Great expectations: Virus mediated gene therapy in neurological disorders

Didu ST Kariyawasam [1][2] MA, Ian E Alexander PhD [3,4], Manju A Kurian PhD [5,6], Michelle A Farrar MBBS PhD [1][2] *

[1] Department of Neurology, Sydney Children's Hospital, Randwick, Sydney, New South Wales, Australia

[2] School of Women's and Children's Health, University of New South Wales Medicine, University of New South Wales, Sydney, New South Wales, Australia

[3] Gene Therapy Research Unit, Children's Medical Research Institute, Faculty of Medicine and Health, University of Sydney and Sydney Children's Hospital Network, Westmead, Australia

[4] Discipline of Child and Adolescent Health, Sydney Medical School, Faculty of Medicine and Health, University of Sydney, Westmead, Australia

[5] Developmental Neurosciences, UCL GOS Institute of Child Health, London, UK

6 Department of Neurology, Great Ormond Street Hospital

*Corresponding author

Dr Michelle Farrar

Level 8 Bright Alliance

Sydney Children's Hospital

High Street

Randwick

New South Wales, 2031

Australia

m.farrar@unsw.edu.au

(+61) 293821549

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ABSTRACT

Gene therapy has tremendous potential for the treatment of neurological disorders to transform patient care. The successful application of gene therapy to treat spinal muscular atrophy is a significant milestone, serving to accelerate similar progress in a spectrum of neurological conditions, with more than 50 clinical trials currently underway, across neurodevelopmental, neurodegenerative, muscular dystrophy, epilepsy, chronic pain, and neoplastic diseases. This review provides an overview of the key features of gene therapy, paradigms of delivery and dosing, potential risks and highlights ongoing research to optimize safe and effective delivery of vectors into the nervous system. Examples of the application of gene therapy in various neurological diseases alongside clinical development challenges will be presented. As the development and translation of gene therapies gain pace, success can only ultimately be realised for patients following implementation in the health system. The challenges and controversies of daunting costs, ethics, early diagnosis and health system readiness will require innovative pricing schemes, regulatory policies, education and organisation of a skilled workforce to deliver of high-quality care in clinical practice as we prepare for advanced therapeutics in neurology.

KEY WORDS

INTRODUCTION

The approval of the first gene therapy (GT) to treat young children with spinal muscular atrophy (SMA), by the Food and Drug Administration (FDA) in 2019, was a significant milestone in clinical neurology, highlighting the potential of genetic therapies to modify a spectrum of similarly challenging and devastating neurological conditions. Unprecedented advances in genetics, virology, immunology and cell biology have ushered in a new era of genetic science, encouraging cutting edge research to develop novel and targeted vectors to optimise efficacy, safety and advance applications. The recent progress of gene therapy has meant that an unparalleled number of gene transfer reagents and strategies are now ready to be implemented, attracting widespread stakeholder interest and becoming directly relevant to clinical neurology practice. The science behind these innovative technologies is unique, rapidly advancing and forms the foundation for a new frontier of disease modification. Accordingly, it is imperative that clinicians develop an understanding of the scientific principles that are informing emerging clinical pipelines. Understanding the unique challenges within the field and focussing on solutions to these obstacles is vital to facilitate effective and safe clinical implementation. This review will focus on advances in gene therapy technology, clinical applications, current uncertainties and barriers to translation as we enter a revolutionised treatment landscape for neurological diseases.

Principles and neurological applications of genetic modulation

The goal of gene therapy is to deliver nucleic acids (DNA or RNA) or synthetic derivatives to target cells. Gene therapy technology commonly utilises key components including a vector and enclosed expression cassette. The latter consists of an enhancer/promoter, a transgene with associated polyadenylation signal and in some cases, other elements such as introns or post-transcriptional regulatory elements (Figure 1.). These act to correct the underlying defect

in a host cell, a process defined as 'transduction' of the cell target. A variety of genetic

strategies are available dependant on the condition and causative genetic mutation (Figure 2).

Due to their monogenic nature, an array of neurological diseases are amenable to

modification.

Gene addition and gene silencing strategies

Where disease is caused by loss of function mutations, wild-type cDNA is introduced to

replete therapeutic protein production, a process known as gene addition [1]. Alternatively,

gene knockdown/silencing may be beneficial in instances of a pathological gain of function

mutation. In the latter, the transgene encodes a small interfering RNA (siRNA) that causes

sequence-specific degradation of target mRNA, leading to depletion of mutant protein [2]. For

instance, in Amyotrophic Lateral Sclerosis overproduction of superoxide dismutase 1 (SOD1)

is silenced by a transgene encoding small-length nucleotide chains [3]. These genetic principles

are extrapolated to more complicated, multigenic processes and may also have dual disease

targets. For instance, in Parkinson's disease (PD), characterised by depletion of dopamine

secondary to striatonigral neurodegeneration, transgenes encoding enzymes essential to

biosynthetic dopamine pathways hold potential utility. Several studies have demonstrated

short-term improvements in motor function with intraputaminal introduction of the *AADC*

transgene (encoding AADC enzyme), catalysing levodopa substrate to dopamine [4-6].

Separately, similar gene addition strategies, utilising AAV vectors have shown efficacy in

treatment of aromatic L-amino acid decarboxylase deficiency, a primary neurometabolic

disorder of serotonin and dopamine synthesis, caused by recessively inherited mutations of

the *AADC* gene [7]. An alternate, approach in PD (where derangements across multiple

monoamine pathways exist) has been triple gene therapy [8]. Here, lentiviruses that have large

transgene capacity, transport transgenes encoding three dopamine producing enzymes, to optimise parallel pathways of dopamine production. This methodology has facilitated improvements in motor function and spontaneous locomotor activity in treated non-human primates compared to controls [9]. A dual approach of gene silencing and gene addition is garnering pre-clinical attention to treat conditions such as Oculopharyngeal muscular dystrophy (OPMD). This autosomal dominant mutation is characterised by trinucleotide expansion in the polyadenylate-binding protein nuclear 1 (PABPN1) gene [10]. The resultant protein folds incorrectly, leading to accumulation of insoluble filaments and consequent weakness in proximal limb, pharyngeal and levator palpebrae muscles [11]. Here, a vector carries transgenes for siRNAs, silencing the dominant gain of function mutation whilst a replacement wild-type gene restores normal function [12].

Gene editing strategies

Correction of an underlying gene mutation (gene editing) has gained relevance in proof of concept studies. This emerging technology is based on introduction of a transgene encoding a DNA targeted enzyme (e.g. zinc finger nuclease and CRISPR (clustered regularly interspersed short palindromic repeats)/Cas 9 toolkits). These cause a break in a targeted DNA sequence [13]. Nucleotide and gene sequences are removed, inserted or modified and the DNA break repaired by harnessing endogenous cell repair processes. The utility of this type of gene modulation is seen in clinical phase II/III clinical trials for mucopolysaccharidosis II where these techniques are employed to insert the missing *IDS* gene *in vivo* [14]. Whilst strategies appear safe in preclinical studies, theoretical risks of 'off-target' gene editing (that change physiological processes or facilitate aberrant pathological pathways) exist, and are particularly problematic in cells that are irreplaceable such as in the central nervous system

(CNS)[15]. Alongside such hypothetical sequalae, acceptability and successful translation of gene editing strategies to the clinic will be dependent on disease-centred and individualised risk-benefit analysis.

For conditions that are multigenic, caused by environmental factors or in diseases where the underlying genetic basis of disease is still unknown, an alternative target of gene therapy includes altering expression of downstream proteins, enzymes or growth factors. For example, transgenes encoding anti-tau antibodies, (the hyperphosphorylated version of this protein form neuronal neurofibrillary tangles, accelerating axonal dysfunction and degeneration) have been efficacious in preclinical models of Alzheimer's disease [16]. In PD, vectors carrying neurotrophic growth factor (neurturin) gene protect dopamingeric neurones from degeneration, leading to improvements in motor scores by 36% in a subgroup of patients at 18 months of follow-up [17].

Ex vivo genetic strategies

The above strategies can occur either *in vivo or ex vivo*. *Ex vivo* methods employ cells transduced with a target gene outside the milieu of the patient's body. In the treatment of lysosomal storage diseases, where traditional intravenous enzyme replacement therapy restricts damage to visceral organs, but has limited capacity to cross the blood-brain-barrier (BBB) to prevent neuronal degeneration, autologous haematopoietic stem cells (HSCs) are transduced *in vitro* with a viral vector containing a healthy copy of the defective gene [18]. Gene-modified cells are injected systemically, enter the CNS and become glial cells where they produce supra-normal levels of the deficient enzyme. The enzyme is taken up by mutated cells of the CNS enabling cross-correction of the underlying deficiency and restoration of phenotype [18]. The efficacy of this strategy is shown in in phase I/II trials for

metachromatic leukodystrophy (caused by arylsulfatase A/ARSA enzyme deficiency), where nine treated patients showed stable engraftment, haematopoietic reconstitution with modification of cells and recovery of activity of ARSA activity in the cerebrospinal fluid (CSF). Delay in the progression of CNS demyelination and stable cognitive and motor scores were observed especially in those treated early in their disease course [19].

The key role of vectors in the gene technology arsenal: challenges and potential risks

Adeno-associated viruses (AAVs) have particularly been utilised as vectors in neurological disease (Table 1).

Table 1: Viral vector properties and clinical implications on efficacy and safety

Transgene expression duration: short term = days to weeks, long term = months to years, lifelong = occurs in sensory neurones of peripheral nervous system after Herpes Simplex based viruses establishes latency in dorsal root ganglion.

[a] Pathogenicity after emergence of viral replication ability *in vivo.*

[b] Food and Drug Administration approval of AVXS-101 in 2019 for treatment of spinal muscular atrophy

	Adeno-associated virus	Adenovirus	Simple retrovirus	Lentivirus	Herpes virus
Transgene carrying capacity	< 5kb	< 8kb	8kb	9kb	30 - 40kb
Integration into host genome	No	No	Yes	Yes	No
Target cell population	Mitotic and quiescent cells	Mitotic and quiescent cells	Mitotic	Mitotic and quiescent cells	Mitotic and quiescent cells
Transgene expression duration	Long term (in quiescent cells)	Short term	Long term	Long term	Life-long
Immunogenicity	Moderate	High	Low	Low	High
Insertional oncogenesis risk	Low	Low	Very High	Moderate	Low
Oncolytic potential	No	Yes	No	No	Yes
[a] Risk of human pathogenicity	Negligible	Possible but low risk	High	High	Possible but low risk
Comments on clinical utility	[b] Only vector to be approved and licenced for clinical use in neurological disease	Reduced utility in patients due to significant immunotoxic effects	Reduced utility in patients due to significant oncogenetic potential	*Ex vivo* strategies utilised due to reduced penetrance of BBB in adults	Effective in malignant brain tumours secondary to oncolytic potential

These non-replicating, non-pathogenic viruses are deemed safe in clinical subjects. AAVs are predominantly non-integrating viruses, incorporating the transgene extra-chromosomally (as an episome), reducing the risk of insertional mutagenesis (seen especially in early trials using retroviruses for immunodeficiency syndromes), and conferring theoretical durability in non-replicating cells of the CNS [20].

As AAVs are single-stranded DNA parvoviruses, bioengineering has been used to generate self-complementary genomes (scAAVs) which bypass the rate-limiting steps of second-strand synthesis. These act independently of the host cell machinery to enable more rapid protein expression [21], however limit transgene packaging capacity so are not universally utilised.

Despite many advantages, the universal application of AAV-mediated gene therapy for neurological disease is restricted by disease and patient-specific factors. For example, in conditions caused by large gene defects, the limited carrying capacity of this system to 5kb of DNA, requires alternative approaches to be investigated [22]. In Duchenne muscular dystrophy (DMD), caused by deletion of 2.2 Mb dystrophin, preclinical trials show efficacy in systemically administered micro/mini dystrophin forms (equating to small nucleotide sequences), with removal of subdomains that appear non-essential to amelioration of phenotype [23]. Alternatively, two viruses, each encoding part of the transgene may be utilised to deliver large genes. This dual AAV system, whilst less efficient, allows full-length reassembly of the gene *in vivo* through recombination of homologous areas or ligation of transcripts at the RNA level [24]. Viral vectors with a larger transgene carrying capacity such as lentiviruses have also been investigated in such diseases (Table 2).

Table 2: Ongoing viral gene therapy clinical trials for neurological disorders

Search strategy and selection criteria: Data for this table were identified by searches of publicly available clinical trial databases available in English that allowed systematic identification of gene therapy trials (5 Feb 2020); Australia and New Zealand Clinical Trials Registry (https://www.anzctr.org.au) , European Union Clinical Trials Register (https://www.ema.europa.eu/en/glossary/european-union-clinical-trials-register), United Kingdom National Institute for Health Research(https://bepartofresearch.nihr.ac.uk/), United States National Library of Medicine at the NIH (clinicaltrials.gov) and Gene Therapy Clinical Trials Worldwide (http://www.abedia.com/wiley/).

 OTC Ornithine transcarbamylase; AACD Aromatic L-Amino Acid Decarboxylase Deficiency; MPS mucopolysaccharidosis; GSD Glycogen storage disease; MLD Metachromatic Leukodystrophy; ALD adrenoleukodystrophy; SMA Spinal muscular atrophy; GAN Giant axonal neuropathy; CMT1 Charcot Marie Tooth type 1; LGMD Limb girdle muscular dystrophy; BMD Becker's muscular dystrophy; DMD Duchenne muscular dystrophy; PD Idiopathic Parkinson's disease; HD Huntington's disease; AD Alzheimer's disease; NCL Neuronal Ceroid Lipofuscinosis; ALS Amyotrophic Lateral Sclerosis; MS Multiple Sclerosis; NMO Neuromyelitis Optica; AAV adeno-associated virus; IV intravenous; IM intramuscular; IT intrathecal/intracisternal; IP intraparenchymal; SC subcutaneous; Newborns ≤ 1 month age; Infants 1 – 12 months age; Children 1 – 10 years, Adolescents 11 – 19 years; Adults > 20 years

[a] Neurotrophic growth-factor

[b] Neuropeptide

Condition	Vector	Administration route	Age of target population	Study phase	Genetic Strategy: Gene
Neurometabolic conditions					
OTC deficiency	AAV	IV	Adults	I/II	Addition: *OTC* gene
AACD Deficiency	AAV	IP	Infants, children	II	Addition: *AADC* gene
MPS type 1	AAV	IV	Children, adolescents, adults	I/II	Gene editing: insertion of *IDUA* gene
MPS type II (Hunters syndrome)	AAV	IT/IV	Infants, children, adults	I/II	Addition: *I2S* gene (cross correction affected cells) Gene editing: insertion of IDS gene
MPS type III (Sanfilippo disease)	AAV	IV/IP	Infants and children	I/II/III	Addition: *SGSH* gene ± SUMF1
MPS type VI	AAV	IV	Children, adolescents, adults	I/II	Addition: *ARSB* gene
Fabry Disease	AAV	IV	Adults	I/II	Addition: *GLA* gene
Pompe Disease	AAV	IV/IM	Children, adolescents, adults	I	Addition: *GAA* gene
GSD type IIb	AAV	IV	Children, adolescents, adults	I	Addition: *LAMP2B* gene
GM1 Gangliosidosis Type II	AAV	IV	Children	I/II	Addition: *GLB1* gene
Gaucher's disease	Retroviral	IV	Infants, children, adolescents, adults	I	Addition: *glucocerebrosidase* gene, ex vivo
Diseases of the white matter					
MLD	Lentivirus /AAV	IV/IP	Children	I/II	Addition: *ARSA* gene, in vivo and ex vivo
ALD	Lentivirus	IV	Children, adolescents	III	Addition: adrenoleukodystrophy gene, ex vivo
Peripheral neuropathies					
SMA	AAV	IV	Newborns, infants, children	III	Addition: *SMN1* gene
GAN	AAV	IT	Children, adolescents, adults	I	Addition e: *GAN* gene
CMT1	AAV	IM	Adolescents, adults	I/II	[a] Addition: *NFT3* gene
Painful diabetic neuropathy	Herpes virus	SC	Adults	I	Addition *GAD67* gene
Muscular dystrophies and myopathies					
LGMD 2E	AAV	IV	Children, adolescents	I/II	Addition: *SGCB* gene
LGMD 2D	AAV	IM	Children, adolescents, adults	I	Addition: *a* sarcoglycan gene
LGMD 2C	AAV	IM	Adolescents, adults	I	Addition: y sarcoglycan gene
X linked myotubular myopathy	AAV	IV	Infants and young children	I/II	Addition: *hMTM1* gene
BMD/IBM	AAV	IM	Adults BMD, Adolescents, adults IBM		Replace: *follistatin* gene
DMD	AAV	IV/IM	Children, adolescents	I/II	Addition: *GALG2*/mini or micro dystrophin/ *follistatin* genes
Neurodegenerative (central nervous system) and movement disorders					
PD	AAV	IP	Adults	I	Addition: [a] *AADC / NTN* [a] *GDFN/* [b] *GABA/* genes
HD	AAV	IP	Adults	I/II	Silence: *mHTT* gene
AD	AAV	IT/IV, IP	Adults	I	Addition: *APOE2 gene/* [a] *NGF, hTERT*
NCL	AAV	IP/IT	Infant, child, adolescents	I/II	Addition: *CLN2/CLN3/CLN6*
ALS	Lentivirus /AAV	IT	Adults	I/II	Addition [a] *GNDF* gene, ex vivo and *SOD1* gene in vivo
Neuroinflammatory Diseases of the Central Nervous System					
MS	Retrovirus	SC	Adults	I/II	Addition: *MBP gene*
NMO	Retrovirus	IV	Adolescents and adults	I	Addition: CD19+ and CD20+ chimeric receptor, ex vivo

A key limitation associated with viral vector choice is the part played by the body's inherent innate and adaptive immunological surveillance system in destroying viral vectors. Whilst exposure and primary immunity rates fluctuate between populations, some studies estimate that 90% of the adult population have been exposed to AAV (mainly respiratory) infections. Pre-existing antibodies to wild type AAV are prevalent in up to 40% of the population and have the potential to neutralise the vector before it reaches target tissues to off-load its genetic cargo [25]. Secondary reductions in efficacy and heightened safety concerns regarding immune-mediated toxicity have meant regulatory guidelines limit access to individuals that have neutralising antibodies below a predetermined threshold titre, for example ≤1:50. Definitive antibody titre limits to guide suitability and consensus on assay standards are not fully determined.

Immune-mediated sequalae are the basis for concerns around cell toxicity and safety associated with GT technology. In a dose finding study of Zolgensma, formerly AVXS 101, (containing *SMN1* transgene under a continuous promoter, carried in a scAAV9 vector), to treat children with infantile SMA , a mild transient asymptomatic transaminitis occurred in 27% children resolving with a short course of oral steroids and close monitoring post infusion [26]. However, in non-human primate and piglet studies intravenous administration of AAV vectors (at comparable doses to human trials), significant systemic and sensory neuron toxicities were noted [27]. Although dose limiting toxicity was not observed in treatment of infants < 8.5 kg under 2 years of age [26], these preclinical findings urge caution and careful monitoring when extrapolated to humans where high doses (in the order of 10^{14} virions/kilogram of bodyweight) are required for effective CNS penetration.

The durability of gene expression is especially important in post-mitotic cells as found in the CNS. Accordingly, sustained gene expression for over four and fifteen years is observed in

the putamen of patients and non-human primates treated for Parkinson's disease respectively [6, 28]. However, long-term outcome data is required to confirm the theory behind the 'one-time' approach that GT proposes. This is especially important when evaluating therapies for targets that undergo regeneration and replication, such as muscle tissue. It must be assumed with the current available technology that repeated administration of vector-transgenes would be less efficacious, secondary to the immune surveillance system that is primed to remove previously detected 'non-self' antigens. In conditions such as the aforementioned DMD where multiple parallel approaches of genetic intervention exist, dosing volume and regime is uncertain, and durability of response unknown, patients should be informed of the potential to be precluded from future, possibly more effective GT trials by participating in current studies.

Suitable levels of transgene product made by the appropriate cell type must be considered so as not to cause phenotypic toxicity and 'off target' gene expression. For example, in preclinical models of Rett's syndrome, replacement and consequent overexpression of the *MECP2* gene attached to a ubiquitous and continually activated promoter leads to impaired learning, memory and ictal features with phenotypic similarities to clinical *MECP2* duplication syndromes [29]. By incorporating cell-specific enhancers/promoters and modulators of transcription and translation into the expression cassette, therapeutic protein production is confined to a target cell and maintained within therapeutic levels [30]. Promoters that 'activate' only in the presence of certain drugs or conditions (for example chemogenetic promoters that turn on in the presence of a specific drug agonist) allows theoretically better control of transgene expression, timing and magnitude and maybe beneficial in disorders where neuropathology is paroxysmal [31]. Clinically, these choices may improve safety and tolerability for individuals.

Alongside demonstrating the emerging utility of these techniques, the onus is on the scientific community to research these findings further and develop ways to circumvent or reduce risks. For instance, in the evolving field of capsid and promoter engineering, endogenous biological characteristics of the vector are modified to improve safety, evade detection by the host immune system, enhance transduction and expression of the transgene and ensure a more targeted tissue specific approach. In preclinical trials, vector modification has promoted > 40-fold increase in genomes delivered to the CNS and improved the homogeneity of biodistribution with >50% of neurons and astrocytes showing effective transduction, while reducing biodistribution and targeting to other organs such as the liver [32].

Although AAVs form the predominant neurological vector, lentiviruses, adenoviral and Herpes Simplex based viral (HSV) vectors all have a part to play in gene therapy for neurological disease (Table 2). Lentiviruses transduce non-dividing and dividing cells, have larger transgene carrying capacity and long-term expression capacity. They insert their nucleic acid package semi-randomly, favouring integration in transcriptionally active genes and therefore may have oncogenetic potential. Due to their large size, lentiviruses have difficulty traversing the extracellular fluid of the brain. Simultaneously, their reduced circulatory survivability means that biodistribution after direct CNS injection or systemic administration respectively, is limited [33]. Thus, they are predominantly utilised in *ex vivo* techniques, for example in autologous transformation of CD34+ cells in patients with leukodystrophy [34]. Previous highly toxic and immunological effects related to the antigenic properties of adenovirus capsids, leading to a fatality in an in vivo trial for ornithine transcarbamylase deficiency, has limited its extensive use [35]. With an intrinsic ability to infect neuronal cells, disseminate across the neuronal network by retrograde/anterograde axonal movement and a large transgene carrying capacity, recombinant replication-defective HSV

vectors have developed to have sustained, safe, targeted transgene expression in neurones and are being evaluated in an array of neuro-specific gene therapy trials [36].

Dosing and delivery paradigms: effect on safety and efficacy

In parallel with development of vector-gene design, the route of administration alters efficacy, safety and patient acceptability of these therapies. Systemic intravenous administration (of AAV's in particular) is the most logistically practical, non-invasive route, however, requires higher doses to ensure adequate numbers of vector-genomes cross the BBB. Biodistribution associated with this route may also include non-targets such as the liver, potentially affecting safety. Dosing directly into the brain (intraparenchymal) or CSF compartments (the latter by intrathecal, stereotactically-targeted regions or intracerebroventricular routes) theoretically reduces immunotoxicity by limiting spread to multiple organs and sequestration of the vector in the liver upon first pass. In patients with symptomatic Parkinson's disease, suppression of neuronal firing in the subthalamic nuclei after GABAergic input, improves symptomology. Accordingly, an AAV vector housing an enzyme (glutamate decarboxylase/GAD enzyme) that converts endogenous glutamate to GABA, when injected directly into the subthalamic nuclei, at six months post intervention improved validated disease rating scores by 36% from baseline [37]. Thus, patients with terminal disease can be symptomatically managed with gene therapy despite underlying degeneration of the nigrostriatal pathways. Whilst intraparenchymal delivery has been well tolerated in clinical trials, the neurosurgical expertise required restricts this methodology to institutions with relevant resources when translated into real world practice, and perhaps to diseases that are refractory and degenerative in nature. Patient acceptability may also be dependent on the hitherto unvalidated theory that gene therapy is a one-time only procedure.

Whilst intrathecal pathways have efficacy in treating diseases of motor and sensory neurones, this route has limitations in terms of tissue tropism and biodistribution in non-human primates, tending to preferentially transduce astrocytic cells and accumulating around perivascular spaces and Purkinje cells of the cerebellum [38]. Furthermore, whilst theoretically evading the hosts' immune surveillance system by administration into the 'protected' site within the BBB, intrathecal delivery still induces an antibody response [38].

Efficacy of treatment response may also be related to timing of intervention. For example, early initiation of GT potentially prevents disease progression in neurodegenerative diseases, with presymptomatic dosing leading to the best outcomes in patients with SMA [39] and the mucopolysaccharidoses [40]. In other diseases such as adult-onset neurodegenerative pathologies, a long latent period prior to emergence of symptoms makes it difficult to determine the therapeutic window. The emergence of GT thus has far-reaching implications; early diagnosis appears imperative for some conditions supporting addition of these disease to newborn screening programmes [41]. In contrast, patients who have progressed along their disease course should be appropriately counselled of difficulties in predicting benefits of intervention, which may primarily be focused on slowing progression. Gene technologies that modify symptoms of refractory disease may be more appropriate in this setting, as seen in many approaches for symptomatic relief of drug-resistant Parkinson's disease [37].

Gene therapy: the expanding potential in neurological disease and barriers to implementation

Gene therapy holds significant potential for disease modification in conditions that are often life-limiting and refractory to conventional medication. As such, an accelerated translational pathway is sought by stakeholders. Accordingly, the US government has removed special

oversight regulations from gene therapy trials and mandated an approach consistent with conventional medications when considering approval status [42, 43]. Whilst we have utilised examples of conditions to illustrate the broad principles of 'classical' gene therapy, the clinical development pipeline encompasses exploration of novel targets and techniques. These examples also exemplify unresolved questions within clinical and research frameworks arising from GT. They include the urgent need for detailed natural history studies and biomarkers of disease progression and treatment response so that we can be trial ready to test the effectivity and efficiency of these novel, advanced therapeutics as they rapidly emerge. Barriers to clinical implementation include the high cost, equity of access and best-practice standards for safe and streamlined administration of these biological agents. Furthermore. approval of GT is region specific, and in many countries, additional regulatory systems govern its use. These oversee biosafety, procurement of gene therapy licences, importation, policy guidelines and codes of practice. Understanding and navigating the often complex, country specific regulations and policies, alongside education and organisation of a skilled workforce to deliver GT, will ensure that healthcare services are prepared for clinical translation of these unique therapeutics. Two hypothetical cases are illustrated, to compare and contrast clinical applications and the associated challenges of gene therapy for two neurodegenerative diseases 1.) the monogenic disease of Spinal muscular atrophy where gene therapy is already being utilised within the therapeutic repertoire and 2.) Parkinson's disease where multiple biomechanisms contribute to the presenting phenotype and gene therapy strategies are still in the clinical trial phase (Case 1 and 2).

Case 1: Clinical pathways for administration of gene therapy for SMA

A hypothetical case is explored to highlight the current evidence base, clinical implications and challenges to implementation of gene therapy for SMA.

A neonate, born at term, screens positive for 5q deletion of exon 7 of the *SMN1* gene through a state-wide newborn screening programme. Diagnostic bloods verify homozygous deletions of exons 7 and 8 in *SMN1* and confirms 2 *SMN2* copy numbers (the neonate is therefore most likely to develop SMA type 1).

On neurological examination the neonate is presymptomatic. EMG shows no signs of active denervation. The neonate is the first child of parents who live in a rural region of the country.

The neonate's treatment will be managed at a tertiary neuromuscular centre 300 km away from home. Treatment options (regulatory and reimbursement dependent) include access to a single dose of intravenous gene therapy with an scAAV9 vector carrying the *SMN1* gene, an (multiple dosing) intrathecal *SMN2* enhancing therapy or supportive care alone.

Clinical considerations and conundrums

- **Should gene therapy be initiated in the presymptomatic phase of SMA?**

  Efficacy of gene therapy for SMA is increased when started early. Of sixteen patients treated before the age of six weeks and within the presymptomatic phase of their disease, all were alive and free of permanent ventilation after (06.0-18.6 month of age at last follow up Dec 31st, 2019) (NCT03505099). Functional motor scores increased rapidly in this group when compared to patients treated after symptom onset (NCT03306277). Likewise, all infants treated with nusinersen in the presymptomatic phase of SMA were alive and without the need for permanent ventilation and experienced improvements in motor milestones in timelines consistent with normal development. There are no head to head trials that determine whether gene therapy is more efficacious than other approved disease modifying agents.

- **Will this neonate be eligible for gene therapy?**

  Existence of pre-existing neutralising AAV9 antibodies (maternally derived/acquired and in titres > 1 in 50) may preclude eligibility for gene therapy in this neonate. Currently, testing for these titres is centralised to few international locations. Development of clinical pipelines for expedient testing and confirmation of results prior to dosing are essential. Pre-infusion assessment of liver and renal function, haematology and signs of active infection are important.

- **How quickly should dosing occur?**

  With many SMA experts in agreement that delays to treatment come at the cost of motor neuron degeneration, expedient timing of dosing is imperative. Dosing cannot occur during periods of illness or active infection. Treatment choices may thus depend on family preference, safety and the availability of local resources required to administer disease modifying therapy in a timely manner. *

- **What are the short-term side effects and cautions with gene therapy and how are these ameliorated? Are there any long term sequalae?**

  The most commonly occurring side effects associated with gene therapy are monitorable and manageable. These include a rise in liver transaminases, thrombocytopaenia and vomiting. Side effects are reduced with the initiation of oral steroids prior to dosing and close post infusion blood monitoring for three months to guide steroid titration. A transient rise in cardiac enzymes (troponin I) can also occur and should be monitored over the same duration. This has unclear clinical implications; however cardiac toxicity has been noted in preclinical studies. Children should be vaccinated against respiratory syncytial virus and other immunisations completed before infusion or deferred till after steroid course is completed. The long term sequalae are unknown but include a small theoretical risk of oncogenesis.

- **Will the effect of gene therapy be sustained?**

  This is unknown. Although preclinical data suggests durability of transduction due to the episomal nature of AAV9, human studies are yet to corroborate this. Ongoing long term follow up data has shown no previously achieved motor milestones have been lost (range 4.1-5.2 years Dec 31st, 2019 in symptomatic patients). If multiple doses are required to maintain efficacy in the future, antibody mediated responses to AAV9 and its genetic cargo must be circumvented. Bioengineering of the vector-gene may facilitate such sequalae in the future.

- **What is the commitment required from the family?**

  The promise of a 'one and done' approach to management (with a single intravenous infusion) appears advantageous, but incorrect. In reality, pre-screening and clinical evaluation prior to dosing and regular (weekly/bimonthly liver function, FBC and EUC tests) post dosing require a significant commitment from families. Psychosocial factors including ability to travel to specialist centres, impact on work and family life and financial cost of time spent attending appointments require consideration and support from appropriate services. This includes ongoing provision of SMA standards of care, especially for symptomatic patients.

- **Will the neonate be cured and is there a role for combination therapy?**

  Timely therapeutic intervention is probably the primary determinant of clinical outcomes. In presymptomatic children, subclinical motor neurone loss may occur prior to treatment initiation such that clinical follow up and management is important. Currently, we have limited data to understand the cellular mechanisms behind SMN repletion. With constraints on financial budgets and risks of cumulative side effects, combination therapy may not be justified without evidence of significant improvements in efficacy.

Conclusions for the clinician

- Gene therapy for conditions such as SMA is a fast-evolving field.

- Shared decision making with families, highlighting the current evidence base and unknown elements ensures that informed, patient and family centred management occurs.

* Jurisdictional variations in reimbursement structure, regulatory approvals and local policies mean that logistical as well as clinical factors play a part in influencing optimal treatment options for an individual.

Case 2: The future of gene therapy for Parkinson's disease
A hypothetical case is explored to highlight the current evidence base, clinical implications and challenges to implementation of gene therapy for Parkinson's disease

A patient in his/her 70's has been diagnosed with Parkinson's disease for the last two years. Symptoms include unilateral rigidity, bradykinesia and tremor which are now significantly affecting the patient's ability to perform activities of daily living.

He/she has associated poor sleep and low mood and suffers intermittently from dizziness secondary to autonomic dysfunction, a known association with the underlying disease. Conventional treatment with Levodopa has been limited by the 'on/off phenomenon' including dyskinesia during dose escalation and breakthrough periods of akinesia between doses.

Clinical considerations and conundrums

- **What is the clinical rationale for treatment with gene therapy in this patient?**

  Due to the multiple, interplaying mechanisms that contribute to Parkinson's phenotype, gene therapy for this disease has a spectrum of viable targets. Clinical rationale for gene therapy could be based on whether disease modification is achievable or whether symptomatic relief alone is the goal. Disease modifying routes could be considered in the early course of disease (when there are low levels of nigral degeneration), to stop disease mediated cell death, thereby influencing disease progression as well as ameliorating symptoms.

- **What are the proposed disease modifying genetic strategies in Parkinson's disease?**

  These involve delivery of growth factor genes to targeted areas of the CNS. AAV-GDNF injected into the putamen was safe and effective at ameliorating parkinsonian behaviours in preclinical small animal and non-human primate models. Based on this, a phase I clinical trial is underway to test safety and efficacy in humans (NCT 01621581). Similar strategies include AAV-NTN that protects dopaminergic neurones from degeneration in preclinical studies. Efficacy has not been replicated between phase I and II clinical trials where primary outcomes of motor function improvement with treatment were unmet.

- **What are the proposed strategies for symptomatic management and how will these benefit patients who have trialled conventional medications with variable efficacy or reduced tolerability?**

  In patients with similarly severe 'on/off' symptoms linked to exogenous levodopa intake an endogenous dopamine synthesis strategy may be helpful. Introduction of three genes, constituting enzymes responsible for dopamine synthesis in one vector has garnered attention. A phase I trial showed that putaminal injections of this triplicate, lentivirus-mediated genetic package was well tolerated, reduced Unified Parkinson Disability Scores (UPDS) by 12 points and decreased the need for medication.

  Similarly, in patients with uncontrollable motor fluctuations with levodopa treatment, an improved response to conventional treatment, improvement in symptomology in the 'off-state' and a modest reduction in UPDRS scores with AAV-AADC therapy was noted.

- **What is the utility of gene therapy for non-motor effects of Parkinson's disease?**

    Gene therapy for Parkinson's disease has focussed on predominantly local injections of vector-gene to sites across the nigro-striatal pathway. Focal administration of viral vectors may ameliorate basal ganglia centred (motor) symptoms. However, the global effects of disease on cognition, autonomic function and mood which significantly impact quality of life, may not be eased using these stratagems and require further evaluation in clinical trials.

- **How far is away is gene therapy from being a viable therapeutic option for our patient, outside a clinical trial?**

    The translational pipeline from proof of concept studies, through preclinical/clinical trials to an approved therapy can be lengthy. In Parkinson's disease, efficacy in preclinical models has not been faithfully translated in the clinical trial domain. Variations in diffusion and retrograde transport of viral vectors between species may account for these findings. Animal models that more accurately reflect the pathophysiology in Parkinson's disease may be more helpful in evaluating genetic therapies. However, maintaining safety must be the central paradigm throughout the pipeline. Although gene therapy for Parkinson's disease remains within the clinical trial phase, with the acceleration of these technologies and the adaptation of successful strategies from Parkinson specific and other neurodegenerative diseases, clinical translation of these therapeutics is becoming a feasible possibility.

Conclusions for the clinician

- Delineation of a patient's disease stage, response to conventional medications and patient-specific goals of therapy are vital to inform the appropriate genetic strategy in a disease where there are multiple biochemical targets.

- Clinicians can facilitate the translational pipeline by updating patient registries, ensuring clinical-trial readiness.

Barriers to implementation: the impact of advanced therapeutics on clinical trial design

Preclinical trials have showed efficacy in preventing neurodegeneration, particularly of the spiny neurons in the striatum in Huntington's disease utilising gene knockdown or gene editing techniques [44]. A theoretically 'ideal' condition in which to apply the strategies of classical gene therapy (due to presence of a long prodromal phase for intervention and one, highly penetrant causative gene mutation), clinical trials are yet to replicate efficacy seen in preclinical models. By utilising clinical outcome measures large patient numbers and long

duration of follow-up are required, to show any meaningful change with treatment; an issue that could be partly addressed by the development of more robust disease biomarkers to measure efficacy of GT. The multi-systemic pathophysiology inherent to Huntington's disease means that recognised co-morbidities concomitantly require investigation to fully assess therapeutic efficacy. In areas such as cognition outcome measures are less well established and trial endpoints not well demarcated. Cumulatively, this increases the complexity and duration of clinical trials for GT.

A conceptual change in trial design is required, concentrating on safety and limited dose escalation over a pharmacologically therapeutic range, whilst sustained transgene expression in non-dividing cells of the CNS, potentially make traditional later trial phases redundant. Limitations in cohort size associated with rare diseases require adaptive and innovative trial designs. In Huntington's disease clinical trials, recruitment enrichment strategies have increased statistical power, reduced numbers required to enrol and improved the probability of seeing therapeutic benefit within a defined time period, in presymptomatic individuals treated with disease modifying agents [45]. Trial recruitment is assisted by comprehensive and up to date patient registries. Establishing collaborative clinical research networks, across national and international boundaries is fundamental to recruit patients with rare disease into trials, accelerate dissemination of trial outcomes into the clinic, and feedback longer term data on efficacy and safety. The drive to develop biomarkers of disease and treatment response has been made more urgent since the emergence of gene therapy techniques. In Huntington's disease, the discovery of a novel biomarker, mutant huntingtin (mHTT) protein in the cerebrospinal fluid has prompted trials to assess its utility in denoting disease progression and predicting treatment response, to circumvent limitations associated with clinical endpoints [46]. Similar difficulties may be applicable across a number of rare,

neurological conditions, whilst overcoming such obstacles is vital to facilitate timely, cost-effective, safe and equitable access to these innovative therapies.

Barriers to implementation: the impact of advanced therapeutics on evaluating cost, capacity and clinical utility in modern healthcare systems

The adoption of orphan drugs for rare diseases continue to challenge the conventional infrastructure of healthcare systems, which have classically depended on providing routine, cost-effective medications for the masses [47]. Fast-tracked for approval by the FDA in 2019 to treat children less than 2 years of age with SMA, the development and clinical implementation of Zolgensma illustrates the successes and challenges that may be used as a template for biologics that are on the horizon. A single centre open label trial showed that all fifteen patients with infantile onset SMA, given a single intravenous dose of drug were alive, without the need for permanent ventilation and had rapid escalation of developmental milestones during the initial 20-month follow-up [26]. In the initial study, 11 children gained head control, 9 developed the ability to roll and two developed a motor trajectory that included walking at the initial data cut off, in stark contrast to natural history cohorts where 95% mortality was expected at this age [26].

High costs associated with these agents initially appear prohibitive to implementation and widespread equitable access. Secondary to fast-tracking of genetic technologies, traditional determinants of drug utility such as long-term efficacy and safety outcomes may be lacking, before the drug is approved. Moreover, evidence of utility beyond the small, homogenous populations used to test efficacy in clinical trials make it difficult to assess true drug value.

For example, whilst significant efficacy is shown in younger patients with severe SMA, questions arise as to if this is replicated in other subgroups. Regulatory bodies, policy makers

and healthcare providers must be ready to collaborate and evaluate the significant up-front costs associated with gene therapy against more subtle measures of efficacy. Public/patient stakeholder engagement should be promoted to ensure meaningful outcomes are being considered against acceptable levels of risk and cost. A potential more global route to improving the cost-benefit of these therapies is to 'harmonise' approval processes across countries [48]. The International Horizon Scanning (Beneluxa) Initiative, a pilot project currently involving 8 European countries, aims to seek successful ways of collaborating on pharmaceutical policy, anticipating the impact of high cost medicines. By utilising a central database to continuously gather data, analyse research and literature and facilitate information sharing about new and developing medicines, the framework serves to enable policymakers to identify future challenges, set priorities, improve insight in expected costs and facilitate timely decision and joint negotiations for lower drug prices.

Differing national structures of reimbursement make it difficult to standardise the threshold at which a therapy is thought cost-effective, independent of approval status. The high cost also generates ethical issues pertaining to equity of access [49]. Ultimately the success of gene therapy can only be realised with equitable, timely and sustainable provision to patients who would most benefit from these novel therapeutics.

Other issues, beside the monetary, arise when we think of translating gene therapy to the clinic. For example, adopting outcome measures from clinical trials may not be relevant, feasible or cost-effective when translated to the clinic. Thus, decisions need to be made about how healthcare services monitor efficacy in real-world settings. A proposed route is to collaborate and standardise outcome measures across treatment centres, with an emphasis on

establishing meaningful functional endpoints. Combing data together, the limits of treatment efficacy may be better determined.

The global shortage in GMP grade manufacture of viral vectors at doses required for clinical use, compounded by jurisdictional differences in regulations for reagent development may prevent or delay access to therapies and research. Accordingly, there is a critical need to establish and upscale facilities to produce clinical grade viral vectors and advance the potential for precision medicine. The prospect of custom designed GRT for ultra-rare disease necessitates dynamic, flexible and cost-effective approaches to small vector manufacture, clinical trial readiness, bioethical guidance and regulatory oversight.

Centralising administration of these agents may be the way to build capacity into healthcare service models, streamline cost-effectiveness and promote best practice for safe administration and monitoring of side-effects and efficacy. However, indirect costs to the patient such as having to travel vast distances to have access to therapy must be balanced against this model of service provision.

Novel gene therapy targets

Novel non-monogenic targets of gene therapy have been developed in pre-clinical studies for management of neurological conditions which have multigenic, environmental or no clear aetiology. Noteworthy examples include epilepsy, chronic pain and glioblastoma multiforme.

Parallel pathways to adjust the epileptogenic milieu and neuronal excitability of the central nervous system reduce seizure burden in preclinical models of epilepsy, independent of aetiology. Approaches include introducing transgenes for neuroinhibitory peptides and/or their receptors [50], preventing degradation of inhibitory neurotransmitters by gene knockdown

of the corresponding proteolytic enzyme [51] and modulation of excitatory/inhibitory ion channel expression [52].

Temporal evolution of disease plays a part in directing the most effective gene therapy technique to be utilised. In developmental epilepsies such as Dravet's syndrome, (caused by mutations in the *SCN1A* gene), early neuronal network dysfunction leads to drug refractory infant-onset seizures and epileptic and developmental encephalopathy by 2 years of age. Non-germ line gene editing using CRISPR/Cas-9 techniques, that corrects the underlying genetic mutation could be especially beneficial in modifying disease trajectory if employed early enough to prevent abnormal networks from emerging and becoming entrenched [53]. Genetic editing at latter stages is likely to have missed the physiological 'developmental therapeutic window' for maximum effectivity [53]. Strategies of neuronal tone modulation as described above may be more valuable in this instance and are also amenable to patients with low burden of disease and long periods of normal brain function between episodic attacks.

Similarly, the manifold pathways leading to manifestation of chronic pain have uncovered novel targets for intervention. For instance, introduction of glutamic acid decarboxylase enzyme transgene associated with an replication deficient HSV vector converts glutamate to the main inhibitory neuropeptide Gamma-aminobutyric acid (GABA) in the dorsal root ganglion [54]. This reduces pain behaviours in murine models, showing sustained effect after one intrathecal injection and efficacy despite late treatment [54]. Immune mediated triggers for chronic pain are well known. Exploiting this, one of the more promising antidotes for chronic pain is the intrathecal delivery of a transgene for Interleukin 10 (IL-10) [55].

Inherent characteristics of viral vectors can be harnessed in the management of highly malignant brain tumours such as glioblastoma multiforme (GBM)[56]. Acting as "suicide" genetic therapies, oncolytic viruses such as adenovirus and HSV-1 replicate quickly and preferentially infect high-turnover tumour cells [57, 58]. Virions cause tumour cell death by

directly triggering oncolysis and immune modulated removal of infected tumour cells. Oncolytic vectors can also carry transgenes that facilitate conversion of prodrugs into cytotoxic chemotherapeutics [59]. This strategy triggers an apoptotic cascade that ends with tumour cell death from within, whilst sparing normal cells and extending median survival to some extent [59].

CONCLUSIONS

The rapid emergence of GT promises to irrevocably change the therapeutic landscape, altering the trajectory for an array of devastating neurological diseases. With the prospect of GT moving closer to clinical reality, a truly personalised medicine approach is envisaged, with genetic strategy, vector choice, mode and timing of administration dependant on not only the underlying disease pathophysiology but the patient-specific genetic mutation, immune profile and disease stage. Developments in vector and therapeutic cassette design will facilitate targeted therapy, improve transduction and sustained transgene expression, aiding in circumvention of safety concerns regarding 'off-site' genetic modulation and immune-mediated toxicity. However, uncertainties remain, should be addressed and patients and their families appropriately counselled. Downplaying the 'curative' label given to these agents is important, particularly as therapies administered to symptomatic individuals may not fully restore function secondary to irreversible neuronal pathology. Instead stability or improvements in function and subsequent quality of life measures may be more relevant but still valuable for patients. Uncertainties also include paucity of medium and long-term safety and efficacy data, with emphasis on concomitant ongoing surveillance required to support health outcomes.

The impact of these innovative technologies will not only be felt at the patient-clinician interface. Challenges remain in providing sustainable, clinically appropriate, and equity of access to patients that would benefit from these therapeutics. Healthcare models should anticipate a paradigm shift from one of supportive care to a more proactive approach, as the natural history changes with disease-modifying therapeutic intervention. With this comes a need to identify disease early or presymptomatically to optimise outcomes, concomitantly changing the traditional implications of high cost drugs within resource restricted healthcare settings. A similar paradigm shift is anticipated in the set-up of preclinical and clinical trials, ensuring that outcomes are expeditious, streamlined and applicable to patient populations in real-world settings. We propose that appraising factors of benefit against cost, safety and infrastructure elements will be challenging for individuals alone. A wider strategy that engages stakeholders across patient-groups, policy, science, drug development and clinical spheres may form the foundation for a more comprehensive and patient-orientated assessment of acceptable risks, meaningful endpoints and limits of treatment.

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DK and MF planned the manuscript. DK executed and prepared the first and subsequent drafts of the manuscript. DK, MF, IA and MK contributed to manuscript revision. All authors read and approved the submitted version.

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The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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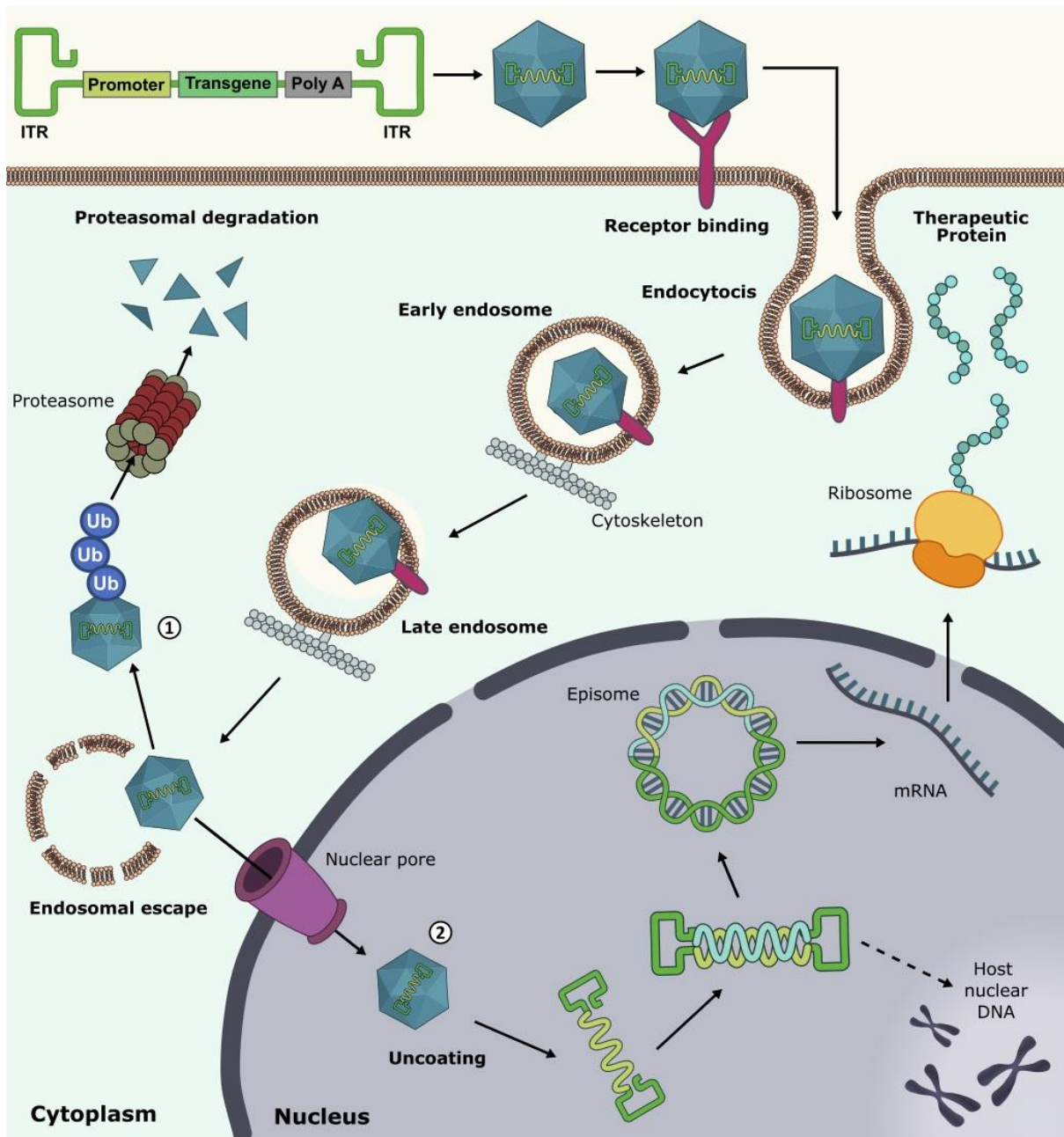
Figure Legends

Figure 1: Components of the vector-transgene delivery system and the process of host cell transduction.

Example illustrates some of the pathways of vector delivery and cell transduction process for Adeno-associated (AAV) viral vectors

Vector: A vehicle or delivery system that carries genetic material, Transgene: exogenous nucleic acid (DNA, RNA or synthetic derivatives) introduced to a eukaryotic host cell, Enhancer/promoter: regulatory DNA sequences that controls the extent, duration and cell specificity of transgene activity, Polyadenylation signal: Forms a chain of adenine nucleotides that increases the stability of transgene mRNA, Episome: DNA element that sits

and acts extra-chromosomally of the hosts nuclear DNA. Due to the lack of integration with host DNA, regulatory host cell processes continue with-out disruption. Episomal vectors reduce the risk of insertional mutagenesis, subsequent oncogenesis and loss of physiological cell function.

AAV virions binds to the host cell membrane. Tissue/cell tropism is modulated by serotype of the virus i.e. antigenic differences in capsid proteins that recognise specific host cell receptors. AAV vectors have broad inherent tissue tropism, targeting cells in the central nervous system, kidneys, liver and eyes. The vector-transgene is endocytosed and carried in an endosome. Virion release occurs in the cell cytoplasm and undergo one of two processes 1.) Ubiquitylation marks the virion for proteosomal degradation 2.) Virions enter the nucleus through a nuclear pore and are uncoated. Double-stranded DNA strand is formed that is competent for transcription. The transgene adopts an episomal location in most cases (0.1-1% of the transgene is integrated into the host's DNA). After transcription and translation of the transgene, a therapeutic protein product is formed. On completion of this process, the cell is defined as being functionally 'transduced'. Ongoing advances in AAV-based gene delivery technology are likely to deliver more efficient vectors that can be given at lower doses.
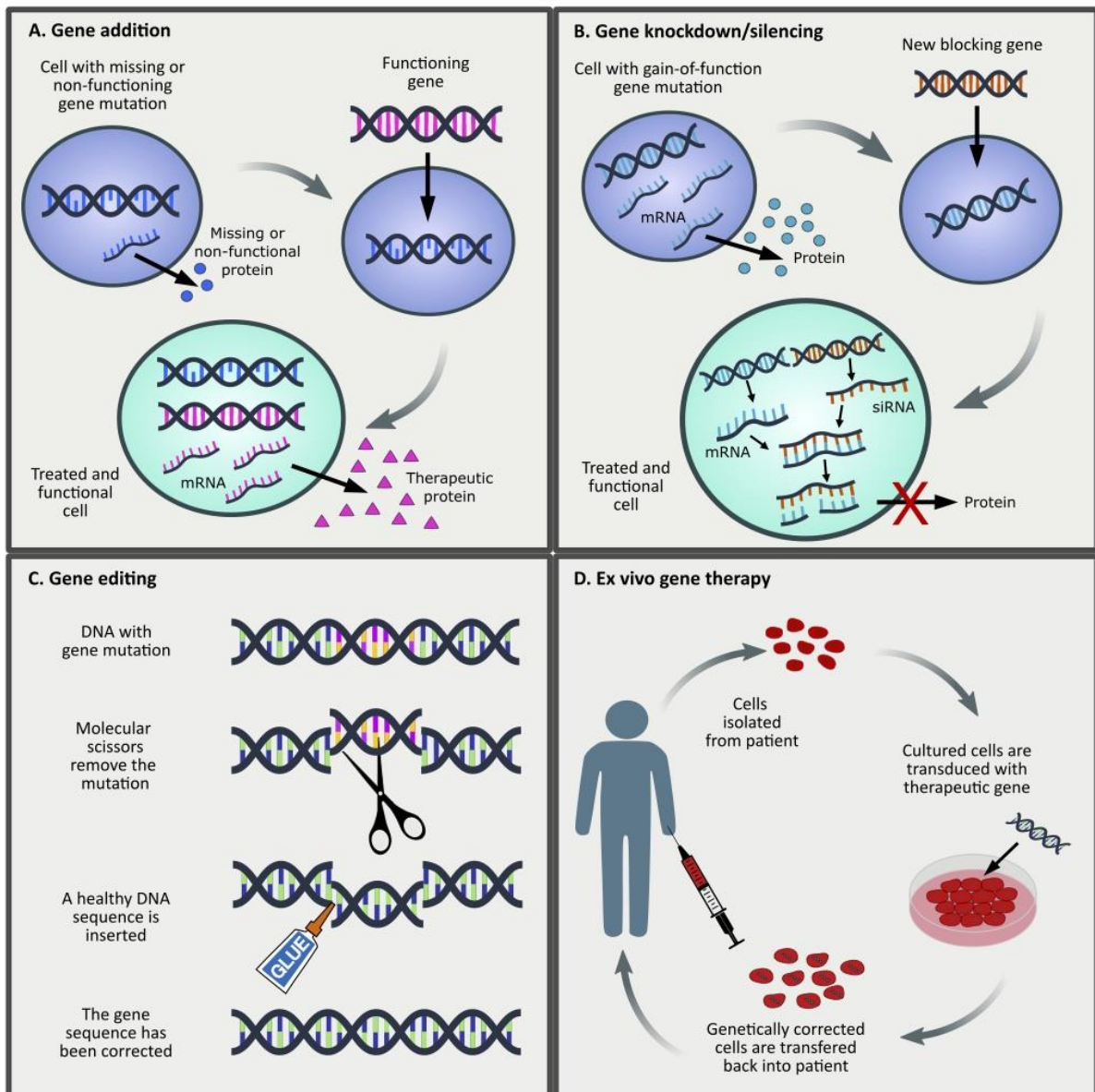
Figure 2: Genetic strategies in gene therapy

A: In loss of function mutations, a transgene is introduced so that the host cell can form a specific therapeutic protein, B: In gain of function mutations expression of the mutant gene is targeted. In the example shown the transgene encodes a small interfering mRNA (shRNA which is converted to siRNA in the target cells) that targets and degrades specific host cell mRNA sequences, preventing excessive translation of the protein, C: Genetic sequences can be inserted, removed or modified by [a] CRISPS/Cas-9 toolkits or Zinc finger nucleases, and the break repaired to form a functional DNA sequence, D: *Ex vivo* techniques where an

individual's own (usually haematopoietic) cells are extracted and genetically modified *in vitro*. Genetically modified cells are autologously reintroduced. Strategies A, B and C can occur *in vivo* or *ex vivo*. The global shortage in GMP grade manufacture of viral vectors at doses required for clinical use, compounded by jurisdictional differences in regulations for reagent development may prevent or delay access to therapies and research. Accordingly, there is a critical need to establish and upscale facilities to produce clinical grade viral vectors and advance the potential for precision medicine. The prospect of custom designed GRT for ultra-rare disease necessitates dynamic, flexible and cost-effective approaches to small vector manufacture, clinical trial readiness and regulatory oversight.