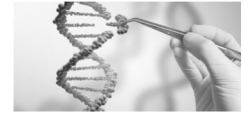


## RPE65 & Advances in Retinal Gene Therapy

IAN DANFORD

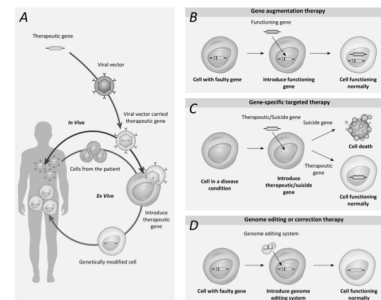
## Gene Therapy

- ▶ In broad strokes:
  - ▶ Introduction of functioning genes into cells and tissues hampered by a mutated, defective gene.
- ▶ Diseases being treated with gene therapy currently tend to be defined by cells either not expressing an important gene (actually absent) or producing a dysfunctional copy of a gene (functionally absent).
- ▶ More difficult to "fix" a disease caused by a gene that is only partially effective or too effective.



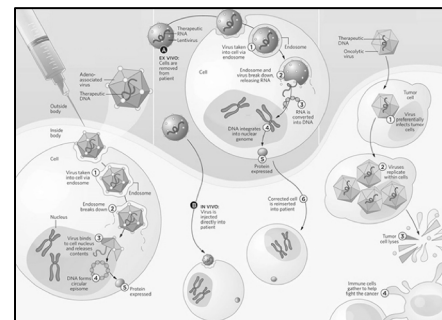
## 3 Basic Types of Gene Therapy

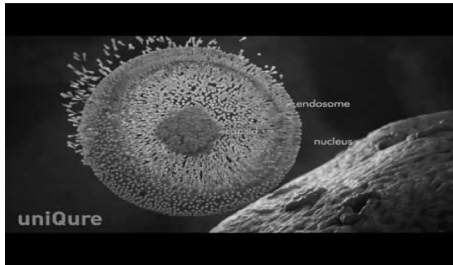
- ▶ "Gene Augmentation"
  - ▶ Introduce a normal, functioning gene to substitute for a non-functioning or under functioning gene
- ▶ "Gene-Specific Targeting Therapy"
  - ▶ Genetic material (DNA, RNA) introduced to indirectly alter inappropriate gene activity
- ▶ "Genome Editing"
  - ▶ Directly repair mutated genes to become normal functioning genes (CRISPR)



## Inserting Genes

- ▶ Gene therapy uses "vectors" to package and deliver functional DNA into cells without the functional gene.
- ▶ Researchers are discovering many different kinds of vectors, but viruses have been the most effective—particularly the Adeno-associated viruses (AAV).
- ▶ AAV works well for gene therapy because:
  - ▶ It does not cause disease
  - ▶ The immune system tends to not react to it strongly
  - ▶ It does not insert the gene into the patient's DNA (next slide)

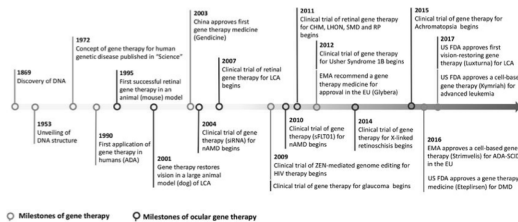




## Eye and Gene Therapy

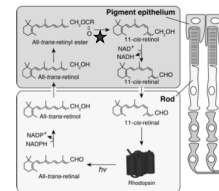
- ▶ Over half of clinical gene therapy trials target retinal diseases.
- ▶ The eye offers some advantages for the development of successful gene therapies:
  - ▶ Relatively directly accessible for examination and follow-up (compared to assessing bone marrow, etc)—doesn't biopsy, blood draws, etc to assess retinal health.
  - ▶ Enclosed structure and small—compared bone marrow, etc
  - ▶ Blood-retinal barrier prevents transmission of gene therapy products to the rest of the body

## History and Timeline of Ocular Gene Therapy



## RPE65 Gene

- ▶ The best known and most successful example of retinal gene therapy is treatment of **Leber's Congenital Amaurosis Type 2 (LCA2)** by replacing mutant **RPE65** with a normal copy of the gene.
- ▶ RPE65 gene encodes for the enzyme all-trans retinyl ester isomerase.
- ▶ Without this enzyme there is accumulation of All-trans-retinyl ester which leads to rapid visual decline and can over time lead to cell death of the photoreceptors and RPE cells.



## Leber's Congenital Amaurosis

### Epidemiology

- ▶ Birth prevalence of LCA is **2-3 per 100,000 births**.
- ▶ Onset of severe vision loss **at birth or within first year of life (in most cases)**.
- ▶ **Most common cause of inherited blindness in childhood.**
- ▶ Is the cause of blindness in more than **20%** of children attending schools for the blind.
- ▶ **RPE65** mutations account for **3-16%** of LCA cases.



## Leber's Congenital Amaurosis

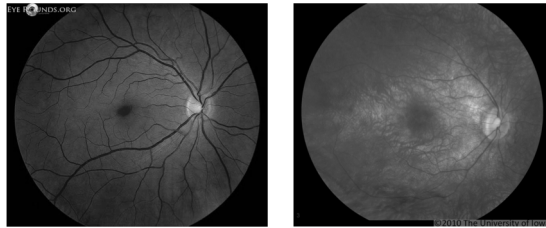
### Clinical Findings

- ▶ Visual acuity usually around 20/200 to count fingers.
- ▶ Sluggish pupils
- ▶ Nystagmus
- ▶ Night blindness
- ▶ Light sensitivity
- ▶ Oculodigital sign\*
- ▶ Early on fundus exam usually appears normal...



## Leber's Congenital Amaurosis Type 2

Fundus Findings



## RPE65 Gene Therapy

Proof of Principle in Animal Model

- ▶ In 2001 a preclinical study with the Briard dog model of LCA2 (predisposed to RPE65 -/- and consequent blindness) was performed
- ▶ Showed marked visual improvement using the AAV-mediated delivery of RPE65.
- ▶ This triggered the development of clinical trials in humans with LCA2.



## RPE65 Gene Therapy

Clinical Trials in Humans

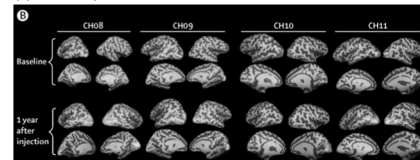
- ▶ In 2008 several clinical trials (phase I) found visual improvement after gene therapy with RPE65.
- ▶ In one of the seminal studies, all 12 subjects safely had stable improvement in vision and retinal function
- ▶ These 12 patients had received subretinal injections of AAV2-hRPE65v2 in their worse seeing eye.
- ▶ Visual improvement was durable for at least 3 years—observation still ongoing.



## RPE65 Gene Therapy

Clinical Trials in Humans

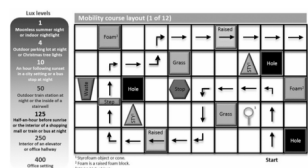
- ▶ Functional MRI studies revealed increased visual cortex activation and improved function and structure of visual pathway in patients who had received gene therapy in both eyes.



## RPE65 Gene Therapy

Clinical Trials in Humans

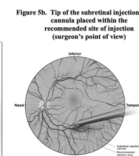
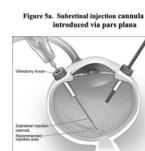
- ▶ Multi-luminance Mobility Test
  - ▶ Standardized obstacle course that study participants maneuvered through before and after treatment at various luminance levels.
  - ▶ Has served as an inclusion/exclusion criteria as well as a primary endpoint in RPE65 gene therapy studies.



## RPE65 Gene Therapy

The Surgery

- ▶ Pars plana vitrectomy
- ▶ Use of a extremely small [41 gauge] needle to inject vector into subretinal space.
- ▶ Use of intraoperative OCT helps confirm injection site and avoid too much tension on macula.







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