

# Sickle cell gene therapy term paper

[Science](#), [Genetics](#)



## **Introduction**

An article published by the Cochrane Collaboration and posted in the Cochrane Library in 2012 caught my attention. The article stated that no quasi-randomized or randomized clinical trials of gene therapy for sickle cell disease have been documented. The article further stated that no conclusions and recommendations are attributed to the application of gene therapy on sickle cell disease (Olowoyeye & Okwundu, 2013). This statement inspired me to evaluate the current developments in gene therapy that targets sickle cell disease. Sickle cell disease is one of the oldest genetic disorders and yet few attempts have been made to develop gene therapy. This paper therefore seeks to analyze current literature and evaluate the current state with respect to the development of gene therapy for sickle cell disease.

A genetic mutation refers to an alteration in the base sequence of DNA. There are various forms of mutation, but the most common is called substitution or point mutation, and it involves single base pairs. Mutations in the genes that code for the protein globin are critical in the formation of sickle cell disease. Globin is a protein that is pivotal in the transportation of oxygen in the body. Globin is a critical component of the hemoglobin, a metalloprotein complex that is involved in the transfer of oxygen from lungs to the rest of the body. A genetic mutation that results to sickle cell disease involves a single mis-sense mutation, whereby the base Adenine is changed to Thymine. Consequently, this causes the amino acid sequence of the globin in that glutamic acid is replaced by valine. The function of a protein significantly depends on its structure, thus any change in the structure of the

protein affects the function of that protein. With regard to sickle cell disease, the change in amino acid sequence changes the structure of hemoglobin from a round shape to a C-like/sickle shape. This affects the ability of hemoglobin to transport oxygen. The sickle shape impedes the entry and exit of hemoglobin to and from capillaries. Gene therapy aims at replacing the defective gene with the correct one so that the protein formed has the correct functional structure. Many scientists have attempted to develop gene therapy for sickle cell anemia.

## **Review and Synthesis**

Pawliuk et al (2001) argue that sickle cell disease is a common autosomal recessive disorder across the globe. Defective hemoglobin usually polymerizes into long fibers after undergoing deoxygenation with red blood cells. This causes red blood cells to assume the sickle shape, become adhesive and rigid. This phenomenon triggers anemia, microcirculation occlusion, and infarction, as well as organ damage. Pawliuk et al (2001) contend that human gamma globin, when compared to beta globin is a strong inhibitor of the polymerization of the abnormal hemoglobin (HbS). Beta globin has been found to be only effective at a high concentration. Pawliuk et al (2001) proposed that a gene therapy for sickle cell disease, which involves forced expression of gamma globin or a hybrid of both gamma and beta globins. The researchers conducted their study using transgenic mice. These researchers hoped to introduce hematopoietic stem cells in adult RBCs. After a long-term expression of the genes (ten months), the researchers realized that in two models of the mice, there was a significant reduction in sickling and dehydration. Pawliuk et al (2001) argue that the ability to achieve a

stable transfer of vectors expressing core elements of locus control region of human beta globin protein proves to be challenging. In their study, they overcame this obstacle by using responsive elements and RNA splicing of human HIV virus. This was due to the fact that responsive elements and RNA splicing of human HIV virus had been used to ameliorate beta-thalassemia. Pawliuk et al (2001) argue that before conducting human trial using their approach, it essential to achieve large scale lentiviral production that is devoid of replication-competent retrovirus. In addition, there is a need to have bone marrow reconstitution using transduced stem cells, but in the absence of toxic myeloablation.

On the other hand, Bank (2008) conducted a review of various literatures to elucidate the possibility of developing a gene therapy for sickle cell disease and beta-thalassemia. Bank (2008) argues that a point mutation in the genes that code for Beta globin lead to the scarcity of beta globin as well as a reduction in the adult hemoglobin. Bank (2008) contends that there are two approaches that can be used to improve the function of beta globin using gene therapy. The first approach can involve the use of homologous recombination to correct the defect in Beta globin. The second approach may involve the introduction of a new and normal Beta globin gene. Option one is more advantageous than the second option because it retains the beta-globin in its natural environment. Option one has been successfully used in mice. However, its use in human trials is hampered by the low frequency at which homologous recombination occurs. Bank (2008) and Fischer (2007) contend that gamma retrovirus vectors, which contain Maloney viral components has registered success in other applications like in

the treatment of combined immunodeficiencies. The curative gene is usually transduced in the patient's defective gene and this leads to immune reconstitution of the associated T-lymphocyte, as well as cure. Bank (2008) regrets that no such combination is available for erythroid progeny that expresses beta-globin. In addition, Bank (2008) argues that another obstacle involves the ability to enhance the expression of the corrective beta globin genes than that of the curative genes especially in immune disorders.

In another commentary, Caplen (2002) echoes the findings of the study conducted by Pawliuk et al (2001). In February, 2002 Natasha Carlen, of the National Institute of Health, reported the above study and its limitations concluding that although these findings were exciting, several issues had to be addressed before clinical studies could begin on humans. Caplen regrets that turning the promising findings of the Pawliuk et al study into a new therapy is challenging. Caplen (2002) seconds the recommendations put forward by Pawliuk et al, which call for an extensive evaluation of the clinical safety of the lenti-virus vector system as well as ensuring efficient gene transduction. This will ensure that a high number of modified red blood cells are obtained.

In addition, Sjecklocha et al (2011) argue that gene therapy for sickle cell anemia calls for efficient delivery of a gene product that is tightly regulated as well as stably expressed. In their study, Sjecklocha et al (2011) employed a non viral sleeping beauty transposon model. The researchers used SB 100X hyperactive transposase to transduce two sets of genes: hybrid IHK-beta-globin transgene/Ds Red into human cord CD34+ cells. The researchers realized that the transduced hybrid IHK-beta-globin transgene differentiated

successfully into different lineages. All the lineages showed transgene integration. This study showed that mature erythroid expressed hemoglobin can be expressed by primary human CD34 plus cells that have been transduced with constructs while maintaining tight erythroid specific expression. Analysis of transgene content and expression of beta globin transduced cells show that each of the nucleofected trails retained the gene in each lineage.

In this study, a non viral transposon called Sleeping Beauty was used to transduce human cord blood cells with a hybrid Beta globin transgene. This was the first time that a non viral vector was used to carry a transgene for gene therapy for sickle cell disease. Even though the study was not aimed directly at curing sickle cell disease, the fact that beta globin can be transduced into non viral vectors could be a good leap toward using this approach in gene therapy of sickle cell disease. Mature erythroid cells were found to have a significantly high beta-globin to gamma globin ratio. This was an indication that the SB transgene had been successfully integrated in beta-globin of erythroid cells. Sjecklocha et al (2011) argue that non viral sleeping beauty transposon system is significant in the transfer of IHK-beta-globin transgene therapy of SCD. Sjecklocha et al (2011) argue that non viral vectors are good systems for genetically modifying cells. Sjecklocha et al (2011) however regret that these systems have significantly low gene transfer efficiency. Sjecklocha et al (2011) recommend the use of transposons in the genetic modification of cells because they are stable and lead to long expression of the transduced genes. In order to achieve maximum efficiency, an enhanced transposon needs to be used and stable

gene transfer efficiencies can be achieved. There is a need to conduct more studies in order for this method to be replicated in human trials.

## **Conclusion**

This analysis has shown that no human clinical trials have been conducted with regard to SCD gene therapy. However, numerous studies have been conducted in laboratory animals, and some of these studies have recorded promising results. Before conducting human trial, it is essential to achieve large scale lentiviral production that is devoid of replication-competent retrovirus. In addition, there is a need to have bone marrow reconstitution using transduced stem cells, but in the absence of toxic myeloablation. In order to achieve maximum efficiency, an enhanced transposon needs to be used and stable gene transfer efficiencies can be achieved. There is a need to conduct more studies in order for this method to be replicated in human trials. The results posted by Pawliuk et al (2001) are very promising. These researchers proposed that a gene therapy for sickle cell disease, which involves forced expression of gamma globin or a hybrid of both gamma and beta globins. The second milestone achievement is that recorded by Sjecklocha et al (2011). This was the first time that a non viral vector was used to carry a transgene for gene therapy for sickle cell disease. Even though the study was not aimed directly at curing sickle cell disease, the fact that beta globin can be transduced into non viral vectors could be a good leap toward using this approach in gene therapy of sickle cell disease.

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