ECCMID Symposium “CRISPR-Cas revolution”

CRISPR-Cas9 based therapy for persistent virus infections (HIV)

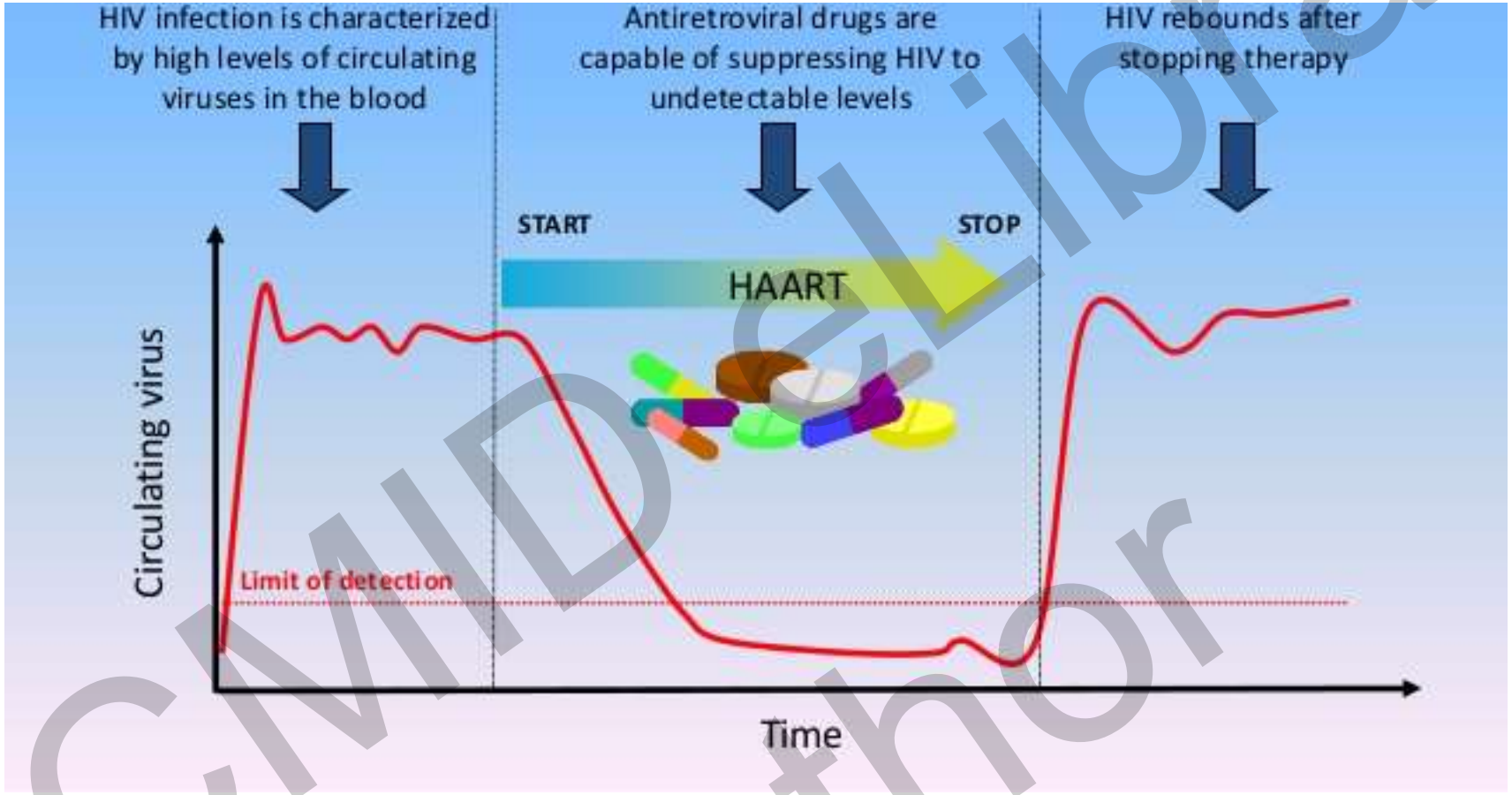
Ben Berkhout
Laboratory of Experimental Virology
University of Amsterdam
The Netherlands

HIV in the Netherlands since 1983 (18 million inhabitants)

We have analyzed some 60,992 patient samples since start epidemic

End 2017: 19,582 HIV+ people in care (approx. 2300 don't know their status)
12,378 MSM, other men (3593) and women (3611)
around 750 new cases in 2017 (PReP started in risk groups)
90% diagnosed, **93%** on ART, **95%** viral suppression

Research towards a cure:
Anti-HIV gene therapy (RNAi)
CURE approaches (CRISPR-Cas)

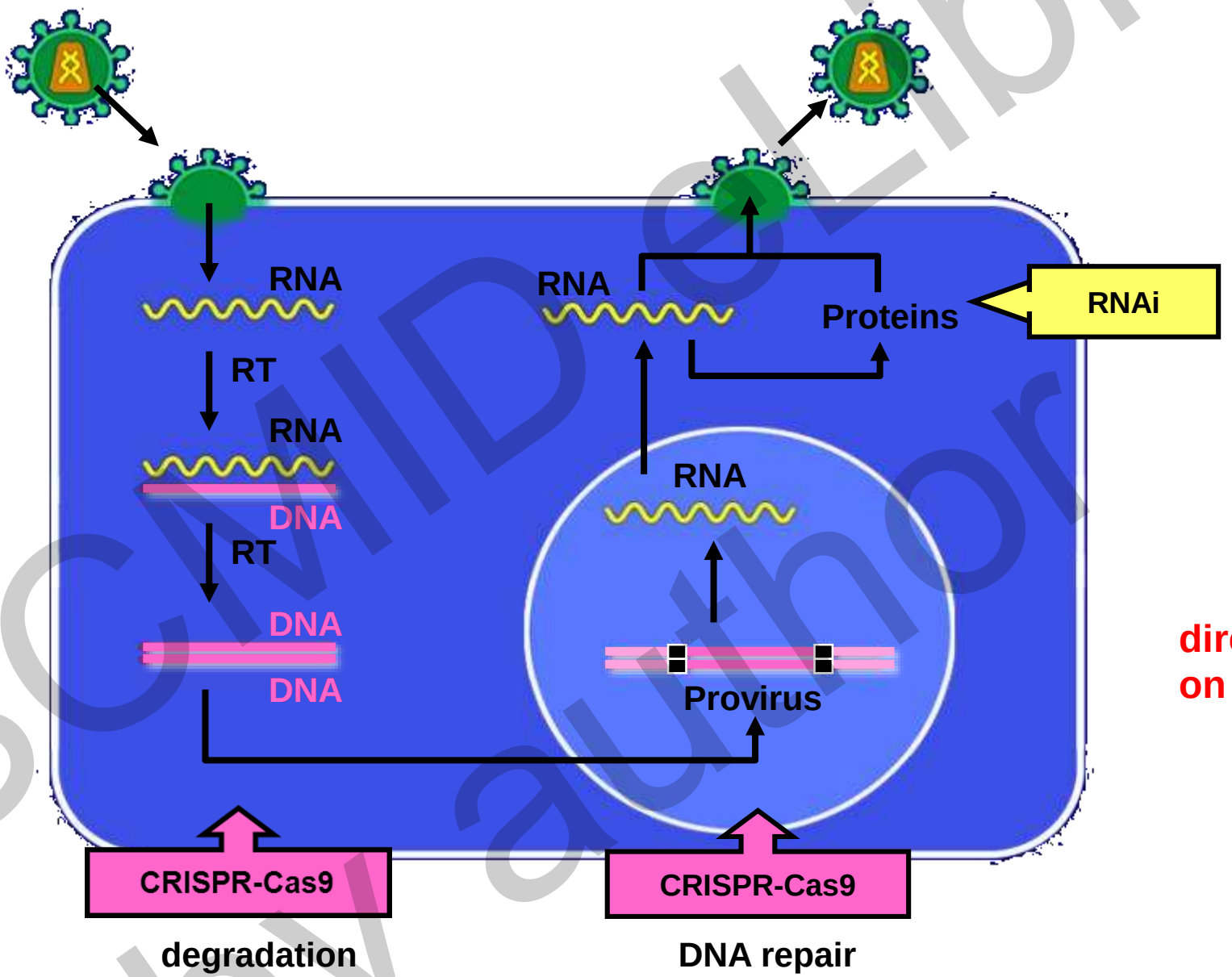


Combination drug therapy saves lives: Durable HIV control possible

But HIV rebounds rapidly after stopping therapy > **life-long therapy**

Alternative: Gene therapy for durable therapeutic effect (**<u>single treatment</u>**)
- Protect cells against HIV, use lentiviral vector that stably integrates!
- Express antiviral molecules (RNAi)
- Direct attack on HIV DNA in the reservoir (CRISPR-Cas)

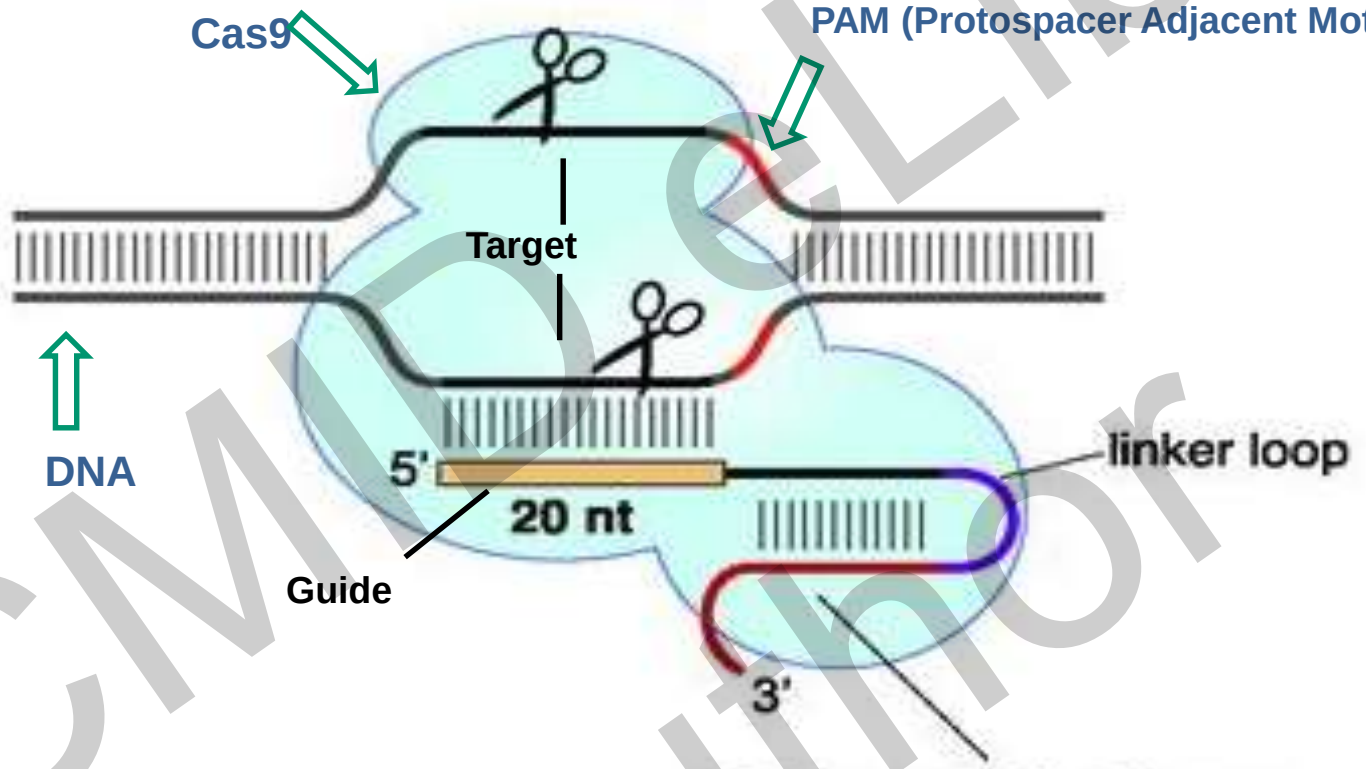
HIV drug therapy a success, but what about a cure
Attack on the HIV reservoir



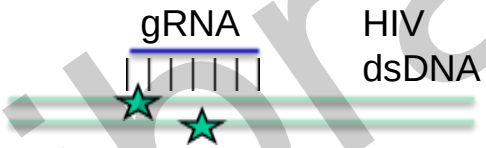
direct attack on reservoir

CRISPR-Cas9

molecule of the year 2015 (Science)



- bacterial origin
- dsDNA endonuclease
- RNA guided (guide RNA)

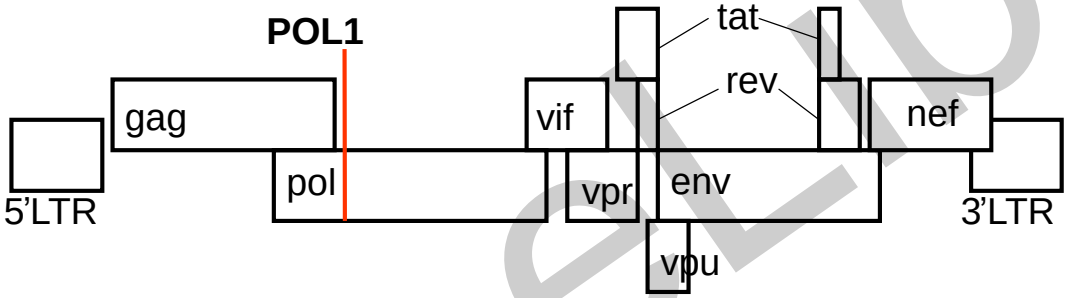


	RNAi	versus	CRISPR-Cas9
Effector	**short hairpin RNA**		**guide RNA**
Target	**RNA (sequence-specific)**		**dsDNA (sequence-specific)**
Result	**cleavage**		**cleavage**
Components	**1 (shRNA)**		**2 (gRNA + CRISPR-Cas9)**
Viral escape	**yes (point mutation in target)**		**no (initial literature)**

Bonus:
direct attack on HIV DNA reservoir

Globally similar antiviral approaches
Yet many **surprising differences**

Cell culture infections: Potent inhibition by some shRNAs
But HIV escape, which can be blocked by a combinatorial attack (3 shRNAs)



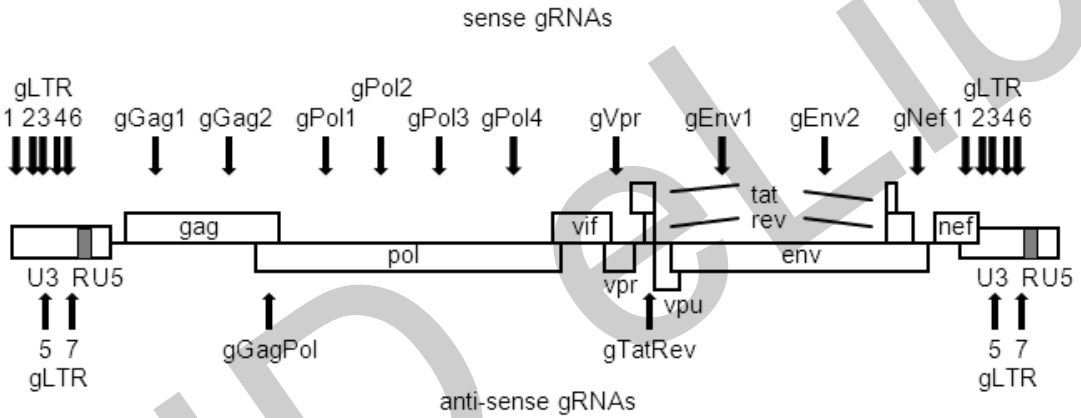
nr	Pol, Protease																		AA sequence					
	A	C	A	G	G	A	G	C	A	G	A	T	G	A	T	A	C	A	G	T	G	A	D	D	T
8x	-	-	-	-	-	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-
4x	-	-	-	-	-	-	-	-	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3x	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2x	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2x	-	-	-	-	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-	N	-	
1x	-	-	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	

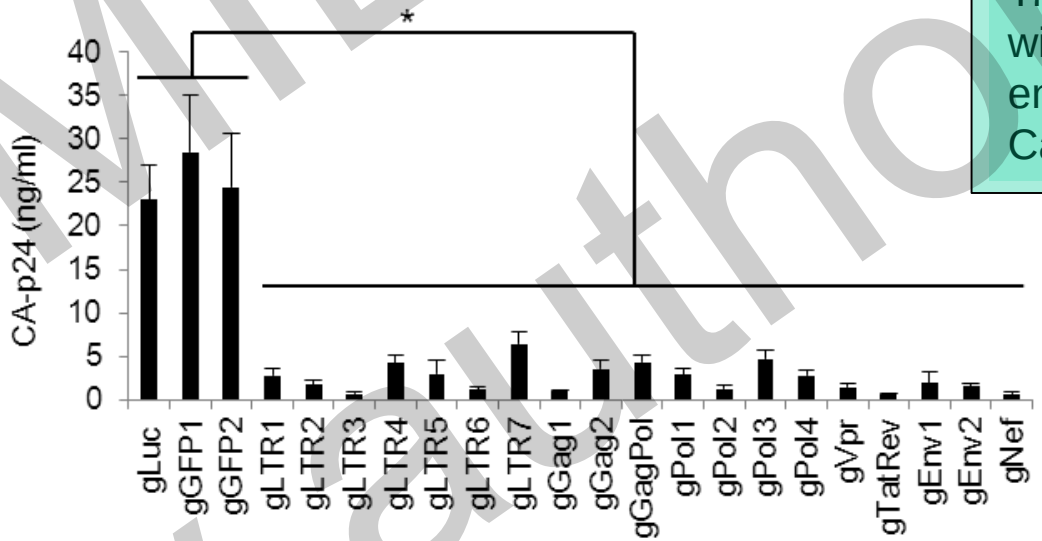
Regular RT-induced mutations
Preference for silent mutations (25/27)

Targeting HIV DNA (conserved sites only)

profound inhibition of viral gene expression by **each gRNA** (unlike shRNAs)

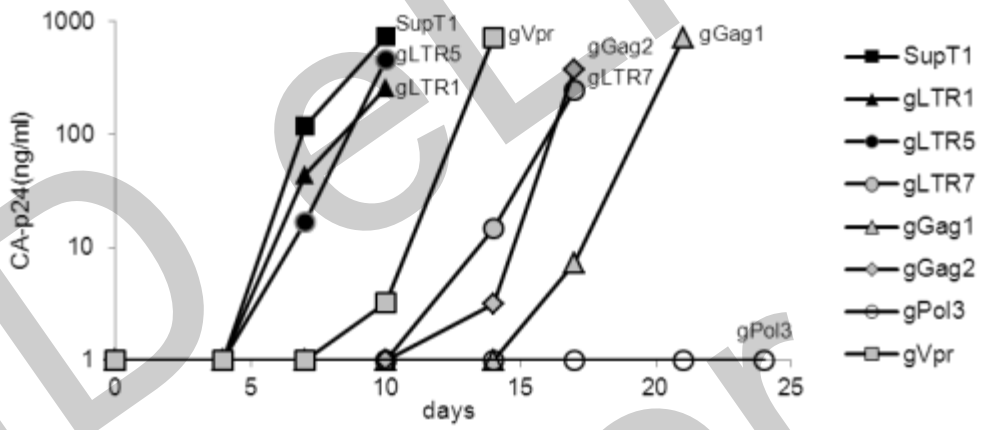
9 kb HIV genome



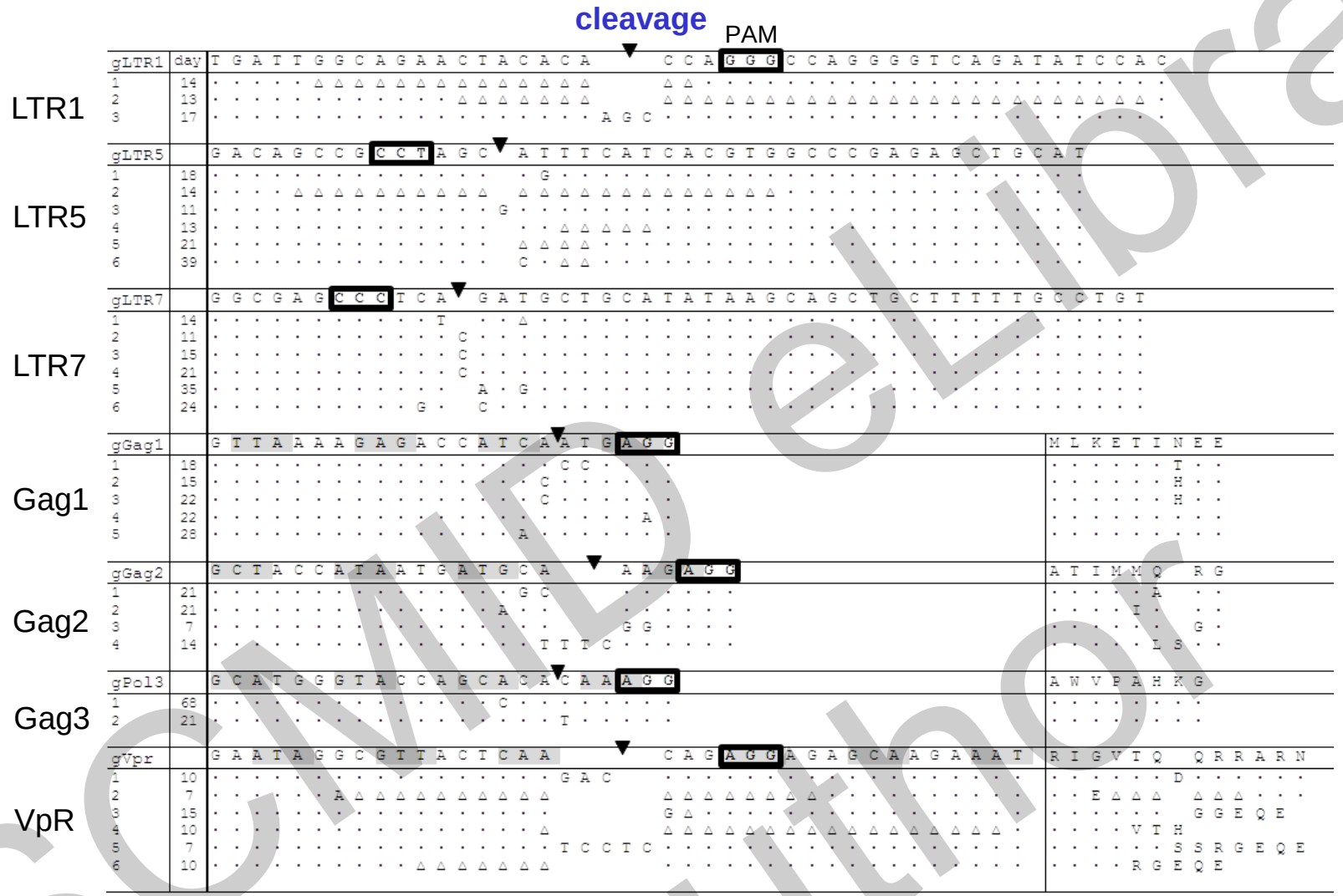


Transfect 293T cells with plasmids encoding CRISPR-Cas, gRNA and HIV

Transduce T cell line
with **LV**-Cas-gRNA
Infect with HIV LAI isolate

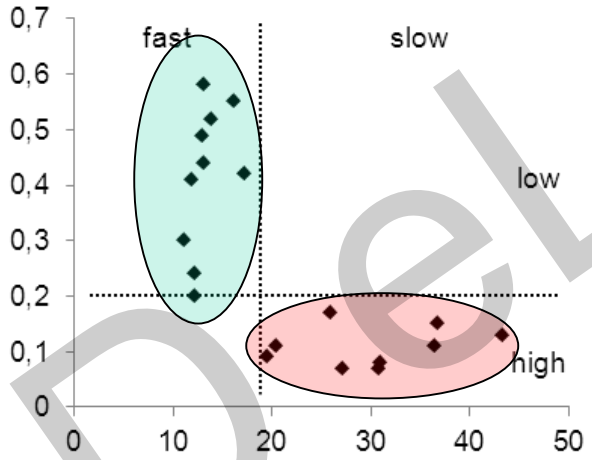


Good inhibition of HIV replication
But sometimes **immediate** virus escape?



Unlike RT-induced pointmutations in RNAi escape,
now **indels** (small insertions/deletions) very popular,
but not in important ORFs (Gag): not compatible with HIV replication

target conservation
(Shannon entropy)



breakthrough replication (days, n=4)

Indels!

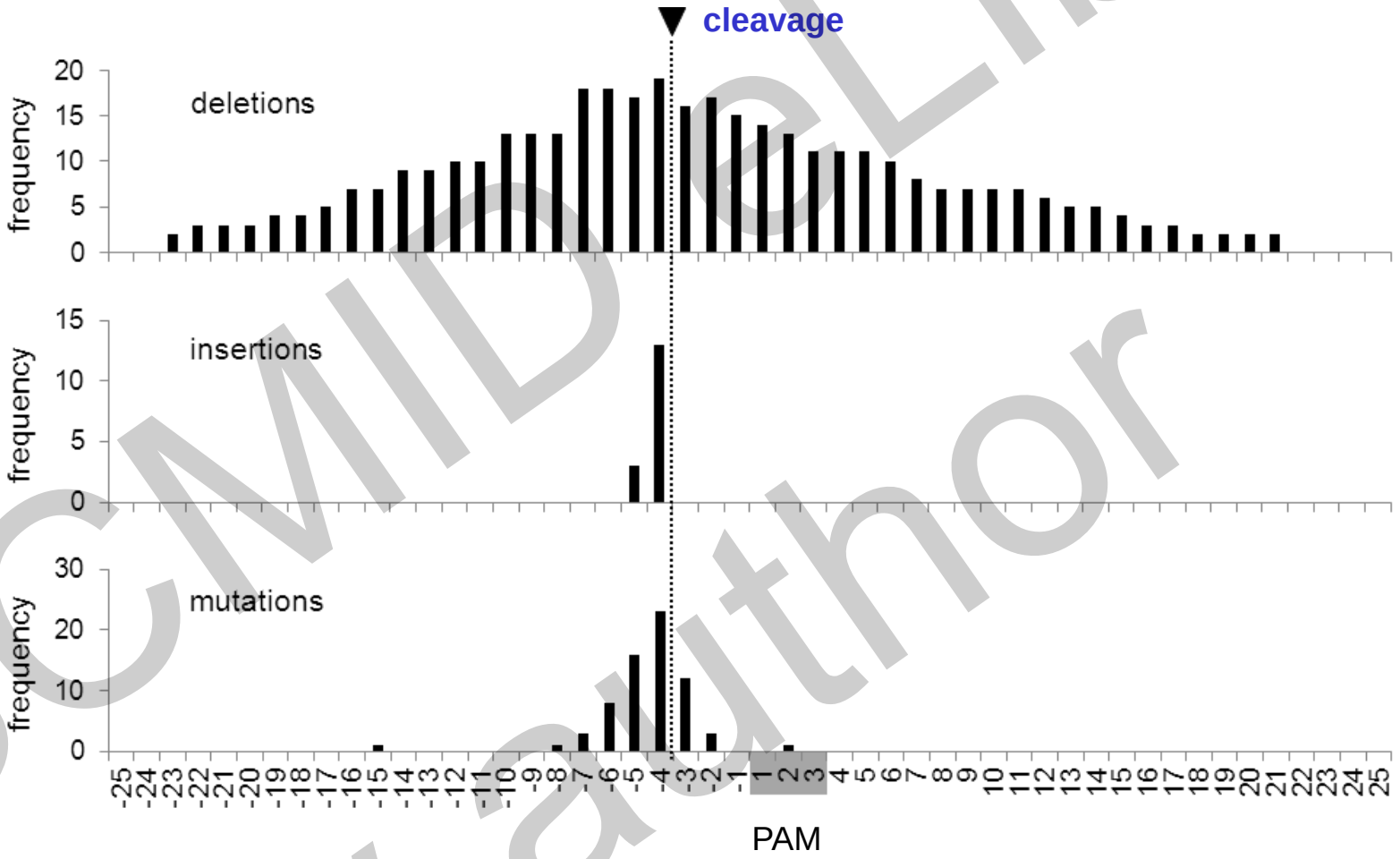
point mutations

Variable targets (high Shannon) = rapid escape by indels ……. **mechanism?**

Well-conserved targets (low Shannon) = slow escape by (regular) point mutations
indels not compatible with function

All occurs around the cleavage site: CRISPR-Cas9 mediates DNA cleavage, but subsequent DNA repair by the **NHEJ pathway** (non-homologous end joining) creates indels that allows viral escape

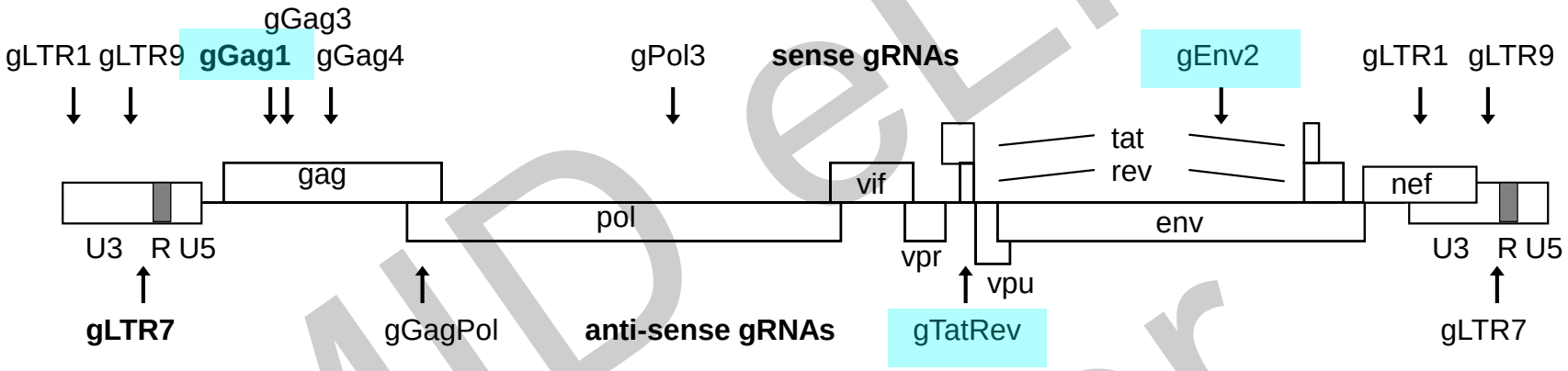
HIV escapes with help from the host (NHEJ)



Combinatorial CRISPR attack

1. Better inhibition
2. Higher threshold for resistance

Possible bonus: **excision** of sequences between 2 cleavage sites (LTRs)



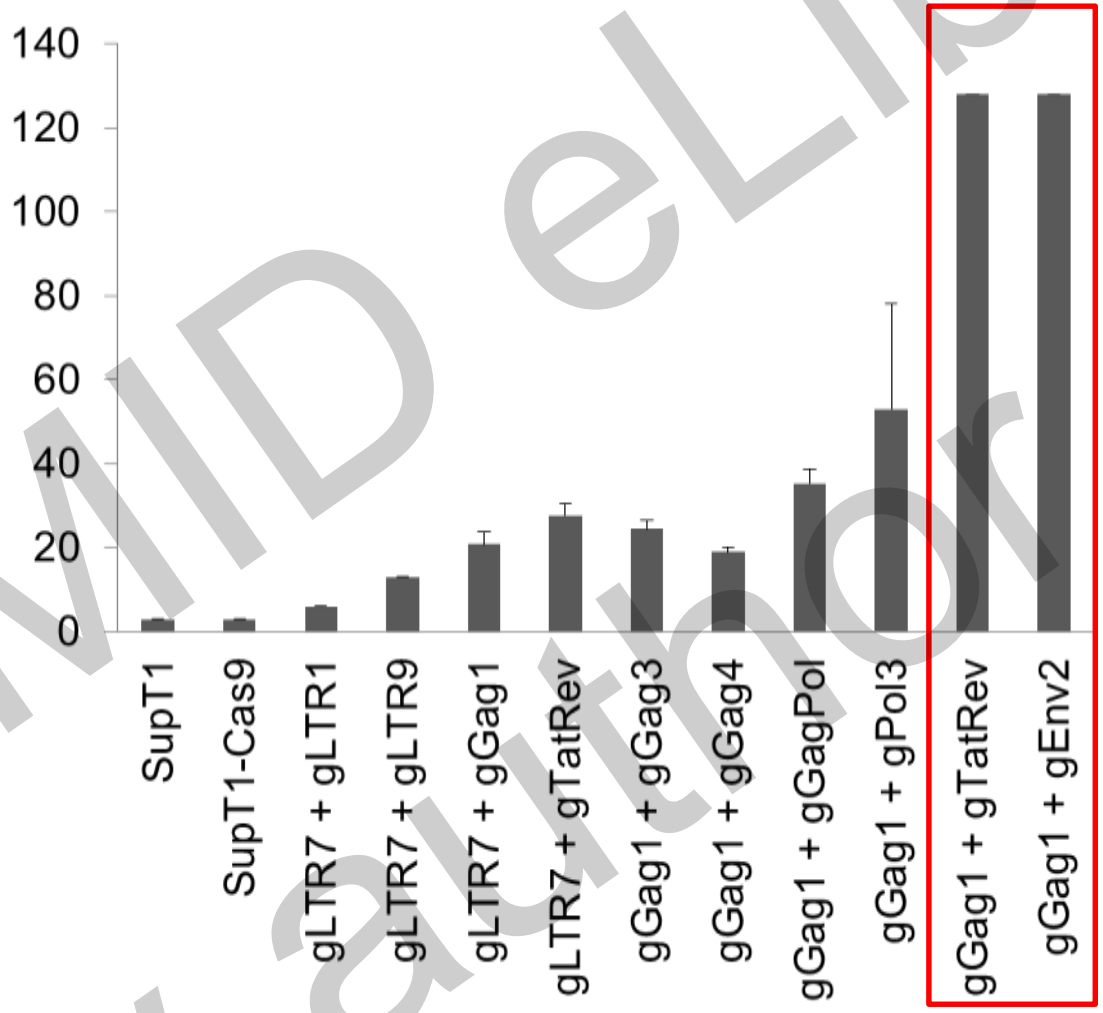
gRNA I	gRNA II	distance (bp)
	gLTR9	22, 8810
gLTR7	gLTR1	322, 9110
	gGag1	988, 8145
	gTatRev	5590, 3542

gRNA I	gRNA II	distance (bp)
	gGag3	91
	gGag4	438
	gGagPol	888
gGag1	gPol3	2796
	gTatRev	4602
	gEnv2	6452

HIV replication: two combi's prevent viral escape

gGag1+gTatRev and gGag1+gEnv2 (very conserved targets)

Time to "HIV breakthrough"
(days, average 4 cultures)



< no escape at end experiment

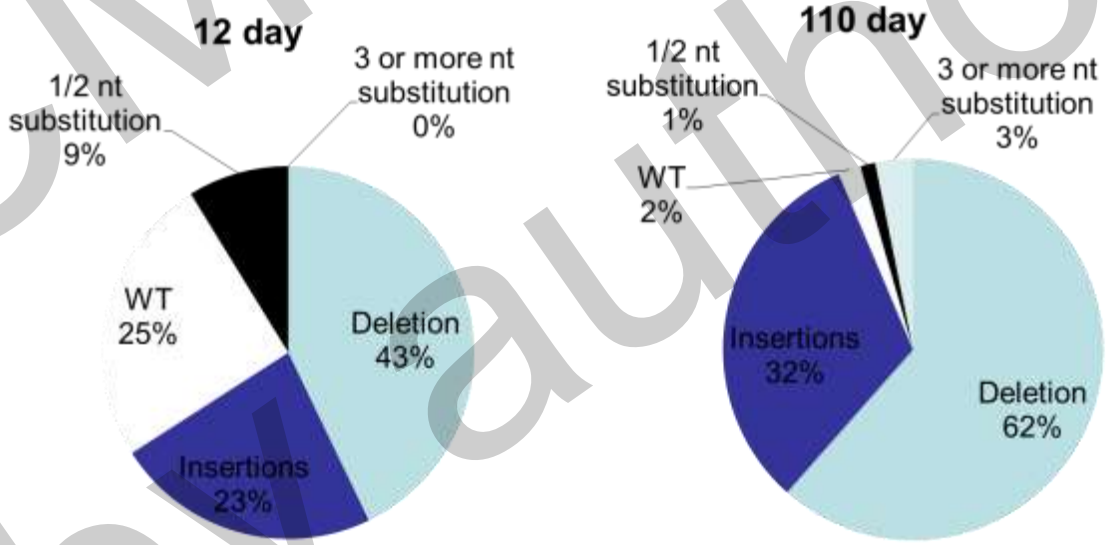
HIV sterilization???

When escape: regular NHEJ signature in both targets

wild-type HIV proviruses disappear over time

gRNAs	target region	wild-type sequence frequency (%)	
		day 12	day 110
gGag1 + gTatRev	gGag1	8 / 21 (38.1)	2 / 38 (5.3)
	gTatRev	7 / 28 (25.0)	0 / 39 (0.0)
gGag1 + gEnv2	gGag1	8 / 21 (38.1)	1 / 37 (2.7)
	gEnv2	0 / 21 (0.0)	0 / 43 (0.0)

A shift from minor to major mutations (repeated Cas action)



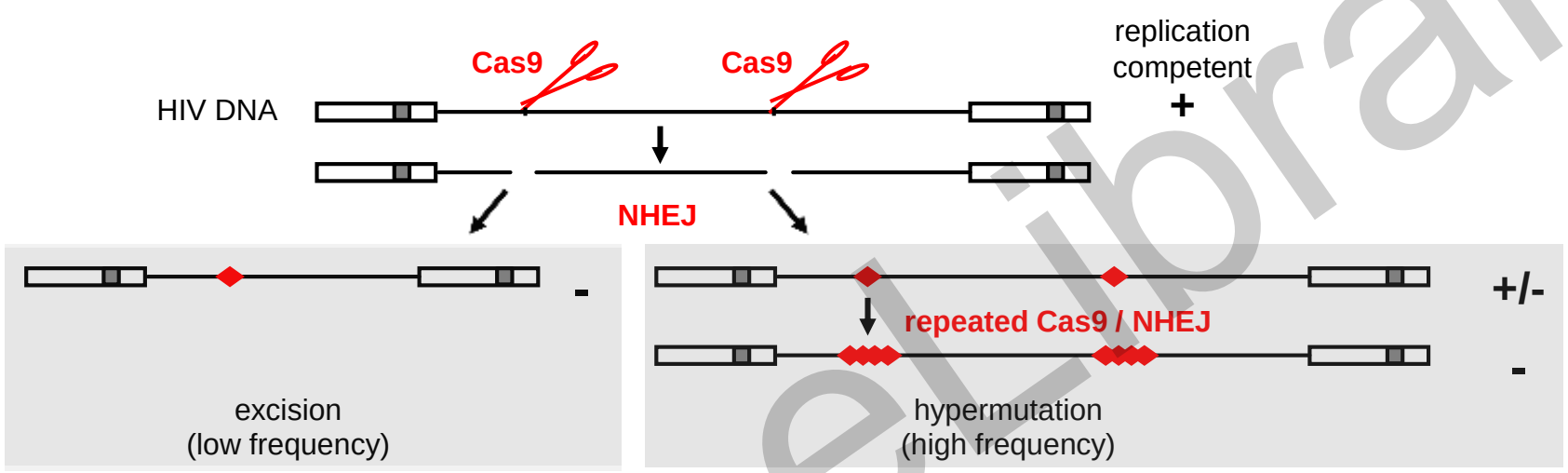
Ultra-sensitive screen for infectious HIV

gRNAs	Culture	virus rescue			
		day 16	day 41	day 69	day 97
gGag1 + gTatRev	1	+	-	-	-
	2	+	-	-	-
	3	+	-	-	-
	4	+	-	-	-
gGag1 + gEnv2	1	+	+	+	-
	2	+	+	-	-
	3	+	+	+	-
	4	+	+	+	-

Cultures of cells were effectively **sterilized!**

gGag1+gTatRev faster than gGag1+gEnv2

The host (NHEJ) helps to inactivate HIV-1



Provirus excision only? ("HIV Excision" Meeting, NIH)
Prominent product in PCR, but in fact occurs surprisingly little

Instead, most HIV genomes are **inactivated by NHEJ-hypermutation !**

Why? DNA repair is simply too fast

1st break repaired before 2nd break occurs

no 2 breaks simultaneously > no excision

We can sterilize HIV …. but can we find the HIV reservoir in humans?

LETTER

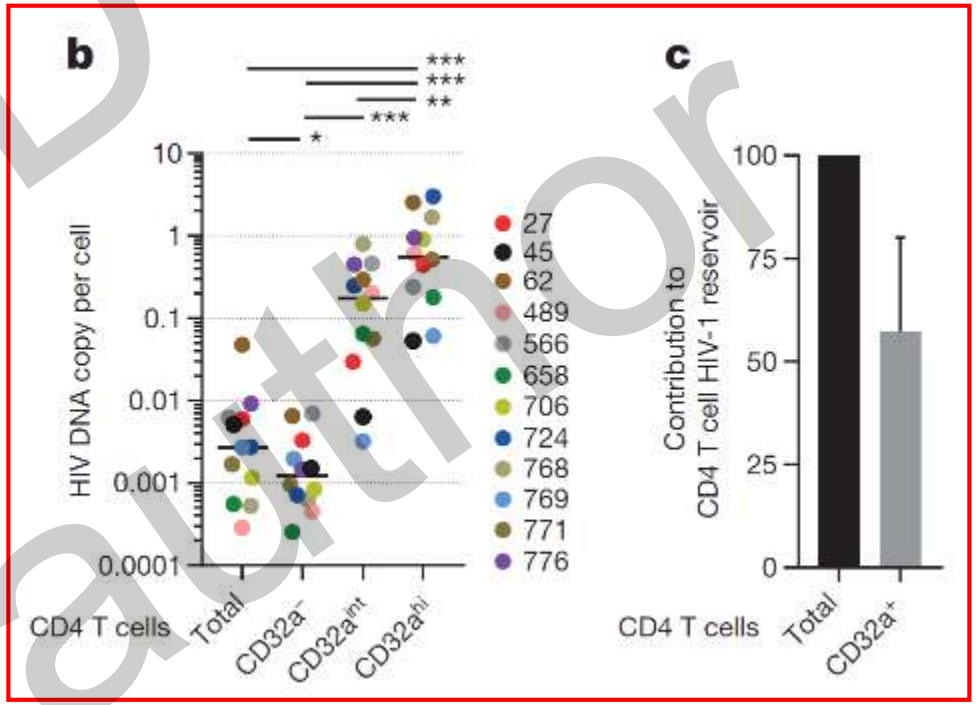
doi:10.1038/nature21710

CD32a is a marker of a CD4 T-cell HIV reservoir harbouring replication-competent proviruses

Benjamin Descours[1*], Gaël Petitjean[1*], José Luis López Zaragoza[2,3,4], Timothée Bruel[2,5], Raoul Raffel[1], Christina Psomas[6], Jacques Reynes[6], Christine Lacabaratz[2,3,4], Yves Levy[2,3,4], Olivier Schwartz[2,5], Jean Daniel Lelievre[2,3,4] & Monsef Benkirane[1]

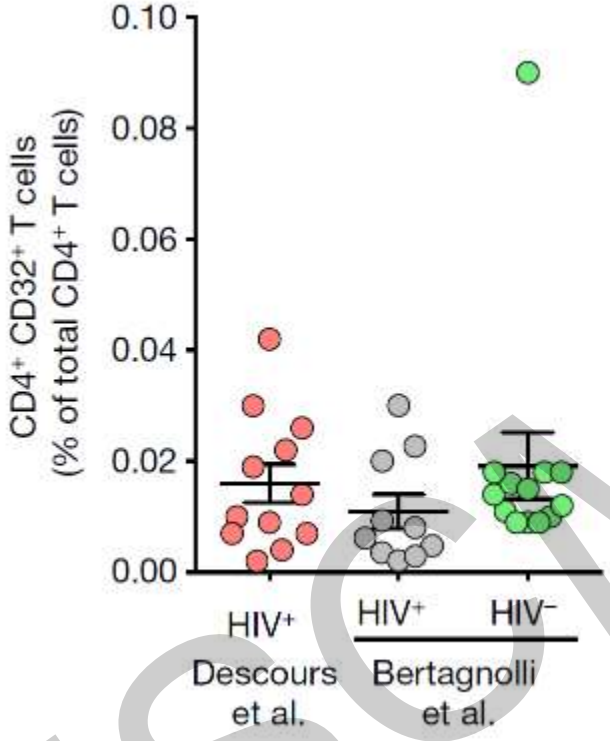
Receptor for IgG antibodies

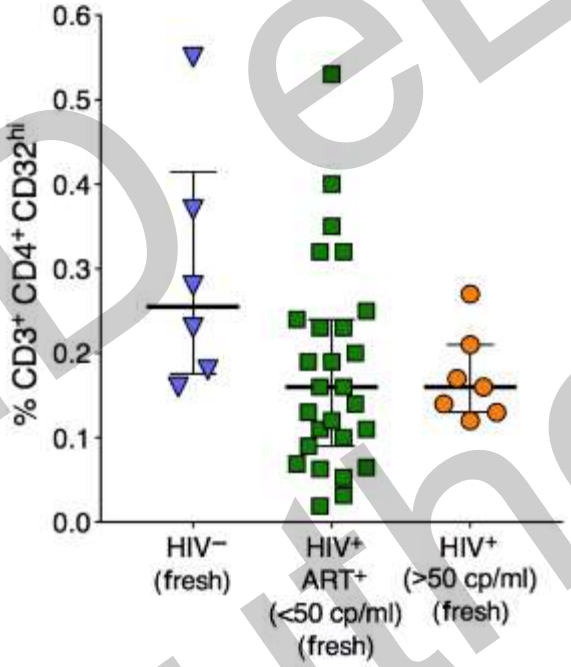
Average HIV DNA enrichment of ~1000-fold

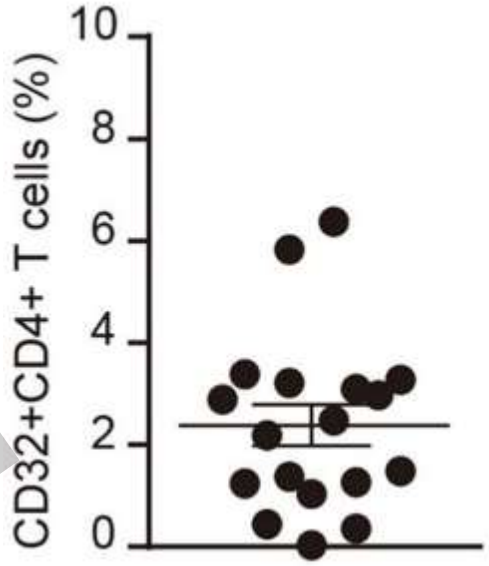


Descours et al, Nature 2017

Several groups have shown CD32 expression on a small subset of CD4+ T cells







Descours Nature 2017; Bertagnolli Nature 2018; Abdel-Mohsen Sci Transl Med 2018; Holgado Front Immunol 2018

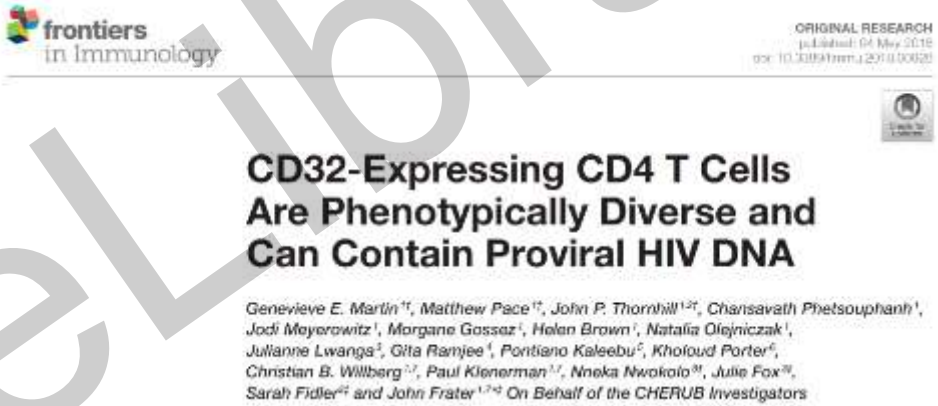
So far nobody could confirm HIV DNA enrichment in CD32+ cells

SCIENCE TRANSLATIONAL MEDICINE | RESEARCH ARTICLE

HIV

CD32 is expressed on cells with transcriptionally active HIV but does not enrich for HIV DNA in resting T cells

Mohamed Abdel-Mohsen,[1*] Leticia Kuri-Cervantes,[2*] Judith Grau-Exposito,[3*] Adam M. Spivak,[4] Racheal A. Nell,[4] Costin Tomescu,[1] Surya Kumari Vadrevu,[1] Leila B. Giron,[1] Carla Serra-Peinado,[3] Meritxell Genescà,[3] Josep Castellví,[5] Guoxin Wu,[6] Perla M. Del Rio Estrada,[7] Mauricio González-Navarro,[7] Kenneth Lynn,[1,2,8] Colin T. King,[9] Sai Vemula,[6] Kara Cox,[6] Yanmin Wan,[10] Qingsheng Li,[10] Karam Mounzer,[8] Jay Kostman,[8] Ian Frank,[2] Mirko Paiardini,[9] Daria Hazuda,[6] Gustavo Reyes-Terán,[7] Douglas Richman,[11] Bonnie Howell,[6] Pablo Tebas,[2] Javier Martinez-Picado,[12,13,14] Vicente Planelles,[4] Maria J. Buzon,[3†] Michael R. Betts,[2†] Luis J. Montaner[1†]



frontiers in Immunology

ORIGINAL RESEARCH

CD32-Expressing CD4 T Cells Are Phenotypically Diverse and Can Contain Proviral HIV DNA

Genevieve E. Martin[1†], Matthew Pace[1†], John P. Thornhill[1,2†], Chansavath Phetsouphanh[1], Jodi Meyerowitz[1], Morgane Gossez[1], Helen Brown[1], Natalia Olejniczak[1], Julianne Lwanga[3], Gita Ramjee[4], Pontiano Kaleebu[5], Khofoud Porter[6], Christian B. Willberg[1,7], Paul Klenerman[1,7], Nneka Nwokolo[8†], Julie Fox[9†], Sarah Fidler[6‡] and John Frater[1,7*‡] On Behalf of the CHERUB Investigators

nature COMMUNICATIONS

ARTICLE

DOI: 10.1038/s41467-018-05157-w OPEN

CD32 expression is associated to T-cell activation and is not a marker of the HIV-1 reservoir

Roger Badia[1], Ester Ballana[1], Marc Castellví[1], Edurne García-Vidal[1], Maria Pujantell[1], Bonaventura Clotet[1], Julia G. Prado[1], Jordi Puig[1], Miguel A. Martínez[1], Eva Riveira-Muñoz[1] & José A. Esté[1]

The Journal of Infectious Diseases

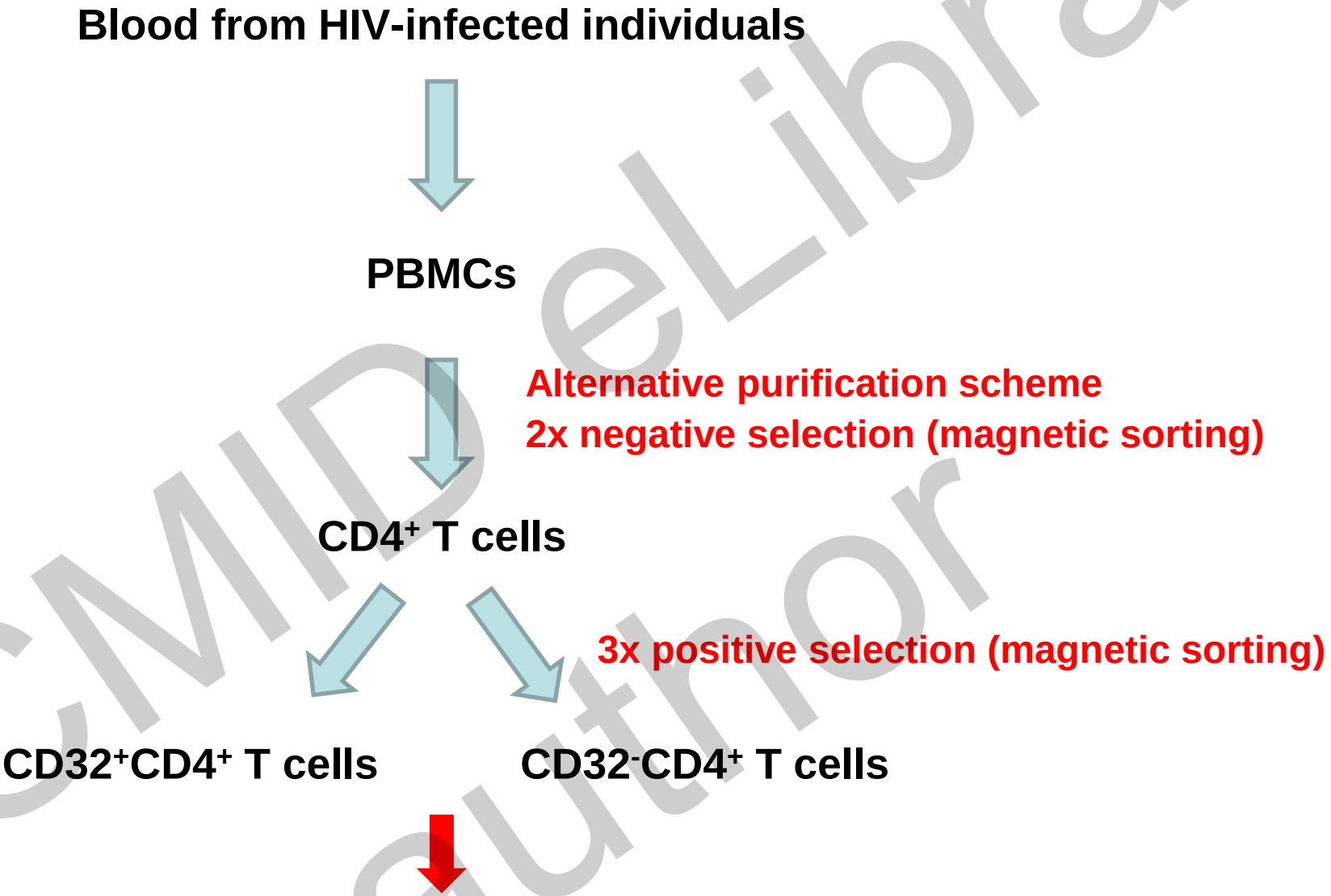
MAJOR ARTICLE

IDSA Infectious Diseases Society of America hivma

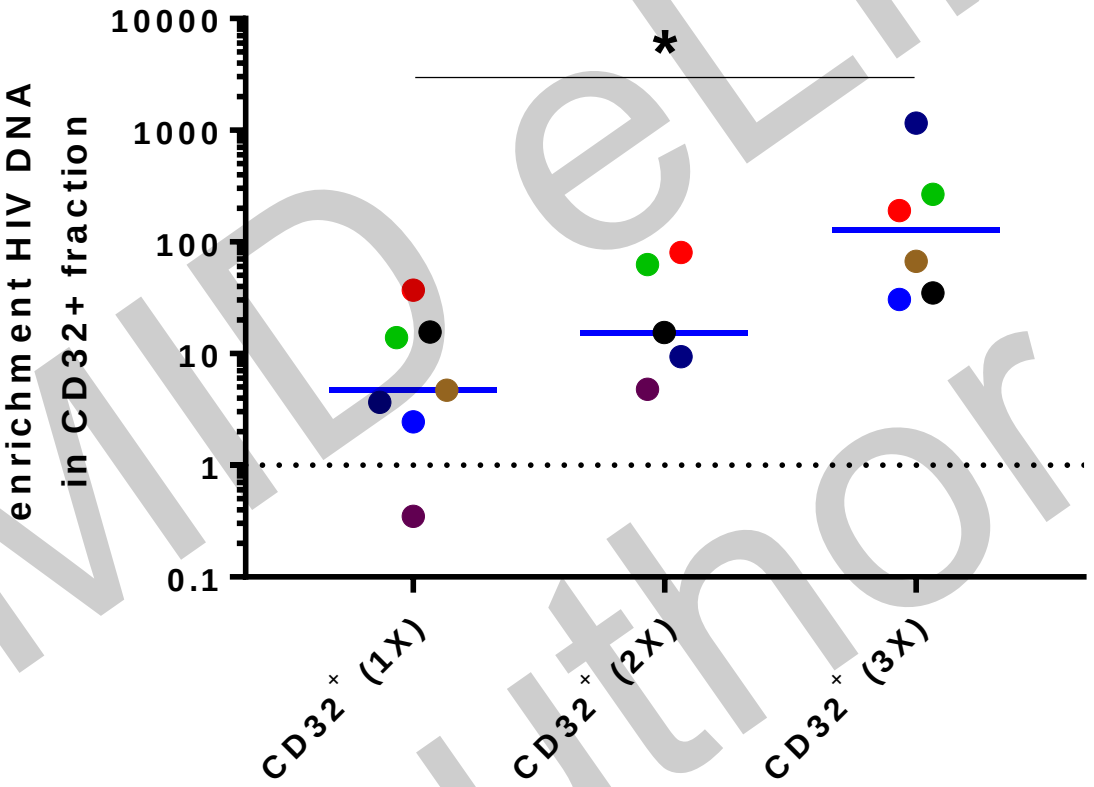
The Latent Human Immunodeficiency Virus (HIV) Reservoir Resides Primarily in CD32^{-}CD4^{+} T Cells in Perinatally HIV-Infected Adolescents With Long-Term Virologic Suppression

Adit Dhummakupt,[1] Lilly V. Siems,[1] Dolly Singh,[1] Ya Hui Chen,[1] Thuy Anderson,[1] Aleisha Collinson-Streng,[1] Hao Zhang,[2] Purvish Patel,[2] Allison Agwu,[3] and Deborah Persaud[1]

- All CD32+ cell purifications based on FACS
- Purity of the CD32 cell fraction a problem



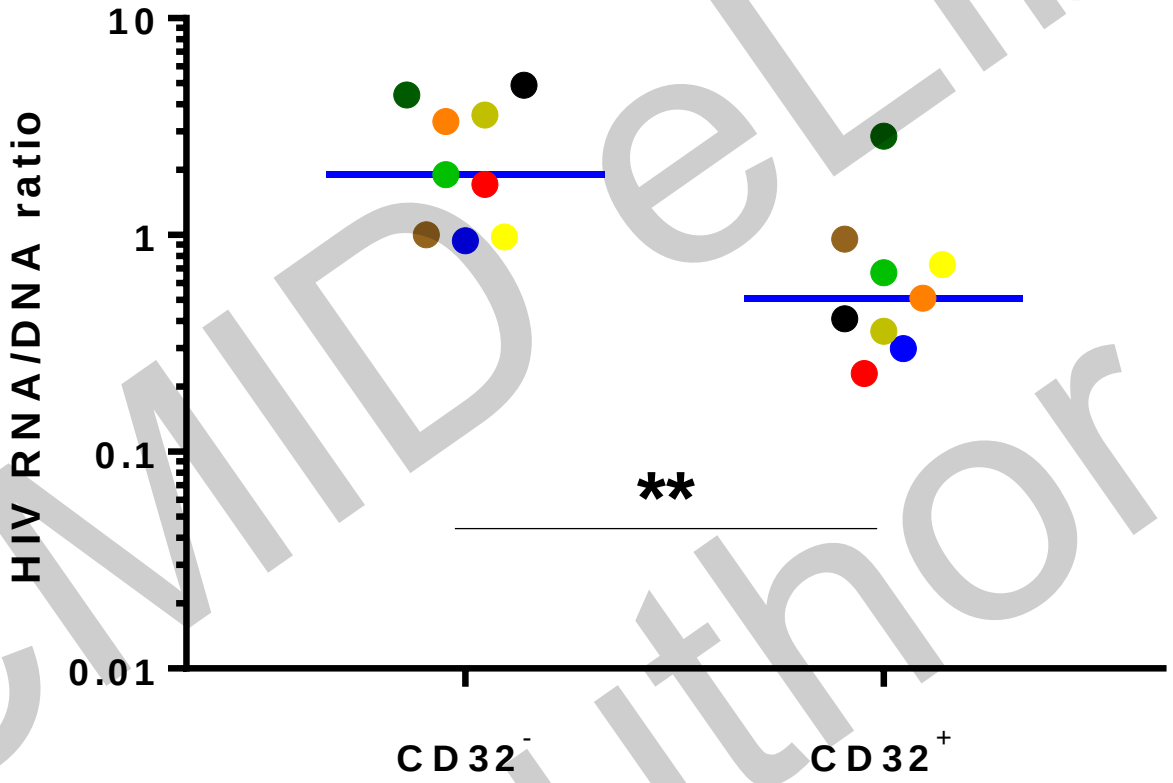
Extra two rounds of CD32+ positive selection resulted in high (up to ~1000-fold) enrichment in HIV DNA in CD32+ fraction



Mean enrichment: **11.1x** **34.5x** **292.4x**

HIV DNA load reached 0.53 copies per cell

No enrichment in HIV RNA was observed and HIV RNA/DNA ratio was significantly lower in the CD32+ fraction, indicating that HIV in these cells is mostly transcriptionally silent



CD32 remains a bona fide candidate marker of the HIV reservoir and a promising target for therapeutic cure strategies

CRISPR-Cas:
Gang Wang
Zhao Na
Bep Klaver
Gilles Darcis
Zongliang Gao
Elena Herrera Carrillo
Yme van der Velden
Caroline Binda
Minghui Fan
Atze Das

CD32:
Gilles Darcis
Neeltje Kootstra
Berend Hooibrink
Margreet Bakker
Suzanne Jurriaans
Kevin Groen
Thijs Montfort
Carine van Lint
Alexander Pasternak

European Conference of Virology - Rotterdam April 2019

www.ecv2019.com

Sponsors:
AIDS Fund
ZonMw

Laboratory of Experimental Virology

Amsterdam UMC
University Medical Centers

Human germline editing in the news

Jianku He (Shenzhen, China)
Out of the blue:
Not published
Not controlled by ethics committees
University employer did not know
Informed consent on vaccination

Protect the unborn child of a HIV-positive father during in vitro fertilization with his sperm and (?) later in life by germline removal of the main receptor for HIV: CCR5

- fertilized oocyte treated with anti-CCR5 CRISPR-Cas
- 9 months later the twins Lulu and Nana were born
- wt gene still present in one baby? (mosaicism)

Prevention?
No absolute protection (CXCR4-using strains)
Safe alternative means available: sperm washing!

Too early for oocyte or baby experiments!

Safe??
It is in germline!
Off target effects excluded?
R5 really not needed in life? (altered West Nile Virus susceptibility ……..)