INVESTIGATING POTENTIAL DRIVER MUTATIONS IN PATIENTS UNDERGOING GENE THERAPY FOR SICKLE CELL DISEASE

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Table of Contents

Preface

Sickle Cell Disease (SCD) is a genetic condition which affects the red blood cells in your body. This condition causes the shape of your red blood cells to change – instead of looking like a disc they become crescent shaped. Because of this change in shape, the blood cells can be too large to fit through blood vessels and can cause immense pain to the individual, known as sickle cell crisis. People with SCD are also at a higher risk of developing conditions such as strokes and heart attacks (Gladwin and Sachdev, 2012). SCD is a relatively common genetic disease, affecting about 12,000-15,000 people in the UK (National Institute of Clinical Excellency). Since its genetic linkage was discovered around 70 years ago, there have not been many major advances in the treatment of SCD. The most potent curative treatment at present is a bone marrow transplant, in which hematopoietic stem cells (cells which are responsible for the creation of red blood cells), HSCs, are transplanted into a patient to increase the number of healthy red blood cells produced. This treatment in many ways should be flawless, however finding a matching donor can be tricky. Through advances in gene technologies, scientists can alter stem cells from the patient to make them healthy instead of using a donor, although there have been significant health and safety concerns. This paper will aim to outline some of the potential risks with gene editing treatment, and will explain how these have been evaluated, and how they could be mitigated in the future.

Haematopoiesis

Haematopoiesis is the umbrella term that describes a set of cell divisions which allow for specification of blood cells from one common ancestor(Kent and Eaves, 2016). The first cells identified in haematopoiesis are hematopoietic stem cells (HSCs). HSCs have the potential to differentiate into any type of blood cell through a series of divisions. This first differentiation could be classed as the most important cell division as it is responsible for determining the fate of all future divisions, and which cells is it possible to create (shown in figure one). At any one time, a person will have between 20,000 and 200,000 HSCs in their bone marrow, which, in an adult, will divide one or two times per year (Mitchell et al., 2022). Haematopoiesis is a highly active process in the body – it is estimated that over one trillion cells are created and destroyed each day (Kent and Eaves, 2016). However, with every division of these cells, there is a random chance that the DNA will replicate incorrectly and cause a mutation. Usually, these mutations happen quite linearly within a person, and mutational burden (the potential systemic effects of these mutations) increases with age (Mitchell et al., 2022). Mutations can also serve as a useful diagnostic tool. It is possible to track these mutations down the pathway of haematopoiesis and construct a ‘family tree’ of sorts to understand which cells are acquiring the most mutations, and if they have a common ancestor (Lyne et al., 2018; Shepherd and Kent, 2019). This is done by taking samples from the patient and analysing the DNA for similarities and differences. In doing this, they can be sorted into a chronological order, based on number of differing mutations from a common ancestor. Figure two captures this and shows how a ‘blood cell family tree’ can be constructed using this method of DNA analysis (Spencer Chapman M, Cull A.H. et al., manuscript in revision, 2022).

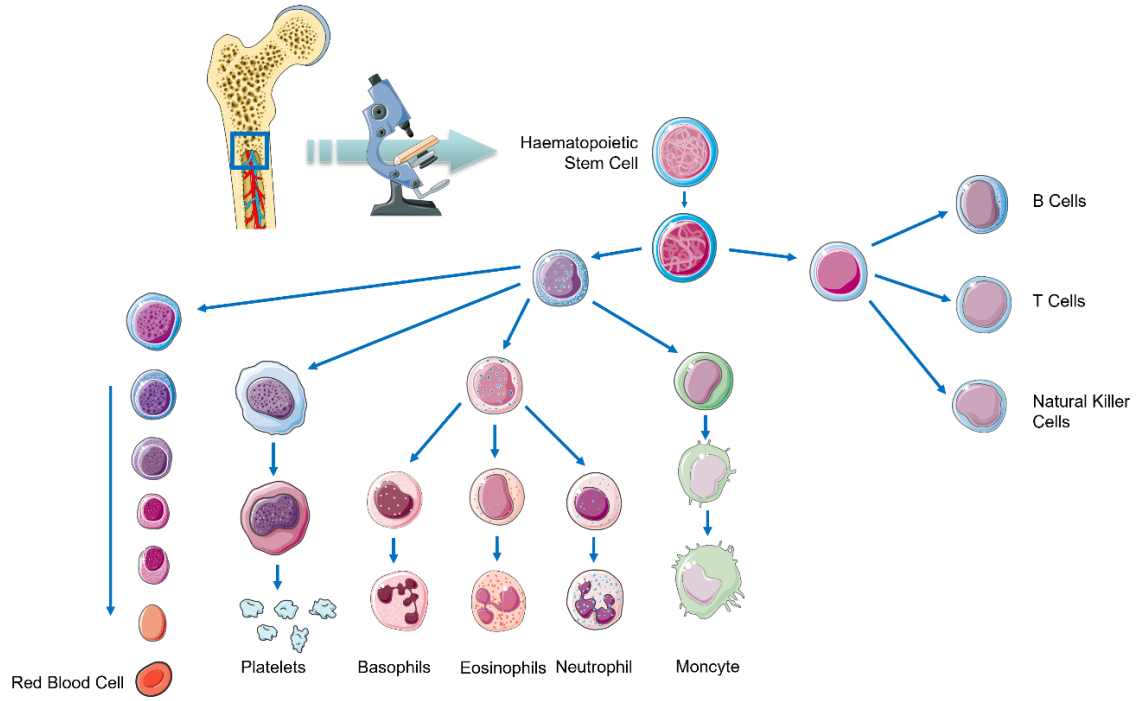

Figure 1 - A diagram showing the most basic pathway of haematopoiesis. Parts of the figure were drawn by using pictures from Servier Medical Art. Servier Medical Art by Servier is licensed under a Creative Commons Attribution 3.0 Unported License (https://creativecommons.org/licenses/by/3.0/).

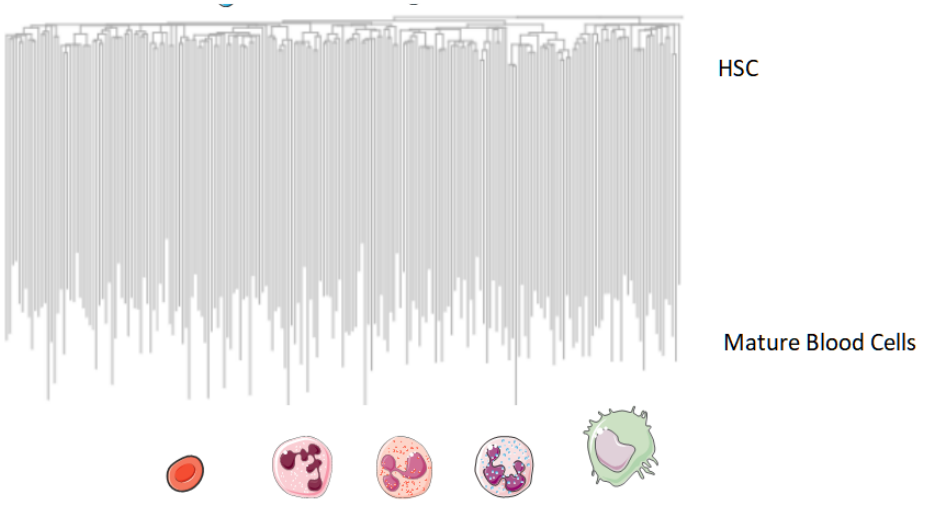

Figure 2 - A diagram showing how a cell family tree can be constructed by looking at mutations accumulated throughout a lifetime – from birth to cell maturity. Each line represents an independent cell lineage pathway, like a traditional family tree. Parts of the figure were drawn by using pictures from Servier Medical Art. Servier Medical Art by Servier is licensed under a Creative Commons Attribution 3.0 Unported License (https://creativecommons.org/licenses/by/3.0/).

Sickle Cell Treatment using Gene Therapy

Ever since the successful analysis of the complete genetic makeup of humans through the Human Genome Project, researchers have been using this information to find cures for some of the worst human diseases. In the case of sickle cell disease scientists have been aiming to target the root cause of the illness – the genetic code itself. The benefit to this method is that it uses modified versions of the patient's cells, so it is less likely that the patient's immune system identifies them as foreign. If the immune system does identify them as foreign, they are more likely to be destroyed by patient cells in the blood. This method of Gene Therapy first requires stem cells to be harvested from the patient. In one phase 2 clinical study, stem cells are harvested chemically using a drug known as Plerixafor. Plerixafor moves stem cells from the bone marrow into the body's blood stream, so that they can be taken out in a similar process to donating blood. These cells are then modified by a lab to correct faults in the cell. In the case of the patients in this study, the patients all had a gene 'switched on' that promotes healthy red blood cells to be produced (Esrick et al., 2021). After these cells have been quality checked, they are ready for implantation. The patient will be given a high dose of chemotherapy to wipe out all the old stem cells which do not function correctly. After this, the new, edited cells will be transplanted into the patient and replace the old cells in the bone marrow. The patient is monitored and then moved to an outpatient clinic to be monitored by clinicians for any adverse effects. Figure 3 summarises the method explained above.

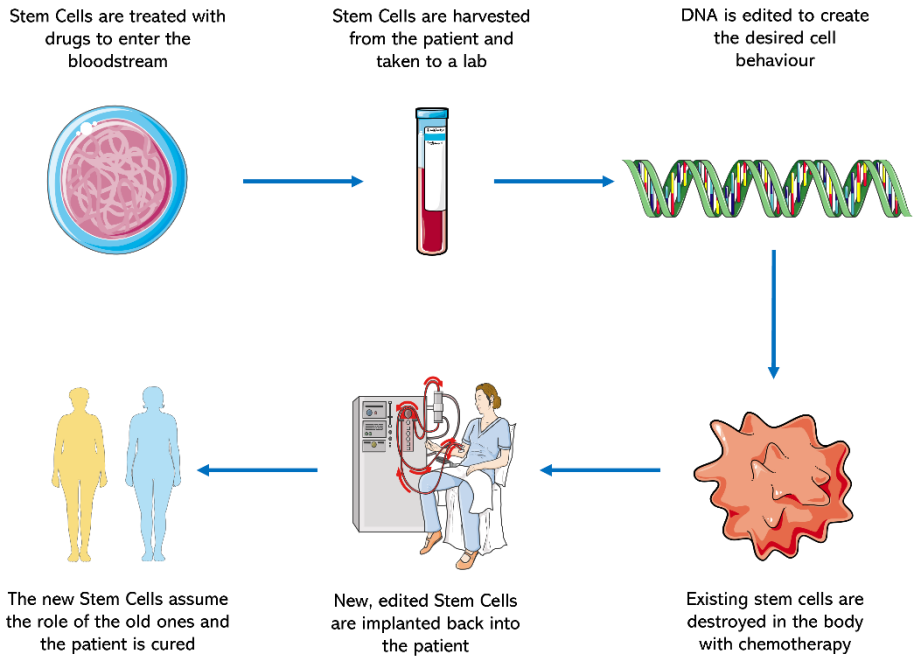

Figure 3 - A flowchart showing the process by which cells are gene edited to cure a patient of Sickle Cell Disease. Parts of the figure were drawn by using pictures from Servier Medical Art. Servier Medical Art by Servier is licensed under a Creative Commons Attribution 3.0 Unported License (https://creativecommons.org/licenses/by/3.0/).

This treatment is great at curing Sickle Cell Disease in patients, however the data shows there are some risks involved with this – the most potent being unwanted mutations in cells. In some patients, certain cancers have materialised, which may be caused by gene therapy (Goyal et al., 2022; Jones and DeBaun, 2021; Hsieh et al., 2020). There are a couple of working hypotheses for this. It is possible these cells have a predisposition to be less efficient at replicating, and so more mistakes are made which would cause more mutations. Damage could have been caused when the natural environment of these cells changed. Human cells can change how they function when placed in different environments, so this change may have increased the mutational rate of stem cells. It is also possible that cells may remain from before the chemotherapy treatment, which are now mutating as they have been damaged, and not killed in the process. Finally, the patient's body could be asking these cells to work harder and divide more to replenish what it believes is lost stem cells, leading to a higher mutational risk. It is also possible that Sickle Cell Disease mutations across all cell types may cause cells to have a higher mutation rate and therefore, when these cells are under stress, they cause more than the normal number of mutations. All these theories listed point towards the process of gene therapy being the cause of these spontaneous mutations. It is important to understand these mutations in detail, and what potential they have for causing disease and cancers.

Driver Mutations Associated with Gene Therapy

The methods and theories above all aid researchers with identifying some of the adverse effects present in patients who have had this gene therapy. The work of this lab consisted of identifying the mutations present in the cells of patients who had undergone the therapy detailed in the paragraph above, and then assessing if these are "driver mutations". Driver mutations are changes in genetic code that make a person more likely to contract a disease or illness (usually cancer). Seven potent mutations were identified which may drive cells to become cancerous, some of which are explored in more detail below.

TP53 (Tumour Protein P.53)

TP53 is a gene responsible for making the protein P53. Within the scientific field, this is the most common tumour suppressor protein when thinking about drivers of cancer. Most people have two functional copies of this protein, but it has been shown that even having one dysfunctional copy can be a detriment to a patient ((US), 1998). P53 plays a fundamental role in sensing cellular changes and damage and mitigating cell stress. However, more importantly, P53 is one of the main proteins in regulating cell division – it is important in ensuring that the cell divides properly, and controls cell activity to ensure this happens. P53 is also crucial in cell death, meaning that if the cell deems the damage irreversible or too severe for the patient, P53 regulates the controlled and programmed death of this cell to prevent the cell from causing more damage. When TP53 is in a mutated form it may cause instability throughout the whole cell, as well as deletion of other tumour suppressors and an increase in the number of cancers driving mutations irrespective of other factors. Its link to cancer has been established well in a paper from the Baylor College of Medicine. They concluded that TP53 mutations link to poorer survival rates in people who have cancers (Donehower et al., 2019). This research also shows that over 91% of all cancers seen in this study lose both TP53 genes, not just one. The benefit to this research is that you can use TP53 to predict prognosis in these patients as it was noted that four genes, when used in compliment with TP53 expression, can be a strong marker for the outcome of cancer diagnosis (Donehower et al., 2019). It seems likely that the mutation in TP53 relating to gene therapy comes from an increased demand on the cells to replenish the cell population. It is also possible, however, that these mutations occur because of the chemotherapy given to wipe out all existing stem cells (Li et al., 2020). This is an avenue that needs investigating in the future.

CDKN2A

CDKN2A is an important gene responsible to produce two proteins – p16 an p14. Both play a critical role in tumour suppression and help stop cells diving too often during periods of bodily growth, and from replicating too often when a person reaches old age (a process called senescence). p16 is specifically important in regulating the cell cycle (Cánepa et al., 2007). p16 acts as a 'checkpoint moderator', its function important in controlling whether a cell can progress to the next stage in the cycle. It does this by binding to two proteins, each which control a separate aspect of the cycle. It has been proven that a higher number of cells which produce p16 lead to negative consequences, such as increased tumour formation, kidney damage and heart conditions (Baker et al., 2016). An increased level of CDKN2A in the body could lead to a higher number of p16 protein being expressed, which would cause more tumours to form, leading to more cancerous complications for these patients.

DNMT3A

DNMT-3A is an important gene which is responsible for encoding the protein **DN**A **M**ethyl**T**ransferase **3a**. This protein is responsible for adding one carbon and 3 hydrogen atoms (a methyl group) to a specific fragment of DNA to stop genes from being expressed and leading to the creation of a protein. When DNA methylation is upregulated, it causes gene silencing to be expressed in cells. This means there is higher regulation in the function of a cell as there are less protein types being produced. Specifically in HSCs, DNMT3a plays a role in promoting differentiation into the different pathways shown in Figure 1 (Challen et al., 2011). Lack of DNMT3a production can lead to a higher risk of developing Acute Myeloid Leukemia – without this protein, cells may not have adequate regulation of genes being coded into proteins, causing the cells to be produced immaturely, and divide too rapidly (Holz-Schietinger, Matje and Reich, 2012). Cells that are immature and divide too fast can usually cause cancers as they are unstable as cells, and so do not have the correct cancer-preventing proteins in place when they divide.

PPM1D

PPM1D is a less well-known gene which, in healthy people, is important for the regulation of the cell-under-stress response. It acts on the protein p53, which has been mentioned above, and supresses its activity. This means that cells are less likely to die through programmed cell death (apoptosis). PPM1D also is responsible for preventing growth in many cells when they are under stress, as when cells are under stress, it is important that they do not replicate, as they have a higher chance of making cancerous mistakes. It has been shown in many studies that PPM1D has a role in cancer development, and that it can be used as a successful biomarker for cancer progression within the body (Deng et al., 2020). Interestingly, it has also been shown to be more frequent in patients who have undergone high doses of chemotherapy, begging the question as to if these mutations have been caused by the therapy that has been given to patients to cure their Sickle Cell Disease (al Hinai et al., 2021). Because of this, it is hard to use this as a marker in these patients for if they will develop cancers after the therapy they have had, or if this is a result of the higher chemotherapy dosage.

Moving Forward

The data collected throughout this study has proven that there are some definite trends in the development of driver mutations in people who have had a course of curative Gene Therapy for Sickle Cell Disease. The four mutations detailed above prove significant short- and long-term risk to patients, leaving them susceptible to many chronic illnesses of the blood. The most important outcome for these patients is to make sure they are being given diagnostic tests to identify some of the serious illnesses (such as leukaemia) before it becomes fatal. Moving forward on a wider, research-based scale, it becomes evident that there is a lack of knowledge as to where the problem lies which changes the implanted cells in such a way that they are prone to driver mutations materialising. Although the work from this lab suggests that the reason for this is positive selection of cell type, this has not been proven in an in vivo or in vitro setting. It is also important to be aware that there is significant benefit in studying these patients over a longer period. Gene therapy treatment for sickle cell disease, is still on-going as it remains the best option for these patients. Solely considering short term benefit, and immediate quality of life for these patients shows that the treatment is a safe, and effective method to cure Sickle Cell Disease (Esrick et al., 2021). However, it is unclear if the people who have developed these driver mutations will develop various blood cancers and leukemia, or if the mutations will lie dormant and have no systemic effect to the person throughout their life. Because of this, longitudinal studies, with long follow up periods at regular intervals would be useful. Combining this with new, innovative diagnostic tests for these cancers, it could become an essential tool for the prevention of the development of serious blood-based diseases. In time, experiments will need to be devised to effectively analyse each step of the Gene Therapy process to isolate the root of the problem, to ensure future recipients of this quality-of-life boosting treatment are not also disposed to lack of protection from some of the harshest blood-based malignancies known to man.

References

Baker, D. J. et al. (2016). Naturally occurring p16(Ink4a)-positive cells shorten healthy lifespan. *Nature*, 530 (7589), pp.184–189. [Online]. Available at: doi:10.1038/NATURE16932 [Accessed 31 August 2022].

Cánepa, E. T. et al. (2007). INK4 proteins, a family of mammalian CDK inhibitors with novel biological functions. *IUBMB life*, 59 (7), pp.419–426. [Online]. Available at: doi:10.1080/15216540701488358 [Accessed 31 August 2022].

Challen, G. A. et al. (2011). Dnmt3a is essential for hematopoietic stem cell differentiation. *Nature genetics*, 44 (1), pp.23–31. [Online]. Available at: doi:10.1038/NG.1009 [Accessed 5 September 2022].

Deng, W. et al. (2020). The role of PPM1D in cancer and advances in studies of its inhibitors. *Biomedicine & Pharmacotherapy*, 125, p.109956. [Online]. Available at: doi:10.1016/J.BIOPHA.2020.109956 [Accessed 5 September 2022].

Donehower, L. A. et al. (2019). Integrated Analysis of TP53 Gene and Pathway Alterations in The Cancer Genome Atlas. *Cell reports*, 28 (5), pp.1370-1384.e5. [Online]. Available at: doi:10.1016/J.CELREP.2019.07.001 [Accessed 29 August 2022].

Esrick, E. B. et al. (2021). Post-Transcriptional Genetic Silencing of BCL11A to Treat Sickle Cell Disease . *New England Journal of Medicine*, 384 (3), pp.205–215. [Online]. Available at: doi:10.1056/NEJMOA2029392/SUPPL_FILE/NEJMOA2029392_DATA-SHARING.PDF [Accessed 29 August 2022].

Gladwin, M. T. and Sachdev, V. (2012). Cardiovascular Abnormalities in Sickle Cell Disease. *Journal of the American College of Cardiology*, 59 (13), pp.1123–1133. [Online]. Available at: doi:10.1016/J.JACC.2011.10.900 [Accessed 5 September 2022].

Goyal, S. et al. (2022). Acute Myeloid Leukemia Case after Gene Therapy for Sickle Cell Disease. *New England Journal of Medicine*, 386 (2), pp.138–147. [Online]. Available at: doi:10.1056/NEJMOA2109167/SUPPL_FILE/NEJMOA2109167_DISCLOSURES.PDF [Accessed 29 August 2022].

al Hinai, A. S. A. et al. (2021). PPM1D mutations appear in complete remission after exposure to chemotherapy without predicting emerging AML relapse. *Leukemia 2021 35:9*, 35 (9), pp.2693–2697. [Online]. Available at: doi:10.1038/s41375-021-01155-y [Accessed 5 September 2022].

Holz-Schietinger, C., Matje, D. M. and Reich, N. O. (2012). Mutations in DNA methyltransferase (DNMT3A) observed in acute myeloid leukemia patients disrupt processive methylation. *The Journal of biological chemistry*, 287 (37), pp.30941–30951. [Online]. Available at: doi:10.1074/JBC.M112.366625 [Accessed 5 September 2022].

Hsieh, M. M. et al. (2020). Myelodysplastic syndrome unrelated to lentiviral vector in a patient treated with gene therapy for sickle cell disease. *Blood Advances*, 4 (9), p.2058. [Online]. Available at: doi:10.1182/BLOODADVANCES.2019001330 [Accessed 29 August 2022].

Jones, R. J. and DeBaun, M. R. (2021). Leukemia after gene therapy for sickle cell disease: insertional mutagenesis, busulfan, both, or neither. *Blood*, 138 (11), pp.942–947. [Online]. Available at: doi:10.1182/BLOOD.2021011488 [Accessed 29 August 2022].

Kent, D. G. and Eaves, C. J. (2016). Adult Hematopoiesis. *Encyclopedia of Immunobiology*, 1, pp.15–25. [Online]. Available at: doi:10.1016/B978-0-12-374279-7.01003-1 [Accessed 29 August 2022].

Li, B. et al. (2020). Therapy-induced mutations drive the genomic landscape of relapsed acute lymphoblastic leukemia. *Blood*, 135 (1), pp.41–55. [Online]. Available at: doi:10.1182/BLOOD.2019002220 [Accessed 29 August 2022].

Lyne, A. M. et al. (2018). A track of the clones: new developments in cellular barcoding. *Experimental Hematology*, 68, pp.15–20. [Online]. Available at: doi:10.1016/J.EXPHEM.2018.11.005 [Accessed 29 August 2022].

Mitchell, E. et al. (2022). Clonal dynamics of haematopoiesis across the human lifespan. *Nature 2022 606:7913*, 606 (7913), pp.343–350. [Online]. Available at: doi:10.1038/s41586-022-04786-y [Accessed 29 August 2022].

Shepherd, M. S. and Kent, D. G. (2019). Emerging single-cell tools are primed to reveal functional and molecular heterogeneity in malignant hematopoietic stem cells. *Current opinion in hematology*, 26 (4), pp.214–221. [Online]. Available at: doi:10.1097/MOH.0000000000000512 [Accessed 29 August 2022].

(US), N. C. for B. I. (1998). *The p53 tumor suppressor protein*. [Online]. Available at: https://www.ncbi.nlm.nih.gov/books/NBK22268/ [Accessed 29 August 2022].

Prevalence | Background information | Sickle cell disease | CKS | NICE. [Online]. Available at: https://cks.nice.org.uk/topics/sickle-cell-disease/background-information/prevalence/ [Accessed 5 September 2022].