The Potential Applications of Combining Stem Cells and Gene Therapy in Medicine

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PASS WITH DISTINCTION

Research Paper based on lectures at Medlink 2008

Abstract

'Gene Therapy meets stem cells. That is the wave of the future'[1]

Stem cells are an exciting new aspect of biology with many future applications and the potential to revolutionise medicine. It could be the technology to provide new cures for many currently incurable diseases. In this paper I will look at the effects of combining stem cells with gene therapy and any possible scientific and ethical problems that may arise from this.

Introduction

Stem Cells are the 'building blocks for every type of cell in the body, capable of maturing into any tissue type' according to the Cambridge Stem Cell Initiative (2009). On division by mitosis, each Stem Cell has the potential to become any other cell in the body.

James Thomson, a US scientist at the University of Wisconsin in Madison first isolated embryonic stem cells in November 1998 by removing them from discarded embryos at a fertility clinic. In his report published 5th August 1998 and entitled '*Embryonic Stem Cell Lines Derived from Human Blasocysts*,' Thomson immediately realised the applications for these cells in 'human developmental biology, drug discovery, and transplantation medicine' (Thomson et al. 1998)

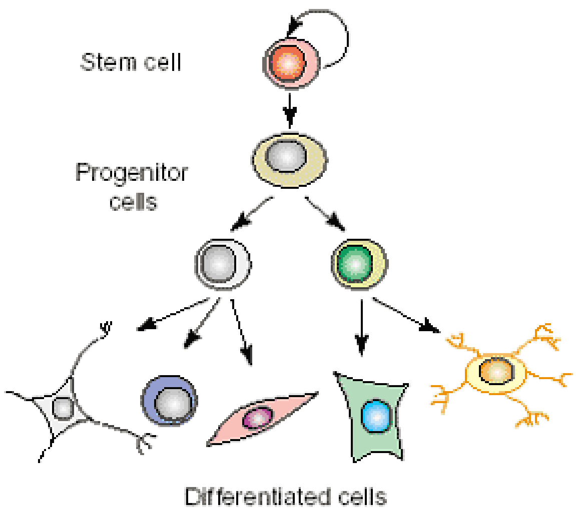


Fig. 1 - Diagram to show Stem Cell undergoing Mitosis

Stem Cells theoretically have the ability to divide without limits and 'occur as a self-renewing population' (John Bryant, Biological Sciences Review Feb 2009) as when the original cell divides by mitosis, another undifferentiated stem cell is produced as well as the daughter cell (see fig. 1).

There are three types of stem cell: Adult (Somatic) stem cells, embryonic stem cells and foetal stem cells which are commonly found in the blood in the umbilical cord after birth.

Adult (Somatic) Stem Cells

These are comparatively scare in the body and cannot differentiate into the wide range of cells that foetal and especially embryonic stem cells can.

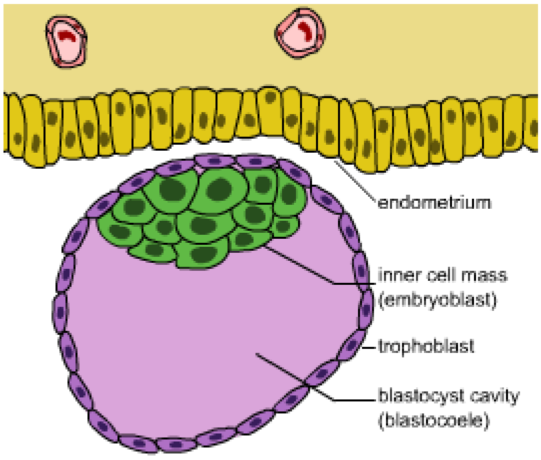
Foetal Stem Cells

These are most commonly found in the blood in the umbilical cord after birth and can differentiate into a wider variety of cells than Adult stem cells

[1] Peter Aldhous – New Scientist 13th June 2007

Embryonic Stem Cells

Fig. 2 Diagram of a Blasocyst



These can theoretically differentiate into any cell in the body. They are derived from embryos fertilised in vitro when the embryo is 4-5 days old and at the stage of a Blasocyst. The Blasocyst is between 0.1 and 0.2 mm in diameter. (see fig. 2) The stem cells are found in the inner cell mass or embryoblast. If the embryonic stem cells aren't isolated, they will quickly arrange themselves in three layers: the endoderm, the mesoderm and the ectoderm. However if they are removed from the blasocyst and grown in cell culture conditions, they 'remain as a self renewing stem cell population.' (John Bryant, Biological Sciences Review Feb 2009)

Stem Cells have the ability to replace and repair cells or tissue which have been damaged or destroyed by disease. 'Sooner or later, we no longer are able to repair and replace these cells with anything like the rate of their loss through disease or injury.' (Eve Herold, Stem Cells and the New Future of Medicine, USA Today, March 2003) Stem cells have the ability to correct this problem.

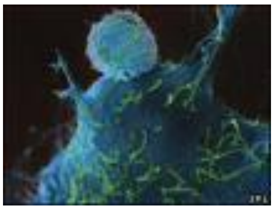


Fig. 3 Cancerous White Blood Cell

Stem cell therapies are already being used in medicine today to treat Leukaemia. Leukaemia is a cancer of the white blood cells (see Fig. 3) called Leukocytes. These are made in the bone marrow and release into the blood stream to mature. If these become cancerous, they cannot fight off infection and disrupt the functions of the major organs. Chemotherapy can be used to kill all of these cancerous cells but if it can't eliminate them all, a bone marrow transplant must be used. The patient's bone marrow cells are replaced with healthy ones from a donor after radiation has destroyed the existing Leukocytes. The healthy stem cells from the bone marrow are released back into the blood stream and these should begin producing healthy Leukocytes again to replace the abnormal ones.

Since stem cells were discovered, much research has been carried out on their possible applications including the idea of the idea of growing organs from stem cells has been researched. This would help patients in need of transplants overcome the fact that there is a shortage of donors and also prevent the effects of rejection. Theoretically it is possible to grow a complete organ in a laboratory using stem cells, differentiating them into the different tissues that make up the organ. Although scientists haven't yet found a method for differentiating stem cells into specific cells or growing complete structures in a lab, advances have been made with the first bronchus transplant using the aid of stem cells. A donor's cartilage was used and the patient's own epithelial cells were grown over this. Then stem cells from the patient were used to produce cartilage to replace that of the donor. The transplant was successful. The operation was done in the University of Barcelona's Hospital Clinic by Professor Paolo Macchiarini on the 12th June 2008. Professor Martin Birchall from the University of Bristol who worked with the University of Barcelona by carrying out the stem cell engineering said that he believes it will eventually be possible to use the technique for all kinds of transplants.

Discussion

Until very recently the research into the applications of stem cells and gene therapy have been researched separately. Now they are starting to be combined to provide possible cures for many previously incurable diseases. In this paper I will be looking into two possible applications for this potential new therapy: the treatment of breast cancer and a genetic disease, cystic fibrosis.

Although both stem cell therapies and gene therapies are fairly new aspects of science and remain experimental procedures, combining the two could lead to treatments and cures for many diseases, many of which are currently untreatable.

Gene therapy is defined, by the Oxford English Dictionary, as the 'introduction of normal genes into cells in place of missing or defective ones in order to correct genetic disorders'. The success of gene therapy is dependent on the introduction of the 'therapeutic transgene into the appropriate human target cells, but also on the ability of the gene to function properly in the cell.' (Stem Cell Information, The National Institute of Health's resource for Stem Cell Research) There are currently two strategies for introducing this vector into the patient. (see Fig. 4) Viruses are often used to perform this task either by Direct Delivery or Cell Based delivery. In the process of Direct Delivery the 'disease causing' genes are removed and placed with the desired genes. When a virus replicates, it replaces the host's DNA with its own so the virus would 'infect' the host cell with the normal gene, replacing

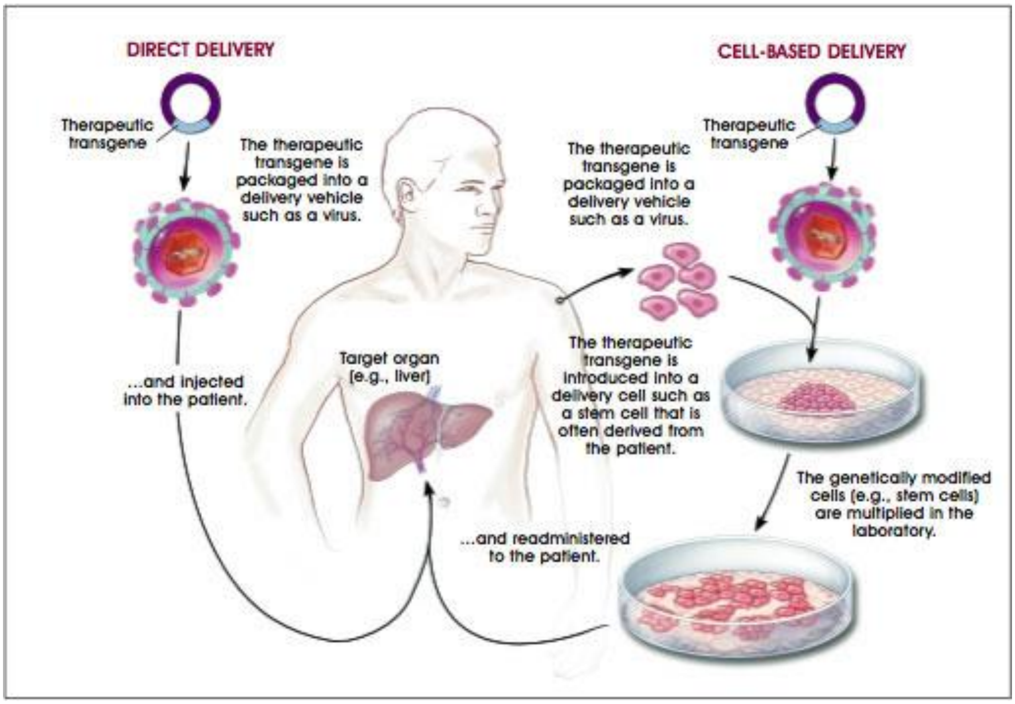


Fig. 4 – Diagram to show the different methods of delivering therapeutic transgenes into patients

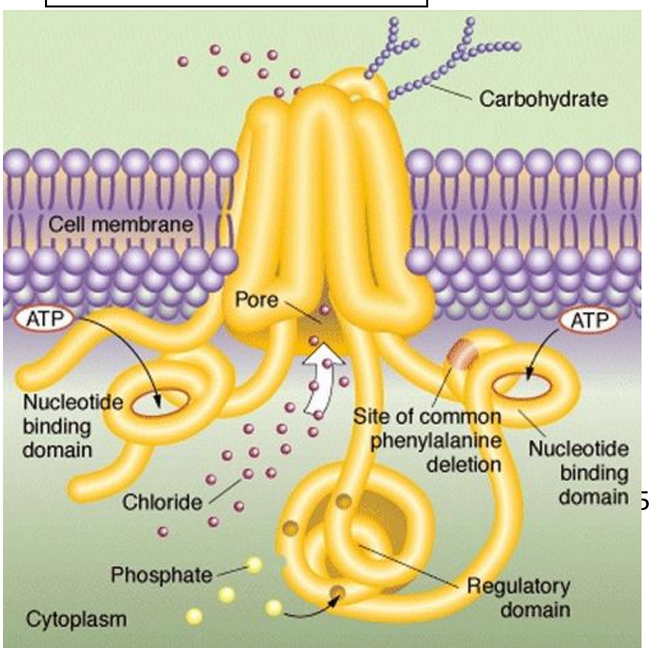
the abnormal one. However this method is quite inaccurate. Cell Based Delivery involves the use of living cells such as stem cells to introduce the therapeutic transgenes into the body. These cells are allowed to multiply and then are reintroduced back into the patient.

Stem cells would provide a safer option for introducing the desired genes into the patient. The work of the Whitehead Institute for Biomedical Research, Cambridge by J. Hana et. al has shown the potential of combining stem cells with gene therapy in medicine. By changing skin cells into induced pluripotent stem cells (iPS) using a retrovirus, these cells can differentiate into other cells like a stem cell. Any mutations in the genes of these cells are corrected and then they are transplanted back into the patient. In the body they replicate rapidly, replacing the damaged DNA in the body with healthy DNA. These will continue to replicate for the rest of the patient's life. Stem cells will not be rejected by the body's immune system and they do not have a limited lifespan or limited ability to divide so therefore have the potential to correct gene mutations. This means that they have the possibility to correct genetic diseases. Stem cells also have the ability to self renew and therefore, repeated administrations of stem cells could be dramatically reduced. Using this research in the future it may be possible to correct diseases due to mutations by correcting them in the patient's stem cells and allowing the stem cells to divide correcting the gene throughout the body.

Cystic Fibrosis

1 in 2500 babies is born with Cystic Fibrosis every year in the UK. It is a recessive genetic disorder caused by a gene mutation. The disease is much more common in people of Caucasian decent than those of African or Caribbean heritage. 1 in 25 white Europeans are carriers of the gene. The disease is usually diagnosed in the first year of life. It affects the internal organs, mainly the lungs and digestive system. Symptoms include a persistent cough, breathing difficulties, and recurring chest infections. Although in 3 out of 20 cases there are no problems with the digestive system, usually children with Cystic Fibrosis have poor weight gain, a bloated abdomen or constipation. Cystic Fibrosis is usually diagnosed by 'sweat test' as sufferers from the disease have abnormally high levels of salt in their sweat. A genetic test can also be performed to confirm that there is a mutation present. Today all babies are screened for Cystic Fibrosis six days after birth by taking a small 'heel prick' blood test. Babies with Cystic Fibrosis have unusually high levels of a chemical called immunoreactive trypsinogen. If this test is positive, the sweat test and a genetic test may be performed to confirm that the patient is suffering from the disease. If the disease is diagnosed early, prognosis can be improved by commencing treatment immediately.

Fig. 5 - Diagram of the Cystic Fibrosis Transmembrane Conductance Regulator



Cystic Fibrosis is caused by a mutation leading to a deletion of the phenylalanine 508 amino acid on chromosome 7 in 70% of cases. This is responsible for clearing the airway of dust and mucus, a process which is done by pumping chloride ions into the mucus, through a cystic fibrosis transmembrane conductance regulator (CFTR) lowering the water potential and stimulating osmosis from the surrounding cells into the mucus causing it to become less viscous and easier for the cilia to clear (see Fig 5). In cystic fibrosis cases no chloride ions are pumped into the mucus. This means the mucus remains thick and the cilia cannot clear it.

Current treatments include physiotherapy to clear the lungs of mucus and to reduce infection, medication to fight infection and clear mucus and enzyme tablets to help digest food. Despite this life expectancy is currently only around 31 years and every day 3 people die with this disease. New treatments are currently being researched and developed including an inhaled spray to introduce normal copies of the gene to the lungs and drugs to correct the abnormal salt/water regulation of the cells. The Cystic Fibrosis Trust is currently funding research to 'find an effective treatment for CF through gene therapy and [they] believe that it is going to be the nearest thing to a cure in the foreseeable future.'

Curing Cystic Fibrosis and other genetic diseases could be another application for gene therapy combined with stem cells. The National Academy of Sciences (PNAS) believes that undifferentiated adult stem cells from the bone marrow could be corrected for the faulty gene in cystic fibrosis and then differentiated into airway epithelial cells replacing the ones with the faulty CFTR with fully functional cells.

Hereditary breast cancer

Cancer is a malignant or invasive growth or tumour caused by uncontrollable cell division. Breast cancer consists of changes in the ducts, lobules (milk producing glands), lymph channels and other parts of the breast.
Symptoms may include some of the following:

- Change in the shape and/or size of the breast
- Redness of the skin
- Discharge from the nipple (may be bloody)
- A lump or thickening in the breast area or around the armpit (a malignant lump is likely to be hard, oddly shaped and difficult to move)

Many people with a family history of breast cancer and who know they possess the particular genetic mutation may opt to have a preventative mastectomy even if there are no abnormalities. They can also reduce the risk factors including:

- Genetic factors
- Age
- Hormone Replacement Therapy (HRT)
- Prolonged of the contraceptive pill
- Daily alcohol intake
- Being overweight after menopause

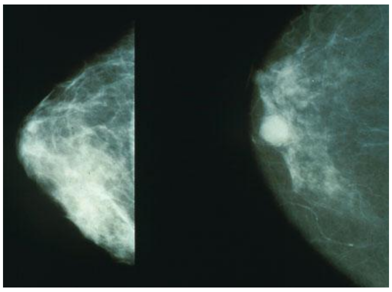


Fig. 6 - Mammography showing a healthy breast (left) and a cancerous breast (right)

Breast cancer is diagnosed by either a clinical examination when a clinician indentifies any lumps, a biopsy when tissue is removed from the suspected tumour and examined under a microscope for cancerous cells or a scan. Several different scans are used including MRI, CT, ultrasound or a mammography (see Fig. 6)

Current treatments of breast cancer

Different treatments are used to treat breast cancer depending on the patient and their individual cancer ie. the stage and type of the cancer. Surgery is one of the options that either removes the cancer but not the breast in a partial mastectomy or removes the breast and the cancer in a total mastectomy. Radiation therapy involves using high energy external x-rays or using a radioactive substance internally through needles or wires to kill the cancerous cells. Chemotherapy involves 'the treatment of disease with a chemical agent… [by] tinkering with the cancer cells' DNA or RNA and thus the ability of these cells to reproduce.' (John Diamond) Hormone therapy acts by to removing or blocking the action of hormones that aid cancer cells. All of these treatments have very undesirable side effects and are not 100% effective. Therefore a treatment that is not as traumatic and distressing for patients would be very much welcomed. This is where the use of stem cells combined with gene therapy could be very useful in the treatment of hereditary breast cancer. In the future, cancer could be treated directly by correcting the mutations that lead to cancer or by solely studying the differentiation of stem cells scientists could gain a better understanding of how and why the abnormal differentiation that leads to cancer occurs.

Between 5-10% of all breast cancer cases are caused by mutations on the BRCA1 gene. This mutation mostly causes Basaloid Breast Cancer (BBC) which has a poor prognosis because the breast cancer cells do not possess hormones or proteins that can be easily targeted by therapy. The function of the BRCA1 gene is to control cell growth and suppress tumours so if these genes contain mutations, the chance of healthy cells becoming cancerous increases and there is a 7 times greater risk of developing cancer before menopause. The mutation in these genes is more common in families of Ashkenazi or Eastern European Jewish origin and gives an 85% chance of developing breast cancer. Although not all cases of breast cancer are caused by this mutation, it can lead to devastating effects killing most women in a family. For Sophie Roell, a member of a family in which this gene commonly caused deaths amongst women, 'growing up without a mother verged on a family tradition.' (Roell, Sophie, Threat of Cancer is my Family's Grim Legacy, Times Newspaper, 14th Feb 2003)

The Function of the Genes PTEN and BRCA1

The mutation of the PTEN gene is another possible cause of breast cancer and is found in roughly 30% of all cancers. PTEN is a tumour suppressant gene that works alongside BRCA to prevent cancer developing. When PTEN is knocked out, strong growth signals

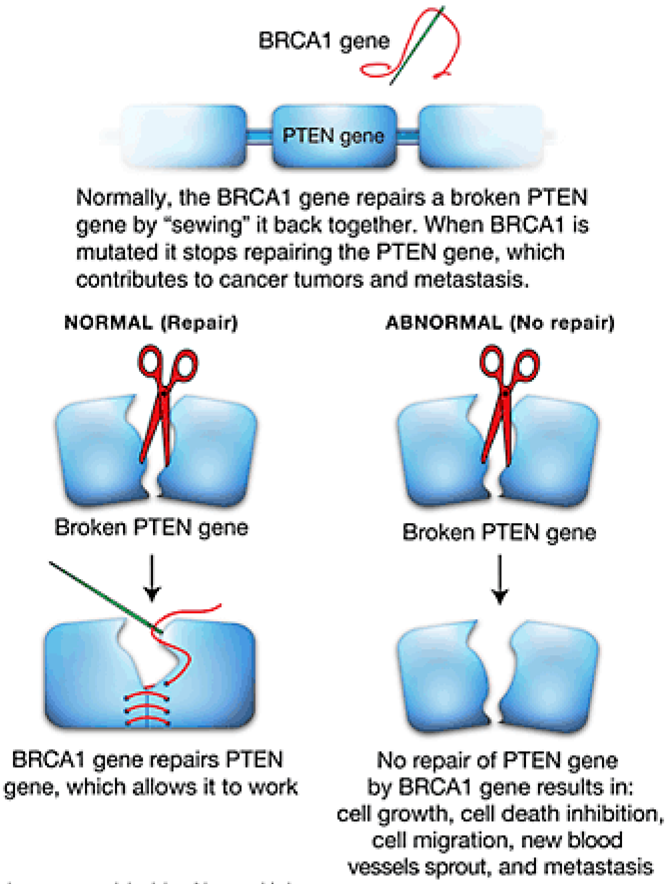


Image provided by Nancy Heim, Columbia University Medical Center ® 2007

Fig. 7 – Diagram showing the function of BRCA1

are sent to cells because the activity of proteins in the cell increases. This is known as the PTEN/P13K pathway. Fig 7 (see previous page) shows the function of the BRCA1 gene alongside PTEN. If PTEN mutates, then BRCA1 will correct the mutation by ‘sewing’ it back together

The Function of LOX

90% of fatalities die to cancer are caused by metastasis, when a cancerous cell breaks away from the primary tumour and moves through the lymph system and blood vessels to another part of the body where it may form a secondary tumour. Research by the Institute of Cancer Research that an enzyme called lysyl oxidase (LOX) is responsible for initiating metastasis. LOX sends out signals to another area of the body, prompting it to prepare a new area where a cancerous cell could settle and begin to divide. Without this preparation, the area would be too dangerous and the cancerous cell would not be able to grow. ‘Drugs to block this enzyme's action could keep cancer at bay.’ (Enzyme Behind Cancer Spread Found, BBC News, 8th March 2009) Using the process of combing stem cells with gene therapy, the genes coding for LOX could be mutated so that the enzyme doesn’t function and therefore would not be able to initiate metastasis. However discussion should be given to whether the treatment is given to everyone at birth to knock the gene out or should the treatment be given when cancer is diagnosed. With the latter option, there is the risk that the treatment could be given too late if the cancer is diagnosed late.

Concerns about Stem Cells

Although stem cells possess the potential to provide cures for many currently untreatable diseases, some scientists are worried about unexpected results if their use is not monitored closely. The main concern is related to the feature of them that makes them so versatile: Embryonic Stem Cells grow very quickly and if not monitored closely and directed the growth could become uncontrollable leading to tumours. Scientists have not discovered a totally reliable method of differentiating stem cells into a particular tissue and there are concerns that the stem cells may differentiate into undesired tissue types. To overcome this scientists are experimenting with a method of partial differentiation when the stem cells are partially differentiated before transplantation into the patient.

Ethical Issues Associated with Stem Cells

The main question for Embryonic Stem Cell research is ‘Does the end justify the means? The ‘end’ is ultimately to ‘achieve the relief of human suffering’ (Louis Guenin)

Opposition to Embryonic Stem cell research maintain the theory that an embryo is a human being from fertilisation onwards as it is genetically unique and therefore has the same ‘moral status’ (Dr. Kathi E. Hanna) and rights to life as a human being even at this point. Therefore the destruction of an embryo, whether it is spare or not is the same as ending the life of another person. The Catholic view presented by Edmund D. Pellegrino is that it is not justifiable to kill a person in order to help another person.

The other side of the argument from the supporters of Embryonic Stem Cell research is that an early human embryo should not be seen as a person and despite the fact that it has the potential to become one. John Bryant points out that 'up to 80% of early embryos do not implant into the lining of the womb and thus do not establish a pregnancy.' However Dr. Hanna also argues that it would be immoral not to use embryos that were to be discarded anyway to find a cure for diseases that are killing thousands of people. Embryonic stem cells may not have to be used, as skin cells could be changed into pluripotent stem cells using a retrovirus.

Another potential ethical hurdle with the process of combining gene therapy with stem cells is that the whole of the patient's genetic code could be changed over time. This could be seen as altering the person or as 'playing God' because the scientist is changing the genetic code of the person. Some people could be worried that the genes of a person could be modified and designed to make a 'superhuman.'

These issues are very important because even if scientists disagree with the ethical issue involved in embryonic stem cell research or are not concerned at altering a patient's genetic code they will not get public support if the public do not fully understand the future applications of this research or the method used. The public may be scared of this new research due to lack of knowledge and the government may reduce or withdraw essential funding due to this. Therefore the public need to be educated and convinced if this treatment is to succeed and is to become available as a treatment.

Conclusion

Although research has been carried out into the possibility of combining gene therapy with stem cells to cure disease caused by mutations it is in its early stages and few applications have been researched. In this paper I have taken the current research into the method of this therapy and applied it to cure two diseases caused by mutations but this method could potentially be applied to any disease caused by a mutation. This method could also be made specific to each individual patient by correcting the particular genes that cause each individual cancer making treatment more effective. The research is in its early stages and the long term effects of potentially changing a patient's genome have not been researched.

However resolving the ethical issues is a major obstacle which needs to be overcome before this treatment is commercially available. This could be done by educating the public, showing them the benefits of stem cell research and showing them that not all research has to be carried out on embryonic stem cells. Adult stem cells can be used as a replacement or differentiated cells can be converted back into stem cells using a retrovirus. The public needs to become aware of this before it will readily accept this new treatment.

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