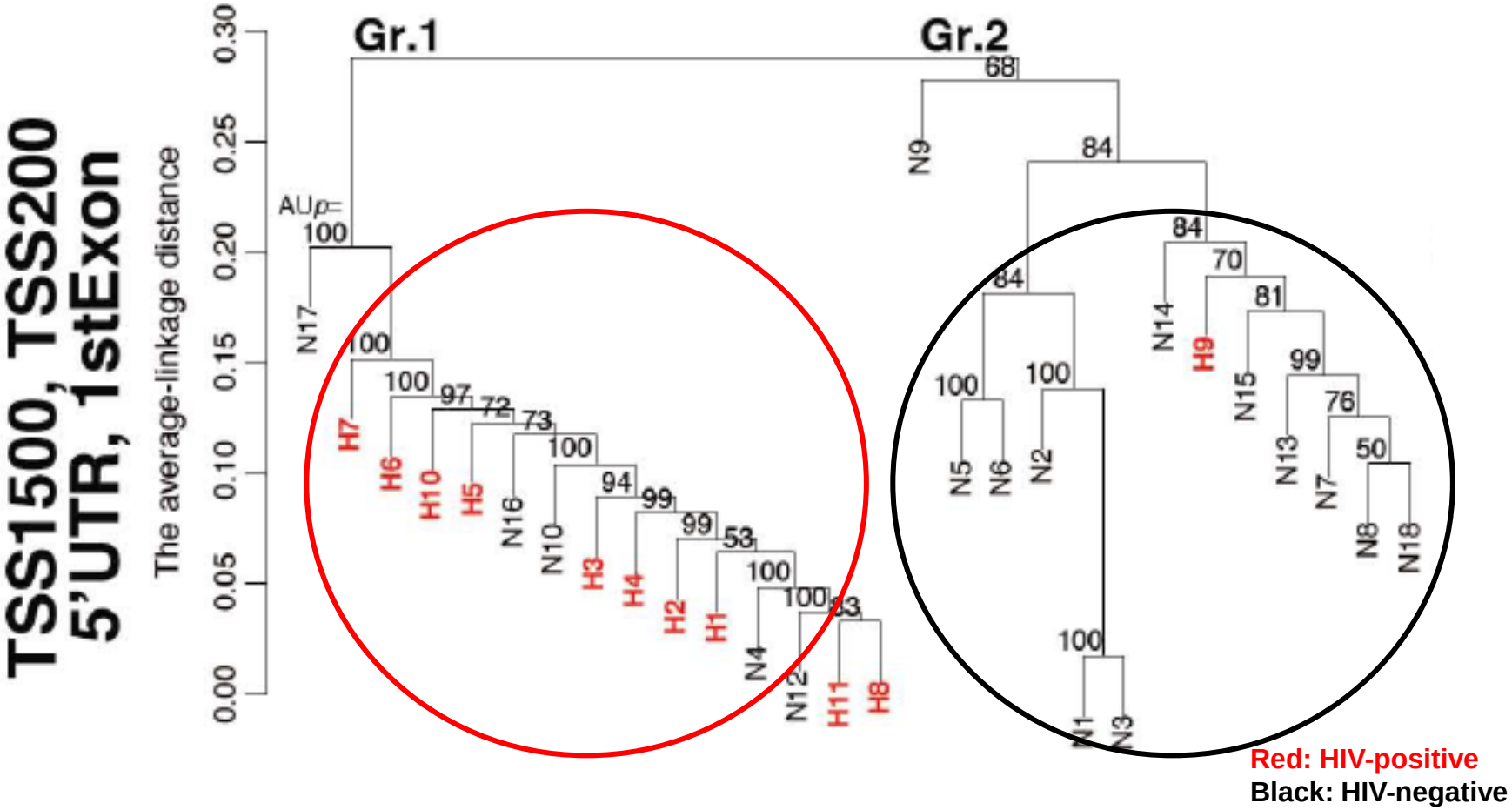
Black: HIV-negative

Methylation patterns adjusted by “Gender” are also different among two groups



Red: HIV-positive
Black: HIV-negative

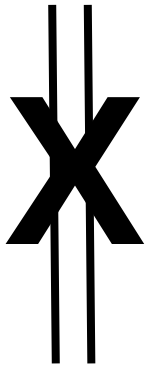
Methylation patterns adjusted by “Age” are also different



Exclusion of age-related targets in 27K microarray analysis

Answer to Question 1

HIV-1 associated lymphomas



Non-HIV-1 lymphomas

DNA methylation pattern in peripheral blood B cells.

Two of 10 HIV-1 positive patients analyzed possessed dramatic changes of DNA methylation pattern.

Question 2

What is the cause of differential patterns of DNA methylation?

- **Most of HIV-associated lymphomas are B cells.**

↓

There are some exogenous factors.

Viral proteins (Tat, Vpr, Nef)
Cytokines

Question 2

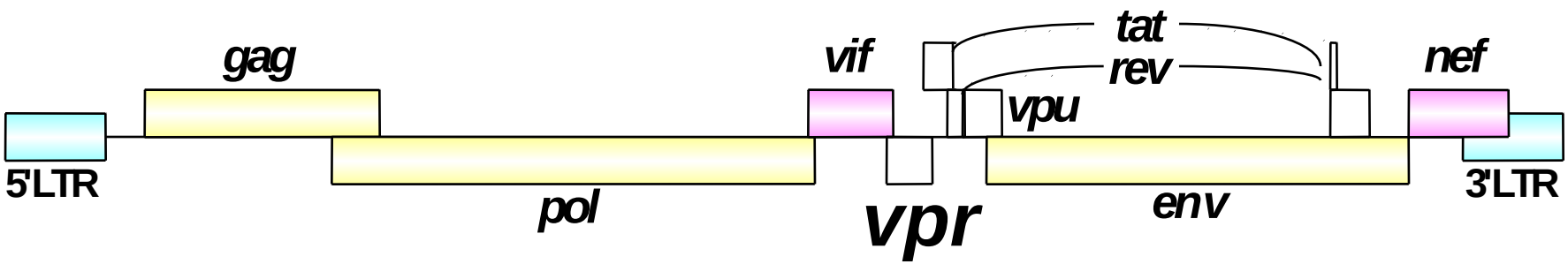
What is the cause of differential patterns of DNA methylation?

- **Most of HIV-associated lymphomas are B cells.**

There are some exogenous factors.

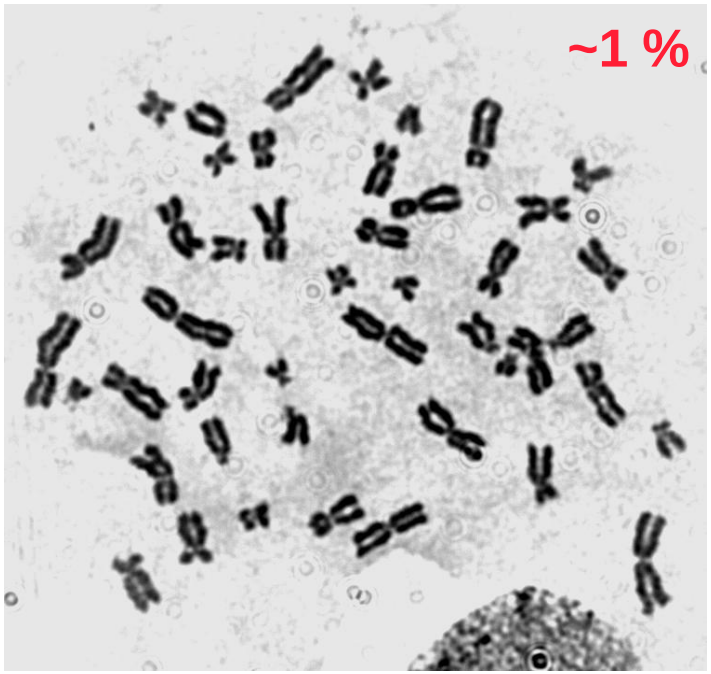
Viral proteins (Tat, Vpr, Nef)
Cytokines

Vpr is a candidate viral protein of malignancy

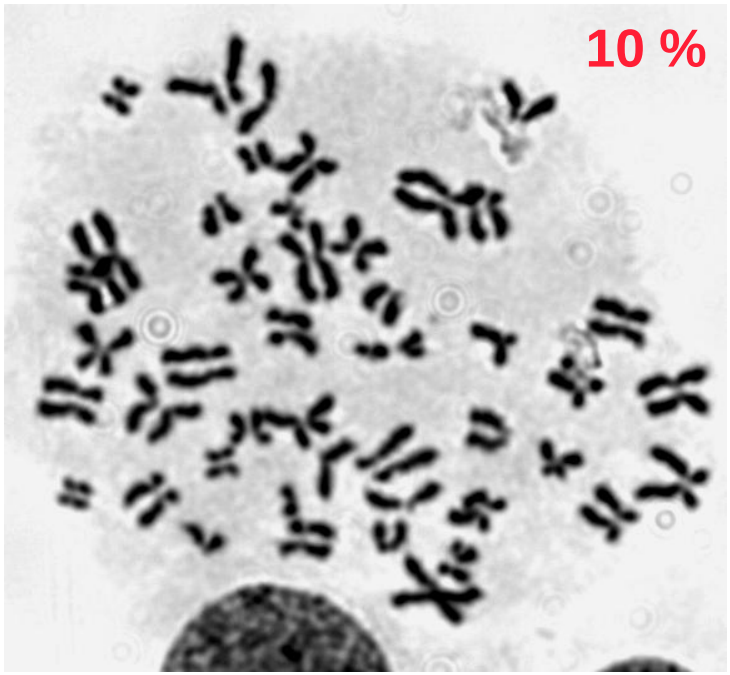


1. A virion-associated nuclear protein of 96 amino acids.
2. Vpr is important for macrophage infection.
3. Vpr induces various genomic instabilities, which include
   - DNA double-strand breaks
   - Premature sister chromatic separation
   - Retrotransposition
4. Vpr is present in circulating blood.

Premature Sister Chromatid Separation in HIV-infected Patient (PCS)



Healthy Volunteer

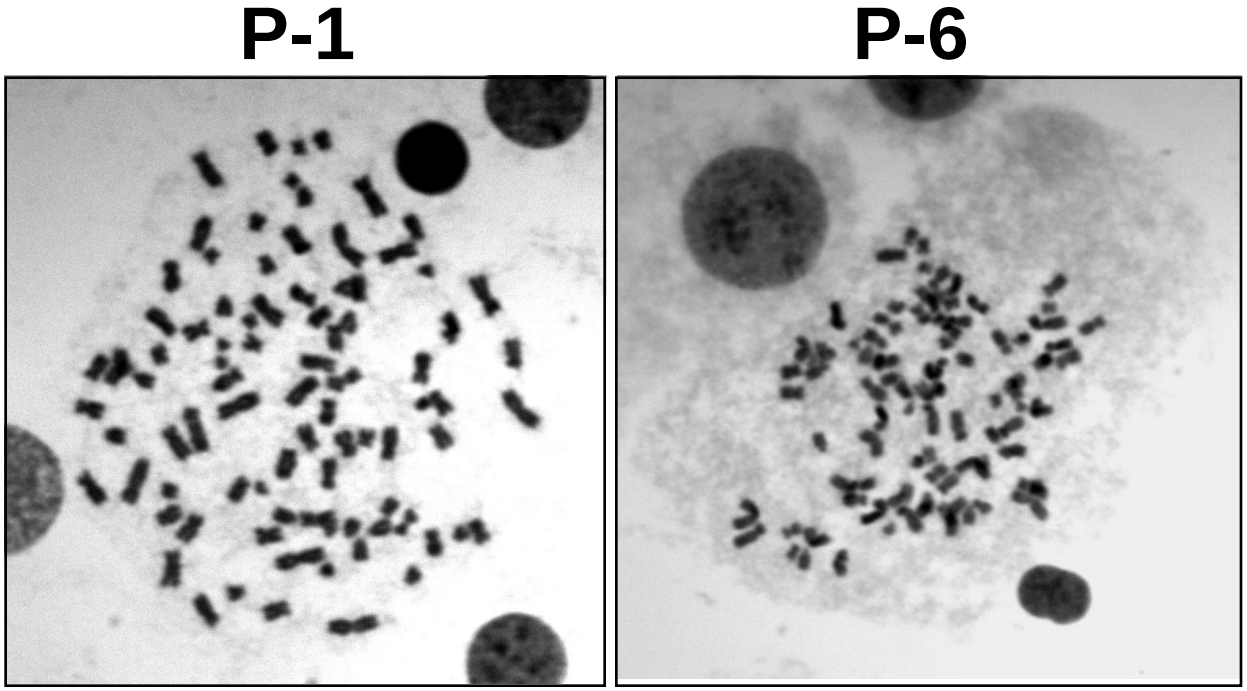


HIV+

“PCS is a hallmark of aneuploidy”

(Shimura et al. AIDS 19, 2005)

Anueploidy in HIV-1 infected cells



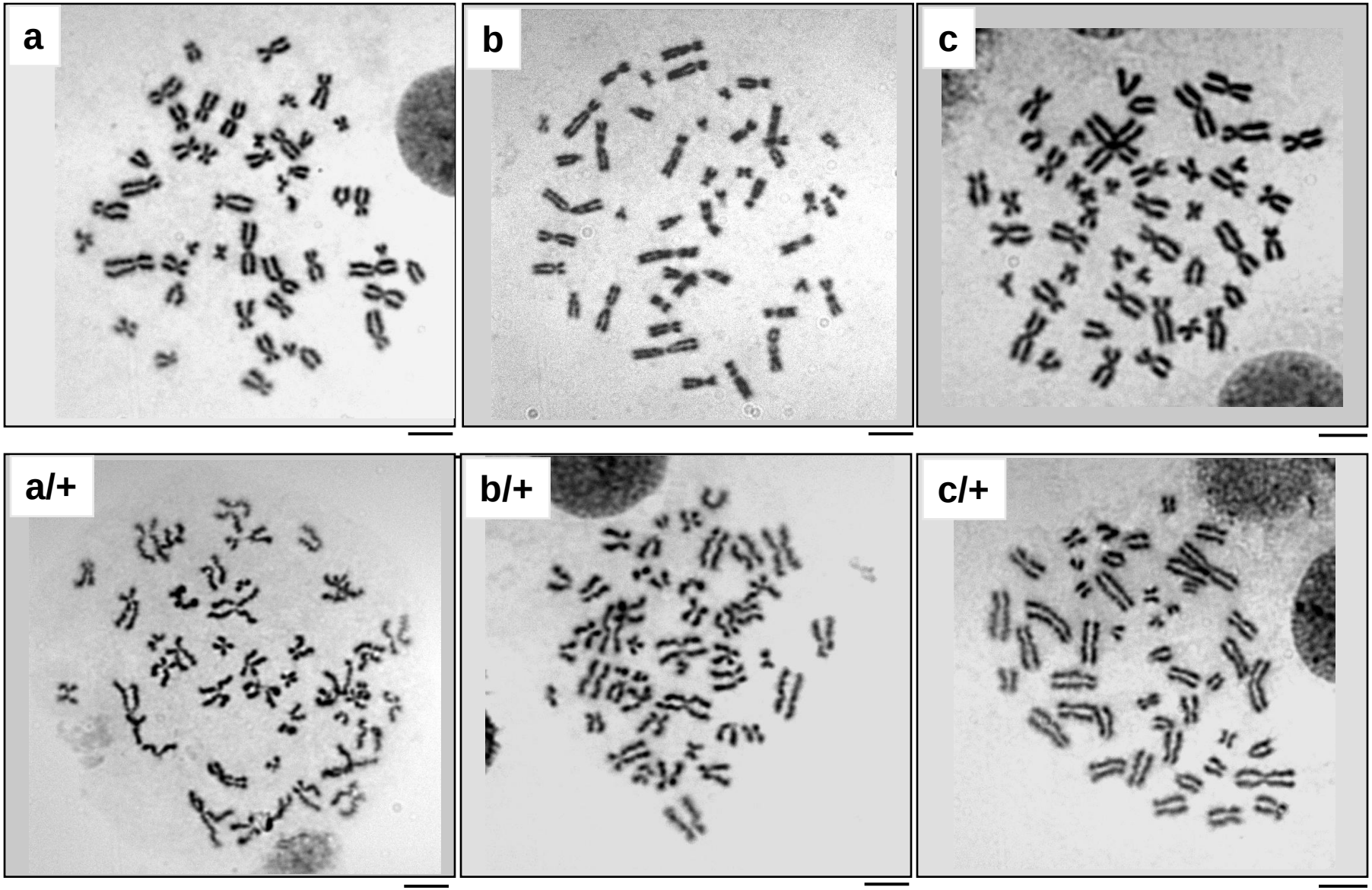
Numbers of chromosomes

85

75

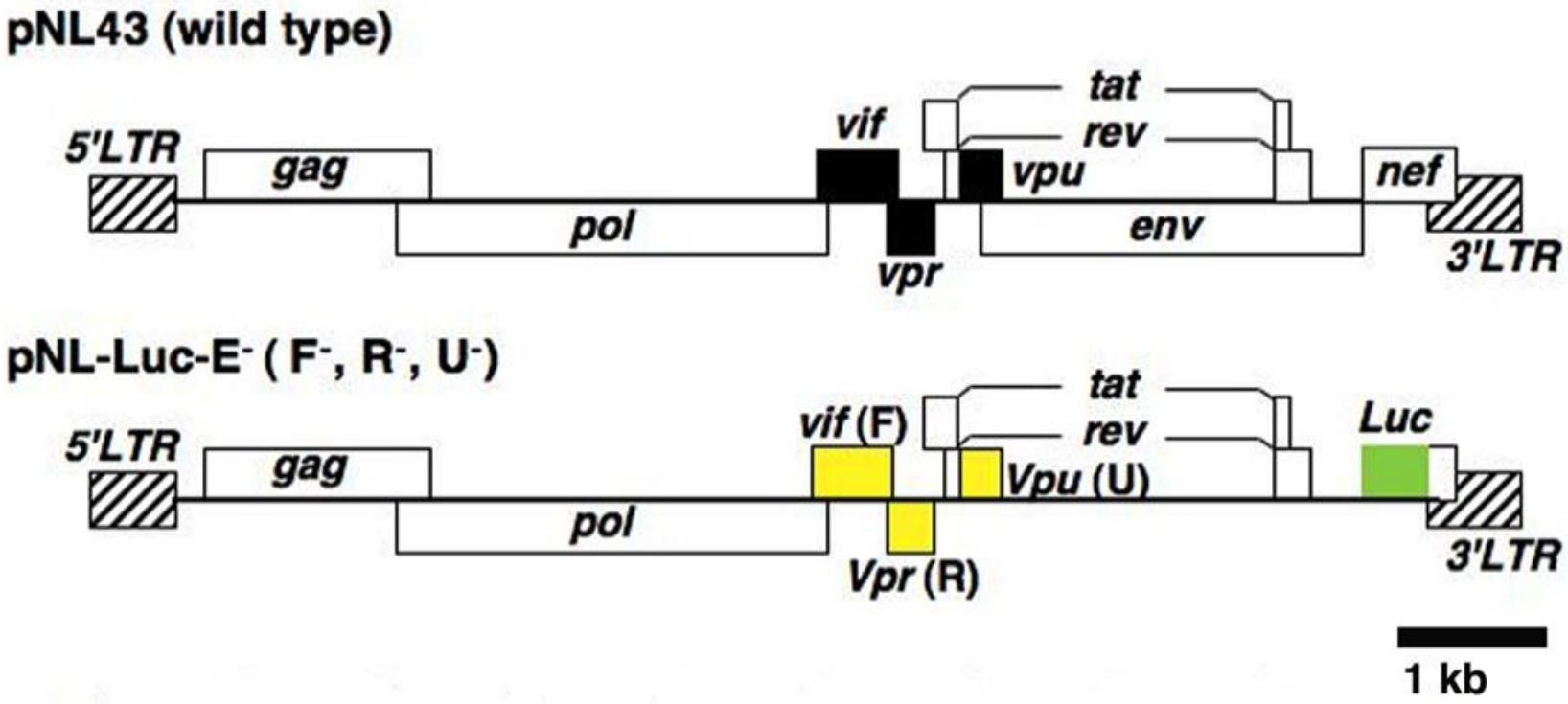
(Shimura et al. AIDS 19, 2005)

HIV-1 Infection in vitro promotes PSC in healthy blood cells

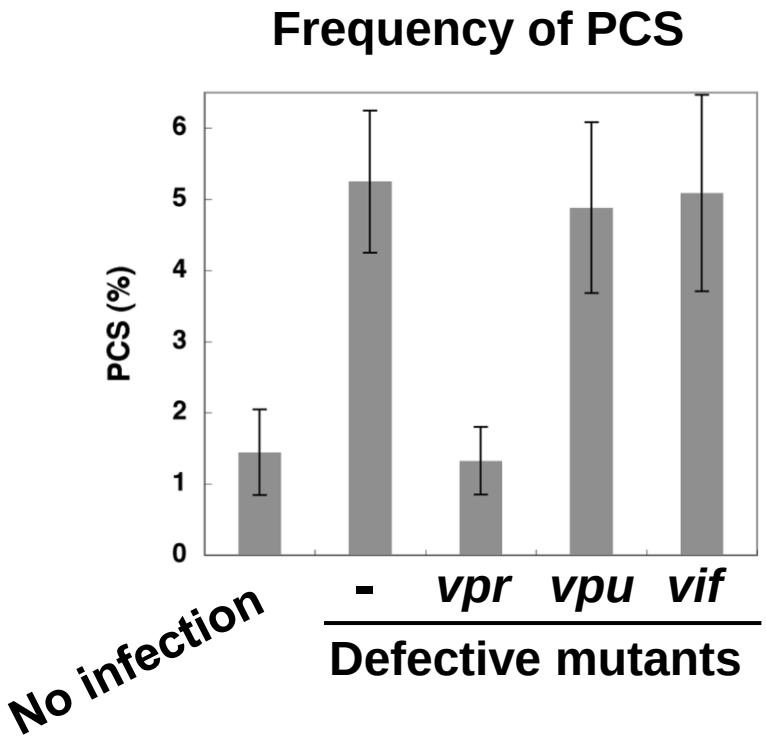


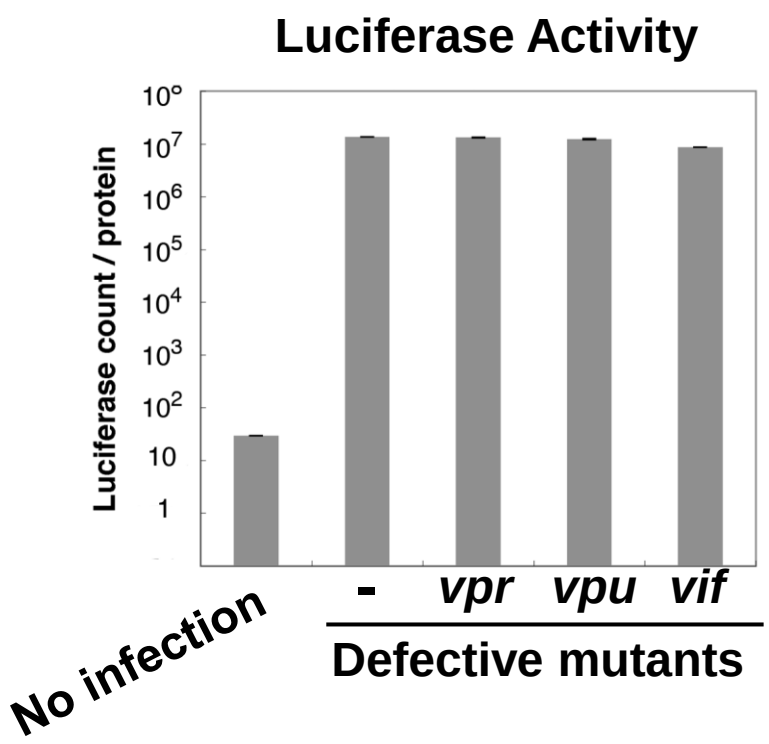
Shimura et al. AIDS 19, 2005

What is responsible gene for PCS ?



vpr is responsible for HIV-induced PCS



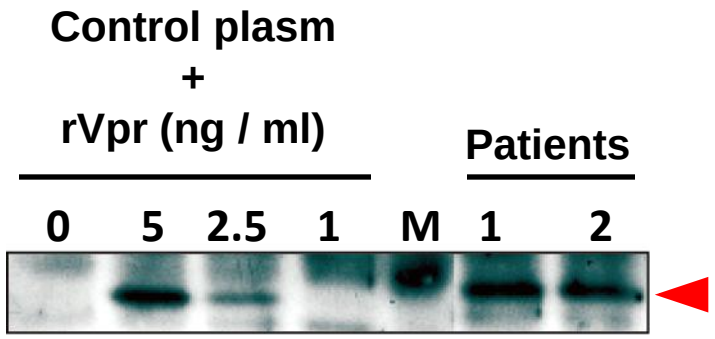


84 hrs post infection

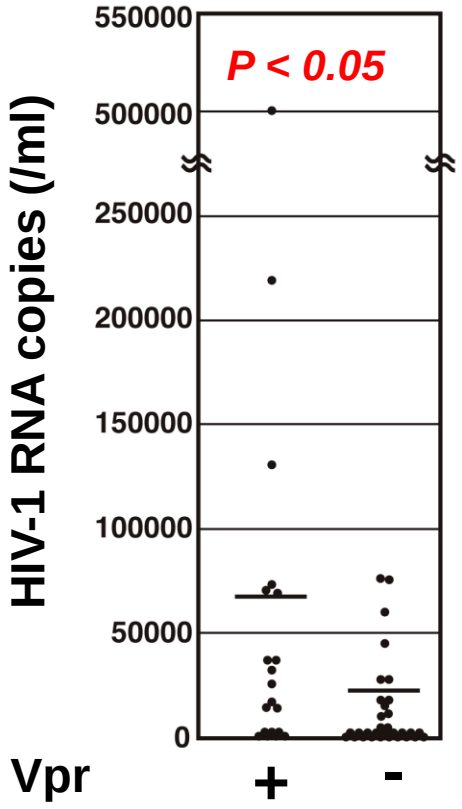
MOI; 0.007

Vpr is present in plasma of HIV-positive patients

(Hoshino S et al., *AIDS Res Hum Retrovir*, 23, 391, 2007)



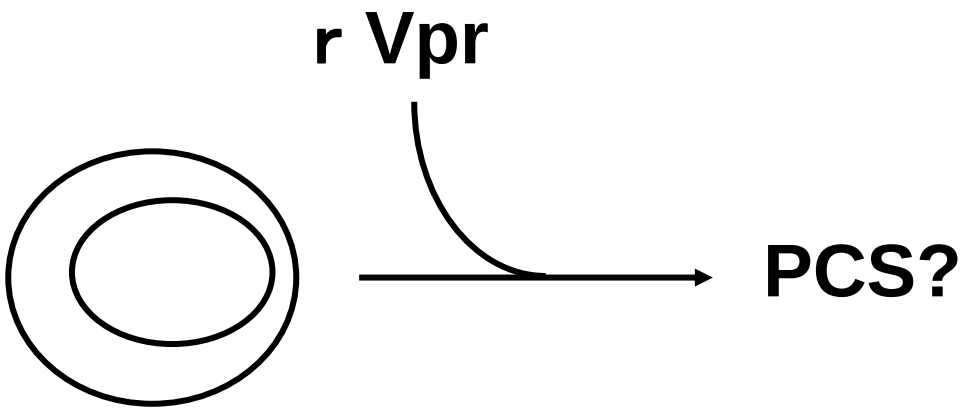
→ Concentration is nM level.

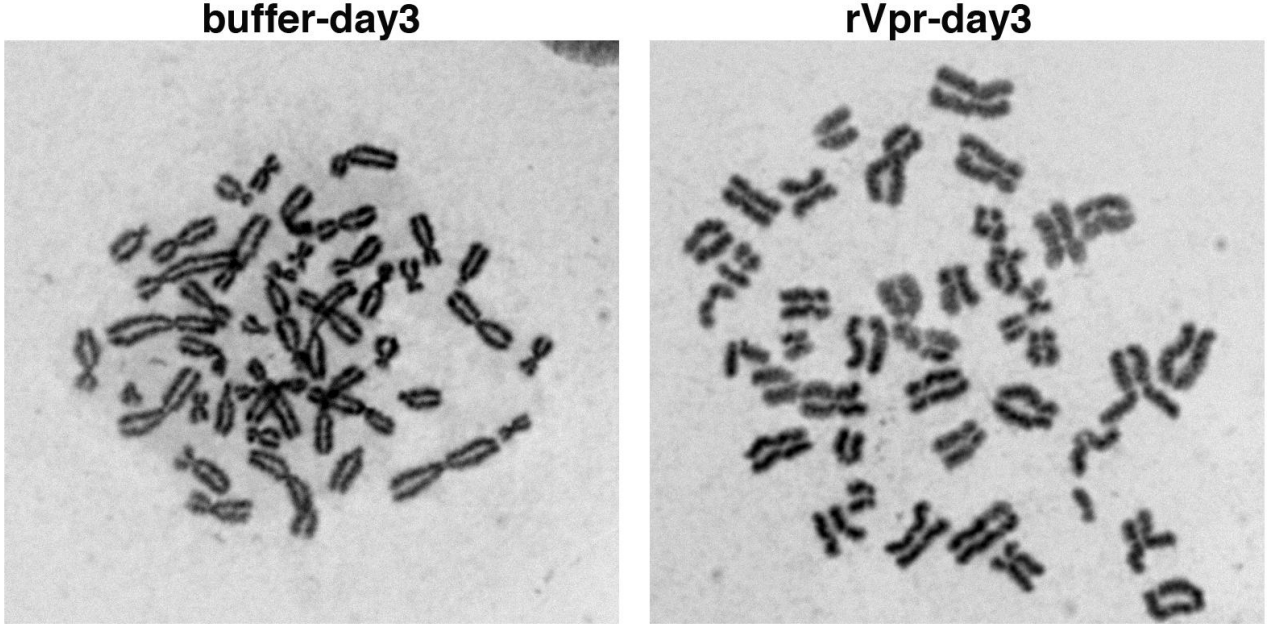


Cases 52 20 32

(～40%)

Exogenously added r Vpr also induces PCS

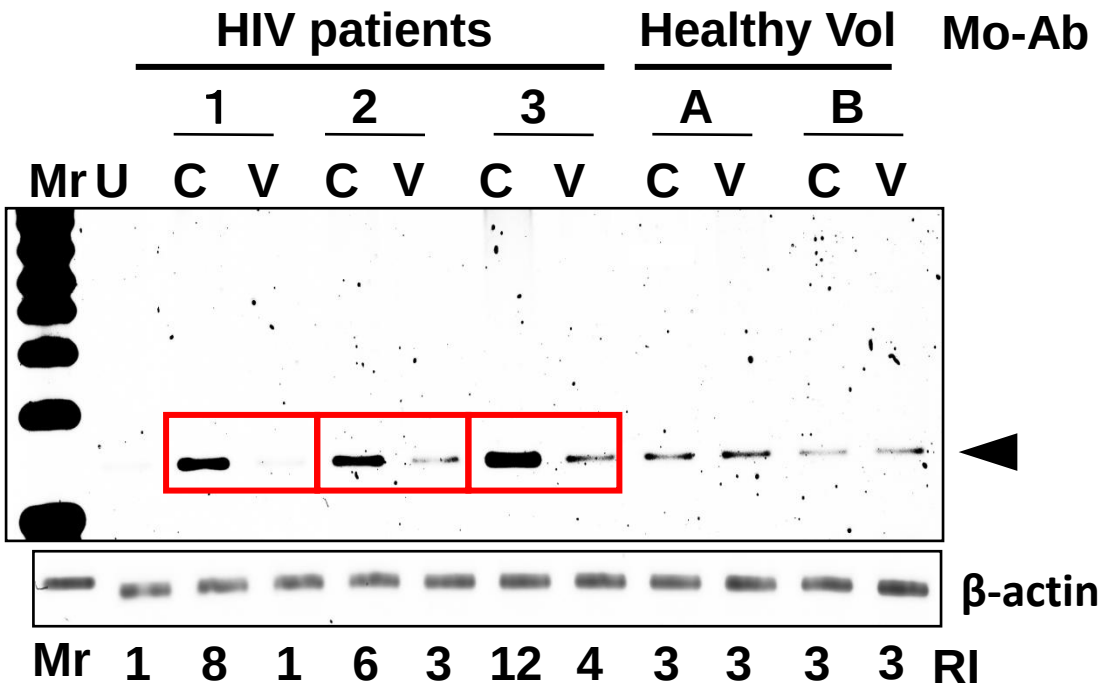




Vpr in patients' plasma is biologically active!

Exp-1. 6 /15 plasma samples were positive for retrotranposition (RTP)

Exp-2. The activity of RTP was neutralized by anti-Vpr MoAb.



C, control IgG; V, anti-Vpr monoclonal Ab x10 dilution

(Iijima K, *Retrovirology*, 2013)

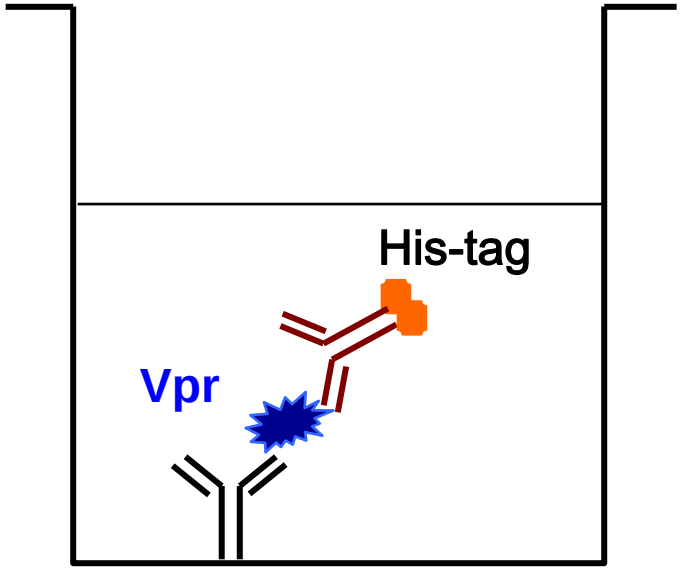
Current Question

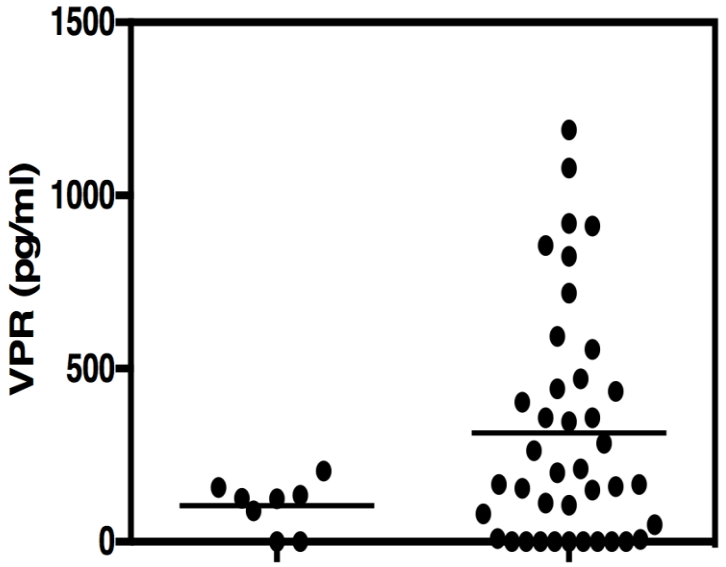
Is plasma Vpr “really”
a candidate factor related to malignancy?

1. Monitoring plasma Vpr.

2. Correlation of Vpr titer and DNA methylation.

Newly developed ELISA system can monitor plasma Vpr





Capture: polyclonal peptide antibody

Detector: recombinant chick-derived MoAb (B34)

16/40 (>40%)

Summary

1. HIV-associated lymphomas are different from non-HIV lymphomas.

2. HIV has Vpr that induces genomic instability.

3. Plasma Vpr is detectable in more than 40% of the patients.

4. Next issue is to compare plasma Vpr and DNA methylation pattern.

5. We can use a humanized recombinant monoclonal Ab to Vpr.

⟶ better prognosis and QOL of the patients

Collaborators

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