Cystic Fibrosis Trust

Research in focus

Alternative ion channels

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Uniting for a life unlimited

Foreword

In cystic fibrosis (CF) the lungs become clogged with thick, sticky mucus. The mucus is hard to clear away and can lead to life-shortening lung infections and inflammation.

In the last 10 years, access to medicines known as CFTR modulators have transformed the lives of many people with CF in the UK[1]. They work by increasing the amount of working CFTR, the protein that is damaged in CF. The medicines make the mucus become thinner and less sticky, and the lungs become cleaner and healthier as a result. However, there are around one in 10 people who are unable to benefit from CFTR modulators due to the form of CF they have, and new treatments are urgently needed.

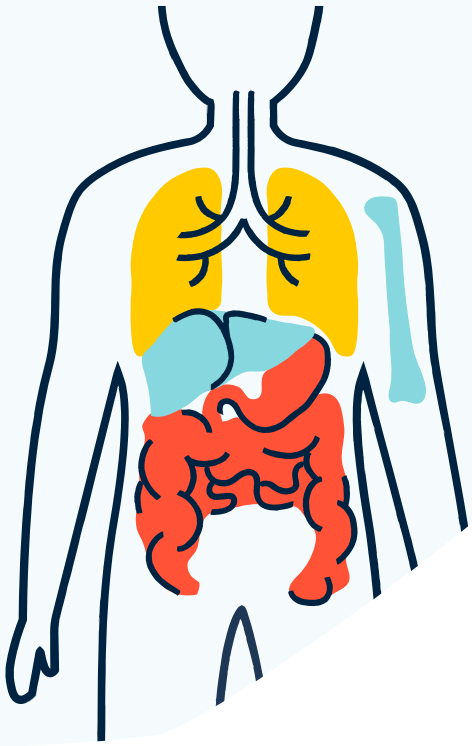
The CFTR protein keeps the mucus healthy by forming a channel or gate to move charged chemicals or 'ions' to the surface of the lungs. When the CFTR protein is faulty, this channel doesn't work and mucus becomes thick and sticky. Alongside the CFTR protein, there are a number of other, alternative ion channels that also transport ions into mucus, keeping it healthy. Researchers around the world are pioneering the development of medicines that act on these alternative ion channels as a treatment for everybody with CF, particularly those who do not benefit from CFTR modulators.

Less is known about how these alternative ion channels work in comparison to the CFTR protein. The Trust is funding a Strategic Research Centre (SRC) to identify potential new medicines that could work for everyone with CF[2]. In this report we highlight the promising laboratory research underway in screening new alternative ion channel medicines.

Dr Lucy Allen, Director of Research, Cystic Fibrosis Trust

What is CF?

Cystic fibrosis is a rare, inherited condition that affects over 10,800 people in the UK[3]. It is caused by defects (mutations) in a gene that makes the CFTR protein, which controls the movement of ions and water in and out of cells. One group of mutations, known as 'class 1 mutations' result in no CFTR protein being made at all[4]. The defects cause the internal organs – especially the lungs and digestive system – to become clogged with thick, sticky mucus. This results in chronic infections and inflammation in the lungs and in the digestive system, blockages, bloating and difficulty breaking down food. Some adults with CF may also develop CF-related diabetes (CFRD) and forms of arthritis, osteoporosis and liver problems that are related to having CF.



Keeping the lungs healthy

When people breathe in dust or bugs, the body has a natural system for clearing them away, known as mucociliary clearance. This is when the cells that line the lungs produce a thin layer of mucus that traps dust, and traps and inactivates any bugs that are breathed in. The mucus is then carried out of the lungs by the presence of tiny beating hairs, called cilia, on the surface of one type of lung cell[5].

The surface of the lungs is kept hydrated and at the correct acid-alkali balance by the transport of charged chemicals or 'ions' into and out of the 'airside' of the lungs, through channels or 'gates' present in airway cells. The channels are made by specific proteins, such as CFTR. It is the CFTR protein which forms a channel to transport chloride and bicarbonate ions out of the cells onto the surface of the lungs. The movement of the ions to the surface of the lungs also causes water to move there too. There are several other ion channels in airway cells, and these all work together, playing their part to keep the lungs clean and healthy.

Thick sticky mucus in cystic fibrosis

In CF, the CFTR protein either isn't made, or doesn't work properly. The chloride and bicarbonate ions and water are not moved as effectively to the surface of the cells, the mucus becomes thick, sticky and harder to clear away, and less able to kill off any bugs trapped in it. This means that mucociliary clearance doesn't work so well.

However, as the CFTR protein works alongside other ion channels in the airways, changing the activity of these alternative ion channels to improve mucociliary clearance could be an effective treatment approach for CF. In comparison to CFTR modulators, that are only available to people with specific CF mutations, a treatment that changed the activity of alternative ion channels would work for everyone with CF. This could make a difference for families like Lizzy's, whose 16-year-old son Isaac has CF.

"CF is very rarely in the background in our house. The last six years have been a constant round of infections, gut and sinus problems. Because of CF, my son can no longer play football or rugby and he's in a lot of pain. He is not eligible for the new CFTR modulator drugs such as Kaftrio as he has two rare, class 1 mutations. This means he never forms the correct CFTR protein in the first place, so needs a very different kind of fix. We desperately need research to continue, to help him and others like him".

Lizzy, Isaac's mum

Isaac

Questions to answer about alternative ion channel medicines for CF

Scientists have identified at least two different alternative ion channels that act in a similar way to CFTR, that help keep the lungs healthy. The Trust is funding a Strategic Research Centre (SRC) led by Dr Mike Gray at Newcastle University, to develop therapies that act on these alternative channels as a treatment for CF[2].

The two channels with similar function to CFTR are formed by proteins called 'SLC26A9' and 'TMEM16A'. The SLC26A9 channel transports chloride, while the TMEM16A channel, like CFTR, transports both chloride and bicarbonate to the surface of the lungs[6].

There are a number of questions that Dr Gray and his international team of co-investigators need to answer, before any potential alternative ion channel medicine can be tested in clinical trials in people with CF. These include:

- **What is the best way to alter the activity of the alternative ion channels?**
- **Where will the new medicines come from?**
- **Will everyone with CF respond in the same way?**

Below we've outlined the research underway in these three areas.

What is the best way to alter the activity of the alternative ion channels?

Researchers have several ways of altering the activity of alternative ion channels. One method is to increase the length of time that the channel gate is 'open', so increasing the flow of ions transported to the surface of the lungs. Another method is to increase the amount, or number, of the alternative channels that are present in the lung cells. Within the SRC, both of these approaches are being explored.

Before testing how to alter the activity of the alternative ion channels, we need a good understanding of exactly how they work in the lungs. Like many of the proteins in our body, these alternative ion channels may have more than one role (picture them as a swiss army knife!). In the laboratory testing phase of developing new medicines, it is important to understand all the effects that a potential medicine might have on a protein, to avoid any unintended consequences.

The exact role of the TMEM16A protein within the lungs is still under debate. SRC-funded researchers in Professor Karl Kunzelmann's lab in Regensburg, Germany have produced new results suggesting that **blocking its activity** reduces the amount of mucus secreted into the lungs, and this improved mucociliary clearance[7].

Researchers at the UK-based biotech company Enterprise Therapeutics have found that medicines that **boost the activity** of TMEM16A can hydrate the airways[8]. A phase 1 clinical trial investigating the safety of this type of medicine in healthy volunteers has recently been completed.

More research on the role of TMEM16A in the lungs is underway to explain how these two sets of results link together. While this is being worked out, the researchers are looking at potential medicines that either boost or block its activity.

Where will the new medicines come from?

The search for new potential medicines which might act on a particular protein within the body often begins by searching through hundreds of thousands of existing chemicals, to give researchers a starting point. Each library of chemicals is tested a number of times, and each time the library of chemicals is tested it is called a screen. As it can be extremely time-consuming to test so many chemicals, researchers try and develop ways of doing these screens quickly and efficiently, these are known as 'high throughput screening' or HTS.

Researchers in Utrecht in The Netherlands, led by Professor Jeff Beekman, have developed a HTS to test potential new drugs in nasal cells taken from people with CF. In the lab, the nasal cells grow into hollow balls of cells called organoids[9]. The cells that form these organoids are similar to the cells that line the lungs, with the same proportions of different ion channels. The organoids are grown in a liquid with all the nutrients they need to stay healthy. When the ion channel activity in the organoids is boosted by a chemical from the screen, water moves to the inside of the organoids, causing them to swell. Researchers can see them swelling down a microscope. If the added chemical does not change the activity of the ion channels, the organoids won't swell.

As part of the Trust-funded SRC, Professor Beekman's lab, together with Dr Mike Gray's group, have tested a chemical library of 1,400 potential medicines in these organoids. These chemicals are medicines that have been shown to be safe in people to treat other conditions, sourced from the US medicine regulator the Federal Drugs Administration (FDA). After two rounds of screening, 13 of these chemicals caused the organoids to swell. These positive responses or 'hits' are currently being tested in more detail, to learn how they caused the swelling.

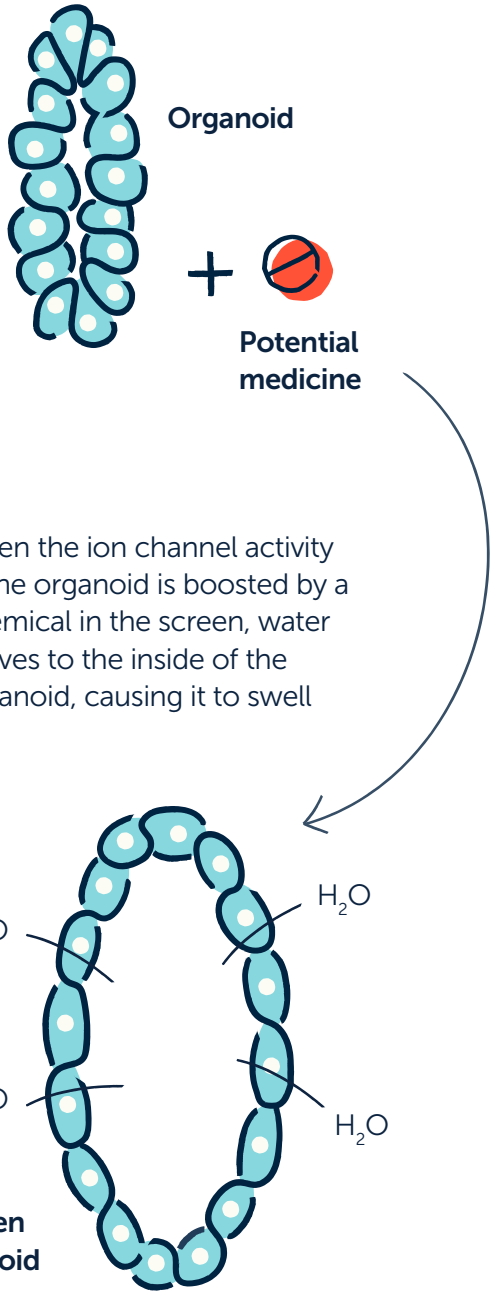
"We were delighted and surprised to find that some of these medicines did cause the organoids to swell – showing that they improve fluid secretion. The organoids were all made from nasal cells taken from people with class 1 mutations who cannot make any CFTR protein, so we know the drugs must be working in other ways.

"Next, we need to work out exactly how these medicines are making the organoids swell. We think it is through their action on alternative ion channels and now we're thoroughly checking if this is the case."

Dr Mike Gray, SRC Principal Investigator, Newcastle University

SRC researchers in Lisbon, led by Professor Margarida Amaral, are also currently testing whether a library of 540 chemicals that are produced naturally in our body, could be used as a medicine to alter alternative ion channel activity in people with CF. Their first screen found eight possible hits so far, and further studies are ongoing.

Nasal cells taken from people with CF grow into hollow balls of cells called organoids in the lab



Will everyone with CF respond in the same way to alternative ion channel medicines?

Everyone with CF has a different experience of the condition, and will respond differently to medicines used to manage and treat it. Using cells obtained from different donors with CF, the SRC-funded researchers are investigating differences in response in the laboratory.

Some of the differences in response to medicines are due to differences in someone's genes. This ranges from people with CF with different mutations in the CFTR gene; to individual variations in our genes that everyone in the population has[10].

The cause of some of these population-wide variations are already known, but it is still important to check for other variations.

Researchers know that there are variations in the gene that makes the SLC26A9 ion channel, and these variants can alter the amount of SLC26A9 protein made[11]. So it is very important to understand the effect of the new potential medicines for each variation of the ion channel.

Developing medicines that act on these alternative ion channels is likely to have the greatest benefit for people with CF who are unable to make any CFTR protein. However, there are signs that these medicines may also boost the activity of CFTR modulators too, and research to study this further is underway.

What's next?

The scientists across the different research groups working together within this Trust-funded SRC have developed innovative methods for screening potential medicines for beneficial activity on these alternative ion channels. Their results so far offer real hope for people unable to benefit from CFTR modulators.

They are currently investigating their screening hits in more detail, to be confident of exactly how these medicines are working. This includes checking whether they are only working on the intended ion channel and not other proteins in different parts of the body, and that altering the ion channel activity has the overall effect of improving mucociliary clearance in the lungs.

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Cystic Fibrosis Trust

About Cystic Fibrosis Trust

Cystic Fibrosis Trust is the charity uniting people to stop cystic fibrosis (CF). We fund vital research, improve care, speak out and race towards effective treatments for all. Cystic Fibrosis Trust is here to make sure everyone with cystic fibrosis can live without limits.

Since 1964, we've supported people with cystic fibrosis to live longer, healthier lives - and **we won't stop** until everyone can live without limits imposed by CF.

For more information:

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