Institution: University College London

Unit of Assessment: 1 - Clinical Medicine

Title of case study: Development, validation and delivery of the first gene therapies for haemophilia and creation of the biopharmaceutical spin-out company Freeline Therapeutics

Period when the underpinning research was undertaken: 2003-2020

Details of staff conducting the underpinning research from the submitting unit:

Name(s):	Role(s) (e.g. job title):	Period(s) employed by submitting HEI:
Amit Nathwani	Professor of Haematology	2003 to present
Edward Tuddenham	Emeritus Professor of Haemophilia	2006 to present

Period when the claimed impact occurred: 2014 to 2020

Is this case study continued from a case study submitted in 2014? N

1. Summary of the impact

UCL research is transforming the lives of people with haemophilia, an inherited blood clotting disorder that causes internal bleeding and significantly affects quality of life for the 800,000 affected males, worldwide. The team at UCL's Cancer Institute pioneered single-dose gene therapy for haemophilia that restores blood-clotting and can be delivered at 1% the cost of conventional treatment. In collaboration with the St Jude Children's Research Hospital, Tennessee, the UCL team successfully trialled the novel therapies which deliver a functional gene to patients' cells allowing them to produce the clotting factor proteins. The therapies have treated over 300 patients in clinical studies to-date. A spinout company, Freeline Therapeutics, launched in 2015 to develop the approach, now employs over 200 people in three countries and has attracted investment of USD278,000,000.

2. Underpinning research

Haemophilia is an inherited bleeding disorder affecting 1 in 5,000 people worldwide and is caused when the body fails to manufacture one of two proteins needed for blood coagulation: factor VIII in the case of haemophilia A and factor IX in the case of haemophilia B. The genes for factor VIII and IX are encoded on the X-chromosome. Consequently, the condition mainly affects males. Females can carry the mutated gene and pass it on, but only express symptoms if two copies of the mutated gene are present. Conventional prophylactic treatment involves injecting protein concentrates derived from blood plasma, every two to three days for the lifetime of the patient to prevent spontaneous bleeding. If left untreated the condition is debilitating and often life-threatening. The treatment is invasive, extremely expensive (GBP150,000 a year), carries a small risk of infection from contaminated blood products, and is only available to 20% of the world's haemophilia patients.

Research led by Tuddenham to clone the genes coding for clotting factors (**R1**) provided the basis for transformative developments in gene therapy for haemophilia. Focusing initially on factor IX, Nathwani led an international collaboration with St Jude Children's Research Hospital in Tennessee, to develop a method for *in vivo* delivery and expression of a replacement gene to rectify the lack of clotting factor protein, using a modified form of the adeno-associated virus (AAV), first in mice and then in primates. This involved cloning a codon-optimised version of the factor IX gene into an AAV2 genome in which all virulent DNA had been removed (**R2**) and designing a tissue-specific promoter to guide protein synthesis (**R3**).

The UCL team led the first ever successful human trial of haemophilia gene therapy in which a single dose of a vector based on the AAV8 virus was injected into a peripheral vein of six patients with severe haemophilia B (showing baseline factor IX activity of less than 1% of normal). The potency of the vector was increased by having two complementary copies in tandem of the gene, such that the single strand would immediately form a stable double helix on entering the target cell. Intravenous delivery was made possible by the AAV8 virus's particular ability to locate the liver, which is the natural site of factor IX synthesis. Treatment resulted in expression of factor IX at 2-11% of the normal level in all participants: even at low doses, patients exceeded the 1% figure needed to effectively reduce bleeding (**R4**). Four additional subjects were recruited in 2012 to receive therapy at a high dose. In a 2014 follow-up study, all ten patients had sustained a dose-dependent level of circulating factor IX of 1-6% of normal value for a median of 3.2 years. No late toxic effects of the therapy were reported (**R5**).

Collaboration between UCL and St Jude also produced a factor VIII vector for haemophilia A, the more common form of the disorder. This task was complicated by the fact that the relevant gene is 4.5 times larger than its factor IX equivalent and therefore more difficult to package into an AAV virus. To overcome this, the team developed a bioengineered form (variant 3) of the gene which was both smaller and more potent. This technology, with Nathwani and Tuddenham listed as inventors, was licensed to the Californian biotech company BioMarin (**R6**), which conducted a successful phase 1/2 dose-escalation study involving nine patients with severe haemophilia A in 2015/16 (**R7**). Industry-led Phase 3 trials have now been undertaken, described in section 4, treating approximately 300 people with moderate/severe haemophilia to date.

3. References to the research

R1. Mannucci PM and Tuddenham EGD.(2001) The Hemophilias- From Royal Genes to Gene Therapy. *N Engl J Med*; 1773-1779. DOI: 10.1056/NEJM200106073442307

R2. 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. (2006) Self-complementary adeno-associated virus vectors containing a novel liver-specific human factor IX expression cassette enable highly efficient transduction of murine and non-human primate liver. *Blood*. Apr 1;107(7):2653-61. DOI: 10.1182/blood-2005-10-4035

R3. Nathwani AC, Gray JT, McIntosh J, Ng CY, Zhou J, Spence Y, Cochrane M, Gray E, Tuddenham EG, Davidoff AM. (2007) 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*. Feb 15;109(4):1414-21. DOI: 10.1182/blood-2005-10-4035

R4. Nathwani,AC, Tuddenham EGD, Rangarajan D, 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 CYC, 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*. December 22; 365(25): 2357–2365. DOI: 10.1056/NEJMoa1108046

R5. Nathwani AC, Reiss UM, Tuddenham EG, Rosales C, Chowdary P, McIntosh J, Della Peruta M, Lheriteau E, Patel N, Raj D, Riddell A, Pie J, Rangarajan S, Bevan D, Recht M, Shen YM, Halka KG, Basner-Tschakarjan E, Mingozzi F, High KA, Allay J, Kay MA, Ng CY, Zhou J, Cancio M, Morton CL, Gray JT, Srivastava D, Nienhuis AW, Davidoff AM. (2014). Long-term safety and efficacy of factor IX gene therapy in hemophilia B. *N Engl J Med*. Nov 20;371(21):1994-2004. DOI: 10.1056/NEJMoa1407309

R6. Patent US 9,504,762 B2 (2016)

R7. Rangarajan S, Walsh L, Lester W, Perry D, Madan B, Laffan M, Yu H , Vettermann C, Pierce GF, Wong WY, Pasi KJ. (2017). AAV5-Factor VIII Gene Transfer in Severe Hemophilia A. *N Engl J Med.* Dec 28;377(26):2519-2530. DOI: 10.1056/NEJMoa1708483

4. Details of the impact

Haemophilia affects approximately 1 in 10,000 males world-wide. It varies in its severity, but symptoms include frequent bruising and joint pain and, if left untreated, can in many cases result in uncontrolled internal bleeding. The condition has a significant impact on quality of life for all those affected. Standard treatments require injections of clotting factors several times per week for life. Although stringent safety procedures are in place for preparation of blood products, the standard treatment carries a theoretical risk of infection with new and emerging viruses.

The UCL team, led by Nathwani and Tuddenham, has pioneered a single-dose gene therapy that allows individuals to produce the missing clotting factor and removes the need for further prophylactic treatment, transforming quality of life and reducing ongoing healthcare costs**.** Successful UCL-led trails in small numbers of patients have been followed by at least six further trials involving approximately 300 patients with severe/moderate haemophilia who, to date, continue to benefit from the therapy. The technology platform has been commercialised and led to multibillion pound investments by large pharmaceutical companies in gene therapies for haemophilia and other genetic disorders. It has also resulted in creation of a spin-out company Freeline Therapeutics, founded by Nathwani, that has trialled an enhanced AAV vector/gene construct that returns Factor IX levels to near normal.

Patient benefits and cost savings
Following the first successful gene therapy trials for haemophilia at UCL, Phase 3 trials led by pharmaceutical companies including UniQure and Biomarin, have treated approximately 300 patients in total to date. The trials demonstrated that in 90% of cases, the gene therapy treatments for haemophilia lead to significant increases in levels of Factor VIII or IX, concomitant reduction in internal bleeds and no need for ongoing treatment. Based on data from the original UCL trial for haemophilia B, in which five of the seven participants who had previously relied on prophylactic treatment were able to stop injecting, with no spontaneous bleeding episodes, the UCL team estimated that trial alone had saved the NHS more than GBP1,500,000 in the first four years (**R5**).

UniQure, a gene therapy specialist, has licensed the factor IX gene cassette developed by UCL and used in the 2011 trial led by UCL, to develop a proprietary factor IX replacement therapy for haemophilia B. Long term follow up data reported in December 2019, showed sustained factor IX activity in all 10 patients four years after treatment with AMT-060 at a level sufficient to eliminate or significantly reduce the risk of bleeding events (**S1**). In a recent Phase 3 trial undertaken by Uniqure and based on the gene therapy construct developed at UCL 54 patients received treatment and by 26 weeks post-therapy, 96.3% had successfully discontinued routine prophylaxis and 72.2% had not experienced any bleeds post treatment (**S2**)**.**

Among pipeline products for haemophilia A, **BioMarin** has trialled a therapeutic programme using a factor VIII vector licensed from UCL and St Jude, involving 135 patients with moderate/severe haemophilia. Three-year data released in 2019 showed that nearly all participants who had received a one-time dose of the vector were no longer having to infuse clotting factors, and that average annualised bleed rates had reduced by 96% (**S3**).

Individuals who have received the gene therapy treatment in trials commonly report that, post treatment they are more active and enjoy greater confidence. One gene therapy recipient said “What I have experienced in the last 3 years is a factor level hovering around 40% and no bleeds at all, and nothing coming close to bleed. Some simple pleasures - not being so stiff in mornings, no limping and pain getting off airplanes or out of movie theatres, not worrying about how far away my fridge is, not having to bring treatments with me on out-of-town trips.” (**S4**)**.** In a recent Haemophilia Society newsletter a gene-therapy recipient said his new Factor IX levels

were “insane” and he hadn’t had any treatment for two years since the therapy. He added: “I don’t think about my haemophilia much anymore.” For another, this created an issue at first. He felt like he had lost part of his identity, saying “I was no longer ‘Paul the haemophiliac’” but added, “I haven’t had any treatment since 2018. I can’t stop smiling” (**S5**).

Spin out company Freeline Therapeutics

In 2015, Professor Nathwani founded a spinout company, Freeline Therapeutics, to develop a commercial AAV-based therapy for haemophilia B, based on UCL’s research. The company, which has its headquarters in Stevenage, UK, now employs 200 FTEs in the UK, Germany and the US. Its product candidate, FLT180a, uses a synthetic AAVS3 vector to enhance the delivery of a highly functional form of the human factor IX gene to a patient’s liver cells. Data from a Phase 1/2 clinical trial of 10 patients released in July 2020 showed that, at the lowest dose, participants with severe or moderate symptoms achieved long-term durability, displaying a level of factor IV activity at 52 and 104 weeks normally associated with the mild form of the disorder. Individuals in the highest-dose group were able to sustain factor IX activity at 50-150% of normal levels (**S6**). The company is now using this vector to develop a pipeline of therapies for other genetic disorders.

Freeline’s work has attracted significant investment, including USD40,000,000 (GBP30,600,000) of Series C funding from Syncona, a FTSE 250 healthcare company, and a further USD80,000,000 (GBP61,200,000) from investment firms Novo Holdings A/S, Eventide Asset Management and Wellington Management Company, among others (**S7**). Following the announcement, Managing Partner, Novo Holdings A/S said: “Our investment strategy is to identify and invest in US and European life science companies which are true leaders in their area of expertise, developing innovative product candidates that significantly advance patient care. We have been impressed by the Freeline platform and its scientific co-founders, as well as their experienced management team, and we are delighted to support Freeline as they continue to build momentum.” An Initial Public Offering on NASDAQ in August 2020 raised an additional USD158,800,000 (**S8**).

Large pharma licencing gene therapy to treat haemophilia A and haemophilia B

Gene therapy is becoming the treatment of choice for haemophilia and major pharmaceutical firms are investing heavily in the field. In recent years, Pfizer has acquired an interest in the haemophilia A candidate SB-525 through a licensing deal with its creator, Sangamo who in 2017, received an upfront payment of USD70,000,000 (**S9**) and a further USD25,000,000 on early completion in 2019**.** At the same time, the world’s largest biotech company, Roche, has acquired Spark Therapeutics, developer of the SPK-8011 platform (also for haemophilia A) (**S10**).

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

S1 uniQure Announces One-Year Follow-Up Data from the Phase IIb Study of Etranacogene Dezaparvovec and Long-Term Follow-Up Data for AMT-060 in Patients with Hemophilia B: https://tools.eurolandir.com/tools/Pressreleases/GetPressRelease/?ID=3677182&lang=en-GB&companycode=nl-qure&v=

S2 Pipe SW et al. (2020) LBA-6 First Data from the Phase 3 HOPE-B Gene Therapy Trial: Efficacy and Safety of Etranacogene Dezaparvovec (AAV5-Padua hFIX variant; AMT-061) in Adults with Severe or Moderate-Severe Hemophilia B Treated Irrespective of Pre-Existing Anti-Capsid Neutralizing Antibodies. 62nd American Society of Hemotology Annual Meeting and Exposition: https://ash.confex.com/ash/2020/webprogram/Paper143560.html

S3 Biomarin confirms timeline for gene therapy of hemophilia putting pressure on rivals: https://www.biopharmadive.com/news/biomarin-confirms-timeline-for-hemophilia-gene-therapy-putting-pressure-on/558279/

S4 Patient Testimonial: My Experience on a Gene Therapy Trial John Konduros, Children's Hospital of Philadelphia's (CHOP), United States. doi:10.1016/j.transci.2019.08.011

S5 Haemophilia Society newsletter. Winter 2020.

S6 Chowdary P. Phase 1/2 interim data from B-AMAZE study of adeno associated virus (AAV) gene therapy (FLT180a) confirms progress towards achieving Factor IX levels in the normal range for patients with severe or moderately severe haemophilia B. Presented at the 13th Annual Congress of the EAHAD, February 2, 2020; The Hague, Netherlands: https://www.ashclinicalnews.org/online-exclusives/flt180a-shows-efficacy-hemophilia-b-high-doses/

S7 Freeline Closes $120 Million Series C Financing Round: https://www.freeline.life/investors-media/newsroom/freeline-closes-120-million-series-c-financing-round/

S8 Freeline Therapeutics Rings the Nasdaq Opening Bell in Celebration of its IPO: https://www.albion.vc/news/freeline-therapeutics-rings-nasdaq-opening-bell-celebration-its-ipo

S9 Sangamo Therapeutics and Pfizer announce collaboration for Hemophillia A gene therapy: https://www.pfizer.com/news/press-release/press-release-detail/sangamo_therapeutics_and_pfizer_announce_collaboration_for_hemophilia_a_gene_therapy

S10 Roche concludes acquisition of Spark Therapeutics, Inc. to strengthen presence in gene therapy: https://www.roche.com/media/releases/med-cor-2019-12-17b.htm