British HIV Association
BHIVA

Dr Geoff Nichol

Sangamo BioSciences, USA

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Sangamo BioSciences, USA

COMPETING INTEREST OF FINANCIAL VALUE ≥ £1,000:	
Speaker Name	**Statement**
Dr Geoffrey Nichol	Is an employee and US Section 16 Officer at Sangamo BioSciences; receives salary and holds shares and share options in Sangamo BioSciences
Date	**October 2014**

CCR5 knockout gene therapy trials

Geoff Nichol MB ChB FRACP
Executive Vice-President, R&D
Sangamo BioSciences

BHIVA Autumn Conference
2014

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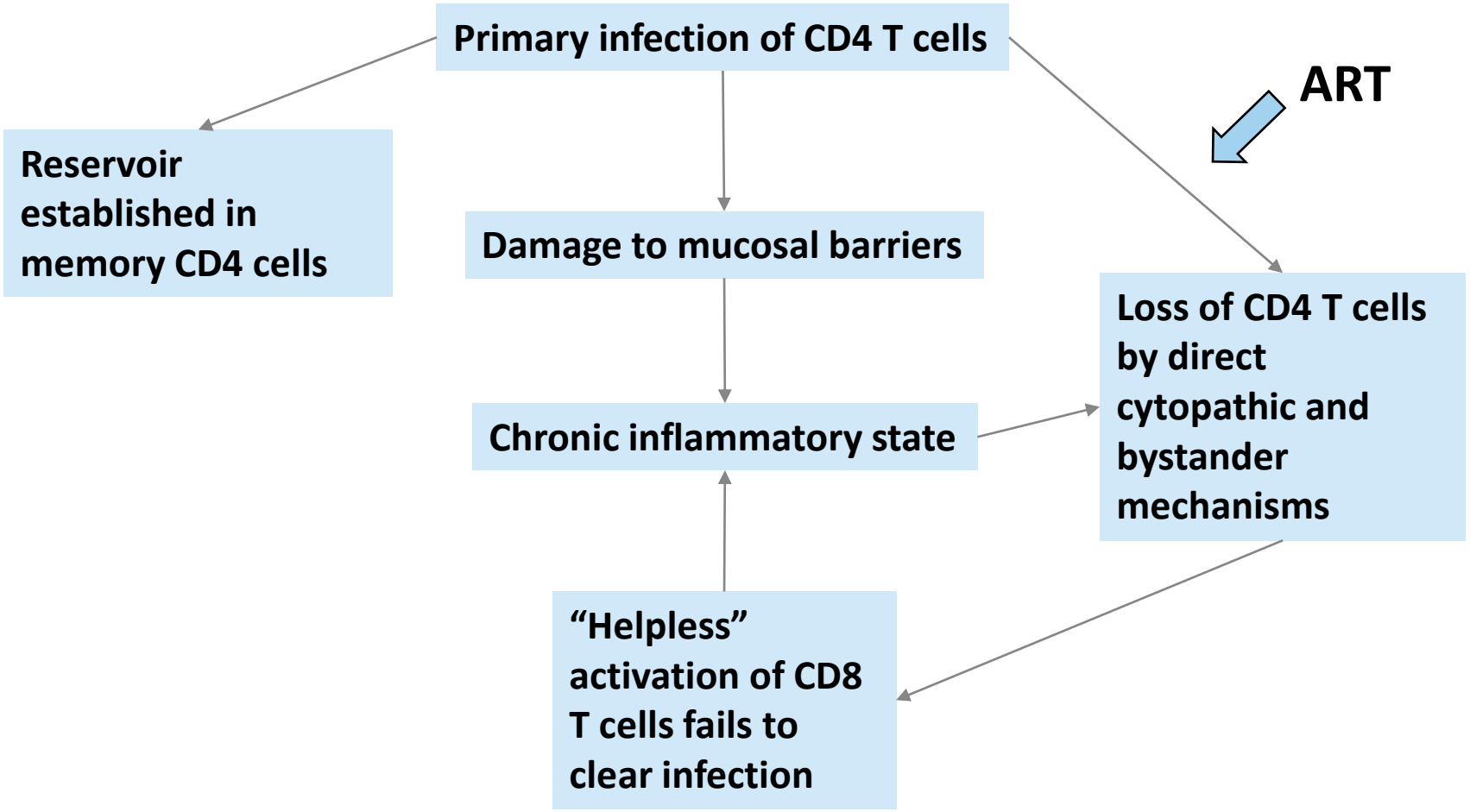
HIV – an infection and an immune system disease



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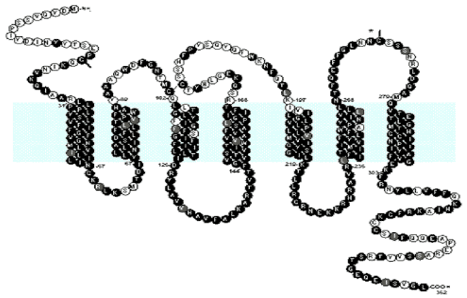
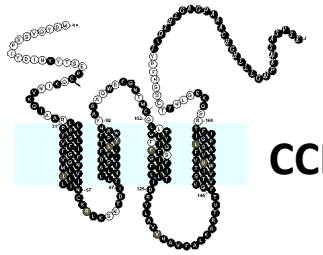
Blocking the narrow door
A lesson from Nature – the CCR5△32 mutation



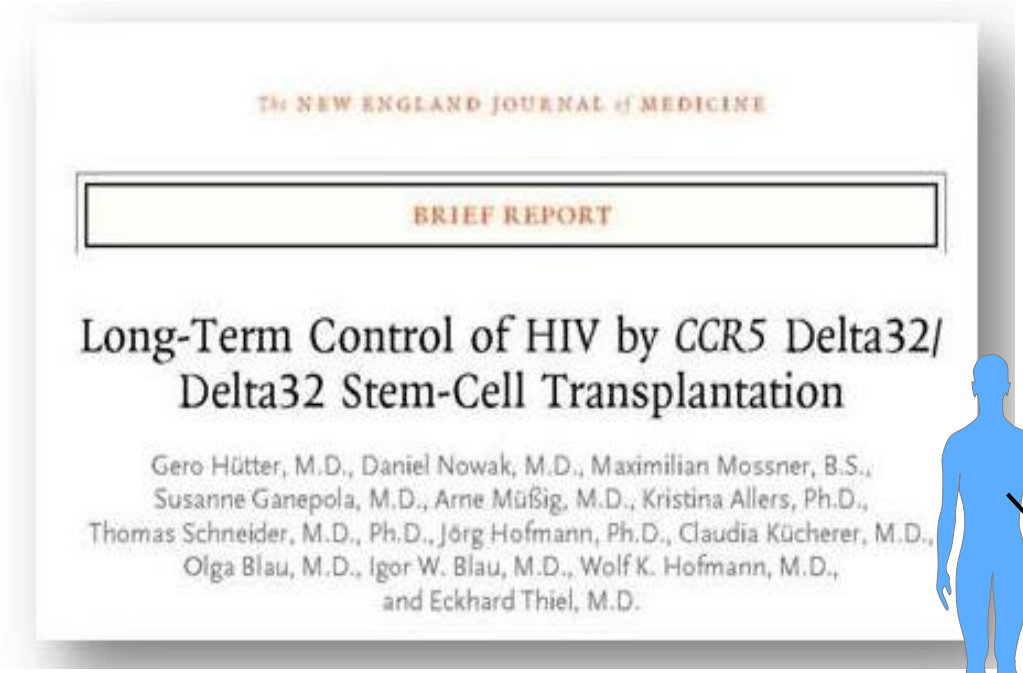
CCR5



CCR5△32

Individuals homozygous for the CCR5△32 allele are highly resistant to HIV-1 infection

“Berlin patient”

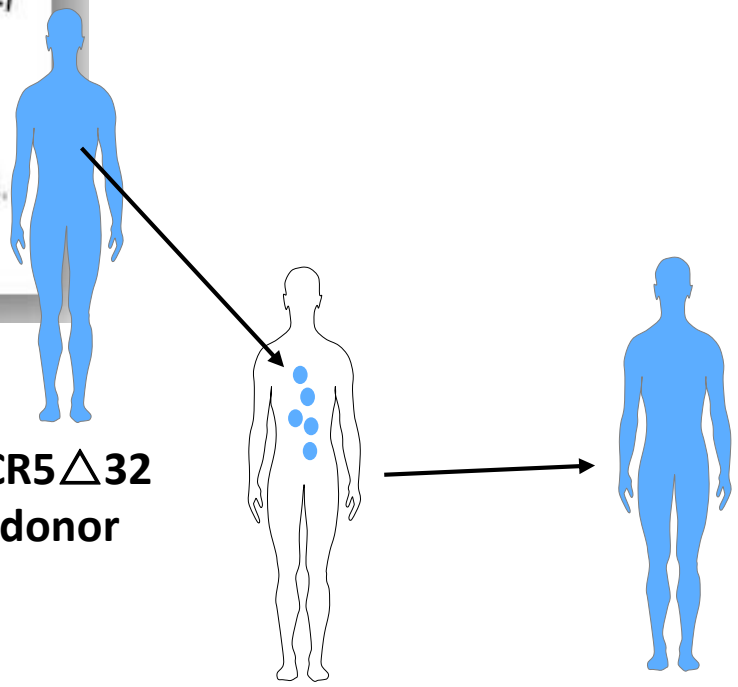


The NEW ENGLAND JOURNAL of MEDICINE

BRIEF REPORT

Long-Term Control of HIV by *CCR5* Delta32/Delta32 Stem-Cell Transplantation

Gero Hütter, M.D., Daniel Nowak, M.D., Maximilian Mossner, B.S., Susanne Ganepola, M.D., Arne Müßig, M.D., Kristina Allers, Ph.D., Thomas Schneider, M.D., Ph.D., Jörg Hofmann, Ph.D., Claudia Kücherer, M.D., Olga Blau, M.D., Igor W. Blau, M.D., Wolf K. Hofmann, M.D., and Eckhard Thiel, M.D.

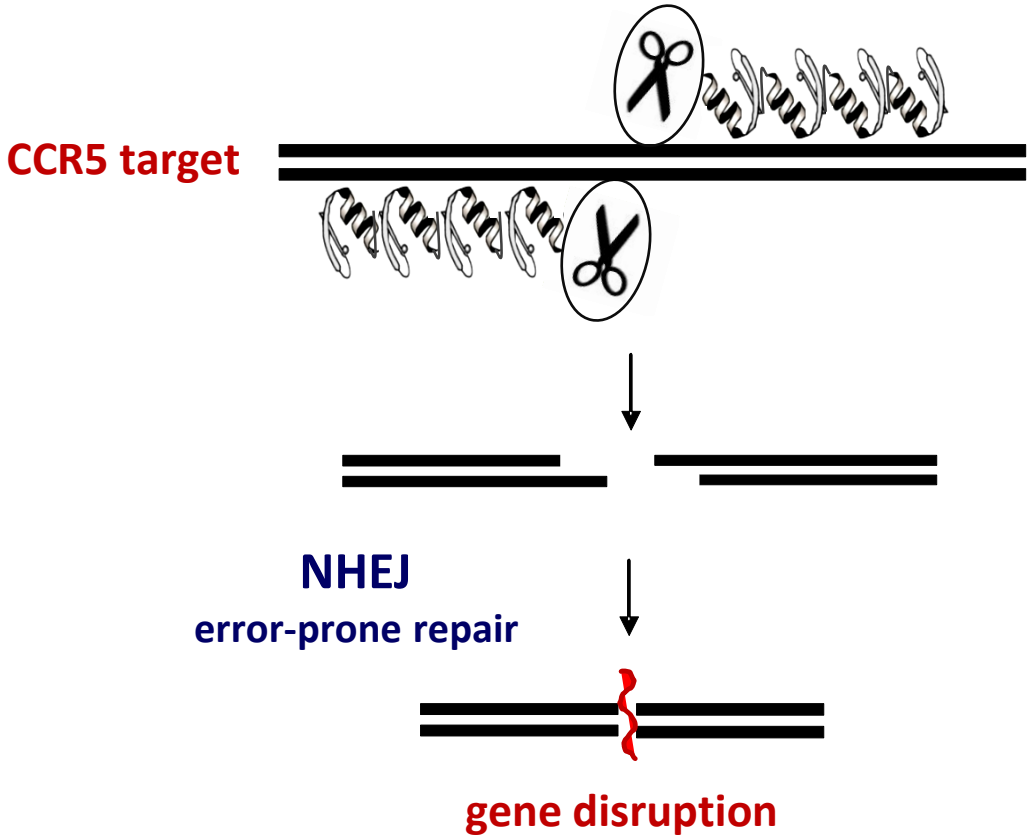


CCR5△32 donor

“Berlin patient”

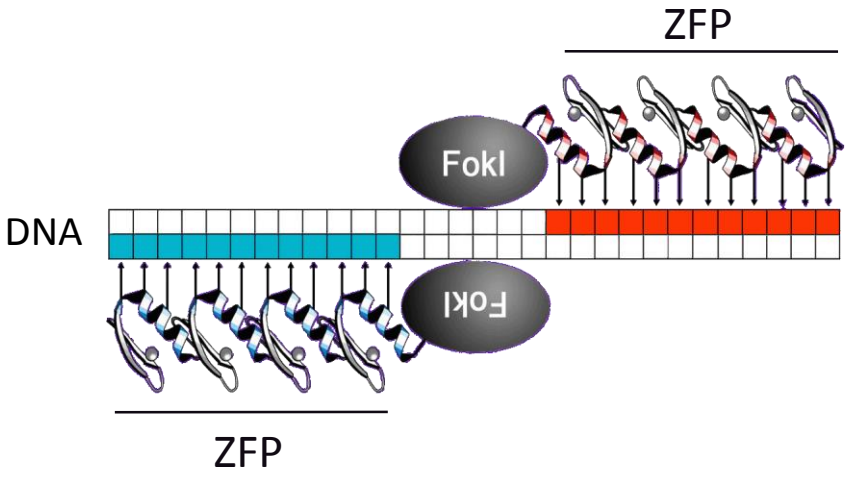
SB-728-T – the product

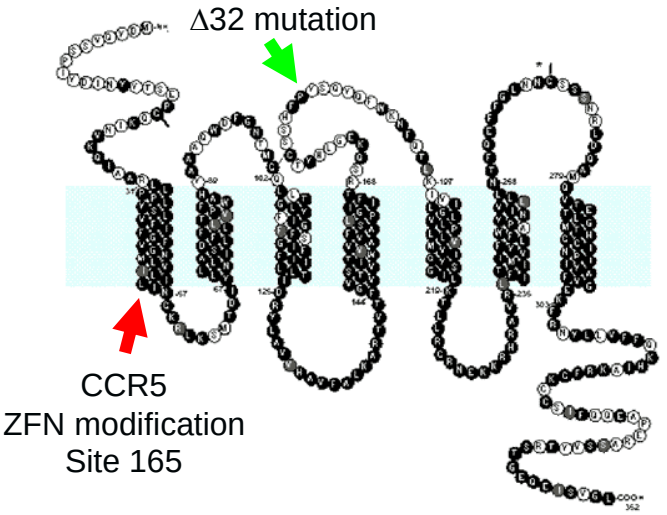
ZFNs cause targeted gene disruption



Zinc finger nucleases (ZFNs)

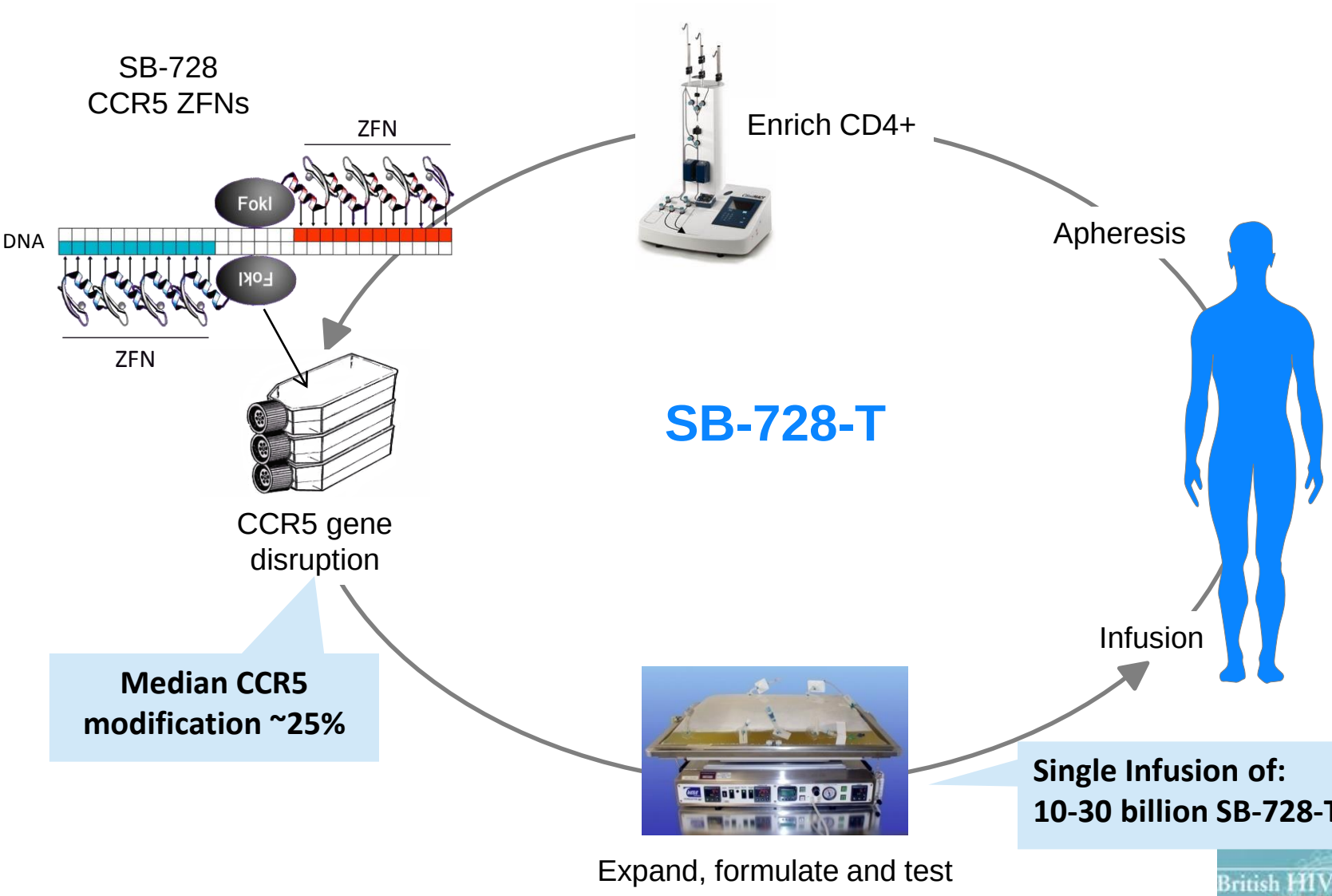
"Designer restriction enzyme"





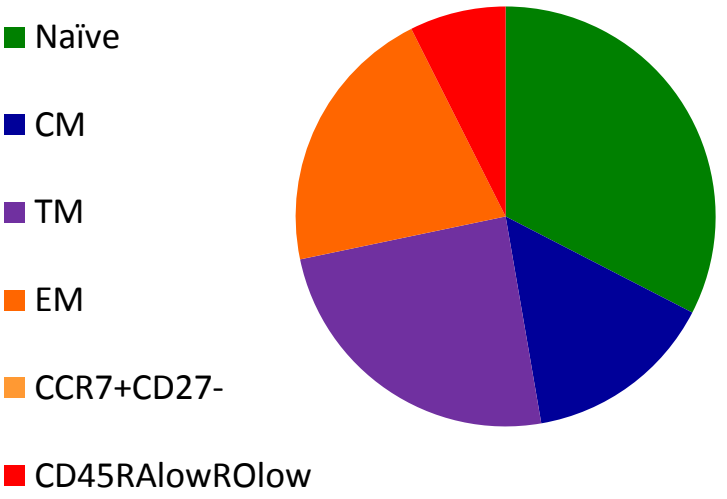
- Delivered with a non-integrating, replication-deficient, chimeric adenoviral 5/35 vector or mRNA electroporation

SB-728-T: Zinc finger nuclease driven CCR5 modified autologous CD4+ T-cells

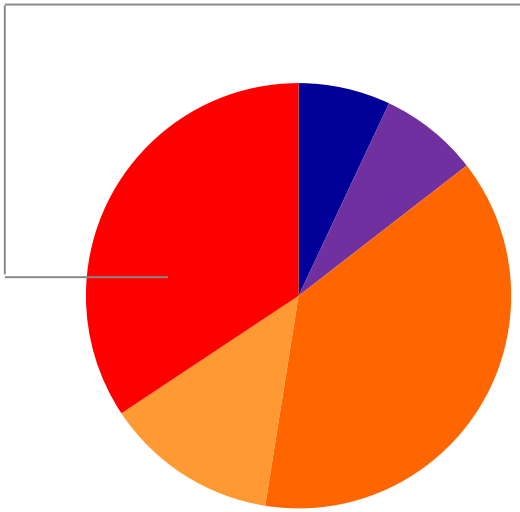


The infused product (SB-728-T) contains T-cells with a stem cell-like phenotype

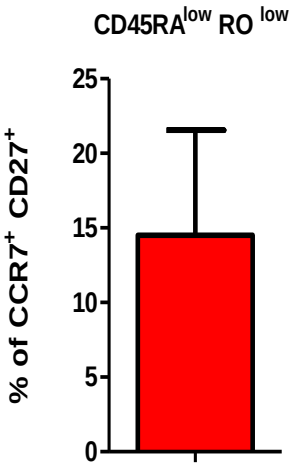
CD4+
pre-CCR5 modification

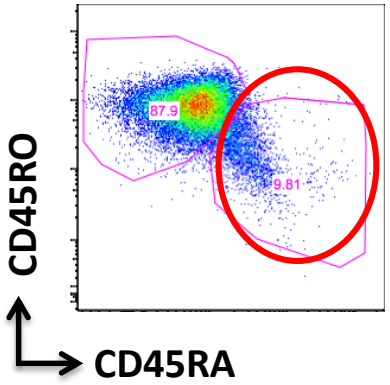


CD4+
SB-728-T



Memory Stem Cell-Like





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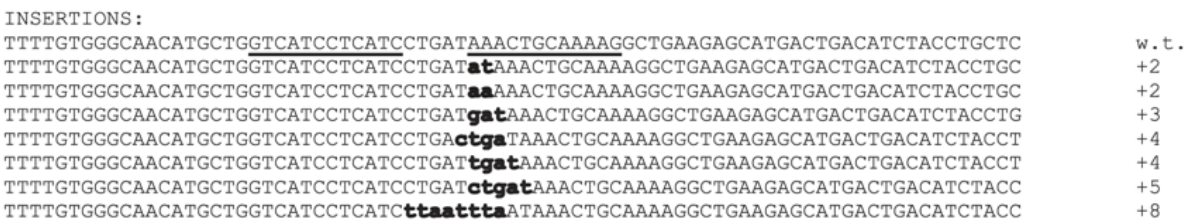
How we assay for CCR5 deletions

- ZFN mediated gene disruption generate a diverse array of short insertions and deletions to the targeted CCR5 locus.



- Most frequently is a 5-bp insertion or “Pentamer Duplication” (CTGAT)
  - Approximately 16 to 39% (mean = 23%) of CCR5 allele disruptions

- In clonal studies bi-allelic disruption occurs in about 1/3 of disrupted cells – total CCR5 knockout
  - 2/3 if one allele already has the Δ32 mutation

SB-728 – key exploratory clinical studies

Study	Study Goal
SB-728-T-902 Cohorts 1-3 (n=9) • Immune non-responders (INR) on ART; longitudinal follow-up 3 years	**Reservoir Depletion / Elimination & Immune Reconstitution**
Phase I study at U Penn (n=6) • ART-treated subjects, treatment interruption (TI)	**Demonstrate Immunological Control of Viral Growth without ART**
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SB-728-T-1101 Cohorts 1-5 (n=18) • Cytoxan preconditioning, ART, TI	
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SB-728-HSC (planned) • mRNA electroporation in HSPCs	

SB-728-T – pharmacokinetics and pharmacodynamics

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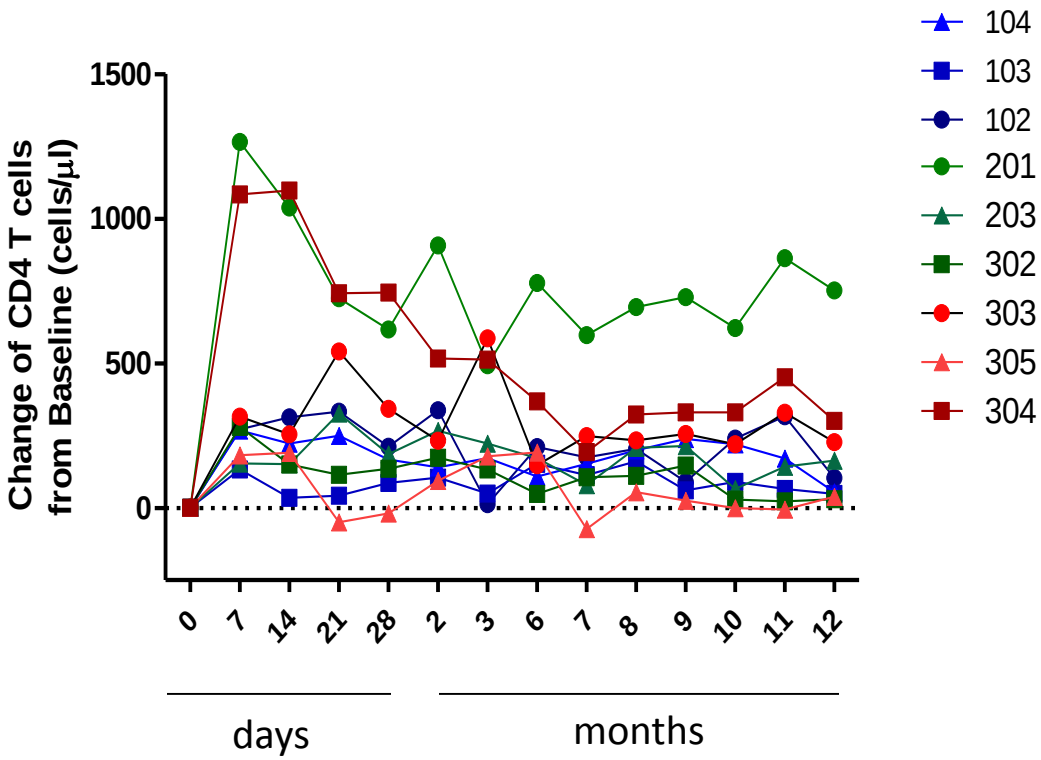
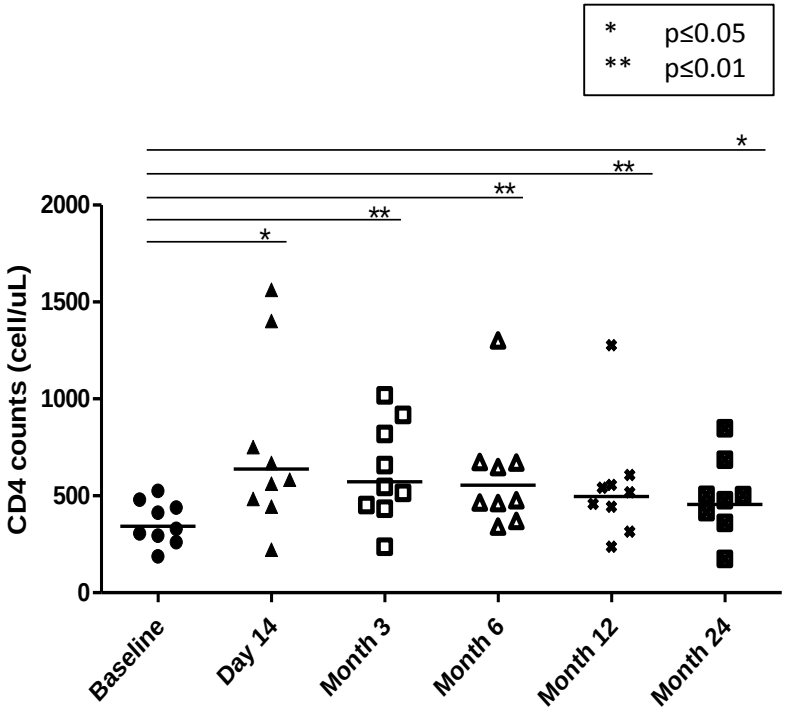
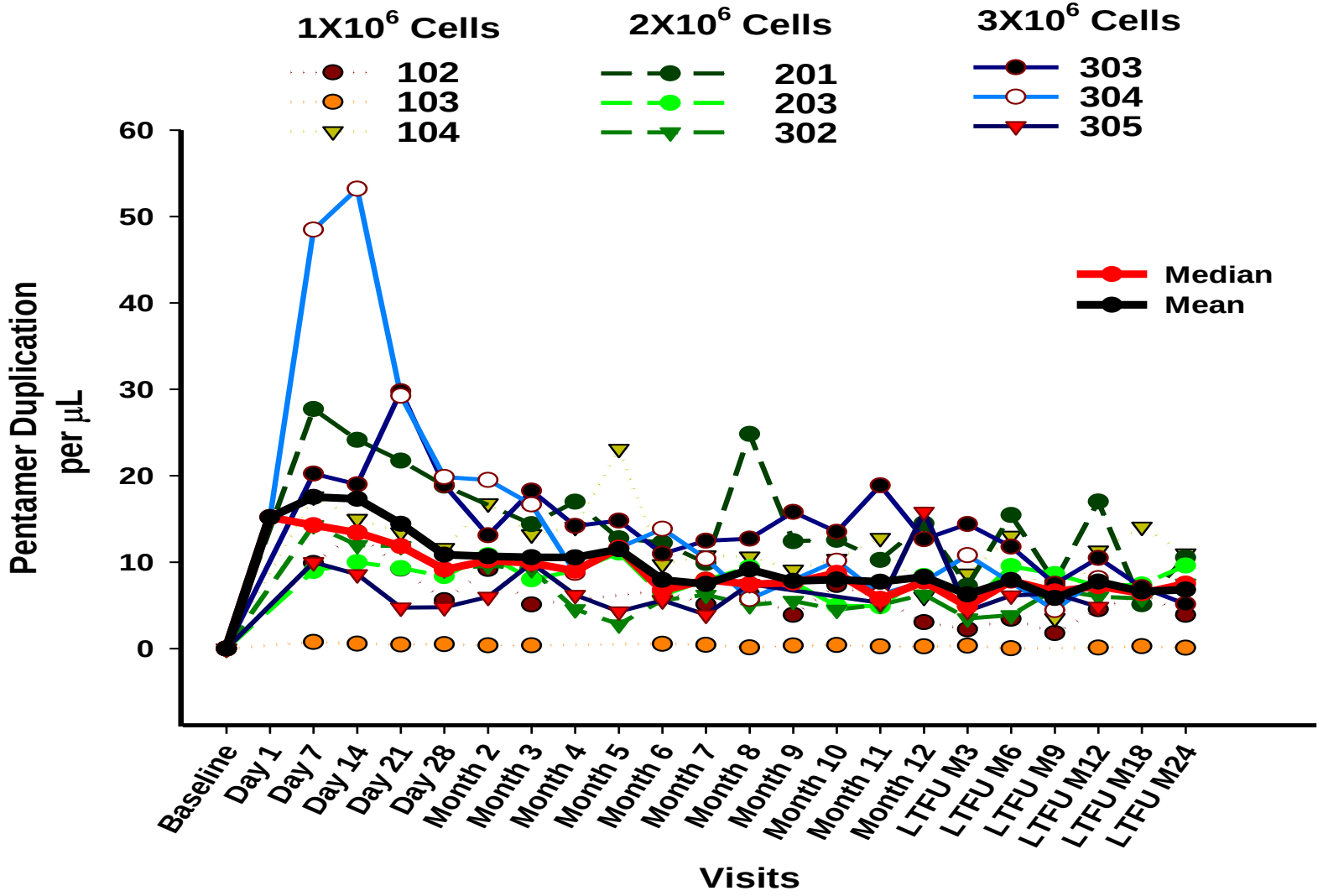
Long-term CD4 T-cell reconstitution post SB-728-T



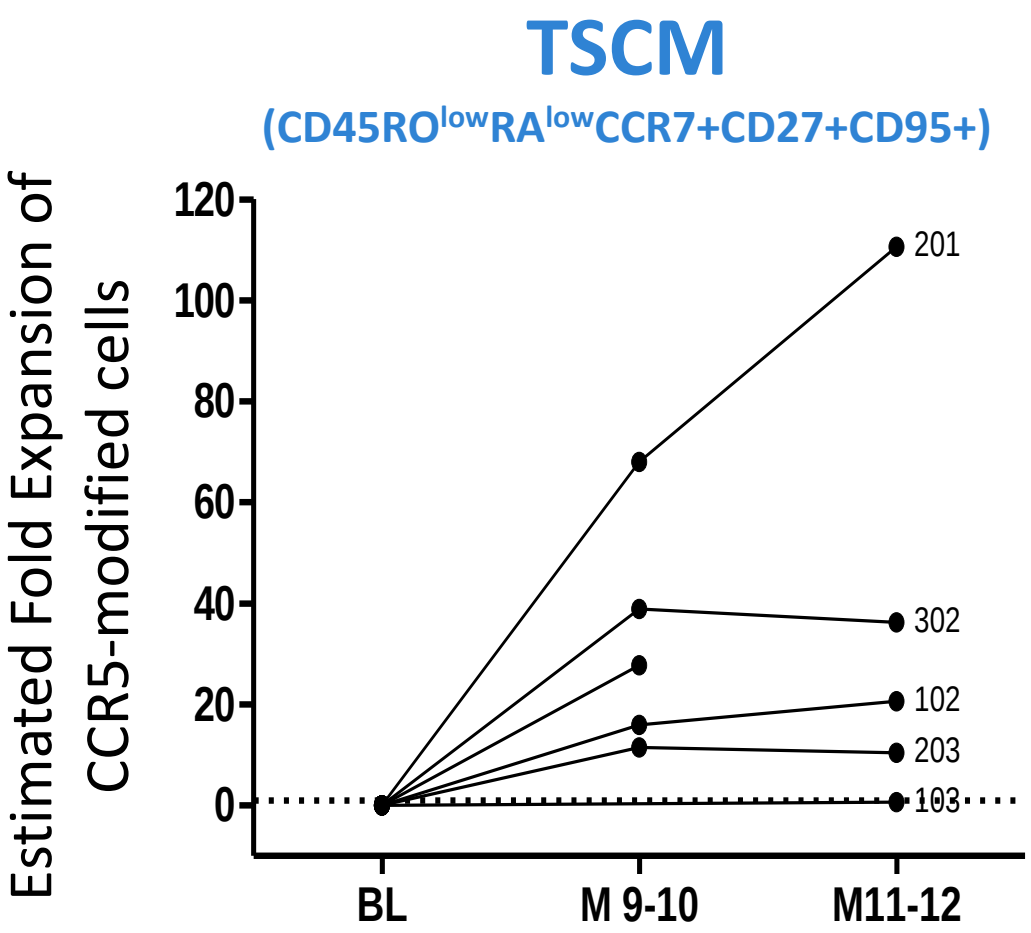


Infusion of CCR5-disrupted cells led to a sustained significant increase in CD4 T cell counts (mean of 103 cells/µL at 12 Months)

Long-term engraftment of CCR5 modified cells

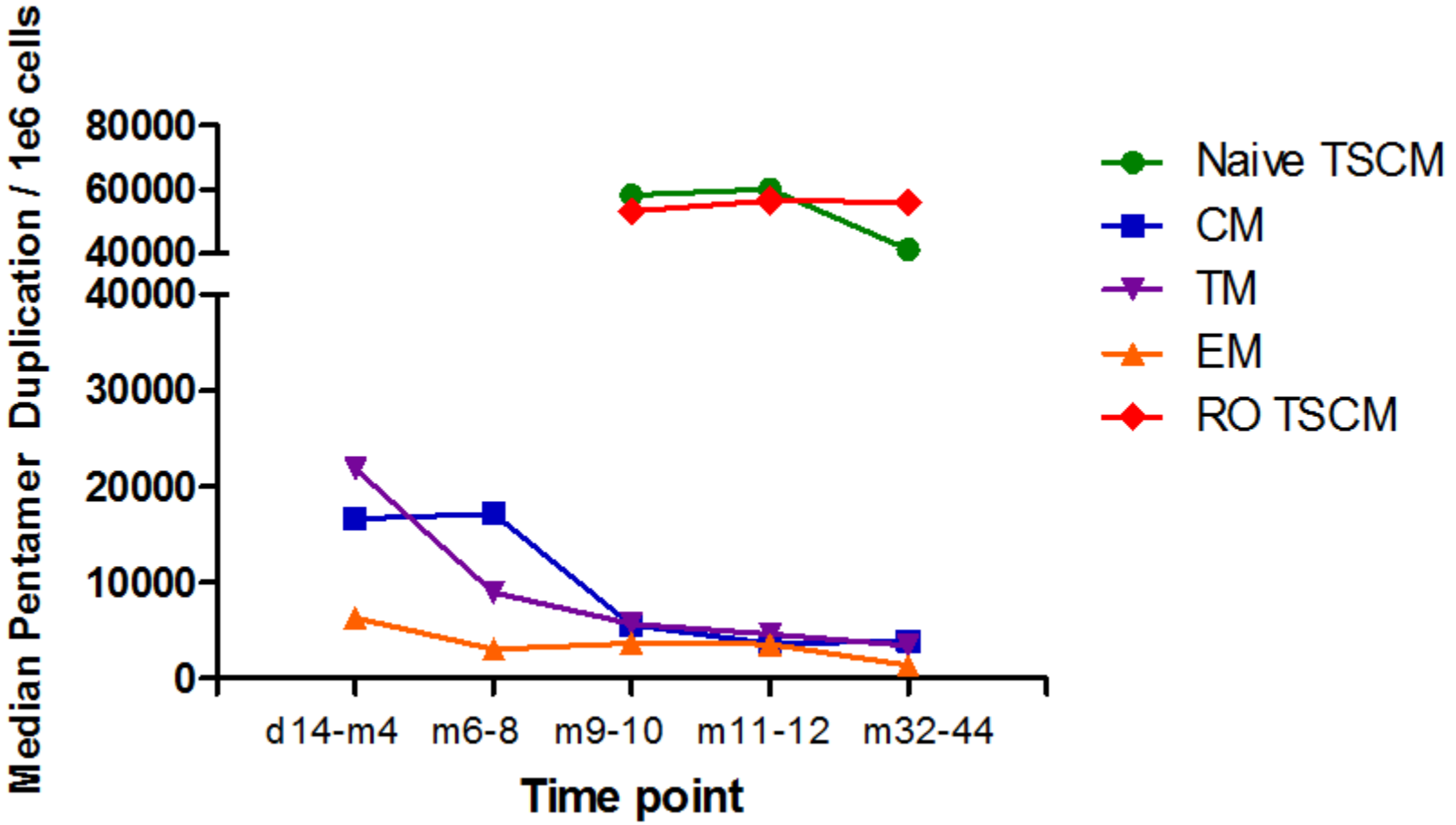


CCR5 modified T-memory stem cells expand and persist up to 12 months

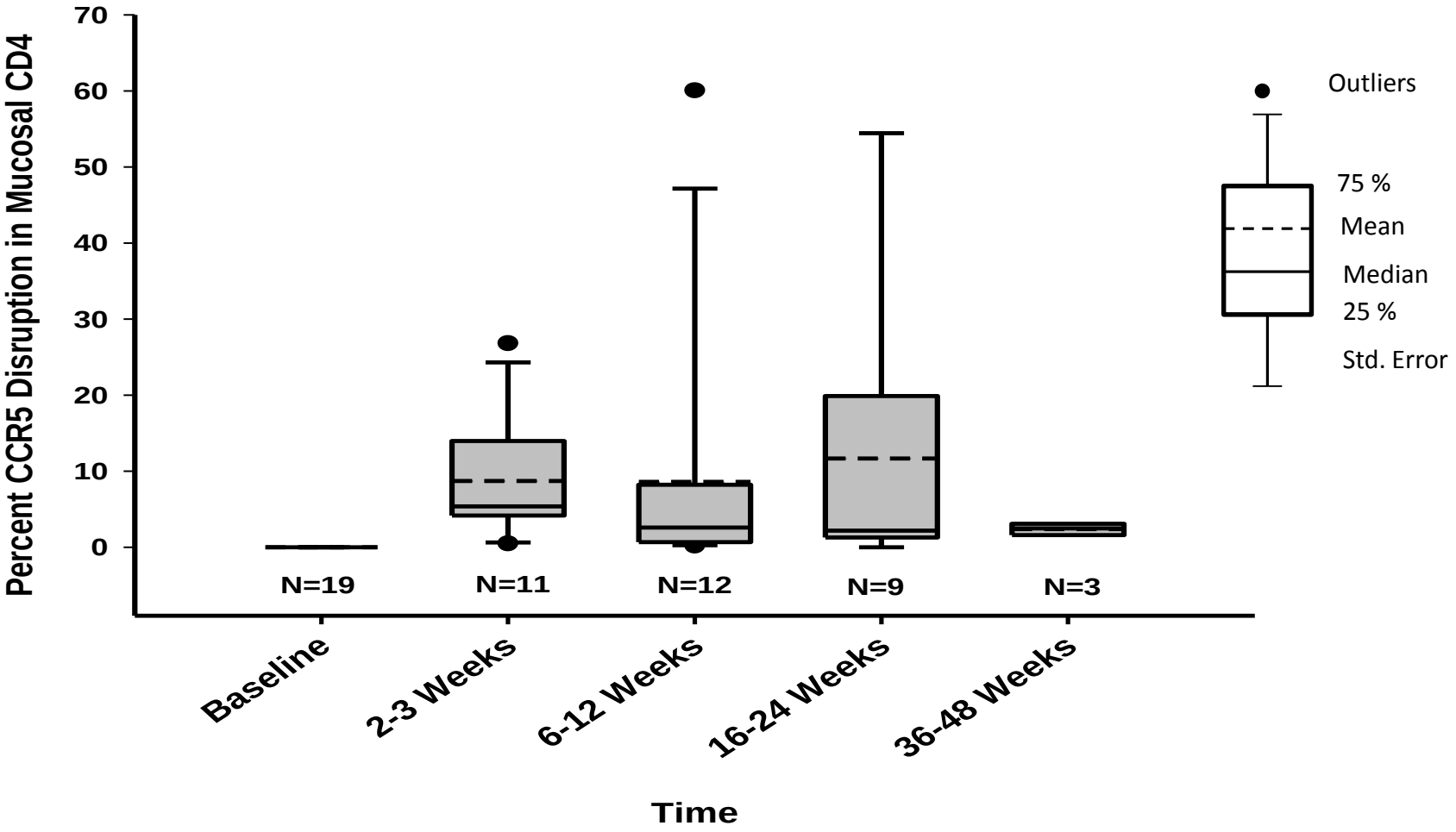


- Median fold expansion of CCR5-modified cells relative to amount infused was **20.7 at Month 12**
- In contrast, fold expansion in modified CM and EM were ~ 3 fold and < 1 fold

CCR5 gene modification level is maintained for three years in the TSCM fractions



SB-728-T traffics to the rectal mucosa



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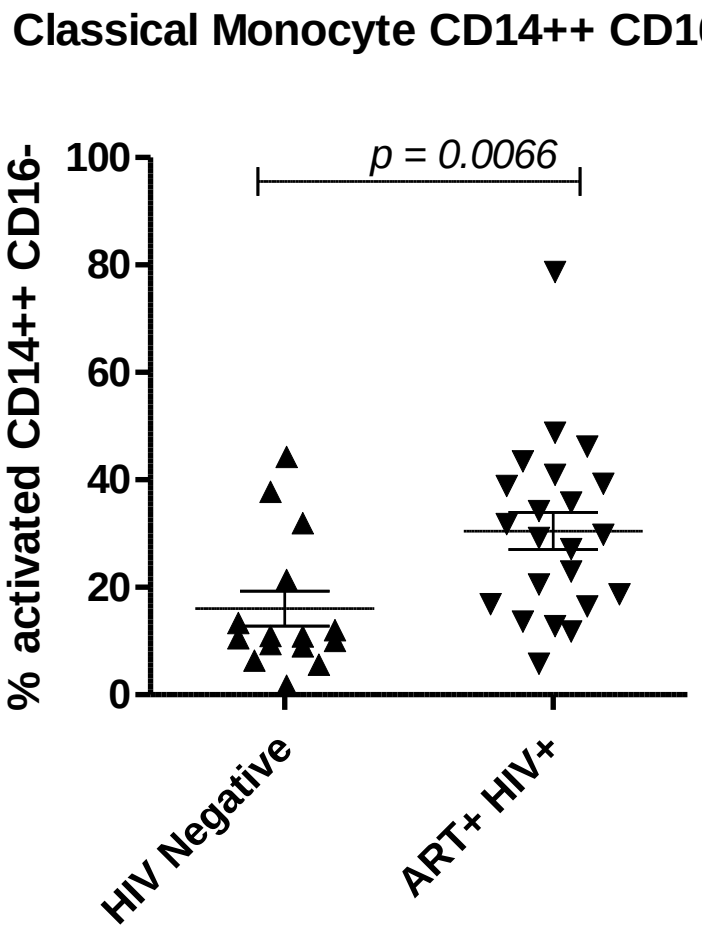
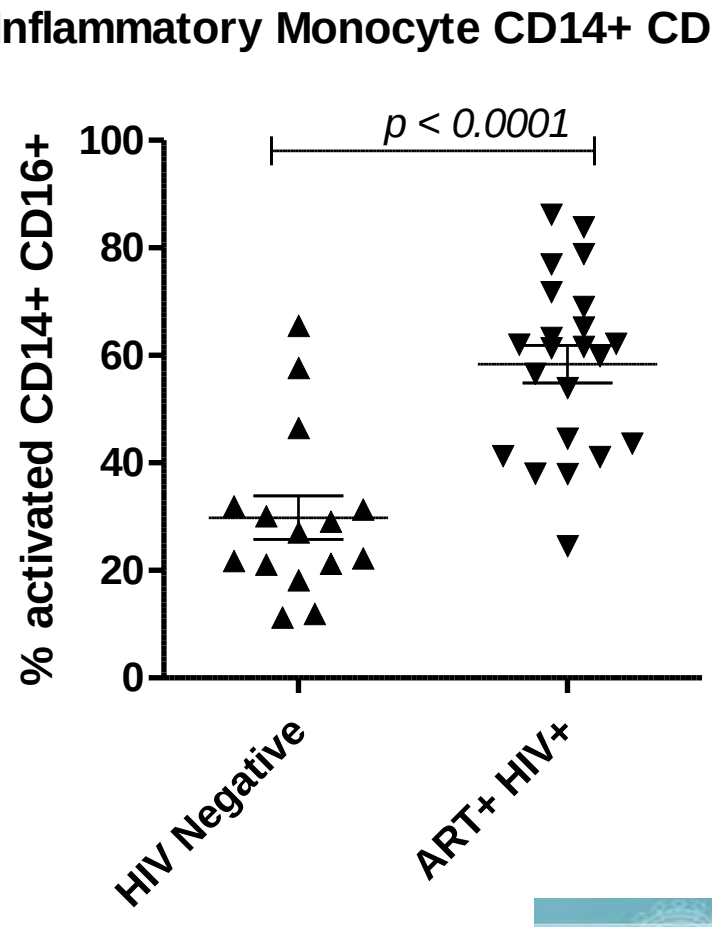
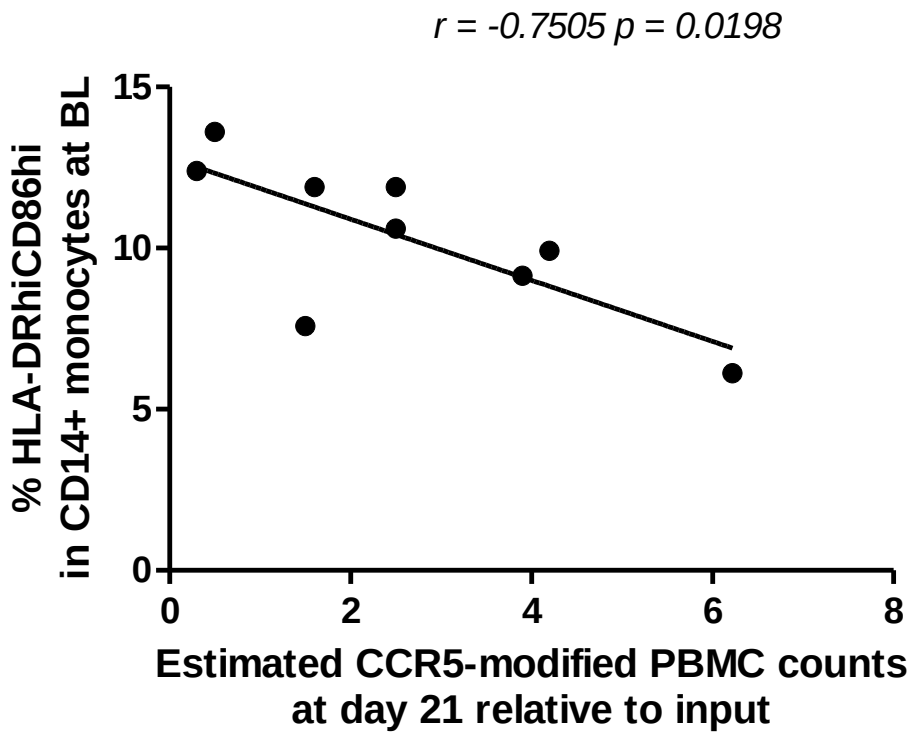
High levels of monocyte activation (DRhiCD86hiCD40hi) in HIV+ subjects at baseline



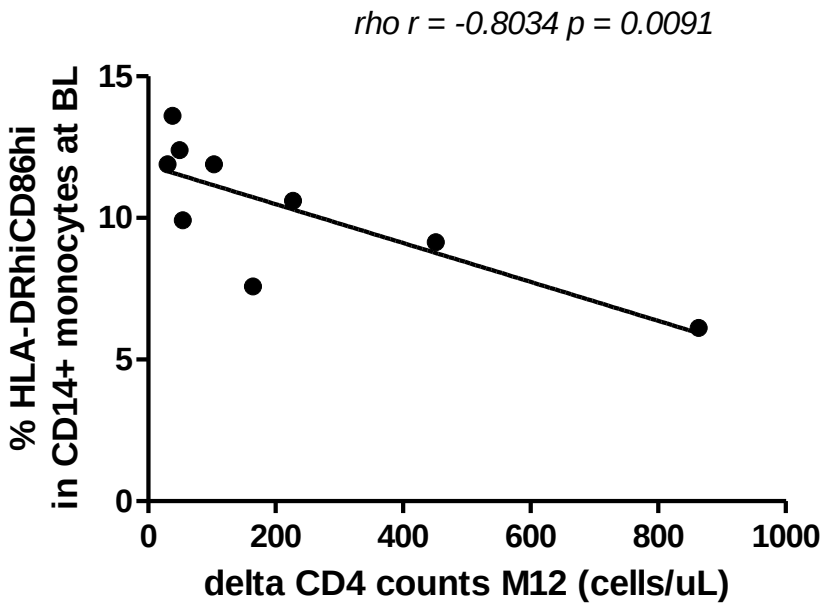


Baseline levels of monocyte activation inversely correlate with levels of CCR5-modified cell engraftment

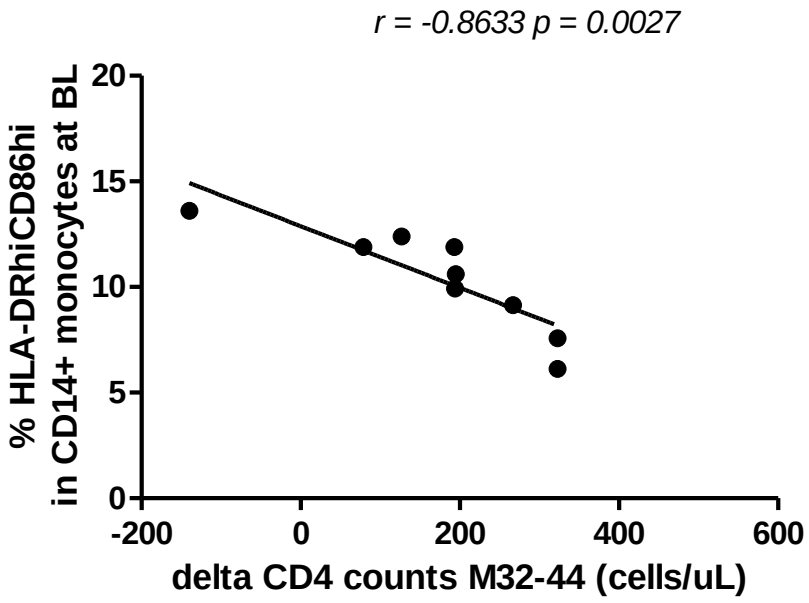


Baseline levels of monocyte activation inversely correlate with levels of CD4 T-cell reconstitution

CD4 Persistence after 1 Year



CD4 Persistence after 3 Years



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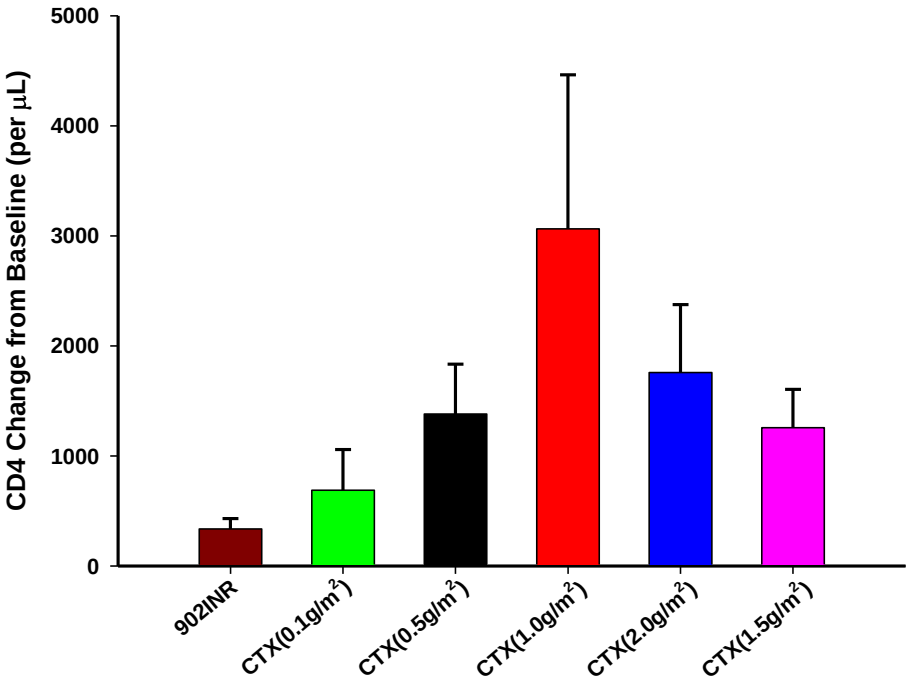
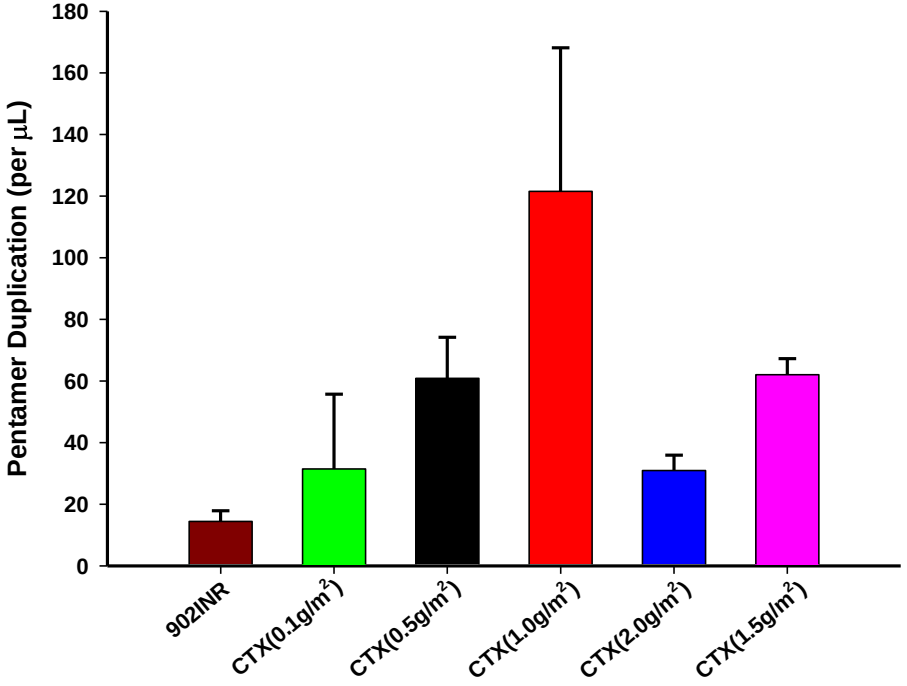
SB-728mR-T 1401 (commencing) • mRNA electroporation, ART, TI	
SB-728-HSC (planned) • mRNA electroporation in HSPCs	

Higher peak CD4 T-cell reconstitution and engraftment of SB-728-T is observed at a dose of 1 gm/m2 CTX

CD4 change from baseline



Pentamer duplication



SB-728-T – effects on viral load during ART interruption

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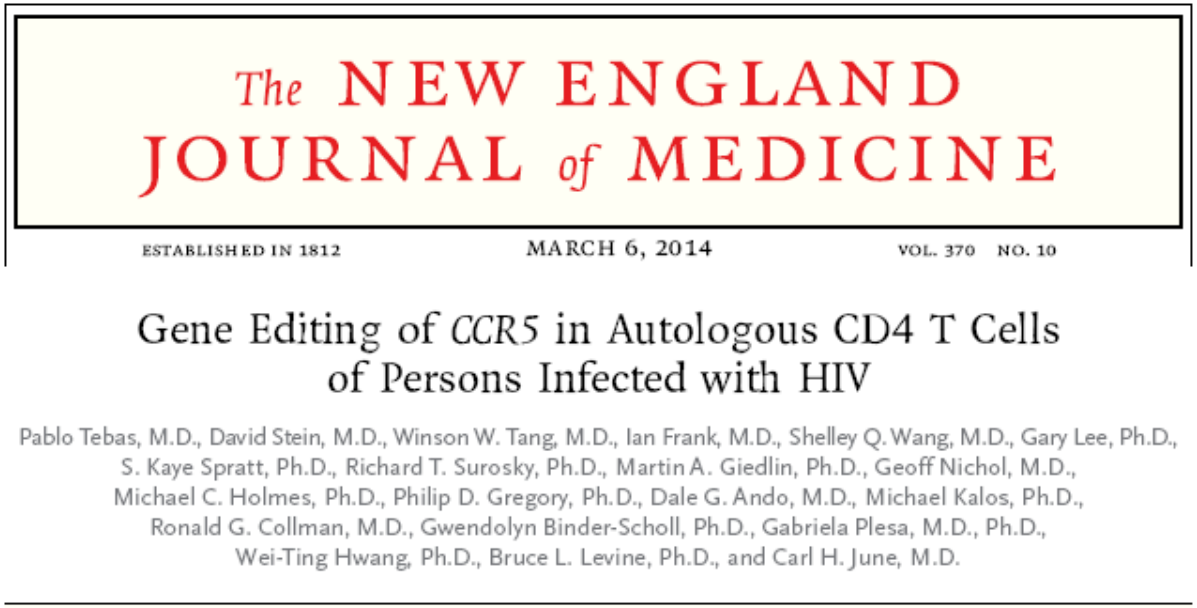
First-in-human study of CCR5 KO published in NEJM 6 March 2014



The NEW ENGLAND JOURNAL of MEDICINE

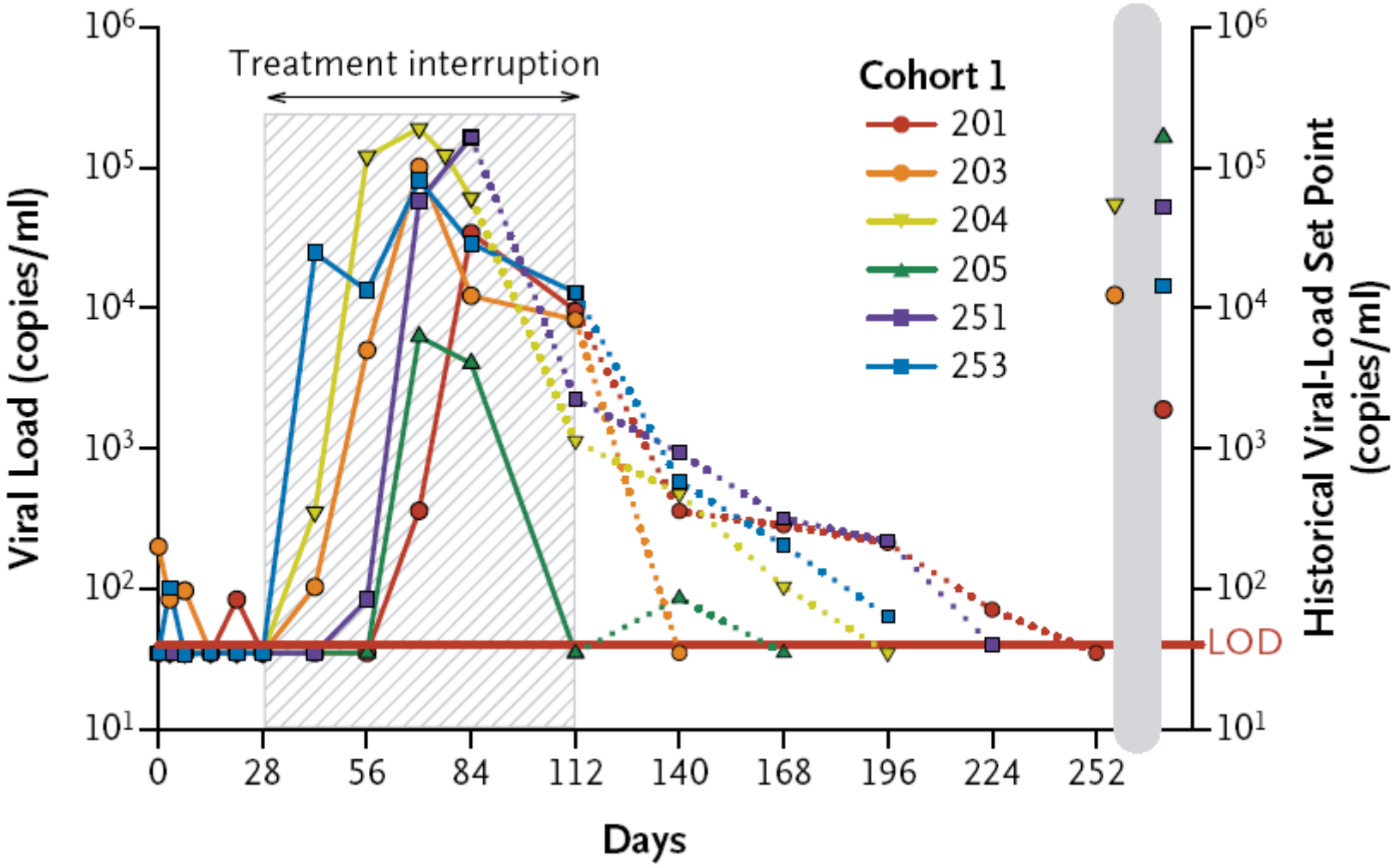
ESTABLISHED IN 1812 MARCH 6, 2014 VOL. 370 NO. 10

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

Pablo Tebas, M.D., David Stein, M.D., Winson W. Tang, M.D., Ian Frank, M.D., Shelley Q. Wang, M.D., Gary Lee, Ph.D., S. Kaye Spratt, Ph.D., Richard T. Surosky, Ph.D., Martin A. Giedlin, Ph.D., Geoff Nichol, M.D., Michael C. Holmes, Ph.D., Philip D. Gregory, Ph.D., Dale G. Ando, M.D., Michael Kalos, Ph.D., Ronald G. Collman, M.D., Gwendolyn Binder-Scholl, Ph.D., Gabriela Plesa, M.D., Ph.D., Wei-Ting Hwang, Ph.D., Bruce L. Levine, Ph.D., and Carl H. June, M.D.

- First genome edited therapy tested in man (ZFN modified CD4+ T cells)
- Infusions generally safe and well tolerated
- Marked increases in total CD4+ T cell levels
- Traffic to GALT (key battle ground of HIV infection)
- Modified cells show a selective survival advantage during ARD interruption
- One subject controlled viral load to below levels of detection prior to reinstating ARD

HIV viral load during treatment interruptions.

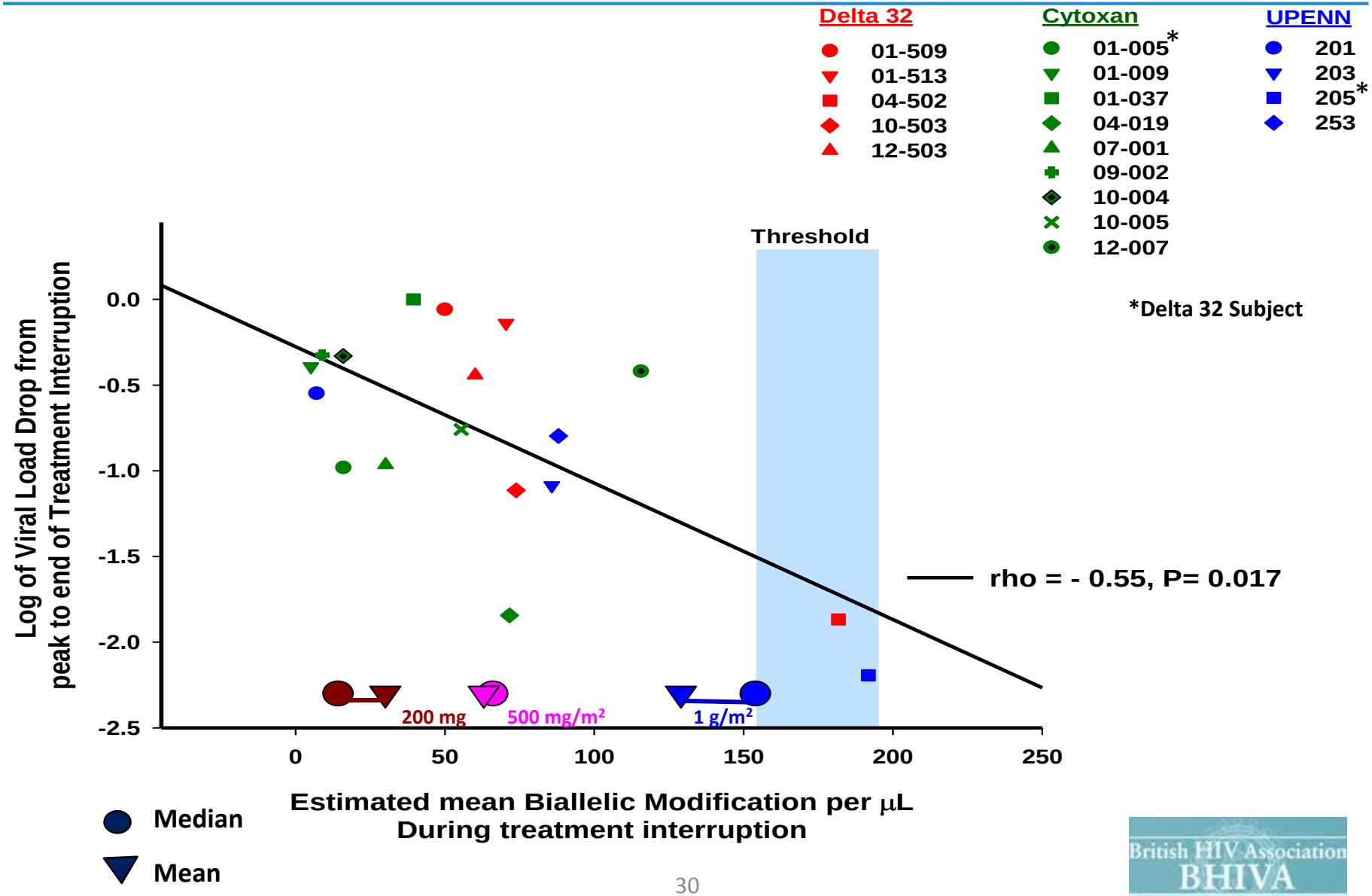


Tebas et al, 2014

British HIV Association BHIVA

Changes in VL correlate with levels of biallelic modification

6-week bi-allelic engraftment following Cytoxan - approaching threshold?



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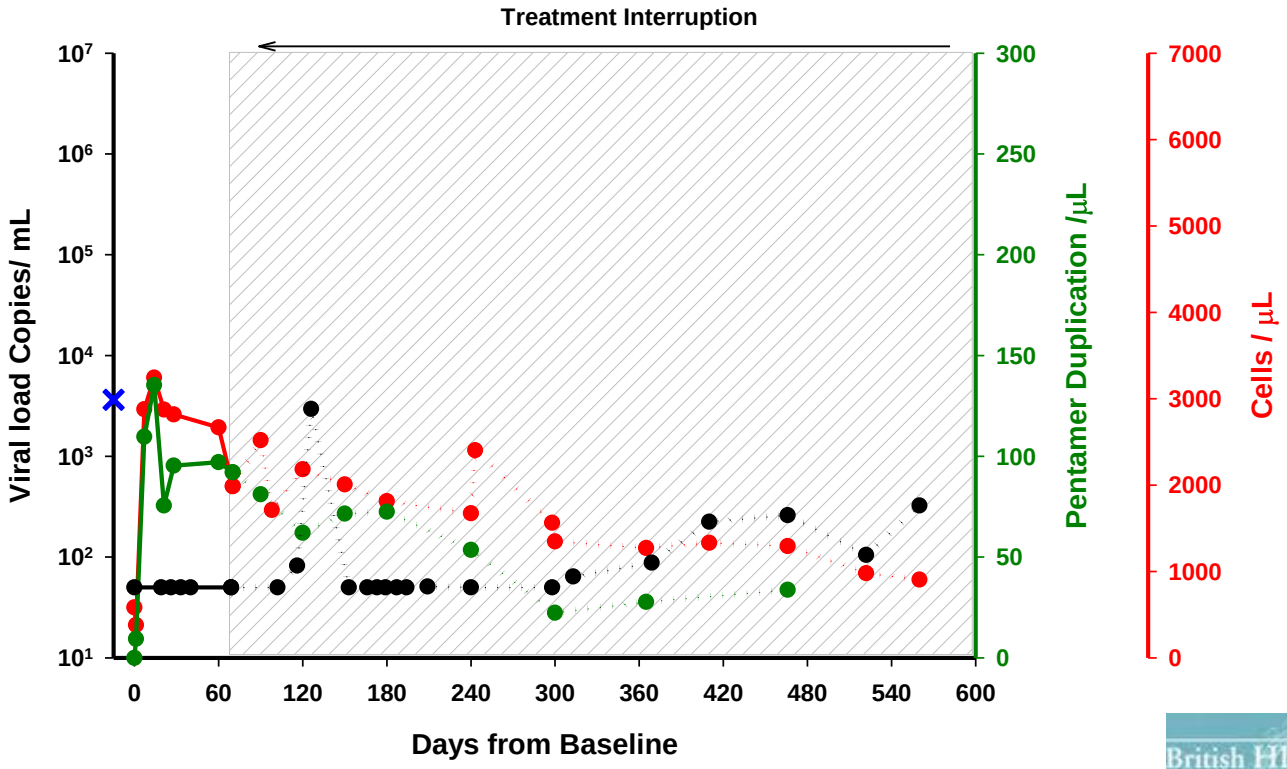
SB-728-HSC (planned) • mRNA electroporation in HSPCs	

Sustained functional control of viral load for more than one year

- Subject 04-502 (SB-728-902 Cohort 5)
  - Viral load controlled for more than 59 weeks (<500 VL copies/mL)
  - Subject remains off ART
  - Durable functional control achieved



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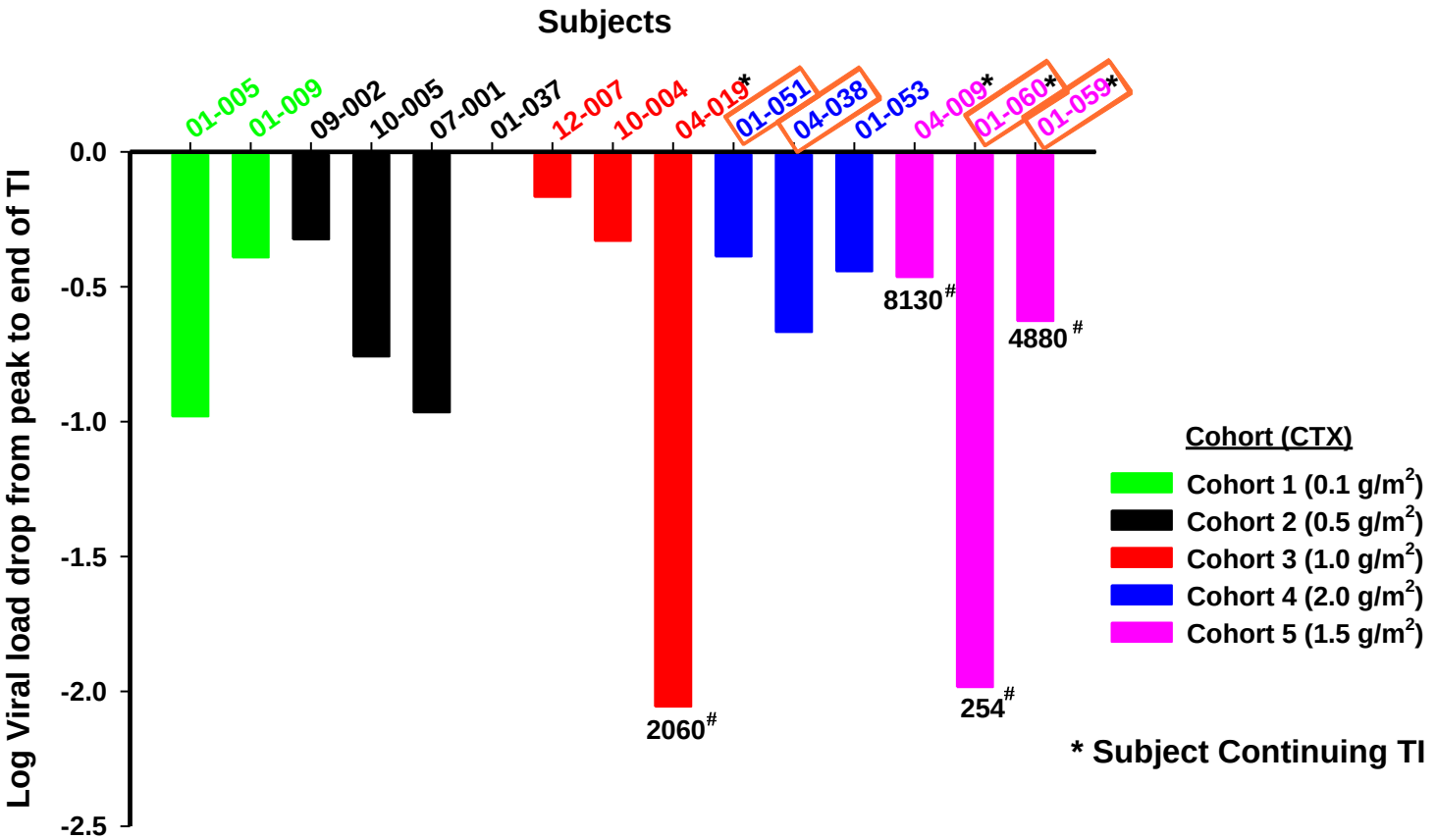
SB-728-1101: Viral load decreases from peak during TI

Four subjects with extended TI with VL <10,000 copies and CD4>500

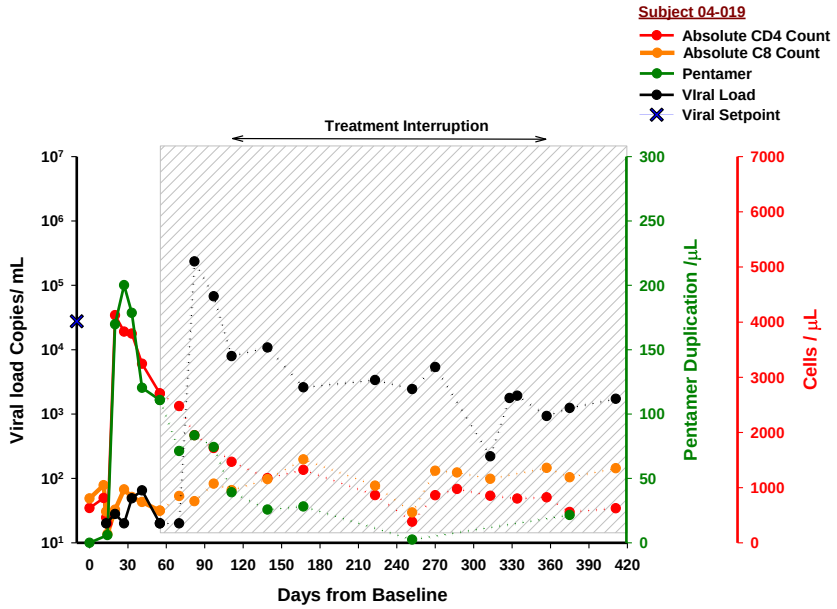


TI= Treatment interruption
Red box: Δ32 Heterozygote
Viral Load: Copies/mL

Meaningful reductions in VL seen during TI in Cytoxan-treated subjects

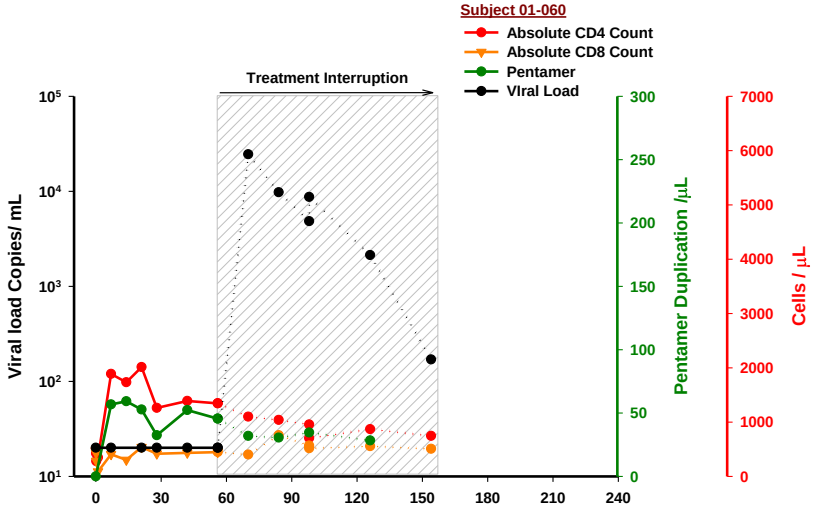
Subject 04-019 (SB-728-1101)

- CTX dose – 1.0 gm/m^2
- >2 log reduction in Viral load (VL)
- Sustained control for 39 weeks
- Subject remains off ART



Subject 01-060 (SB-728-1101)

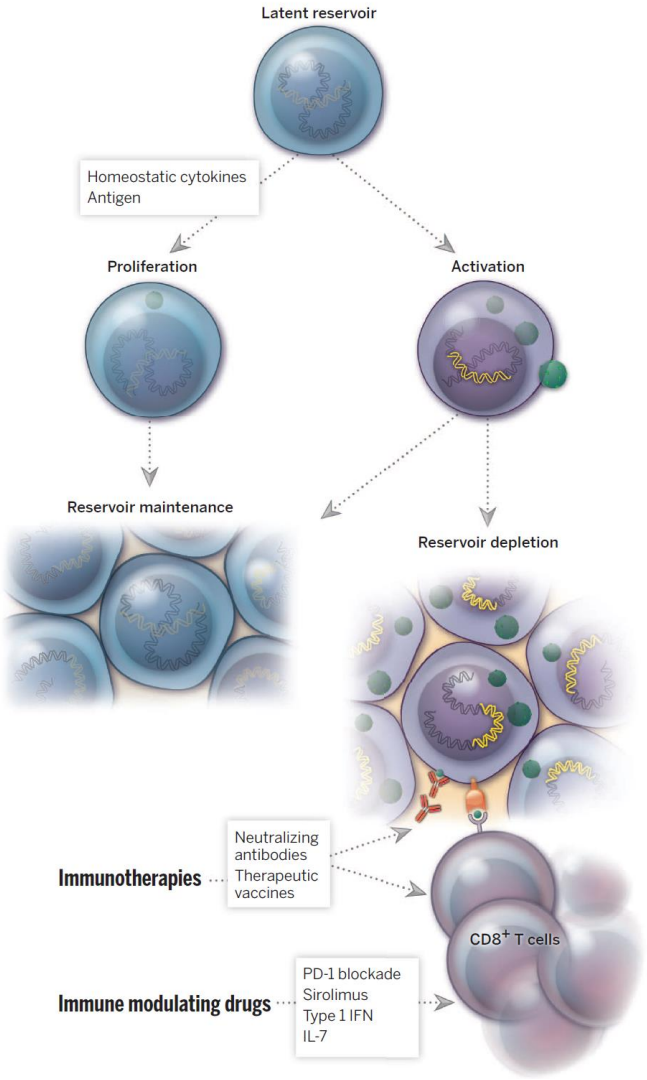
- CTX dose – 1.5 gm/m^2
- >2 log reduction in VL
- Subject remains off ART



SB-728-T – effects on the HIV reservoir

HIV reservoir

- Laid down at time of initial infection
- HIV DNA integrated within CD4 memory cells
- Reservoir size driven by time from infection to start of ART
- Highly stable on chronic ART
- Maintenance is a dynamic process
  - Activation cycling of CD4 reservoir cells creates a target for immunotherapies



Barouch and Deeks, Science 345, 169 (2014)

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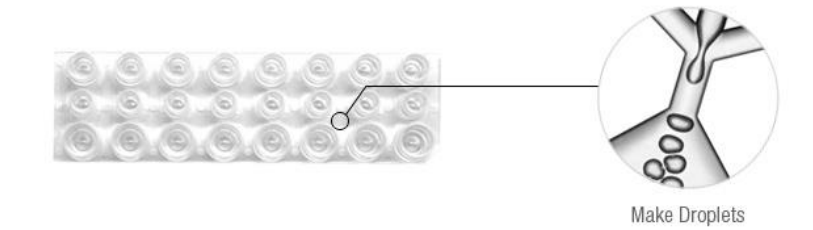
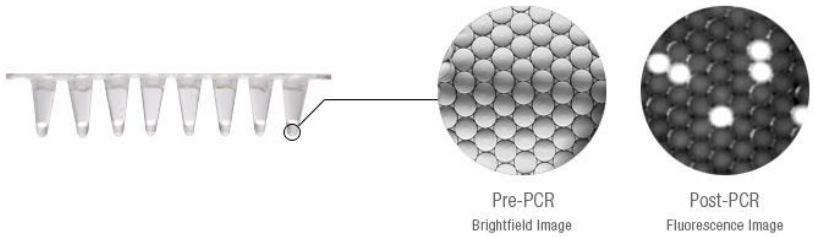
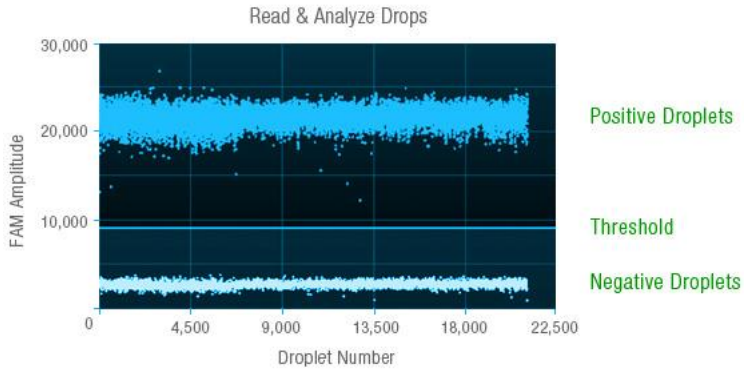
Digital PCR- A new sensitive method to assess HIV proviral DNA





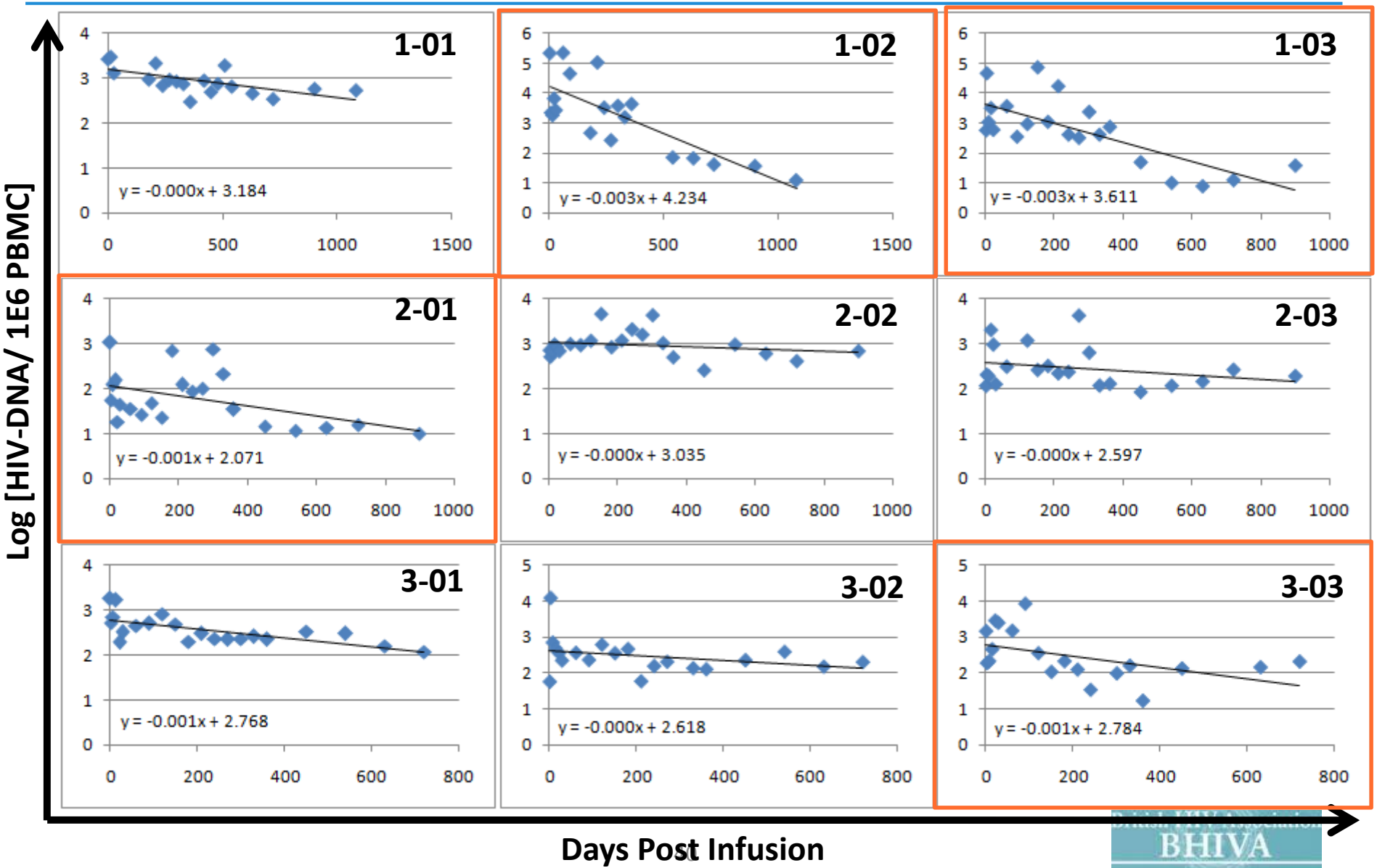


Step 1. "Digitize" sample into 20,000 droplets. Effectively reducing level of background gDNA

Step 2. Run PCR to endpoint. Quantification is no longer dependent upon PCR kinetics

Step 3. Fluorescent analysis drop by drop (yes or no). Copy number is calculated by Poisson distribution

Reduction of PBMC HIV DNA (ddPCR) observed in SB-728-T treated subjects (Median 0.9 log decrease at Month 36)



BHIVA

Raltegravir/maraviroc +/- IL-7 - increased CD4 counts <u>BUT</u> increased HIV pro-viral DNA

Impact of Interleukin 7 and Raltegravir plus Maraviroc intensification on total HIV DNA reservoir: Results from ERAMUNE 01

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Figure 3: Median change from baseline in CD4 counts

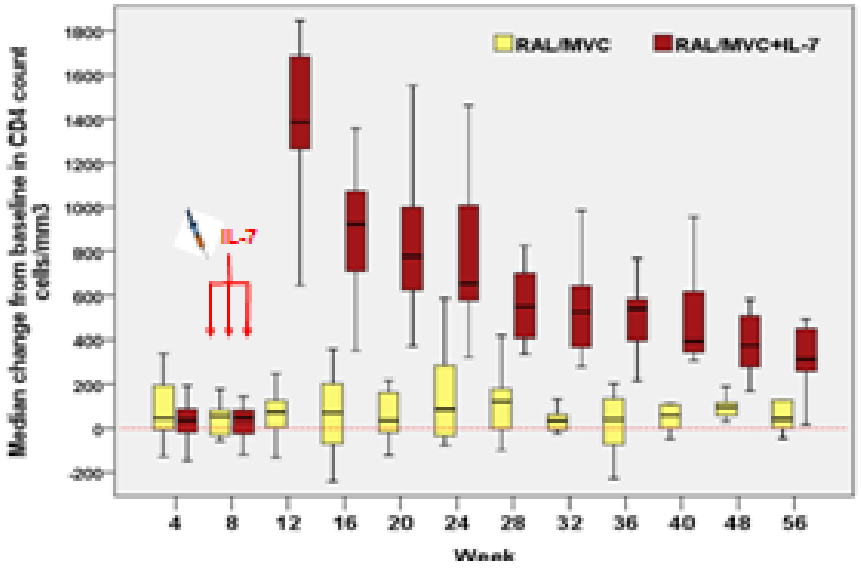
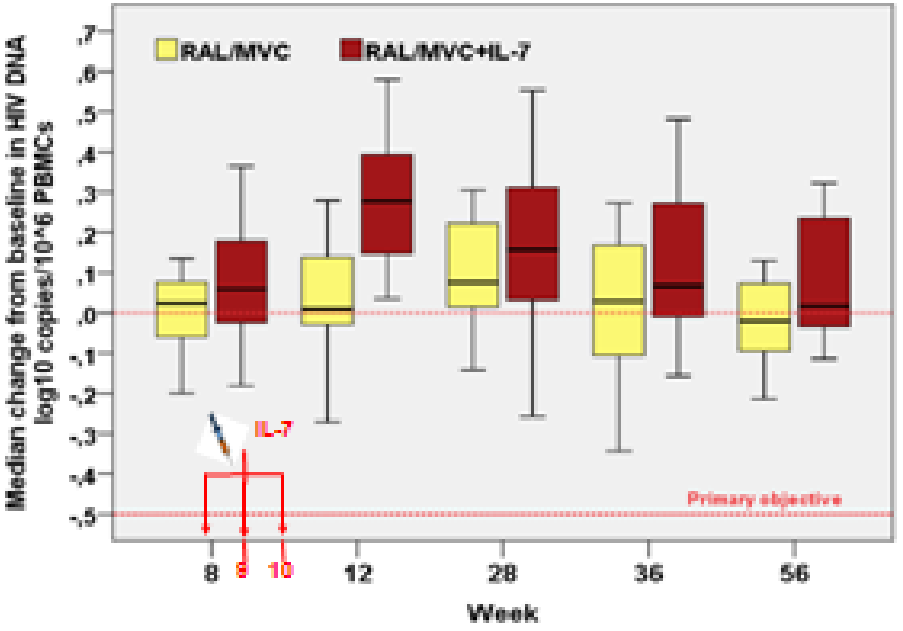


Figure 2: Median change from baseline in HIV DNA in the PBMCs

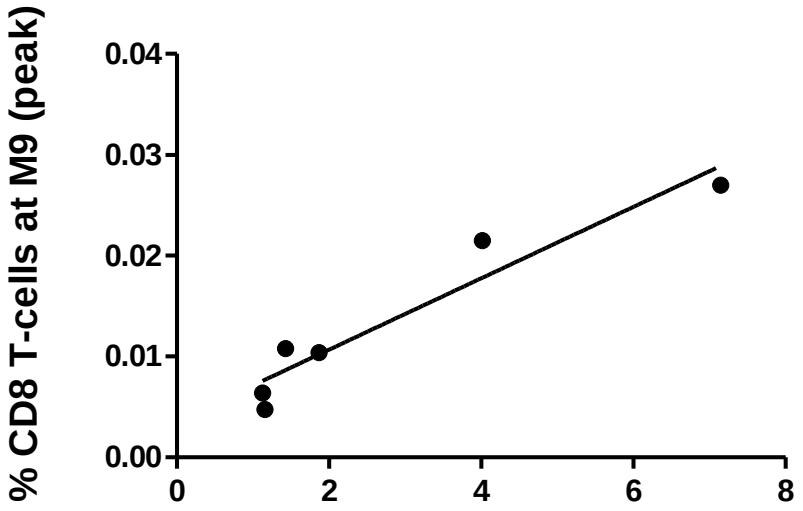


Katlama et al CROI 2013

CD8 T-cells responsive to HIV GAG post-infusion correlate with the decay of CD4 T-cells harboring integrated HIV DNA

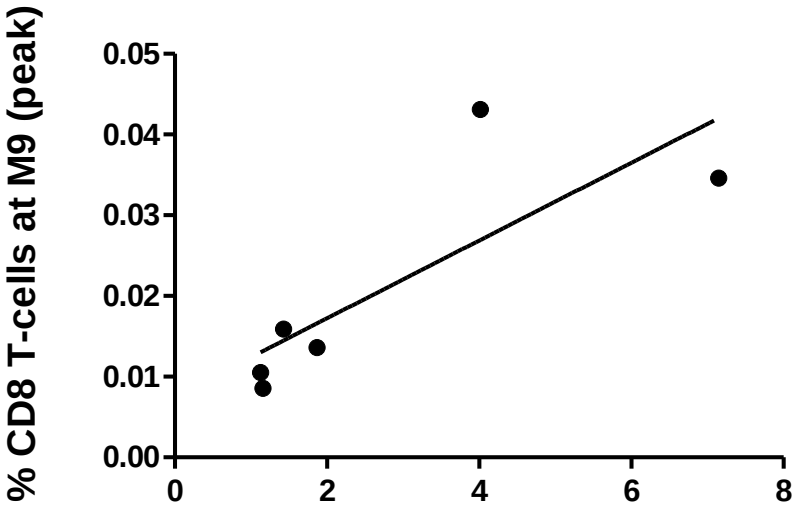
TNF-α

Spearman *r = 0.8857 p = 0.033*



IFN-γ

Spearman *r = 0.8286 p = 0.058*



Reservoir Reduction

Ratio [Integrated HIV DNA (copies/10e6 cells)] BL/M36

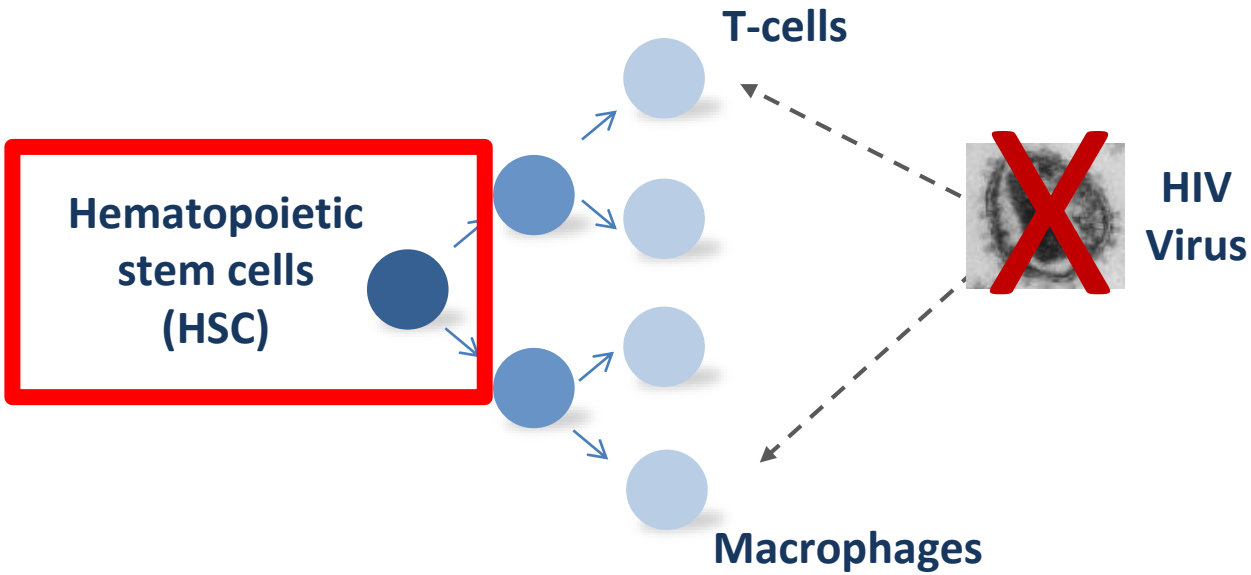
British HIV Association BHIVA

Gene therapy for HIV

SB-728-T - Next steps

- IND for mRNA electroporation of CD4 cells is open – SB-728mR-T
  - Allows potential for retreatment
- Key proof-of-concept Phase II study commencing:
  - Optimal subject population
    - Short time from initial infection to ART
    - Favorable macrophage inflammatory profile
  - Optimal Cytoxan dose (1 g/m^2)
  - 9 subjects in 2 cohorts will receive multiple doses of SB-728-mR-T
    - Cohort 1: SB-728-mR-T infusions of 2 equal doses 14 days apart
    - Cohort 2: SB-728-mR-T infusions of 3 equal doses 14 days apart
  - Objective: define proportion of subjects with functional control outcome
- Reservoir assay work continues

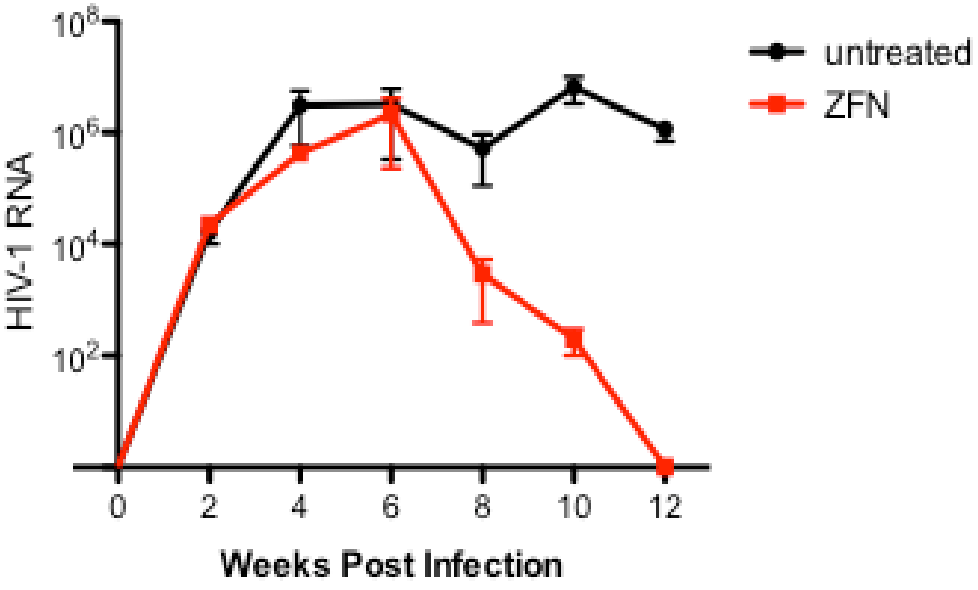
Using ZFNs to protect CD34+ HSCs



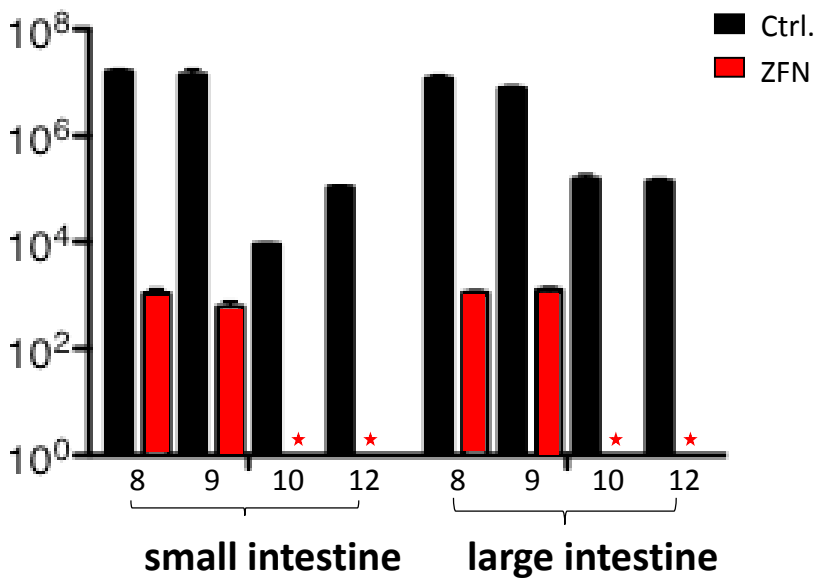
SB-728 CD34+ HSCs in HIV

ZFN-treated HSC mice control R5-tropic HIV-1

blood



gut mucosa



HIV-1_{BaL}

IND in 2014 in collaboration with City of Hope and California Institute of Regenerative Medicine

Summary and conclusions

- Ex vivo CCR5 knockout using ZFNs - a very appealing strategy for treatment of HIV
- T cell program has shown
  - Sustained increase in total CD4 count and CCR-modified CD4 cell engraftment with tissue trafficking
    - Influenced by host factors related to inflammation
    - Optimized by conditioning with Cytoxan 1 g/m^2
  - Control of VL to undetectable or <1000 copies in a CCR5 Δ32 heterozygote for more than 1 year
  - Two subjects with a 2-log decrease in viral load with Cytoxan conditioning, sustained in one case for >39 weeks
  - Downward trends in viral reservoir in PBMCs over three years
    - Related to CD8 activation/numbers
- Optimized Phase II program commenced for SB-728-mR-T
- IND open for HSC program in 2014

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