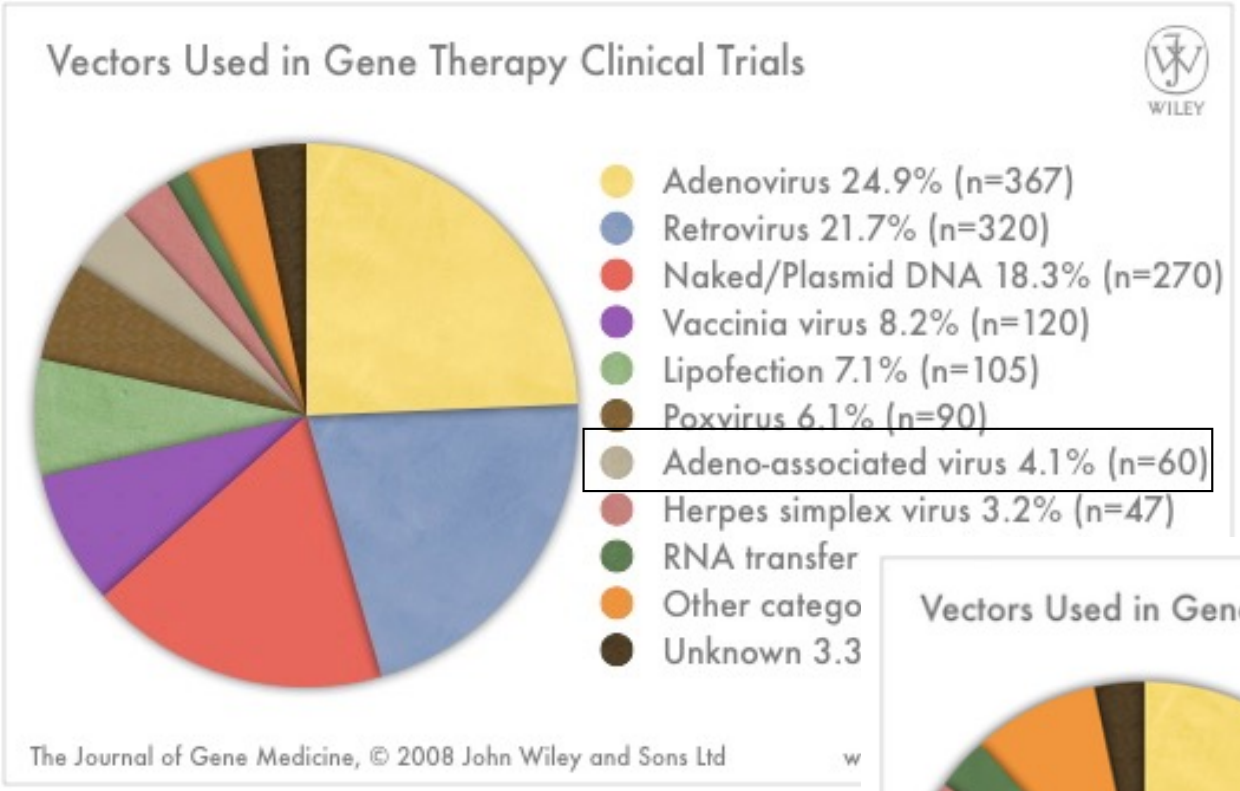
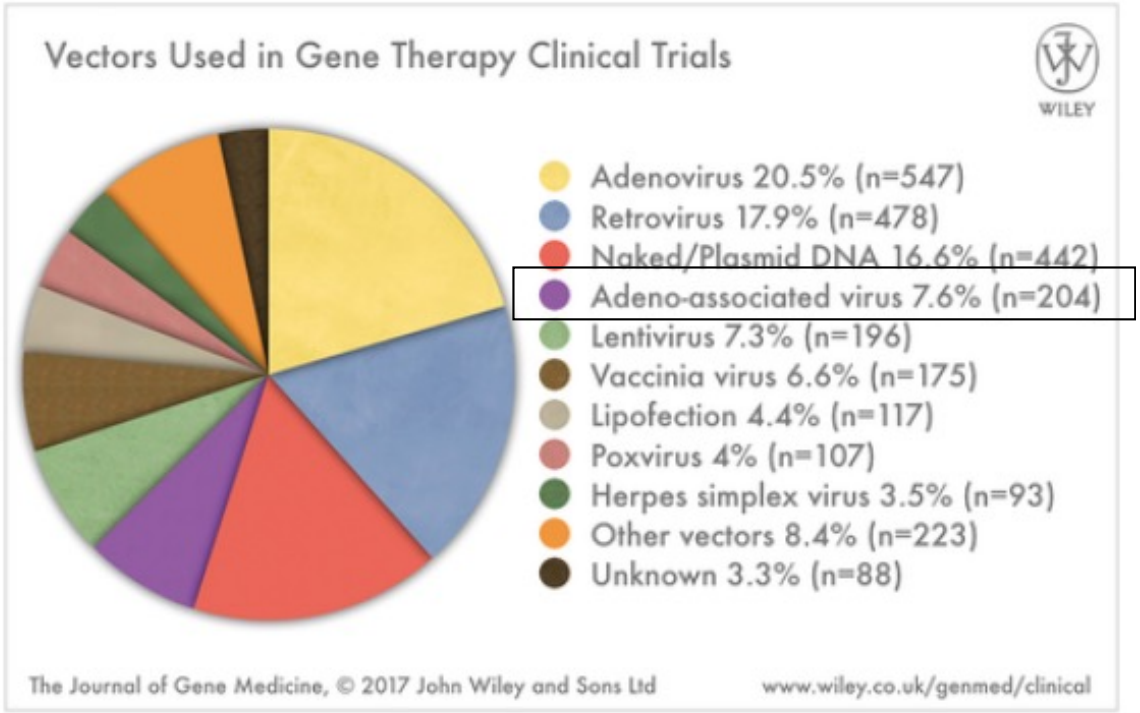
Which vectors for the genes





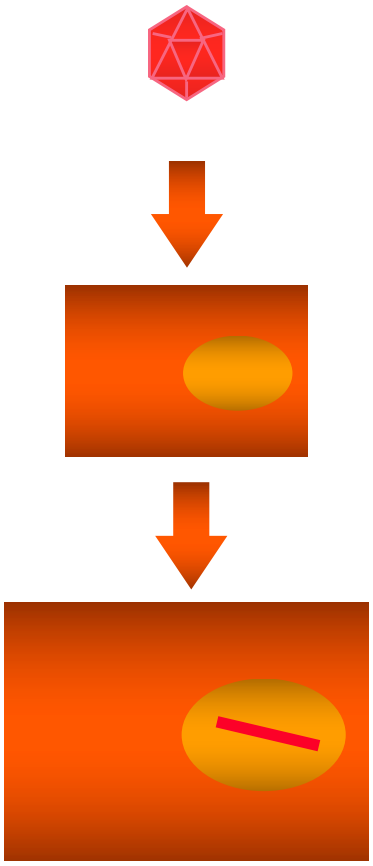
Adeno-associated vectors

-Several serotypes (AAV1-.....): up to 12 characterized
-AAV2 most frequently used
-Isolated in association with adenovirus
-Adeno-associated virus were never associated with any human disease

Non enveloped particle of 20nm diameter
Stable to heat, mild proteolyitic digestion, and nonionic detergents
Dna 25% of the particle mass
Genome: ssDNA
Icosahedral symmetry

AAV (Parvovirus) Life cycle

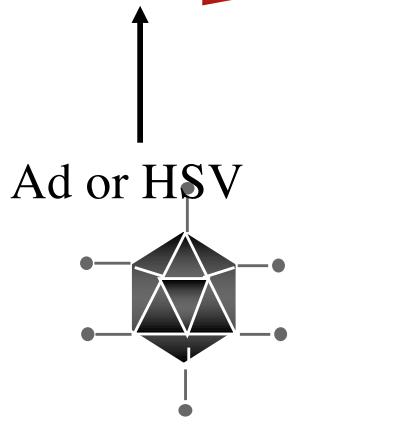
Latent phase



Site-specific integration

In a fraction of infected cells

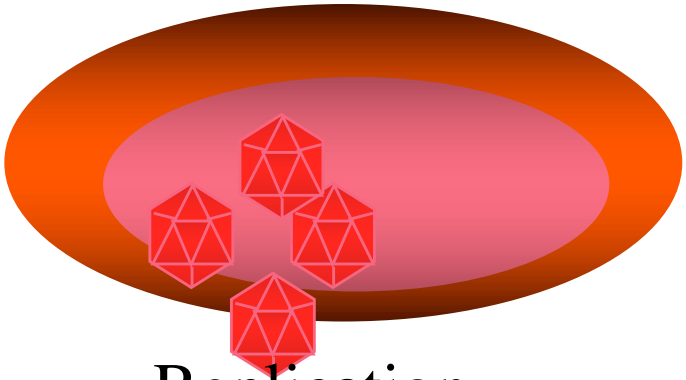
Rescue



Ad or HSV

Lytic phase

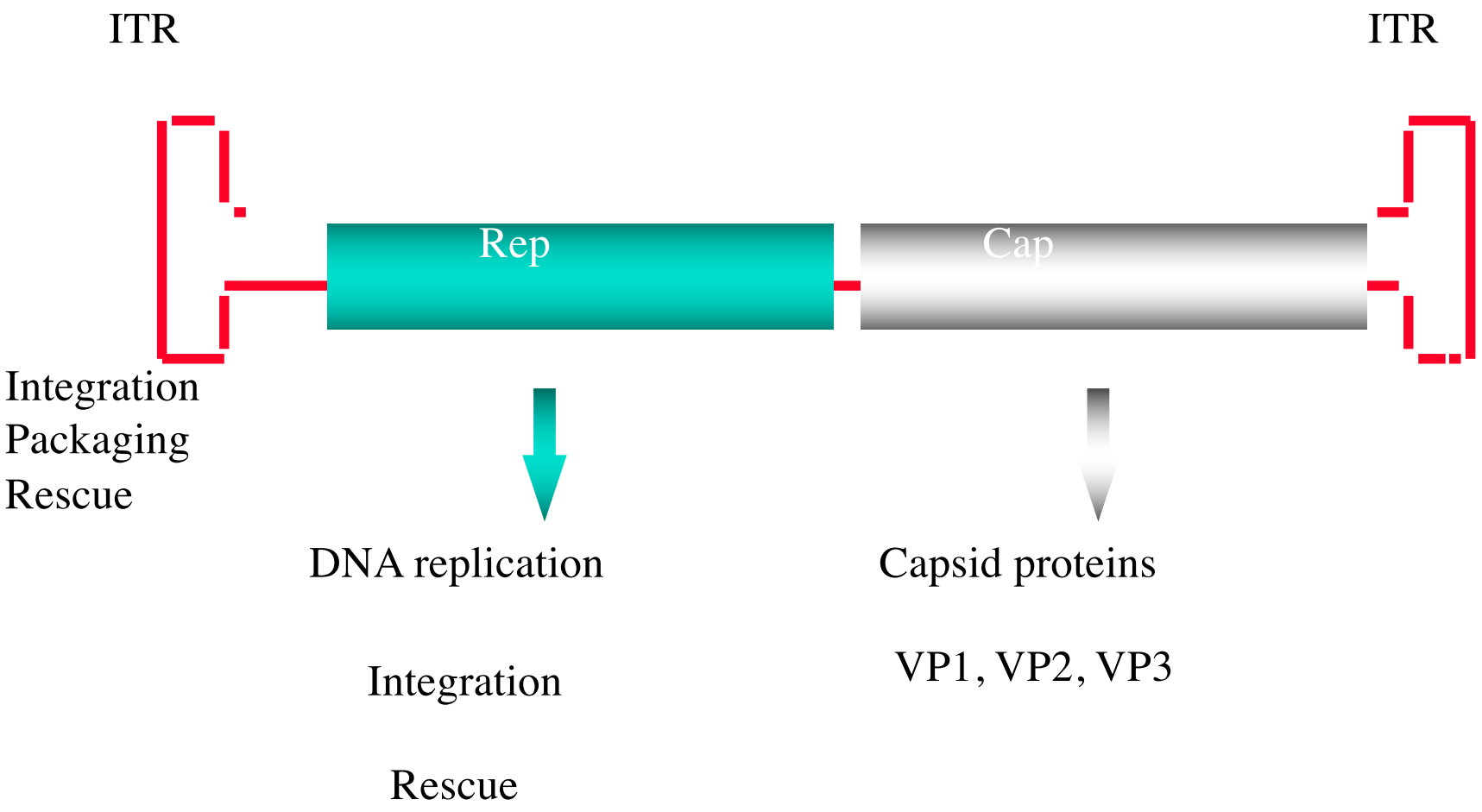
Ad (E1,E2A, E4,VARNA) or HSV



Replication

100000 particles/cells

AAV genome (ssDNA 5 Kb)



AAV genome (ssDNA 4681bp, + or minus)

2 ORFs

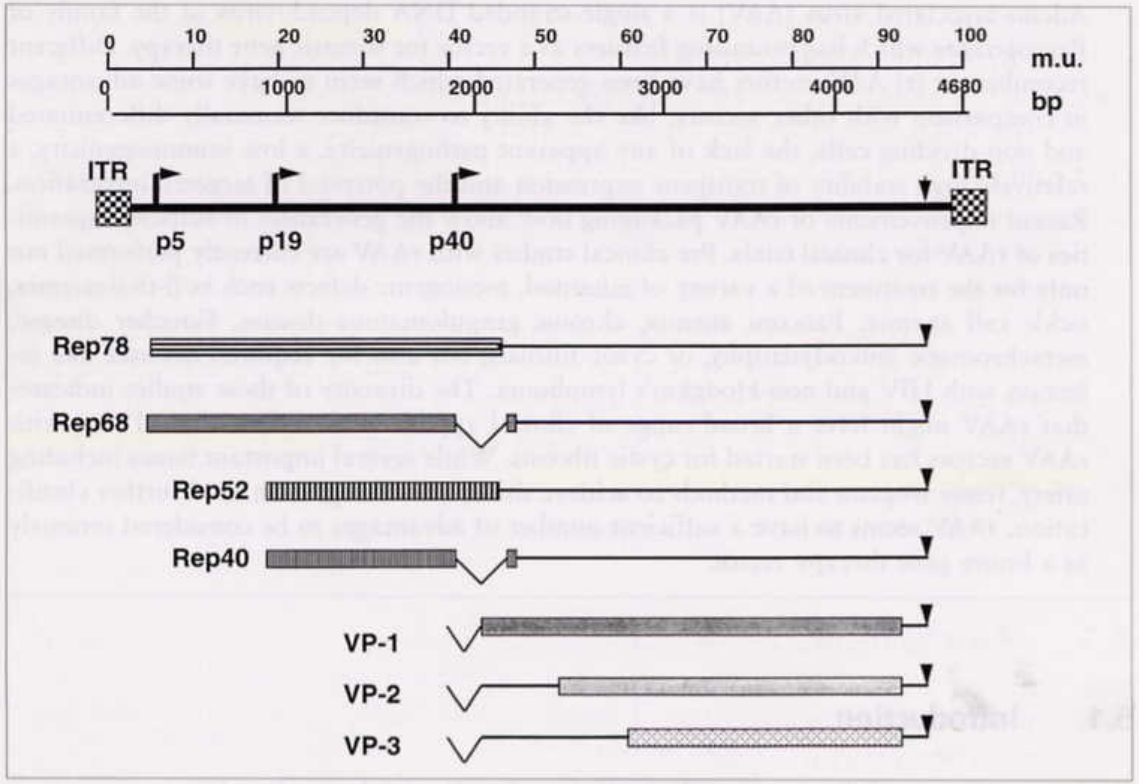
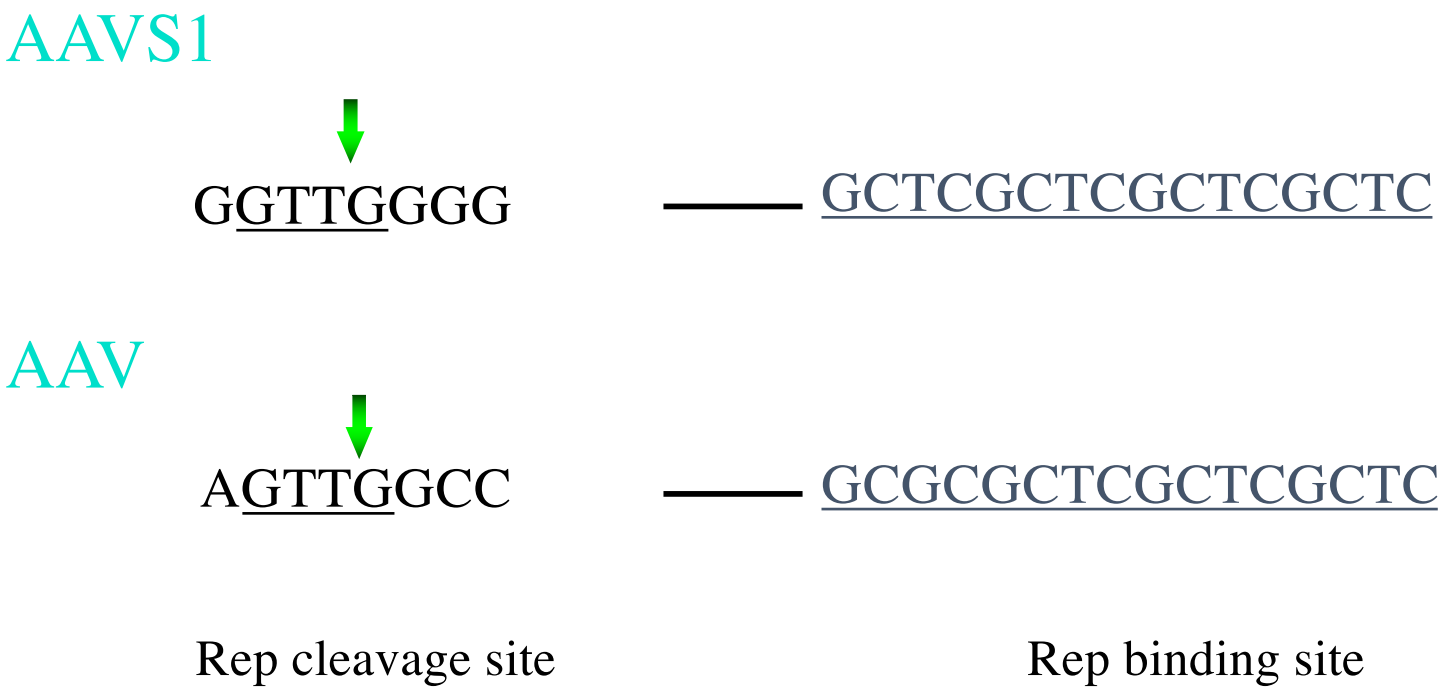


Figure 5-1. Map of the AAV genome (adapted with modifications from Kotin [3]). The AAV genome encompasses 4680 bp, divided into 100 map units (mu). Indicated are the two inverted terminal repeats (ITRs), the three viral promoters at map position 5 (p5), 19 (p19) and 40 (p40) and the common polyadenylation signal at map position 96 (vertical arrow). The open reading frames are represented by rectangles, untranslated regions by solid lines and the introns by carats. Large Rep proteins (Rep78 and Rep68) under control of the p5 promoter and small Rep proteins (Rep52 and Rep40) driven by the p19 promoter exist in spliced and unspliced isoforms. The cap genes encoding three different capsid proteins (VP-1, VP-2, VP-3) are under control of the p40 promoter.

AAV integration

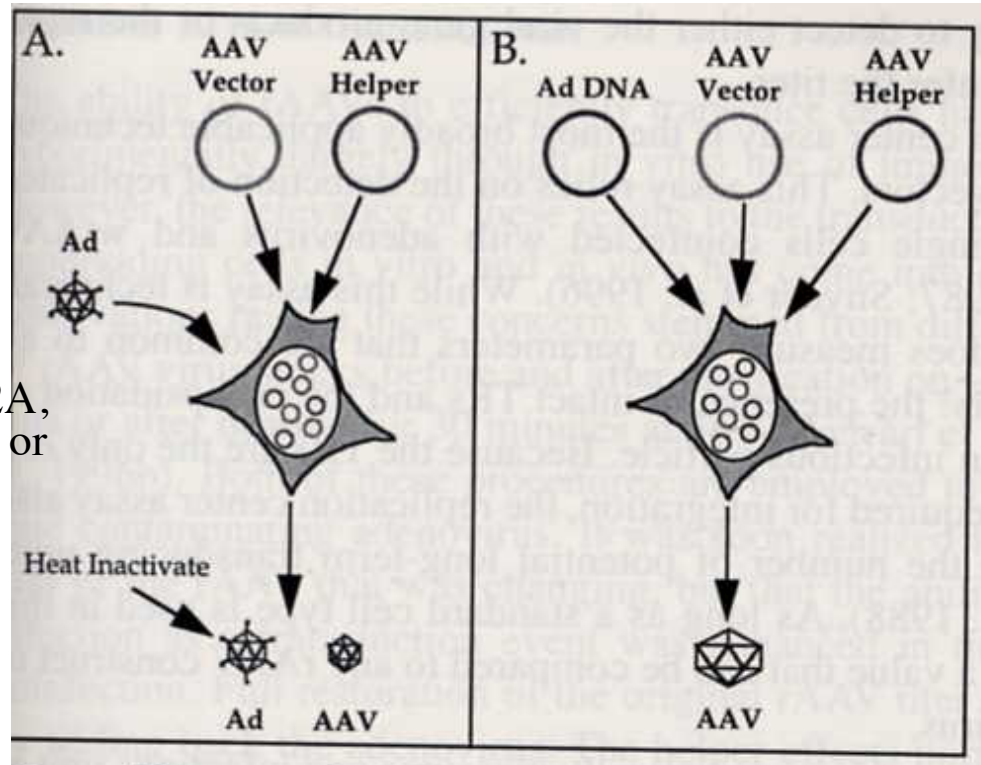
Directed by signals encoded in the primary sequence of chromosome 19, 5' of insertion points (50-70% of integrations,in culture and with wild type AAV, otherwise episomal concatemeres)



Requires viral Rep proteins

AAV vector prep

Or Ad (E1,E2A, E4,VARNA) or HSV



Or a rev-cap producing cell line

Figure 1 Diagram of rAAV production (*A*) Classic Method—the rAAV genome is introduced in *trans* with a plasmid expressing the AAV *rep* and *cap* genes. The Ad helper effect is supplied by Ad infection. (*B*) T.O.A.D. Method—differs from the clasic method in that the Ad helper functions are supplied by a nonreplicating Ad minigenomic plasmid.

AAV-vectors

Advantages

- *Integration/episomal forms*
- No disease related
- *No immunogenicity*
- High titers (10e10)
- Infection of non dividing cells
- Long term gene transfer in lung, CNS, eye, muscle

Disadvantages

- Small size
- Inefficiency of purified AAV
- ssDNA (camptothecin)
- Variable transduction efficiency (1-80%)

AAV modifications

Hybrids:

Retro/adeno

AAV/adeno

Dual AAV vectors (concatemere exploitation)

….

Targeting:

microRNA

peptides in the capsid

AAV hybrids

Promoters

….

AAV tropism

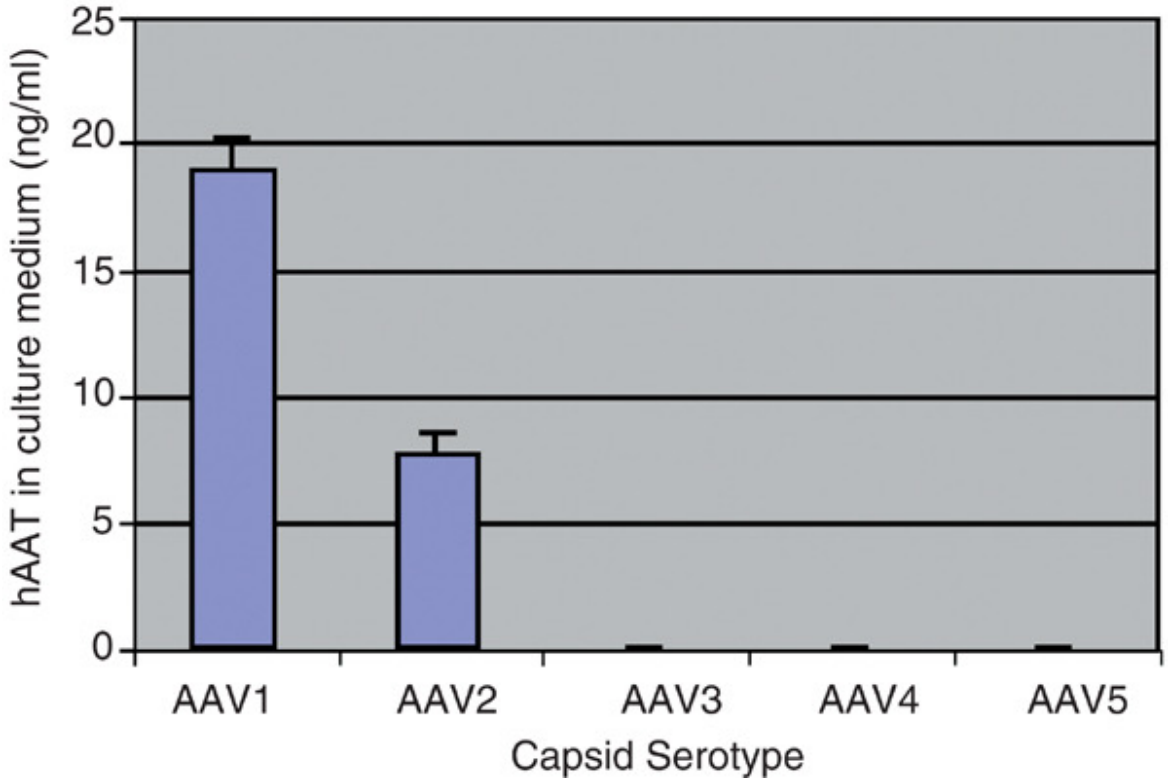
Table 1 Cellular Receptors for AAV Serotypes

	Serotype	Primary receptor	Secondary receptor
Murine muscle	AAV1	Unknown	Unknown
	AAV2	Heparin sulfate	$\alpha_v\beta_5$ Integrin/FGFr
	AAV3	Heparin sulfate	Unknown
Murine muscle/respiratory tract →	AAV4	O-linked sialic acid	Unknown
	AAV5	N-linked sialic acid	PDGF receptor
Murine muscle	AAV7	Unknown	Unknown
Murine liver	AAV8	Unknown	Unknown

Endocytosis/ to the nucleus in 15'

2004 Gene and cell ther

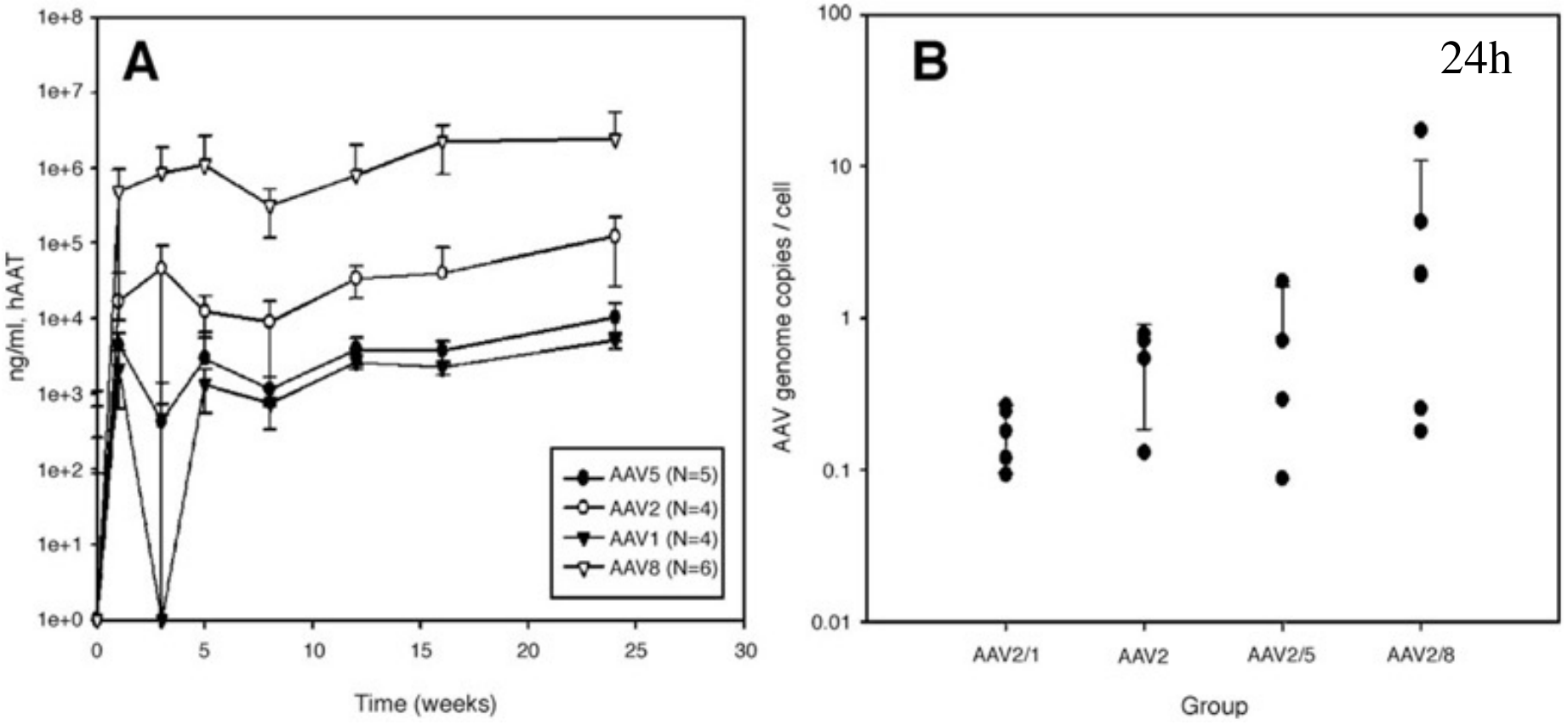
Gene transfer into **pancreatic islets**



AAT:alpha anti trypsin

Loiler et al Gen ther 2003

Injection into the portal vein of C57Bl/6 mice to target the **liver** with equal doses of 9.6 10^{10} vector particles per animal



Mol ther 2005

Cellular tropism and transduction properties of seven adeno-associated viral vector serotypes in adult retina after **intravitreal** injection

- **Premise:**

rAAV for gene therapy, and clinical trials have begun in patients with genetically linked retinal disorders.

- **Exp:**

Intravitreal injection is optimal for the transduction of retinal ganglion cells adult rat retina after intravitreal injection.

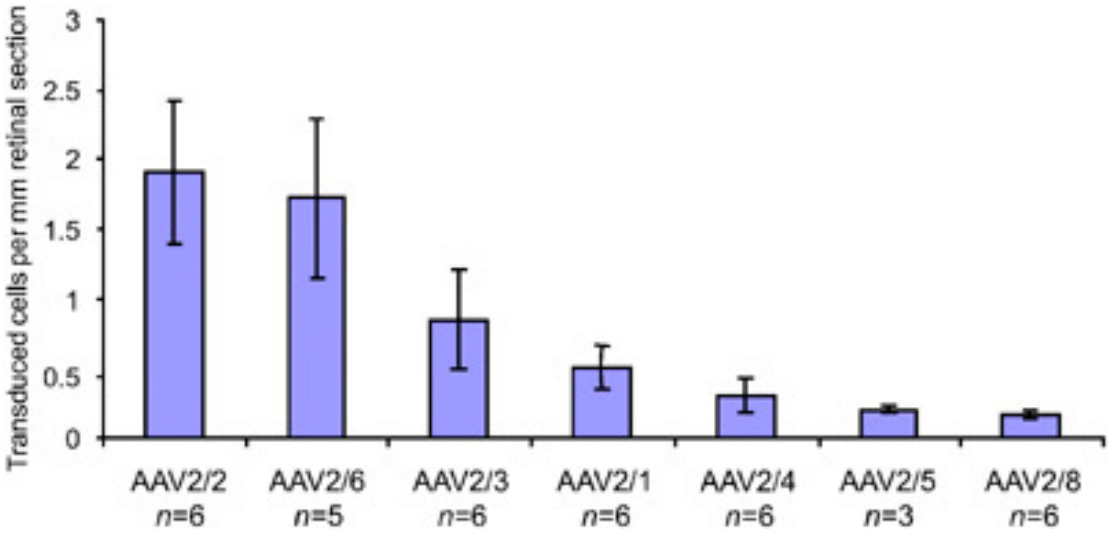
- **Results:**

-rAAV2/2 and rAAV2/6 transduced the greatest number of cells, whereas rAAV2/5 and rAAV2/8 were least efficient.

-Most vectors primarily transduced RGCs; however, rAAV2/6 had a more diverse tropism profile, with 46% identified as amacrine or bipolar cells, 23% as RGCs and 22% as Müller cells. Müller cells were also frequently transduced by rAAV2/4. The highest photoreceptor transduction was seen after intravitreal rAAV2/3 injection.

Hellstrom et al Gene Ther 2009

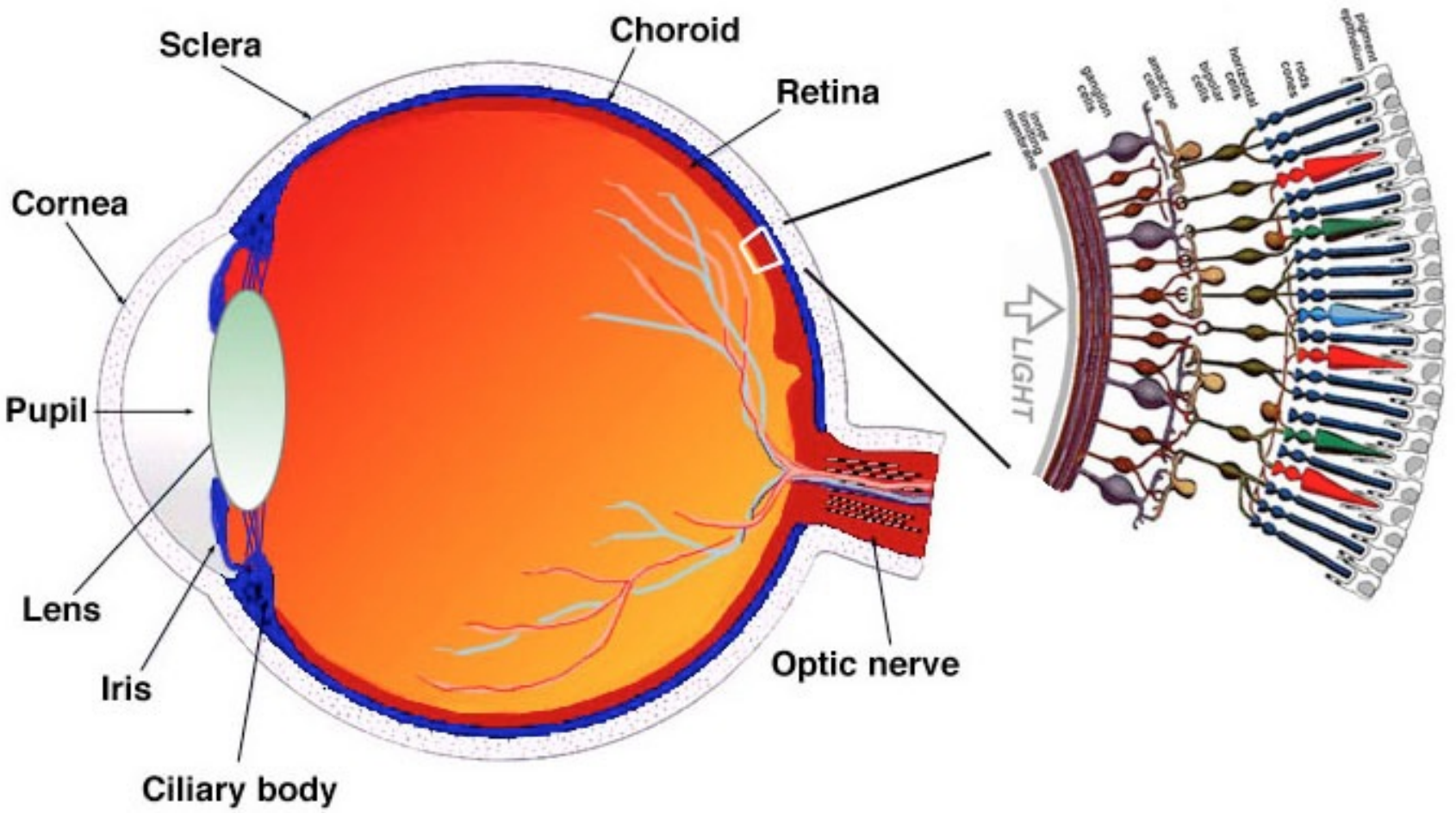
rAAV2/2 and rAAV2/6 transduced the greatest number of cells, whereas rAAV2/5 and rAAV2/8 were least efficient.



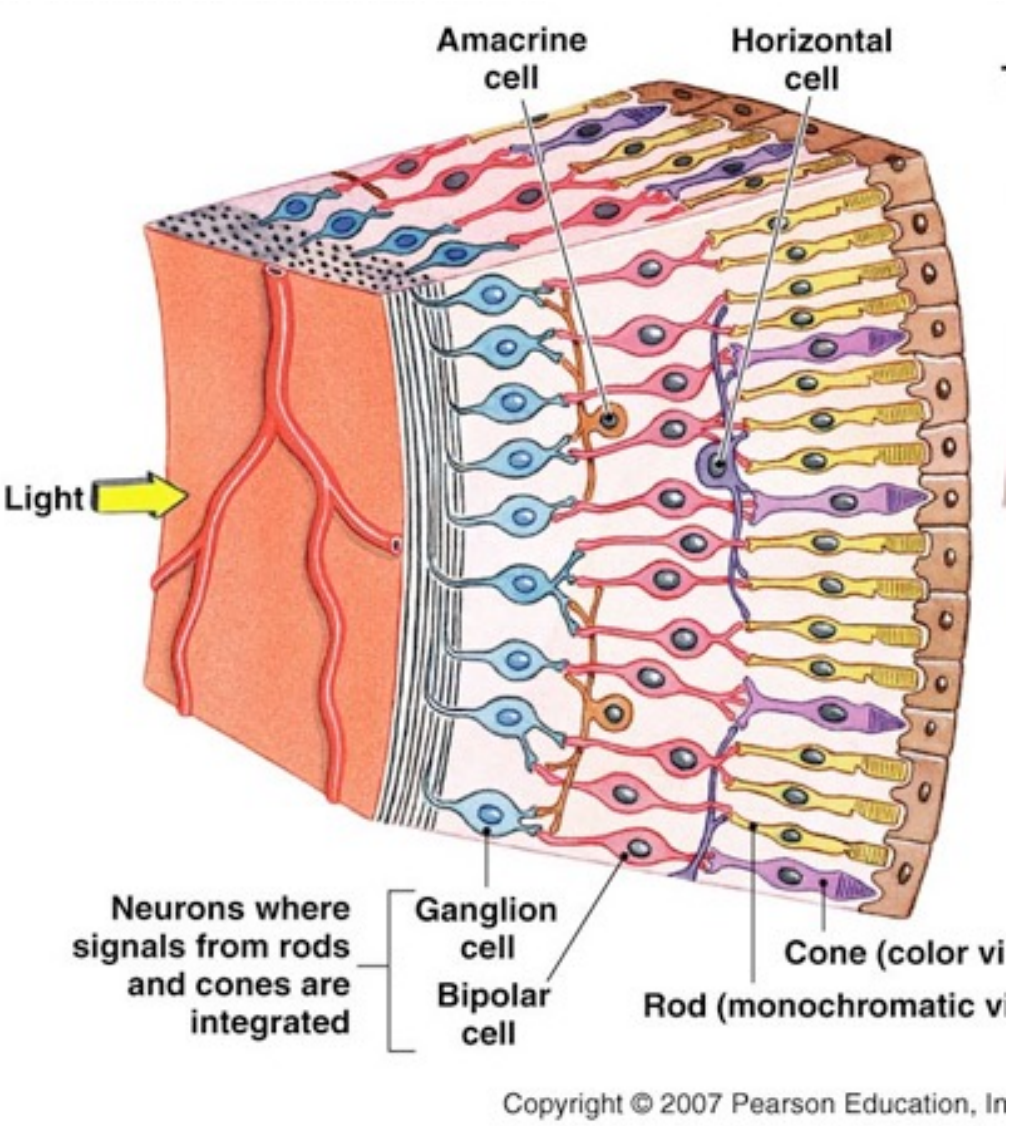
Transduction efficiency of the seven rAAV serotypes after an intravitreal, titre-matched (2.6 10^9 genomic copies) injection in adult Wistar rats, assessed 10 weeks later.

Gene Ther 2009

Human eye and enlargement of the retina



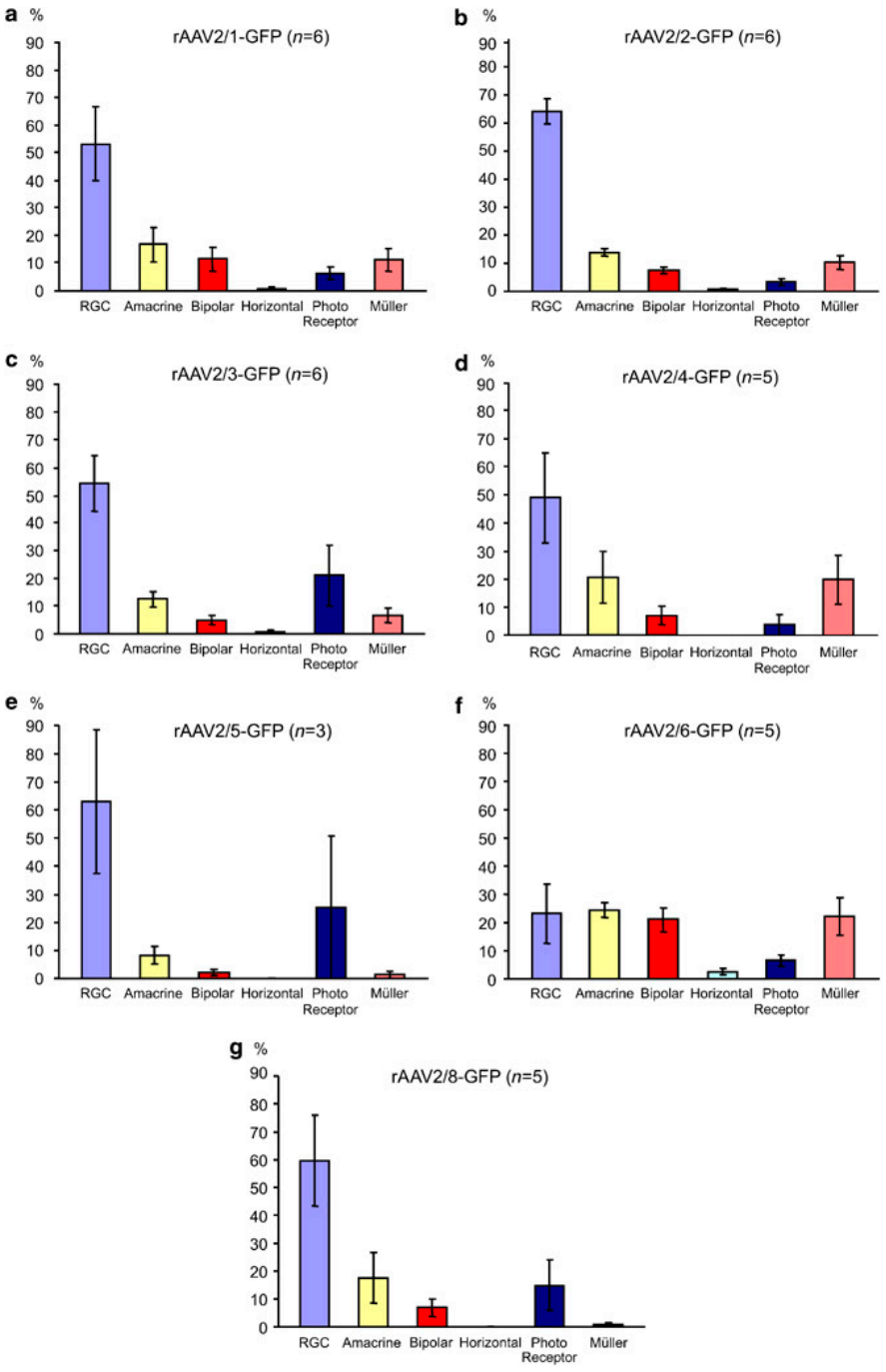
Organization of the retina



+ Muller glia

The proportion of transduced cells of each type for each AAV serotype.

-Most vectors primarily transduced RGCs; however, rAAV2/6 had a more diverse tropism profile, with 46% identified as amacrine or bipolar cells, 23% as RGCs and 22% as Müller cells (glia). Müller cells were also frequently transduced by rAAV2/4. The highest photoreceptor transduction was seen after intravitreal rAAV2/3 injection.



Gene Ther 2009

AAV clinical success?

Safety and Efficacy of Gene Transfer for Leber's Congenital Amaurosis

Premise: Leber's congenital amaurosis (**LCA**) is a group of inherited blinding diseases with onset during childhood. *One form of the disease, LCA2, is caused by mutations in the retinal pigment epithelium–specific 65-kDa protein gene (**RPE65**).*

Exp: subretinal delivery of a recombinant adeno-associated virus (**AAV2**) carrying RPE65 complementary DNA (cDNA) (ClinicalTrials.gov number, NCT00516477 [ClinicalTrials.gov]). **Results:** **Three patients** with LCA2 had an acceptable local and systemic adverse-event profile after delivery of AAV2.hRPE65v2. Each patient had a modest improvement in measures of retinal function on **subjective tests of visual acuity**. In one patient, an asymptomatic macular hole developed, and although the occurrence was considered to be an adverse event, the patient had some return of retinal function. Although the follow-up was very short and normal vision was not achieved, this study provides the **basis for further gene therapy** studies in patients with LCA.

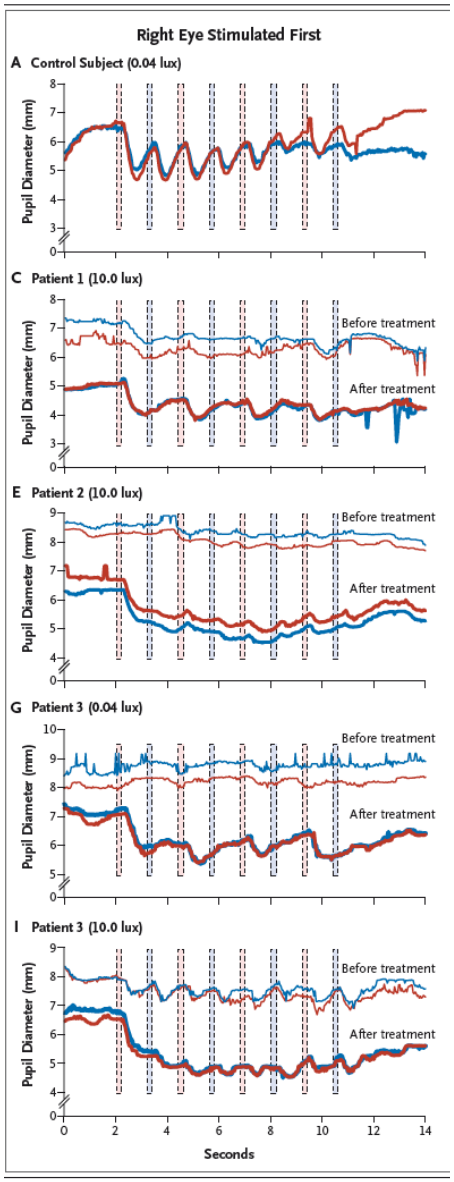
NEJM 2008

Efficacy: pupillary light reflex

ctrl

before treatment

after treatment



for Patient 1 at baseline and 4.75 months after injection (Panels C

Injection in the Right Eye of the Three Patients

VECTOR DNA ONLY IN A TEAR OF PATIENT 1 AT DAY 5 P.I.

NEJM 2008

Video 1. Subretinal Injection of Recombinant Adeno-Associated Virus Vector 2.hRPE65v2 in Patient 3.

Video 2. Obstacle Course: Patient 3.

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https://www.nejm.org/doi/full/10.1056/NEJMoa0802268

NEJM 2008

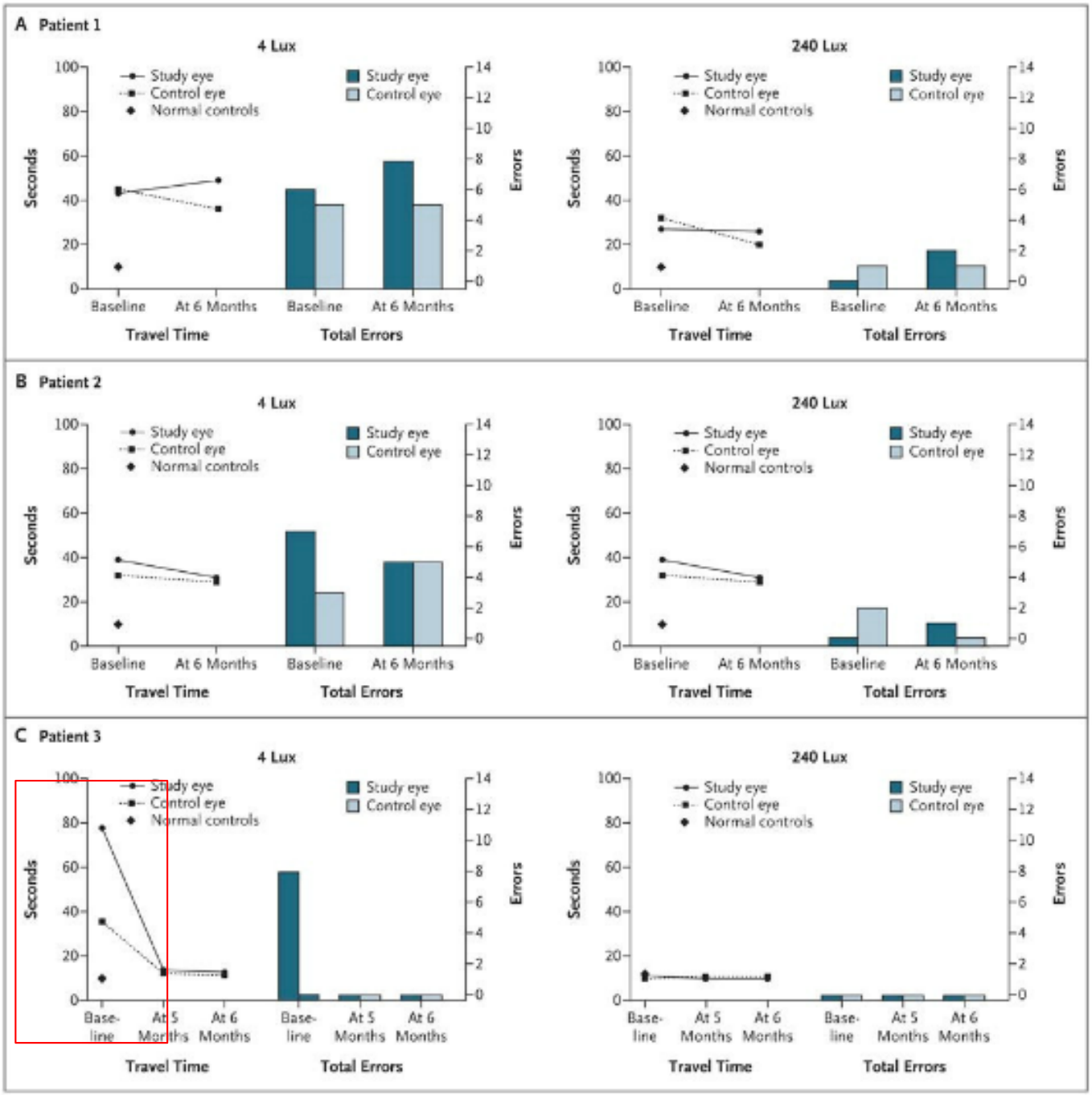

Figure 3. Assessment of Visual Mobility.

Visually guided mobility was assessed at the Pedestrian Accessibility and Movement Environment Laboratory at University College London (see Fig. 1 in the **Supplementary Appendix** and **video**). Panels A, B, and C show data for Patients 1, 2, and 3, respectively, at ambient illumination levels of 4 lux and 240 lux. Average travel times for completing the course (±1 SD) for five control subjects are indicated.

NEJM 2008

Conclusions from **Effect of Gene Therapy on Visual Function in Leber's Congenital Amaurosis**

James W.B. Bainbridge, Ph.D et al

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*The results of this study suggest that subretinal administration of recombinant adeno-associated virus vector is **not associated with immediate adverse events** in patients with severe retinal dystrophy and that adeno-associated virus–mediated RPE65 gene therapy can lead to **modest improvements** in visual function, even in patients with advanced degeneration. Our findings provide support for the development of further clinical studies in children with RPE65 deficiency; these children are more likely to benefit than adults.*

NEJM 2008

AAV-2 treatment for Leber's Congenital Amaurosis

Trial Number	Sample Size	Date Initiated	Phase	Surgery Site	Primary Measure	Primary Result	Remarks	Publications
NCT00516477	12	October 2007	I/II	Consortium of CHOP, UPENN, TIGEM and SUN	Safety and tolerability of subretinal administration of AAV2-hRPE65v2 Promotor – CBA	Safe, efficacious and well tolerated	Allocation: N/A Intervention Model: Single Group Study type – Interventional Assignment Masking: None (Open Label) Primary Purpose: Treatment Intervention- Unilateral injection	Maguire et al 2008, 2009 Simonelli et al 2010 Testa et al 2013
NCT00481546	15 (8 years and older)	October 2007	I	UFL, UPENN	Primary safety endpoint - standard ocular examination Toxicity assessment by measurement of vision, hematology, and serum chemistries, assays for vector genomes **Subretinal** injection of **rAAV2-CBSB-hRPE65** Promotor – **CBA shortened**	No serious ocular or systemic adverse events	Allocation: N/A Intervention Model: Single Group Study type – Interventional Assignment Masking: None (Open Label) Primary Purpose: Treatment One or two, uniocular, subretinal injections; relative doses: 0.3X (Cohort 1), 0.6X (Cohort 2), 0.45X (Cohort 3), 0.9X (Cohorts 4 and 5)	Cideciyan et al 2008, 2009, 2013; Hauswirth et al 2008; Jacobson et al 2012

Padhy et al, Application of Clinical Genetics, 2020 Review

AAV-2 treatment for Leber's Congenital Amaurosis

Trial Number	Sample Size	Date Initiated	Phase	Surgery Site	Primary Measure	Primary Result	Remarks	Publications
NCT00821340	10	January 2009	I	Hadassah Hospital, Jerusalem, Israel	Primary outcome measures – ocular and systemic safety Secondary outcome measures – visual function, quantified before and after vector administration **Subretinal** injection of **rAAV2-CBSB-hRPE65**	An increase in vision was present in the treated area as early as 15 days after the intervention	Allocation: N/A Intervention Model: Single Group Study type – Interventional Assignment Masking: None (Open Label) Primary Purpose: Treatment	Banin et al 2010
NCT01496040	3 cohorts of 3 patients each (total 9, aged between 6–50 years)	September 2011	I/II	Nantes University Hospital, France	Safety evaluation **Subretinal** injection of **rAAV2/4.RPE65** Promotor – **RPE 65**	rAAV2/4.RPE65 vector was well tolerated and safe. Visual function improvement varied between patients	Allocation: N/A Intervention Model: Single group Assignment Masking: None (Open Label) Primary Purpose: Treatment	Le Meur G et al 2018
NCT00749957	12 (6 years and older)	December 2008	I/II	Casey eye institute, Portland	Primary outcome measures – Ocular or Non-ocular Adverse Events Secondary outcome measures – Changes in Visual Fields, and Best Corrected Visual Acuity **Subretinal** injection of **rAAV2-CB-hRPE65** Promotor – **CBA**	Not associated with serious adverse events improvement in one or more measures of visual function was observed in nine of 12 patients Greatest improvements in visual acuity were observed in younger patients with better baseline visual acuity	Allocation: N/A Intervention Model: Parallel Assignment Study type – Interventional Assignment Masking: None (Open Label) Primary Purpose: Treatment	Weleber et al 2016

Padhy et al, Application of Clinical Genetics, 2020 Review

AAV-2 treatment for Leber's Congenital Amaurosis

NCT00643747	3	2007	I/II	Moorefield's, UCL, London, UK	Primary outcome measures – intraocular inflammation Secondary outcome measures – Visual function (microperimetry and dark adapted perimetry) **Subretinal** injection of **rAAV2/2.hRPE65p. Hrpe65** Promotor – **hRPE 65**	No serious adverse events No clinically significant change in visual acuity or in peripheral visual fields on Goldmann perimetry in any of the three patients No change in retinal responses on electroretinography	Allocation: N/A Intervention Model: Single Group Study type – Interventional Assignment Masking: None (Open Label) Primary Purpose: Treatment **SAFETY TRIAL**	Bainbridge et al 2008 (Preliminary result of the trial) Bainbridge et al 2015 (Longterm outcome of above clinical trial at 3 years with additionally recruited patients)
	12 (5 years to 30 years)	2007	I/II	Moorefield's, UCL, London, UK	An Open-label Dose Escalation Study Four participants were administered a lower dose of the vector, and 8 were administered a higher dose **Subretinal** injection of **rAAV2/2.hRPE65p. Hrpe65** Promotor – **hRPE 65**	Varied extents of improvements in retinal sensitivity in six participants for up to 3 years (peak at 6 to 12 months after treatment and then declining) No associated improvement in retinal function (on ERG) Three participants had intraocular inflammation, and two had clinically significant deterioration of visual acuity	Allocation: N/A Intervention Model: Single Group Study type – Interventional Assignment Masking: None (Open Label) Primary Purpose: Treatment **SAFETY AND EFFICACY TRIAL**	

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AAV-2 treatment for Leber's Congenital Amaurosis

Trial Number	Sample Size	Date Initiated	Phase	Surgery Site	Primary Measure	Primary Result	Remarks	Publications
NCT01208389	12 (8 years and older)	September 2010	I/II Follow on	CHOP, UPENN	Adverse events as a measure of safety and tolerability Primary outcome measures: safety and tolerability Secondary outcome measures: changes in visual function as measured by subjective, psychophysical tests and by objective, physiologic tests **subretinal** administration of **AAV2-hRPE65v2** Promotor – **CBA**	Results (through 6 months) including evaluations of immune response, retinal and visual function testing, and functional magnetic resonance imaging indicate that re-administration is both safe and efficacious after previous exposure to AAV2-hRPE65v2	Allocation: N/A Intervention Model: Single Group Study type – Interventional Assignment Masking: None (Open Label) Primary Purpose: Treatment One time, subretinal administration of vector in 300 microliters to the contralateral, previously uninjected eye	Bennett et al 2012
NCT00999609	31 (3 years or older)	November 2013	III	CHOP, UPENN, University of Iowa	Safety and Efficacy Study Primary outcome measures: Multi-luminance Mobility Testing – MLMT (Bilateral) Secondary outcome measures – Full-field Light Sensitivity Threshold (FST) Testing Multi-luminance Mobility Testing (Monocular) Visual Acuity	Functional vision improvement measured using MLMT, FST at 1 year	Allocation: Randomized Intervention Model: Parallel Assignment Study type – interventional Masking: None (Open Label) Primary Purpose: Treatment Study arms-Interventional: Subretinal voretigene neparvovec-rzyl No Intervention: Control	Russel et al 2017

Padhy et al, Application of Clinical Genetics, 2020 Review

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

EMA/823783/2018
EMEA/H/C/004451

Luxturna (*voretigene neparvovec*)

Luxturna consists of a virus that contains normal copies of the ***RPE65*** gene. When Luxturna is injected into the eye the virus carries the ***RPE65*** gene into the retinal cells and enables them to produce the missing enzyme. This helps the cells in the retina to function better, slowing down the progression of the disease.

The type of virus used in this medicine (adeno-associated virus) does not cause disease in humans.

Luxturna received a marketing authorisation valid throughout the EU on 22 November 2018.

FDA approval: December 2017

Spark therapeutics

QUESTIONS?