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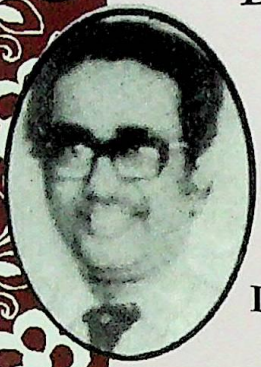

Dr. Arunasalam Sivapathasundaram Memorial Lecture – 2022

(Former Consultant Paediatrician, Teaching Hospital, Jaffna)

"Curing incurable: Development of genetic-based therapies for thalassaemia"

by

Professor Sachith Mettananda,
MBBS(Col) MD-Paed(Col),
DPhil(Oxon) FRCPCH (UK) FRCP(Edin),
Professor of Paediatrics,
University of Kelaniya

on

Monday 21st March, 2022 at 3.00 p.m

at

Hoover Auditorium, Faculty of Medicine, University of Jaffna.

Dr. Arunasalam Sivapathasundaram
Memorial Lecture 2022

Curing incurable: Development of genetic-based therapies for thalassaemia

Professor Sachith Mettananda,
MBBS(Col) MD-Paed(Col) DPhil(Oxon) FRCPCH(UK) FRCP(Edin),
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21 March 2022

Message from the Vice Chancellor

Late Dr. Arunasalam Sivapathasundaram a clinician and a paediatrician was punctual, disciplined, meticulous, and kind. His clinical skills were excellent, and his commitment to patient care is unmatchable. He was a friendly colleague to all his juniors but would not hesitate to be stern with his subordinates if the patient care was compromised. He is one of the rare examples of clinicians who went the extra mile to uplift the facilities of hospitals where they work. His fight for the construction of the 1200-bed Teaching Hospital for Jaffna is an excellent example.

He took a great interest in the activities of the Jaffna Medical Association, as well. He often conducted clinical lecture demonstrations and case discussions to disseminate the vast knowledge and experience to junior doctors and medical students. He was elected as the Secretary of the Jaffna Medical Association in July 1987 and functioned efficiently for a short period before his demise. He lost his life during the 1987 conflict and was killed on duty by the IPKF.

We are proud to have Prof Sachith Mettananda, Professor of Paediatrics at the faculty of medicine, University of Kelaniya. He has pioneered several research activities in the firld of paediatric haematology. He won a Commonwealth Scholarship from the British Government to read for a Doctor of Philosophy degree at Weatherall Institute of Molecular Medicine, University of Oxford, UK. He received clinical training in Paediatrics and Paediatric Haematology at John Radcliffe Hospital, Oxford, UK and was elected a Fellow of the Royal College of Paediatrics and Child Health in 2020. He has won many national and international awards for research. These include Commonwealth

Scholarship Award (2012), President's Award for Scientific Publications (2011, 2015, 2016 and 2018) and Ten Outstanding Young Persons (TOYP) Award for Medical Research (2016). He has also won the Vice Chancellors Award for the Outstanding Researcher in the University of Kelaniya in 2017, 2019 and 2021.

I thank Prof Sachith Mettananda for agreeing to deliver the late Dr. A. Sivapathasundaram memorial oration for the year 2022 on the title Curing incurable: Development of genetic-based therapies for thalassaemia. I believe this talk will create awareness and find a permanent cure for this disease.

All glories to Almighty.

Professor Sivakolundu Srisatkunarajah,

Professor in Mathematics,

Vice Chancellor,

University of Jaffna.

Curing incurable: Development of genetic-based therapies for thalassaemia

It gives me immense pleasure to deliver the Dr Arunasalam Sivapathasundaram Memorial Lecture this year, which I consider a great honour. It is one of the rare opportunities for a paediatrician in this country to pay tribute to a great paediatrician who made the ultimate sacrifice while treating the poor and innocent children of Sri Lanka. I thank the Faculty of Medicine, University of Jaffna, for inviting me for this lecture.

Arunasalam Sivapathasundaram was born on the 23rd of November 1939 at Puloly, Point Pedro, in a family of six children. He had his primary education at Vadamaradchi Hindu Girls' College and secondary education at Hartley College, Jaffna, where he excelled in his studies, oratory and drama.

He obtained the Diplomas in Child Health of Sri Lanka and London in 1970 and 1975, respectively, followed by the Membership of the Royal College of Physicians in the UK in 1977. During his career, he served in many parts of Sri Lanka, including Ratnapura, Balangoda, Ragama, and Lady Ridgeway Hospital for Children, Colombo, before assuming duties as the Consultant Paediatrician at Base Hospital Point Pedro in 1974. He was appointed as a Consultant Paediatrician to the Teaching Hospital, Jaffna, in 1983, where he served for four years before his untimely demise in 1987.

As a clinician and a paediatrician, Dr Arunasalam Sivapathasundaram was punctual, disciplined, meticulous, and

kind. His clinical skills were excellent, and his commitment to patient care is unmatchable. He was a friendly colleague to all his juniors but would not hesitate to be stern with his subordinates if the patient care was compromised. He is one of the rare examples of clinicians who went the extra mile to uplift the facilities of hospitals where they work. His fight for the construction of the 1200-bed Teaching Hospital for Jaffna is an excellent example.

He took a great interest in the activities of the Jaffna Medical Association, as well. He often conducted clinical lecture demonstrations and case discussions to disseminate the vast knowledge and experience to junior doctors and medical students. He was elected as the Secretary of the Jaffna Medical Association in July 1987 and functioned efficiently for a short period before his demise. It was indeed a great loss to the children and public of Jaffna and Sri Lanka when he was killed on the 22nd of October 1987 while at work in the hospital premises in front of many to whom he had been a rescuer.

I consider it a great honour to pay tribute to such a vibrant personality by delivering this lecture titled 'Curing incurable: development of genetic-based therapies for thalassaemia.'

Introduction

Despite advances in clinical medicine, several diseases remain incurable or partly curable at present. This is particularly true for many genetically inherited diseases we encounter in children. They include diseases affecting the cardiovascular system like inherited cardiomyopathies, neurological diseases like spinal muscular

atrophy, immunological diseases like severe combined immunodeficiency and haematological diseases like haemoglobinopathies. Today, in this lecture, I intend to take you through a journey in which we made an attempt to cure one of the incurable paediatric haematological diseases, thalassaemia,using an extremely novel pathway of genome-based therapeutics.

Thalassaemia is a genetic disorder of haemoglobin synthesis

I understand this audience is composed of clinicians, scientists and academics from diverse backgrounds therefore, I would start by giving a basic introduction to thalassaemia. Thalassaemia is a genetic disorder of haemoglobin synthesis due to mutations in one of the globin genes which forms haemoglobin (1). Adult haemoglobin, haemoglobin A, which is themain oxygen carrier molecule in human red blood cells (RBC), is a tetramer of two α- and two β-globin polypeptide chains. In thalassaemia, the synthesis of one of these globin chains is impaired due to autosomal recessively inherited mutations of globin genes leading to a profound reduction in haemoglobin within RBCs. Based on the type of globin genes affected, thalassaemia is divided into two forms: α-thalassaemia and β-thalassaemia. α-Thalassaemia is caused by deletions of the β-globin genes located in chromosome 16, whereas β-thalassaemia is caused by mutations in the β-globin gene in chromosome 11(2).My talk today will centre around β-thalassaemia, where we attempted to devise a cure.

β-Thalassaemia is an incurable disease

β-Thalassaemia, which was first described by the American haematologist, Thomas B. Cooley in 1925, is one of the most

common genetic diseases in the world(3). It is particularly common in the tropical belt, which extends from the Mediterranean through the middle east to South and Southeast Asia(4). Being in this tropical belt, β-thalassaemia is a common disease in Sri Lanka, too. The first report of thalassaemia in Sri Lanka comes from the famous paediatrician Professor C.C.de Silva, who described four children with thalassaemia in his publication in 1951(5). Thalassaemia was originally confined to North-Western and North Central Provinces of Sri Lanka, however, due to population migration, it is now found in every corner of the country, including the Jaffna peninsula(6). The carrier prevalence of β-thalassaemia in Sri Lanka is 1 in 40, and we see 50-60 births of children with thalassaemia each year(7).

Due to the impaired synthesis of haemoglobin A, children with thalassaemia are brought for medical attention around six months of age. These patients develop progressively worsening severe anaemia from around 3-4 months of life, leading to symptoms such as lethargy, poor feeding, fatiguability and shortness of breath when their haemoglobin reaches very low levels. The haemoglobin can go down to levels as low as 3-4 g/dL, which could be fatal if untreated (8).

The anaemia in β-thalassaemia is chronic and lifelong. Consequently, children with β-thalassaemia require blood transfusions every 2-5 weekly to maintain their haemoglobin at safe levels(9). They are transfusion-dependent for life. Regular blood transfusions are associated with several unavoidable complications that includetransfusion - transmitted infections such as HIV and hepatitis, and febrile, allergic or haemolytic transfusion reactions,

which can sometimes be life-threatening(10). Nonetheless, the most troublesome complication of chronic blood transfusion is iron overload. With each pack of blood, 200 mg of iron is infused into the body, which does not have an effective mechanism of excreting iron. Thus, iron gets accumulated in body organs, including the liver, pancreas, other endocrine organs and the heart(11). Although part of this iron can be removed using iron chelator medication, none of the current iron chelator medicationsis effective in optimally chelating iron deposited in organs. This leads to progressive dysfunction of organs leading to complications of thalassaemia, which include growth failure, hypothyroidism, hypoparathyroidism, diabetes mellitus, cirrhosis, and cardiomyopathy. Due to these inevitable complications, patients with β-thalassaemia have a poor quality of life and die prematurely(12, 13).

Bone marrow transplantation (BMT) is the only curative treatment available for thalassaemia at present. However, it is not available to a majority of patients due to a lack of suitable donors. The idealbone marrow donor is a human leukocyte antigen (HLA) identical sibling. However, most patients with thalassaemia do not have siblings as their parents were advocated not to have further children due to the 25% risk of recurrence ofthe disease in the second child. Even if some patients with thalassemia have siblings, only one-fourth of them will be HLA matched. Thus, only a limited number of patients with thalassaemia havesuitable donors limiting theusefulness of BMT as a cure(14). Additionally, BMT is associated with complications like graft failure and graft versus host disease, which can sometimes be fatal. Hence, most patients with thalassaemia in

Sri Lanka and all over the world do not undergo BMT and succumb to their illness in their late twenties and thirties(15).

Based on the facts that I just presented,it is clear that there is a desperate need for new therapies to cure thalassaemia. In the remainder of my talk, I will describe a series of pre-clinical, invitro and translational research studies that we performed to find a cure for this life-limiting disease.These research studies were commenced as part of my PhD atthe University of Oxford and were continued in Sri Lanka with the support of UK collaborators since my return to Sri Lanka in 2015.

Excess α-globin is the primary pathophysiological problem in β-thalassaemia

To comprehend the rationale of genetic-based curative therapies presented in this talk, it is important to have a clear understanding of the pathophysiology of β-thalassaemia. In healthy human RBCs, the α- and β-globin chains that combine to form haemoglobin are synthesised at equal rates to maintain the 1:1 equilibrium of α- and β-globin chains. As described before, in β-thalassaemia, thesynthesis of α-globin chains in human RBCs is impaired due to autosomal recessively inherited mutations of the β-globin gene. However, the synthesis of β-globin chains continues normally, leading to an excess of α-globin chains in RBCs. These free α-globin chains, which are insoluble and toxic, precipitate in RBCs and their precursors to cause cell destruction in the circulation and within the bone marrow(16). This destruction is the primary cause of anaemia in patients with β-thalassaemia (Figure 1).

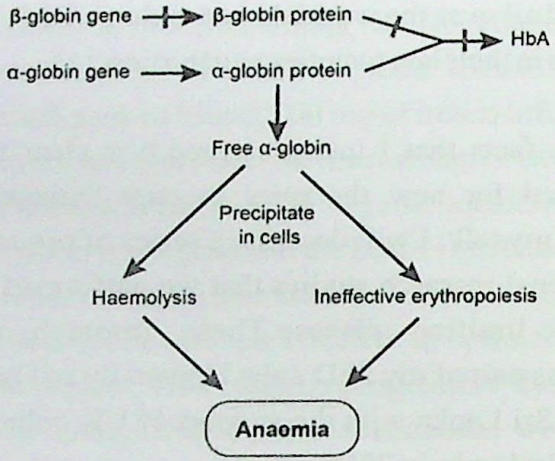

Figure 1 – Pathophysiology of β-thalassaemia

Considering the central role of excess α-globin in causing anaemia in β-thalassaemia, we hypothesisedthat controlled reduction of α-globin would be beneficial in β-thalassaemia. Reducing the excess α-globin should reduce RBC destruction, improve the severity of anaemia and decrease the need for RBC transfusion in patients with β-thalassaemia.

Natural reduction of α-globin is beneficial in patients with β-thalassaemia

To examine the existing clinical data supporting our hypothesis, we performed a comprehensive literature survey on a large number of observational, case-control and cohort studies of β-thalassaemia. In particular, we evaluated the beneficial effects of having a natural reduction of α-globin in patients with β-thalassaemia. A natural reduction of α-globin is seen in patients who have α-thalassaemia trait where one or two out of four α-globin genes are mutated. α-Thalassaemia trait is asymptomatic and does not lead to any

significant haematological abnormalities. Due to the high prevalence of both α- and β-thalassaemia in communities, the co-existence of α-thalassaemia trait is commonly found in patients with β-thalassaemia. In our review, which we publish in Blood, we found that co-inheritance of α-thalassaemia trait in patients with β-thalassaemia is associated with milder disease, older age at presentation, less severe anaemia and significantly reduced need for blood transfusions (16). Furthermore, a reasonable proportion of patients with β-thalassaemia who co-inherit α-thalassaemia trait were completely asymptomatic and lived normal lives in the community without coming to medical attention at all. These findings validated our hypothesis that therapeutic reduction of α-globin has the potential to ameliorate the disease severity of β-thalassaemia.

Understanding the regulation of human α-globin gene

The next obvious question is how can we achieve a reduction of α-globin therapeutically? Is it possible to reduce the amount of α-globin produced in RBCs of a living organism? To answer these questions, we need to have a detailed understanding of the regulation of the expression of human α-globin. For this, we again reviewed the available information on the regulation of α-globin gene, which we published in *Annals of New York Academy of Science* (17).

The expression of any gene in the human body is controlled by three inter-related regulatory mechanisms. They are enhancers, transcription factors and epigenetic mechanisms (Figure 2). With regards to the regulation of the α-globin, enhancers and epigenetic mechanisms are the two most important regulatory mechanisms.

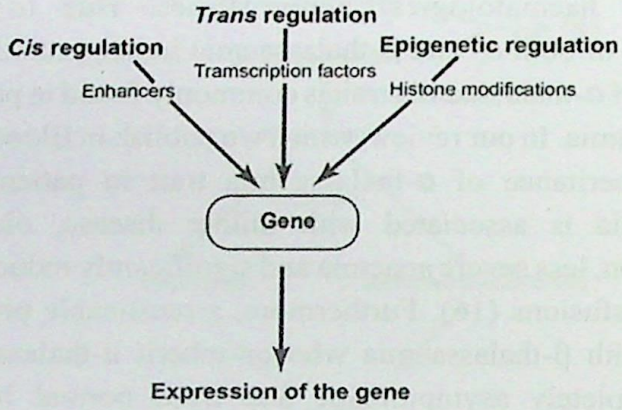

Figure 2 – Regulation of human genes

What are enhancers? Enhancers are specific DNA sequences of the genome that facilitate the expression of a gene to produce the specific protein encoded by that gene. The main enhancer of α-globin is located in the same chromosome as of α-globin, approximately 40-kilobases proximalto the gene. Several laboratory, animal and human studies have revealed that this enhancer of α-globin is critical for the normal expression of α-globin. In the absence of this enhancer, the synthesis of α-globin is markedly reduced in human RBCs.

The other important regulatory mechanism governing the expression of α-globin is epigenetic regulation. Epigenetics explain how different cells in the human body which have the same set of genes in their nucleimaintain different characteristics. In a simpler way, we know that every human cell has all genes required to produce every protein of the human body. However, only a limited number of relevant proteins are produced in each human cell. For example, globin genes are present in all human cells, including the

brain, muscle and intestinal cells. However, haemoglobin is only produced in RBC and is never produced in the brain, muscle or intestinal cells. The reason for this is that in non-erythroid cells, globin genes are silenced through chemical modifications of DNA and its associated histone proteins. These silencing mechanisms are referred to as epigenetics.

Following these studies,it was clear that if we need to modify the expression of human α-globin we musteither alter the enhancers or the epigenetics of α-globin. Is this therapeutically possible? If so, what approaches can we take? More importantly, how can we test any of the new approaches that we aspire to? Can we test these straight in humans? Or animals? The definite answer is 'no'. None of the new medicinesor therapeutic approaches can be tested directly on humans or animals. They must first be tested in pre-clinical in-vitro models. i.e.,they should be tested in human cells in laboratories before proceeding into animal experiments or phase 1 clinical trials in humans.

Development of the in vitro human erythroid cell culture system

For this purpose, we developed a human erythroid cell culture system in the laboratory at the University of Oxford. Basically, we grew erythroid cells in the laboratory from human haematopoietic stem cells (HSCs). HSCsare the cells that are responsible for the generation of RBCs and all other blood cells in the body. They are found in the bone marrow however, a small proportion of HSC scan be found in the peripheral circulation as well.Using a specialised technique, we separated these HSCs from peripheral blood of normal individuals and patients with thalassaemia and differentiated them into RBCs in tissue culture plates over a period of 21 days in a

culture medium containing erythropoietin, the hormone responsible for RBC formation in the human body. This culture system produced a large number of live RBCs, exactly similar to how they are produced in the bone marrow. It provided us with a very valid system to check the effect of medicinesand other therapeutic pathways that might reduce the expression of α-globin. The development of this new cell culture system is published in our paper in *Experimental haematology(18).*

Identification of epigenetic inhibitor drugs that silence α-globin

Next, we embarked on our attempts to reduce α-globin in RBCs generated using this culture system. Our first attempt was to change the epigenetic regulation of α-globin. Here we decided to test the effects of a new group of compounds which are known as epigenetic inhibitors. We treated RBCs using several epigenetic drugs in tissue culture plates with an aim to identify a drug that decreases α-globin production without altering the production of β-globin. The expression levels of globin genes were measured using the real-time PCR technique. This study identified two potential drugs that would decrease the production of α-globin.

Table 1 – α/β globin ratios in cells treated with epigenetic drugs compared to untreated cells. The potential drugs are in bold font

Epigenetic inhibitor	Mean α/β globin ratio (Compared to untreated)n=3	Standard deviation	p-value (compared to untreated)
CXD101	3.25	0.21	0.04
(+)- JQ1	6.45	1.55	0.03
(-)- JQ1	1.30	0.10	0.04
PFI-1	2.47	1.70	0.27

CBP/BRD4(0383)	1.36	0.67	0.45
UNC0638	3.12	0.76	0.04
IOX1	0.37	0.03	0.02*
IOX2	1.03	0.11	0.71
5-Azadeoxycytidine	3.34	2.01	0.18
Sodium Valproate	1.57	0.78	0.33
K00135	0.74	0.27	0.24
SMARCA	0.91	0.15	0.39
I-BET	2.55	0.64	0.05
Rucaparib	3.34	1.31	0.09
BAZ2B	1.19	0.12	0.11
Chaetocin	0.64	0.04	0.05*
5-Iodotubercidin	1.07	0.59	0.86
Olaparib	1.14	0.59	0.73
Entinostat	1.09	0.59	0.81
Trichostatin A	1.70	0.24	0.15
Vorinostat	0.70	0.42	0.50
Methylstat	1.75	0.42	0.09
Bromosporin	1.54	0.21	0.05
CBP probe (0113)	2.23	0.88	0.14
RVX-208	1.12	0.23	0.47
SRT1720	1.22	0.77	0.67
EX527	1.23	0.33	0.35
GSK343	1.84	1.14	0.33
C646	1.23	0.55	0.55
Sodium butyrate	2.85	1.14	0.11
Tranylcypromine	0.67	0.13	0.04*
SGC0946	0.98	0.33	0.94
UNC1215	0.87	0.57	0.73
SET7/9-1	0.91	0.07	0.15
SET7/9-2	0.89	0.29	0.58

The first drug, IOX1is an epigenetic inhibitor drugthat inhibits histone demethylase enzyme, which is responsible for the removal of methyl groups from histone proteins. It is a new chemical entity thatis not yet approved by the FDA nor entered into clinical trials. Further experiments showed that IOX1 significantly decreased the production of α-globin without altering the expression of β-globin in a dose-dependent mannersuggesting that IOX1 has a specific action on α-globin but not on β-globin (Figure 3). Cells treated with IOX1 were morphologically similar to untreated cells confirming that the treatment does not have toxicity in cells. Similarly, the expression levels of most other genes in the erythroid cells treated with IOX1 weresimilar to the untreated cells. These observations confirmed that IOX1 selectively reduces the production of α-globin without altering the production of β-globin or having cellular toxicity, thus confirming it as a suitable compound to therapeutically reduce α-globin in patients with β-thalassaemia. We published these findings in our paper in the journal Haematologica(19).

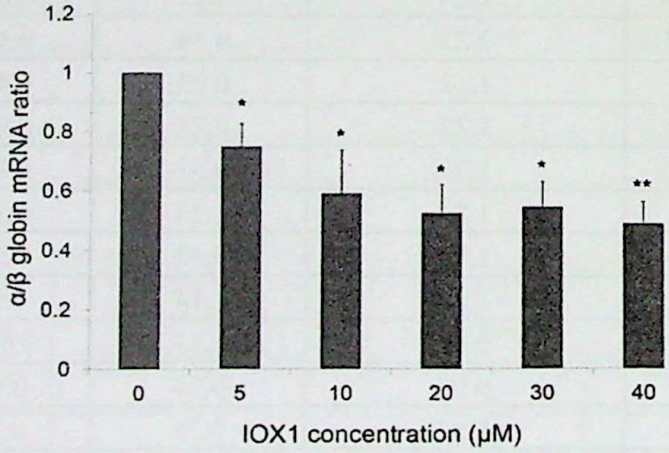

Figure 3 – Dose-dependent reduction of α*-globin by IOX1*

The second candidate drug, vorinostat, is a histone deacetylase inhibitor that blocks the removal of acetyl groups from histone proteins. It is currently licenced for the treatment of advanced cutaneous T-cell lymphoma and multiple myeloma. Similar toIOX1, vorinostat reduced the expression of α-globin in a dose-dependent manner in human RBCs. In addition to this action, vorinostat induced the expression of α-globin. Increasing *y*-globin has an additional advantage in patients with β-thalassaemia as *y*-globin can combine with α-globin to form fetal haemoglobin and further reduce the functional excess of α-globin in RBCs (Figure 4). Cells treated with vorinostat were comparable to control cells in morphology and the expression profile of other genes. These results showed that vorinostat exerts synergistic beneficial effects for patients with β-thalassaemia by reducing α-globin whilst increasing *y*-globin in RBCs. We published our findings in Scientific Reports(20).

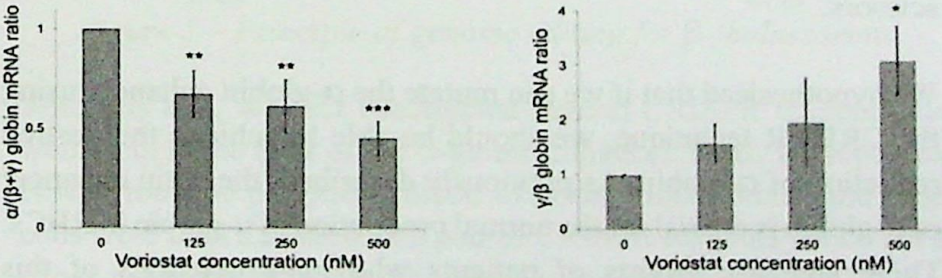

Figure 4 – Dose-dependent reduction in α-globin and induction of y-globin by vorinostat

Genome editingof α-globin enhancer

In our next approach, we examined the ways to alter the α-globin enhancer to decrease expression of the α-globin gene. Here, we usedan extremely noveltechnique ofg enetic-based therapeutics, 'genome editing'. Genome editing refers to altering the DNA sequence of a pre-determined location of the human genome to producea desirable change in a cell of our interest. This can be done using three different molecular medicine techniques, but the CRISPR technology, which was invented in 2013, is believed to be the most powerful genome-editing technique to date. In fact, CRISPR is considered one of the greatest inventions in modern medicine. Its inventors Emmanuelle Charpentier and Jennifer Doudna were awarded the Noble Prize in 2020 for this ground-breakinginvention. The basic principle of CRISPR genome editing is to mutate a specific gene or an enhancer of a gene by cutting at a pre-determined target site of the human genome, thus creating adeletion. In simple terms, CRISPR reagents work like molecular scissors.

We hypothesised that if we can mutate the α-globin enhancer using the CRISPR technique, we should be able to achieve the desired reduction of α-globin. As previously described, the main enhancer of α-globin is crucial for the normal production of α-globin in RBCs. There are case reports of patients who have deletions of this enhancerhaving α-thalassaemia despite having normal α-globin genes(21). Therefore, we thought, if we could create a deletion of this enhancer by genome editing in human RBCs, we would be able to decrease the α-globin production to levels beneficial in patients with β-thalassaemia. The ultimate goal is to harvest HSCs from patients with β-thalassaemia, genome edit them in laboratories to

create a deletion of α-globin enhancer and transplant them back to the same patient as an autologous BMT (Figure 5). Hypothetically, erythroid cells generated from these stem cells should have decreased levels of α-globin which should lead to reduced RBC destruction and to a potential 'cure' of β-thalassaemia.

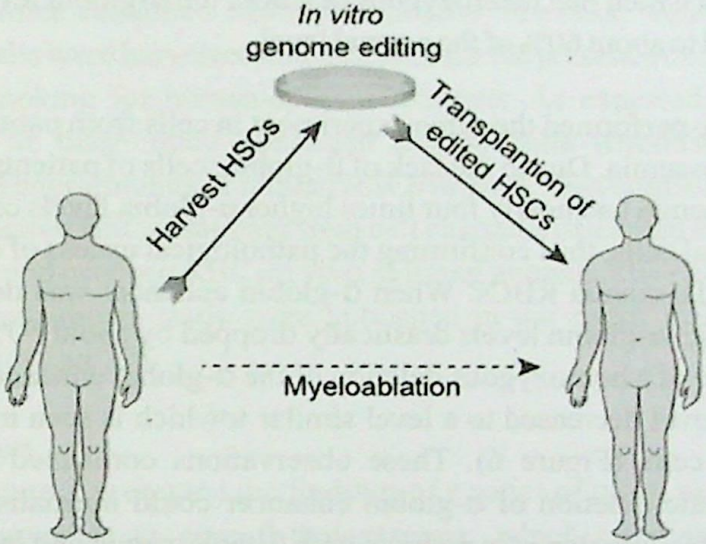

Figure 5 – Principle of genome editing for β-thalassaemia

With this aim, we first constructed several CRISPR reagents that would cut at the ends of the α-globin enhancer. Next, we separated HSCs from the peripheral blood of normal individuals, and these cells were then treated with a pair of CRISPR reagents which cut at either end of the enhancer. The aim was to create two cuts in DNA on either side of the α-globin enhancer to create a deletion of the enhancer. The efficiency of this process is not 100% therefore, some cells would have a homozygous deletion (i.e.,α-globin enhancer is deleted in both copies of the chromosome), whereas the others have a heterozygous deletion (i.e.,α-globin enhancer is deleted only in one chromosome).These genome-edited HSCs were then

differentiated into RBCsusing the previously described culture system and examined for the effect of genome editing by RT-PCR.As expected, homozygous deletion of the α-globin enhancer resulted in a statistically significant reduction in α-globin production to approximately 13% of the normal levels. Similarly, in the cells which had heterozygous deletion, the α-globin levels were reduced to about 60% of the normal level.

Then we performed the same experiment in cells from patients with β-thalassaemia. Due to the lack of β-globin, cells of patients with α-thalassaemia had nearly four times higher α-globin levels compared to normal cells, thus confirming the pathological excess of α-globin in β-thalassaemia RBCs. When α-globin enhancer was deleted in one allele, α-globin levels drastically dropped by about 50%. In the presence of a homozygous deletion of the α-globin enhancer, the α-globin level decreased to a level similar towhich is seen in normal control cells (Figure 6). These observations confirmed that the therapeutic deletion of α-globin enhancer could normalise the α-toβ- globin imbalance in patients with β-thalassemia and invariably leads to less severe disease or a 'cure' of thalassaemia.

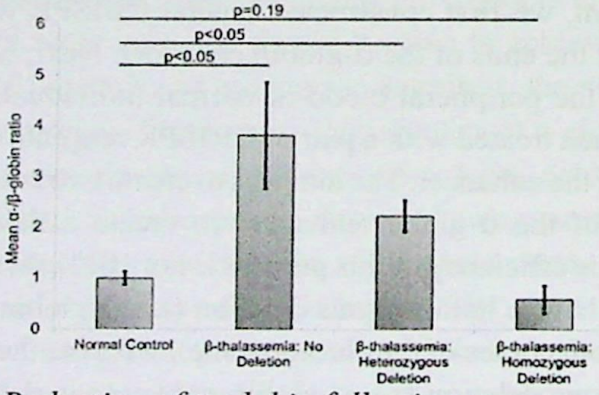

Figure 6 – Reduction of α-globin following genome editing of α-globin enhancer of β-thalassaemia RBCs

After these successful in vitro experiments, the obvious question was, would these results remain the same in vivo? Could genome-edited cells remain viable in the body? To examine this, we performed animal studies in mice. Here we transplanted genome-edited human HSCs to bone marrows of fourimmunodeficient mice. All four mice remained healthy, and after 12weeks, their bone marrow cells were harvested and analysed for the presence of human cells by looking for human-specific antigens. As expected, bone marrows of these mice contained human cells demonstrating successful engraftments of human HSCs in mice. This confirms that genome-edited HSCs maintain stem cell characteristics, can survive long term and produce RBCsin vivo in living animals.The results of the genome editing study were published in our paper in Nature Communication(22).

Conclusion

In this lecture, I presented the findings of a series of novel genome-basedtherapeutics to cure β-thalassaemia, which remains alife-limiting disease in a majority. Firstly, by studying the available data from several observational, case-control and cohort studies, we demonstrated that reduction of α-globin is beneficial and can reduce the need for blood transfusions in patients with β-thalassaemia. Next, by examining the regulation of the α-globin gene, we showed that modification of enhancers or epigenetic environment of α-globin are the most feasible areas to target therapeutically. Then I presented how the in vitro RBC culture system was developed to test the effects of medicines and different therapeutic modalities that target α-globin. Using this culture system, we identified two potential drugs, IOX1 and vorinostat, that reduce α-globin in RBCs, which can be used to devise a cure for thalassaemia. Finally, I

showed the successful utilisation of CRISPR genome editing of α-globin enhancer in HSC to reduce the expression of α-globin in erythroid cells.

There are many advantages for these approaches over the existing management of thalassaemia. The pharmacological approach with epigenetic drug targeting has the obvious advantage of being easy to use as a pill. If a pill that reduces the blood transfusion requirement can be developed using our findings, it will be a tremendous discovery forpatients in developing countries like ours. To achieve this goal, major optimisations should be done to both IOX1 and vorinostat before they can be used in phase 1 clinical trials. The support of the pharmaceutical industry is required for such optimisations.However, as thalassaemia is considered a disease of the poor, it gets a low priority in drug development pharmaceutical research which mostly concentrate on the return on investment.

The main advantage of the CRISPR genome editing approach is that it alleviates the need for HLA-matched sibling donors,which is the main limiting factor for BMTnow. When this therapy is fully developed, it will be available to all patients with β-thalassaemia. Also, as transplanted cells are autologous,this approacheliminates the need for immunosuppression and minimisesthe serious adverse effects of BMT.

However, several outstanding challenges must be addressed before CRISPR genome editing becomes standard therapy for β-thalassemia. Although we can target the genome editing tools to our desired site (i.e., α-globin enhancer) with high precision, CRISPR genome editing tools can introduce off-target mutations in unwanted

sites of the genome. Genetic modifications are permanent, and these deleterious off-target mutations might create cells with oncogenic potential or functional impairment.Therefore, genome editing techniques should be optimised to improve the precision of editing and to minimise off-target mutations before they can be introduced into clinical trials.

Despite several challenges of genome editing, CRISPR genome editing has reached clinical trials for thalassaemia even now. A Swiss-American biotechnology companyCRISPR Therpeutics which works in collaboration with Harvard University, recently published the preliminary results of the first-everphase 1 / 2 clinical trial that utilised CRISPR genome editing to induce fetal haemoglobin production in patients with thalassaemia. In this trial, a 19-year-old girl with thalassaemia who underwent autologous BMT with genome-edited HSCs was transfusion independent for 21 months, confirming the therapeutic utility of genome editing in thalassaemia.

In conclusion, during my talk, I hope I was able to show you how new scientific technologies can effectively betransformed into therapeutics to cure an incurable disease. In this example of thalassaemia, both epigenetic drug targeting and CRISPR genome editing of enhancers showed considerable promise in devising a cure. Therefore, with the advancement of scientific discoveries in epigenetics and genome editing, it is reasonable to believe that most incurable diseases we see in our children at present will be easily curable in the very near future.

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