

## Gene Therapy for Sickle Cell Disease: Start of a New Era

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### Abstract

This manuscript reviews treatment of Sickle Cell disease over time. The application of allogeneic stem cells proved the sickle cell disease could be permanently corrected and cured but limited to those with a compatible donor. The use of autologous cells for therapy is the dawn of a new era where the need for a third party is removed and eliminates that ethical issue. With sickle cell disease screening for the most high-risk patients is now available. The use of autologous genetically modified cells is equally as effective as those treated with allogeneic cells and makes it possible for the first time to treat all symptomatic children and adults suffering from sickle cell disease. This is the beginning of a new era for the treatment of hemoglobinopathies.

**Keywords:** Gene therapy, Autologous cells, Sickle Cell disease, Stem cell therapy, Transcranial Doppler ultrasound, Viral vectors

Sickle cell disease (SCD) is the consequence of the formation of hemoglobin S (HbS), typically resulting from homozygosity or compound heterozygosity for pathogenic variants in the sickle cell gene. Under low oxygen pressure, sickle hemoglobin molecules affected by sickle cell pathogenic variants interact with one another to deform the red cell and give the cell its classic “sickle” appearance. This defect in the beta chain forms a hump that fits into another complementary spot on another hemoglobin molecule, thereby allowing them to hook together and form tetrahedral crystals. These ridged aggregates go on to precipitate out of solution and lead to collapse of erythrocyte and result in loss of cellular function and ensuing anemia. The main determinant of cell deformation is the rate and extent of HbS formation [1].

Currently the only cure for SCD is a stem cell transplant. E. Donnall Thomas was the first to perform a successful transplant in 1957 [2], pioneering the way for the first transplant for sickle cell disease and leukemia in 1958 at St Jude Children’s Hospital in Memphis, Tennessee [3]. Currently more than 24,000 stem cell transplants are

performed annually for a variety of indications [3].

There is substantial variation in the phenotype of SCD. The Cooperative Study of Sickle Cell Disease in the USA [4] found that 39% experienced no pain whilst 1% had 6 or more hospitalizations per year. Variability in other complications such as stroke, chest syndrome, and premature death were also observed. The most impactful modifier of SCD-related complications appears to be the concentration of fetal hemoglobin and co-inheritance of alpha-thalassemia. Fetal hemoglobin typically varies from 1 to 30% and is inherited as a quantitative genetic trait. Three major loci have been identified that contribute to 50% of the variation in SCD [1].

This variability in SCD phenotype means that therapeutic intervention requires individualization of treatment for affected individuals. Specifically, not all people with SCD will require intensive therapy to mitigate symptoms and improve health and lifestyle. Accordingly, a screening method is needed to determine which children should undergo a stem cell transplant or other intensive therapies.

Adams et al. [5] reported on the value of transcranial Doppler ultrasound for the prediction of stroke in 130 children with SCD. They divided subjects into two groups: one group would undergo regular transfusions and the other group received standard/usual care. In the usual/standard cohort, ten of 67 were diagnosed with cerebral infarction, with one resulting from an intracerebral hematoma whilst only one cerebral infarction occurred in the 63 subjects making up the transfusion cohort. This represented a ninety-two percent difference in risk of stroke ( $p < 0.001$ ). Two conclusions can be derived from this study: Doppler ultrasound can well detect a high-risk group and transfusion is protective for stroke [5].

Transcranial Doppler (TCD) ultrasound was later compared with MRI of the brain to determine which imaging technology was more accurate in predicting stroke [6]. MRI is considered to be the comparative standard for evaluation. Naffea et al. [6] found that TCD was nearly as accurate in at MRI in predicting risk for stroke. However, TCD is a far more facile and economic technology to use in this regard and is, as such, an important first-line option for delineating risk for stroke in individuals with SCD. TCD can thus be used to identify those affected individuals who would most benefit from stem cell therapy [6]; in this regard, MRI could be utilized to confirm abnormal ultrasound (TCD) results.

We now know that the benefits of blood transfusion therapy for symptomatic SCD far outweigh the risks and that there is a reasonably reliable screening method to identify patients at increased risk for stroke resulting from the congenital anemia. As such, the next question that needs to be addressed is whether the outcome in children treated with allogeneic stem cells demonstrate a significant and more clinically relevant improvement in quality of life along with fewer complications than those treated with usual care or blood transfusion.

Bernaudin and colleagues [7] reported on the long-term follow-up of 234 children with SCD who received myeloablative conditioning and matched-sibling transplantation, indeed one of the largest studies of long-term follow up of such children treated in this manner. This 24-year study evaluated the use of antithymocyte globulin (ATG), which reduces the incidence of rejection and was routinely instituted in cases described later in the series. Death, non-engraftment or rejection (donor cells  $< 5\%$ ) were considered to be primary adverse events, the 5-year event-free survival was 97.7% with the median length of follow-up being 7.9 years. Event-free survival was primarily associated with chronic-graft-versus-host disease (GvHD), but most individuals with GvHD did not experience any of the primary adverse events. There were no SCA events (e.g, pain crises, chest syndrome) in those with mixed chimerism, even in those where the donor

cell fraction were only at 15-20%. Those that underwent myeloablative transplantation with matched-sibling donor had a higher event-free survival of 98% compared to those that had non-myeloablative conditioning at a rate of 88% [7].

This study also demonstrated that the time to reach satisfactory engraftment is shorter with bone marrow transplantation (BMT) than with cord blood donation (CBD). As such, BMT shortens the risk window and improves outcomes, with a difference in time for engraftment of about 20 days [7]. Mortality was low and further decreased when ATG was instituted. In fact, the mortality rate decreased from 6% to 1.4% with the inclusion of ATG, thus leading to its routine inclusion by the end of the study period. In addition, the use of ATG reduced the GvHD from 10.5% to 5.4%.

Chimerism relates to the ratio of donor cells to host following stem cell transplantation. Complete engraftment was defined as  $>95\%$  donor cells; rejection was classified as  $<5\%$  donor cells. Low engraftment was defined as 5-50% donor cells with high engraftment being classified as 50-95%. At one year, 54% of subjects had complete chimerism, 44% had mixed chimerism and 2% showed rejection [7].

Long term chimerism of  $>70\%$  was observed in one hundred and twelve subjects. Of the 92 with mixed chimerism, there was rejection of the graft in 4.3% of such individuals. The time of loss of the graft was variable at 1.5, 2, 2.3 and 9-years post-transplant. None of those with mixed chimerism had any SCA-related vaso-occlusive crisis or acute-chest syndrome but 5 eventually developed anemia. As long as chimerism remains higher than 50% there were no reported cases of anemia [7].

This study also affirmed that the transplantation process has an adverse effect on gonadal functioning. Of those females who were pubertal and underwent stem cell transplantation, all required hormone replacement therapy. Nine of 32 pre-pubertal girls who underwent transplantation did undergo normal pubertal changes with menstruation. Because of this, it is suggested that female children should be offered fertility preservation. Indeed, one of the girls who provided ovarian tissue prior to transplantation went on to have a spontaneous uneventful pregnancy, delivering at 38 weeks of gestation. She was then re-implanted with an additional fragment of ovarian tissue and subsequently conceived a second child. In contrast, all boys who were pre-pubertal and of pubertal age who underwent transplantation had normal testosterone levels and bone age test results. Three of these children have gone on to father a child. Regardless, it is still recommended to offer fertility preservation to boys who will undergo stem cell transplantation.

We previously published in 2020 [8] that the ability to use autologous cells for the amelioration of SCD symptoms is in place. One of the primary reasons to pursue the use of autologous genetically modified cells is that there are so few patients that have access to a compatible donor [7,8]. In addition, the process of conditioning the bone marrow of recipients increases the risk of death [7]. To this end, Ribeil and collaborators [9] reported their experience with treating a patient with lentiviral *ex vivo* gene modified autologous cells. The male child had homozygous sickle cell disease. His course before transplants was complicated by numerous vaso-occlusive crisis including two episodes of acute chest syndrome and bilateral hip osteonecrosis. In addition, he had undergone a cholecystectomy and splenectomy. By age of nine he was on a regimen of transfusions with chelation and had failed hydroxyurea therapy [9].

The bone marrow-enriched CD34+ cells that were to be used for transplantation were transduced with Lentiglobin BB305. Myeloablation was performed prior to transplantation. CD34+ cells were infused after two days of a washout period. Red-cell transfusions continued until a large population of HbA T87Q (25 to 30% of total hemoglobin) was detected. The patient was monitored for any form of engraftment toxic effects [9]. Neutrophil engraftment was achieved by day 38 and sufficient platelet levels were detected at 91 days after transplantation. Hemoglobin levels were stable between 10.6 and 12.0 g per deciliter at 6 months post transplantation [9]. The patient had side effects from the conditioning procedure that included neutropenia anemia and thrombocytopenia and a *Staphylococcus epidermis* infection, all of which resolved. After discharge, he developed lower limb pain at 3 months and transient increases of alanine aminotransferase, aspartate aminotransferase and Y-glutamyltransferase between 5 and 8 months after transfer; as before, all these events spontaneously resolved.

The child was discharged from the hospital on day 50 after transplant and was, at 15 months, free of any clinical SCA events. His course contrasted very favorably to his history prior to the transplant and all pain medications were eventually discontinued. An MRI of his head performed at 8 months following transplantation showed no progression of a white matter hypodensity and a lower limb MRI at 14 months failed to show any recent bone tissue damage. The patient reported full participation in normal academic and physical activities. Elevated iron levels continued to decrease despite discontinuation of the chelation agent. Laboratory values for liver and kidney function were normal and there was progressive normalization of erythropoiesis [9].

Soni and collaborators [10] reported their experience using the same transplantation method as Ribeil [9], along

with using the same vector. As with the experience reported by Ribeil and colleagues [9], the two male patients, aged 13 and 42, reported a similar salutary outcome post transplantation, with neutrophil engraftment was 37% and 16% respectively [10].

These two manuscripts [9,10] provide powerful supportive evidence for the clinical use of autologous cells in the treatment of children and adults with SCD. It appears that bone marrow conditioning is a source of morbidity and mortality for these individuals; accordingly, developing an effective therapy that uses autologous cells but does not require bone marrow conditioning would considerably improve outcomes. As we previously wrote, the need to incorporate genetics and genomics into current therapeutic interventions for SCD and other hemoglobinopathies has finally joined with the technological capabilities to provide these interventions safely and effectively [8].

Obstetricians and gynecologists often function as primary care providers for young women; as such, the assessment, diagnosis and early care for young women with SCA would be a part of their ongoing obstetrical and gynecological care. The considerable variability in disease expression as well as recent advancements in therapeutic interventions must be disseminated to all clinicians who care for reproductive-aged women. Most at-risk groups are screened for sickle cell disease; now that the disease is amenable to more successful and less harsh treatments, the role of pregnancy termination for the management of fetuses affected with SCA has been greatly reduced. Indeed, the use of ones' own cells profoundly reduces and perhaps eliminates any ethical dilemma of involving a third party for treatment.

Finally, the availability of salutary therapeutic options for a cure for SCA makes fetoscopic therapy unnecessary, thus reducing the need for a highly morbid prenatal intervention. This also removes the need for consideration of prenatal therapies which would potentially raise ethical concerns given our history as exemplified by the Tuskegee experience. Besides our experience with SCA also is applicable to the many hemoglobinopathies that will be candidates for similar future therapies. We must continue to strive to make the findings of the Human Genome Project a seminal component of therapeutic interventions in diseases associated with genetic and genomic perturbations.

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