GENE THERAPY FOR NEURODEGENERATIVE DISEASES

from genetic leukodystrophies to Alzheimer disease

- X-linked adrenoleukodystrophy (ALD)
- Metachromatic leukodystrophy (MLD)
- Alzheimer disease (AD)

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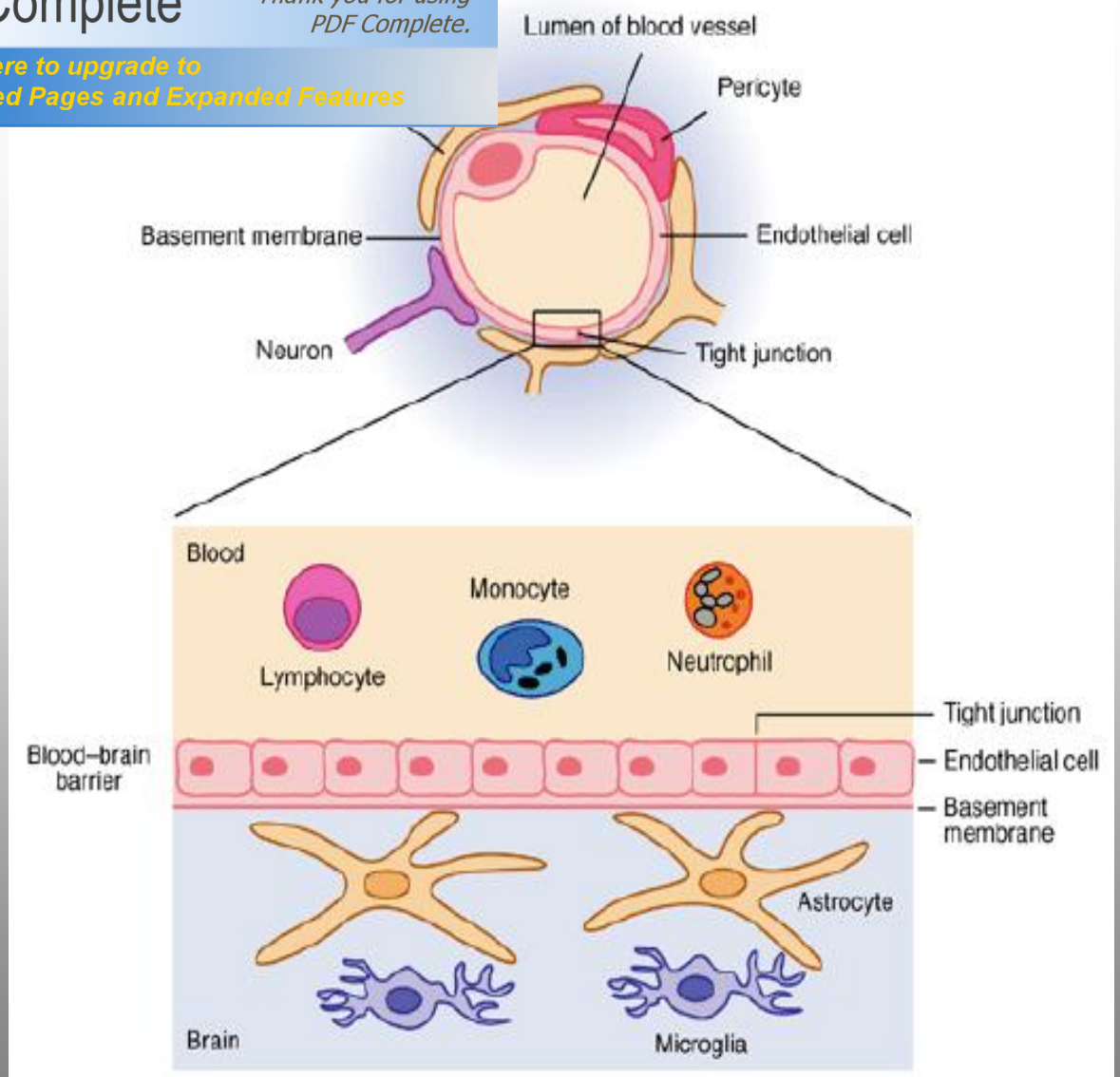
e delivery to the Central Nervous System

Specific issues

- the brain is a complex organ: interconnections between neuron, glia and other cells

- the target areas:
  - discrete brain region: Parkinson, Huntington
  - large portion of the brain: lysosomal storage diseases (-> leukodystrophies)

- target cells: neurons, sub-population of neurons, glia, endothelial cells

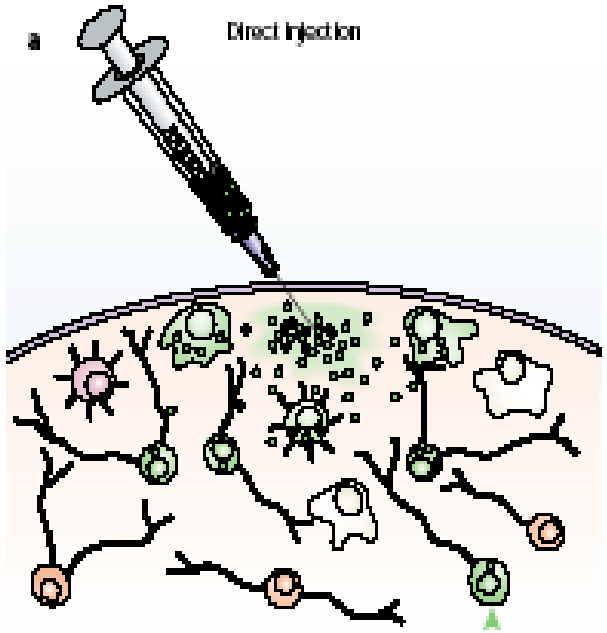


The blood–brain barrier (BBB)

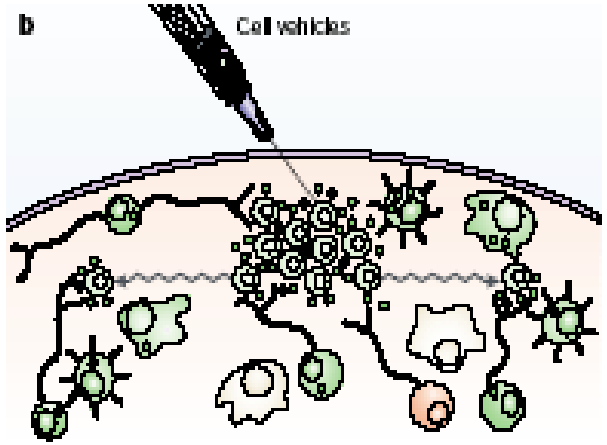
Expert Reviews in Molecular Medicine © 2003 Cambridge University Press

BBB protects the brain from pathogens
Hampers the delivery of therapies to the CNS

Modes of gene/protein delivery to the brain



- taken up by the cells at the injection site or diffuse away
- anterograde / retrograde transport to distant sites
- volume constraints / number of stereotactic injections

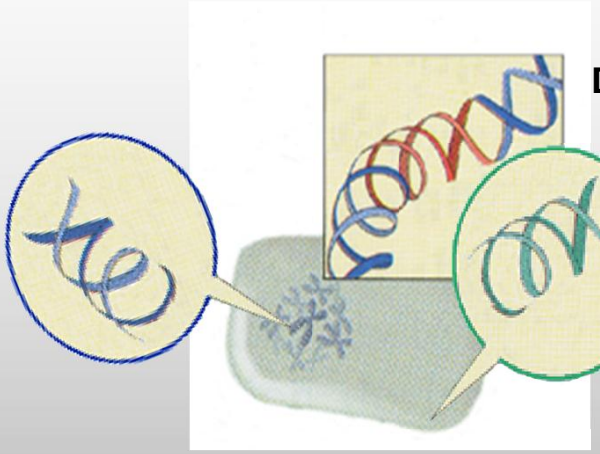


- astrocytes, macrophages, fibroblasts, neural precursor cells
  some cells can migrate away from the injection site and (neural precursor cells) be incorporated into the cytoarchitecture of CNS
- Hematopoietic cell transplantation

IE THERAPY APPROACHES

STRATEGIES

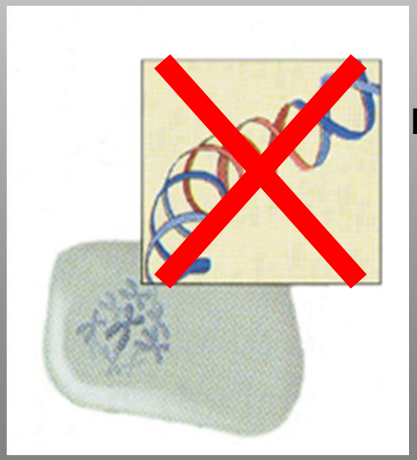
Gene replacement (loss of function)



Defective gene

Symptomatic treatment

Down-regulation of disease genes (siRNA)



Defective gene

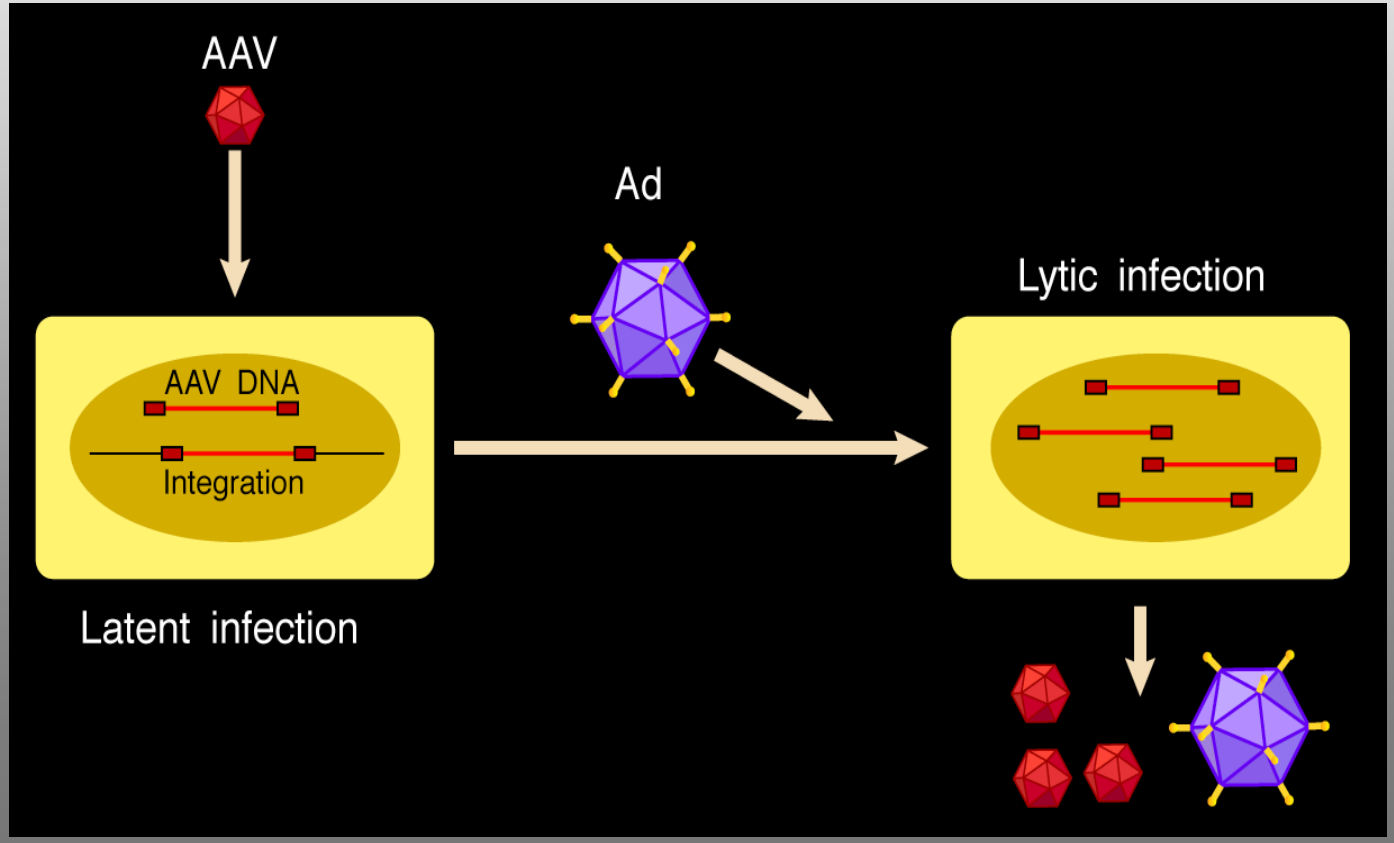
Gene correction

rum of vectors used in basic research
s the few being used in clinical trials

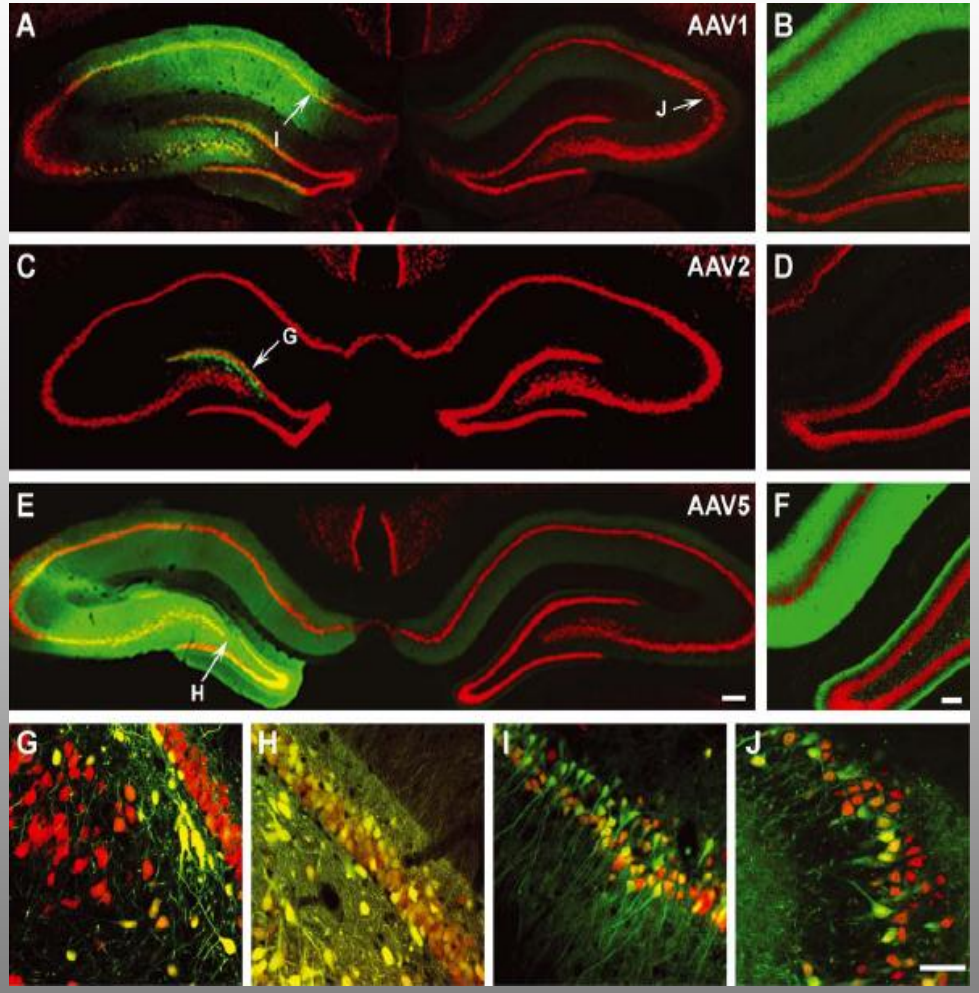
Vector	Virion type	Particle size	Titers (transducing units ml^{-1})	Transgene capacity (maximum)
MoMLV	Retrovirus (RNA)	100 nm	10^{6}–10^{9}	7–8 kb
Lentivirus	Retrovirus (RNA)	100 nm	10^{6}–10^{9}	7–8 kb
Recombinant adenovirus	Adenovirus (dsDNA)	80–120 nm	10^{10}–10^{12}	20 kb
'Gutless' adenovirus	Adenovirus (dsDNA)	80–120 nm	10^{9}	Up to 36 kb
Adeno-associated virus	Parvovirus (ssDNA)	20–30 nm	10^{9}–10^{13}	4.5 kb
Sindbis	RNA (alphavirus) (ssRNA)	60–65 nm	10^{7}	6 kb*
Poliovirus replicon	Picornavirus (ssRNA)	30 nm	10^{9}	6 kb*
HSV amplicon	Herpesvirus (dsDNA)	120–300 nm	10^{9}	Up to 150 kb
HSV recombinant virus	Herpesvirus (dsDNA)	120–300 nm	10^{11}	30–50 kb

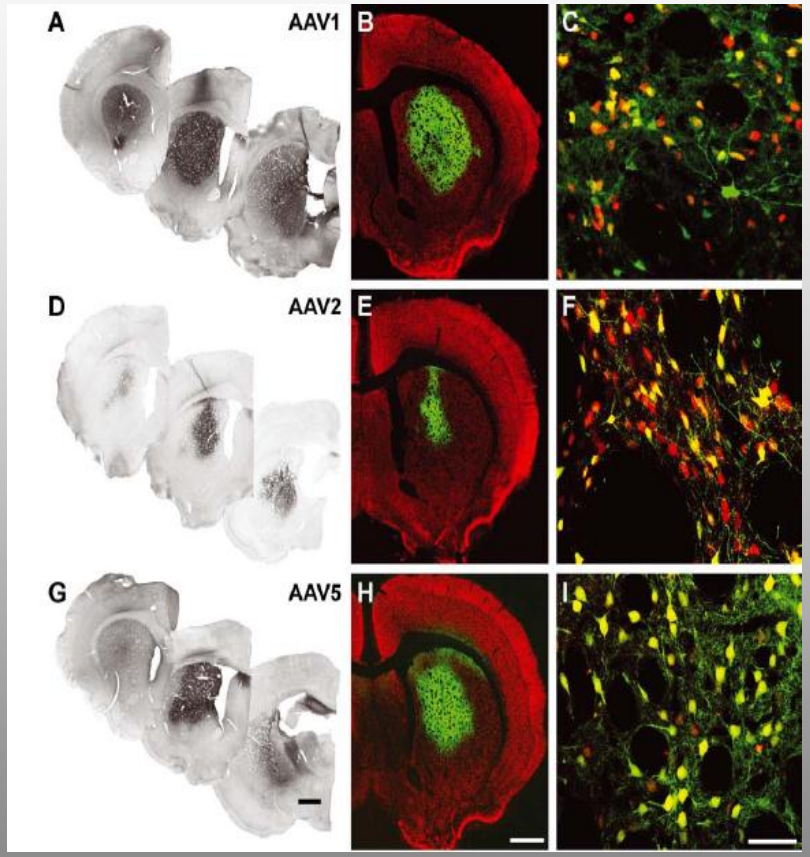
Adeno-associated virus (AAV)

- A20 nm diameter
- Å**Linear s/s DNA genome**
- Å**Maximum insert size - 5 kb**
- Å**Defective parvovirus dependent on helper virus for replication**
- Å**Wild type virus integrates into chromosome 19**
- Å**Non-pathogenic**



CTION EFFICIENCY OF AAV SEROTYPE

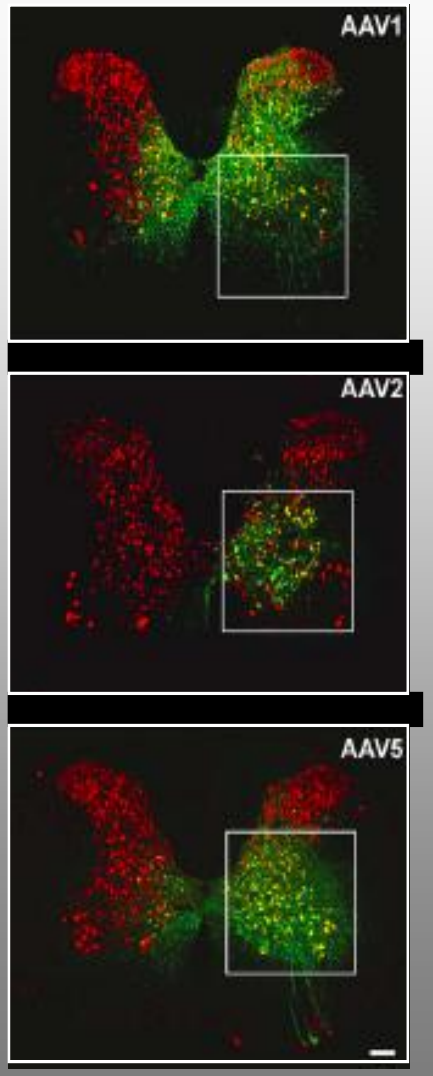


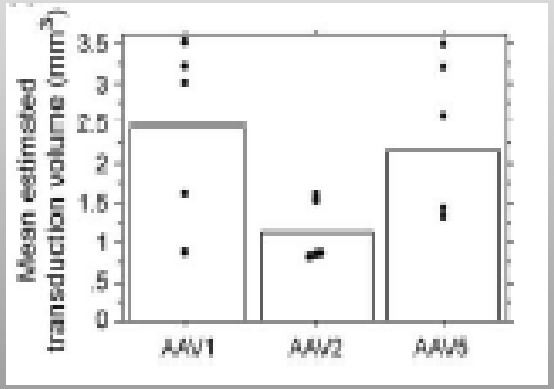


Burger et al. (2004) Mol Ther. 2:302-3

AV: diffusion depends on serotypes

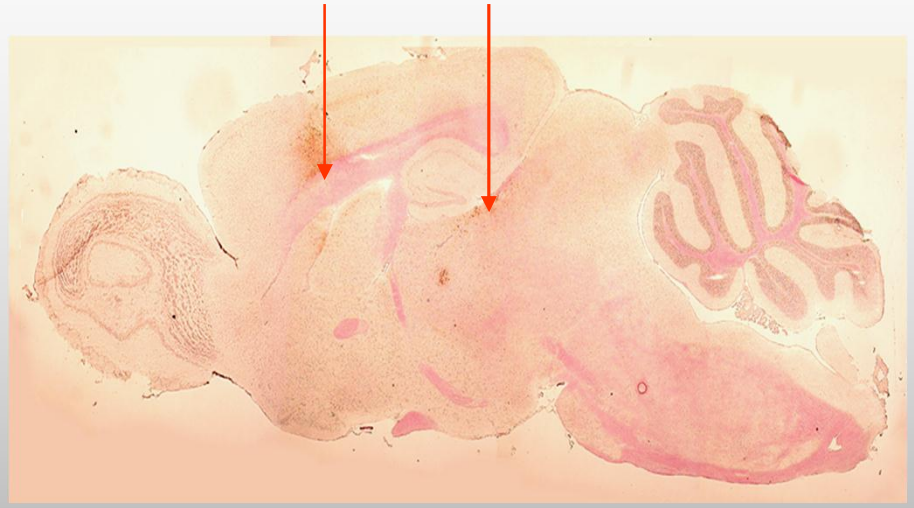
In the spinal cord



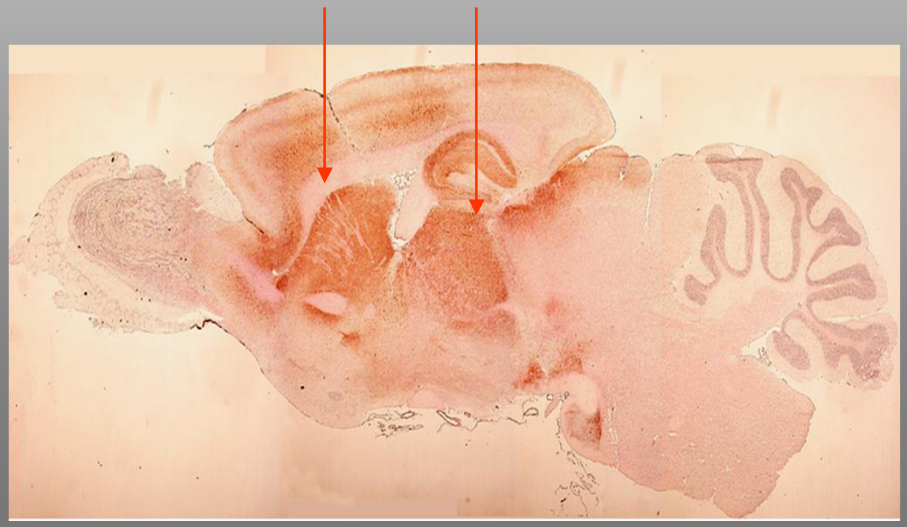


vectors: a clear advantage in term of diffusi

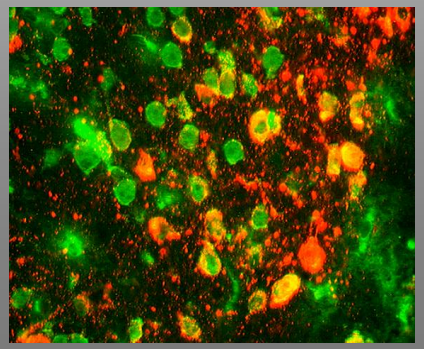
AAV2-PGK-ALD



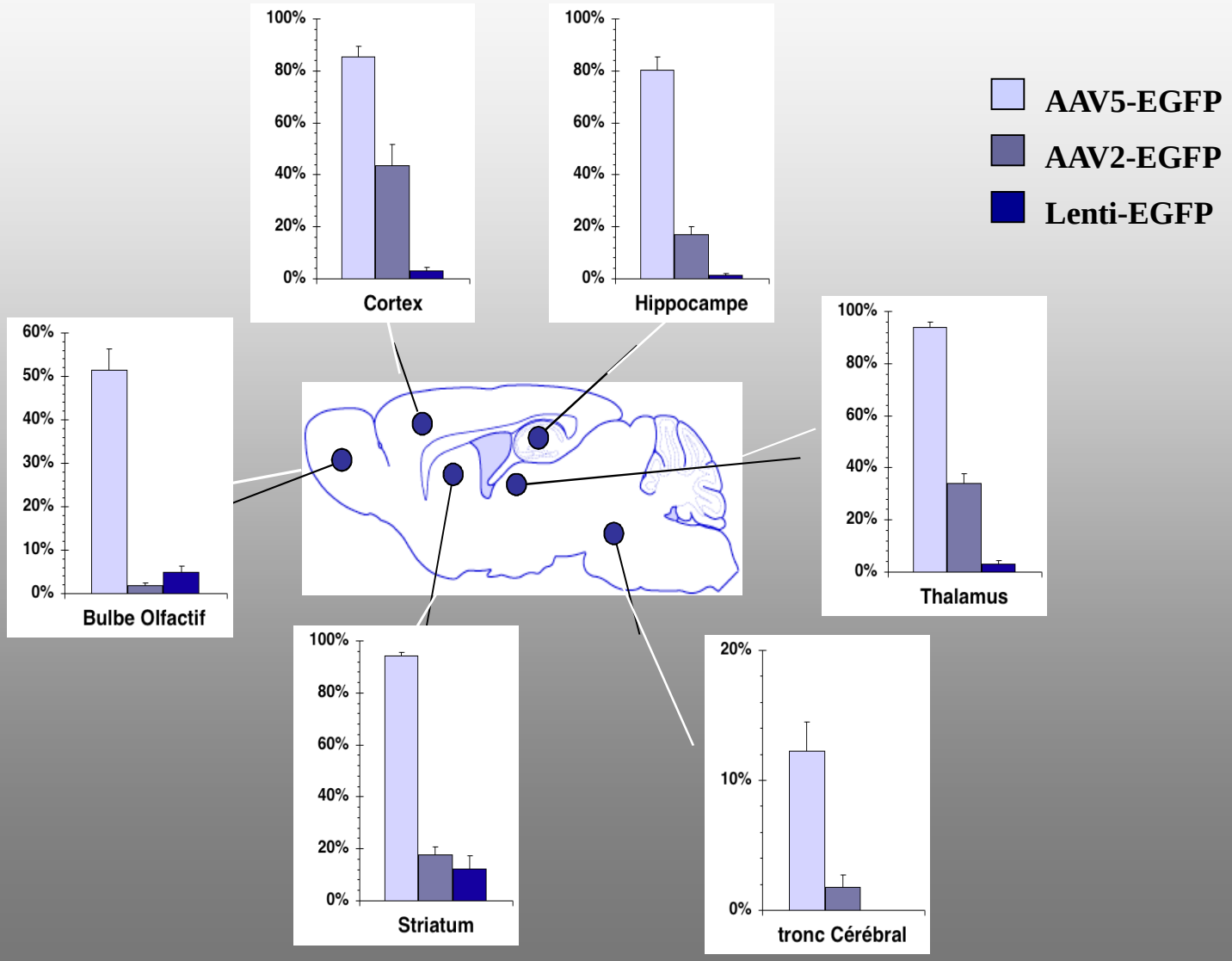
AAV5-PGK-ALD



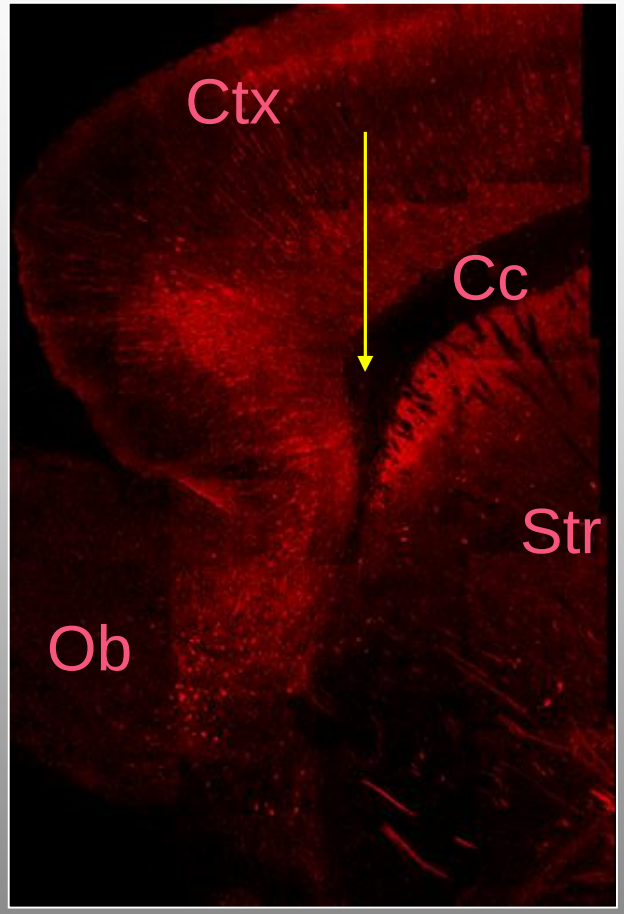
NeuN + ALDP



AAV vector : neuronal tropism



y vectors do not like oligodendrocytes

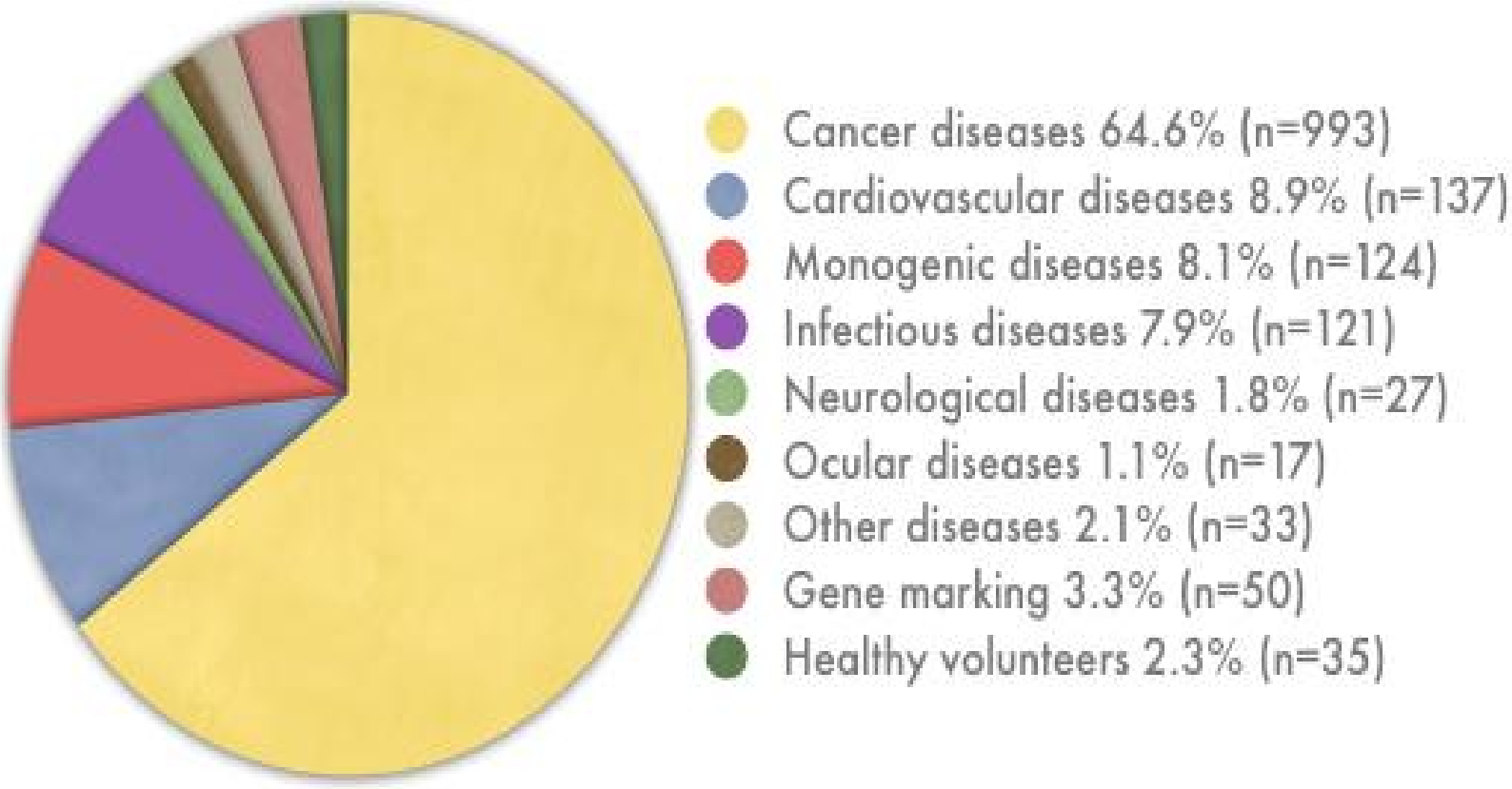


Needs to develop new vector envelops to target oligo ++ (antibody, ligands to specific receptor, chemical modifications..

Potential clinical applications

- Å**stroke**
- Å**spinal cord injury**
- Å**neurodegenerative diseases (Parkinson, Huntington, Alzheime**
- Å**CNS lysosomal storage diseases**
- Å**brain tumors**
- Å**pain**

Indications addressed by Gene Therapy Clinical Trials



 www.wiley.co.uk/genmed/clinical

APY CLINICAL TRIALS IN THE CNS

Symptomatic treatments

Amyotrophic Lateral Sclerosis

Å**Genetically engineered encapsulated cells producing CNTF**
Aebischer et al. (1996) Nat. Med. 2: 696-699

Å**Zinc finger DNA binding protein** (ZPF-TF, transcription factor), naked DNA, intramuscumlar administration

Huntingtonôs Disease

Å**Genetically engineered encapsulated cells producing CNTF**
Bachoud-Lévi et al. (2000) Hum. Gene Ther. 11: 1723-1729;
Bloch et al. (2004) Hum. Gene Ther. 15: 968-975

Alzheimerôs Disease

Å**Autologous fibroblasts infected with an NGF-expressing retroviral vector (MoMLV)** Tuszynski et al. (2005) Nat. Med. 11: 551-555
Å**AAV-NGF (0401-623)**

Y CLINICAL TRIALS IN THE CNS

Symptomatic treatments

Parkinson's Disease

- **AAV-hAADC-2 (0307-593)** Eberling et al. (2008) Neurology 70: 1980-1983
- **EIAV-TH-AADC-GTPCH** Jarraya et al. (2009) Science Transl. Med. 1: 2ra4
- **AAV-GAD (104-469)** Kaplitt et al. (2007) Lancet 369: 2097-2105
- **AAV-Neurturin (0501-689)** Marks et al. (2008) Lancet Neurol 7: 400-408
- **AAV2-GDNF**

APY CLINICAL TRIALS IN THE CNS

Gene replacement

Canavan (childhood leukodystrophy)

Å**Cationic liposome containing an ASPA plasmid DNA (9711-222)**

Å**AAV-ASPA (0001-381)** Janson et al. (2002) Hum. Gene Ther. 13: 1391-1412;
McPhee et al. (2006) J. Gene Med. 8: 577-588

Late infantile neuronal ceroid lipofuscinosis a form of Batten disease (lysosomal storage disease)

Å**AAV-CLN2 (tripeptidyl peptidase I) (0312-619)**
Worgall et al. (2008) Hum. Gene Ther. 19: 463-474

X-Linked Adrenoleukodystrophy

Å**HIV-ALD**

Cartier et al. (2009) Science 326: 818-823

GENE THERAPY FOR NEURODEGENERATIVE DISEASES

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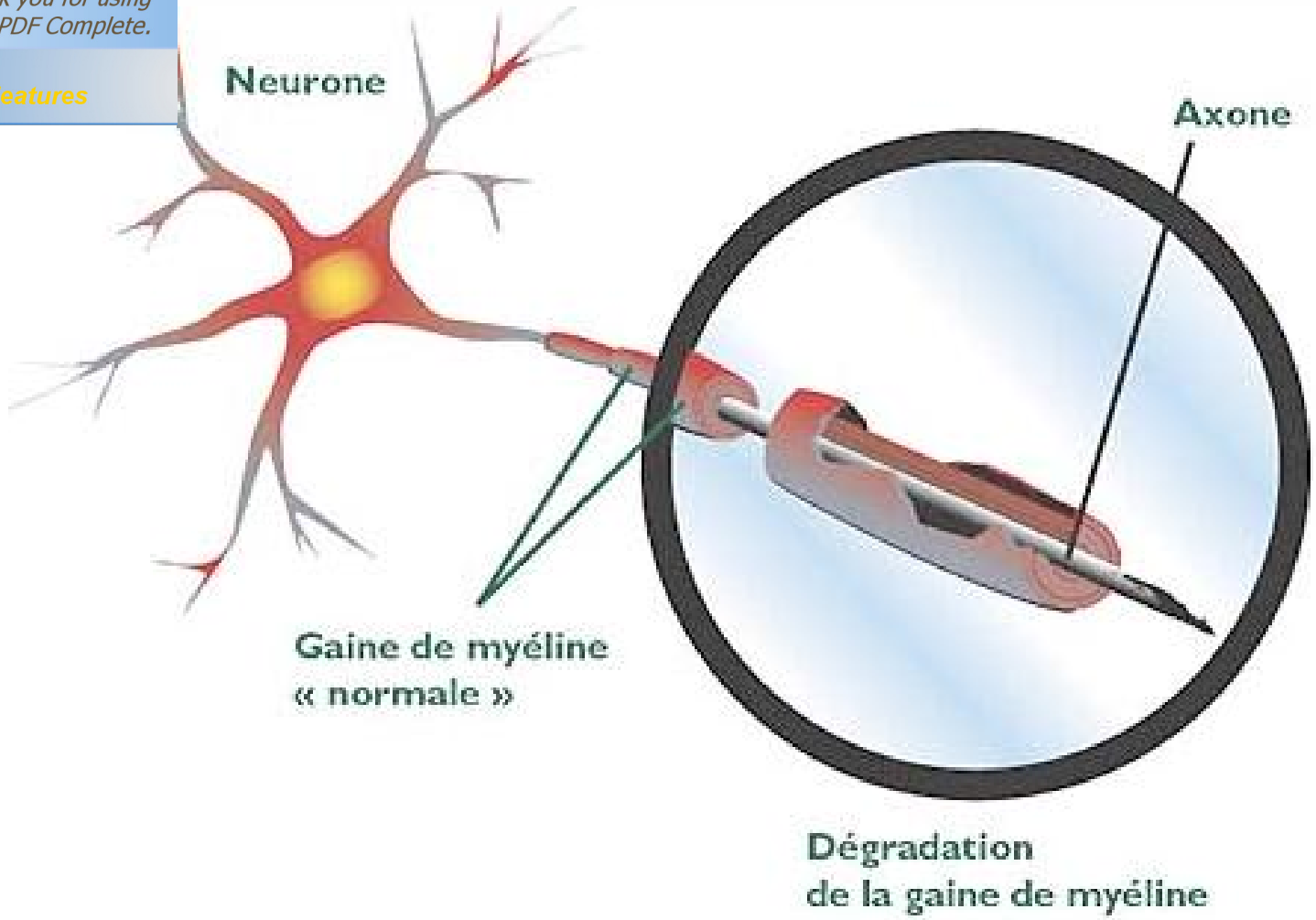
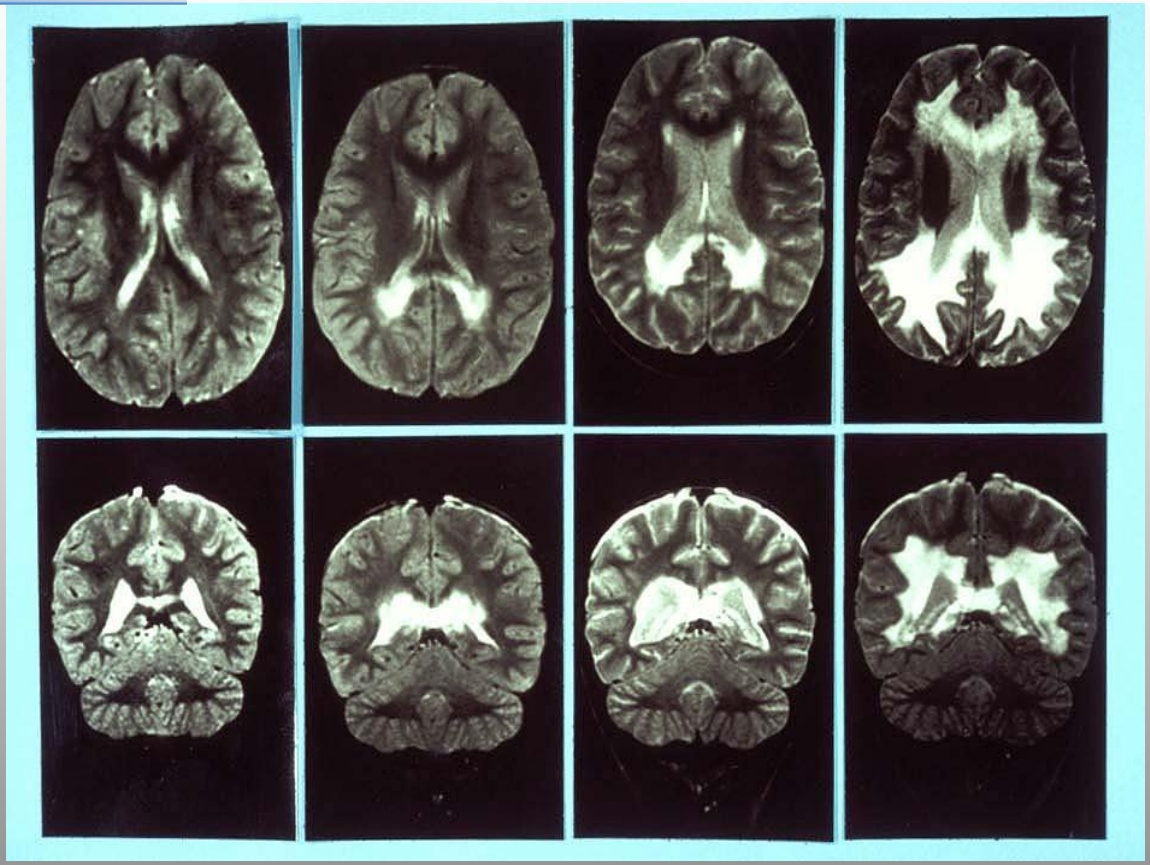
- Alzheimer disease (AD)

N Cartier
INSERM U986 / MIRCen CEA

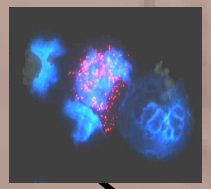


leucodystrophies

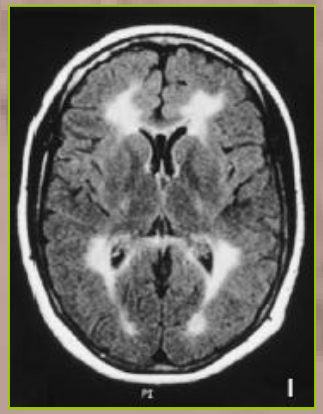


Gene Therapy strategies for neurodegeneratives diseases : Genetic Leukodystrophies

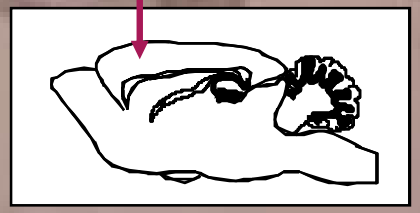
Adrenoleukodystrophy (ALD)



HSC ex vivo gene therapy



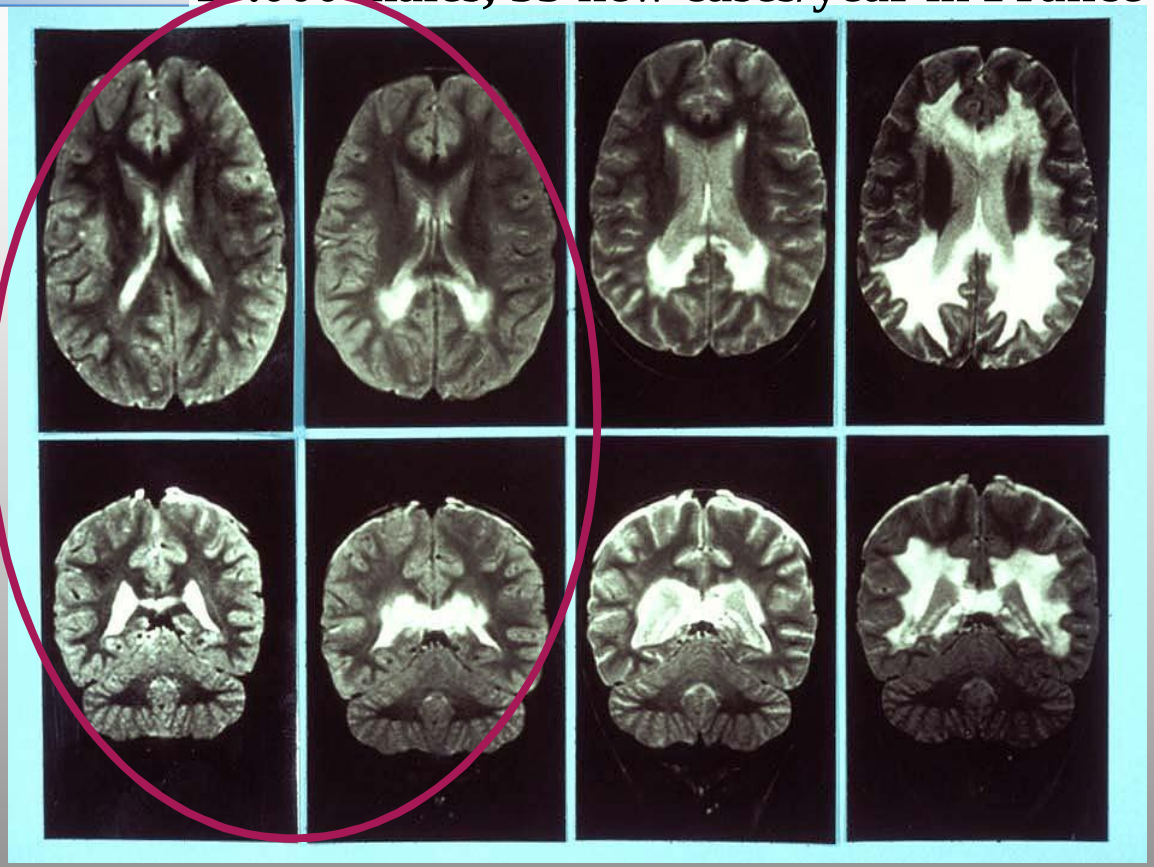
Metachromatic leukodystrophy (MLD)



In situ direct AAV gene transfer
to the brain

Adrenoleukodystrophy

17.000 males, 35 new cases/year in France



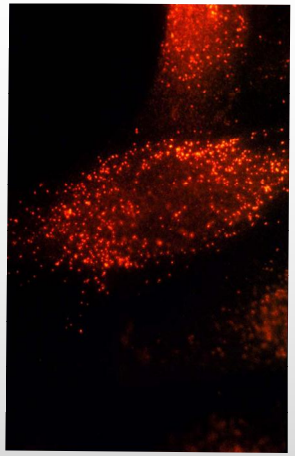
Åno neurological symptoms

Åmild cognitive deficits

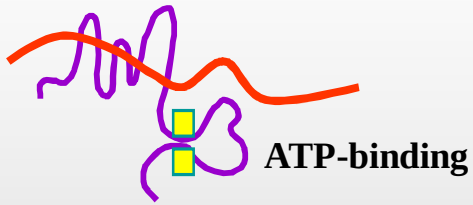
12-18 months

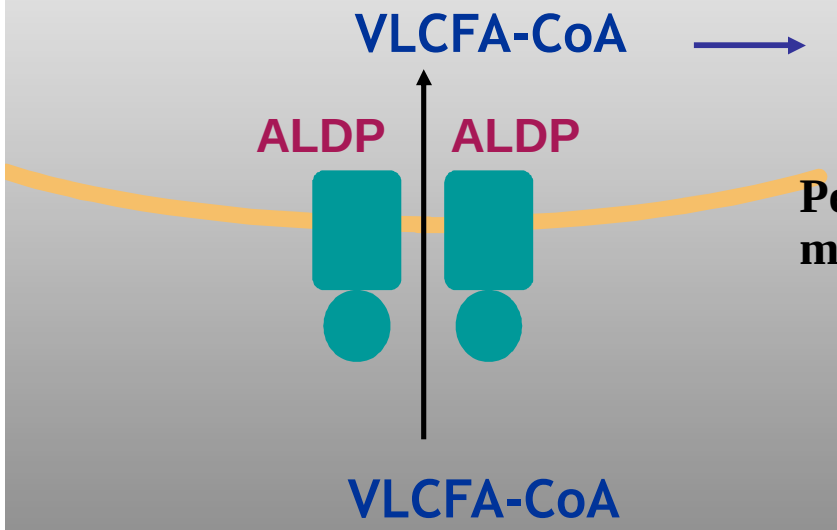
Åvisual deficits
Åauditive deficits
Åpyramidal signs
Åcerebellar signs
Åseizures

D gene and the ALD protein



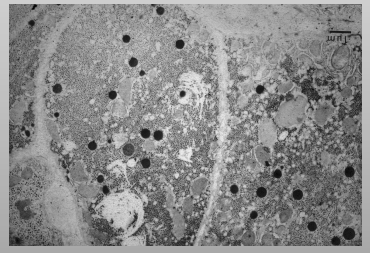
ÅABC peroxysomal transporter



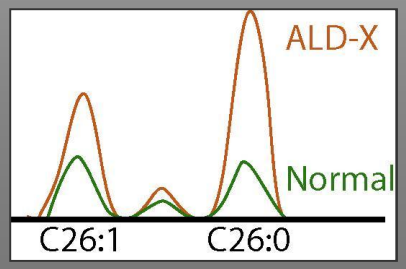


ß-oxidation

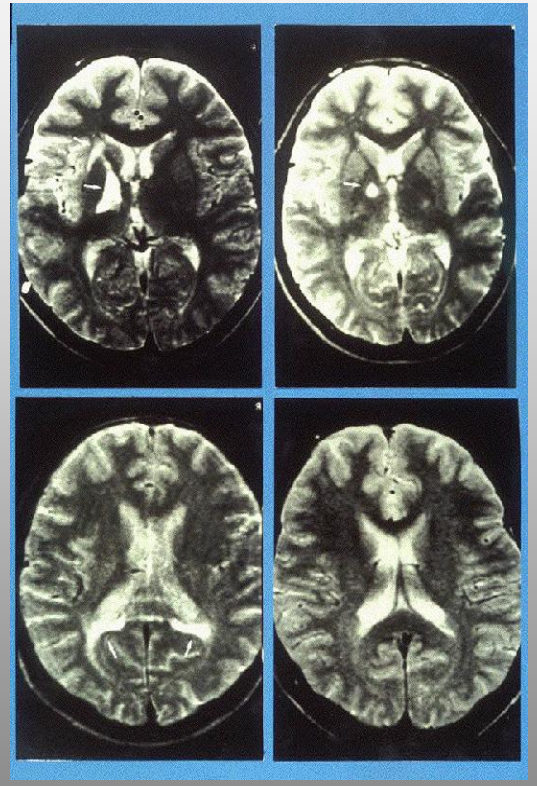
Peroxisomal membrane



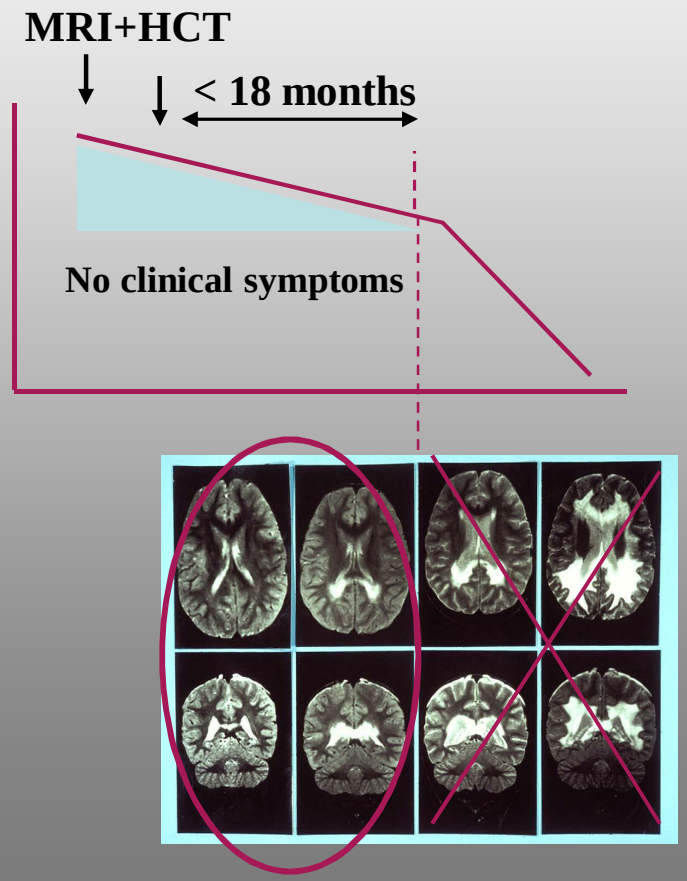
ALD : Accumulation of VLCFA in blood and tissues



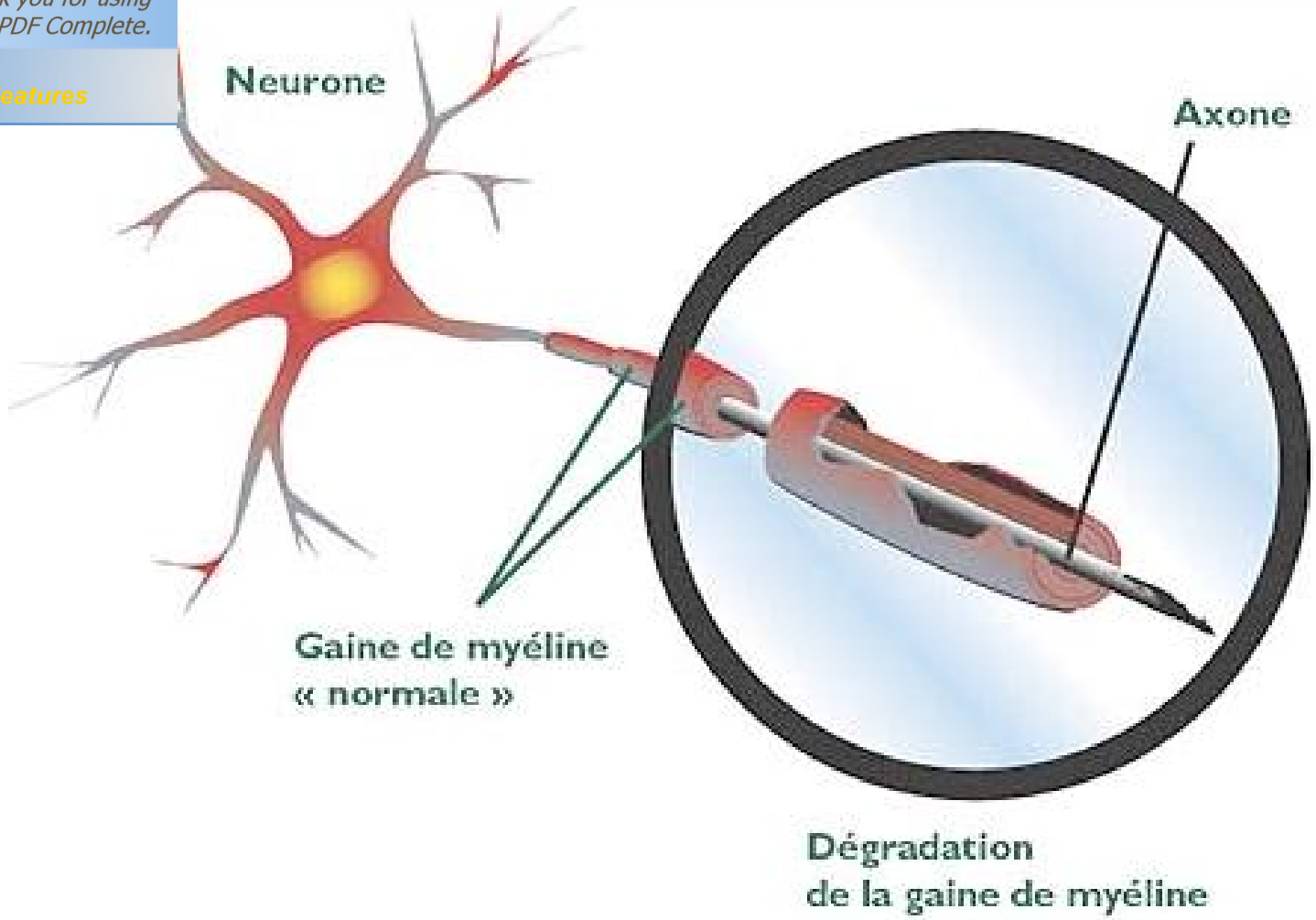
poietic stem cell transplantation (HSCT) can arrest cerebral demyelination in ALD

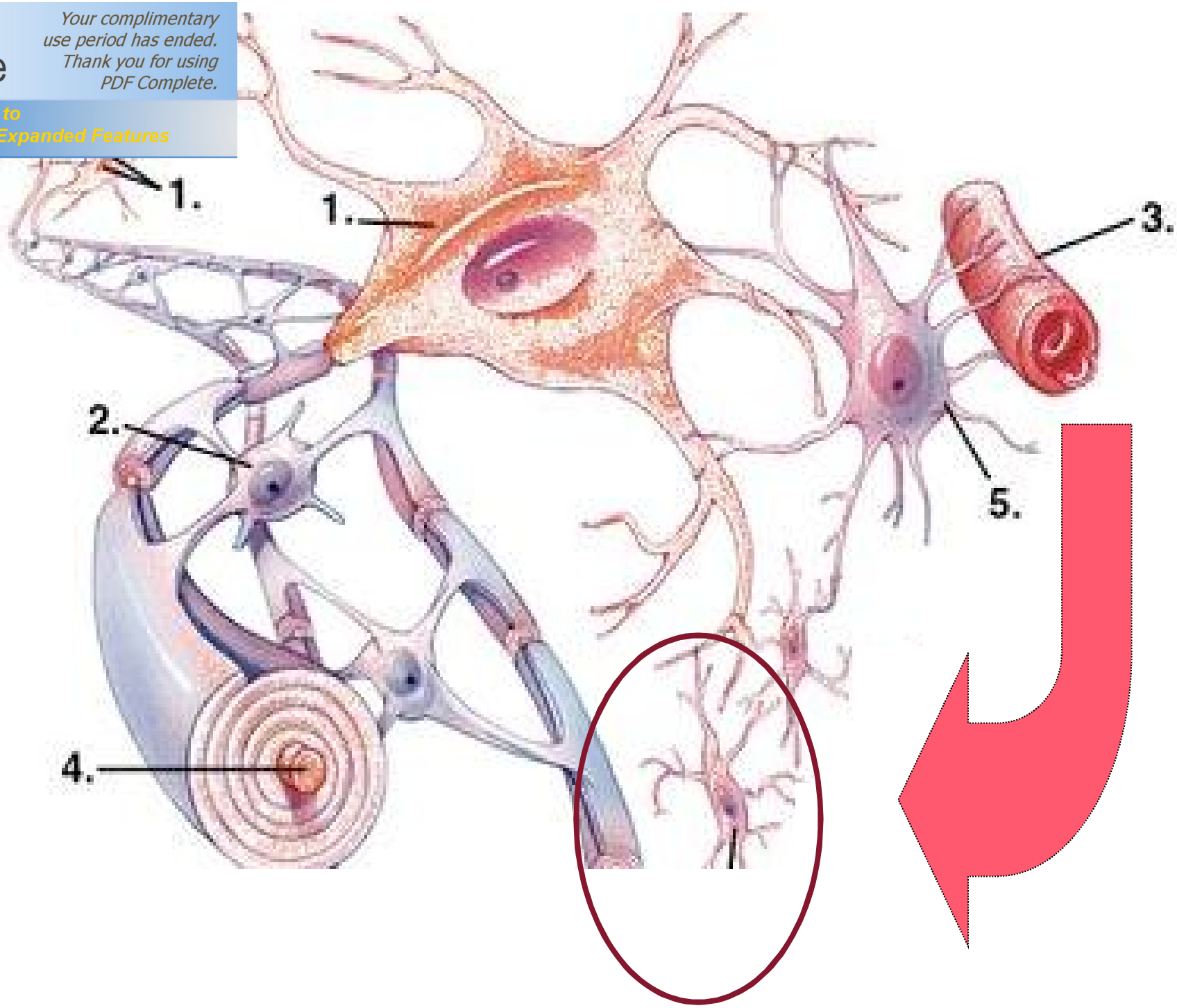


Natural evolution of the disease



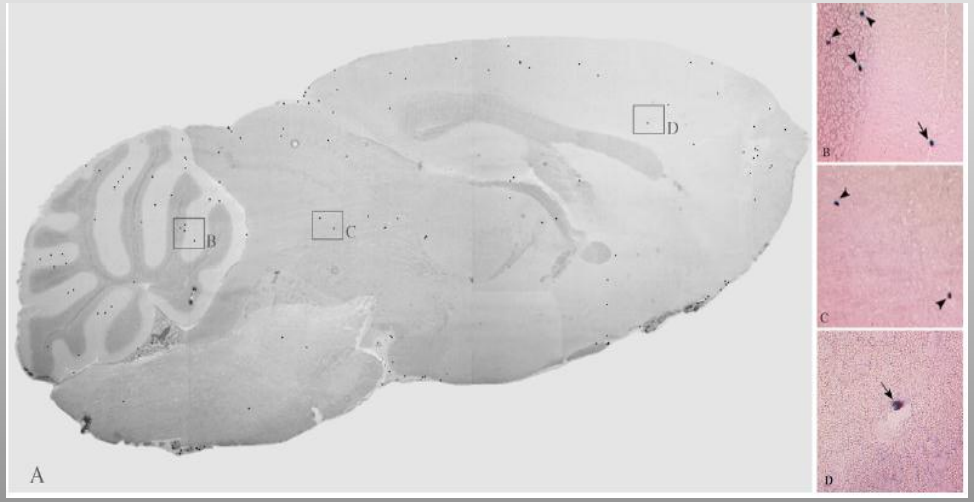
N Engl J Med 1990
Lancet 2000

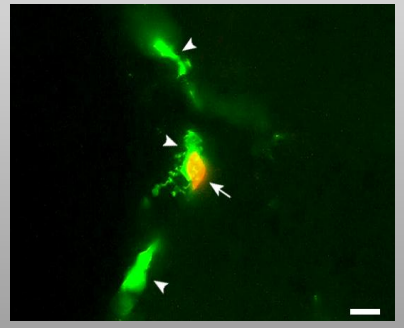




cement of brain microglia originates om hematopoietic precursors

- ✓ present in the bone marrow after hematological reconstitution following BMT that go into the blood and then penetrate into the brain



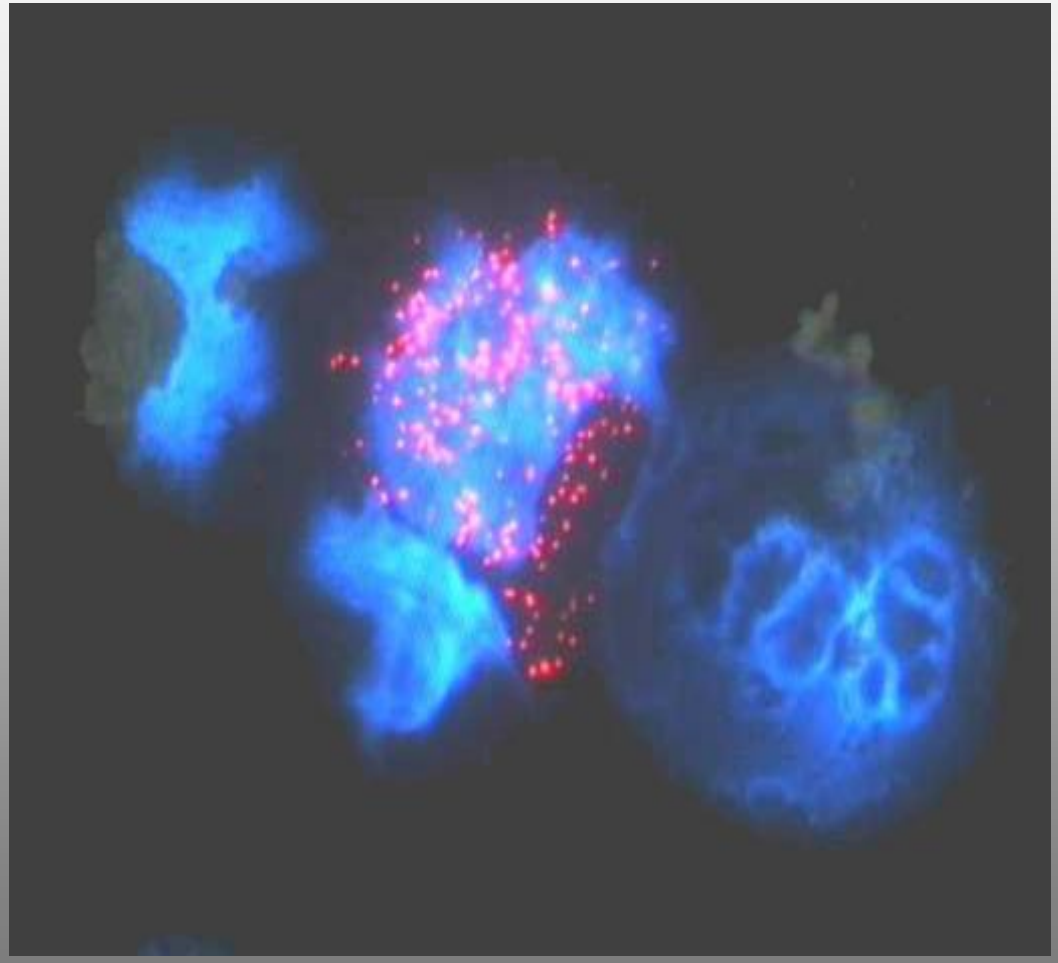


- ✓ The replacement of brain microglia is a relatively slow process
  Some time is needed until a sufficient number of normal microglia cells in the brain can stop the demyelinating process

Why HSC gene therapy in ALD ?

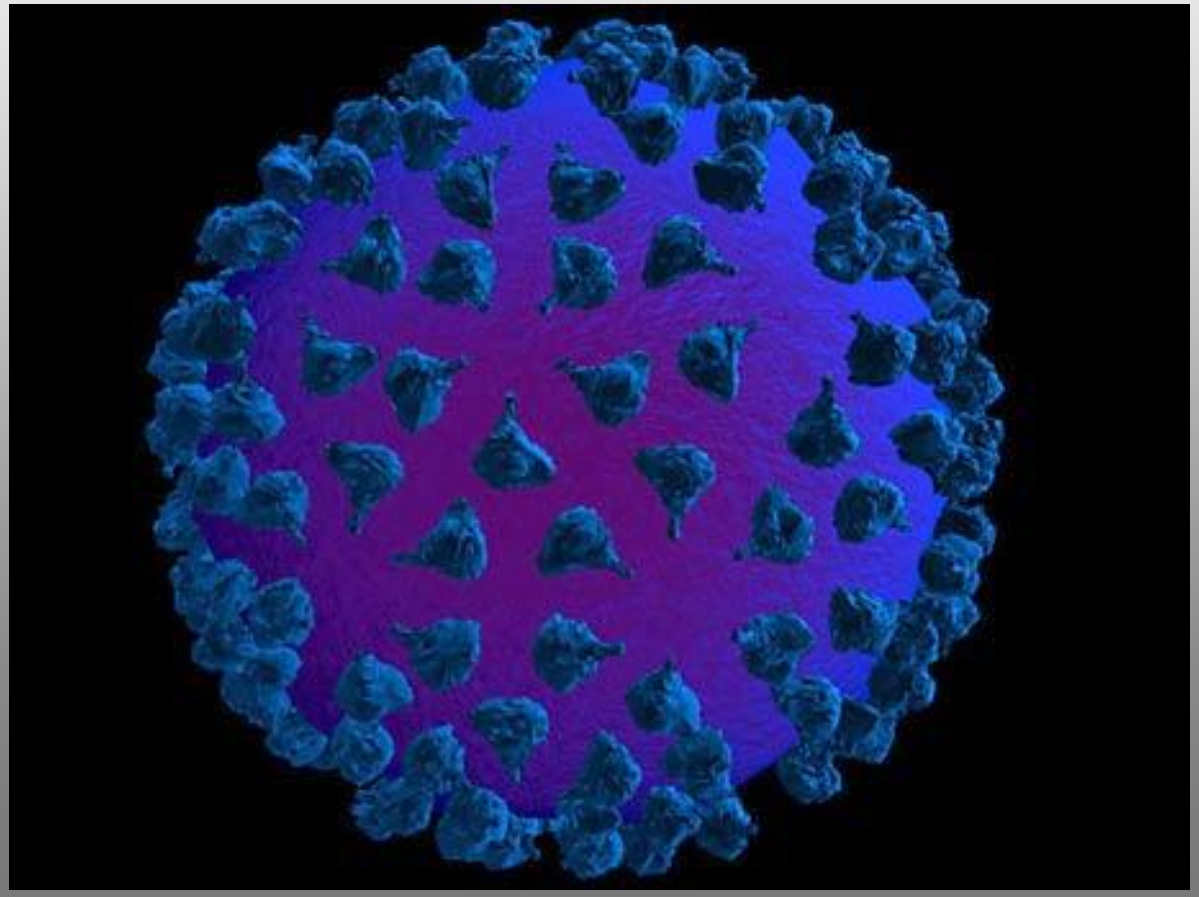
- ✓ **Lack of matched URD or cord blood**
- ✓ **Mortality risk of allogeneic BMT in children remains close to 20%**
- ✓ **Mortality risk of allogeneic BMT in adults is up to 30-40%**
- ✓ **Any complication of BMT delays the efficacy of BMT in X-ALD ++ (GVH, delayed hematopoietic reconstitution)**

Corriger les propres cellules de la moelle osseuse des enfants



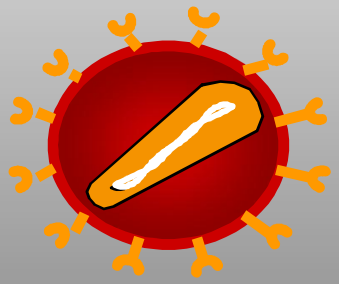
COMMENT ?

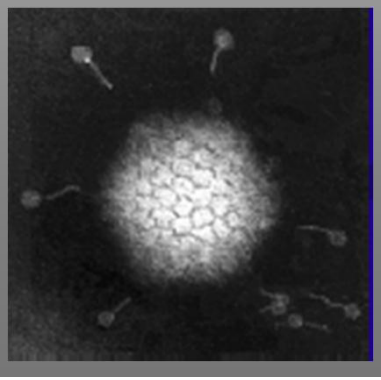
On n’a toujours pas trouvé mieux queé .



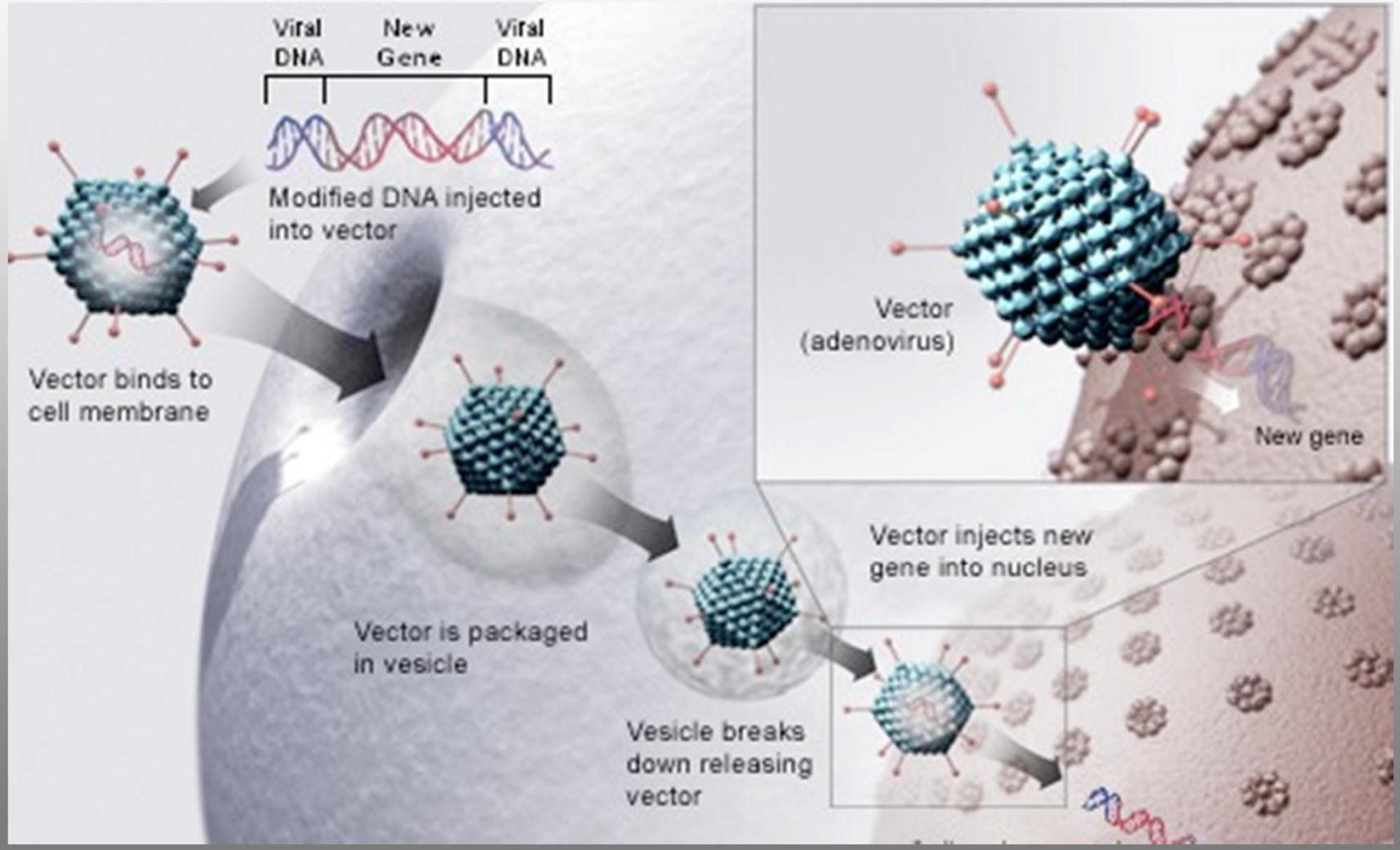
Capside

Enveloppe

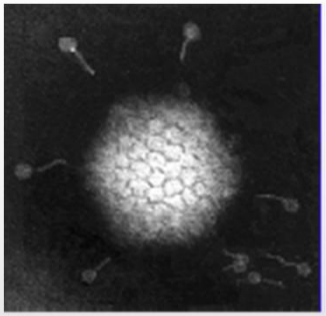


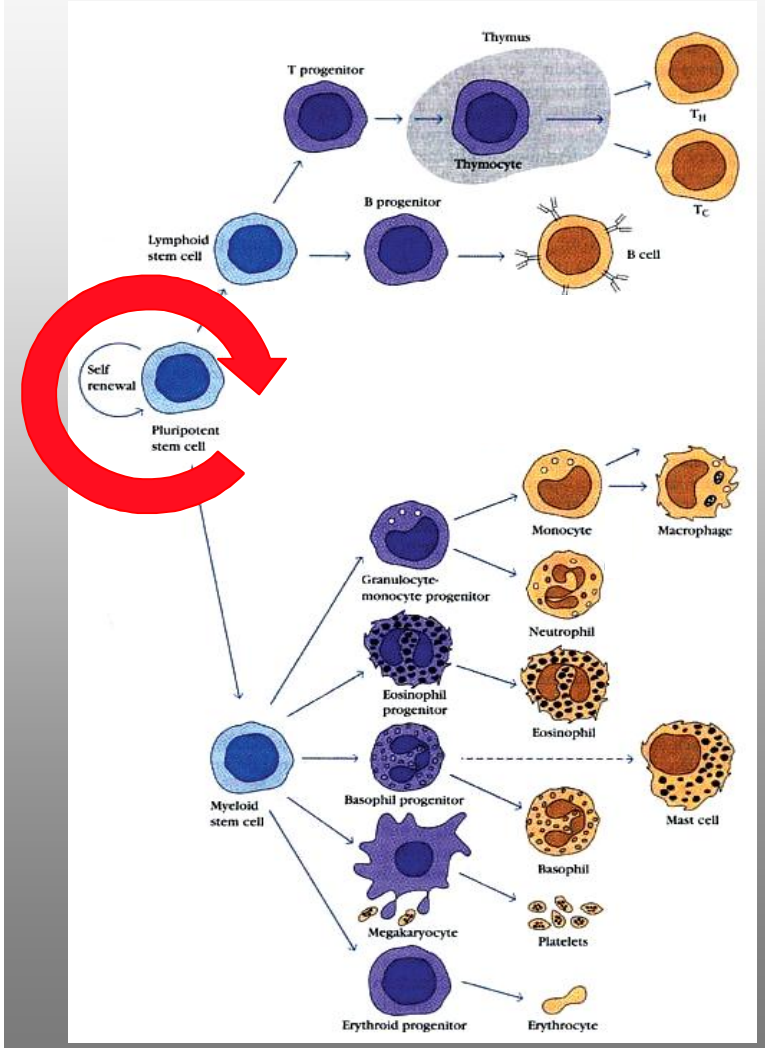


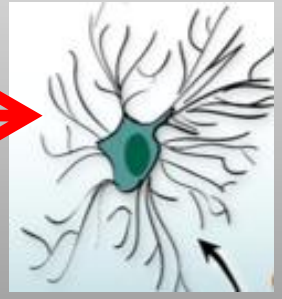
érer un gène à l'aide d'un vecteur



Which vector ?

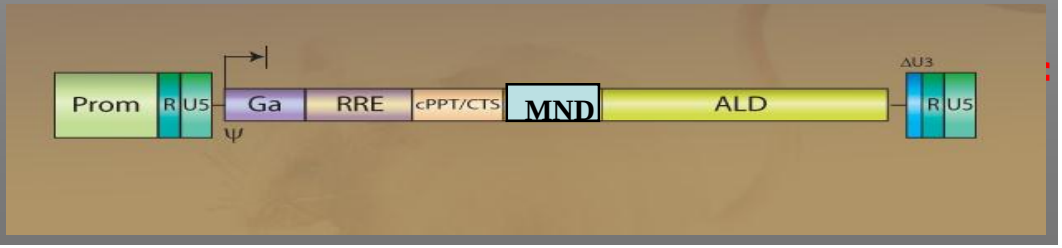




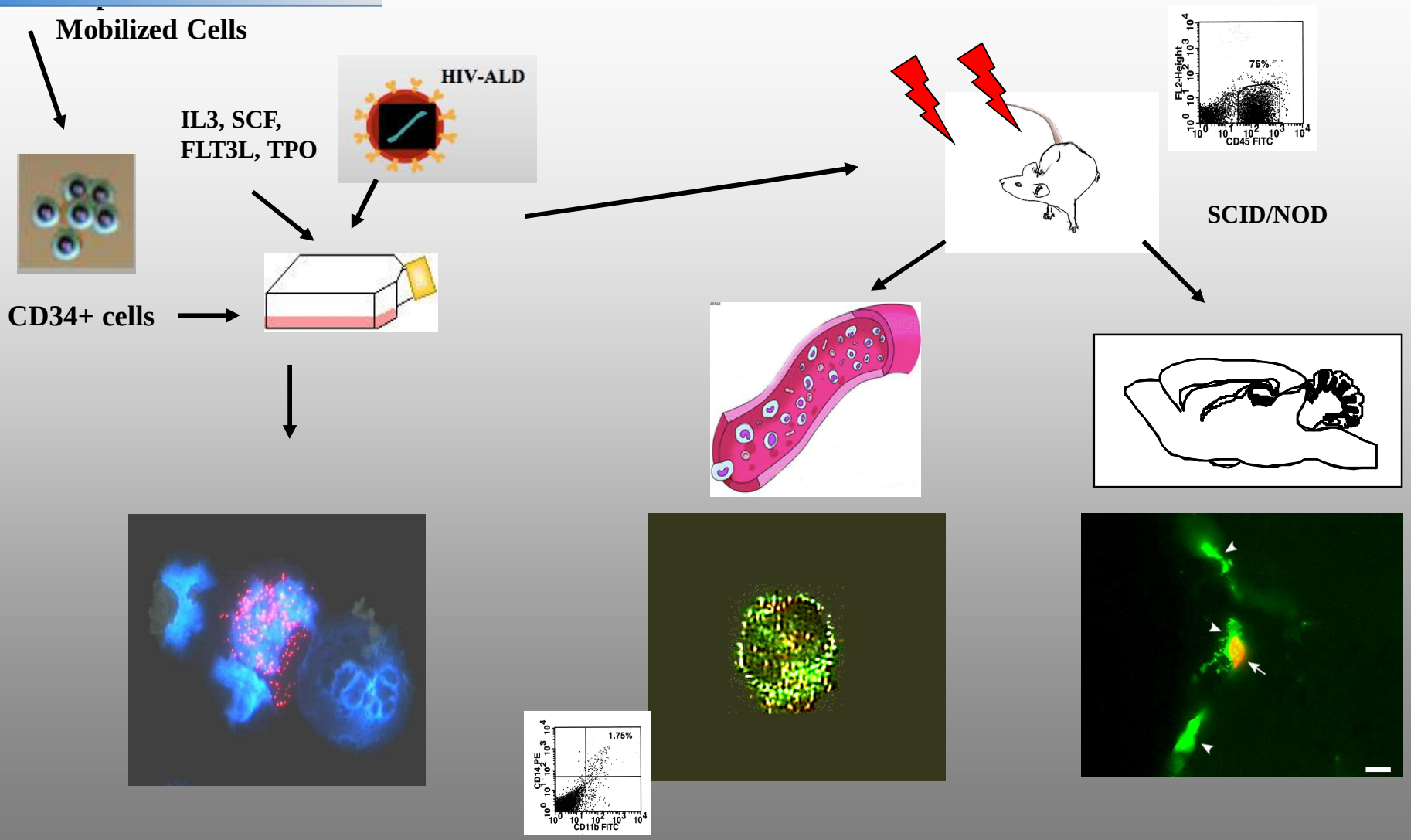


- Need for an integrative vector
- Classical retroviral vector

HIV1-derived lentiviral vec



Lentiviral ALD gene transfer in CD34+ cells from ALD patients



45-60%
(ALDP+,CD34+)

Human monocyte
(ALDP+, CD68+)

Human brain microglia
(ALDP+, RCA+)

lanning towards clinical trial in ALD (2002-2005)

- Development and Production of the clinical grade lenti-ALD vector (Cell Genesys Inc, USA)
- Viral safety issues: vector and transduced CD34+ cells
- Risk of insertional mutagenesis
- Scale-up of the transduction protocol to the clinical conditions
- AFSSAPS: pre-IND and discussions

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AFSSAPS: pre-IND and discussions with experts ++++

- Scale-up of the transduction protocol to the clinical conditions
- Final authorization from AFSSAPS : december 2005

Phase I/II study
gene transfer to CD34$^+$ cells followed by autologous transplantation

5 ALD patients (5-15y) with cerebral demyelination, candidate for HSCT, without HLA-matched donor or UCB

ÅCD34+ cells from ALD patients are purified from PBC, transduced ex vivo, frozen until re-infusion (for RCL assays). Re-infusion of > 4 x 10^6/kg

--> 3 x 10^6/kg non-transduced CD34$^+$ cells/kg are preserved (in case of graft failure)

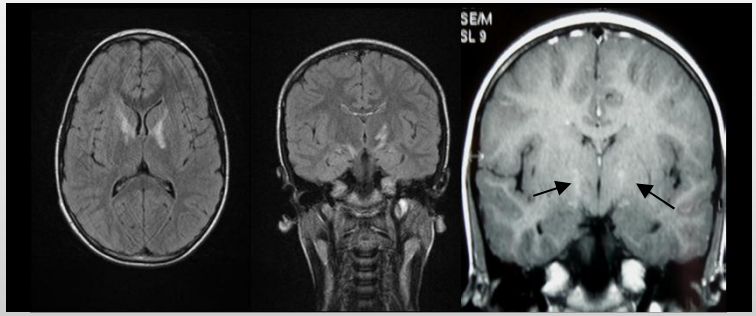
Åmyeloablating conditioning

busulfan (4 mg/kg x 4 days: D_{-10} to D_{-7})
cyclophosphamide (50mg/kg/day x 4 days: D_{-5} to D_{-2})
followed by re-infusion of Ó4 x 10^6/kg transduced CD34+ cells (D_0)

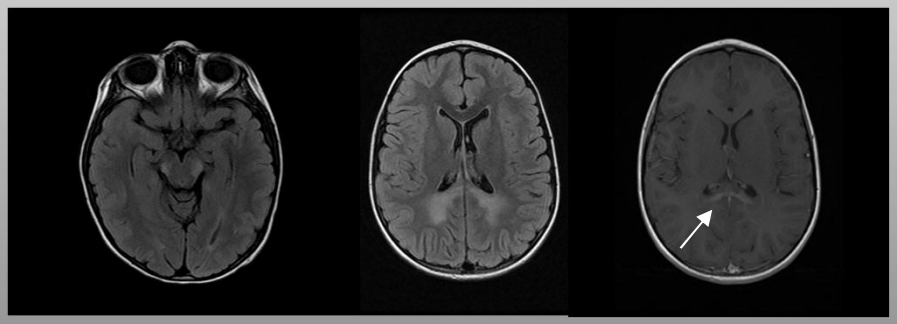
SC gene therapy in X-ALD

Å **3 patients treated**

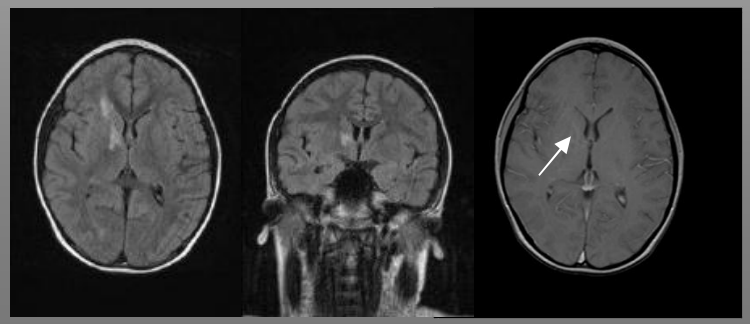
patient 1 : LS score = 2,25
gadolinium +
7 1/2 y, ALD protein -



patient 2 : LS score = 7
gadolinium +
7 y, ALD protein -



patient 3: LS score = 2
gadolinium +
7 y, ALD protein + decreased



Endpoints

- Safety
- Gene marking and transduction of HSC
- Neurological outcome

I - Safety

C gene therapy in X-ALD : Safety

- Re-infused with:
  4.6x10^6 CD34+/kg (P1), 7.2x10^6 CD34+/kg (P2), 8x10^6 CD34+/kg (P3)

- Procedure was well tolerated without complications

- Hematological recovery at day 15, complete for immune functions at 12 months (P1, P2 & P3)

- normal cellularity of bone marrow aspirate
  P1, P2, P3 at 12 months and P1 & P2 at 24 months

- Safety of lentiviral vector up to 36 months (P1), 30 months(P2) and 16 months (P3)

  - all RCL tests negative
    (gag-pol, HIV1 western-blot, HIV1-ELISA x 2, VSV.G DNA)

  - Polyclonal hematopoietic reconstitution without detectable genotoxic effects due to lentiviral vector insertion in or close to genes

Safety

Any detectable signs of genotoxicity ?

✓Lentiviral vector integrates into the genome like murine gamma retrovirus

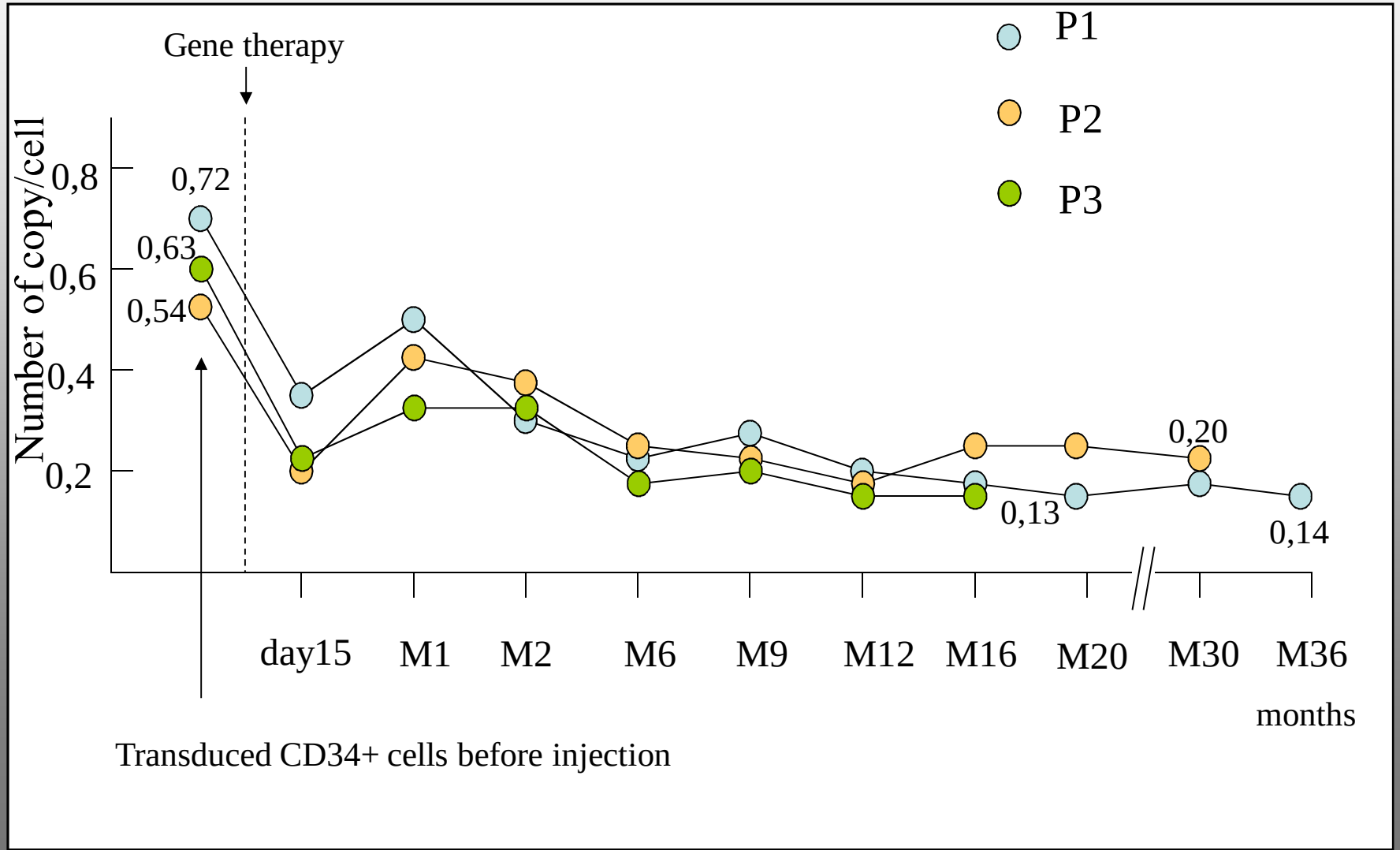
✓Insertion of any DNA fragment into, close to or even at distance of gene may modify gene expression (proto-oncogenes, miR, SNPs acting in cis or trans on gene expression) etc..) resulting in the emergence of « dominant clones »

✓Tracking the frequency of insertion site retrieval allows to assess potential genotoxicity effect:

if a dominant clone emerge, the retrieval frequency for lentiviral insertion in such cell clone must be more frequent

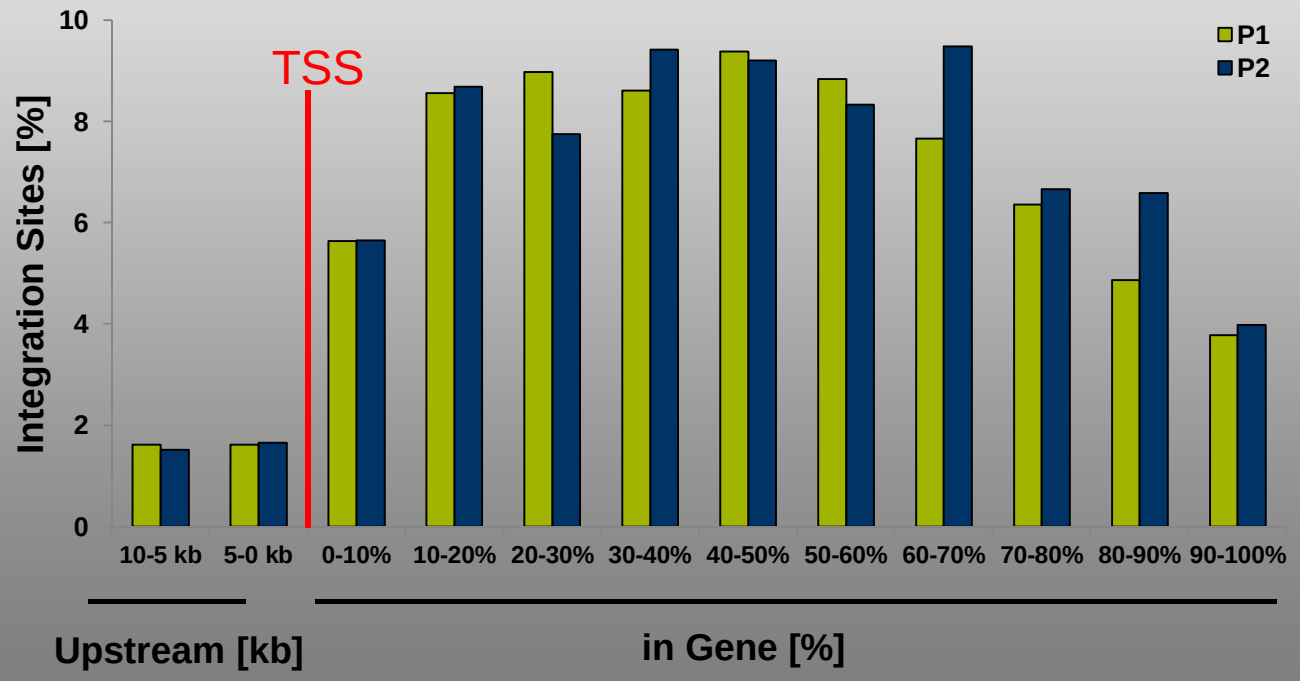
Safety

Number of integrated vector copy post-transplant in peripheral monocytes/lymphocytes



Integration in Gene Coding Regions

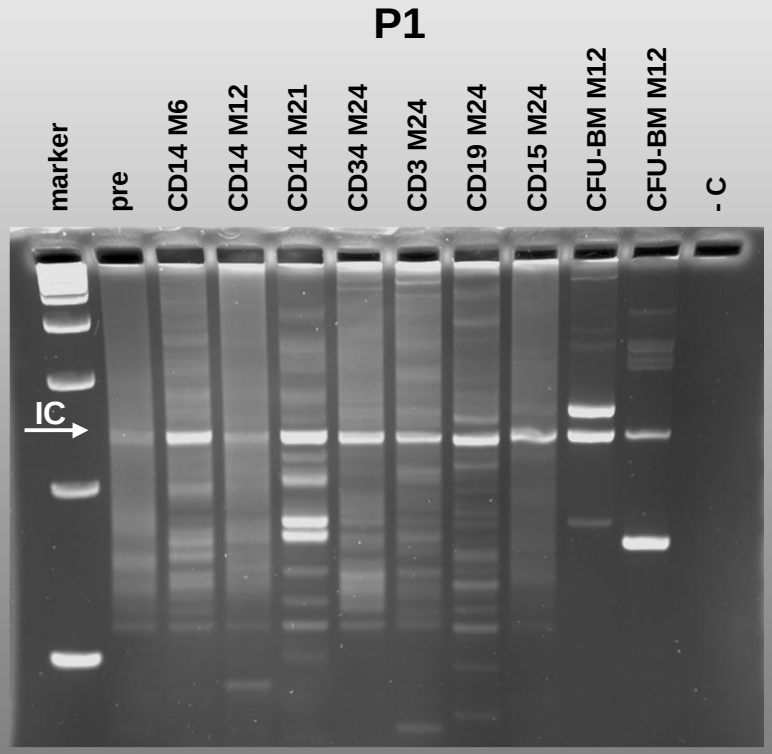
	P1 [#]	P1 [%]	P2 [#]	P2 [%]
Unique exactly mappable IS	**2217**		**1380**	
pre-transplant cells	501		486	
post-transplant cells	1719		898	
IS in Refseq Genes	1612	**72.71**	1046	**75.80**
IS in Refseq Genes +/- 10 kb	1774	80.02	1144	82.90

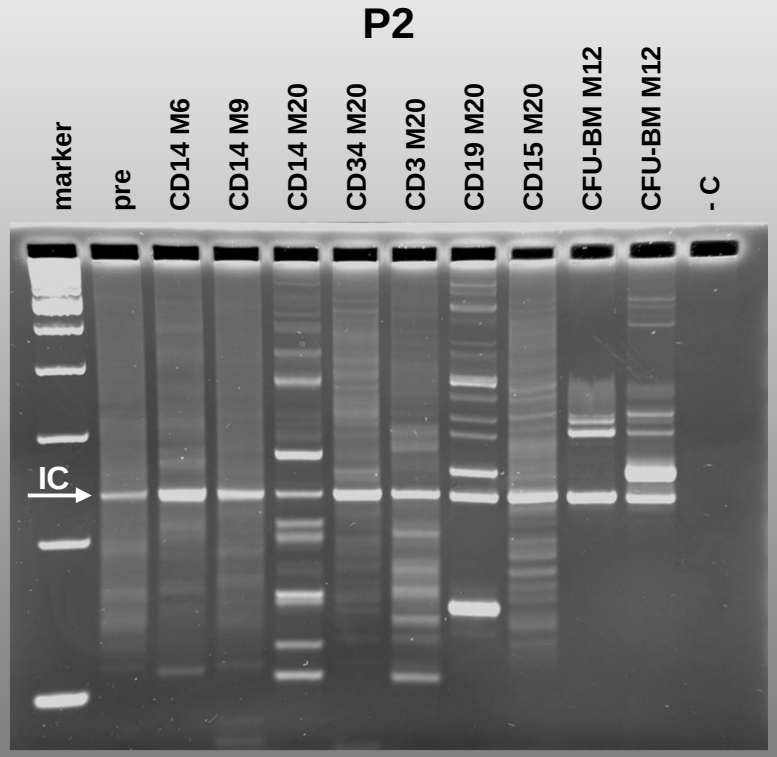


→ **Characteristic lentiviral integration profile**

Safety
Clonality of Hematopoietic Repopulation

Samples: Transduced CD34+ cells prior to re-infusion (pre)
Cell sub-populations 6 to 24 months after transplantation
BM-derived colony forming units (CFU-GMs)





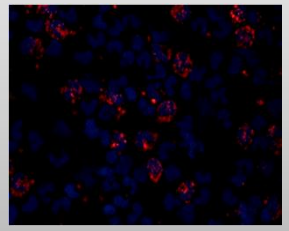
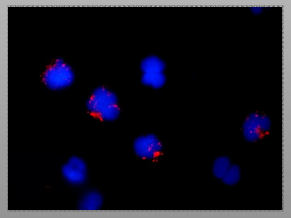
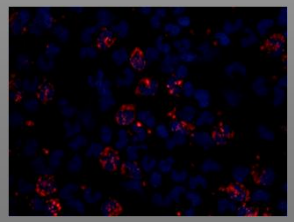
Polyclonal hematopoietic repopulation in both patients

Objectives

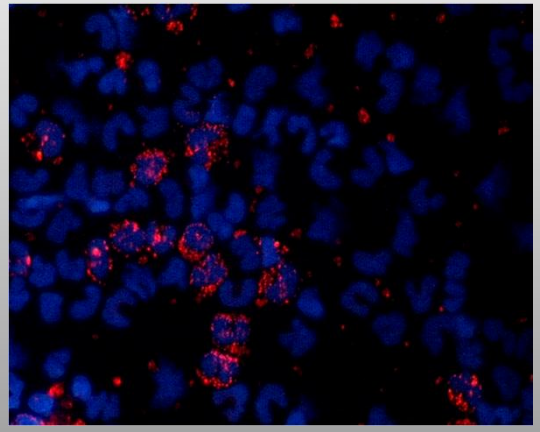
- safety
- expression of recombinant ALD protein in leucocytes ?

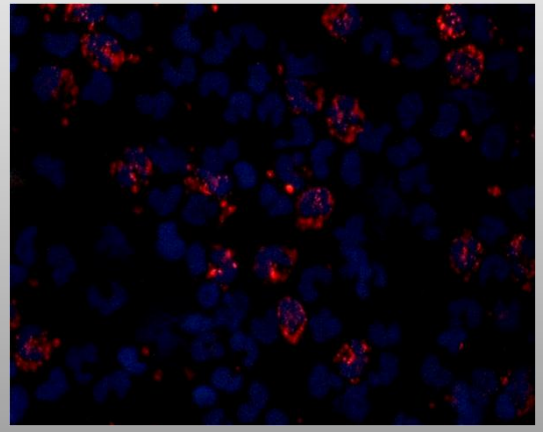
  correction of stem cells ?
- efficiency on the neurological disease

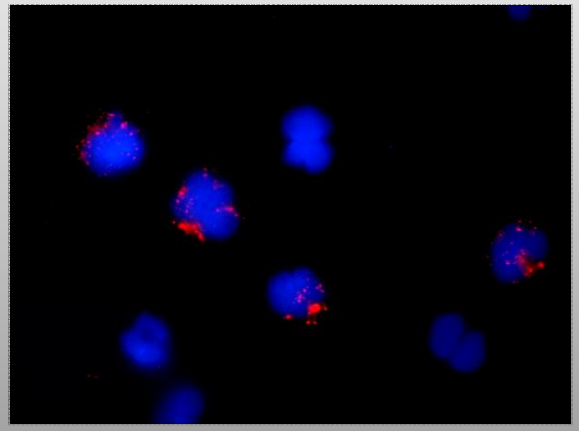
Expression ?
sduced CD34+ cells before re-infusion

	% of ALDP+ before freezing	% of ALDP+ after thawing		nb of vector copy/cell	nb of RNA copy/cell
P1	50,6	51		0,72	5400 endogenous: 1320
P2	33	34		0,54	ND
P3	42	44		0,63	ND

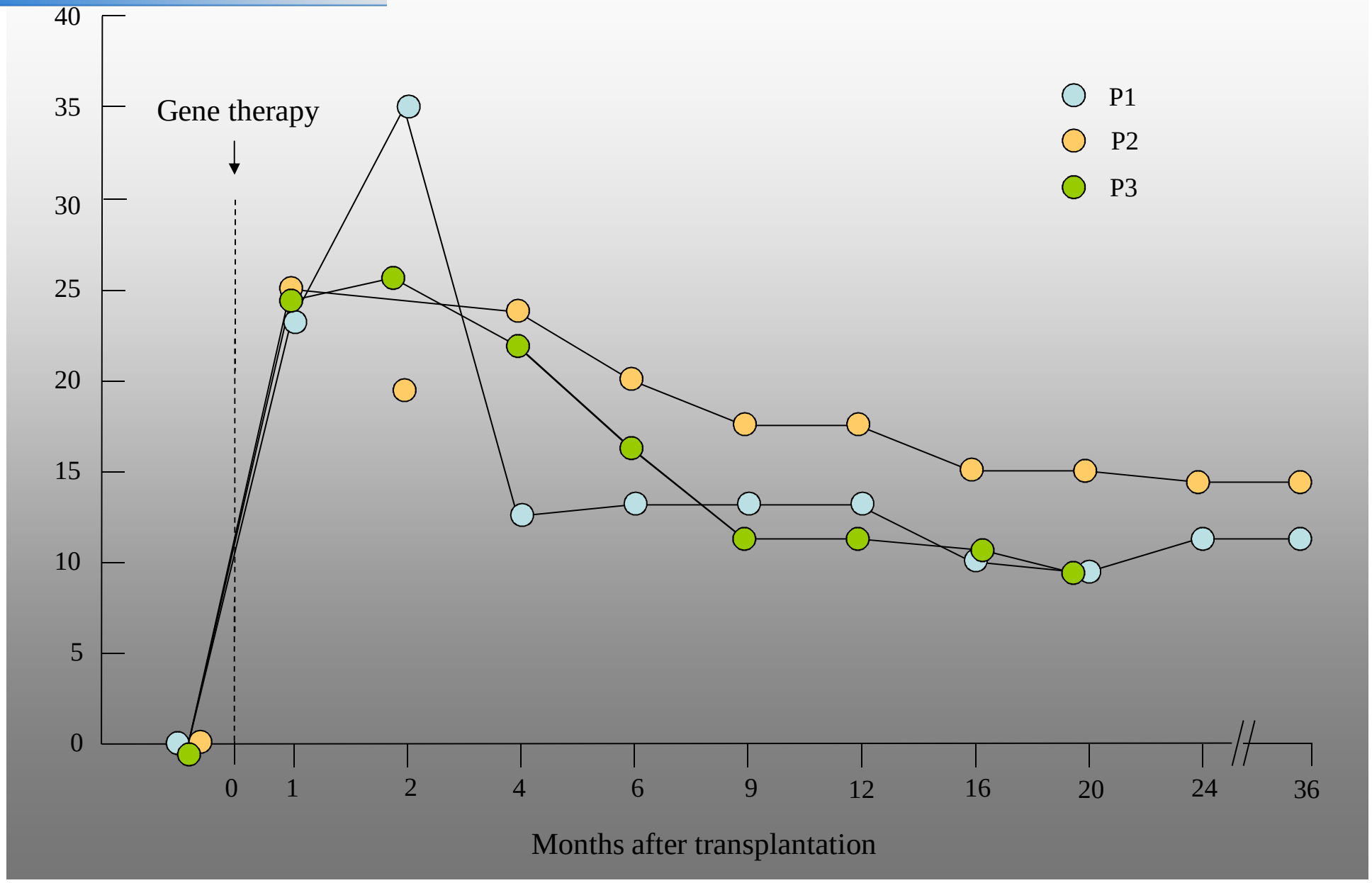
Gene marking : ALD protein is expressed in peripheral blood cells of treated patients

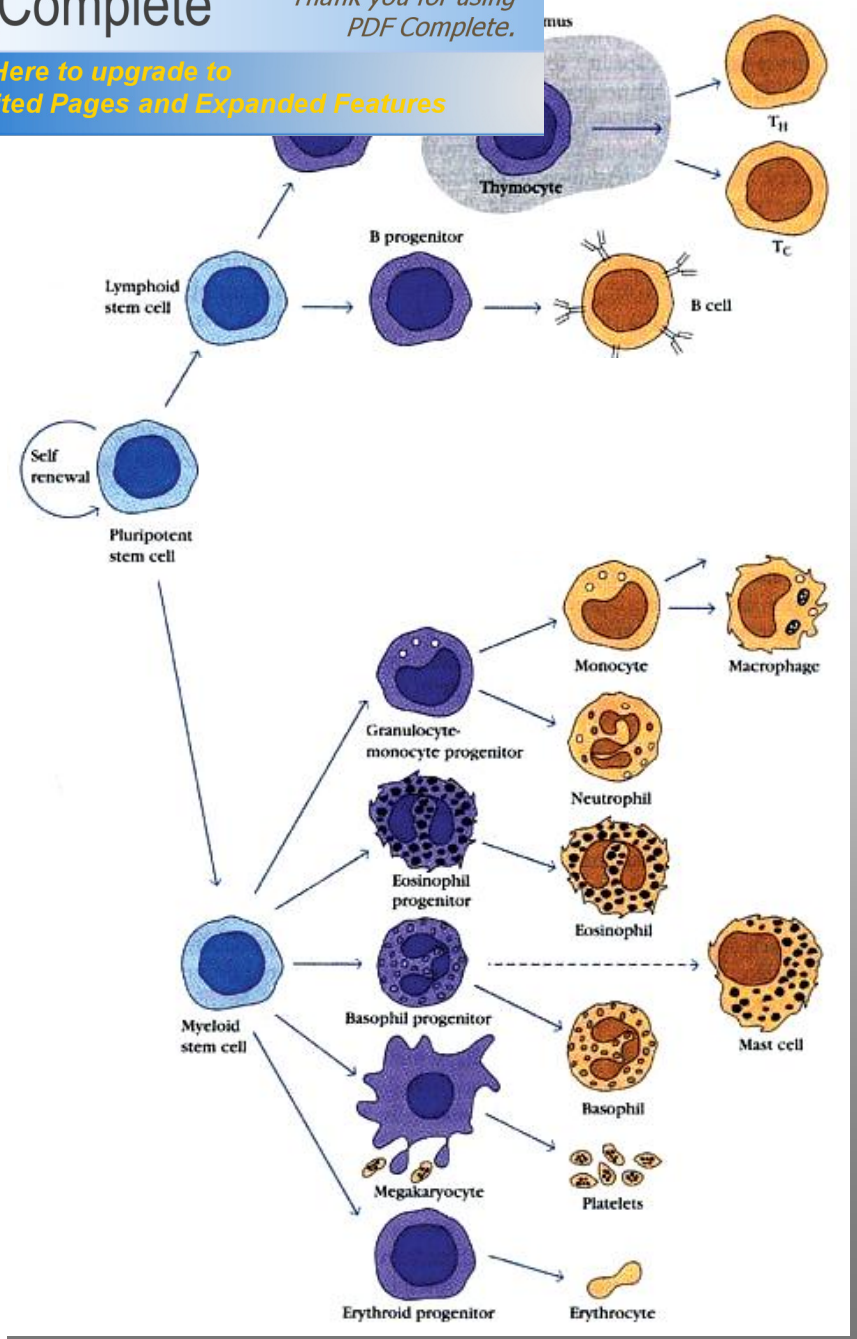






centage of ALD protein positive monocytes/lymphocytes eripheral blood after HSC gene therapy



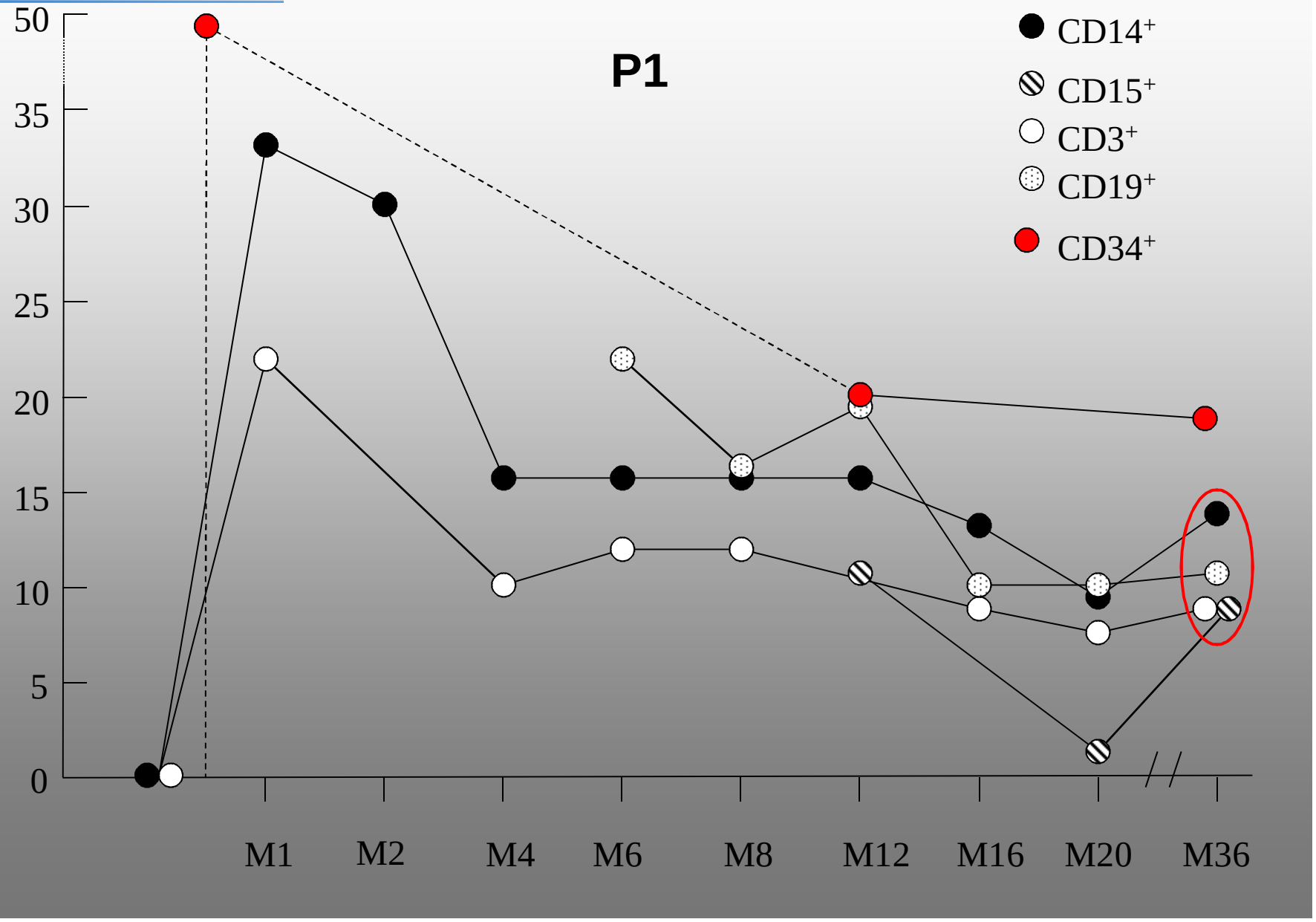


Have Hematopoietic Stem Cells been corrected ?

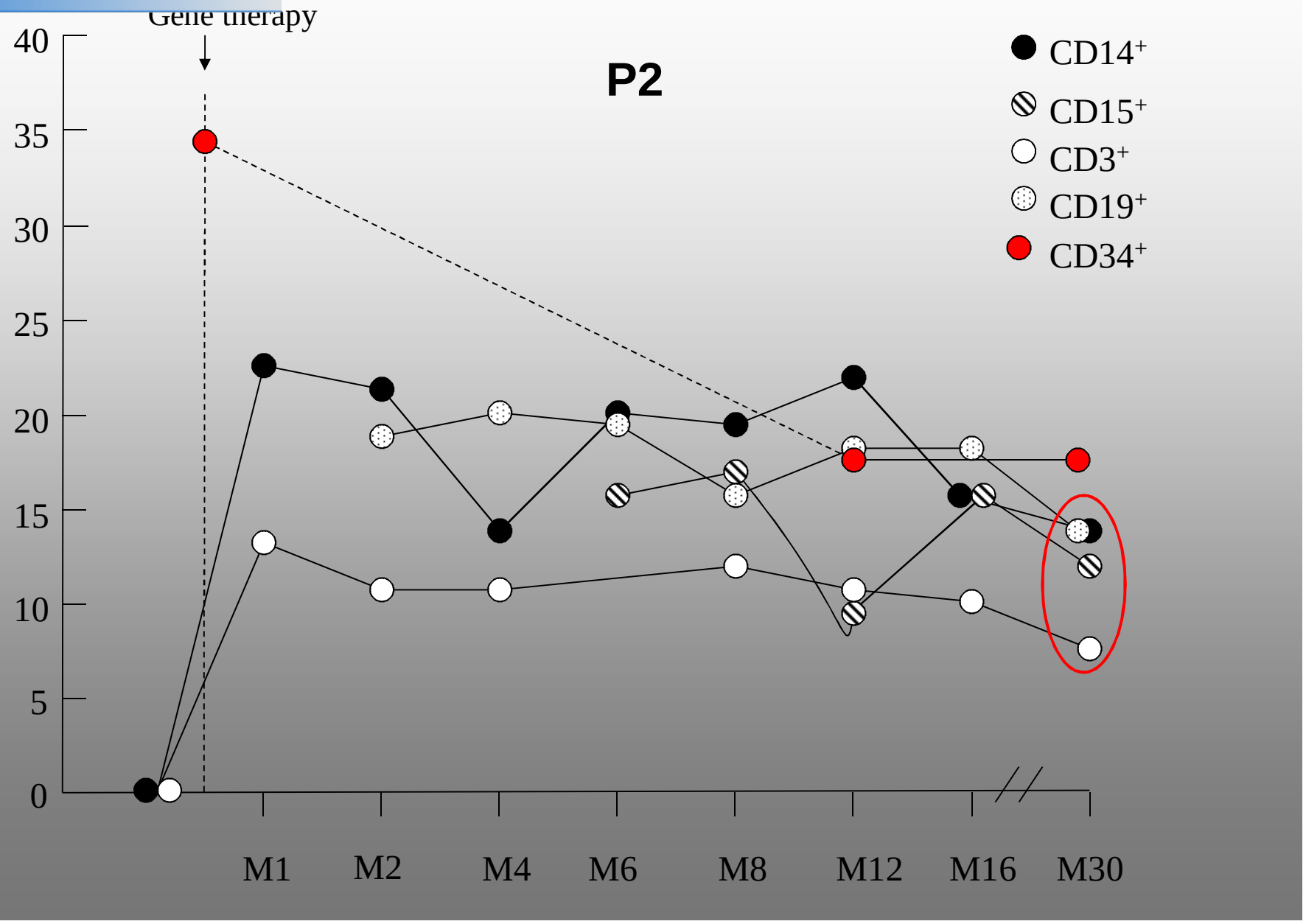
- **Expression in cells with short half-life ?**
  monocytes et granulocytes (3-4 days)
- **Expression in bone marrow CD34+ cells ?**
- **Presence of Common insertion sites**
  in lymphoid and myeloid cells ?

tes, granulocytes, T and B lymphocytes expressing ALD protein

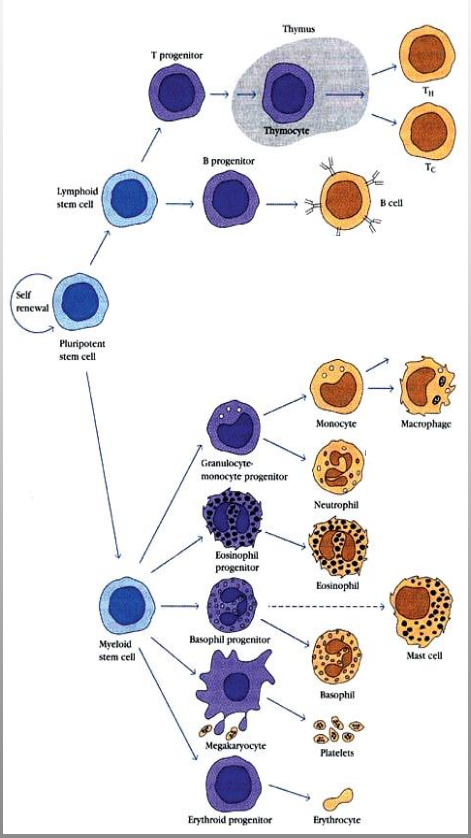
ne therapy



s, granulocytes, T and B lymphocytes expressing ALD protein



ns of lentiviral vector
n at least 4%
of both lymphoid and myeloid cells



	light blue: IS identified in myeloid cells (CD14 and/or CD15 and/or CFU)
	mid blue: IS identified in myeloid and lymphoid cells at the same timepoint
	dark blue: IS identified in lymphoid cells (CD3 and/or CD19)

Next	M6	M9	M12	M17	M21	M24
RefSeq Gene	156	374	379	498	218	221
ADRBK2						
ARHGEF3						
ARID3A						
BLID						
BRE						
BUB1B						
C11orf49						
C17orf57						
C1orf112						
C2CD3						
CCDC21						
CDC25A						
CFH						
CNTN5						
CRADD						
ERLIN1						
FCHSD2						
FRMD8						
GLCE						
GP6						
GTF2A1						
HIST1H2BC						
HLA-DMB						
HOOK1						
INPP5A						
KIAA0776						
KIAA1128						
KIAA1267						
KIAA1303						
KIFC1						
LOC93349						
LRRK2						
LYL1						
LYPLAL1						
MAPK1						
MBOAT5						
MBTD1						
MECP2						
MEIS1						
MLZE						
M-RIP						
MTHFD2L						
MYO9A						

Endpoints

- Safety
- Gene marking and transduction of HSC
- Neurological outcome

Neurologic outcome

Patient P1 (M36)

- developed mild right hemiparesis and aggravated frontal syndrome at M7
- partial regression of hemiparesis at M12 with nearly complete reversal at M16
- improvement of frontal syndrome from M12 to M24, stable since
- no changes in verbal IQ (108); decrease of performance IQ (99 -> 75)

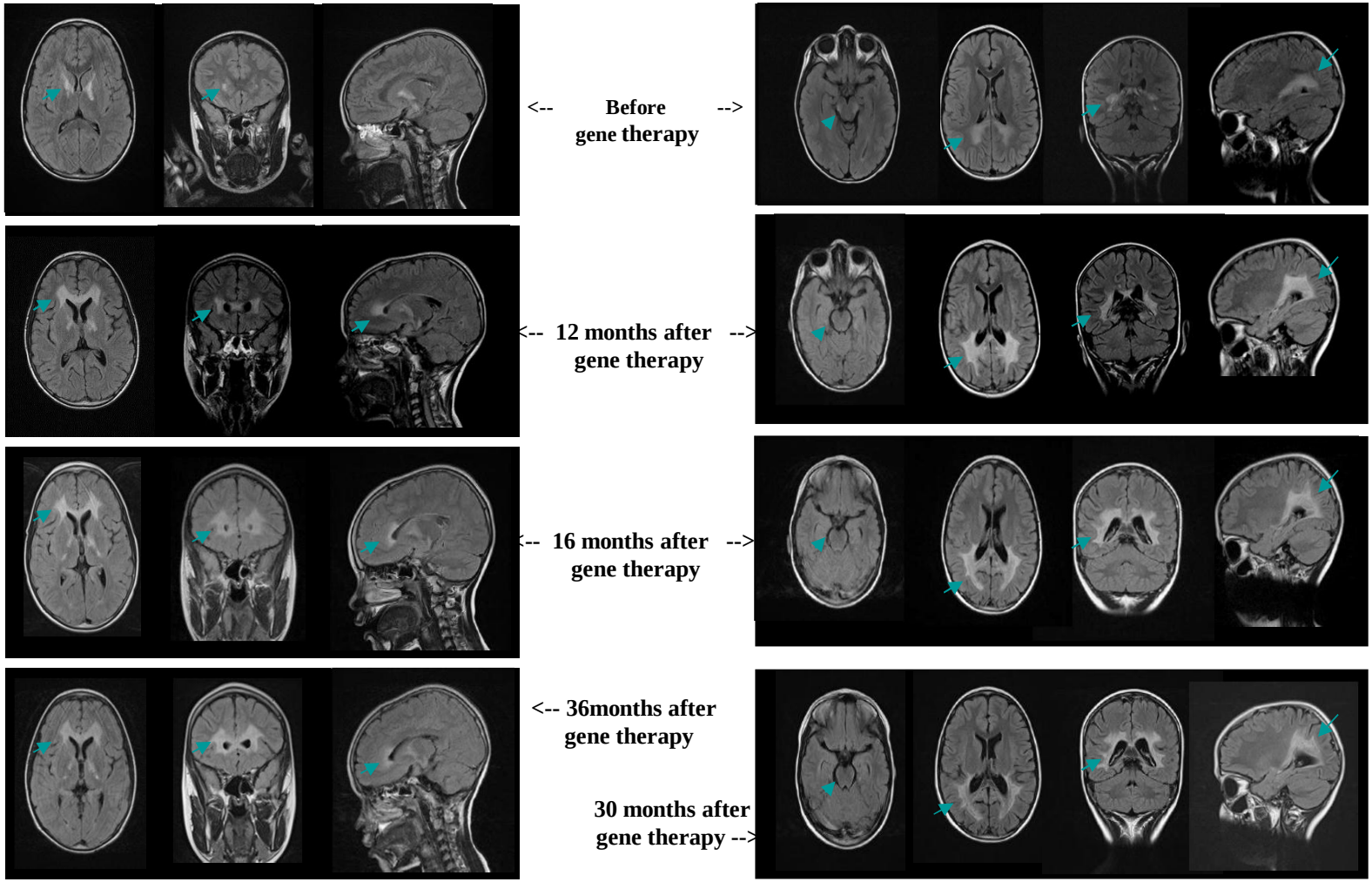
Patient P2 (M30)

- no neurologic signs, excepting bilateral inferior quadranopsia without decreased visual acuity
- no cognitive deficits, excepting moderate decrease of visuo-spatial performances in respect to other cognitive functions
- normal IQ: VIQ= 110, PIQ=114

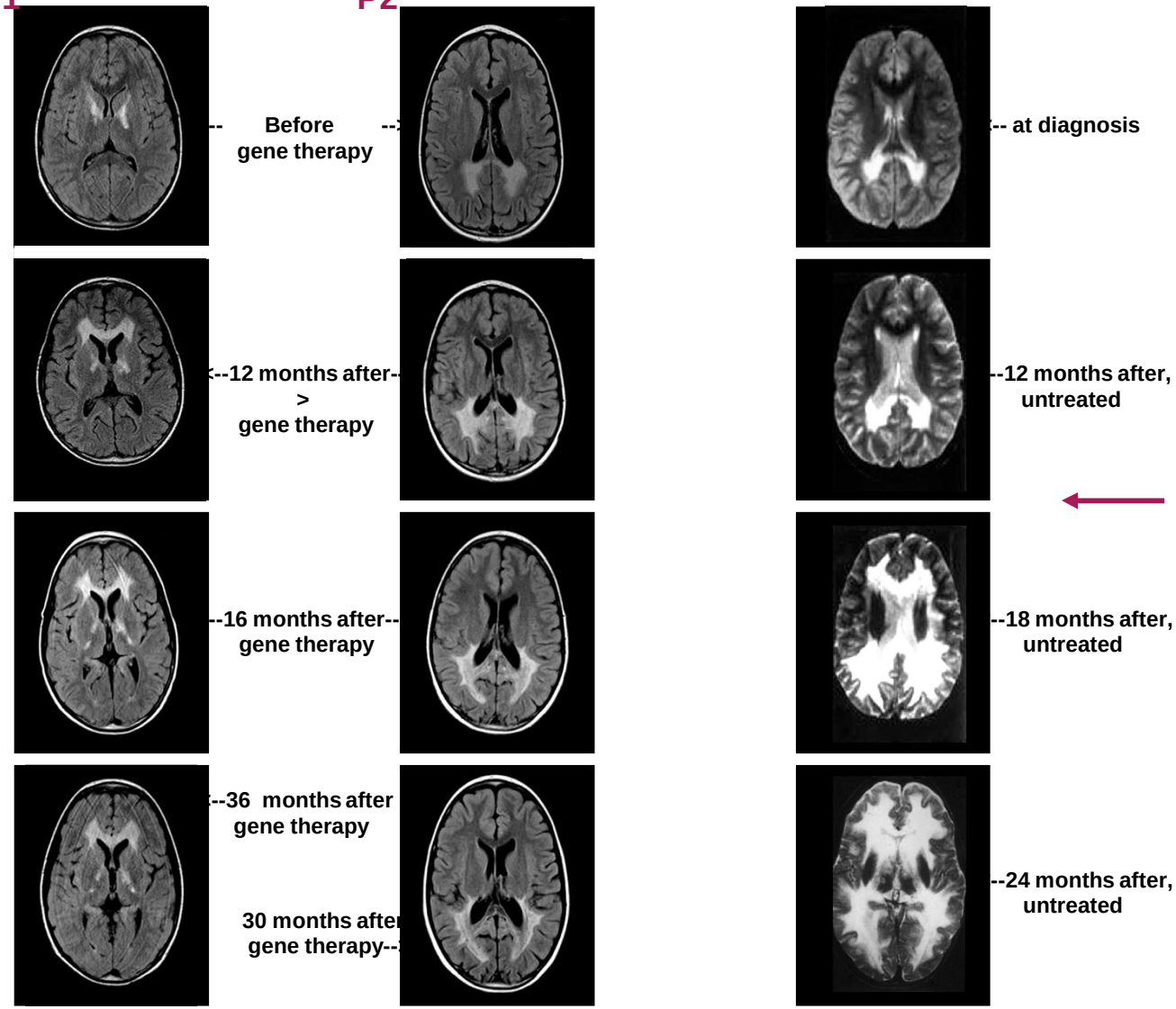
Patient P3 (M15)

- no neurologic symptoms
- « the jury is still out »

lesions progressed and then stablized
« classical » successfull allogeneic HCT



C gene therapy versus no treatment



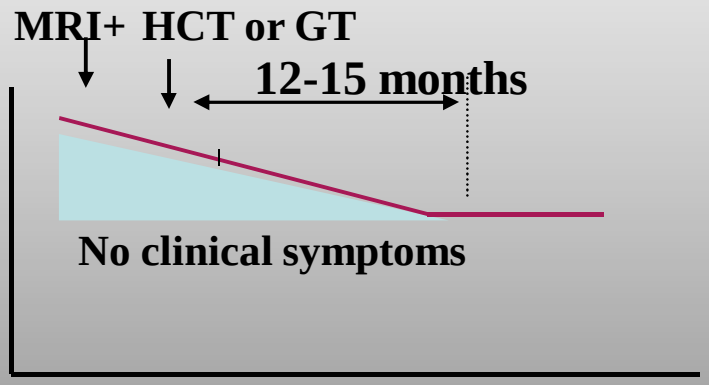
Untreated ALD patient

neurologic outcome in HSC gene therapy

Åvery similar to that observed after allogeneic HCT
(100% chimerism, uncomplicated)

→ No progression of the lesions after M12

evolution
of the disease



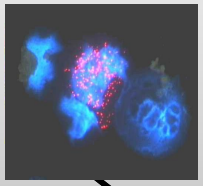
Åonly difference : delayed disappearance of gadolinium enhancement
(reflecting neuro-inflammation)
likely due to lower percentage of corrected microglia
after HSC gene therapy

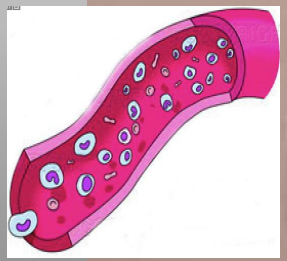
conclusions

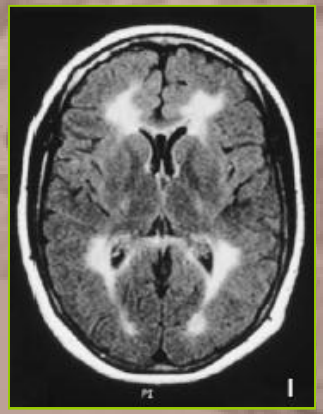
- first demonstration that lentiviral vector efficiently transduces HSC in the absence of selective advantage
- no safety concern with respect to HIV infection (mobilization of the vector and recombination)
- no safety concern with respect to insertional mutagenesis risk / genotoxicity
- first successfull gene therapy trial for a severe disease of the CNS

rapy for genetic leukodystrophies

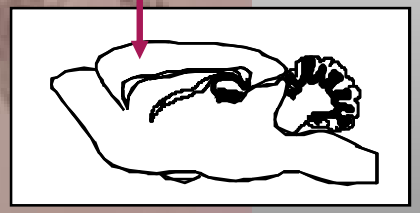
Adrenoleukodystrophy (ALD)







Metachromatic leukodystrophy (MLD)

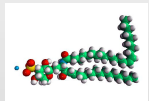


- X-linked adrenoleukodystrophy (ALD)
- Metachromatic leukodystrophy (MLD)
- Alzheimer disease (AD)

Metachromatic Leukodystrophy (MLD)

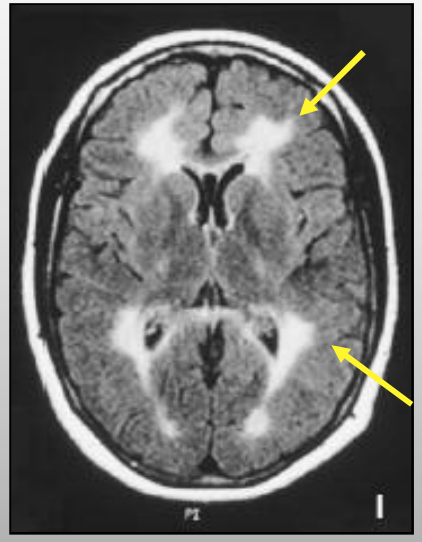
Lysosomal storage disease due to the deficiency of ArylSulfatase A (ARSA)

- 1/40 000
- ↗ Sulfatides (cerebroside-3-sulfates)

  ↓ ARSA

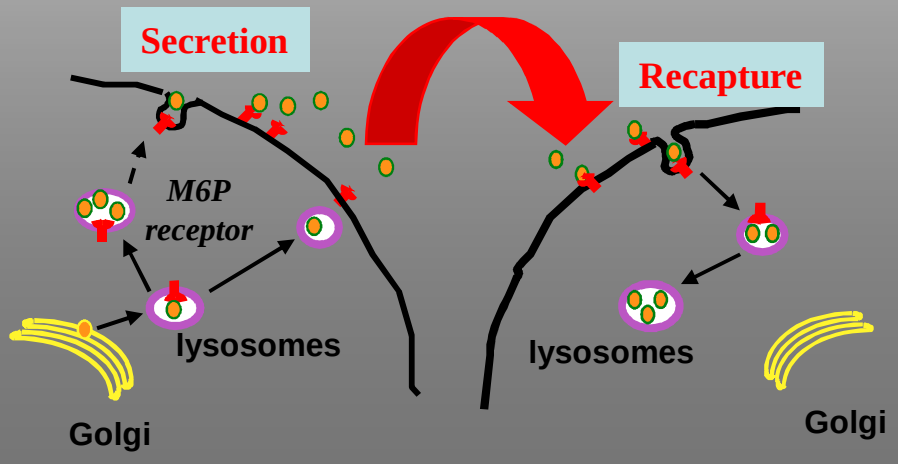
  galactosyl-ceramides



- CNS and PNS demyelination
- Late infantile form (60%)

  Early Juvenile and Late Juvenile forms

  Adult form
- ARSA can be secreted/recaptured

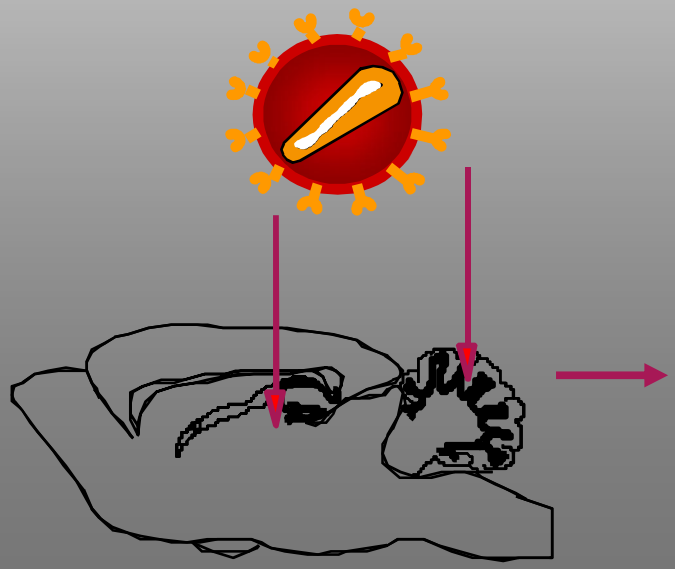


e rationale of brain gene therapy in MLD

The benefit of allogenic hematopoietic stem cell transplantation is limited to late juvenile (> 6 years) and adult forms of MLD

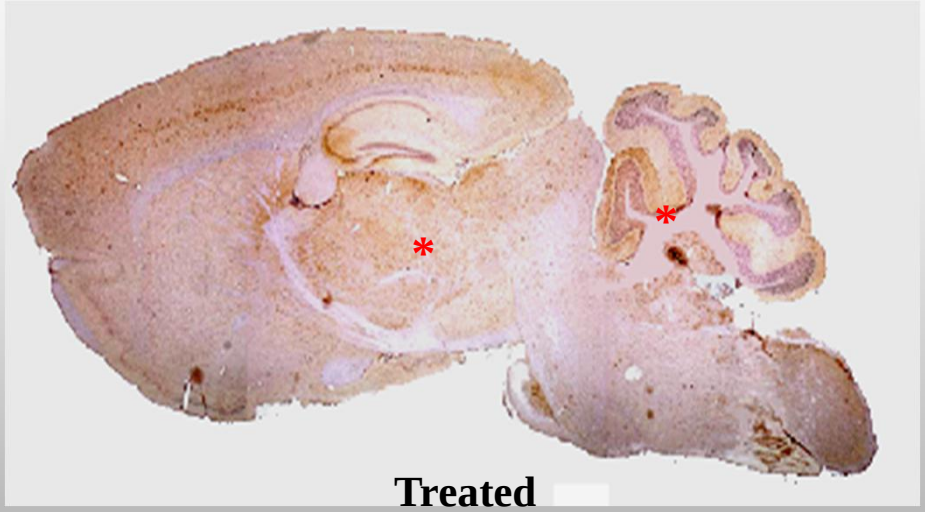
Enzyme replacement therapy ?
lysosomal enzymes do not cross the blood-brain-barrier

→ **Delivering rapidly ARSA enzyme into the brain appears the only potential therapeutic approach to have chance to result in a clinical benefit in rapidly progressive forms of MLD**

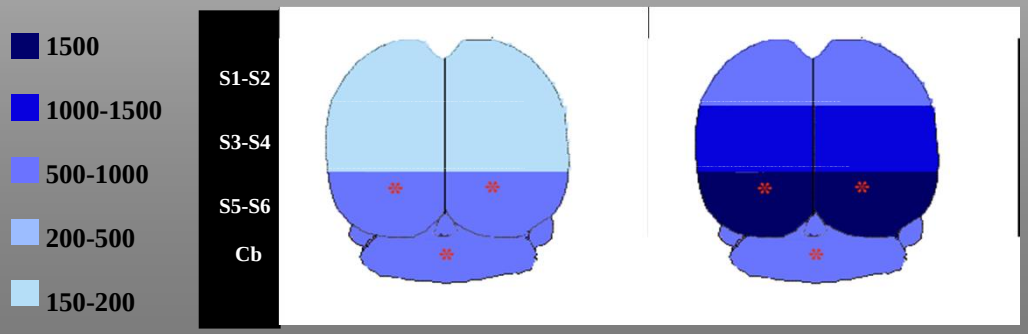


→ ***In situ* AAV gene therapy**

racerebral injections of AAV2/5-ARSA
s strong expression and diffusion of recombinant ARSA



ARSA levels (ng ARSA/mg protein on ELISA)

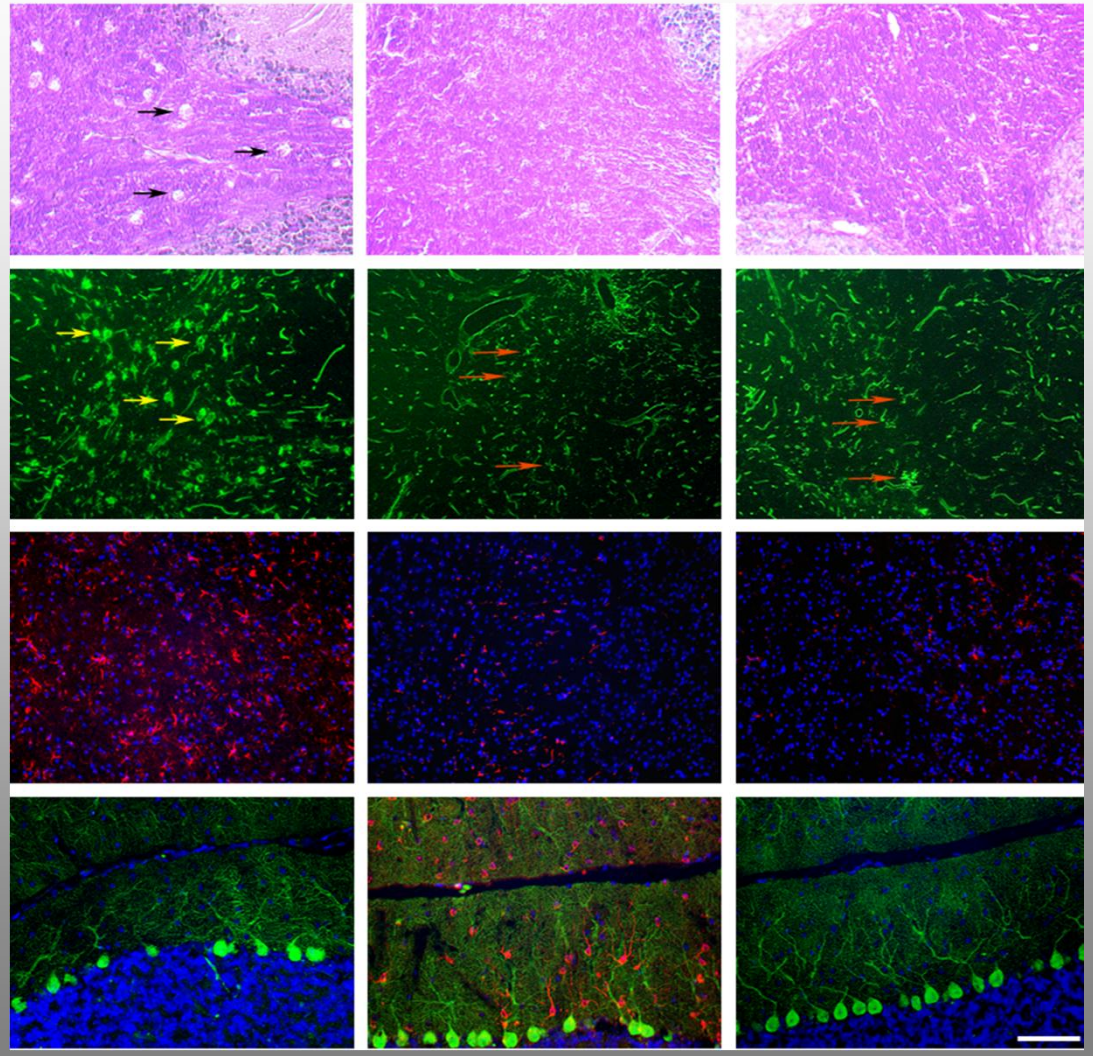


* Injection sites

Human brain: 100-150

Sevin C et al., Hum Mol Genet, 2006
Sevin C et al., Gene Ther, 2007

ection of AAV2-5/ARSA improves brain pathology



18months

PAS reactivity

Microglial activation

Astrogliosis

Purkinje cell degeneration

MLD **MLD treated** **control**

Sevin C et al., Hum Mol Genet, 2006
Sevin C et al., Gene Ther, 2007

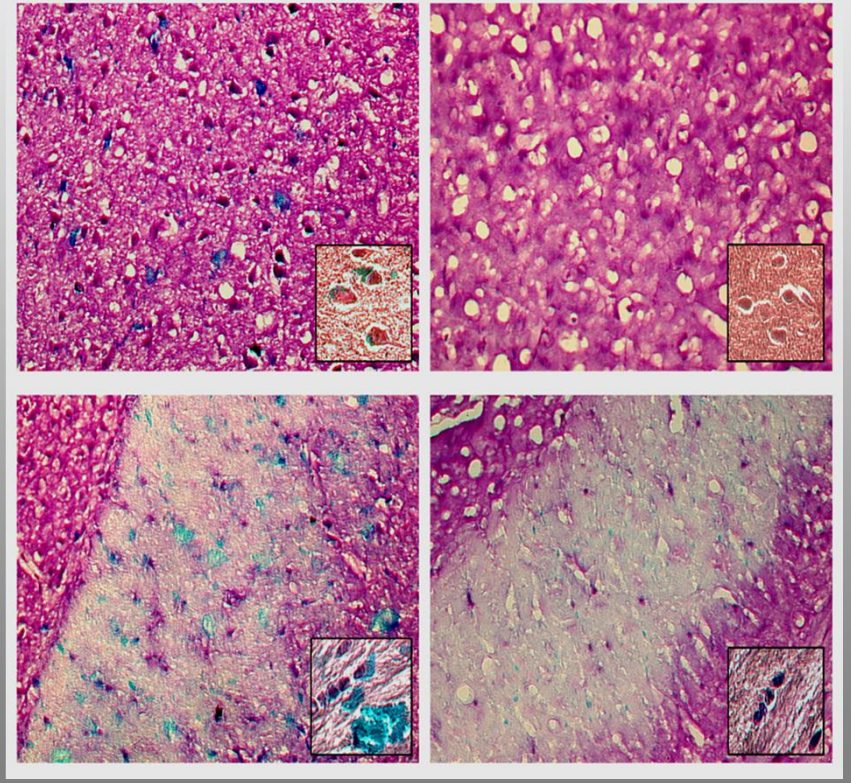
erebral injection of AAV5/ARSA fatide storage and motor impairmant

Sulfatide storage (Alcian blue)

Untreated **Treated**

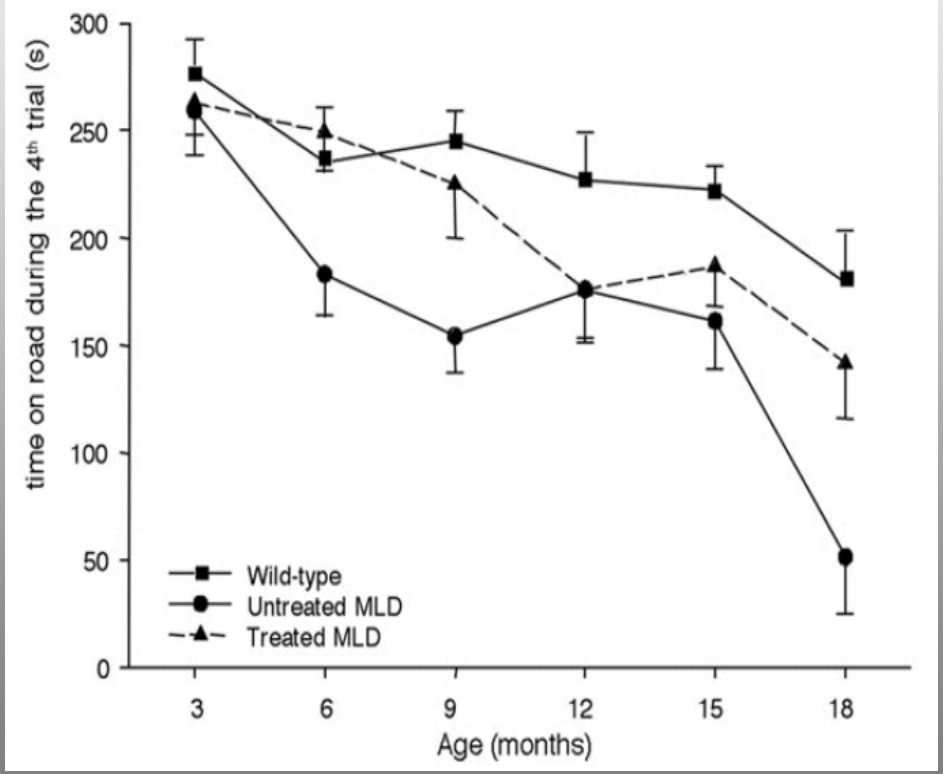
Cortex

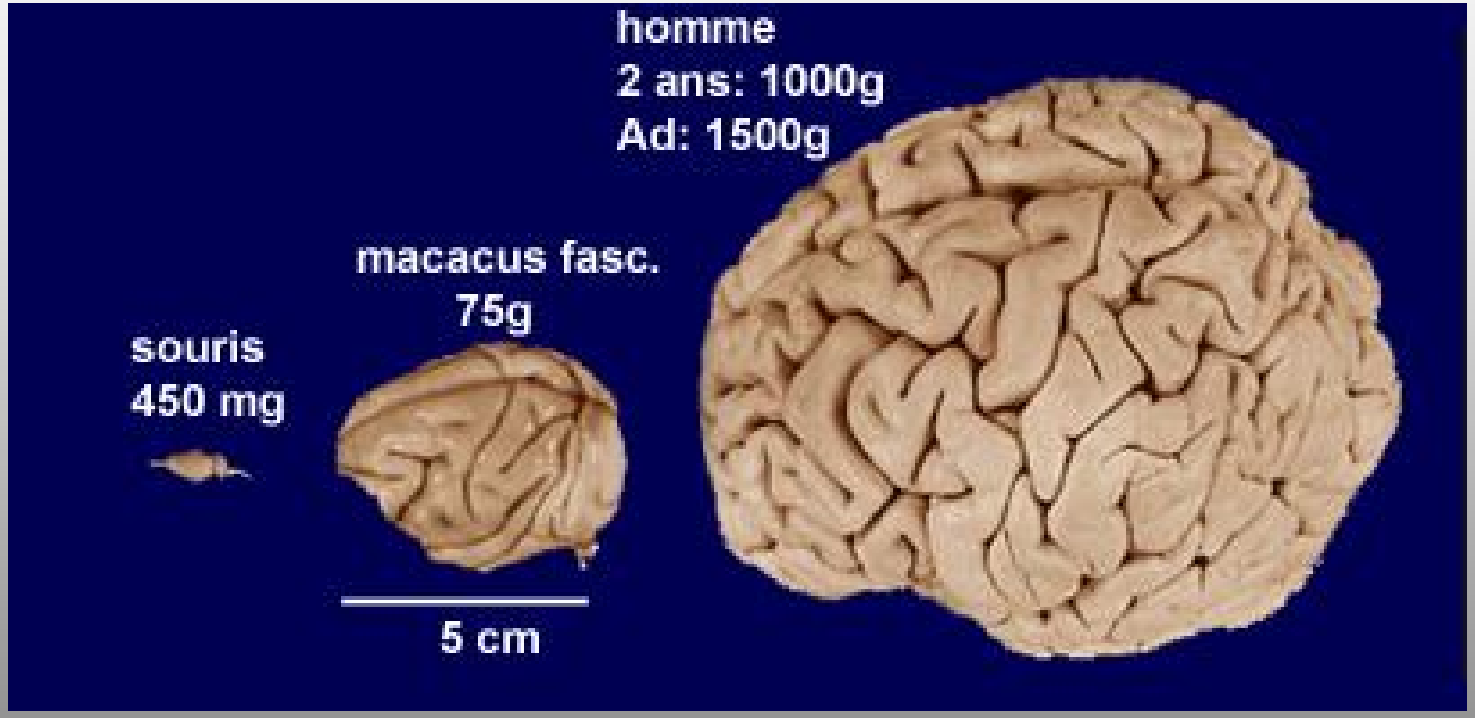
White Matter



18 months

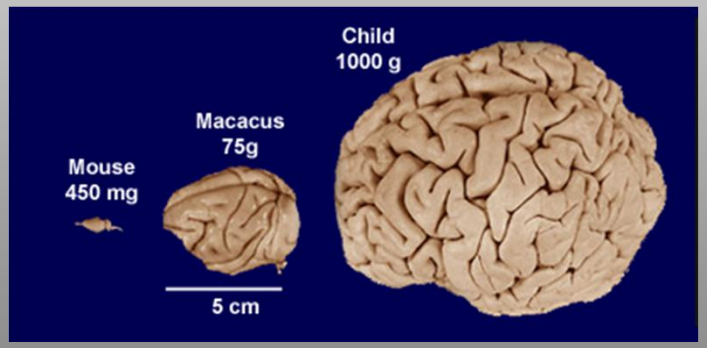
Rotarod

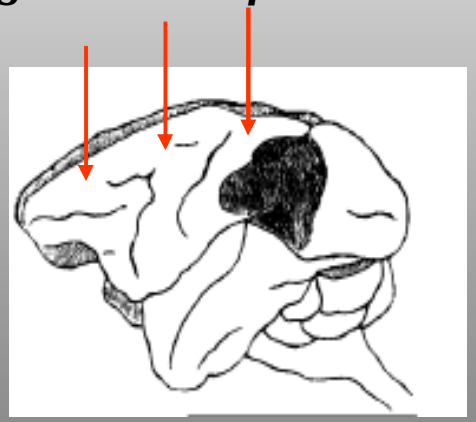




inical evaluation in large animal

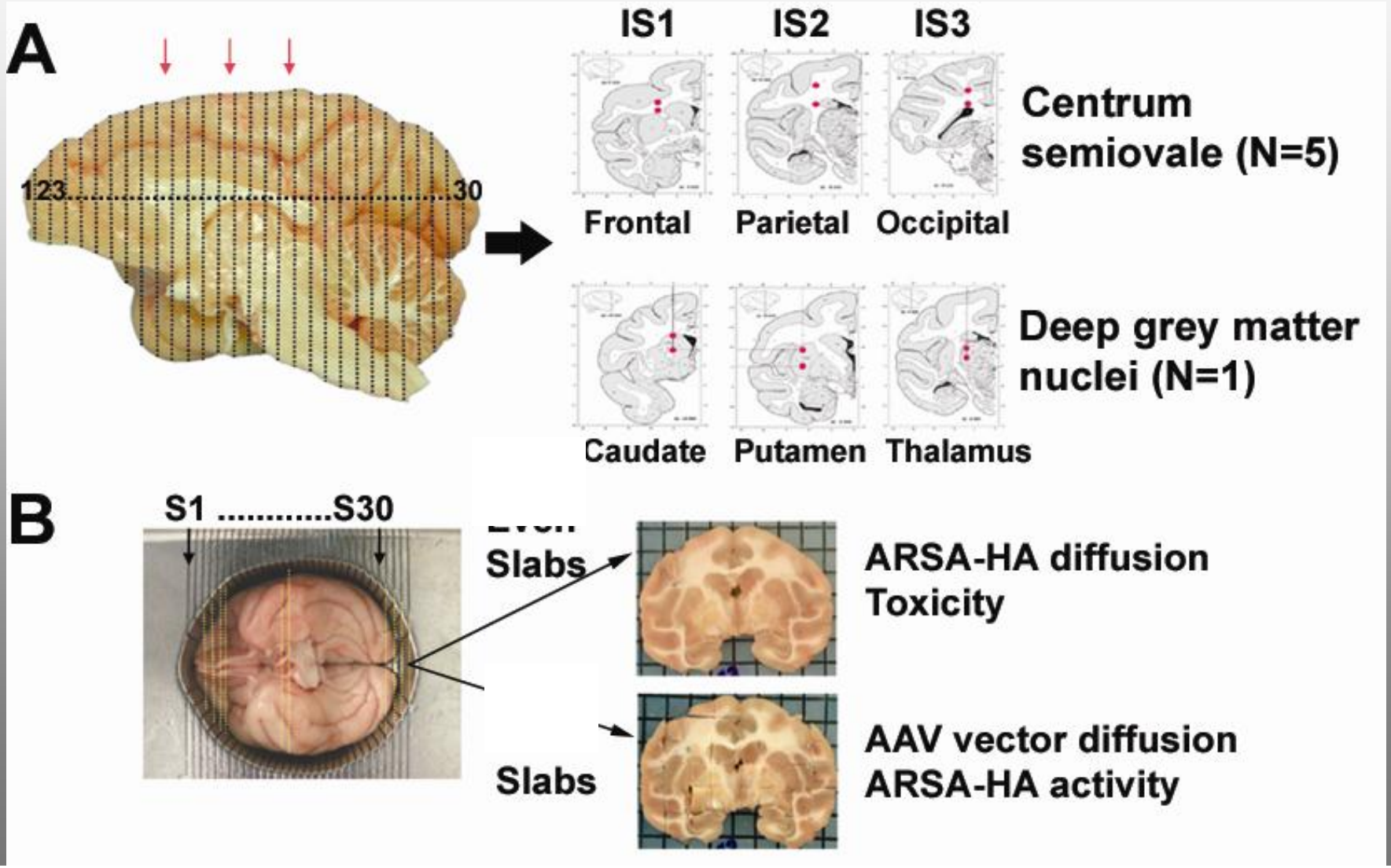
Singe Macacus fascicularis



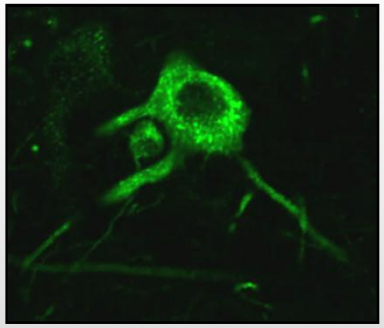


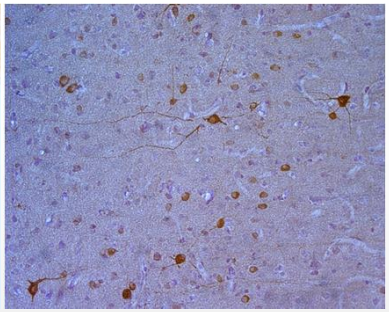
- Diffusion
- Tolerance

y and tolerance in monkeys (AAV5/ARSA-HA)

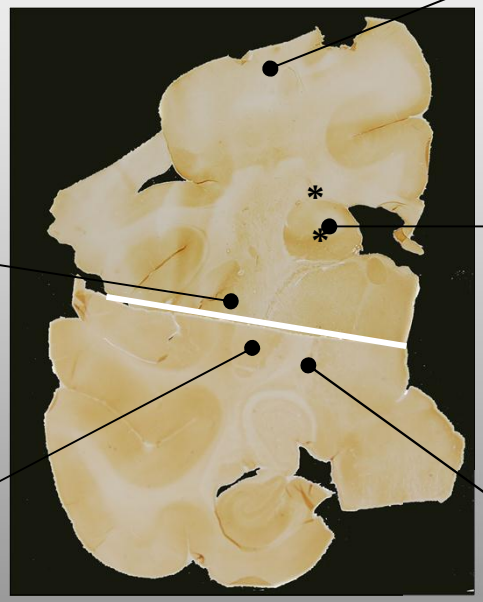


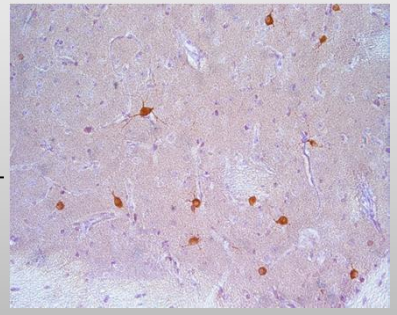
sion of ARSA-HA
emisphere



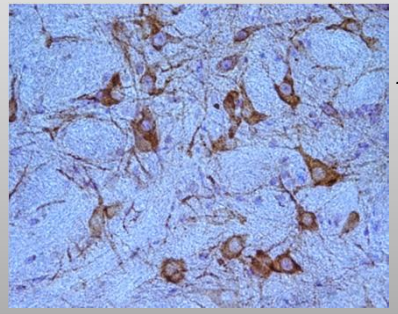


Cerebral cortex

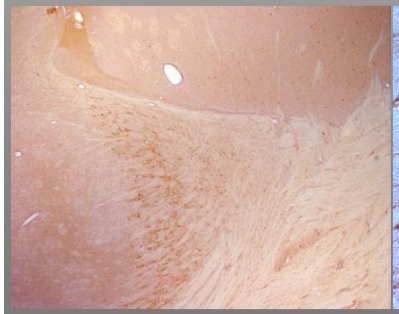


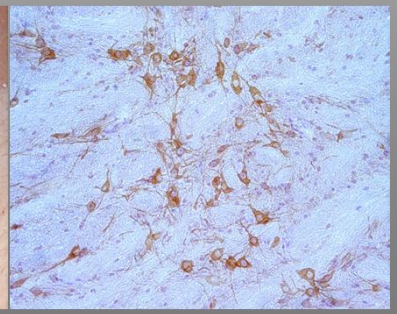


Caudate nucleus

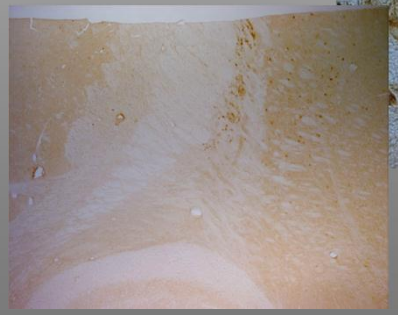


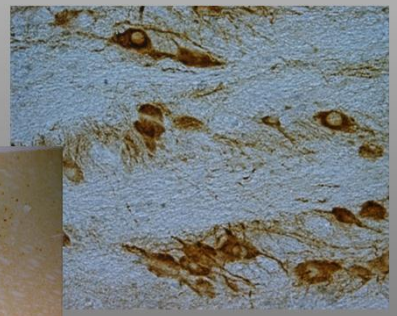
Putamen





Pallidum

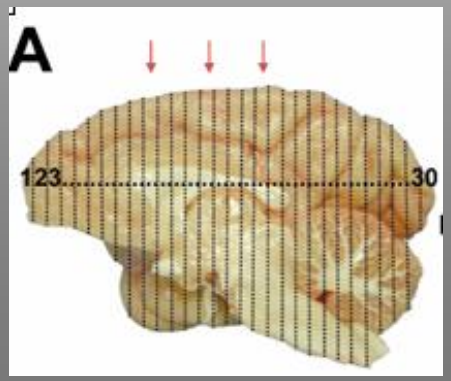




Zona incerta

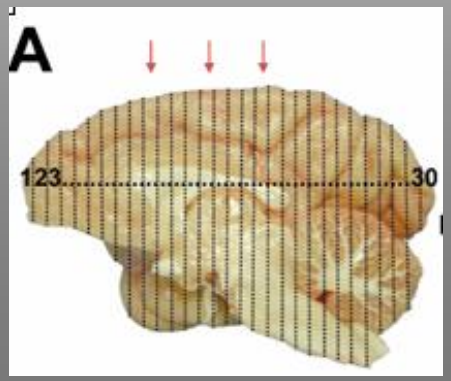
Diffusion of the AAV5-ARSA vector

Monkey	Antero-posterior diffusion (mm)	Mean VGC (and maximum value at injection site)	Diffusion volume* (cm^3)	% of the injected hemisphere containing AAV vector**
Prim1	32	8.0 ± 2.3 (138)	12.1	37.2 %
Prim2	38	4.0 ± 0.9 (137)	13.3	40.8 %
Prim3	45	2.3 ± 0.6 (86)	14.1	43.3 %
Prim4	45	1.7 ± 0.5 (47)	12.0	36.9 %
Prim5	48	5.3 ± 1.5 (238)	15.1	46.4 %
Prim6	36	2.5 ± 0.7 (42)	12.8	39.3 %

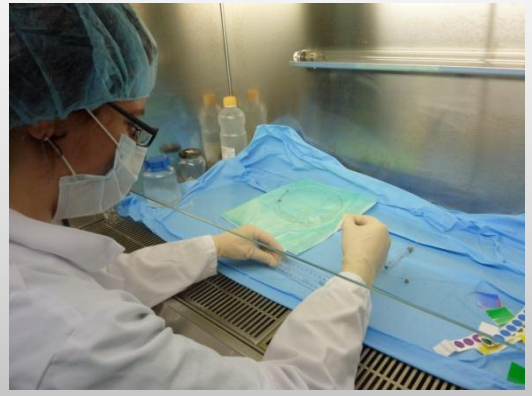


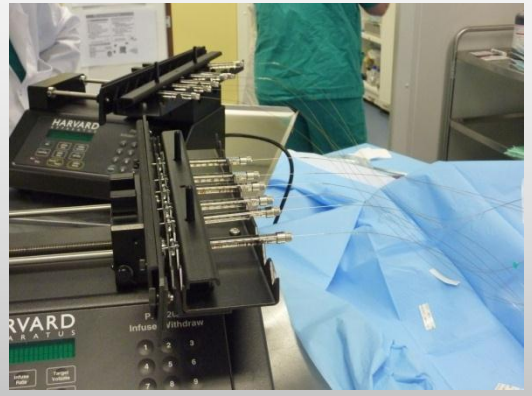
e AAV-driven therapeutic protein activity

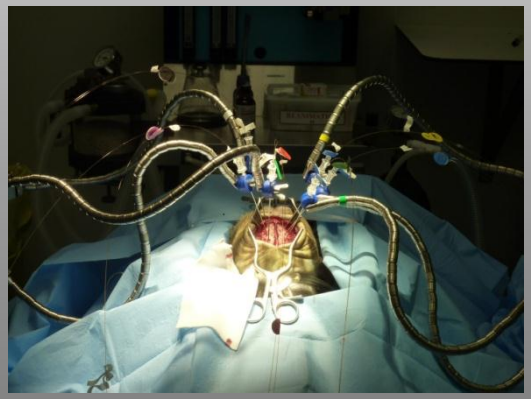
	recombinant ARSA activity	Volume (cm^3) of ARSA overexpression	Hemisphere % of ARSA overexpression
Prim1	1.38 ± 0.01 (0,013)	18.1	55.7 %
Prim2	1.21 ± 0.05 (0,027)	16.3	50.2 %
Prim3	1.24 ± 0.06 (0,029)	19	58.5 %
Prim4	1.14 ± 0.04 (0,042)	21.1	64.9 %
Prim5	1.12 ± 0.03 (0,027)	18.1	57.7 %
Prim6	1.16 ± 0.05 (0,049)	21.1	64.9 %

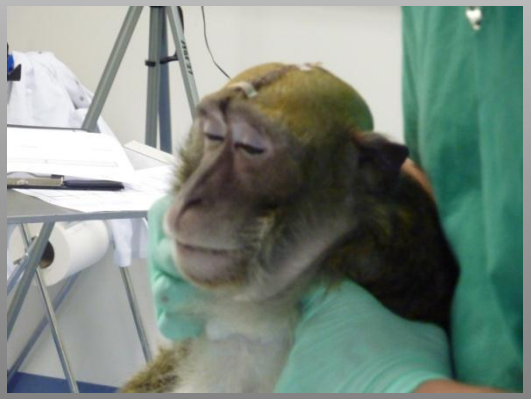


jection protocole in primates



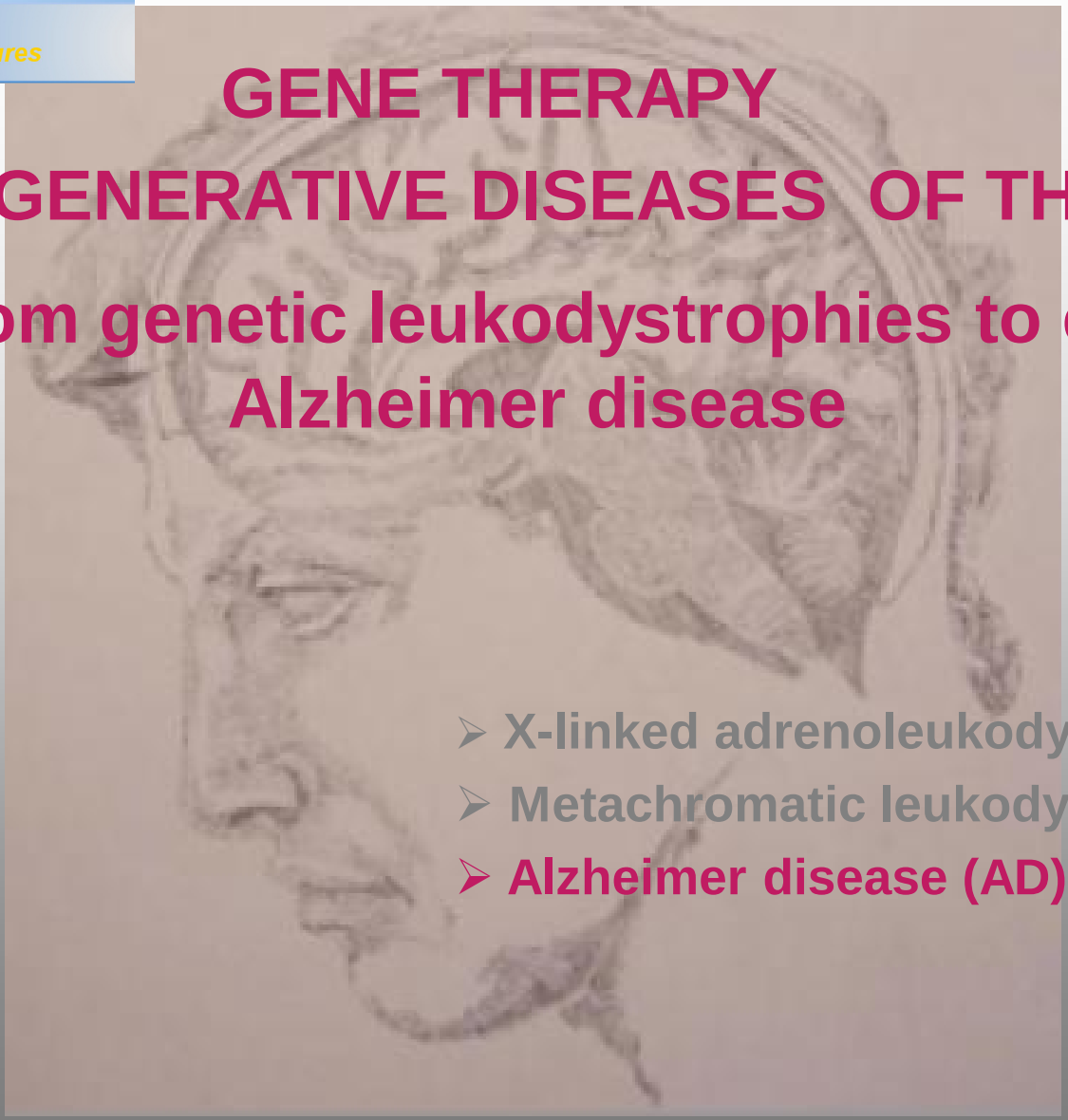






GENE THERAPY FOR DEGENERATIVE DISEASES OF THE CNS

from genetic leukodystrophies to é Alzheimer disease

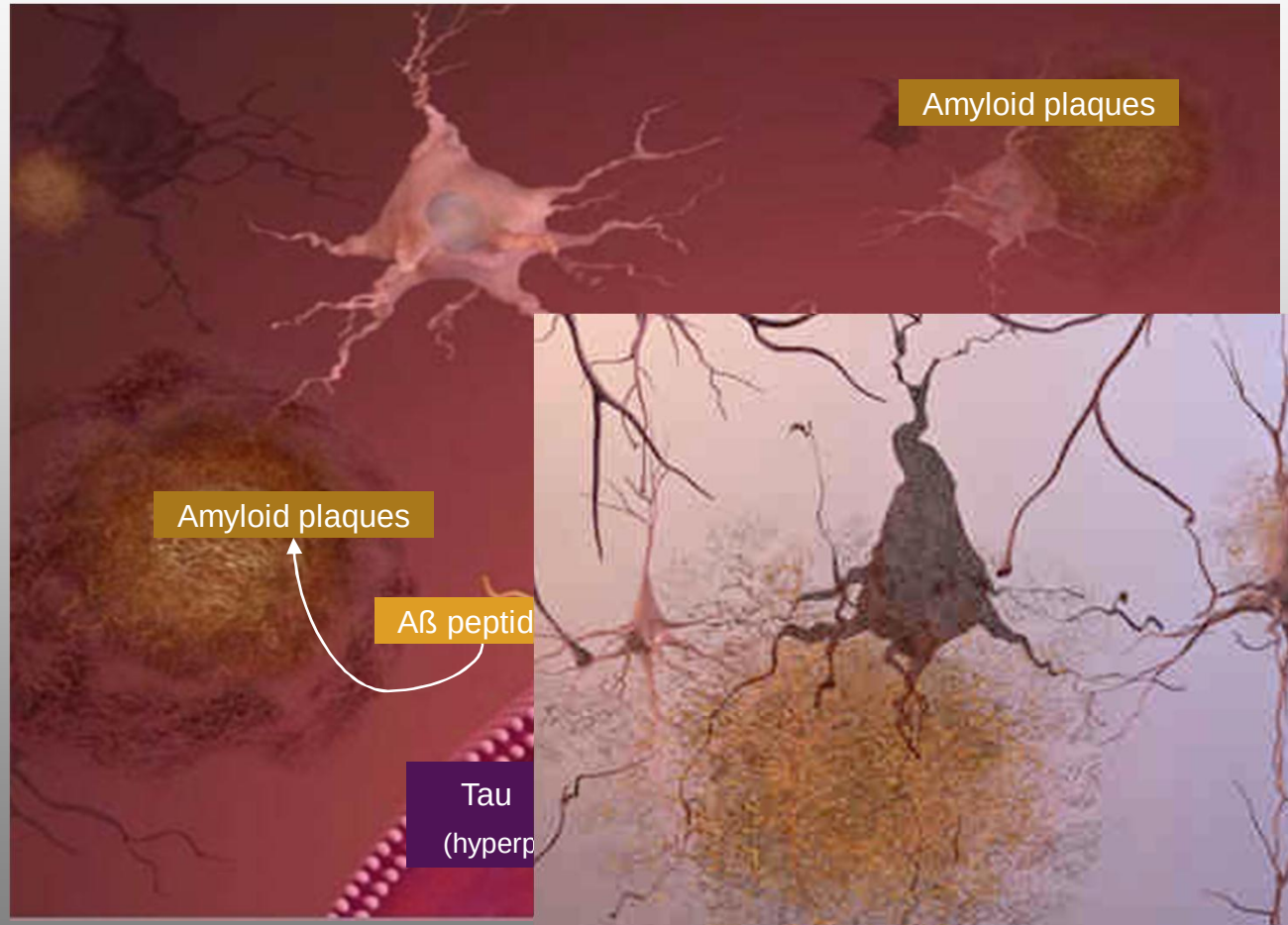


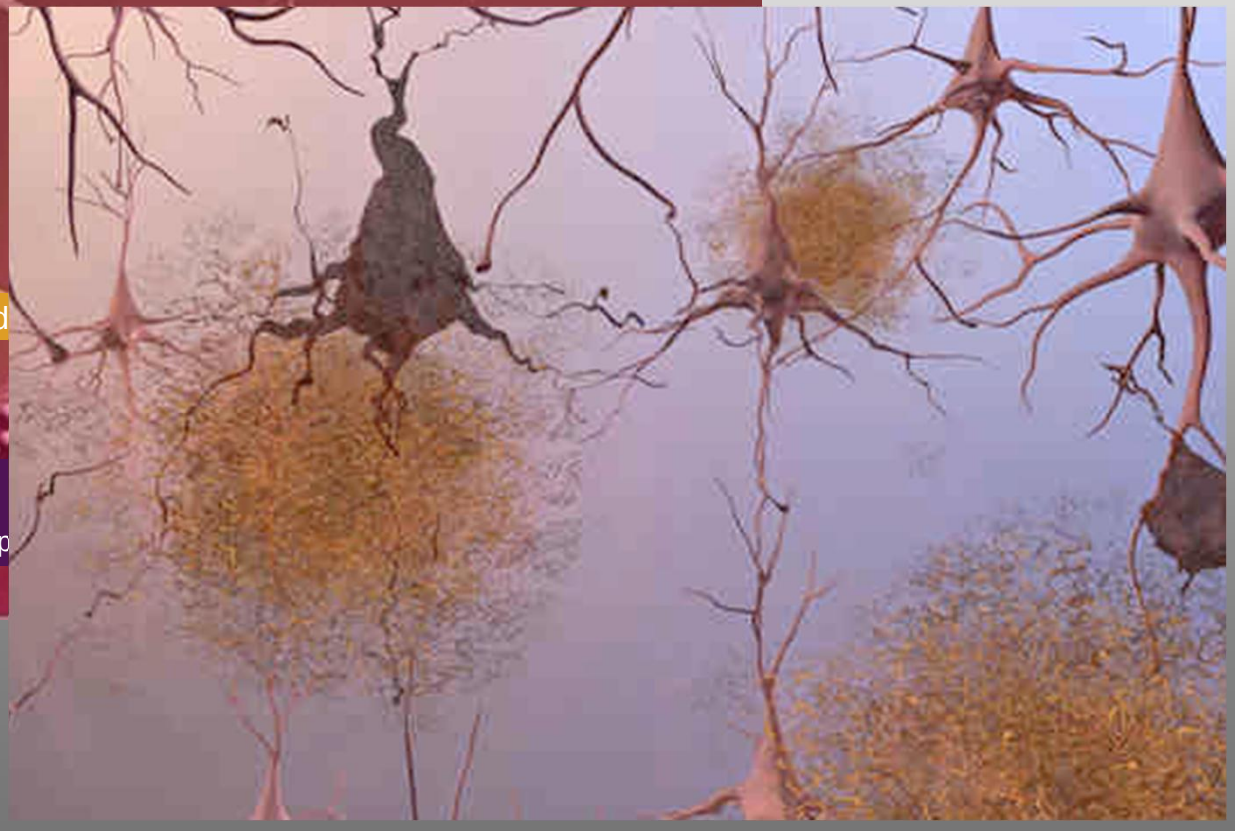
- X-linked adrenoleukodystrophy (ALD)
- Metachromatic leukodystrophy (MLD)
- Alzheimer disease (AD)

Cholesterol metabolism and Alzheimer Disease

CYP46A1 as a therapeutic target in Alzheimer's disease

ER DISEASE CHARACTERISTIC LESIONS



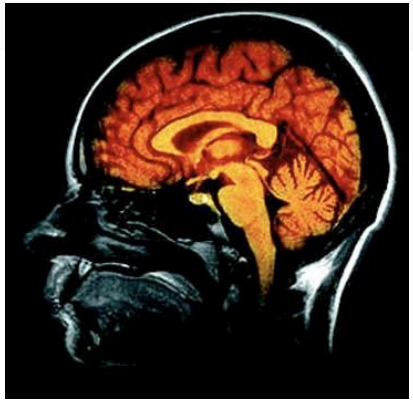


Three genes identified in the familial forms of AD:

- **APP** (Amyloid Precursor Protein)

- **Presenilin 1 et 2** (PS1, PS2)

REBRAL CHOLESTEROL METABOLISM



AcetylCoA

HMGCR

Cholesterol

ABCA1

HDL

ApoE

LDLR

NEURON

Cholesterol

Synaptic vesicles

Cholesterol-24-hydroxylase

(CYP46A1)

24-OHC

excess

ABC

HDL

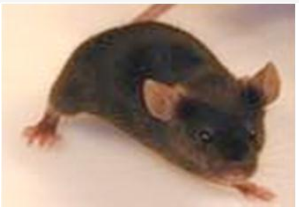
ApoE

CSF

p46 A1 as a potential target for AD

INTRACEREBRAL STEREOTACTIC INJECTION OF AAV5-CYP46A1

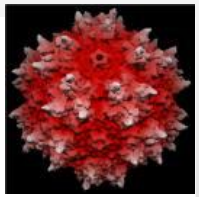
APP23 mouse



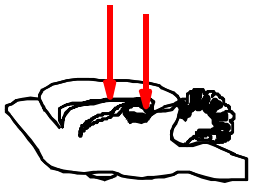
- $hAPP_{751}$ containing the swedish double mutation
- First cognitive deficits as soon as 3 months of age
- First amyloid deposits at 6 months

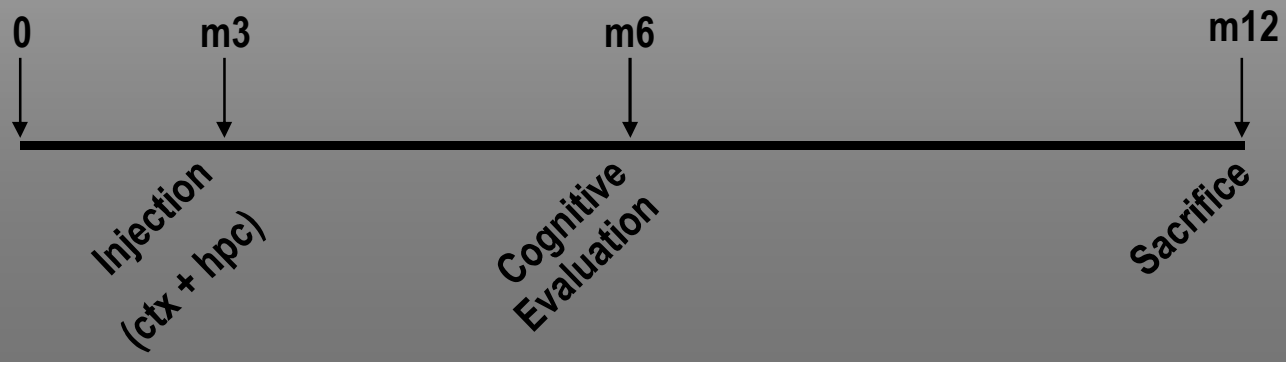
(Sturchler-Pierrat et al. 1997)

ADENO ASSOCIATED VECTORS

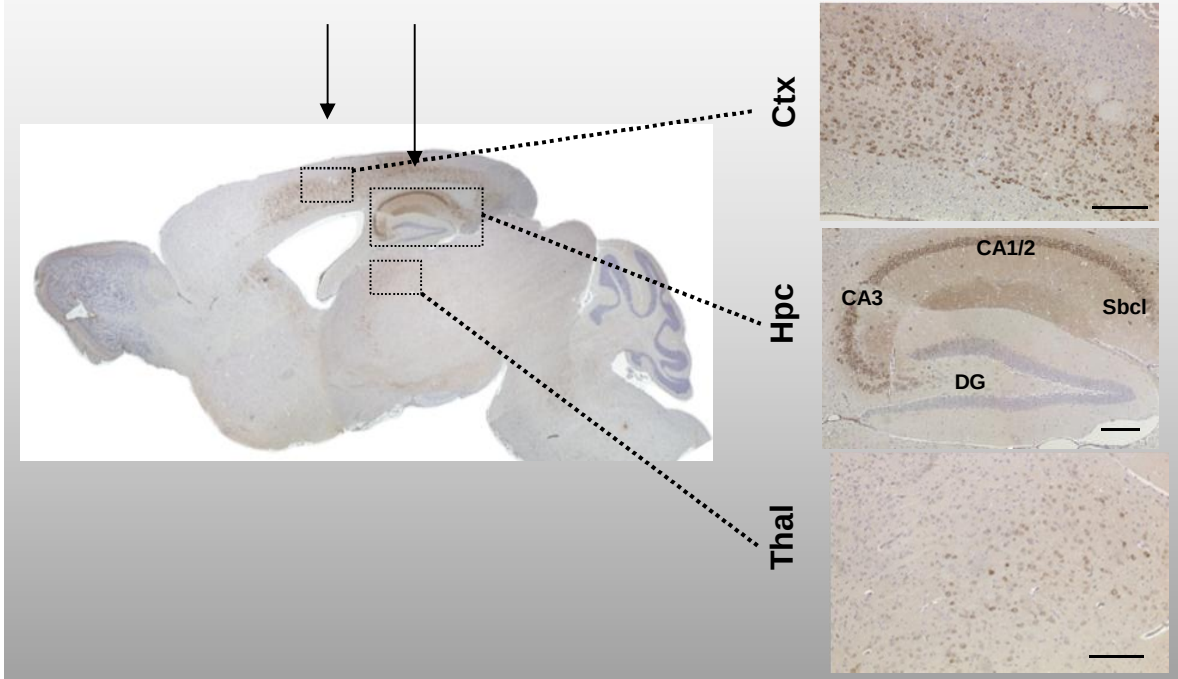


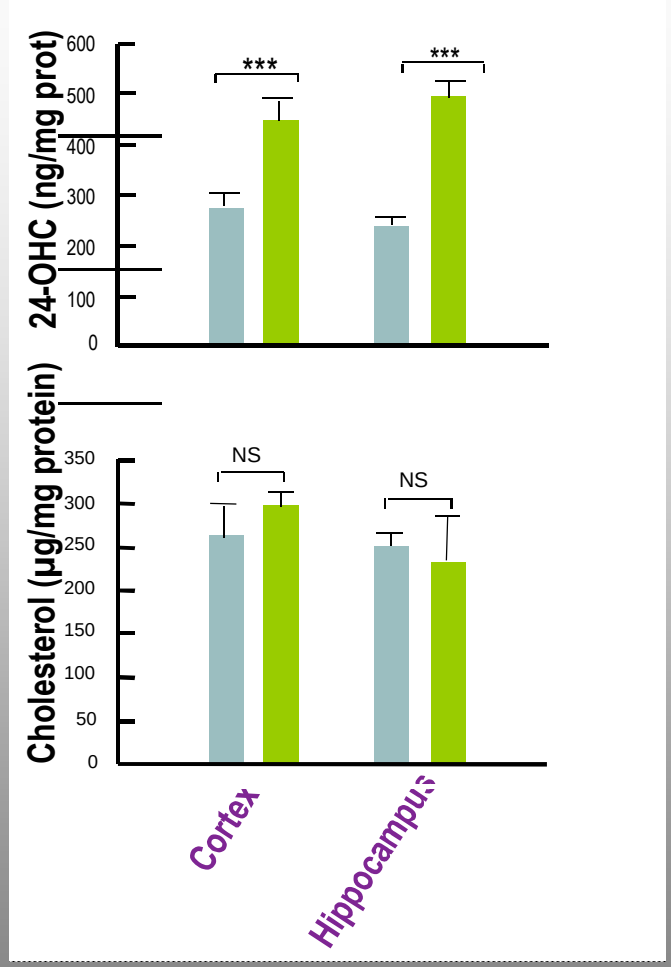
- AAV5-wtCYP46A1 (AAV-CYP)
- AAV5-mtCYP46A1 (AAV-control)





AV-CYP46 vector results in a significant increase of the 24-OHC content



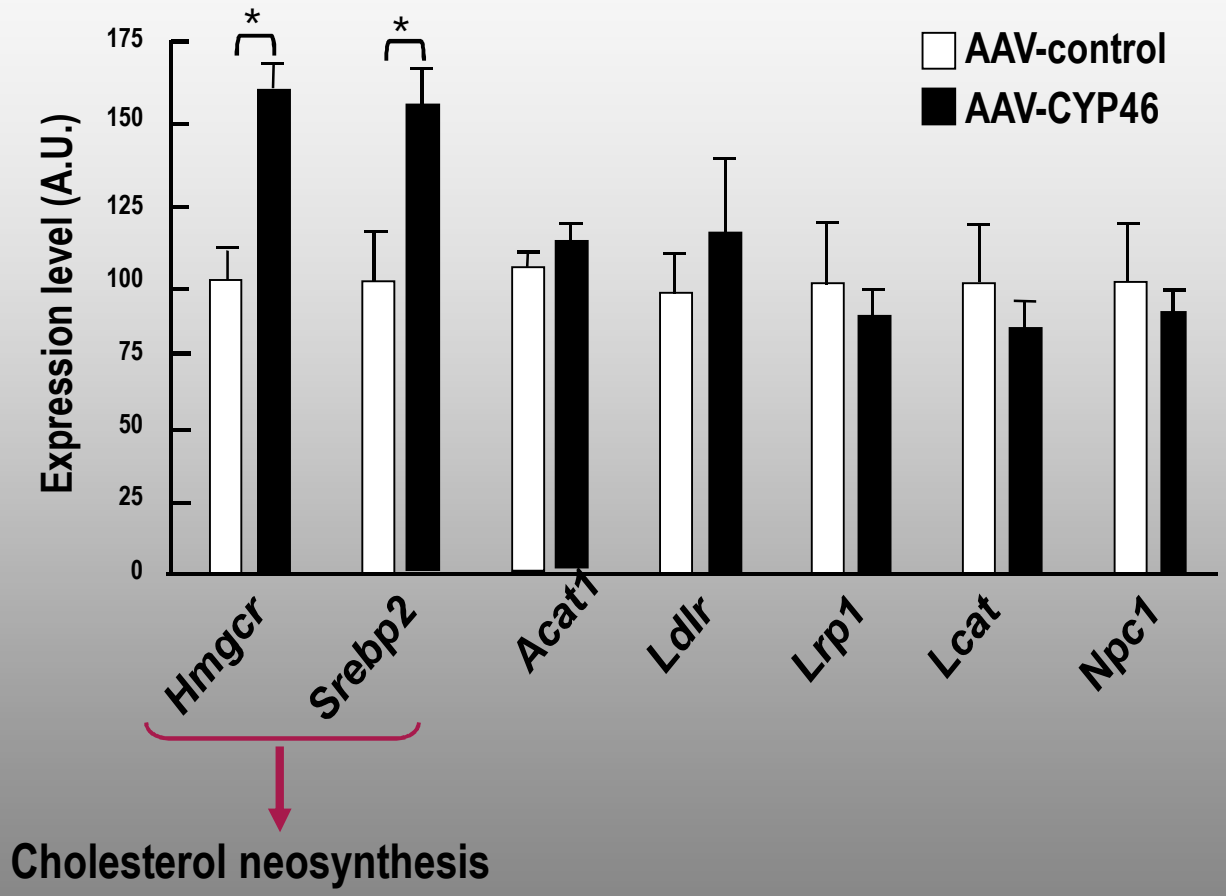


AAV-control

AAV-CYP46

ON OF CHOLESTEROL METABOLISM RELATED GENES

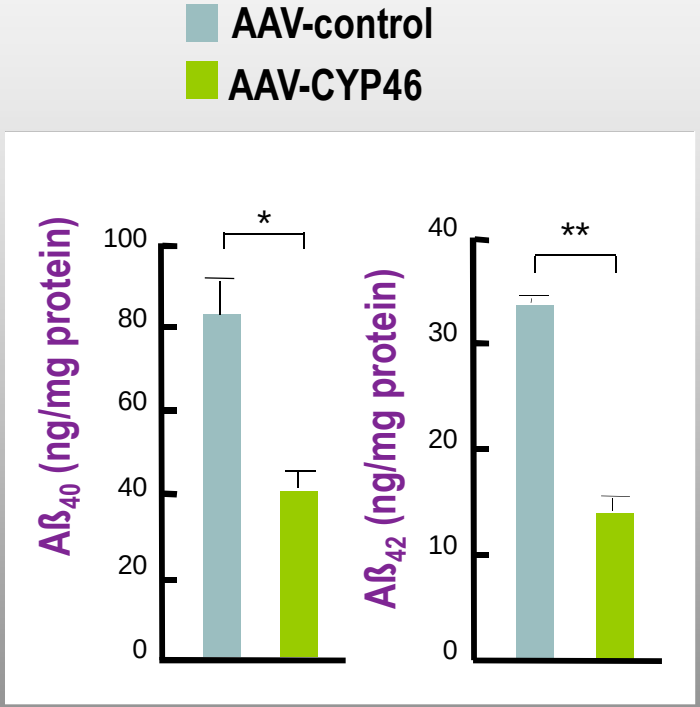
IN VIVO



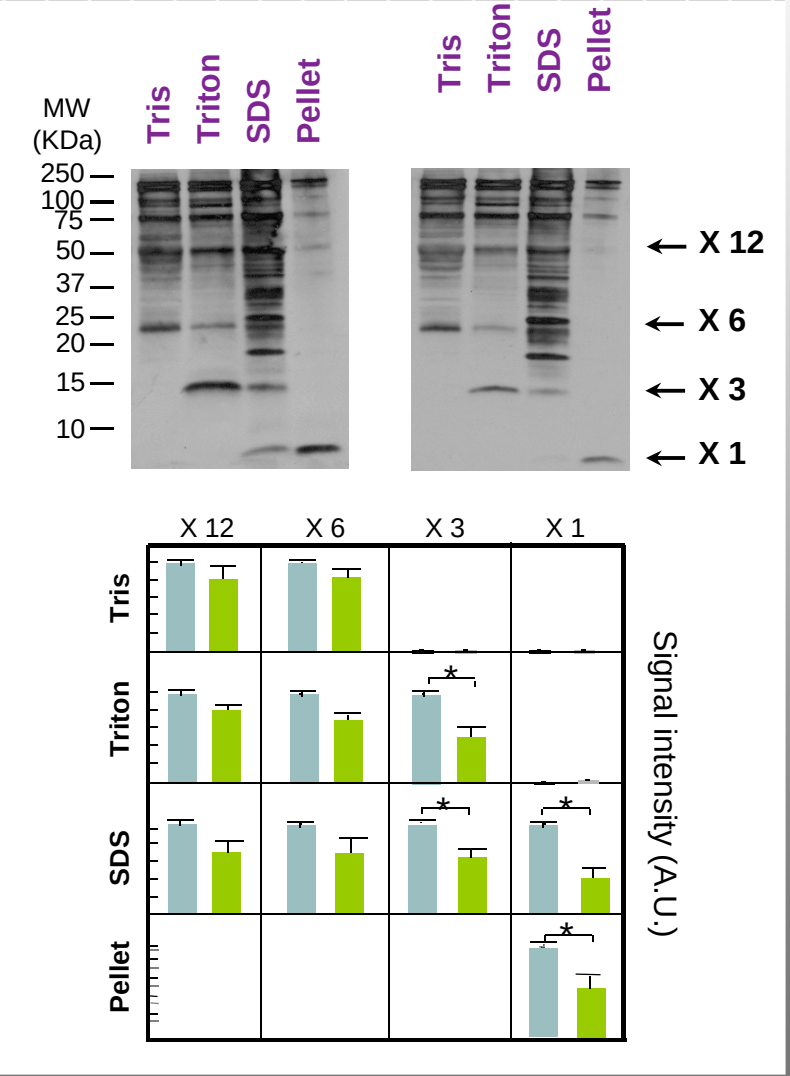
The injection of AAV-CYP46 vector induces a significant increase of the expression level of *Hmgcr* and *Srebp2*

and amyloid trimers are decreased in treated mice

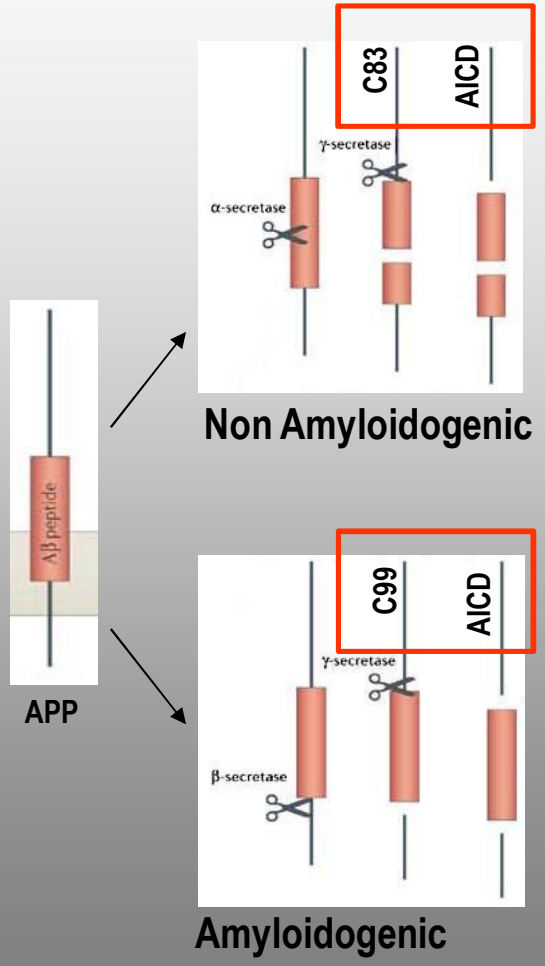
Aß$_{40/42}$ CONTENT

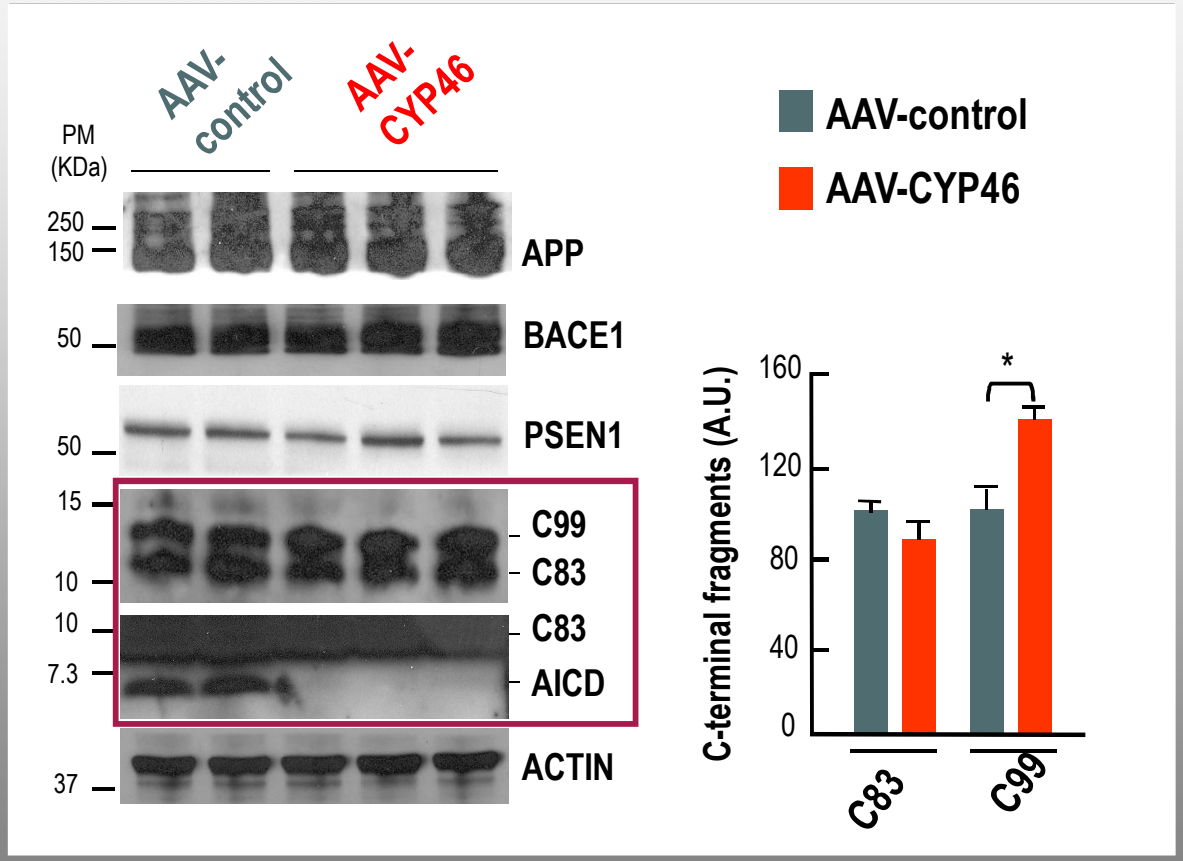


Aß OLIGOMERS



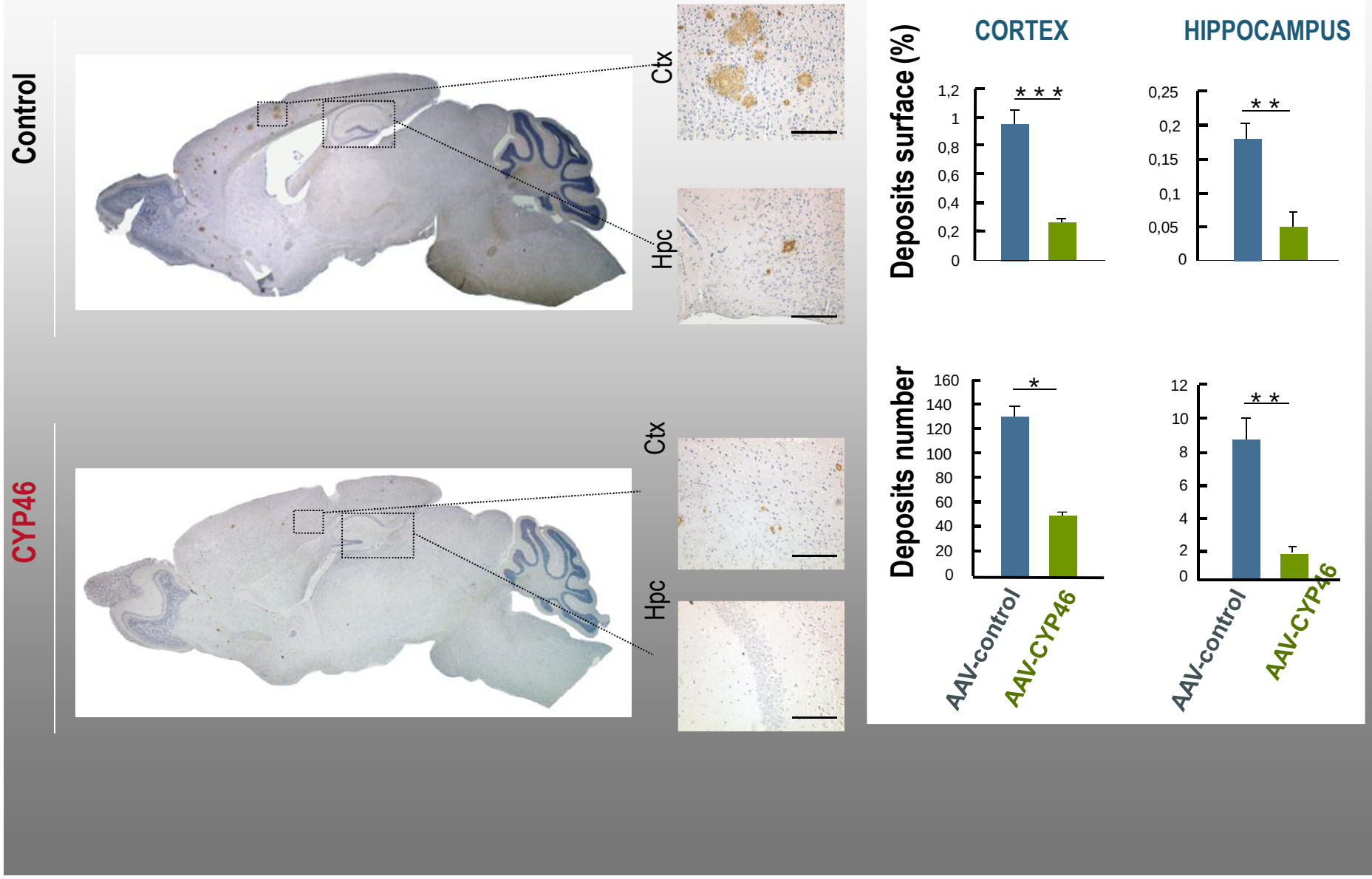
APP CLIVAGE PRODUCTS



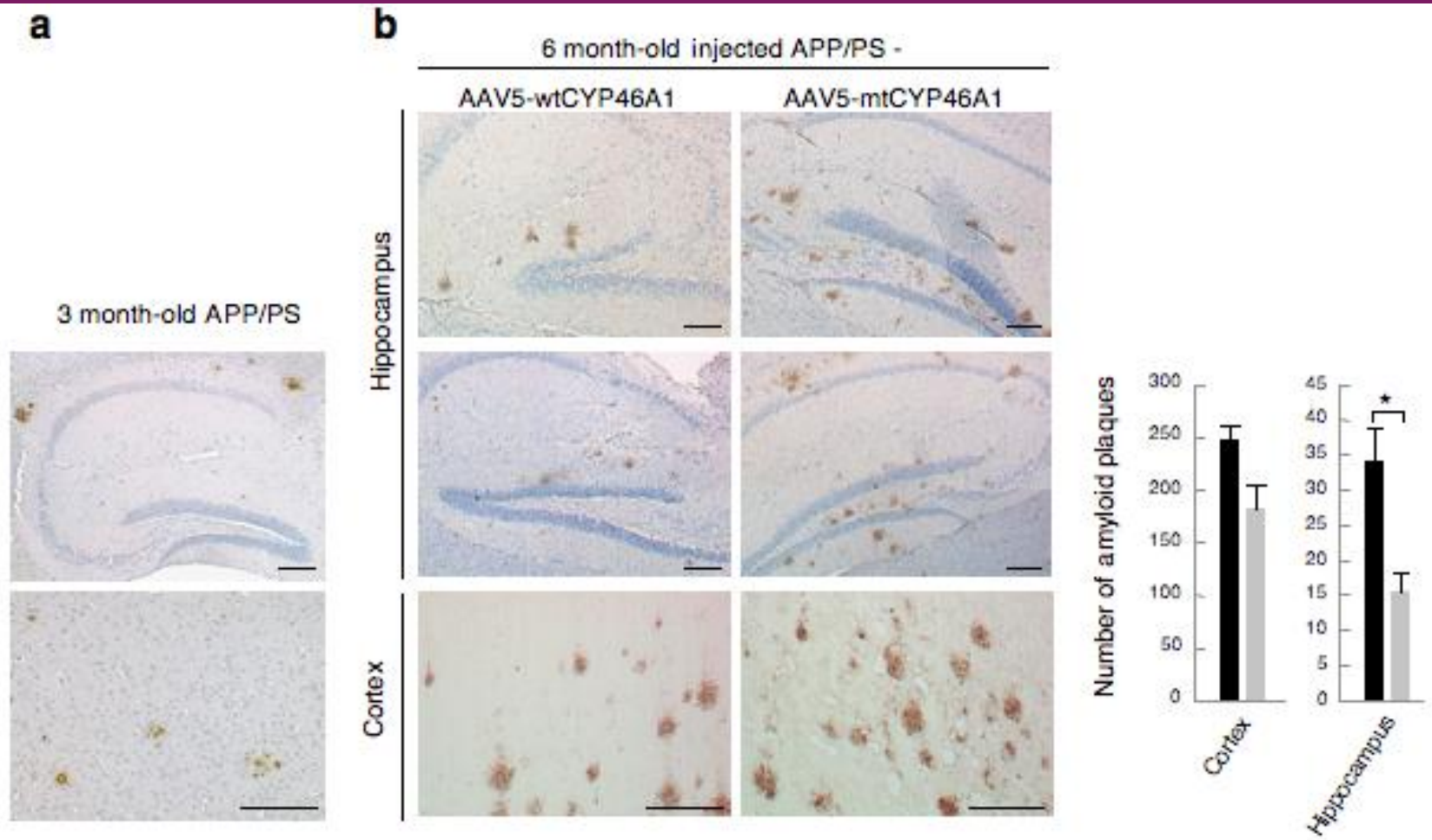


The overepression of CYP46 is associated with a significant decrease of AICD production

ression prevents amyloïd deposits in APP23 mice

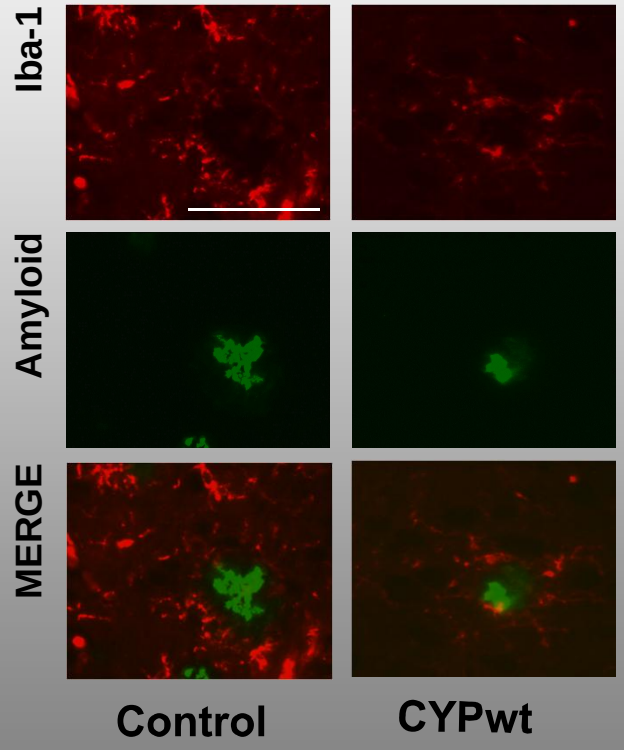


expression reduces existing amyloïd deposits in APP23-PS mice

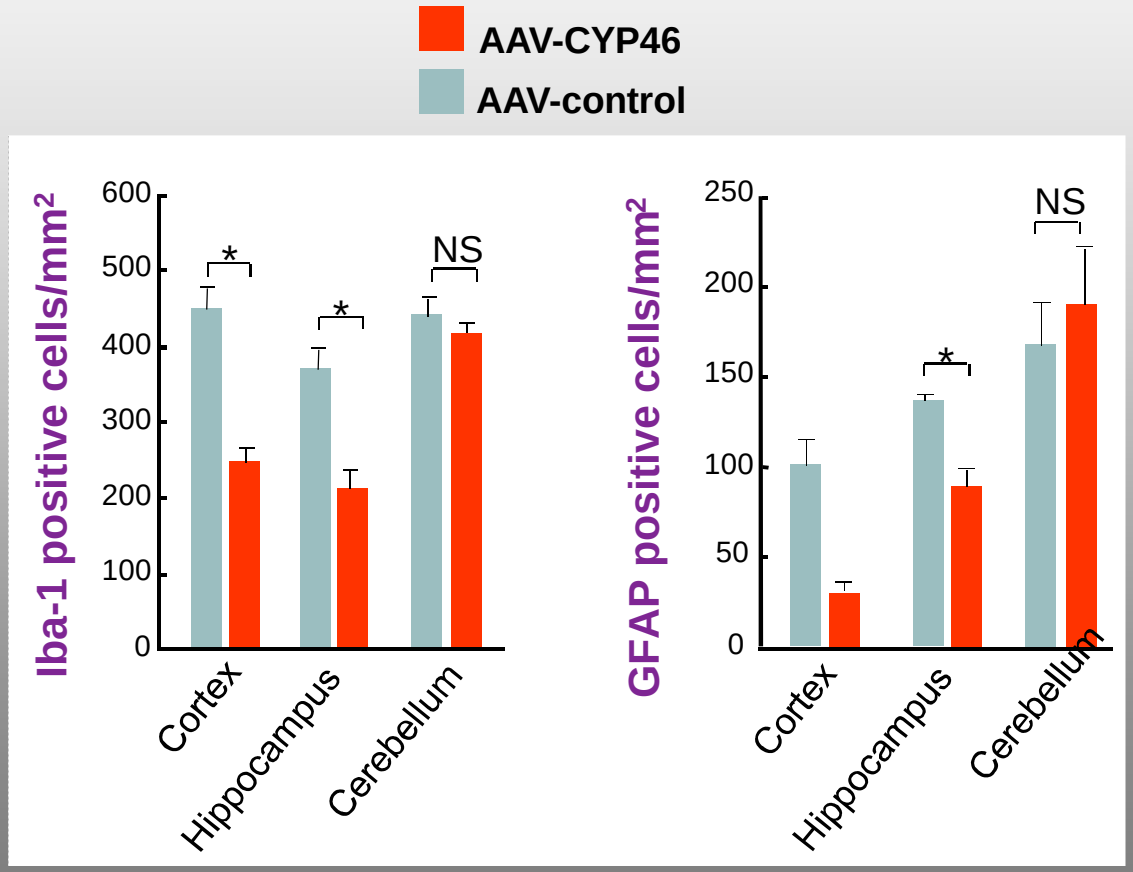


EFFECT ON INFLAMMATION

MICROGLIOSIS



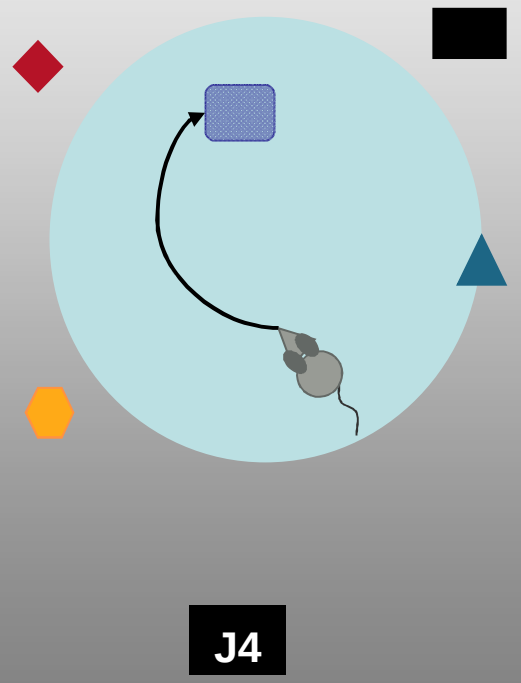
ASTROCYTOSIS

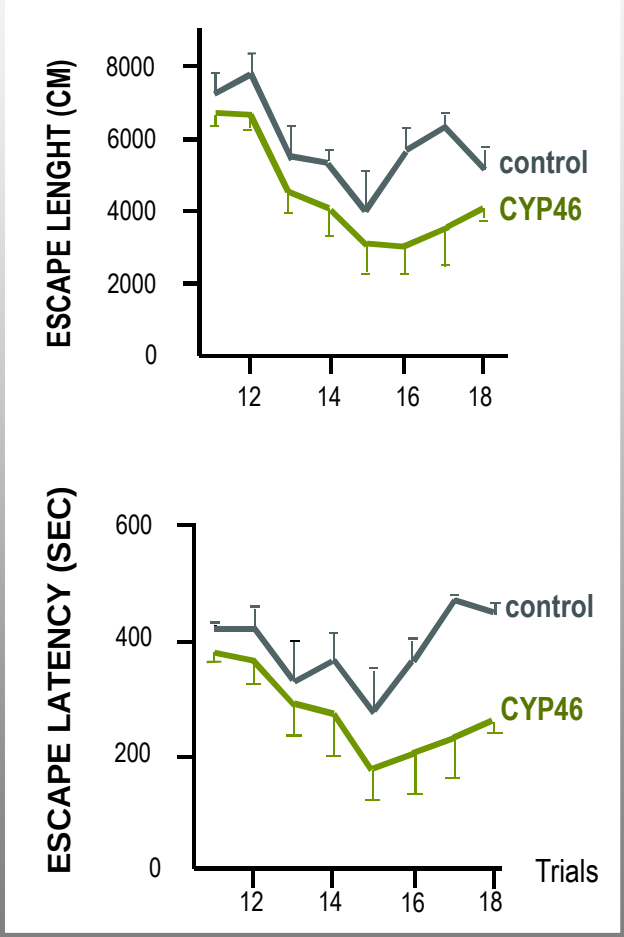


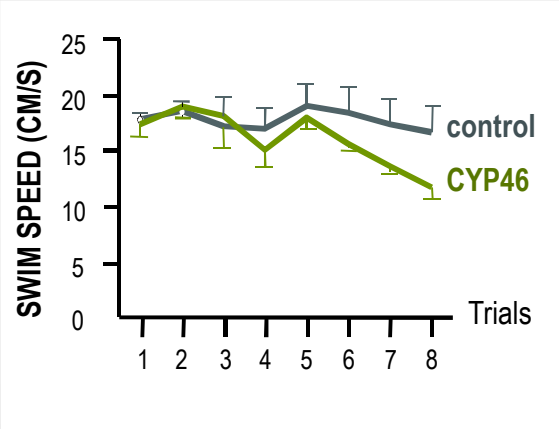
⇒ Decrease of inflammatory cells

COGNITIVE EVALUATION

Morris water maze







⇒ AAV-CYP46 injected APP23 mice show an improvment of their cognitive performances

CONCLUSION

OVEREXPRESSION OF CHOLESTEROL-24-HYDROXYLASE IS ASSOCIATED WITH:

- Decreased Aß40/42 production
- Decreased amyloid plaques and Aß oligomers in APP23 mice (preventive and curative)
- Decreased inflammation
- Improvement of cognitive deficits
- Decreased AICD, suggesting that the γ-secretase cleavage is affected
- Lower cholesterol content in lipid rafts with a displacement of APP and PSEN1 out of lipid rafts
- no major modification of lipid metabolism

AS A THERAPEUTIC TARGET FOR AD : Further steps

- **Proof of concept**
  - **Underlying mechanism of the role of CYP on amyloid pathology**

    in vivo : microarray APP23/APP23 CYP

    in vitro : N2A-APP-CYP subcellular localisation of the different key actors coll MC Potier, C Duyckaerts
  - **Therapeutic potential**
    - in a more agressive model APP/PS KI : coll B Delatour, C Duyckaerts
    - CYP and Tau pathology : coll Luc Buee
    - Large animal model : coll N Deglon, P Hantraye

s thérapeutiques innovantes pour Alzheimer ?

- Marché actuel : médicaments peu efficaces
- Médicaments en phase II et III prévisionnels connus
- Nouveaux médicaments dans le pipeline connus

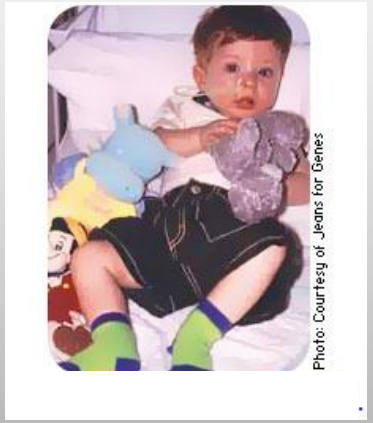
Quelle est la place de la thérapie génique aujourd'hui ?

Challenge : conduire les investisseurs et les pharmas à reconsidérer la TG

- **Nouvelle modalité thérapeutique**
- **même si paraît plus complexe que les traitements classiques**

ave been important years for gene therapyé .

Parkinson



Déficits immunitaires

Rétinite pigmentaire RP65

Adrénoleucodystrophie

nature
www.nature.com/nature
Vol 461 | Issue no. 7268 | 29 October 2009

Gene therapy deserves a fresh chance

Initial interest in gene therapy waned after the technology failed to live up to expectation. Progress made since has received little attention, but suggests that the pervading sense of disillusionment is misplaced.

In the early 1990s, when the first human trials got under way, it seemed to many that the era of gene therapy was at hand: the techniques of modern molecular biotechnology would make it possible to repair genetic defects by inserting healthy DNA directly into a patient's cells. The excitement was short-lived. Lasting effects proved difficult to obtain in early trials, and the community quickly grew sceptical. Then, in 2003, when it was announced that several gene-therapy patients in a Paris-based clinical trial had developed leukaemia, and that one of them had died, the mood became bleak. Subsequent reports of successful and effective gene-therapy trials have done little to lift the prevailing sense of doom. For most researchers, gene therapy now seems like a dead end.

But it doesn't have to be a dead end — not if scientists shift their perspective on the risks of gene therapy to be more in line with that of clinicians.

Scientists are trained to focus on understanding the systems that they study in great detail. And when they devise therapeutic interventions — for example, harnessing a viral shell to insert a therapeutic gene into a patient's DNA — they naturally want those systems to be engineered with equally great care, and for them to be as near to risk-free perfection as possible.

Clinicians, by contrast, care for real patients in real time, which makes treatment decisions a matter of pragmatism. How do the risks stack up against the benefits for each available alternative — given that the risks are never zero? Clinicians are certainly not cavalier about their patients' well-being, but they may well end up prescribing a therapy that has a poorly understood mechanism and potentially large side effects because it gives the patient the best odds of recovery or survival. If they — and patients — had shied away from such dangers in the past, life-saving interventions such as organ grafts and bone-marrow transplants might never have been developed.

From that perspective, the fact that, collectively, the Paris trial and others carried out since have produced positive results in some 20 patients out of a total of two dozen looms at least as large as the handful of leukaemia cases. To clinicians, such results suggest a treatment that is risky, but potentially life-saving — a new option for people for whom there are no alternatives.

However, this was not the view that prevailed. When the viral delivery vehicle itself turned out to be responsible for the leukaemia cases in the Paris trial, scientists deemed the trial a failure. Bad press ensued, proposals for gene-therapy clinical trials came under increased regulatory scrutiny and standards for demonstrating safety were set higher than for other approaches. Unsurprisingly in such a climate, the biotechnology and pharmaceutical industries gradually dropped out of the gene-therapy pursuit. This corporate disinterest slowed clinical progress: academic centres are ill-equipped to make gene-therapy vectors of clinical grade and scale, and research funding is typically insufficient to support clinical trials. More insidiously, it has become harder to recruit young talent to a field that is perceived as falling short of its promises.

> "The results suggest a treatment that is risky, but potentially life-saving."

To reverse this trend, it is time for researchers and industry to refresh their perspective on gene therapy and to consider its successes with as much intensity as its setbacks. The focus on adverse events has had positive consequences: researchers dissected the exact molecular mechanisms that led to cancer, designed better vectors, devised animal models to test these vectors and developed sophisticated assays for monitoring patients. As a result, both scientists and clinicians now have a battery of extraordinarily refined tools for preclinical and clinical studies of gene therapy. The field is ripe for further successes. ■

tter perspectives é .

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11/06/2009

Time to take gene therapy seriously

Your writers

Mark Henderson is Science Editor of The Times, and a double winner of the Norwich Union /

Gene therapy isn't going to suitable for every genetic disease in the near future. The problems of carrying replacement genes to the many cells affected by systemic conditions such as cystic fibrosis are still extremely challenging. It is making considerable progress, though, and it deserves better support from the pharmaceutical industry and other research investors.

POSTED BY MARK HENDERSON ON NOVEMBER 6, 2009 IN GENETICS , MEDICINE | PERMALINK | POST TO TWITTER BOOKMARK ...

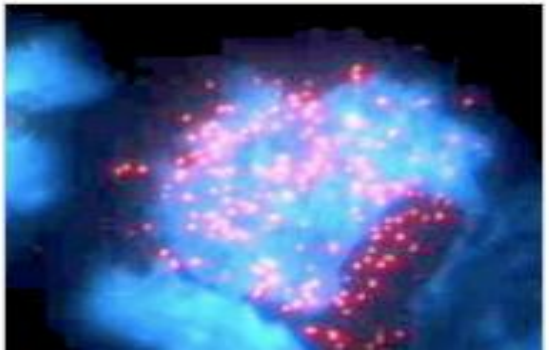
After Setbacks, Small Successes for Gene Therapy

By GINA KOLATA
Published: November 5, 2009

The New York Times

Not long ago, gene therapy seemed troubled by insurmountable difficulties. After decades of hype and dashed hopes, many who once embraced the idea of correcting genetic disorders by giving people new genes all but gave up the idea.

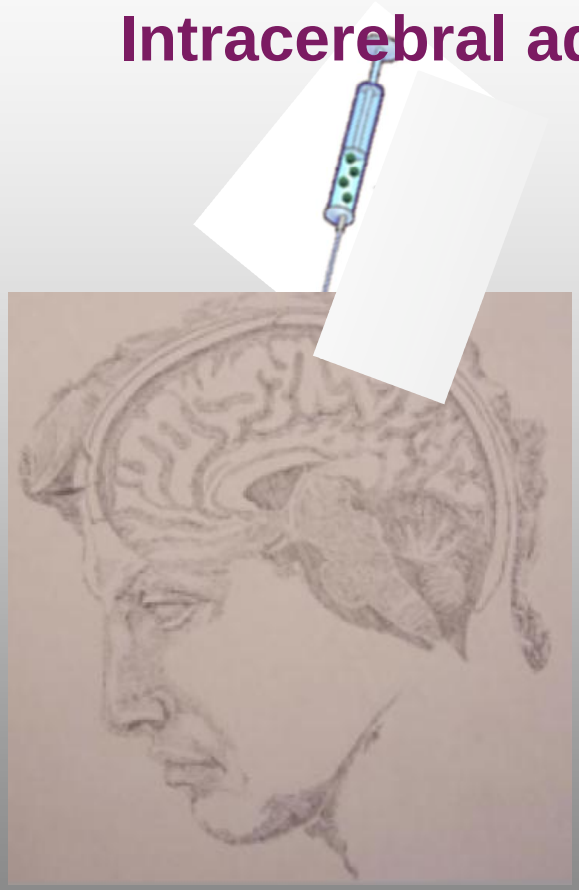
Enlarge This Image

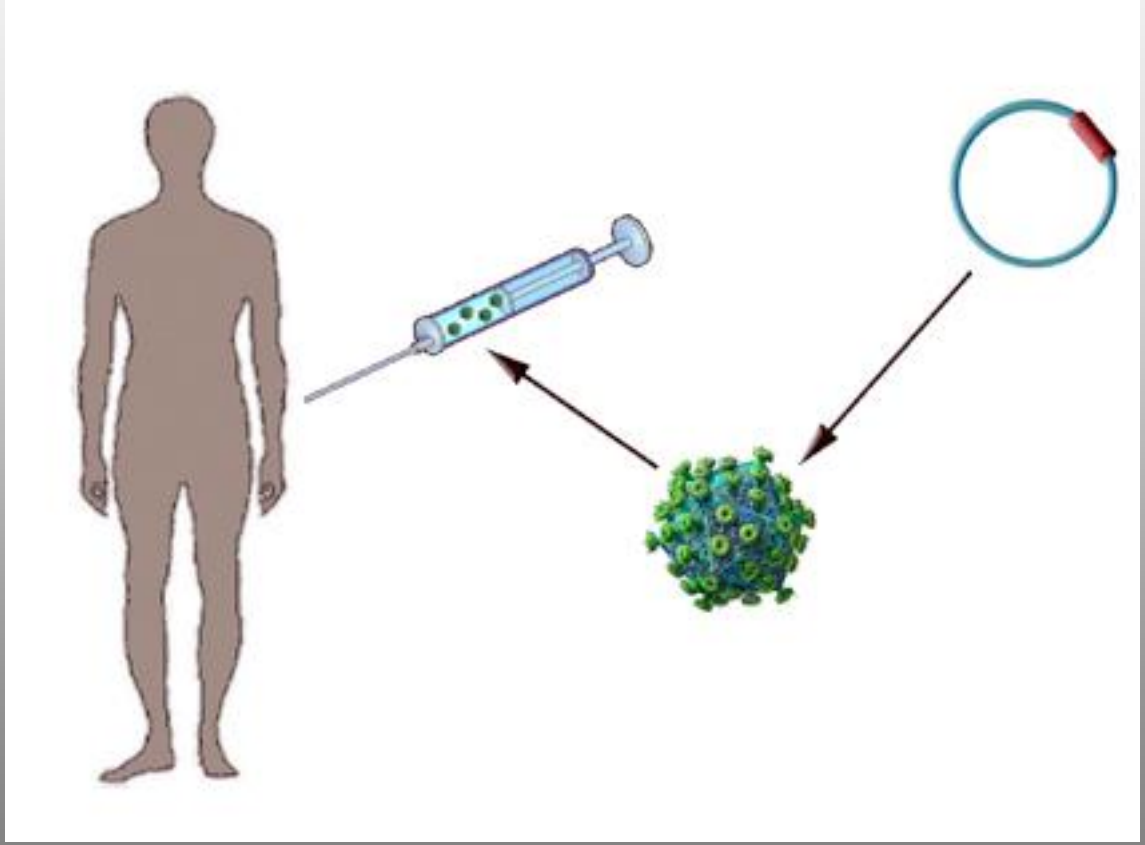


But scientists say gene therapy may be on the edge of a resurgence. There were three recent, though small, successes — one involving children with a fatal brain disease, one with an eye disease that causes blindness and one with children who have a disease that destroys the immune system.

erapy strategies for neurodegenerative diseases

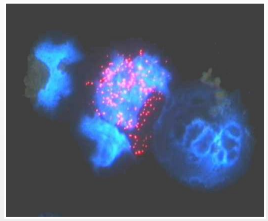
Intracerebral administration, and how in the future ?





The ALD teams

Inserm
Institut national de la santé et de la recherche médicale



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J Cossec
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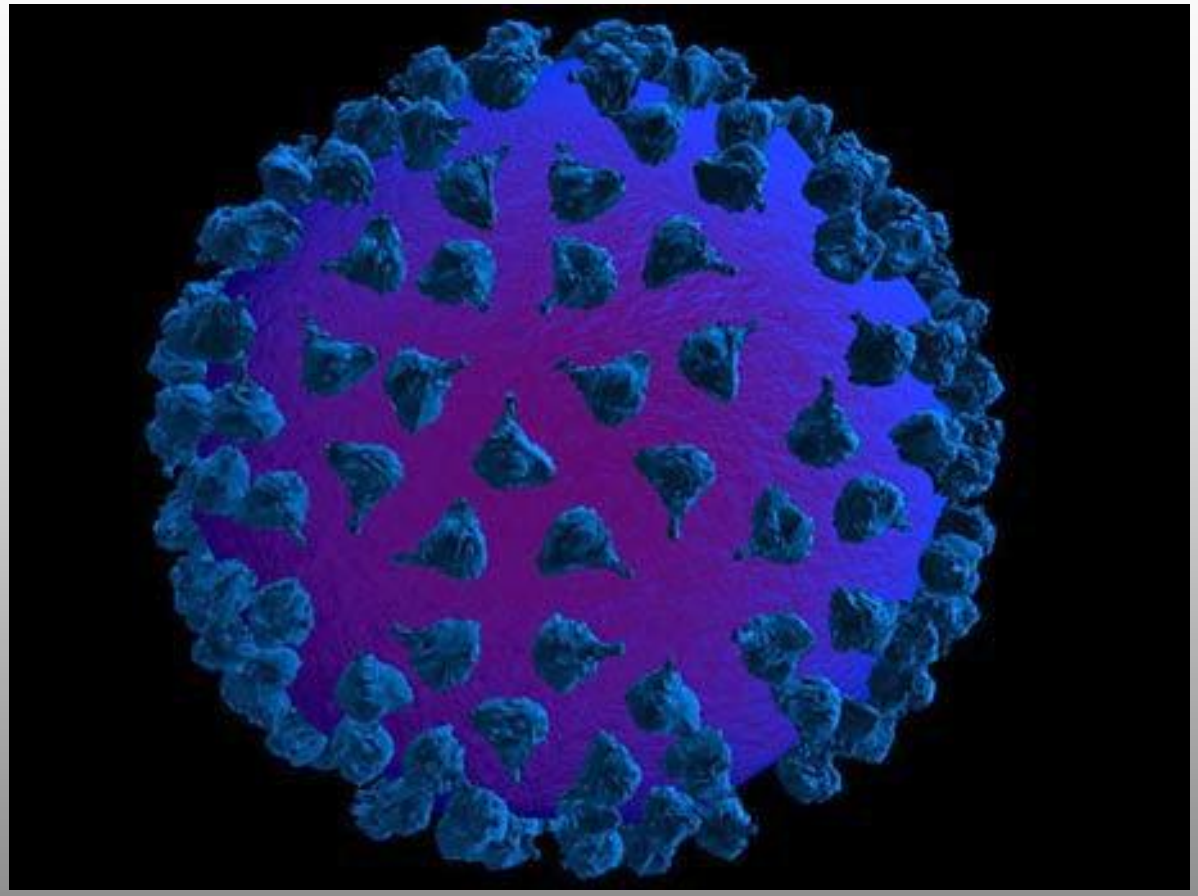

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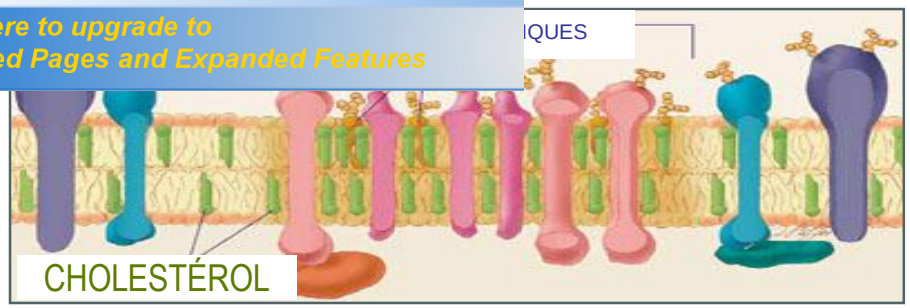
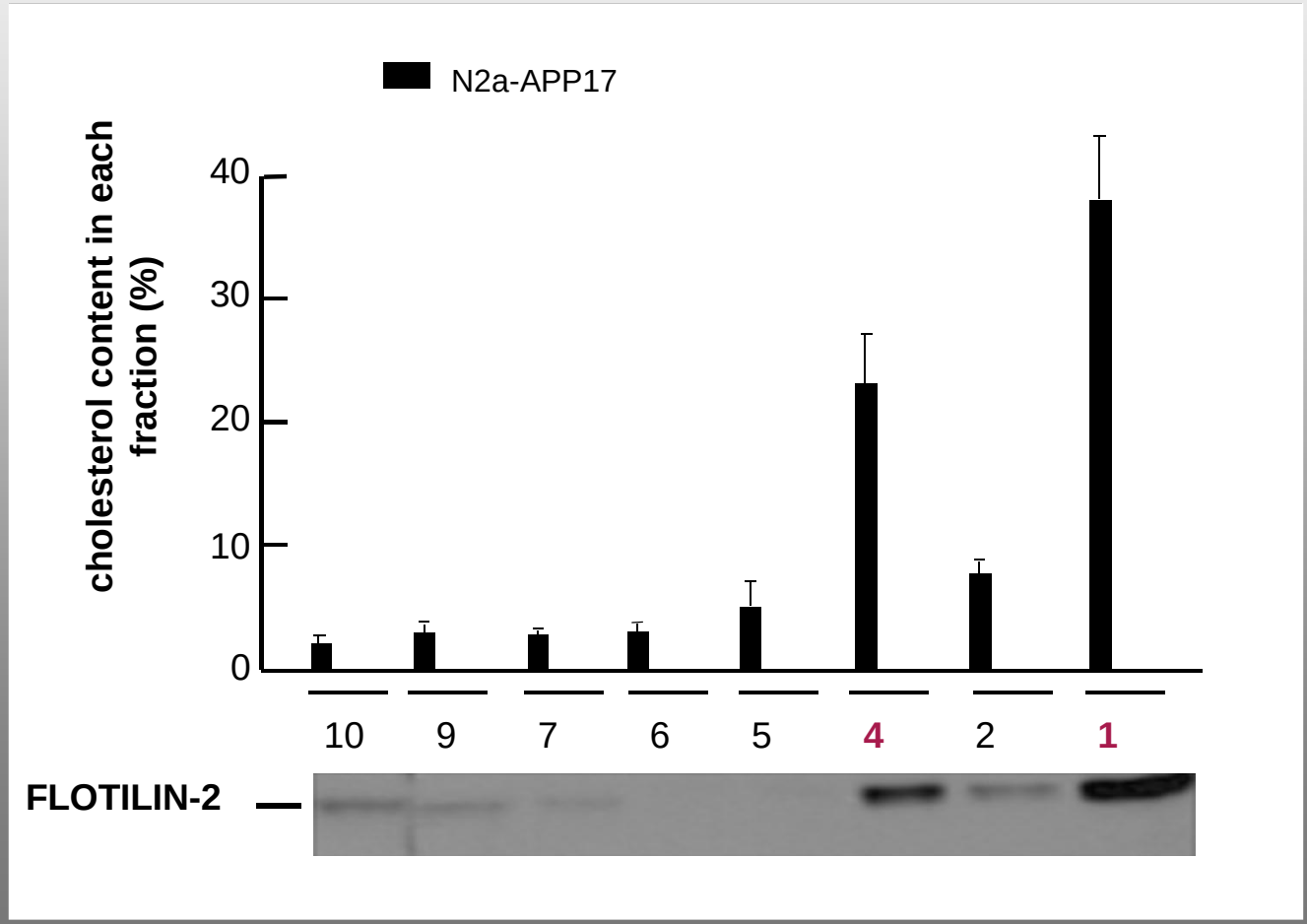
TRACELLULAR CHOLESTEROL DISTRIBUTION



DRM analysis

(Triton X-100 extraction and purification on a density gradient)

(flotillin-2 positive fractions)



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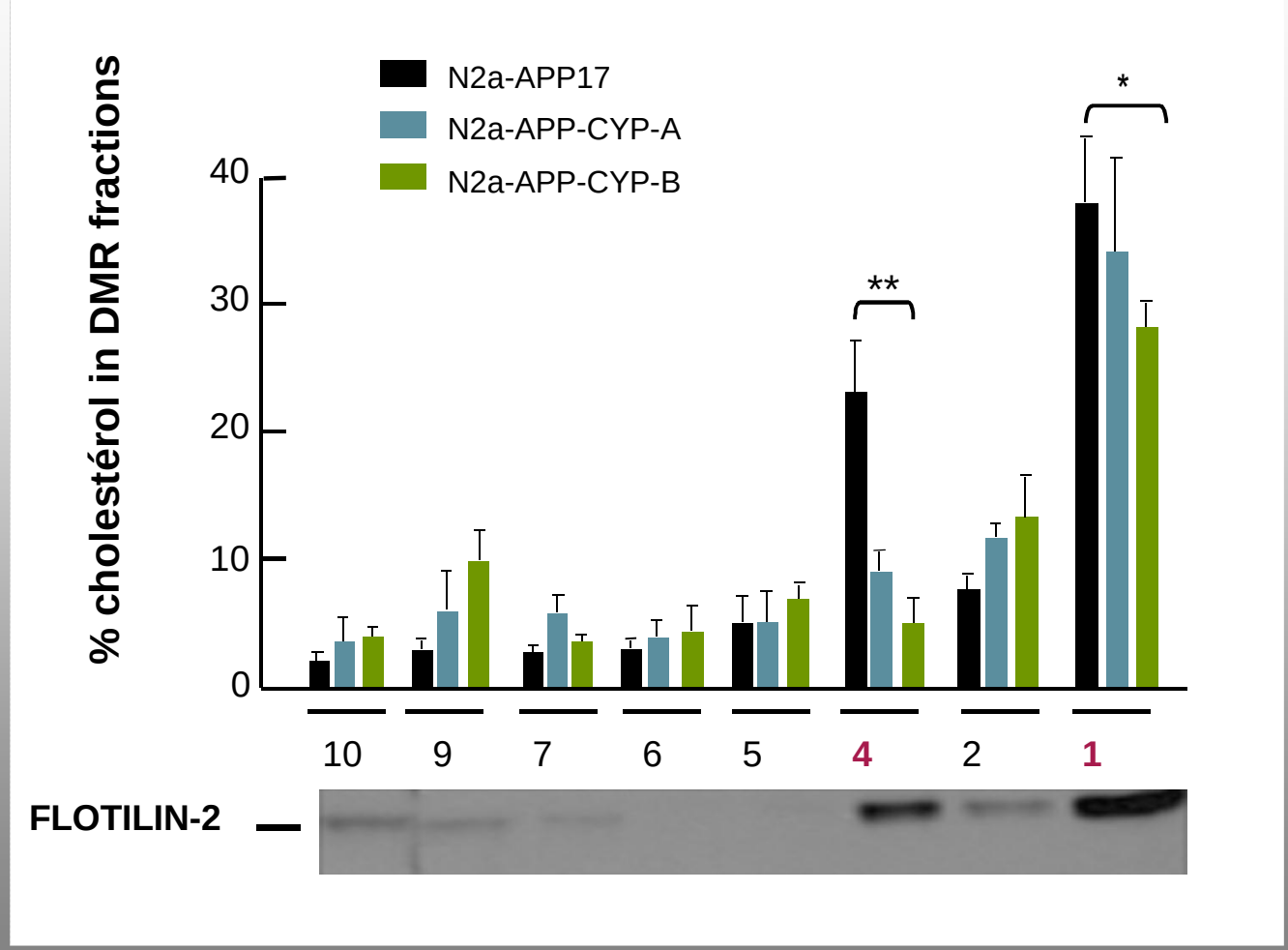
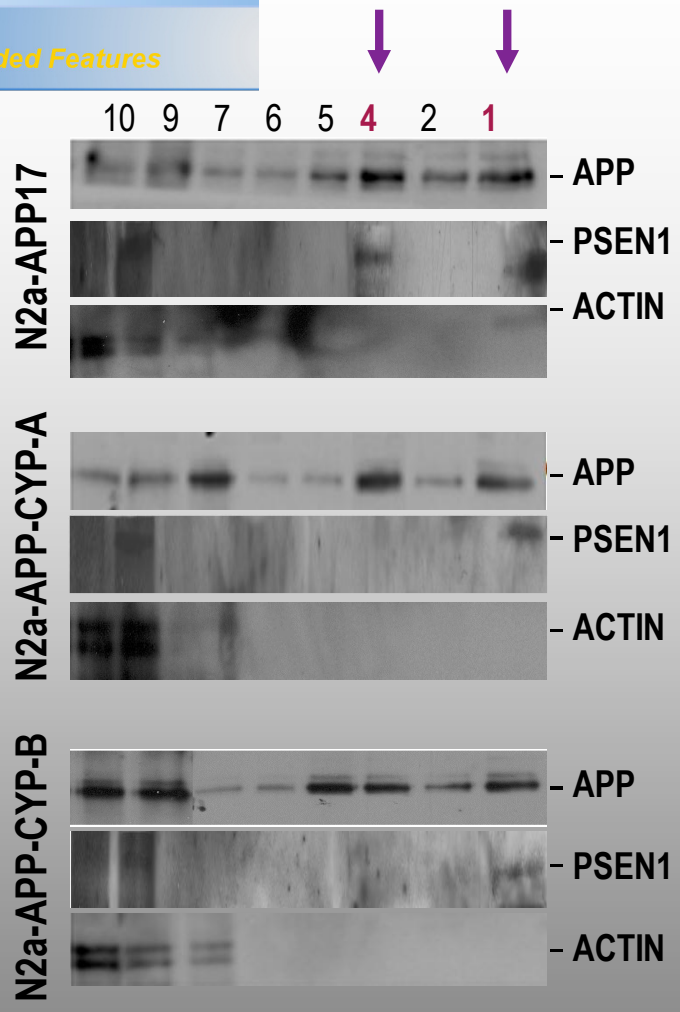
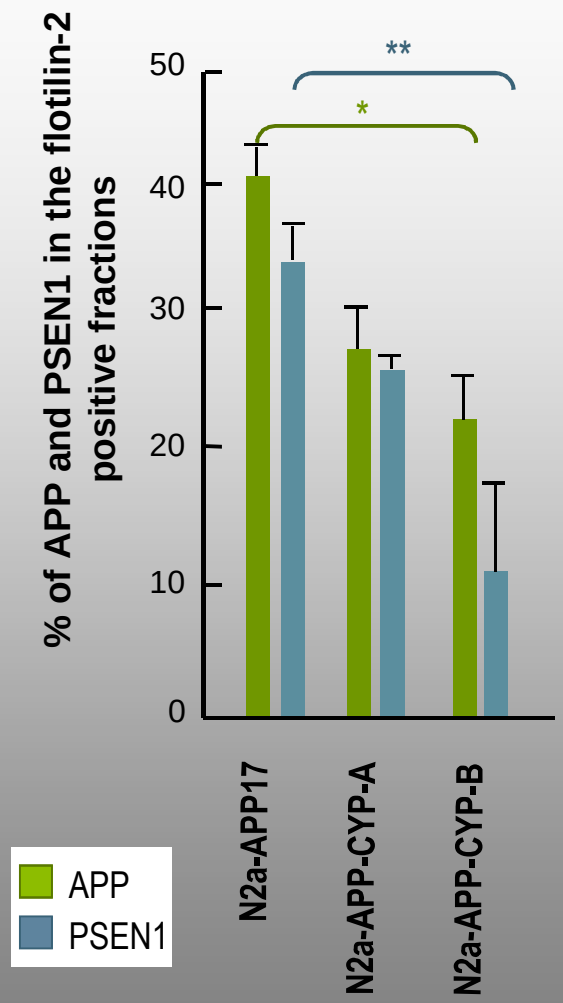


A decrease in the cholesterol content is observed in lipid rafts

D PSEN1 PROTEINS IN THE DIFFERENT FRACTIONS





APP and PSEN1 are displaced out of the “lipid raft” fractions

AS A THERAPEUTIC TARGET FOR AD : Further steps

- **Proof of concept**
  - **Underlying mechanism of the role of CYP on amyloid pathology**

    in vivo : microarray APP23/APP23 CYP

    in vitro : N2A-APP-CYP subcellular localisation of the different key actors coll MC Potier, C Duyckaerts
  - **Therapeutic potential**
    - in a more agressive model APP/PS KI : coll B Delatour, C Duyckaerts
    - CYP and Tau pathology : coll Luc Buee
    - Large animal model : coll N Deglon, P Hantraye

2009 recent progresses in gene therapy

Parkinson

Déficit en ADA, DICS-X

Rétinite pigmentaire RP65

Adrénoleucodystrophie

nature
www.nature.com/nature
Vol 461 | Issue no. 7268 | 29 October 2009

Gene therapy deserves a fresh chance

Initial interest in gene therapy waned after the technology failed to live up to expectation. Progress made since has received little attention, but suggests that the pervading sense of disillusionment is misplaced.

In the early 1990s, when the first human trials got under way, it seemed to many that the era of gene therapy was at hand: the techniques of modern molecular biotechnology would make it possible to repair genetic defects by inserting healthy DNA directly into a patient's cells. The excitement was short-lived. Lasting effects proved difficult to obtain in early trials, and the community quickly grew sceptical. Then, in 2003, when it was announced that several gene-therapy patients in a Paris-based clinical trial had developed leukaemia, and that one of them had died, the mood became bleak. Subsequent reports of successful and effective gene-therapy trials have done little to lift the prevailing sense of doom. For most researchers, gene therapy now seems like a dead end.

But it doesn't have to be a dead end — not if scientists shift their perspective on the risks of gene therapy to be more in line with that of clinicians.

Scientists are trained to focus on understanding the systems that they study in great detail. And when they devise therapeutic interventions — for example, harnessing a viral shell to insert a therapeutic gene into a patient's DNA — they naturally want those systems to be engineered with equally great care, and for them to be as near to risk-free perfection as possible.

Clinicians, by contrast, care for real patients in real time, which makes treatment decisions a matter of pragmatism. How do the risks stack up against the benefits for each available alternative — given that the risks are never zero? Clinicians are certainly not cavalier about their patients' well-being, but they may well end up prescribing a therapy that has a poorly understood mechanism and potentially large side effects because it gives the patient the best odds of recovery or survival. If they — and patients — had shied away from such dangers in the past, life-saving interventions such as organ grafts and bone-marrow transplants might never have been developed.

From that perspective, the fact that, collectively, the Paris trial and others carried out since have produced positive results in some 20 patients out of a total of two dozen looms at least as large as the handful of leukaemia cases. To clinicians, such results suggest a treatment that is risky, but potentially life-saving — a new option for people for whom there are no alternatives.

However, this was not the view that prevailed. When the viral delivery vehicle itself turned out to be responsible for the leukaemia cases in the Paris trial, scientists deemed the trial a failure. Bad press ensued, proposals for gene-therapy clinical trials came under increased regulatory scrutiny and standards for demonstrating safety were set higher than for other approaches. Unsurprisingly in such a climate, the biotechnology and pharmaceutical industries gradually dropped out of the gene-therapy pursuit. This corporate disinterest slowed clinical progress: academic centres are ill-equipped to make gene-therapy vectors of clinical grade and scale, and research funding is typically insufficient to support clinical trials. More insidiously, it has become harder to recruit young talent to a field that is perceived as falling short of its promises.

> "The results suggest a treatment that is risky, but potentially life-saving."

To reverse this trend, it is time for researchers and industry to refresh their perspective on gene therapy and to consider its successes with as much intensity as its setbacks. The focus on adverse events has had positive consequences: researchers dissected the exact molecular mechanisms that led to cancer, designed better vectors, devised animal models to test these vectors and developed sophisticated assays for monitoring patients. As a result, both scientists and clinicians now have a battery of extraordinarily refined tools for preclinical and clinical studies of gene therapy. The field is ripe for further successes. ■

nature Vol 462|12 November 2009

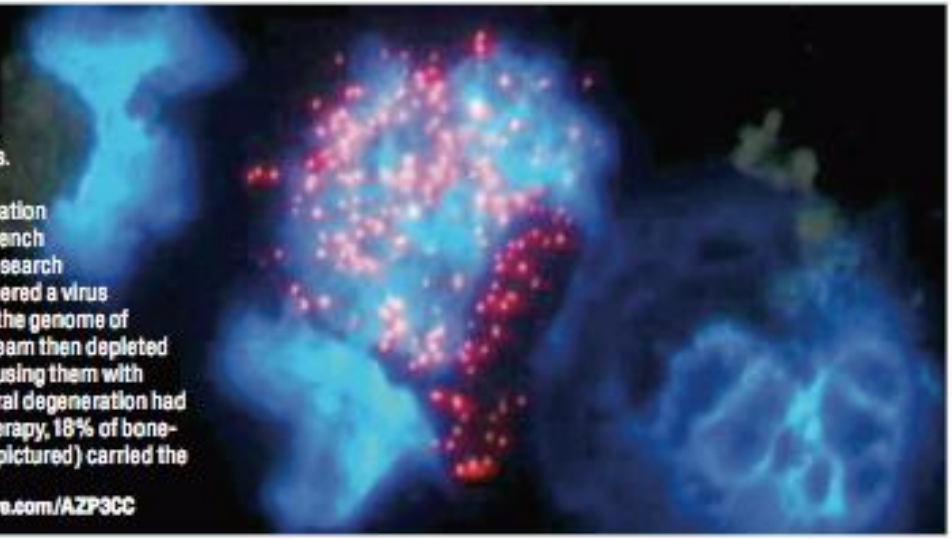
RESEARCH HIGHLIGHTS

Nerve repair

Science 326, 818–823 (2009)

Researchers have slowed a fatal brain disease by inserting a gene into stem cells and then transplanting them into two patients. X-linked adrenoleukodystrophy (ALD) is a neurodegenerative disease caused by a mutation in the *ABCD1* gene. Patrick Aubourg of the French National Institute for Health and Medical Research (INSERM) in Paris and his colleagues engineered a virus to insert a functioning copy of the gene into the genome of the patients' bone-marrow stem cells. The team then depleted the patients of their bone marrow before infusing them with the repaired cells. Sixteen months later, neural degeneration had stopped in both patients. Two years after therapy, 18% of bone-marrow stem cells in one patient (four cells pictured) carried the working gene (cell with red dots).

For a longer story on this research, see go.nature.com/AZP3CC



P. AUBOURG

DE NOUVEAUX ESPOIRS EN THÉRAPIE GÉNIQUE

5 NOVEMBRE

Deux médecins français publient dans la revue « Science » les résultats positifs du premier traitement d'une maladie neurodégénérative par thérapie génique.

LE SENS

L'équipe menée par Nathalie Cartier et Patrick Aubourg vient d'emporter un franc succès dans la lutte contre l'adrénoleucodystrophie, une maladie génétique du cerveau qui touche un nouveau-né sur 2 000 en France. En agissant sur le gène responsable, cette thérapie novatrice a permis d'enrayer, en un peu plus d'un an, la progression de la maladie

PARITÉ HOMMES-FEMMES : FAUT-IL VRAIMENT UNE LOI !

6 NOVEMBRE

Le ministre du Travail, Xavier Darcos, incite les partenaires sociaux à négocier sur l'égalité professionnelle hommes-femmes et promet une loi pour 2010.

LE SENS

N'en jetez plus ! Les entreprises doivent déjà négocier d'ici le 1er janvier un plan d'action en faveur des seniors, sous peine de sanctions financières. A cette date, les pénalités pour celles qui n'emploient pas assez de salariés handicapés seront durcies. Depuis quelques semaines, les DRH des grands groupes sont également pressés d'agir contre le

TRANSPORT AÉRIEN : LA COURSE À LA TAILLE CONTINUE

12 NOVEMBRE

Iberia et British Airways concluent un accord de fusion qui signe la naissance du troisième opérateur aérien européen dans un contexte de crise du secteur.

LE SENS

Après de longues fiançailles, les deux partenaires espagnol et britannique ont finalement décidé de se marier sous le régime de la communauté. Mais il faudra encore un an pour border définitivement le contrat et célébrer la noce : d'ici à la fin 2010 si tout se passe bien. Sur le Vieux Continent, ce mariage conclut la longue phase

Ardipithecus

[Image © 2005 J

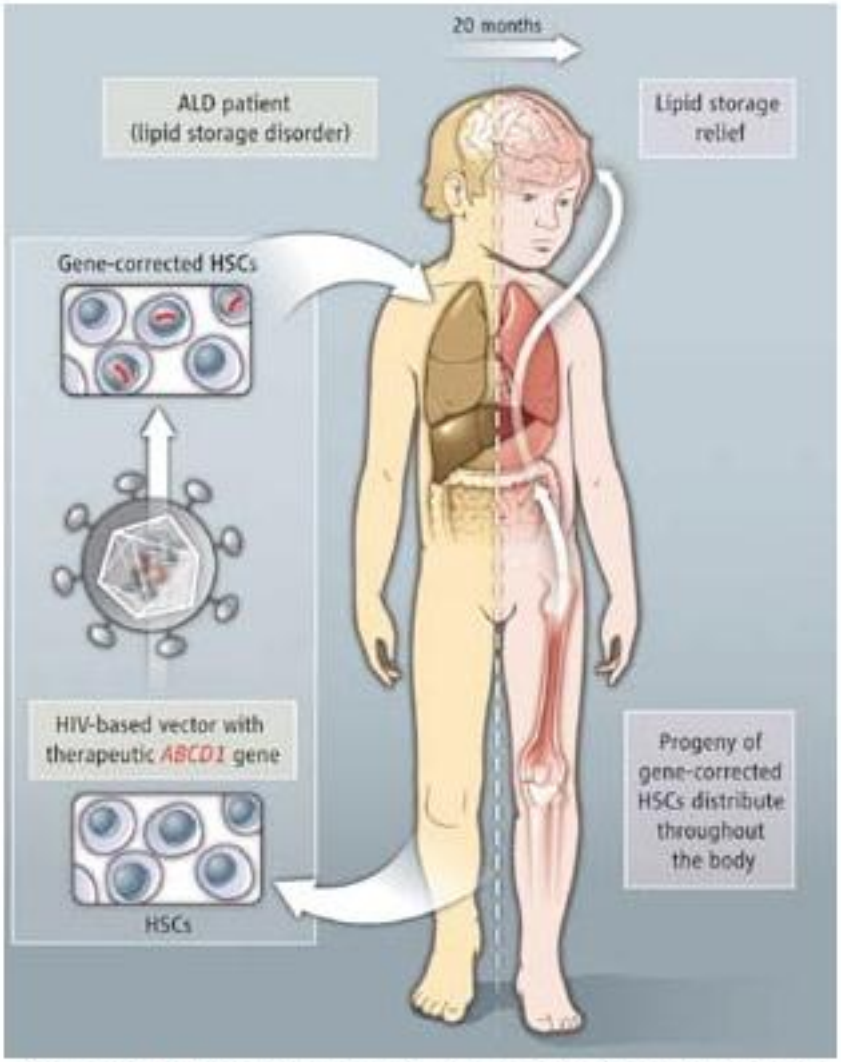
Science's list of the nine other groundbreaking achievements from 2009:

Pulsars Detected by Fermi: NASA's *Fermi* Gamma-Ray Space Telescope helped to identify previously unknown pulsars—highly magnetized and rapidly rotating neutron stars—and shed light onto their unique gamma-ray emissions.

Rapamycin: Researchers found that tinkering with a key signaling pathway produces life-extending benefits in mice—the first such result ever achieved in mammals. The discovery was particularly remarkable because the treatment did not start until the mice were middle-aged.

Graphene: In a string of rapid-fire advances, materials scientists probed the properties of graphene—highly conductive sheets of carbon atoms—and started fashioning the material into experimental electronic devices.

Plant ABA Receptors: Solving the structure of a critical molecule that helps plants survive during droughts may help scientists design new ways to protect crops against prolonged dry periods,



Progeny of HSCs that were engineered to carry the correct version of a gene (through the integration of a lentiviral vector) distribute throughout the body. Cartier et al. show that some cells replaced diseased microglia in the brain and relieved lipid storage in patients suffering from ALD. This image relates to an article that appeared in the 6 November 2009 issue of *Science*, published by AAAS. The study, by Dr. Nathalie Cartier-Lacave of INSERM in Paris, France and colleagues, was titled, "Hematopoietic Stem Cell Gene Therapy with a Lentiviral Vector in X-Linked Adrenoleukodystrophy."

du gène thérapeutique permet la diminution des lésions neuropathologiques et du phénotype clinique dans le modèle animal

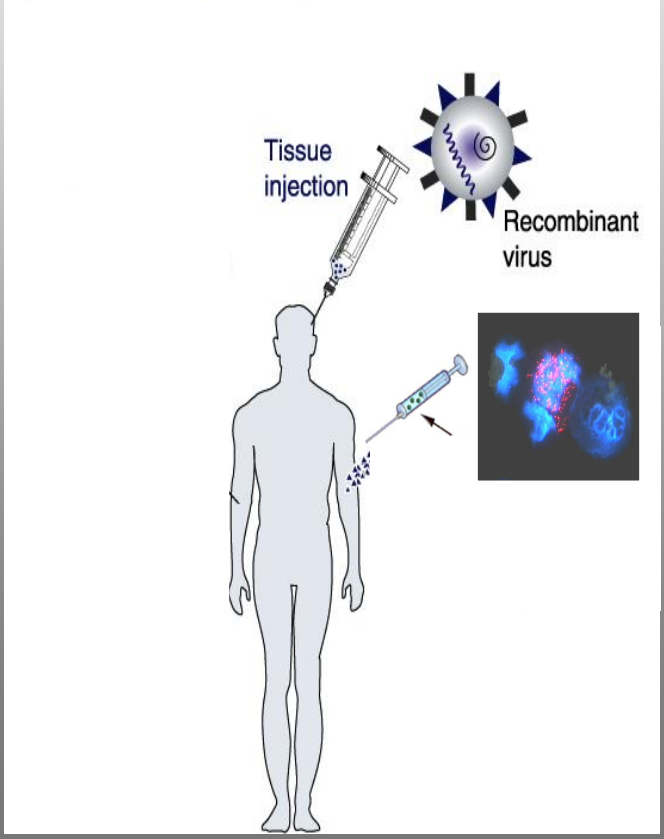
Ce gène est une cible thérapeutique ++++

compléter les données précliniques

pour proposer une thérapie génique

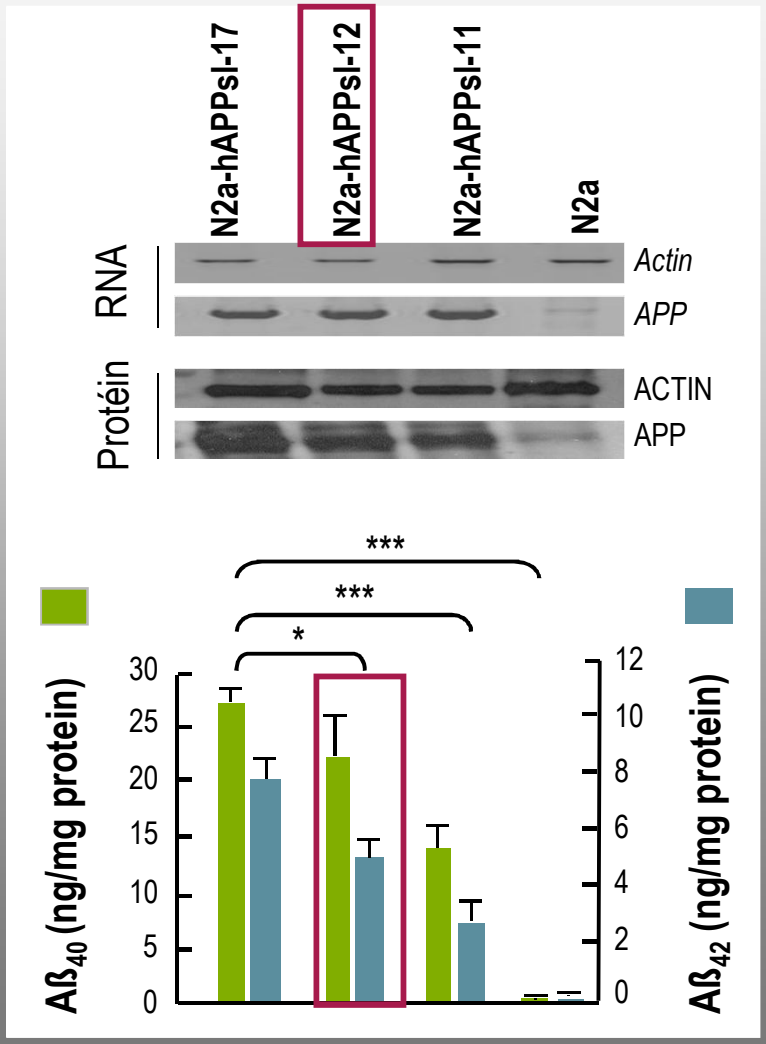
chez des patients Alzheimer

sévèrement et précocément atteints



IN VITRO STUDY

Murin neuroblastoid N2a cells overexpressing APPsl



on of CYP46 is associated with decreased Aß secretion

IN VITRO

Stable CYP46 Overexpression inN2A-APP