BIOSKETCH

NAME **POPPLEWELL, Linda**	**POSITION TITLE Lecturer in Biomedical Sciences**

EDUCATION/TRAINING

INSTITUTION AND LOCATION	**DEGREE**	**YEAR**	**FIELD OF STUDY**
University of Southampton, Hampshire, United Kingdom	B.Sc Hons.	1988	Physiology and Biochemistry
University of Southampton, Hampshire, United Kingdom	Ph.D.	1993	Biology

A. Positions

1994-1996: Clinical Biochemist, Kings College Hospital, London, United Kingdom
1998-1999: Post-doctoral research assistant, Royal Holloway University of London, Egham, United Kingdom
2005-2012: Senior post-doctoral research assistant, Royal Holloway University of London, Egham, United Kingdom
2012-2015: Research Officer, Royal Holloway University of London, Egham, United Kingdom
2015-present: Lecturer in Biomedical Sciences, Royal Holloway University of London, Egham, United Kingdom

B. Selected peer-reviewed publications

1. **Popplewell L**, Koo KY, Leclerc X, Duclert A et al. (2013). Gene Correction of a Duchenne Muscular Dystrophy Mutation by Meganuclease-Enhanced Exon Knock-in. Human Gene Ther 24(7):692-701. doi: 10.1089/hum.2013.081
2. Koo T, **Popplewell L**, Athanasopoulos T, Dickson G. (2014). Triple trans-splicing AAV vectors capable of transferring the coding sequence for full-length dystrophin protein into dystrophic mice. Hum Gene Ther. 25(2):98-108. doi: 10.1089/hum.2013.164.
3. Jarmin S, Kymalainen H, **Popplewell L**, Dickson G (2014). New developments in the use of gene therapy to treat Duchenne muscular dystrophy. Expert Opin Biol Ther. 2014 Feb;14(2):209-30. doi: 10.1517/14712598.2014.866087.
4. Athanasopoulos T, Jarmin S, Foster H, Foster K, Kang J, Koo T, Malerba A, **Popplewell L**, Scherman D, Dickson G (2014). Genetic therapies of muscle disease: Duchenne muscular dystrophy. Chapter in 'Advanced textbook on Gene Therapy, Gene Transfer and Genetic Pharmacology'. Published by Imperial College Press. ISBN 978-1-84816-828-2 (HB), ISBN 978-1-908977-28-1 (PB).
5. Lu-Nguyen NB, Jarmin SA, Saleh AF, **Popplewell L**, Gait MJ, Dickson G (2015). Combination Antisense Treatment for Destructive Exon Skipping of Myostatin and Open Reading Frame Rescue of Dystrophin in Neonatal mdx Mice. Mol Ther. 23(8):1341-8. doi: 10.1038/mt.2015.88.
6. Kawecka K, Theodoulides M, Hasoglu Y, Jarmin S, Kymalainen H, Le-Heron A, **Popplewell L**, Malerba A, Dickson G, Athanasopoulos T. (2015). Adeno-Associated Virus (AAV) Mediated Dystrophin Gene Transfer Studies and Exon Skipping Strategies for Duchenne Muscular Dystrophy (DMD). Curr Gene Ther. 15(4):395-415. PMID:26159373.
7. Massouridès E, Polentes J, Mangeot PE, Mournetas V, Nectoux J, Deburgrave N, Nusbaum P, Leturcq F, **Popplewell L**, Dickson G, Wein N, Flanigan KM, Peschanski M, Chelly J, Pinset C. (2015). Dp412e: a novel human embryonic dystrophin isoform induced by BMP4 in early differentiated cells. Skelet Muscle. 2015 Nov 14;5:40. doi: 10.1186/s13395-015-0062-6.

8. Moore M, Vallese D, Dickson G, **Popplewell L** (2015). Exciting Developments in CRISPR/Cas9-mediated Genome Engineering Approaches for Duchenne Muscular Dystrophy. Cell and Gene Therapy Insights 1(2), 215-230.
9. Marsollier AC, Ciszewski L, Mariot V, **Popplewell L**, Voit T, Dickson G, Dumonceaux J. (2016). Antisense targeting of 3'end elements involved in DUX4 mRNA processing is an efficient therapeutic strategy for Facioscapulohumeral Dystrophy: a new gene silencing approach. Hum Mol Genet. 2016 Jan 19. pii: ddw015. [Epub ahead of print]. PMID:26787513
10. Klein P, Oloko M, Roth F, Montel V, Malerba A, Jarmin S, Gidaro T, **Popplewell L**, Perie S, Lacau St Guily J, de la Grange P, Antoniou MN, Dickson G, Butler-Browne G, Bastide B, Mouly V, Trollet C. (2016). Nuclear poly(A)-binding protein aggregates misplace a pre-mRNA outside of SC35 speckle causing its abnormal splicing. Nucleic Acids Res. 2016 Aug 9. pii: gkw703. [Epub ahead of print] PMID: 27507886
11. Lu-Nguyen N, Malerba A, **Popplewell L**, Schnell F, Hanson G, Dickson G (2017). Systemic intravenous administration of antisense therapeutics for combinatorial dystrophin and myostatin exon splice modulation improves muscle pathology of adult mdx mice. Mol Ther Nucleic Acids. 2017 Mar 17;6:15-28. doi: 10.1016/j.omtn.2016.11.009.
12. Hiller M, Falzarano MS, Garcia-Jimenez I, Sardone V, Verheul RC, **Popplewell L**, Anthony K, Ruiz-Del-Yerro E, Osman H, Goeman JJ, Mamchaoui K, Dickson G, Ferlini A, Muntoni F, Aartsma-Rus A, Arechavala-Gomeza V, Datson NA, Spitali P. (2018). A multicenter comparison of quantification methods for antisense oligonucleotide-induced DMD exon 51 skipping in Duchenne muscular dystrophy cell cultures. PLoS One. 2018 Oct 2;13(10):e0204485. doi: 10.1371/journal.pone.0204485. eCollection 2018.
13. Koo T, Lu-Nguyen NB, Malerba A, Kim E, Kim D, Cappellari O, Cho HY, Dickson G, **Popplewell L**, Kim JS. (2018). Functional Rescue of Dystrophin Deficiency in Mice Caused by Frameshift Mutations Using Campylobacter jejuni Cas9. Mol Ther. 2018;26(6):1529-1538. doi: 10.1016.
14. Benedetti S, Uno N, Hoshiya H, Ragazzi M, Ferrari G, Kazuki Y, Moyle LA, Tonlorenzi R, Lombardo A, Chaouch S, Mouly V, Moore M, **Popplewell L**, Kazuki K, Katoh M, Naldini L, Dickson G, Messina G, Oshimura M, Cossu G, Tedesco FS. (2018). Reversible immortalisation enables genetic correction of human muscle progenitors and engineering of next-generation human artificial chromosomes for Duchenne muscular dystrophy. EMBO Mol Med. 2018 Feb;10(2):254-275. doi: 10.15252/emmm.201607284.
15. Harish P, Malerba A, Lu-Nguyen N, Forrest L, Cappellari O, Roth F, Trollet C, **Popplewell L**, Dickson G. (2019). Inhibition of myostatin improves muscle atrophy in oculopharyngeal muscular dystrophy (OPMD). J Cachexia Sarcopenia Muscle. 2019 May 7. doi: 10.1002/jcsm.12438.
16. Lu-Nguyen N, Ferry A, Schnell FJ, Hanson GJ, **Popplewell L**, Dickson G, Malerba A. (2019). Functional muscle recovery following dystrophin and myostatin exon splice modulation in aged mdx mice. Hum Mol Genet. 2019 Sep 15;28(18):3091-3100. doi: 10.1093/hmg/ddz125.

C. Research Support

Awarding body	Period covered	Amount of funding
Motor Neurone Disease Association	01/10/12- 30/09/15	£88,696
Muscular Dystrophy Campaign	01/10/13-30/09/16	£240,393
Muscular Dystrophy Campaign	01/10/13-30/09/16	£80,000
Rosetree's Trust	01/08/14-31/07/17	£60,000
Duchenne Parent Project	01/05/14-30/04/16	€188,000
Action Duchenne	01/01/15-31/12/17	£199,000
Muscular Dystrophy UK	01/10/15-30/09/20	£250,000
Action Duchenne	01/07/16-31/12/16	£37,627
Muscular Dystrophy UK	01/11/16-30/10/19	£218,450
Muscular Dystrophy UK	01/07/17-30/06/21	£358,850
Muscular Dystrophy UK	01/10/17-30/09/21	£113,857
Jesse's Journey	01/09/18-31/08/20	£116,462
Gilbert Family Foundation	01/02/19-30/01/22	£484,052
Industry-funded contract	01/01/19-28/02/19	£297,500
Industry-funded contract	01/01/19-30/07/19	£195,000
Industry-funded contract	01/02/19-31/01/20	£148,000

D. Most significant contributions to science

(i) Antisense oligonucleotide-induced exon skipping of out-of-frame exons to restore the DMD *transcript reading frame as a therapy for Duchenne muscular dystrophy.*

Royal Holloway University of London and others have described the skipping of out-of-frame exons on the *DMD* transcript. LP was involved in the optimisation of antisense oligonucleotide for the targeted skipping of exons that would have the highest Duchenne muscular dystrophy patient applicability. These sequences have been patent protected and are being developed in both non-conjugated and cell-penetrating peptide conjugated form through collaboration with pharmaceutical companies. The antisense oligonucleotide developed for the targeted skipping of exon 53 is now in clinical trial. On the basis of the recent FDA conditional approval of exon 51 antisense oligonucleotide and the superior skipping seen with the exon 53 antisense oligonucleotide, accelerated approval is anticipated.

Outputs:

1. Arechavala-Gomeza V, Graham I, **Popplewell L**, et al. (2007). Comparative analysis of antisense oligonucleotide sequences for targeted skipping of exon 51 during dystrophin pre-mRNA splicing in human muscle. Hum Gene Ther 18: 798-810. PMID:17767400.
2. Kinali M, Arechavala-Gomeza V, Feng L, Cirak S, Hunt D, Adkin C, Guglieri M, Ashton E, Abbs S, Nihoyannapoulos P, Garralda M, Rutherford M, McCulley C, **Popplewell L**, et al. (2009) Local Restoration of Dystrophin Expression in Duchenne Muscular Dystrophy: A Single Blind, Placebo-controlled Dose Escalation Study Using Morpholino Oligomer AVI-4658. Lancet Neurology 8: 918-928. doi: 10.1016/S1474-4422(09)70211-X.
3. **Popplewell L**, Adkin C, Arechavala-Gomeza V, et al. (2010). Comparative analysis of antisense oligonucleotide sequences targeting exon 53 of the human *DMD* gene: implications for future clinical trials. Neuromus Disord 20: 102-10. doi: 10.1016/j.nmd.2009.10.013.
4. Patents:
    - US Application No. 12/556,626 - DMD Exon 53 Oligomers - This application has been granted. US patent No 8,084,601, granted December 27, 2011
    - US Application No. 13/204,326 - DMD Exon 45 Oligomers - This application has been granted. US patent No 8,324,371, granted December 4, 2012
    - US Application No. 13/307,825 - DMD Exon 44 Oligomers - This application is currently being examined and has been accepted in part.
    - US Application No. 13/307,926 - DMD Exon 46 Oligomers - This application is currently being examined.

(ii) Antisense oligonucleotide-induced exon skipping of out-of-frame exons to disrupt the MSTN transcript reading frame as an anti-muscle wasting therapy for Duchenne muscular dystrophy.

By disrupting the transcript reading frame, antisense oligonucleotides can be used to knockdown expression of the muscle growth antagonist, myostatin. Other strategies including AAV-ProMyo gene addition, immunological knockdown of myostatin, antibody blockade of the ActIIb receptor, antisense oligonucleotide disruption of the ActIIb receptor have been used with mixed results; we were the first to describe the use of antisense oligonucleotide to knockdown myostatin expression itself and to good effect and with high specificity. The work has been extended to use a cocktail of antisense oligonucleotides, one to restore dystrophin protein expression and the other to myostatin expression. Our recently accepted paper describes enhanced dystrophin restoration with myostatin knockdown, suggesting that a combination treatment is required for maximum efficacy.

Outputs:

1. Kang JK, Malerba A, **Popplewell L,** Foster K, Dickson G (2011). Antisense-induced myostatin exon skipping leads to muscle hypertrophy in mice following octa guanidine morpholino oligomer treatment. Mol Ther 19: 159-64. doi: 10.1038/mt.2010.212.
2. Malerba A, Kang JK, McClorey G, Saleh AF, **Popplewell L**, et al. (2012). Dual Myostatin and Dystrophin Exon Skipping by Morpholino Nucleic Acid Oligomers conjugated to a Cell-penetrating Peptide Is a Promising Therapeutic Strategy for the Treatment of Duchenne Muscular Dystrophy. Mol Ther Nucleic Acids. 2012 Dec 18;1:e62. doi: 10.1038/mtna.2012.54
3. Lu-Nguyen NB, Jarmin SA, Saleh AF, **Popplewell L**, Gait MJ, Dickson G (2015). Combination Antisense Treatment for Destructive Exon Skipping of Myostatin and Open Reading Frame Rescue of Dystrophin in Neonatal mdx Mice. Mol Ther. 23(8):1341-8. doi: 10.1038/mt.2015.88.

4. Lu-Nguyen N, Malerba A, **Popplewell L**, Schnell F, Hanson G, Dickson G (2017). Systemic intravenous administration of antisense therapeutics for combinatorial dystrophin and myostatin exon splice modulation improves muscle pathology of adult mdx mice. Mol Ther Nucleic Acids. 2017 Mar 17;6:15-28. doi: 10.1016/j.omtn.2016.11.009.

(iii) Full DMD *gene correction through homology-directed repair.*

We have been the only group to fully correct the *DMD* gene using gene editing. All other work has used non-homologous end joining to induce either small InDels as the result of a single double strand break or a large deletion as the result of two double strand breaks. This has important implications in the development of a gene editing as a therapy for Duchenne muscular dystrophy. Non-homologous end joining is error-prone and could result in an alteration in the amino acid sequence of the expressed dystrophin. Additionally the expressed dystrophin will be truncated with perhaps compromised functionality. The full repair we describe was of a specific DMD deletion and was achieved through targeting with an endonuclease within intron 44, 5' of a mutation hotspot and supply of a homologous repair template. This work has developed the field substantially since it has the potential to effectively cure a mutation that causes Duchenne muscular dystrophy, and with adaptation could be made applicable to a high proportion of patients.

Outputs :

1. **Popplewell L**, Koo KY, Leclerc X, Duclert A et al. (2013). Gene Correction of a Duchenne Muscular Dystrophy Mutation by Meganuclease-Enhanced Exon Knock-in. Human Gene Ther 24(7):692-701. doi: 10.1089/hum.2013.081
2. Patent submitted:
    - US Application No PCT/EP2013/064942 for design of repair template.
3. Koo T, Lu-Nguyen NB, Malerba A, Kim E, Kim D, Cappellari O, Cho HY, Dickson G, **Popplewell L**, Kim JS. (2018). Functional Rescue of Dystrophin Deficiency in Mice Caused by Frameshift Mutations Using Campylobacter jejuni Cas9. Mol Ther. 2018;26(6):1529-1538. doi: 10.1016.

(iv) AAV gene addition therapy for Duchenne muscular dystrophy.

The laboratory of Prof George Dickson, of which LP was a part, was the first to describe the codon optimisation of a microdystrophin for delivery using an AAV vector, which lead to much higher levels of microdystrophin protein expression and greater amelioration of disease phenotype in the mdx mouse model of the disease. Others are now incorporating this into their AAV dystrophin replacement vectors and work in all labs is now concentrating on improvements in the design of the microdystrophin to enhance functionality. On the basis of excellent results in the GRDM dog model of the disease, clinical trials are now being planned on which LP is acting as lead PI. Since the microdystrophin will have compromised activity, even with improved design, we have also developed a triple trans-splicing AAv vector system, where the whole DMD gene is delivered in three separate vectors. We were the first to report the restoration of full length dystrophin protein in the mdx mouse using this strategy. This work has paved the way for further optimisation of these vectors such that amelioration of the disease phenotype is achieved. AAV gene addition therapy for Duchenne muscular dystrophy would have applicability to all patients and a single treatment should result in longterm dystrophin protein expression so holds great hope as a therapy for Duchenne muscular dystrophy.

Outputs:

1. Koo T, Malerba A, Athanasopoulos T, Trollet C, Boldrin L, Ferry A, **Popplewell L**, Foster H, Foster K, Dickson G. (2011). Delivery of AAV2/9-Microdystrophin Genes Incorporating Helix 1 of the Coiled-Coil Motif in the C-Terminal Domain of Dystrophin Improves Muscle Pathology and Restores the Level of α1-Syntrophin and α-Dystrobrevin in Skeletal Muscles of mdx Mice.Hum Gene Ther. 22(11):1379-88. doi: 10.1089/hum.2011.020.
2. Koo T, **Popplewell L**, Athanasopoulos T, Dickson G. (2014). Triple trans-splicing AAV vectors capable of transferring the coding sequence for full-length dystrophin protein into dystrophic mice. Hum Gene Ther. 25(2):98-108. doi: 10.1089/hum.2013.164.