Fattori di patogenesi e di protezione

La genetica

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Considerations on the genetic characteristics of HIV infected hosts

On the base of current knowledge, none of the studied genetic polymorphisms seem to be necessary and sufficient to explain lack of disease progression

It is likely that the condition of LTNP is due to multifactorial mechanisms, possibly independent

The genetic identikit of LTNP is still largely uncovered

Main features of GISHEAL LTNP

	National Cohort								TOTAL			
	ALT				ELVIS							
	N	Median	P25	P75	N	Median	P25	P75	N	Median	P25	p75
Men	39				60				99			
Women	11				34				45			
Age at date of GISHEAL enrolment (yrs)	50.0	38.0	34.2	43.2	92.0	37.9	32.9	42.5	142.0	38.0	33.1	42.7
Duration of known HIV seropositivity (yrs)	50.0	9.4	8.6	10.2	94.0	9.7	7.7	14.3	144.0	9.7	8.1	11.3
Duration of follow-up (yrs)	50.0	4.2	2.2	7.7	94.0	3.9	1.1	11.4	144.0	4.2	1.5	10.3
CD4 at date of GISHEAL enrolment	50.0	720.5	618.0	818.0	94.0	770.5	610.0	1033	144.0	743.0	614.0	978.0
RNA at GISHEAL enrolment in year	50.0	4,000	135.0	30,000	74.0	3,144	94.0	10,809	124.0	3,644	115.0	12,950

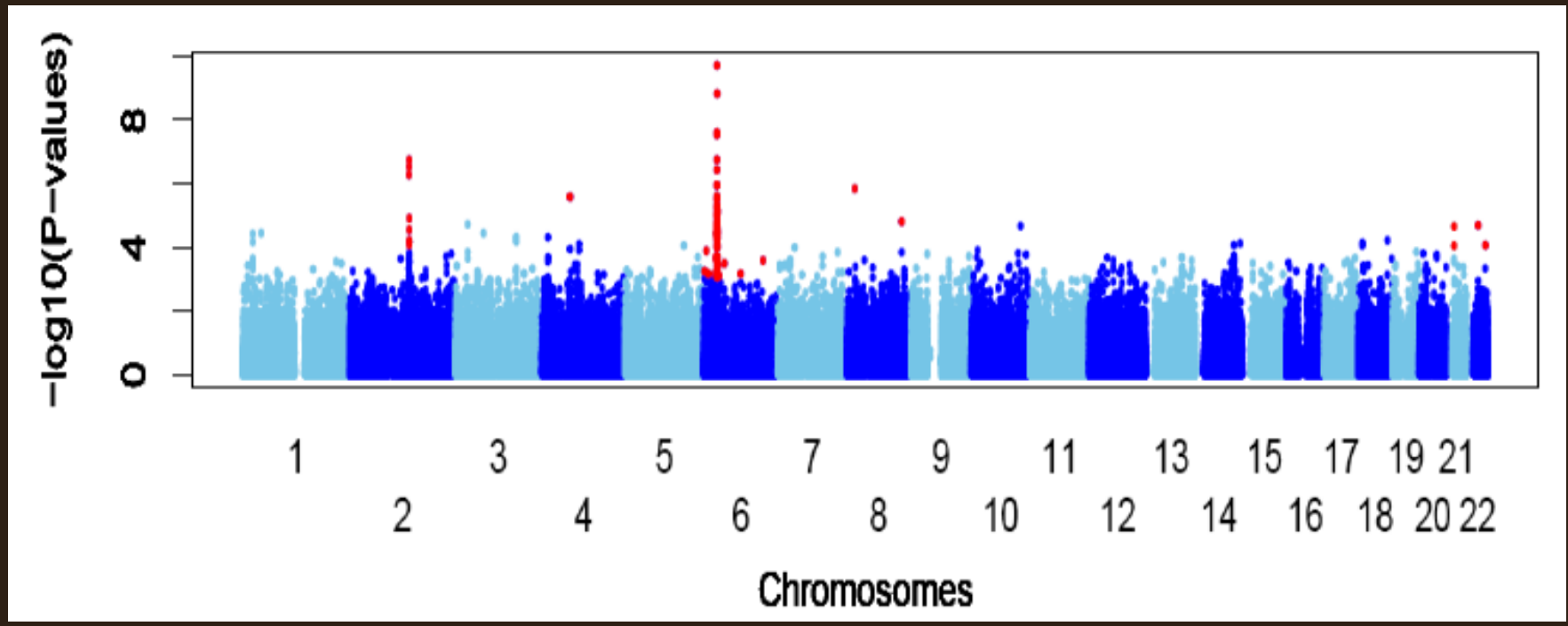
Guergnon et al, JID 2012

Control cohort: PRIMO cohort

- 605 subjects French seroconverters
- Antiretroviral ART-naive at enrolment
- Predominantly males (87.8%) and predominantly infected by sexual contact (96.0%)
- Median age at enrolment was of 34 years

Guergnon et al, JID 2012

GWAS study of the GISHEAL LTNP Manhattan plot



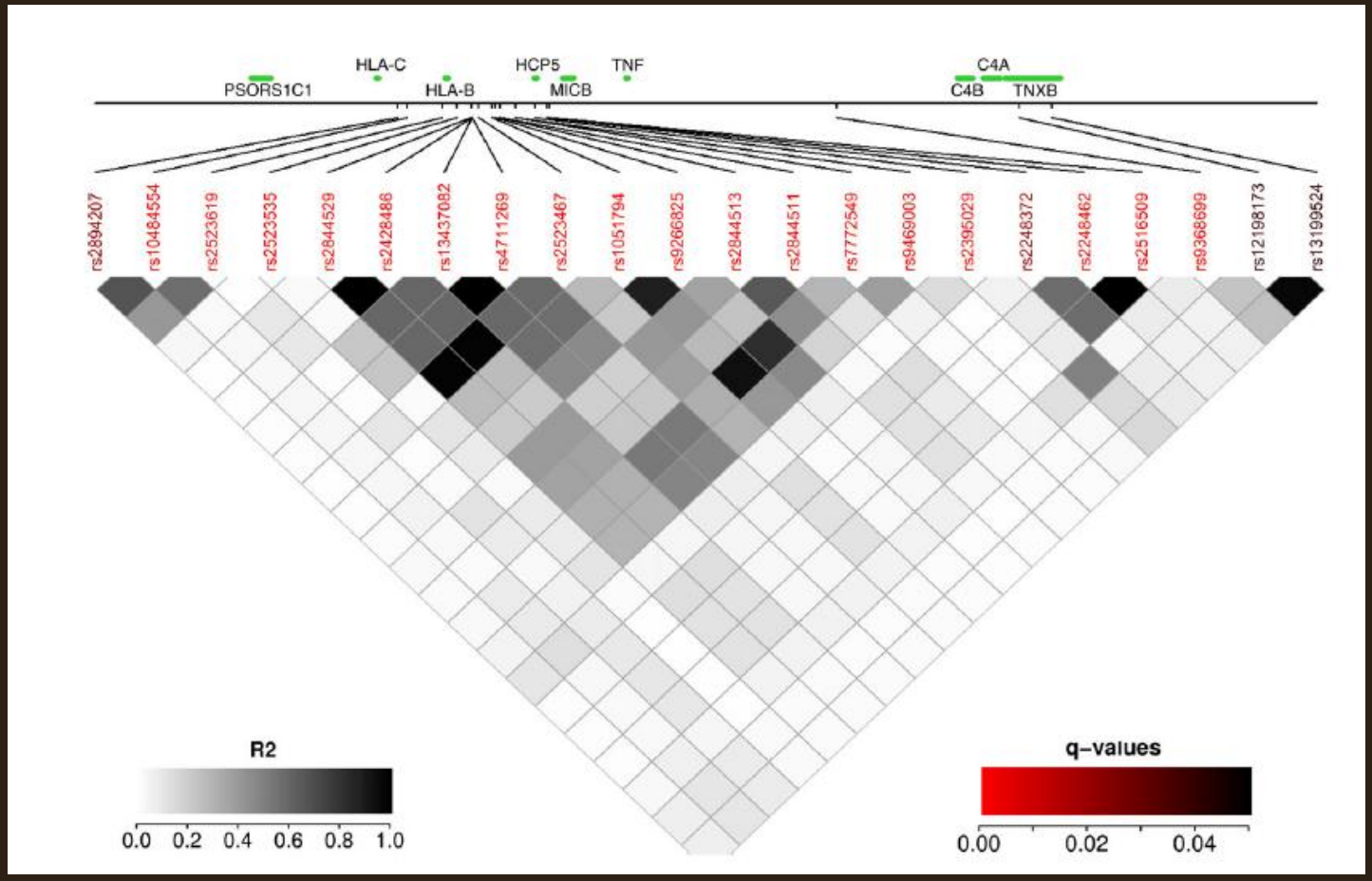
Guergnon et al, JID 2012

Most Significant SNPs Associated With LTNP Condition in the GISHEAL Cohort

SNP Name[a]	Chromosome No.	Position	Gene	Alleles	FDR[b]	P^b	LTNPs		PRIMO Cohort		LTNP-PRIMO Ratio[c]
							MAF	Minor Allele	MAF	Minor Allele	
rs2395029	6	31539759	Intragenic, HCP5	T/G	3.15×10^{-7}	1.61×10^{-11}	0.118	G	0.026	G	+
rs9368699	6	31910520	Intergenic, upstream C6orf48	T/C	2.93×10^{-6}	2.99×10^{-10}	0.125	C	0.031	C	+
rs13437082	6	31462539	Intergenic, HLA-B/MICA	T/C	5.37×10^{-6}	8.70×10^{-10}	0.455	T	0.265	T	+
rs4711269	6	31462798	Intergenic, HLA-B/MICA	T/C	5.37×10^{-6}	1.10×10^{-9}	0.455	T	0.266	T	+
rs1051794	6	31487088	Intragenic, MICA	A/G	2.36×10^{-4}	6.02×10^{-8}	0.476	A	0.305	A	+
rs7772549	6	31515622	Intergenic, MICA/HCP5	T/C	1.98×10^{-3}	6.06×10^{-7}	0.479	C	0.318	C	+
rs2516509	6	31557973	Intergenic, HCP5/MICB	A/G	2.26×10^{-3}	8.06×10^{-7}	0.382	G	0.234	G	+
rs10484554	6	31382534	Intergenic, MICA/HCP5	T/C	2.42×10^{-3}	1.36×10^{-6}	0.254	T	0.128	T	+
rs2844513	6	31496193	Intergenic, MICA/HCP5	T/C	2.42×10^{-3}	1.25×10^{-6}	0.295	C	0.484	C	–
rs2844511	6	31497763	Intergenic, HCP5/MICB	T/C	2.42×10^{-3}	1.22×10^{-6}	0.194	T	0.379	T	–

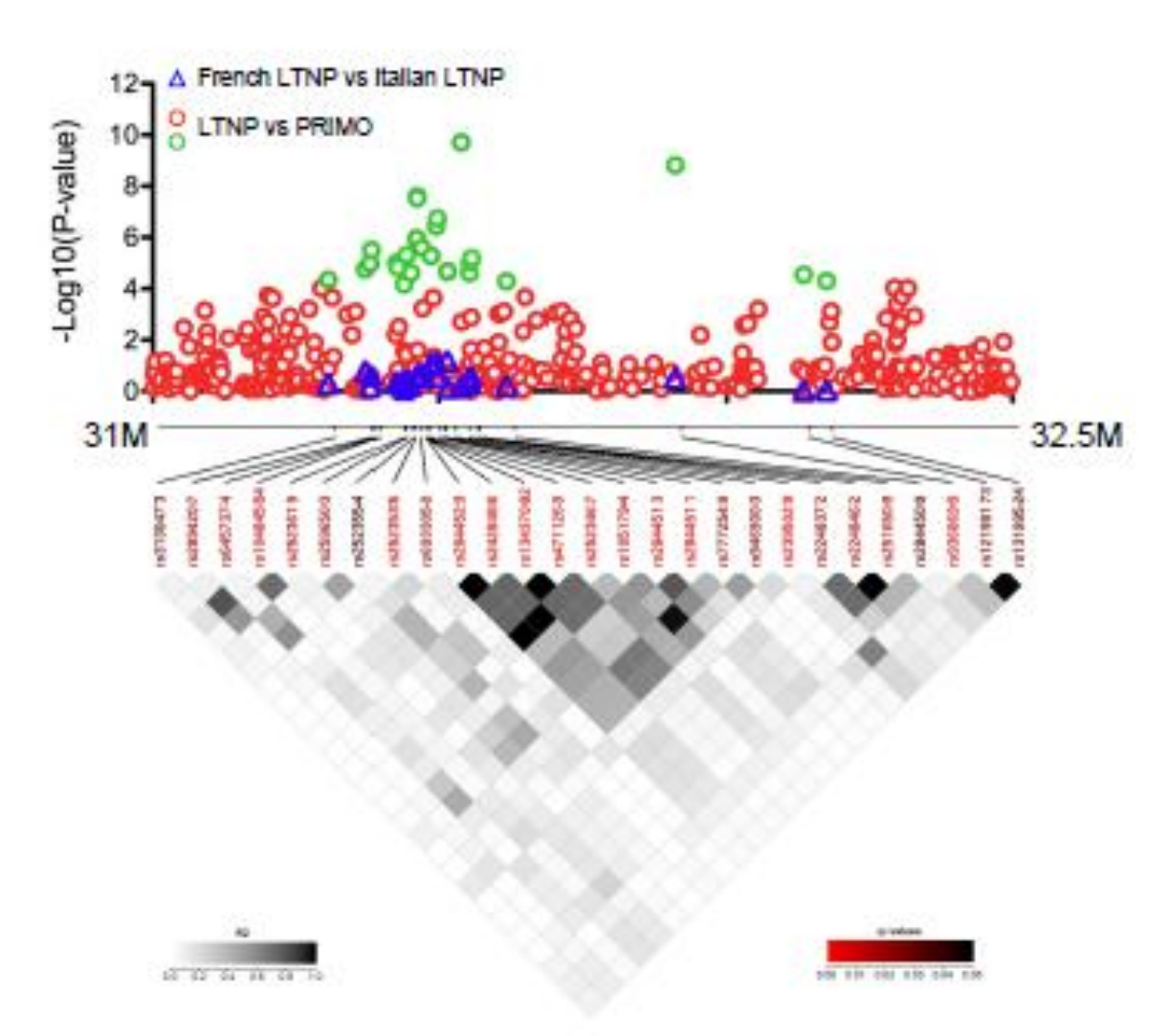
Guergnon et al, JID 2012

Linkage disequilibrium matrix for the major SNPs in the major histocompatibility complex region



Guergnon et al, JID 2012

Distribution of -Log10 for the SNPs located in the MHC region and LD matrix for the MHC major SNPs



Guergnon et al, JID 2012

HLA-B*57

- HLA-B*27 and HLA-B57 are the strongest factors associated with low levels of plasma HIV-RNA and of blood cell–associated HIV-DNA
- HIV DNA is thought to represent HIV reservoirs and to predict disease course
- These MHC genes rapidly induce exceptionally robust and persistent HIV-specific, highly avid, polyfunctional CD8 T-cell responses that specifically correlate with cell-associated HIV-DNA cell-associated HIV-DNA is mostly concentrated in CD4 T cells, a highly heterogeneous population
- T_{CM} are probably the main CD4 subpopulation involved in virus control.

Immune Responses Driven by Protective Human Leukocyte Antigen Alleles From Long-term Nonprogressors Are Associated With Low HIV Reservoir in Central Memory CD4 T Cells

Benjamin Descours,[1,a] Veronique Avettand-Fenoel,[2,a] Catherine Blanc,[3] Assia Samri,[1] Adeline Mélard,[2] Virginie Supervie,[4] Ioannis Theodorou,[1] Guislaine Carcelain,[1] Christine Rouzioux,[2] Brigitte Autran,[1] and the ALT ANRS CO15 Study Group

- Remarkably lower infection level of central memory CD4 T cells was an exclusive feature that distinguished the HLA-B27/B57 HIV reservoirs
- T_{CM} protection was correlated with preservation of T_{CM} counts, which correlated positively with the magnitude of HIV Gag-specific CD8 T cells

CID 2012

Central memory CD4 T cells

- It would be fundamental to demonstrate that these results can extend to the lymphoid tissues
- However, infection levels in PBMCs and mucosal compartments are strongly correlated, and HIV-specific CD8 T cells are also highly concentrated in lymphoid organs.
- It can be hypothesized that the ratio of HIV-specific effector CD8 T cells to HIV-infected cell in tissues might remain similar.

Descours et al, CID 2012

Potential functional cure strategies to reduce HIV reservoirs by reinforcing the immune response to protect central memory CD4 T lymphocytes

- T_{CM} count preservation was observed after SIV challenge in vaccinated macaques with potent anti-Gag CD8 T cells
- A similar T_{CM} protection was recently reported in natural hosts of SIV as sooty mangabeys, in association with a lower CCR5 induction after cell activation.
- Because these monkeys usually have poor antiviral immunity and elevated pVL, it is possible that the magnitude of T_{CM} reservoirs exquisitely reflect various mechanisms of disease control, independently of the pVL.

Descours et al, CID 2012

Intergenic rs13437082

- The third most prevalent SNP in our study
- In stronger linkage with the region spanning from MICA to MICB than with the HLA-C to HLA-B region
- In this MHC region, 3 LTNP-associated SNPs were in strong linkage with one another, although they were in very weak linkage disequilibrium with SNPs in the rest of the MHC locus.
- The role of MICA/MICB gene variation needs deeper investigation and may provide new insights for the role of natural killer cell–related pathways in HIV disease non progression

Natural killer cells

- NK cells are central to virus-specific responses
- NK cell involvement in continuous active control of viremia in LTNP or EC patients is however still partly disputed.
- Involvement of a protective genetic background for HLA:KIR interaction involves only some patients, only a fraction of NK cells, and has been suggested to rather involve KIR-expressing HIV-specific CTLs

Natural killer cells and HIV

- The relevance of NK cell function in the setting of HIV-controller status has been suggested by genetic studies showing the association between HLA-Bw4$_{80I}$ DNA carriage and specific KIRs
- NK cell-associated control of HIV replication in vitro occurs with KIR3DS1^{+} NK cells in a HLA-Bw4$_{80I}^{+}$ target cell genetic background
- However this has not been subsequently reproduced in vivo in EC/LTNP cohorts. Overall, various combinations of these mechanisms appear to be involved in the successful control of HIV replication in some LTNP and EC patients

Natural killer cells and HIV

- Involvement of the activating NK receptors in disease progression was shown by the demonstration that HIV infection is associated to profoundly decreased expression of Natural Cytotoxicity Receptors
- This in turn leads to an impaired cross-talk between NK cells and DC resulting in an altered DC editing.
- Moreover, rates of $CD4^{+}$T-cell loss after antiretroviral treatment interruption are inversely associated with NCR expression on NK cells before ART discontinuation

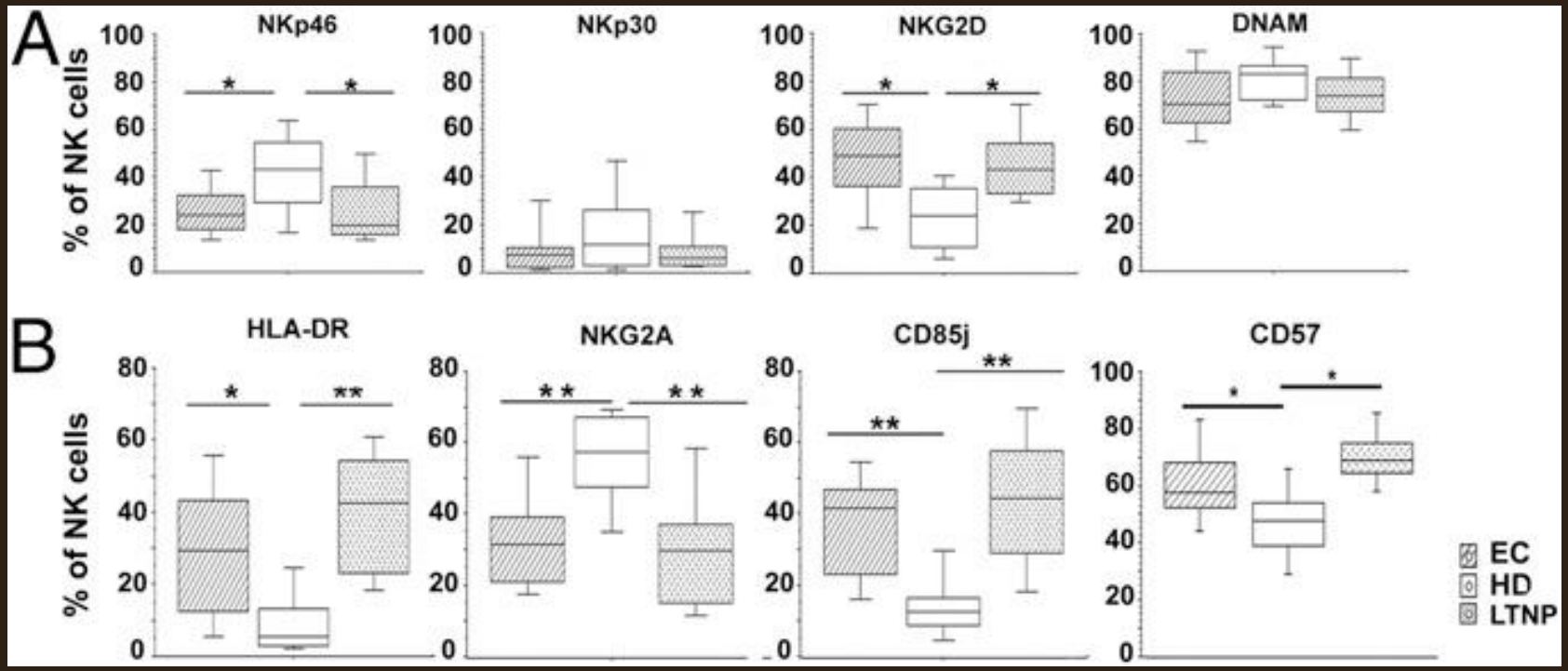
Natural killer cells and HIV

- Activating NK cell receptor expression and functional modulation in peripheral NK cells obtained from a cohort of HIV controller patients.
- The data provide evidence that differences in inducibility/modulation of NK cell NCR may offer clues on how to achieve successful disease-free HIV-1 control.

Marras et al, PNAS 2013

Natural killer cells in HIV controller patients express an activated effector phenotype and do not up-regulate NKp44 on IL-2 stimulation

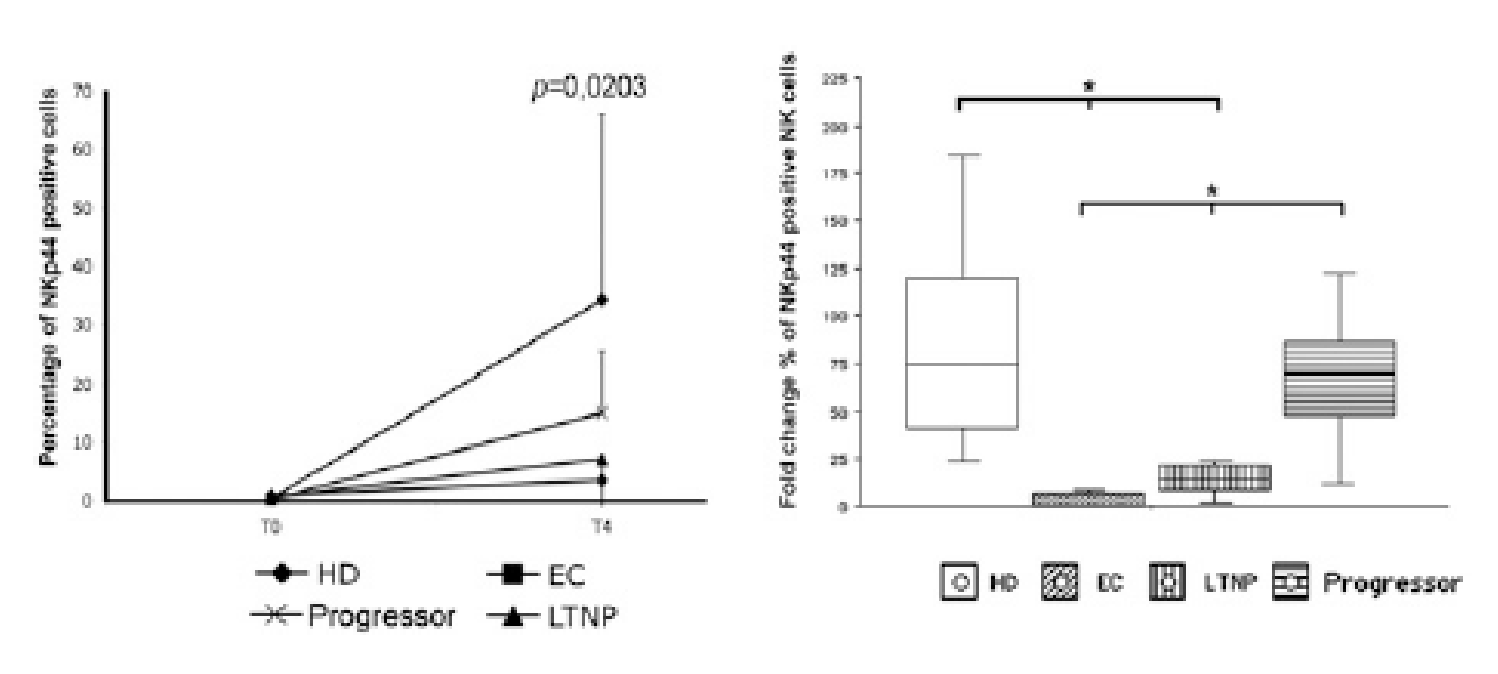
Flow cytometric analysis of peripheral NK cells in HIV-1–positive patients, ECs/LTNPs, and HDs



Marras et al, PNAS 2013

Natural killer cells in HIV controller patients express an activated effector phenotype and do not up-regulate NKp44 on IL-2 stimulation

IL-2–induced expression of NKp44 by purified NK cells in vitro



Marras et al, PNAS 2013

Natural killer cells and HIV

- The NCR expression and regulation on NK cells of EC/LTNP represents a distinctive characteristic not seen in HD or aviremic HIV-progressors on ART
- It is plausible that unique individual NCR regulation is an additional parameter affecting the outcome of host-pathogen or host–tumor interactions.
- Its association with NK cell activation and increased proportions of effector-type NK cells is consistent with mechanisms of HIV control in EC/LTNP with conserved DC-NK cell crosstalk and downstream $CD8^{+}$CTL HIV-specific responses.

Marras et al, PNAS 2013

Natural killer cells and HIV

- These observations agree with the reported continuous $CD8^+$CTLs functional control of low-level HIV replication in all EC patients and support the notion that maintenance of EC status may require combined NK cell-$CD8^+$CTL inhibition of replicating HIV

Marras et al, PNAS 2013

MHC class III

- Finally, one SNP in strong association with the LTNP condition is rs9368699
- In this regard, a recent study suggested that this SNP was preferentially associated with the LTNP condition rather than with the spontaneous control of viremia
- This is probably the most robust marker of the LTNP condition ever found, pointing to the genetic differences between LTNPs and HIV controllers.
- Of interest, genes including heat shock proteins, HLA-B associated transcripts, and complement components map in the vicinity of this SNP according to the linkage disequilibrium and are therefore new candidates contributing to the LTNP condition.

TLR9

- TLR9 is localized in the intracellular endolysosomal compartment, it is activated by unmethylated CpG motifs within single strand DNA that are present in bacteria and viruses.
- TLR9 is expressed on pDC, B cells and monocytes/macrophages
- CpG DNA is recognized in the endosome following nonspecific uptake into the cells and the activation of TLR9 leads to the induction of MyD88 dependent signaling pathway.
- Stimulation of TLR9 triggers B-cell proliferation and secretion of antibodies, secretion of Type 1 cytokines and chemokines

TLR9 and HIV

- In the context of HIV infection, the role of SNPs in TLRs may provide relevant information on HIV pathogenesis because the in vitro activation and signaling through TLR9 (and TLR4, TLR2) might enhance HIV replication .
- Genetic variants of TLR9 have been associated with the rate of disease progression in adults
- Specific variants of TLR9 were found to be associated with risk of mother-to-child transmission of HIV-1
- Functional study demonstrated a critical role of the G allele of the rs352140 SNP on TLR9 expression

Bochud et al; Soriano et al; Pine et al; Ricci et al.

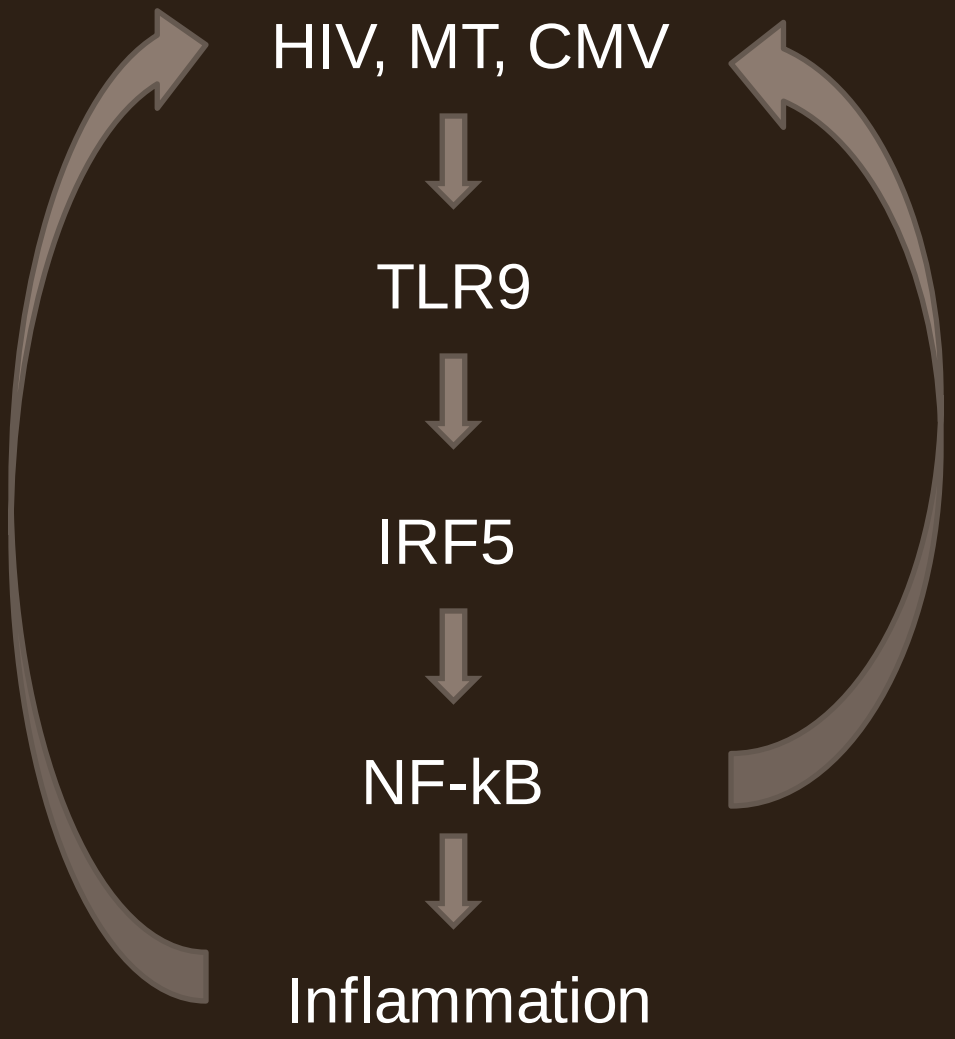
TLR9 rs352140 A is associated with HIV rapid progression

99 HIV+ Long Term Non Progressors

163 HIV+ Progressors

rs352140	PR	LTNP	P-Value
AA	58 (35.6 %)	23 (23.2 %)	0.025
AG+GG	105 (64.4%)	**76** (76.8 %)	

TLR9 rs352140

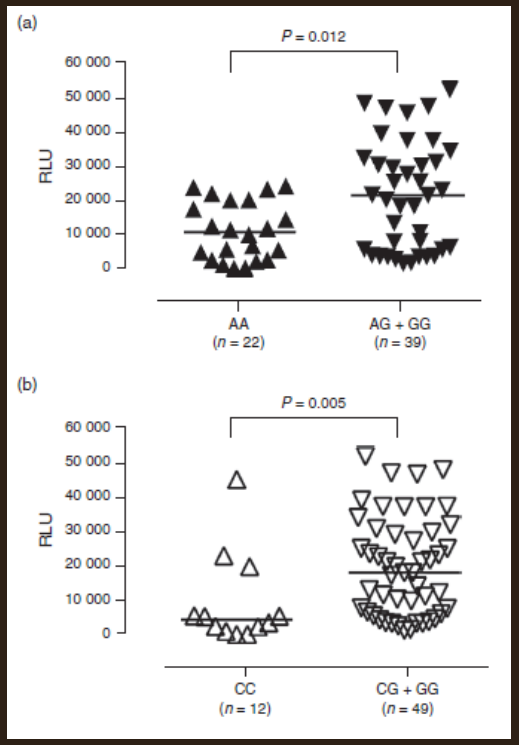


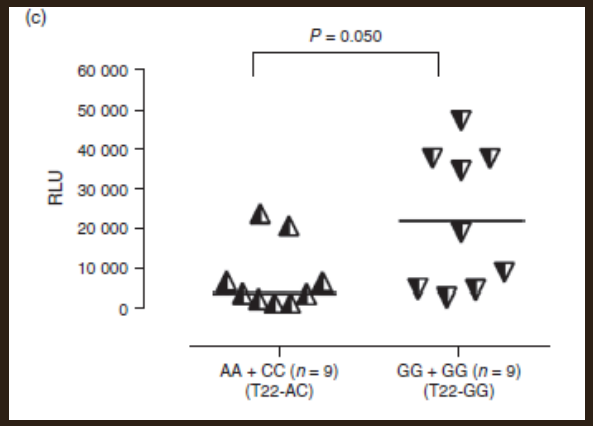
Identification of Human *TRIM22* Single Nucleotide Polymorphisms Associated with Loss of HIV-1 Transcription and Advanced HIV-1 Disease

- TRIM22 is an interferon-induced protein that inhibits HIV-1 transcription and replication *in vitro*
- Two SNPs, rs7935564A/G and rs1063303C/G characterize the coding sequence of human *TRIM22* gene
- TRIM22 N155/T242, T22-N155/R242 and T22-D155/T242 inhibit basal HIV-1 LTR-driven transcription in 293T cells; in contrast, T22-D155/R242 enhanced basal HIV-1 transcription, but exerted inhibitory effects upon cell stimulation with phorbol myristate acetate plus ionomycin.
- *In vivo*, homozygotes T22-D155 and heterozygotes N/D155 were more frequent in advanced disease progression as compared to LTNP or to normal progressors or to uninfected individuals whereas T22-N155 did not significantly associate with HIV-1 disease progression.
- Although, the rs1063303C/G distribution was similar among the groups, an rs7935564G/rs1063303G (D155/R242) haplotype was found more frequently in AP vs. LTNP

Ghezzi et al, AIDS 2014

Identification of TRIM22 single nucleotide polymorphisms associated with loss of inhibition of HIV-1 transcription and advanced HIV-1 disease





Association between TRIM22 SNP-1and SNP-2 genotypes and HIV-1 replication in primary human phytohemagglutinin blasts.

Ghezzi et al, AIDS 2014

Identification of TRIM22 single nucleotide polymorphisms associated with loss of inhibition of HIV-1 transcription and advanced HIV-1 disease

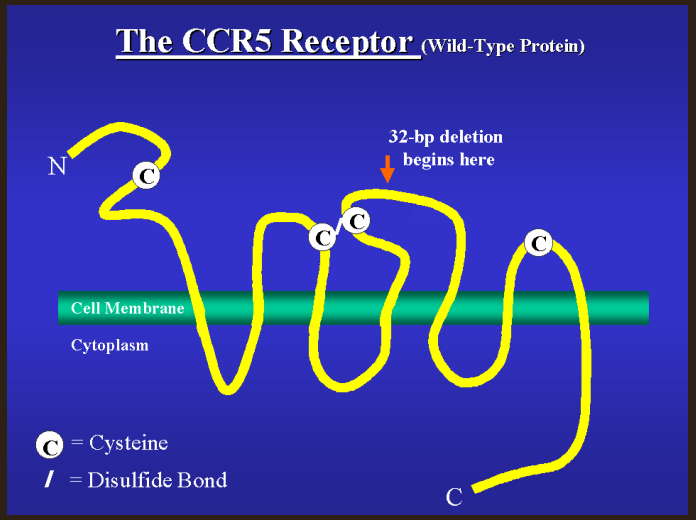
Association of genotype with severity of HIV-1 disease

SNP ID	Severity of HIV-1 disease[a]	Genotype frequency			*P* value[b]	*P* value[b] (dominant model[c])
SNP-1		AA *n* (%)	AG *n* (%)	GG *n* (%)		
	APs ($n=57$)	10 (18)	35 (61)	12 (21)	Reference	Reference
	NP1 ($n=14$)	6 (43)	7 (50)	1 (7)	0.090	0.040
	NP2 ($n=62$)	20 (32)	32 (52)	10 (16)	0.170	0.060
	LTNPs ($n=95$)	35 (37)	50 (53)	10 (10)	0.020	0.010
	HIV-1-negative ($n=182$)	72 (40)	80 (44)	30 (16)	0.009	0.002
SNP-2		CC	CG	GG		
	APs ($n=57$)	17 (30)	23 (40)	17 (30)	Reference	Reference
	NP1 ($n=14$)	3 (21)	10 (72)	1 (7)	0.120	0.530
	NP2 ($n=62$)	15 (24)	37 (60)	10 (16)	0.090	0.480
	LTNPs ($n=95$)	28 (30)	44 (47)	22 (23)	0.280	0.990
	HIV-1-negative ($n=182$)	46 (26)	93 (52)	40 (22)	0.230	0.530

Ghezzi et al, AIDS 2014

CCR5Δ32

- A 32 base pair deletion in the CCR5 gene (CCR5-Δ32), leading to a truncated gene product, has been shown to confer marked protection against HIV-1 infection in homozygous individuals, while infected heterozygotes show delayed progression of their infection



Moore JP, et al *AIDS Res Hum Retroviruses.* 2004; Dean M et al. *Science.* 1996; Liu R, et al *Cell* 1996; de Roda Husman et al *Ann Intern Med* 1997.

Gene therapy

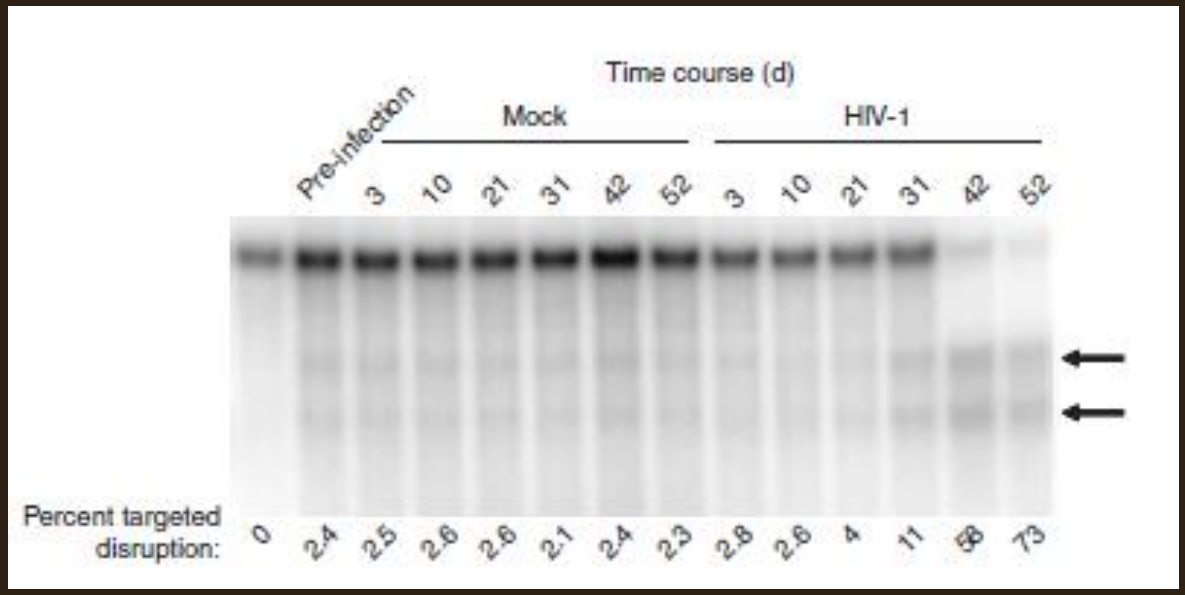
- Various gene therapy approaches to block *CCR5* expression are being evaluated, including *CCR5*-specific ribozymes11,12, siRNAs13 and intrabodies14
- The targeted cell populations include both mature T cells and CD34+ HSPCs. Loss of *CCR5* in HSPCs appears to have no adverse effects on hematopoiesis
- An alternative approach is the use of engineered ZFNs to permanently disrupt the *CCR5* open reading frame
- ZFNs comprise a series of linked zinc fingers engineered to bind specific DNA sequences and fused to an endonuclease domain

Gene therapy

- Concerted binding of two juxtaposed ZFNs on DNA, followed by dimerization of the endonuclease domains, generates a double-stranded break at the DNA target.
- Such double-stranded breaks are rapidly repaired by cellular repair pathways, notably the mutagenic nonhomologous end-joining pathway, which leads to frequent disruption of the gene due to the addition or deletion of nucleotides at the break site
- A significant advantage of this approach is that permanent gene disruption can result from only transient ZFN expression

Establishment of HIV-1 resistance in CD4+ T cells by genome editing using zinc-finger nucleases

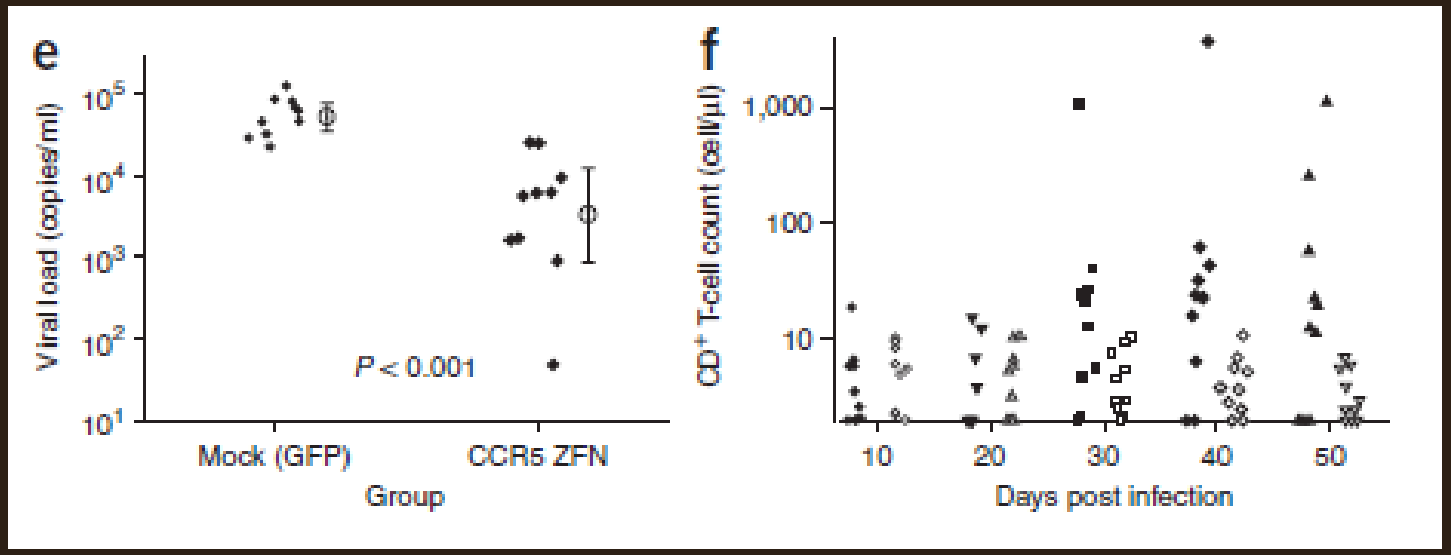
- An HIV-resistant genotype was generated *de novo* using engineered zinc-finger nucleases (ZFNs) to disrupt endogenous CCR5.
- Transient expression of CCR5 ZFNs permanently and specifically disrupted ~50% of CCR5 alleles in a pool of primary human CD4+ T cells.



Perez et al Nat Biotech, 2008

CCR5 ZFN

- Genetic disruption of CCR5 provided robust, stable and heritable protection against HIV-1 infection *in vitro* and *in vivo* in a NOG model of HIV infection.
- HIV-1-infected mice engrafted with ZFN-modified CD4+ T cells had lower viral loads and higher CD4+ T-cell counts than mice engrafted with wild-type CD4+ T cells, consistent with the potential to reconstitute immune function in individuals with HIV/AIDS by maintenance of an HIV-resistant CD4+ T-cell population.

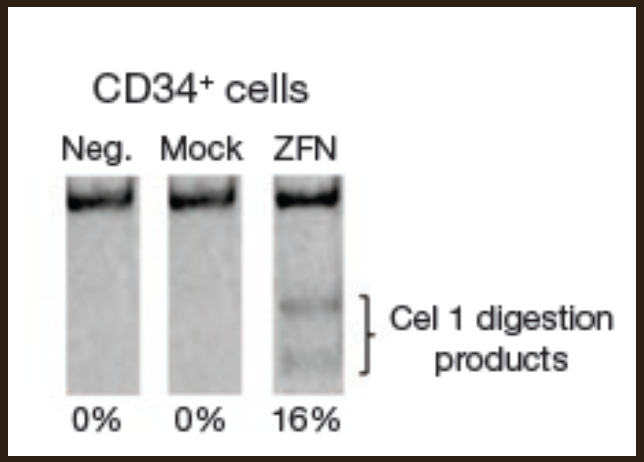


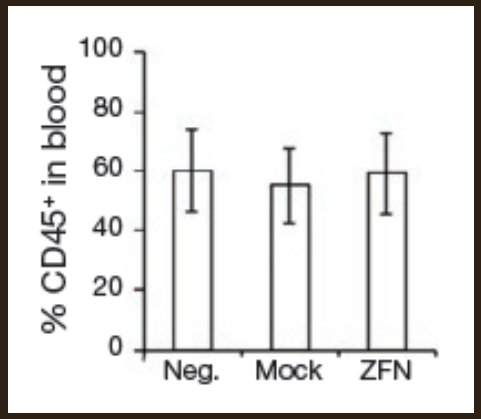
Perez et al Nat Biotech, 2008

Disruption of *CCR5* in HSPC

- However, disruption of *CCR5* in HSPCs is likely to provide a more durable anti-viral effect and to give rise to *CCR5*−/− cells in both the lymphoid and myeloid compartments that HIV-1 infects.

- Delivery of *CCR5*-specific ZFNs to human CD34+ HSPCs and transplant of the modified cells into NSG mice, which support both human hematopoiesis and HIV-1 infection

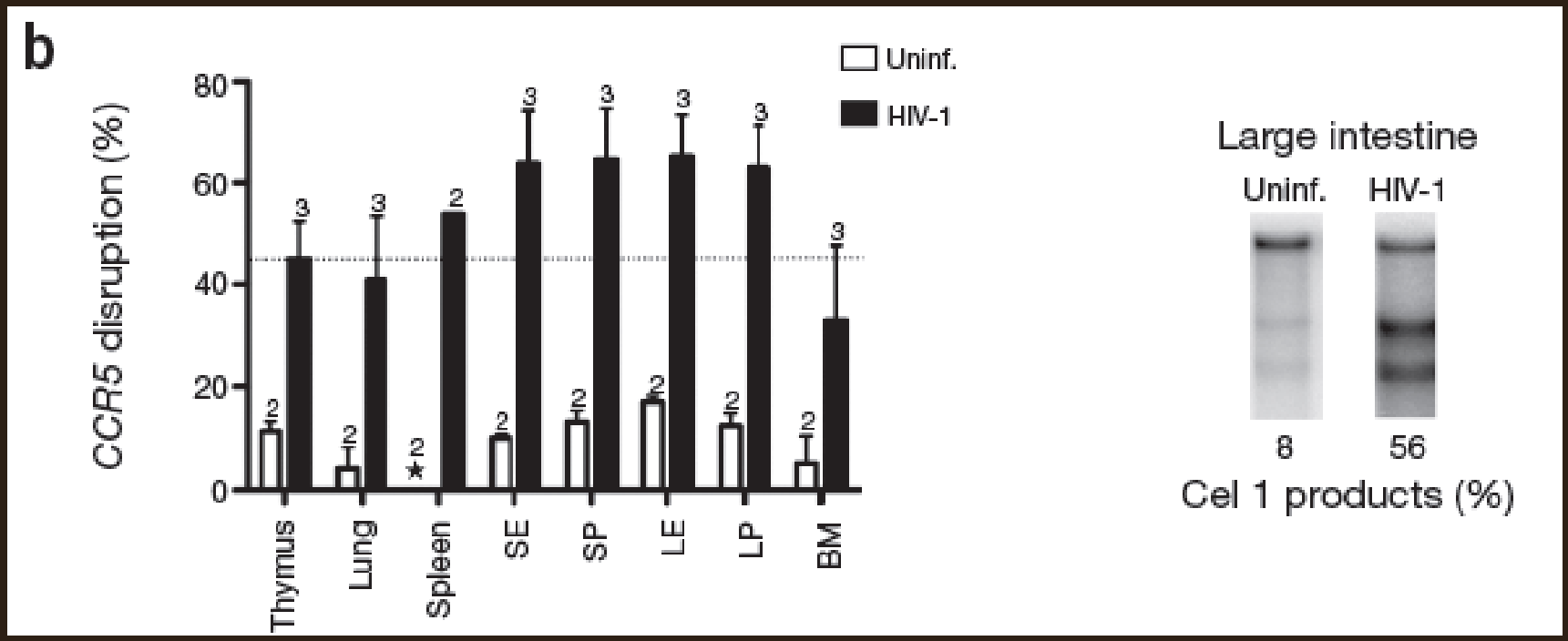




Holt N et al: 2010 Nature biotechnology

Disruption of *CCR5* in HSPC

Infection of the mice with a CCR5-tropic strain of HIV-1 leads to rapid selection for CCR5– human cells

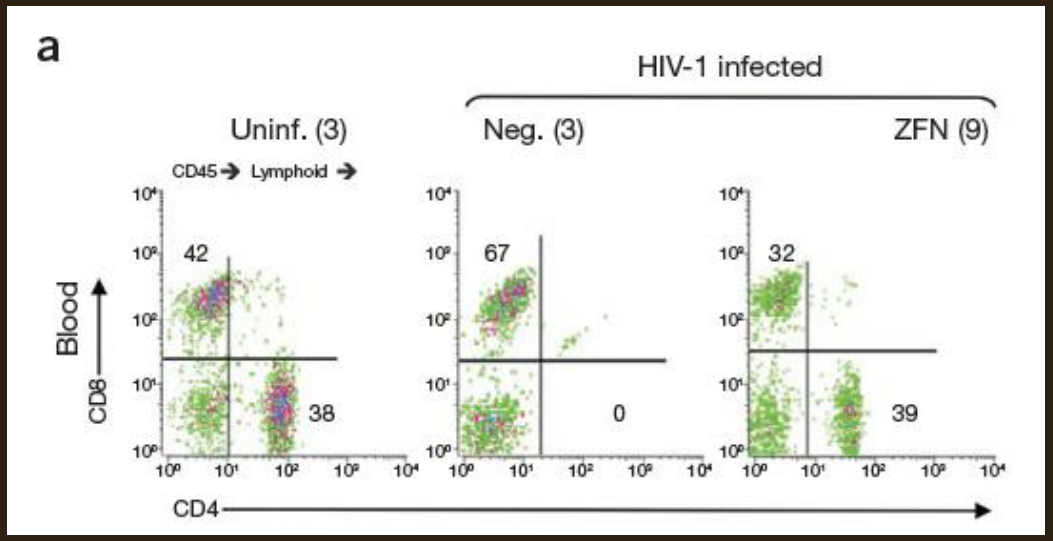


Holt N et al: 2010 Nature biotechnology

Disruption of *CCR5* in HSPC

Infection of the mice with a CCR5-tropic strain of HIV-1

significant reduction in viral load



protection of human T-cell populations in the key tissues that HIV-1 infects

Holt N et al: 2010 Nature biotechnology

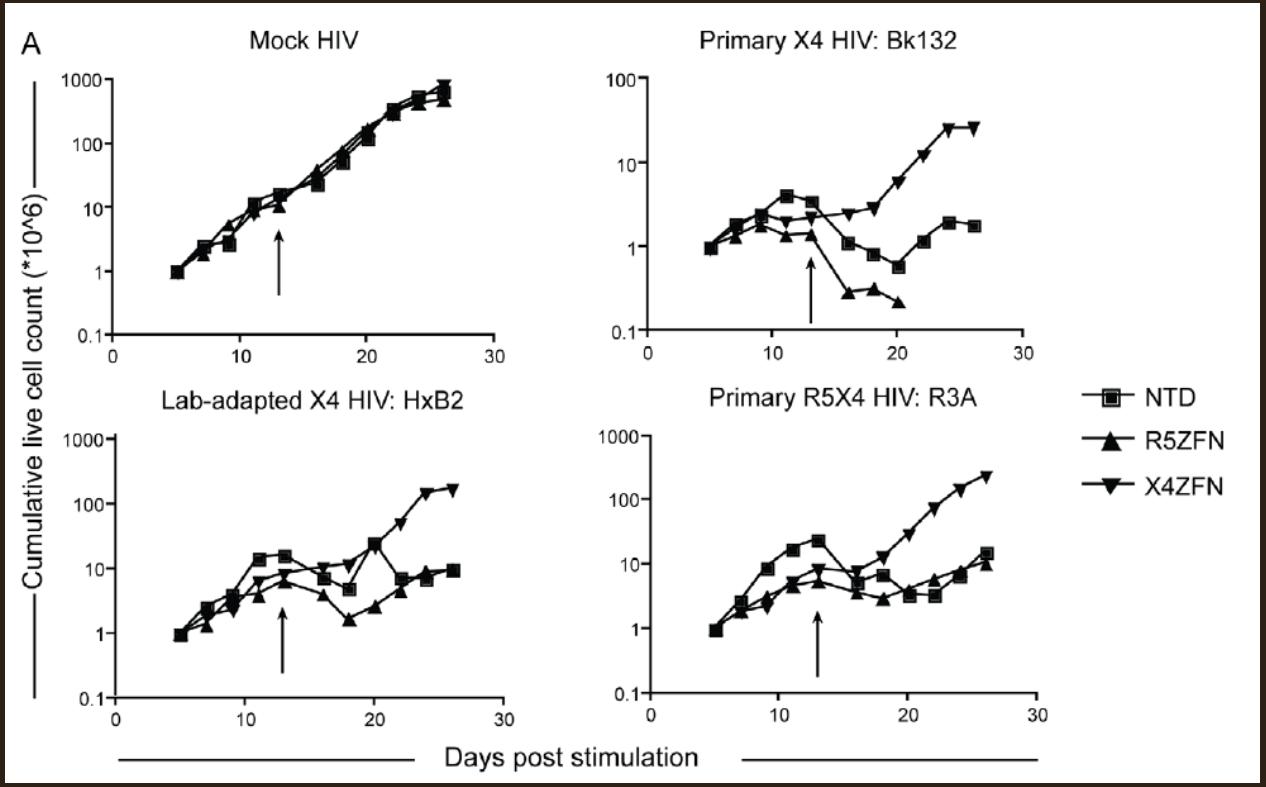
Engineering HIV-Resistant Human CD4+ T Cells with CXCR4-Specific Zinc-Finger Nucleases

- The introduction of CCR5-specific zinc-finger nucleases into human CD4+ T cells prior to adoptive transfer raises the need to protect cells from virus strains that use CXCR4 (X4) in place of or in addition to CCR5 (R5X4).
- Engineering of a pair of zinc finger nucleases that, when introduced into human T cells, efficiently disrupt CXCR4 by cleavage and error-prone non-homologous DNA end-joining.

Wilen G. B. et al. 2011 PLoS Pathogens

CXCR4-Specific Zinc-Finger Nucleases

- The resulting cells proliferated normally and were resistant to infection by X4-tropic HIV-1 strains. CXCR4 could also be inactivated in CCR5Δ32 CD4+ T cells, such cells were resistant to all strains of HIV-1 tested.



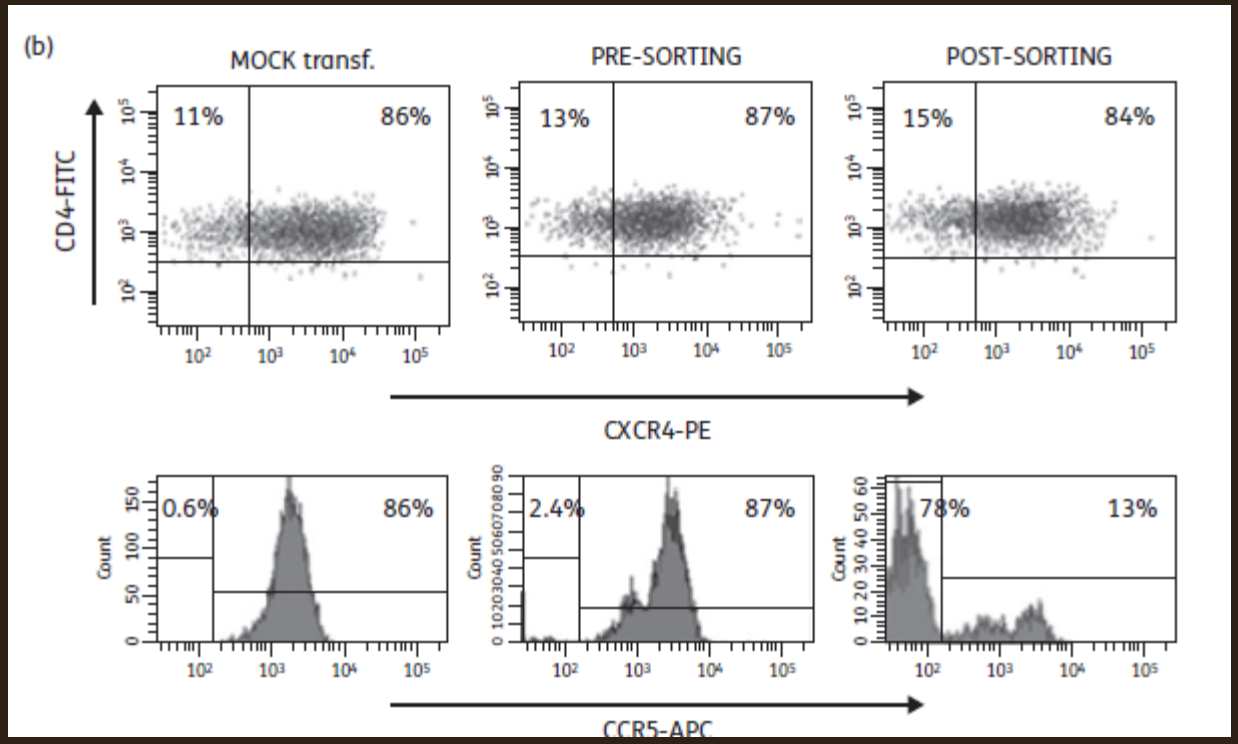
Wilen G. B. et al. 2011 PLoS Pathogens

CXCR4-Specific Zinc-Finger Nucleases

- Loss of CXCR4 also provided protection from X4 HIV-1 in a humanized mouse model, though this protection was lost over time due to the emergence of R5-tropic viral mutants.
- These data suggest that CXCR4-specific ZFNs may prove useful in establishing resistance to CXCR4-tropic HIV for autologous transplant in HIV-infected individuals.

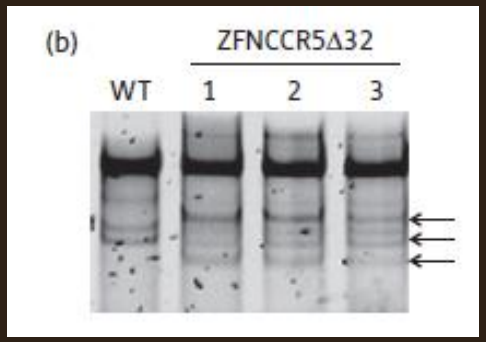
Gene editing using a zinc-finger nuclease mimicking the CCR5Δ32 mutation induces resistance to CCR5-using HIV-1

Cytometry plots of mock-treated cells and cells transfected with ZFNCCR5D32 plasmids



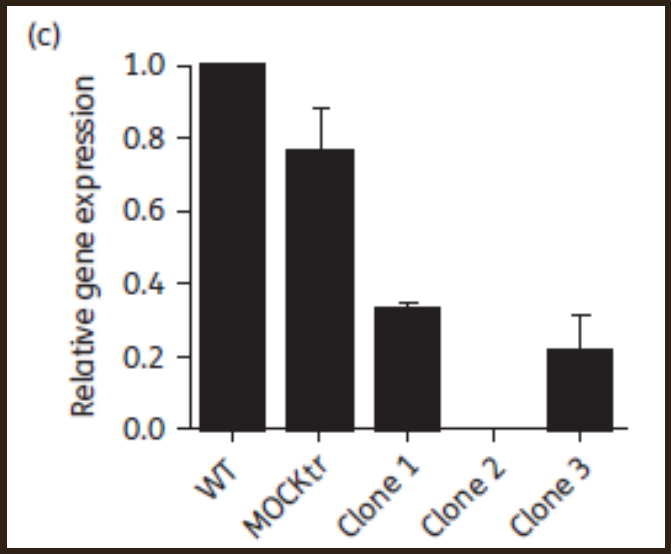
Roger Badia et al., J Antimicrob Chemother, 2014

Gene editing using a zinc-finger nuclease mimicking the CCR5Δ32 mutation induces resistance to CCR5-using HIV-1



Surveyor mutation detection assay of the individual clones obtained

mRNA expression in the selected clones

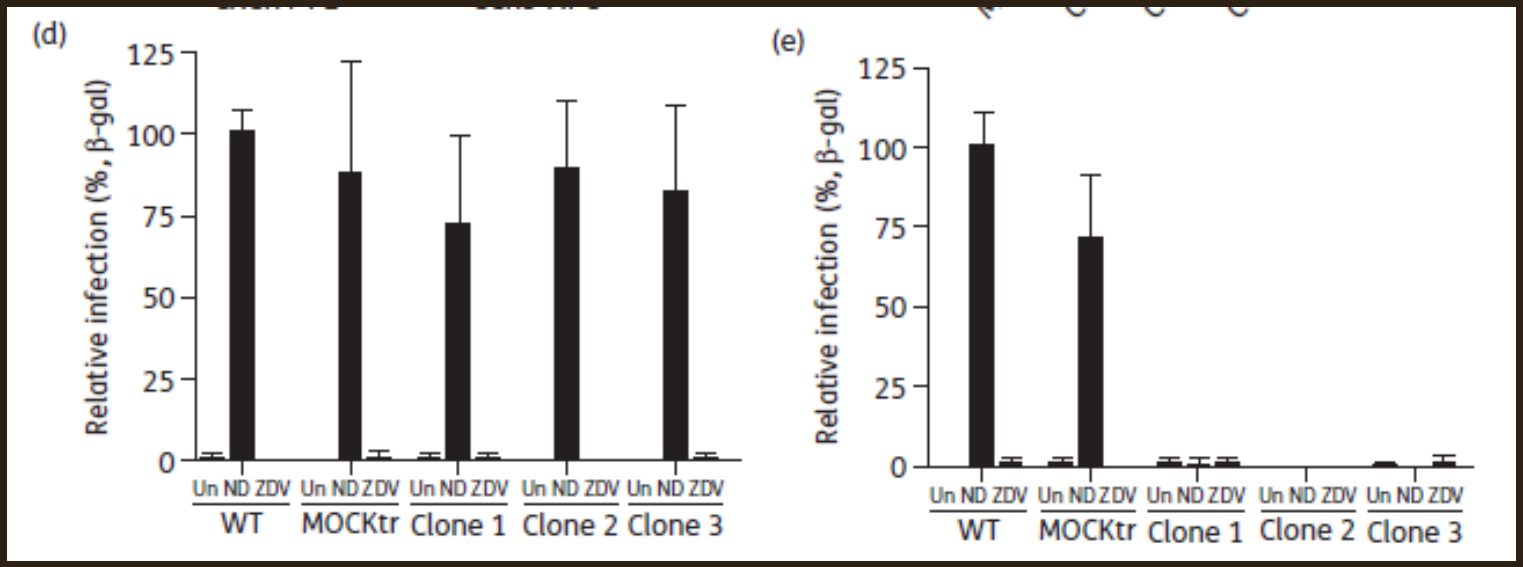


Roger Badia et al., J Antimicrob Chemother, 2014

Gene editing using a zinc-finger nuclease mimicking the CCR5Δ32 mutation induces resistance to CCR5-using HIV-1

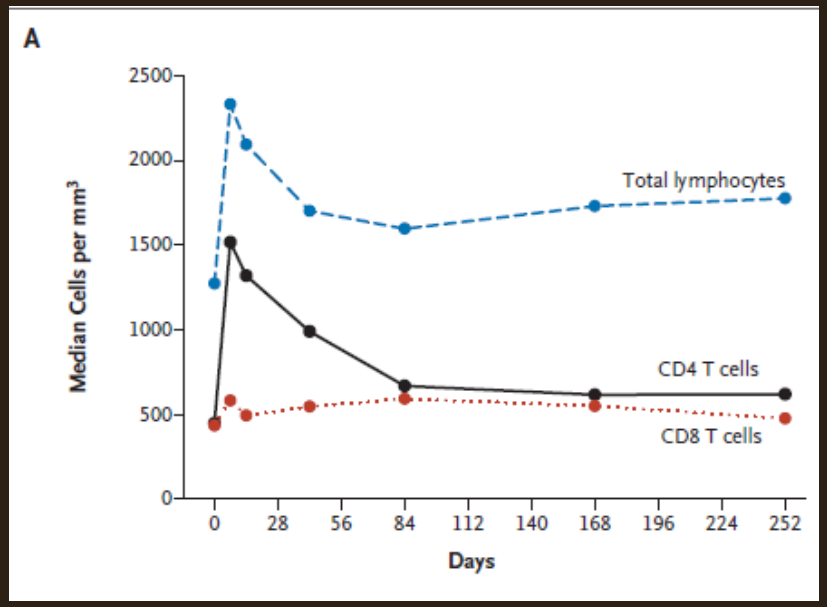
Percentage of HIV NL4-3 replication

Percentage of HIV BaL replication



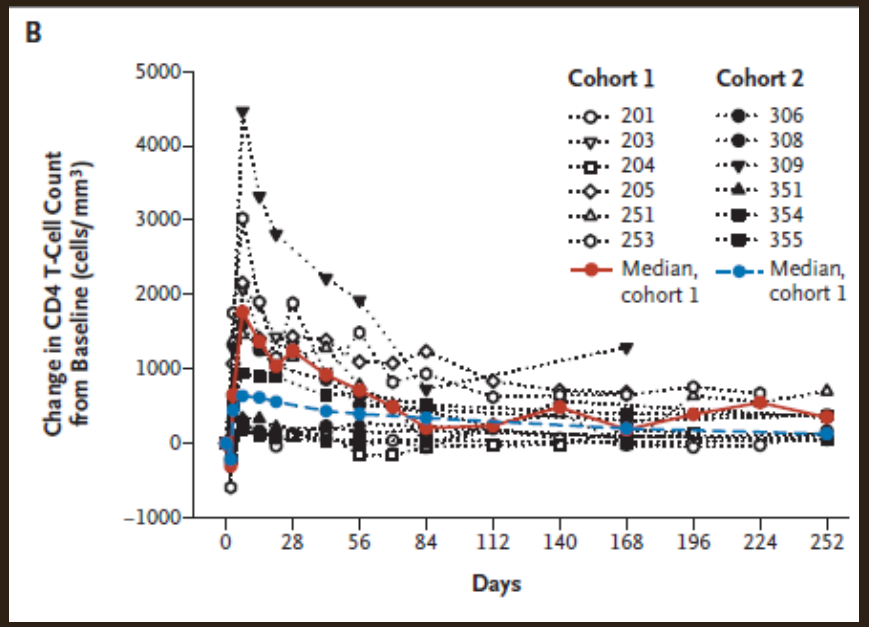
Roger Badia et al., J Antimicrob Chemother, 2014

Gene Editing of *CCR5 in Autologous CD4 T Cells* in HIV Infected patients



Median total lymphocyte, CD4 T-cell, and CD8 T-cell values

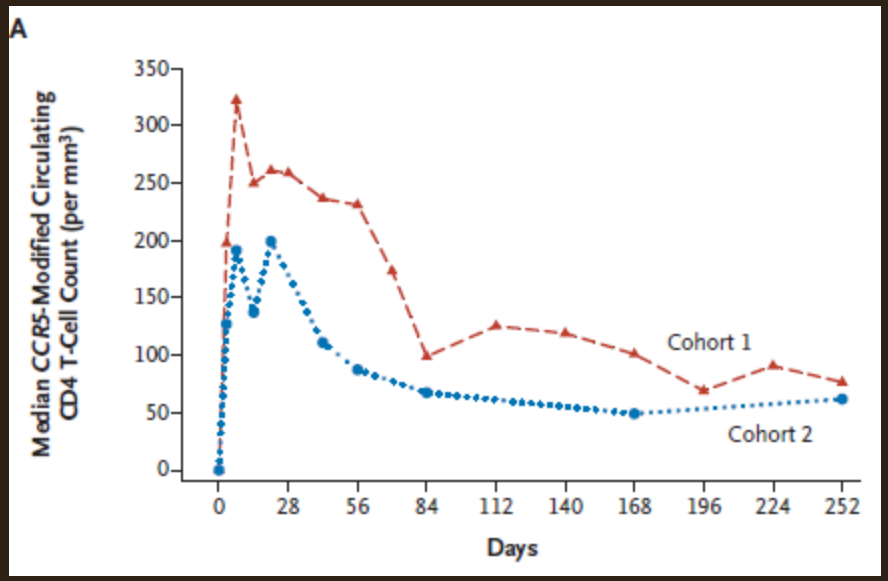
Change in CD4 T-cell count from baseline



Pablo Tebas et al., N Engl J Med 2014

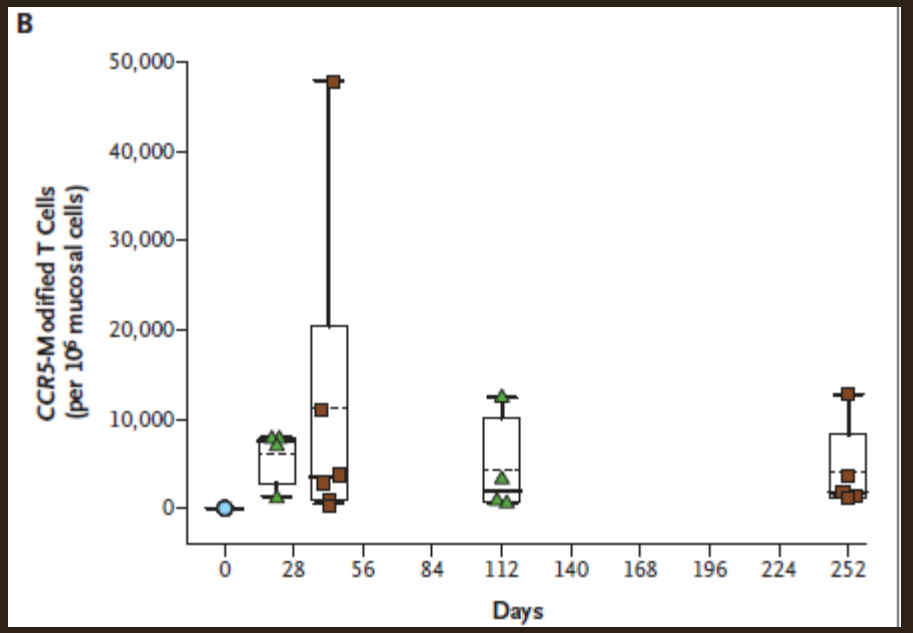
Gene Editing of *CCR5 in Autologous CD4 T Cells* in HIV Infected patients

CCR5-Modified CD4 T Cells in the Circulation and Mucosal Tissues



Median absolute number of *CCR5-modified circulating* CD4 T cells

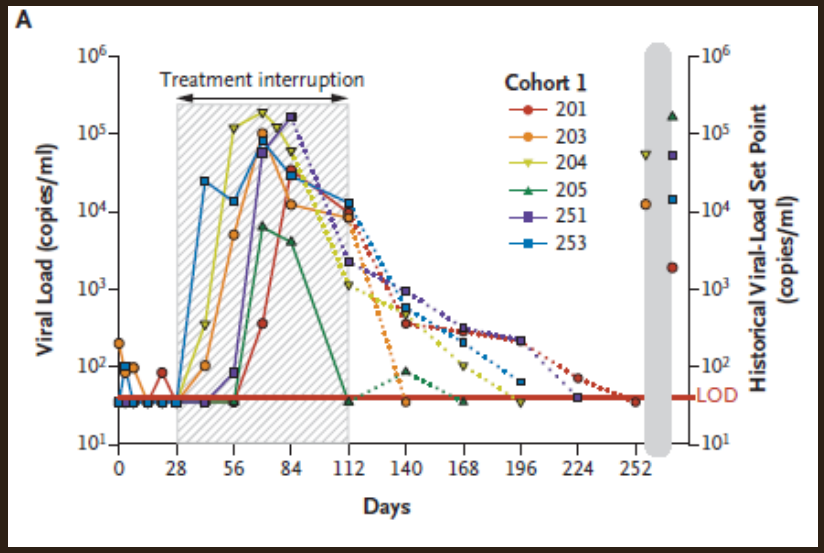
CCR5-modified cell traffic to rectal mucosal tissues



Pablo Tebas et al., N Engl J Med 2014

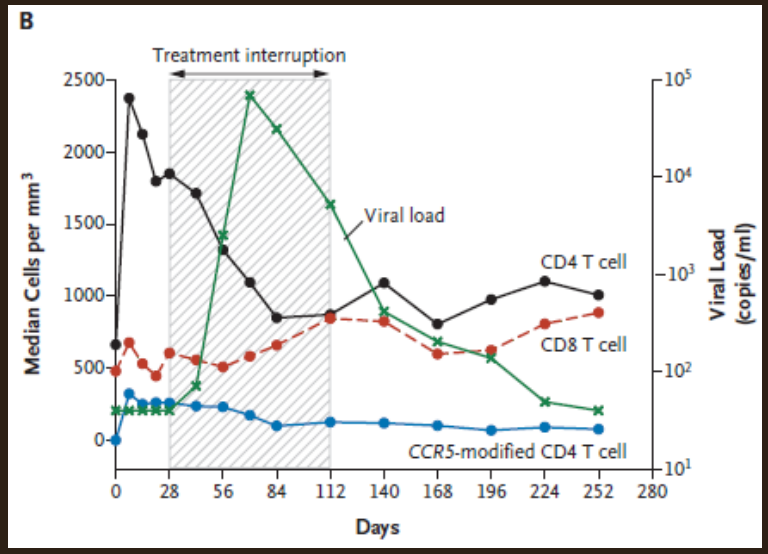
Gene Editing of *CCR5 in Autologous CD4 T Cells* in HIV Infected patients

Changes in Viremia during Treatment Interruption



HIV viral loads for the six patients in cohort 1

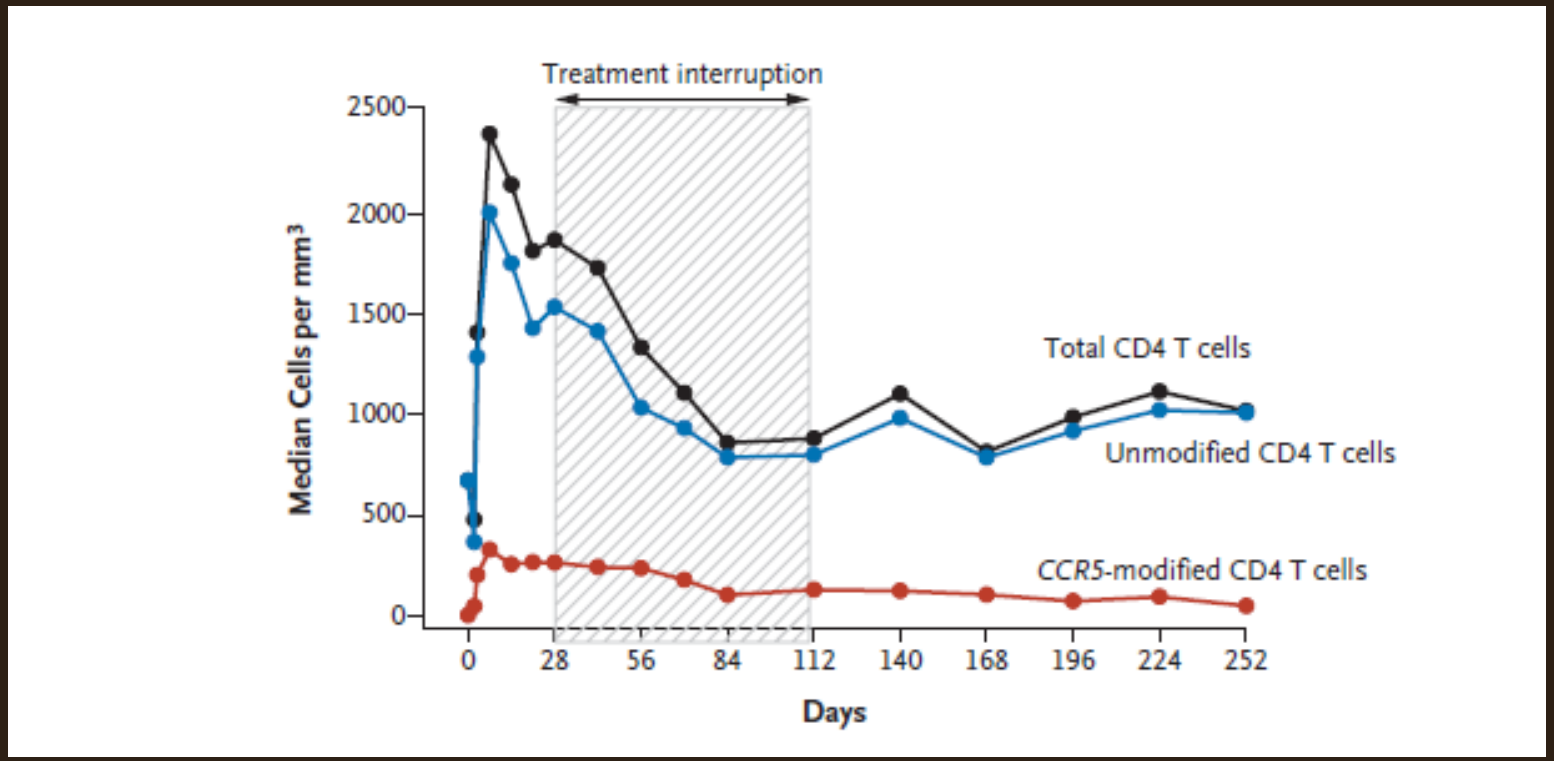
CD4 T-cell, CD8 T-cell, viral load and *CCR5-modified* T-cell counts in cohort 1 during the treatment interruption



Pablo Tebas et al., N Engl J Med 2014

Gene Editing of *CCR5 in Autologous CD4 T Cells* in HIV Infected patients

CCR5-Modified CD4 T Cells during Treatment Interruption.



Pablo Tebas et al., N Engl J Med 2014

Specificity of cleavage of ZFN

- Two new papers raise questions about the purported specificity of these genome modification tools.
- They show that ZFNs, in addition to cleaving at their desired sites, can also have unexpected cleavage effects *in vivo* that cannot be predicted using conventional *in silico* analyses.
- These findings could have important consequences for the safe use and optimization of ZFNs in gene therapy.

Pattanayak, V., et al *Nat. Meth.* 2011
Gabriel, R. *et al. Nat. Biotechnol.* 2011

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Dante Gabriel Rossetti: Pandora (1879)