An Overview of Nonclinical Considerations in Translating AAV Gene Therapies to the Clinic

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Scope

- General planning for preclinical assessments of gene therapy investigative drugs for retinal & neurodegenerative diseases
- Retinal Gene Therapy Points to Consider (PTC)
- Neurodegenerative Disease Gene Therapy PTC
- CRISPR CAS Gene Editing – basic concepts and genotoxicity

General planning for preclinical assessments of gene therapy investigative drugs

Focus on AAV

Regulatory Guidance

Human Gene Therapy for Retinal Disorders

Guidance for Industry

US Department of Health and Human Services

FDA CBER

January 2020

Human Gene Therapy for Neurodegenerative Disorders

Draft Guidance for Industry

US Department of Health and Human Services

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Preclinical Assessment of Investigational Cellular and Gene Therapy Products

Guidance for Industry

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November 2013

Long Term Follow-Up After Administration of Human Gene Therapy Products

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US Department of Health and Human Services

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January 2020

Questions to be Addressed Before Initiating Nonclinical Toxicology Studies

- Who is the intended patient population?
- What gene therapy (GT) product will be used clinically? (vector type, promoter, transgene, etc.). How will the clinical product be manufactured?
- What is the intended clinical route of administration and how will the vector be delivered?
- What is the level of pharmacologic effect anticipated to be clinically beneficial?
- Is repeat dose administration necessary and if so, how far apart will the injections be administered?
- What species is pharmacologically relevant?
- Is there an appropriate animal model of disease/injury that can be used?
- What are the kinetics of transgene expression in the selected species?

Recommendations for Preclinical Studies to Support Retinal/ND Gene Therapy (GT) (1)

- Preclinical in vitro and in vivo proof-of-concept (POC) studies are recommended to establish feasibility and support the scientific rationale for a clinical trial.
  - Animal species and/or models selected should demonstrate a biological response to the investigational GT product that is similar to the expected response in humans.
- Biodistribution studies should be conducted to assess the distribution, persistence, and clearance of the vector and possibly the expressed transgene product, from the site of administration to target and non-target tissues, including applicable biofluids, as feasible.

Recommendations for Preclinical Studies to Support Retinal/ND GT (2)

- Toxicology studies for an investigational GT product should
  - incorporate elements of the planned clinical trial (e.g., dose range, ROA, dosing schedule, evaluation endpoints, etc.) to the extent feasible.
  - be sufficiently comprehensive to permit identification, characterization and quantitation of local and systemic toxicities, their onset (i.e., acute or delayed) and potential resolution, and relationship to dose.
  - characterize any abnormal ophthalmic/CNS findings or lesions (frequency, severity, potential cause, and clinical significance).
  - characterize Inflammatory or immune responses to assess potential attribution to the vector or transgene.

Recommendations for Preclinical Studies to Support Retinal/ND GT (3)

- Rodent models of retinal disorders are often used to generate POC data.
  - However, due to differences in ocular size and anatomy in rodents as compared to the human eye, animals with more “human-like” eyes, such as rabbits, pigs, or nonhuman primates, may also provide applicable safety information. Inclusion of the larger animals also facilitates relevant experience with the surgical procedures and delivery systems intended for clinical use.
  - However, due to differences in anatomy in rodents as compared to the central and peripheral nervous systems in humans, animals with larger brains or spinal columns, such as pigs or nonhuman primates, may provide additional safety information and facilitate dose extrapolation. Inclusion of larger animals may also allow for the evaluation of the surgical dosing procedures and delivery device systems intended for clinical use.

Summary

- IND enabling studies should
  - use the Phase I clinical formulation/manufacturing process.
  - replicate the route of clinical administration including any devices used.
  - demonstrate POC.
  - identify a MED.
  - evaluate potential toxicity over a range that encompasses doses anticipated to be efficacious.
  - identify a safe starting dose.
  - be adequately designed to identify potential adverse effects and to follow those effects to resolution or stability and collect ancillary data (e.g., serum samples, CSF samples, aqueous samples, vitreous samples) to determine the etiology of effects (e.g., are they immune mediated or due to some other cause).
  - characterize biodistribution from the site of administration.

Retinal Gene Therapy

Points to Consider

Key questions to think about for retinal GT

- What is the retinal cell type of interest?
- What is the intended route of administration and delivery instrumentation?
  - Subretinal; intravitreal; suprachoroidal; subinternal limiting membrane.
- How will dose be extrapolated from nonclinical species to humans?
- What is a “human-like” eye?

Retinal Gene Therapy Products in Development

Indication	Gene	Vector	Affects
Choroideremia (x-linked)	Rab escort protein	AAV2-REP1 4D-110	Choroid, RPE
X-linked Retinitis Pigmentosa	Retinitis Pigmentosa GTPase Regulator	AAV8-RPGR AAV2/5-RPGR AAV5-RPGR rAAV2tYF-GRK1-RPGR	Rod PR
MERTK-associated Retinitis Pigmentosa	MER Receptor Tyrosine Kinase	rAAV2-VMD2-hMERTK	RPE/rods
Autosomal recessive Retinitis Pigmentosa	PDE6A - subunit of the rod cGMP-phosphodiesterase		Rods
LCA	retinoid isomerohydrolase	AAV2-REP65 (Luxturna)	RPE
X-linked retinoschisis	RS1 gene	AAV8-scRS/IRBPhRS) gene	Macula (cones)
Achromatopsia	cyclic nucleotide gated channel alpha 3 cyclic nucleotide gated channel beta 3	AAV2/8-hG1.7p.coCNGA3 AAV2/8-hCARp.hCNGB3	Cones
Diabetic ME	Anti-VEGF Fab	AAVCAGsCD59 RGX-314	Endothelial cells

Retinal Transduction by AAV Serotypes

Serotype	Subretinal Administration	Intravitreal Administration
1	RPE	
2	RPE and Photoreceptors	Penetrates ILM and transduces inner retina
3		
4	RPE	
5	RPE and Photoreceptors	
6		
7	RPE and Photoreceptors	
8	RPE and Photoreceptors, Müller cells	Penetrates ILM
9	RPE and Photoreceptors, Müller cells	Penetrates ILM

Vectors need to enter the target cell nucleus

Rod and cone nuclei are in the outer nuclear layer

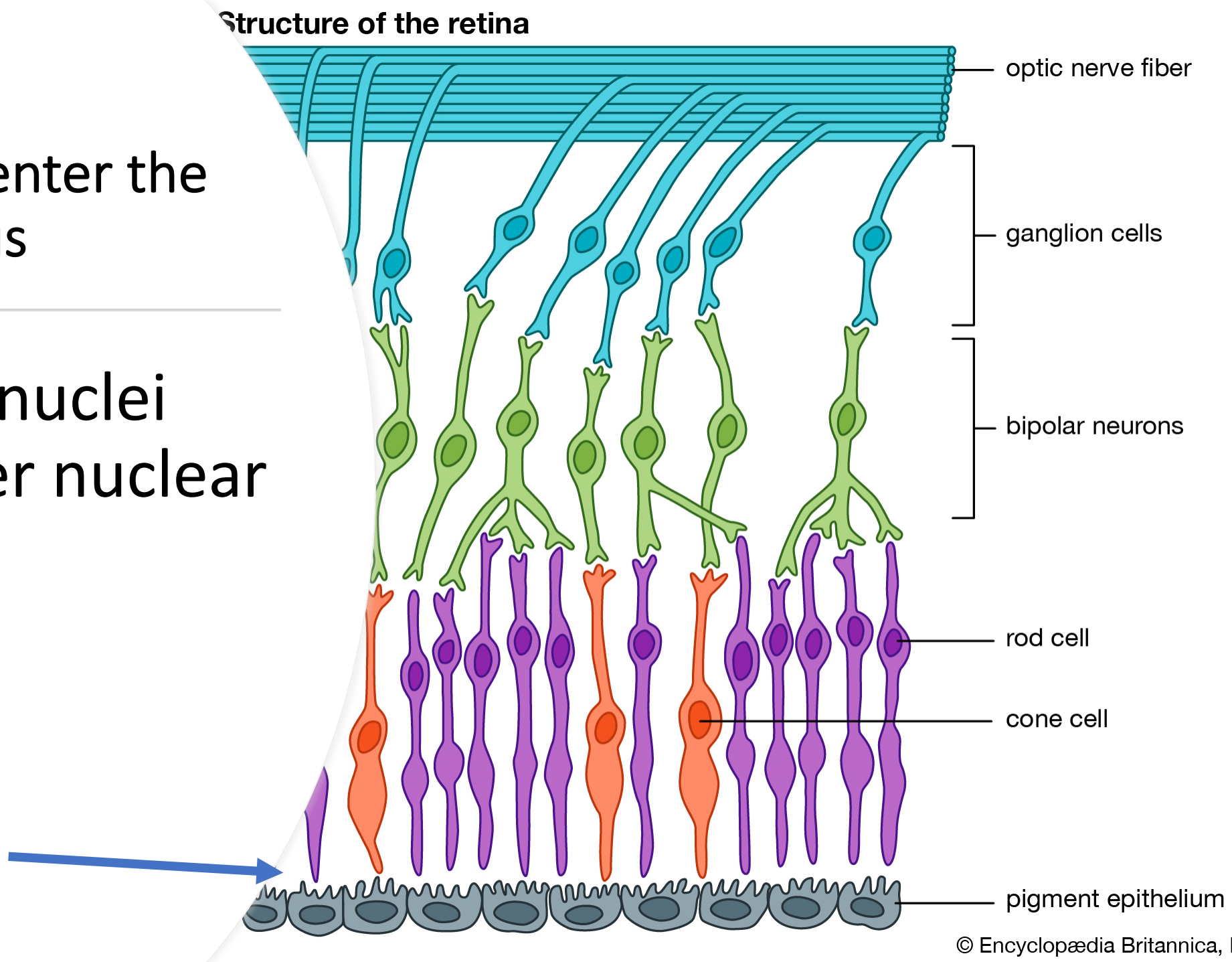
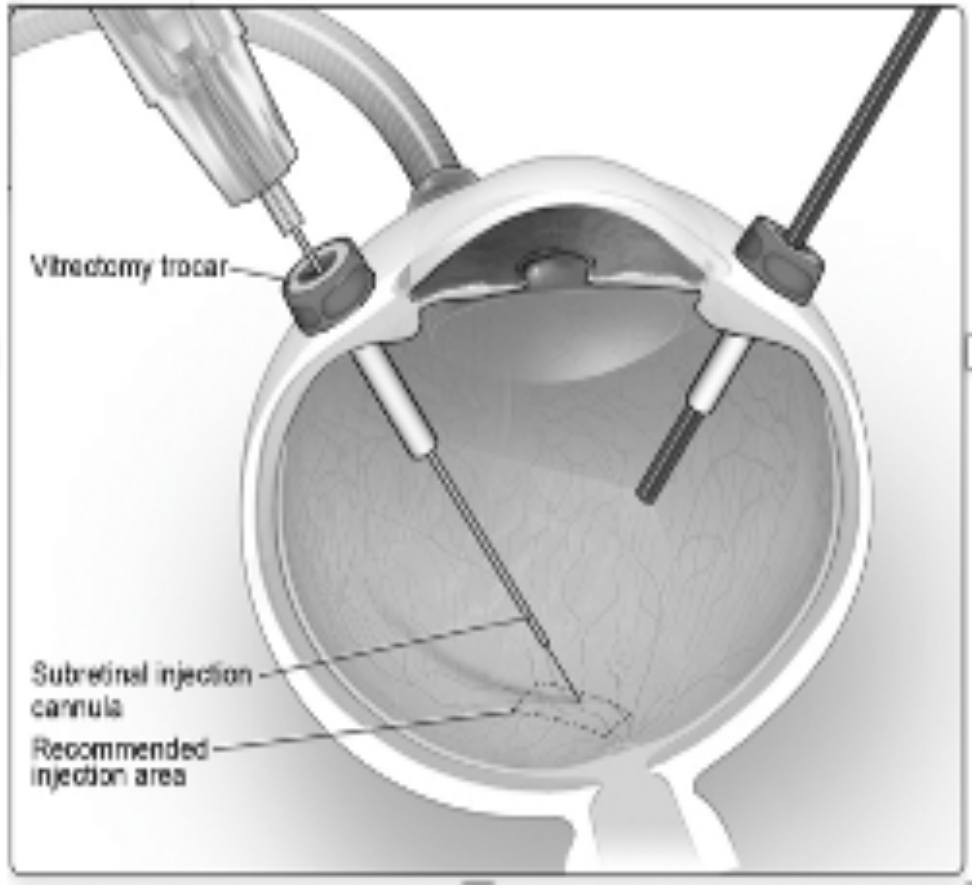
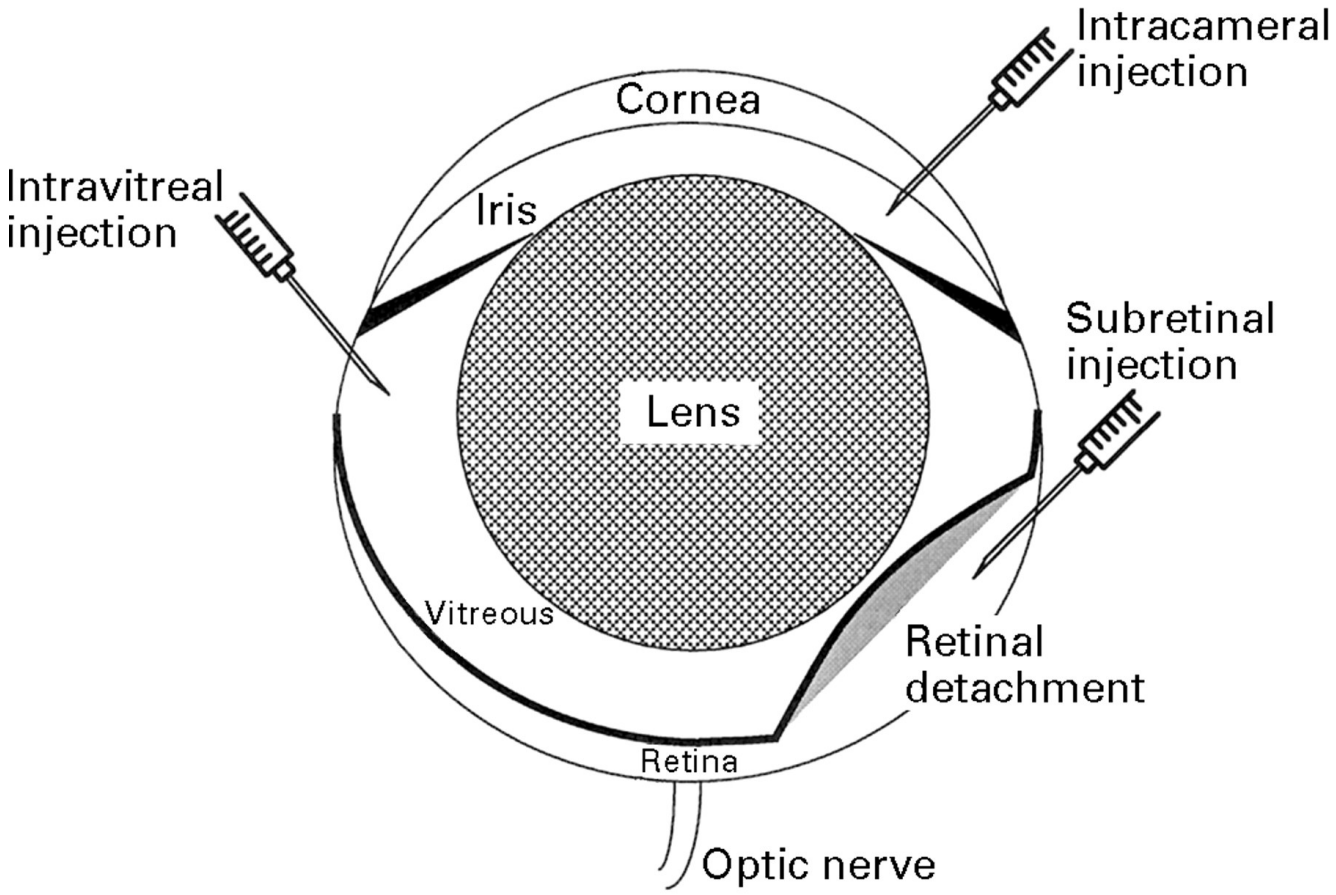
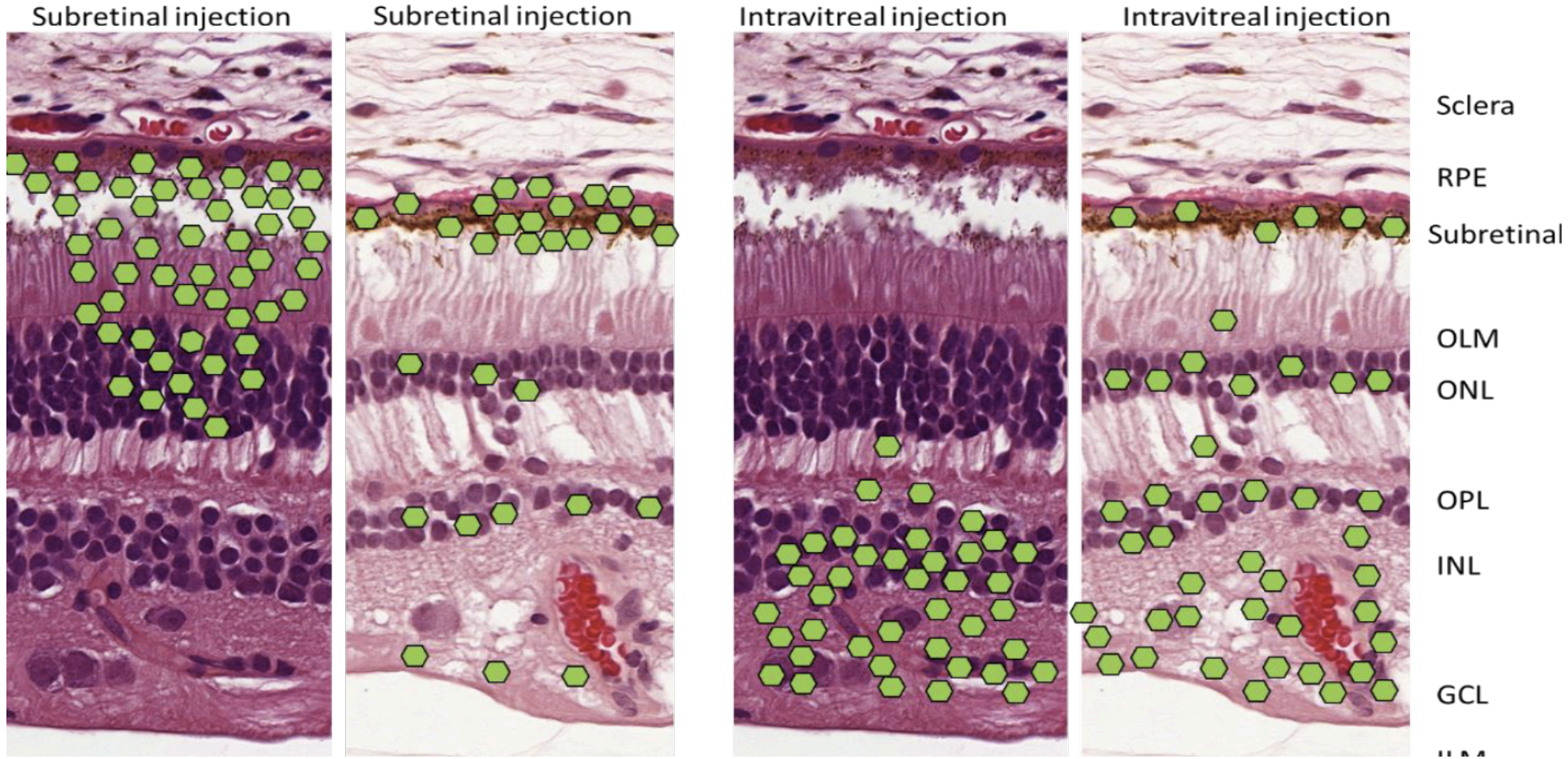


Illustration of ROA Challenges





Conceptual Contributions of ROA and Disease State to rAAV Retinal Penetrance



Buck and Wijnholds Intl J Mol Sci 2020

Summary

- Targeting photoreceptor nuclei is most readily achieved by subretinal administration.
- Subretinal administration involves creating a partial vitrectomy and a subretinal bleb (a focal retinal detachment) – this involves insertion of multiple ports through the sclera and cannot be replicated, but can be approximated, in large animal eyes.
- IVT administration is not optimal for targeting cells in the outer retina (at least in healthy animals).
- Targeting endothelial cells or delivering a gene that results in a secreted protein can be achieved by IVT or subretinal delivery.
- IVT administration is much less technically challenging and carries a lower risk of causing retinal tears or detachment.
- The retinal inner limiting membrane constitutes a significant barrier to AAV penetration; toxicity studies using IVT administration should use a species where the ILM thickness is closer to the thickness in humans.

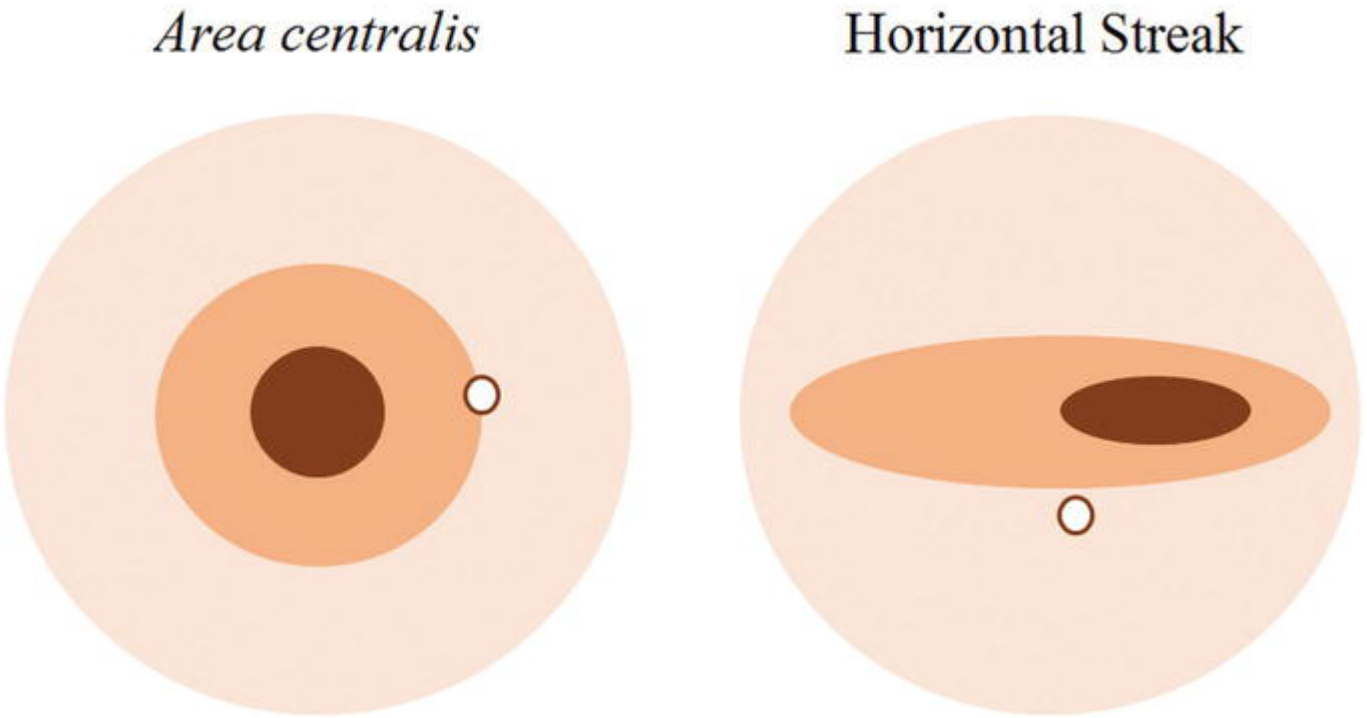
Comparative Ocular Anatomy

What is a “human-like” eye?

Parameter	Human	NHP	Dog	Pig	Rabbit	Rodent
Blink rate	Every 5s	Every 6s	Every 10-20s	Every 20-30s	Every 6m	Every 5m
Nictitating Membrane	No	No	Yes	Yes	Yes	Yes*
Central corneal thickness (mm, avg)	0.54	0.42	0.5-0.66	0.8	0.36	0.16-0.2
Aqueous humor volume (µL)	310	123	770[1]	No data	287	13.6 (rat) 5.9 (mouse)
Vitreous volume (mL)	4	1.8-2.0	1.7 ± 0.86	2-3	1.5-1.8	0.013-0.054 (rat) 0.0053 (mouse)
Retinal Vasculature	Holangiotic	Holangiotic	Holangiotic	Holangiotic	Merangiotic ON myelination extends horizontally on either side of ONH	Holangiotic
Inner limiting Membrane thickness (nm)	1500 nm	100-2000 nm (age dependent)	?	?	40 nm	20 nm
Retinal Specialization	Area centralis (aka macula) + fovea	Area centralis (aka macula) + fovea	Visual Streak or area centralis	Visual Streak and area centralis	Visual Streak	Visual Streak
Ratio Rods:cones (single values represent average across whole retina)	20:1	15-30:1	23-40:1	8:1	15:1	30:1

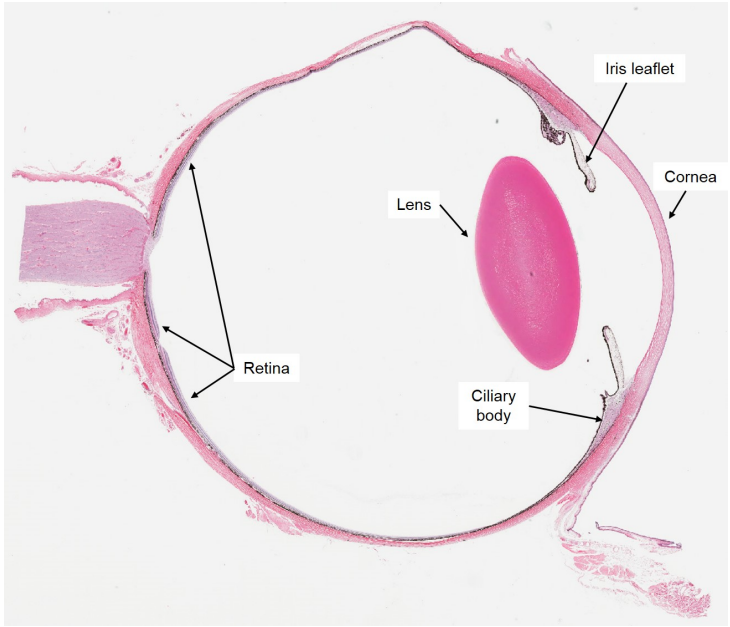
*essentially nonfunctional

Retinal Specializations: areas of higher cell density; some species have a fovea which is a further specialization of the area centralis

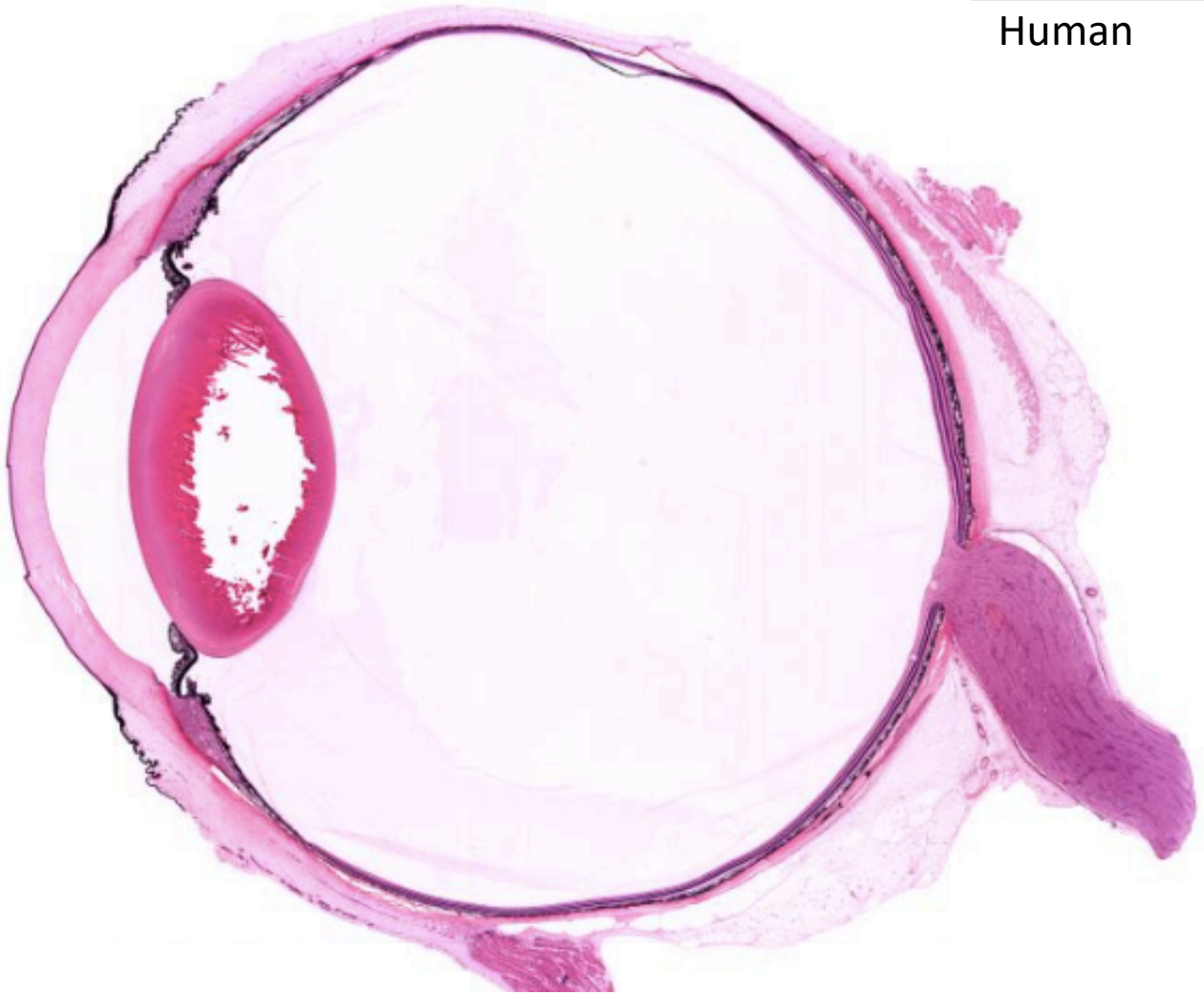


Fovea contains dense populations of cones, favoring greater visual acuity in this region

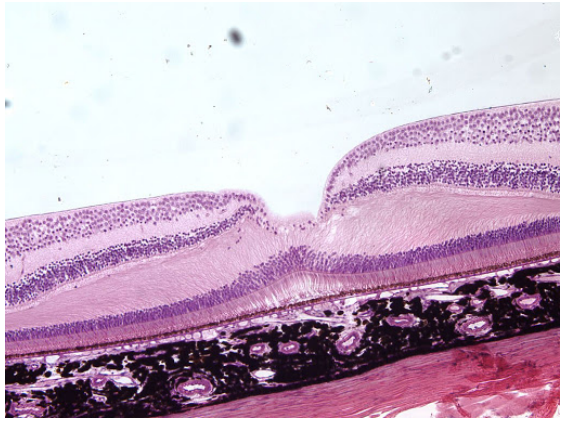
Nonhuman Primate



Human



Macula with fovea

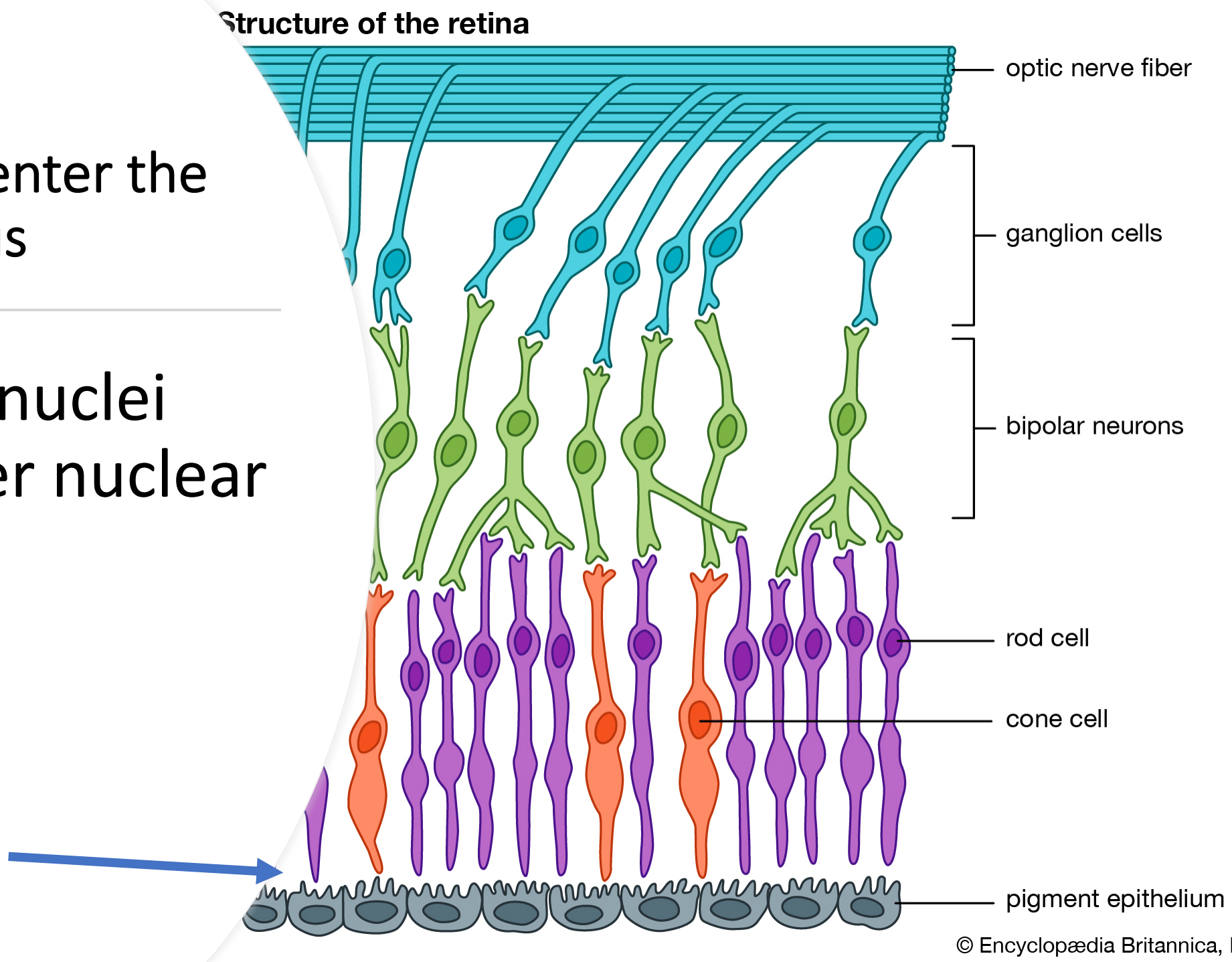


Retinitis Pigmentosa (RP)

- A class of diseases involving progressive degeneration of the retina, typically starting in the mid-periphery and advancing toward the macula and fovea. Typical symptoms include night blindness followed by decreasing visual fields, leading to tunnel vision and eventually legal blindness or, in many cases, complete blindness.
- **The bulk of RP is due to mutations in genes expressed in rods,** although cones die secondarily to rods.

Vectors need to enter the target cell nucleus

Rod and cone nuclei are in the outer nuclear layer



Diabetic Retinopathy

Reference	Type of vector	Promoter	Transgene	Regulation of transgene	Target	Transduced retinal cell	Animal model	Administration route
(Sun et al., 2019) [15]	AAV	CAG	HGFK	Igk leader	Endothelial cell	N/A	OIR mouse	Intravitreal
(Tu et al., 2018) [16]	scAAV2	CMV	CAD	Null	Endothelial cell	N/A	OIR mouse	Intravitreal
(Biswal et al., 2014) [17]	scAAV2	GFAP	Endostatin	HRSE-HRE	Endothelial cell	Müller cell	OIR mouse	Intravitreal
(Haurigot et al., 2012) [18]	AAV2	CAG	PEDF	Null	VEGF	Ganglion, amacrine, horizontal cell	Transgenic mouse overexpressing IGF-I	Intravitreal
(Pechan et al., 2009) [19]	AAV2	CMV	sFlt-1	Null	VEGF	N/A	OIR mouse	Intravitreal
(Lai et al., 2005) [20]	AAV2	CMV	sFlt-1	Null	VEGF	N/A	Transgenic mouse overexpressing VEGF (trVEGF029)	Subretinal
(Jiang et al., 2009) [21]	Lipofectamine	N/A	HIF-1α siRNA, VEGF siRNA	Null	HIF-1α & VEGF	N/A	OIR mouse	Intravitreal
(Lamartina et al., 2007) [22]	Adenovirus	CMV/IRES-M2	sFlt-1	Doxycycline	VEGF	Müller cell	OIR rat	Intravitreal
(Ideno et al., 2007) [23]	AAV5	CMV	sFlt-1	Null	VEGF	N/A	SDT rat	Subretinal
(Le Gat et al., 2003) [24]	Adenovirus	CMV	ATF, Endostatin	Null	uPA/uPAR	N/A	OIR mouse	Intravitreal
(Igarashi et al., 2003) [25]	Lentivirus	CAG	Angiostatin	Null	Endothelial cells	N/A	OIR mouse	Intravitreal
(Gehlbach et al., 2003) [26]	Adenovirus	CMV	sFlt-1	Null	VEGF	N/A	OIR mouse	Periocular
(Auricchio et al., 2002) [27]	AAV2/1, AAV2/2	CMV	PEDF, TIMP3, Endostatin	Null	Endothelial cells	N/A	OIR mouse	Subretinal
(Bainbridge et al., 2002) [2	AAV2	CMV	sFlt-1	Null	VEGF	Ganglion cell layer, inner nuclear layer	OIR mouse	Intravitreal

Screenshot

Achromatopsia

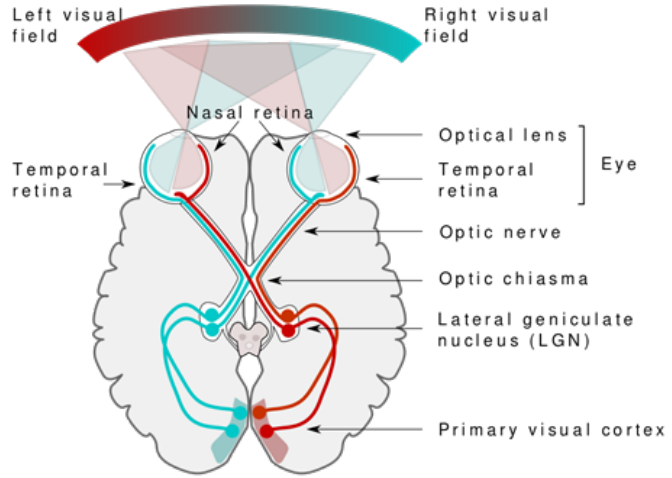
- An autosomal recessive retinal disease involving loss of cone function that afflicts approximately 1 in 30,000 individuals.
- Patients with achromatopsia usually have visual acuities lower than 20/200 because of the central vision loss, photophobia, complete color blindness and reduced cone-mediated electroretinographic (ERG) amplitudes.
- Mutations in three genes have been found to be the primary causes of achromatopsia, including *CNGB3* (beta subunit of the cone cyclic nucleotide-gated cation channel), *CNGA3* (alpha subunit of the cone cyclic nucleotide-gated cation channel), and *GNAT2* (cone specific alpha subunit of transducin).

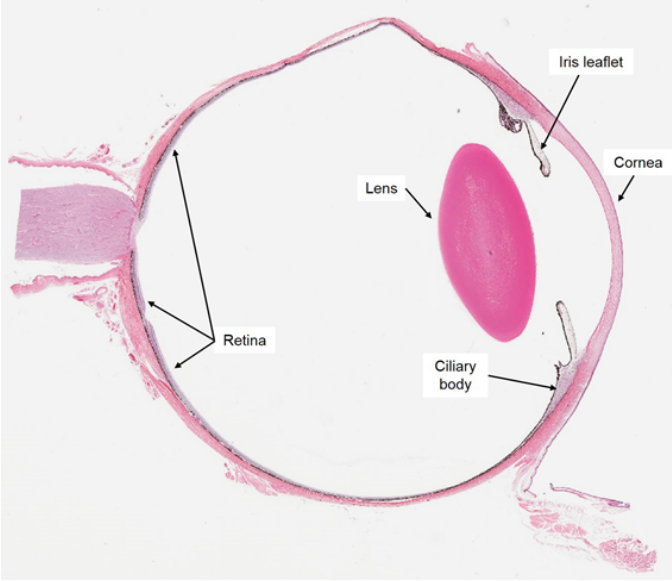
SPECIES SELECTION SUMMARY

- For subretinal administration where the target is the RPE or the rods or any other cell type that is relatively evenly distributed across the retina, any larger species in which the clinical administration procedure can be approximated might be justified.
- For subretinal administration where the target is the cones, the species choice will be influenced by whether the administration procedure is modified to target the fovea, which is highly rich in cones. In this case the only appropriate large animal species in which the clinical administration procedure can be approximated, and the fovea is present, is the NHP.
- For intravitreal administration, a species in which the ILM thickness approximates that in humans would be desirable so that the level of vector penetration can be presumed to be similar – in this case the best data are available for the NHP but other species with eyes of similar size (pig, dog) might be acceptable.

OTHER ADVANTAGES OF A LARGE EYE

- Enables gathering biodistribution date from the visual pathway in the brain.
- Facilitates collection of vitreous and/or aqueous samples to look for vector, levels of expressed transgene (for secreted proteins like the anti-VEGF Fab) and antibody responses to the vector and transgene.





Neurodegenerative Diseases Gene Therapy

Points to Consider

Key questions to think about for GT for neurodegenerative diseases

- What is the cell type of interest and where is it located?
- What is the intended route of administration and delivery instrumentation?
  - Intrathecal (via cisterna magna, C1-C2, lumbar puncture); intraparenchymal; intravenous NOTE: AAV does not cross the BBB readily in species larger than mice hence the frequent use of intraparenchymal or IT routes.
- How will dose be extrapolated from nonclinical species to humans?

Nervous System Transduction by AAV Serotypes

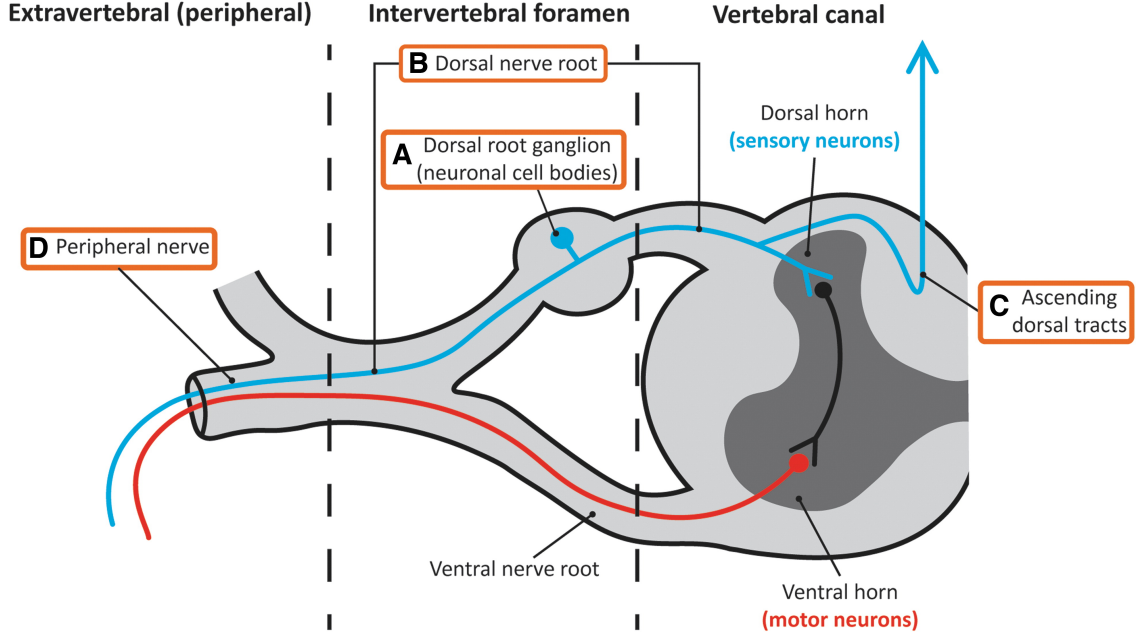
Serotype	Neurons	Astrocytes	Microglia
1	***	*	**
2	*		
5	**	*	
6	**		
7	**	**	**
9	***		

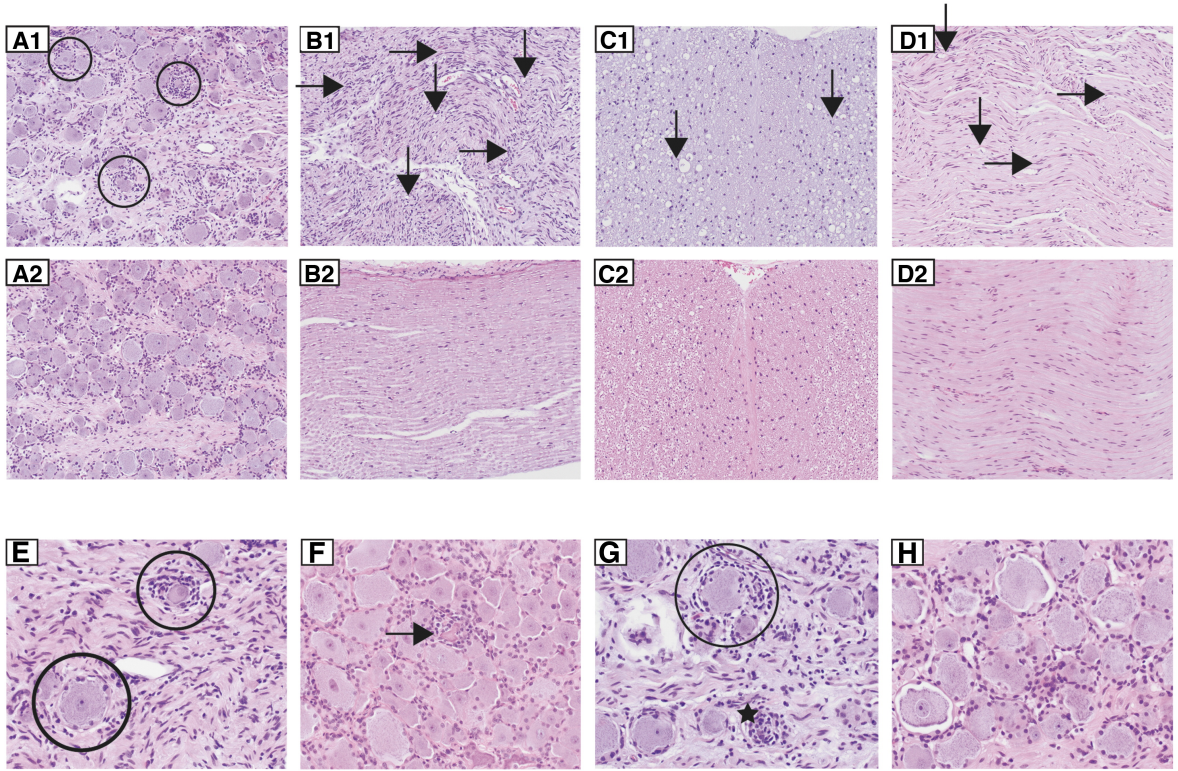
Haggerty DL et al Molecular Therapy Methods and Clinical Development 17:69-82, 2020

FDA Places Partial Clinical Hold on Zolgensma Trial Over Safety Concerns

The U.S. **Food and Drug Administration (FDA)** placed a partial clinical hold on clinical trials for intrathecal administration of Zolgensma, **Novartis'** multi-million dollar gene therapy treatment for spinal muscular atrophy due to safety concerns.

The Swiss giant announced the hold in a **filing** with the U.S. Securities and Exchange Commission this morning. Novartis said the announcement follows a communication its subsidiary AveXis, the developer of Zolgensma, made to health authorities and clinical trial investigators. The communication was made after AveXis found a small hitch in a preclinical study. The company discovered animal findings that showed dorsal root ganglia (DRG) mononuclear cell inflammation, which were sometimes accompanied by neuronal cell body degeneration or loss. What this means clinically is not yet known, Novartis said in its filing. Novartis said this had not been seen in prior animal studies with Zolgensma.





Adeno-Associated Virus-Induced Dorsal Root Ganglion Pathology

Hordeaux et al. Human Gene Therapy 2020 Aug; 31(15-16):808-818

AAV-Related DRG Changes

- Dose dependent.
- Not prevented by immunosuppression though there may be mitigation of associated clinical signs.
- May be associated with subtle clinical signs (e.g., loss of tail reflex) but in extreme cases has been associated with pain and hind limb lameness.
- Usually seen in monkeys (but may be seen in dogs and pigs) – samples are biased because most studies are done in monkeys. Not seen in rodents.
- Predominantly seen after IT administration; may also be seen after IV administration of strongly neurotropic AAV (e.g., AAV9) at high doses.

Impact

- Even if the AAV is being administered by some other route other than IV, it is helpful to collect DRGs and spinal cord to examine histologically, or supply data to justify why this was not done.
- Can result in clinical holds if there is no NOAEL or if proposed clinical doses exceed the NOAEL.
- Should be expected at doses of 1E13 and higher administered intrathecally in cynos.

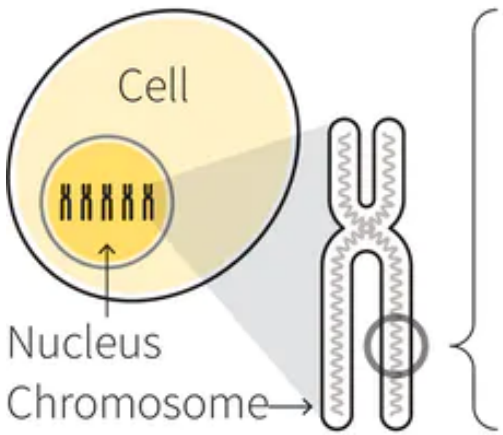
CRISPR Editing is All About DNA Repair Mechanisms

Assessing Genotoxicity for CRISPR Cas based Therapeutics

Gene editing

A DNA editing technique, called CRISPR/Cas9, works like a biological version of a word-processing programme's "find and replace" function.

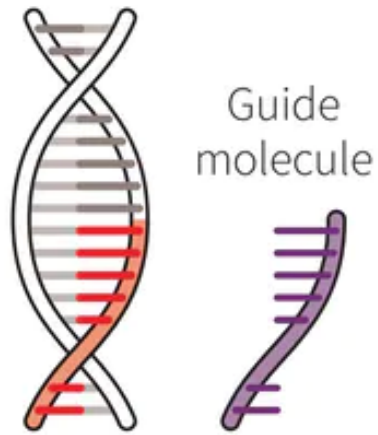
HOW THE TECHNIQUE WORKS



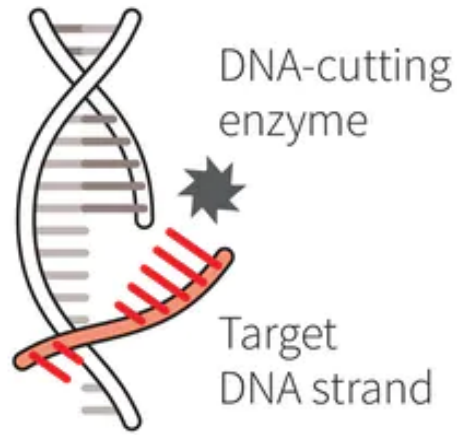
A cell is transfected with an enzyme complex containing:

Guide molecule

DNA-cutting enzyme



A specially designed synthetic guide molecule finds the target DNA strand.



An enzyme cuts off the target DNA strand.

The amended DNA strand repairs itself.

Sources: Reuters; Nature; Massachusetts Institute of Technology

Staff, W. Foo, 20/05/2016

REUTERS

Two primary DNA repair mechanisms

Non-homologous end-joining (NHEJ): Template independent, error prone: Enzymes can stitch the dangling ends back together, which often results in one or more bases—the building blocks of DNA—being added or deleted (indels).

Homology-directed repair (HDR): Error free pathway. Other enzymes can patch the break with a single strand of DNA that matches the DNA sequence upstream and downstream of the cut. A complementary DNA strand is created to complete the double-strand repair.

EXAMPLE

- Leber's Congenital Amaurosis 10 (LCA10) due to deep intronic bi-allelic mutation in CEP290 gene that creates a cryptic splice donor site.
- EDIT-101, an AAV5 delivered CRISPR/Cas9 administered subretinally, contains two guides that target the ends of the CEP290 locus containing the mutation. The DSB created results in either a deletion or an inversion of the mutated region. This prevents aberrant splicing and restores translation of the functional protein (Maeder et al, Nature Med 2019; 25:229-33).

Assessing Genotoxicity for Gene Edited Products

- Characterize on-target editing (the desired outcomes).
- Characterize off-target editing (Biased/Unbiased). Biased methods rely on looking for potential similarities to the guide sequences. Unbiased methods don't make assumptions about where off-target sites might be.
  - In silico (biased)
  - In vitro (DNA) e.g. Digenome-Seq } unbiased
  - Cell-based e.g. GUIDE-Seq
- Characterize gross chromosomal changes (Giemsa banded karyotyping, FISH, UDiTaS (UniDirectional Targeted Sequencing) - deletions, translocations.
- Tumorigenesis– curated risk assessments of affected sites; ex vivo proliferation assays; clonogenicity analyses; in vivo tumorigenicity with/without serial transplantation to increase the number of generations.
- Proposed approach should be discussed with CBER pre-IND.

CRISPR CAS Summary

- CRISPR CAS relies on making very specific targeted cuts and then utilizing the endogenous DNA repair mechanisms to restore the edited DNA to a functional sequence (editing).
- Genotoxicity assessment of gene editing products is a weight of evidence argument that incorporates multiple types of evaluations to look at the potential consequences of off (and on) target editing.
- Get buy in from CBER to proposed genotoxicity assessments.

WHY

- SUBRETINAL INJECTION OF LUXTURNA
- Evelyn's story: Our experience with ZOLGENSMA

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