



## **Stem Cell Therapy for Cystic Fibrosis?**

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This is a very exciting time for researchers working on new treatments for cystic fibrosis. It is 20 years since 1989, when Lap-Chee Tsui and his collaborators in Toronto identified the CFTR gene, and its common mutation,  $\Delta F508$ , the underlying cause of CF. After the gene was discovered, researchers had high hopes for a dramatic advance, using genes as medicines ("gene therapy"). During the past twenty years, there have been many advances, but the early hopes for gene therapy have not (yet) been achieved.

### **We Continue to Hope Gene Therapy Will Work**

There is renewed hope as groups in the U.K. and the U.S.A. find better ways to get a normal copy of the CF gene into the airways of young adults living with CF. We all hope these new trials of gene therapy will work. Those who attended the Australian CF Conference in Brisbane at the end of August, 2009, heard the latest results from the U.K. from Professor Eric Alton. However, we also know that the body has very strong defences against "foreign" DNA: after all, when we eat food, there is DNA in those three meals a day, and yet we don't turn into the animals and plants we eat. Our cells are programmed to destroy "foreign" DNA very efficiently!

And now scientists are making a concerted effort to the ultimate goal: using an artificial gene, a sequence of DNA which contains a healthy CFTR gene with all its control signals that could correct the defect causing CF. The healthy CFTR gene will be coated within a fatty material - known as a liposome - to help it sneak past patients' immune systems. These will be inhaled as aerosol droplets, in sessions that will last for hours, so that when a liposome brushes against a lung cell it will dissolve on its membrane, slipping its cargo, the tiny sliver of DNA, inside the cell. "It sounds simple," says researcher Steve Hyde from Oxford University, "but it has taken 80 researchers working flat out for eight years to design and make these droplets, and to show in mice that they can avoid immune defences and deliver healthy protein to lungs for months rather than days."

Just getting the gene into the right configuration for coating with liposome took five researchers a total of five years of cutting and pasting tens of thousands of DNA sequences, each one tested to make sure it works and is safe. Even then, there may be unexpected setbacks or immune reactions, so alternative gene variants and coatings are being designed just in case! "This is something we have worked on for 20 years already, and it will see us through to the end of our careers," adds co-researcher Deborah Gill. "Sometimes I am the optimistic one and Steve is cautious. Then the roles reverse. We keep each other going."

### **Human Stem Cells**

There is another approach, using stem cells to introduce a functional copy of the CF gene inside a cell that can integrate into a patient's tissue and correct the problems.

Human stem cells can come from many sources. The most flexible cells, that are known to be able to give rise to every cell type in our bodies, are embryonic stem cells (ES cells), but some people have an ethical objection to using embryos in this way. Most people accept embryo research (using human embryos that are "left over" from IVF, and would otherwise be thrown away), but there is a scientific reason why ES cells are unlikely to be used for treatment. These cells are from another individual, and because they are not matched to the immune system of the person with CF, would be rejected. ES cells are so primitive that they not only form normal cells, but can also cause cancer, which doesn't matter in the lab but could harm a patient. ES cells are the stem cell equivalents of those little white mice used by scientists; they are very useful in the lab for experiments, but will probably never be used for therapy.



Realistically, two kinds of stem cells might be used to treat CF and avoid the problem of immune rejection. One kind could be prepared from a person living with CF. Or cells can come from an unaffected relative or closely matched volunteer (perhaps from newborn babies from the public cord blood bank, matched to the person with CF).

### **Cells from a Person with Cystic Fibrosis**

We all have stem cells in our bodies, in very large numbers at birth, and fewer as we get older. The stem cells easily give rise to cells from the tissue from which they are taken: skin stem cells like to give skin, while liver stem cells give liver and blood stem cells, blood. New techniques now allow scientists to “turn” skin cells into much more primitive cells that resemble those from embryos (called “induced pluripotent stem cells”, or iPS). These cells, which can in theory be made from anyone with CF, can almost certainly give rise to lung cells, and if they are from the patient they will be “matched” and will not be rejected. However, they will still contain the faulty CF gene that does not work properly, so in addition to making the stem cell, it will also be necessary to correct the defect. And iPS cells must be shown to be safe and not cause cancers before they can be used therapeutically.

Our research group (Drs Faten Zaibak and Caitlin Filby, now in new labs at the Murdoch Childrens Research Institute, and working closely with Dr Ngaire Elwood who directs the Cord Blood Bank) has recently shown that it is possible to put an entire human gene into a stem cell, integrate it into the cell’s DNA, and get what seems to be normal function. The gene that was used (when mutated) causes another disease, Friedreich ataxia, because that gene is a bit smaller and easier to use than the CFTR gene. Faten and Caitlin have now obtained a full length human CFTR gene, and will try the same approach with stem cells for CF. If this can be done, those cells could almost certainly be put back into the individual and not be rejected.

### **Stem Cells from Cord Blood**

Some children with CF are fortunate, because their cord blood was saved when they were born. Cord blood contains many stem cells, some of which can be grown indefinitely. These cord blood units from children with CF may turn out to be very precious. As of now, we have advised families NOT to try to grow cell cultures from them, but keep them in storage. It is not yet possible to guarantee that the stem cells will survive when grown in the test tube.

Cord blood has been used for thousands of stem cell treatments (mostly for leukaemia), and there are many units of cord blood stored in the “public banks”. During the past four years, babies who have been born and are siblings of children living with CF have had cord blood collected free in Australia and New Zealand, and a few families have made sure of a match by having IVF and an embryo test prior to pregnancy. Cord blood from a matched sibling is known to be excellent for cell therapy for other diseases, and completely safe provided the collection is sterile, so why not for CF? This has, of course, been the hope of our research group at the University of Melbourne for the past four years.

We think that cells grown from cord blood can make lung cells in the lab, though it is difficult to get them to express the CF protein. The problem will be to get them into the lung! It is not enough to breathe them in; the body’s defences and the sticky mucus in CF lungs will stop them getting into the tissue. We know that injected stem cells go to the lungs via the blood stream, but we have to prove they stay there and are integrated into the tissue. It is essential to be sure that no harm could result when the cells go to other organs as well as the lung.

However, if stem cells from a sibling who does not have CF, but who is immune-identical to the child with CF, help to normalise the lungs when injected, the next step will be to carry out research to see if similar cord blood cells from the cord blood bank can be used if they are matched.

The researchers share a frustration with those living with CF and their families at how long these experiments take: we wish it moved faster, but we have to be certain that there are no hidden problems or side effects before therapy can be attempted.

Lap-Chee Tsui, who headed the Toronto group that found the CF gene, has moved to become Vice-Chancellor of the very illustrious University of Hong Kong, and another of his colleagues, Francis



Collins, has just been appointed to the post of Director of the United States National Institutes of Health, perhaps the most powerful job in the world of medical research. As always, CF research leads in so many ways.

*Professor Bob Williamson recently retired as Director of the Murdoch Childrens Research Institute in Melbourne, after working on cystic fibrosis molecular genetics for about 30 years.*