GENE THERAPY FOR LYSOSOMAL STORAGE DISEASES WITH NEUROLOGICAL IMPLICATIONS

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ABSTRACT

Lysosomal storage diseases (LSD) are a numerous group of diseases, which can present from mild to very severe clinical profile with implication of many organs. The most severe forms present nervous central system (NCS) implications, that cause mental retardation and lifespan shortening. There is no curative therapy for this kind of diseases, and existing treatments are not able of successful correction of CNS impairments. Gene therapy is a curative treatment option, being now evaluated in preclinical and clinical trials, because of its advantage of correcting CNS cells.

LYSOSOMAL STORAGE DISEASES

Main features:
- Caused by a specific hydrolase dysfunctionality
- Cause undigested storage in lysosomes
- Present neurological, renal, cardiovascular, gastro-intestinal, musculo-skeletal, ophthalmological and respiratory problems
- Severe forms cause mental retardation and lifespan shortening

It is a group of >50 different diseases:
- **Mucopolysaccharidosis** (MPS types I - VII)
- **Oligopolysaccharidosis**
- **Lipoidosis**
  - Sphingolipidoses (Niemann-Pick disease, Gaucher disease)
  - Gangliosidosis (Tay-Sachs disease)
  - Leukodystrophies, (Krabbe disease, Canavan disease, Adrenoleukodystrophies)
- **Glycoproteinosis** (Sialidosis, Fucosidosis, Mannosidosis)
- **Neuronal Ceroid Lipofuscinosis** (Infantile NCL, Late-infantile NCL)

TREATMENT OPTIONS

Enzyme Replacement Therapy
Missing enzyme administration in form of recombinant enzyme.
Not able to cross BBB.

Substrate Reduction Therapy
Small molecules administration in order to block undigestible substrate production.

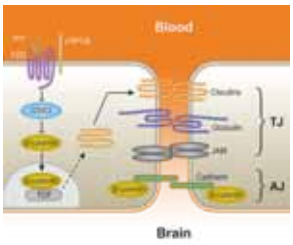
Disadvantages & Obstacles
- These treatments are no curatives, and require chronicity.
- Successful in small number of LSD.
- Not able to complete CNS correction

New treatments necessity
Some new treatments in trials. The most promising for NCS implications is:

Gene Therapy
- Curative treatment
- Introduction of a gene encoding missing hydrolase
- Two approaches: *ex vivo* and *in vivo*
- Able to achieve CNS correction

BLOOD-BRAIN BARRIER

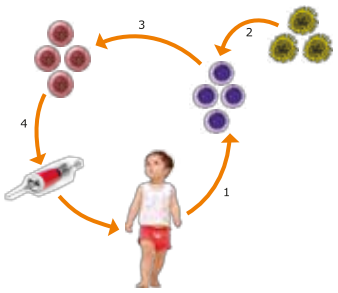
Is the main obstacle to reach CNS for CNS directed therapies.
- Isolates the brain from circulating blood.
- Formed of endothelial cells that present tight junctions between them
- Function: protecting from potentially dangerous agents.



GENE THERAPY APPROACH FOR LSD

EX VIVO GENE THERAPY

Ex vivo gene therapy
Consists on:
1. Isolating patient's cells
2. Culturing them and transducing with suitable vector
3. Selecting of transduced clones
4. Infusing corrected cells back to the patient



Mostly used vectors: **Lentivirus**
- Capacity of transducing dividing and non-dividing cells
- Permanent expression
- Low immunogenicity
- Broad tropism

Main advantage: patient is not exposed to viral capsides and consequently less immune response

Proof of principle for correcting NCS:
- Microglia derives form hematopoietic stem cells (HSC)
- Cross-correction in brain

PRECLINICAL AND CLINICAL TRIALS

Preclinical studies for *in vivo* gene therapy with AAV in LSD

Disease	Animal model	Cells type	Gene	Most outstanding results	Reference
MPS I	mouse	Erythroids	IDUA	Neurological improvements but not totally cure of locomotory activity and memory	Pan et al, 2009
MPS VII	mouse	hMSC	GUSB	ea GUSB serum levels restoration to 40%. 4 month (study duration time) MSC persistence and enzyme expression. No tumor formation observed.	Sands et al, 2008
MLD	mouse	HSC	Arysulfutase A	Enzyme activity full reconstruction. Prevention of motor, learning and neuropathological impairments.	Biffi et al, 2004

IDUA - α - L-iduronidase; GUSB - β-glucuronidase; MLD - Metachromatic Leukodystrophy

Results of clinical trial of ex vivo gene therapy for LSD Cartier et al, 2009

Disease: X-linked Adrenoleukodystrophy (ALD)
Vector: SIN-lentiviral vector, under MSV promoter
Adjuvant treatment: full myeloablation regimen
Patients: 7 and 7.5 years old boys with ALD.

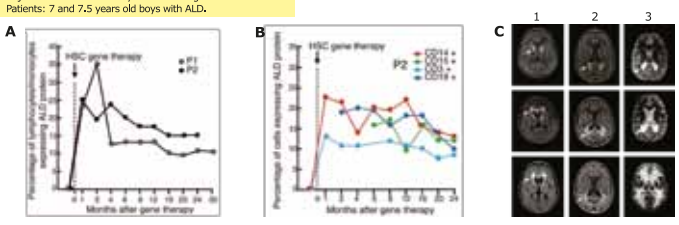


Figure A: Transduced cells percentage in both patients; Figure B: Enzyme expression levels in one of the patients; Figure C: Demyelination progress in two patients (1, 2) and one non-treated control (3).

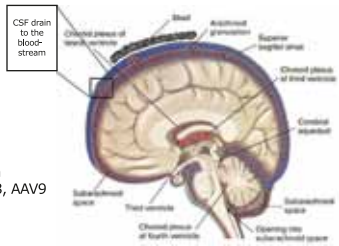
Results:
- stabilization of transduced cells number ~10%
- stabilization of ALD protein expression in different cell types (average ~10%)
- slower progression, stabilization and even regression of impairment

Planned / ongoing clinical trials for LSD *ex vivo* gene therapy

Disease	Clinical phase	Cell type	Gene	Principal investigator
Metachromatic Leukodystrophy	Phase I/II	Autologous CD34+	ARSA	Biffi, Alessandra
X-linked Cerebral Adrenoleukodystrophy	Phase I/II	Autologous CD34+	ALD	Inserm

IN VIVO GENE THERAPY

***In vivo* gene therapy**
- Introduction of the gene directly to the patient
- Exposure to viral elements



Mainly used vectors: **Adeno asociated vectors (AAV)**
- Low immunogenicity
- Broad tropism
- Small vector capacity
- Many different serotypes with different tropism
- For CNS transduction: AAV1, AAV2, AAV5, AAV8, AAV9

Administration routes

Systemic
- Only AAV9 is able to cross BBB
- Higher dose is required
- Important immunogenic problems

Organ-specific
- Intraparenchymal Intracisternal Intraventricular
- Lower doses
- Cerebrospinal fluid possibilities vector distribution and gene correction

PRECLINICAL AND CLINICAL TRIALS

Preclinical studies for *in vivo* approach using AAV in LSD

Disease	Animal model	Serotype	Gene	Administration	Most outstanding results	Reference
GM1	mouse	AVV1	Beta, galactosidase	Intra, thalamic	Thalamus correction, distribution to cerebrum. Storage amelioration. Lifespan increment	Beak et al, 2010
MPS IIIA	mouse	AVV1, AVV8	Sulfamidase	Intramuscular Intravenous	Intramuscular: enzyme production in muscle, but not secre/on into bloodstream. Intravenous (AAV8) GAG storage correction in all /ssues inc-luding CNS. Lifespan increment.	Bosch et al, 2011
Sandhoff	mouse	AVV2	Beta, hexosaminidase	Intra, stratium	Prevention of thalamic neurons loss. Also in contralateral thalamus. Local immune response	Sargeant et al, 2011
MSP IIIA	mouse, dog	AVV9	Sulfamidase	Intra,CSF	Efficient transduction of all brain. Owning to the AAV9 capacity of cross BBB, storage correction was achieved in the rest of organs.	Haurigot et al, 2013

Planned / ongoing clinical trials for LSD in vivo gene therapy with AAV

Clinical trial	Clinical trial	Administration	Gene	Principal investigator
Tay Sachs	Phase II	Intracranial	α, β hexosaminidase	Timothy Cox
Metachroma/c!Leukodystrophy	Phase I/II	No data	Arylsulfatase A	INSERM
Galactosialidosis	Phase I	Intravenous	Protective Protein Cathepsin A	Arthur Nienhuis

patients who develop cerebral demyelinati

Published clinical data: Phase I clinical trial for LINCL using		
Administration	Gene	Principal investigator
Intraparenchymal	CLN2	Ronald Crystal
No data	Arylsulfatase A	INSERM

Results

10 patients aged 8,12, presented from mild to severe forms.
Results: many serious adverse events (60), among them seizure, increased myoclonus, anemia. And non,serious (94), such as thrombocytosis, seizure, vomiting, etc). Authors suggest that these effects are not unequivocally atributed to the vector. But this safety trial outline the multiple adverse effects that should be taken into consideration with this therapy.

CONCLUSIONS

Gene therapy is a curative therapeutic option for many diseases that involve CNS and that have no treatment at the moment. Although still many obstacles have to be overcome and many advancements in this field must be scaled up to humans and tried in clinical trials before gene therapy can become conventional treatment it is very promising therapy for many diseases that have no curative treatment at the moment, such as LSD.