

New Ways to Breathe: Cystic Fibrosis and Gene Therapy

by

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The Case

At age 9, Lucas was doing very well in the classroom, but not so well on the playground. He was small for his age and not very interested in sports. Lucas preferred to burrow into his favorite books while his classmates chased one another and kicked balls around.

There was something about Lucas that his parents and teachers knew that his friends did not. A few months after birth, Lucas had been diagnosed with cystic fibrosis (CF), a genetic disorder that affects 1 in 3600 North Americans. “His body accumulates large amounts of mucus,” the doctor had explained to his parents. “The disease affects several organs, but its effect on the respiratory tract is the most detrimental.”

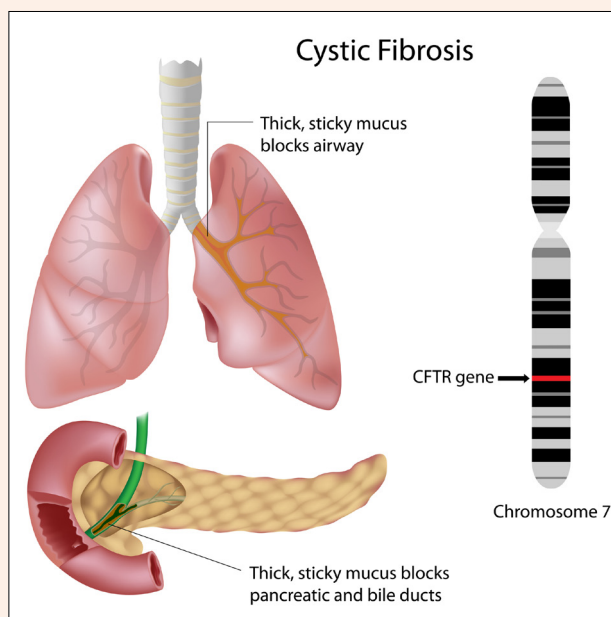
What Causes Cystic Fibrosis?

After Lucas’s diagnosis, the first thing his parents did was to attempt to better understand their son’s condition. They stumbled across the following explanatory segment in a CF foundation website:

The gene responsible for CF symptoms is the cystic fibrosis transmembrane conductance regulator (CFTR). In a healthy person, the CFTR protein functions as an ABC transporter that uses ATP to pump chloride ions out of epithelial cells in the lungs and the gastrointestinal and urinary tracts, keeping extracellular and intracellular salinity balance in check. When an individual’s CFTR is defective, ion concentrations cannot be regulated properly and cells expressing this protein have difficulty transporting water, ultimately leading to a buildup of mucus and bacteria.

There are over 800 possible amino acid mutations known to lead to a malfunctioning CFTR channel. The most common of these is $\Delta F508$, which is a deletion (Δ) of phenylalanine (F) at position 508 of the polypeptide chain. The $\Delta F508$ mutated CFTR proteins may still operate as chloride channels in principle, but they are marked for degradation by chaperone proteins and never reach the cell membrane. For other mutations, the altered CFTR protein may reach the membrane but have decreased functionality.

CF is an autosomal recessive disorder. This means that an individual with one or two normal CFTR alleles will not show symptoms. However, if an individual receives two malfunctioning CFTR alleles from their parents, they will express the CF phenotype.



Genetic testing revealed that both of Lucas’s parents were carriers of $\Delta F508$.

Gene Therapy

Though devastated by the news of Lucas' illness, his parents were thankful that their boy was born in a time when CF was widely studied and much better understood than it had been in the past. They discovered that the average survival age of CF patients is was about 50 years and rising as new research improved treatment. However, Lucas' parents were hopeful that a cure might be found in their son's lifetime.

Then they found gene therapy. After conducting some research by themselves, they approached Dr. John Kay, Lucas's pediatrician.

"Can you tell us more about gene therapy?" asked Lucas's mother.

"It's a promising area of research in finding a cure, ever since they identified the cystic fibrosis gene," said Dr. Kay. "The idea is to insert a functional version of the gene that replaces or enhances the expression of the defective one that your son was born with."

"That...sounds like a cure," said Lucas's father with guarded optimism. "Does it work?"

"Well, to be honest with you, gene therapy for cystic fibrosis has met with several obstacles and issues before. The clinical trials we've had so far are not too encouraging..." At this, Dr. Kay peered intently through his glasses, "But it could be worth trying. There's a clinical trial coming up. You never know."

The Issues with Gene Therapy for Cystic Fibrosis

Lucas's parents decided to do some research before enrolling their son in a gene therapy clinical trial. After consulting Dr. Kay further, they summarized the steps of gene therapy as follows:

1. Functional CFTR gene is placed into a vector.
2. Vector is delivered to its desired target tissue.
3. Vector inserts functional CFTR gene into targeted cells.
4. Functional CFTR protein is produced by expressing the newly inserted gene.

Based on this information, the couple emailed several physicians and researchers recommended by Dr. Kay. Their goal was to find out what obstacles made gene therapy especially hard for CF researchers. The following is a selection of the responses they received:

Dear Sir and Madam,

Thank you for your email. Regarding the vector (**Step 1** on the list that you attached): There are many options regarding the choice and design of vectors for gene therapy, each with its pros and cons. We organize vectors into 2 types: viral and non-viral. The vector that we design must be able to bypass the mucus barrier of the lungs in order to reach the desired target cells. Another challenge is that it cannot be destroyed by immune or digestive processes (Davies & Eric, 2010).

A note about gene insertion (**Step 3**): Different types of vectors will accomplish this differently. For example, within the context of viral vectors, the method that adenoviruses use to insert the functional CFTR into target cells differs from the one used by retroviruses. This is another issue researchers have to consider when designing new vectors.

Hope this helps!

Dr. Jen Forman, Department of Virology, Hospital for Sick Children

Hi,

In recent years, a lot of attention has been focused on vector design when it comes to gene therapy. But a problem unique to CF is what you outlined in **Step 2** (vector delivery). Namely, how would the vector get to the desired target area (i.e., lung mucosal surface) in the first place?

In fact, the method of delivery and composition of the vector-containing drug is crucial in determining how effectively the vector penetrates the pulmonary barrier. Optimal aerosol design and uniform pulmonary deposition are important aspects of the treatment.

I hope this helps. All the best to your son.

Dr. Bruce Souza, Department of Respiriology, St. Joseph's Hospital

Hello,

Regarding protein production (**Step 4** in your list), the immune system tends to react to the new CFTR proteins as foreign substances to be removed. Hence the cells may have to be treated in order to prevent this form of rejection (Riddell et al., 1996). This is one of the major issues with keeping CFTR levels high after administering the gene.

Good luck!

Dr. Wei Lin, Department of Immunology and Microbiology, Western University

The fourth email came from a group of researchers from the MaRS Discovery Institute. They introduced the couple to a promising new innovation that they were working on. Granted, their project was still in its infancy, but the researchers hoped that, in the near future, it would improve the efficacy of gene therapy in a significant way.

Your Challenge

As the team of researchers from MaRS, propose a novel research idea (e.g., element, process, tool) to increase the effectiveness of gene therapy in the treatment of CF. Justify your idea using currently available evidence.

You will be evaluated based on the following criteria:

- *Quality of Idea:* You should demonstrate the novelty and creativity of your idea. Remember that your judge may not know everything about your project's specific topic, so you need to explicitly show your project's ingenuity and originality.
- *Evidence and Analysis:* You should use credible, peer-reviewed sources to argue for the feasibility of your idea.
- *Awareness of Relevant Risks and/or Limitations:* You should demonstrate awareness of potential side effects, unavailable technology, low success rate, etc., and propose mitigation strategies.
- *Report Quality and Style:* There is no defined structure you need to adhere to, but your report should be well-organized and easy to follow. The word limit is 1000 words maximum, not including references.

Guidelines

Step 1: Organizing Your Team

An often overlooked job of a scientist is lab management. While there may not be a "designated leader" in your team, you may still want to partition the research. For example, for the first few days of the challenge, each member can do general research into CF and the big issues with gene therapy. When everyone is on the same page, each member might

focus his or her research on a specific issue and look into experiments done about it. The team can then convene and focus their research on one of these problems.

The specifics of team management are up to you and your team. Practicing this will greatly prepare you for lab courses in the future. However, be warned that unprepared brainstorming sessions are guaranteed to waste time!

Hint: Have someone on the team who keeps up with scientific news or has a broad scientific knowledge outside of the classroom. He/She will be helpful in coming up with interdisciplinary ideas.

Step 2: Researching

You will be consulting a variety of resources to understand the various issues in gene therapy (the ones mentioned in the case are a select few), come up with a novel idea, and back it up. In the beginning, you can use “general” resources such as textbooks and credible websites to get the big idea.

When you need more detail, you can find papers using a variety of online research tools such as Google Scholar, Web of Science and Pubmed. It will be helpful to read review papers first before looking into experiments or clinical studies. Review papers summarize key information about a specific subject and refer to recent developments in the laboratory.

Step 3: Generating the Idea

After you have acquainted yourselves with the subject and issues, you have arrived at the most challenging but exciting part of the exercise: coming up with your idea. The open-ended nature of the challenge provides room for creativity, but also for disaster. Keep in mind the following pointers:

1. *Be specific:* If you answer “Use nanoparticles,” you will need to elaborate more. For example, what kind of nanoparticles and how?
2. *Be flexible:* There are many issues with gene therapy that you can address besides the ones mentioned in the case. Your idea does not necessarily have to address one specific issue. For example, compound X may both help the vector reach the target cells and prevent an immune response at the same time.
3. *Be creative:* Teams with ideas that stand out will be rewarded. Note that you do not need a 100% feasible idea. Rather, focus your time on finding evidence that argues for the potential of your innovation, and be sure to discuss the risks and current limitations in science associated with it. Interdisciplinary ideas are always impressive. For example, if you recently read that a certain compound protects a certain type of bacteria from T-cells, could we use it in our vector design? Keep your eyes open for recent scientific news and discoveries in other fields. Do note however that these interdisciplinary ideas may be harder to back up with sufficient evidence. It is a risky but rewarding tradeoff!

Step 4: Writing

Having a great idea is not the only thing this challenge is about; you also have to develop it sufficiently. Your written proposal should answer the following:

1. What is your idea?
2. How would your idea work?
3. Why would your idea work?

Besides keeping the above questions in mind, remember to keep the grading criteria listed under “The Challenge” section in mind. The maximum word limit for your submission is 1000 words. Do note that length does not equal quality, so try to explain your idea sufficiently yet concisely. Other than this, the format of the proposal is up to you.

Remember: Include in-text citations and a separate reference page (not included in the 1000 word limit).

