Using induced pluripotent stem cells to find causes and cures for bone marrow failure in children and young adults

Professor Andrew Elefanty

Grant-in-Aid 2018 ongoing

murdoch children's research institute

The work of Professor Elefanty's project represents a collaborative effort between three laboratories at the Murdoch Children's Research Institute; the Blood Development (Andrew Elefanty and Elizabeth Ng) and Immune Development laboratories (Ed Stanley), who have worked together on the blood differentiation and genetic manipulation of human pluripotent stem cells for 18 years, and the Translational Bioinformatics group, headed by Cas Simons, who have the necessary expertise in analysis of genomic sequencing that is required to complement cell and molecular biology skills.

Bone marrow failure is not one disease, but a complex mixture of many disorders that have a similar end point– the inability of the bone marrow to make sufficient blood cells. In some cases, there are mutations in genes that are required for normal blood formation, but in many other instances the cause is not known, and it appears that the immune system functions abnormally and attacks the body's own blood cells.

Professor Elefanty's research aims to explore the causes of bone marrow failure, and therefore to discover new approaches to treatment and cure. His team is comparing the blood forming ability of stem cell lines made from patients with bone marrow failure with lines made from their unaffected relatives. These stem lines are made from bone marrow samples harvested at diagnosis from young patients with bone marrow failure and also from blood samples given by their parents, who serve as 'controls' against which the patients samples can be compared.

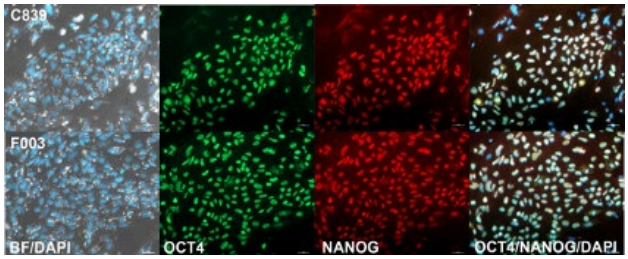
The bone marrow or blood cells from patients and their parents are first turned into stem cells – called induced pluripotent stem cells, or iPSCs – using a Nobel Prize-winning genetic 'trick' involving the transient, enforced expression of four genes. The process takes about a month, leading to the generation in the

laboratory of normal cell lines that are immortal, can be cryopreserved and thawed as required, and can be directed to turn into any cell type in the body, including new blood cells (a process called 'differentiation'). Professor Elefanty explains, "For many years our laboratories have been studying the process of differentiating iPSC into blood stem cells, and we have become progressively more proficient as we understand more about the process. For example, we can now make blood cells from iPSC that resemble the blood cells made during human development – they express the same genes and the cells display similar functions, in some cases including an ability to engraft into the bone marrow of laboratory mice."

This extraordinary progress has enabled Professor Elefanty's laboratory team to perform similar differentiations and analyses on blood cells made from the iPSCs of patients with bone marrow failure. These analyses will include sequencing the genomes of these patients and their relatives to determine whether there are differences in the patient's genes that might explain their inability to robustly make sufficient blood cells. In cases in which genetic abnormalities are found, his team are aiming to determine how this causes the bone marrow to fail, and how to treat or cure the problem. "Eventually, we would like to use our skills in genetic manipulation to 'correct' the genetic problem in the patient iPSCs, and therefore to be able to make non-diseased blood cells to transplant back into these patients."

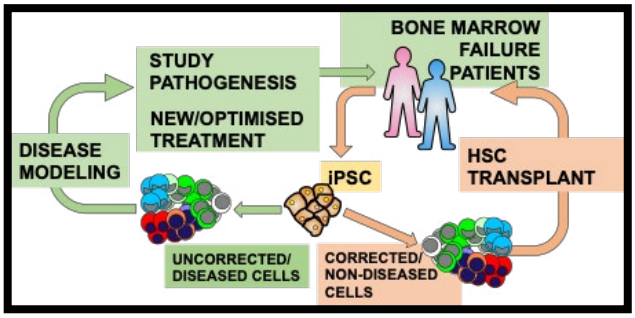
For the larger proportion of patients who do not appear to have a genetic abnormality, Professor Elefanty's work will compare the ability of the patient and parent iPSC lines to differentiate into blood cells – to determine whether the patient samples are intrinsically poor at making blood cells, or whether they are normally fine, and some secondary event (an abnormality in immune cell function, for example) was to blame for their bone marrow failure. This information will be most important, since it is not clear whether there is a problem in blood formation in patients with bone marrow failure even when there is not a clearly identified genetic abnormality.

Currently, iPSC lines from 6 patients with bone marrow



Immunostaining of iPSC lines derived from patient (C839) and parent (FO03), showing robust expression of the pluripotency genes OCT4 and NANOG.

Professor Andrew Elefanty



Applications of patient iPSCs in the study and treatment of Bone Marrow Failure. Correction of underlying mutations or use of non-diseased iPSCs followed by differentiation produces blood stem cell (HSCs) for replacement cell therapy. Diseased iPSC cells facilitate study of pathogenesis and enable screening for new therapies.

DNA samples for patients and their parents for sequencing studies to identify genetic abnormalities are also being performed, and studies to determine whether these patient iPSCs differentiate normally into blood cells or whether this function is impaired are underway. The information generated from the blood forming studies with the results from the DNA sequencing will then be correlated for analysis.

Professor Elefanty's work excitingly contributes to two areas of research and therapy for patients with bone marrow failure. Firstly, the ability to differentiate stem cells enables the study of abnormalities in blood formation in these patients, and subsequently to trial various therapies on cells in the laboratory before they are used in patients. Secondly, if genetic abnormalities can be identified in some patients, there is potential to correct these defects and then generate sources of blood cells in the laboratory that could be used as a transplantation therapy for these patients. These options are shown schematically in the figure, and offer incredible hope in achieving the Maddie's Vision mission.