Establishing an *in vivo* humanised mouse model for telomere related Bone Marrow Failure Syndromes

Professor Tracy Bryan

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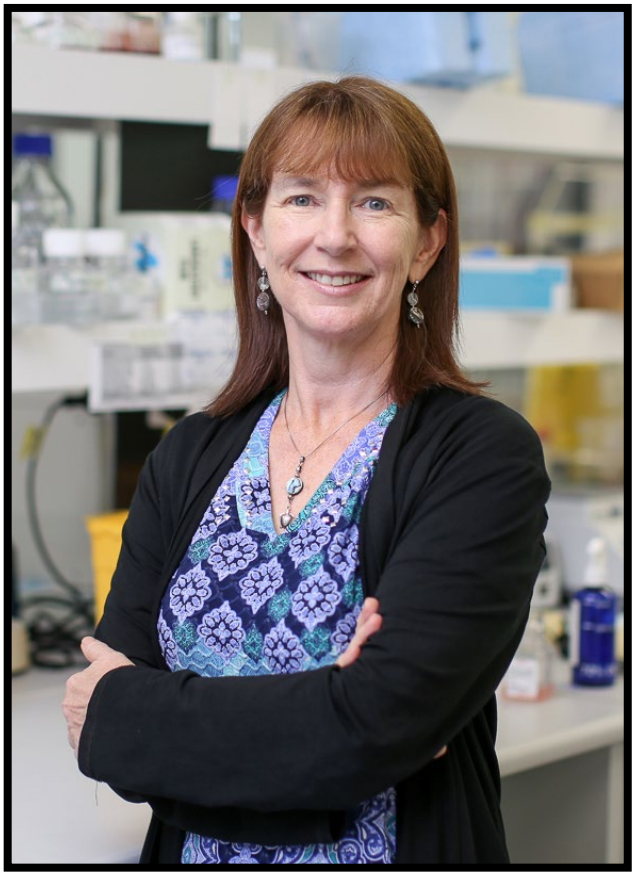
For the last 25 years, Professor Tracy Bryan has been performing research aimed at understanding how the protective tips at the ends of chromosomes, called telomeres, are linked to cancer, aging and other diseases. Her research team is particularly interested in understanding how an enzyme called telomerase functions to prevent telomeres from shortening. This is relevant to cancer, because a majority of cancers produce too much telomerase – this enables them to keep lengthening their telomeres, overcoming the signals that tell healthy cells to stop dividing. However, in patients with inherited mutations in telomerase genes, the telomerase in their stem cells is defective or present at low levels, which means their telomeres become too short and these cells die prematurely.

Many families with inherited BMF caused by short telomeres have not yet had their causative mutation(s) identified. Professor Bryan is therefore collaborating with Drs Lucy Fox and Piers Blombery, and other haematologists around Australia, to determine whether any telomerase mutations found in these families are likely to be causing their disease. Experimental procedures Professor Bryan has developed over the last 20 years to recapitulate the patient's mutation in cancer-derived cells in culture are utilised to determine whether the mutated telomerase is defective. However, these experimental systems have limitations, since telomerase in cancer cells does not always behave the same as telomerase in bone marrow stem cells.

Excitingly, however, gene editing technology is rapidly advancing. Clustered regular interspaced short palindromic repeats (CRISPR) based genome editing of human bone marrow stem cells has matured a lot in recent years. This project aims to use these techniques for setting up a much more clinically-relevant model of telomere-related BMF. Professor Bryan explains, "We are introducing patient mutations into the genomes of blood stem cells from healthy donors, and we can then



Professor Tracy Bryan, CMRI

determine the impact of the mutation on the ability of telomerase to maintain telomeres in the cell type that is most relevant to this disease. This will also allow us to make fundamental discoveries about how short telomeres lead to bone marrow failure, which is not fully understood."

About 30 – 40% of patients with inherited BMF do not know the identity of the mutation causing their disease. The current experiments will assist in providing definitive molecular diagnoses to individual families, allowing them to understand what is causing their disease and helping their clinicians make treatment-related decisions. For example, it is known that patients with short telomeres often experience high levels of toxicity from standard bone marrow transplant procedures, so identifying such patients would allow use of modified procedures that would spare them this toxicity.

Professor Bryan's research is completely aligned with the mission of Maddie's Vision, to ultimately find a cure for bone marrow failure, "Currently, patients with bone marrow failure are given transplanted stem cells. However, the five year survival rate after bone marrow transplant in short-telomere patients remains only approximately 65%, with complications including graft-versus-host disease. It would be preferable to use a gene-corrected version of the patient's own cells for the transplant, but such strategies are challenging in BMF patients. However, very recent progress in the success rate of CRISPR-based gene editing in human blood stem cells raises the exciting possibility that the technology has matured sufficiently to make gene therapy a viable strategy for inherited BMF syndromes. The techniques we are refining while introducing telomerase gene mutations into healthy human stem cells will theoretically be able to be applied in reverse to fix the same mutations in patient cells, and we want to test this as the next stage in this research. Furthermore, these techniques won't only be applicable to telomere-related BMF, but also may be applied to fix genes causing other forms of BMF. We would warmly welcome collaborations with other CRE Executive member researchers to test this!"

Professor Bryan undertook an undergraduate Bachelor of Science degree in biology at Macquarie University in Sydney, before travelling to Johns Hopkins University in the USA to gain hands-on experience

in a world-renowned cancer genetics laboratory. She returned to Australia to do a PhD on the role of telomeres in cancer cells with Professor Roger Reddel at Children's Medical Research Institute (Westmead), before travelling back to the USA for postdoctoral training in the lab of Professor Tom Cech at the University of Colorado, Boulder. Professor Cech's laboratory had just identified the gene for human telomerase and it was a fantastic opportunity for her to be involved in the initial investigations into how telomerase works. While in the Cech laboratory, she developed a huge appreciation for the powerful role of fundamental biochemical analysis of molecular machines, and how much information they impart about disease mechanisms. This has shaped the research she is now undertaking in her own laboratory at the Children's Medical Research Institute, Westmead, Sydney.

When she returned to Australia, she also brought back her husband, whom she had met in the Cech laboratory, Dr Scott Cohen, a fellow telomerase researcher, "Even more than our shared love of science, we also shared a love of hiking in the Colorado mountains, and now our favourite way to unwind is bushwalking in the Sydney area, often with our two dogs."

Professor Bryan's project is very collaborative, involving a large Australian and international team with complementary expertise. The research team at CMRI led by both Professor Bryan and Dr Cohen, brings expertise and technology related to telomeres and telomerase, "Many of the techniques for studying this enzyme were developed or refined by us. We also have fantastic colleagues at our institute, including Associate Professor Karen MacKenzie, whose expertise relates to understanding telomerase specifically in human blood cells. Professor Ian Alexander and Dr Leszek Lisowski at CMRI are among Australia's leading experts in gene therapy approaches for treating disease, and their expertise will be vital for this project. We are also collaborating with Professor Matthew Porteus at Stanford University in the USA, who has developed the most recent techniques for genome editing of human blood cells. Through Maddie Riewoldt's Vision, we have also made linkages with other Australian researchers aiming to apply gene therapy approaches to other subsets of inherited BMF patients, using complementary techniques, so we are very excited about the possibility of pooling all of our expertise and working together towards this goal in the coming years."

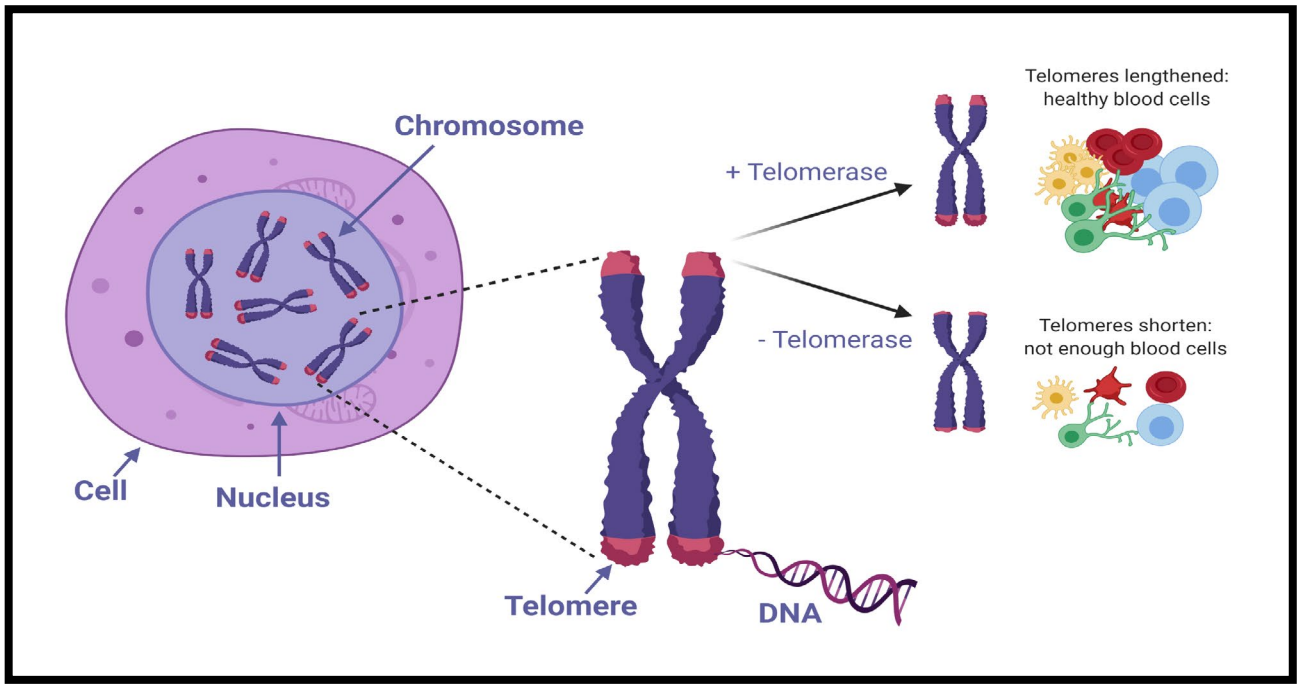


Illustration depicting location of telomeres and function of telomerase