Haematologists at the George Papanicolaou Hospital in Greece are hopeful that, by studying the haematopoietic stem cells of patients with inherited blood disorders, they can pave a path towards safe and successful gene therapy for individuals with thalassaemia.

**THALASSAEMIAS ARE BLOOD-RELATED**

Genetic disorders. Among inherited single-gene disorders such as sickle-cell anaemia or haemophilia, thalassaemia is the most commonly occurring, warranting its designation by the World Health Organization (WHO) as a major public health concern. It arises from a single faulty globin gene, or indeed from its complete absence, and it is characterised by a failure to produce haemoglobin effectively. If conventional treatments fail, patients can be exposed to devastating cardiovascular complications, bone deformities and potentially fatal iron overloads in vital organs.

Though current treatment options, the supporting transfusions and chelation, and the curative allogeneic haematopoietic stem cell transplantation (HSCT) available to thalassaemic patients have progressed, they often come with high risks. Not least among them are iron overloads from repeat blood transfusions and graft-versus-host disease (GVHD) from transplantation. Moreover, lifelong treatment for conventional therapy-compliant sufferers severely compromises their quality of life, while also placing a huge financial burden on national economies.

Director of the Haematopoietic Cell Transplantation Unit (HCTU) and the Gene and Cell Therapy Center at George Papanicolaou Hospital (GPH) in Greece, Dr Evangelia Yannaki is a haematologist working alongside haematology specialists from the University of Washington in the US and the Memorial Sloan-Kettering Cancer Center in New York, USA, to bring the considerable benefits of gene therapy closer to a reality for thalassaemic patients.

**HEMATOPOIETIC STEM CELL TRANSPLANTATION**

Transplantation approaches to treating thalassaemias are centred on grafts as a replacement source of vital HSCs, which are cells giving rise to all types of blood cells in the body, and are derived from genetically compatible sibling donors. Sufferers of classes I or II thalassaemia who are 17 years old or younger and have a sibling donor who is genetically compatible stand an 82–93 per cent chance of being cured.

Thalassaemic patients who fall outside of this narrow margin, however, face lower odds of survival. A lack of genetically matched donors is a reality for many, as are occurrences of GVHD. To counter graft-against-host and host-against-graft immunological reactions that can even occur in fully matched donor-recipient pairs due to the recognition of each other as ‘foreign’, patients must take long-term courses of immunosuppressants, thereby rendering them increasingly susceptible to opportunistic infections. Moreover, in many cases, immunosuppressants cannot effectively prevent or treat these complications.

**ALTERNATIVE THERAPY**

Scientists are now suggesting gene therapy as a possible alternative. In order to treat patients without the need for compatible donors and free them from a dependence on transfusions and chelation, Yannaki intends to one day enter a functioning copy of the β-globin gene into patients’ own HSCs, thereby using gene therapy as a way to restore normal haemoglobin production. However, although the procedure is successful in treating other genetic diseases such as sickle-cell anaemia and haemophilia, there are obstacles that must be overcome before it can be used as a viable treatment for thalassaemia.

In this endeavour, Yannaki has been investigating where best to obtain the large quantities of HSCs that are critical for treating β-thalassaemia with gene therapy. To date, she has discovered that mobilising HSCs into the peripheral blood that circulates the body using granulocyte-colony stimulating factor (G-CSF) and immunostimulant plerixafor offers the best-performing HSC graft source in both murine models of thalassaemia and human patients. These mobilising agents cause the forced movement of HSCs from bone marrow into the blood, where they can be collected via a procedure called cytopheresis.

Until recently, the safety and yield of HSCs provided by thalassaemic patients after mobilisation were largely unknown in the
context of gene therapy. Yannaki and her colleagues have assessed the safety and efficacy of HSC mobilisation using a range of available methods in 40 8-thalassaemic adults. They then tested how these differently mobilised cells behave in culture, and later, genetic correction by lentiviral vector gene transfer of a normal copy of 8-globin and transplantation into immunodeficient mice. The researchers’ goal is to optimise the mobilisation strategy and define the optimal graft for patients with thalassaemia before they embark upon a gene therapy trial.

OLD VERSUS NEW

Mobilising HSCs in the peripheral blood is minimally invasive and able to produce a far greater yield of HSCs than conventional bone marrow harvest, but until plerixafor appeared on the scene, G-CSF was the only option available to do so. Though fairly well tolerated in patients with haematological malignancies and normal HSC donors, G-CSF mobilisation has been associated with incidences of thrombosis and splenic rupture. Furthermore, in some cases, G-CSF simply does not result in effective mobilisation, leading to repeated attempts that arduously extend the period of apheresis – the process by which HSCs are extracted from donor blood.

In two clinical trials, Yannaki and her colleagues sought to determine, for the first time, the safest and most efficacious method of mobilising HSCs in 8-thalassaemic adults who are predisposed to spleen enlargement and thrombosis. Both patients with an intact spleen and those who had undergone a splenectomy (SPL– spleen removal) were enrolled in the studies. For non-SPL 8-thalassaemic adults, the researchers found that G-CSF works tolerably well, but this scenario is dramatically reversed in SPL patients whose treatment results in excessive hyperleukocytosis. In order to bring the abnormal white blood cell count down to a safe level, it is necessary to reduce the G-CSF dose significantly, which leads to inferior HSC yields. To prevent hyperleukocytosis it is possible to preemptively treat asplenic patients with hydroxyurea – an effective drug for combating excessive proliferation of blood cells. Though this has substantial benefits for patient safety and results in good yields of HSCs (such as CD34+), the mobilisation procedure slows to six weeks as a result. In contrast, though inducing mild leucocytosis, plerixafor is well-tolerated, and manages to affect an incredibly rapid mobilisation – within one day – in both SPL and non-SPL patients, thereby appearing to be the fastest and safest option.

A POWERFUL BLEND

Despite the obvious advantages of plerixafor, it has not always proven to be the most efficacious treatment. Indeed, HSC yields have been, on occasion, inferior in comparison with those induced by G-CSF. How then do two perform as a combined agent for mobilisation in patients with thalassaemia? Yannaki’s studies bear witness to what appears to be an incredible synergy between plerixafor and G-CSF, showing their combined effects to be superior to their constituent parts in safety and efficacy. Not only does the combined treatment act with great speed, but it is also possible to obtain high yields of CD34+ from SPL patients in a single apheresis procedure despite the mandatory dose reductions of G-CSF needed to avoid hyperleukocytosis.

Asplenic – if not all – thalassaemic patients should be treated upfront with G-CSF and plerixafor as an alternative to today’s reality of remobilisation procedures, high rates of failure and prolonged aphereses. The benefits of combining the two agents towards a safe and effective gene therapy treatment for 8-thalassaemia are not confined to HSC yields, but extend to the mobilised cells themselves, namely through higher engraftment capacity and 8-globin expression by each copy of the transferred normal gene upon transplantation into immunodeficient mice.

Yannaki’s findings are indicative of a promising future for gene therapy. The process of mobilisation has been optimised and the best source of HSCs determined, both of which should greatly improve the outcome of gene therapy towards safe and effective alternatives for those ill-served by the only treatments available today.