**Nucleic Acid-Based Therapies: A Developing Frontier for Precision Medicine**

**Munir Pirmohamed, MB ChB, PhD, FRCP, FRCP(E), FMedSci**

**Department of Molecular and Clinical Pharmacology, Institute of Translational Medicine, University of Liverpool, Block A: Waterhouse Building, 1-5 Brownlow Street, Liverpool, L69 3GL**

E-mail: munirp@liverpool.ac.uk

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The genomic revolution, heralded by completion of the human genome project, is providing unprecedented knowledge of the underlying genetic basis of disease. In the UK, the 100,000 genomes project, is beginning to uncover the genetic basis of rare diseases, ending the diagnostic odyssey, which faced many families. Precision diagnosis however is only the first step in the development of new precision therapies, where there is a need to understand the molecular basis of disease to enable the development of treatments targeted to those individuals with specific mutations, to repair or overcome the underlying molecular defect.

Understanding of the molecular basis of cystic fibrosis has already led to the development of small molecules, such as ivacaftor, that improve the functioning of the CFTR protein with the *G551D* mutation, which affects 4% of the cystic fibrosis population1. Identification of novel mutations can also allow the re-purposing of existing medicines. An example is the use of high-dose riboflavin in childhood motor neurone, a condition caused by mutations in the riboflavin transporters2, *SLC52A2* and *SLC52A3*.

Over the last year, there have been some dramatic advances using nucleic acid based therapies. Haemophilia A, an X-linked disorder, currently requires frequent infusions of factor VIII to prevent bleeding episodes. A recent dose-ranging study using an adenovirus gene therapy vector showed that factor VIII levels were normalised 52 weeks after a single infusion in 6 out of 7 participants, with a reduction in bleeding episodes and rescue factor VIII infusions3. If the results were replicated in a larger group, this could potentially herald a cure for haemophilia, which would be a remarkable advance. Key to this will be the demonstration of long-term effectiveness and safety, including whether the body mounts immune responses to the viral vector. Gene therapy has had a chequered history, but improved methods of packaging gene inserts and their delivery may finally be on the verge of producing transformational developments, as seen not only with haemophilia A3, but also with sickle cell disease4, junctional epidermolysis bullosa5 and *RPE65*-mediated inherited retinal dystrophy6.

Another form of nucleic acid therapy is the use of antisense oligonucleotides. These are single-stranded oligonucleotides which bind their complementary mRNA affecting splicing and restoring protein synthesis7. This is exemplified by nusinersen sodium, recently licensed for the treatment of spinal muscular atrophy, which works on the *SMN2* gene producing a full-length protein. A single randomised controlled trial showed that treatment early in life reduced mortality and improved motor function8. The cost however is enormous ($750,000 first year, $375,000 per year thereafter) leading to questions about its clinical value. An alternative mode of action of antisense therapy is to prevent the formation of a mutated protein7. This has generated a lot of media excitement with the announcement that the drug, Ionis-HTTRx, reduced the levels of the mutant Huntingtin protein in CSF in a dose-dependent manner in a phase I trial9. The results of the trial have not been published, and of course, it is too early to know whether longer-term therapy will improve symptoms and survival. However, if shown to work clinically, it would be truly transformational.

Finally, genome editing holds enormous promise for the future. It is the ability to edit DNA to remove a mutation, correct a mutation or alter the sequence at a precise location in the genome using engineered nucleases such as the CRISPR-Cas9 system10. Genome editing of embryos is possible as highlighted by a recent study where a mutation in the MYBPC3 gene, which causes hypertrophic cardiomyopathy, was repaired11. However, the ability to change the genome where the genetic alteration could be passed from one generation to another, brings up ethical challenges, with (perhaps unfounded) fears that this could pave the way to the development of “designer babies”. Another concern relates to the specificity of the CRISPR-Cas9 system, and whether it may lead to off-target mutations10. Somatic gene therapy is perhaps less of a concern ethically, and is already finding application in malignancies. T cell therapies using chimeric antigen receptors (CAR) have been used successfully in malignancies such as acute lymphoblastic leukaemia (ALL) leading to the first successful FDA approval of tisagenlecleucel, a CAR-T cell therapy targeting CD19 in ALL, at a list price of $475,000 for a one-time infusion12.

In conclusion, there have been remarkable advances in nucleic acid based therapies over the last few years, providing treatments for diseases of unmet need. Over the next few years, it will be important not to over-hype the promise of these technologies, but to adopt a more realistic approach which allows proper assessment of efficacy and develop monitoring strategies to assess long-term safety especially since only small numbers are likely to be treated, at least initially. A key challenge will be affordability of these novel therapies, particularly since many may be cost-ineffective if conventional health economic models are applied. [798 words].

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**References**

1. Ramsey BW, Davies J, McElvaney NG, et al. A CFTR potentiator in patients with cystic fibrosis and the G551D mutation. *N Engl J Med* 2011;365(18):1663-72. doi: 10.1056/NEJMoa1105185

2. Johnson JO, Gibbs JR, Megarbane A, et al. Exome sequencing reveals riboflavin transporter mutations as a cause of motor neuron disease. *Brain* 2012;135(Pt 9):2875-82. doi: 10.1093/brain/aws161

3. Rangarajan S, Walsh L, Lester W, et al. AAV5-Factor VIII Gene Transfer in Severe Hemophilia A. *N Engl J Med* 2017;377(26):2519-30. doi: 10.1056/NEJMoa1708483

4. Ribeil JA, Hacein-Bey-Abina S, Payen E, et al. Gene Therapy in a Patient with Sickle Cell Disease. *N Engl J Med* 2017;376(9):848-55. doi: 10.1056/NEJMoa1609677

5. Hirsch T, Rothoeft T, Teig N, et al. Regeneration of the entire human epidermis using transgenic stem cells. *Nature* 2017;551(7680):327-32. doi: 10.1038/nature24487

6. Russell S, Bennett J, Wellman JA, et al. Efficacy and safety of voretigene neparvovec (AAV2-hRPE65v2) in patients with RPE65-mediated inherited retinal dystrophy: a randomised, controlled, open-label, phase 3 trial. *Lancet* 2017;390(10097):849-60. doi: 10.1016/S0140-6736(17)31868-8

7. Mustonen EK, Palomaki T, Pasanen M. Oligonucleotide-based pharmaceuticals: Non-clinical and clinical safety signals and non-clinical testing strategies. *Regul Toxicol Pharmacol* 2017;90:328-41. doi: 10.1016/j.yrtph.2017.09.028

8. Finkel RS, Mercuri E, Darras BT, et al. Nusinersen versus Sham Control in Infantile-Onset Spinal Muscular Atrophy. *N Engl J Med* 2017;377(18):1723-32. doi: 10.1056/NEJMoa1702752

9. Orgill M-A. Drug lowers deadly Huntington’s disease protein 2017 [Available from: <http://www.ucl.ac.uk/consultants/uclc-news/brain> accessed 7 January 2018 2017.

10. Komor AC, Badran AH, Liu DR. CRISPR-Based Technologies for the Manipulation of Eukaryotic Genomes. *Cell* 2017;168(1-2):20-36. doi: 10.1016/j.cell.2016.10.044

11. Ma H, Marti-Gutierrez N, Park SW, et al. Correction of a pathogenic gene mutation in human embryos. *Nature* 2017;548(7668):413-19. doi: 10.1038/nature23305

12. Bach PB, Giralt SA, Saltz LB. FDA Approval of Tisagenlecleucel: Promise and Complexities of a $475000 Cancer Drug. *JAMA* 2017;318(19):1861-62. doi: 10.1001/jama.2017.15218