**Fondation Mérieux Conference:**
Humanized models to study immunity and to accelerate the development of new solutions for human health

*Veyrier-du-Lac, France, April 2017*

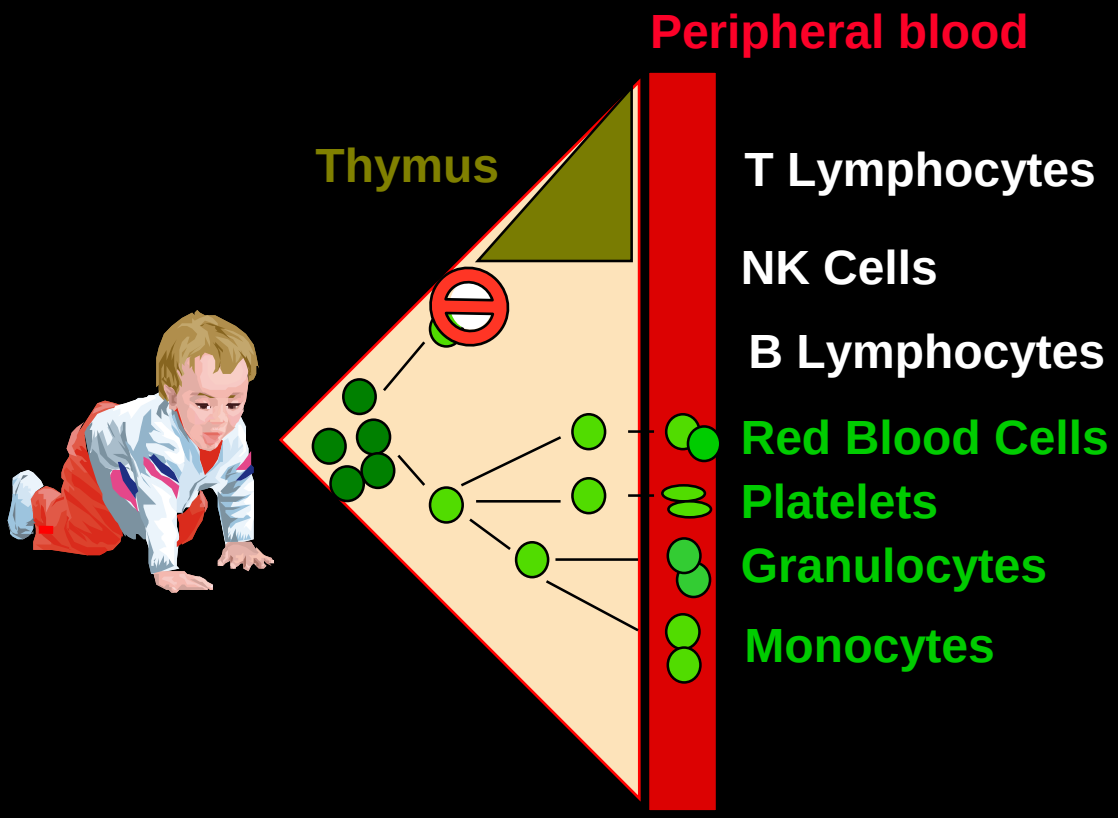
# *Genetic engineering of human hematopoiesis and its preclinical modeling in hematochimeric mice*

*Luigi Naldini, MD, PhD*

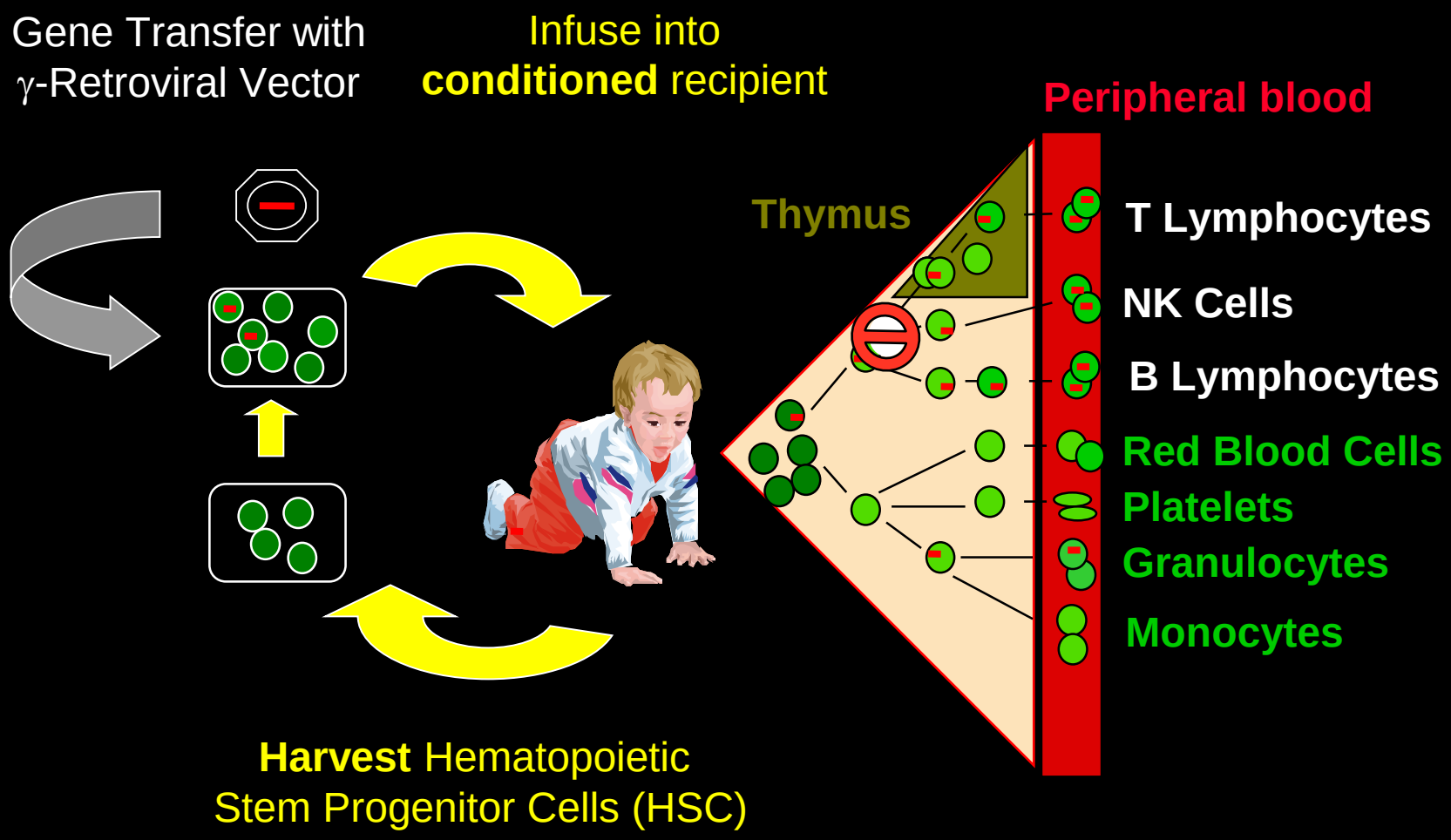
# The Promise of HSC Gene Therapy

- *Increasing power of gene transfer technologies*
  - Allows: correcting genetic bases of disease
  - instructing novel functions to target cells
- *Improved stem cells manipulation & transplant*
  - exploits regenerative potential of stem cells
- *Make possible to design new therapies*
  - for monogenic diseases, cancer & infection

# A Seminal Study:
# ADA-SCID HSC Gene Therapy



# A Seminal Study:
# ADA-SCID HSC Gene Therapy




*Aiuti et al., Science 2002*

# Early Clinical Testing of HSC Gene Therapy

- *Seminal work with γ-Retroviral Vectors in Primary Immunodeficiencies*
  - Low gene transfer but efficacy in selected diseases
  - Leukemia triggered by vector insertion in some patients
  - *ADA-SCID HSC gene therapy became 1st ex vivo gene therapy drug on the market in 2016*

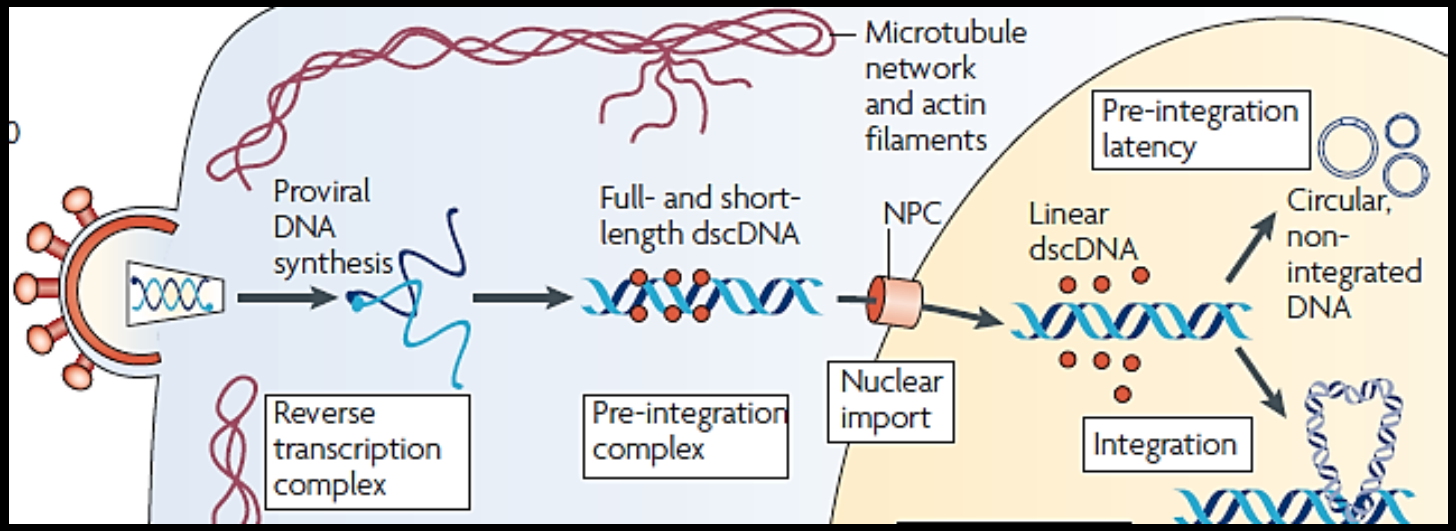
# Challenges to Broader Application of HSC Gene Therapy

- *Achieve efficient HSC gene transfer*
  - Low gene transfer by early γ-retroviral vectors
  - Ex vivo manipulation may affect HSC function
  - Process yield sufficient to allow polyclonal engraftment & rapid multilineage reconstitution
- *Regulate transgene expression*
  - Unregulated expression may cause toxicity
- *Alleviate risk of insertional mutagenesis*
  - Random vector integration may occasionally activate oncogenes

# More Efficient Vectors Derived from HIV

*Nuclear Translocation of Viral Genome*

γ-retroviruses → *Mitosis Dependent* →
- Infect only dividing cells
- Prolonged ex vivo culture



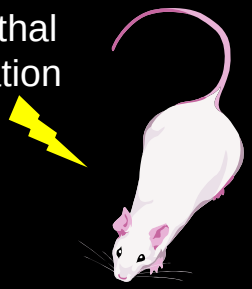

HIV (Lentivirus) → *Nuclear Transport Dependent* →
- Can infect nondividing cells
- Short ex vivo culture

*Naldini et al, Science 1996*

# Human HSC Gene Transfer by Lentiviral Vectors

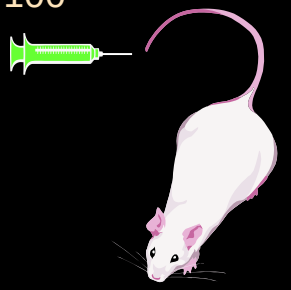
SCID/NOD mouse

Sublethal irradiation



Cord blood CD34+ cells

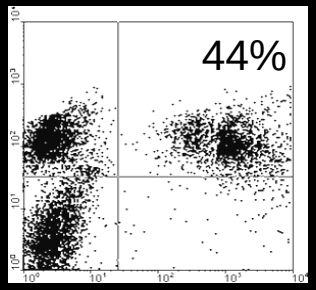
**LV-GFP**
IL-6 20 ng/ml
TPO 20
SCF 100
Flt3-L 100



>12 weeks

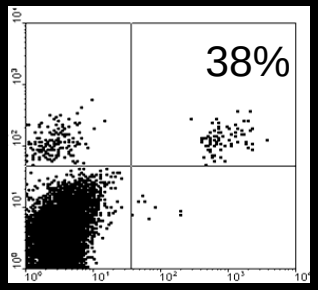
- *Hematochimeric Engraftment in SCID mice*
  - Long-term reconstitution & secondary transplant
  - polyclonal reconstitution & self-renewal

Primary recipient

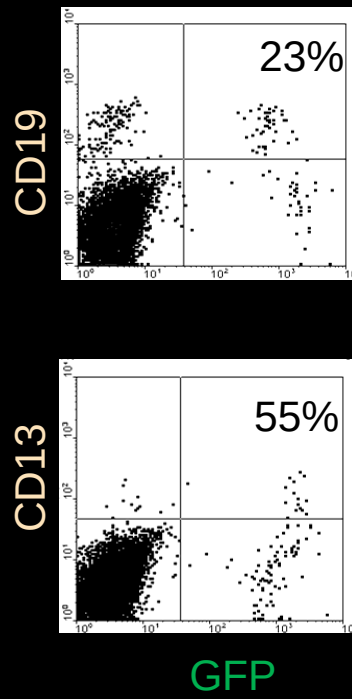



48 %CFCs GFP+

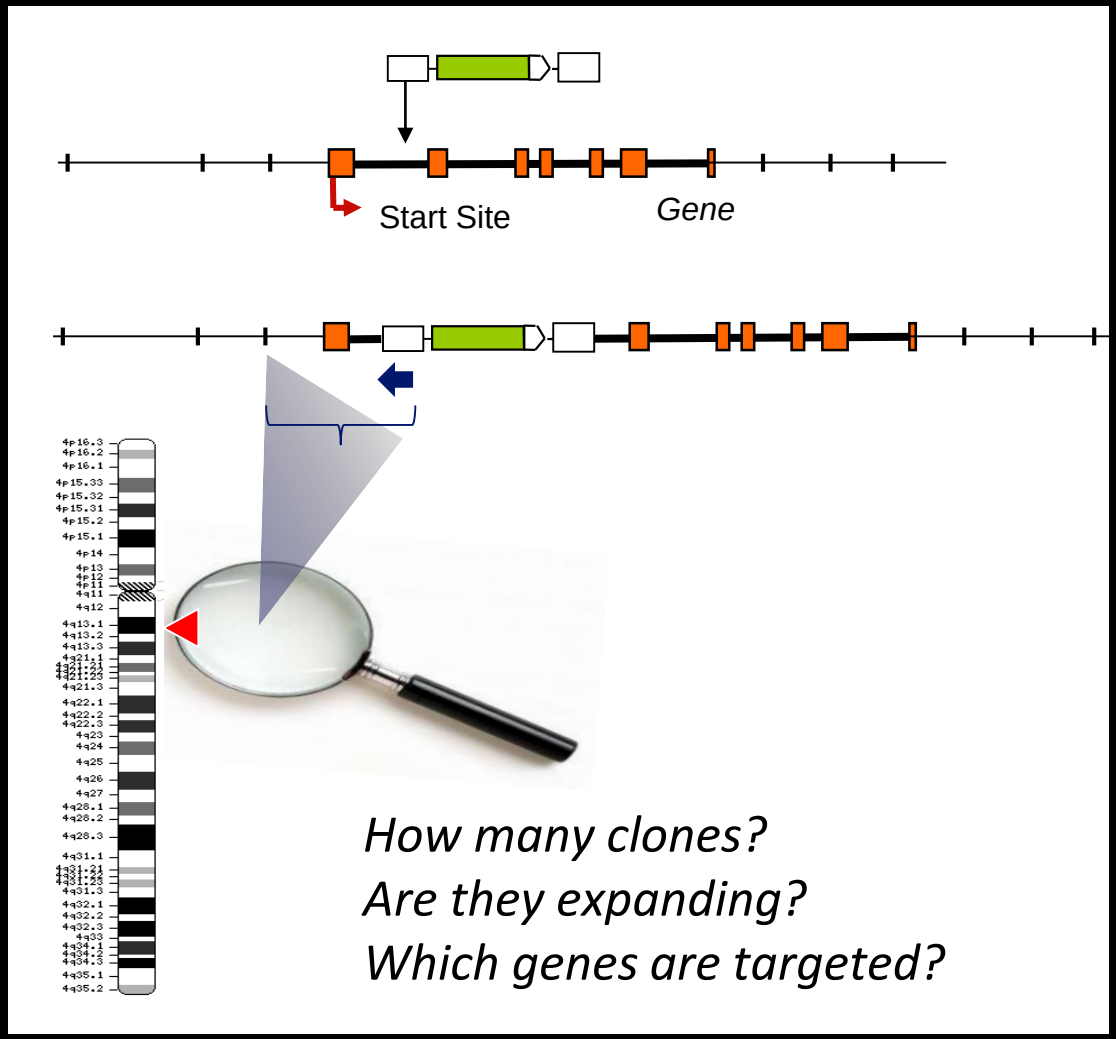
Secondary recipient




50 %CFCs GFP+



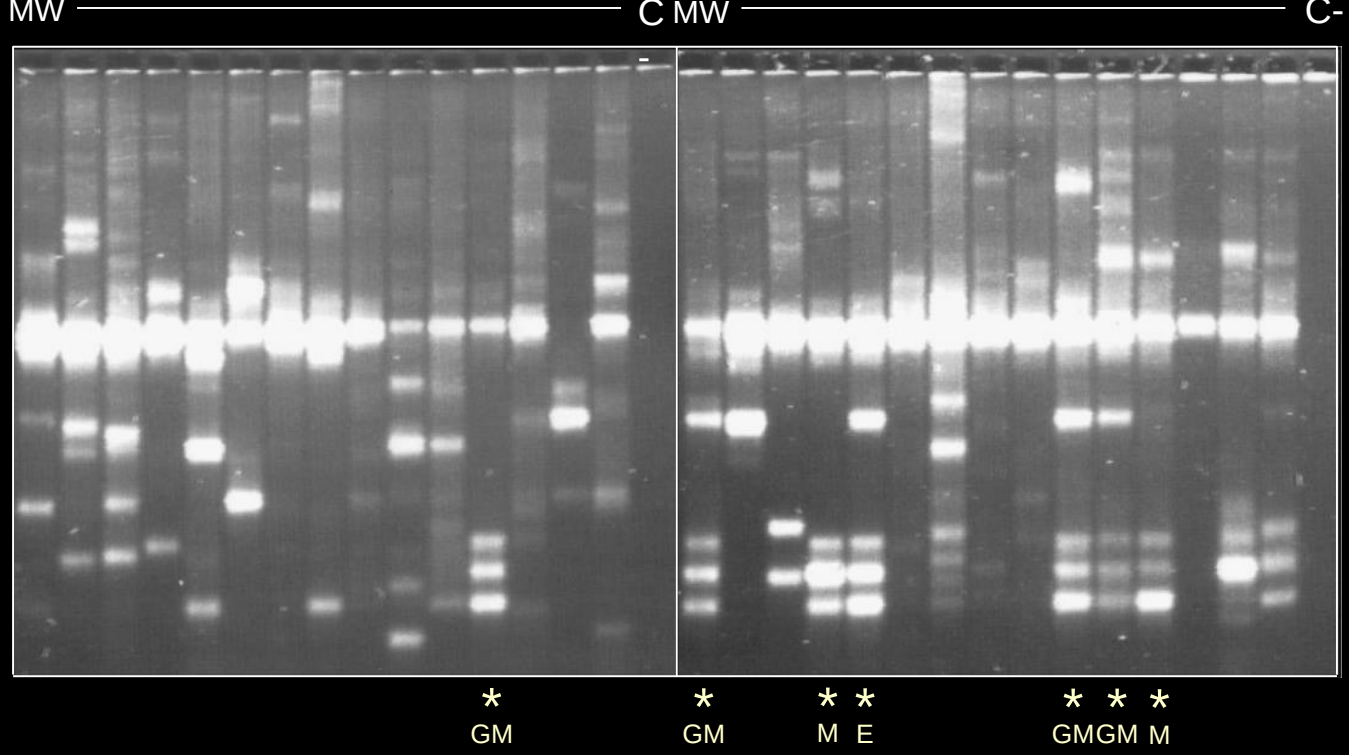
# Vector Integration Site Analysis



# Self-Renewal & Multipotency of Transduced SRC

Bone Marrow DNA


CFC DNA

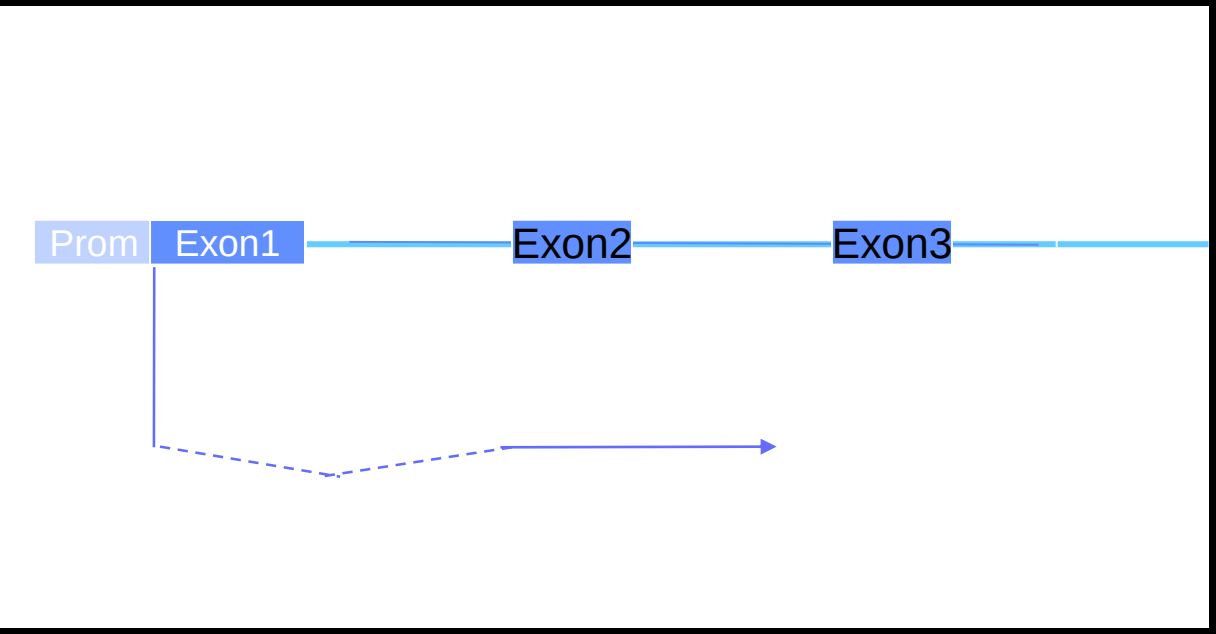



| *Sample* | *Proviral/Genomic Sequence (5' - 3')* |
|---|---|
| *BM 2°* | ATCTCTAGCA*GGAAGTCCACAATT*CCTAACTGCTGTGCCATGAATTCAGA |
| *CFC 1°* | ATCTCTAGCA*GGAAGTCCACAATT*CCTAACTGCTGTGCCATGAATTCAGA |
| *CFC 2°* | ATCTCTAGCA*GGAAGTCCACAATT*CCTAACTGCTGTGCCATGAATTCAGA |

*Ailles et al., Mol Ther 2002*

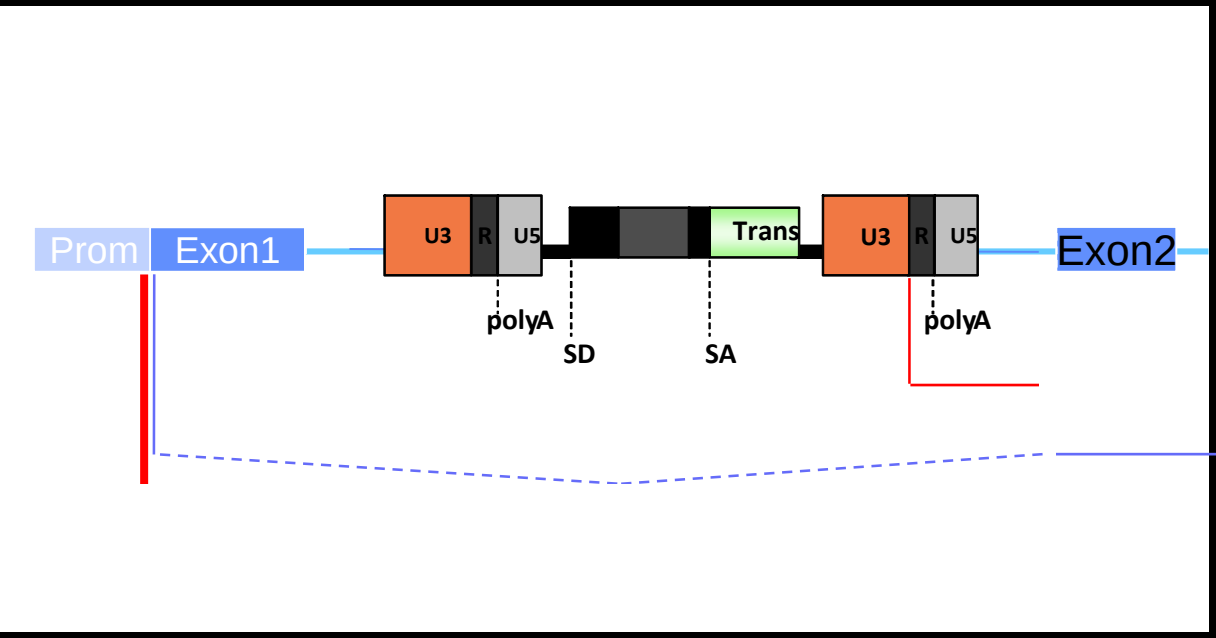
# Insertional Mutagenesis in HSC Gene Therapy

- *Random γ-retroviral vector integration near cancer gene may activate its oncogenic potential*



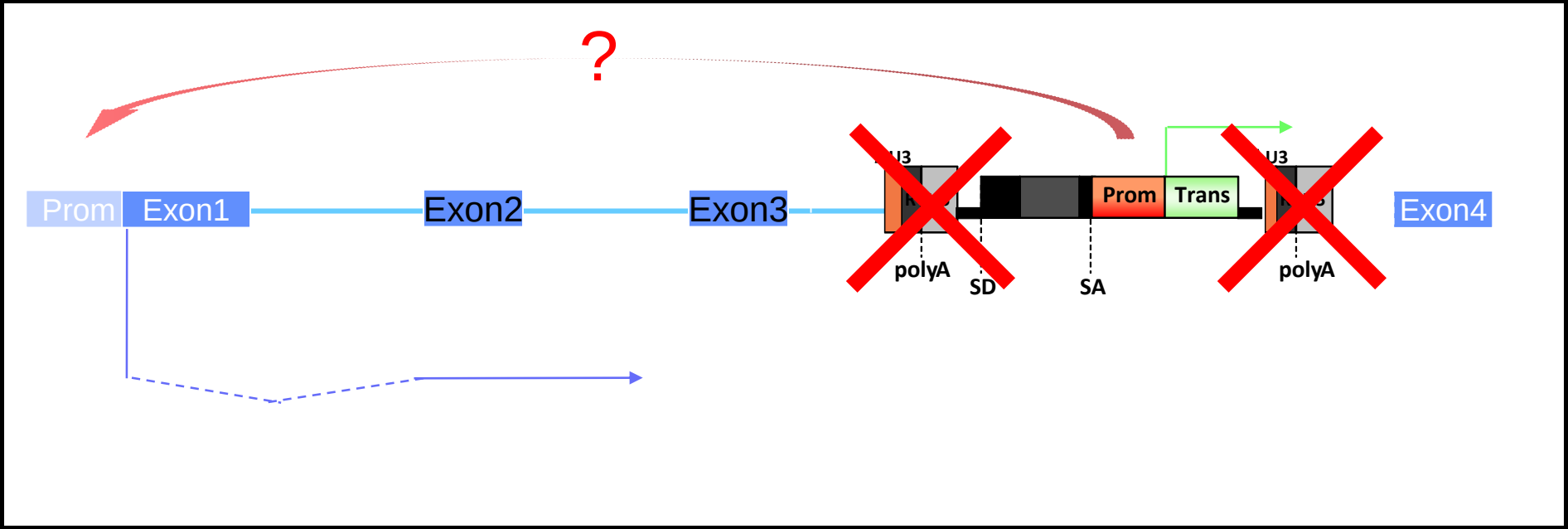
# Insertional Mutagenesis in HSC Gene Therapy

- *Random γ-retroviral vector integration near cancer gene may activate its oncogenic potential*



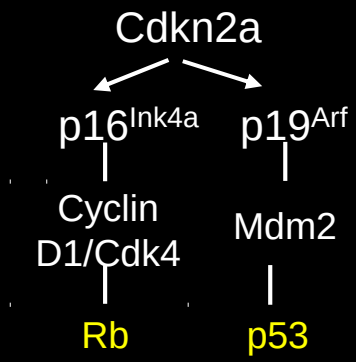

- *Insertional bias for promoter & growth-related genes*
- *Strong enhancer prormoter in LTR*

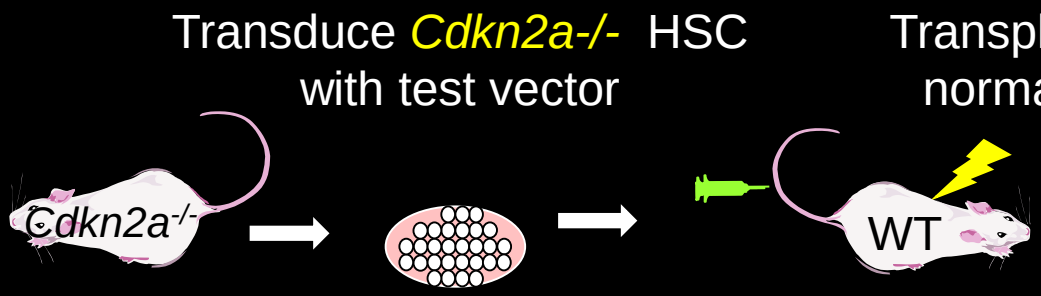
# A Safer Vector Derived from HIV



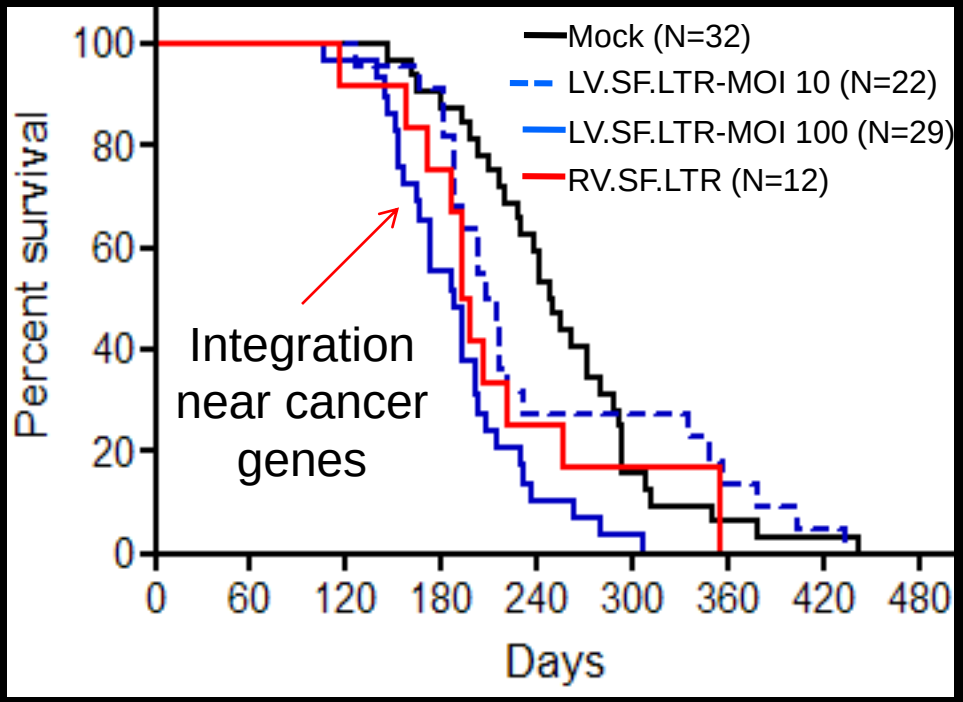

- *Insertional bias for the body of expressed genes*
- *Transcriptionally inert (self-inactivating, SIN) LTR*
- *Moderate internal promoter*

# A Safer Vector: Studies in Tumor Prone Mice

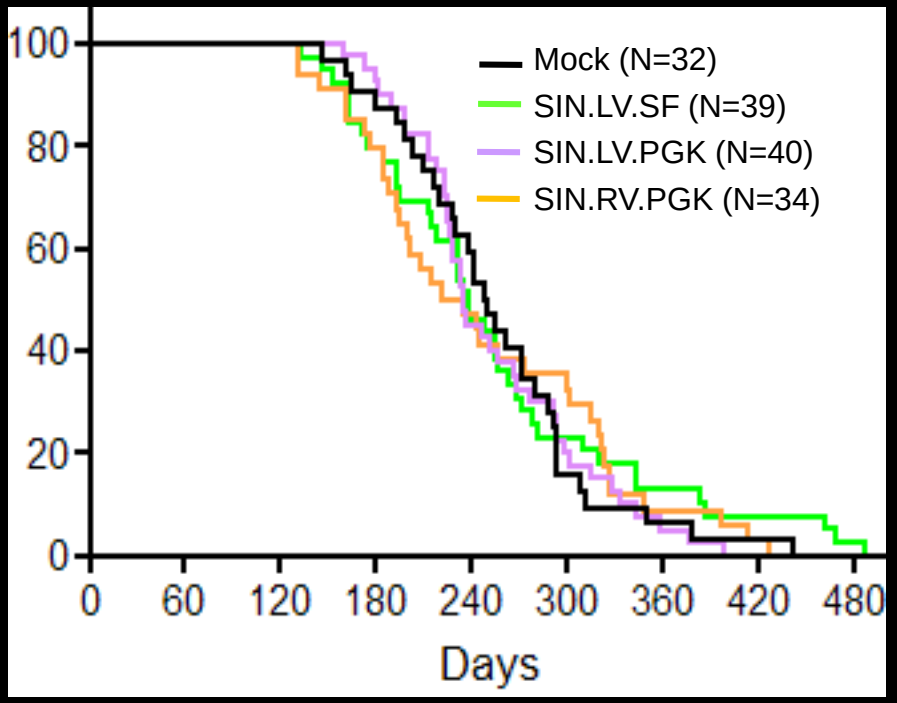



Active LTR vectors




Self-Inactivating LTR vectors

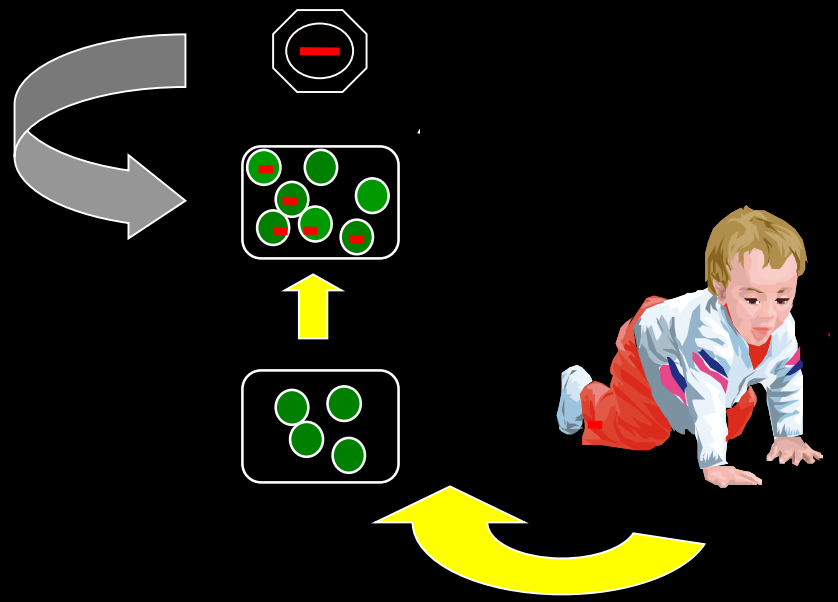



*Montini et al., Nature Biotech. 2006; J. Clin. Invest. 2009*

# Advancing Applications of HSC Gene Therapy

*Immuno-hematological diseases*

Gene Transfer with
**Lentiviral Vector**



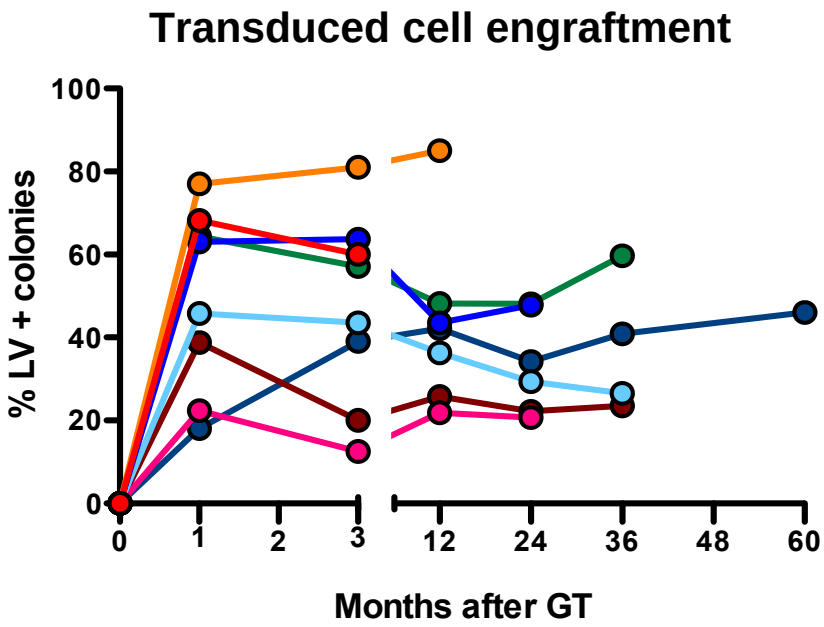
Harvest Hematopoietic
Stem Progenitor Cells

*Storage diseases*

# LV HSC Gene Therapy Trials at Tiget

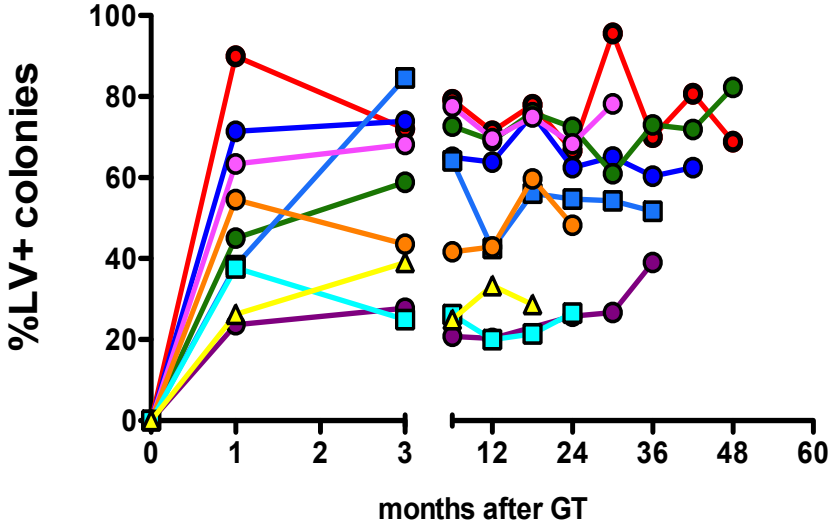
- *Wiskott Aldrich Syndrome*
  - PID with platelets deficiency, eczema & autoimmunity
  - Allogeneic HSC curative but high morbidity if mismatched
  - $\gamma$-RV HSC gene therapy efficacious but most patients later developed leukemia
- *Metachromatic Leukodystrophy (MLD)*
  - Storage disease (ARSA) affecting CNS & PNS
  - Dysmyelination and neurodegeneration
  - No treatment available

# Myeloid Gene Marking in WAS and MLD Patients




**WAS**

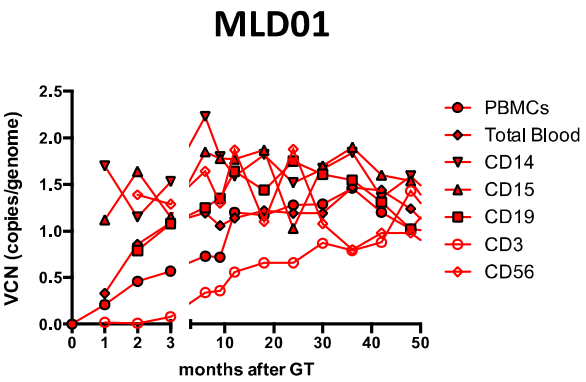
- WAS10_01
- WAS10_02
- WAS10_03
- WAS10_04
- WAS10_06
- WAS10_07
- WAS10_08
- WAS10_09

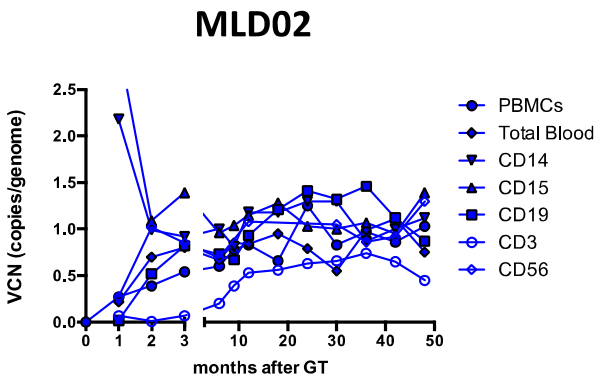


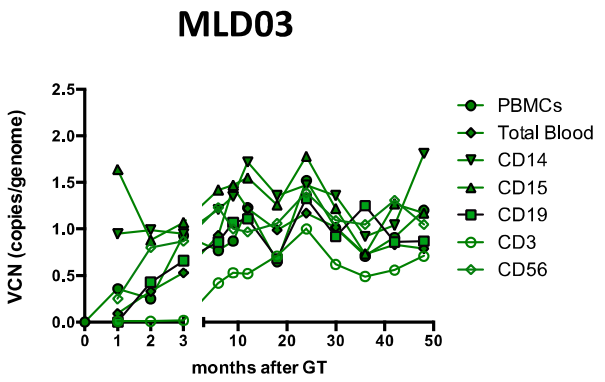

**MLD**

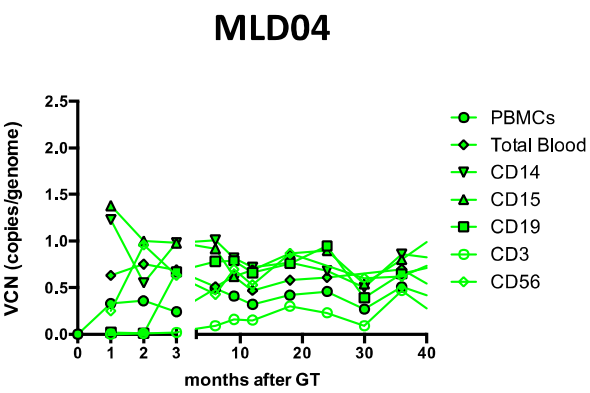
- MLD01
- MLD02
- MLD03
- MLD04
- MLD05
- MLD06
- MLD07
- MLD08
- MLD09

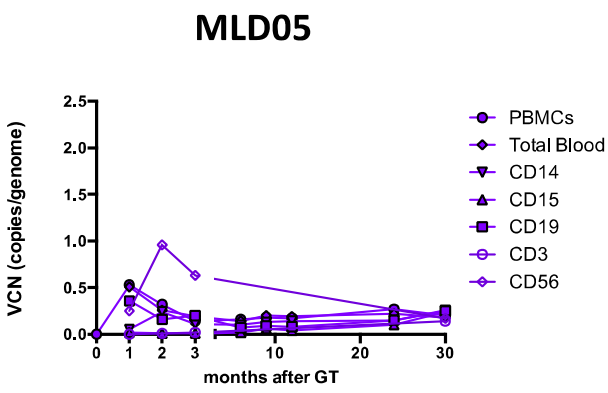
# Multi-Lineage Gene Marking

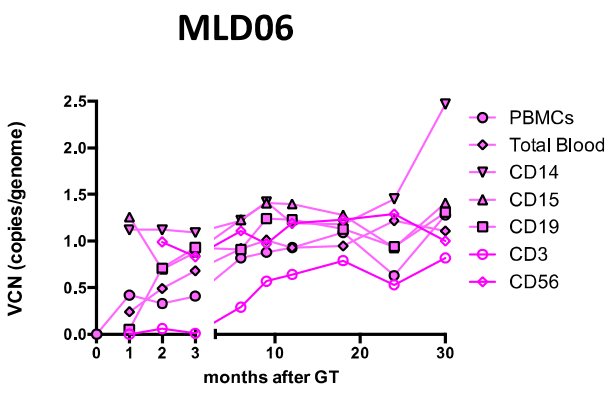


# Genome-wide Analysis of LV IS in Patients

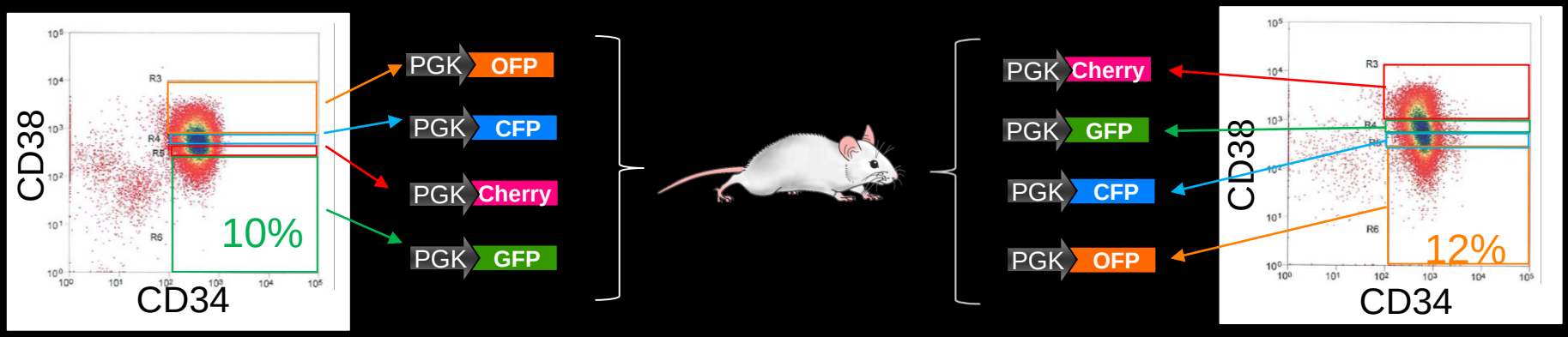
- *Highly polyclonal stable reconstitution*
- *No dominant or expanding clones*
- *No selection for insertions at cancer genes*
- *Confirms prediction of improved safety from pre-clinical models*

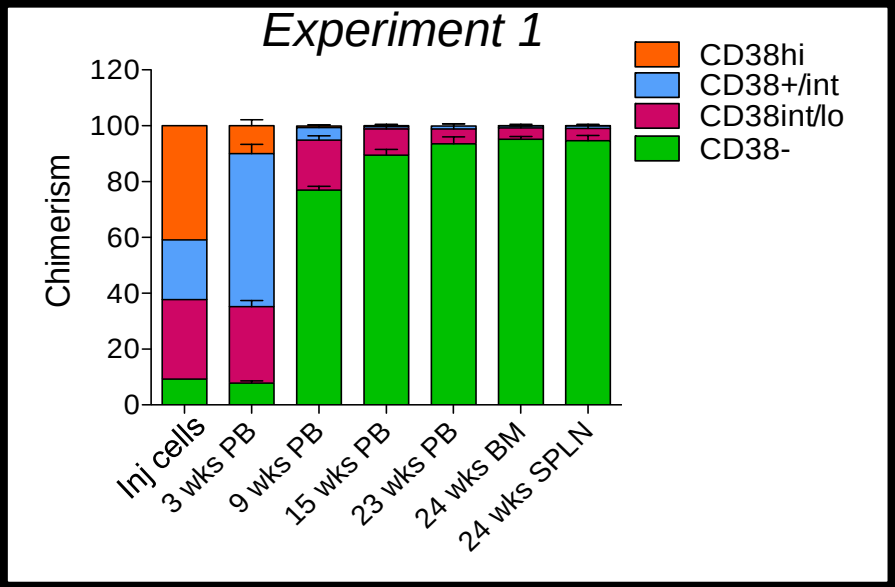
→ *No evidence of genotoxicity*

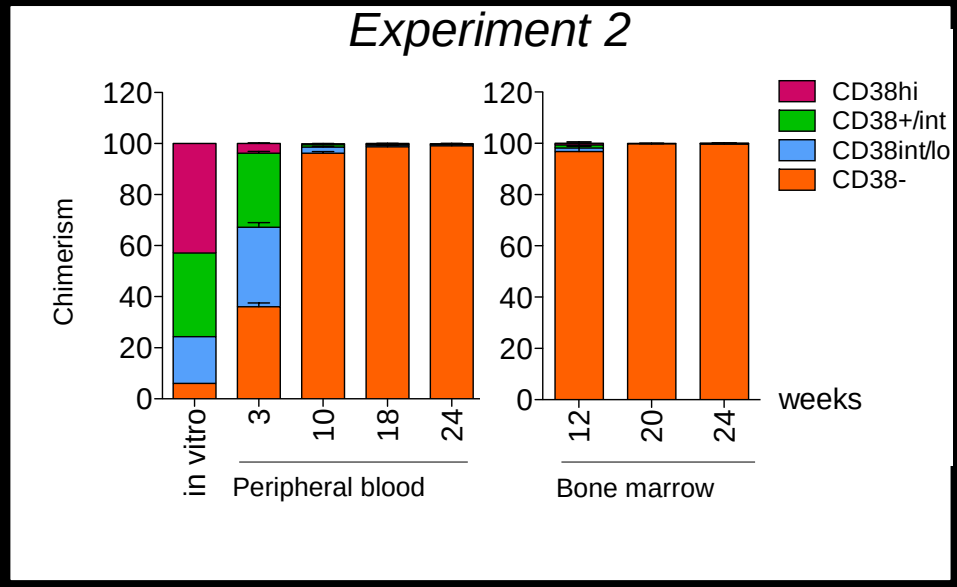
*Aiuti et al., Science, 2013; Biffi* Montini* et al, Science 2013*
*A. Calabria, E. Montini et al., unpublished*

# Short vs. Long-Term Contribution to Hematopoiesis

*FACS-sort CD38 subpopulations from CD34⁺ G-CSF MPB* → *Differentially mark by LV* → *Mix back together and xenotransplant*

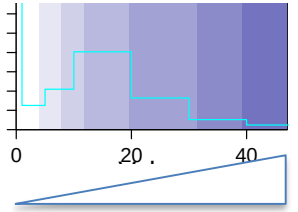


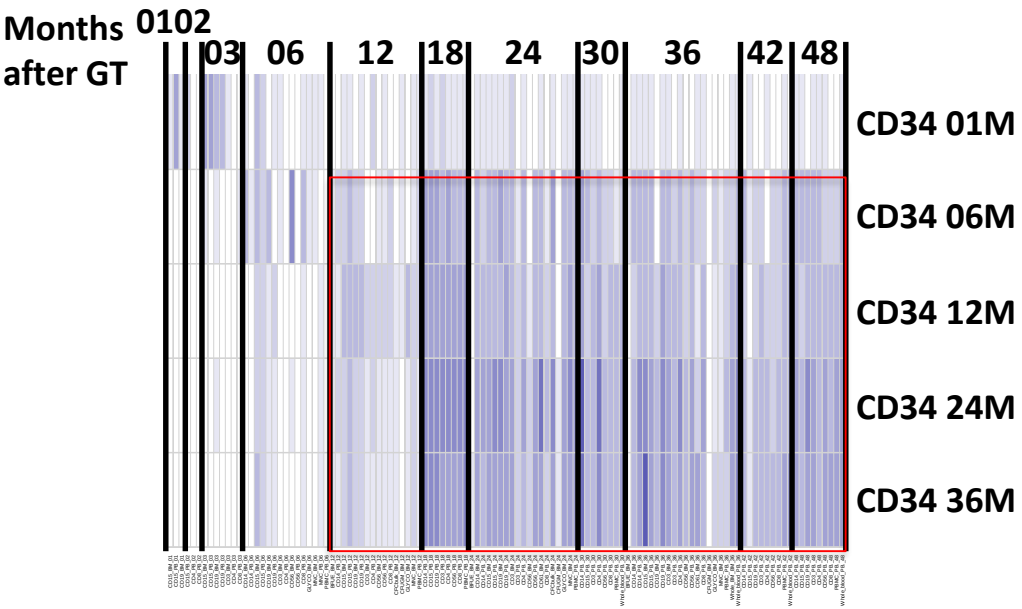




*Zonari & Gentner, Stem Cell Reports 2017*

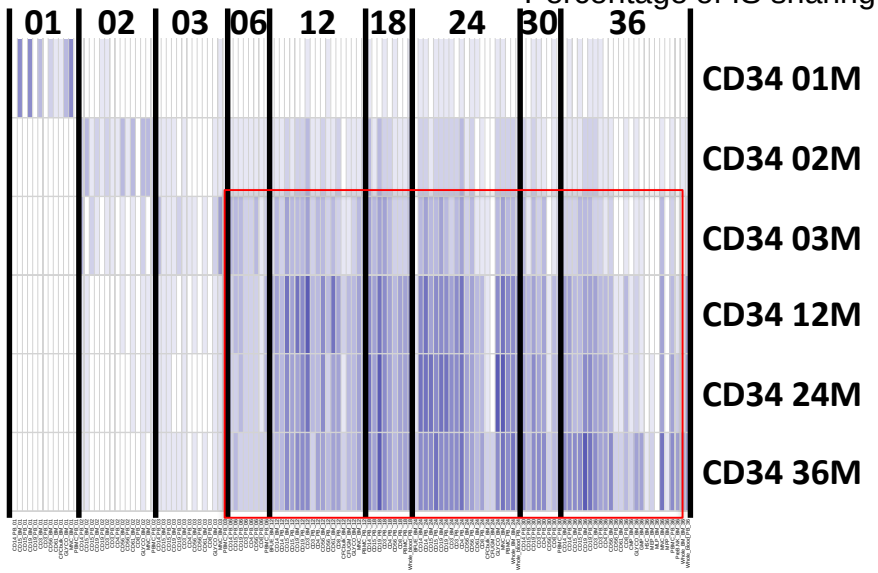
# Shared IS among CD34 and other lineages



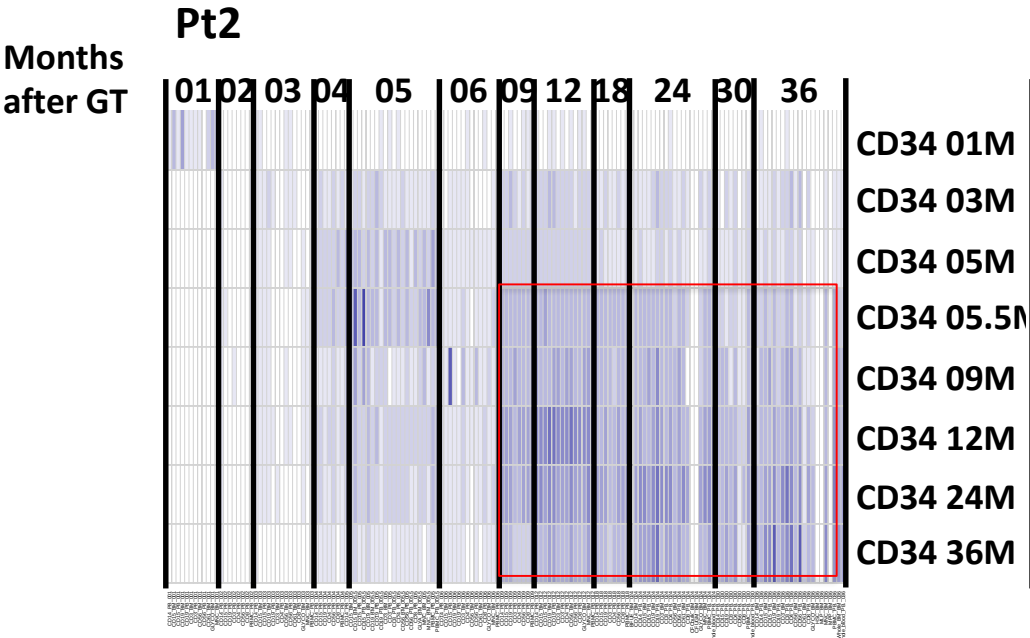

## WAS Pt1



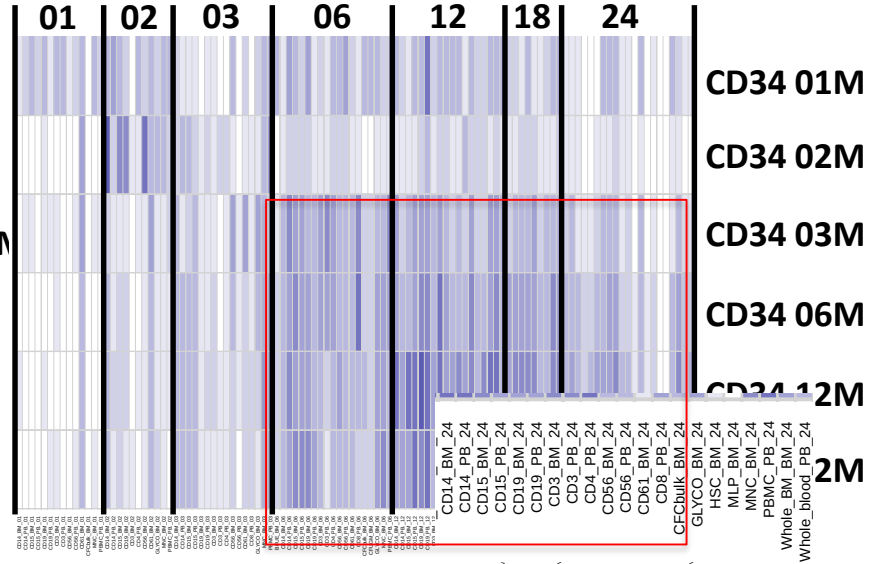

## Pt3




## Pt2




## Pt4



# Clonal Tracking of Hematopoiesis in Humans

- *Early post-transplant reconstitution mostly driven by short-lasting progenitors*
- *Followed by stable multi-lineage output from long-lasting HSC*

- *LV transduced HSC robustly engraft*
- *LV provide the means to safely engineer human hematopoiesis to near completion*

*Biasco et al., Cell Stem Cell 2016; Montini, unpublished*

# HSC Gene Therapy of WAS: Summary

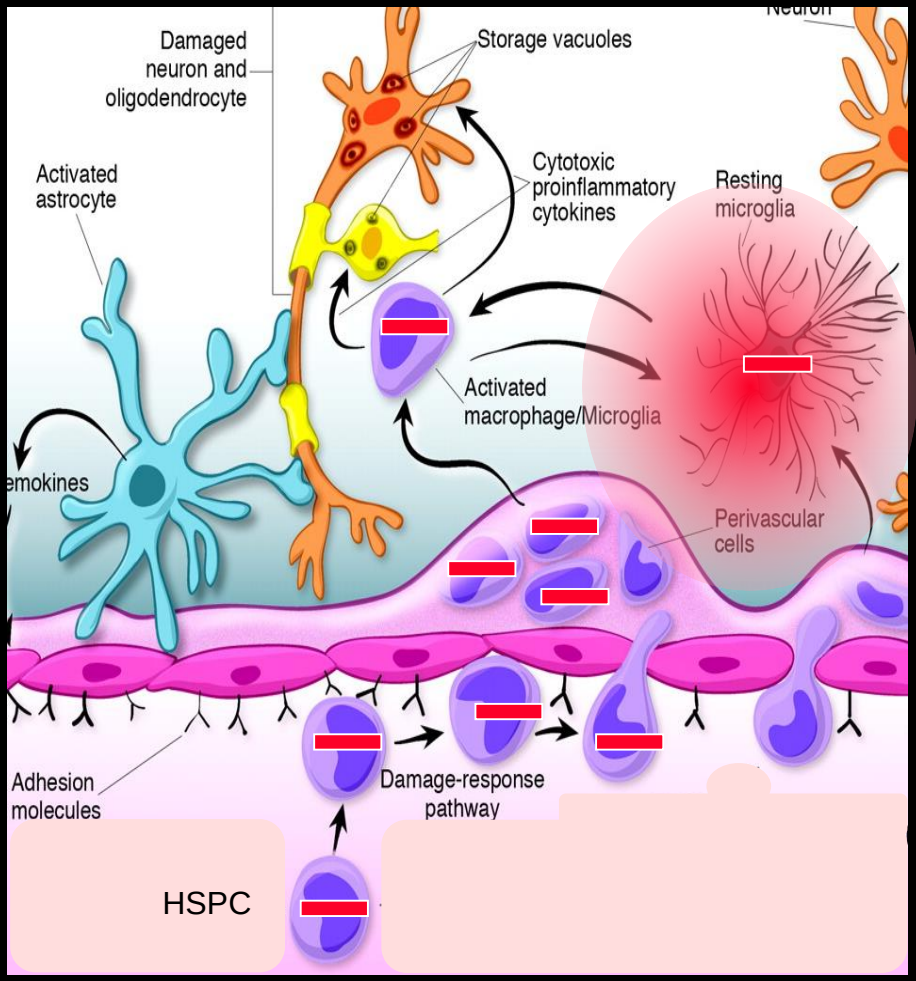
- *Persistent clinical benefit and safety*
  - 8 patients: all alive FU 0.8-6 years
  - Improvement in immune function, platelet number
  - Reduced bleeding, eczema, infection, autoimmunity
  - *Benefit comparable to successful allogenic HSC*

*Aiuti et al., Science, 2013; Biasco et al, Cell Stem Cell 2016*

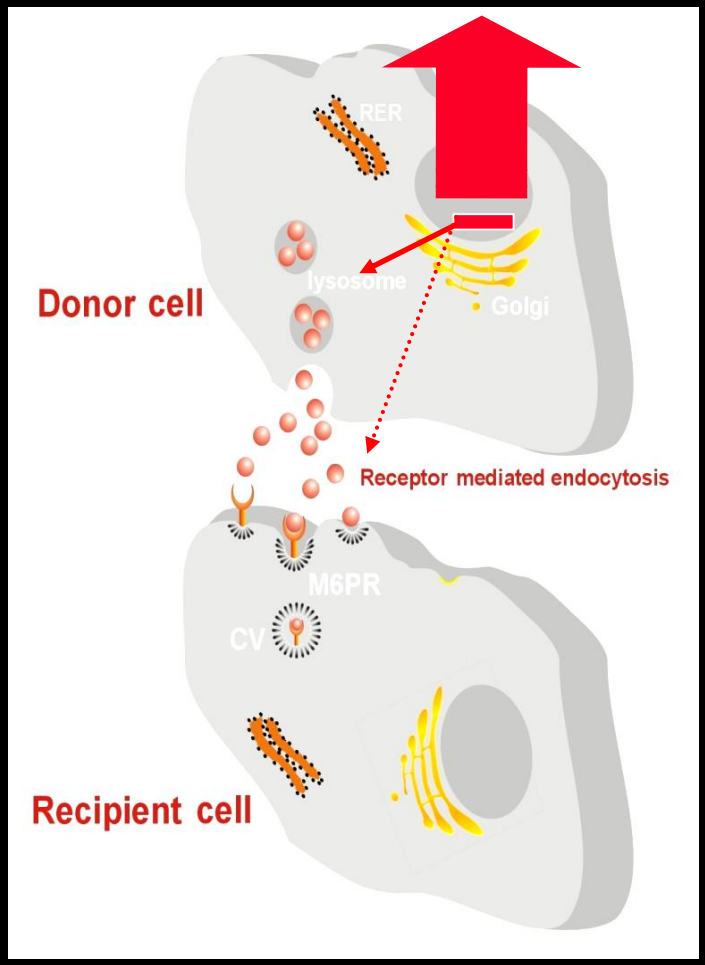
# Rationale for HSC Gene Therapy of MLD

CNS

Blood



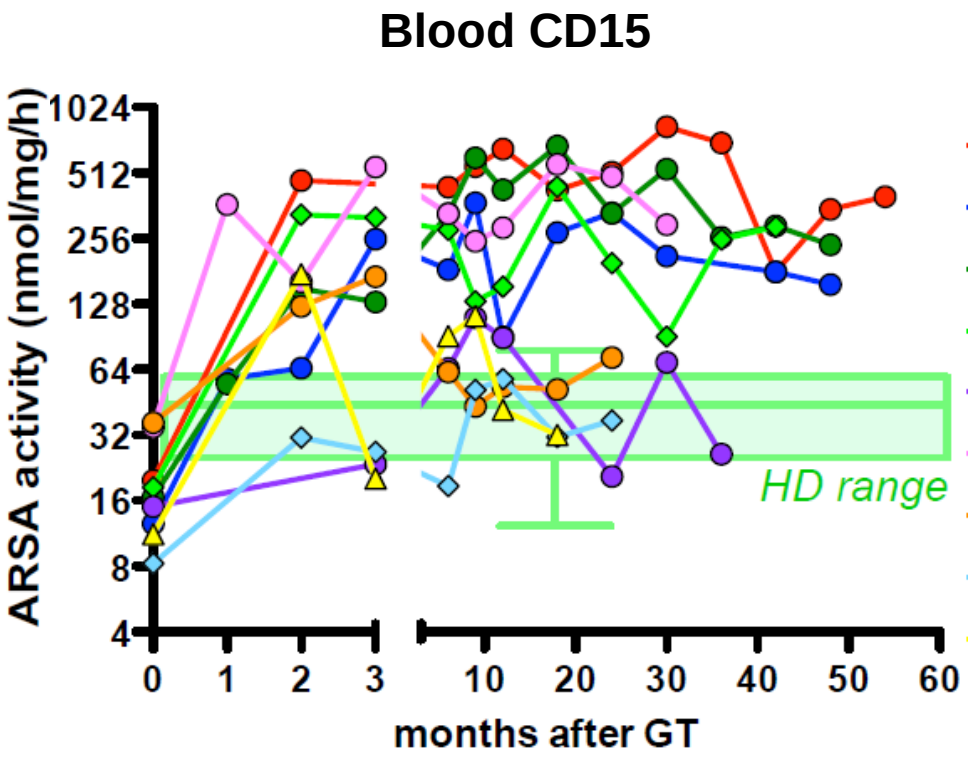

After conditioning, some microglia reconstitution from infused progenitors

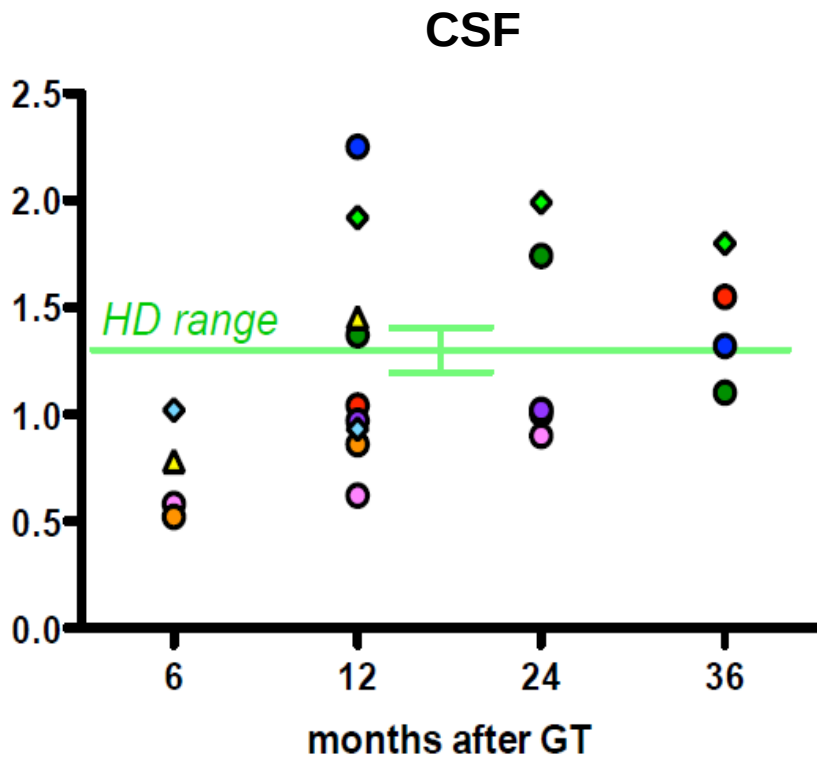



Cross-correction of resident cells

*Biffi et al., J. Clin Inv. 2004 and J. Clin Inv. 2006; Capotondo et al., PNAS 2012*

# ARSA Activity Reconstitution in MLD Patients

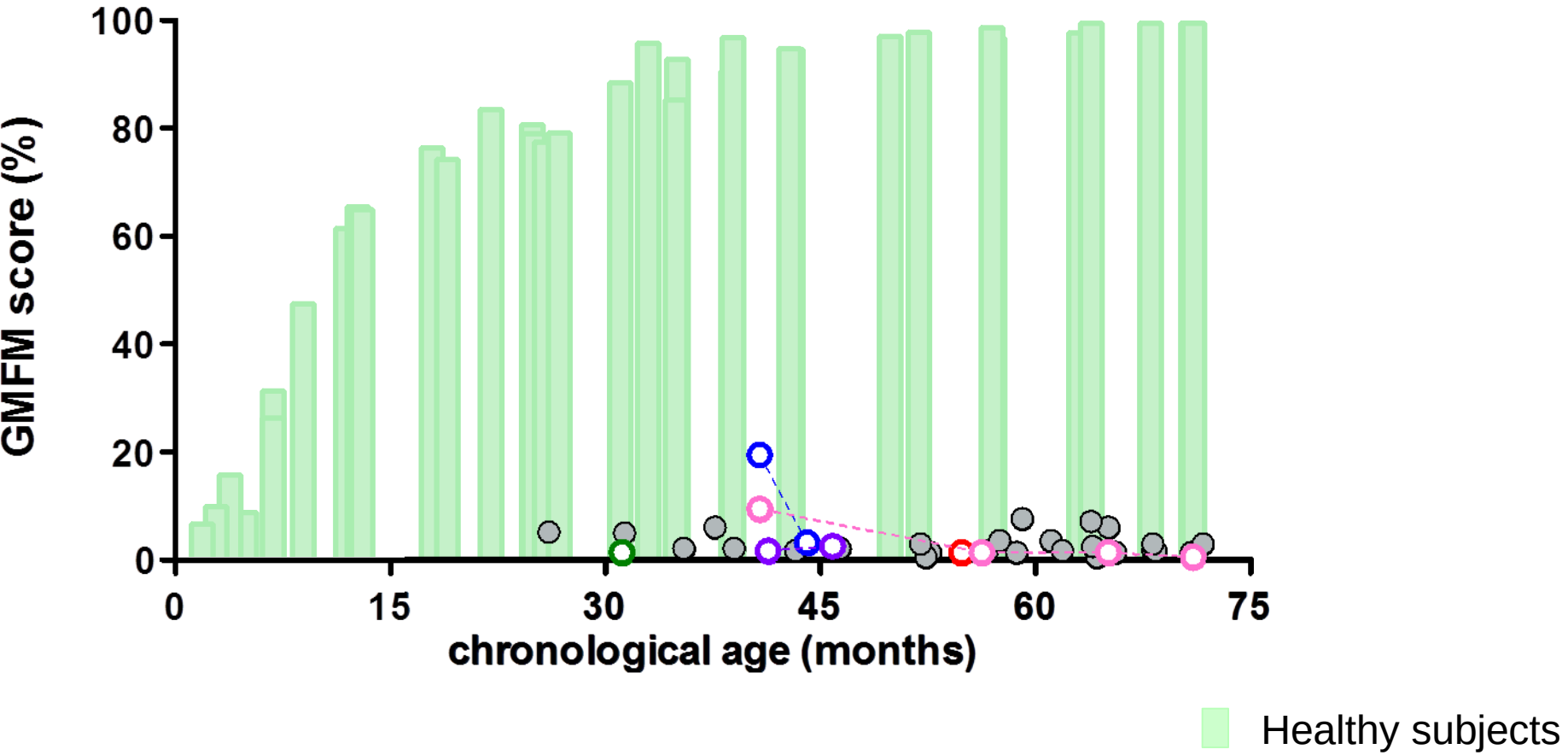



- MLD01
- MLD02
- MLD03
- MLD04
- MLD05
- MLD06
- MLD07
- MLD08
- MLD09

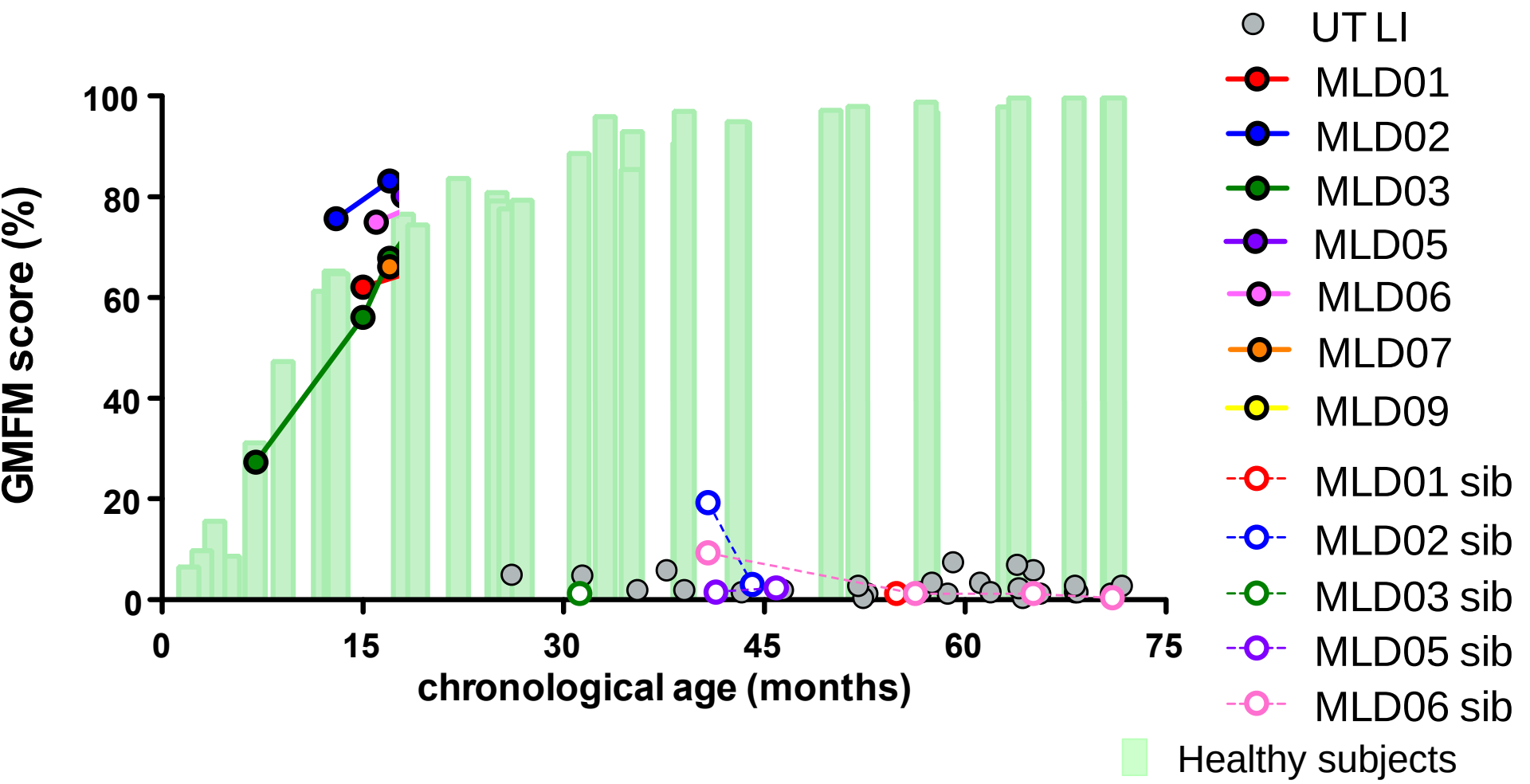
# Late Infantile MLD: Disease Evolution

## GMFM scale: evaluation of motor skills

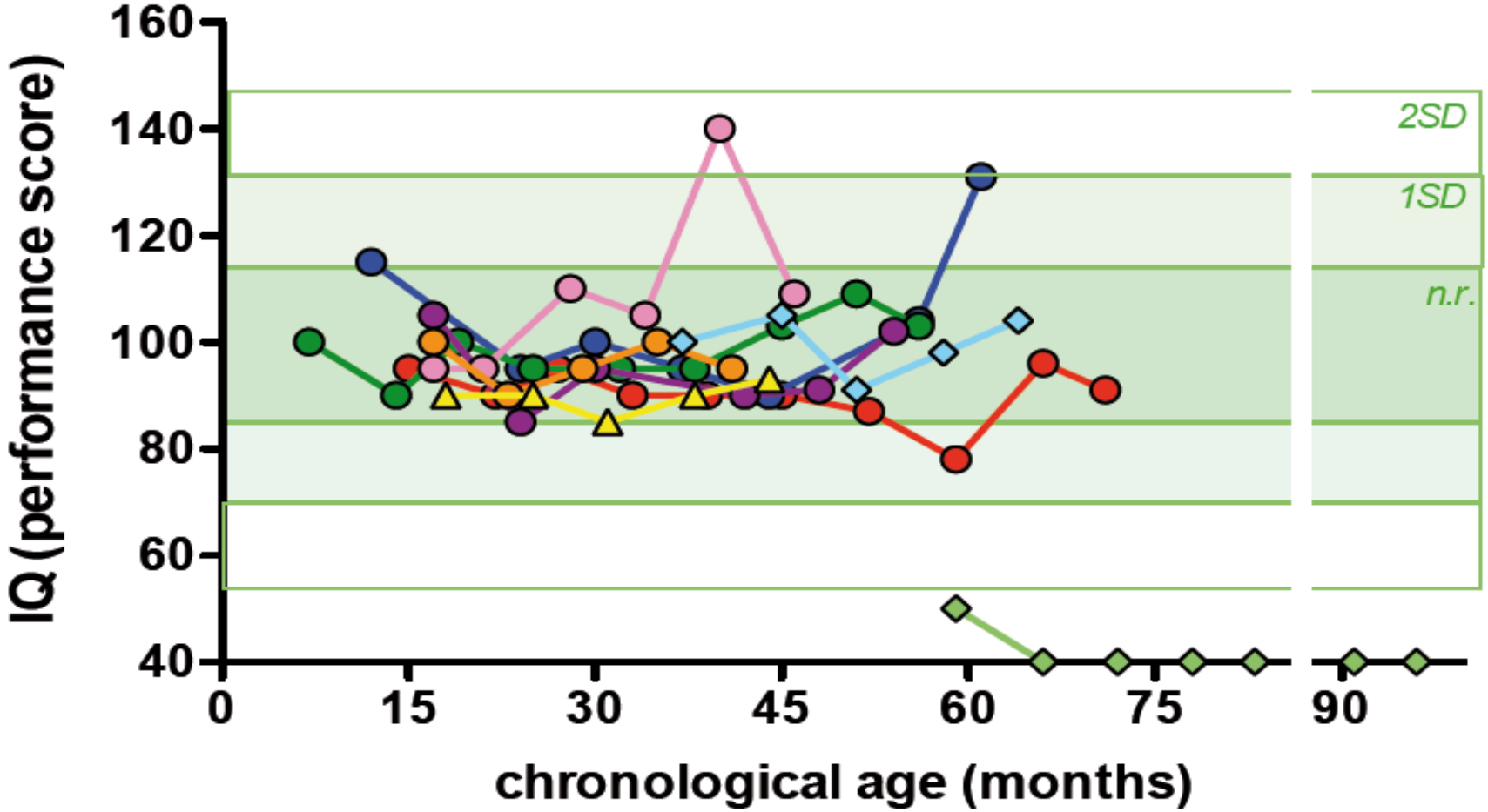


# Clinical Benefit of HSC Gene Therapy

## GMFM scale: evaluation of motor skills



# Neuropsychological Evaluation




*Bayley scale for infant development*

# HSC Gene Therapy of MLD: Summary

- *Well tolerated and safe (current follow-up)*
- *Reconstitution of ARSA activity in hematopoietic cells and CNS (CSF)*
- *No Disease Onset or Progression*
  - *In pts treated as pre- or early symptomatic*

*Biffi, Montini et al, Science 2013; Sessa, Lorioli &Biffi, Lancet 2016*

# Contributors: MLD & WAS Clinical Trials

*MLD*

*Alessandra Biffi, Maria Sessa*

*L. Lorioli, F. Fuma*

*S. Acquati, D Red*

*S. Canale, M. Ces*

*T. Plati, M. Gabald*

*A Rovelli, Monza*

*Integration Stud*

*Eugenio Montini, A*

*F. Benedicenti, Lu*

*DKFZ, Heidelberg*

*C. von Kalle, M. S*

*Univ. Perugia*

*S. Martino, F. Mor*

*WAS, CRU & UTMO*

*Alessandro Aiuti*

# Current Clinical Testing of HSC Gene Therapy

- *Seminal work with γ-RV in PID*
  - ADA-SCID 1st ex vivo gene therapy product on the market
- *Expanded applications using Lentiviral Vectors*
  - Stable high-level polyclonal reconstitution with transduced cells (up to 9yr follow-up)
  - No evidence of genotoxicity to date
  - Persistent clear clinical benefit in most treated patients
- *SR-Tiget trials: MLD, WAS, β-thalassemia*
  - 24, 9, 7 pts; up to 6 yr follow-up; up to 80% stable marking
- *Similar findings in multiple trials and sites*

*Aiuti et al., Science, 2013; Biffi* Montini* et al, Science 2013*
*Biasco et al, Cell Stem Cell, 2016; Sessa et al., Lancet, 2016*

# A Future Outlook for HSC Gene Therapy

- Autologous HSC GT may become preferred to allogeneic HSC transplant in several diseases
  - mixed chimerism sufficient for full benefit
- With further improvements
  - faster hematopoietic reconstitution (better preserve progenitors)
  - increased HSC input (improved harvest, decreased manipulation, ex vivo HSC expansion)
  - milder conditioning regimens (non mutagenic)
- With more precise genetic engineering

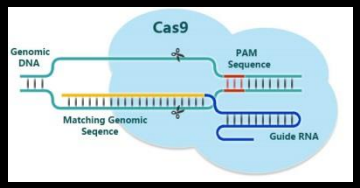
# Targeted Gene Editing

- *Edit DNA sequence to correct mutations*

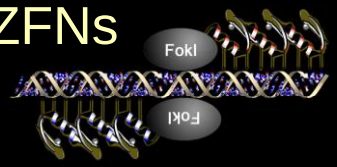

A. Lombardo

# Exploiting Artificial Nucleases (ANs) for Targeted Genome *Editing*

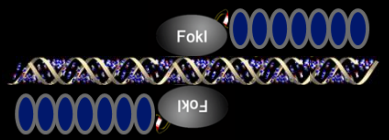
CRISPR/Cas9




ZFNs



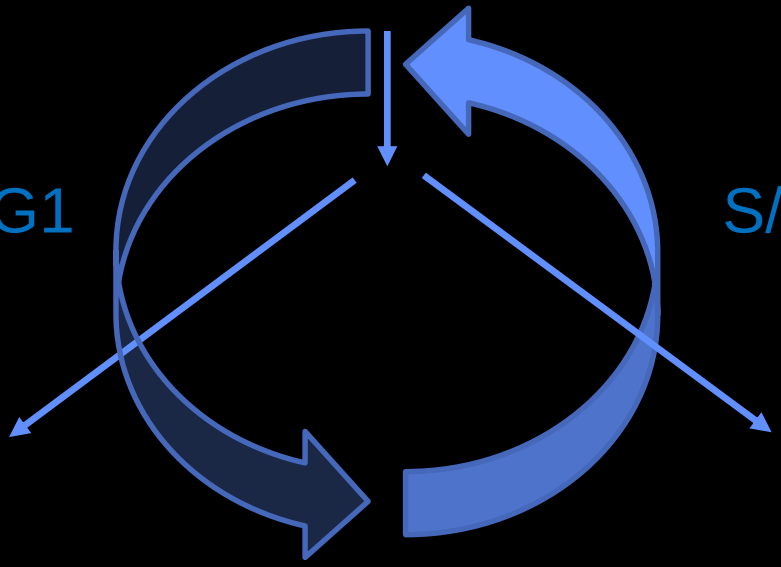

TALENs




DNA DSB at target site

*NHEJ*
Non Homologous End Joining

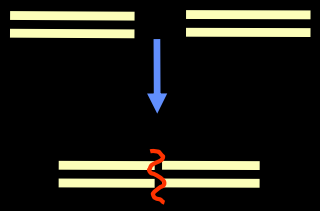
G1



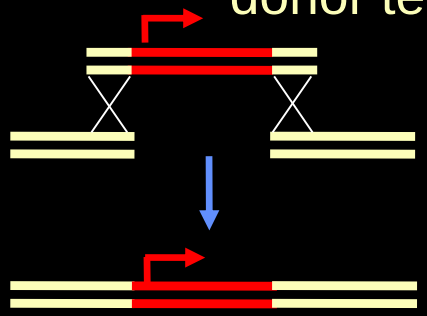
S/G2

*HDR*
Homology Driven Repair

Exogenous donor template



Gene Disruption



Transgene Insertion / Gene Correction

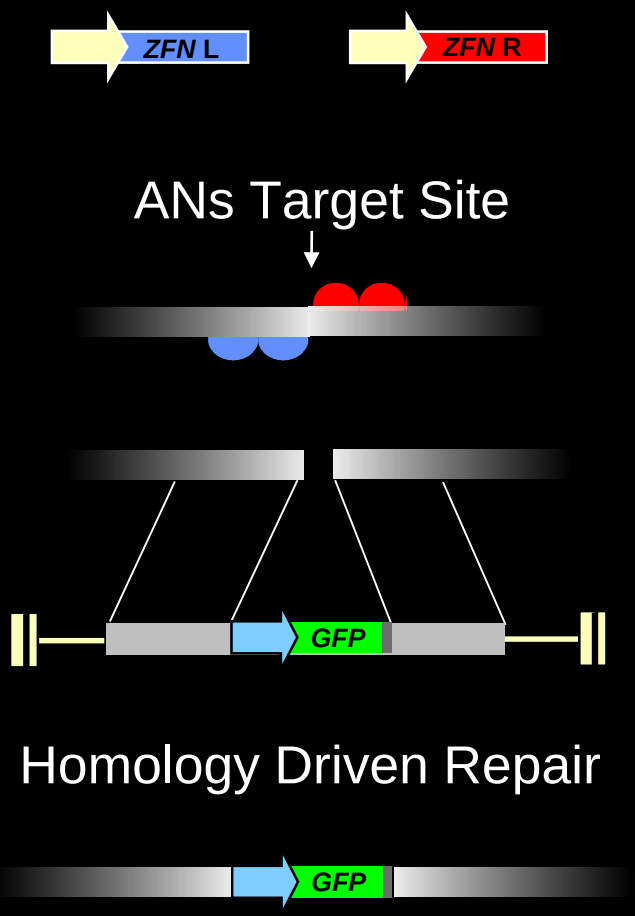
# Therapeutic Potential of Targeted Gene Editing in HSC Gene Therapy

- *in situ gene correction vs. gene replacement*
  - restores gene *function* and *expression control*
  - may abrogate risk of genome-wide insertional mutagenesis
  - genotoxic risk limited to off target activity
    - circumscribed to small fraction of genome
    - challenging to comprehensively define
      - hit-and-run nature & low sensitivity
    - potential for bi-allelic hits

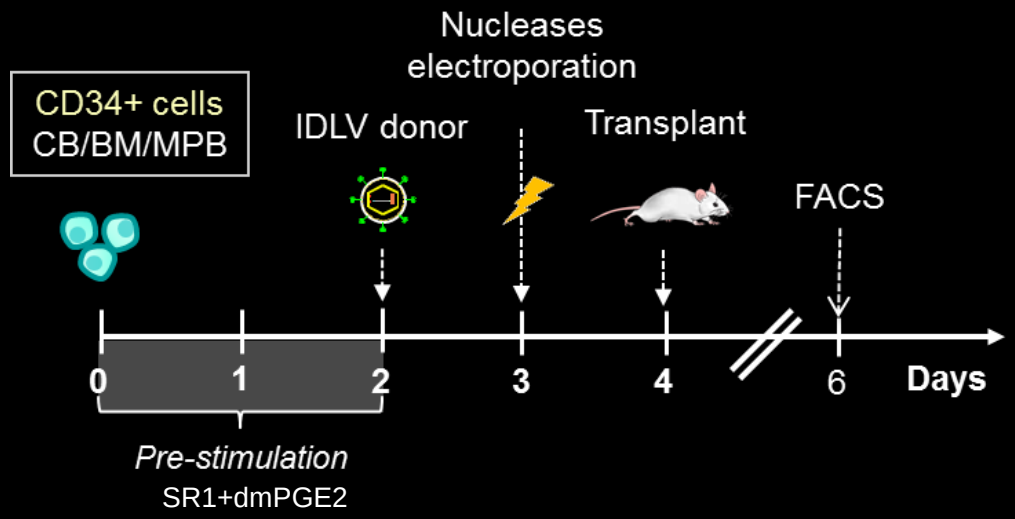
# Delivering the Gene Editing Machinery

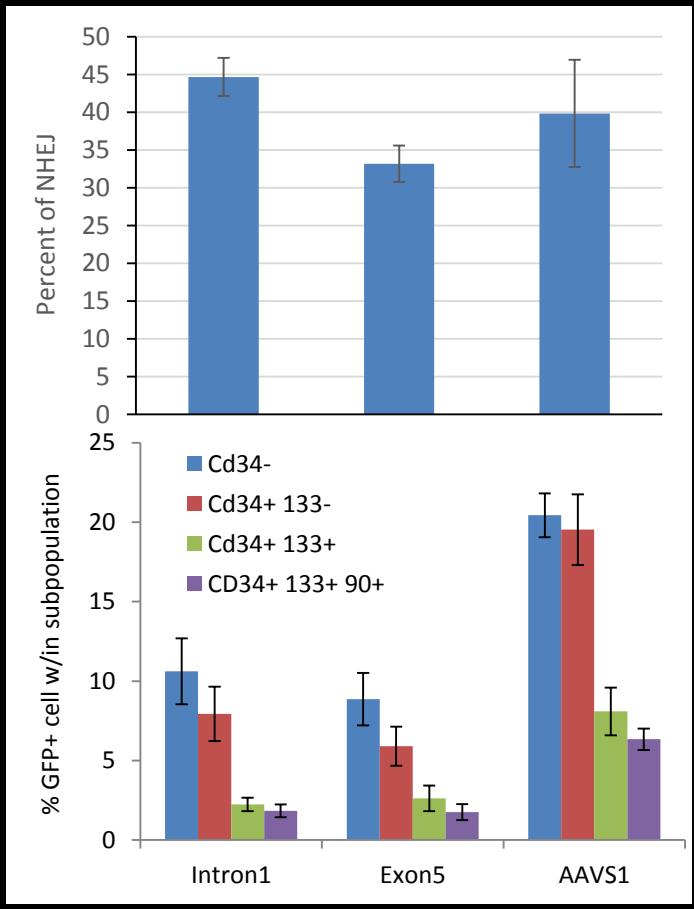
*mRNA, RNP*
*Integrase Defective LentiVector (IDLV)*
*Adenoviral 5/35*
*Artificial Nucleases*
*(ZFNs, TALENs, CRISPR/Cas9)*

*IDLV, AAV6,*
*plasmid DNA or oligo*
*with target site homologies*
*as donor template for HDR*



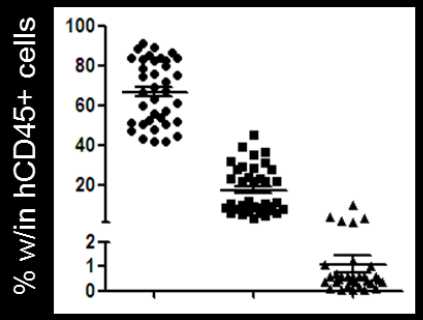
# Targeted Gene Editing of Human HSPC



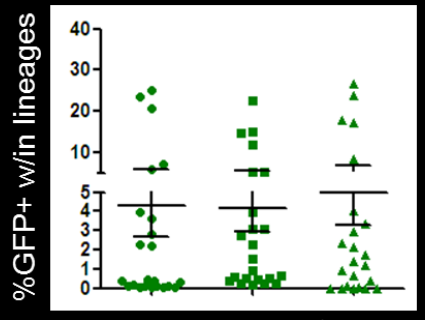




- Efficient disruption (NHEJ) vs. insertion by HDR
- HDR constrained in more primitive cells
- High specificity of integration
- Long-term multi-lineage engraftment of edited HSPC

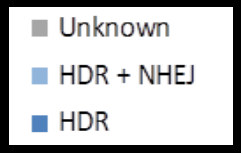
*Human engraftment*

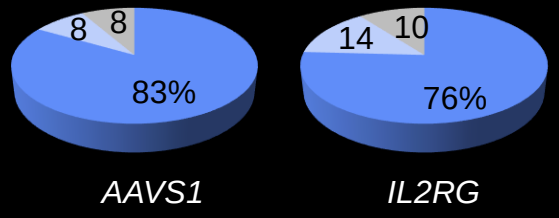



*GFP in subpopulations*




PCR on CFU



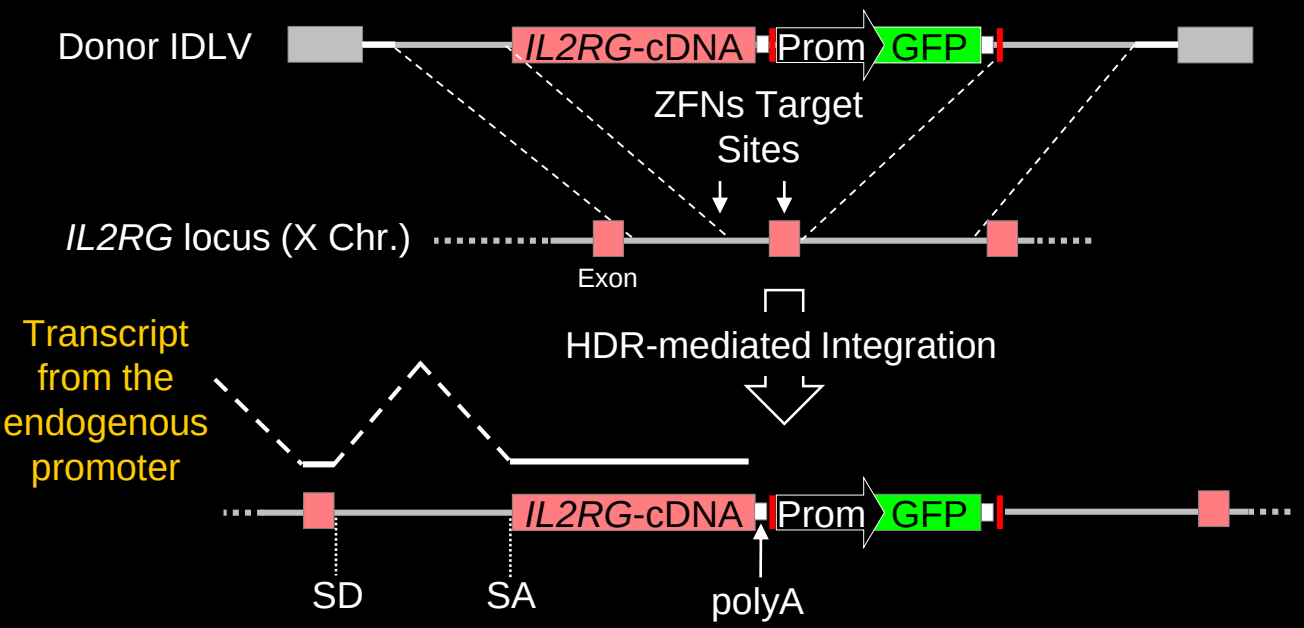




*Genovese et al., Nature 2014*

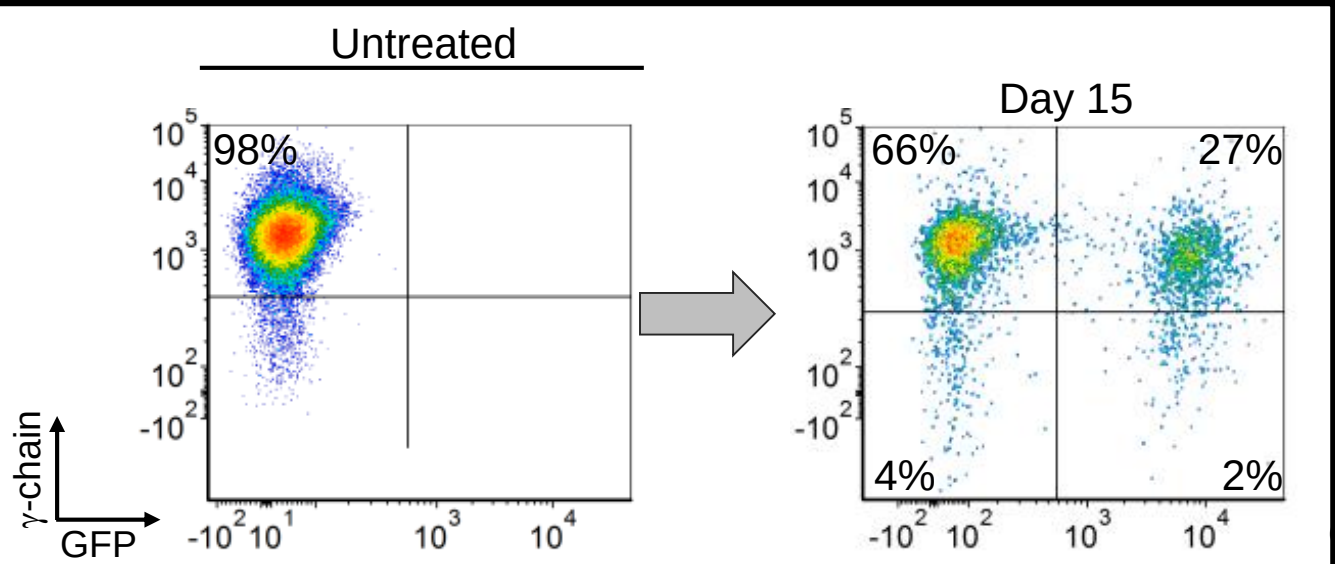
# Therapeutic Potential of Targeted Gene Editing in HSC Gene Therapy

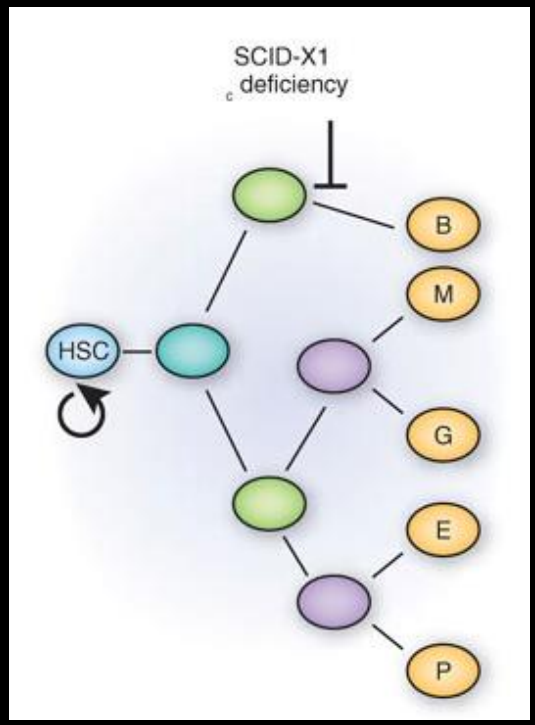
- *HDR-mediated editing constrained in HSC*
  - quiescence, apoptosis, limited DNA repair
  - low yield of edited HSPC may impact safety
- *Rationale for first clinical testing*
  - Primary immunodeficiencies such as *IL2RG, RAG1/2, CD40L, BTK deficiency*
  - unregulated expression may pose risk of transformation or malfunction
  - selective advantage of gene corrected cells may compensate low editing efficiency

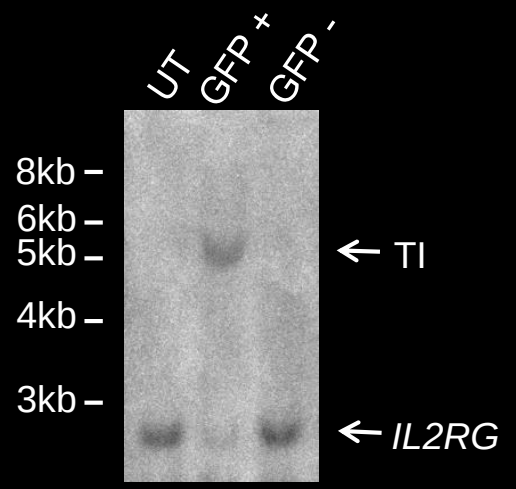
# Correction of *SCID-X1* Causing Mutations




Primary T cells from male donor



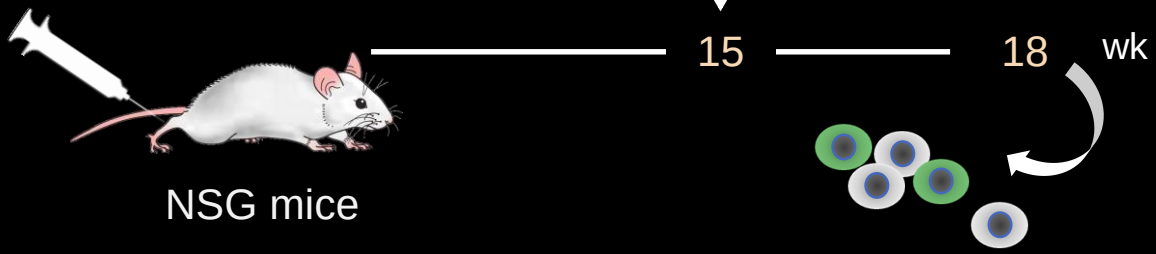



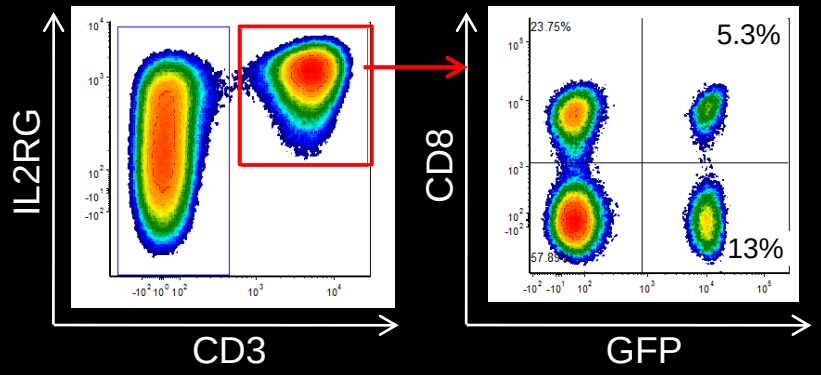



# Functional T Cells from *IL2RG* Edited HSC

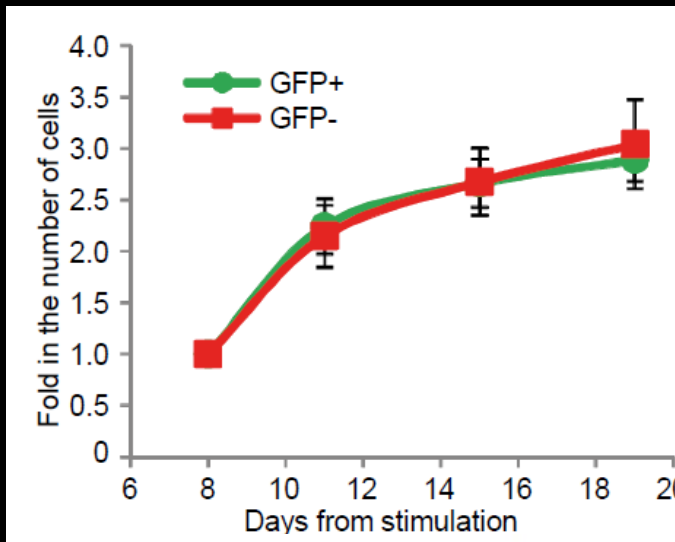
Gene Edited
♂ cord blood
HSPC

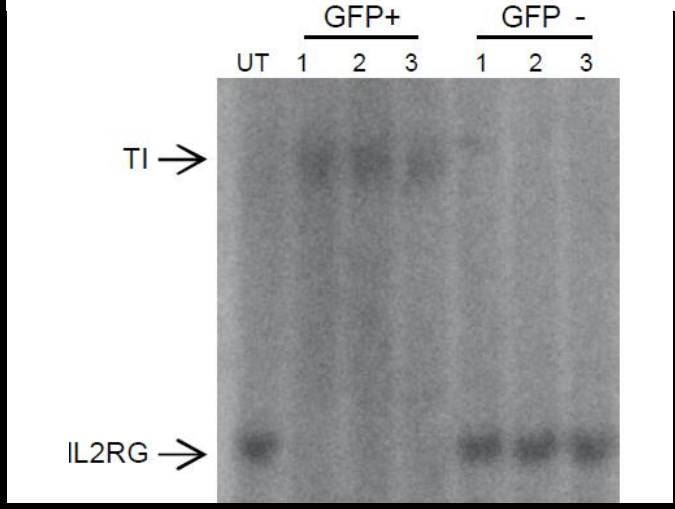
Human lymphoid-promoting cytokines
from tumor challenge




*ex vivo T-cell growth*



# Gene Correction of SCID-X1

- *Normal output & function of T-cell progeny from edited HSPC*
  - *Molecular evidence of correction of progenitors from patients*

- *Selective advantage may provide therapeutic benefit even from few edited HSPC*
  - *Can we model therapy in mice to instruct clinical trial design?*

*Genovese et al., Nature, 2014 & Schiroli et al., unpublished data*

# Summary: Modeling SCID-X1 Gene Correction

- *Low fraction of wild-type HSPC correct disease*
- *Edited progenitors in murine cell product*
  - rescue lymphoid compartments
  - T cells persist and can mount immune response
- *Safety concerns on low input of corrected cells*
  - Suboptimal immune reconstitution w/out conditioning
  - Potential for replicative stress oncogenesis
  - Conditioning and engraftment of up to 10% corrected HSC is protective and fully rescues the disease phenotype

*G. Schiroli, P. Genovese et al., submitted manuscript*

# Conclusion: SCID-X1 Gene Correction

- *Required threshold level of edited HSPC to fully rescue disease phenotype in mice (10%)*
  - within reach of optimized protocols using clinically relevant human HSC sources and scale
- *Partial myeloablative conditioning advisable to*
  - allow engraftment of edited HSC
  - protect from replicative stress (and transformation risk?) of corrected thymic lymphoid progenitors
- *Rationale for clinical translation established*

# Conclusions: Cell and Gene Therapy

- *Novel pharmacology*
  - Gene-based delivery of biotherapeutics
    - by gene transfer or editing
    - stable, regulated, targeted
- *Exploits powerful biological processes*
  - Information transfer by genome & epigenome
  - Regenerative potential of stem cells
  - Homing & trafficking by body cells as smart agents
- *Clinical evidence of clear benefit (cure) for*
  - some monogenic diseases,
  - potential for treating cancer and infections

# Cell & Gene Therapy: the Challenges Ahead

- *Safety*
  - "Live" biological drugs: unprecedented complexity long-term effects, germline & environment
  - Limited understanding of stem cells biology
- *Bedside delivery*
  - Need for multidisciplinary expertise and
  - new biological readouts of therapy
- *Society*
  - Personalized medicine:
    - manufacturing, quality standards
    - marketing pipeline, cost and sustainability

# Sponsors & Contributors

*Giulia Escobar*
*Luigi Barbarossa*
*Pietro Genovese*

*San Raffaele Institute*
*Eugenio Montini, A. Calabria*
*Chiara Bonini, E. Provasi*