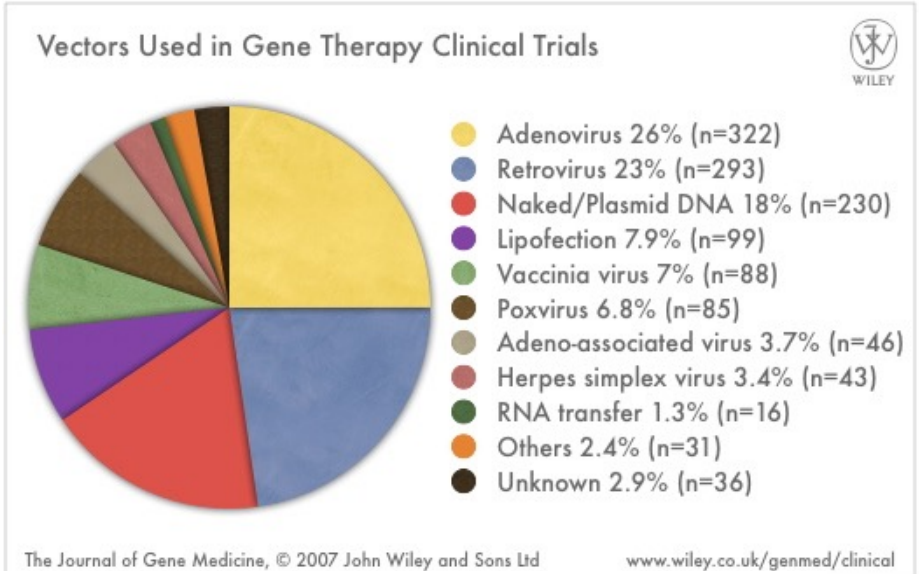
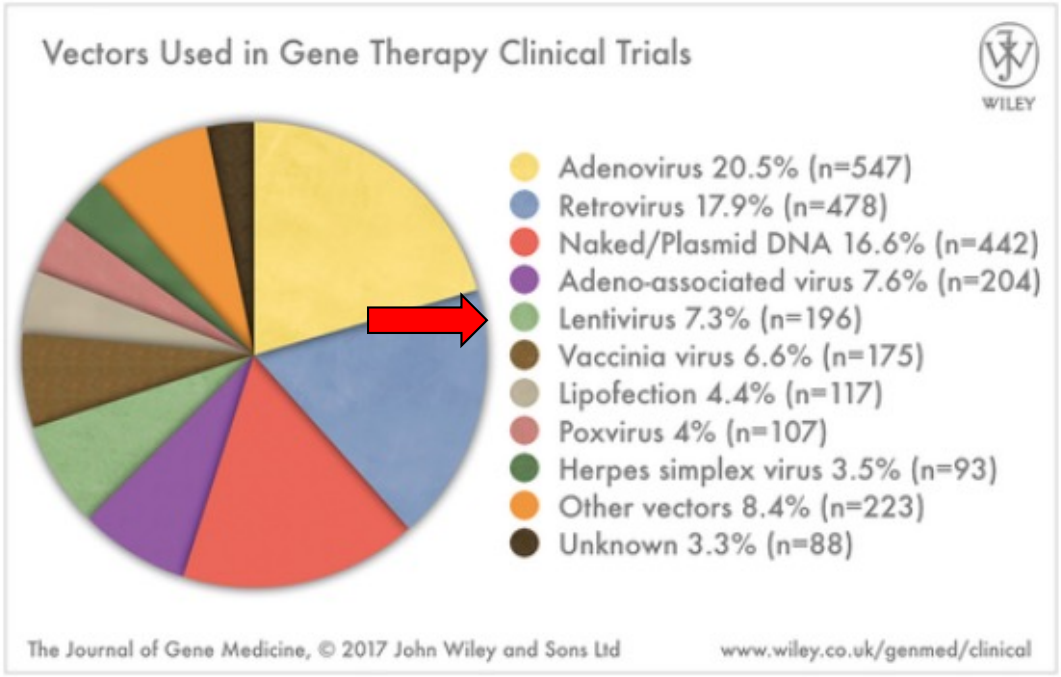
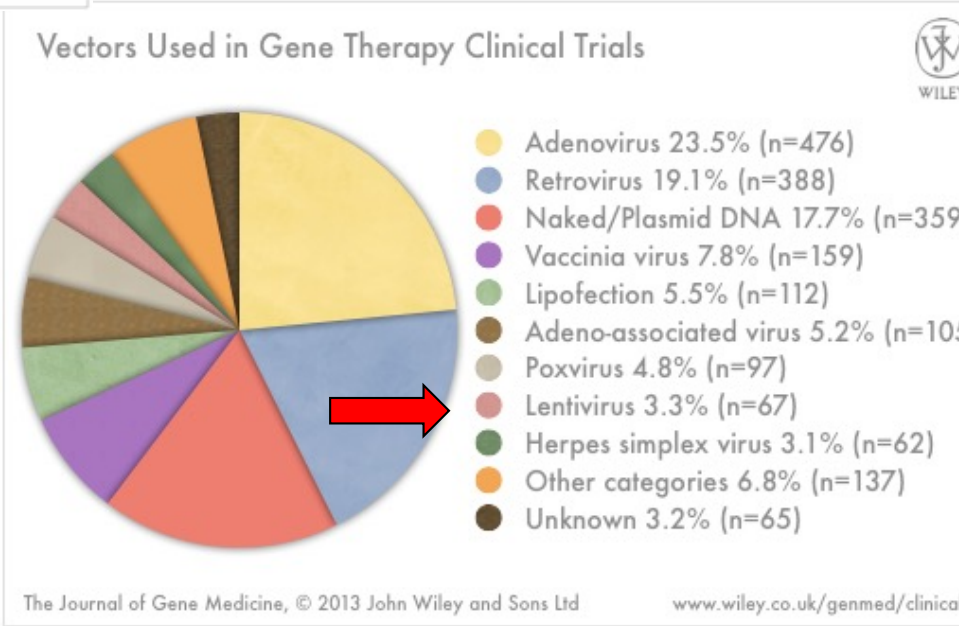
Which vectors for the genes







Science 23 August 2013: Vol. 341 no. 6148

Lentiviral Hematopoietic Stem Cell Gene Therapy Benefits Metachromatic Leukodystrophy
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Ex-vivo approach LV-PGK ARSA vector in HSC from patient

GSK, Fondazione Telethon and Fondazione San Raffaele to collaborate on gene therapy for rare diseases

LV-HSC correction in **mouse model**

J Clin Invest. 2004

Correction of metachromatic leukodystrophy in the mouse model by transplantation of genetically modified hematopoietic stem cells

Alessandra Biffi1, Michele De Palma1, Angelo Quattrini2, Ubaldo Del Carro2, Stefano Amadio2, Ilaria Visigalli1, Maria Sessa2, Stefania Fasano3, Riccardo Brambilla3, Sergio Marchesini4, Claudio Bordignon1,5 and Luigi Naldini1,5

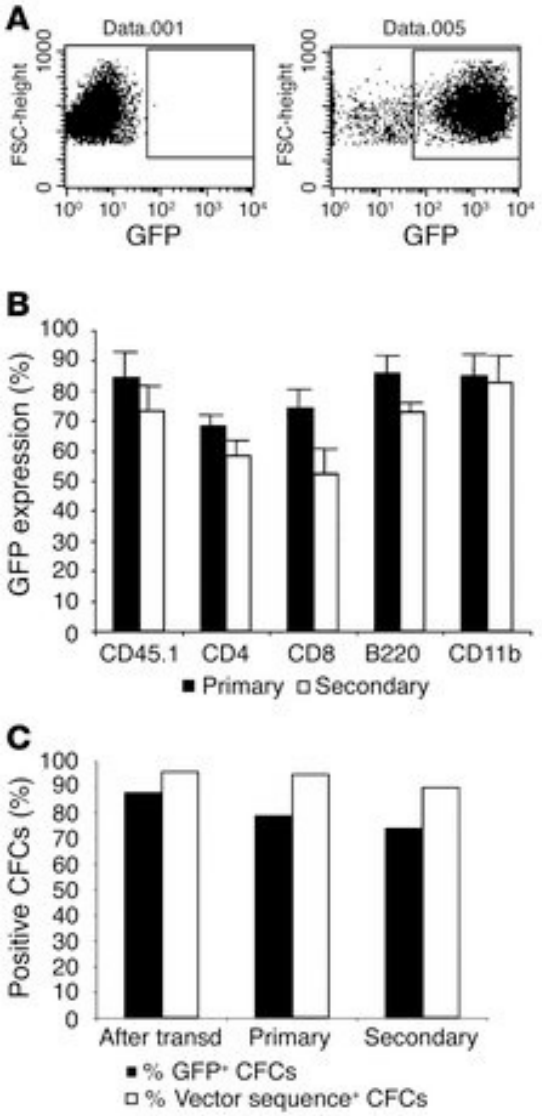
Metachromatic leukodystrophy(MLD)

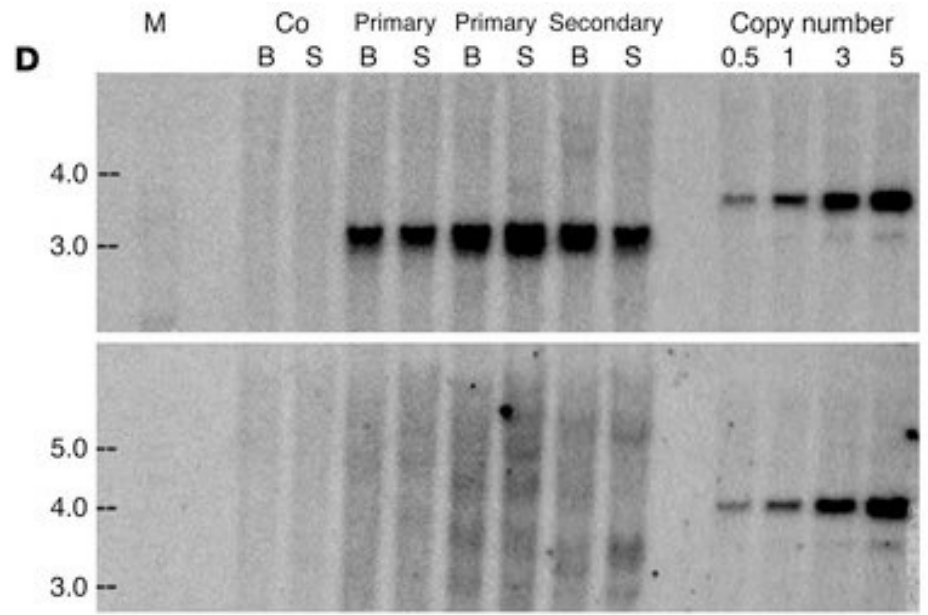
-Arylsulfatase A (ARSA) deficiency: lysosomal storage disease
-Autosomal recessive disease
-Causes accumulation of the enzyme substrate sulfatide and sphingolipids in microglia and specific neurons (CNS) and in Schwann cells and macrophages (PNS)
-Causes demyelination and neurodegeneration, severe progressive cognitive and motor impairment

LV-HSC with GFP, 3 months after BMT

progenitors

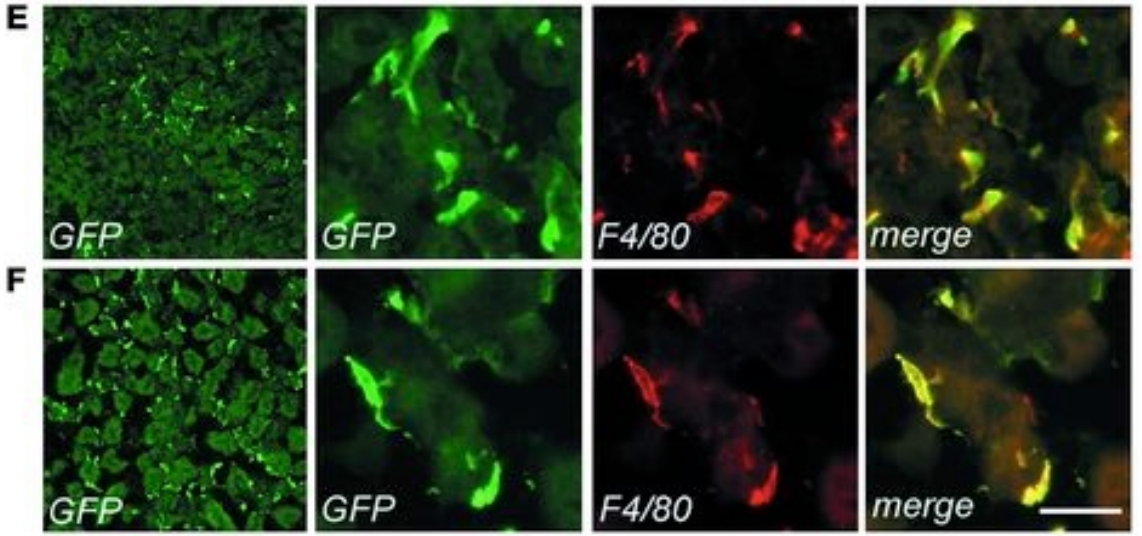
FACS





>=5 copies

No dominant clone



liver

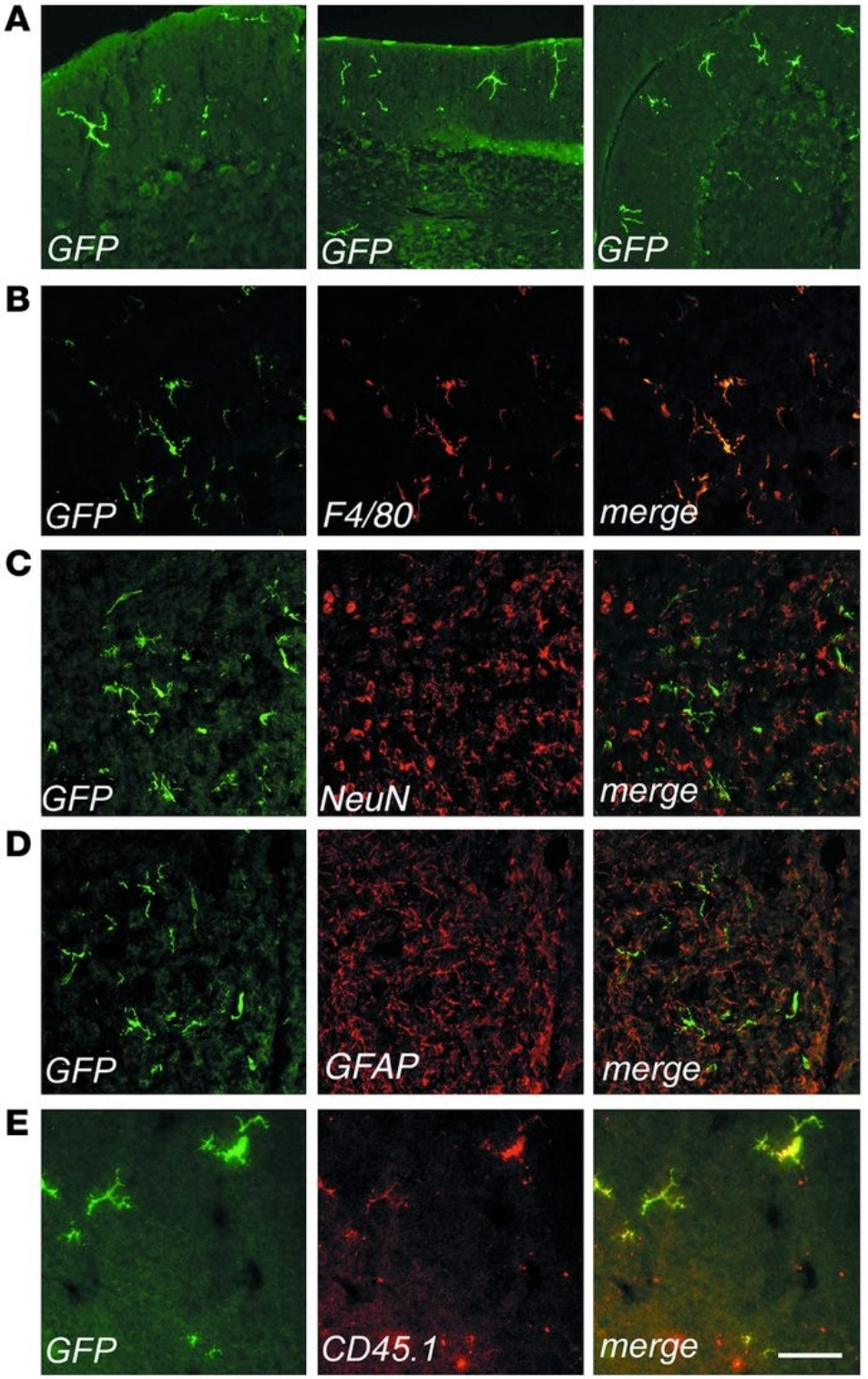
kidney

F4/80 indicates blood cells

LV-HSC in the CNS

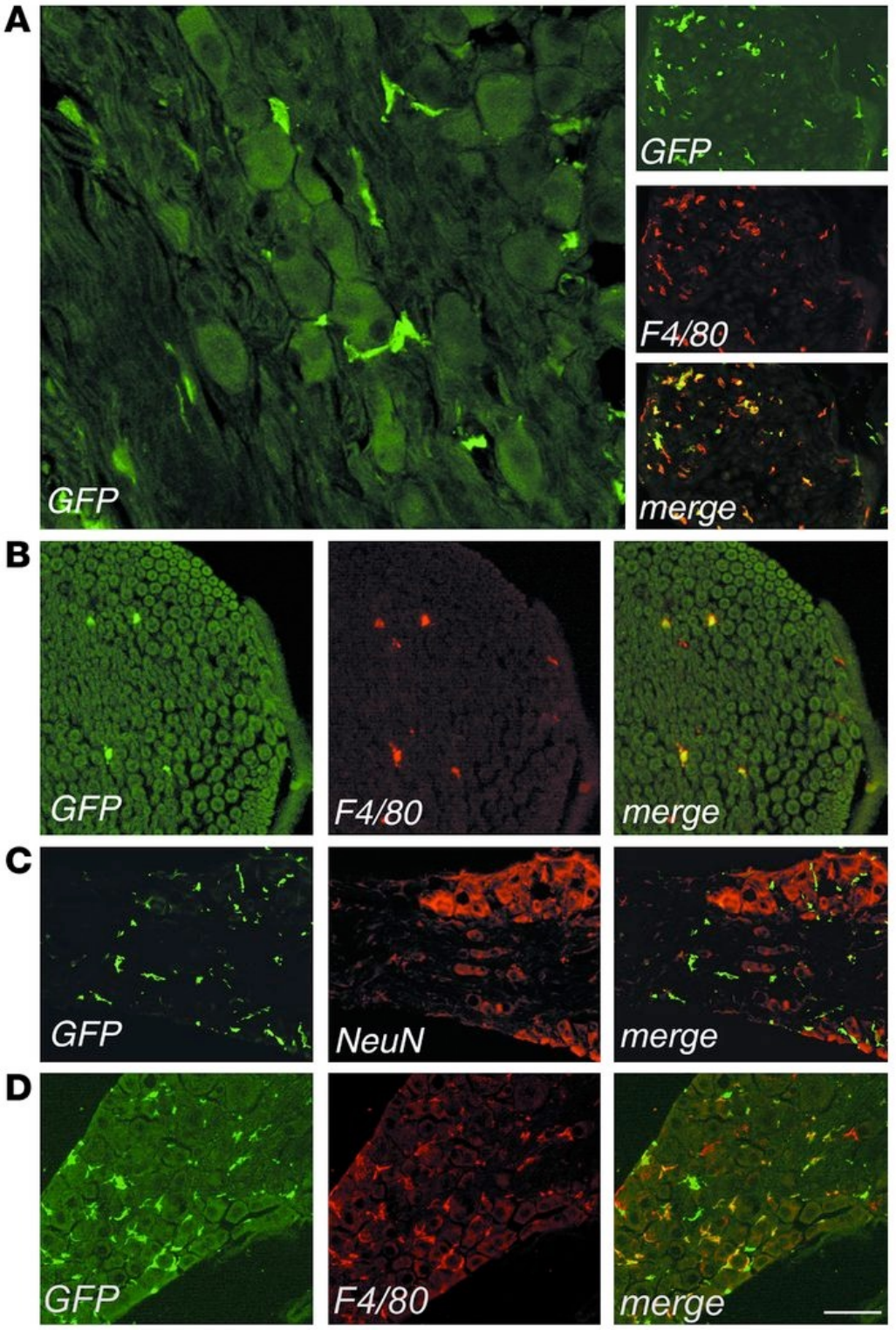
(A) cerebellums of transplanted mice, analyzed at **3 months** (left panel, scale bar: 200 μm) and 6 months (middle and right panels, scale bar: 300 μm) after BMT.
(B–E) brain sections from transplanted mice **9 months** after BMT, immunostained as indicated.

Postive cells are those injected not the resident neurons or glial cells



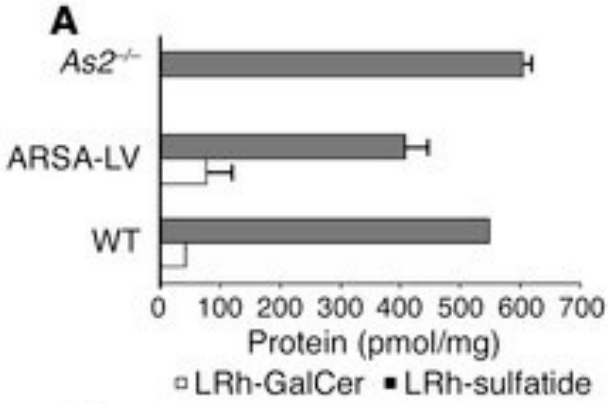
LV-HSC in the PNS

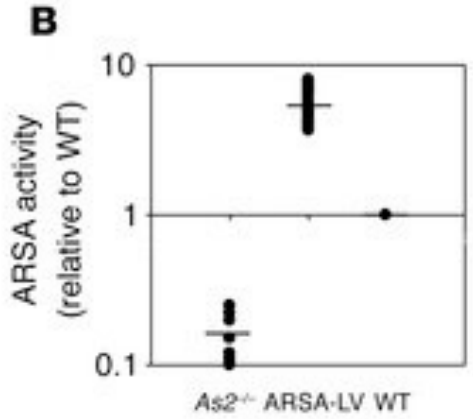
Representative cryostatic sections of the dorsal root ganglion (A), sciatic nerve (B), and acoustic ganglion (C) of a transplanted mouse, **6 months** after BMT . (D) Vector-expressing cells in the PNS of a representative secondary transplant recipient. Cryostatic section from the dorsal root ganglion 4 months after BMT,



ARSA activity and neurophysiology (PNS)

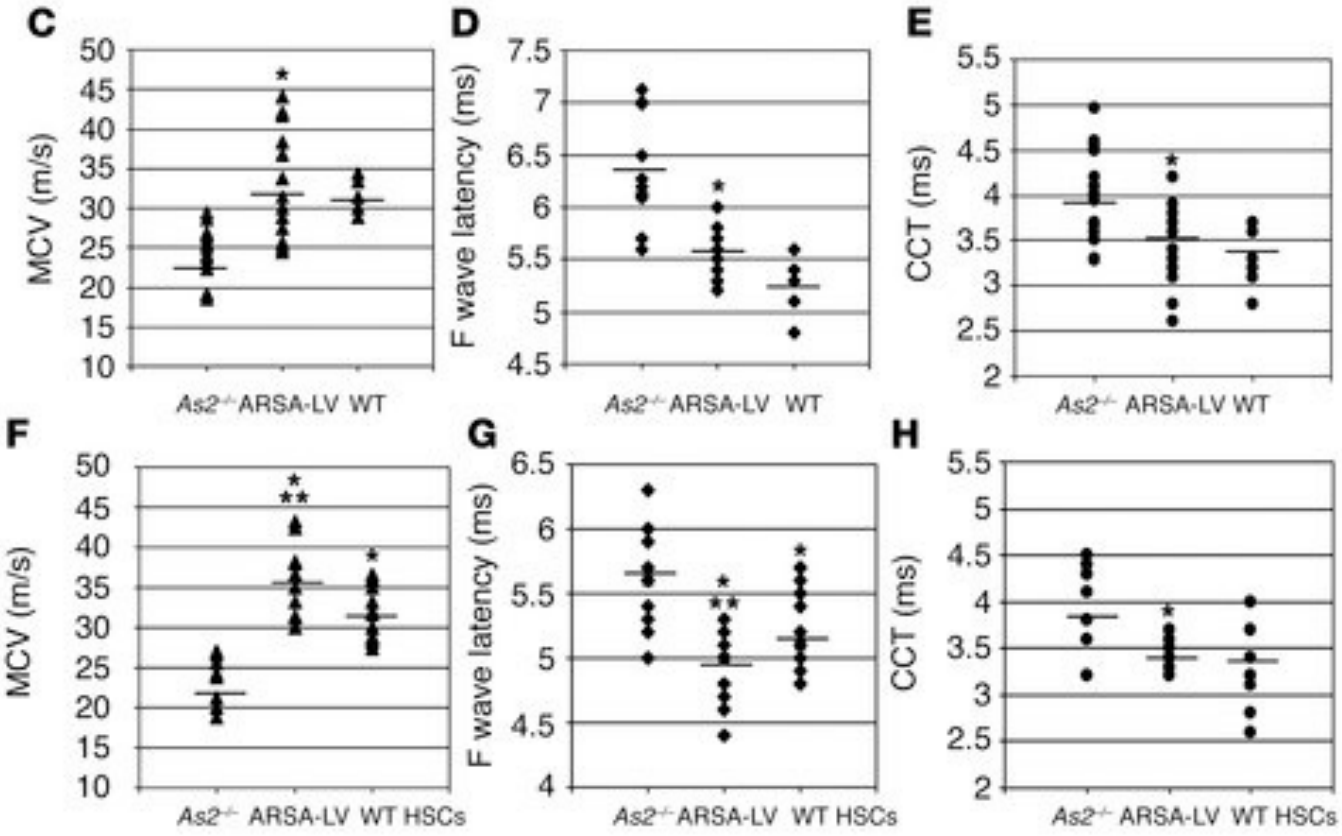
7 months pst BMT, sulfate metabolism





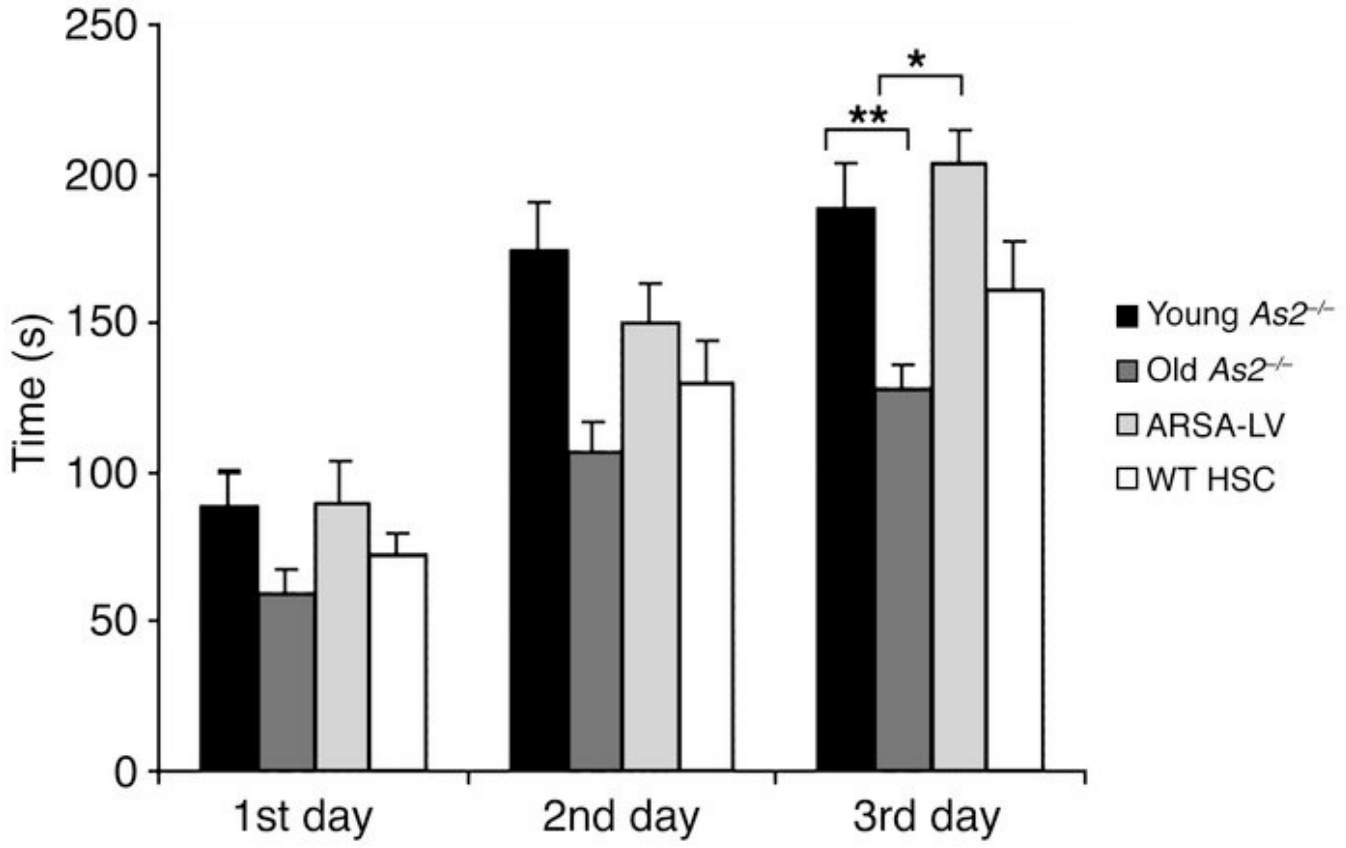
PBMCs

Motor conduction in 8 and 12 months old mice



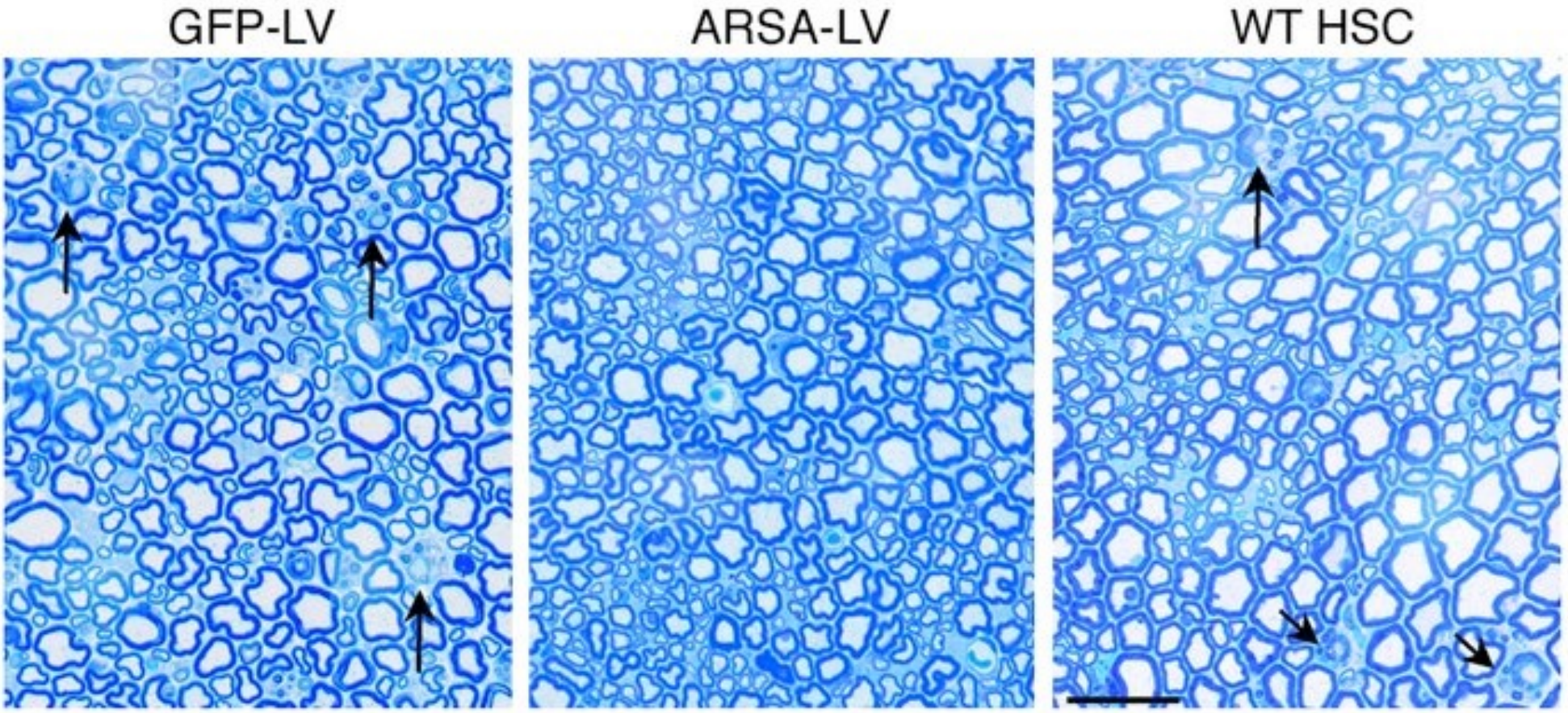
Motor learning and coordination -CNS

Latencies to fall off the rotarod were recorded



Twelve-month-old mice transplanted with ARSA-transduced or WT HSCs and two cohorts of untreated $As2^{-/-}$ mice of 3 and 12 months of age were tested on an accelerating rotarod apparatus (n = 12–30 per group).

Protection from lipid accumulation



Long-term protection from lipid storage and demyelination in transplanted MLD mice. Toluidine Blue–stained sections of the **sciatic nerve** of representative 12-month-old MLD mice transplanted with GFP-LV– and ARSA-LV–transduced As2–/– HSCs, or with WT HSCs. Several **demyelinated fibers** (arrows) and **metachromatic granules in Schwann cells** are present in mock-treated and WT HSC–transplanted mice, whereas they are almost absent in mice transplanted with gene-corrected cells

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Safety of vector batches

Third generation LV-PGK-ARSA in stimulated CD34+ cells

Test	Specification	Batch #		
		08087	09015	09021
Physicochemical and identity				
Osmolality (mOsm/Kg)	290-350	315	306	313
pH EP 2.2.3	7.0-8.0	7.6	7.7	7.6
ARSA transgene sequence	Corresponding	Corresponding	Corresponding	Corresponding
Vector integrity	Corresponding to the reference	Corresponding	Corresponding	Corresponding
Lentiviral proteins	Corresponding to the reference	Corresponding	Corresponding	Corresponding
Potency and bioactivity				
Infectious Titer (TU/ml)	$\geq 2x10^8$	$6.4x10^8$	$4.0x10^8$	$2.7x10^8$
Physical titer (HIV Gag p24 Antigen) (ng/ml)	FIO	$1.1\ x10^4$	$1.0x10^3$	$8.2x10^3$
Infectivity (Transducing unit/ng p24)	$\geq 2x10^4$	$5.6x10^4$	$3.8x10^4$	$3.3x10^4$
Transgene function (ARSA activity, fold to untransduced)	$\geq$ 5 fold untransduced cells	22	14	16
Microbial purity and safety				
Sterility EP 2.6.1	Negative	Negative	Negative	Negative
Mycoplasma EP 2.6.7 (cultural assay)	Negative	Negative	Negative	Negative
Endotoxin EP 2.6.14 (quantitative assay) (EU/ $2x10^8$ TU)	≤ 25	3	21	7
In vitro Adventitious viruses	Negative	Negative	Negative	Negative
In vivo Adventitious viruses	Negative	Negative	Negative	Negative
RCL	Negative	Negative	Negative	Negative
Process and product impurities				
Host cell proteins (ng/ $2x10^8$ TU)	FIO	22	36	44
Plasmid residual DNA (VSV-G) (copies/$2x10^8$ TU)	$\leq 4x10^8$	$0.6x10^8$	$1.6x10^8$	$1.9x10^8$
Large T antigen (protein contamination) (ng/ml)	$\leq$LOQ (*)	$\leq$LOQ	$\leq$LOQ	$\leq$LOQ
Large T antigen Residual DNA (copies/$2x10^8$ TU)	$\leq 2.0 \times 10^5$	$0.7x10^4$	$2.3x10^4$	$1.5x10^4$
Benzonase contamination (ng/ml)	≤ 0.2	< 0.1	<0.1	<0.1
E1A DNA (copies/$2x10^8$ TU)	$\leq 2.0 \times 10^5$	$1.7x10^4$	$3.4x10^4$	$4.3x10^4$
Total residual DNA (µg/$2x10^8$ TU)	FIO	0.9	1.9	1.5
BSA contamination (µg/$2x10^8$ TU)	FIO	0.4	0.7	0.8
Vector cross-contamination	$\leq 10^5$pp / 10^{10}pp	$\leq 10^5$pp / 10^{10}pp	$\leq 10^5$pp / 10^{10}pp	$\leq 10^5$pp / 10^{10}pp

Transplant details

2 LV-transduction round

Presymptomatic patients with affected siblings

	MLD01	MLD02	MLD03
Cell dose ($CD34^+$ cells/kg)	$11x10^6$	$7.0x10^6$	$7.2x10^6$
VCN (copies/genome)	2.5	2.5	4.4
Transduction efficiency (%)	97	90	93
ARSA activity (fold to HD)	>10	>10	>10
BU total dose (mg/kg)	10.4	14.6	10.4
Neutropenia (days post-GT)	+9 to +38	+9 to +45	+11 to +37

Table S5. Transplant details. VCN: vector copy number, measured after 14 days of culture; HD: healthy donor; BU: busulfan. Transduction efficiency was measured by quantitative PCR performed on individual colonies obtained from colony forming cell – CFC – assay.

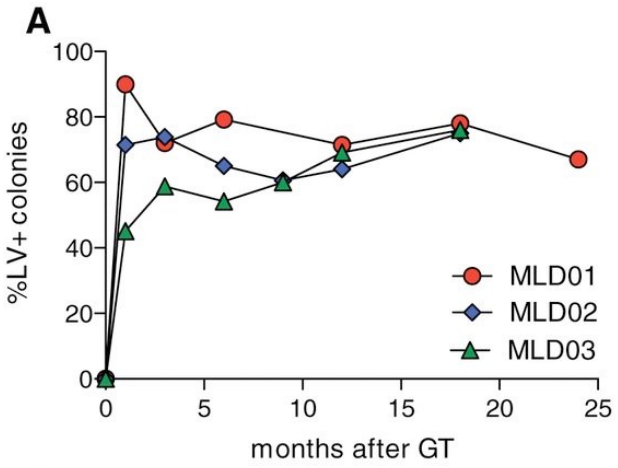
Patients

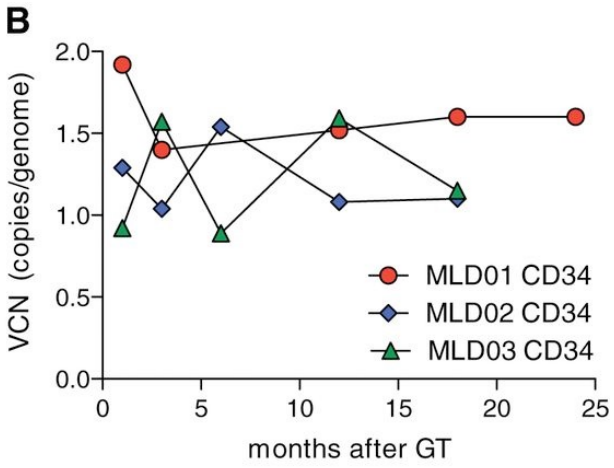
	MLD01	MLD02	MLD03
Leukocyte ARSA activity (nmol/mg/h)(before HSC-GT)	12	7.3	4.7
ARSA gene mutations	c.821C>T$^{LI(20)}$ (p.Thr274Met) c.821C>T^{LI} (p.Thr274Met)	c.730C>T$^{LI(21)}$ (p.Arg244Cys) c.731G>A$^{LI(21)}$ (p.Arg244His)	c.443C>G^{UK} (p.Pro148Arg) c.443C>G^{UK} (p.Pro148Arg)
Age at expected onset (onset in the affected sibling/s)	18 months	24 months	15 months
Age at HSC-GT	16 months	13 months	7 months
Age at last follow up	39 months	30 months	25 months
Symptoms at HSC-GT	no	no	no
NCV index at HSC-GT	-11.5	-2.3	-6.7

Table S3. Treated patients' characteristics. HSC-GT: HSC gene therapy. LI: late infantile-associated mutation; UK: unknown/not previously described mutation.

Gene marking in patients after HSC-GT

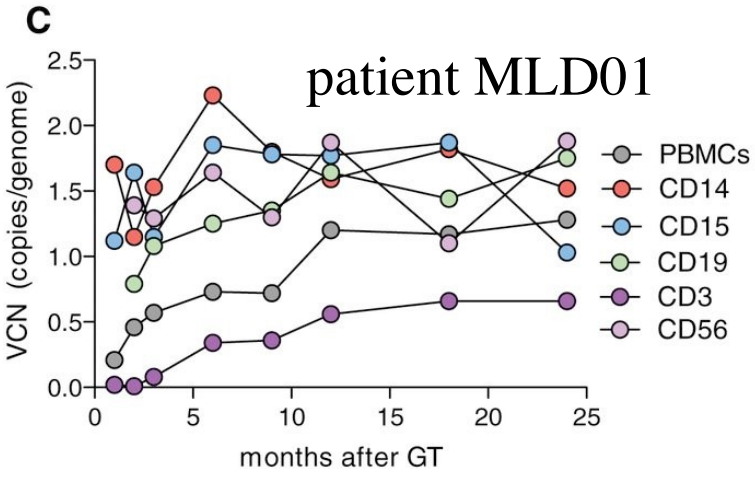
PB- and BM-derived cells percentage (%) of LV+ colonies on total tested colonies.

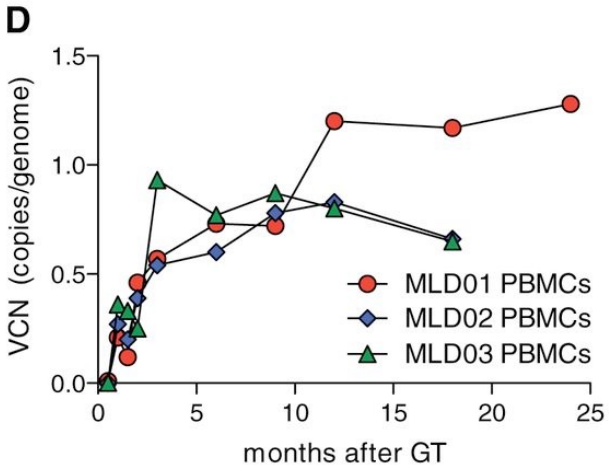




LV copies/host genome

LV copies /host geno me



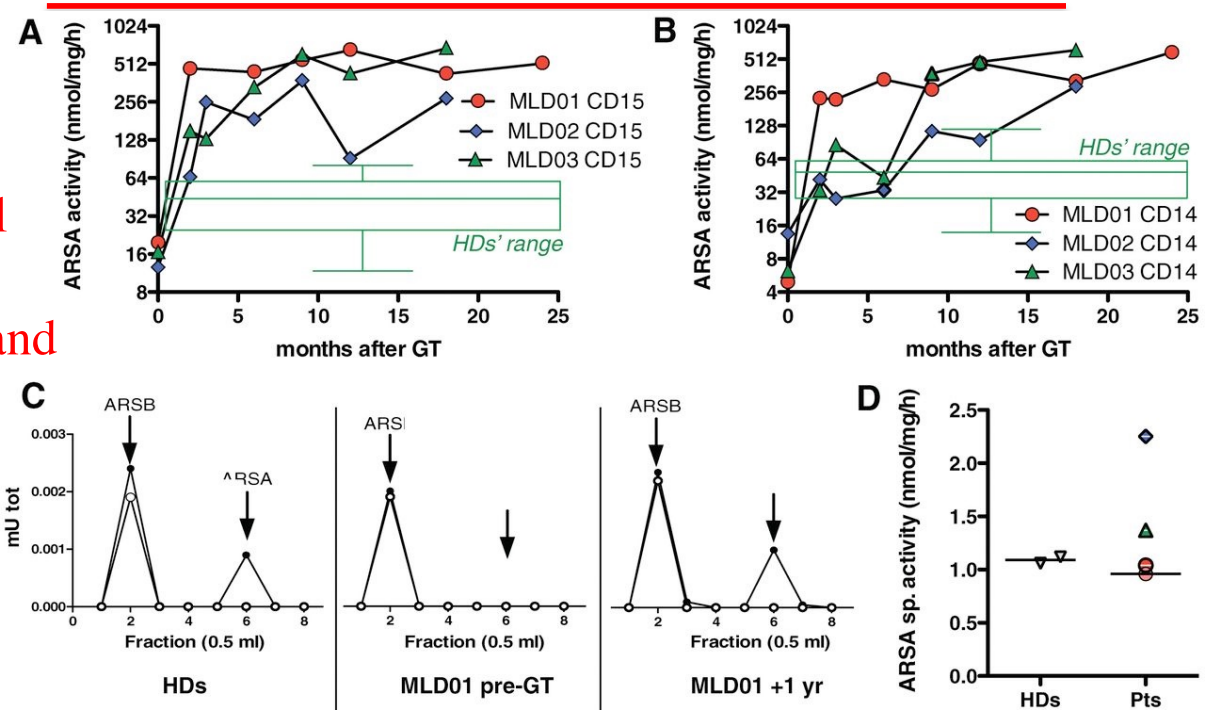


LV copies/hos t genome

ARSA expression in patients after HSC-GT

ARSA activity measured with the *p*-nitrocatechol sulfate (PNC) assay

Stable and above normal ARSA expression in hematopoietic lineages and efficient delivery and bioavailability in CNS

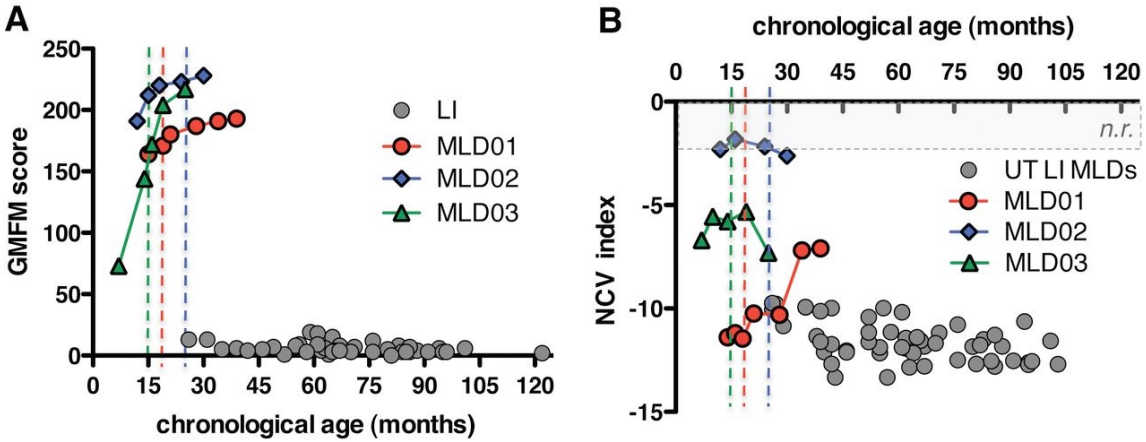


Representative DEAE cellulose-chromatography analysis on cerebrospinal fluid (CSF) from a pool of four HDs, of a MLD patient **before** treatment and of the same patient 1 year after gene therapy.

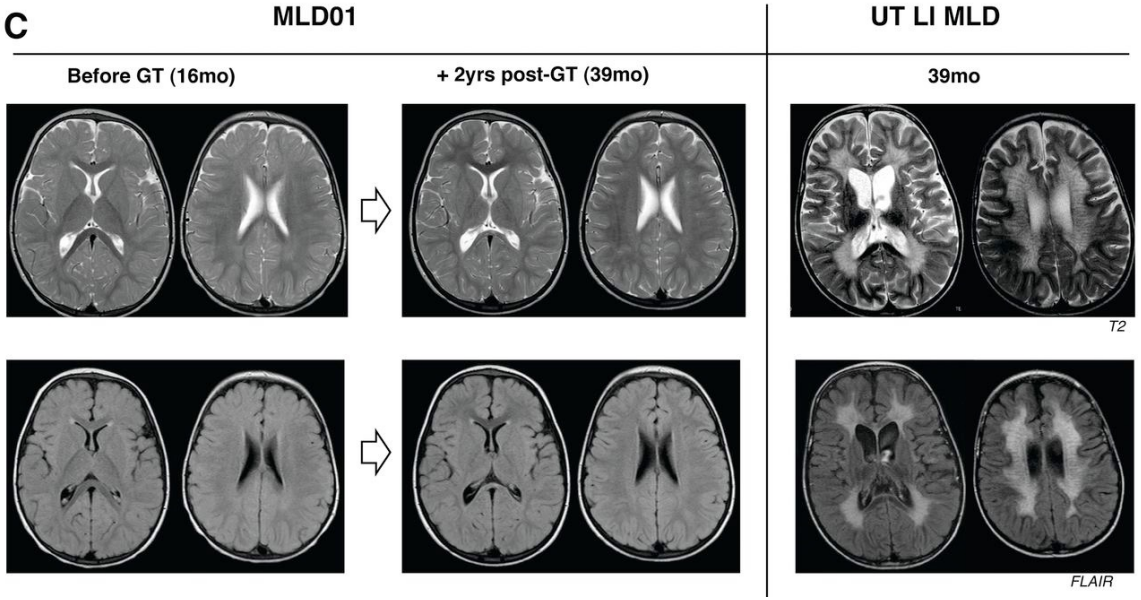
Specific activity (toward MUS) of the ARSA enzyme isolated from the CSF

Clinical follow up of MLD patients after HSC-GT

Gross Motor Function Measure (*GMFM*) score (A) and nerve conduction velocity index (B)



Axial T2 weighted fast spin-echo Magnetic Resonance images (top) and FLAIR MR images (bottom) obtained from patient MLD01 at baseline (before GT) and at +2 years after treatment, and corresponding (equivalent) images of an age-matched untreated patient with LI-MLD

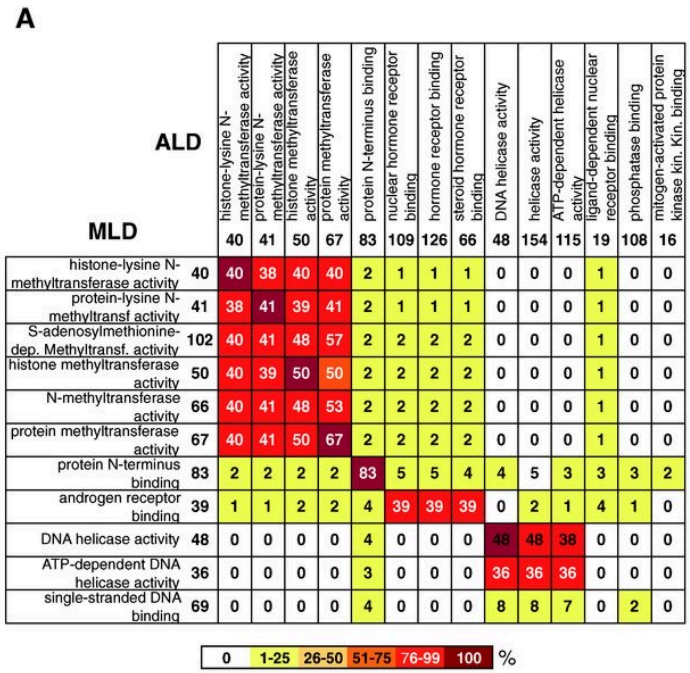


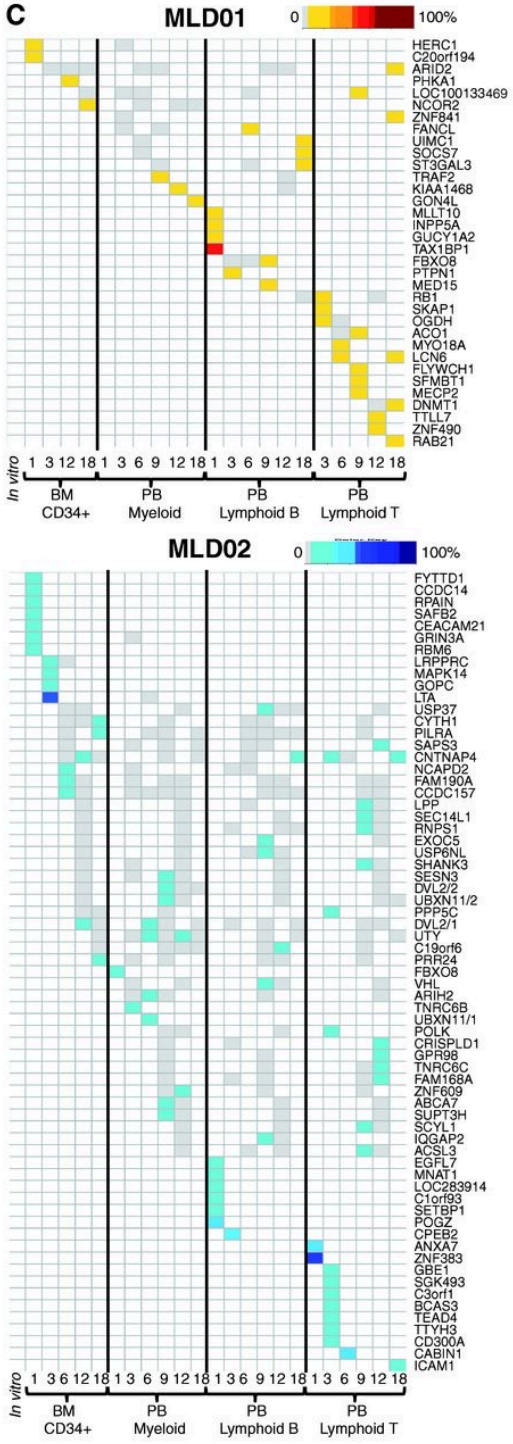
LV genomic integration profile.

A: analysis on ISs from three patients with MLD and two patients with ALD who had been treated with HSC-GT. NB LV80% IS within genes

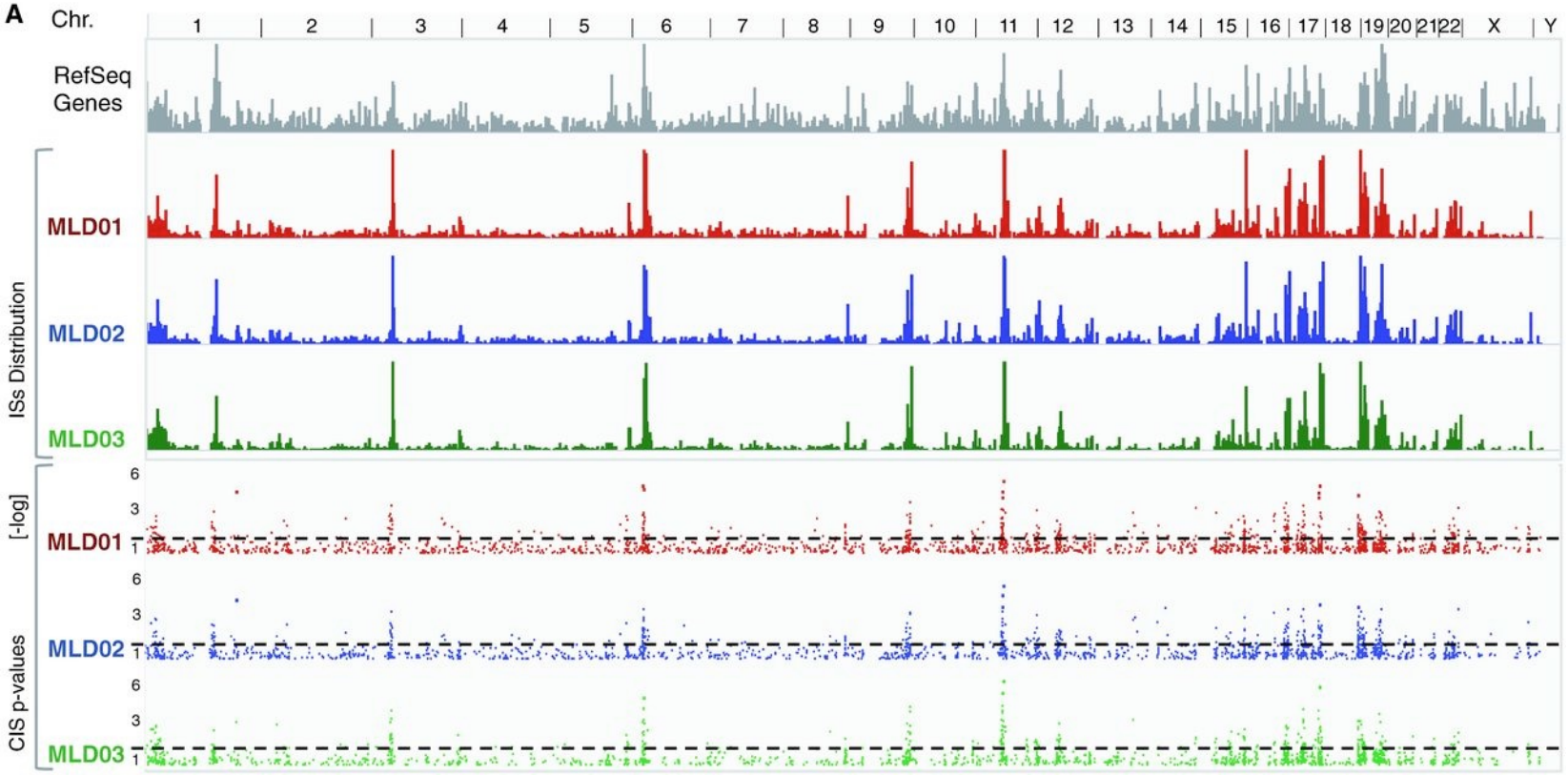
>800 LAM PCR on the 3 MLD patients
Different cell types
Mapped 14482 sites for MLD01
11077 for MLD02
10959 for MLD03

C No cloning dominance over time

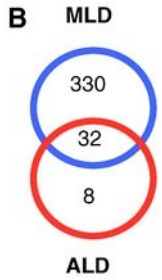




Frequency distribution along chromosomes



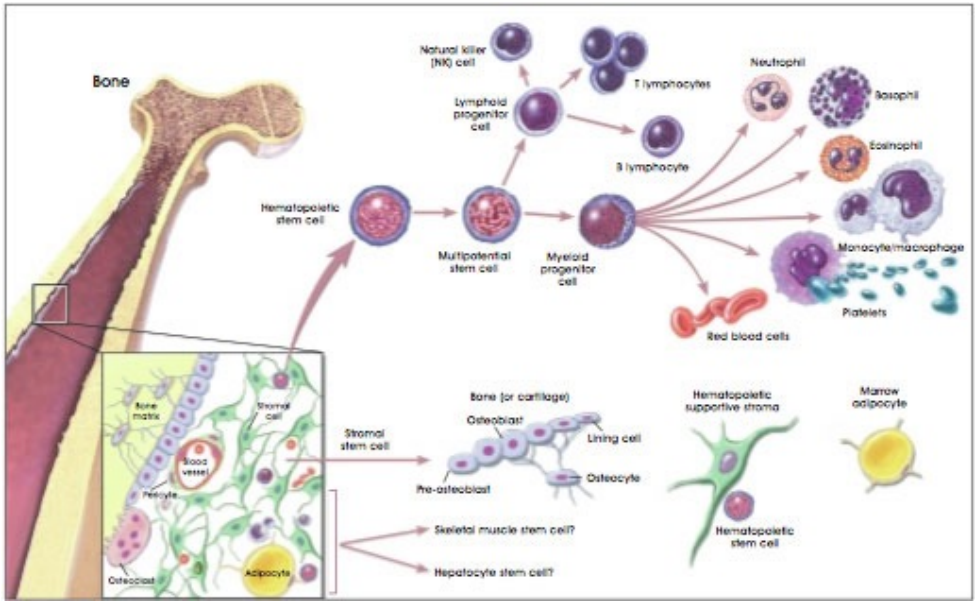
Common insertion site analysis



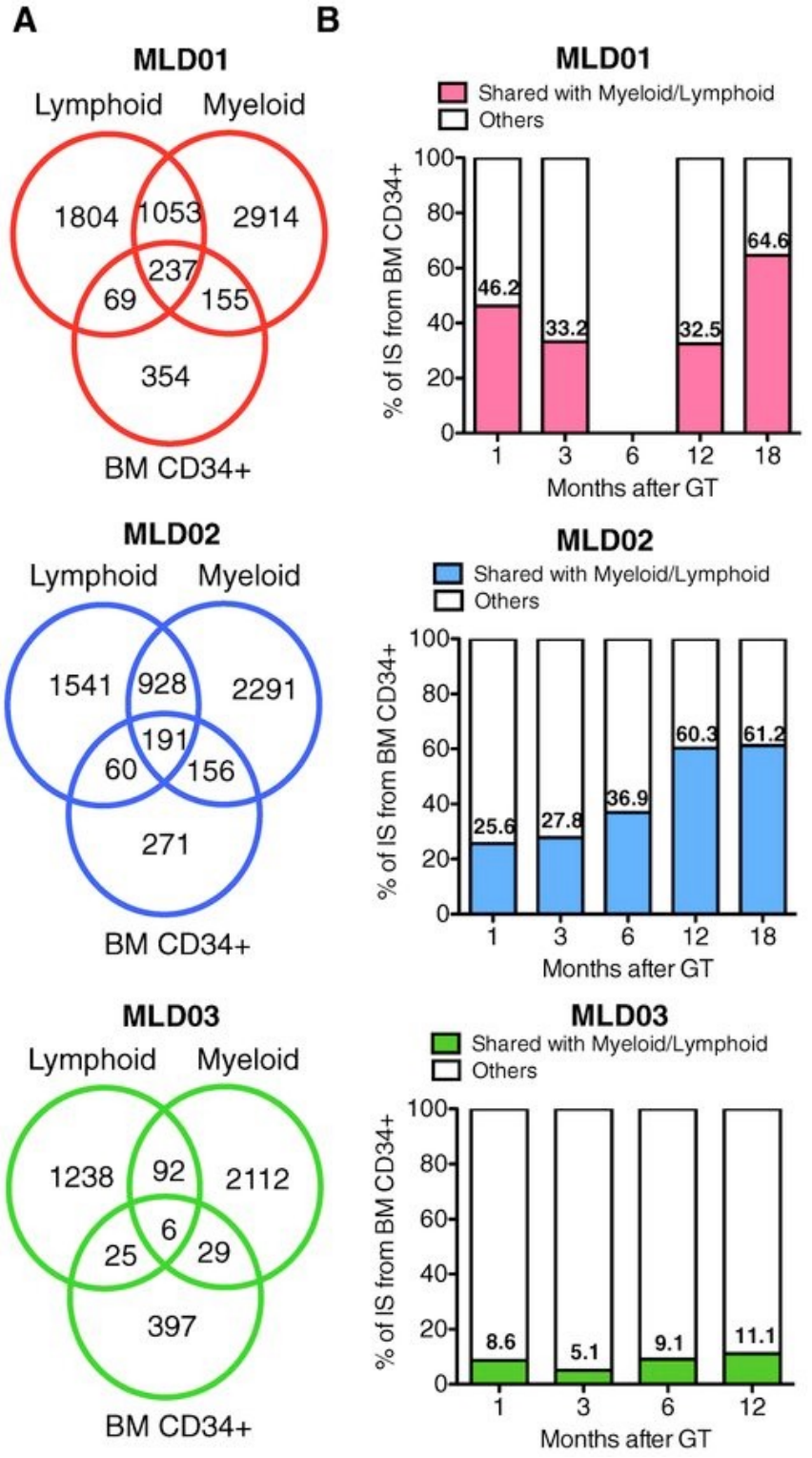
Venn diagrams showing the overlap between CIS genes in the MLD and ALD HSC-GT trials

Stem cell marking and clonal dynamic

Sustained clonogenic activity of engrafted cells-efficient transduction and engraftment of HSCs



Several IS shared among progenitors and mature myeloid and lymphoid cells indicating efficient transduction and engraftment of HSCs



Science 23 August 2013: Vol. 341 no. 6148- Phase I-II trial . Conclusions

- Ok transgene expression
- Ok engraftment
- Ok integration
- No side effect
- Blocked degeneration

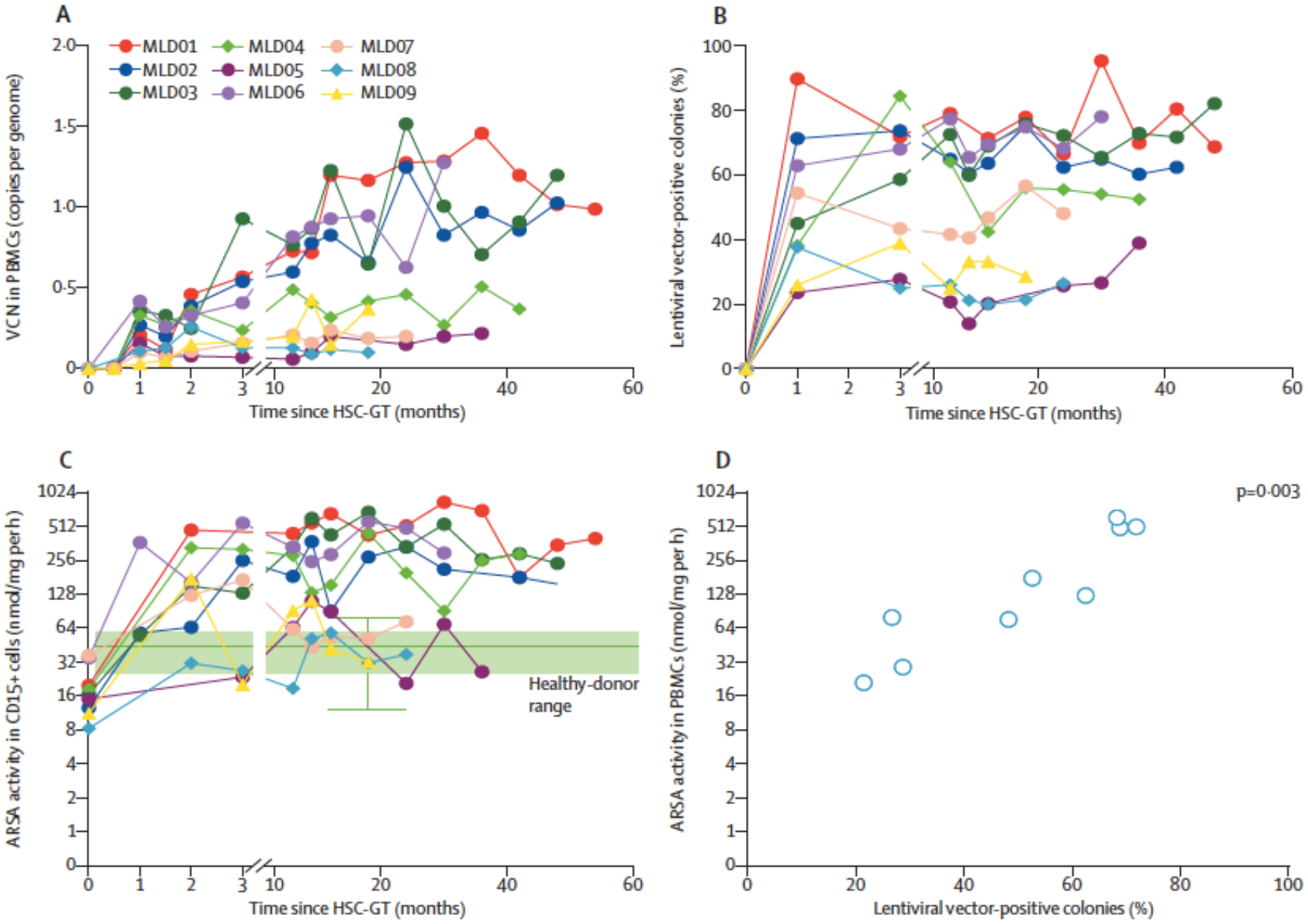
- Follow up needed

Clinical Trial Follow up

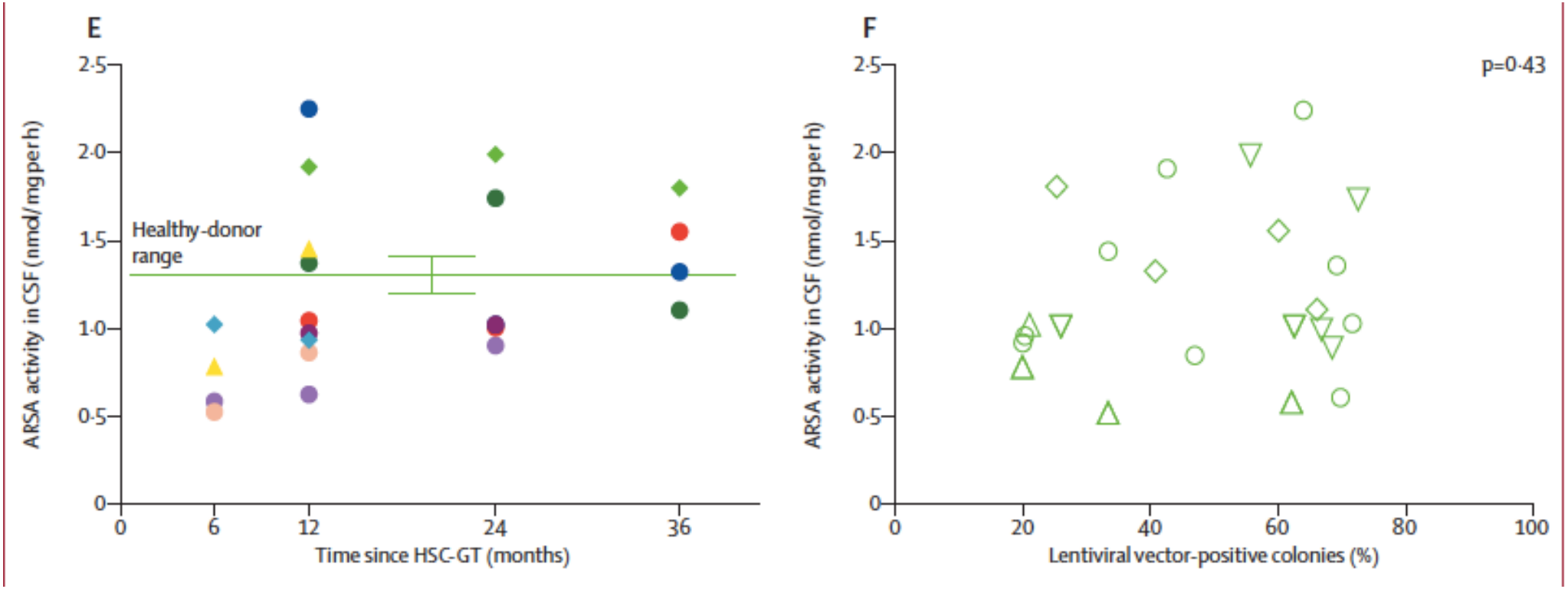
Sessa M, Lorioli L, et al., Naldini L. Biffi A. Lentiviral haematopoietic stem-cell gene therapy in early-onset meatchromatic leukodystrophy: an ad-hoc analysis of a non-randomised, open-label, phase ½ trial. The Lancet 2016

9 patients enrolled 2010-2013

Clinical Trial Follow up



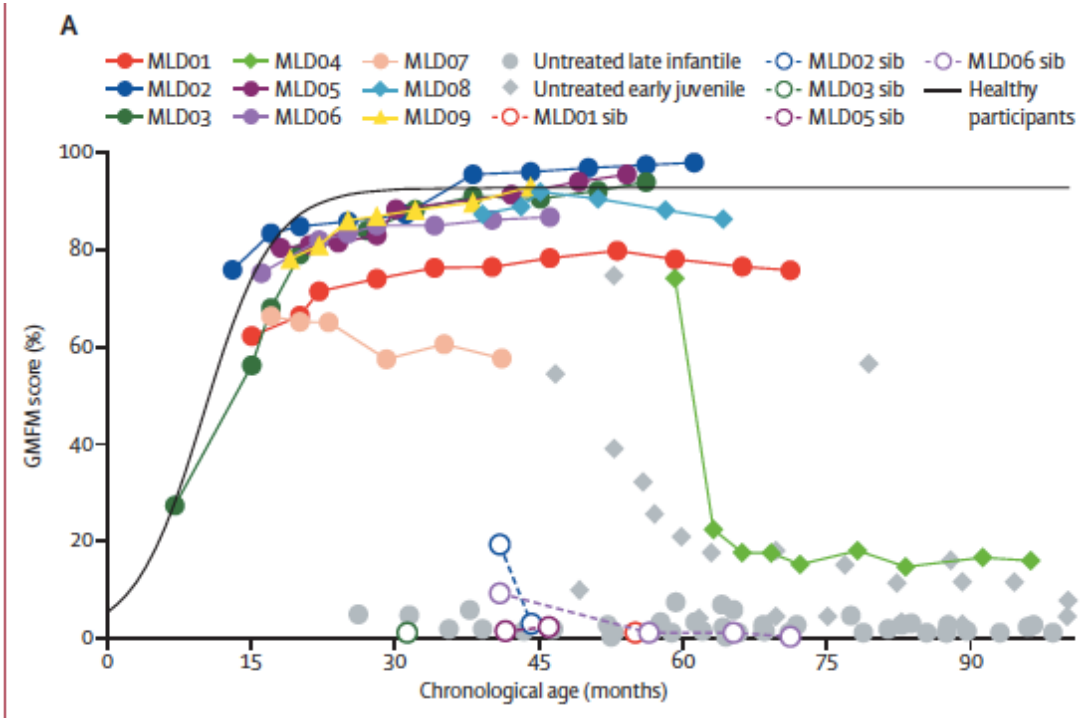
Clinical Trial Follow up



Cerebrospinal fluid

Clinical Trial Follow up

Gross Motor Function Measure (*GMFM*)
score



Conclusions

All 9 enrolled patients survived
High level of transduced cells engraftment (thanks to high transduction efficacy of HSC by lentiviral vectors and ablative regimen)
All patients experiment therapeutic benefit (before or very early after symptom onset), protection from massive CNS demyelination, amelioration of PNS morphology and function. Robust level of ARSA production in HSC and their progeny and for efficient enzyme delivery in nervous system allowing sulphatides removal.

Further follow up

Extended Clinical trial up to 20 patients

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

EMA/559368/2020
EMEA/H/C/product number

Libmeldy (*autologous CD34+ cell enriched population that contains haematopoietic stem and progenitor cells transduced* ex vivo *using a lentiviral vector encoding the human arylsulfatase A gene*)

Fondazione Telethon, Ospedale San Raffaele e l'azienda produttrice, Orchard Therapeutics

Libmeldy received a marketing authorisation valid throughout the EU on 17 December 2020.

Science 23 August 2013: Vol. 341 no. 6148

Lentiviral Hematopoietic Stem Cell Gene Therapy in Patients with Wiskott-Aldrich Syndrome

Alessandro Aiuti1, et al, et Luigi Naldini

LV third generation in CD34+ cells, BMT

Wiskott-Aldrich Syndrome

WASP deficiency (intracellular key regulator of actin polymerization)

X linked mutation in WAS gene
Recessive

Bone marrow disease – recurrent infection, low platelet counts (thrombocytopenia)

HSPC transplantation from an HLA-identical donor is the treatment of choice

9 patients

3 reported

Table 1

Characteristics and treatment of the three WAS patients.

WASP expression analysis was performed on PB lymphocytes by FACS. Patient 1 also received G-CSF mobilized peripheral blood (MPB)-derived $CD34^+$ cells, previously collected as back-up, to achieve the target HSPC dose. A&W, alive and well; CMV, cytomegalovirus; ENT, ear, nose, throat; GI, gastrointestinal; GE, gastroesophageal; HHV-6, human herpes virus type 6; HSV, herpes simplex virus; IVIG, intravenous immunoglobulins; URTI, upper respiratory tract infection; UTI, urinary tract infection; VZV, Varicella zoster *virus*.

	Patient 1	Patient 2	Patient 3
Infectious manifestations	Recurrent ENT	Pneumonias, colitis arthritis/cellulitis, URTI, UTI	Pneumonia with respiratory distress, URTI, otitis
Pathogens	VZV, CMV, HSV, EBV	CMV, HHV-6, candida	*Pneumocystis jirovecii*, CMV
Thrombocytopenia manifestations	Skin petechiae	Skin petechiae, GI bleeding	Skin petechiae, GI bleeding, epistaxis
Eczema	Moderate-severe	Moderate-severe	Severe
Other	Developmental disorder, allergy	Failure to thrive, elevated inflammatory indexes/vasculitis, hepatosplenomegaly	GE reflux/food aversion (fed by nasogastric tube), allergy
WAS mutation	Exon 10: C>T 995 (R321X)	IVS10del11nt	37C>T (R13X)
WASP expression	<5%	<5%	<5%
Zhu score	3	4	4
Age at treatment (years)	5.9	1.6	1.1
Infused $CD34^+$ cells ($\times 10^6$/kg)	3.66 (BM) + 5.25 (MPB)	14.1	10.2
Vector copies/genome	1.9 (BM) - 1.4 (MPB)	2.4	2.8
Transduction efficiency (CFC)	92% (BM) - 88% (MPB)	97%	100%
Follow-up (months)	32	23	20
Current clinical conditions	A&W, no eczema, no major bleeding or petechiae, off IVIG	A&W, no eczema, no major bleeding or petechiae	A&W, no eczema, no major bleeding or petechiae

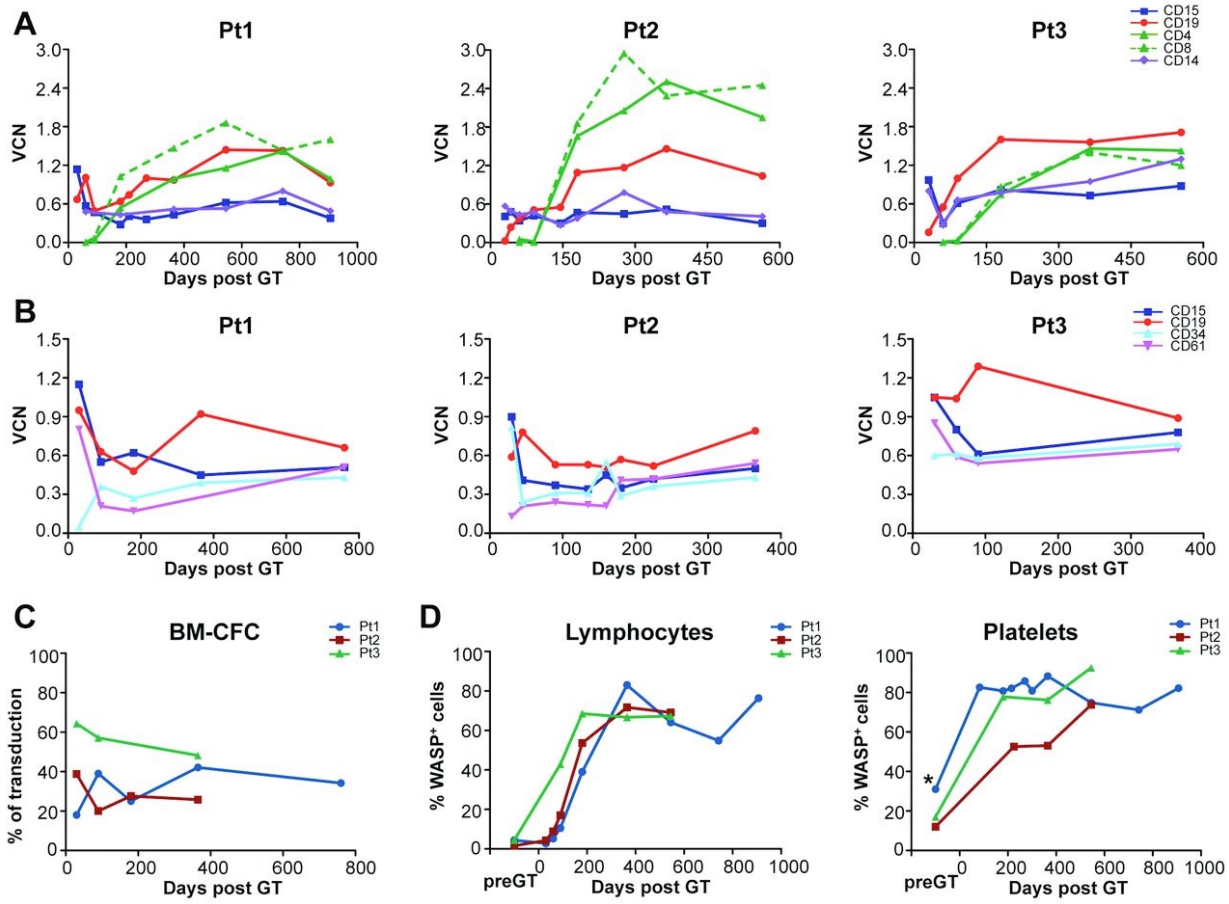
Engraftment of transduced cells and WASP expression after gene therapy

VCN per genome was evaluated by qPCR at different time points (up to 2.5 years)

WAS protein expression measured by cytofluorimetric analysis

PB

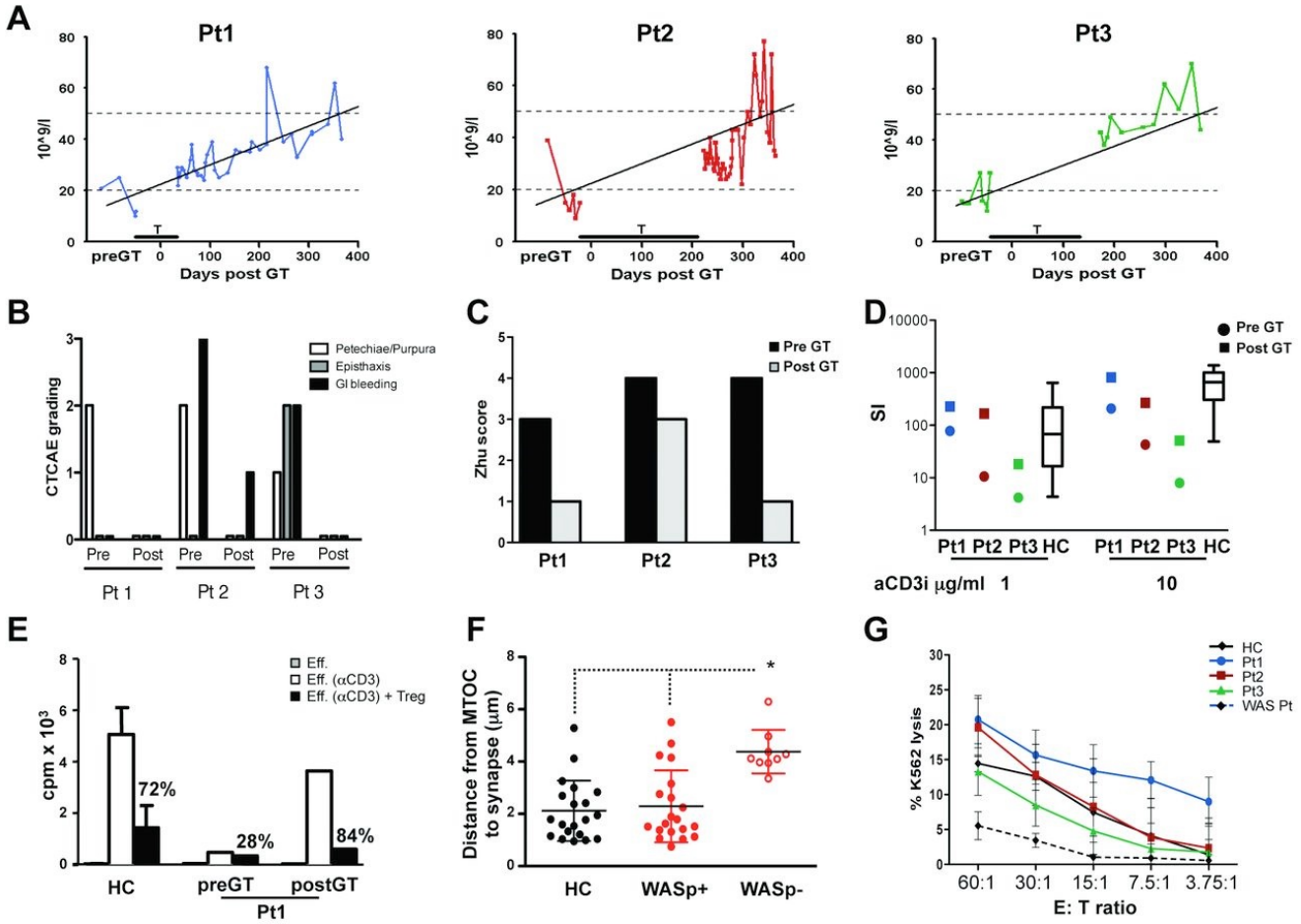
BM



Clinical features and immune function of WAS patients after gene therapy

A. Platelets counts

B. Summary of bleeding events, C. disease score, D. TCR driven proliferation, E-G blood cell activity



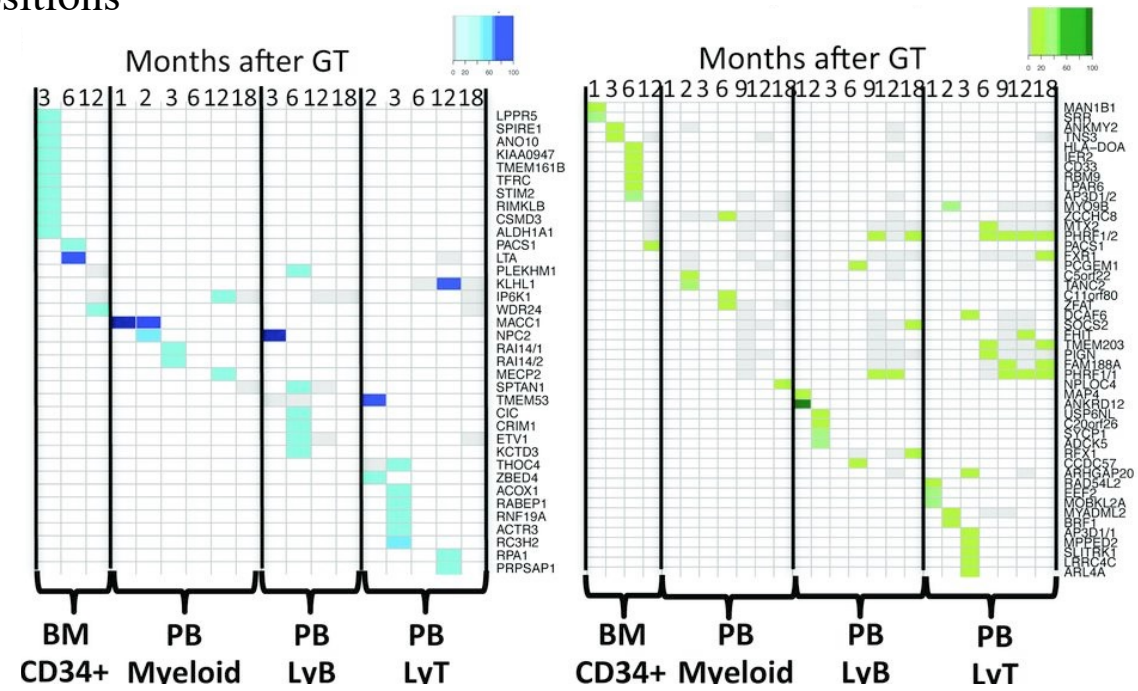
effector T cells

Formation of NK immunological synapse.

NK cytotoxic activity

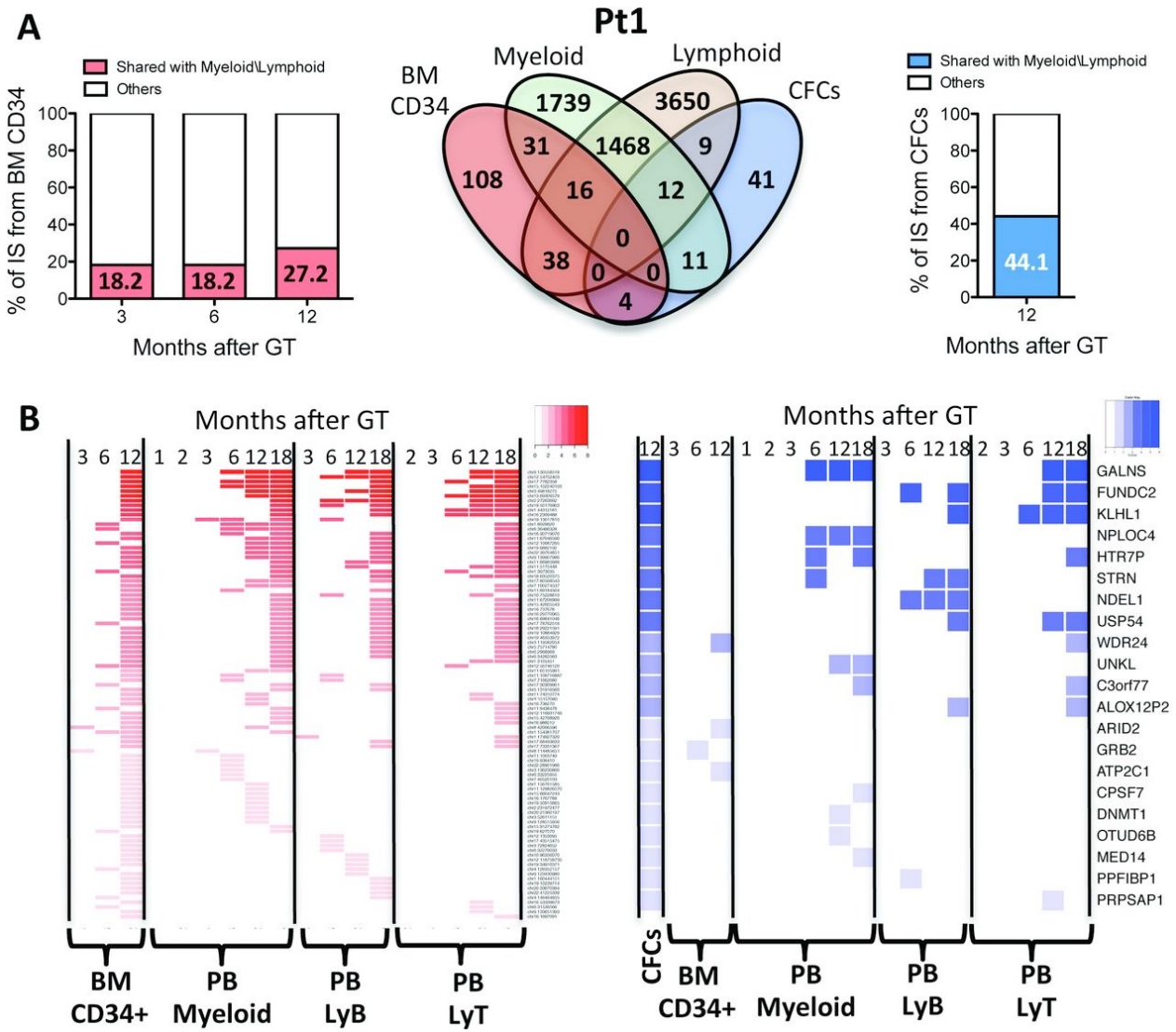
Long-term polyclonal engraftment of gene-corrected HSPC, assessed by longitudinal integration site profiling

LAM PCR and next generation sequencing detected >2,400,000 IS sequences mapped to 33,363 unique chromosomal positions

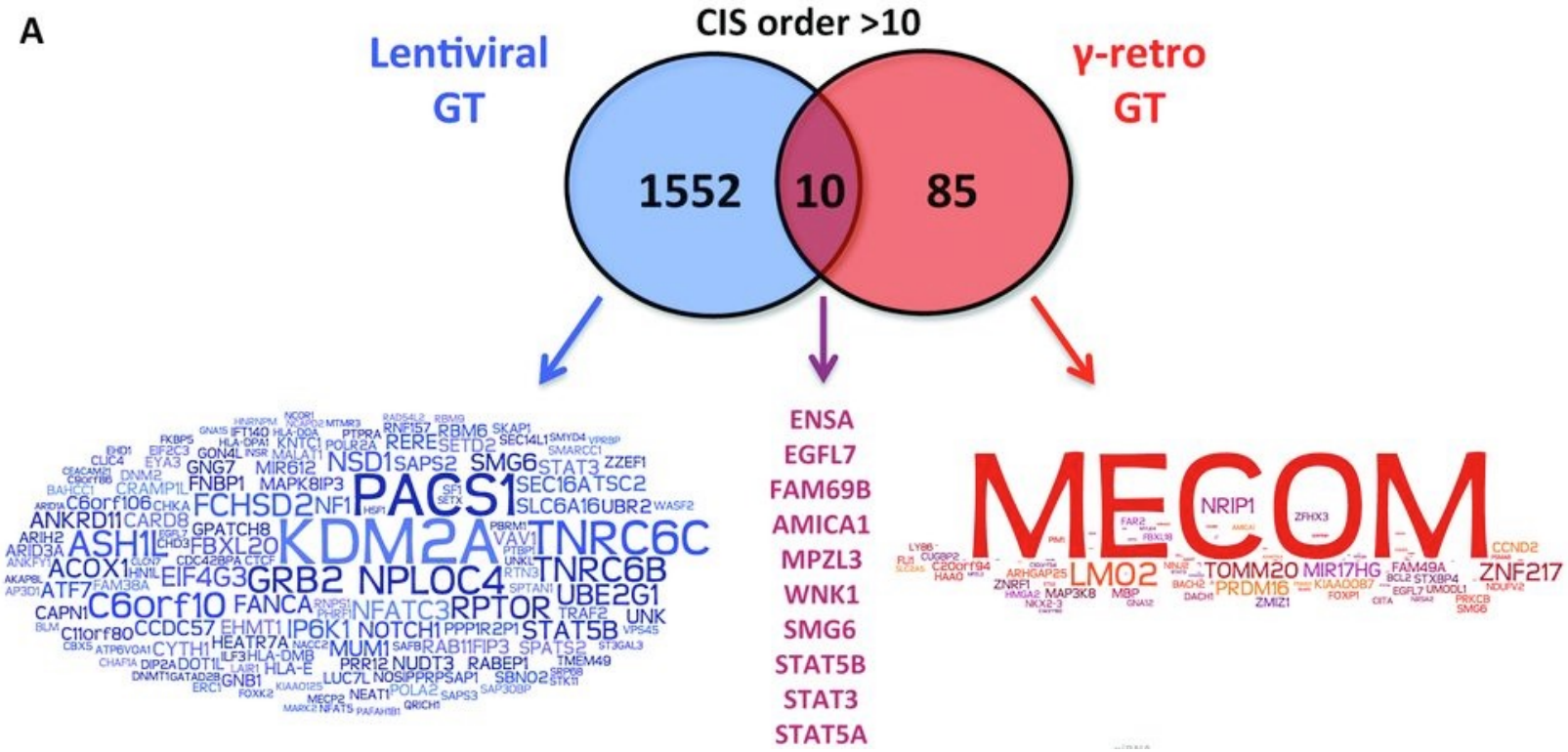


Clones from diff cells at different time points=> LAMPCR => Seq => insertion sites=>frequency

Multilineage engraftment and activity of gene-corrected HSPC



Common insertion sites and oncogenic hits in lentiviral versus γ-retroviral gene therapy



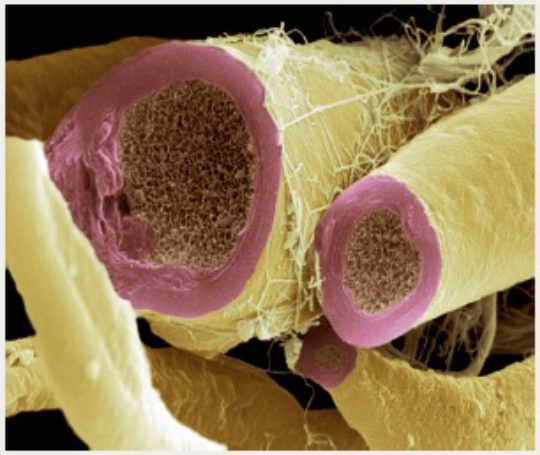
Science 23 August 2013: WAS Phase I-II trial Conclusions

- Platelets ok
- Lymphoid cells ok
- Protection from bleeding and resolution of eczema
- HSC engraftment, nultilineage
- No insertion in cancer prone sites
- No dominant clone

Long term follow up needed

Gene therapy treatment for Cerebral Adrenoleukodystrophy

First gene therapy for adrenoleukodystrophy



Myelinated nerve cells. Credit: Science Photo Library / Alamy Stock Photo

Bluebird Bio has earned a marketing authorization from the European Commission for its single-dose gene therapy Skysona (elivaldogene-autotemcel; Lenti-D) to treat a severe form of adrenoleukodystrophy. The go-ahead is for patients younger than 18 years with cerebral adrenoleukodystrophy (CALD) for whom a matched hematopoietic stem cell donor is not available. CALD is a rare X-linked neurodegenerative disease caused by a mutation in the *ABCD1* gene;

NATURE BIOTECHNOLOGY | VOL 39 | NOVEMBER 2021 | 1317–1331 | www.nature.com/naturebiotechnology

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Skysona (*elivaldogene autotemcel*)

An overview of Skysona and why it is authorised in the EU

What is Skysona and what is it used for?

Skysona is a medicine used to treat children under 18 years of age with early cerebral adrenoleukodystrophy (CALD). CALD is a rare inherited disorder in which there is a change (mutation) in the *ABCD1* gene. The mutation prevents the production of an enzyme called ALDP

Skysona received a marketing authorisation valid throughout the EU on 16 July 2021.

The NEW ENGLAND JOURNAL of MEDICINE 2017

ORIGINAL ARTICLE

Hematopoietic Stem-Cell Gene Therapy for Cerebral Adrenoleukodystrophy

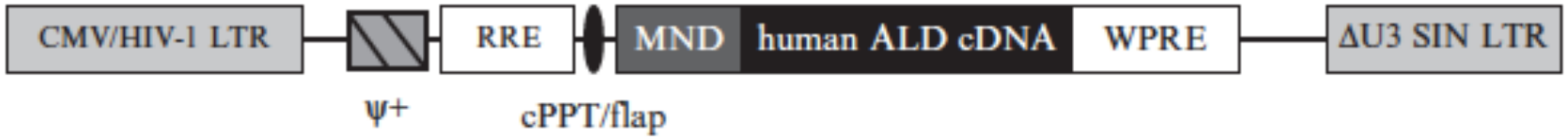
Florian Eichler, M.D., Christine Duncan, M.D., Patricia L. Musolino, M.D., Ph.D., Paul J. Orchard, M.D., Satiro De Oliveira, M.D., Adrian J. Thrasher, M.D., Myriam Armant, Ph.D., Colleen Dansereau, M.S.N., R.N., Troy C. Lund, M.D., Weston P. Miller, M.D., Gerald V. Raymond, M.D., Raman Sankar, M.D., Ami J. Shah, M.D., Caroline Sevin, M.D., Ph.D., H. Bobby Gaspar, M.D., Paul Gissen, M.D., Hernan Amartino, M.D., Drago Bratkovic, M.D., Nicholas J.C. Smith, M.D., Asif M. Paker, M.D., Esther Shamir, M.P.H., Tara O'Meara, B.S., David Davidson, M.D., Patrick Aubourg, M.D., and David A. Williams, M.D.

Gene therapy treatment for Cerebral Adrenoleukodystrophy

-ALD or CALD is an X-linked diseases
-Characterized by demyelination and neurodegeneration, that leads to loss of neurologic function and death

- Caused by deficiency in ALD protein encoded by ABCD1 gene
- ALD is a transporter implicated in metabolism of VLCFA (very long chain fatty acids
- HCT allogenic

- Gene therapy: ex vivo transduction of CD34+ positive cells with lenti-D

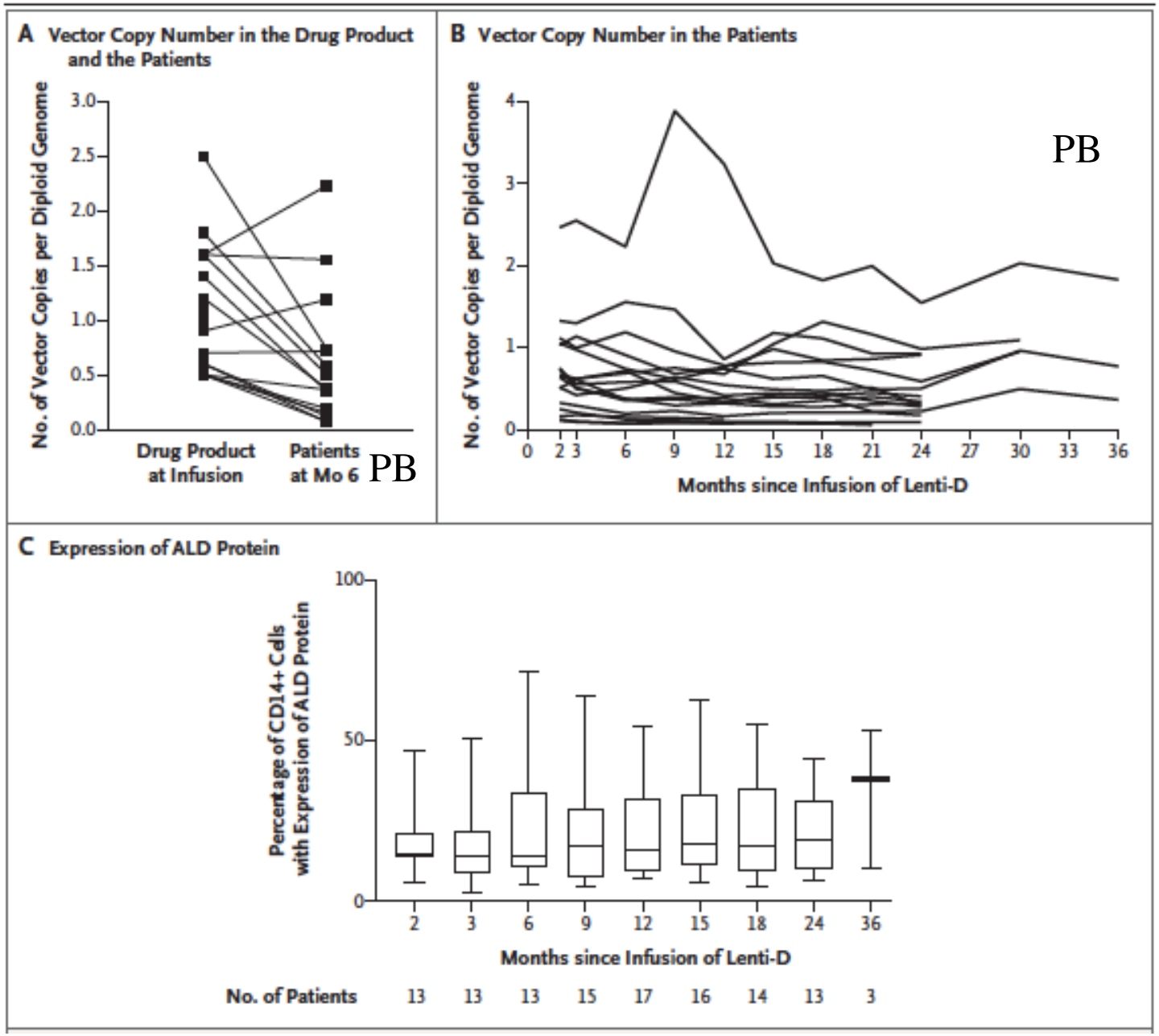


Gene therapy treatment for CALD. Protocol

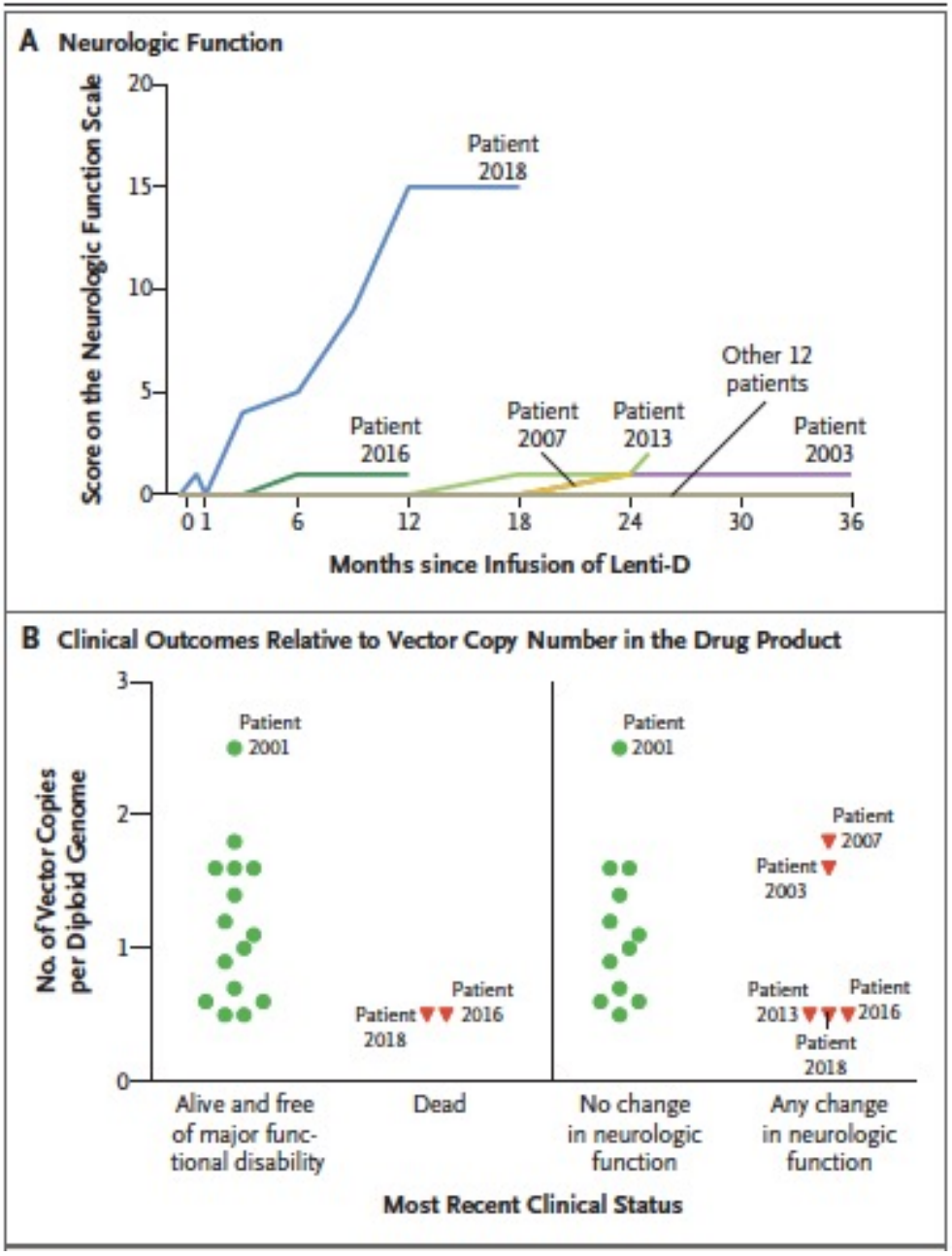
Table 1. Baseline Characteristics of the Patients and the Drug Product.

Characteristic	Value
Patients	
No. enrolled*	17
Age at enrollment (yr)	
Median	6
Range	4–13
Loes score†	
Median	2.0
Range	1.0–7.5
Score on neurologic function scale‡	
Median	0
Range	0
Time from consent to infusion of drug product (days)	
Median	67.0
Range	58.0–89.0
Drug product	
Vector copy number (vector copies/diploid genome)	
Median	1.0
Range	0.5–2.5
Dose (CD34+ cells/kilogram of body weight)	
Median	10,500,000
Range	6,000,000–19,400,000

Gene therapy treatment for Cerebral Adrenoleukodystrophy. Gene marking and ALD expression



Gene therapy treatment for Cerebral Adrenoleukodystrophy. Clinical Follow up



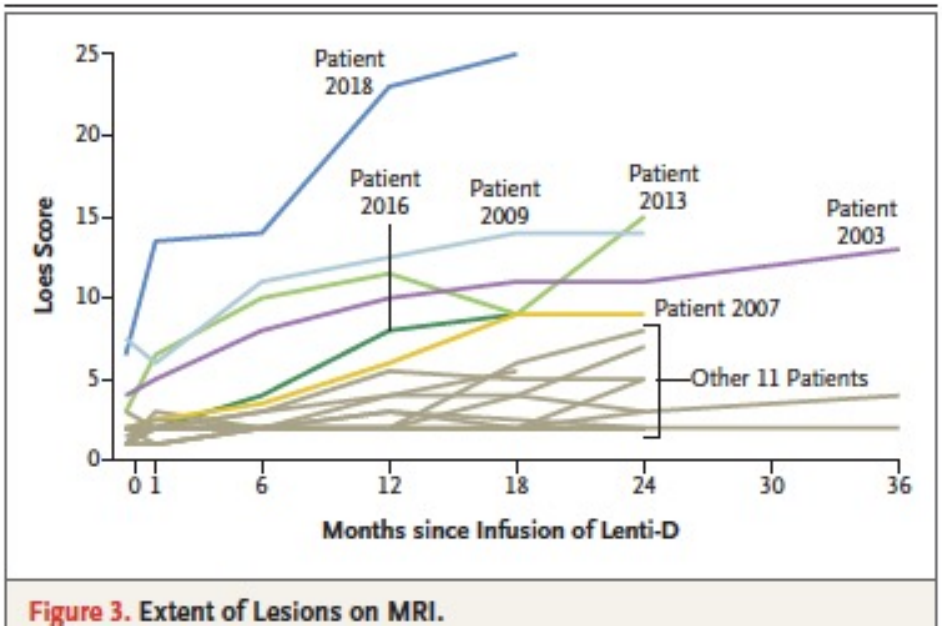


Figure 3. Extent of Lesions on MRI.

Gene therapy treatment for Cerebral Adrenoleukodystrophy. Conclusions

15/17 treated patients therapy was effective, stable neurologic functions and are free of major functional disabilities
2 patients died from disease complications (had severe symptoms at time of infusion)
LAM-PCR analyses don't evidence clonal expansions no preferential integration site in protoncogenes

More longer and more wider studies has brought to EMA approval