Table of Contents

10 / 02 / 25

Evaluating Gene Therapy as a Treatment for ABCA3-Related Congenital Surfactant Deficiency

Why I Chose this Project

I spent some time shadowing in neonatal intensive care units, as I want to become a neonatologist. It was here I met a baby with a fatal genetic lung disease, her only chance? A neonatal lung transplant. She is my inspiration for my project.

Why it Matters

Congenital surfactant deficiency is a fatal genetic condition caused by mutations (in this project ABCA3 Mutations). Without a lung transplant these babies typically die <1 year of life. Neonatal lung transplants are notoriously hard to come by and only have a 5 year survival rate of 50%. A new treatment is needed if these babies have any chance of survival. If successful it will allow patients to live, decrease strain on healthcare systems and eliminate their need for further treatments. It'll open the door as well for research on gene therapy for other surfactant disorders and genetic conditions

10 / 02 / 25

What is Congenital Surfactant Deficiency

An umbrella term for a group of genetic diseases characterized by insufficent production or dysfunction of surfactant. While rare it's associated with severe respiratory distress in full term infants and early onset lung disease

Causes Include:

- Mutations in ABCA3 (project focus)
- Mutations in surfactant proteins, typically B and C.

Symptoms and Clinical manifestations

- Severe work of breathing
- Cyanosis
- Cold limbs, fingers and toes
- Tachypnea
- Grunting
- Low oxygen levels
- Tachycardia
- Mottling

In rare cases, CSD may not be identified until late infancy, childhood, or early adulthood. This typically manifests as childhood interstial lung disease or adult pulmonary fibrosis. ABCA3 patients are usually critical from birth but later onset is possible.

Continued on next page

10 / 02 / 25

Treatments

There is no cure, current treatments focus on sustaining life until a lung transplant becomes available.

- Oxygen therapy
- Surfactant replacement therapy
- Nutritional support
- Inhaled corticosteroids | system steroids
- Macrolide antibiotics
- Non invasive ventilation (CPAP, BiPAP)
- Mechanical ventilation (conventional, high frequency oscillation, jet).
- ECMO

Diagnosis

Diagnosis is through genetic testing, lung biopsy, or electron microscopy.

Numbers

- 1 in 20,000-40,000 live births affected by ABCA3 related disease.
- 1 in 33-70 individuals are a carrier of a ABCA3 mutation.
- 10% transplant candidates get new lungs.
- 30% of transplant ~~candidates~~ patients make it to 10 years.
- ABCA3 accounts for up to 50% of CSD cases.

10 / 10 / 25

ABCA3 Gene

Focus: understand the ABCA3 gene and how mutations affect it.

- The ABCA3 gene is located on chromosome 16 at 16p13.3 (p indicates the petite arm and 13.3 and band).
- Negative strand meaning its transcribed from the complementary DNA strand.
- It's 33.6 Kb, it's mRNA is 6.8Kb.

There is over 200 known mutations, they can be:
- Missense- single amino acid change.
- Nonsense- early stop codon.
- Frameshift- insertion or deletion of nucleotides that is not a multiple of 3. Everything downstream changed.
- Splice- change in DNA sequence that disrupts signals that tell the cell where to cut and join RNA to create mature mRNA).
- General insertions\deletions.

These mutations can disrupt surfactant production by:
- preventing ABCA3 protein from being made.
- causing the proteins to misfold.
- damaging it's ability to transport lipids.

In order for disease, two mutations are required. Carriers may be asymptomatic or have mild lung issues.

Shulenin et al. (2004) examined lung tissue from mutated patients and observed abnormal lamellar bodies. This suggests ABCA3 is important for proper lamellar body formation.

4

10 / 10 / 25

ABCA3 Protein

Focus: to understand how the gene and protein are connected, how the protein works, and how the mutations affect protein function.

- The A3 gene encodes a 1,704 amino acid polypeptide. It's a ATP-binding cassette subfamily A member 3. meaning it deals primarily with lipid transport.
- It's a phospholipid transporter located on the membrane of lamellar bodies.

How is it made?
- It's produced in type II alveolar cells. After it's sent to the endoplasmic reticulum to be folded into the correct shape.
- Then it moves to the golgi apparatus where it undergoes glycosylation (addition of sugar molecule).
- Finally it gets cut at one end- N-terminal, to remove an extra piece.
- Before undergoing glycosylation the protein weighs 190KD. The final, working version weighs 150KD.

- ABCA3 covers 10-20% of lamellar body membranes, it's very abundant.
- It has a two transmembrane domain and a two nucleotide-bonding domain.
- Localization signal sequences direct the protein to lamellar bodies.

Continued on next page

5

10 / 17 / 25

ABCA3 Protein

What does it do?
- The ABCA3 protein acts kind of like a bridge between the cytoplasm and interior lamellar body.
- It uses ATP (energy) to change it's shape, then translocates "flips" lipids across the membrane.
- The amphipathic (hydrophobic and philic sides) structure of lipids may it difficult for them to cross lamellar membrane hydrophobic core.

What do mutations do?
- restrict the protein from maturing and reaching lamellar bodies.
- creates non-functional proteins.
- causes small, disorganized or absent lamellar bodies.

6

10 / 19 / 25

Surfactant

Focus: to understand what makes up surfactant, how it works and the effect of mutations.

What is it made of?
Surfactant is a complex mixture of 80% phospholipids, 10% neutral lipids, and 10% surfactant proteins.
- SP-A: regulates surfactant secretion.
- SP-B: spreads surfactant.
- SP-C: Stabilizes lipid layer
- SP-D: binds to pathogens.

How is it secreted?
- It's excreted by exocytosis: the lamellar body then fuses with plasma membrane of type II alveolar cells. Surfactant is released into lining fluid.
- Once secreted contents undergo structural changes. Lamellar body contents unfold into a lattice structure called tubular myelin.
- It spreads into a single layer at the air-liquid interface. This is the surface active agent.

Up to 90% is recycled but it does degrade overtime. It's taken up by type II cells via endocytosis and cleared by alveolar macrophages.

Type II Alveolar Cells

10 / 26 / 25

Focus: to understand what alveolar cells are and what there function is.

What are they?
- Also known as ~~type II pneumocytes alveolar epithial~~ epithelial type II cells.
- Make up 17% of the alveolar wall and 5% of alveolar surface.
- Alveolar fluid is (roughly) 35ml per adult lung.

What do they do?
- Responsible for production, storage and secretion of pulmonary surfactant.
- Help trigger cytokines and antimicrobial peptides.
- Smaller then type I cells but more numerous. They can seperate into type I after injury to help lungs repair and regenerate.

Anything else?
- Appear at 24 weeks gestation, surfactant production increases dramatically during third trimester.
- Labour increases surfactant production.
- Women at risk for preterm labour may be given corticosteroids, these help mature type II cells and boost surfactant production.

8

Lamellar Bodies

10 / 28 / 25

Focus: to understand what lamellar bodies are, how they're created and how mutations affect them.

Lamellar bodies are secretory organelles found mainly in type II alveolar cells.
- Oval shaped 0.2-1.1 micrometers.
- Not visible under light microscopy unless stained so transmisson electron microscopy is the gold standard for visualization.
- Membrane bound, filled with layers of surfactant proteins and phospholipids.
- Lamellar bodies are transported through the cytoplasm to the type II cell.

How are they formed?
- Begins in the golgi apparatus where lipids and proteins are directed into multivesecular bodies that will become lamellar bodies.
- ABCA3 protein moves phospholipids into the developing lamellar body.

Effects of mutations
- ABCA3 mutations mean the protein is unable to transport and support lamellar body formation. Resulting in small or absent bodies.

CRISPR-Cas9

11 / 01 / 25

Focus: to understand the basis of CRISPR gene therapy and delivery methods.

What is CRISPR
- Clustered regularly interspaced short palindromic repeats.
- Originally discovered in naturally occuring bacteria and was adapted for laboratory use.
- Gene editing tool that allows scientists to modify the DNA of living organisms.

What is Cas9
- DNA cutting ~~protein~~ enzyme, molecular scissors.

How does it work?
- Made of two parts, a small strand of RNA programmed to look for ~~for~~ a specific DNA sequence (PAM) gRNA And Cas9, an enzyme that cuts a double strand of DNA.
- Once CRISPR has entered the cell it works to locate the DNA it's been told to find. It uses a PAM sequence, once found, cRNA binds to complementary DNA sequence.
- Cas9 cuts both DNA strands 3 base pairs up from the PAM.
- Once DNA is broken the cell works to repair it. A donor DNA template can be introduced into the cell. The template aligns with the cut DNA and the cell copies the correct sequence into the genome.

10

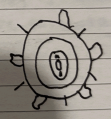
Gene Therapy Delivery Methods

11 / 02 / 25

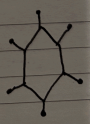
Electroporation
- uses electrical impulses to create openings in the phospholipid bilayer of the cell membrane.
- non viral vector
- risk of cell death, especially in neonates.
- suitable for ex-vivo

Adeno-associated virus
- Popular for in-vivo
- low immunogencity
- too small for ABCA3 cargo

Lentivirus Vectors
- Can carry large cargo
- Modified to infect more cells
- Integrates itself into cell DNA
- high immunogenicity though typically mild.



Adenoviral vectors
- Large carrying capacity
- Can infect wide range of cells
- High risk of immune response
- Non integrating



Hypothesis

11 / 22 / 25

The ABCA3 gene encodes a lipid transporter protein in type II alveolar cells and is essential for proper surfactant production and lamellar body formation. Mutations cause congenital surfactant deficiency leading to neonatal RDS and intersitial lung disease.

Gene therapy is a novel treatment option for many conditions and shows promise for treating ABCA3-related disease. It would allow for correction of mutations, providing a long term solution.

The actual hypothesis
Cells that are treated with gene therapy to repair ABCA3 will produce substantially more surfactant then mutated cells.

The Experiment

11 / 22 / 25

What am I doing?
My project aims to assess the viability of using gene therapy to treat ABCA3-related congenital surfactant deficiency. Additionally I will design a potential CRISPR based treatment.

The procedure will be split into 3 parts:
Backround research: this is where I did a comprehensive review of available scientific literature to better understand the components of my project.

Data analysis: I started thinking a lot here about will this work? how will this work? what could go wrong?

Application: In this section I suggested further directions and proposed my treatment.

Variables:
Controlled:
- Confirmed ABCA3-related congenital surfactant deficiency, information only collected from credible sources.

Independant: Type of treatment given.

Dependant: Amount/quality of surfactant produced.

13

The Experiment

11 / 22 / 25

Materials
Zotero: to create and manage citations.

Microsoft excel: for data analysis and creating visuals.

Scientific databases: for collecting information.

Publicly available images

Google docs: for formatting before placing into online project.

The Disease

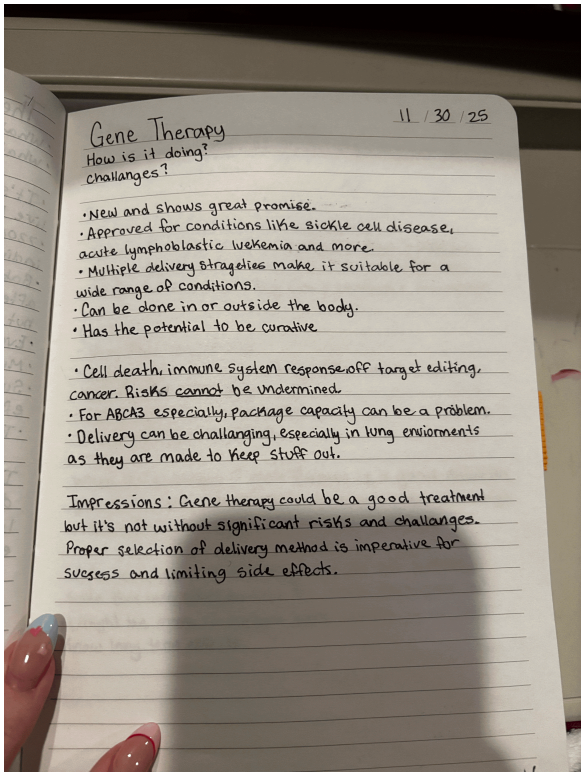
11 / 30 / 25

- What stands out to me?
- What can be done

- It's the most common form, 1 in 20,000-40,000 live births.
- 7200 mutations most are missense or private to individuals\families.
- Babies with two of the same mutation die shortly after birth. Those with two different may live longer but develop interstial lung disease.
- Even with mild disease survival past 20 is very rare.
- Mutations alter lamellar bodies.
- Surfactant through an endotracheal tube is not very effective.
- Treatments are life preserving until a lung transplant.

Impressions: a new treatment is needed, it's a devastating disease with very few treatment options. Lung transplant is the only definitive treatment and even with survival is low.

15



Gene Therapy

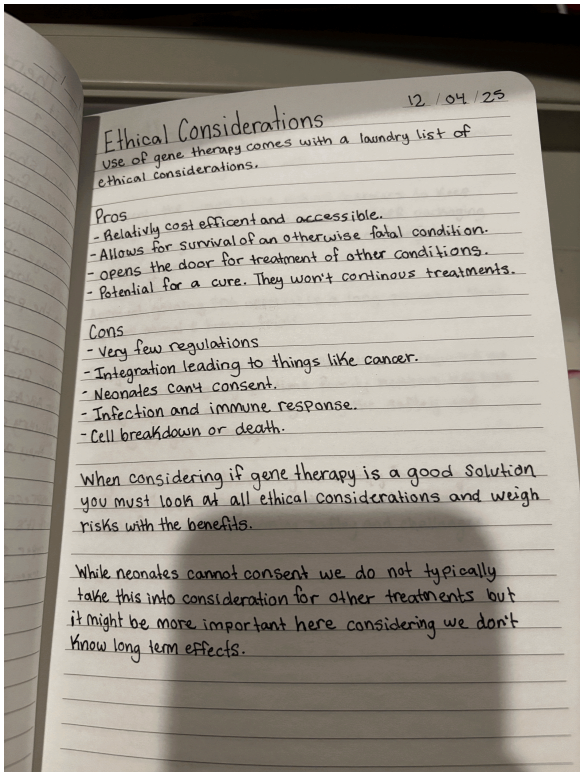
11 / 30 / 25

How is it doing?
Challanges?

- New and shows great promise.
- Approved for conditions like sickle cell disease, acute lymphoblastic leukemia and more.
- Multiple delivery strategies make it suitable for a wide range of conditions.
- Can be done in or outside the body.
- Has the potential to be curative

- Cell death, immune system response, off target editing, cancer. Risks cannot be undermined.
- For ABCA3 especially, package capacity can be a problem.
- Delivery can be challanging, especially in lung enviorments as they are made to keep stuff out.

Impressions: Gene therapy could be a good treatment but it's not without significant risks and challanges. Proper selection of delivery method is imperative for sucsess and limiting side effects.



Ethical Considerations

12 / 04 / 25

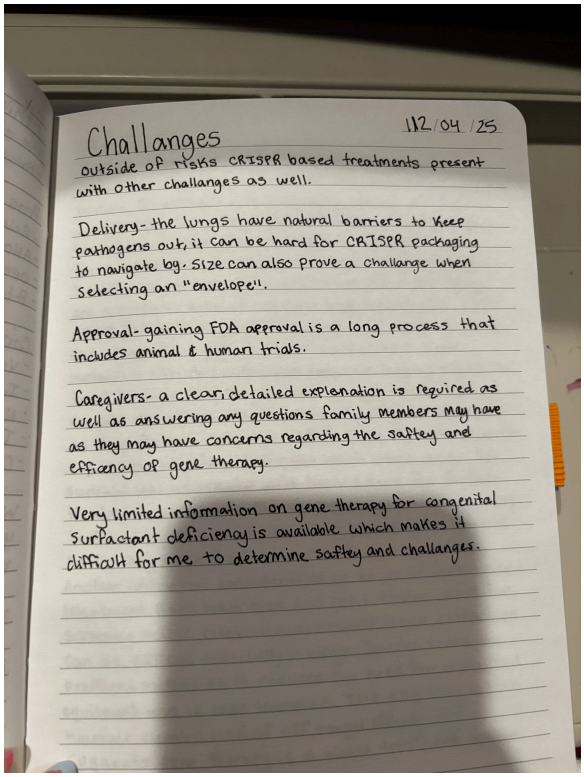
Use of gene therapy comes with a laundry list of ethical considerations.

Pros
- Relativly cost efficent and accessible.
- Allows for survival of an otherwise fatal condition.
- opens the door for treatment of other conditions.
- Potential for a cure. They won't continous treatments.

Cons
- Very few regulations
- Integration leading to things like cancer.
- Neonates can't consent.
- Infection and immune response.
- Cell breakdown or death.

When considering if gene therapy is a good solution you must look at all ethical considerations and weigh risks with the benefits.

While neonates cannot consent we do not typically take this into consideration for other treatments but it might be more important here considering we don't know long term effects.



Challanges

12 / 04 / 25

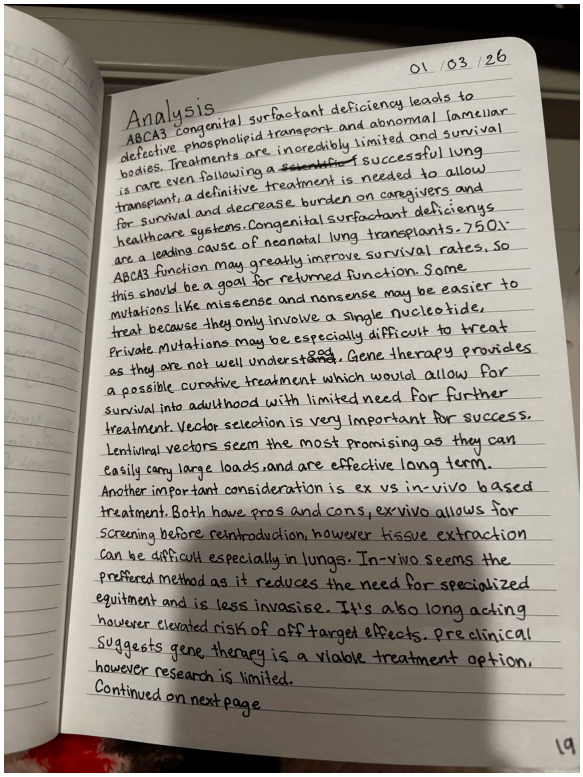
Outside of risks CRISPR based treatments present with other challanges as well.

Delivery- the lungs have natural barriers to keep pathogens out, it can be hard for CRISPR packaging to navigate by. Size can also prove a challange when selecting an "envelope".

Approval- gaining FDA approval is a long process that includes animal & human trials.

Caregivers- a clear, detailed explenation is required as well as answering any questions family members may have as they may have concerns regarding the saftey and efficency of gene therapy.

Very limited information on gene therapy for congenital surfactant deficiency is available which makes it diffault for me to determine saftey and challanges.



Analysis

01 / 03 / 26

ABCA3 congenital surfactant deficiency leads to defective phospholipid transport and abnormal lamellar bodies. Treatments are incredibly limited and survival is rare even following a ~~scientific~~ successful lung transplant, a definitive treatment is needed to allow for survival and decrease burden on caregivers and healthcare systems. Congenital surfactant deficienys are a leading cause of neonatal lung transplants. 750.- ABCA3 function may greatly improve survival rates. So this should be a goal for returned function. Some mutations like missense and nonsense may be easier to treat because they only involve a single nucleotide. Private mutations may be especially difficult to treat as they are not well ~~understand~~understood. Gene therapy provides a possible curative treatment which would allow for survival into adulthood with limited need for further treatment. Vector selection is very important for success. Lentiviral vectors seem the most promising as they can easily carry large loads, and are effective long term. Another important consideration is ex vs in-vivo based treatment. Both have pros and cons, ex-vivo allows for screening before reintroduction, however tissue extraction can be difficult especially in lungs. In-vivo seems the preffered method as it reduces the need for specialized equitment and is less invasise. It's also long acting however elevated risk of off target effects. Preclinical suggests gene therapy is a viable treatment option. however research is limited.

Continued on next page

01/03/26

Neonates with severe or homozygous mutations die shortly after birth, those who survive into childhood or adulthood suffer from idiopathic lung disease and significantly reduced lifespan. This places considerable stress not only on families, but healthcare professionals proffesinals and systems. While gene therapy carries risks, benefits greatly outweigh them. Before it can be applied in a clinical setting however more research is needed to assess efficency in different mutations and to determine eligibility criteria; however it shows great promise as a future treatment.

01/06/26

Application

This disease desperatly needs a cure. It's effects are devastating.

I have designed a possible CRISPR based gene therapy for treatment of congenital surfactant deficiency. When you compare it to current treatments it preforms better in almost every category. My therapy uses lentilviral vectors due to there superior size and use in-vivo. Neonates under developed immune systems also lowers immunogenicity, and in-vivo reduces the stress placed on their fragile bodies. My other consideration is time, since ABCA3 mutated patients experience shortened lifespans timely administration of treatment is important, in-vivo lowers time it takes for treatment.

Below is an outline for my treatment stragety

- The lentivirus RNA is edited to contain gRNA, Cas9 enzyme, PAM sequence, and a donor dna template.
- The lungs should be pretreated with mucolytics to thin mucus and improve accessibility.
- The lentivirus will then be introduced to the patient through an endotracheal tube. There it will bind to cells.
- After fusing with the cell membrane, it will release it's RNA into the cytoplasm.
- Inside the cell, the RNA will be converted to double stranded DNA, enter the nucleus and integrate into the genome.

Continued on next page

Comparisons

01/06/26

- The gRNA binds to Cas9 and locates the DNA sequence adjacent to PAM and binds.
- Cas9 cleaves, creating a double stranded break in the DNA.
- The cell will now work to comp repair the break. A healthy donor template can be used for something called homology directed repair. The cell will copy the correct sequence into the genome. HDR will have to compete with the cells fasten natural repair which will reduce efficency but is still better then nothing.

So how does it compare?

Cost:
lung transplant- $1.2 million
nicu stay- $3000+ \ day
Surfactant replacement- $6751 dose
*This is only some of the costs
Gene therapy- $1-3 million

Surfactant Production:
lung transplant- good but surfactant does degrade.
Surfactant replacment- very limited.
gene therapy- most or complete restoration.

Longevity:
lung transplant; 5-15 years
gene therapy; almost regular lifespan

Comparisons

01/06/26

Time in hospital:
Current (standard) treatment- until death (several months)
Lung transplant- 1-3 weeks + time before.
Gene therapy- 3-6 weeks.
Need for future treatments:
Lung transplant- high
Gene therapy- low or unneeded

While much of this is hypothetical it still represents the likely outcomes.