

Zinc Finger Nuclease-based Stem Cell Therapy for AIDS

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Pre- and Post-clinical studies

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ZFN technology



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Pre-clinical studies



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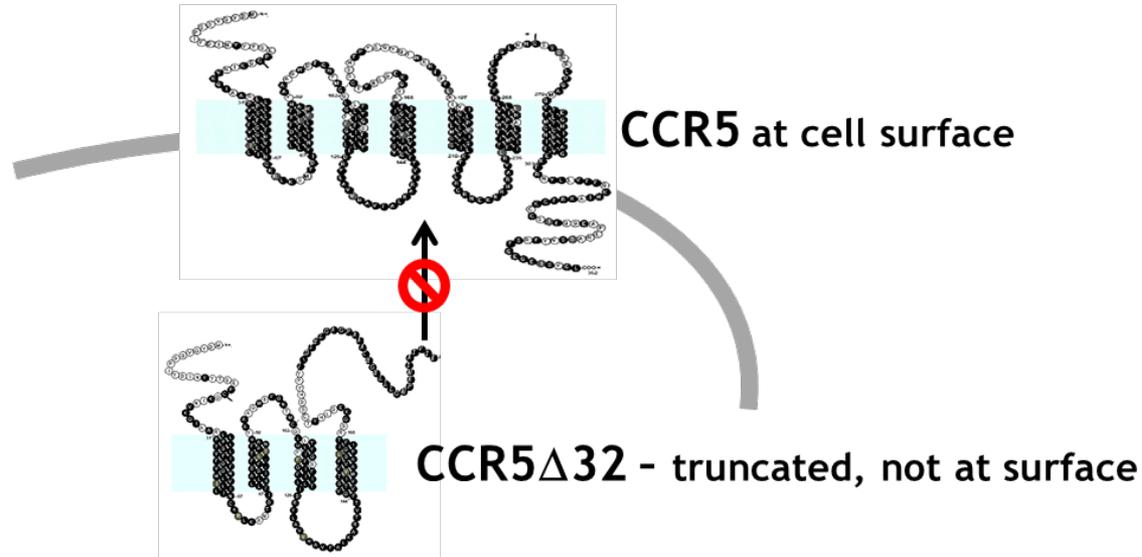
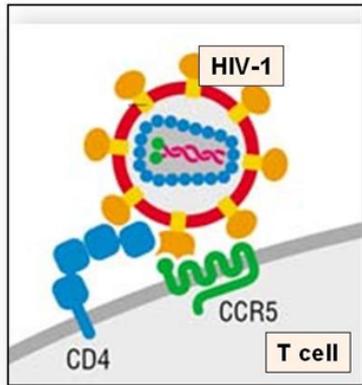
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Clinical trial



CCR5 antagonists – taking a lesson from Nature



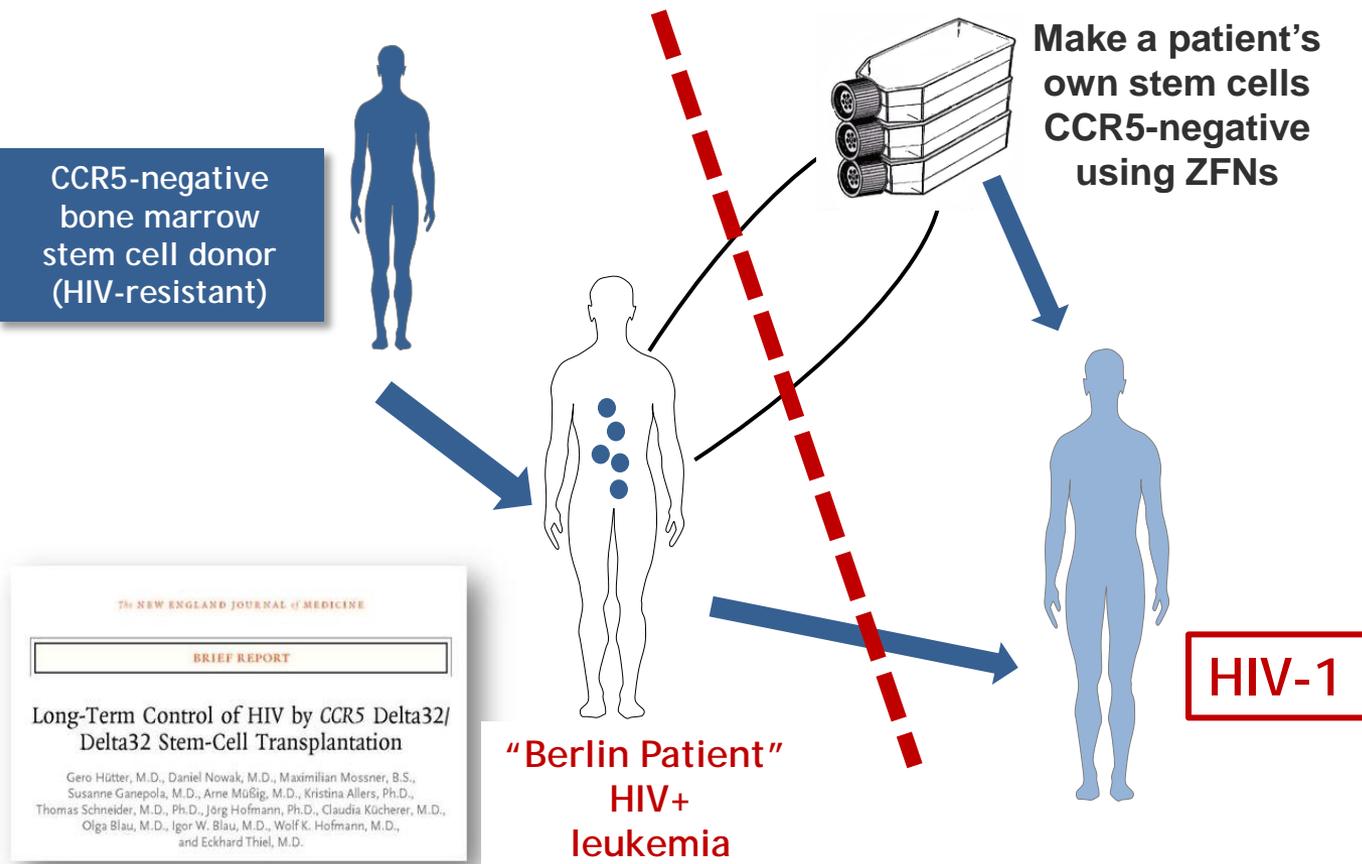
- HIV enters cells after binding to both CD4 and “co-receptor”.
- Most – but not all – strains of HIV use CCR5, and some use CXCR4.
- ~1% population are CCR5 Δ 32 homozygous, and profoundly HIV-resistant

Maraviroc



Study Rationale

SB-728mR-HSPC, genetically modified hematopoietic stem/ progenitor cells for induction of an HIV-1 resistant immune system



THE NEW ENGLAND JOURNAL OF MEDICINE

BRIEF REPORT

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

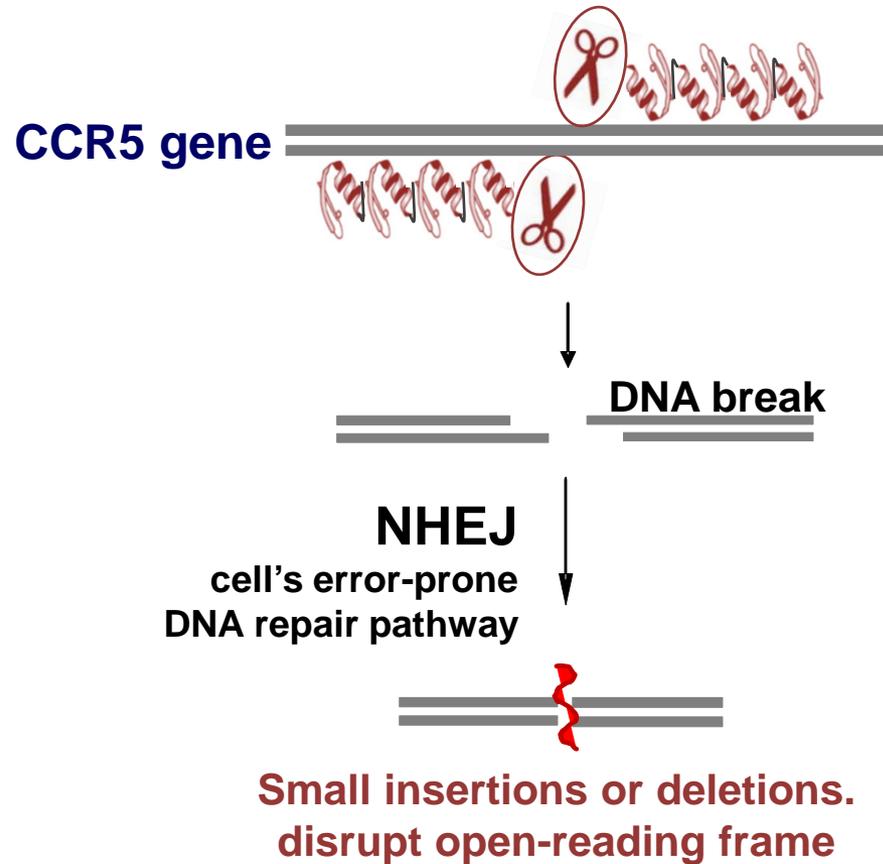
Gero Hütter, M.D., Daniel Nowak, M.D., Maximilian Mossner, B.S., Susanne Ganepola, M.D., Arne MüSig, M.D., Kristina Allers, Ph.D., Thomas Schneider, M.D., Ph.D., Jörg Hofmann, Ph.D., Claudia Kücherer, M.D., Olga Blau, M.D., Igor W. Blau, M.D., Wolf K. Hoffmann, M.D., and Eckhard Thiel, M.D.



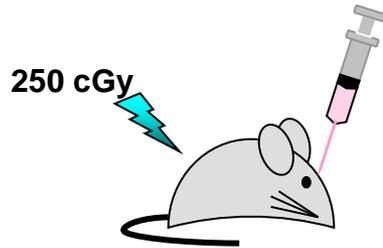
ZFN-mediated disruption of CCR5 in adult HSPC



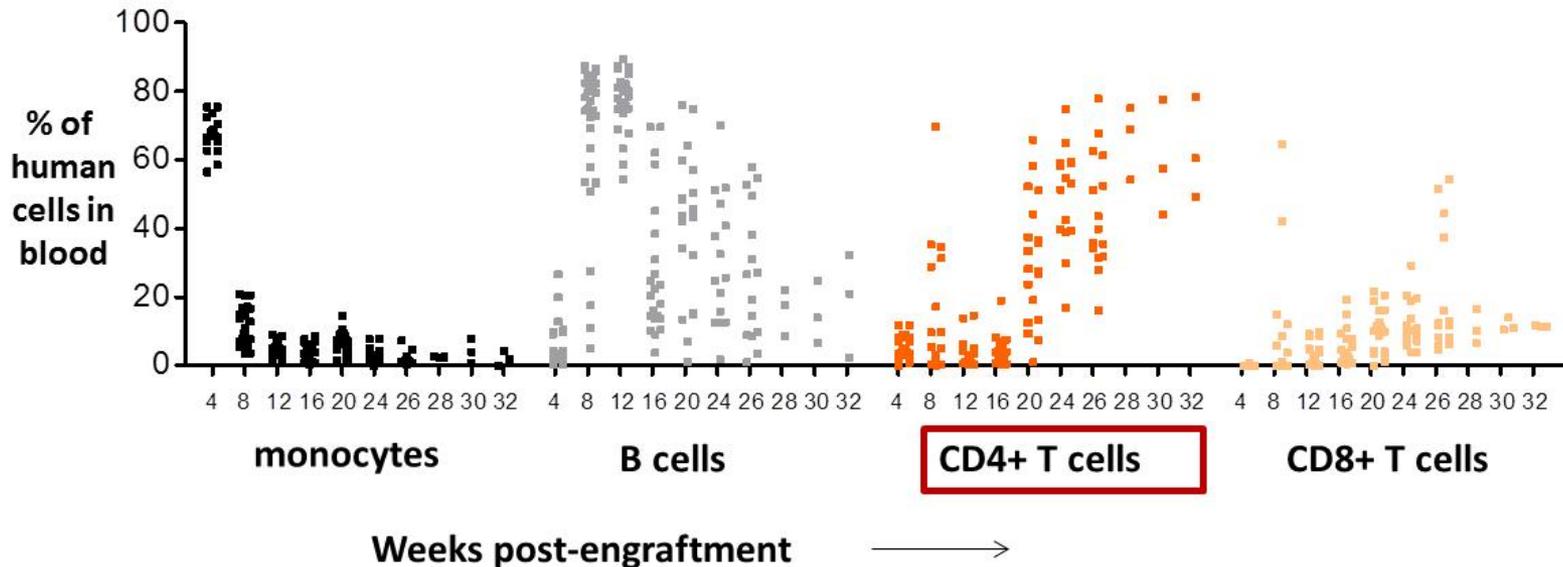
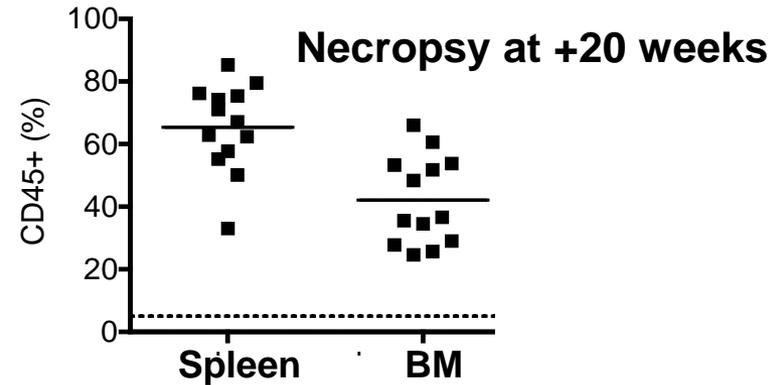
Residues at tip are engineered to recognize a specific 3 base sequence



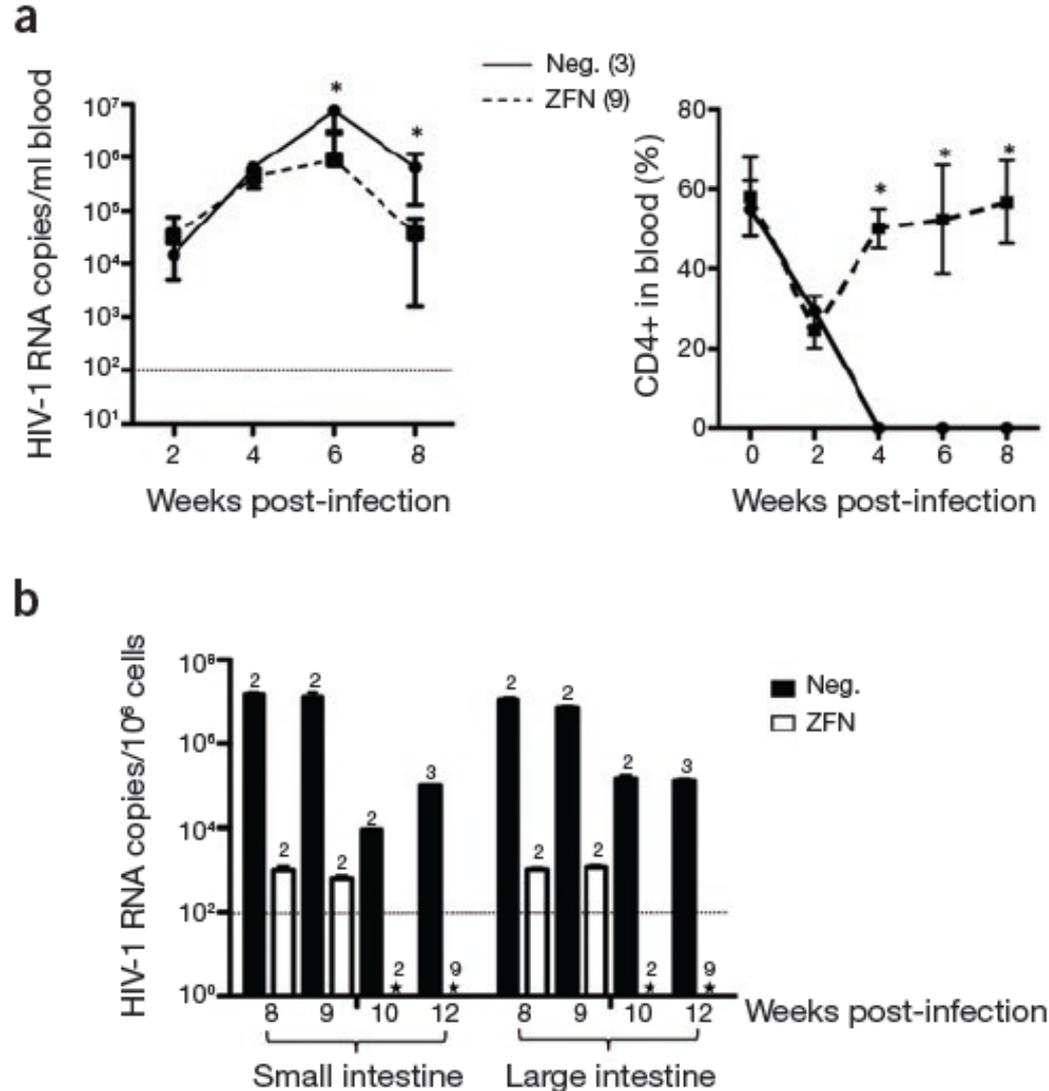
Humanized NSG mouse model to evaluate adult mobilized CD34+ HSPC



NSG adult (8 wks)



Proof of Concept Studies

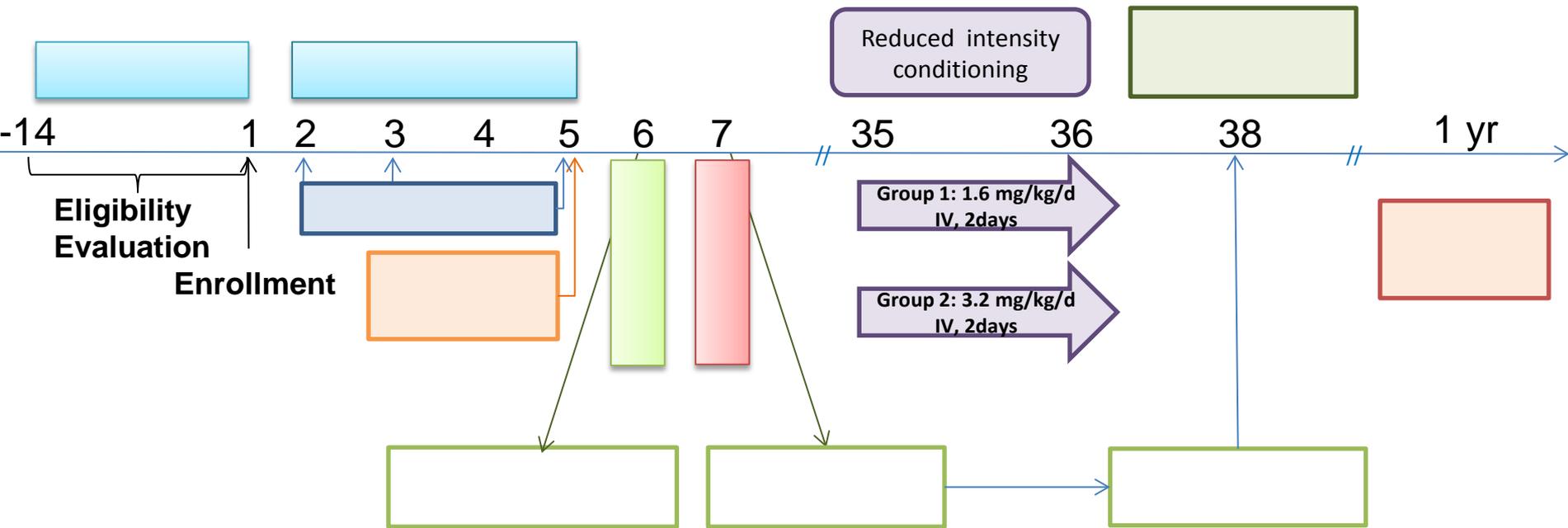


Clinical Trial Summary for SB-728mR-HSPC

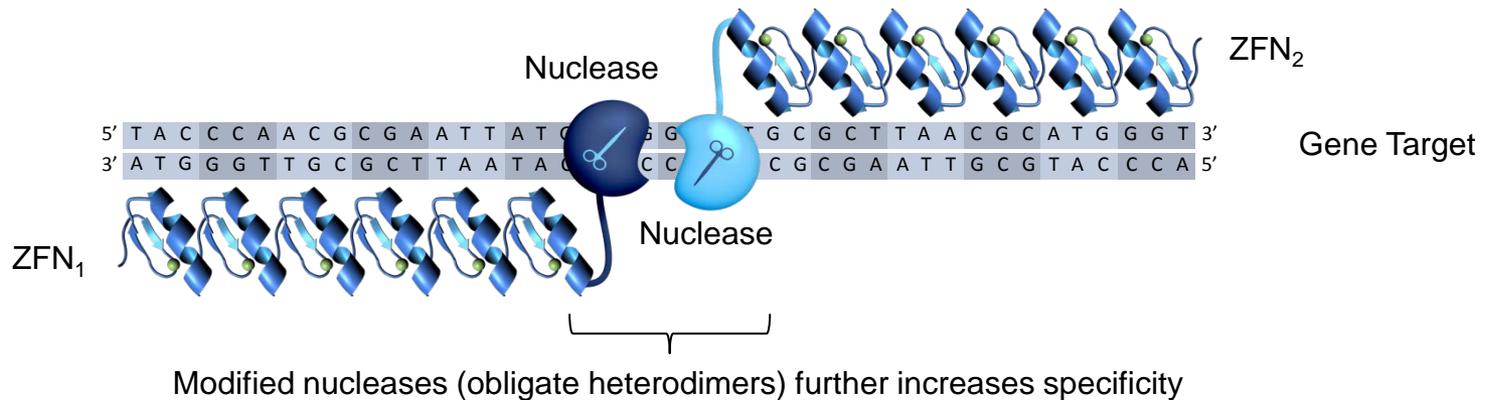
- **Product:** Purified, autologous HSPC, modified with SB-728mR to disrupt the CCR5 gene
- **Eligibility:** HIV-1/AIDS patients, aviremic while on cART, with suboptimal CD4 count (200-500 cells/ μ L)
- **Primary Endpoints:** Safety & tolerability assessed by grading of AE/SAE according to NCI CTCAE v4.0.
- **Secondary Endpoints:**
 - Detection of CCR5 modified HSPC in bone marrow over time
 - Measurement of WBC and platelet count at 3 and 12 months
 - Changes in CD4+ T-cell counts and CD4/CD8 ratio over time
 - Detection of CCR5 modified CD4 T cells in peripheral blood over time
 - Detection of CCR5 modified CD4 T cells in rectal mucosa at 12 months and end of ATI
 - Change in plasma HIV-1 RNA and PBMC proviral DNA during ATI
 - Pharmacokinetics of busulfan



Clinical Trial Schema

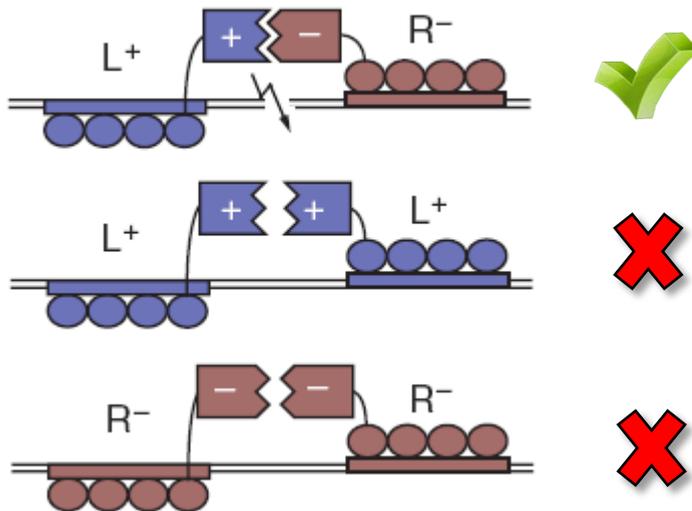
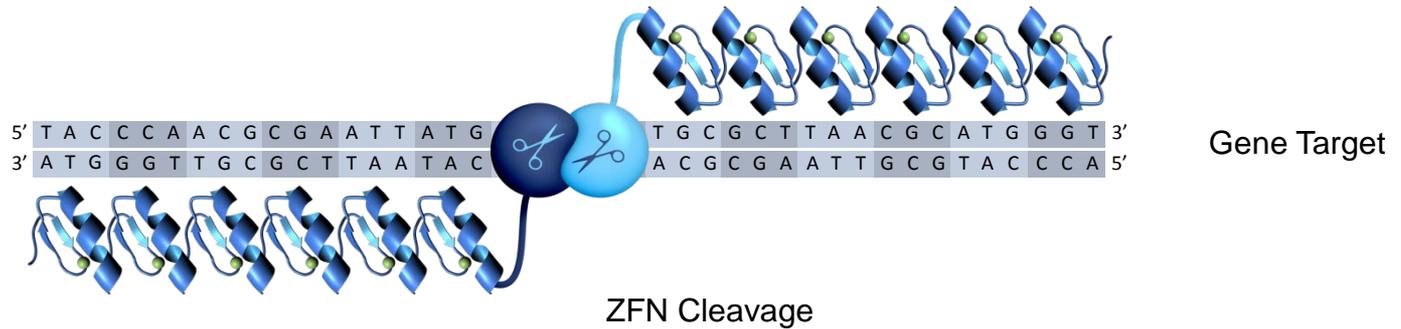


ZFP Nucleases (ZFNs) edit genes



- Contains two domains:
 - Nuclease domain of FokI restriction enzyme
 - Designed zinc finger protein (ZFP)
- Cleaves as a dimer
- May be engineered to cleave virtually any sequence
- Effectively a “designer restriction enzyme”
- Strict spatial / orientation requirements for cleavage

ZFP Nucleases (ZFNs) edit genes



Miller et al. NBT 2007
An improved zinc-finger nuclease architecture for highly specific genome editing

Doyon et al. NMEH 2010
Enhanced zinc-finger nuclease activity with improved obligate heterodimeric architectures

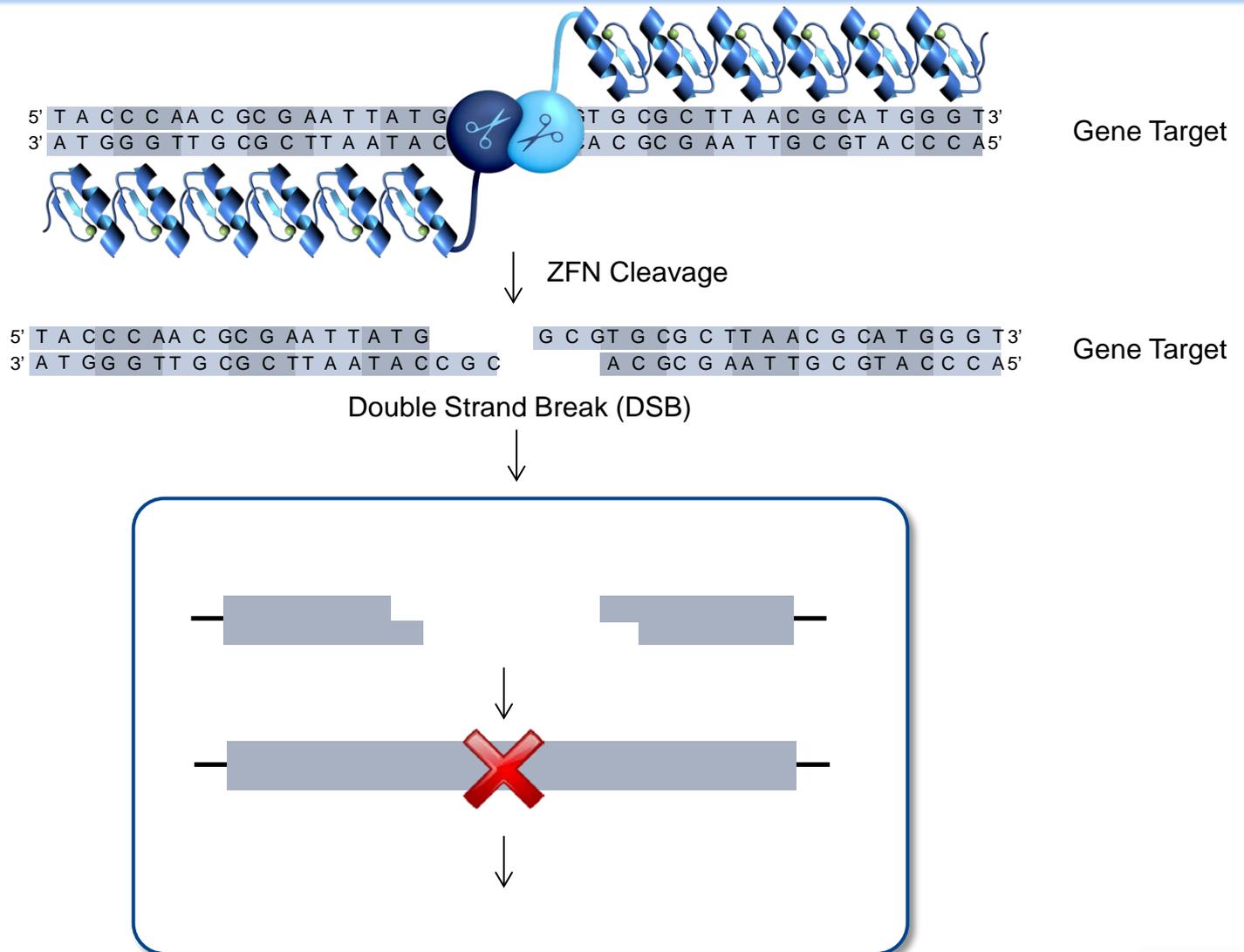


Factors Affecting ZFN Specificity

- **High DNA binding specificity intrinsic to wt and engineered C₂H₂ zinc finger DNA binding domains**
- **The absolute requirement for both ZFN monomers to be resident on the DNA target to generate an active nuclease with catalytic activity**
- **The dimerization interface of the FokI domain is weak resulting in independent monomer DNA binding**
- **Use of obligate heterodimer FokI domains which reduce the possibility of unwanted cleavage by homodimerization**
- **Strict spatial / orientation requirements for cleavage**
- **Fidelity of the natural DNA repair pathways**



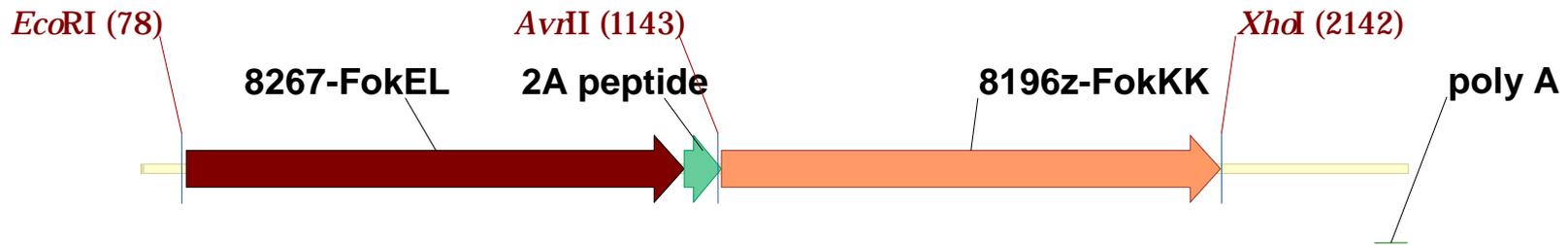
ZFP Nucleases (ZFNs) edit genes



NHEJ = Non-homologous End Joining



SB-728mR: Advantages of mRNA delivery



- Bicistronic mRNA encoding both CCR5 ZFNs synthesized in vitro using T7 RNA polymerase
- Well tolerated (low cytotoxicity)
- Drives high levels of target gene disruption
- Provides highly transient expression
- Eliminates the potential for insertional mutagenesis
- Eliminates the use of viral vectors in cell manufacturing
- CMOs have established cGMP-compliant methods for manufacturing mRNA



MaxCyte GT Instrument: Scalable Electroporation Device

- Scalable: $5e5$ - $1e10$ total cells ($>300e6$ CD34+ HSPC)
- Chemically defined buffers/media (no added 'biologicals')
- cGMP compliant, sterile closed system
- **Regulatory: Drug Master File on record with FDA and previously reviewed by the NIH-RAC and Health Canada**
- Currently being used in several clinical trials and late-stage pre-clinical studies evaluating the safety and biological activity of cell therapy products

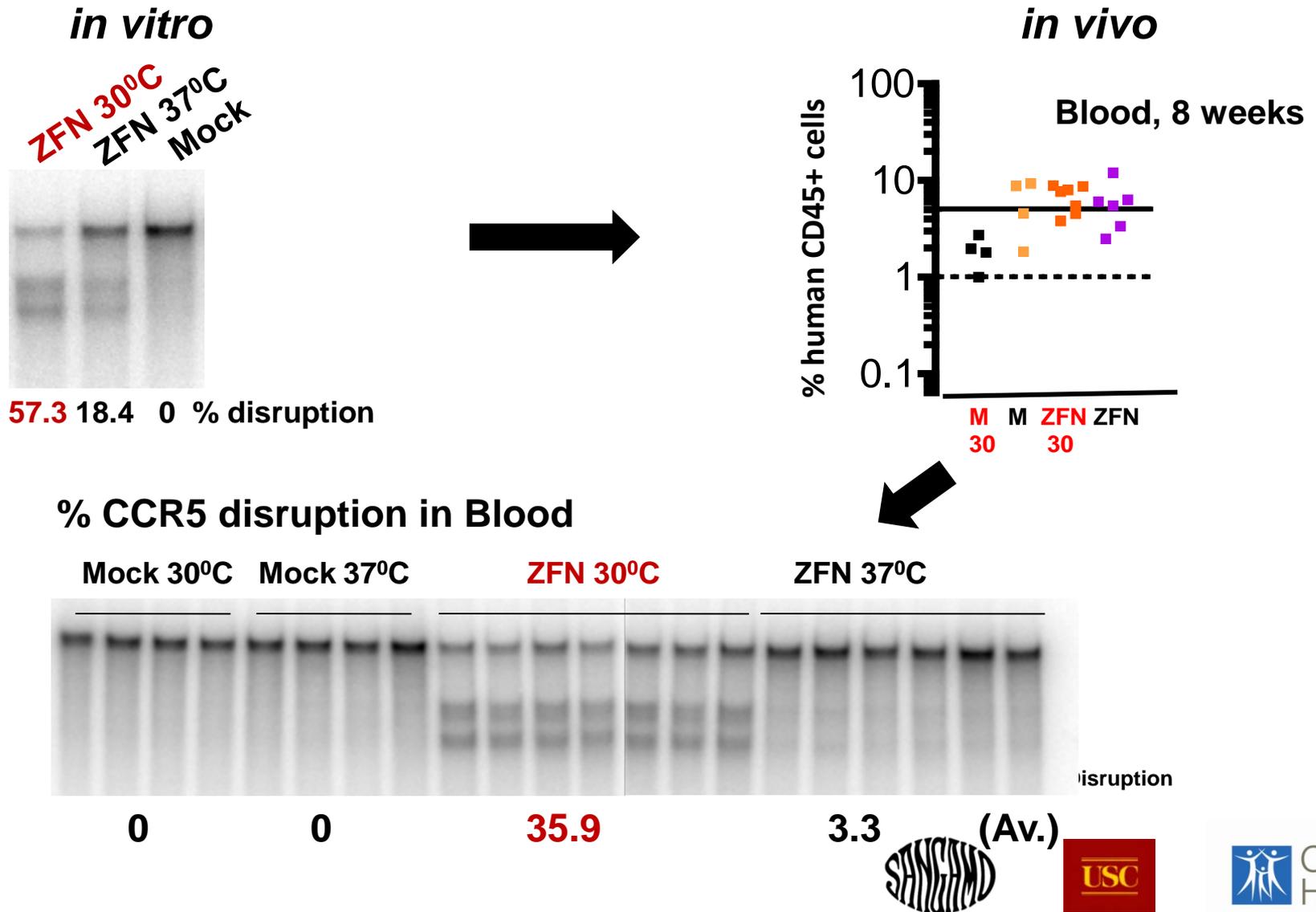


Optimized Large-Scale Electroporation Process - Disrupting CCR5 in HSPCs via ZFN mRNA delivery

- CD34s isolated from mobilized leukopaks using the CliniMACS
- Pre-stimulation
 - CD34+ HSPC pre-stimulated with hematopoietic cytokines for 24 hours at 37°C
- Electroporation
 - Cells washed and resuspend in EP buffer
 - mRNA added and cells electroporated
- Post-transfection
 - Cells cultured at 30°C overnight (16-18 hours)
 - Transient 30°C incubation increases ZFN-mediated gene disruption (Doyon Y, et al. (2010). *Nat Methods*. Jun; 7(6):459-60.)
 - Cells returned to 37°C and cultured for 2 days
- Cells formulated and cryopreserved (40-60% of CCR5 alleles disrupted)

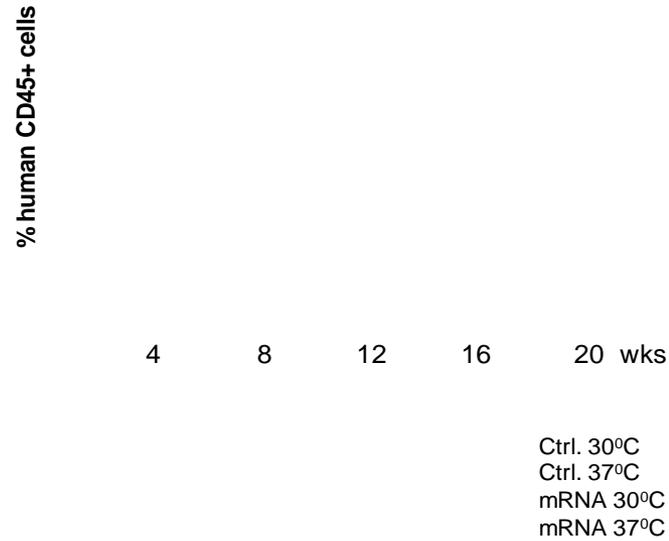


HSPC Electroporated with ZFN mRNA at Large-Scale - Progeny Maintain CCR5 Disruption In Vivo

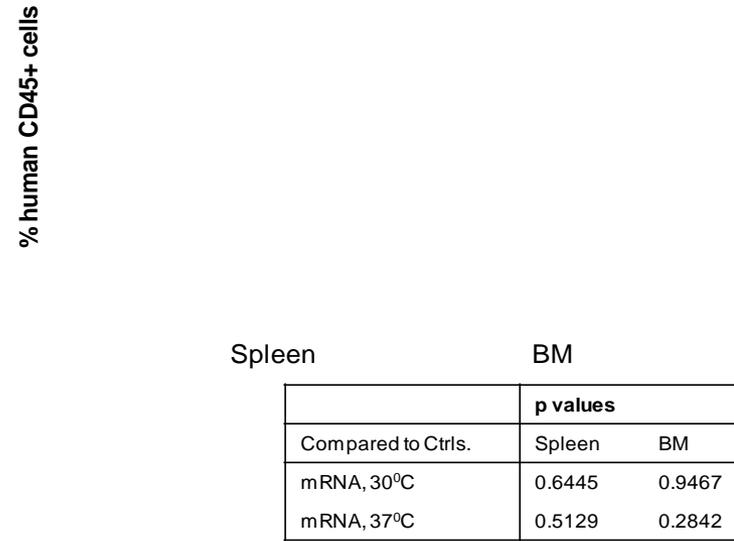


HSPC Electroporated with ZFN mRNA at Large-Scale - No Effect on Engraftment of NSG Mice

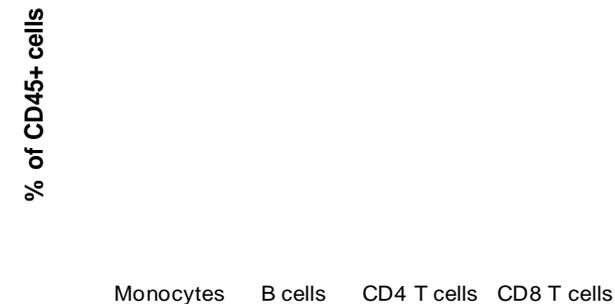
A Peripheral blood engraftment, indicated times



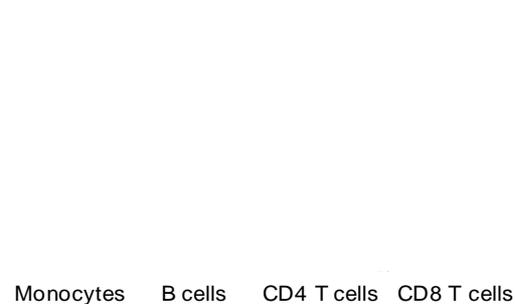
B Tissue engraftment @ 20 weeks



C Spleen - lineages



D Bone marrow - lineages



Evaluating ZFN Specificity and Non-Clinical Safety



Summary: IND-Enabling Safety Studies

Objective

- Evaluating ZFN specificity in CD34+ HSPC:
 - Monitor changes at the top 23 off-target sites by deep sequencing in CD34+ HSPC
 - DSB quantitation by immunostaining with 53bp1/H2AX
- Standard in vitro safety assays:
 - Soft-agar transformation assay in human fibroblasts
 - Karyotype analysis of SB-728 treated HSPC
- Tumorigenicity in NSG mice
 - Patient dose derived from 3 separate donors
 - 5-month study duration to look for tumor formation



Other assays for ZFN specificity

Perez, EE. et al. Establishment of HIV-1 resistance in CD4+ T cells by genome editing using zinc-finger nucleases. *Nat. Biotechnol.* **26**: 808-16, (2008).:

- SELEX-based analysis of the top 15 sites genome-wide with the highest homology to the experimentally determined consensus

Gabriel, R. et al. An unbiased genome-wide analysis of zinc-finger nuclease specificity. *Nat. Biotechnol.* **29**, 816–823 (2011).:

- *In vivo* approach – laborious but useful (more suited to high risk applications)
- Provides an unbiased approach to identify potential ZFN off-target sites
- Surveys whole genome with no requirement for prior knowledge of ZFN binding specificity



Assessing the Specificity of ZFN action

Molecular assessment for ZFN specificity:

- SELEX-based analysis of the top 15 sites genome-wide with the highest homology to the experimentally determined consensus (Perez et al., NBT 2008)
- An additional 8 sites identified by mapping the location of non-integrating Lentiviral vector integration in cells also treated with the CCR5 ZFNs (Gabriel et al., NBT 2011)
- Direct DNA sequencing assay of Top 23 sites ($\geq 10,000$ sequence reads per site)

Cell-based assessment:

- Quantification of DSB formation over time by immunohistochemistry using antibodies against proteins involved in DNA repair

Biological assessments:

- Demonstrate normal human fibroblasts treated with the CCR5 ZFNs maintain anchorage-dependent growth in a soft agar transformation assay
- Confirm normal karyotype of SB-728mR-treated HSPC



Tumorigenicity Studies: Evaluating a Patient Dose of SB-728mR Treated CD34+ HSPC

- Engraft a whole patient dose (~150 million) of SB-728mR maximum dose treated cells from 3 donors (50 million cell per donor) in NSG mice, plus 1/3 control HSPC
- Maintain animals for 5 months (20 weeks), with weekly observations, and weighing; blood at 4 and 12 weeks for standard panel, plus huCD45 cell frequency.
- 20 weeks evaluation chosen:
 - Multi-lineage development observed over the 20 weeks
 - Maximum level of human cell engraftment occurs 8-12 week;
 - EBV-transformed cells grow out in NSG mice within 8 weeks
 - Previous engraftment studies using HSPC carrying translocations associated with leukemia exhibited tumors by 5 months
- At necropsy, gross pathology, and major organs harvested, weighed, and evaluated by histopathology. Performed by independent team.



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