Gene therapy and HIV cure

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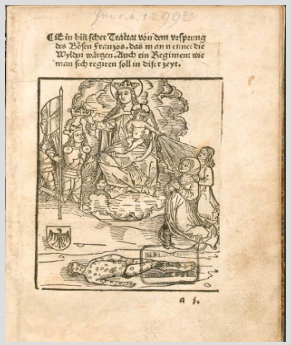
Progress in the treatment of infectious diseases

Syphilis



1496

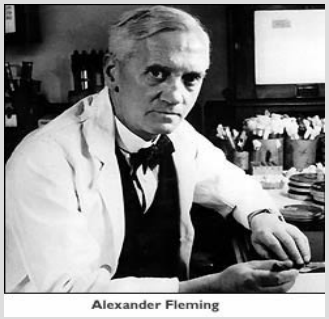
1689

August v. Wassermann

1906

Paul Ehrlich

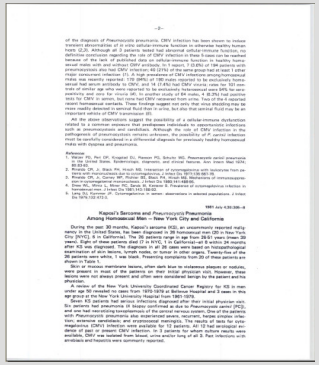
1909



Alexander Fleming

1941

HIV



1981

Luc Montagnier

1983

Hiroaki Mitsuya

1985

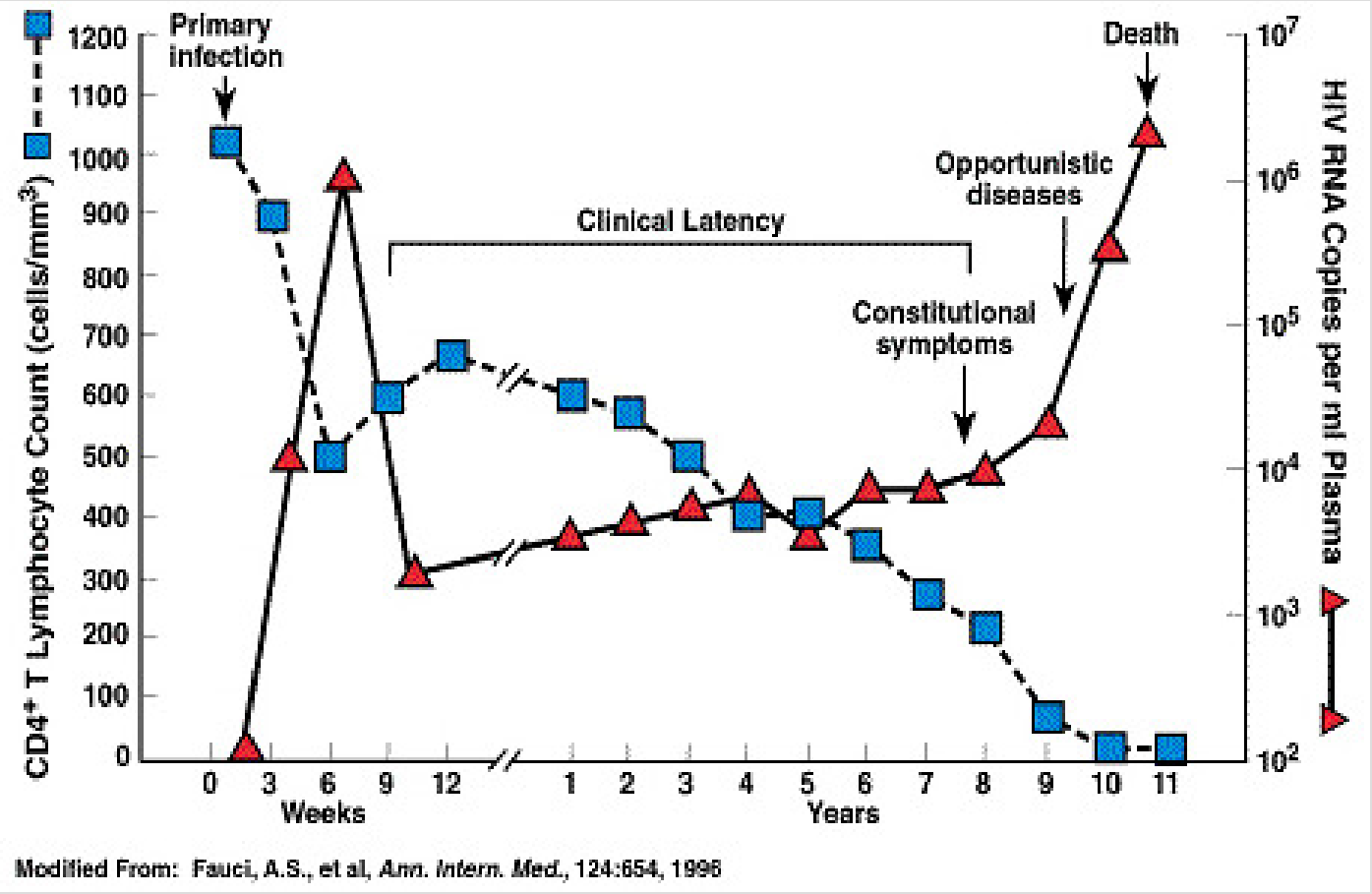
HAART

1996

?

2013

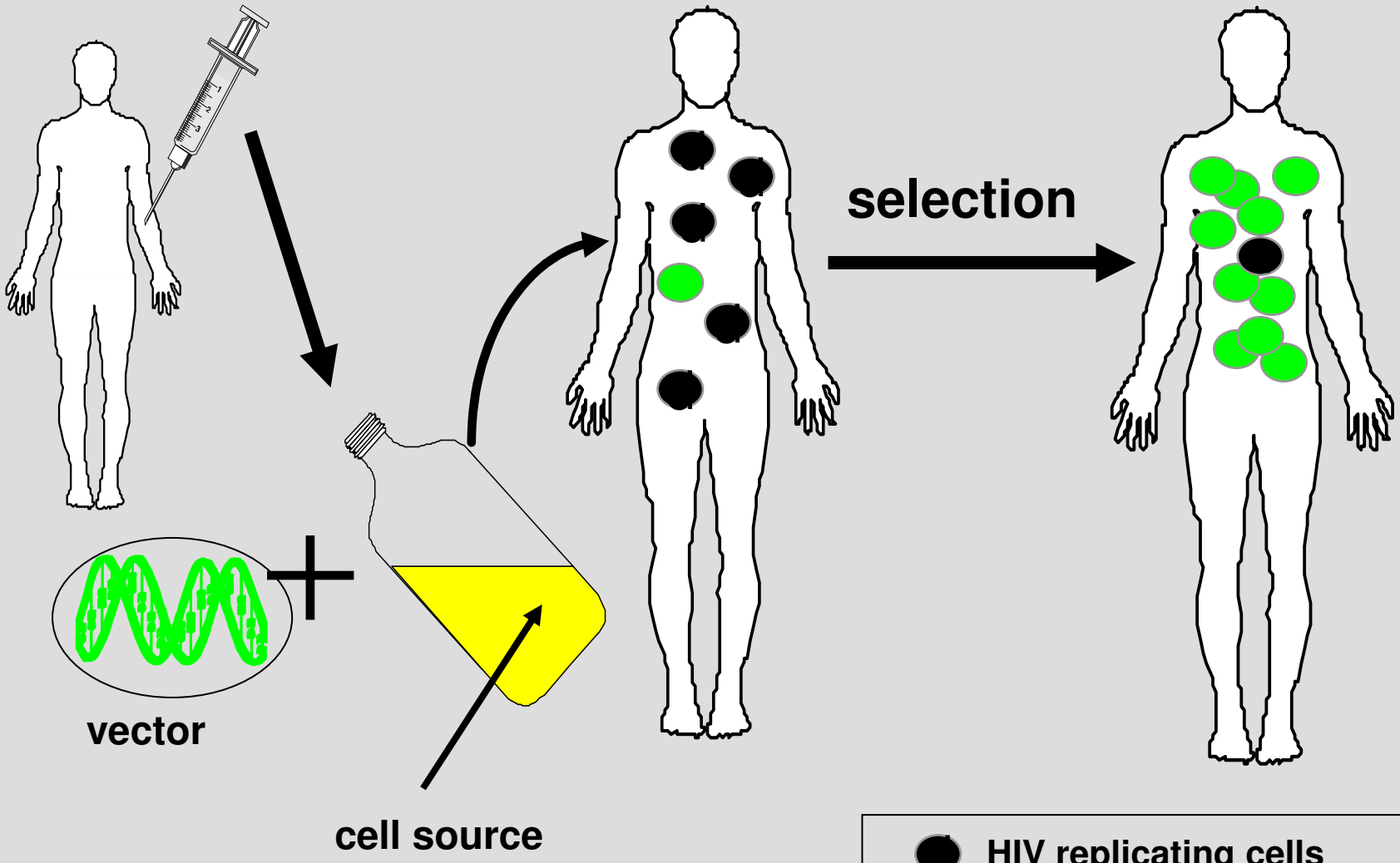
Why cell therapy against HIV?



CD4 decline is responsible for HIV symptoms

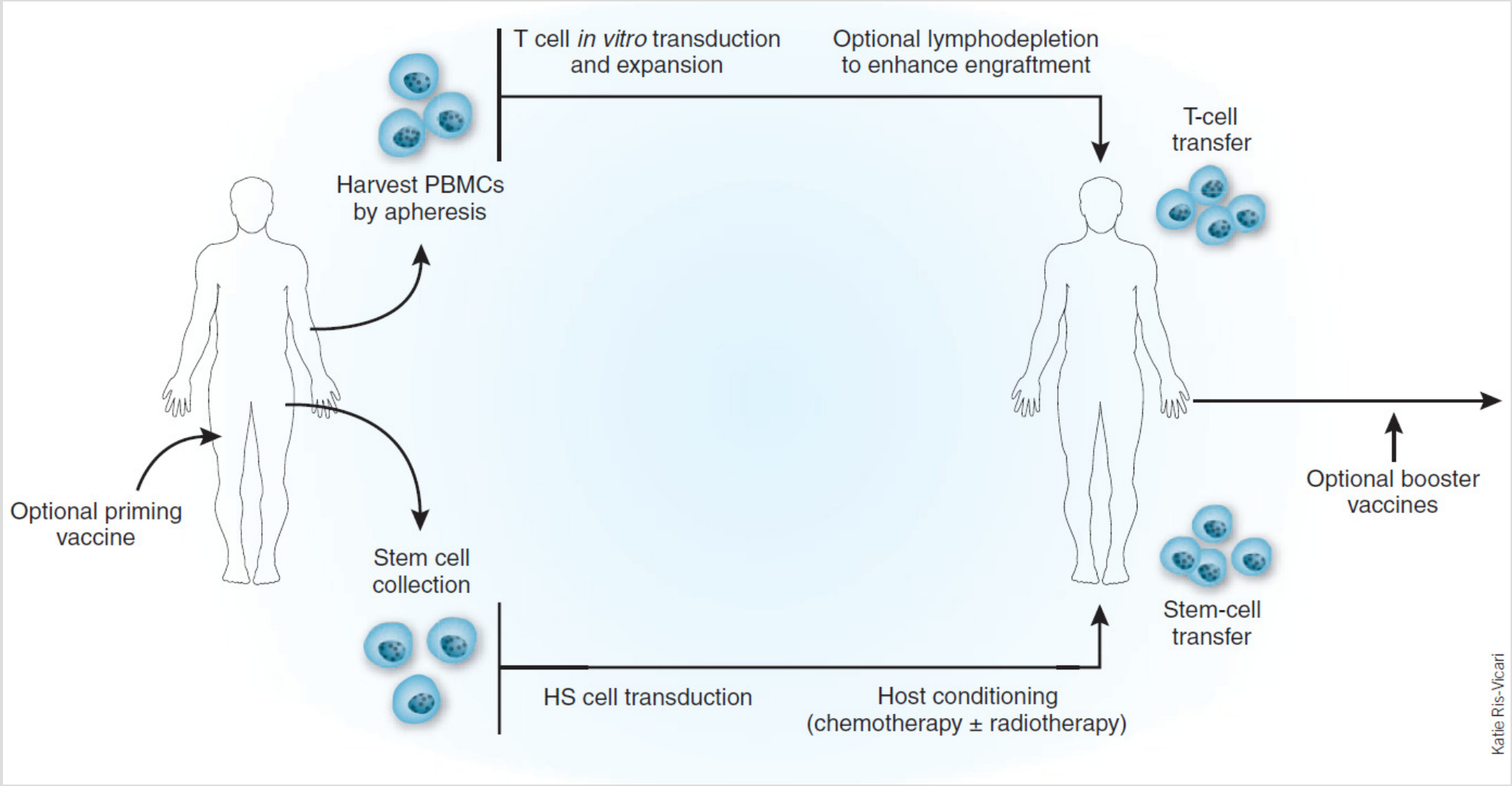
can AIDS be prevented by CD4 substitution?

Principle of enrichment of transduced cells



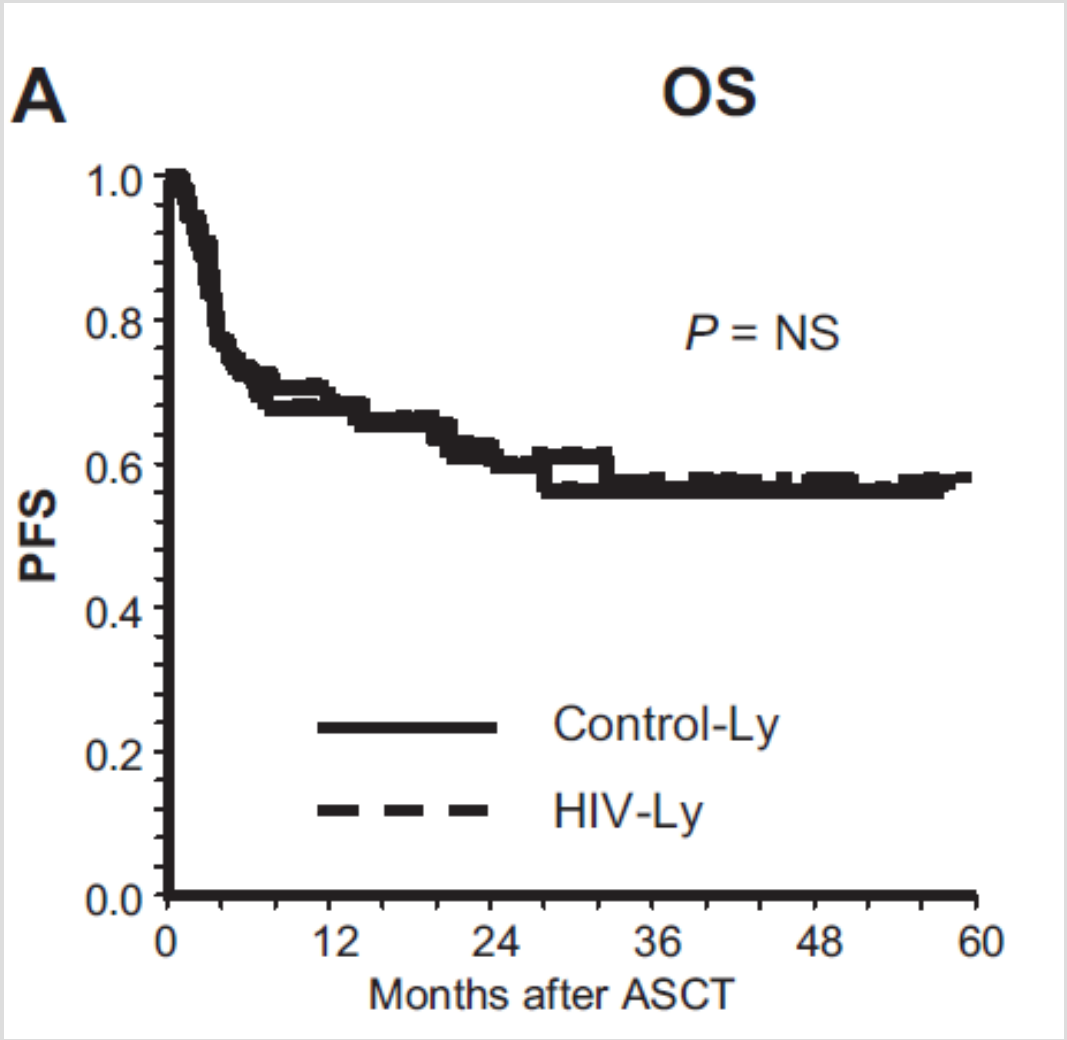
von Laer et al. AAAMC 6: 121-140, 2007.

Two sources for HIV cell therapy



Nat Biotech 2007 25:1449

Autologous transplantation in HIV



[Diez-Martin, Blood 2009]

no difference in survival after auto SCT in HIV+ vs. HIV-neg.

no increased risk for auto stem cell/gene therapy approaches

From HIV cell to HIV gene therapy

J.M. Hassett
1983 first stem cell transplantation in an HIV patient, Mt. Sinai, NY

David Baltimore suggested gene therapy for HIV infection in 1988

sCD4: Truncated secreted CD4
CCR5-RNAi: RNAi targeting CCR5
Anti-CCR5 ribozyme
Intracellular scFV against CXCR4, CCR5
M87o: Membrane-anchored fusion inhibitory peptide
Secreted neutralizing human mab 2F5 Membrane-anchored anti-gp41 scFv
ScFv-RT Intracellular single-chain Fv to RT
ScFv to IN: Intracellular single-chain Fv to IN

Anti-hCyclinT1 intrabodies
Td-Tat: Transdominant HIV Tat protein
scFV to Tat
siRNA to Tat
Tar decoy
RevM10: Transdominant HIV Rev protein
Dominant negative Sam68, a Rev homolog
scFv to Rev
RRE decoy
Rev cofactor eIF-5A mutants
Ribozymes
Small-guide RNAs directing specific cleavage of HIV RNA by tRNase ZL
Antisense RNA

Antisense to packaging signal
Transdominant HIV gag protein
Alpha1 antitrypsin
ER retained CD4 chimera
F12-vif: Transdominant Vif

2011: targets of HIV gene therapy

Detection of the HIV resistance gene: CCR5-delta32

Cell, Vol. 86, 367–377, August 9, 1996, Copyright ©1996 by Cell Press

Homozygous Defect in HIV-1 Coreceptor Accounts for Resistance of Some Multiply-Exposed Individuals to HIV-1 Infection

Rong Liu,* William A. Paxton,* Sunny Choe,* Daniel Ceradini,* Scott R. Martin,* Richard Horuk,† Marcy E. MacDonald,‡ Heidi Stuhlmann,§ Richard A. Koup,* and Nathaniel R. Landau*
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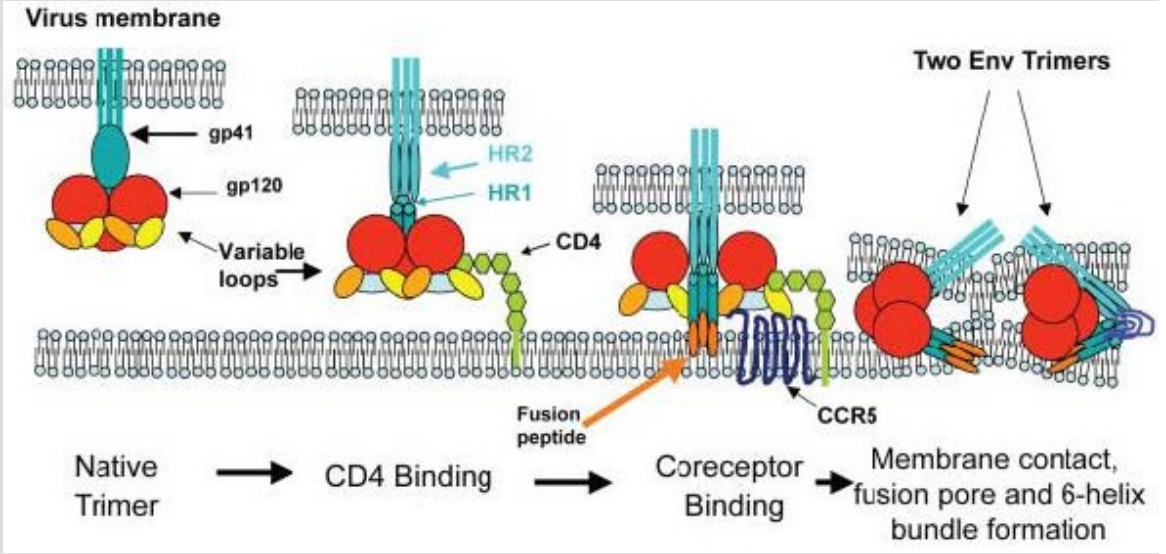
designated EU2 and EU3, required about 1000-fold more virus to establish infection than contol cells from unexposed donors. While a small fraction of the cells did become infected with this high inoculum, the virus failed to replicate further. Analysis of the early events of the viral replication cycle showed that macrophage-tropic HIV-1 isolates failed to enter or fuse to the CD4+ cells of these two individuals (Dragic et al., 1996). Thus, the resistance of these individuals to sexual transmission of HIV-1 was likely to have resulted from the inability of their cells to support entry of macrophage-tropic virus.

HIV-1 can broadly be divided into macrophage- or T-tropic isolates (Gartner et al., 1986; Koyanagi et al., 1987; Fisher et al., 1988). Macrophage-tropic nonsyncytium-inducing (NSI) isolates infect primary macrophages but fail to infect transformed T-cell lines, while T-tropic syncytium-inducing (SI) strains have the reciprocal tropism. Both classes of HIV-1 efficiently infect CD4+



CCR5-Δ32 deletion

- inactive receptor
- frequency 10-20% (1% homozygous)*
- CCR5-Δ32/Δ32 resistant against R5
- in vivo highly resistant against X4 and dual

*Europe

CCR5-delta32 protects against HIV transmission

conversion rate from R5 to X4 of 7-8% /year

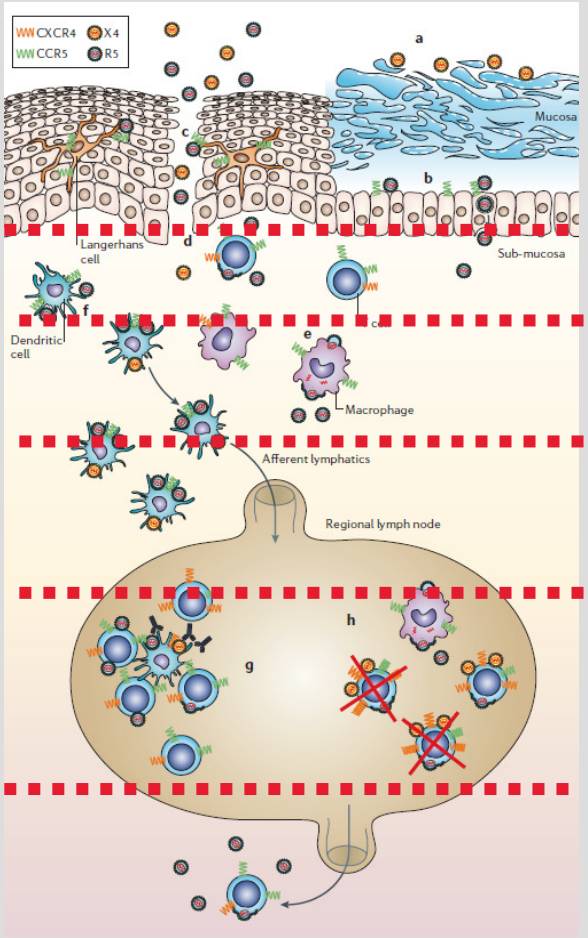
[Koot,J Infect Dis 1999]

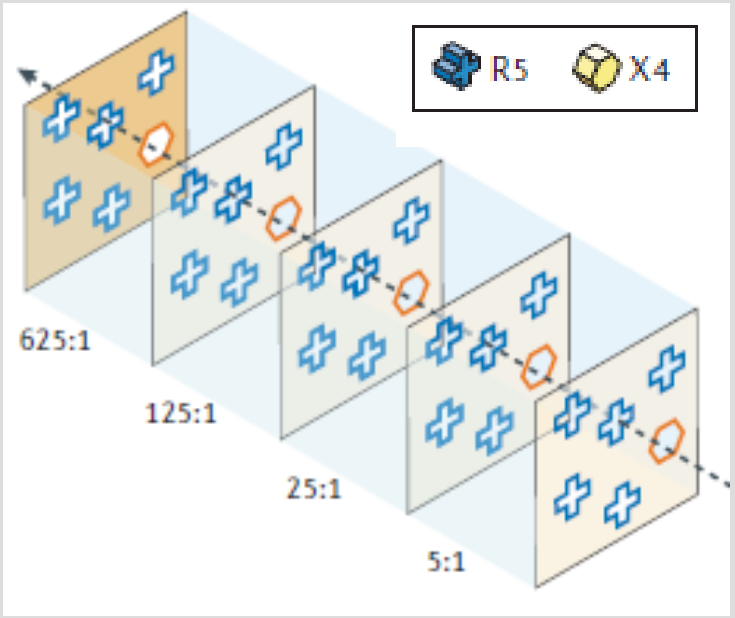
	Seronegative Number	Seropositive Number
Genotypes		
CCR-5/CCR-5	582	645
CCR-5/Δccr-5	114	78
Δccr-5/Δccr-5	8	0
Total	704	723

[Samson, Nature 1996]

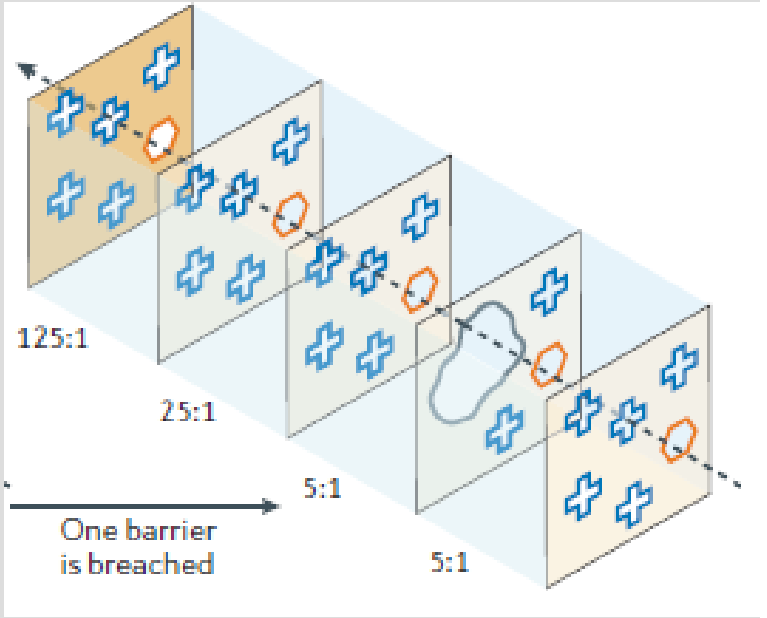
CCR5-d32/d32 individuals get frequently exposed by non CCR5 using strains but almost never get infected!

CCR5-delta32 protects against HIV transmission





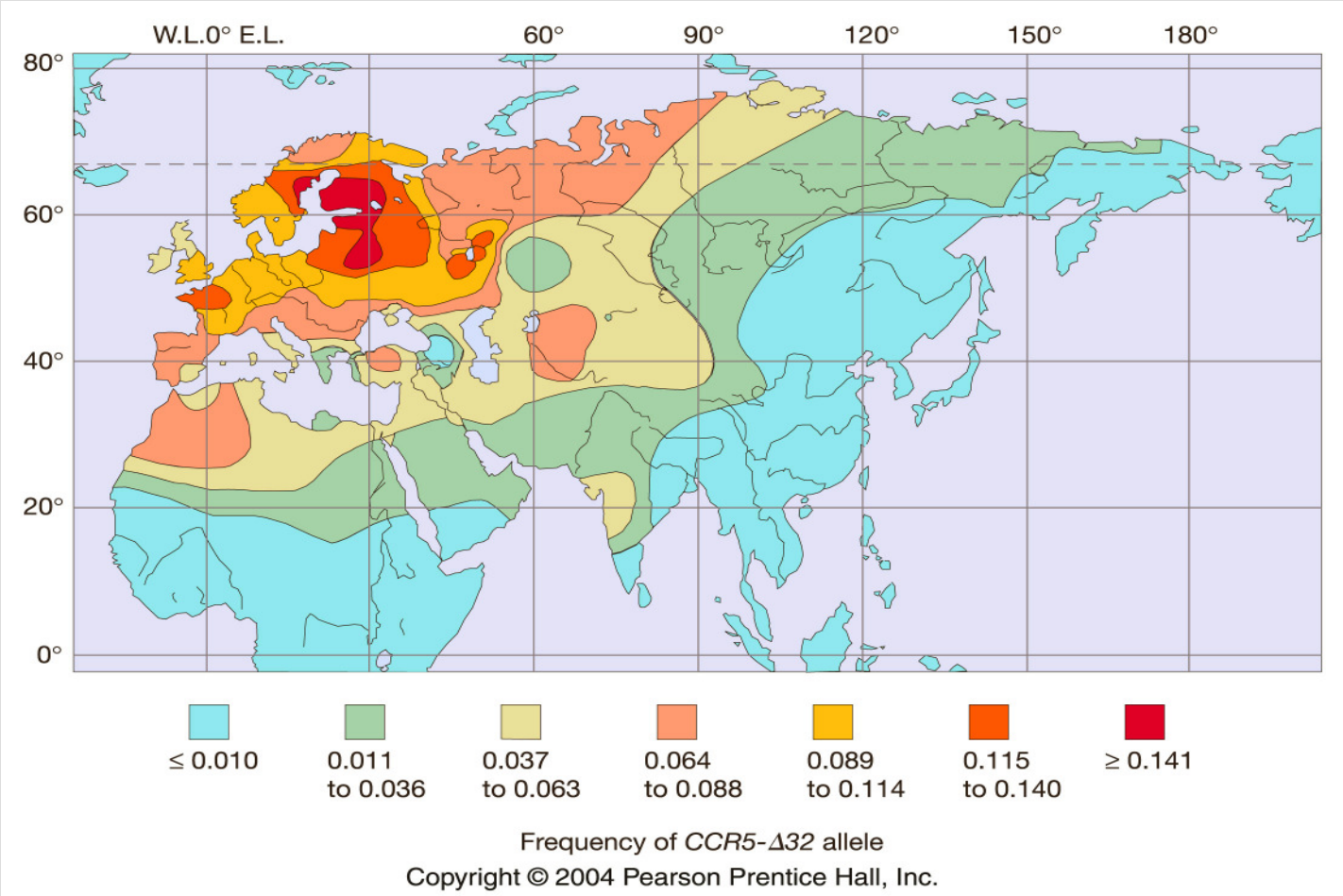
R5:X4 = 3,125:1



R5:X4 = 625:1

[Margolis, Nat Rev 2006]

Frequency of the CCR5-delta32 allele



Frequency of *CCR5-Δ32* allele

highest frequency in Northern Europe

unknown selective advantage for d32 deletion

absent in Africans, Asians and Indians

History of CCR5 targeted cell therapy

1996 first description of the CCR5-delta32 deletion
[Liu, Cell 1996]

2001 Robert Chow founded STEMCYTE, Inc. build up a cord blood bank with over 10.000 CCR5 screened donors

2008 Chow group published a pre-match of probable HIV+ recipients
[Chen, Biol Blood Marrow Transplant 2008]

2007 First successful allo HSCT after CCR5 donor selection
[Hütter, NEJM 2009]

2011 Disruption of CCR5 in zinc finger nuclease-treated CD4 T cells (Sangamo trial)
[CROI 2011]

2012? FDA approval for CCR5 knock down in lentiviral treated stem cells
[personal communication Calimmune]

Case report: patient´s history

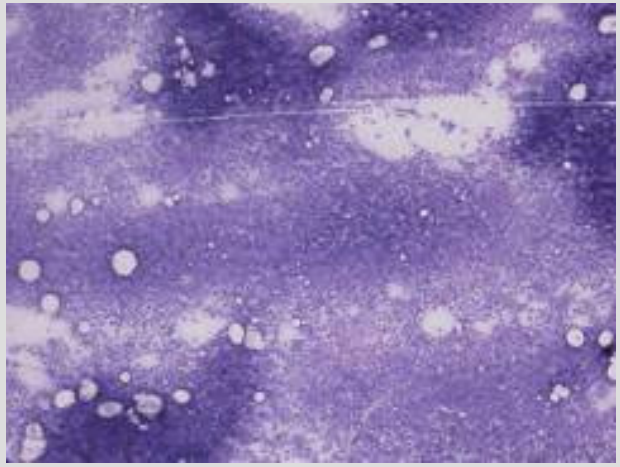
40-year-old patient

HIV infection mid ´90s
HAART since 2002

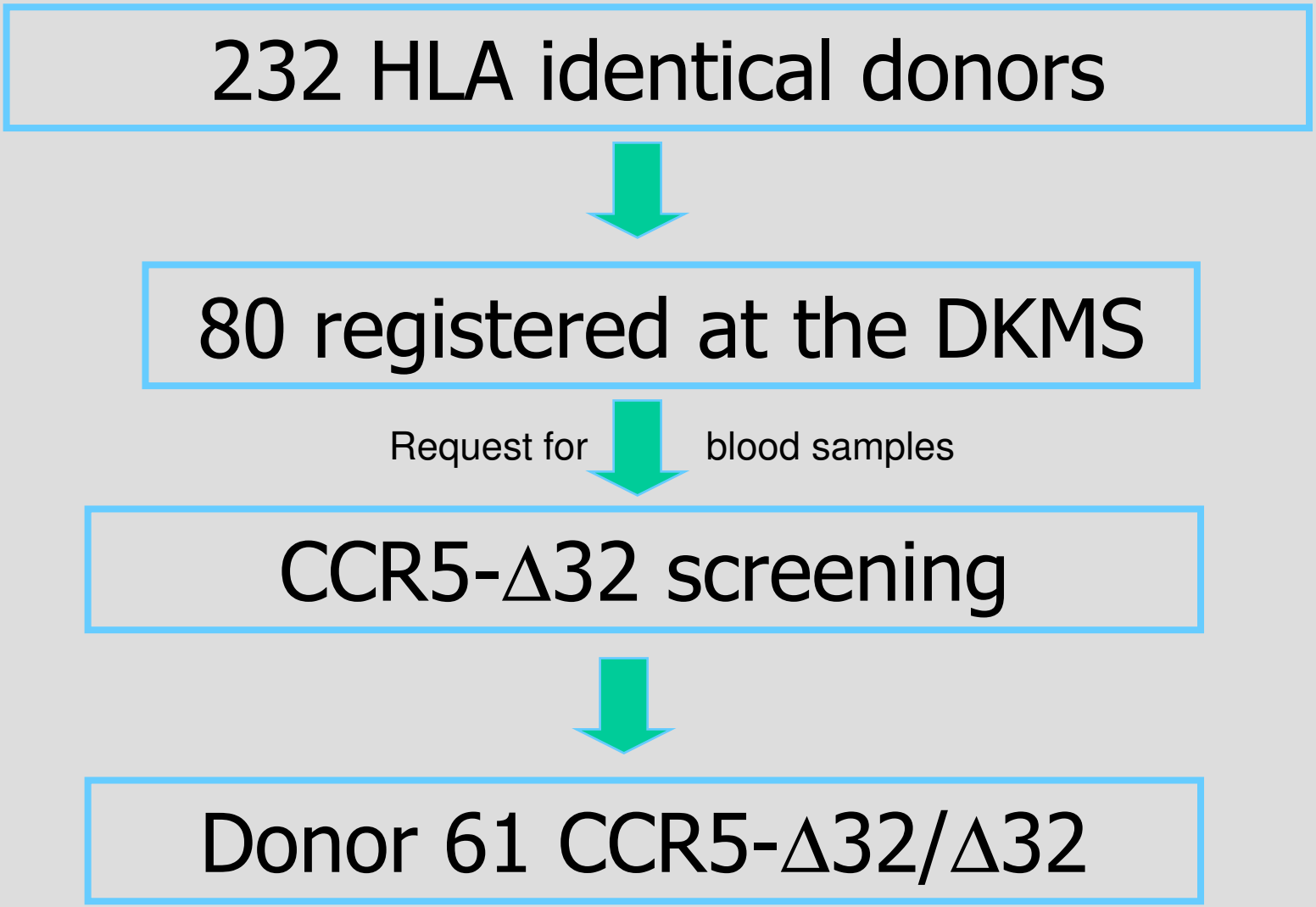
- HIV-1 RNA < detection limit
- $CD4^+$ T-cells 300-400 /µl
- No AIDS defining illnesses

Spring 2006 weakness
June ´06 anemia
July ´06 pancytopenia

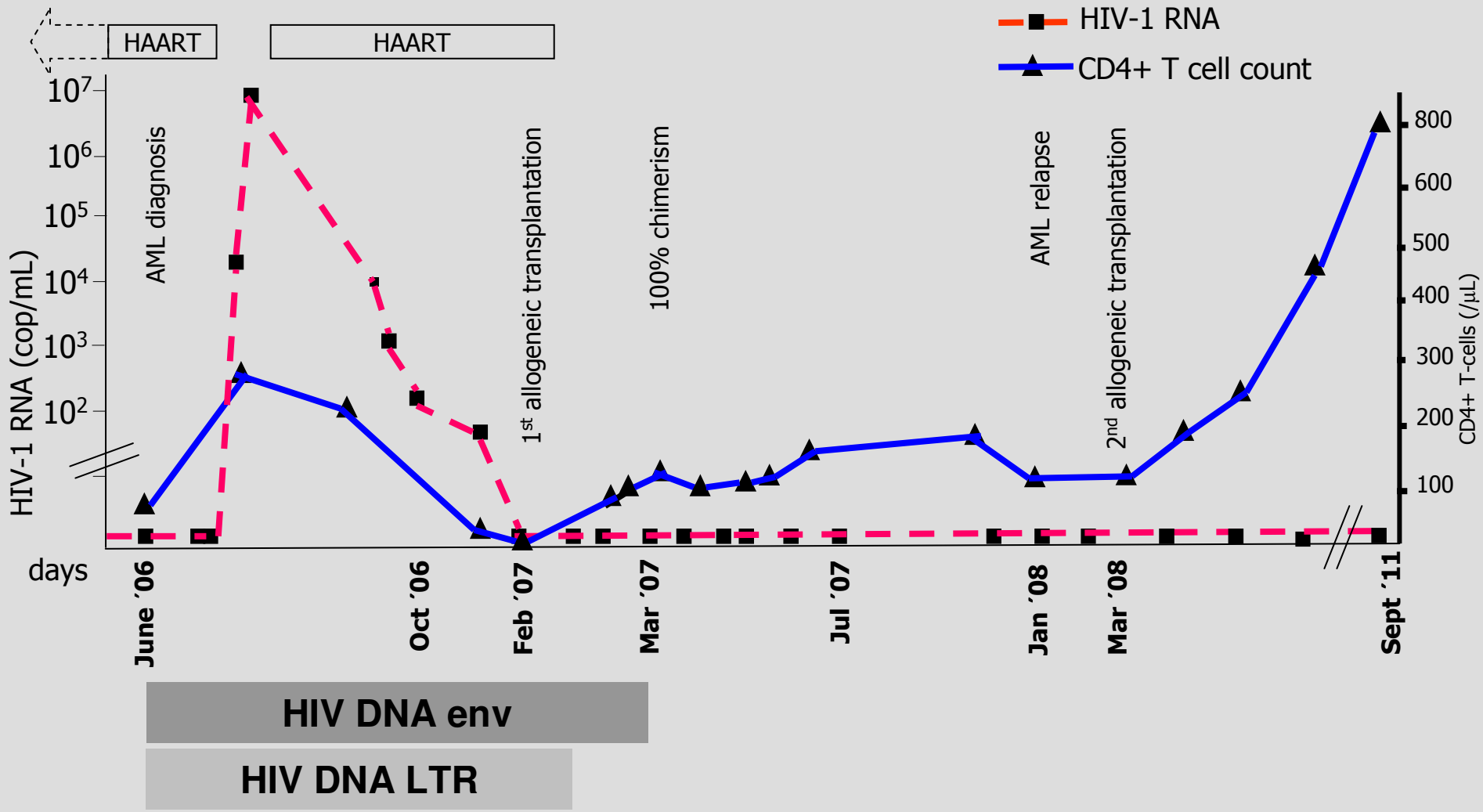
Donor request:
232 HLA identical!



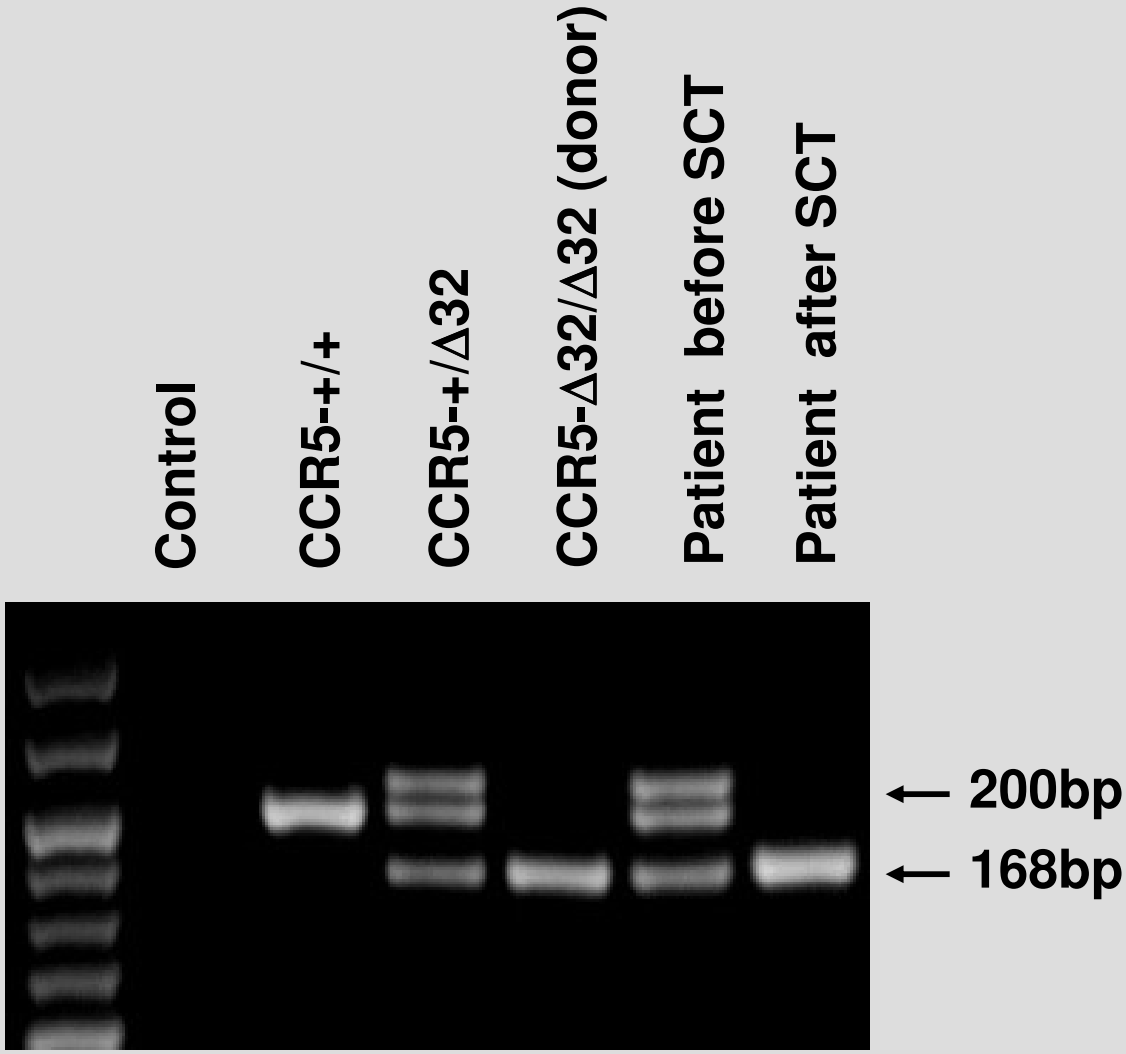
Donor search and genotyping



HIV-1 & T-cell reconstitution after CCR5-delta 32 allo SCT



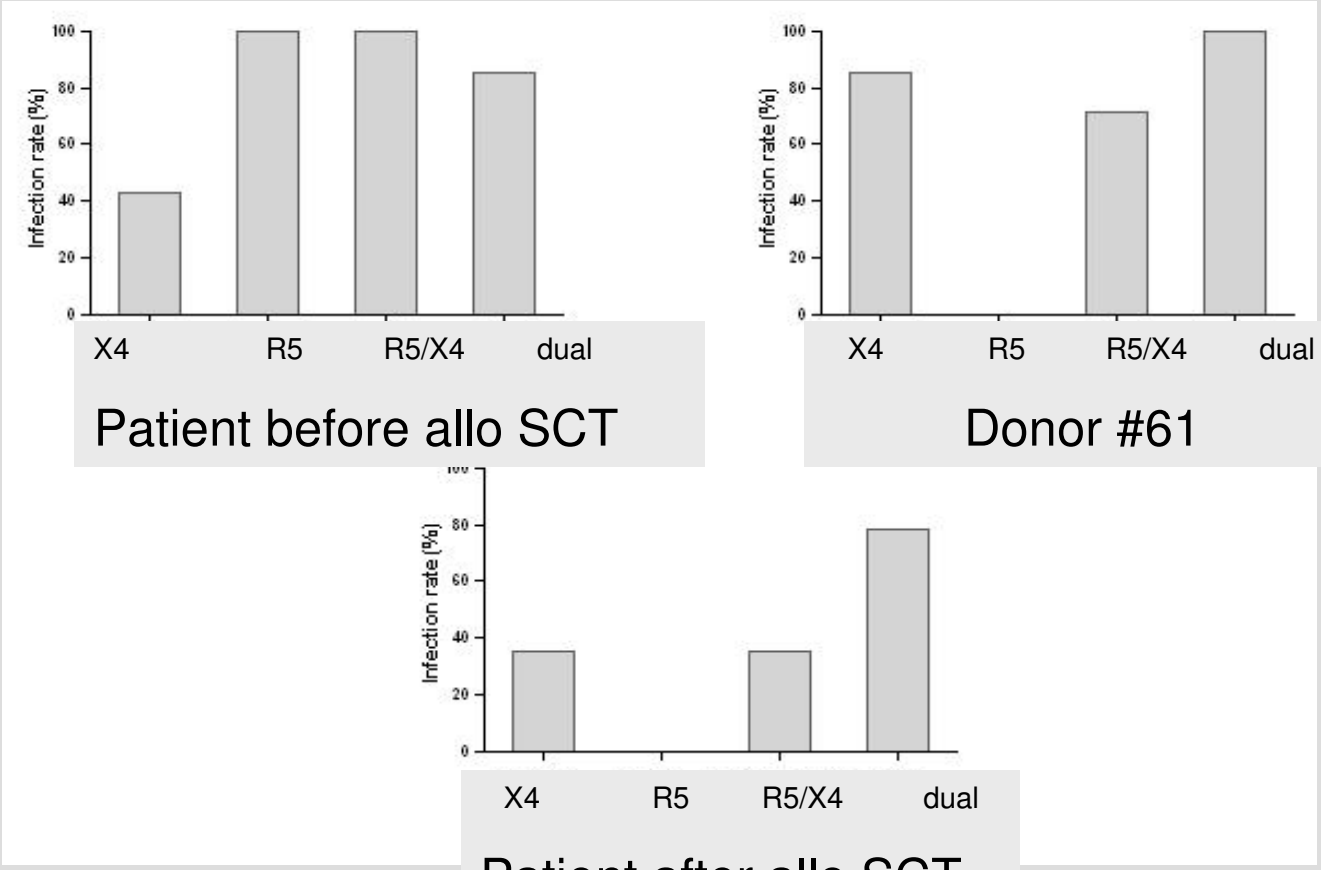
Development of the CCR5-Δ32 genotype



The patient was already CCR5-d32 heterozygous before SCT

Complete change of CCR5 genotype after 2 months

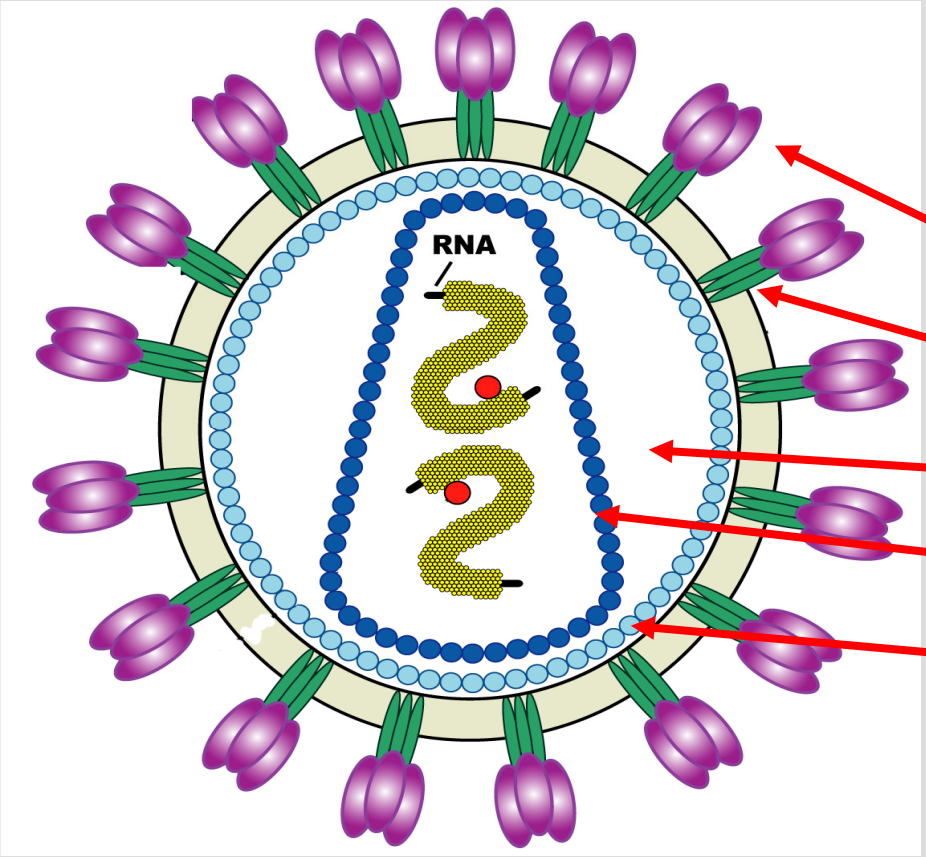
Susceptibility of donor´s and patient´s lymphocytes against HIV



donor's resistance has moved to the patient

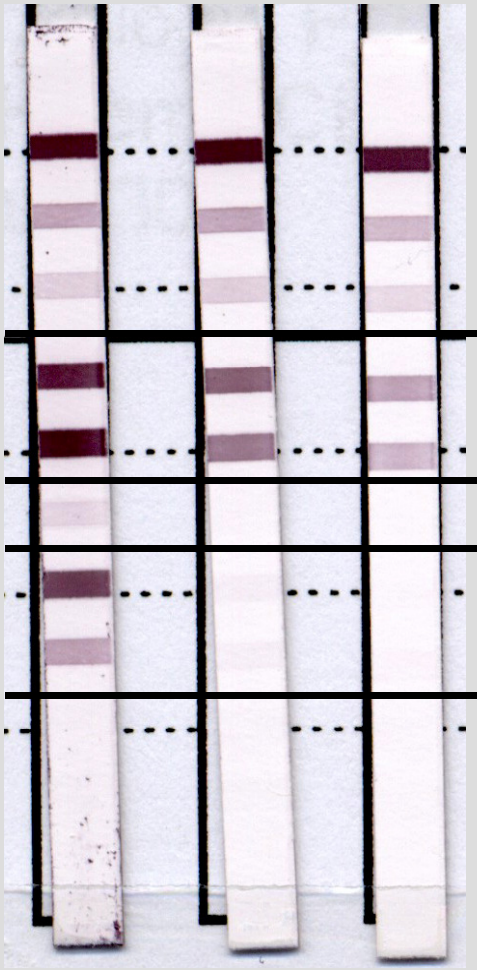
complete resistance against CCR5 using strains

Serodeconversion



p120
p41
p31
p24
p17
HIV-2

Pretransplant
22 months after SCT
44 months after SCT



Controls
HIV env
HIV pol
HIV gag

Lessons we have learned from case No. 1

Virus persisted only a short time in peripheral blood

Virus may persist in reservoirs for a long time but did not replicate

Although high selective pressure, no X4 has emerged

Without replication, the reservoir is probably cleared in <2 years

AB against HIV only against surface antigens (similar to HBV)

Questions arising from this case

1. Is there something special with the virus?
2. What was the tropism of patient's virus?
3. Are there reservoir cells left?
4. Why did HIV not rebound?
5. Is the patient sterilizing cured?

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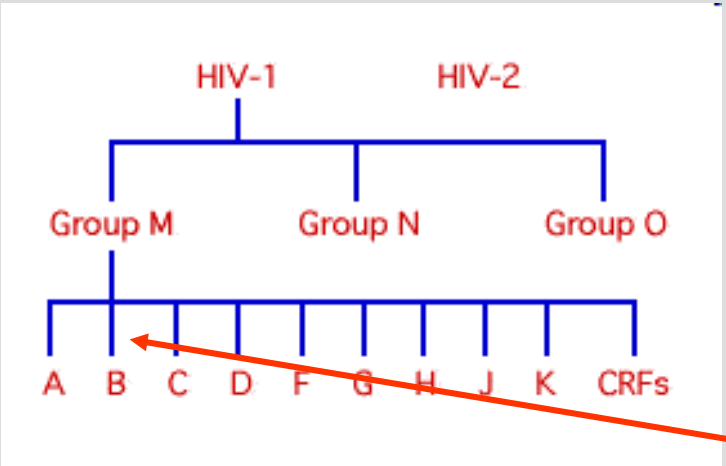
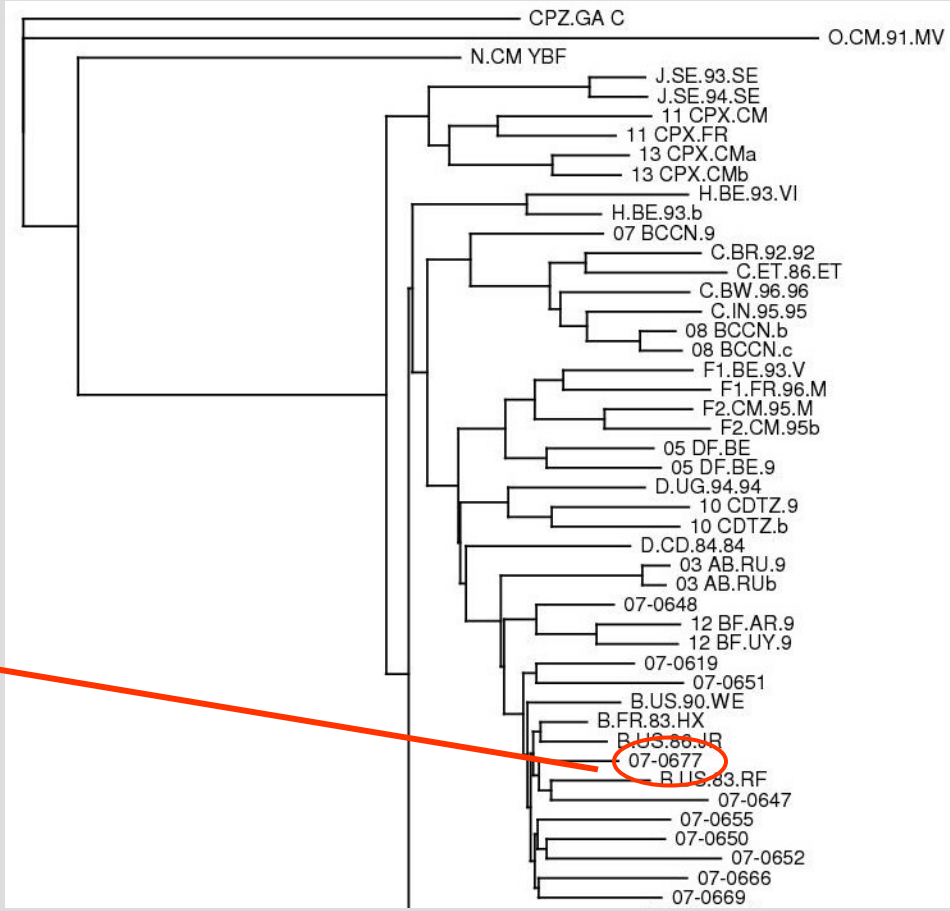
5. Is the patient sterilizing cured?

Determination of patient´s virus type





*env*6537s and 7254as/*env*5as

Questions arising from this case

1. Is there something special with the virus?
2. What was the tropism of patient's virus?
3. Are there reservoir cells left?
4. Why did HIV not rebound?
5. Is the patient sterilizing cured?

Determination of the patient´s virus tropism

Bio-assay

Trofile™

n.a.

geno2pheno

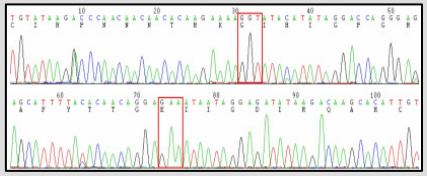
PSSM

R5

34-50% sensitivity

89-96% specificity

11/25
net charge rule

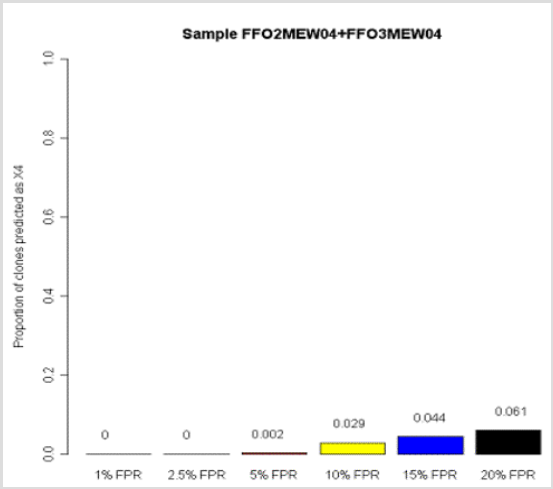


R5

30% sensitivity

93% specificity

454 ultra deep sequencing



97.1% R5; 2.9% X4

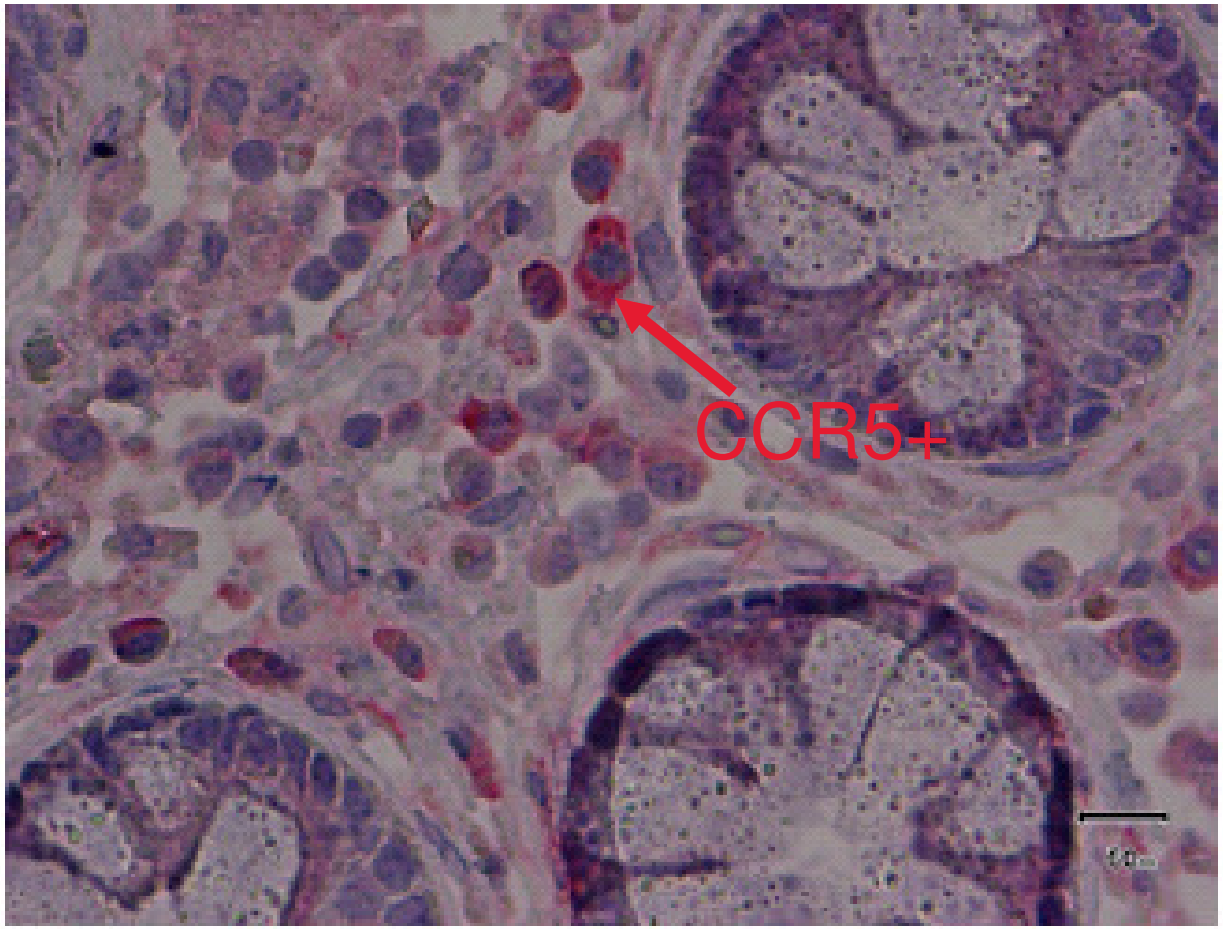
10% false prediction ratio

Patient harbored a small X4 proportion before transplant!

Questions arising from this case

1. Is there something special with the virus?
2. What was the tropism of patient's virus?
3. Are there reservoir cells left?
4. Why did HIV not rebound?
5. Is the patient sterilizing cured?

Immunohistology of intestinal mucosa (day +159)

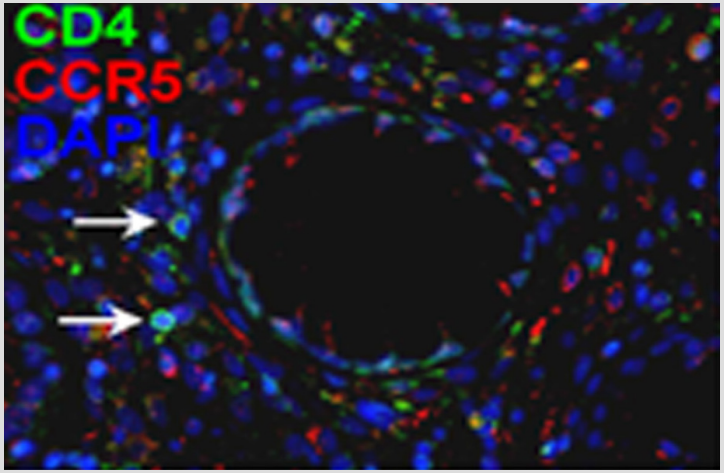
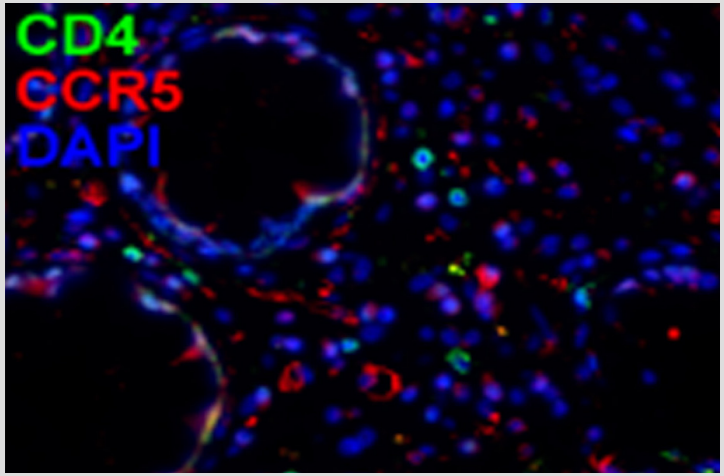
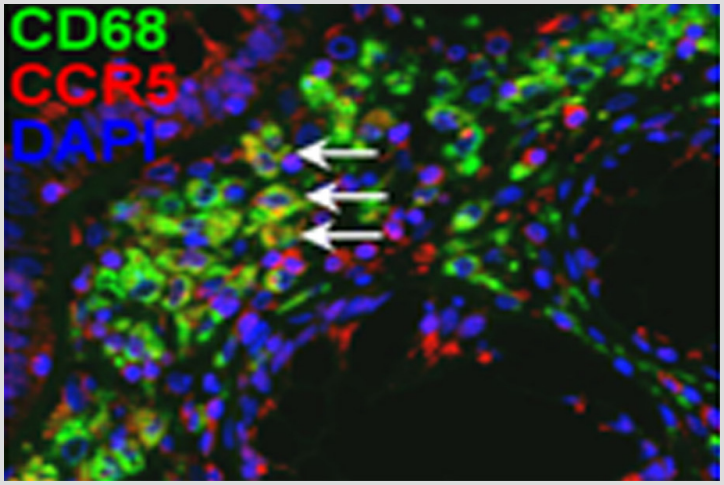
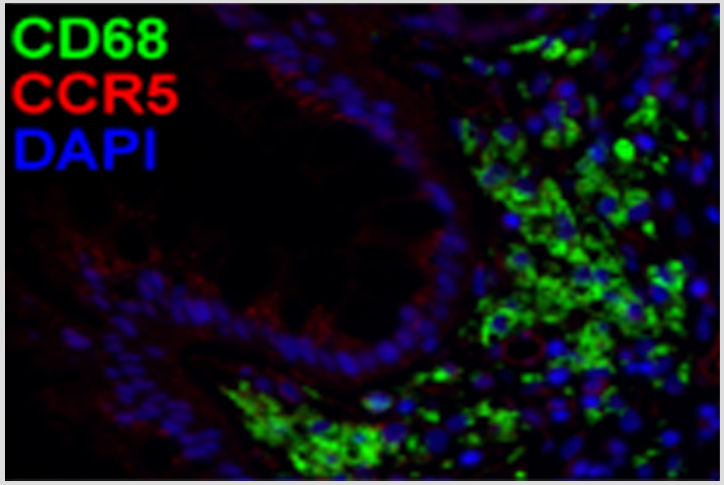


CCR5+ macrophages still detectable ½ year after SCT

these macrophages are „typical" reservoir cells

however, tissue proviral HIV tests were all negative

CCR5 tissue chimerism (gut)

	6 mo. after Tx	2 years after Tx
T-lymphocytes		
macrophages		

HIV reservoir outside immune system

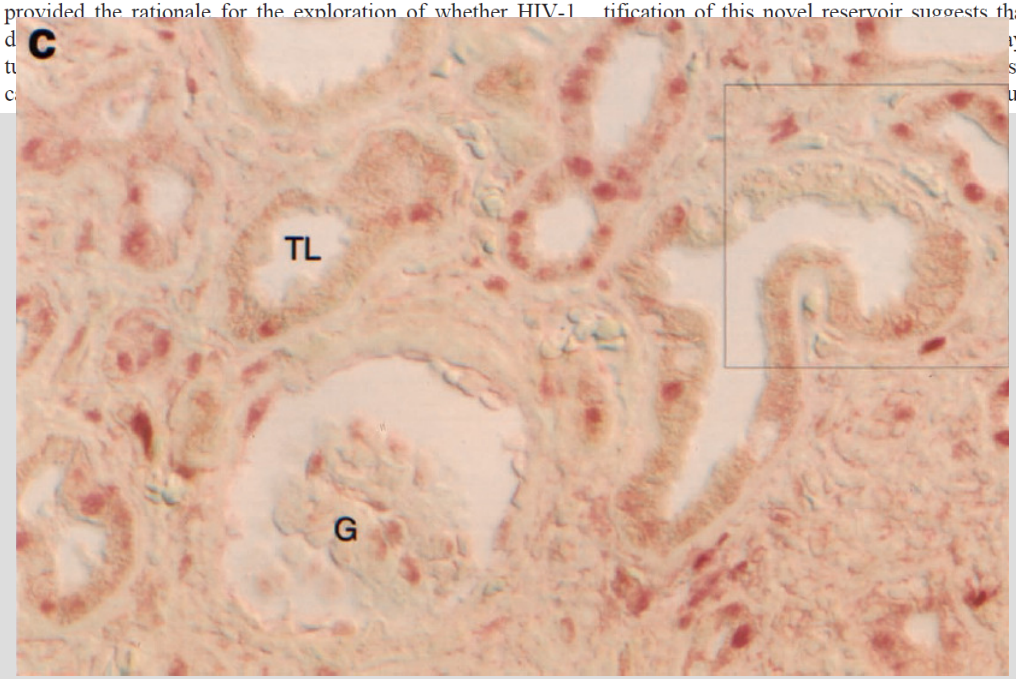
J Am Soc Nephrol 11: 2079–2087, 2000

Renal Epithelium Is a Previously Unrecognized Site of HIV-1 Infection

LESLIE A. BRUGGEMAN,* MICHAEL D. ROSS,* NOZOMU TANJI,§ ANDREA CARA,† STEVEN DIKMAN,‡ RONALD E. GORDON,‡ GODFREY C. BURNS,‖ VIVETTE D. D'AGATI,§ JONATHAN A. WINSTON,* MARY E. KLOTMAN,† and PAUL E. KLOTMAN*

*Divisions of *Nephrology and †Infectious Diseases and ‡Department of Pathology, Mount Sinai School of Medicine; §Department of Pathology, Columbia University College of Physicians and Surgeons; and ‖Department of Medicine, St. Vincent's Hospital and Medical Center, New York, New York.*

Abstract. The striking emergence of an epidemic of HIV-related renal disease in patients with end-stage renal disease provided the rationale for the exploration of whether HIV-1 active replication in renal tissue. Infiltrating infected leukocytes harbored more viral mRNA than renal epithelium. Identification of this novel reservoir suggests that effectively tar- y be critical for sease. Thus, renal usly unrecognized



HIV may persist in non immune cells

probably not sufficient for viral replication

however, these cells will not get replaced by hematopoietic stem cells

Questions arising from this case

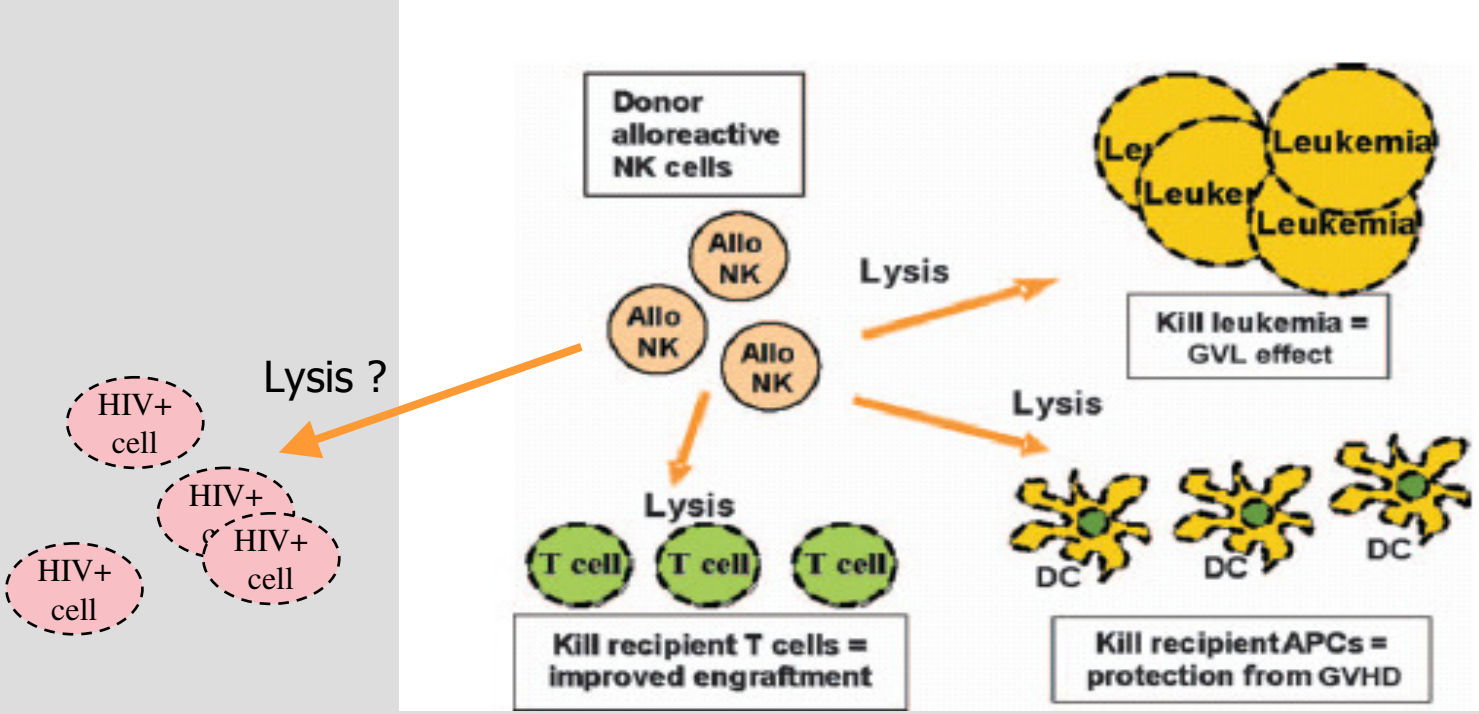
1. Is there something special with the virus?
2. What was the tropism of patient's virus?
3. Are there reservoir cells left?
4. Why did HIV not rebound?
5. Is the patient sterilizing cured?

Immunological theory

donor alloreactive NK cells have multiple effects in the host

they are responsible for eradication of leukemic blasts

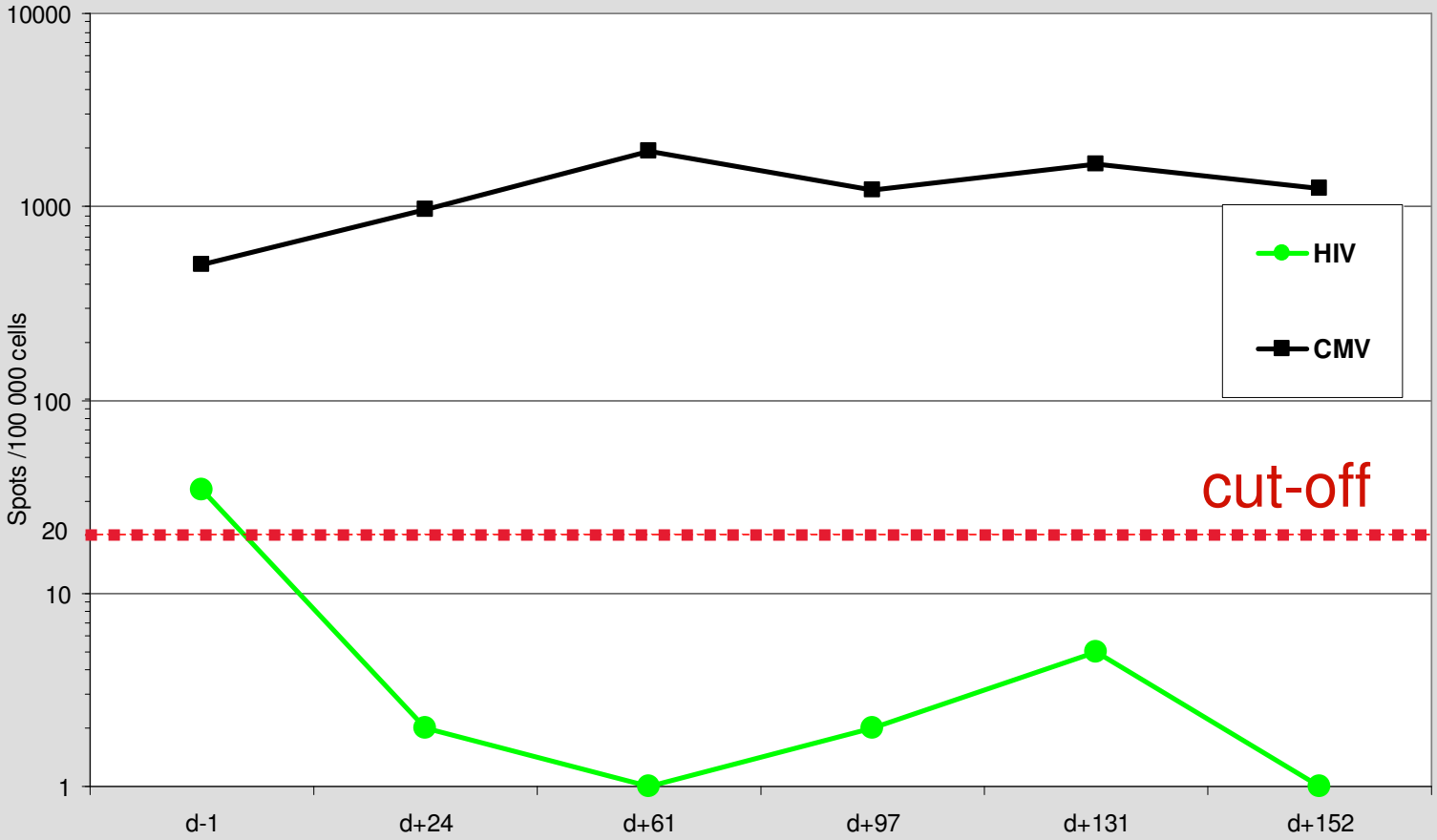
HIV reactive T-cells are well known with unclear effect of HIV maintenance



[mod. From Ruggeri, Immun Rev 2006]

HIV clearance by alloreactive NK cells?

p24 specific T-lymphocytes

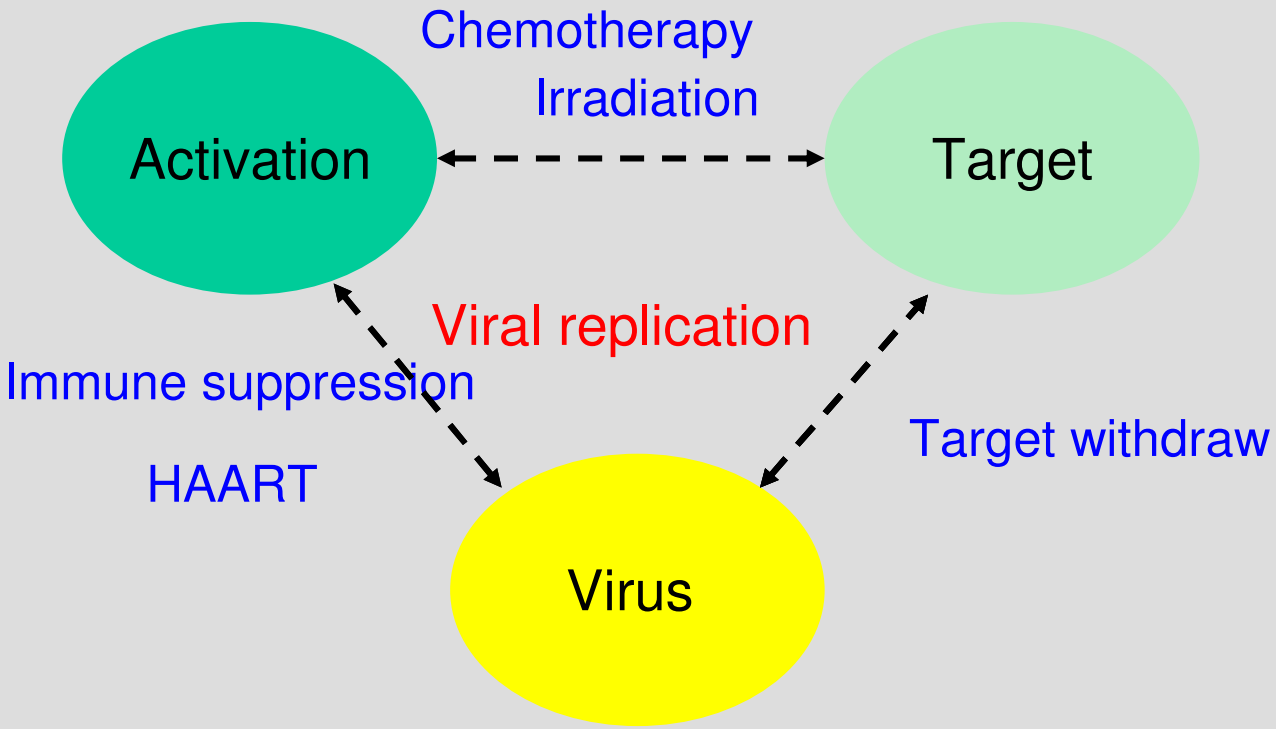


disappearance of p24 specific T-cells

no effect of immune suppression against CMV specific T-cells

no evidence for eradication by HIV specific T-cells

Stoichiometrical theory



Transplantation may have set back the time as it was during first exposure

reduction of infected CD4+ T cell by allogeneic transplantation

re-colonization of CCR5-delta32 deficient T-cells

CCR5 preferred during first infection (gatekeeper)

Questions arising from this case

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The “cure” question…

…or, when is a cure a cure?

Facts:

- No replication despite HAART discontinuation
- No HIV provirus
- Sero de-conversion
- CD4 cell increase

functional cure = no HIV symptoms and no HIV medication

sterilizing cure = no trace of HIV anywhere

Eradication?

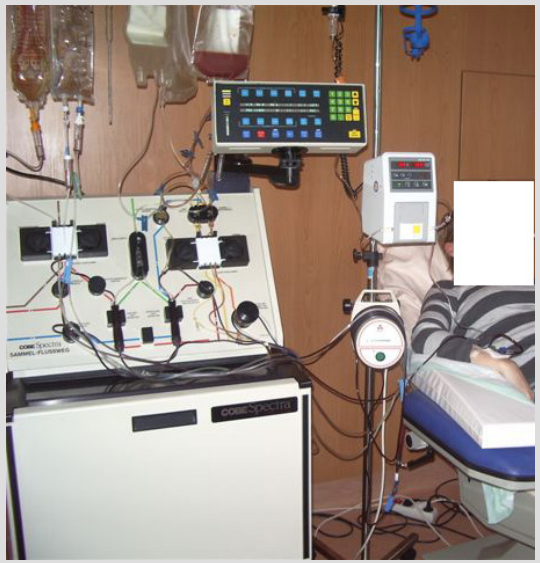
Frank Maldarelli, NCI

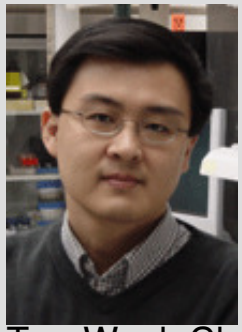
Single copy assay negative 2009

Steven Deeks, UCSF

2011 …there is something in the plasma….

Calculated hypothetical virus load





Tae-Wook Chun, NHI

apheresis of leucocytes

141 wells with $10x10^6$ cells

$\Rightarrow$ quantitative co-culture
$\Rightarrow$ all 141 wells negative

HIV_{cal} < 1 infected cell / 1,4 bn CD4+ T-cells

$\approx$ < 4 infected cells in whole blood volume

Traces of HIV in the „Berlin patient“?

We have performed a series of studies looking for virus and have not found anything definitive

Steven Deeks, UCSF

Important questions in the case of…

- same molecular DNA sequence?
- new infection?
- which cells harbored the material
- how valid are these tests?

The search for patient No. 2

gender	*age*	*diagnosis*	*location*	*registered donors*	*donors CCR5 –d32 tested*			*status*
					(+, +)	(+, -)	(-, -)	
male	adult	NHL	Freiburg, Germany	>1	ND			died before Tx
female	3	DBA	Heidelberg, Germany	120 +1*	103	17	1*	stopped
male	adult	MDS	Lausanne, Switzerland	1	1			Tx Aug 2010
male	29	NHL	Mainz, Germany	1	1			Tx Nov 2009 with (+;+)
male	15	leukaemia	Jerusalem, Israel	3 +3*			3*	Jan 2010, died after Tx
male	50	CMML	Berlin, Germany	60	25	5		Tx in April 2010 with (+;+)
male	adult	NHL	Mannheim, Germany	ND	nd			Tx cancelled
male	14	KS	Dublin, Ireland	?	Nd			stopped
male	42	AML	Hamburg, Germany	1	1			Tx 2011
male	adult	NHL	Frankfurt, Germany	35	stopped			stopped
male	adult	leukaemia	Münster, Germany	30	7	2	1	ongoing
?	?	?	?	1*			1*	Ongoing

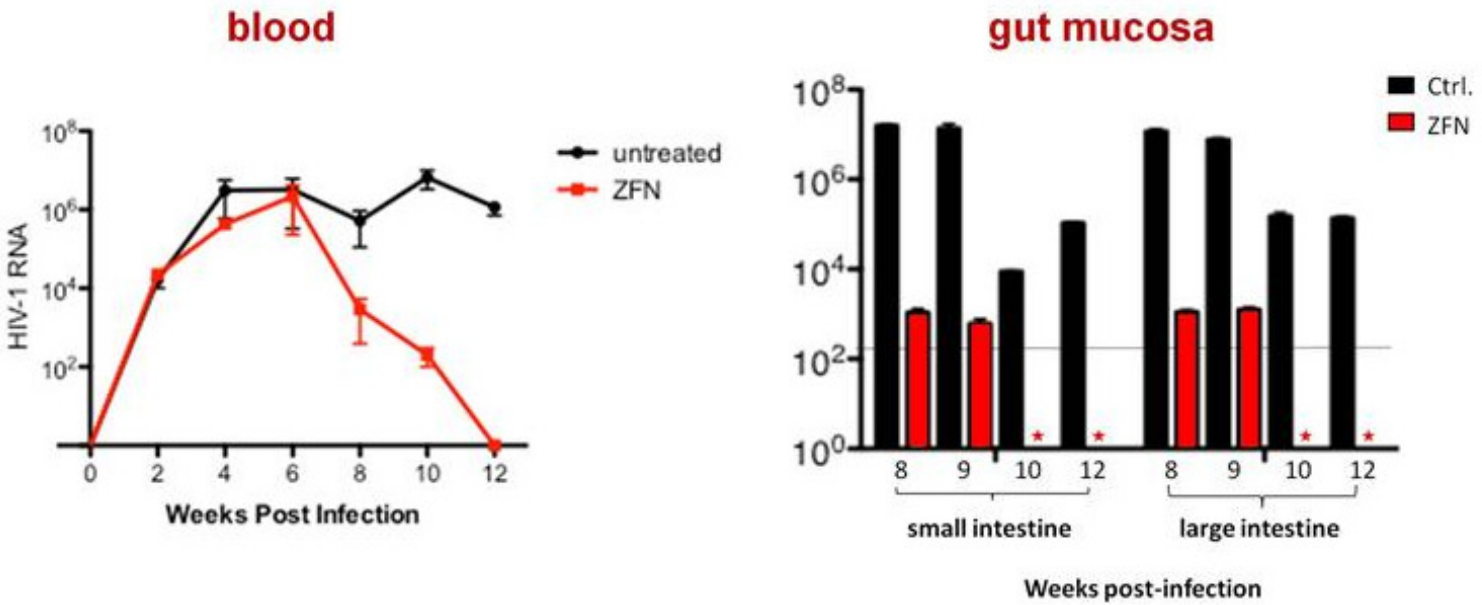
*cord blood

Classes of HIV gene therapy

Class	Phase targeted	Prevents infection	Protects from viral CPE	Protects from CTL	Selective advantage
I	Early, including integration	Yes	Yes	Yes	Yes
II	Viral gene expression	No	Yes	Partially	Yes
III	Assembly, release of infectious particles	No	No	No	No

von Laer et al. J Gene Med 2006

ZFN CCR5-modified HSC control HIV



Engraftment of CCR5-negative HSC and presence of HIV-resistant T-cells reduces HIV-1 to undetectable levels in both the peripheral blood and gut mucosa

[Paula Cannon 2011]

zinc-finger technology

1.

Endogenous gene targeted for disruption

2.

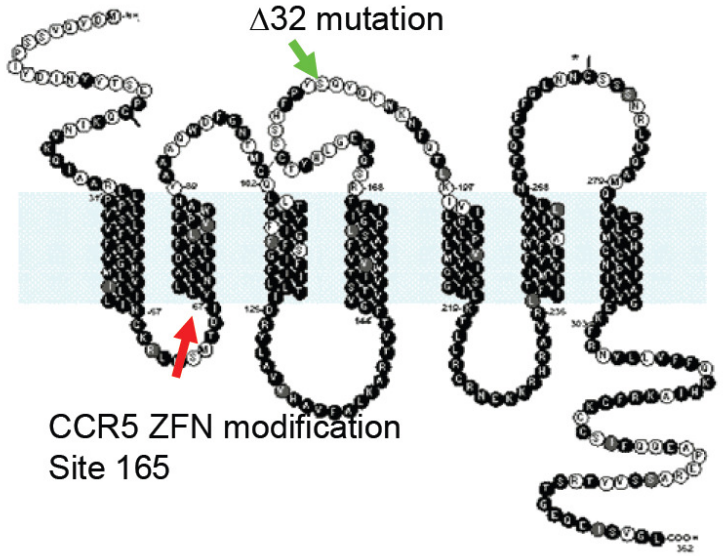
ZFNs dimerize and introduce a double stranded DNA break in the gene

3.

Break repaired by non-homologous end-joining (NHEJ) – resulting in loss of genetic information

4.

Gene disrupted



ZFN pairs targeted to region upstream of the Δ32 mutation

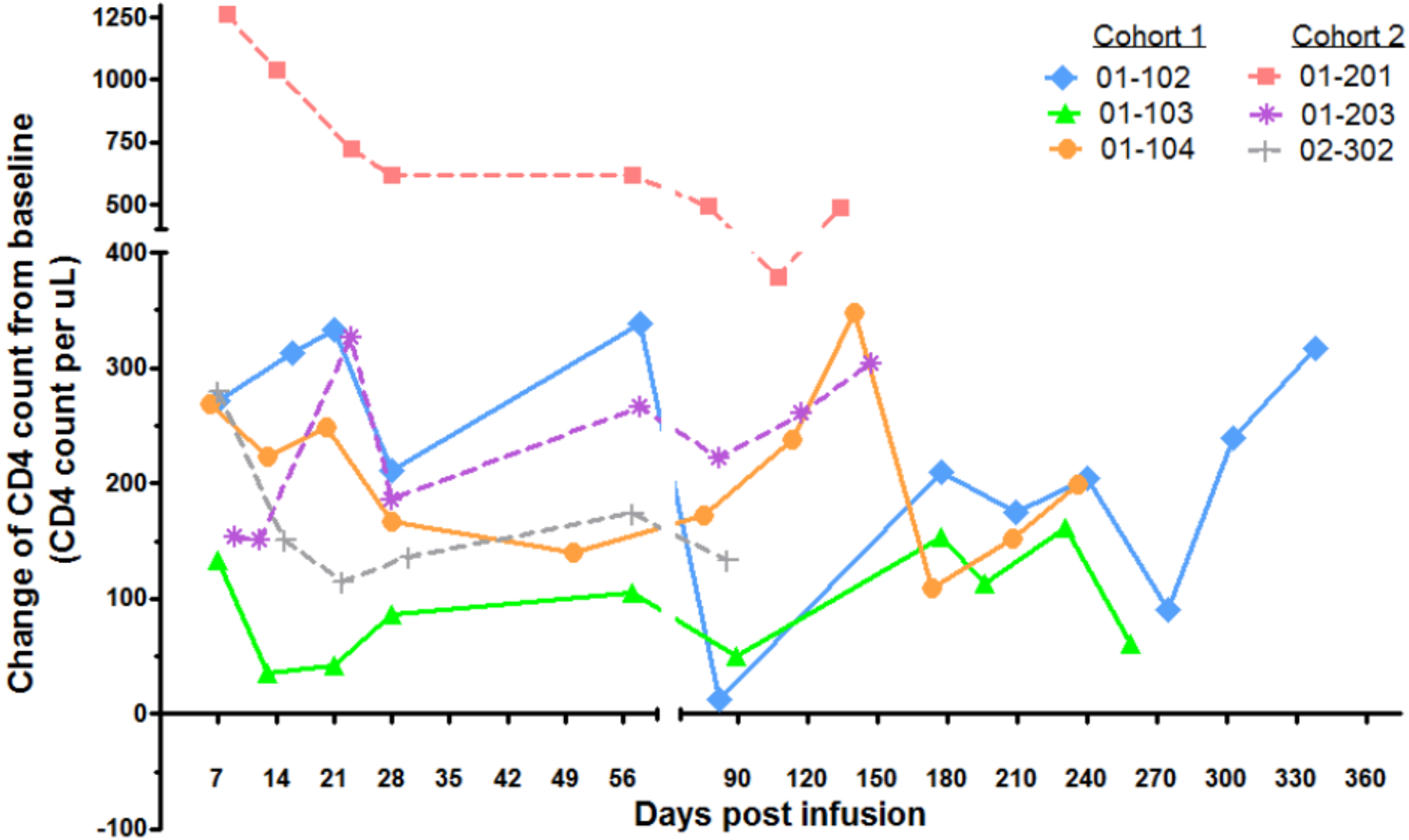
[Lalezari CROI 2011]

Sangamo Phase I (SB-728-0902)

Sangamo SB-728-0902 Phase 1 Study Design

- Open label, single-dose study
- Study population – HIV+ subjects on HAART
  - Aviremic
  - CD4 T-cells 200 - 500 cells/mm^3
- Single infusion of SB-728-T
  - Cohort 1 (N=3): 0.5 - 1.0 x 10^{10} cells
  - Cohort 2 (N=3): 2.0 x 10^{10} cells
  - Cohort 3 (N=3): 3.0 x 10^{10} cells

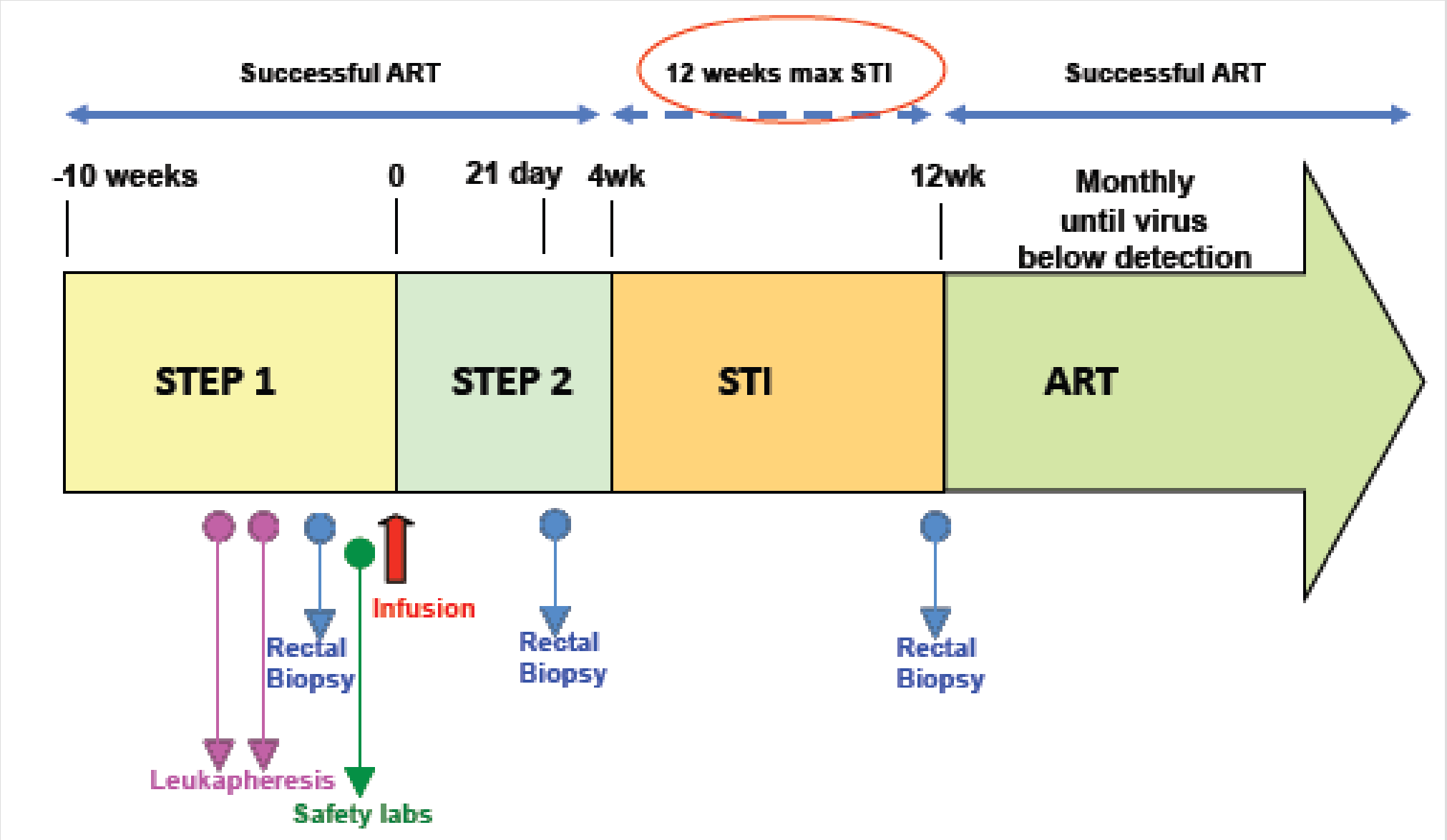
Sangamo Phase I (SB-728-0902)



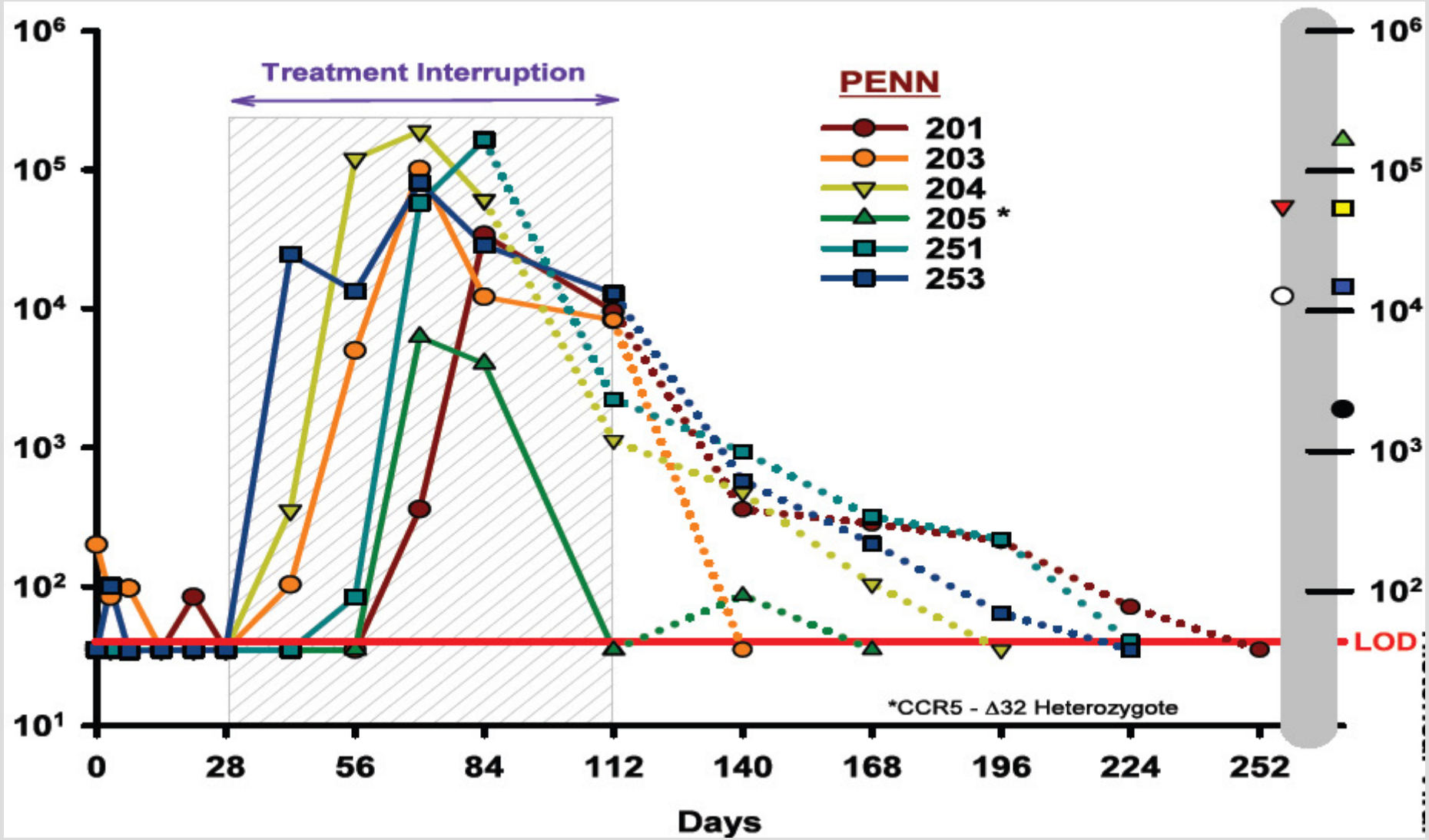
The Sangamo trial (SB-728-0902)

- **Improved and sustained increase in total CD4+T-cell counts seen in 5/6 subjects**
- **Normalization of CD4:CD8 ratios seen in 3/5 subjects**
- **ZFN-modified T-cells engraft, expand, and persist in peripheral blood**
  - ZFN-modified CD4+ T-cells detected at frequencies up to 7-fold higher (median 2.9) than predicted input on day 14
  - Expansion of ZFN-modified T cells in PBMC may be due to cell proliferation and/or altered distribution
- **ZFN-modified T-cells engraft and persist in rectal mucosa**
  - Engraftment and persistence of ZFN-modified T cells in rectal mucosa demonstrated normal homing to this important tissue

The Sangamo trial SB-728-T



The Sangamo trial SB-728-T



The Sangamo trial SB-728-T

Durable increases in total CD_4 T-cells; normalization of CD_4:CD8 ratio

Expansion of CD_4 T-cells associated with increases in IL-2, -7 and -15

Engrafts, expands, persists (>1 yr) in Blood and GALT

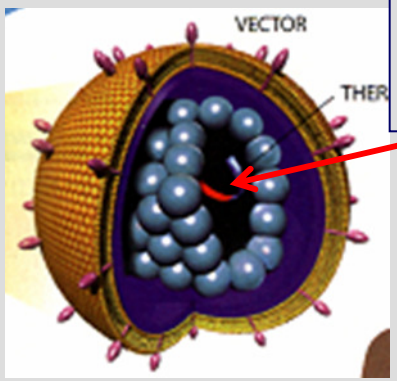
May reduce HIV-RNA during HAART interruption in subjects with CD_4 >450 cells/mm^3

- HIV-RNA in a $\Delta 32$ heterozygous subject became undetectable
- Biallelic CCR_5 modification correlates with HIV-RNA suppression

Limited increases in HIV Proviral DNA during STI that reverses with reinstitution of HAART

The Calimmune approach

Calimmune Aims to Treat Hematopoietic Stem Cells and T Cells to Engineer a Population of Blood Cells Protected From HIV



Lentiviral backbone (self inactivating) that contains a short hairpin RNA to CCR5 plus other anti-HIV agent(s)

Down regulates CCR5 preventing HIV attachment and blocking entry of HIV to target cells

- Anti-HIV gene containing CD34+ blood stem cells engraft in marrow producing T cells and monocyte/macrophages for long term protection

- Anti-HIV gene containing CD4+ T cells circulate through the body for short term protection

The Calimmune approach

Pre-Clinical Results (Proof of Concept In Animals)

- Safe and effective in mice and monkeys
- 10 years of safety studies in monkeys
- 5-7 fold reduction in CCR5 receptors in early monkey studies
- 10 fold reduction in CCR5 in human cell models
- Consistent > 3 log inhibition of HIV in multiple models
- Blocks HIV entry and decreases HIV load

... to come to an end...

Case No. 1 as a proof of principle

Gene therapy techniques improve

Most promising: Targeting the viral entry mechanism

Current trials are promising but on a very early stage

Many open questions:
CD4 cells or stem cells?
Which time-point of HIV infection?
CCR5 knock down alone sufficient?
Which technique is the best?
….

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amfAR
MAKING AIDS HISTORY