Overcoming rAAV Production Barriers & Non-viral vectors

Robert M. Kotin

University of Massachusetts Medical School

Generation Bio

Disclosures

Generation Bio – Scientific Founder
and Head of Discovery

Inventor on US and European patents assigned to NIH and UMMS

Consultant to Gene Therapy Companies

National **Heart Lung and Blood** Institute
Division of Intramural Research

Association
Monégasque
Contre les
Myopathies

Duchenne
Parent Project
France

Viral and non-viral gene therapy platforms

rAAV

- Two regulatory approved products:
  - Glybera™ uniQure
  - Luxturna™ Spark
  - Other advanced programs
- Vectors derived from non-pathogenic dependoparvoviruses
- No virus coding sequences
- Efficiently transduces a wide range of tissue / cell types
- Demonstrated safety in clinical studies

ceDNA

- Scalable production in *Sf*9 cells
- AAV-like properties:
  - Persistent expression
  - Episomal
- No capsid -> no nAbs
- Re-administrable
  - Titrate to achieve effect
  - Re-dose to maintain therapeutic effect

rAAV gene therapy
drug development challenges

CMC

- Vector production
  - Non-clinical
  - GMP
- Quality
- Know-how
- Economics

Production

Production

Production

The best vector is useless if it can not be produced in meaningful quanitites

Everything else

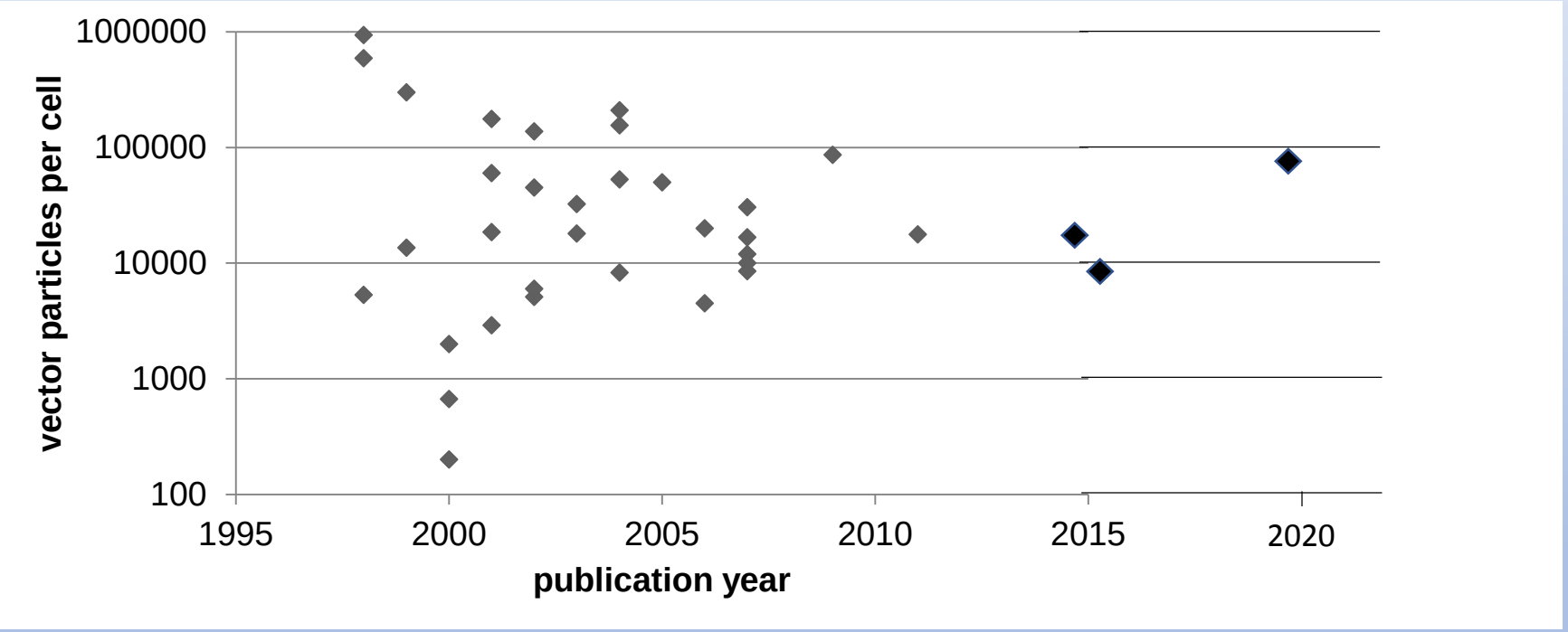
- AAV capsid capacity
- Pre-existing and cross-reacting neutralizing Abs
- Practically limited to single-administration
- Patient-to-patient variability
- Animal models non-predictive

AAV vector dose ranges for clinical studies

- Ocular
  - Intravitreal 10^9 - 10^{12}
  - Subretinal $<10^{12}$
- CNS
  - Intrastriatal – 10^{13}
  - Intrathecal / intraventricular – 10^{13} - 10^{14}
- Systemic
  - Liver - $\leq 10^{15}$ ($\geq 10^{13}$ per kg)
  - CNS - $\leq 10^{15}$ ($2x10^{14}$ per kg)
  - Skeletal muscle - $\leq 10^{15}$ ($\geq 10^{13}$ per kg)

Vector doses expressed as vector genomes (vg) represent filled particles

rAAV Production by Publication Year



Bioprocessing Requirements

Volumetric vs Adherent Cell Processes

log vg	log cell#	Vol (ml or L)	# bioreactors	15cm plates	Area cm^2	m^2	km^2
10	5	0.1	**1 tube**	0.005	1	0.0001	1.00E-10
11	6	1	**1 tube**	0.05	10	0.001	1.00E-09
12	7	10	**1 tube**	0.5	100	0.01	1.00E-08
13	8	100	**1 flask**	5	1000	0.1	1.00E-07
14	9	1000	**1 flask**	50	10000	1	1.00E-06
15	10	10	**1 benchtop**	500	100000	10	1.00E-05
16	**11**	100	**1 wave bag**	**5000**	1000000	100	1.00E-04
17	**12**	1000	**1 bioreactor**	**50000**	10000000	1000	1.00E-03
18	**13**	10000	**1 bioreactor**	**500000**	100000000	10000	1.00E-02
19	**14**	100000	**1 bioreactor**	**5000000**	1E+9	100000	1.00E-01
20	**15**	1000000	**1 fermenter**	**50000000**	1E+10	1000000	1.00E+00
21	**16**	10000000	?	**500000000**	1E+11	1E+7	1.00E+01
22	**17**	100000000	?	**5000000000**	1E+12	1E+8	1.00E+02
23	**18**	1000000000	?	**500000000000**	1E+13	1E+9	1000

Assumptions

a. Yield = 10e5vg/cell

b. Cell density = 10e5/cm(2)

c. Cell density = 10e6/cm(3)

Two widely used rAAV production processes

- Human Embryonic Kidney (HEK) 293 cells

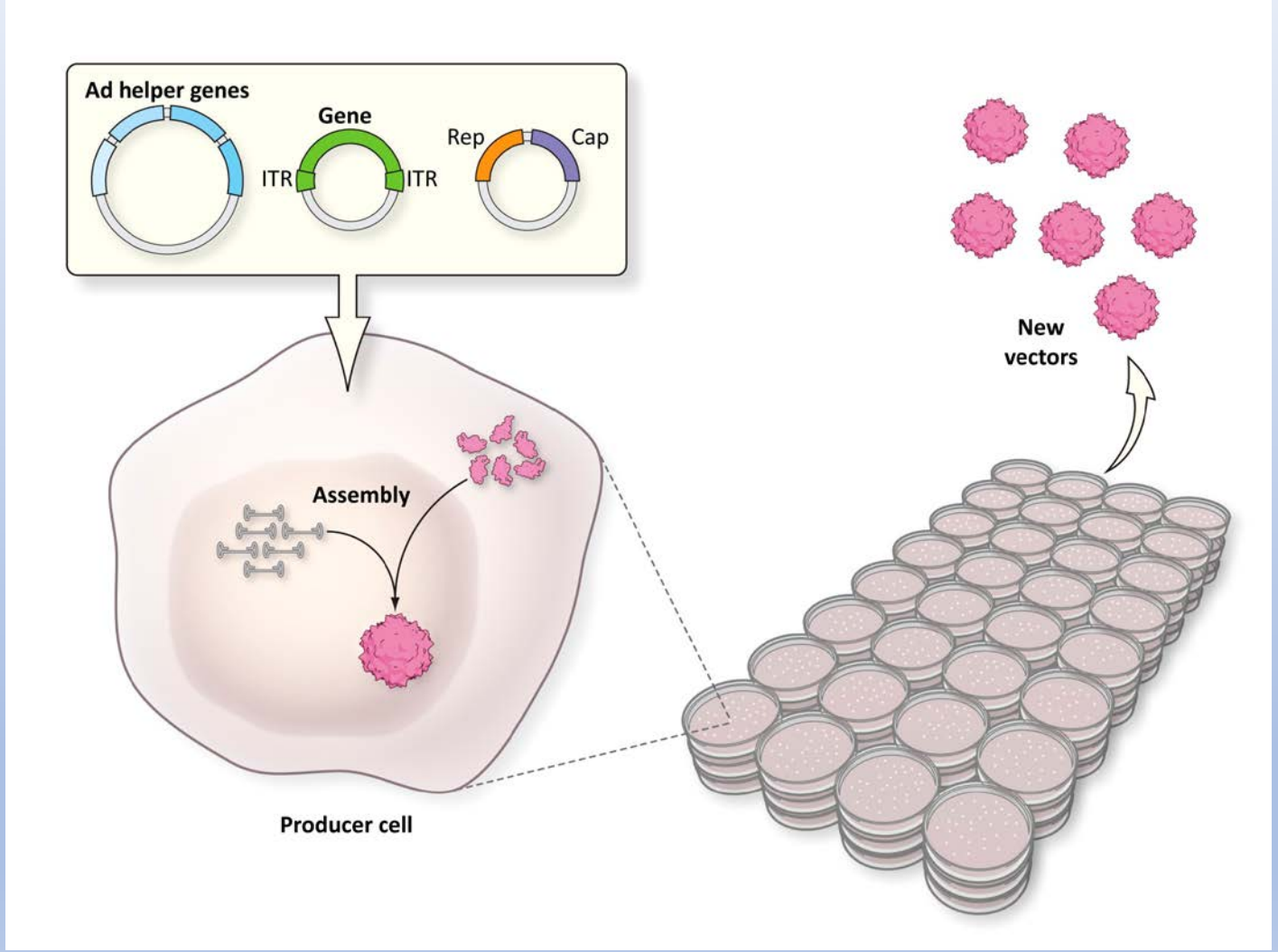
- *Spodoptera frugipeda (Sf*9*)* cells + baculovirus

All processes are transient - AAV and adenovirus (helper virus) gene products required (Cytotoxicity associated with both virus gene products)

Chemical Transfection
Human Embryonic Kidney (HEK) 293 cells

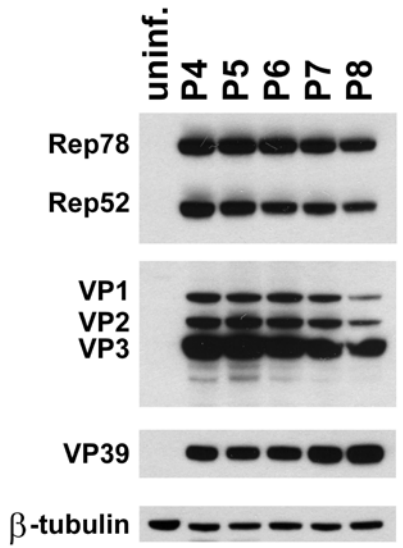
Transfection:

- Transfection efficiency
  - Inorganic media, e.g. CaPi
  - Organic media, e.g., Lipids
- No cell-to-cell spread
  - Primary cells only
- Adherent cells
  - Limited expansion
- Plasmids
  - Cost – expensive
  - Quality (*E. coli* product)
  - Non-bacterial sources
  - Stable and easily stored
- Vector quality
  - No engineering of *cap* gene



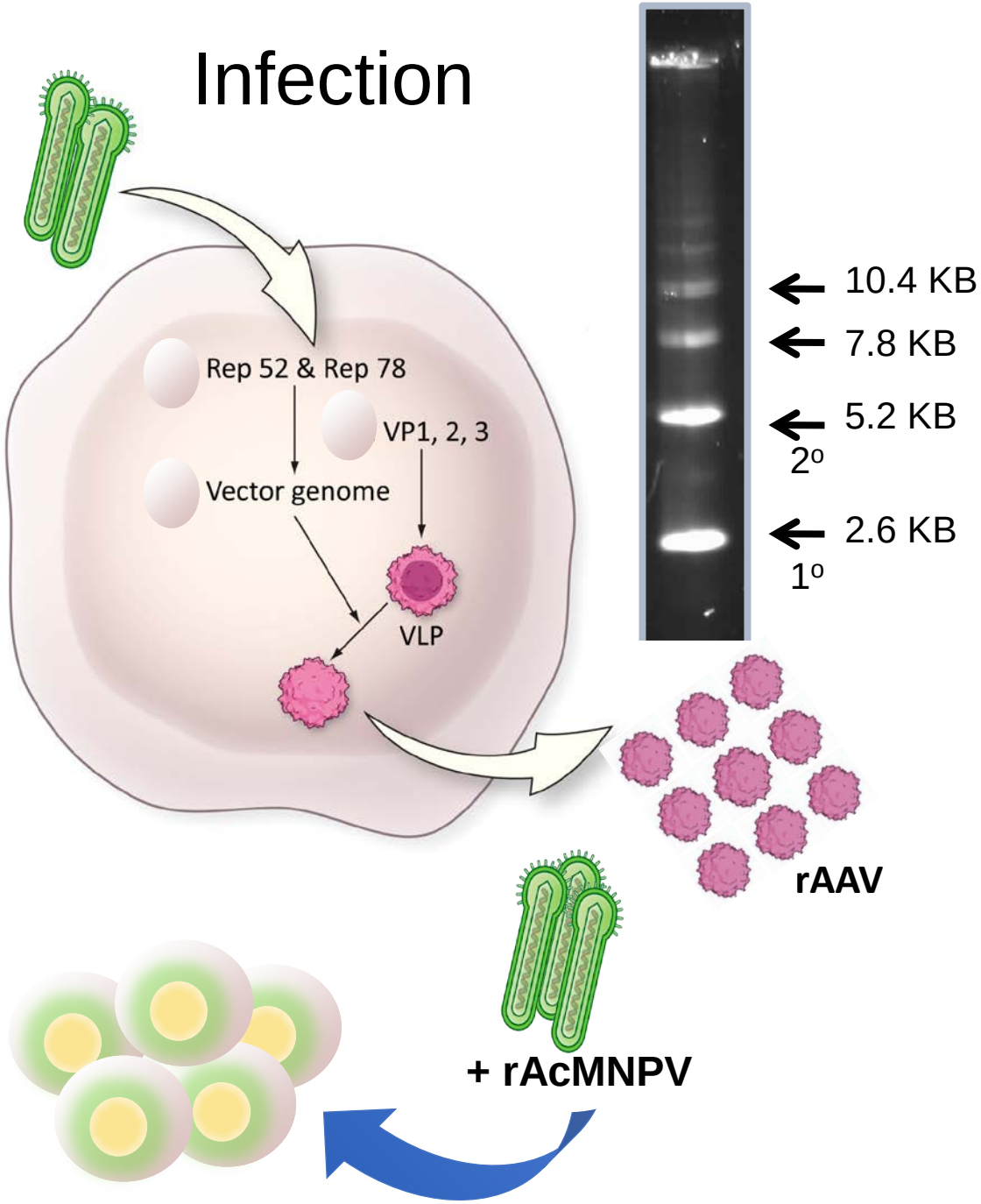
Spodoptera frugipeda (Sf9) cells + baculovirus

- Baculovirus
  - Cell-to-cell spread –
  - Low MOI : 2° and 3° infections
- Volumetric expansion
  - Relatively low cost
  - BEVs and rAAV produced in same cells
  - Genetic and physical stability
- Vector quality
  - Requires optimization of *cap* gene expression



2x rAcMNPV

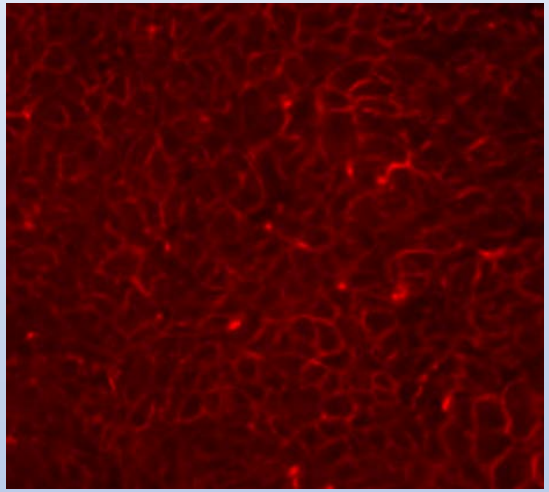
Bac-Rep/Cap
Bac-rAAV



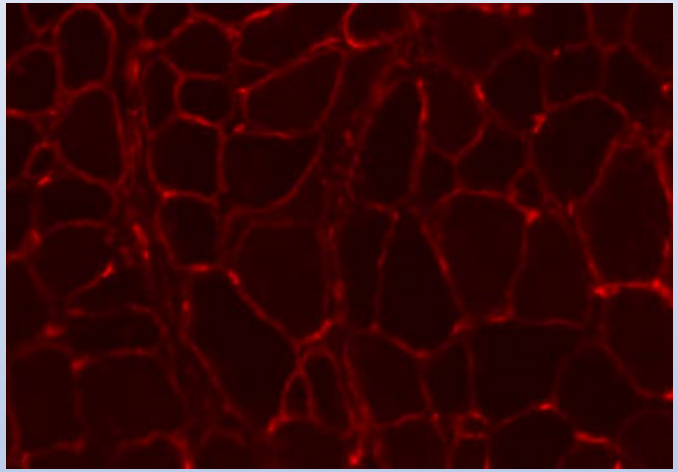
NHLBI / UPenn DMD non-clinical study: Systemic Delivery of rAAV9-U7smOPT
H. Lee Sweeney & Meg Sleeper

- Produced rAAV9-U7smOPT in 500 L bioreactors
- Down-stream process utilized novel V_HH immuno-affinity ligand
- High dose cohort $1x10^{15}$ vg per kg
- Skeletal muscle and heart biopsies
- Cardiac function assessed by echocardiography
- Animals survived up to 18 mos post treatment

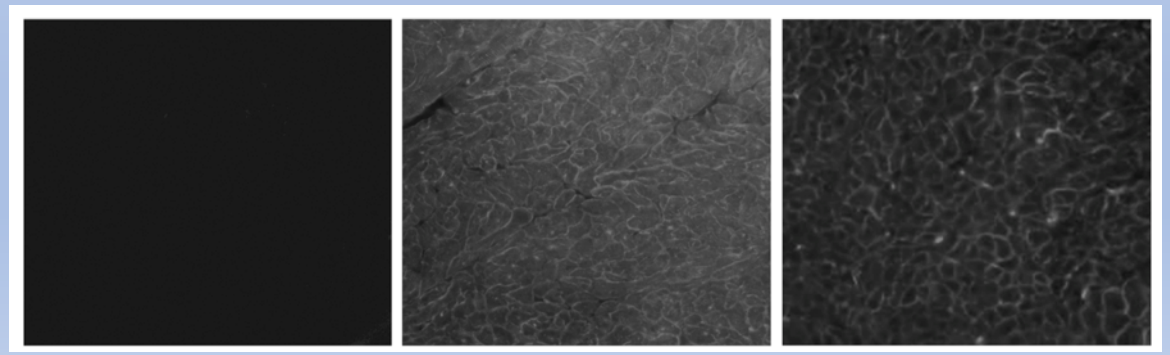
Systemically administered rAAV9-U7smOPT
3.5 x 10^{15} vg 4 weeks post-treatment



Cardiomyocytes



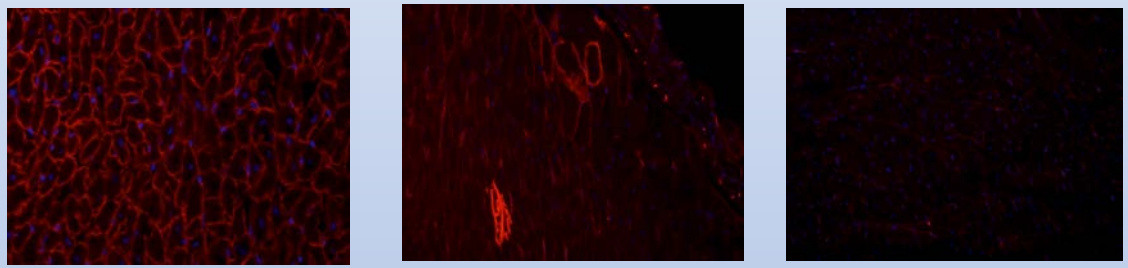
Skeletal muscle



Untreated Direct injection Systemic

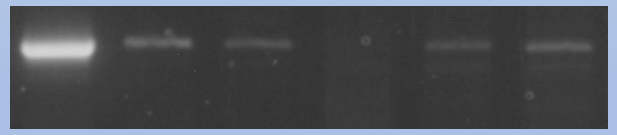
Systemically administered rAAV9-U7smOPT

3.5 x 10^{15} vg
1 year post-treatment



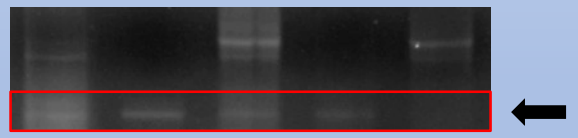
Normal GRMD Untreated

Vector DNA



C H H xH Q TA

rtPCR



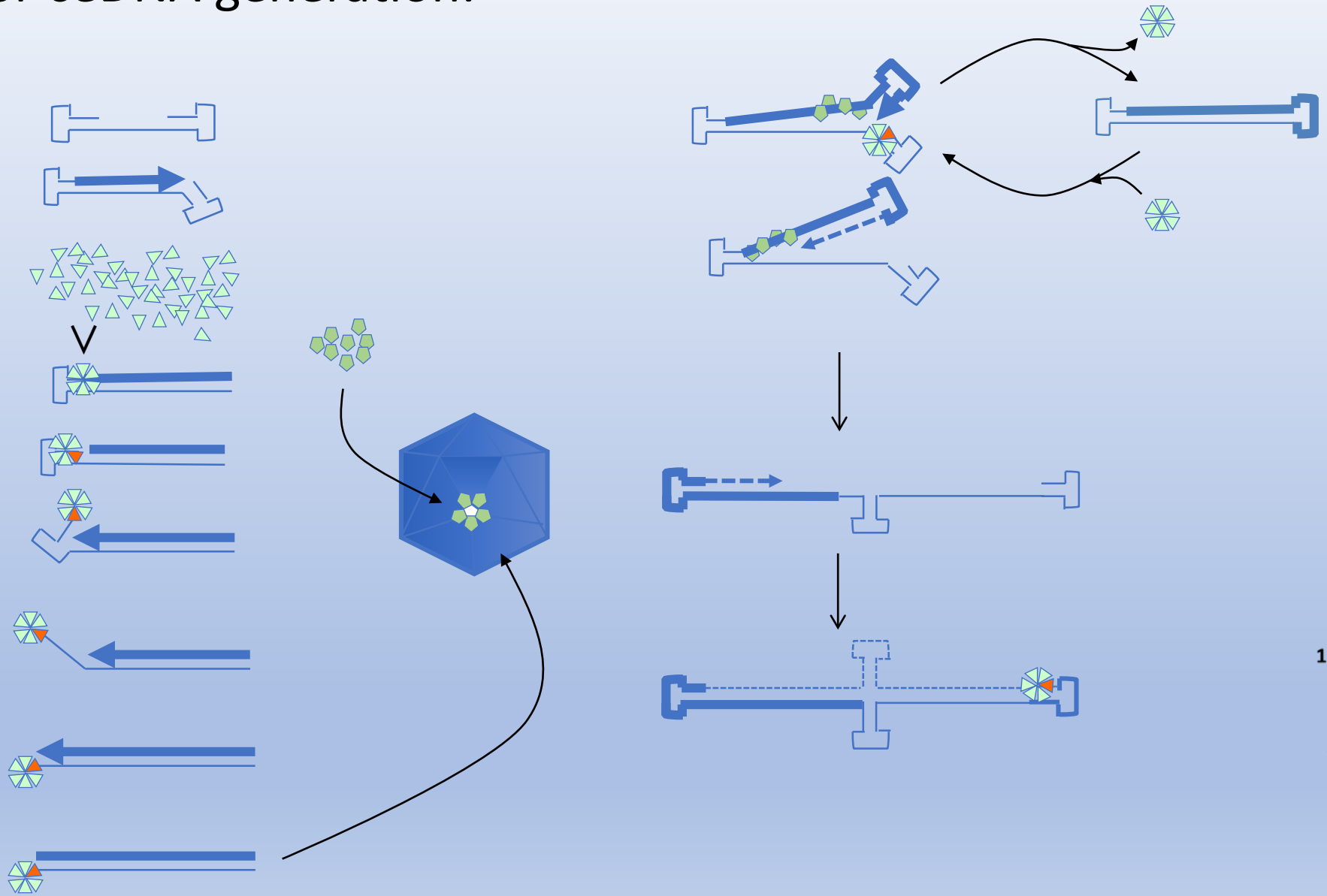
Different sections LV

UMMS / Industry Partnership
Large-scale vector manufacturing

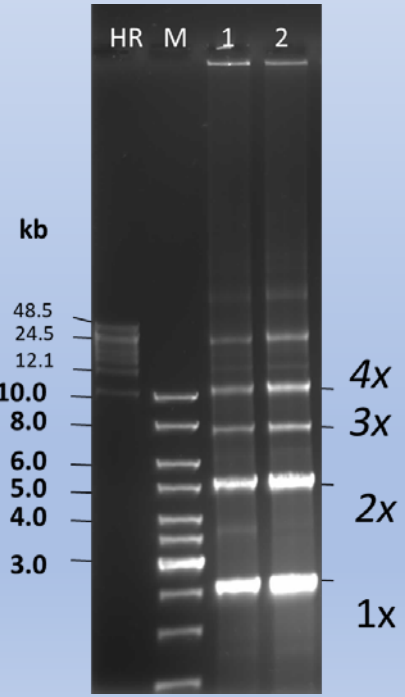
- Combines the expertise of the UMMS virology / vectorology with the engineering expertise of the industry partner.
- Provides sufficient GLP vector to enable large-animal, dose escalation studies
- Transfer technology and materials to cGMP production facility

ceDNA - closed-ended duplex DNA for non-viral gene transfer

Model for ceDNA generation:



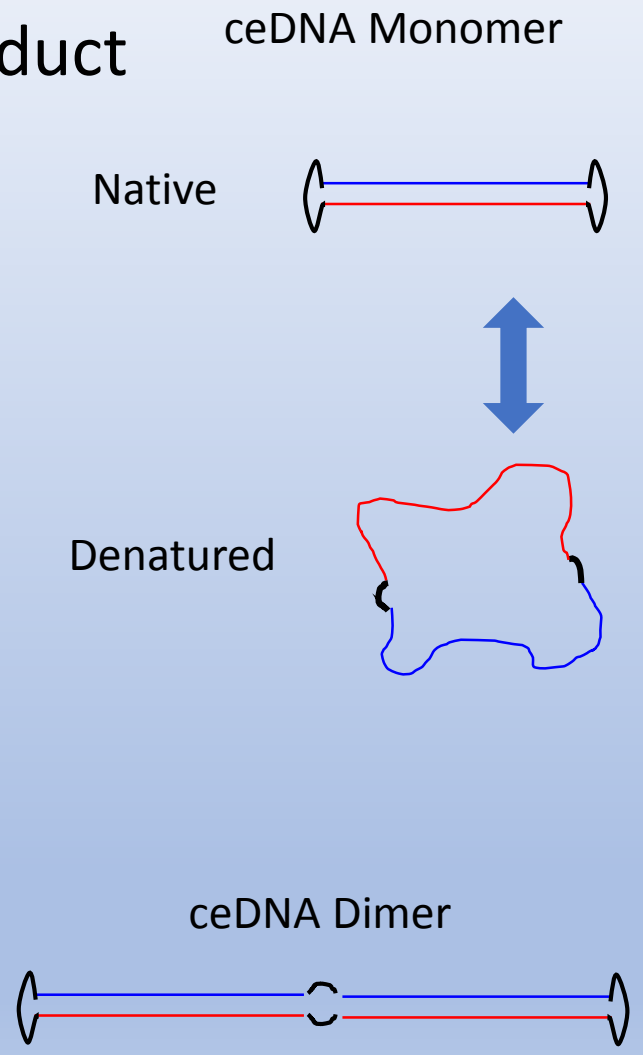
ceDNA conformers

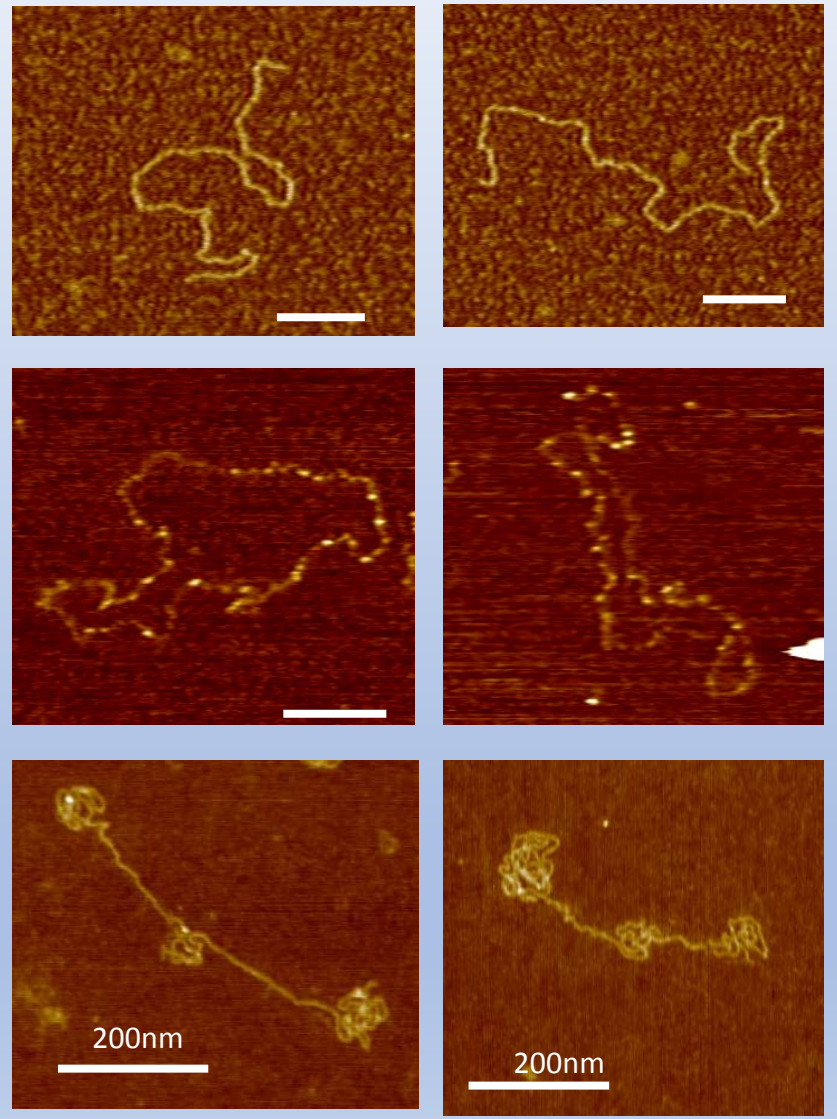


ceDNA: Closed-ended DNA

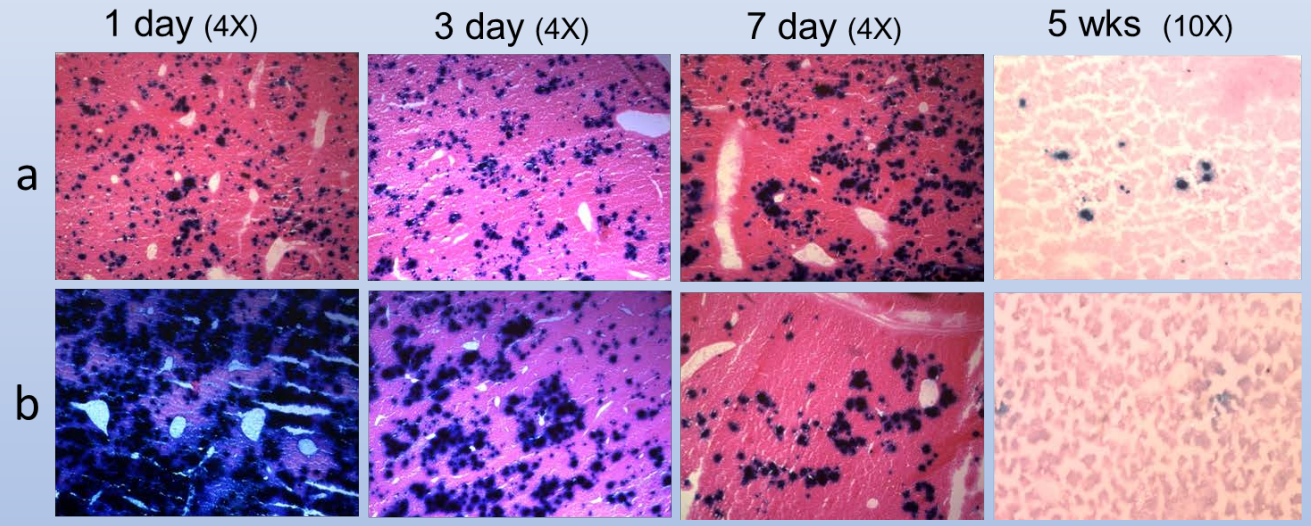
Visualization by Atomic Force Microscopy:

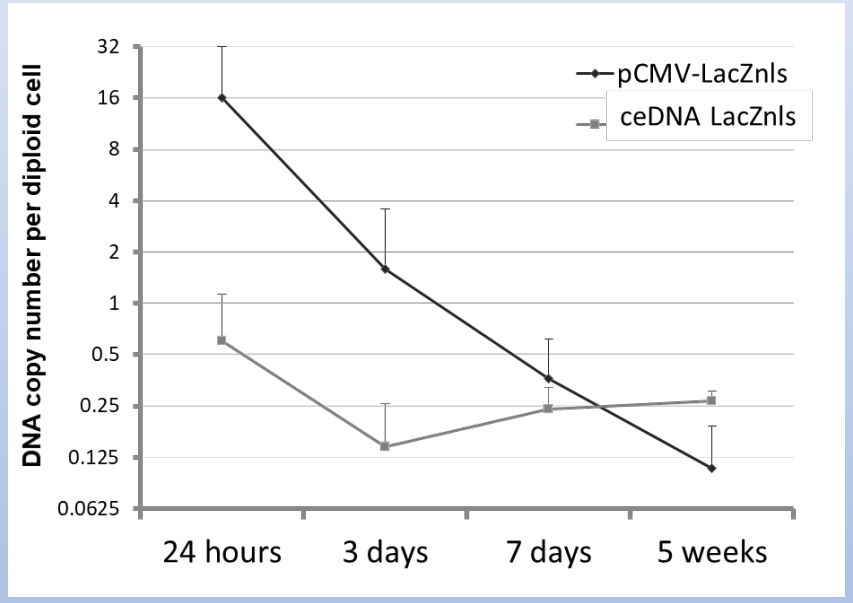
Accumulates as an end-product of AAV DNA replication





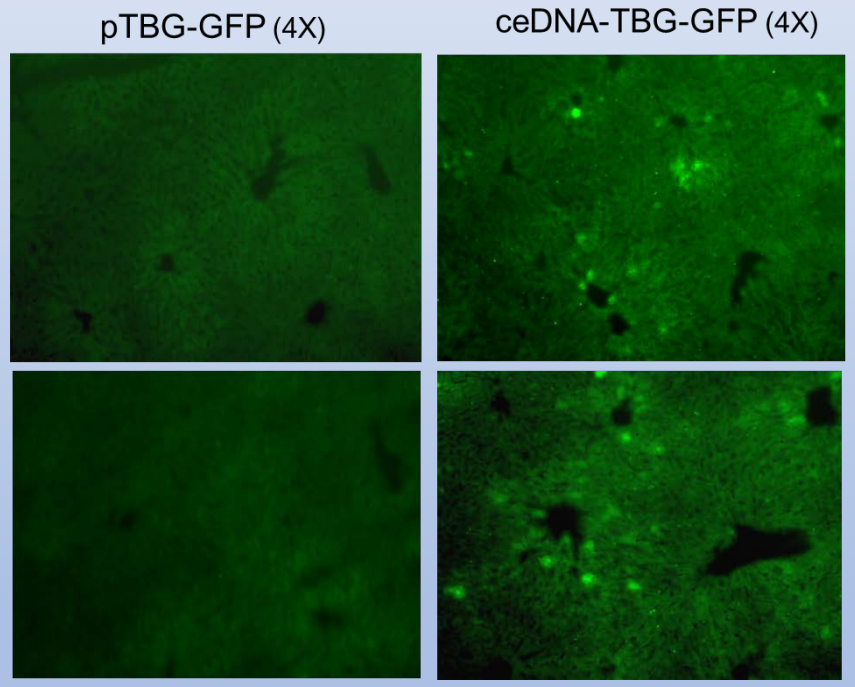
In vivo
Hydrodynamic Tail-vein Injections

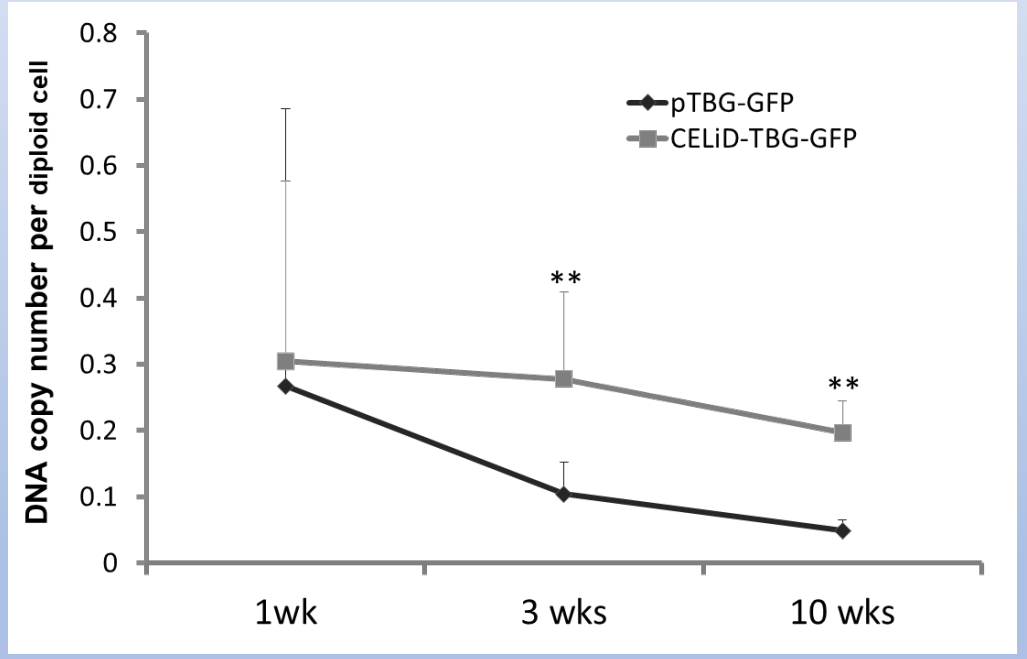




Production and Characterization of Novel Recombinant Adeno-Associated Virus Replicative-Form Genomes: A Eukaryotic Source of DNA for Gene Transfer
Li, Dimitriadis, Yang, Li, Yuan, Qiao, Beley, Smith, Garcia, & Kotin
https://doi.org/10.1371/journal.pone.0069879

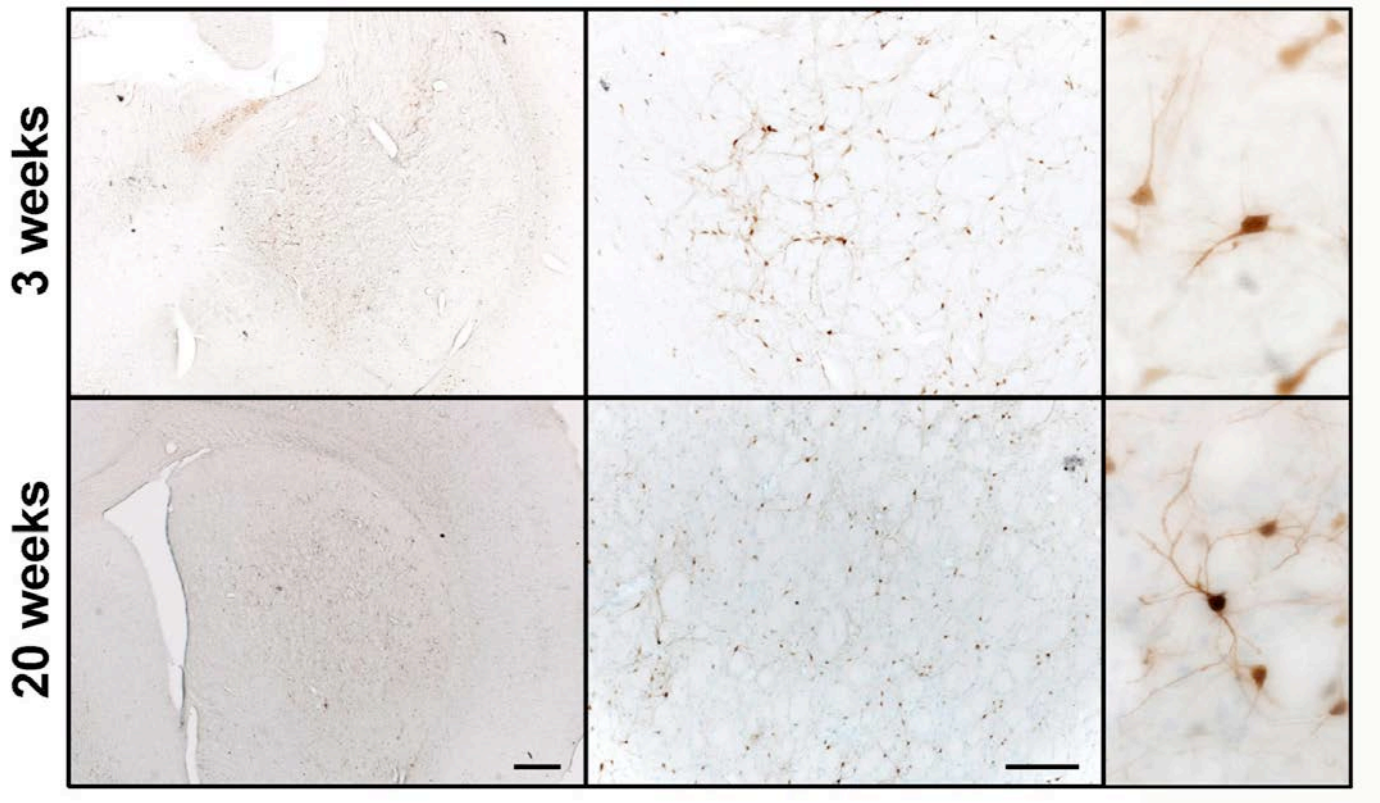
In vivo
Hydrodynamic Tail-vein Injections





Production and Characterization of Novel Recombinant Adeno-Associated Virus Replicative-Form Genomes: A Eukaryotic Source of DNA for Gene Transfer
Li, Dimitriadis, Yang, Li, Yuan, Qiao, Beley, Smith, Garcia, & Kotin
https://doi.org/10.1371/journal.pone.0069879

In vivo
Rat brain convection enhance delivery



Lluis Samaranch
Krys Bankiewicz
UCSF

Brief summary of the experiment:
ceDNA-GFP + jetPEI
Unilateral (right) striatal injection by CED
Volume: 10uL
N=2 rats per survival time (3 and 20 weeks)

ceDNA non-viral gene therapy

Challenges

- Formulation
  - Tissue targeted delivery
  - Lipoplex and polyplex encapsulation
- Innate immunity
- Transcytosis

Attributes

- Produced in *Sf*9 cells
  - GMP compatible
- Capsidless vectors
- ceDNA re-dosable
- No prokaryotic DNA methylation
- No non-vector DNA
  - No plasmid-derived DNA (e.g. ori, *bla)*
- No endotoxins
- No ends

Viral and non-viral gene therapy platforms

- rAAV vector development
  - 1989 - AAV2 prototype vector (Samulski, Chang & Shenk)
  - 1998 - AAV4 and AAV5 first trans-encapsidated vectors (Chiorini, Yang, Liu, Safer & Kotin)
  - 2000s - Hundreds of natural variants (Gao, et al…Wilson Lab 2002)
- First rAAV clinical study publication (1996)
- ceDNA – (2013)
  - Many chemistry formulation options

Acknowledgements

UMMS

Sylvain Cecchini

Guangping Gao

Terry Flotte

Université de Versailles Saint-Quentin

Luis Garcia Lab

NIH

Lina Li

Rick Smith

Tamas Virag

Yu Yang

Emilios Dimitriadis

UCSF

Krys Bankiewicz

Lluis Samaranch