Institution: University College London

Unit of Assessment: 1 – Clinical Medicine

Title of case study: Advances in gene therapy lead to successful treatment of haemophilia

1. Summary of the impact

Haemophilia, an inherited bleeding disease, is treated by frequent and extremely expensive infusions of recombinant versions of the missing factors. Advances in gene therapy have now been achieved at UCL, with successful treatment of Haemophilia B in 10 severely affected patients. The novel factor IX expression cassette has been patented and licensed to an industrial partner (UniQure). Savings to the NHS in excess of £1.5m have already been made and increase every month. Pre-clinical advances have also been made in Haemophilia A, and the factor VIII expression cassette has been patented and licensed to an industrial partner (BioMarin).

2. Underpinning research

Haemophilia is an inherited deficiency of one of the clotting factors that achieve haemostasis and prevent uncontrolled bleeding. Since the cloning of the factor VIII gene, which involved a seminal contribution from Professor Edward Tuddenham at the UCL Cancer Institute, and the later cloning of other clotting factor genes, the standard treatment for haemophilia has been with the infusion of recombinant versions of the missing factor. This approach is very expensive to the NHS (£400m per annum) and beyond the means of 80% of patients living in the developing world.

In 1999, Tuddenham began a collaboration with Dr Amit Nathwani (also UCL Cancer Institute) to develop gene therapy for Haemophilia A and B with the intention of overcoming this problem and providing improved disease control. The initial studies focussed on Haemophilia B (factor IX deficiency) because the factor IX gene is smaller than the factor VIII gene and is easier to package. Extensive iterative preclinical studies performed in collaboration with St Jude Children's Research Hospital, in Memphis, TN, USA led to highly improved in vivo delivery and expression of the factor IX gene in mice and then in primates. This was achieved by incremental technical modifications including codon optimisation of the factor IX gene, the development of a self-complementary gene construct and design of a small tissue specific promoter **[1, 2]**.

Good Manufacturing Practice protocols for vector purification were developed and in 2010 studies in patients commenced **[3]**. In the initial report, the vector was infused at one of 3 doses into the peripheral vein of six severely affected haemophilia B patients with baseline factor IX activity <1% of normal. Stable adeno-associated virus (AAV)-mediated endogenous expression of factor IX at 2-6% of normal levels was observed in all participants for a period of at least three years (follow-up still on-going). Four additional subjects were recruited in 2012 at the high dose level and they have stable expression of factor IX at 5% of normal.

Building on the expertise developed during the haemophilia B work, a new expression cassette for factor VIII has been designed that overcomes the size barrier to incorporation in AAV and also allows highly efficient synthesis compared to all previous expression vectors **[4, 5]**. Final pre-clinical toxicology studies are now ongoing to support a Phase I/II trial of factor VIII gene transfer for Haemophilia A.

3. References to the research

[1] Nathwani AC, Gray JT, Ng CY, Zhou J, Spence Y, Waddington SN, Tuddenham EG, Kemball-Cook G, McIntosh J, Boon-Spijker M, Mertens K, Davidoff AM. Self-complementary adeno-associated virus vectors containing a novel liver-specific human factor IX expression cassette enable highly efficient transduction of murine and nonhuman primate liver. Blood. 2006 Apr 1;107(7):2653-61. http://dx.doi.org/10.1182/blood-2005-10-4035

[2] Nathwani AC, Gray JT, McIntosh J, Ng CY, Zhou J, Spence Y, Cochrane M, Gray E, Tuddenham EG, Davidoff AM. Safe and efficient transduction of the liver after peripheral vein infusion of self-complementary AAV vector results in stable therapeutic expression of human FIX in nonhuman primates. Blood. 2007 Feb 15;109(4):1414-21. http://dx.doi.org/10.1182/blood-2006-03-010181

[3] Nathwani AC, Tuddenham EG, Rangarajan S, Rosales C, McIntosh J, Linch DC, Chowdary P, Riddell A, Pie AJ, Harrington C, O'Beirne J, Smith K, Pasi J, Glader B, Rustagi P, Ng CY, Kay MA, Zhou J, Spence Y, Morton CL, Allay J, Coleman J, Sleep S, Cunningham JM, Srivastava D, Basner-Tschakarjan E, Mingozzi F, High KA, Gray JT, Reiss UM, Nienhuis AW, Davidoff AM (2011). Adenovirus-associated virus vector-mediated gene transfer in hemophilia B. N Engl J Med. 2011 Dec 22;365(25):2357-65. http://dx.doi.org/10.1056/NEJMoa1108046

[4] McIntosh J, Lenting PJ, Rosales C, Lee D, Rabbanian S, Raj D, Patel N, Tuddenham EG, Christophe OD, McVey JH, Waddington S, Nienhuis AW, Gray JT, Fagone P, Mingozzi F, Zhou SZ, High KA, Cancio M, Ng CY, Zhou J, Morton CL, Davidoff AM, Nathwani AC. Therapeutic levels of FVIII following a single peripheral vein administration of rAAV vector encoding a novel human factor VIII variant. Blood. 2013 Apr 25;121(17):3335-44. http://dx.doi.org/10.1182/blood-2012-10-462200

[5] Ward NJ, Buckley SM, Waddington SN, Vandendriessche T, Chuah MK, Nathwani AC, McIntosh J, Tuddenham EG, Kinnon C, Thrasher AJ, McVey JH. Codon optimization of human factor VIII cDNAs leads to high-level expression. Blood. 2011 Jan 20;117(3):798-807. http://dx.doi.org/10.1182/blood-2010-05-282707

4. Details of the impact

Haemophilia B patients have a defective factor IX gene and cannot make the protein factor IX, which is essential for normal blood clot formation. They suffer from frequent, often life-threatening, bleeding episodes that occur without any apparent injury. Current treatment involves injection of factor IX protein concentrates every two to three days for the life-time of the patient. This treatment is invasive, extremely expensive (£150,000 per year) and only available to 20% of the world's haemophilia patients who live in high income countries. Importantly, this treatment is not curative and patients continue to bleed despite prophylactic factor IX treatment which by necessity is administered intermittently, resulting in significant periods when the plasma level is below 1% of normal.

Gene therapy overcomes these limitations by replacing the damaged factor IX gene with a normal copy, thus allowing the patient to make factor IX protein continuously without the need for regular injections. Our recent study showed that a single injection of our novel AAV vector into six patients with severe haemophilia B resulted in an increase in blood FIX levels from undetectable levels before gene therapy to between 1-6% of normal in all participants for a period that now extends to over three years.

Four of the six patients have been able to stop regular injections with FIX protein and still remain free of spontaneous bleeding episodes. This is despite engaging in activities such as playing football and running a marathon that had previously been associated with bleeding. The first patient commented "*This type of solution is something permanent and can make a lot of difference*". The sixth patient said "*I have not needed any of my normal treatment, either preventative or on-demand as a result of an injury. Previously, I used to infuse at home three times a week …. I play football, run and take part in triathlons - and previously I might have had to infuse both before I took part and possibly after as well. Not having to do that has been absolutely brilliant*" **[a]**.

This, the first gene therapy success for haemophilia in the world, has been lauded by experts in the field **[b]**. The results have been widely covered by the media, including the BBC and the New York Times amongst other news outlets across the world **[c]**. It has also been covered on patient-facing

websites **[c]**.

There has been a significant saving to the NHS just from the first six patients treated through reduction or elimination of the need for FIX protein concentrates, amounting to more than £1.5m, a figure which increases with each month that passes. This approach has recently been extended to four more patients who were all treated at the high dose level and are expressing factor IX at 5-8% level over a follow-up period of 9-18 months.

Indeed, the same approach is now being developed by Nathwani and Tuddenham for haemophilia A, the most common severe inherited bleeding disorder affecting approximately 1 in 5,000 males. Patents for the novel factor VIII expression cassette has been filed **[e]** and recently licensed to BioMarin for *[Text removed for publication]* **[f]**. This development is prompting increased investment in research, and is accelerating clinical trials for gene therapy of haemophilia A. *[Text removed for publication].*

5. Sources to corroborate the impact

[a] Statements provided by patients to UCL's News Editor. Contact details provided.

[b] Our findings have been widely discussed in the scientific literature:

- Ponder KP. Merry christmas for patients with hemophilia B. N Engl J Med. 2011;365:2424-2425. http://doi.org/10.1056/NEJMe1111138
- Vandendriessche T, Chuah MK. Clinical progress in gene therapy: sustained partial correction of the bleeding disorder in patients suffering from severe hemophilia B. Hum Gene Ther. 2012;23:4-6. http://dx.doi.org/10.1089/hum.2011.221
- Landis MW, Quong JN. A major advance in gene therapy for hemophilia. Cancer Discovery 2011;2:11. http://dx.doi.org/10.1158/2159-8290.CD-RW2011-61
- Evans-Molina C. A New FIX for Hemophilia B. Science Translational Medicine 2012;4:5. http://doi.org/10.1126/scitranslmed.3003668
- Ponder KP. Hemophilia Gene Therapy: A Holy Grail Found. Molecular Therapy 2011;19 3:427 http://doi.org/10.1038/mt.2011.13

[c] Media coverage of our work:

- BBC News. 'Haemophilia gene therapy shows early success.' http://www.bbc.co.uk/news/health-16107411
- New York Times. 'Treatment for Blood Disease Is Gene Therapy Landmark.' http://www.nytimes.com/2011/12/11/health/research/hemophilia-b-gene-therapy-breakthrough.html?_r=0

[d] News of our research was reported on NHS Choices: http://www.nhs.uk/news/2011/12December/Pages/haemophilia-b-christmas-disease-gene-therapy.aspx

[e] Patents:

- Patent no: US8030065. IMPROVED EXPRESSION OF FACTOR IX IN GENE THERAPY VECTORS
- Patent no: 0911870.4. Optimised Coding Sequence and Promoter

- Patent no: 1210357.8. Codon Optimised Factor VIII V3

[f] BioMarin press release: http://investors.bmrn.com/releasedetail.cfm?ReleaseID=742285 Licensing details can be corroborated by Director BioPharm, UCL Business. Contact details provided.

[g] *[Text removed for publication].*