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Institut Thématique Multi-Organismes
Technologies pour la santé

Enjeux technologiques en thérapie génique à l'aide de vecteurs viraux AAV : de la preuve de concept préclinique à la bioproduction

Oumeya ADJALI, MD, PhD
Email: oumeya.adjali@inserm.fr

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UMR 1089

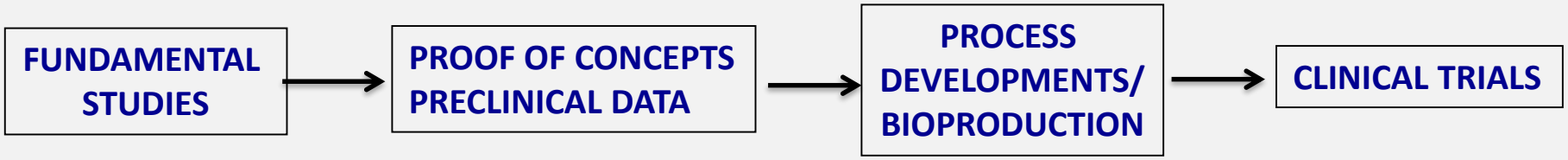
TRANSLATIONAL GENE THERAPY FOR GENETIC DISEASES

Adeno-Associated-based viral vectors (AAV)
Retinal and Muscular Gene Therapy

Inserm
La science pour la santé
From science to health

UNIVERSITÉ DE NANTES

AAV gene therapy product development: from fundamental to applied science



- **THERAPEUTIC GENE**
- **VECTOR DESIGN**
- **rAAV DELIVERY**
- **DOSE FINDING IN PRECLINICAL MODELS**

- **rAAV PROCESS DEVELOPMENTS**
- **rAAV CHARACTERIZATION**
- **TRANSFER TO GMP**
- **SUPPORT CLINICAL TRIAL**

UMR 1089: A unique translational expertise in gene therapy

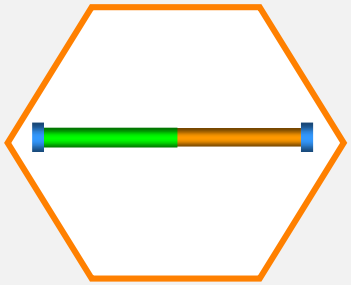
SINCE 2006: ISO 9001

Système de Management certifié

Immunomonitoring in phase I/II/III clinical trials

Recombinant Adeno-Associated Virus-based vectors (rAAV)

WT AAV



-Parvovirus family

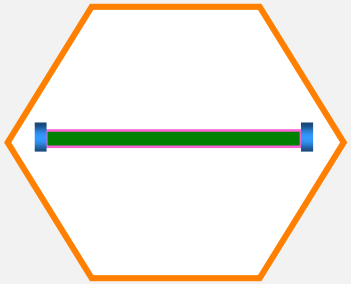
-Dependovirus: Replication-defective in the absence of a helper virus (Adénovirus, herpes virus, papillomavirus…)

-Non pathogenic

- Viral genome = single stranded DNA, 4700 bases

-Icosahedral Capsid: VP1, VP2, VP3 proteins

rAAV



- All proteins deleted except the viral capsid

-*In vivo*, a single injection of rAAV can sustain long term expression of the therapeutic transgene (>10 years)

-Viral genomes mainly episomal, non integrative

rAAV vectors: a wide gene transfer platform

-At least 12 natural serotypes identified with specific tropisms

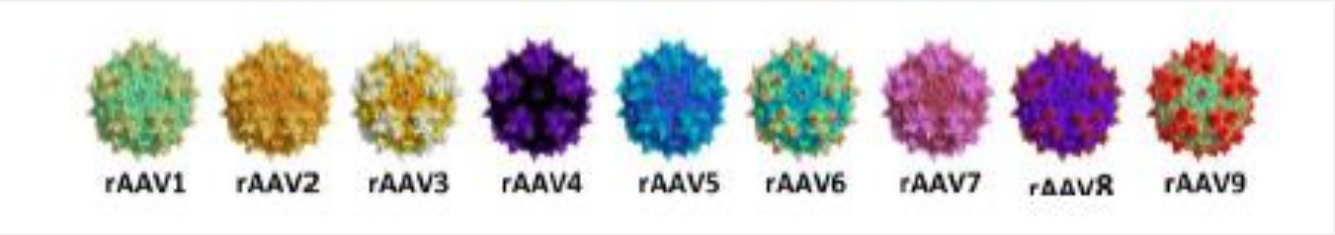


Table 2 Summary of current and emerging AAV vectors suitable for specific clinical applications

Organs	Disease targets	AAV serotypes and isolates
Liver	Hemophilia, α-1 antitrypsin deficiency, ornithine transcarbamylase deficiency	AAV8
Heart	Congenital heart failure, cardiomyopathies	AAV1, AAV6, AAV9
Skeletal Muscle	Muscular dystrophies, α-1 antitrypsin deficiency, lipoprotein lipase deficiency, lysosomal storage disorders	AAV1, AAV6, AAV9
Lung	Cystic fibrosis, α-1 antitrypsin deficiency	AAV5
CNS	Parkinson's, Alzheimer's, Batten's, Canavan's, epilepsy, amyotrophic lateral sclerosis, spinal muscular atrophy, Rett syndrome, lysosomal storage disorders	Intracranial: AAV1, AAV5, AAV8 Systemic: AAV9
Eye	Leber's congenital amaurosis, macular degeneration	AAV4, AAV8

From Asokan *et al. Mol. Ther.* 2012

rAAV vectors: a wide gene transfer platform

-At least 12 natural serotypes identified with specific tropisms

-**2nd generation rAAV with improved properties**: self complementary rAAV (double stranded DNA genomes), Site-directed mutagenesis, chimeric capsids, Selected AAV variants with directed evolution strategies…etc

Table 2 Summary of current and emerging AAV vectors suitable for specific clinical applications

Organs	Disease targets	AAV serotypes and isolates	Emerging vector candidates
Liver	Hemophilia, α-1 antitrypsin deficiency, ornithine transcarbamylase deficiency	AAV8	AAV2 (Y→F), AAV7, AAV-HSC15/17
Heart	Congenital heart failure, cardiomyopathies	AAV1, AAV6, AAV9	AAVM41, AAV2i8, AAV9.45
Skeletal Muscle	Muscular dystrophies, α-1 antitrypsin deficiency, lipoprotein lipase deficiency, lysosomal storage disorders	AAV1, AAV6, AAV9	AAV7, AAV2.5, AAV6 (Y445F/Y731F), AAV2i8, AAV9.45
Lung	Cystic fibrosis, α-1 antitrypsin deficiency	AAV5	AAV6.2, AAV2.5T, AAV-HAE1/2
CNS	Parkinson's, Alzheimer's, Batten's, Canavan's, epilepsy, amyotrophic lateral sclerosis, spinal muscular atrophy, Rett syndrome, lysosomal storage disorders	Intracranial: AAV1, AAV5, AAV8 Systemic: AAV9	For systemic use: AAVrh.10, AAV Clone 32/83
Eye	Leber's congenital amaurosis, macular degeneration	AAV4, AAV8	AAVShH10, AAV2 (Y→F), AAV8(Y733F)

From Asokan *et al. Mol. Ther.* 2012

rAAV: Most promising platform for *in vivo* gene transfer

Evidence of Multiyear Factor IX Expression by AAV-Mediated Gene Transfer to Skeletal Muscle in an Individual with Severe Hemophilia B

Haiyan Jiang,[1,*] Glenn F. Pierce,[1,*] Margareth C. Ozelo,[2] Erich V. de Paula,[2] Joseph A. Vargas,[1] and Peter Smith[1] Jürg Summer[1,*] Alvin Luk[1,*] Catherine S. Manno[3]

850 000 $

FDA News Release December 19, 2017

FDA approves novel gene therapy to treat patients with a rare form of inherited vision loss

Luxturna is the first gene therapy approved in the U.S. to target a disease caused by mutations in a specific gene

LAND
EDICINE

VOL. 365 NO. 25

Mediated Gene Transfer
B

B., B.S., M.D., Savita Rangarajan, M.B., B.S.,
B., B.Chir., Pratima Chowdary, M.B., B.S.,
, B.S.N., James O'Beirne, M.B., B.S., M.D.,
dip Rustagi, M.D., Catherine Y.C. Ng, M.S.,
Mark A. Kay, M.D., Ph.D., Junfang Zhou, M.D., Yunyu Spence, Ph.D., Christopher L. Morton, B.S., James Allay, Ph.D.,
John Coleman, M.S., Susan Sleep, Ph.D., John M. Cunningham, M.D., Deokumar Srivastava, Ph.D.,
Etiena Basner-Tschakarjan, M.D., Federico Mingozzi, Ph.D., Katherine A. High, M.D., John T. Gray, Ph.D.,
Ulrike M. Reiss, M.D., Arthur W. Nienhuis, M.D., and Andrew M. Davidoff, M.D.

The NEW ENGLAND JOURNAL of MEDICI

ESTABLISHED IN 1812 NOVEMBER 2, 2017 VOL

Single-Dose Gene-Replacement Therapy for Spinal Muscular Atrophy

J.R. Mendell, S. Al-Zaidy, R. Shell, W.D. Arnold, L.R. Rodino-Klapac, T.W. Prior, L. Lowes, L. Alfano, K. Berry, K. Church, J.T. Kissel, S. Nagendran, J. L'Italien, D.M. Sproule, C. Wells, J.A. Cardenas, M.D. Heitzer, A. Kaspar, S. Corcoran, L. Braun, S. Likhite, C. Miranda, K. Meyer, K.D. Foust, A.H.M. Burghes, and B.K. Kaspar

uniQure

UNIQURE obtains recommendation for market approval in Europe of GLYBERA®

July 20, 2012

1M $

rAAV vectors are already in the market

24/05/2019

NOVARTIS

Our Company Our Focus

AveXis receives FDA approval for Zolgensma®, the first and only gene therapy for pediatric patients with spinal muscular atrophy (SMA)

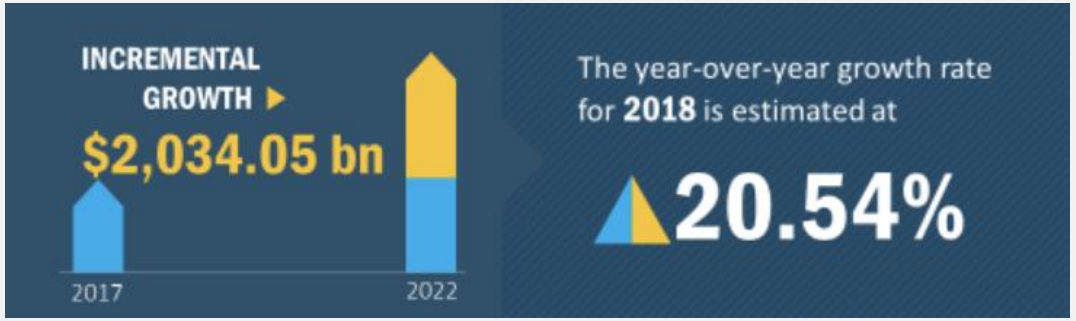
May 24, 2019

ACCUEIL › MONDE

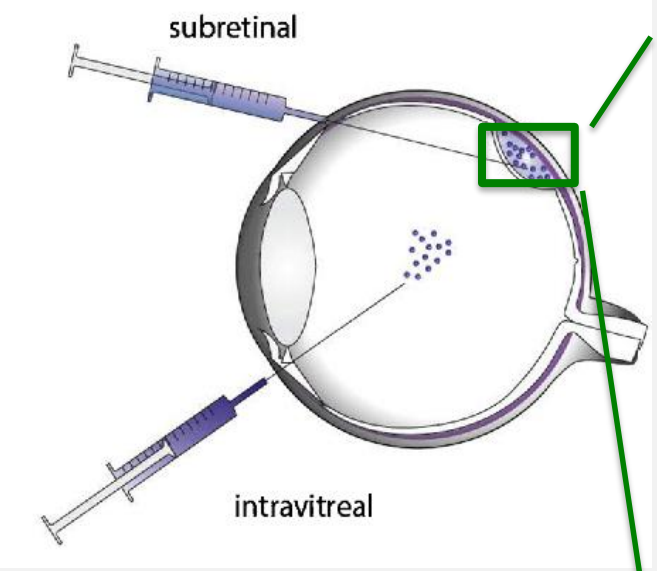
Zolgensma: Le médicament à 2,1 millions de dollars de Novartis autorisé aux Etats-Unis

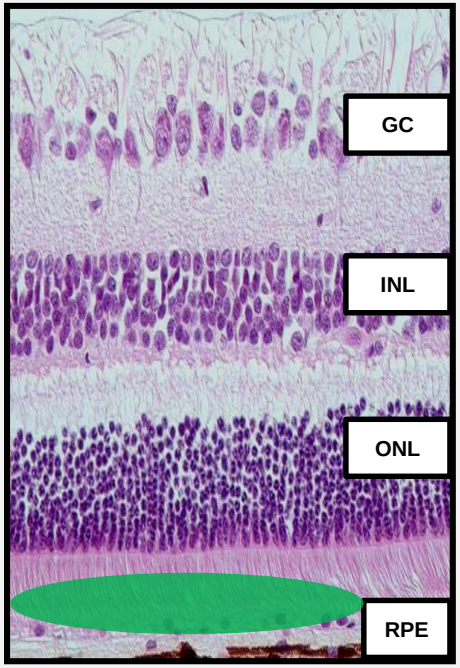
EXCESSIF Le traitement le plus cher du monde est destiné aux enfants atteints d'atrophie musculaire spinale

Gene Therapy market competitivity

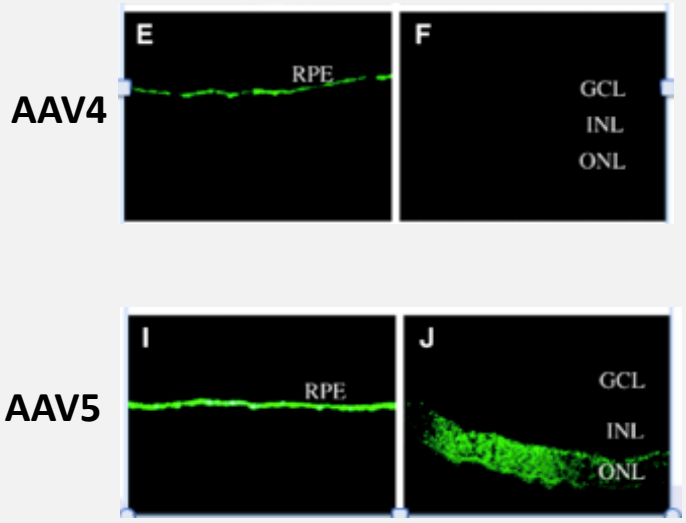


rAAV-based retinal gene therapy





Canine model



Weber *et al. Mol. Ther*. 2003

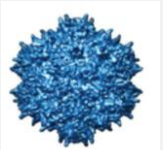
The AAV4 serotype results in exclusive transduction of the RPE layer (rat, dog and nonhuman primate model)

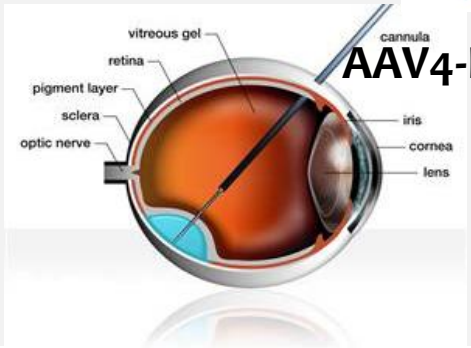
AAV4-RPE65-based clinical trial for Leber Congenital Amaurosis

ANSM authorization

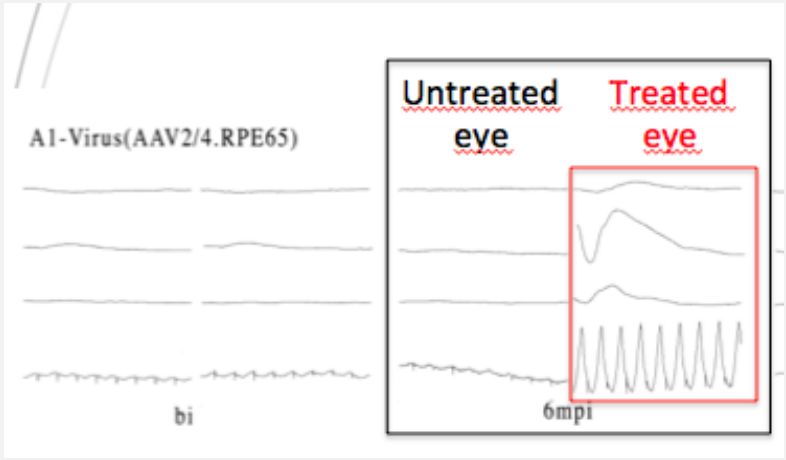
2003-2007	*2007-2009*	*2009-2010*	*2011-2013*
Proof of concept (Canine model, Boisbonne center)	**Toxicological studies**	**Clinical grade vector batch (ABG)**	**Clinical trial (CHU, Nantes)**

Fabienne Rolling group, Le Meur *et al.* 2007





AAV4-RPE65



Le Meur et al. 2007

Mol Ther. 2018 Jan 3;26(1):256-268. doi: 10.1016/j.ymthe.2017.09.014. Epub 2017 Sep 19.

Safety and Long-Term Efficacy of AAV4 Gene Therapy in Patients with RPE65 Leber Congenital Amaurosis.

Le Meur G[1], Lebranchu P[2], Billaud F[3], Adjali O[4], Schmitt S[5], Bézieau S[5], Péréon Y[6], Valabregue R[7], Ivan C[3], Darmon C[4], Moullier P[4], Rolling F[4], Weber M[8].

AAV5-PDE6b-based clinical trial for PDE6b-related retinal dystrophy

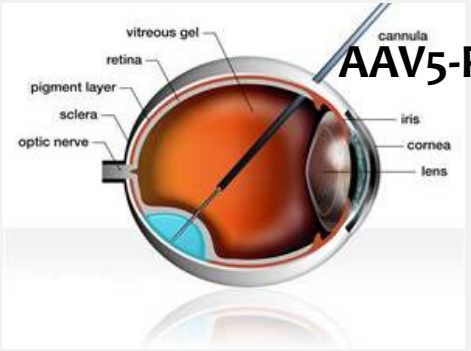
2010-2014 — **Proof of concept (Canine model, Boisbonne center)**

2015 — **Toxicological studies**

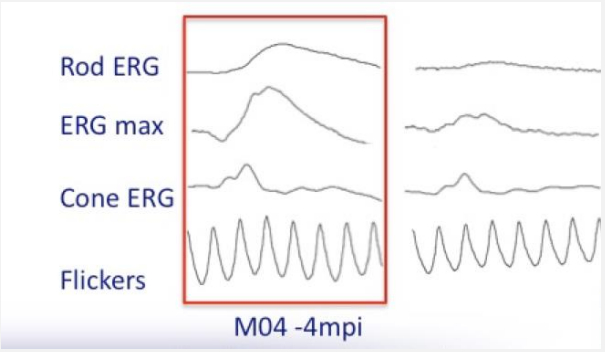
2016 — Clinical grade vector batch (ABG)

2017-Present — Clinical trial (CHU, Nantes)

ANSM authorization :Jul 2017



AAV5-PDE6b

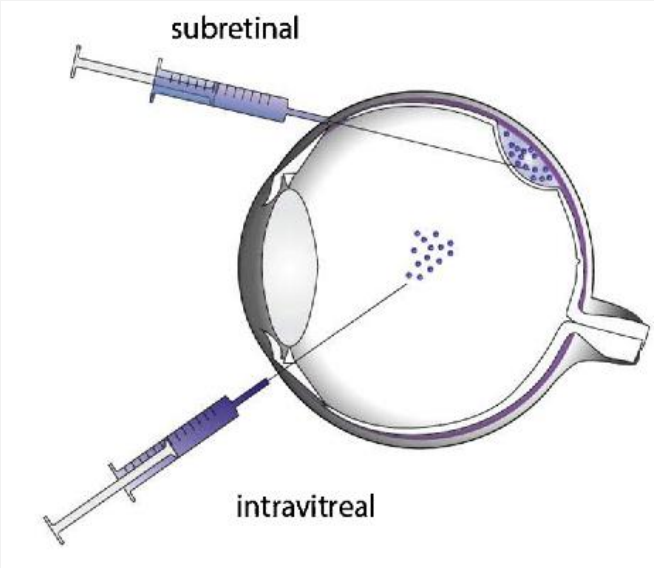


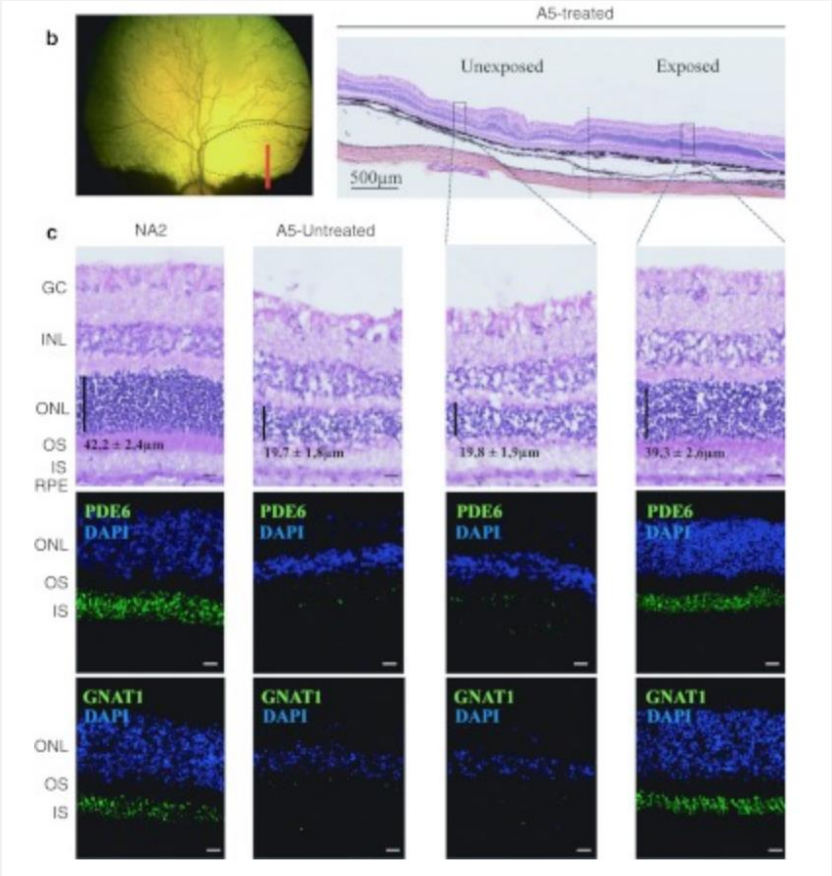
Petit L. et al. Mol Ther 2012
Pichard V. et al. Mol Ther 2016

4 patients already injected (CHU Nantes, sponsor : HORAMA Biotech, Licence 14160A40, 2015)

How to better target retinal cells ?

F. Rolling, V Pichard, T Cronin et al.





Pichard V. et al. Mol Ther 2016

Ameline B, Tshilenge KT *et al.* 2017

Subretinal gene transfer

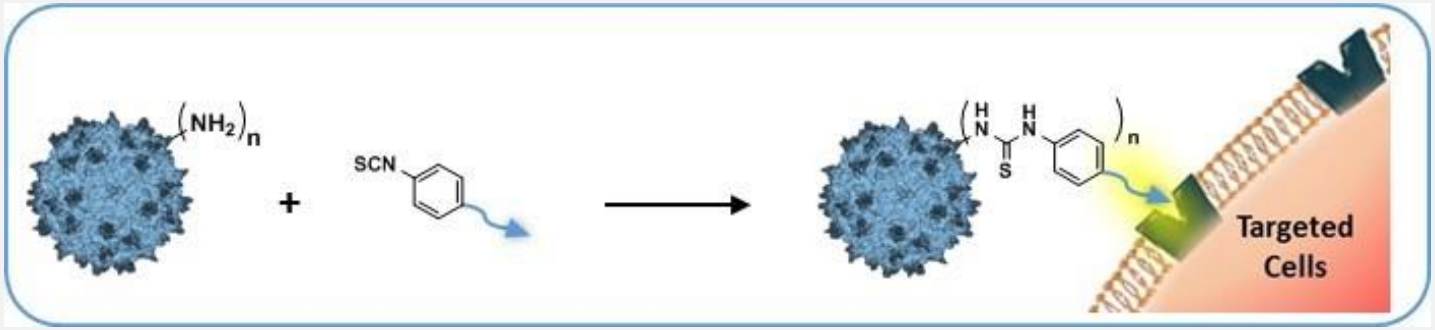
- **Invasive surgical method (retinal detachment, inflammation)**
- **Restricted gene transfer area**

Intravitreal delivery with new AAV variants (i.e AAV2 7.8, Delkara *et al.* 2013)

NExT Gen AAV: When Chemistry assists Biology

Chemical modification of AAV capsid

E. Ayuso and M. Mevel (UMR 1089), D. Deniaud (CEISAM) et al.

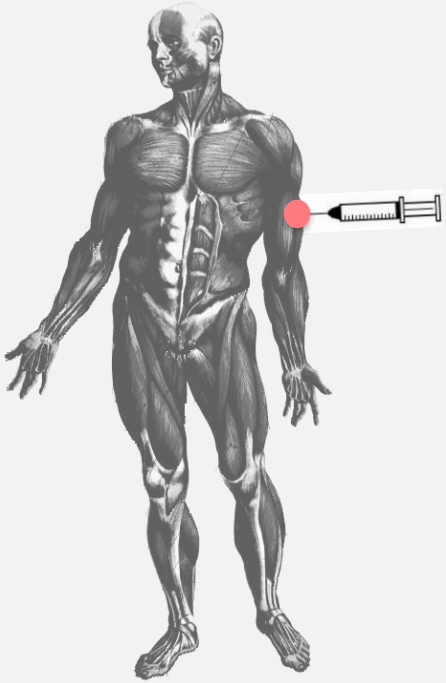


- **2 patents, 1 licence (retinal application)**

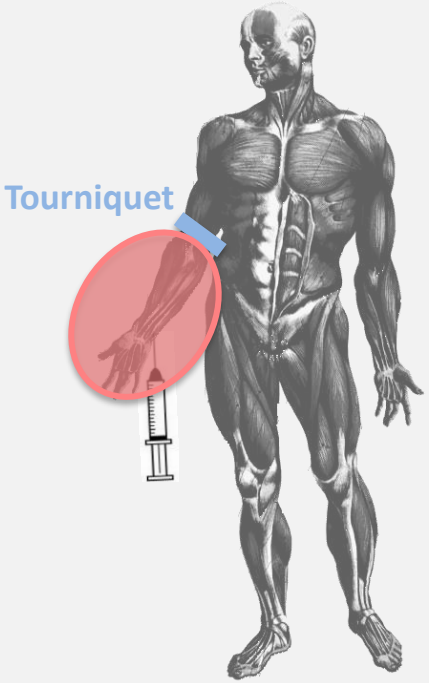
Mevel *et al. Chemical Sciences*, in revision

rAAV-based muscular gene therapy

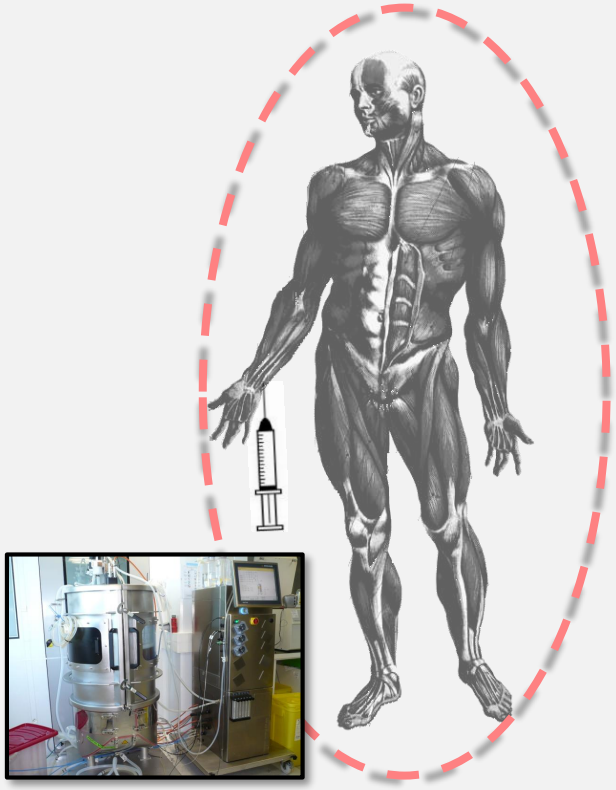
Intramuscular (IM) injection



Locoregional (LR) Injection



Systemic injection



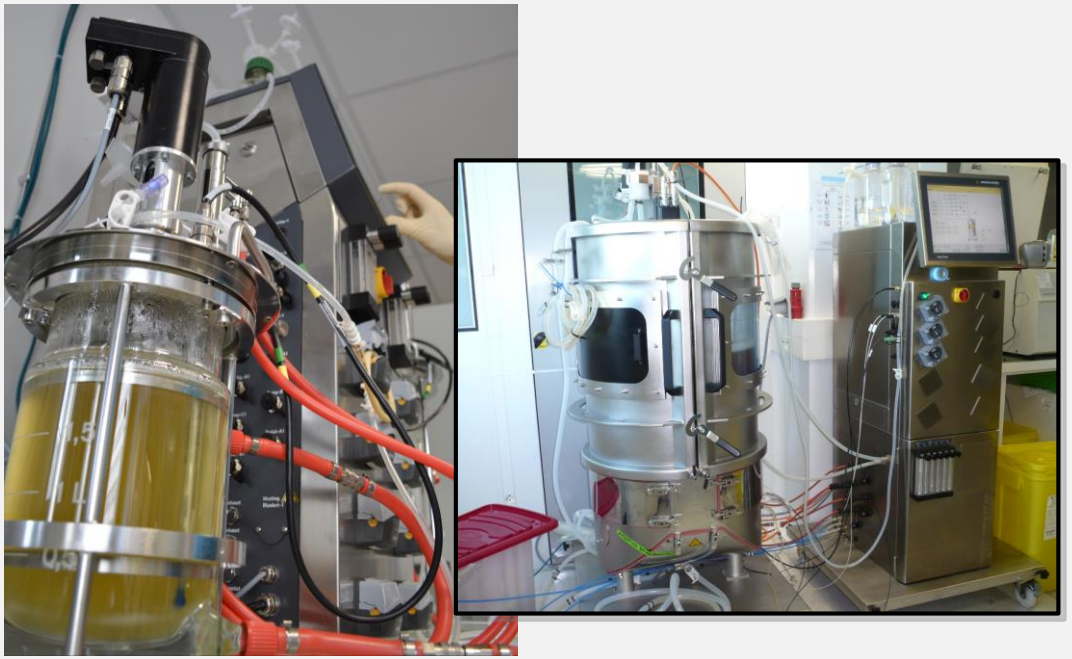
Past strategies

Current-Future

Systemic injection requires high rAAV vector doses (up to $1x10^{14}$ vg/kg)

Moving from small to large scale AAV biomanufacturing processes

Culture plates, 293 adherent cells



Bioreactors: up to 50L in the lab

Baculovirus system (Insect cell suspension)

293 suspension systems

No bioproduction standards

Evaluation of rAAV8-Spc5.12-cµDys (cMD1) in GRMD dogs – Systemic treatment

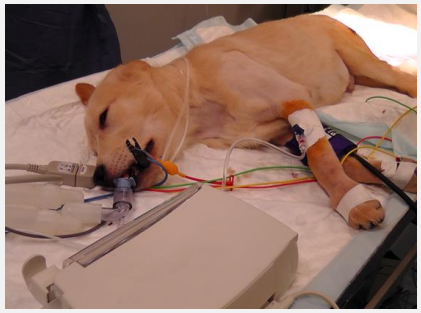
C Le Guiner *et al.*

2-month old GRMD dogs

rAAV8-canine_µDys

Intravenous injection in one cephalic vein

No immunosuppression



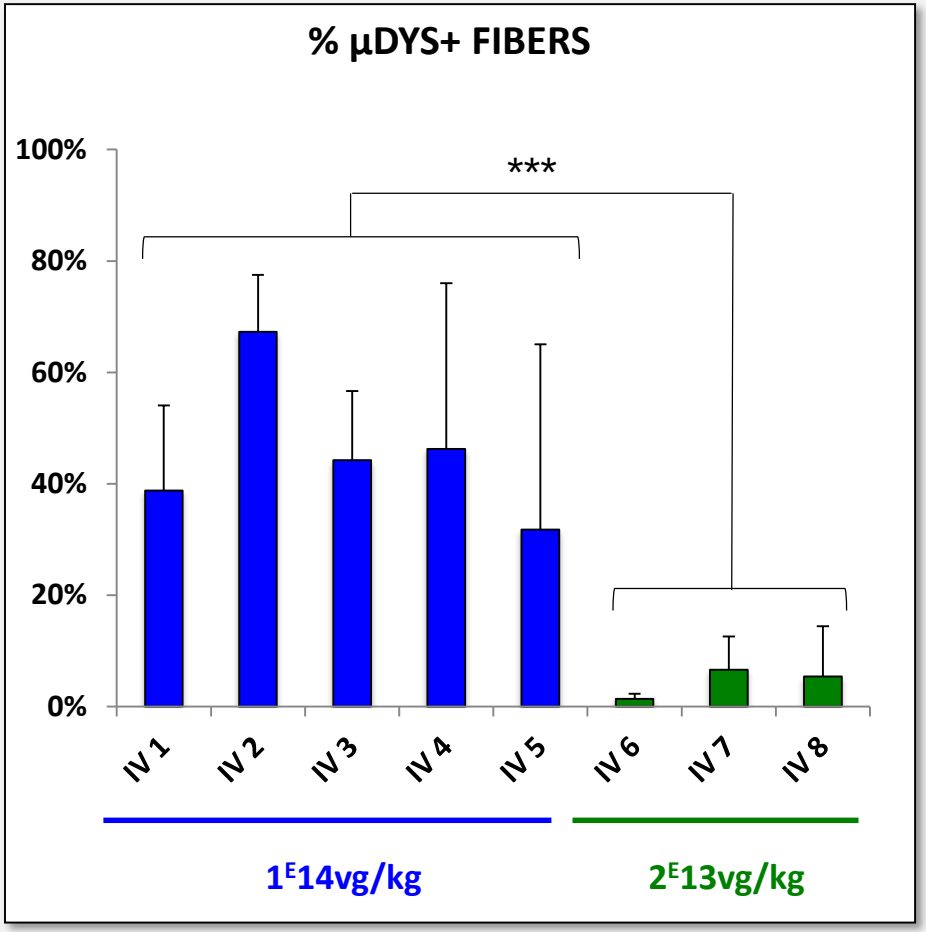
Dose	Timing of follow-up	Name of the dog	Age at injection	Follow-up duration after injection
$1^{E}14$vg/kg	LONG TERM	IV1	2 months	24 months
		IV2	2.5 months	24 months
	≈ 8 MONTHS POST-INJECTION	IV3	2 months	7.5 months
		IV4	2 months	8.5 months
		IV5	2.5 months	8 months
$2^{E}13$vg/kg	≈ 8 MONTHS POST-INJECTION	IV6	2 months	8.5 months
		IV7	2 months	8.5 months
		IV8	2.5 months	6.5 months

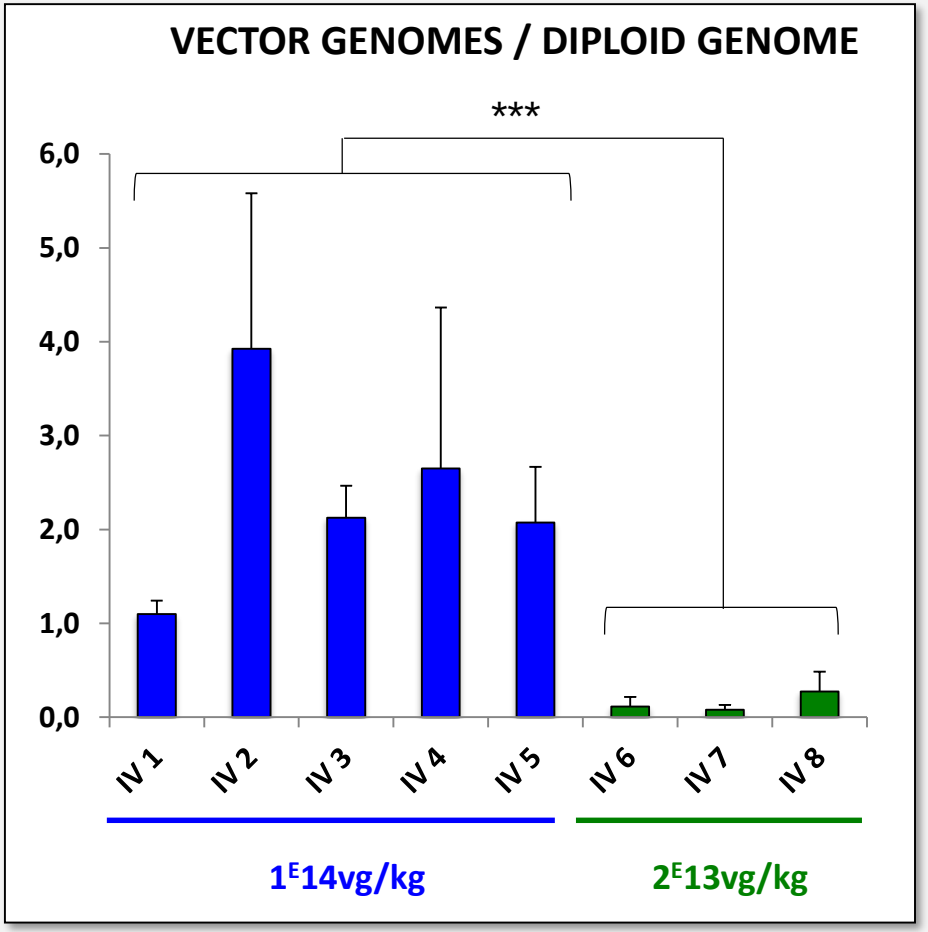
+ Controls dogs: non injected GRMD dogs + healthy dogs

Le Guiner *et al.* Nat Com 2017

Intermediate muscular biopsies

3.5 months post-injection
n=4 biopsies / dog



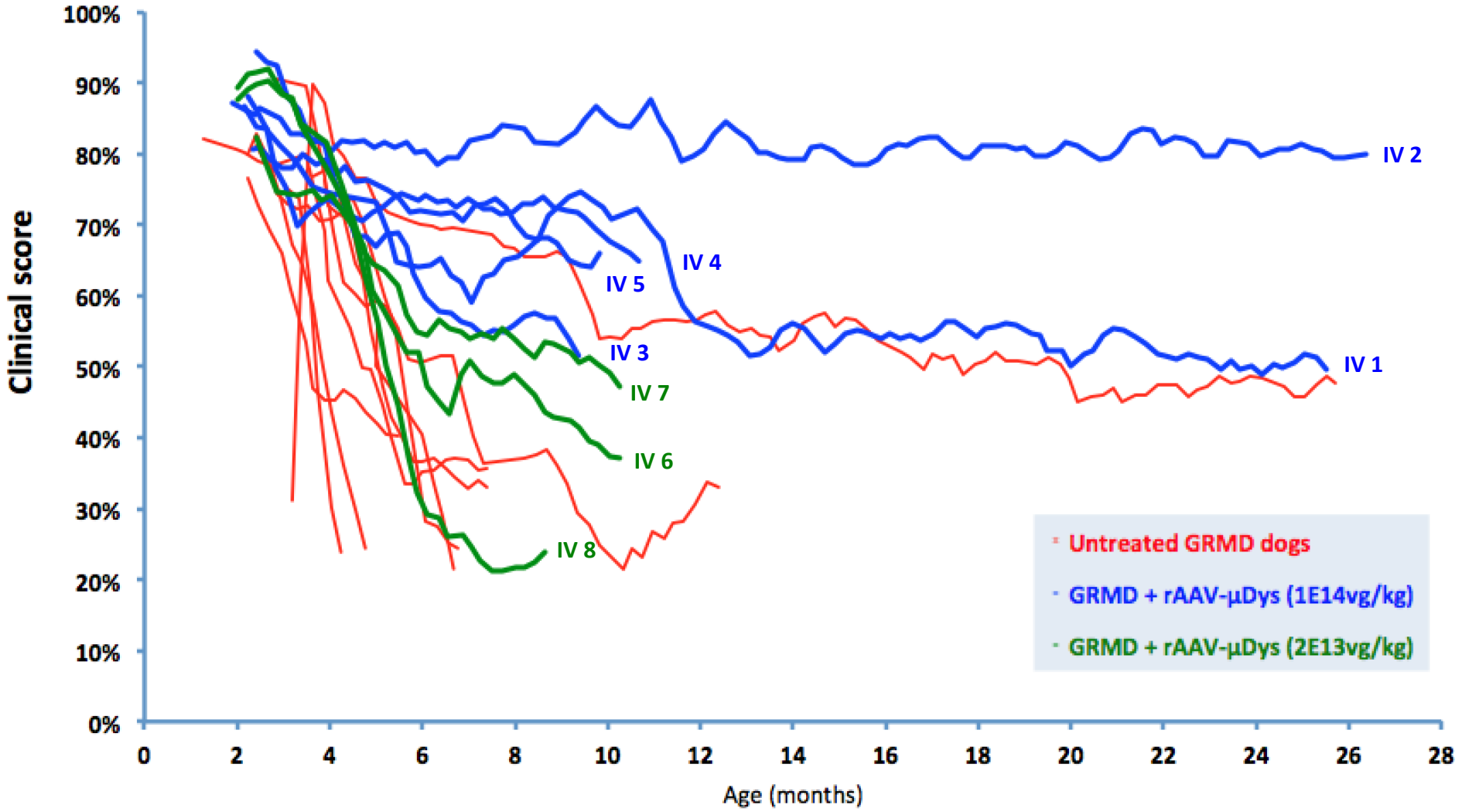


Clinical monitoring

→ Weekly clinical exams

→Scoring of different criteria: *dysphagia, breathing, muscular firmness, general activity…*

→ 100% = healthy dog

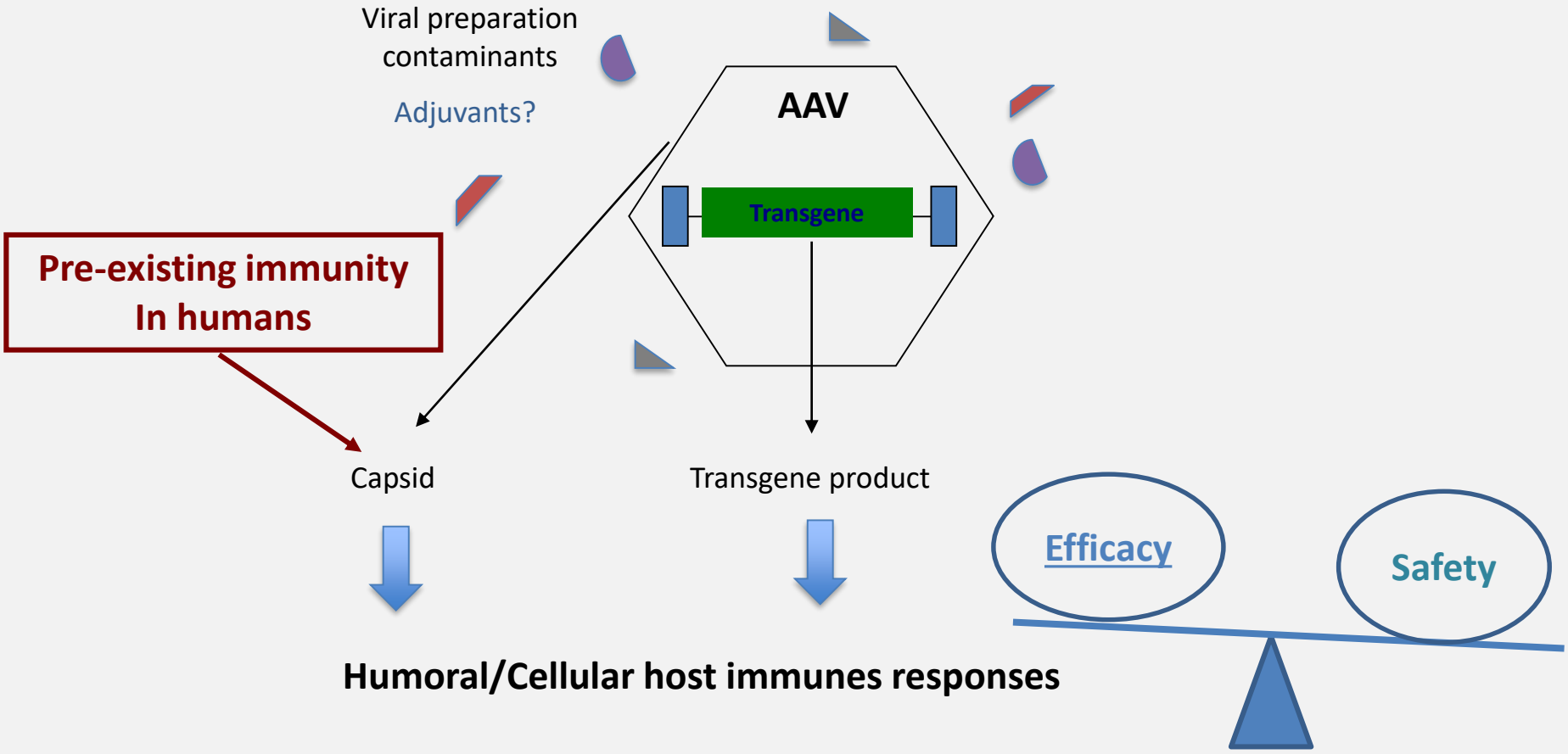


Atlantic Gene Therapies

6 month-old GRMD dog

Untreated

Systemic vector delivery raises immunogenicity issues

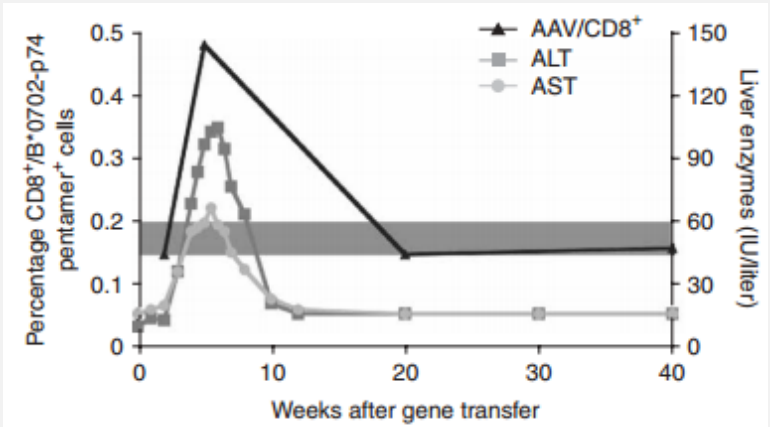


Systemic phase I/II clinical trials: exclusion of positive individuals

Pre-existing cellular immunity can be reboosted by rAAV vectors

	AAV2	AAV4	AAV5	AAV8	AAV9
Positive donors	6% (5/80)	6,8% (4/59)	0% (0/93)	21% (19/91)	49% (29/59)

Xicluna et al. Unpublished



Mingozzi *et al.* (Nature Medicine, 2007)

In vivo evidence of dose-dependent capsid-associated hepato-toxicity in rAAV-treated hemophilia patients

Costricosteroids are sufficient to control these responses

Inflammatory tissues (muscle, liver ?)

rAAV-based muscular gene therapy

10^{14} vg/kg GRMD

$5x10^{15}$ vg/patient 50kg → 1 bioréacteur de 200L

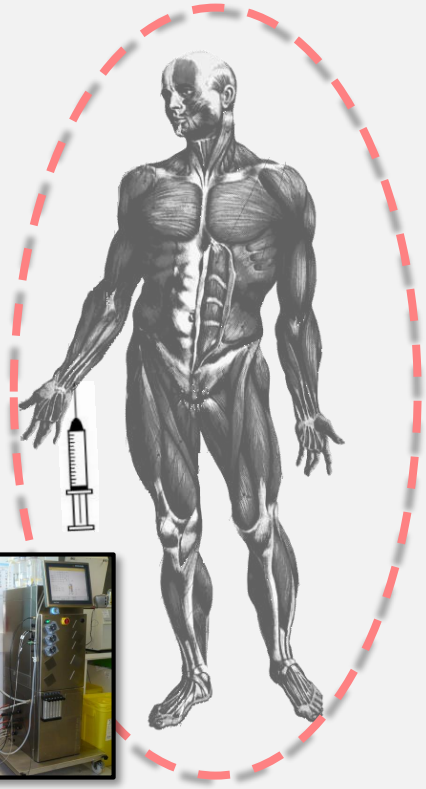
$5x10^{16}$ vg/10 patients → 1 bioréacteur de 2000L
(Phase (I/II)

$1.5x10^{17}$ vg/30 patients → 1 bioréacteur de 6000L
(Phase II pivot)

AMM

$1x10^{19}$ vg/500 patients → 1 bioréacteur de 100 000L
ou
500 bioréacteur de 200L
≈ 15% de la population Duchenne française

Systemic injection



Current-Future

Largest challenge for gene therapy: Bioproduction

Isite Nantes

NeXT
HEALTH AND ENGINEERING

Health and Industry of Future

Le Conseil de l'innovation lance deux grands défis sur la ...

https://www.industrie-techno.com › Innovation

18 avr. 2019 - Le troisième Conseil de l'innovation a lancé le 17 avril deux nouveaux **grands défis**, sur la cybersécurité et la **bioproduction**. Parallèlement, les ...

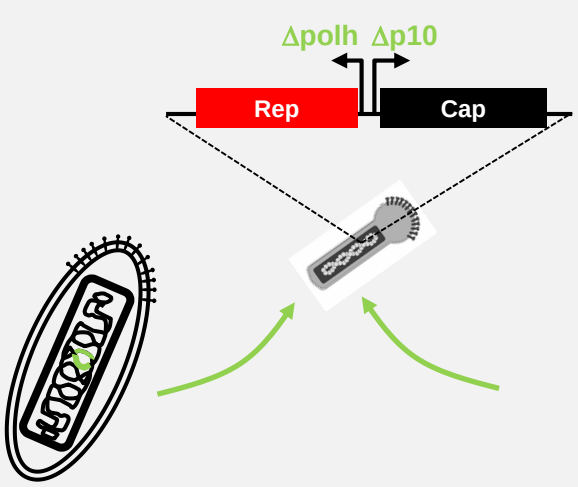
NExT Gen AAV: Back to Virology

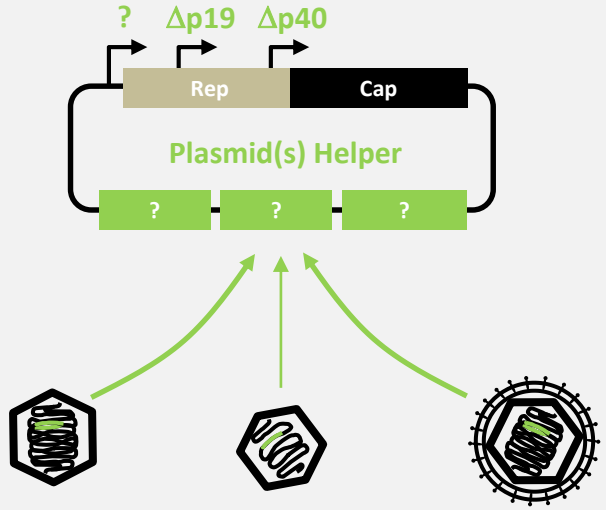
Design of new helper plasmids in insect and mammalian cells

M. Penaud *Budloo et al.*

Baculovirus Optimized System

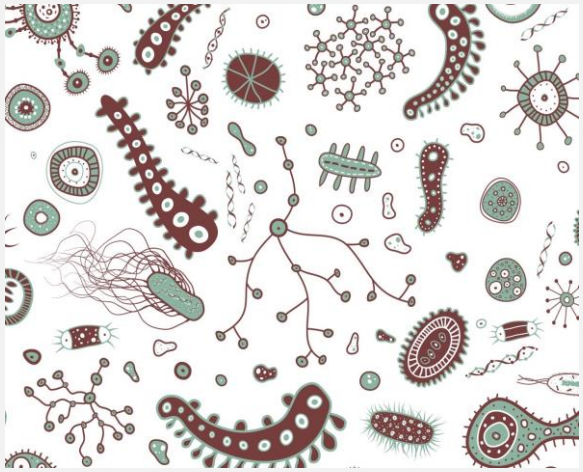
Maammalian cell Optimized System





✓**Improve DNA replication and encapsidation in AAV vectors**

rAAV preps may do not look like…



Adrien Leger, former PhD student

What contaminants ?
What is the proportion of “true rAAV products”

Need of standard QC assays and titration methods

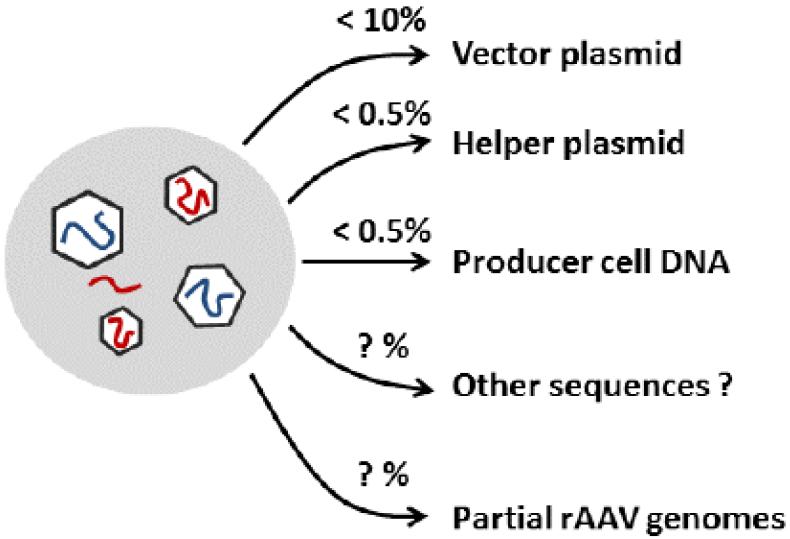
Hum Gene Ther. 2014 Nov;25(11):977-87. doi: 10.1089/hum.2014.057.

Manufacturing and characterization of a recombinant adeno-associated virus type 8 reference standard material.

Ayuso E[1], Blouin V, Lock M, McGorray S, Leon X, Alvira MR, Auricchio A, Bucher S, Chtarto A, Clark KR, Darmon C, Doria M, Fountain W, Gao G, Gao K, Giacca M, Kleinschmidt J, Leuchs B, Melas C, Mizukami H, Müller M, Noordman Y, Bockstael O, Ozawa K, Pythoud C, Sumaroka M, Surosky R, Tenenbaum L, van der Linden I, Weins B, Wright JF, Zhang X, Zentilin L, Bosch F, Snyder RO, Moullier P.

Characterization of DNA contaminants in rAAV batches

E Ayuso, M. Penaud-Budloo, E Lecomte et al.

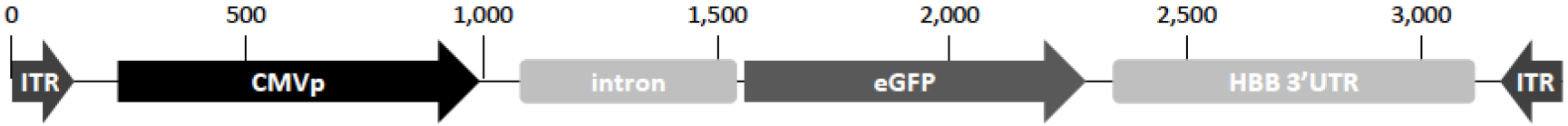


- **Transfer of antibiotic resistance genes**
- **Potentiate Immune response / Silencing (prokaryotic sequences, uCpG)**
- **Oncogenes (E1, E4, cellular genes)**
- **Viral genes (adenovirus genes, rcAAV)**
- **Lower efficiency**

Developement of a high-throughput sequencing- based charcaterization method

Lecomte *et al.* 2015

Example: Analysis of rAAV.rh10-GFP lots produced in insect cells

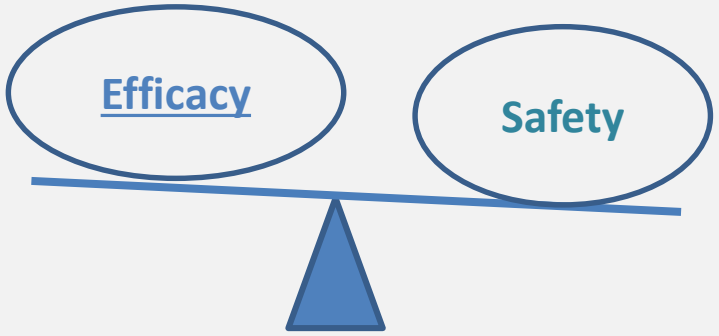


%	CsCl	CsCl	AVB	AVB
Reference Sequence	-DNase	+DNase	-DNase	+DNase
rAAV genome	98.91	98.28	98.35 98.58	97.80
Baculovirus backbone	1.03	1.61	1.56 1.34	2.07
rep-cap	0.05	0.08	0.07 0.06	0.10
Sf genome	0.01	0.03	0.02 0.02	0.03

rAAV are promising but complex therapeutic products with remaining challenges

(13 different capsids *vs* n Chimeric capsids) X (Homologous ITRs *vs* Heterologous ITRs) X (Single stranded AAV *vs* Self complementing AAV) X (Transfection *vs* Stable cell lines *vs* Herpes *vs* Baculovirus) X (CsCl *vs* Affinity column *vs* Ion exchange column)

X (Buffer (should we care ?)
Stability (should we ? How ?)
Titer (vg ? ip ? How ?)
Purity (How ? Spec ?)
Sterility (should we ? What ?)) X (Expression cassette) X (Dose) X (Mode of delivery) X (Target organ) X (Specy) X (Capsid metabolism) ...



From bench to bedside and back again !

Development of rAAV vectors with higher therapeutic index

-Reduction of manufacturing cost

-Reduction of immunotoxicity issues

-New Generation vectors with improved tropism

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Journées annuelles du laboratoire, 20 et 21 Juin 2019

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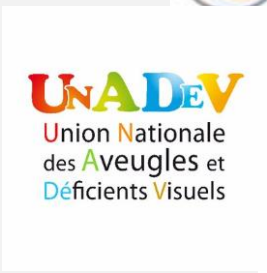
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