RAC 135th Meeting, Sept 11, 2013

Lentiviral Hematopoietic Stem Cell Gene Therapy of Metachromatic Leukodystrophy and Wiskott-Aldrich Syndrome

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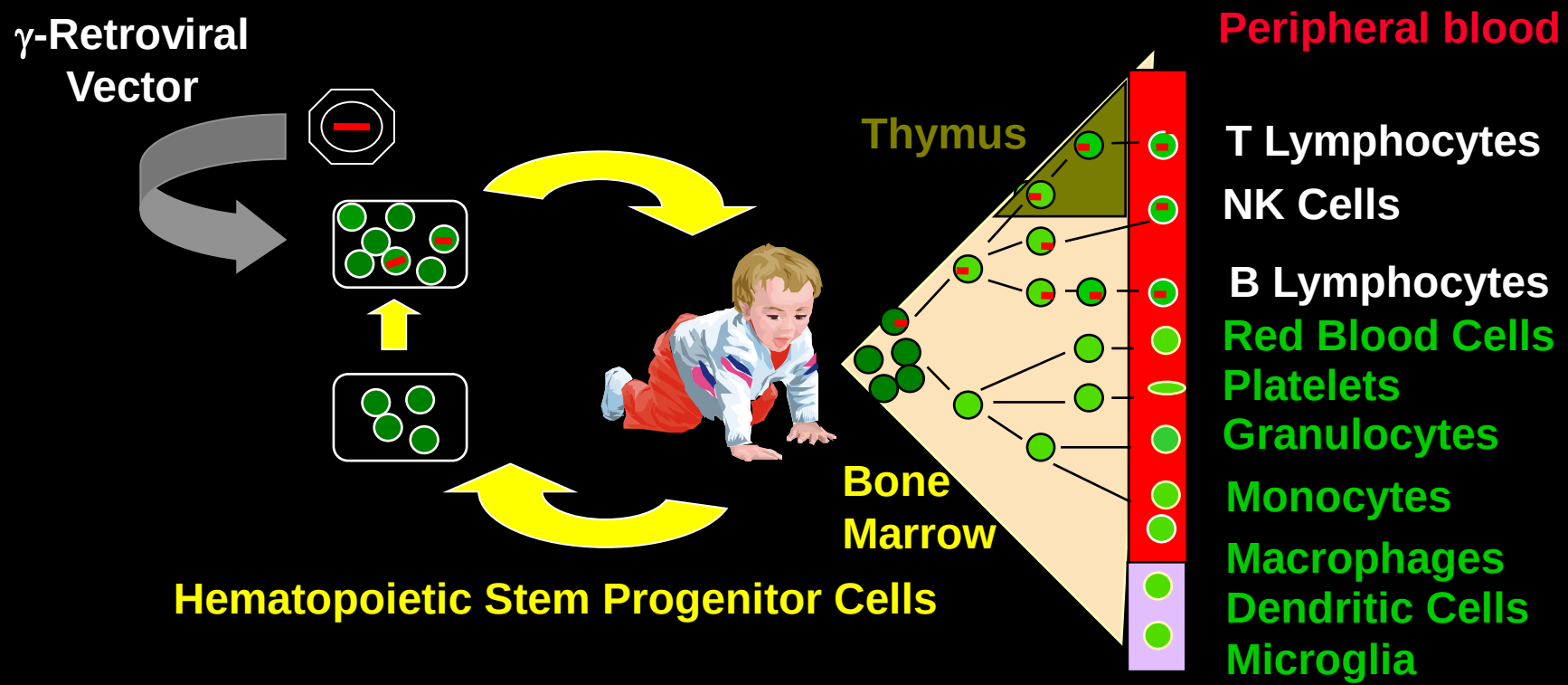
Hematopoietic Stem Cells (HSC)-based Gene Therapy of Primary Immunodeficiencies



- *Successful long-term immune reconstitution in phase I/II clinical studies with most patients alive & well (up to 13 yr follow up)*
- *Key to the success: growth-advantage of corrected lymphocytes*
- *Vector-related leukemia in some patients and some diseases*

Advancing Applications of HSC Gene Therapy

- *Increase efficiency of gene transfer*
  - Vector choice
- *Regulate transgene expression*
  - Ectopic or constitutive expression may cause toxicity
- *Alleviate risk of insertional mutagenesis*
  - Random integration may activate oncogenes

Lentiviral Vectors for Gene Transfer

- *Increased efficiency of gene transfer*
- *Robust expression & improved regulation*
- *Lower concerns for genotoxicity*

Naldini et al, Science 1996; Follenzi et al., Nature Genetics 2003
Montini et al., Nature Biotech. 2006 & J. Clin. Invest. 2009

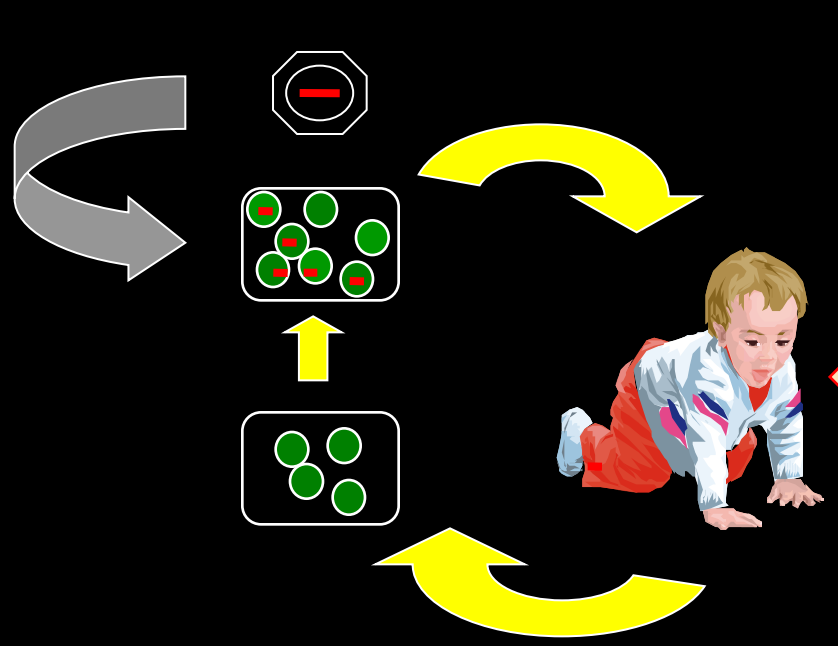
Advancing Applications of HSC Gene Therapy

Immuno-hematological diseases

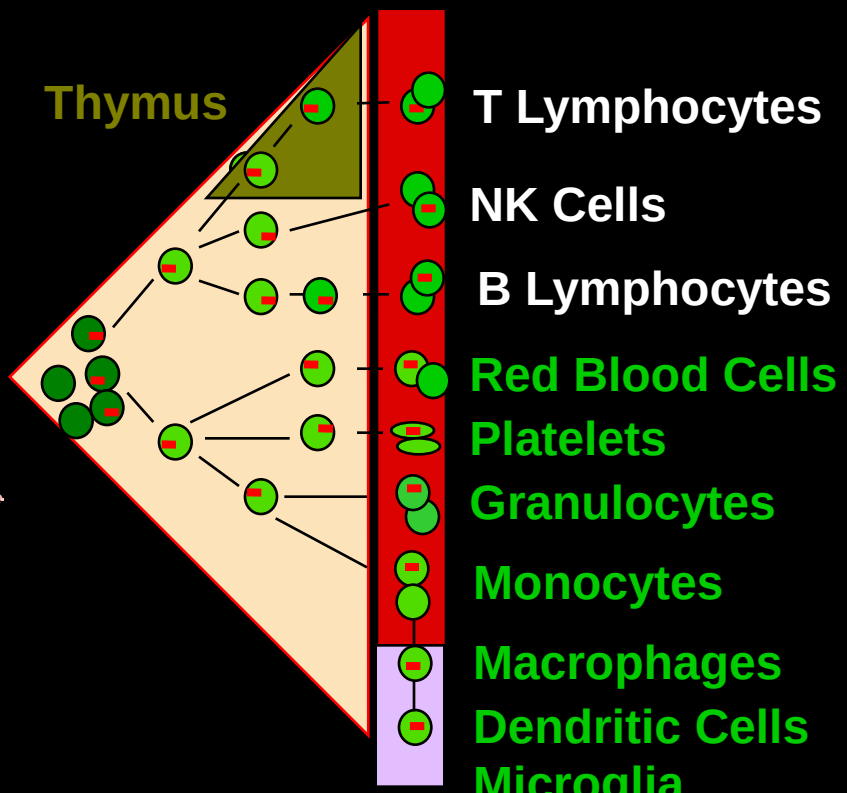
B-Thal
Cavazzana et al.
Nature 2010

Transduce with
Lentiviral Vector

Infuse into
conditioned recipient



Harvest Hematopoietic
Stem Progenitor Cells



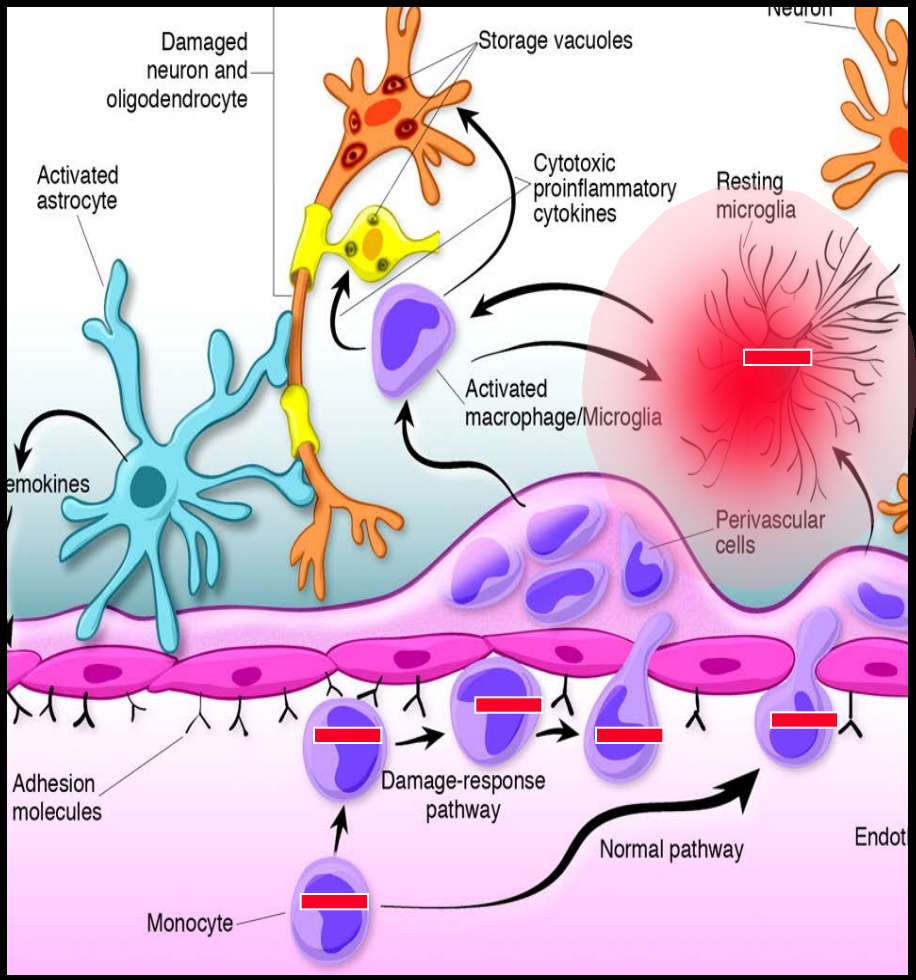
Storage diseases

ALD
Cartier et al
Science 2009

Rationale for HSC Gene Therapy of Lysosomal Storage Diseases Affecting CNS

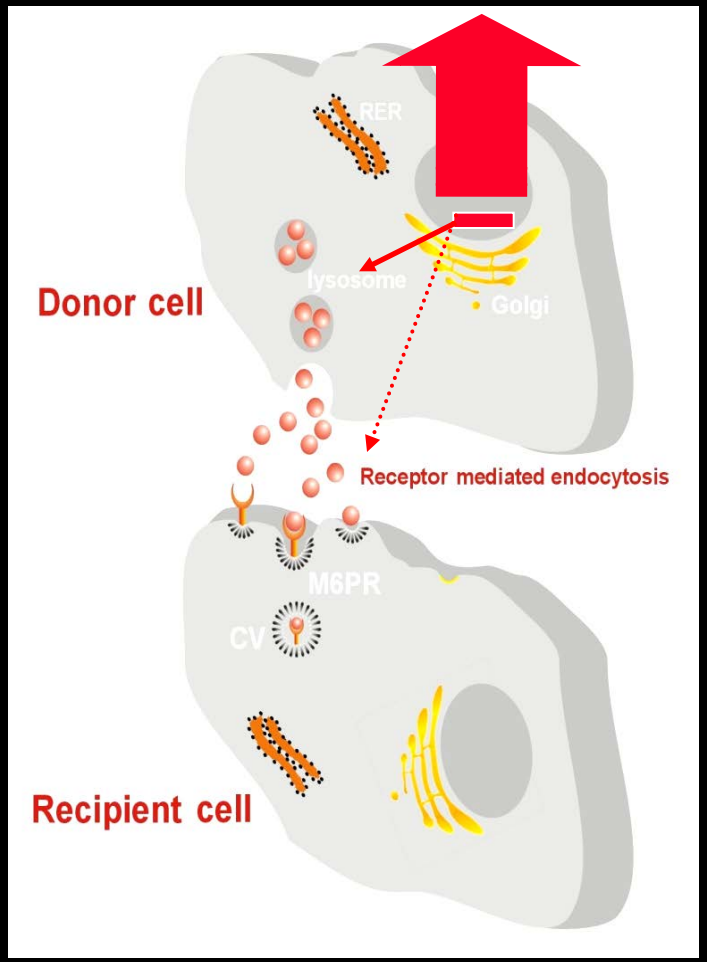
CNS

Blood



Progressive microglia reconstitution with gene corrected cells

→ *Efficient HSC gene transfer*

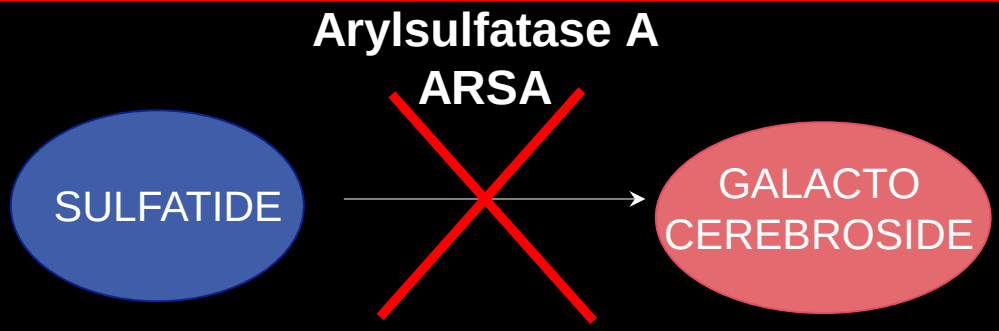


Cross-correction of resident cells

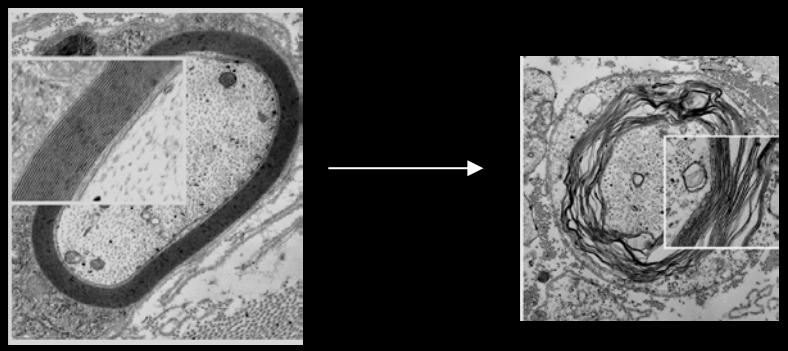
→ *Enzyme overexpression*

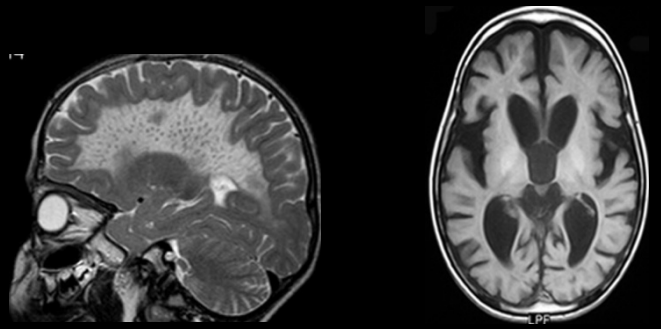
Metachromatic Leukodystrophy

- *Pathogenesis*
  - Genetic deficiency of
  - Storage of sulfatide
  - Microglia activation, degeneration of oligodendrocytes and neurons, severe dismyelination

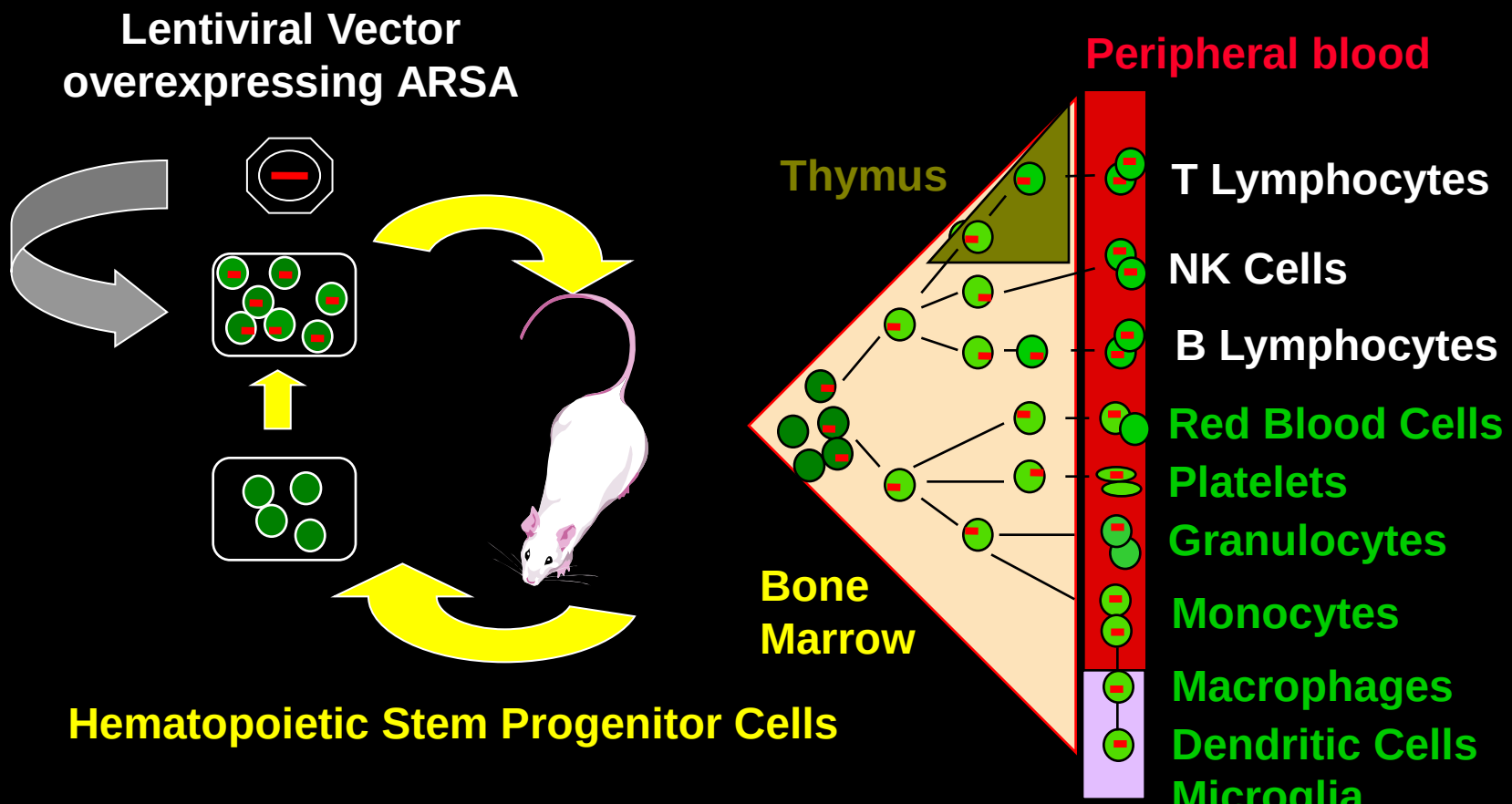


- *Prognosis*
  - fatal within 10 years from onset
- *Therapy*
  - mainly supportive; BMT ineffective



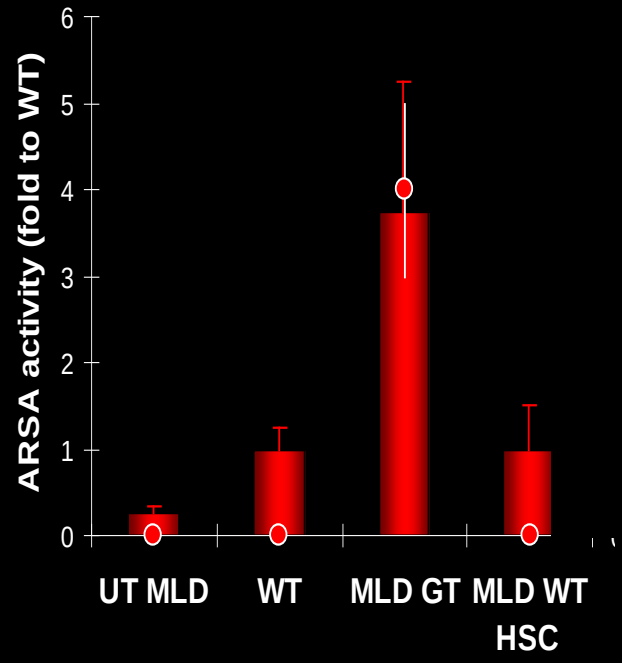


HSC Gene Therapy of MLD Mice

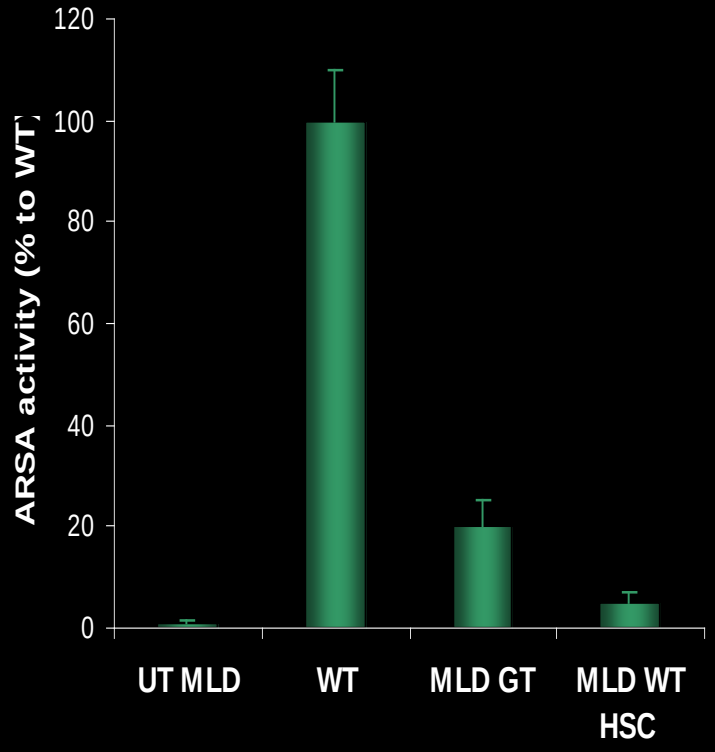


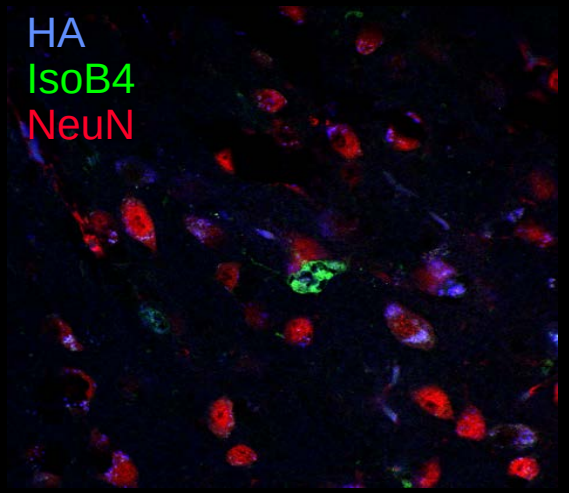
ARSA Reconstitution & Cross-correction in CNS

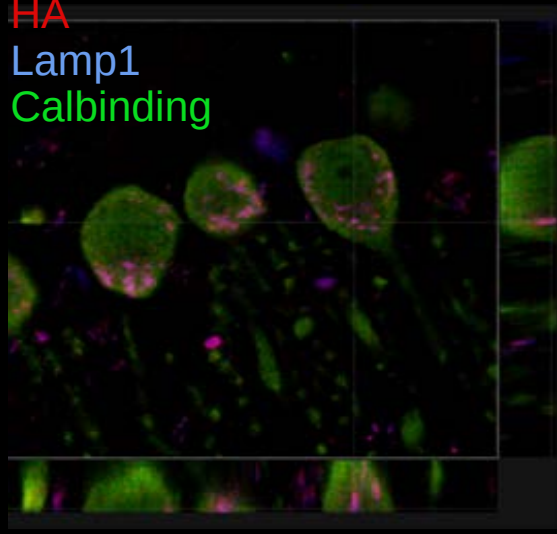
Blood Mononuclear Cells



Brain







HSC Gene Therapy of MLD in Mouse Model

- Supra-normal enzyme reconstitution in hematopoietic cells
- Cross-correction of CNS cells from microglia
- Prevention and correction of disease manifestations

→ *Gene-corrected autologous HSC more effective than wild-type HSC with dose-dependent benefit*

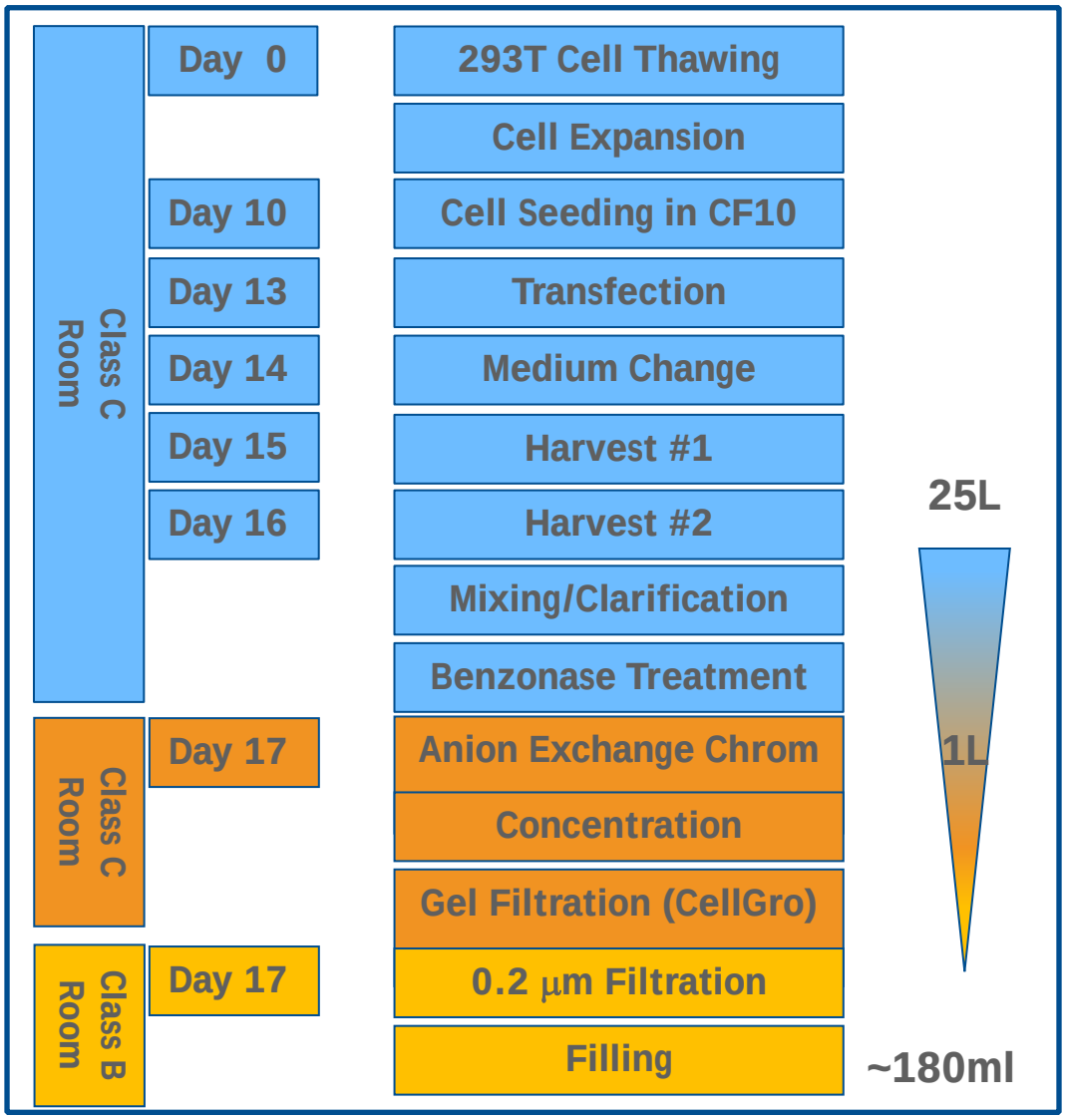
→ *High HSC gene transfer efficiency required*

Biffi et al., J. Clin Inv. 2004 and J. Clin Inv. 2006

HSC Gene Transfer: Preclinical Studies

- *Can we transduce human HSC*
  - to high frequency
  - without affecting engraftment and stem cell properties
  - with low risk of insertional mutagenesis ?
- *Produce high quality GMP LV*
- *Test HSC gene transfer by repopulation of human hematochimeric SCID mice*

Large scale GMP LV manufacturing - MOLMED
Process Overview and Final Yield



ARSA LV (n=3) Range
Total Tranducing Units
4.6E10 - 1.1E11
Total Physical Particles
1.5E13 - 2.5E13
Titre (TU/mL)
2.7E08 - 6.4E08
Infectivity (TU/ng p24)
3.3E04 - 5.6E04

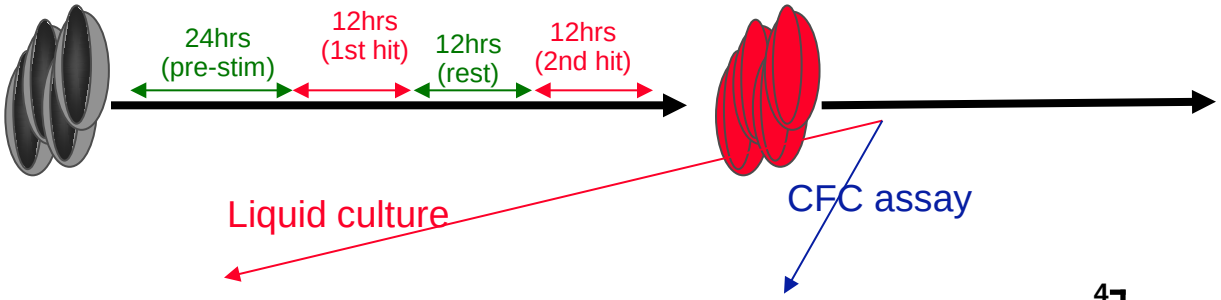
Purified LV: QC strategy

Identity/Potency Tests
Infectious Titer
Physical Titer
Infectivity
Vector integrity
Transgene function
Vector sequence
Lentiviral proteins
pH
Osmolarity

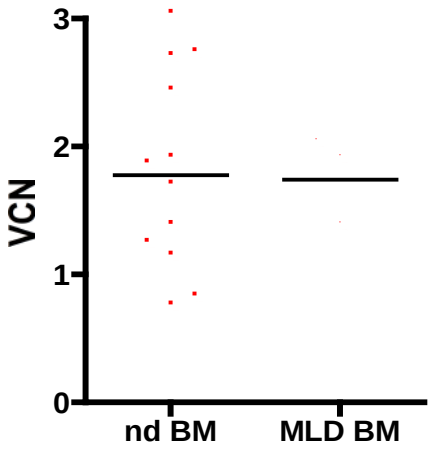
Impurity Tests
Bovine Serum Albumin
Benzonase
Host cell proteins
Large T Antigen protein
Total residual DNA
E1A residual DNA
LTA residual DNA
Plasmid residual DNA
Vector cross-contamination

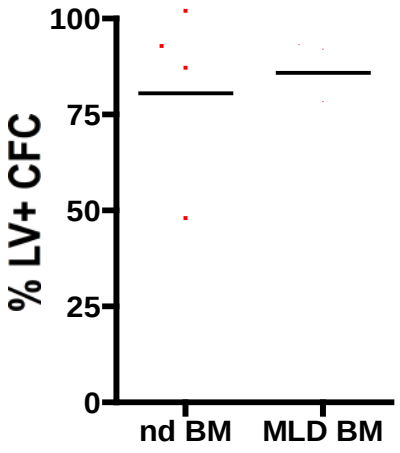
Safety Tests
Endotoxin
Sterility
Mycoplasma
Adventitious viruses
Replication Competent Lentiviruses

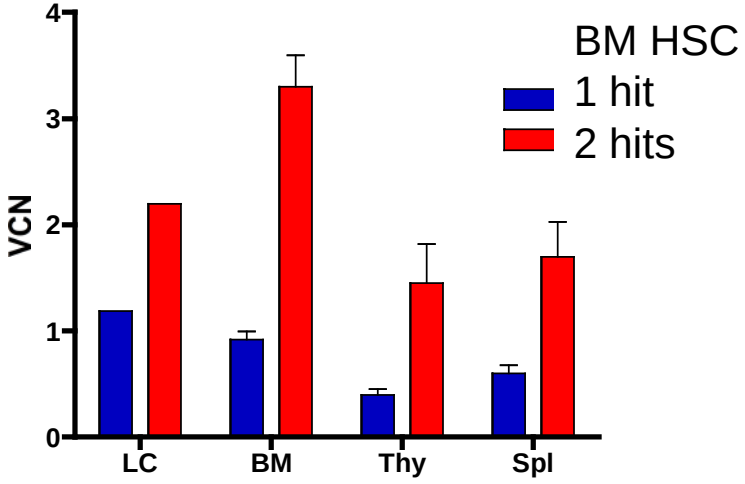
Efficient BM HSC Transduction by GMP LV



Rag2$^{-/-}$*Il2rg*$^{-/-}$







Vector Titer 4.4 ±1.2 x10^8 T.U.^{CEM}/ml Infectivity 4.2 ± 0.7 x10^4 T.U./ng p24

M.O.I. 100 T.U./ml, 10^6 CD34+ cells/ml

Serum-free CellGro with IL-3, TPO, SCF, Flt3-L in retronectin-coated bags

HSC Gene Therapy of MLD: Clinical Testing

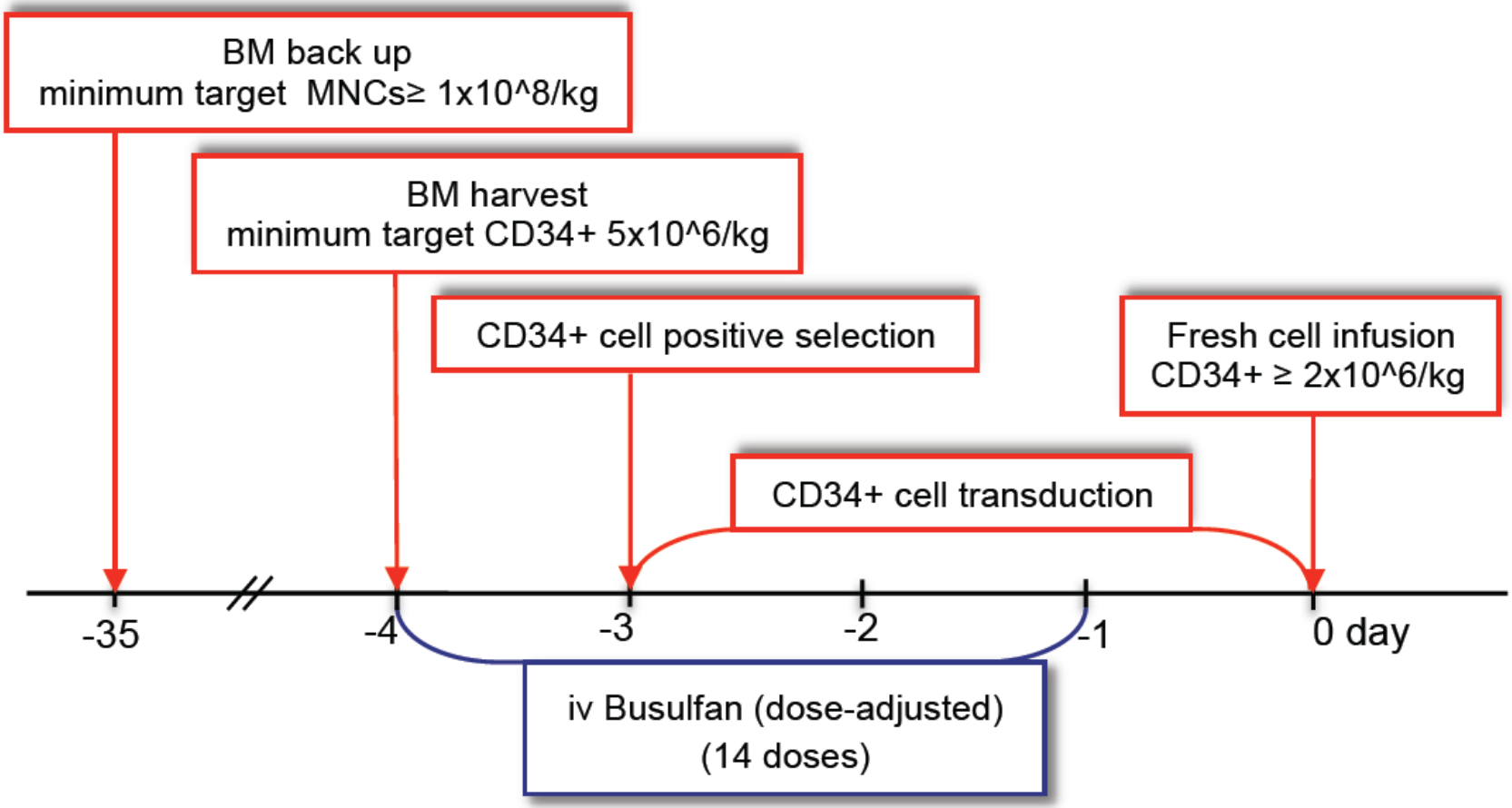
Phase I/II clinical trial

- *Autologous HSC*
- *3rd generation LV encoding ARSA*
  - *Self-inactivating LTR*
  - *Internal PGK promoter*
- *Busulfan-based conditioning*

Clinical P.I. Alessandra Biffi, Maria Sessa

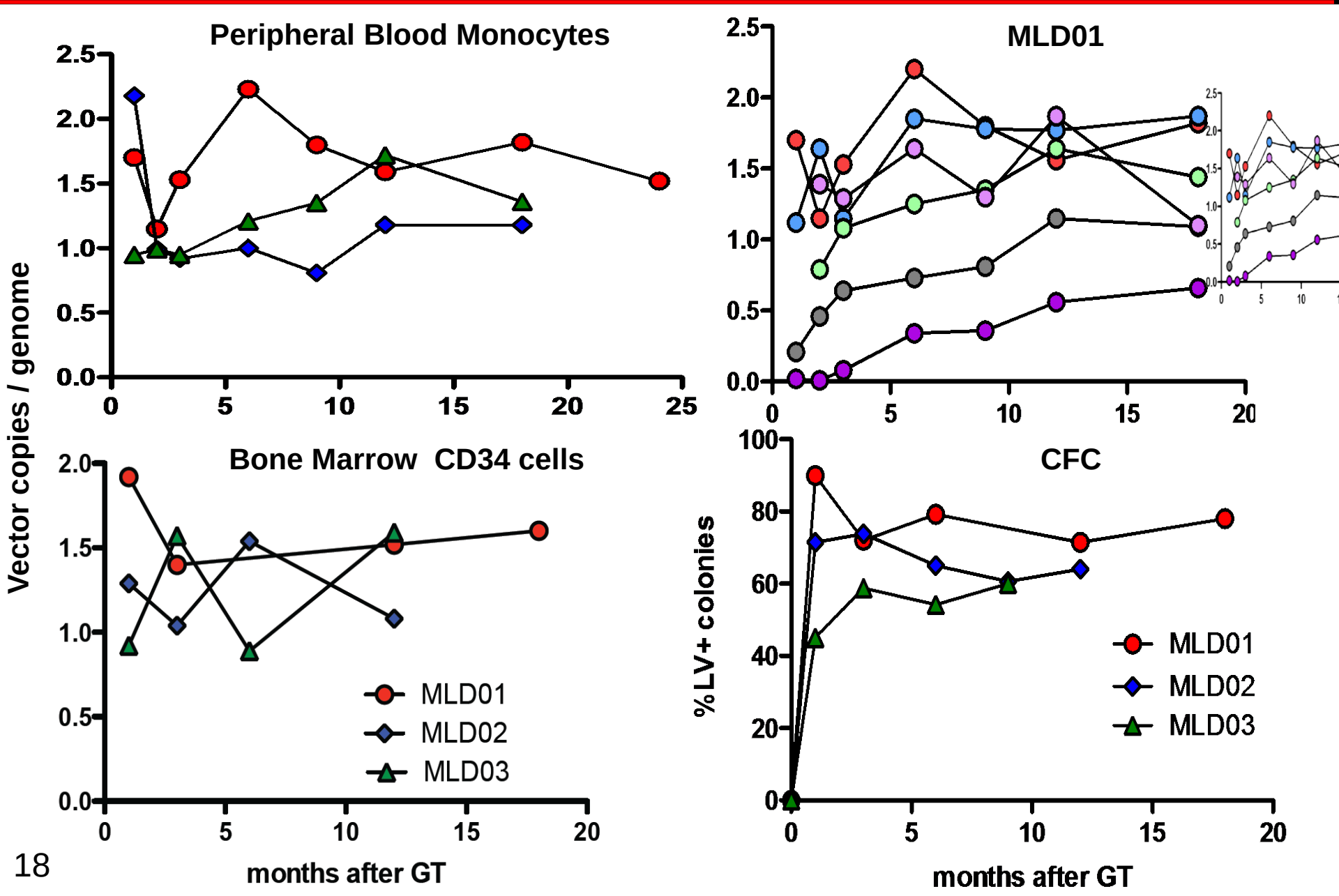
Clinical protocol approved on December 2009
IMPD approved on March 2010
1st patient treated on May 2010, 9 patients treated up to now

HSC Gene Therapy of MLD: Treatment Plan

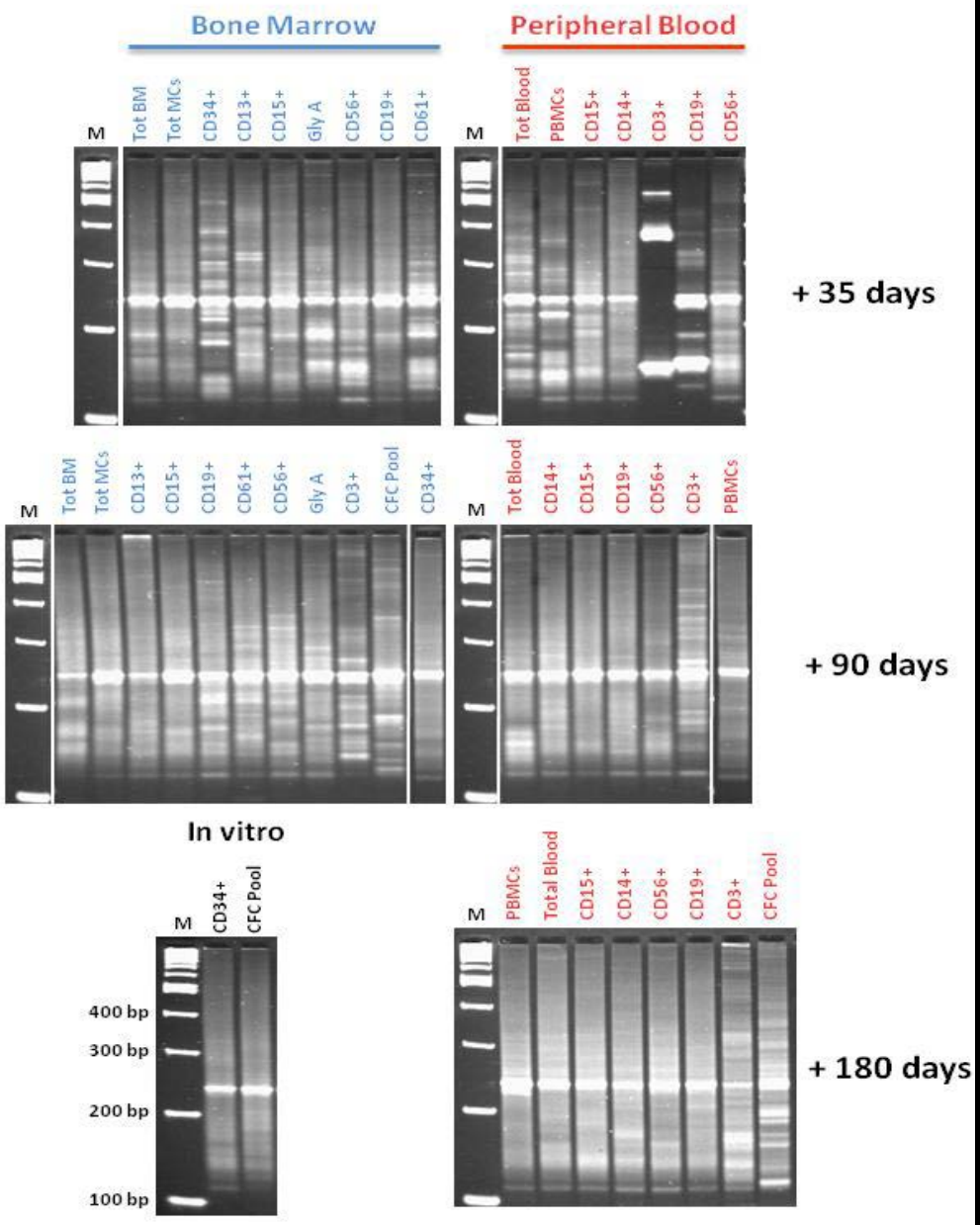


	MLD01	MLD02	MLD03
DOB	6/2/09	16/1/10	10/7/10
Symptoms at Tx	No	No	No
Tx Date	7/5/10	18/2/11	25/2/11
Age at Tx	15 ms	13 ms	7 ms
(Expected) onset at	18 ms	24-27 ms	15 ms
Cell dose	$11x10^6$ /kg	$7.0x10^6$ /kg	$7.2x10^6$ /kg
VCN	2.5	2.5	4.4
Transduction %	97	90	93
ARSA expression	>10x ND	>10x ND	>10x ND
BU dose (mg/kg)	10.4	14.6	10.4
Neutropenia	up to +38	up to +45	up to +37

Stable High-Level Gene Marking in Patients

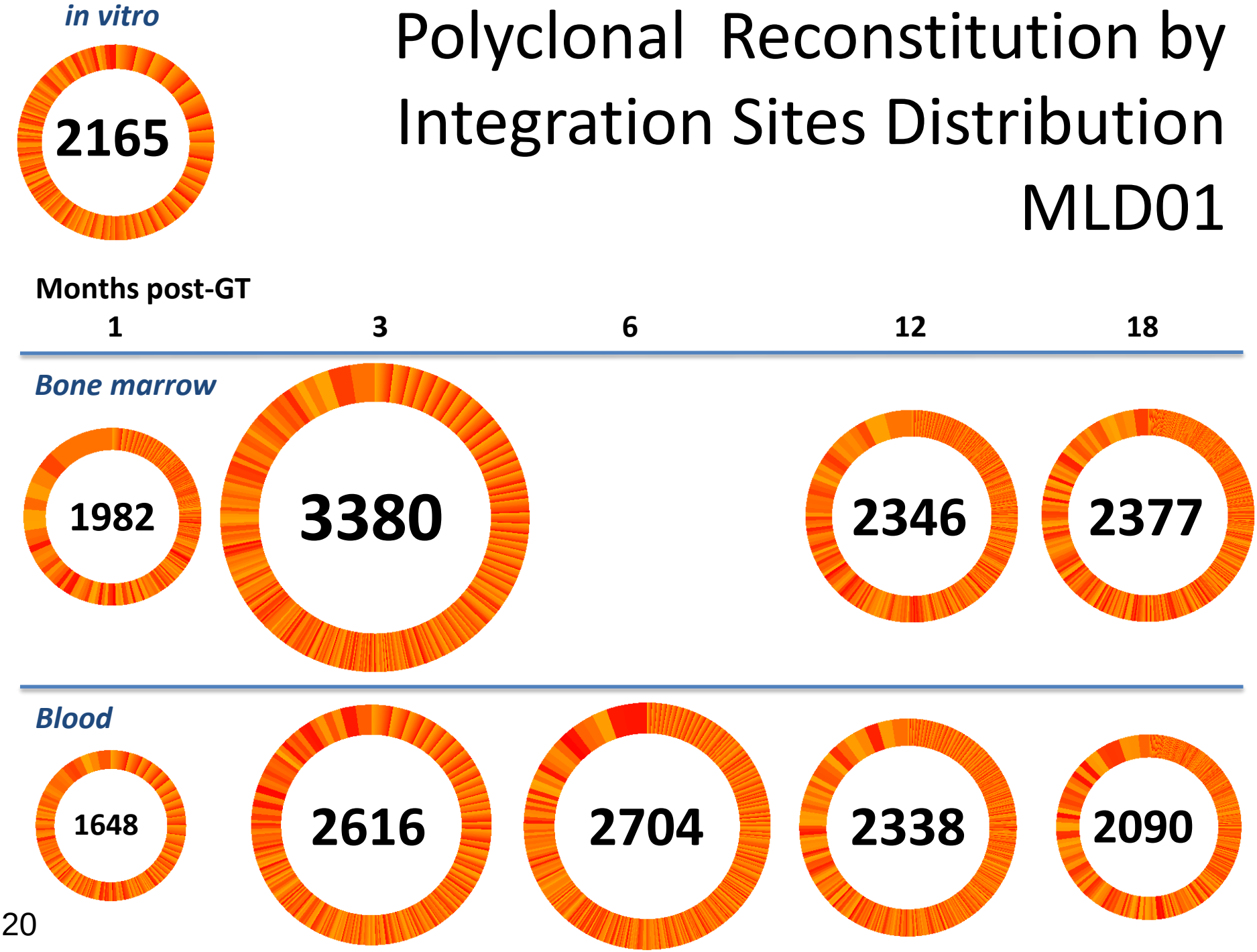


Integration Site Analysis

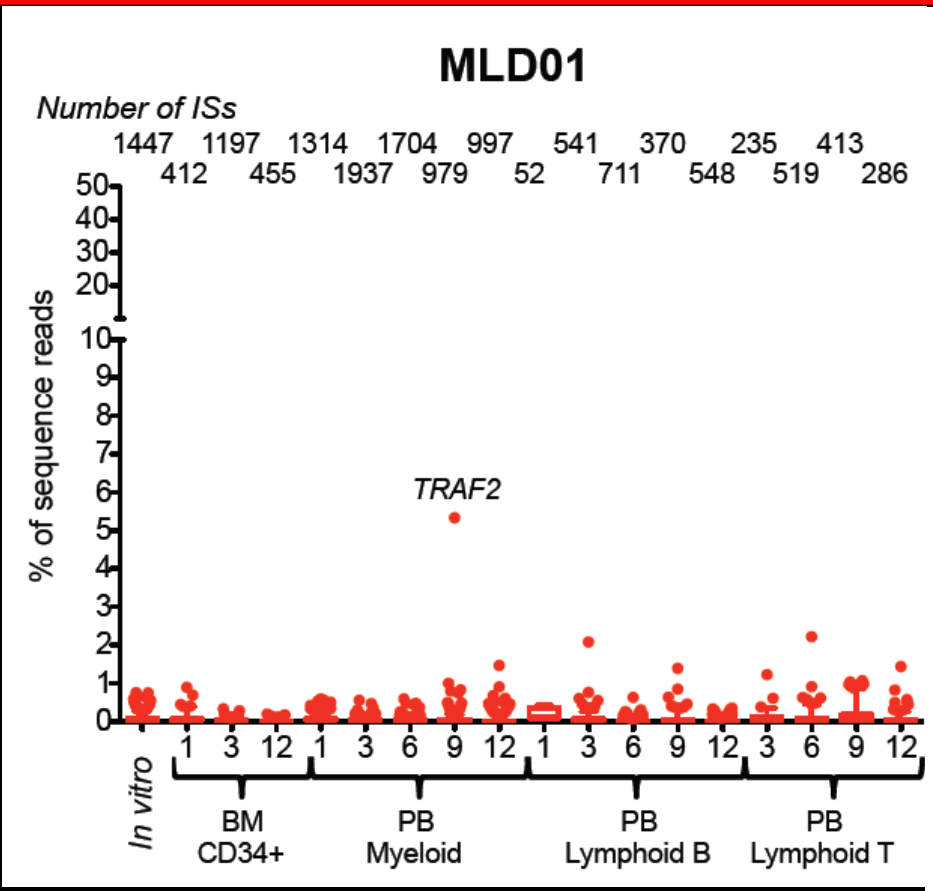


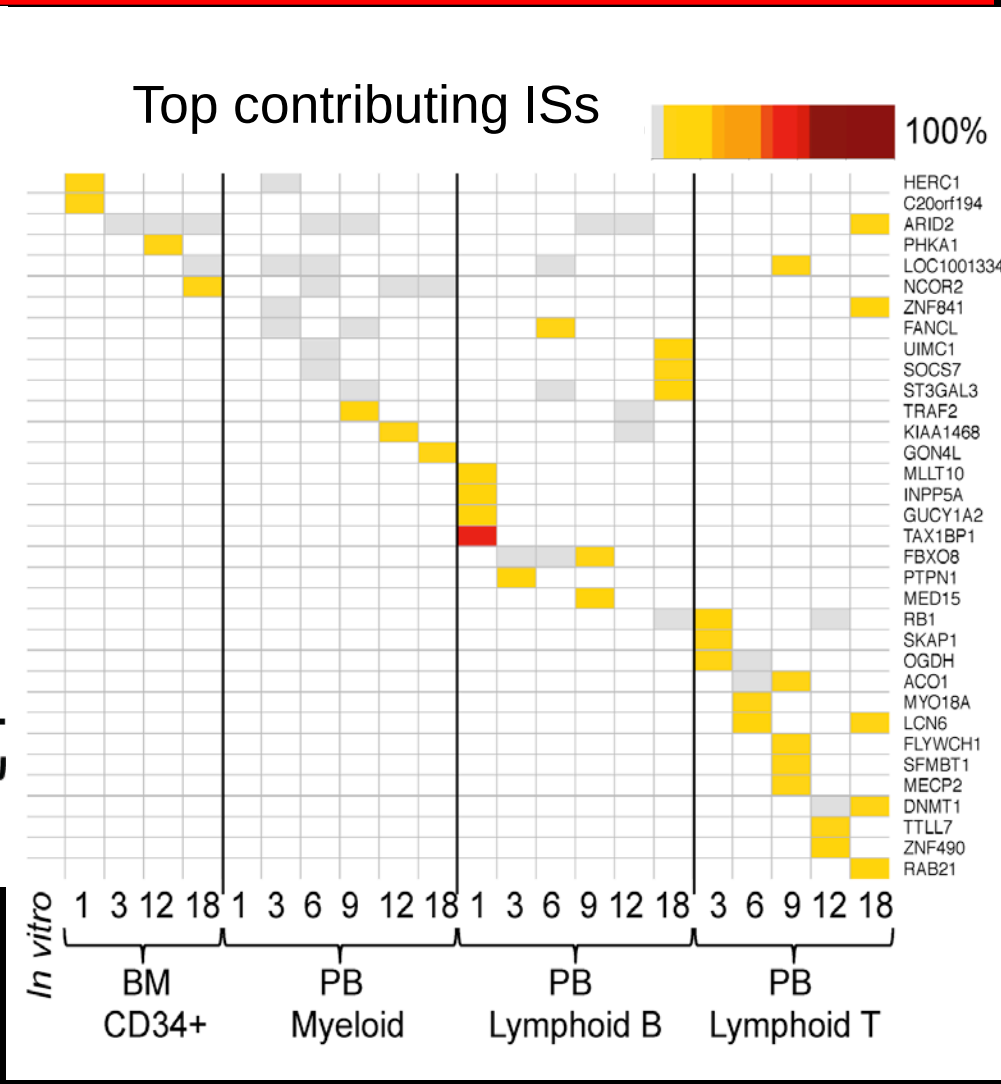
- *9-14,000 unique IS retrieved per patients up to 1.5 yr follow up*
- *Far from saturation*
- *Highly polyclonal reconstitution*

Polyclonal Reconstitution by Integration Sites Distribution MLD01



Relative Abundance of Integration Sites





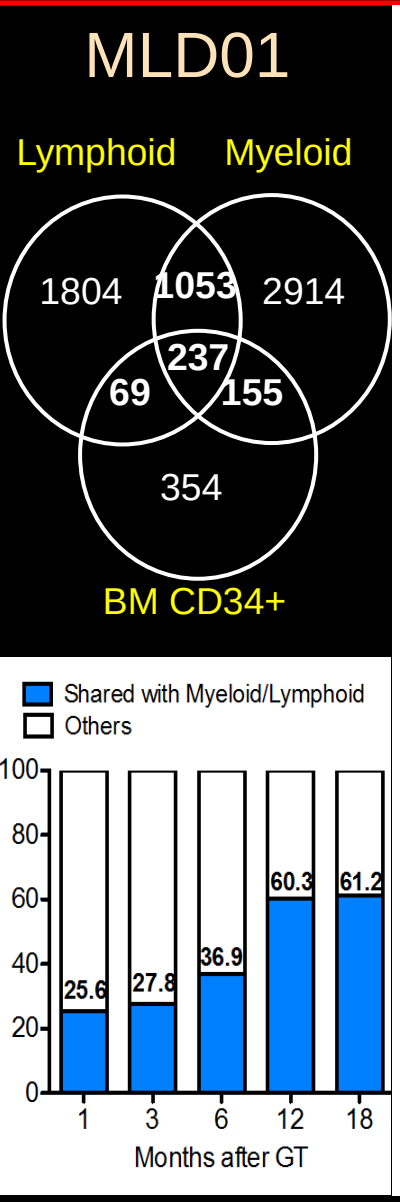
- *No evidence of sustained clonal expansion*

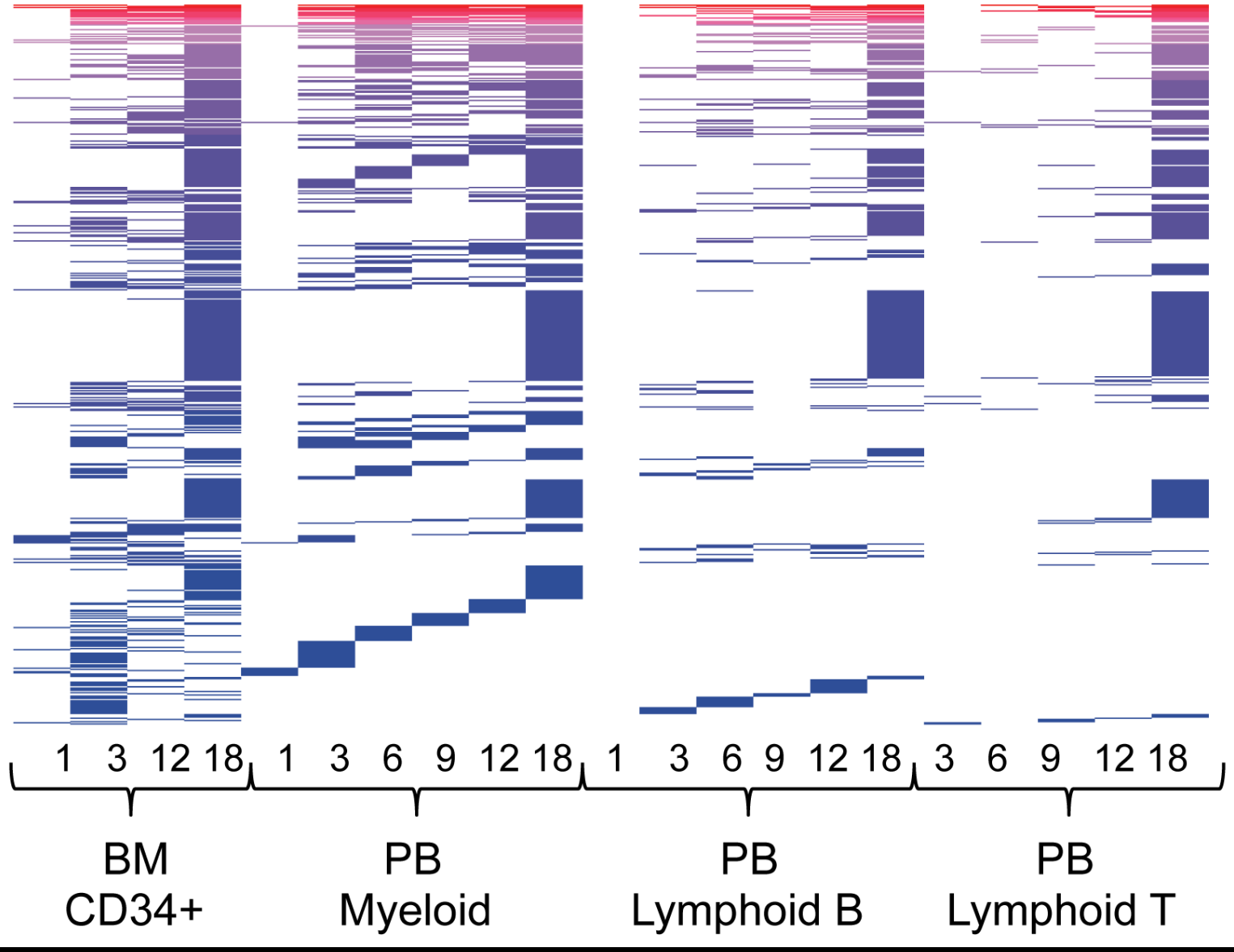
Gene Classes Enriched in LV Integrations

MLD		ALD: histone-lysine N-methyltransf. 40	Protein-lysine N-methyltransf 41	histone methyltransferase 50	protein methyltransferase 67	protein N-terminus binding 83	nuclear hormone recep bind 109	hormone receptor binding 126	steroid hormone recept bind 66	DNA helicase 48	helicase 154	ATP-dependent helicase 115	ligand-dep nuclear recept bind 19	phosphatase binding 108	Mitogen-activ. prot kin. bind 16
histone-lysine N-methyltransf	40	40	38	40	40	2	1	1	1	0	0	0	1	0	0
protein-lysine N-methyltransf	41	38	41	39	41	2	1	1	1	0	0	0	1	0	0
S-adenosylmeth methyltransf	102	40	41	48	57	2	2	2	2	0	0	0	1	0	0
histone methyltransferase	50	40	39	50	50	2	2	2	2	0	0	0	1	0	0
N-methyltransferase	66	40	41	48	53	2	2	2	2	0	0	0	1	0	0
protein methyltransferase	67	40	41	50	67	2	2	2	2	0	0	0	1	0	0
protein N-terminus binding	83	2	2	2	2	83	5	5	4	4	5	3	3	3	2
androgen receptor binding	39	1	1	2	2	4	39	39	39	0	2	1	4	1	0
DNA helicase	48	0	0	0	0	4	0	0	0	48	48	38	0	0	0
ATP-dependent DNA helicase	36	0	0	0	0	3	0	0	0	36	36	36	0	0	0
single-stranded DNA binding	69	0	0	0	0	4	0	0	0	8	8	7	0	2	0
protein transporter	93	0	0	0	0	0	0	0	0	0	0	0	0	1	0

0%	1-25%	26-50%	51-75%	76-99%	100%

Shared Integration Sites among Lineages





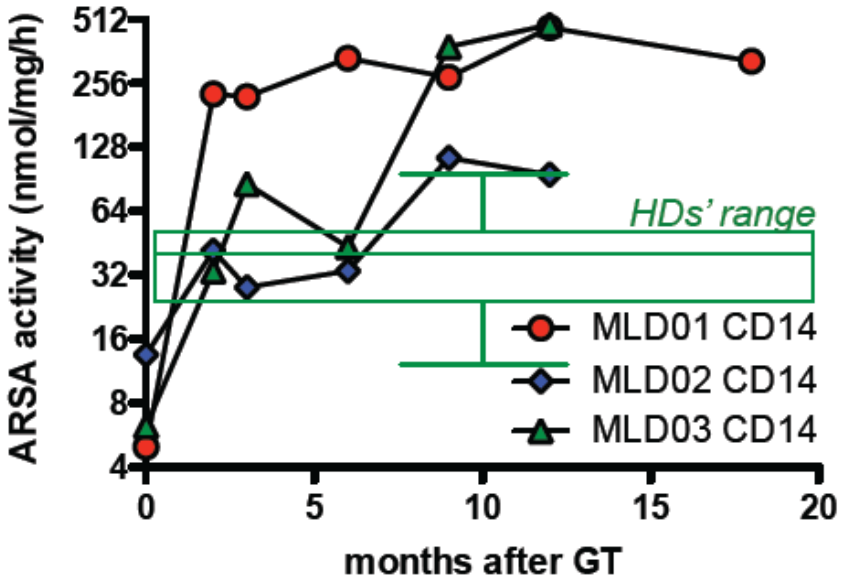
Evidence of HSC gene transfer

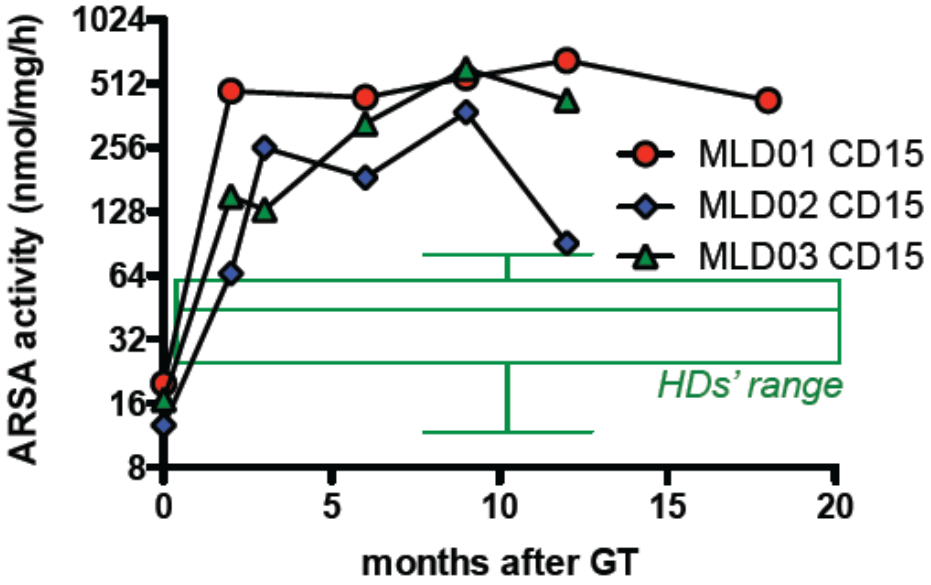
Lentiviral-mediated HSC Gene Transfer

- *Stable high-level marking of hematopoiesis*
- *Polyclonal reconstitution without evidence of genotoxicity*
- *bona fide HSC transduced and engrafted at high frequency*

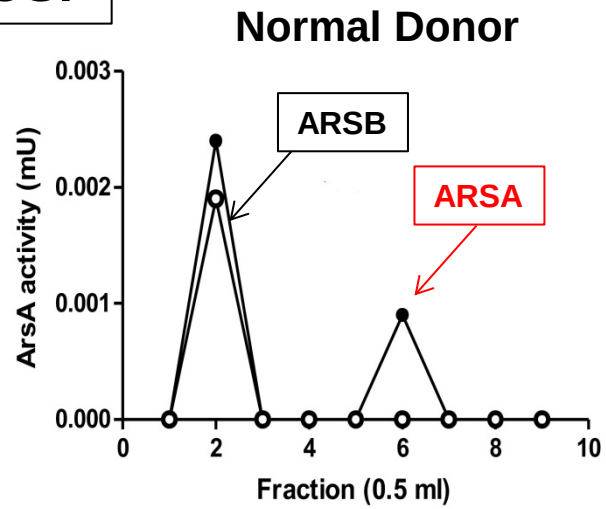
- *Provides the means to engineer human hematopoiesis to near completion*

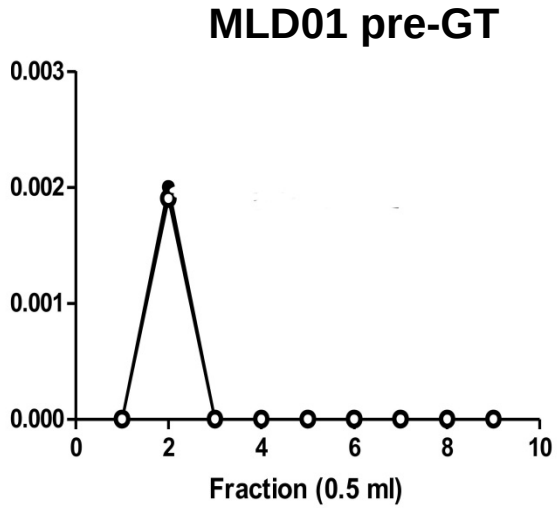
ARSA Activity Reconstitution in MLD Patients

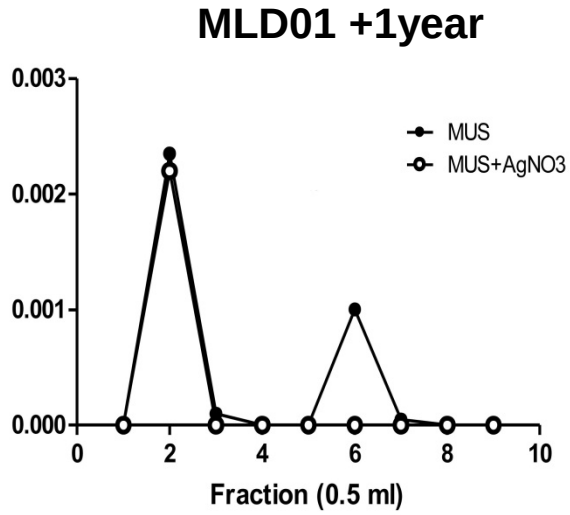




CSF

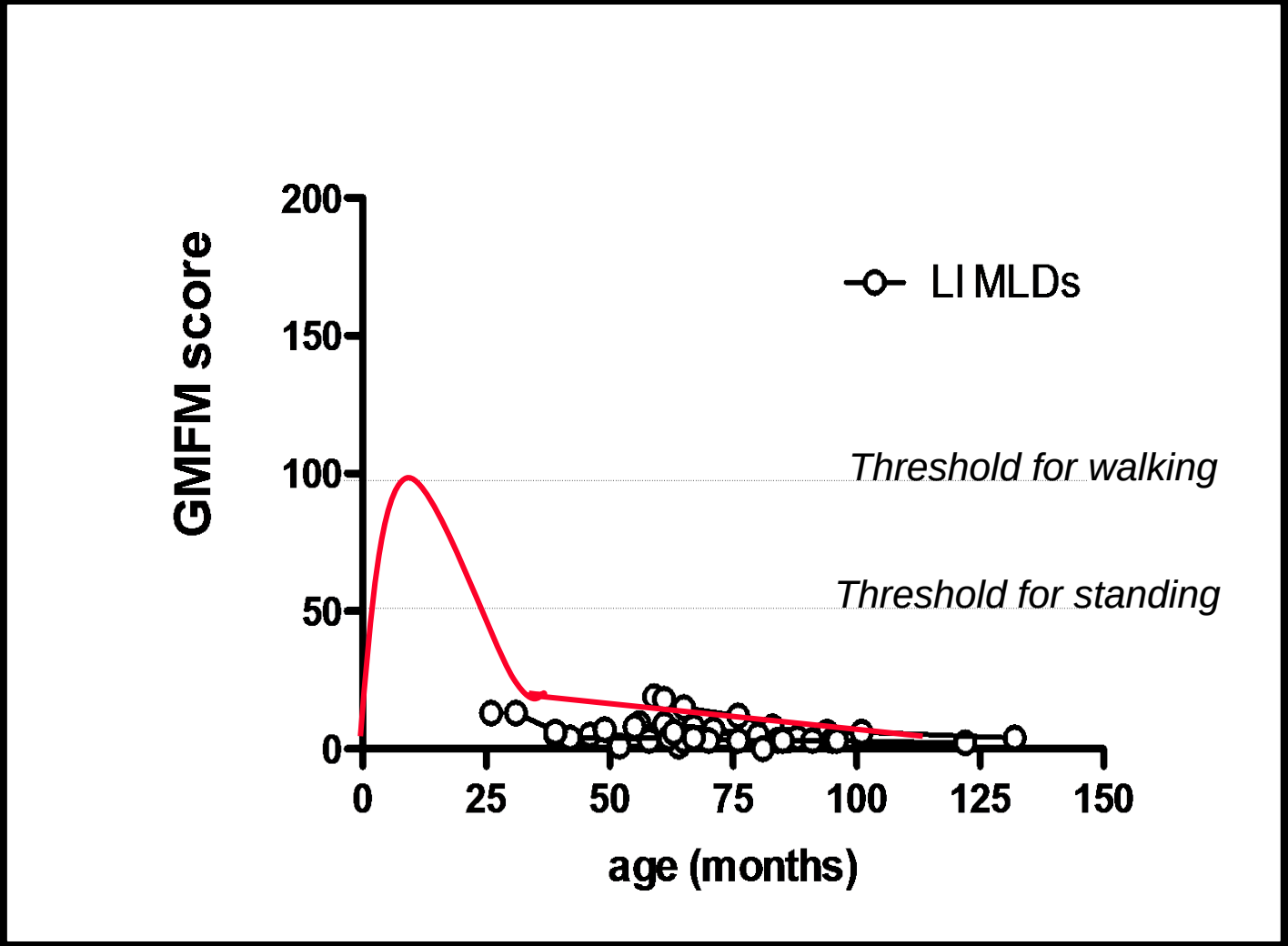






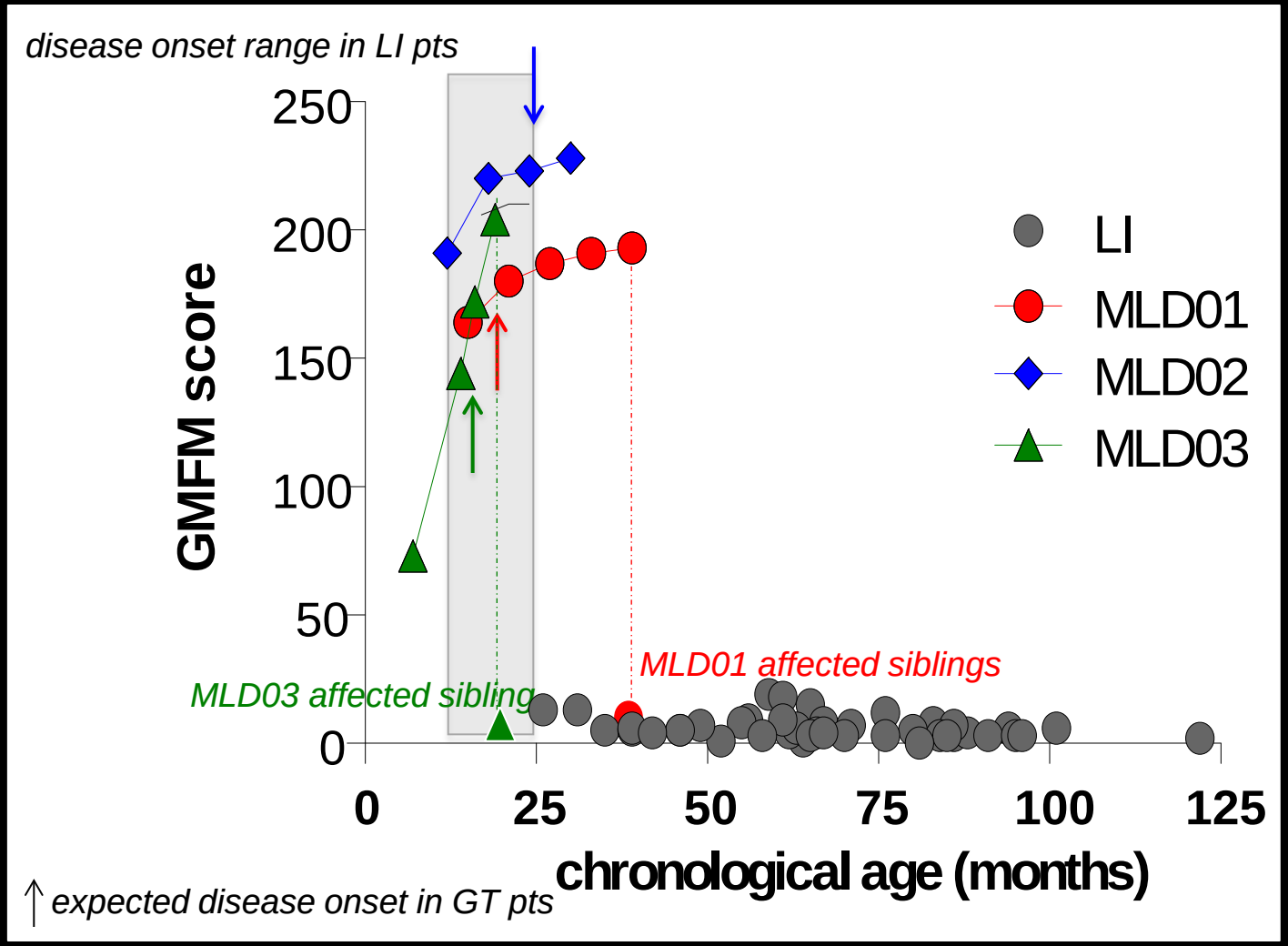
Late Infantile MLD: Disease Evolution

GMFM scale: evaluation of motor skills



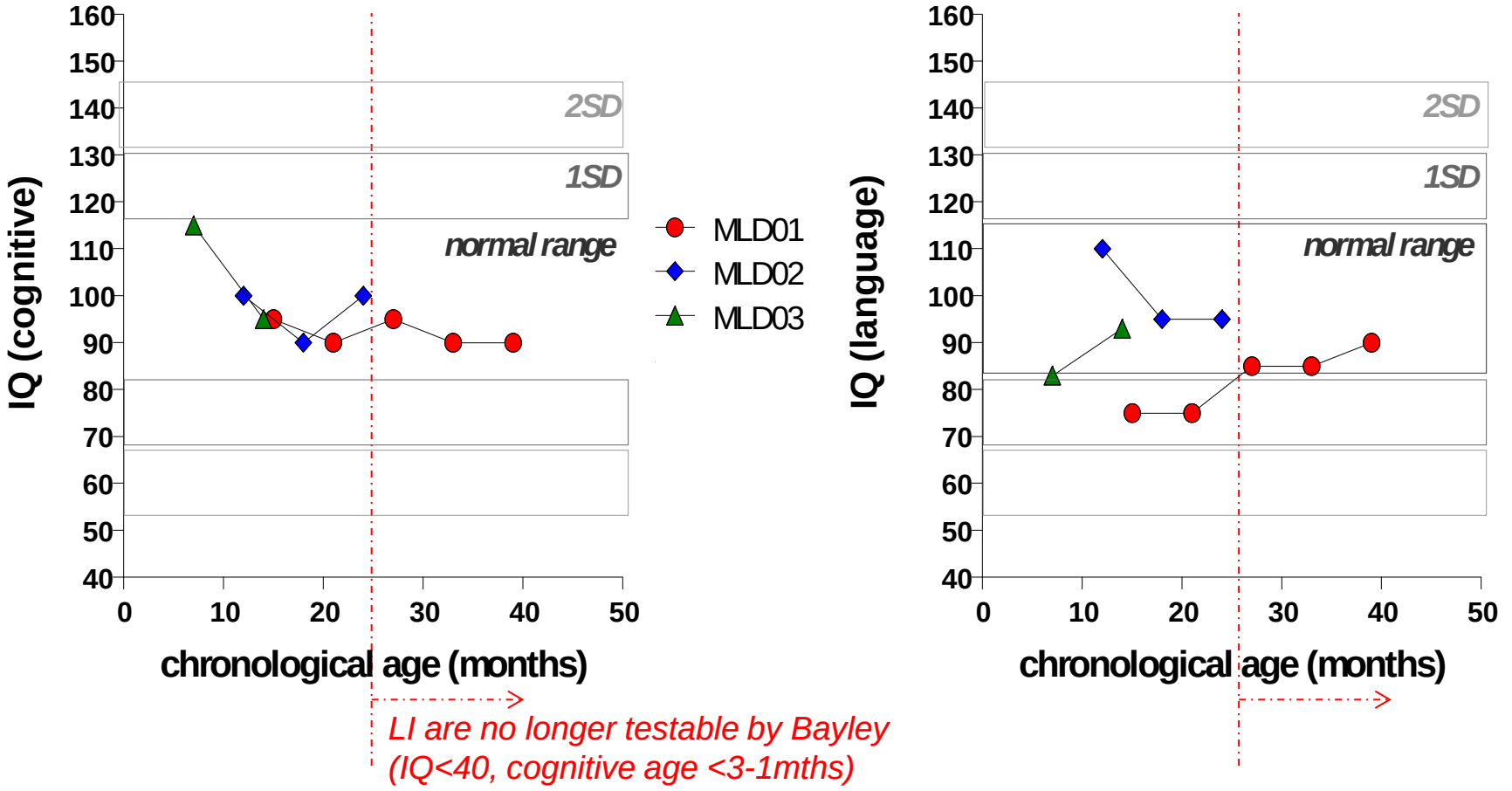
Clinical Benefit (LI) . I

GMFM scale: evaluation of motor skills

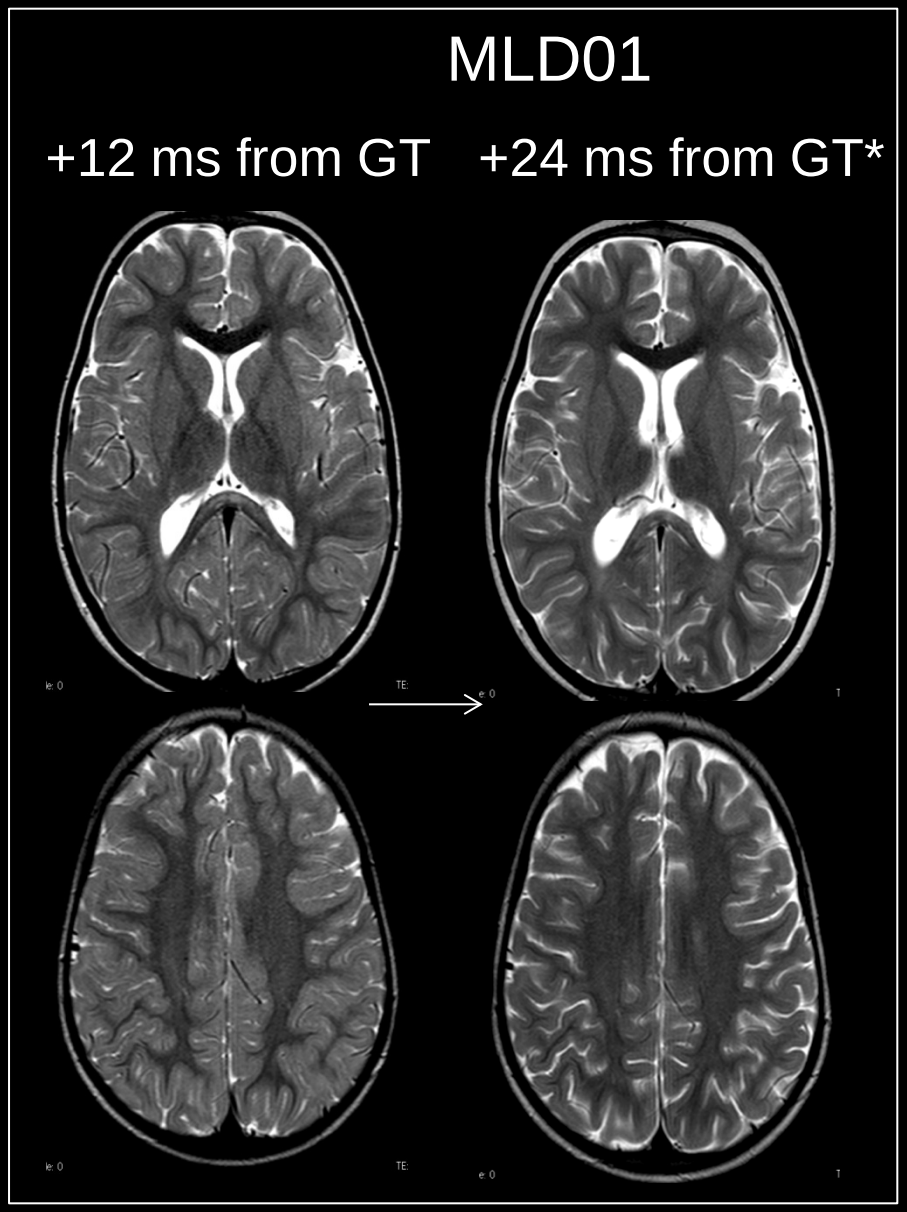


Clinical Benefit (LI) . II

Neuropsychological Evaluation
(Bayley scale for infant development)



MLD01: Prevention of Cerebral Demyelination



HSC Gene Therapy of MLD: Summary

- *Completed treatment of 9 pts*
- *Well tolerated and safe (current follow-up)*
- *Sustained transduced cell engraftment at unprecedented levels*
- *Polyclonal reconstitution with safe vector integration profile*
- *Restoration of enzyme activity in hematopoietic cells and CNS (CSF)*
- *Therapeutic benefit*

Biffi, Montini* et al., Science, 2013*

Wiskott-Aldrich Syndrome (WAS)

- *X-linked primary immunodeficiency with microthrombocytopenia, eczema, autoimmunity and lymphoid malignancies.*
- *Defect in cytoskeleton / growth regulator*
- *Allogenic HSC is curative but*
  - High morbidity & mortality if mismatched
  - Avaliable to few patients
- *HSC gene therapy attempted with γ-RV*
  - Efficacious
  - 50% of patients later developed leukemia triggered by insertional mutagenesis

Current HSC Gene Therapy Trials at TIGET

- *Metachromatic Leukodystrophy (MLD)*

- *SIN LV PGKp-ARSA*
- *Conditioning*
  - Fully myeloablative (dose-adjusted Busulfan in 14 administrations up to 10-14 mg/kg)

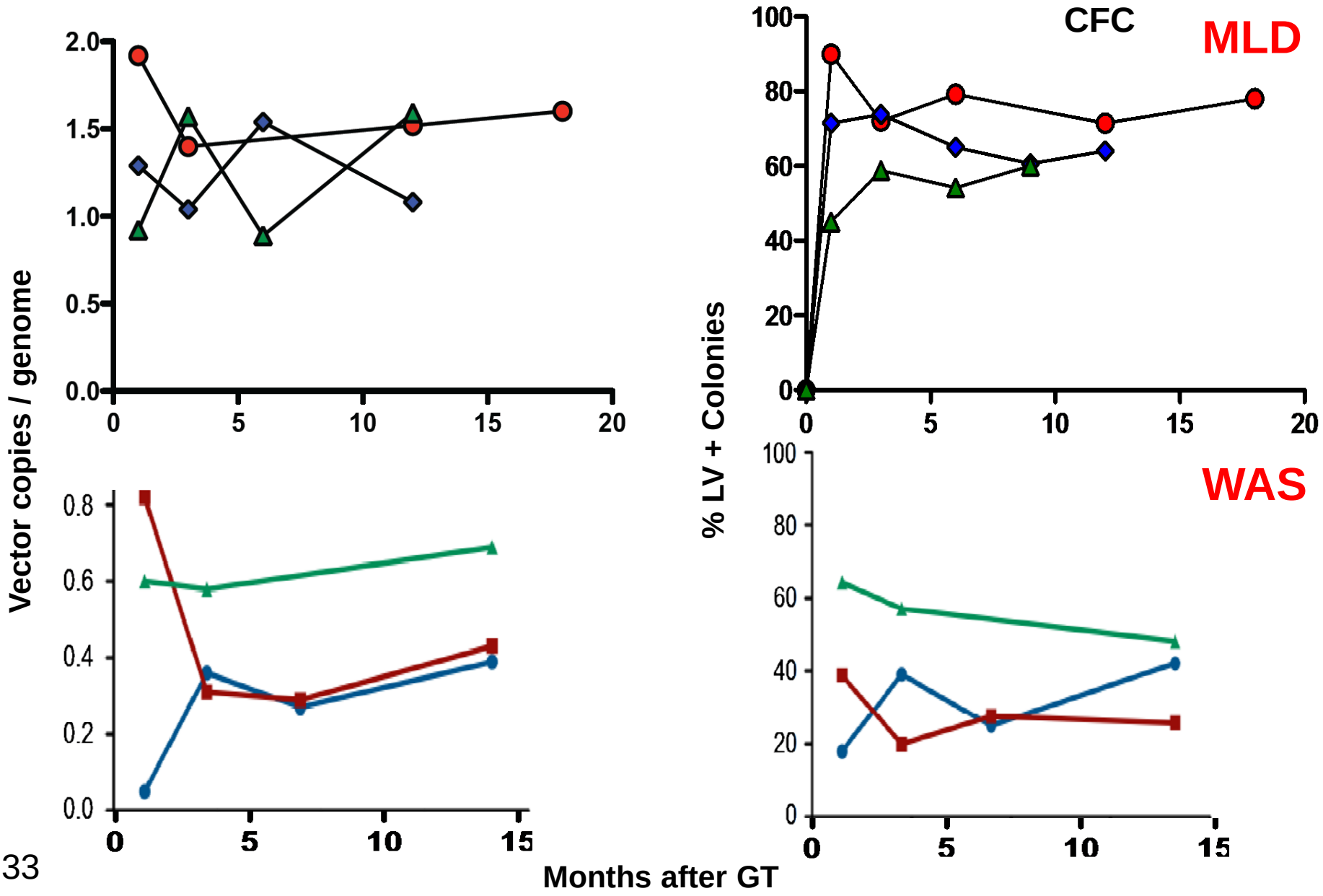
- *Clinical P.I. Biffi, Sessa*

- *Wiskott Aldrich Syndrome (WAS)*

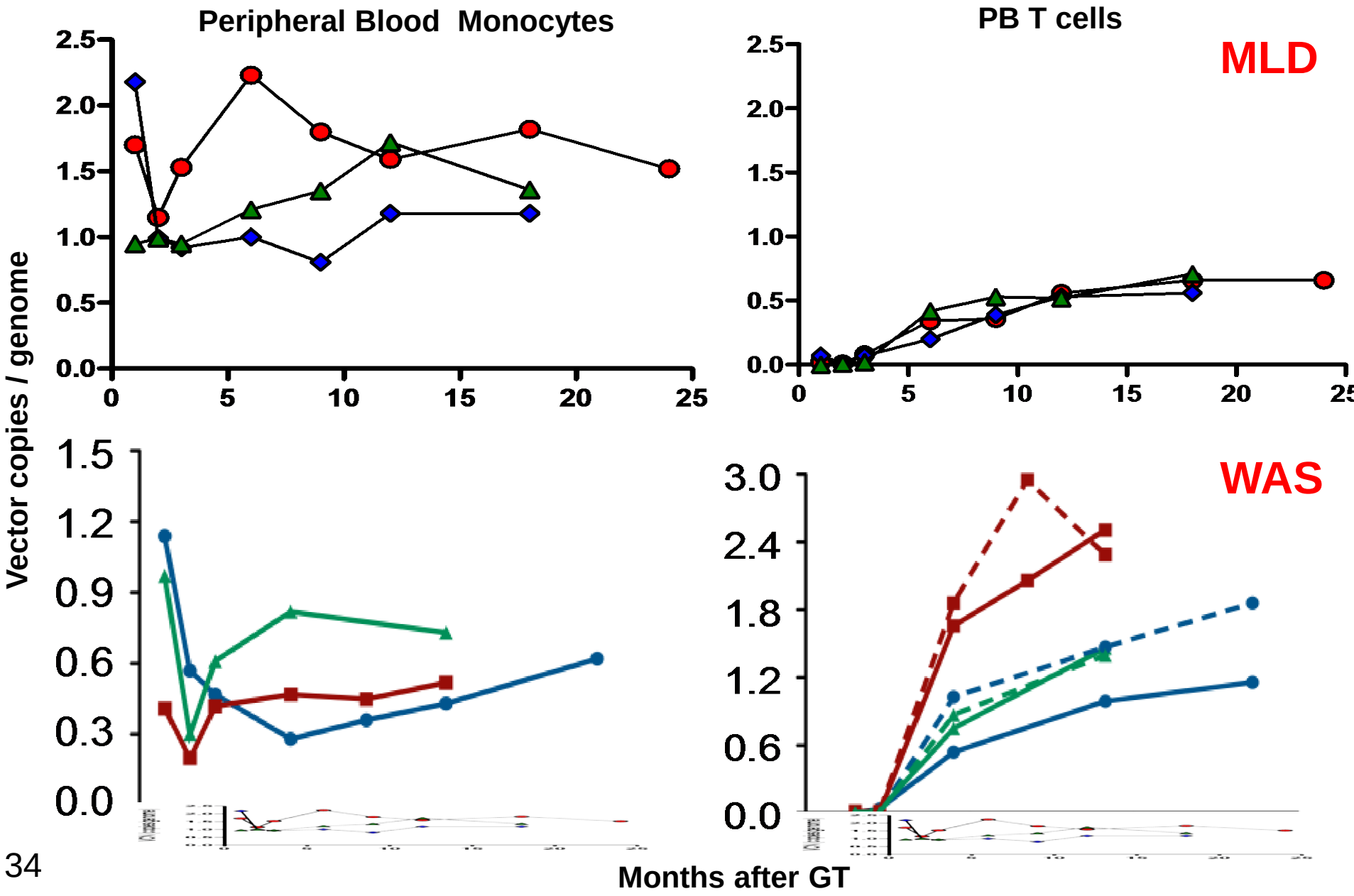
- *SIN LV WASp-WAS*
- *Conditioning*
  - Reduced intensity + immunodepletion (antiCD20 mAb, Busulfan 8 mg/kg, Fludarab
- *Same ex vivo transduction protocol*

- *Clinical P.I. Aiuti*

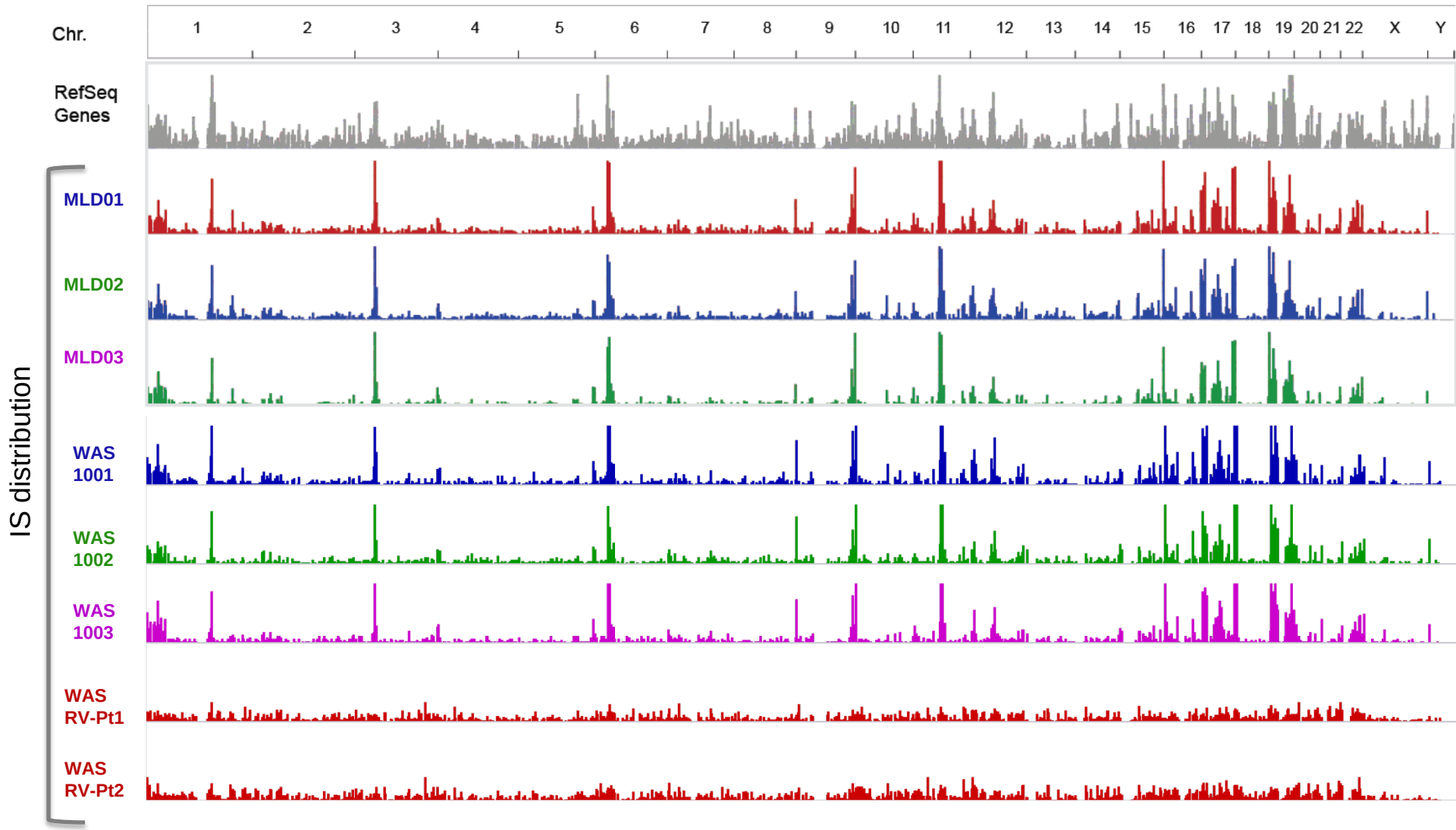
Gene Marking in MLD vs. WAS Patients



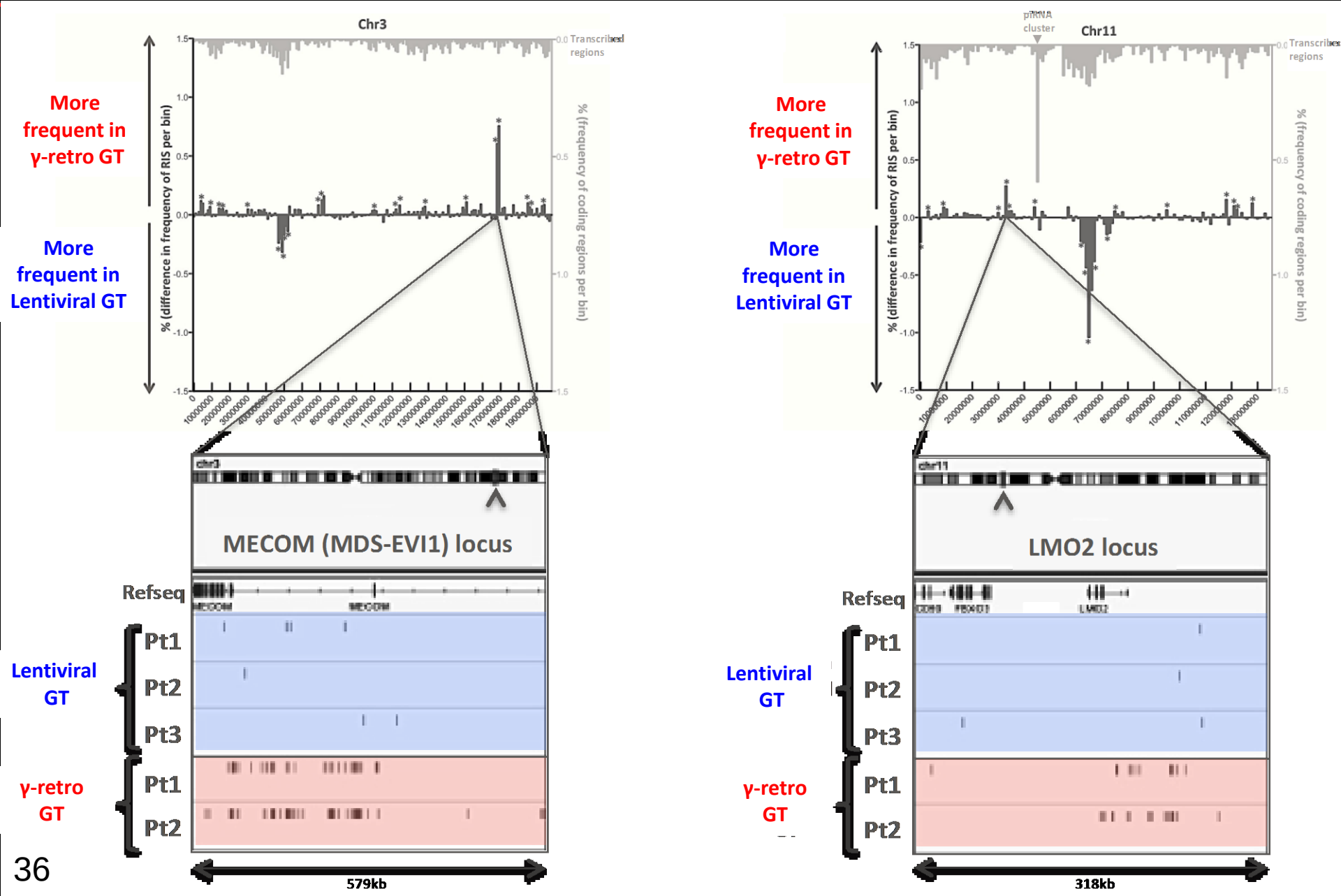
Gene Marking in MLD vs. WAS Patients



Genomic Distribution of Vector Integration



WAS LV (Tiget, IT) vs. γRV (Klein, FRG) Trial



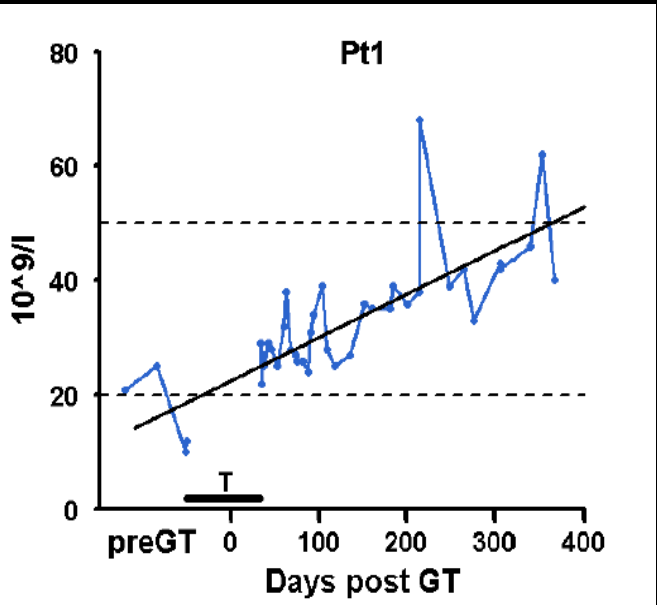
Common Integration Sites (CIS) in WAS LV (Tiget, IT) vs. γRV (Klein, FRG) Trial

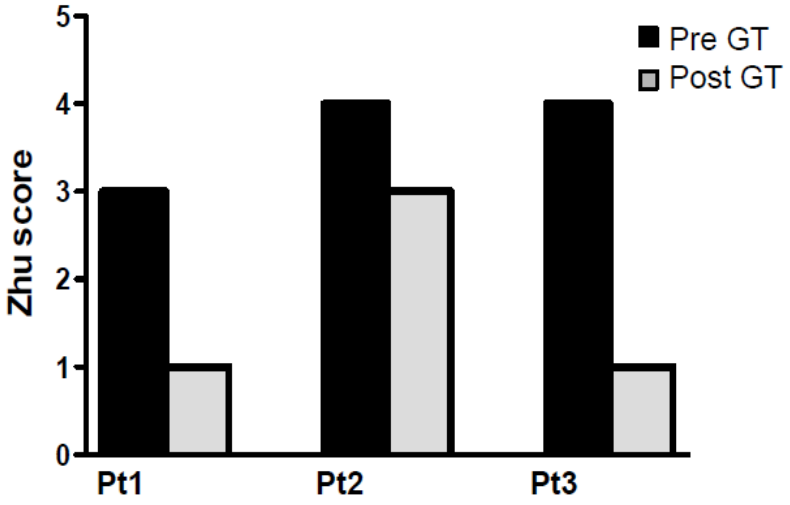
ENSA
EGFL7
FAM69B
AMICA1
MPZL3
WNK1
SMG6
STAT5B
STAT3
STAT5A

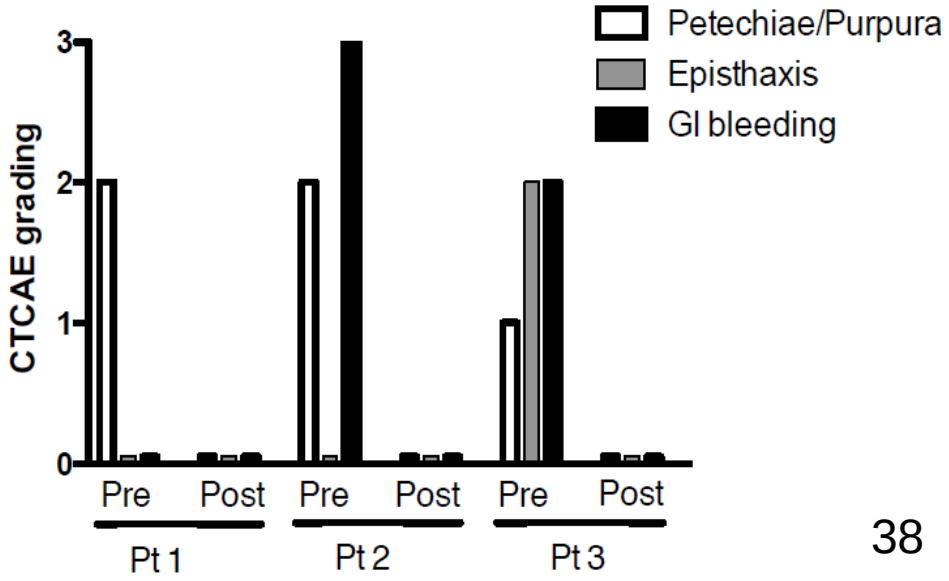
Patient's Benefit of WAS Gene Therapy

In all patients:

- Immune reconstitution
  - T & B cell counts normalized
  - Improved T cell function
  - Protection from severe infections
- Resolution of eczema
- Protection from bleeding







HSC Gene Therapy of WAS: Summary

- *Completed treatment of 6 pts*
- *Well tolerated and safe (current follow-up)*
- *Stable HSC gene marking at 30-50%*
  - *per choice of milder conditioning regimen*
- *Vector integration profile clearly distinct from RV-based gene therapy at same follow-up*
  - *Improved safety predicted*
- *Therapeutic benefit comparable to allogenic HSC transplant*

Aiuti et al., Science, 2013

Rationale for HSC Gene Therapy

When HSCT is effective

- *Use patient's own vs. donor cells*
  - available to all patients
  - lower transplant morbidity: no GvH disease, milder conditioning → WAS

When HSCT is not effective

- *Engineer cells*
  - increase therapeutic gene dosage
  - deliver functional enzyme to CNS → MLD

MLD

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