



SUMMARY OF FINAL REPORT

Neuron-validated approaches for developing Friedreich's ataxia therapeutics

Principal researchers: Dr Richard Wade-Martins, University of Oxford and Dr Filip Lim, Universidad Autonoma de Madrid

Dates of project: July 2007 – July 2009

Background and aims:

The project is a close collaborative interaction between the laboratories of Dr Wade-Martins (Oxford) and Dr Lim (Madrid) jointly funded by Ataxia UK and the Friedreich's Ataxia Research Alliance (FARA).

Our focus has been on the development of *in vitro* neuronal models based on the construction and use of novel *FRDA* genomic DNA locus expression vectors (see below). These cellular models offer a platform for the analysis of the regulation of *FRDA* gene expression, for the screening of compounds able to up-regulate frataxin expression and finally for the identification of factors involved in GAA repeat instability.

The Oxford aims were focused on the construction and development of high capacity herpes simplex virus genomic DNA expression vectors for:

- a) Investigation of global changes in gene expression using microarrays following vector transduction
- b) Investigation of regulation of *FRDA* transcription
- c) Construction of *FRDA* transcription reporter vectors
- d) Construction of GAA repeat expression/expansion vectors

Summary of Results:

We have developed a luciferase-based assay for the quantitative read-out of expression of frataxin protein from the whole genomic locus of the Friedreich's ataxia (FA) gene, *FRDA*, in human neuronal-like cells. This will allow us to investigate mechanisms underlying the regulation of gene expression of the normal gene, or the disease-associated locus with expanded GAA repeats in intron 1, and ultimately provide a method of high-throughput screening of compounds to alleviate *FRDA* transcriptional repression.

Lay summary of the results:

Friedreich's ataxia (FA) is a disease caused by a mutation in the gene coding for frataxin (*FRDA*), in particular a 3-base pair sequence (GAA) is repeated up to 1700

times in the mutated *FRDA* gene. It is still not known how this expansion occurs and why it is pathological. The use of a suitable nerve cell (or neuronal) model to study the effect of the GAA mutation is of vital importance for the identification of effective therapeutic strategies for FA. Although FA is mainly a disease affecting neurons, most of the current studies are performed on blood or skin cells, isolated from FA patients and thus carrying the GAA mutation.

Here we describe the development of cells derived from neurons or nerve cells that recapitulate the effect of the GAA mutation observed in Friedreich's ataxia. While many research groups use short artificial versions of the frataxin gene, we used the full length of the gene as it occurs naturally on human chromosomes, which allows a deeper understanding of the mechanisms causing FA, by closely resembling what happens in mutated cells. We modified the full-length gene to include the GAA mutation, added a reporter gene called 'luciferase' which flashes light when activated, and finally we introduced this mutated full length frataxin gene into cells derived from neurons. When the gene is active we record the flashes of light to monitor how the mutant and normal copies work.

The neuronal cell model developed in our laboratory represents a novel tool which will provide easier understanding of the mechanisms causing FA and allow identification of new therapies for Friedreich's ataxia.

Benefits to people with ataxia arisen/likely to arise from this research:

The neuronal cell model developed in our laboratory represents a novel tool which will provide easier understanding of the mechanisms causing FA and allow identification of new therapies for Friedreich's ataxia.

Publications arisen from this project:

Our laboratory pioneered the development of the iBAC vector system and we have just published a review article in *Molecular Therapy*, the leading gene therapy journal, discussing the development of the technology:

Lufino M, Edser PA, **Wade-Martins R**. (2008) Advances in High-capacity Extrachromosomal Vector Technology: Episomal Maintenance, Vector Delivery, and Transgene Expression. *Molecular Therapy* 16(9):1525-38.

In preparation

Lufino M, Ferreira da Silva A, Alegre-Abarrategui J, **Lim F**, and **Wade-Martins, R**. (2010) A novel genomic DNA-reporter neuronal cell model for the study of Friedreich's ataxia allows rapid quantitative read-out of frataxin expression levels from the expanded locus. Manuscript in preparation.

Gimenez-Cassina A, **Wade-Martins R**, Gomez-Sebastian S, Corrona, J-C, **Lim F and Diaz-Nido, J**. (2011) Infectious delivery and long-term persistence of transgene expression in vivo by a 135 kb iBAC-*FRDA* genomic DNA expression vector Manuscript in revision.

Conferences/ meetings where this research has been presented:

Invited Conference Presentations:

Wade-Martins R. (2008) "High capacity episomal genomic DNA delivery vectors for gene expression *in vitro* and *in vivo*". *Tripartite Meeting in Gene and Cell Therapy: Irish Society for Gene and Cell Therapy, British Society for Gene Therapy & International Society for the Cell and Gene Therapy of Cancer 2008* Cork, Ireland in June 2008.

Reported in:

Guinn B, Casey G, Collins S, O'Brien T, Alexander Y, Tangney M. (2008) Tripartite Meeting in Gene and Cell Therapy: Irish Society for Gene and Cell Therapy, British Society for Gene Therapy & International Society for the Cell and Gene Therapy of Cancer 2008. *Hum Gene Ther.* 2008 Jul 24. [Epub ahead of print].

Lufino M, Alegre-Abarrategui J, Lim F, and **Wade-Martins R.** (2009) "Genomic DNA expression vectors for functional molecular genetic studies on Friedreich's ataxia in neuronal cells *in vitro* and *in vivo*". *Euro-Ataxia Research Meeting, September 2009, Valladolid, Spain.*

Lufino M, Alegre-Abarrategui J, Lim F and **Wade-Martins R.** (2009) "A novel genomic DNA-reporter gene expression vector for the study of Friedreich's ataxia in neuronal cells". *Friedreich's Ataxia Therapeutics Symposium, FARA, July 2009, Philadelphia, PA, USA.*

Lufino M, Lim F and **Wade-Martins R.** (2010) "Friedreich's ataxia cellular models and gene replacement therapies based on genomic DNA locus vectors". *Ataxia UK Research Evening, February 2010, London, UK.*

Conference Platform Presentations:

Lufino M, Alegre-Abarrategui J, Lim F and **Wade-Martins R.** (2009) "Development of iBAC-FRDA-Luciferase fusion vector for the detection of frataxin expression". *Selected for Fairbairn Award competition. British Society for Gene Therapy (BSGT), April 2009, University of London, UK.*

In November 2008 Dr Wade-Martins was invited to Beijing, China, to speak at an Anglo-Chinese workshop on models of neurodegenerative disease.

Conference Poster Presentations:

Lufino M, Alegre-Abarrategui J, Lim F and **Wade-Martins, R.** (2009) "A novel genomic DNA-reporter gene model for the study of Friedreich's ataxia in neuronal cells". *American Society of Gene Therapy (ASGT), May 2009, San Diego, USA.*

Research Presentations:

Dr Wade-Martins has presented his work on neurological disorders at several invited seminars at research institutions in the UK and abroad in 2007-2010, for example at The Institute of Neurology, Queen's Square, London; University of Lund, Sweden; Imperial College, London; St George's Hospital London; Brunel University, Uxbridge; Department of Genetics, University of Cambridge; MRC Centre for Developmental and Biomedical Genetics, University of Sheffield;

Institute of Genetics and Molecular Medicine Institute, University of Edinburgh; and the Institute for Brain Repair, University of Cambridge.

For more support or information please contact: Ataxia UK, Lincoln House, Kennington Park, 1 – 3 Brixton Road. London SW9 6DE

Website: www.ataxia.org.uk.

Helpline: 0845 644 0606 Tel: +44 (0)20 7582 1444 Fax: +44 (0)20 7582 9444

Email: helpline@ataxia.org.uk