Project Details	
Project Code	MRC22NMHBr Kemp
Title	In vivo Haematopoietic Stem Cell Gene Therapy for People with Friedreich's Ataxia.
Research Theme	Neuroscience & Mental Health
Summary	Friedreich's ataxia is an incurable neurological disorder, typically presenting in late childhood. Children born with the condition experience progressive accumulation of nervous system damage and neurological disability. The project will be to develop an in vivo gene therapy approach for people with Friedreich's ataxia that offers the prospect of a universal, safe, and rapidly translatable treatment and a major advance for the genome editing field.
Description	Friedreich's ataxia (FA) is an incurable neurological disorder, typically presenting in late childhood. It is the commonest hereditary ataxia, affecting at least 1 in every 50,000 people. People with the condition experience progressive accumulation of neurological disability with impaired muscle coordination, weakness and difficulties with speech, hearing, vision and sensation. It is caused by a genetic mutation in the frataxin gene (FXN), which carries the genetic code for a protein called frataxin; a mitochondrial protein involved in iron-sulphur cluster biosynthesis and antioxidant protection. There have been numerous studies looking at possible new therapies for FA, but, as yet people with FA remain without any treatment to limit disease progression. Our studies have provided evidence that myeloablative allogeneic haematopoietic stem cell (HSC) transplantation offers the prospect of a disease-modifying treatment for people with FA. When used in clinical practice, however, allogeneic transplantation is associated with significant morbidity and mortality, in addition to challenges of finding an appropriate immunologic matched stem cell donor. To avoid the major limitations of allogeneic HSC transplantation, protocols for HSC gene therapy, involving transplantation of autologous ex vivo lentiviral vector-transduced HSCs into myeloablated recipients are being investigated. We wish to further mitigate the dangers associated with myeloablative high-dose chemotherapy conditioning regimens, and the need for highly specialised cell culture and clinical facilities. We therefore aim to develop a novel in vivo HSC gene therapy approach. To achieve this, HSCs from the bone marrow will be mobilised into the peripheral blood of FA patients and genetically corrected through intravenous injection of CRISPR/Cas9 modified helper-dependent adenoviral vectors. We will carry out experimental work packages to address three specific research objectives (RO): RO1 (Year 1) - Optimisation of CRISPR/Cas9-mediated GAA gene editing of the FXN gene locus in fibroblasts and lymphoblastoid cells derived from people with FA. CRISPR RNAs will be designed and tested for their ability to excise the GAA expansion, situated within Intron 1 of the FXN gene, in cultured fibroblasts and lymphoblast cells derived from people with FA (these cells are already available in our laboratory). This work will be conducted with Professor Nick Allen at the Genome Editing and Transgenics Facility at Cardiff University. The effects of GAA excision on restoring frataxin expression (qPCR/western blotting), mitochondrial function (aconitase & SDH activity assays) and cellular bioenergetics

	(Seahorse XFp Analyzer) will be analysed. RO2 (Year 2) - Generation of helper-dependent adenovirus (HDAd5/35++) - CRISPR/Cas9 vectors. Helper-dependent adenoviral vectors (HDAd5/35++) that target human CD46, a receptor that is expressed on primitive HSCs, will be generated for subsequent experimental use. Using state-of-the-art cloning and viral culture techniques, therapeutic HDAd5/35++ particles will be designed and constructed by the student using HDAd plasmids containing optimised CRISPR/Cas9 machinery and fluorescent reporter and/or positive (e.g. Puromycin; P140K) selection cassettes. This work will be carried out under the guidance of Professor James Uney's laboratory. RO3 (Year 3) - Develop a clinically relevant transduction protocol for stable FXN GAA correction to isolated human FA HSCs. Here the student will provide proof-of-principle of clinically relevant HDAd transduction and FXN GAA excision of isolated human HSCs (FA CD34+ cells). Under the guidance of Dr Kemp, using advanced culture techniques, the student will investigate the safety, efficiency, and stability of GAA excision to human HSCs and their progeny. The effects of viral delivery to HSCs on cell proliferation, differentiation, frataxin levels, and mitochondrial function will be determined.
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