

Institution: Royal Holloway, University of London
Unit of Assessment: A5 Biological Sciences
Title of case study: The Development of Genetic Therapies for Duchenne Muscular Dystrophy
1. Summary of the impact <p>Professor Dickson's research group at Royal Holloway has pioneered the enabling technologies for the development of genetic therapies for the incurable disease Duchenne Muscular Dystrophy (DMD). Dickson's group has, (i) cloned replacement copies of the normal DMD gene, (ii) identified a natural substitute for the defective gene, and (iii) demonstrated that synthetic DNA can be used to correct the defective gene. The work has created impact on health and welfare through the development and clinical trials of a series of investigational medicinal products for this hitherto incurable disease, several clinical trials, and impact on commerce through industrial investment and licensed patents.</p>
2. Underpinning research <p>The impacts reported here result from research carried out in the School of Biological Sciences at Royal Holloway by Professor George Dickson and his team in the period from 1995 onwards. The research is on the molecular biology and pathology of the nervous and skeleto-muscular systems and the development of genetic therapeutics for the treatment of muscular dystrophies, in particular DMD. DMD is a heritable condition affecting one in 3,500 newborn boys and caused by a defect in the gene encoding the protein dystrophin. A dysfunctional copy of the dystrophin gene causes progressive muscle weakness, wasting and fatigability. Most DMD patients become wheelchair-dependent and have a life expectancy of less than three decades. The research that underpins the development of these genetic therapies rests on three fundamental discoveries made by Dickson's team at Royal Holloway. Key researchers in Dickson's team that contributed to this research were (in brackets the time of their employment at Royal Holloway) I. R. Graham (1998-2009), K. Foster (2003-2012), and L.J. Popplewell (1998-1999, 2005-present).</p> <p>Development of antisense oligonucleotide drugs (AOs) targeting the dystrophin mRNA for exon skipping therapy of DMD: In 1996, Dickson was first to demonstrate that AOs could be used to induce therapeutic exon skipping in animal models of DMD [1]. Exon skipping re-opens the genetic reading frame in the mutated DMD mRNA and restores expression of dystrophin protein, albeit in a slightly smaller but highly functional form. The outcome in animal studies has been the effective cure of the DMD condition [2] and the Dickson team has optimised AOs for the human disease, on the basis of which four patents have been granted, and which has led to drugs development and demonstrated proof of concept [3].</p> <p>Development of functional recombinant genes encoding dystrophin for gene therapy of DMD: Dickson has pioneered lab-based cloning of dystrophin genes for gene addition therapy in DMD. Optimised systems have been developed encoding both the full wild-type dystrophin protein and micro-dystrophins compatible with viral delivery vectors [4, 5]. These recombinant genes developed by Dickson group are functional and complement dystrophin deficiency, and have been distributed and used in labs across the world developing DMD gene therapies, including for the first human clinical trial of gene therapy in DMD patients conducted in 2002 which showed proof of concept [5]. Studies have since shown that the optimised microdystrophin gene therapy vectors from the Dickson lab are highly functional and yield sustained improvements in the pathology of dystrophin-deficient muscles [5,6].</p> <p>Identification of utrophin, an embryonic paralogue of dystrophin, with therapeutic potential in DMD: Before the census period, Dickson's group recorded the first description of a distinct human embryonic transcript with homology to the DMD gene. This was instrumental in the full characterisation of the embryonic homologue of dystrophin, now called utrophin. Since, they have established that utrophin and dystrophin are involved in the development or maintenance of junctional folds at the postsynaptic motor endplate of the muscle fibers, and that absence of both proteins leads to ultrastructural defects [7]. As utrophin is capable of functional compensation for</p>

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dystrophin, it is an important pharmaceutical target for new treatments for DMD. With other groups (notably Davies, Oxford), drugs to reactivate embryonic utrophin expression gene in adults were developed.

The research has been underpinned by recurrent research contract income over the REF census period in excess of £3M, from various agencies including the European Commission, Department of Health, Wellcome Trust, Medical Research Council, and various national and international Muscular Dystrophy and Medical Research Charities. (eg UK Muscular Dystrophy Campaign; Association Française contre les Myopathies). Since 2008, the research has generated 26 impact relevant peer-reviewed publications and 7 patent filings, four of which have been granted (see section 3).

3. References to the research (Key researchers in bold)

1. Dunckley, MG, Manoharan, M, Villiet, P, Eperon, IC, **Dickson, G** (1998). "Modification of splicing in the dystrophin gene in cultured Mdx muscle cells by antisense oligoribonucleotides". *Human Molecular Genetics* 7: 1083–90. doi:10.1093/hmg/7.7.1083
Result first reported in 1996 as: Dunckley, MG, Eperon, IC, **Dickson, G**. (1996) Modulation of pre-mRNA splicing in the Duchenne muscular dystrophy gene. *Biochemical Society Transactions* 24,276S. doi:10.1042/bst024276s
2. Malerba A, Sharp PS, **Graham IR**, Arechavala-Gomez V, **Foster K**, Muntoni F, Wells DJ, **Dickson G**. (2011) Chronic systemic therapy with low-dose morpholino oligomers ameliorates the pathology and normalizes locomotor behavior in mdx mice. *Mol Ther.* 19:345-54. doi:10.1038/mt.2010.261
3. **Popplewell LJ**, Trollet C, **Dickson G**, **Graham IR** (2009). Design of phosphorodiamidate morpholino oligomers (PMOs) for the induction of exon skipping of the human DMD gene. *Mol. Ther.* 17:554-61. doi:10.1038/mt.2008.287
4. Foster H, Sharp PS, Athanasopoulos T, Trollet C, **Graham IR**, **Foster K**, Wells DJ, **Dickson G**. (2008). Codon and mRNA sequence optimization of micro-dystrophin transgenes improves expression and physiological outcome in dystrophic mdx mice following AAV2/8 gene transfer. *Mol Ther.* 16:1825-32. doi:10.1038/mt.2008.186
5. Romero NB, Braun S, Benveniste O, Leturcq F, Hogrel JY, Morris GE, Barois A, Eymard B, Payan C, Ortega V, Boch AL, Lejean L, Thioudellet C, Mouro B, Escot C, Choquel A, Recan D, Kaplan JC, **Dickson G**, Klatzmann D, Molinier-Frenckel V, Guillet JG, Squiban P, Herson S, Fardeau M. (2004) Phase I study of dystrophin plasmid-based gene therapy in Duchenne/Becker muscular dystrophy. *Hum Gene Ther.* 15:1065-76. doi:10.1089/hum.2004.15.1065
6. Koo T, Okada T, Athanasopoulos T, Foster H, Takeda S, **Dickson G**. (2011) Long-term functional adeno-associated virus-microdystrophin expression in the dystrophic CXMDj dog. *J Gene Med.* 13:497-506. doi: 10.1002/jgm.1602
7. Deconinck AE, Rafael JA, Skinner, JA, Brown SC, Potter, AC, Metzinger, L, Watt DJ, **Dickson, G**, Tinsley JM, and Davies, KE. (1997). Utrophin-dystrophin-deficient mice as a model for Duchenne muscular dystrophy. *Cell* 90,717-727. doi:10.1016/S0092-8674(00)80532-2

Patents:

- Oligomers (DMD- Exon 53); Inventors: Popplewell, Graham and Dickson; Assignee: Royal Holloway and Bedford New College; US patent No 8,084,601, granted December 27, 2011.
- Oligomers (DMD Exon 45); Inventors: Popplewell, Graham and Dickson; Assignee: Royal Holloway and Bedford New College; US patent No 8,324,371, granted December 4, 2012.
- Oligomers (DMD Exon 44); Inventors: Popplewell, Graham and Dickson; Assignee: Royal Holloway and Bedford New College. US Patent No, 8,461,325, granted June 11, 2013.
- Oligomers (DMD Exon 46); Inventor: Popplewell, Graham and Dickson; Assignee: Royal Holloway and Bedford New College. US Patent No. 8,552,172, granted in part October 8, 2013.

4. Details of the impact

The fundamental discoveries made by Dickson and his team have an impact on health through the development of novel drugs for DMD. The impact has been realised through clinical trials delivered by other teams, conglomerates of teams and companies. **Beneficiaries:** the impact has benefitted

those affected by Duchenne muscular dystrophy, parents of children affected by DMD, and carriers of the defective gene. The research has had impact on pharmaceutical companies who have invested in research and development through clinical trials, and licensing agreements.

Impact on health, area 1: Development and subsequent trials of antisense oligonucleotide drugs (AOs): AO drugs target the dystrophin mRNA and have been developed for exon skipping therapy of DMD. We will describe nine clinical trials, held since 2009, that have either been completed or are ongoing, testing the oligomer drugs in DMD patients.

Two phase I/II trials were held in the UK and two phase II studies in the US, all on AVI-4658 (Eteplirsen), sponsored by Sarepta Therapeutics [8]. The Sarepta trials have met their primary endpoints successfully showing safety and improved clinical outcomes in DMD patients, biochemical efficacy. The trials showed that Eteplirsen was well tolerated and there were no clinically significant treatment-related adverse events, serious adverse events, hospitalizations or discontinuations [9,10]. The phase II trials show an increase in novel dystrophin, absence of adverse events and continued benefits lasting for 84 weeks [11]. The safety and biochemical efficacy indicate the suitability of Eteplirsen to become a disease-modifying drug for Duchenne muscular dystrophy [10]. The trials indicate that 70-80% of sufferers can benefit from this treatment, indicating it could be applied to ~0.5M individuals who suffer from this disease across the world (~4000 in the UK)[12].

Five trials are sponsored by Prosensa and GlaxoSmithKline on products PRO044 (phase I/II completed), PRO045(phase I/II ongoing), PRO051 (phase I/II, III completed) and PRO053 (phase I/II ongoing) [13]. In the trials the compound PRO051 (Drisapersen) has been shown to restore dystrophin expression, was well tolerated and has a beneficial therapeutic effect on DMD patients after 12 [14] and 48 weeks of treatment [12]. The phase III trial has been completed in June 2013 and has shown a definite outcome in that it did not meet its primary endpoint [15].

Impact on health, area 2: Development and subsequent trials of functional recombinant genes encoding dystrophin for gene addition therapy of DMD: Recombinant dystrophin and micro-dystrophin genes engineered in the Dickson laboratory have been distributed around the global research community, and have led to significant pre-clinical testing and pending clinical trials in DMD patients. Two phase I clinical trials of DMD gene therapy, in 2002 [5] and 2009 [16], have been conducted on dystrophin gene therapy for DMD. The 2002 trial showed low levels of dystrophin, and no side effects or any cellular or humoral anti-dystrophin responses. The 2009 trial also provided evidence of gene expression. Dystrophin-specific T cells were detected in two patients before vector treatment [16]. This field continues to move forward, and Dickson has now developed highly-optimised microdystrophin gene vectors, showing 30-fold improvement in expression, which will go into new clinical trials. This therapy would be applicable to all DMD patients (~0.7M worldwide/ ~6000 in the UK).

Impact on health, area 3. Identification and clinical trials of utrophin, an embryonic paralogue of dystrophin, with therapeutic potential in DMD: The utrophin gene and protein is currently a major pharmaceutical target for the identification of drugs to treat DMD therapy. Proof of principle has been delivered [17] and a phase I clinical trial has been conducted in human volunteers for the safety and pharmacodynamic/kinetics of utrophin activator drugs. This has confirmed the safety of the drug and shown that sufficiently high concentrations can be achieved [18]. Currently phase 2 trials are planned. This type of therapy would be applicable to all DMD patients (~0.7M worldwide/ ~6000 in the UK).

Impact on commerce, investment in research. Over the period of assessment this research has been funded in excess of £3M from governmental agencies and medical charities. Following the trials for exon skipping therapy, Dickson and co-workers are evaluating a range of chemical modifications, conjugates and derivatives of AOs in order to design refined medicinal products with improved bio-activity, bio-safety and pharmacokinetic profiles. Royal Holloway holds four patents granted on the basis of this research, and intellectual property licensing option agreements exist with major biotechnology companies resulting in first stage commercial income to a value of £380k [19].

5. Sources to corroborate the impact (indicative maximum of 10 references)

8. *Confirmation of timing, completion status and location of the trials:* (1) Safety and Efficacy Study of Antisense Oligonucleotides in Duchenne Muscular Dystrophy ([NCT00159250](#)) (completed successfully), (2) Dose-Ranging Study of AVI-4658 to Induce Dystrophin Expression in Selected Duchenne Muscular Dystrophy (DMD) Patients ([NCT00844597](#))

- (completed successfully) (3) Efficacy Study of AVI-4658 to Induce Dystrophin Expression in Selected Duchenne Muscular Dystrophy Patients ([NCT01396239](#)): (completed successfully) (4) Efficacy, Safety, and Tolerability Rollover Study of Eteplirsen in Subjects With Duchenne Muscular Dystrophy ([NCT01540409](#)): (ongoing in extension phase) *can be found at* <http://clinicaltrials.gov/ct2/results?term=AVI-4658&Search=Search>
9. *This source reports the outcome of clinical trial* [NCT00159250](#). Kinali M, Arechavala-Gomez V, Feng L, Cirak S, Hunt D, Adkin C, Guglieri M, Ashton E, Abbs S, Nihoyannopoulos P, Garralda ME, Rutherford M, McCulley C, **Popplewell L**, Graham IR, **Dickson G**, Wood MJ, Wells DJ, Wilton SD, Kole R, Straub V, Bushby K, Sewry C, Morgan JE, Muntoni F (2009). Local restoration of dystrophin expression with the morpholino oligomer AVI-4658 in Duchenne muscular dystrophy: a single-blind, placebo-controlled, dose-escalation, proof-of-concept study. *Lancet Neurol.*; 8:918-28. doi: 10.1016/S1474-4422(09)70211-X
 10. *This source reports the outcome of clinical trial* [NCT00844597](#) *and corroborates the claim of the suitability and potential of Eteplirsen to become a disease modifying drug for DMD:* Cirak S, Arechavala-Gomez V, Guglieri M, Feng L, Torelli S, Anthony K, Abbs S, Garralda ME, Bourke J, Wells DJ, **Dickson G**, Wood MJ, Wilton SD, Straub V, Kole R, Shrewsbury SB, Sewry C, Morgan JE, Bushby K, Muntoni F (2011) Exon skipping and dystrophin restoration in patients with Duchenne muscular dystrophy after systemic phosphorodiamidate morpholino oligomer treatment: an open-label, phase 2, dose-escalation study. *Lancet.* 378: 595-605. doi: 10.1016/S0140-6736(11)60756-3
 11. *This report details the outcome of* [NCT01396239](#) *and* [NCT01540409](#) *after 84 weeks.* <http://investorrelations.sareptatherapeutics.com/phoenix.zhtml?c=64231&p=iro-NewsArticle&ID=1831214>
 12. *This reports details the outcome of* [NCT01396239](#) *after 24 weeks and supports the claim that Eteplirsen can benefit 70-80% of DMD sufferers.* Mendell J, Rodino-Klapac LR, Sahenk Z, Roush K, Bird L, Lowes LP, Alfano L, Gomez AM, Lewis S, Kota J, Malik V, Shontz K, Walker CM, Flanigan KM, Kean JR, Allen HD, Shilling C, Melia KR, Sazani P, Saoud JB, Kaye EM; The Eteplirsen Study Group (2013). Eteplirsen for the treatment of duchenne muscular dystrophy. *Ann Neurol.* doi: 10.1002/ana.23982.
 13. *This source lists the clinical trials sponsored by Prosensa:* <http://www.prosensa.eu/patients-and-family/duchenne-muscular-dystrophy/clinical-trials>
 14. *This study reports efficacy of PRO051 and improvement after 12 weeks in trial* [NTR1241](#): Goemans NM, Tulinius M, van den Akker JT, Burm BE, Ekhardt PF, Heuvelmans N, Holling T, Janson AA, Platenburg GJ, Sipkens JA, Sitsen JM, Aartsma-Rus A, van Ommen GJ, Buyse G, Darin N, Verschuuren JJ, Campion GV, de Kimpe SJ, van Deutekom JC. (2011) Systemic administration of PRO051 in Duchenne's muscular dystrophy. *N Engl J Med.* 364(16):1513-22. doi: 10.1056/NEJMoa1011367.
 15. *Report on the primary outcome of the phase 3 trial for Drirapersen:* <http://www.gsk.com/media/press-releases/2013/gsk-and-prosensa-announce-primary-endpoint-not-met-in-phase-iii-.html>
 16. *This reports details the outcome of* [NCT00428935](#). Mendell JR, Campbell K, Rodino-Klapac L, Sahenk Z, Shilling C, Lewis S, Bowles D, Gray S, Li C, Galloway G, Malik V, Coley B, Clark KR, Li J, Xiao X, Samulski J, McPhee SW, Samulski RJ, Walker CM. (2010) Dystrophin immunity in Duchenne's muscular dystrophy. *N. Engl. J. Med.* 7;363(15):1429-37. doi:10.1056/NEJMoa1000228.
 17. *Corroborates proof of concept for Utrophin upregulator SMT C1100:* Tinsley JM, Fairclough RJ, Storer R, Wilkes FJ, Potter AC, Squire SE, Powell DS, Cozzoli A, Capogrosso RF, Lambert A, Wilson FX, Wren SP, De Luca A, Davies KE (2011). Daily treatment with SMTC1100, a novel small molecule utrophin upregulator, dramatically reduces the dystrophic symptoms in the mdx mouse. *PLoS One.* 6;6(5):e19189. doi: 10.1371/journal.pone.0019189.
 18. *Report on clinical trial results for Utrophin upregulator SMT C1100:* <http://tinyurl.com/summitplcutrophintrial>
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