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Therapies for Lysosomal Storage Diseases

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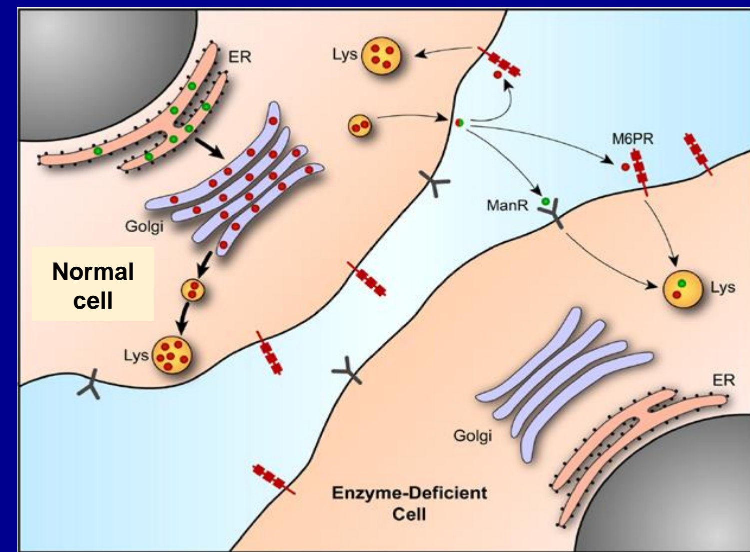
Outline

- Principle of cross correction
- ERT – Basis, historical, manufacturing, limitations, future
- HSCT – Basis, Historical, limitations, future
- SRT – Basis, limitations, future
- Chaperone therapy - Basis, limitations, future
- How do we tackle un cross-correctable diseases?
- Gene therapy – Ex vivo vs in vivo, route, different vectors, Eg of AAV intracranial, AAV9 iv, MLD, MPSIIIA
- Future - Combination therapy

The principle of cross-correction

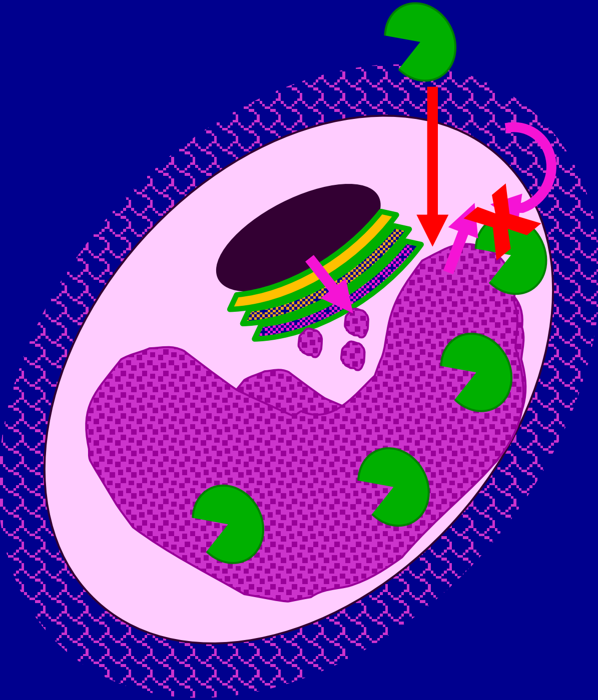


Sands 2006 Mol Ther

- Lysosomal enzymes are glycosylated in the ER
- They have a secretory signal and are further modified in the Golgi with mannose -6-phosphates at certain positions
- M-6-P receptors target most enzyme to the lysosome where it becomes active at acidic pH
- Some enzyme is secreted
- M-6-P and/or mannose receptors on the cell surface scavenge enzyme from interstitial spaces via endocytosis
- endosomes fuse with lysosomes – active enzyme

Treatments for LSDs

- Cellular substrate production and lysosomal recycling
- Enzyme Replacement Therapy
  - Enzyme delivered into the bloodstream can be taken up by affected cells and correct the disease
  - The blood brain barrier limits enzyme delivery to the brain making it ineffectual for neuronopathic diseases with little residual enzyme activity
  - Cross correction won’t work in LSDs where enzyme is not secreted



ERT Historical

- Neufeld describes cross-correction in 1968
- ERT first attempted for Pompe using enzyme from Asperigillus and later human placenta (*deBarsy '73*)
- First successful trial was in 12 Gaucher type I patients in 1991 that led to licencing of Ceredase/alglucerase – purified from human placenta (*Barton NEJM 1991*)
- Discovery that enzymatic processing of beta glucocerebrosidase exposed monosaccharides that hugely improved uptake into cells (*Furbish 1981*)
- Targeted in particular at macrophages as this is where most disease is in Gaucher type I
- Mannose tagging or exposure is very effective for MR recognition but less effective for M-6-P where M-6-P tags are more useful

ERT – Manufacturing

- Enzymes produced in mammalian cells have M6P tags
- Enzymes produced in yeast or bacteria have incorrect glycosylation that can lead to immune responses
- Artificial enzyme production is therefore usually in rodent (CHO cells) or human/primate cell lines
- Purified from media –often post processed to either expose (MR) or add residues (both) to increase uptake.
- Very expensive process
- Taliglucerase (Gaucher) produced in carrot cell lines – much cheaper but potential immunogenicity
- Enzymes for NPB, MPSVII all in trial

Enzyme Replacement Therapy

Natural Enzyme	Disease	Trade name/ enzyme	Company	EMEA approval	FDA approval	Latin America
α-L-Iduronidase	MPSI	Aldurazyme/Laronidase	Genzyme	2003†	2003	some
Iduronate-2-sulphatase	MPSII	Elaprase/ Idursulfase	Shire	2007†	2006	Yes
GALNS	MPSIVA	Elosulfase α	Biomarin	2014†Ψ	2014	Yes
Arylsulphatase B	MPSVI	Naglazyme/galsulfase	Biomarin	2006†	2005	Yes
β-glucuronidase	MPSVII	rhGUS	Ultragenyx	In trial†		
α galactosidase A	Fabry	Fabrazyme/ Agalsidase β Replagal/ Agalsidase α	Genzyme Shire	2001† 2001	2003 N/A*	Yes No
Acid α glucosidase	Pompe	Myozyme/ Alglucosidase α Lumizyme/ Alglucosidase α2	Genzyme Genzyme	2006† N/A‡	2006 2010	No No
Acid β glucosidase	Gaucher (I) ψ	Ceredase/alglucerase Cerezyme/imiglucerase Vpriv/Velaglucerase α Elelyso/taliglucerase α	Genzyme Genzyme Shire Pfizer/Protalix	1994 1997 2010 Refused	1991 1994 2010 2012	N/A Yes Yes 2012
Lysosomal acid lipase	LAL deficiency	Kanuma/sebelipase α	Alexion/ Synageva	2015	2015	No

* Not approved in US Ψ Conditional approval ‡ Lumizyme considered by FDA to be a different enzyme

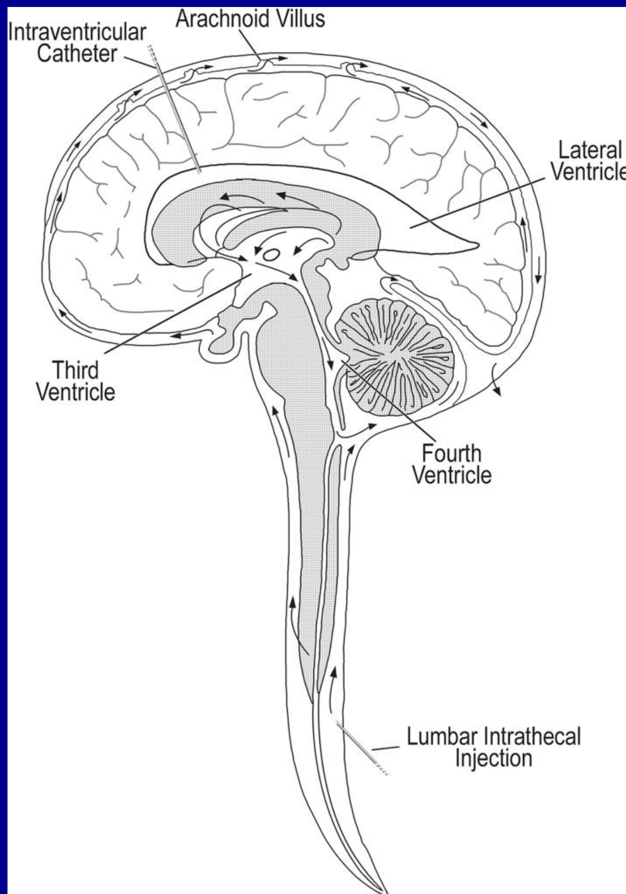
† Manchester unit was a trial centre which contributed to market authorisation of these drugs

Ψ Cerezyme also indicated for non neuronopathic Gaucher (III) in europe (2003)

What are the limitations of ERT?

- £150,000/patient/year in the UK
- Earlier treatment is better
- The blood brain barrier means that enzyme and many drugs can't pass from the bloodstream into the brain – this is where they are mainly needed in neuronopathic LSDs
- The joints and growth plate of the bone are poorly connected to the bloodstream –creating a barrier for MPSI, II, IV, VI and VII diseases.
- MR vs M6P uptake is very rapid – ½ life minutes vs hours
- Functional antibody responses can limit efficacy – *Patel 2012 MGM 106 301-9*– Pompe, *Saif 2012 Hematologica 97:1320-8* – MPS I

ERT - physically bypassing the BBB



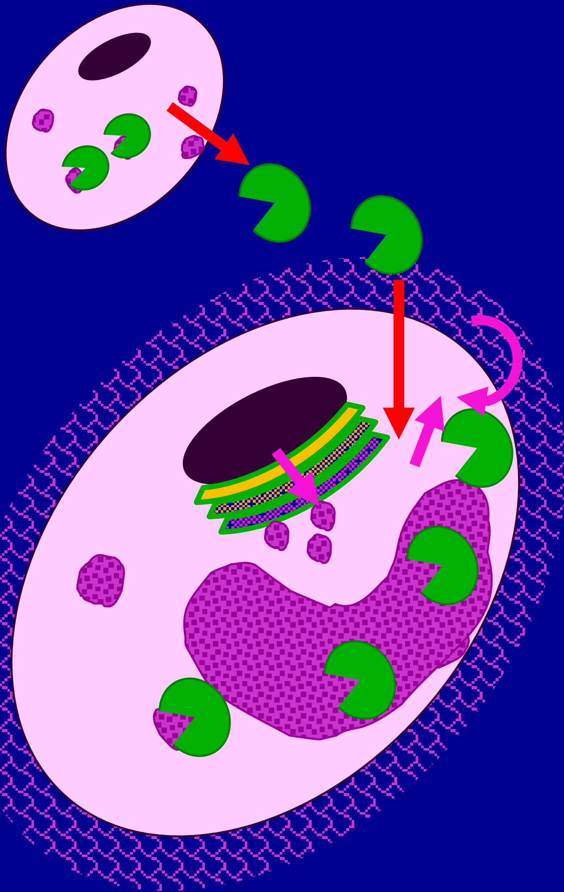
- ICV vs lumbar port delivery
- Enzyme delivery lumbar port – trials in MPSII and IIIA – Shire
- MPSIIIA discontinued due to no change in efficacy – despite detection of enzyme in CSF
- Cerliponase alfa (TPP1) (Brineura) ICV catheter - CLN2 – FDA/EMA approved 2017 – Biomarin (biweekly infusion)

Cohen-Pfeffer Ped Neurol 2017 67:23-35

ERT – Future

- Novel enzyme or substrate reduction therapies aim to circumvent these barriers
  - Either bypass barrier by physical injection/intervention
    - Enzyme delivery lumbar port –trials in MPSII and IIIA (latter dropped) Shire
    - Cerliponase alfa (TPP1) (Brineura) ICV catheter - CLN2 – FDA/EMA approved 2017 - Biomarin
  - In some cases over-production of enzyme or drug may improve delivery
    - Improved circulation time Rowan/Sly MGM 2012 MPSVII
  - Modify enzymes to improve receptor uptake/so they can cross the BBB or bones
    - Fusion to IGF2 for increased M6P uptake – phase II Pompe Biomarin
    - Combined IGF2/ICV delivery BMN250 phase I/II MPSIIIB Biomarin
    - Fusion to proteins transported across BBB – Insulin receptor antibody fusions – armagen MPSI and II in trial
    - Modified carbohydrate structure to "enhance" muscle uptake ATB200 pompe – amicus (co-delivered with a chaperone AT2221)
  - Tolerisation regimens to limit antibody responses
    - Either limit immunogenicity of enzyme or induce tolerance via drugs

Treatments for LSDs



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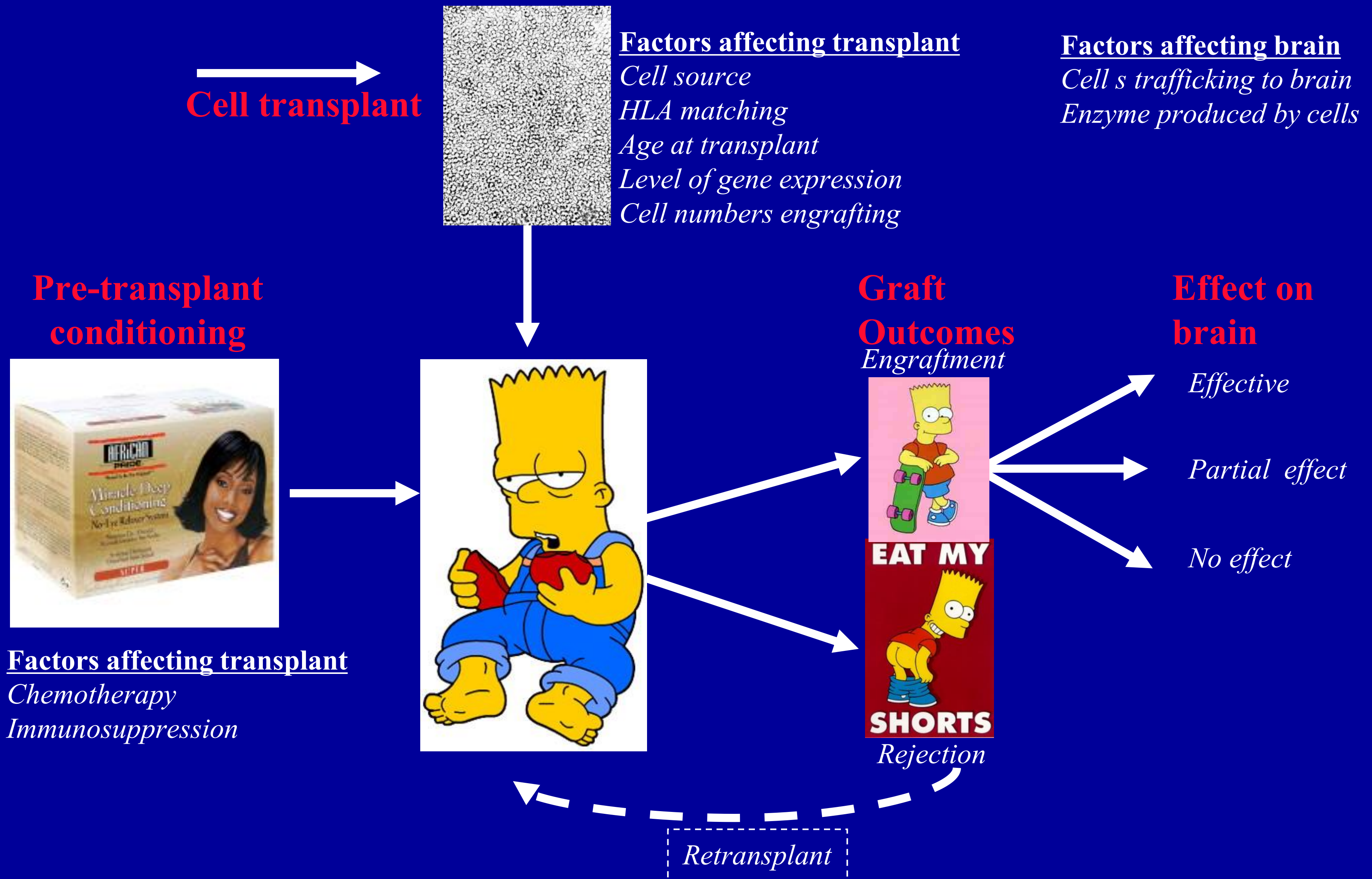
- Hematopoietic Stem Cell Transplant
  - Delivery of enzyme from blood cells
  - Monocytes traffic to the brain and release enzyme
  - MPSIH, alpha mannosidosis, Niemann pick CII

Haematopoietic Stem Cell Transplantation (HSCT) for neurological diseases



Haematopoietic stem cell therapy

❖ In HSCT donor cells repopulate the blood system and release enzyme which cross-corrects affected cells

❖ Blood cells traffic into the brain becoming microglial cells and secrete enzyme, cross-correcting neuronal cells

❖ HSCT has transformed the management of diseases like MPSIH - much more effective than ERT in these diseases

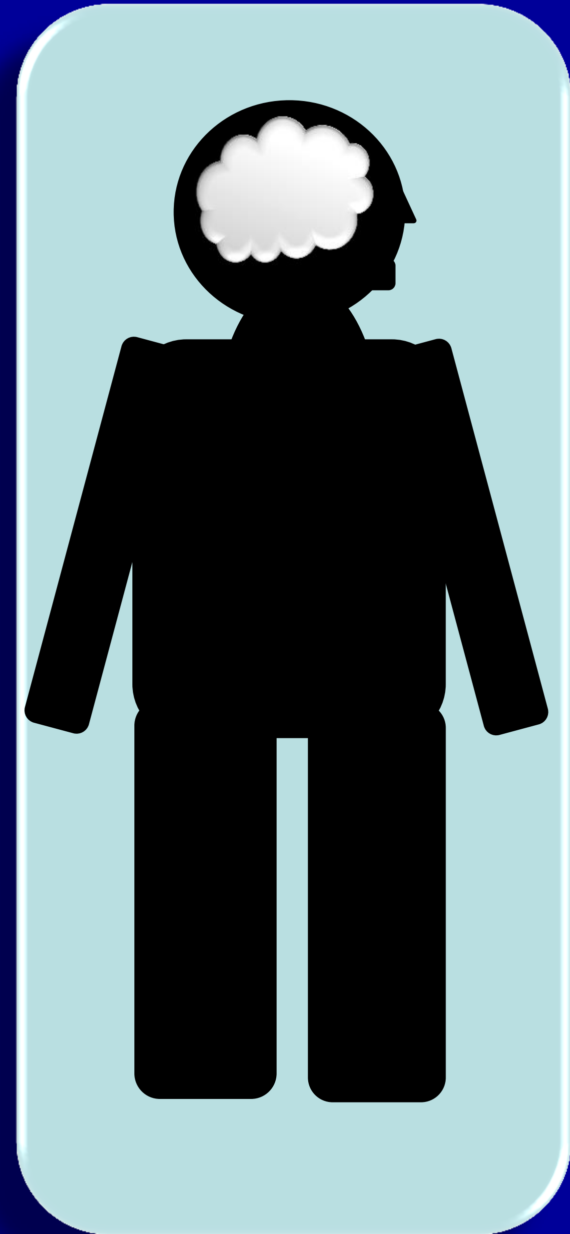
Limitations

❖Few LSDs indicated for standard HSCT therapy – MPSIH, MPSVII, alpha mannosidosis, Krabbe (presymptomatic), GLD (late onset), Wolman

❖Early intervention is critical

❖Some risk of morbidity/mortality – now generally <10% - this makes it optional in MPSIH/S, MPSVI, Fucosidosis, Farber, Gaucher (non neuronopathic & norbottnian), NPC

❖ Insufficient brain enzyme produced in some diseases – MPSIIIA and IIIB – Sanfilippo disease



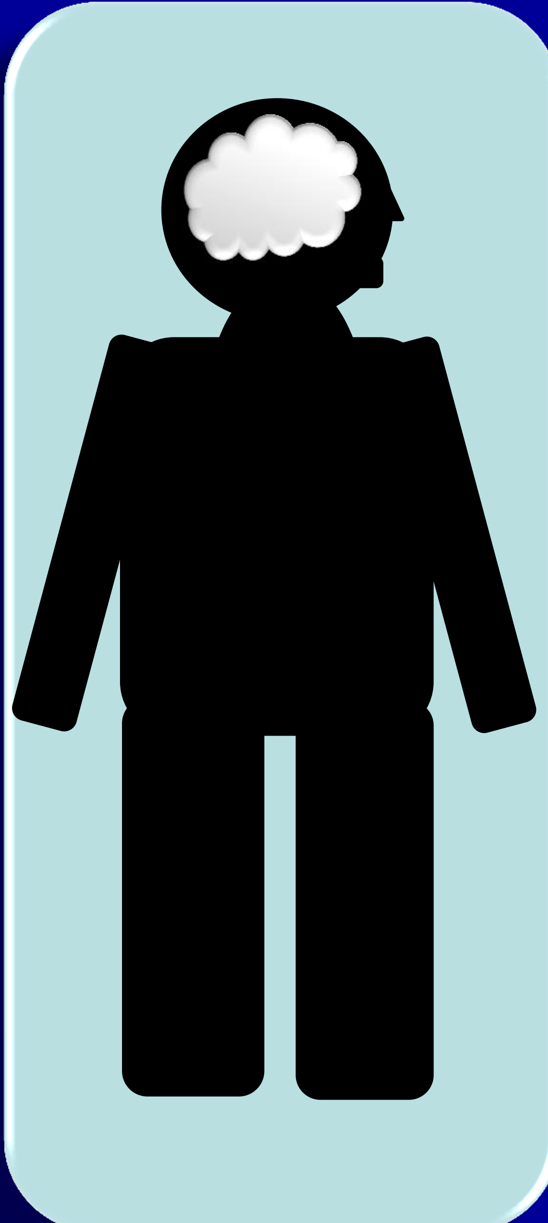
HSCT Historical

- First bone marrow transplants in 1959
- First metabolic transplant described by Hobbs 1981 for MPSIHurler
- 2007 Discovery that RIC is a risk factor for engraftment in MPSI
- Post 2007 survival mostly >90%

What Lysosomal diseases are treatable by HSCT?

Table 1: Guideline for indications (Peters et al. (2); Boelens et al. (3))

Disease	Indication
Mucopolysaccharidoses (MPS)	
MPS I	
- Hurler	Yes
- Hurler-Scheie	No **
MPS VI; Maroteaux-Lamy	
- Severe phenotypes	No **
MPS VII; Sly	Yes
Other MPS	No
Leukodystrophy	
X-linked adrenoleukodystrophy	
- Cerebral	Yes
Metachromatic leukodystrophy	
- Juvenile subtype	In development***
- "Late subtype"	Yes
Globoid leukodystrophy	
- Early infantile subtype (Krabbe's disease)	Yes
- Late onset type	Yes
Other inborn errors of metabolism	
Fucosidosis	In development***
α-mannosidosis	Yes
Aspartylglucosaminuria	In development***
Farber's lipogranulomatosis	In development***
Gangliosidosis	
- GM1	In development***
- GM2	No
Gaucher	
- Type I	No **
- Type III	No/ Yes *
Mucolipidosis I	In development***
Neuronal ceroid lipofuscinosis (NCL)	
- NCL 1	No
- NCL 2	No
Niemann-Pick	
- Type B	Yes
- Type A and C	In development***
Osteopetrosis Exclude neuronopathic osteopetrosis (e.g. in OSTM1) and carbonic anhydrase type II deficiency. Be cautious in case of mild or transient phenotype: discuss with experts	
- Malignant infantile subtype	Yes *
- Wolmans disease	Yes
Adenosine-deaminase-deficiency	Yes
Purine-nucleoside-phosphatase-deficiency	Yes
Mevalonic aciduria	In development***



Boelens 2008 EBMT Transplantation Handbook 41: 544-53

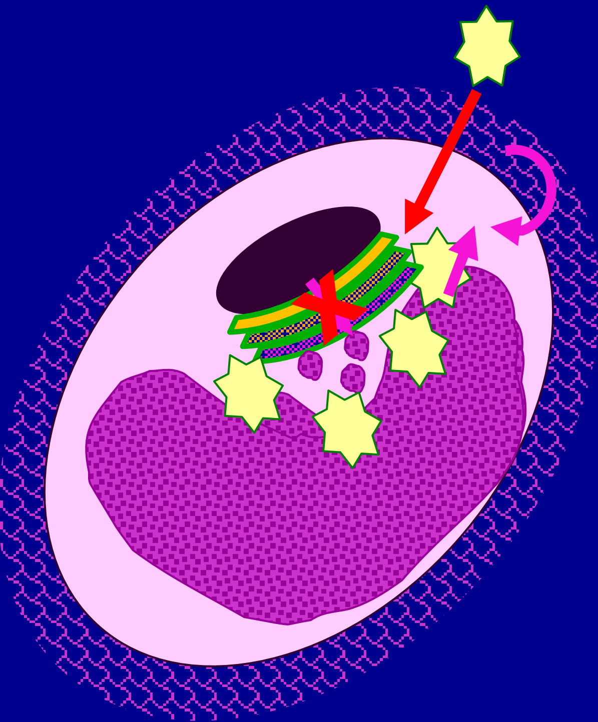
Two classes of inherited diseases that cannot easily be treated

1. Diseases where diffusable protein can complement cells Distribution may be an issue – ie LSDs
   - Eg MPS IIIA/B, MLD, Krabbe (Toxic substrate)
   - Distribution of protein across BBB or to avascular sites

2. Diseases where defect is a membrane protein, cannot traffic to its site of action, or into the correct cells
   - Batten disease, OTC deficiency, Tyrosinemia type1, MPSIIIC
   - Requires tissue specific cellular replacement (stem or progenitor cells), fusion of BM derived cells with selection, gene therapy
   - Most genetic diseases…

Huge cost burden of palliative care

Treatments for LSDs



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- Hematopoietic Stem Cell Transplant
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- Substrate Reduction Therapy
  - Reduction of primary storage material or rerouting degradation down alternate pathways
  - Miglustat/Zavesca - Gaucher/Niemann Pick C

Substrate reduction therapy

- Substrate reduction can be achieved by reducing production of undegraded substrate OR rerouting degradation down alternative pathways
- Candidate drugs must be able to reduce substrate without causing toxicity to the patient
- The more selective the drug – the less likely it is to have major side effects
- The drug must also be able to reach all affected cells – including those in the brain
- Oral delivery is a big advantage over weekly/monthly enzyme
- Not likely to raise antibody responses

Miglustat/Zavesca

- Iminosugar inhibiting glucosylceramide synthase
- Blocks first step in glycosphingolipid production
- Developed as a treatment for Gaucher disease type I- reduces production of glycosphingolipids (substrates stored in Gaucher) (*Cox Lancet 2000*)
- Now clinically approved and can help to stabilise disease and slow disease progression
- Also able to stabilise disease in patients formerly on ERT
- Approved in Niemann Pick C patients with secondary storage of GSLs (Europe)
- Ongoing clinical trial suggesting improvements in peripheral and brain disease – disease stabilisation
- Trialed in MPSIII patients – shown to have no benefit (*Guffon J peds 2011*)

Genistein

- Tyrosine kinase inhibitor, weak oestrogenic compound
- Genistein can be purified from soya beans as a food supplement OR synthesised in its pure aglycone form
- It blocks GAG production in patient cells in culture from all MPS types tested so far - *Piotrowska 2006 Eur J Hum Genet*
- Nontoxic, can be taken orally, *- McClain 2005 Food and Chem Toxicol*
- 10% crosses blood brain barrier - *Tsai 2005 J Chromat A*

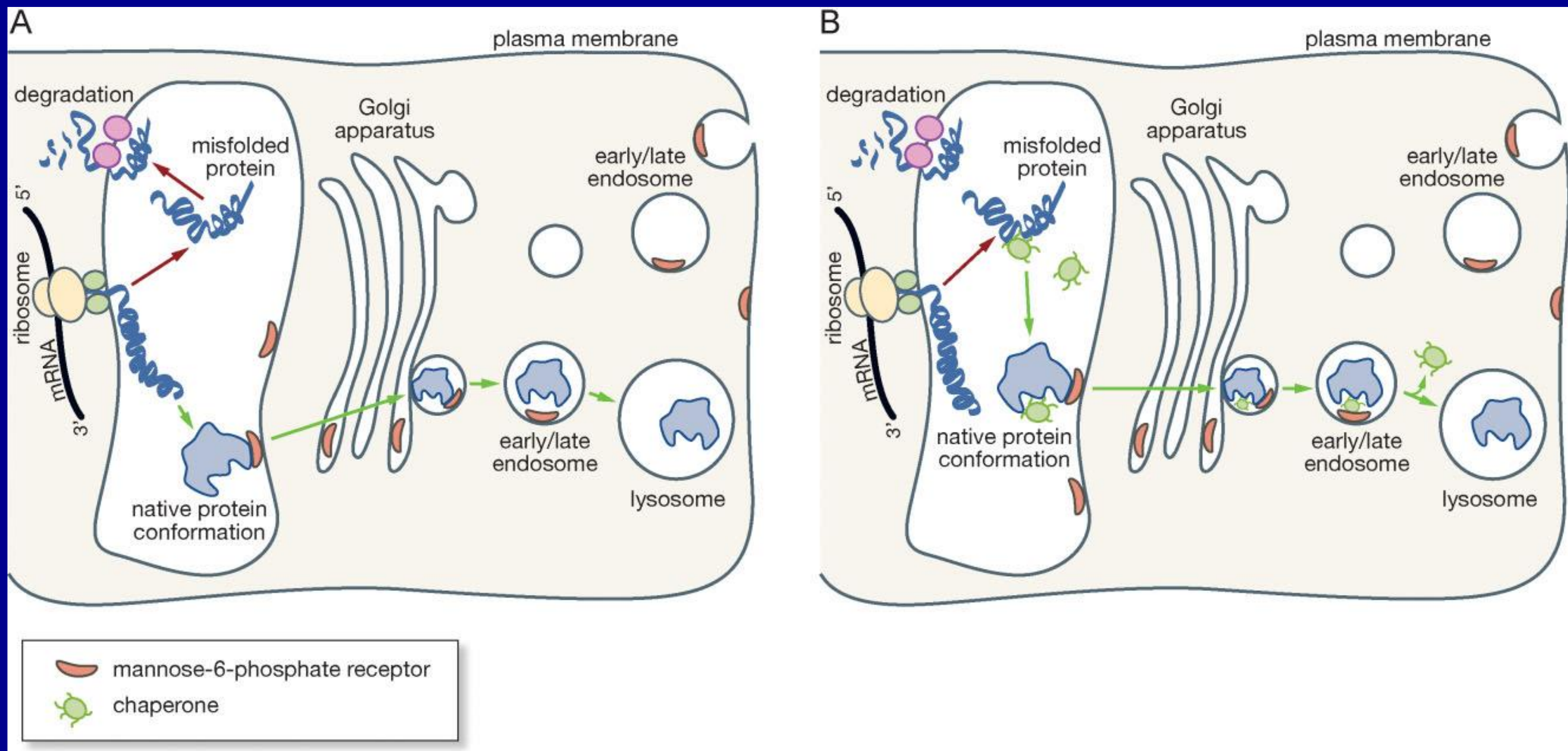
- Long-term evaluation of high dose (160mg/kg/day) genistein aglycone in MPSIIIB mice shows ability to reduce brain GAGs by 35%, neuroinflammation by 15% and correct abnormal behaviour *Malinowska 2010 PLoSOne*

- Several low dose (10mg/kg/day) trials run (*De Ru 2012 Annal Neurol*) best case showed urine GAG reduction but no behavioural effect
- Increased glucuronidation in humans leads to lower plasma levels of active compound
- 160 mg/kg/day phase III investigator led trial started in Aug 2014 in Manchester in patients with MPSIIIA, B and C

SRT – limitations

- Reduction of substrate production can never cure disease
- Primary role to delay symptom onset
- Low drug toxicity and BBB permeability vital
- Surprisingly – may prove to be synergistic with enzyme or gene therapy approach

Chaperone therapy



- The enzyme missing in any LSD can be due to a number of different kinds of mutations in the DNA of the gene
- Some mutations result in a misfolded protein and the cell degrades it
- Chaperones are molecules that bind to and help proteins to fold correctly – some pharmacological agents can perform this function
- Oral administration and ability to cross the BBB are big advantages over enzyme

From: Parenti 2009 EMBO Mol Med 1, 268-279

SRT and Chaperones

Natural Enzyme	Disease	Trade name	Company	EMEA approval	FDA approval
Acid β glucosidase	Gaucher (I)	Miglustat/ Zavesca (NB-DNJ) SRT/chaperone	Actelion	2002	2003
Acid β glucosidase	Gaucher (I) Subset	Cerdelga/ Eliglustat (SRT)	Genzyme	2015Ψ	2014
Cholesterol transporter protein NPC-1	Niemann Pick C-1 (and 2)	Miglustat/ Zavesca (SRT/chap)	Actelion	2009†	N/A
α galactosidase A	Fabry	Galafold/ Migalastat (NB-DGJ) chaperone	Amicus	2016	Filed*
SGSH, NAGLU, HGSNAT	MPSIIIA,B,C	Genistein (SRT)	None	In trial 2014†	

Imino sugars such as NB-DNJ – Miglustat and 1-deoxynojirimycin (NB-DGJ) can function as chaperones –(NB-DNJ also functions as an SRT agent)

* Not approved in US – more data wanted by FDA

Ψ Conditional approval

† Manchester unit - clinical trial centre for drug indication

Limitations of chaperone therapy

- It is a therapy that will only work on a subset of patients with protein misfolding mutations
- But… you have two gene copies per cell – usually with different mutations - so there is more chance
- Patients with gene mutations that do not cause misfolding will not benefit
- Less attractive to pharmaceutical companies because of limited market

Gene Therapy

- Gene addition– non-viral and viral vectors
- Gene repair – CRISPR/Cas9, ZFNs, homologous recombination
- Gene inhibition – siRNA, miRNA
- Cell killing – cancer strategies – often similar to gene addition

Gene addition/augmentation

- Most widely used approach
- Remove viral genes and package RNA/DNA therapeutic gene and promoter in their place
- Gene expression can be episomal from a plasmid – usually transient, or more stably from a viral vector.
  - Transcribed and translated in the cytoplasm/ER
  - Adeno associated viral vectors
- Alternatively by random or directed integration into the host's genome
  - Transcribed in the nucleus, translated in the cytoplasm/ER
  - Retro/lentiviral vectors

Routes of delivery

Direct delivery

- Intravenous, intracranial, intraventricular, intraocular
- Targeting specificity often achieved by delivery to site of interest
- AAV vectors are main choice due to high titres
- Limitations
  - Immunogenicity, preexisting immunity, scale-up

Ex vivo

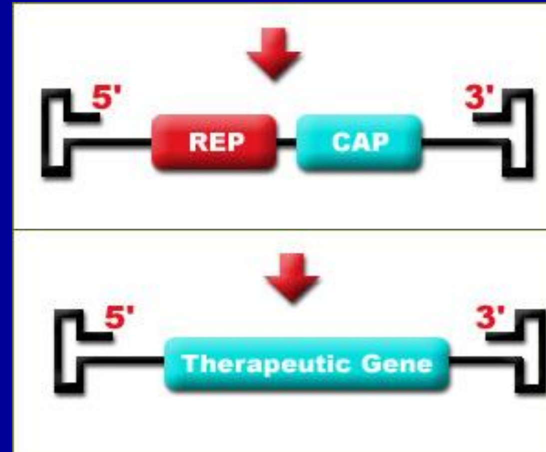
- Transduce cells outside of the body and reintroduce them – typically stem cells (HSCs best example) - Lentiviral vectors
- No direct vector exposure so less immunogenic
- Purified stem cells provide unlimited self-renewal capability
- Limitations
  - Cells normally require a space to re-engraft - hence damage to target organs to achieve engraftment

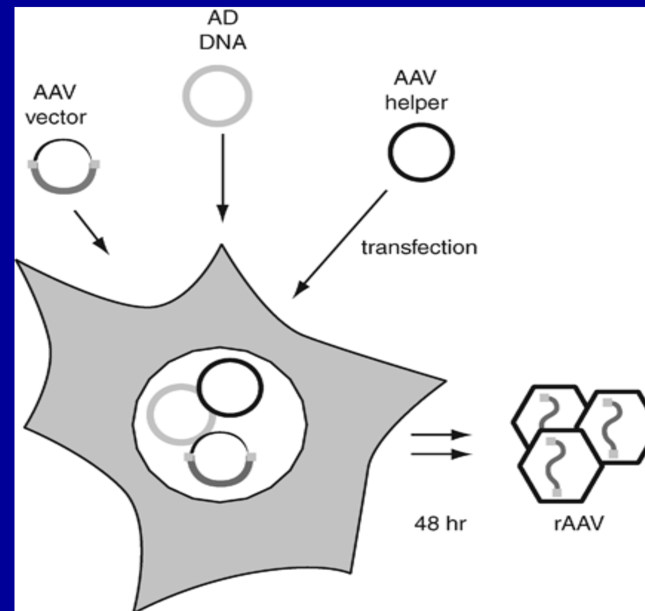
Gene Therapy Vectors compared

Features	Adenovirus	Retro/lentivirus (LV)	Adeno Associated Virus (AAV)	CRISPR/Cas9 Gene editing
Maximum insert size	10 – 30 kb *	7 – 7.5 kb	3.5 – 4.5 kb	Few bp via guide RNA – OR deletion
Concentration (pfu ml^{-1})	$>10^{14}$	$>10^{9}$	$>10^{14}$	N/A – sometimes delivered via viral vector
Integration	Very low frequency	Yes	Occasional	Yes
Duration of expression	Short	Long/permanent	Long	Long/permanent
Advantages	Very efficient infection Well characterised	Long- term expression Lentis infect non-dividing cells very efficiently	Small genome Low toxicity High titres	Corrects gene in situ – appropriate regulation
Disadvantages	Inflammatory response Toxicity Likely to have preexisting host immunity * - gutless vectors	Insertional mutagenesis (low risk from LVs) Small packaging size No infection into non dividing cells (except for LVs)	Insertional Mutagenesis (rare) Small packaging size Inflammatory response	Off-target editing common Poor ability to edit stem cells – ie ex vivo Poor in vivo capabilities

Adeno-associated viral vectors

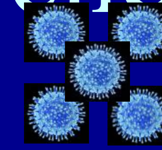
- ssDNA vectors
- More than 10 serotypes with infectious profile for different tissues
- Simple gene structure
- Rep, Cap ITRs
- Small packaging capacity max 4.5 kb
- Mostly episomal / occasional integrations– all long term expressors
- Great for immune privileged sites like the retina
- Some serotypes are good for liver, muscle or brain transduction – long lived expression



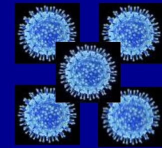


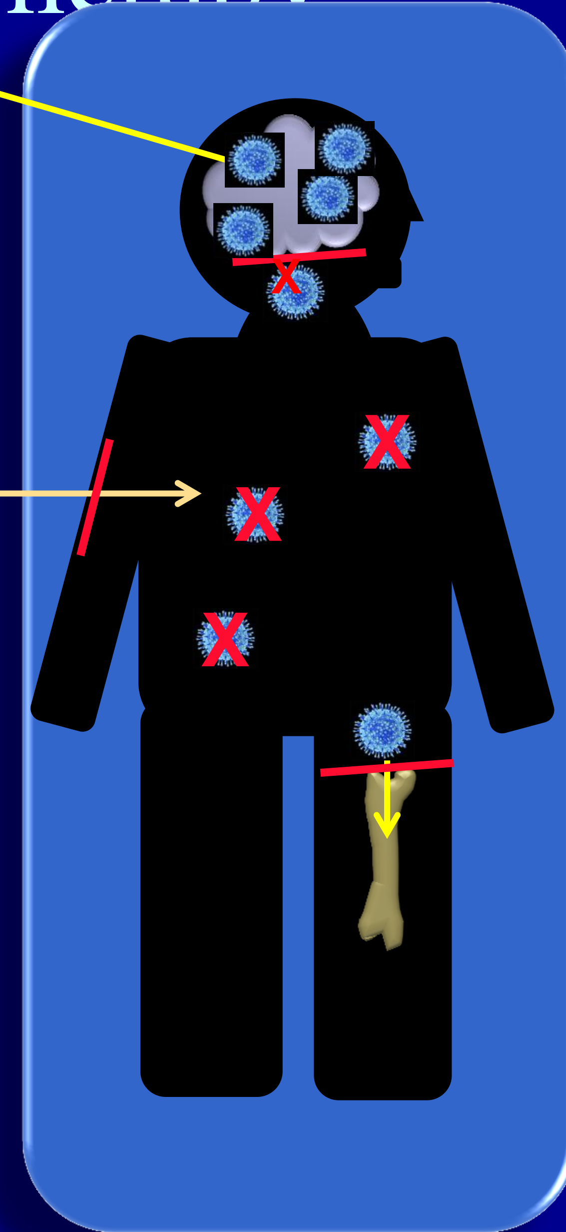
AAV mediated Gene Therapy

- Direct injection of an AAV gene therapy vector to overexpress a missing gene
- IV – AAV9 can cross the BBB - many AAVs are eliminated by the immune system – high doses required
- Brain - targeted intraparenchymal, intrathecal, intraventricular injections – usually multiple
- Serotype 9 and Rh10 are common for brain



- Pros
- In targeted cells - very high gene expression
- Long-term correction
- Potential to be transformative
- Immediate effect





- Cons
- Difficult to distribute vector widely - even ventricular
- Scale-up problem for both IV (high dose) and brain delivery (low volume)
- Immune reactions require immune suppression
- Pre-existing antibodies in some, mean stratification of patients beyond LSD subtype
- Potential for long-term drop-off in expression
- Cost could be very high for one off treatment

AAV Gene Therapy in haemophilia B

- AAV8 (sc) IV delivery to 10 patients with severe Factor IX deficiency (<1%)
- Highest dose – vector immune responses controlled by glucocorticoids
- 18-50 months later steady levels of 1-6% of normal were achieved
- Reduced Factor IX use and bleeding episodes

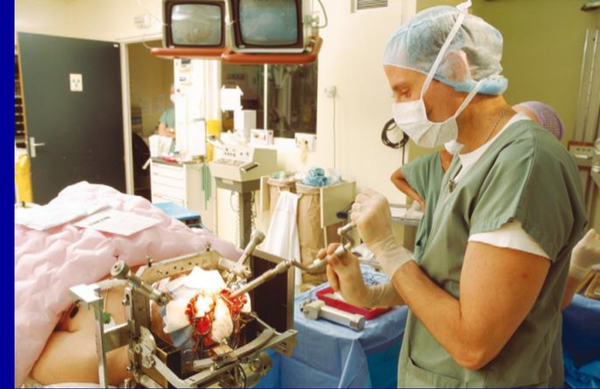
Sebastian Misztal -It's been amazing. I've had no side effects and I don't have to inject myself twice a week, which was not pleasant.

Nathwani 2011 NEJM 365: 2357-65

Nathwani 2014 NEJM 371: 1994-2004

- Glybera - first licenced gene therapy – is an AAV1 for LPLD

AAV Gene Therapy in neurodegenerative diseases

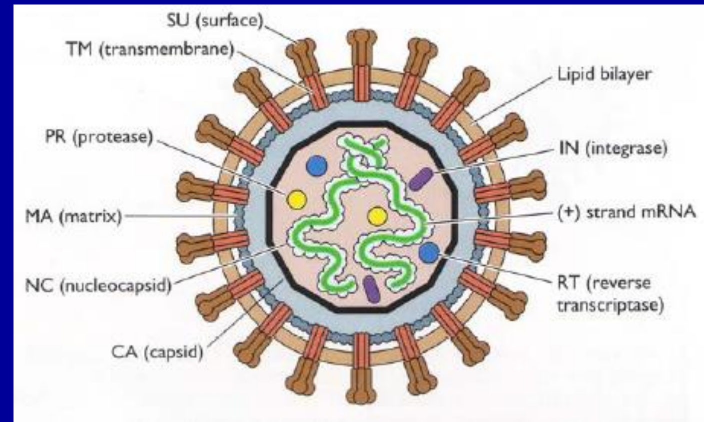


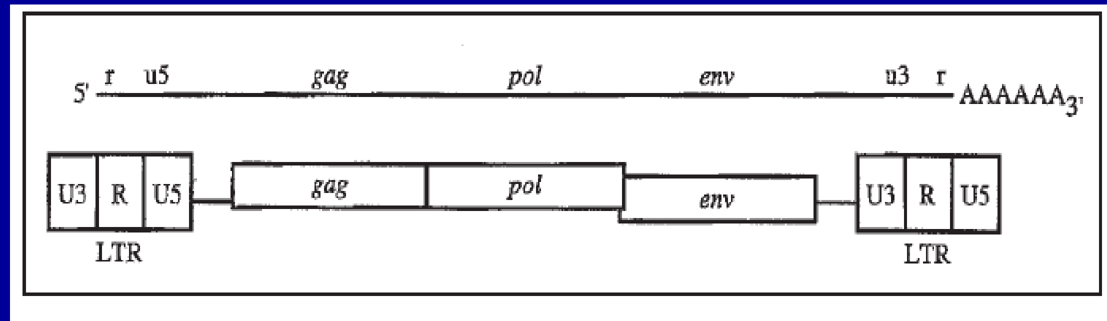
- Sanfilippo disease IIIA/IIIB
  - Direct brain injection of AAV 2/rh10 SGSH at 12 sites
  - Possible stabilisation of disease – Tardieu Hum GT 2014 25: 506
  - Direct brain injection of AAV 2/5 NAGLU at 16 sites
  - Biochemical/neurological improvement ESGCT – Tardieu 2015
- Batten disease
  - Direct brain injection of AAV 2 CLN2 at 6 sites
  - Stabilisation of disease progression in some patients
- Parkinson disease
  - Direct injection of AAV GAD – double blinded (45 patients)
  - 23% improvement in treated vs 12% in untreated
- Intracranial injection has limited volume and spread
  - Intraventricular, cisterna magna or intrathecal (CSF fluid filled spaces in brain and spinal cord) may be more effective
  - Solution: Image guided convection enhanced delivery in sheep- better scale-up

Worgall 2008 Hum GT 19:463-74 LeWitt 2011 Lancet Neurol 10:309-19, Tardieu 2014 Hum Mol Ther 25:506

Retroviral/lentiviral Vectors

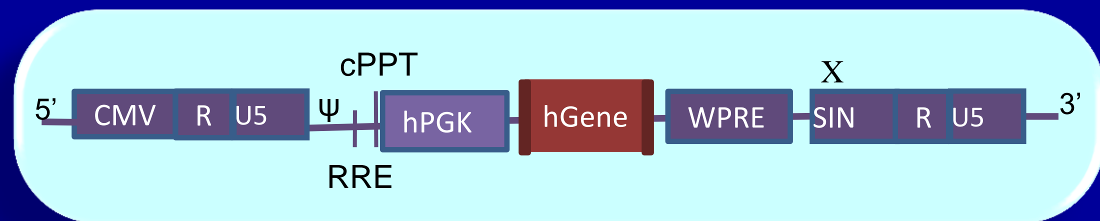
- RNA viral genome
- Reverse transcription and random integration – LTRs
- Lentiviruses can infect stem cells
- Viral envelope gives specificity – eg HIV-1 to CD4+ cells





- Making a vector
- Delete viral genes
  - gag, pol, env
- Insert therapeutic gene
- SIN vectors - replace U3
- promoter with CMV
- Internal mammalian promoter

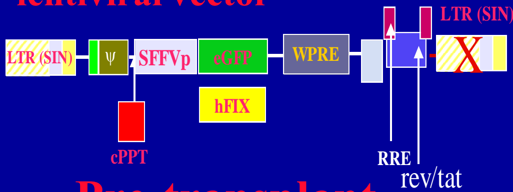
Ψ

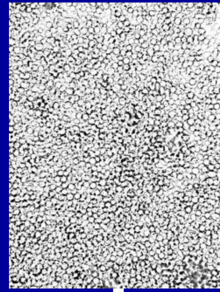


Retro/lentiviral mediated *ex vivo* HSC gene therapy

Transfection with Retro or lentiviral vector

Cell transplant





Factors affecting transplant
Cell source
Cell numbers engrafting
Age at transplant
Number of transduced cells
Level of gene expression- vector
Genotoxicity

Factors affecting Brain
Cell trafficking
Enzyme secretion by microglia

Autologous CD34 Cell Apheresis

Pre-transplant conditioning

Factors affecting transplant
Chemotherapy
Immunosuppression

Graft Outcomes

Engraftment

Rejection

Effect on disease
Effective
Partial effect
No effect

Autologous rescue

Haematopoietic Stem Cell Gene Therapy Clinical Trials for Neurological Diseases

- Retroviral vector transduction pre-2000 was relatively inefficient due to inability to transduce CD34+ HSCs
- Improved cytokine mixes led to retroviral success in X-SCID and WAS
- Without GT most would be dead
- Pre SIN vectors 99 patients 12 leukemias, 2 deaths
- Post SIN vectors 35 patients, no leuks, no deaths

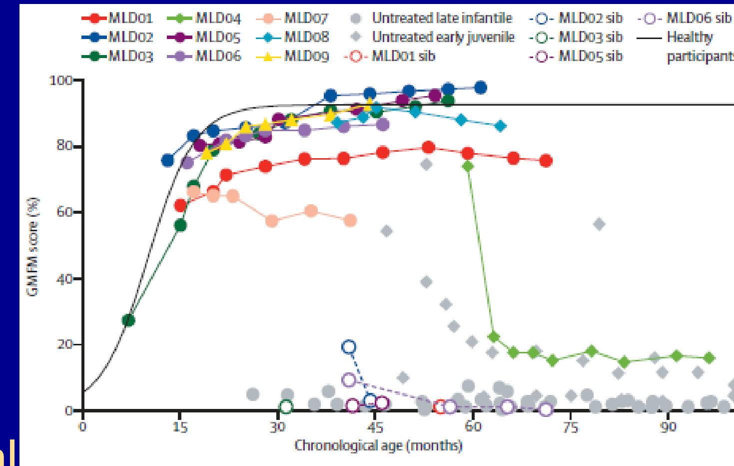
Table 1. Overview of Hematopoietic Stem Cell Gene Therapy Clinical Trials by Vector.

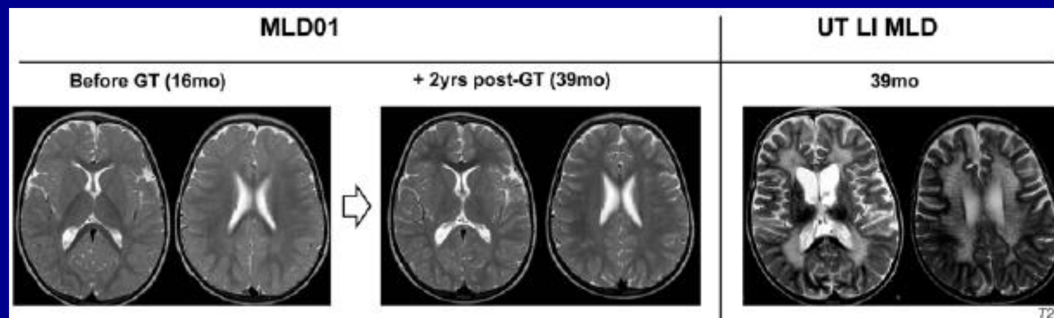
Disease	Vector	Patients	Locations	Outcomes	Vector Related SAEs	References
Pre-2000	LTR γ-retrovirus	Several trials		Insufficient HSC transduction	None reported	Blaese *et al.*, 1995; Malech *et al.*, 1997
ADA-SCID	LTR γ-retrovirus	42 on 3 trials	Italy, UK, USA	29 off ERT	None reported	Aiuti *et al.*, 2009; Candotti *et al.*, 2012; Gaspar *et al.*, 2011b
ADA-SCID	SIN Lentivirus	7 on 1 trial	UK, USA	<1 year follow-up	None reported	Mukherjee and Thrasher, 2013; Gaspar Pers. Comm.
X-SCID	LTR γ-retrovirus	24 on 3 trials	France, UK, USA	Significant clinical benefit to young patients (17/19); older patients did not see benefit (0/5)	5 developed T-ALL, 1 died	Gaspar *et al.*, 2011a; Hacein-Bey-Abina *et al.*, 2010
X-SCID	SIN γ-retrovirus	8 on 1 trial	UK, USA	T Cell recovery (Preliminary)		Mukherjee and Thrasher, 2013
CGD	LTR γ-retrovirus	12 on 5 trials	USA, Germany, Switzerland, UK, Korea	Transient benefit in most, 3 with high engraftment mediated by transformation	3 developed MDS, 1 died	Bianchi *et al.*, 2009; Grez *et al.*, 2011; Kang *et al.*, 2010; Ott *et al.*, 2006
CGD	SIN Lentivirus	1 on 1 trial	Switzerland, Germany, France, UK	<1 year follow-up		Mukherjee and Thrasher, 2013
WAS	LTR γ-retrovirus	10 on 1 trial	Germany	Long-term correction	4 developed T-ALL	Boztug *et al.*, 2010
WAS	SIN Lentivirus	5 on 1 trial	UK, USA, France, Italy	Multilineage correction in 3 patients reported to date	None reported	Aiuti *et al.*, 2013; Mukherjee and Thrasher, 2013
β thalassemia	SIN Lentivirus	1 on 1 trial	France	Transfusion independent	None reported	Cavazzana-Calvo *et al.*, 2010
X-ALD	SIN Lentivirus	4 on 1 trial	France	Stabilisation of neurological disease in 2 patients reported to date	None reported	Cartier *et al.*, 2009
MLD	SIN Lentivirus	9 on 1 trial	Italy	Significant neurological benefit in 3 patients reported to date	None reported	Biffi *et al.*, 2013; Biffi Pers. Comm.

Bigger 2014 Disc Med 17: 207

Lentiviral stem cell gene therapy in MLD patients

- Autologous BM HSC transduced with ARSA expressing LV
  - 3 pre-symptomatic Late Infantile patients (7-16mo old)
  - 18-24 months post Tx 45-80% transduced cells
  - Polyclonal integration - no clonal dominance
  - ARSA activity >normal in PBMCs, 1-2 fold CSF
  - Gross motor function increased to almost normal
  - MRI – no progression (unlike untreated LI MLD)

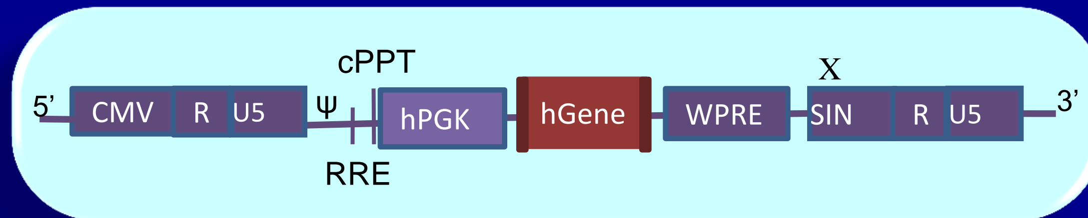




  - All had IQs within normal range 80-100 – normally <40 in MLD patients

Biffi – Science 2013, Lancet 2016

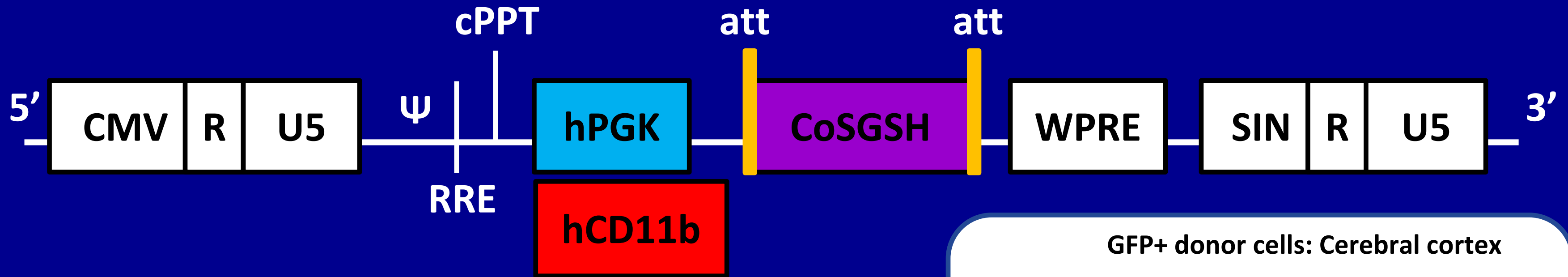
How do we improve?: The right amount of enzyme in the right place

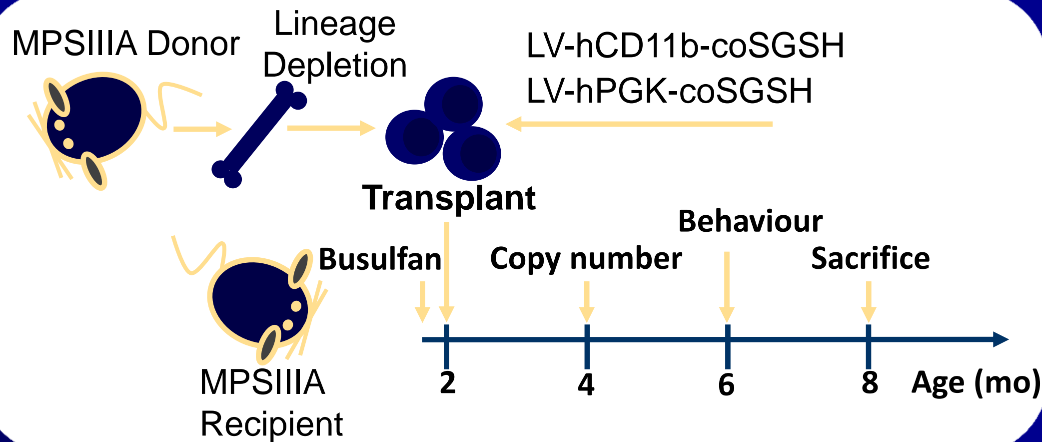


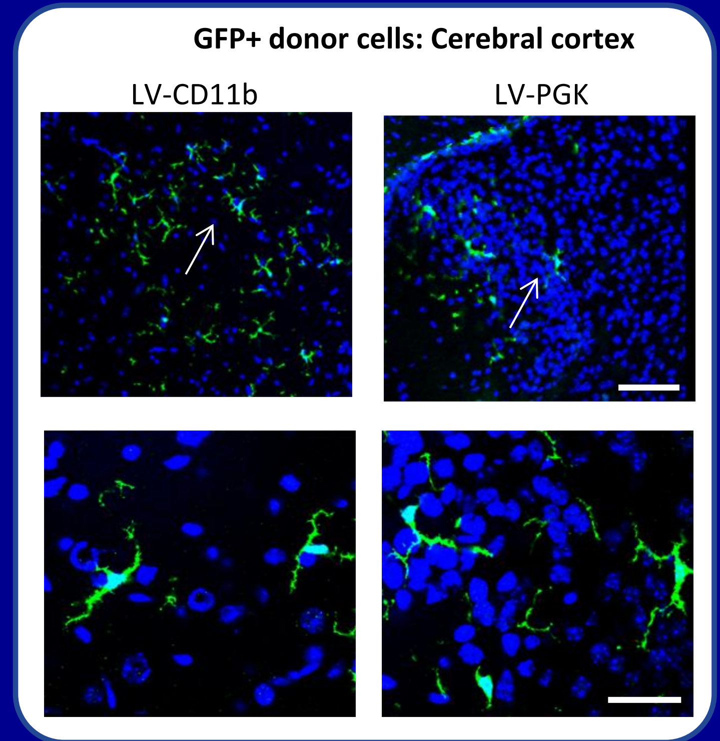
- pCCL ubiquitous vector used for MLD trial
- but…
- Krabbe disease demonstrates toxicity of overexpressed GALC in HSCs
  - *Visigalli 2010 Blood*

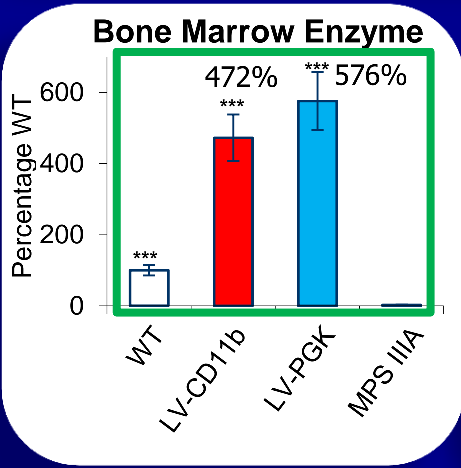
- Targeting enzyme to the right cells improves safety/efficacy
  - miRNA restriction to non-HSC lineages *Gentner 2010 Sci Trans Med*
- Myeloid specific expression for monocytes/microglia in the brain *Sergijenko Mol Ther in press*

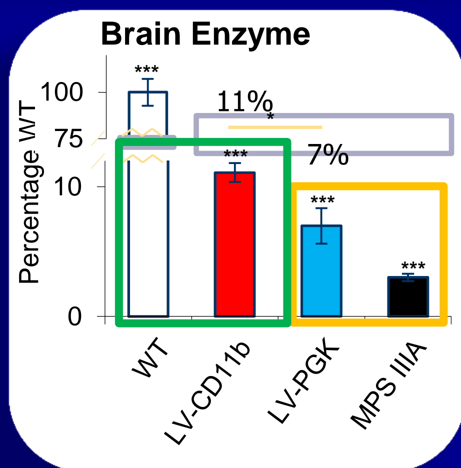
Monocyte specific (CD11b) LV-HSC Gene Therapy in MPSIIIA









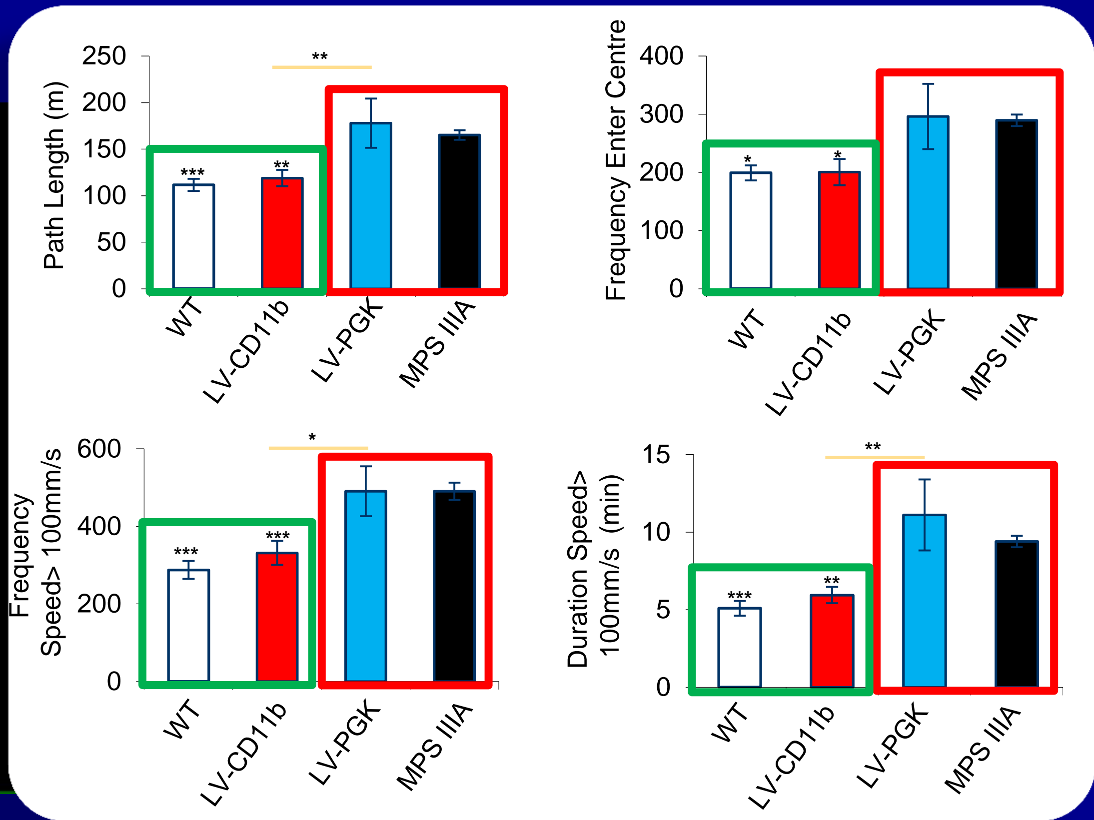


LV-CD11b increases brain enzyme specificity over LV-PGK

Monocyte trafficking to brain and engraftment as microglia is effective regardless of promoter

Sergijenko Mol Ther Jun 7 E pub

Monocyte LV-HSC Gene Therapy corrects hyperactivity



MPS IIIA mice, like the patients are hyperactive.

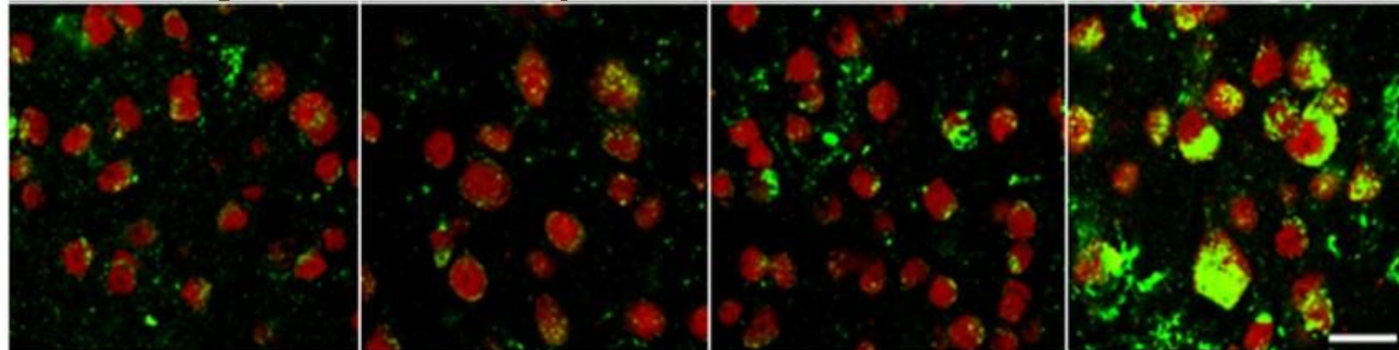
LV-CD11b corrects hyperactive behaviour

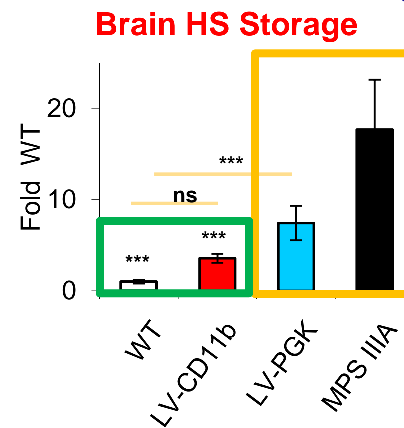
LV-PGK has no significant effect

Sergijenko Mol Ther Jun 7 E pub

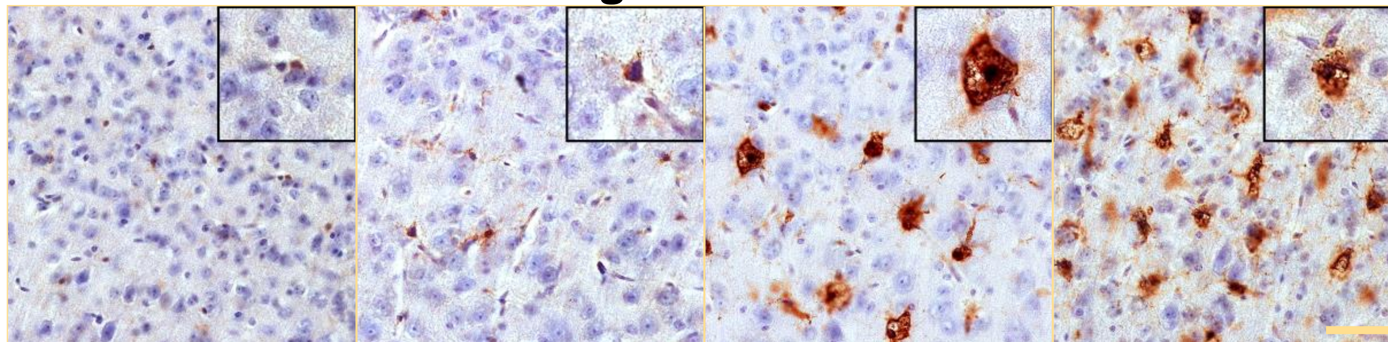
Monocyte LV-HSC GT normalises HS & neuroinflammation

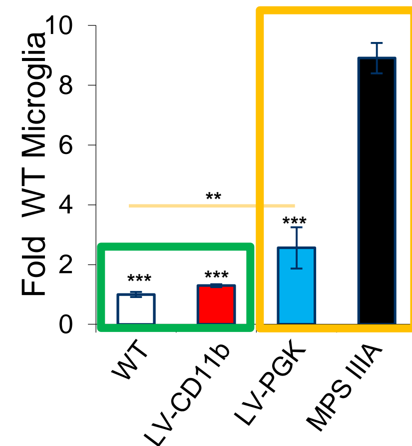
LAMP2: Lysosomal Compartment





Isolectin B4: Cortex Microglia





LV-PGK is still significantly elevated over WT

LV-CD11b normalises storage and neuroinflammation

Phase I/II clinical trial planned for 2015

Sergijenko Mol Ther Jun 7 E pub

Treatment model for LV-HSC Gene Therapy in MPS IIIA

- Full scale transduction optimisation - frozen product optimal
- Programme and GMP vector licenced to Orchard Therapeutics in April 2016
- Clinical trial planned in Manchester – CI: Rob Wynn, CoI: Simon Jones

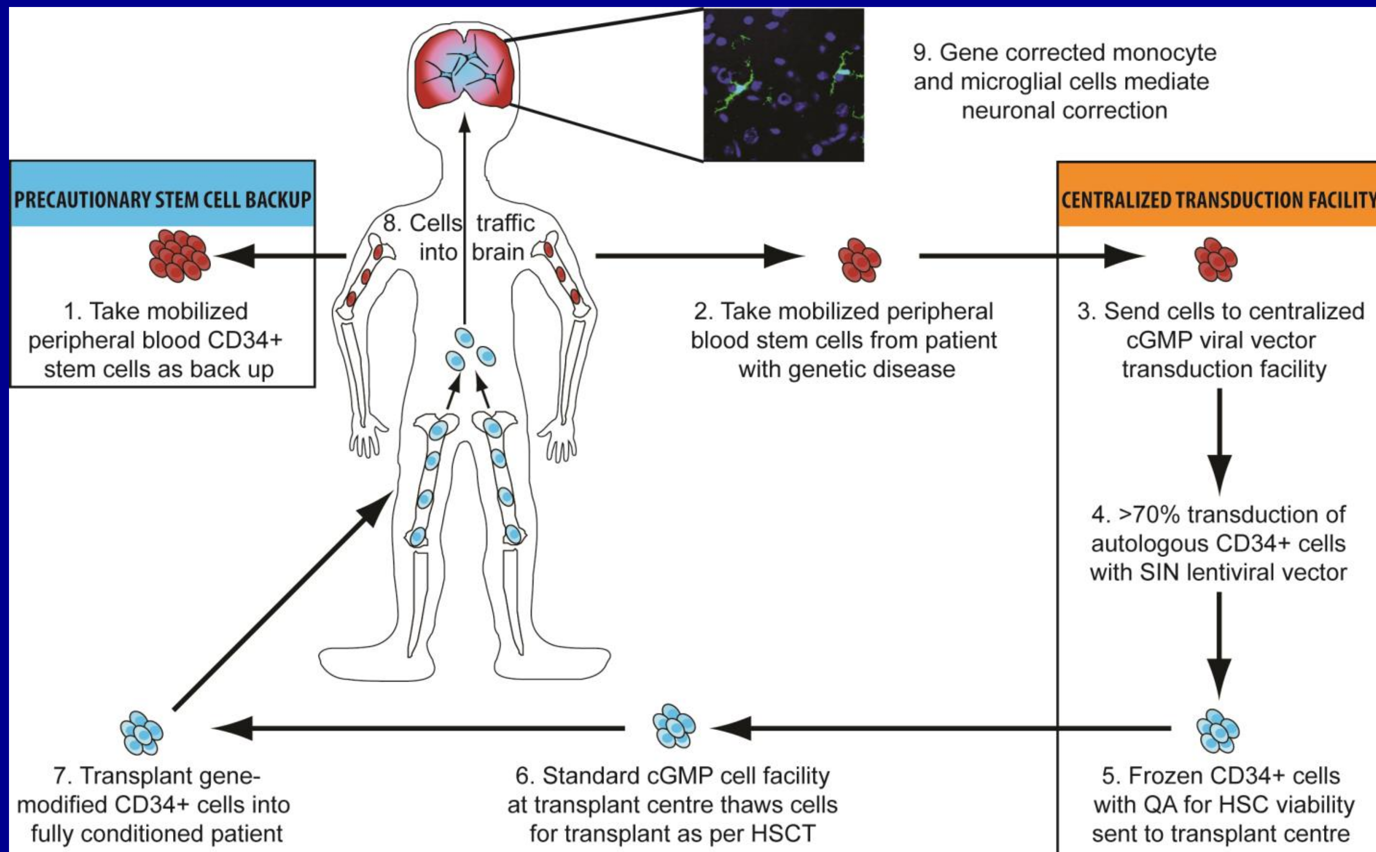
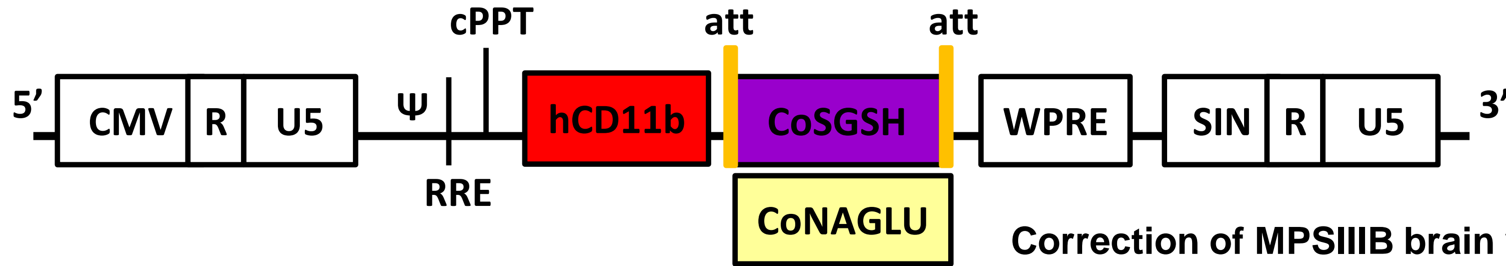
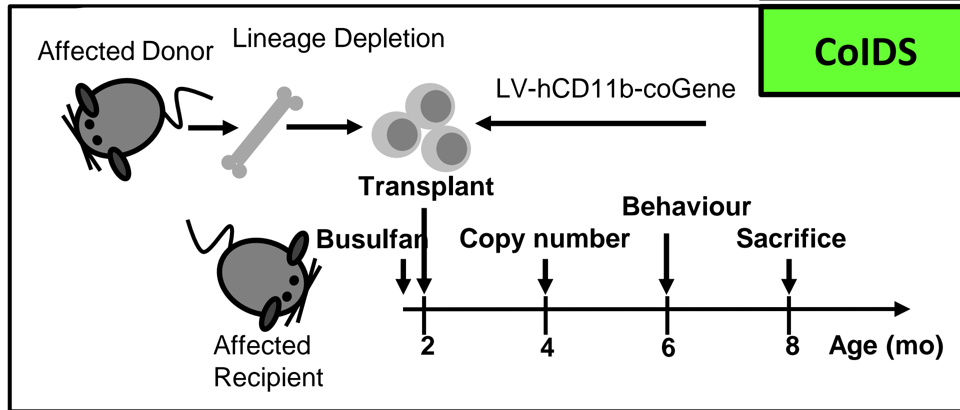
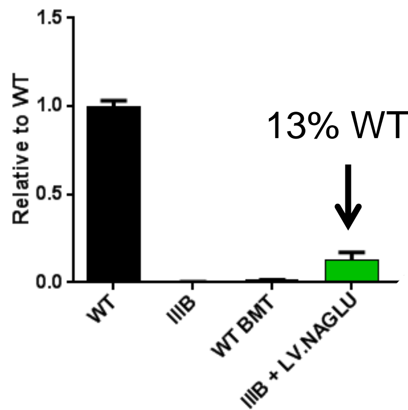


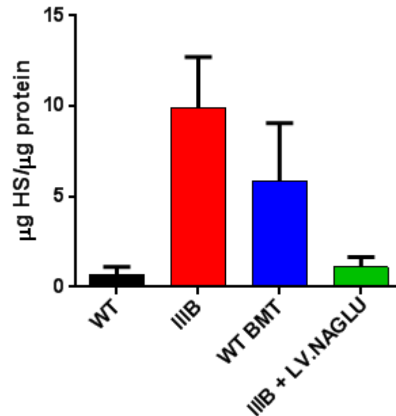
*Figure from Bigger and Wynn Discovery Medicine April 2014

LV-HSC brain correction of MPSIIIB – Holley



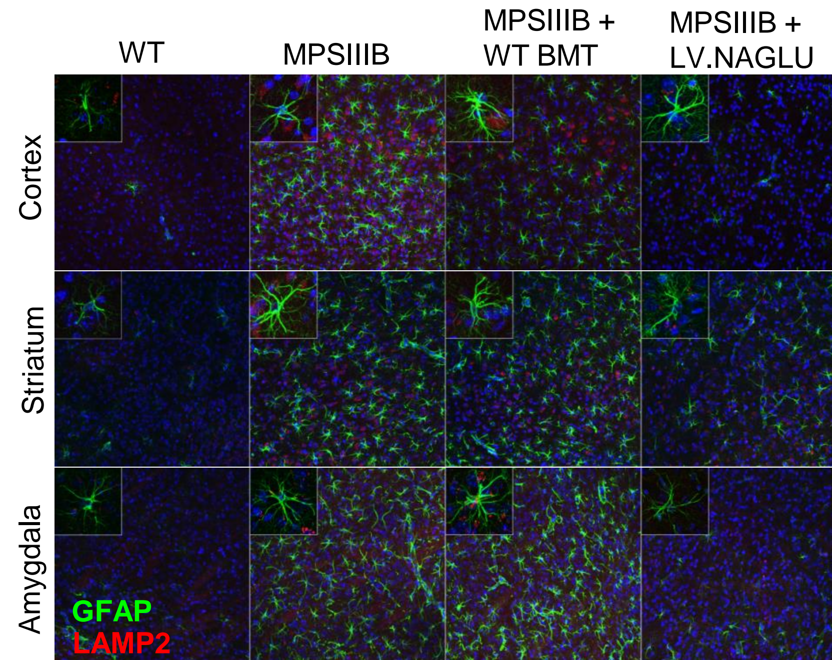






LV-NAGLU increases brain enzyme and corrects HS

Correction of MPSIIIB brain with LV-NAGLU

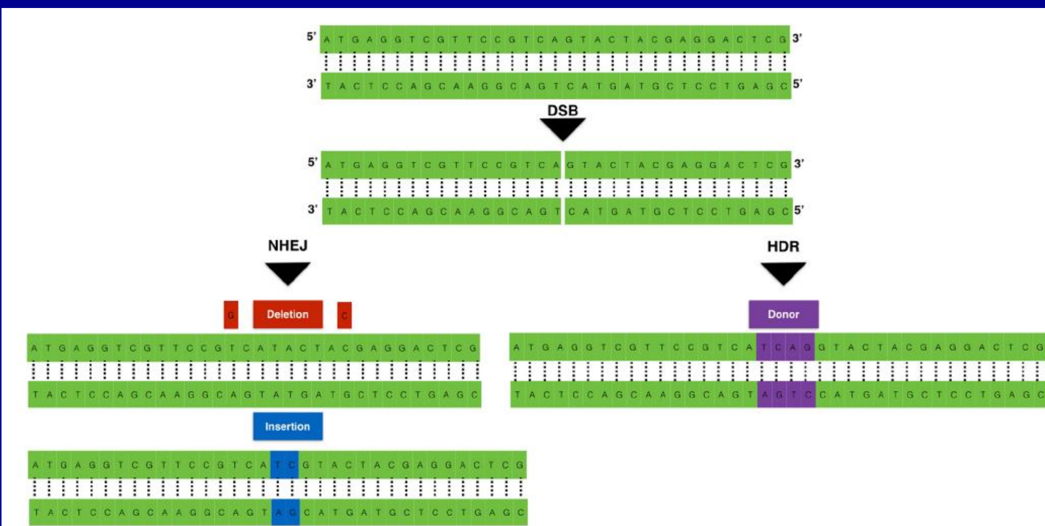


Astrocytosis normalised by LV-NAGLU

Holley, Ellison in submission

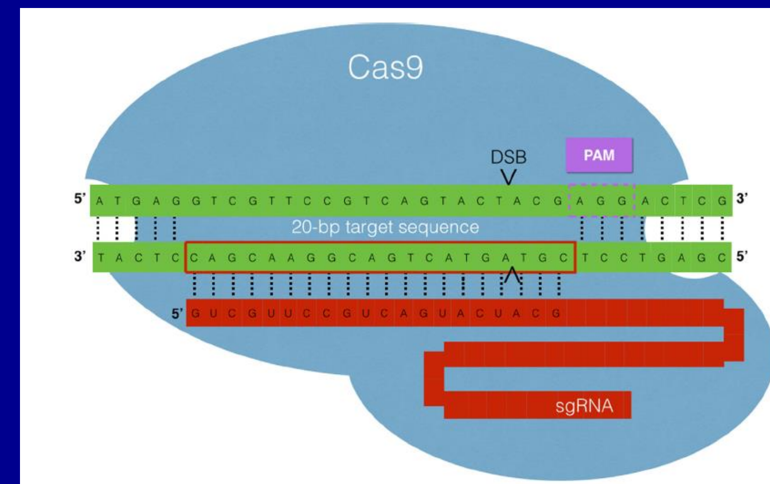
Gene Repair

- Zinc Finger Nucleases (ZFN), Transcription activator-like effector nucleases (TALEN), CRISPR/Cas9 RNA guided endonuclease system
- All introduce a DS break at a targeted location with the guide of homologous binding proteins or RNA



DS breaks repaired by Non-homologous end joining OR homology directed repair (template)

Cas9 endonuclease targets a 20bp sequence based on a single guide RNA with homology to the DNA target



LaFountaine 2015 Int J Pharmaceut 494:180

Gene Repair – route to trial

- Delivery of any gene editing approach has generally been via viral vectors (integration deficient LV) or AAV
- CRISPR/Cas9 system is 4.3kb (Cas9/guideRNA)– just within AAV packaging capacity
- Poor transduction/editing in stem cells limits ex vivo approaches
- HDR is much less efficient – need to improve delivery and off target effects in all systems
- NHEJ is by far the most efficient – thus phaseI trials of targeted deletion of CCR5 binding locus for HIV via ZFN targeting of CD4+ T cells ex vivo and reintroduction (Sangamo) are viable (Tebas NEJM 2014 370:901)

LaFountaine 2015 Int J Pharmaceut 494:180

The Future

- Strategies to increase enzyme delivery to target organs – O'Leary Sun 9:30, Bigger 8:30
- Novel substrate inhibitors/chaperones
- Stop codon read through
- Anti-inflammatories – Helen Parker Sat 11:50
- Unknown – cyclodextran in NPC
- Gene therapy clinical outcomes
- Combination therapies
- Tolerance to ERT – Liao Sun 11:00

LSD treatment – what price therapy?

- Morquio enzyme Vimizim initially refused in UK
- Cost £394,680 pa/pp – more as patients grow
- Incidence
  - ~1/250,000
- Benefit
  - Undoubted benefit, but subtle – improved 6 min walk test, increased height, reduced skeletal issues – almost certainly increased lifespan