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Neuromuscular disease: Gene transfer for children: What we know now

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Abstract

Successful gene transfer therapy (GTT) provides a functional copy of a gene to appropriate tissues for affected patients. While technically difficult, GTT holds great promise for treating and even curing previously fatal diseases. GTT for Spinal Muscular Atrophy is available commercially and ongoing studies continue to show it is safe and effective. Subclinical liver dysfunction is more common in older, heavier children receiving higher viral loads. Human trials support preclinical studies which show that early timing of therapy is important. GTT for Duchenne Muscular Dystrophy has required strategic approaches to create mini- and micro-dystrophin genes that will fit into available viral vectors. There are multiple ongoing studies that demonstrate good safety and efficacy overall. GTT for X-Linked Myotubular Myopathy is being studied in an ongoing trial that has shown improvement in respiratory function (including ventilator independence), neuromuscular function, and histopathological evaluation. Three patients with severe cholestatic liver dysfunction have died. Evaluation is ongoing to better understand these events. While GTT for neuromuscular disorders holds significant promise, it is not without risks and requires in-depth knowledge of the disease, abundant pre-clinical work, careful patient education, and ongoing patient care. There are several key questions that must be considered regarding the feasibility of expanding GTT to new disorders. These examples illustrate how advances in GTT benefit children on a population level and may themselves benefit from early detection by newborn screening (NBS). By becoming involved in advocacy at state and federal levels, families and physicians can impact NBS policy and implementation regarding these disorders.

Keywords: Gene transfer therapy; gene therapy; Duchenne muscular dystrophy; spinal muscular atrophy; X-Linked myotubular myopathy; centronuclear myopathy; newborn screening; Pompe disease

Introduction

Successful gene transfer therapy (GTT) provides a functional gene copy to affected patients. GTT holds great promise for treating and even curing previously fatal diseases. Here we review the state of GTT for spinal muscular atrophy (SMA), Duchenne muscular dystrophy (DMD), and X-linked myotubular myopathy (XLMTM). We review considerations for GTT for new disorders, and status of newborn screening (NBS) for neuromuscular disorders. While our purpose here is to review the state of the science, concerns remain that the costs involved for approved gene transfer products will be an obstacle for widespread availability.

GTT for SMA and DMD: Where are we now?

Untreated, SMA (types 1 and 2) and DMD are progressive fatal neuromuscular disorders for which GTT is either approved (SMA) or under investigation (DMD). The different size and distribution of the two genes require different strategies in the development of GTT.

Spinal muscular atrophy

SMA is an autosomal recessive disorder caused by loss of function mutations in the survival motor neuron-1 (*SMN1*) gene [1]. Phenotype severity correlates with survival motor neuron-2 gene (*SMN*) copy number. Patients with only two copies typically survive without ventilation to a median of eight months [2].

With only eight exons [3], full length *SMN1* can be transferred with adeno-associated virus, serotype 9 (AAV9) vector [4] (Table 1).

Prior studies

The AAV9 viral vector, with tropism for the central nervous system, allowed the first successful *SMN1* gene transfer in mice. These studies also demonstrated the importance of timing, with less recovery if the dose was given later [5].

The first human trial (Phase I/II single ascending dose) enrolled 15 symptomatic infants with SMA type 1 under nine months of age, following safety and time to death or chronic artificial ventilation (over 16 hours per day) [4]. All 15 patients survived beyond 20 months without reaching either endpoint. In contrast, natural history studies show only 8% of untreated patients would be alive and ventilator-independent at that age [6]. Eleven of the 12 in the higher-dose group showed significant improvements. Two of the youngest patients were able to walk. Except for the oldest patient, all achieved and maintained a CHOP INTEND Score greater than 40, and hence were able to use their arms and sit up; key functions which allow an increased measure of independence [4]. Sitting up is never achieved in untreated patients with SMA 1 [7].

Regulatory status and ongoing studies

GTT via onasemnogene abeparvovec-xioi (Zolgensma) for children under two years of age with SMA type 1 was approved by the United States Food and Drug Administration (FDA) in May 2019 [8], and by the European Medicines Agency for children under 21 kg with SMA type 1 or up to three copies of the *SMN2* gene as of May 2020 [9].

Additional studies in SMA include confirmatory multicentre studies with Zolgensma in symptomatic infants in the United States [10] and Europe [11], and a multicentre intrathecal delivery trial using lower doses in older children with SMA type 2 (currently on FDA hold) [12].

SMA gene therapy update – Ohio Registry

Five child neurology sites in Ohio have tracked the outcomes of commercial GTT with onasemnogene abeparvovec-xioi for children with SMA less than two years old and with fewer than five *SMN2* copies. This includes asymptomatic infants identified with NBS as well as symptomatic children, about 50% of whom had been previously treated with nusinersen. This is an antisense oligonucleotide, targeting SMN2 pre-mRNA, encouraging production of SMN without actual gene transfer [13]. Thus, this mixed treatment cohort is distinct from those previously reported [14].

Liver function tests were more often elevated in older, heavier children who received higher weight-based doses, and required longer corticosteroid dosing post-GTT, although no patient was obese. Only two of the nine patients were under the age of six months, both having difficulty with adherence to corticosteroids, and with elevations in transaminases. One child transitioning from nusinersen had particularly high elevations in gamma-glutamyl transpeptidase (GGT). Despite this, no patients developed clinical signs or symptoms of liver dysfunction [14].

For 19 patients, the CHOP INTEND scores showed stabilisation or improvement: 89% (17 patients) improved by at least one point and 70% (12 patients) by at least three points in four months. Several children achieved or maintained a score over 40 [14].

This study emphasised the need for a strong multidisciplinary team, including primary care providers, pharmacists, social workers, and a dedicated nurse practitioner alongside specialists from neuromuscular, pulmonary and complex care teams.

Duchenne Muscular Dystrophy

X-linked DMD is caused by mutations in the DMD gene encoding dystrophin [15]. In affected boys, DMD causes progressive muscle degeneration that is evident clinically, pathologically, and with MRI- or ultrasound-imaging. While the large DMD gene with 79 exons cannot be transferred in its entirety in a single viral vector, individual exons may vary in significance [16, 17]. One patient with a mild form of Becker Muscular Dystrophy was missing 46% of the exons and remained ambulatory into his 60s [18]. These findings led to a number of strategies to design mini- and micro-dystrophin genes small enough to fit into adeno-associated virus (AAV) vectors (Table 2).

Gene therapy studies

Pfizer has launched a Phase IB single ascending dose trial of a mini-dystrophin gene, looking at safety and tolerability in nine children initially, ages 6–13 years old on daily corticosteroids [19]. Initial findings presented on May 15, 2020 at the American Society of Gene and Cell Therapy meeting showed that at the high dose, 35% mini-dystrophin expression was present at two months and 52% expression at 12 months. Three patients demonstrated improvement in functional assessments and had 8% improvement in MRI fat fraction. The main adverse events were atypical haemolytic uraemic syndrome in 40% of patients, and nausea and vomiting. A Phase 3 trial is actively enrolling patients [20].

Solid Bioscience launched a mini-dystrophin Phase I/II ascending dose trial that enrolled six children ages 4–17 and has not completed recruitment [21]. The mini-dystrophin gene was inserted into AAV9, with the creatinine-kinase 8 (CK8) muscle-specific promoter SGT-001, and the nitric oxide synthase-binding site. The main adverse event has been complement activation with thrombocytopenia and anaemia. One seven-year-old child in the high-dose group also suffered acute kidney and cardiopulmonary injury although two additional children tolerated the same dose well. This trial is currently on clinical hold by the FDA.

The Nationwide Children's Hospital, with support from Sarepta Therapeutics, performed an open-label Phase I/II study of four boys initially (age 4–7 years) with confirmed DMD mutations between 18–58 exons, taking twice-weekly corticosteroids for five to 23 months. The eligible mutation locus was limited

Table 1. Study summaries of Onasemnogene abeparvovec-xioi (Zolgensma) for SMA (AAV 9 vector, *SMN1* gene)

Trial	Findings
Preclinical [5] *Status: Completed*	- Successful transfer in mice. - Early administration is important
Phase I/II [4] *- Intervention: Single ascending dose, intravenous* *- Population: 15 symptomatic infants with SMA 1* *- Status: Completed*	- 100% survival to 20 months without constant ventilation (8% if left untreated [6]) - 92% able to use their arms and sit up (0% if left untreated [7])
Phase IV (FDA approved) [14] - Intervention: Intravenous - Population: children under two years old with less than five *SMN2* copies. Included asymptomatic infants and patients with prior nusinersen treatment (50%) - Status: Completed	- 89% improvement in CHOP INTEND by at least one point in four months - 70% improvement in CHOP INTEND by at least three points in four months - Several children able to use their arms and sit up - Liver function tests were more affected in patients with higher weight-based doses, poor corticosteroid adherence, and recent transition from nusinersen, although none developed clinical dysfunction - Strong multidisciplinary team needed for success
Phase I [12] - Intervention: Intrathecal - Status: on FDA hold	See citations
Phase II [10, 11] - Intervention: Intravenous - Status: Completed	

due to concern that novel introduction of exons 3–17 increased the risk of an immune response against the protein itself [22]. This trial used an AAV derived from rhesus monkeys, serotype 74, (AAVrh74) and a muscle hybrid creatine kinase 7 (MHCK7) promoter for strong expression in both myocardium and striated muscle [23]. Mice showed dose-dependent gene expression in over 60% of skeletal muscle fibres, heart and diaphragm [24].

The four boys each received a single dose (2.0 x 1014 viral genomes per kilogram [vg/kg]) of the viral vector containing micro-dystrophin. North Start Ambulatory Assessments (NSAA) and serum creatinine kinase (CK) values were monitored. At one year, the patients had an increase in NSAA scores (average 5.5 points), and a decrease in their baseline CK levels (average of 67.3%). A gastrocnemius biopsy 90 days after treatment showed an average of 81.2% of fibres positive for micro-dystrophin, and Western Blot analysis confirmed high levels of expression [23].

This study did not have any serious adverse events. The clinically important laboratory results included three of the four patients having elevated GGT within the first two weeks, and one patient having an increase in GGT after prednisone taper. Three of the four patients had transient nausea within the first week [23].

Sarepta therapeutics is sponsoring an ongoing phase IIb placebo delayed trial in 40 boys aged 4–7 years, in which the placebo group will receive GTT after one year [25].

The ASPIRO gene therapy trial in XLMTM: update on safety and efficacy findings

XLMTM is a subtype of centronuclear myopathy and a progressive neuromuscular disorder affecting about one in every 40–50,000 newborn boys [26]. XLMTM is caused by a number of mutations [27] in the MTM1 gene-encoding myotubularin,

Table 2. Study summaries of mini-/micro-dystrophin for DMD (AAV vectors, edited *DMD* gene).

Stage	Findings
Preclinical [24] - Status: Completed	- dose-dependent gene expression in mice including skeletal muscle fibres, heart, diaphragm
Phase Ib (Pfizer) [19] - Intervention: Single ascending dose - Population: Initially nine children aged 6–13 years on daily corticosteroids - Status: Active (recruitment completed)	- 35% protein expression at two months - 52% protein expression at 12 months - 33% patients had functional improvement with 8% improvement on MRI fat fraction - 40% patients had atypical HUS, nausea, and vomiting
Phase I/II (Solid Bioscience) [21] - Intervention: Ascending dose - Population: six children aged 4–17 years - Status: FDA hold	- adverse events include thrombocytopenia, anaemia, acute kidney injury,cardiopulmonary injury
Phase I/II open label (Sarepta Therapeutics) [23] - Population: Initially four boys aged 4–7 years on corticosteroids with mutations between exons 18–58 - Status: Completed	- Increase in NSAA scores by average 5.5 points over one year - 67.3% decrease in baseline CK levels over one year - 81.2% expression on 90 day gastrocnemius biopsy - 75% had elevated GGT, though none had complications - 75% had transient nausea in the first week
Phase IIb (Sarepta Therapeutics) [25] - Intervention: Placebo delayed - Status: Active (recruitment completed)	See citations
Phase III (Pfizer) [20] - Status: Enrolling	

which dephosphorylates second messenger lipids [28] for muscle growth and differentiation, as well as cellular organisation and function [29, 30, 31]. This disorder causes facial, extraocular, respiratory and general weakness without myocardial involvement. Children are never able to sit up or walk [32] and require respiratory support, feeding tubes and supportive surgeries [27, 33, 34]. There is a high mortality rate of 50% by 18 months and 88% by 10 years [26, 33, 34]. Muscle biopsies may show abnormal fibre size variation, central nucleation, and muscle fibre atrophy [35].

GTT for XLMTM was initially studied in the canine model using a recombinant adeno-associated virus serotype 8AAV (rAAV8), and the muscle-specific desmin promoter to express canine myotubularin. An effective dosing range was established and demonstrated tolerance, improved motor function, muscle strength and increased lifespan [36].

The ASPIRO Trial

The ASPIRO trial is an ongoing Phase I/II single ascending dose-delayed control using the same vector and promoter with the human version of the MTM1 gene, known as Audentes Therapeutics 132 (AT132). It was preceded by a natural history study known as INCEPTUS, from which a number of patients were enrolled. The INCEPTUS study was a prospective cohort observational study of 34 patients from July 2016 to September 2019, looking at respiratory function, neuromuscular function and quality of life [37] (Table 3).

The ASPIRO trial includes boys aged less than five years (or previously enrolled in INCEPTUS) with genetically confirmed XLMTM and on ventilator support without clinically significant liver disease. As of the most recent datacut on 8 July 2020, 23 patients had been treated. Data from all patients has been analysed for safety.

Follow-up data were available on six of six patients on the lower dose ($1x10^{14}$ vg/kg) and 10 of 17 patients on the higher dose ($3x10^{14}$ vg/kg) and were further analysed for efficacy regarding respiratory function, neuromuscular function, and histopathological changes [38].

Respiratory function

Markers of respiratory function included the number of daily hours of ventilator dependence and maximal inspiratory pressure. As of 8 July 2020, both treatment groups showed significant ($p < 0.0001$) improvements compared to untreated control patients in both measurements, and five of six patients treated with the lower dose had achieved ventilator independence. While the higher-dose cohort had a shorter follow-up period, one patient had also achieved ventilator independence [38].

Neuromuscular function

Evaluation included neuromuscular function using the CHOP INTEND score as well as major motor milestones reached and maintained. The ASPIRO trial found a significant, ongoing change from the baseline score in the lower dose cohort within four weeks, with five of six patients attaining a score of greater than 50, the mean score in healthy infants [7]. The higher dose cohort achieved a significant, ongoing change from baseline in about two weeks, with six of 10 achieving a score greater than 50. One control patient also achieved a score greater than 50, although other controls generally did not improve significantly from baseline [39].

Regarding milestones, five of the six patients in the lower dose cohort were walking independently as of 8 July 2020 (allowing for ankle-foot orthosis in one subject), as was one of the 10 patients in the higher dose cohort, despite the shorter follow-up period for the latter group. No control patients were able to walk independently [38].

Histopathological changes

Muscle biopsies were obtained at 24 and 48 weeks after vector administration and tested for vector copy number, myotubularin protein expression, and XLMTM pathology score on a scale from 0 (normal) to 5 (severe). All patients in both groups for whom data is available continued to have detectable vector copies. Median myotubularin protein expression for both dose levels at both time points was greater than 75% of normal. In both dose cohorts, median XLMTM pathology scores improved from baseline [38].

Safety profile

All patients who received AT132 were monitored for treatment-emergent adverse events (TEAE). One patient in the lower dose group had serious AT132-related TEAEs, while eight in the higher dose group had AT132-related TEAEs [38].

Three patients died, all of whom were older, heavier patients in the higher dose group. They developed severe, progressive cholestatic liver dysfunction characterised by persistent hyperbilirubinaemia and histological findings of intrahepatocellular and canalicular cholestasis, reactive changes in the periportal regions and bile ducts, and secondary fibrosis but without parenchymal inflammatory cellular infiltrates. These subjects all had some evidence of pre-existing intrahepatic cholestasis which has been described in XLMTM [40]; about 50% of study patients had some evidence of hepatobiliary disease.

Conclusions

The ASPIRO trial shows promising results with improvements in ventilator dependence—including the unprecedented milestone of ventilator independence in some patients—and CHOP INTEND scores which are not typically seen in XLMTM. Further investigation is ongoing into the pathophysiology of hepatic dysfunction in the three patients who died.

Is gene replacement a viable option for my favourite disease?

GTT is increasingly considered as a potential therapy for inherited disorders. While the concept seems straightforward and the technology is increasingly well established, there are several questions that arise when considering if GTT is appropriate and feasible for a disorder, some of which are considered here.

Is the natural history of the disease understood? Is the disease serious enough to undertake the risk of systemic delivery?

Detailed knowledge of the natural history of the disease is required for design of a clinical trial, contributes to understanding the risks of treatment, and helps to determine whether the benefit of the treatment outweighs the risk and cost. The importance of understanding natural history was well demonstrated by the experience with SMA, discussed above.

When a disorder has significant mortality, morbidity, or healthcare resource utilisation, such as SMA type 1, families are willing to take more risk, while with milder or more variable disorders, the balance of risk vs. benefit may be less clear.

Is there an alternative treatment option?

For some disorders, alternative treatment options may be equally effective with lower risk and development costs. For example, both biochemical treatments [41, 42, 43] and GTT [44, 45] are under development for limb-girdle muscular dystrophy (LGMD) type R9. If both are shown to be effective, patient preference might depend on efficacy, side effects and costs.

Is there an appropriate therapeutic gene to deliver?

In recessive disorders, there is a missing or abnormal gene to replace, but gene replacement therapy for autosomal dominant disorders—such as myotonic dystrophy or facioscapulohumeral dystrophy—is much more complex. In dominant disorders there is already one normal copy of the target gene, and haploinsufficiency is an uncommon mechanism of disease. One strategy might be to direct gene replacement at key misregulated, downstream genes [46, 47].

Table 3. Study summary of AT132 for XLMTM (AAV8 vector, MTM1 gene).

Stage	Findings	Low Dose Cohort	High Dose Cohort
Phase I/II (ASPIRO) [38]	Significant improvement in respiratory function	Yes	Yes
	Ventilator independence	83%	10%
- Intervention: Single ascending dose	CHOP INTEND Score 50	83% (4 weeks)	60% (2 weeks)
- Population: 23 boys less than five years old (or from INCEPTUS) on ventilator support without significant liver disease	Walking independently (allowing for orthotics)	83%	10%
- Status: Active	Myotubularin protein expression	$>$ 75% typical	$>$ 75% typical
	XLMTM pathology score improvement	Yes	Yes
	AT132-related TEAE	0	8
	Serious AT132-related TEAE	1	0
	Death	0	3 (hepatic dysfunction)

Is the affected tissue accessible?

The distribution of the gene therapy relies on both the vector characteristics and route of administration. After oral or topical delivery, neither of which is currently technically feasible for neurological diseases, intravenous injection is the most straightforward method of delivery, particularly when widespread dissemination is desired (for example, intramuscular injection of every muscle would not be practical). However, peripheral vectors are exposed to the immune system with its potential antibody response, and the viral load can be limiting with larger body sizes.

If the central nervous system is targeted, direct administration via intracerebroventricular, intracerebral, or intrathecal routes may bypass these obstacles and allow higher viral loads, but these are quite invasive. An example in development is the intrathecal delivery of onasemnogene abeparvovec-xioi to patients with SMA who are more than two years old. Intravenous delivery in older, larger children with SMA is limited by toxicity. Intrathecal delivery might allow higher viral and gene doses delivered to the motor neurons without the systemic toxicity. A phase 2 trial is currently on clinical hold by the FDA [12]. Some disorders affect both the CNS and the rest of the body and could potentially not be reached by targeted therapy, which further complicated gene delivery. A good example of this is Friedreich ataxia, which involves the spinal cord and deep nuclei in the brain, but also the peripheral nerves, heart, as well as potentially other organ systems. In this type of disorder, multiple vectors and routes of administration might be needed.

Is the gene product functional in postnatal life, or when the gene can be delivered?

If the missing gene has critical windows of activity, gene replacement therapy might not work or might even be toxic if administered outside that window. For example, the double homeobox 4 gene (DUX4) is normally active in early prenatal life but leads to facioscapulohumeral muscular dystrophy (FSHD) when expressed postnatally [48].

Does dosage matter?

In some cases, gene dosage is tightly regulated, and overexpression of the target gene results in disease. For example, if deleted, the peripheral myelin protein 22 (PMP22) gene causes hereditary neuropathy with liability to pressure palsies. However, if duplicated, it causes Charcot-Marie-Tooth disease type 1A (CMT1A) [49]. Similarly, loss of function of the methyl CpG-binding protein 2 (MECP2) gene on one allele is responsible for Rett syndrome and is at least partially reversible in mice with GTT [50]. However, overexpression due to MECP2 duplication also causes a pathological syndrome [51]. Dosage might be controllable with careful drug design.

Will the gene to be replaced fit into an appropriate vector?

The most common vectors for GTT are AAVs, often AAV9, which can only hold about 4.7 kilobases (kb) of genetic material. This led to the development of mini- and micro-dystrophins for DMD gene therapy as discussed above.

A different strategy is being investigated for dysferlin-related muscular dystrophies (LGMD type 2B and Myoshi myopathy). The *dysferlin* gene is also too large to fit into AAVs. Rather than a mini-dysferlin, a dual vector strategy has been proposed, in which two different gene therapy products are administered, each containing half of the gene, with a 1 kb homologous region to allow a full-length gene to be reconstituted in the patient's cells [52, 53].

Is the affected tissue stable over time or is there rapid turnover?

Due to expected immune response to the viral vector, gene therapy can only currently be given as a one-time treatment, which means it is important that the therapeutic genomes remain in living and active cells. Some cell types are naturally good targets for this, such as the anterior horn cells targeted by SMA treatments, as they are stable over time. In contrast, to maintain the benefits in muscle cells, the therapy would ideally transfect both the muscle cells and satellite cells [54].

Will gene transfer introduce a completely novel protein, resulting in immune rejection?

Immune rejection of not just the viral vector but the novel protein of interest has been an ongoing concern [22]. In humans, however, the immune reaction has typically been to the virus more than the novel protein [55].

Conclusions

While GTT for neuromuscular disorders holds significant promise, it is not without risks and requires in-depth knowledge of the disease, abundant pre-clinical work, careful patient education, and ongoing patient care.

The promise of gene therapy and NBS

Selecting disorders for newborn screening

Newborn screening (NBS) was established in the 1960s in the United States to screen for phenylketonuria (PKU). Once an effective early treatment was established, rapid identification of newborns with PKU became a public health emergency, which justified the expansion of governmental authority and finances to facilitate NBS. However, this expansion raised questions about consent and familial autonomy including the right not to know. NBS remains a complex issue to be addressed prior to each disorder being added to the Federal Recommended Universal Screening Panel (RUSP). Working through these issues can cause significant delay in adoption of new disorders to the RUSP, as was true of Sickle Cell Disease [56].

NBS in the United States is a public health system involving blood spot testing, and several point-of care tests, including hearing screening. There are five major components: (1) the screening itself, determining who and what is tested, (2) follow-up for positive results, (3) confirmatory diagnostic testing, (4) management of the disorder, including potentially gene therapy, and finally (5) self-evaluation of the system and quality improvement [56]. The system is designed to prioritise the interests of affected newborns, while still being of benefit to unaffected newborns, families, and the public.

The RUSP was created by the American College of Medical Genetics in 2006 at the request of the federal Advisory Committee on Heritable Disorders in Newborns and Children (ACHDNC). This committee includes various stakeholders, including physicians, laboratory personnel, patient advocates, public health officials, economists, and ethicists. The original RUSP covered 29 core conditions and 25 secondary conditions. Core conditions were selected on the basis of (1) being detectable with testing but not examination 24–48 hours after birth, (2) having a sensitive and specific test, and (3) having a 'proven benefit' to early detection and treatment [57].

In order to add a new condition to the RUSP, it must be nominated to the ACHDNC, which then votes on performing an evidence review. The evidence review examines information about the disorder, testing, treatment, and the economic impacts thereof. Based on this information, the ACHDNC votes whether to recommend to the United States Department of Health and Human Services that the condition be added to the NBS panel. From there, states must determine—via their own unique process—if and how to implement federal recommendations [58].

Pompe Disease

Pompe Disease is an autosomal recessive deficiency of acid alpha glucosidase with an incidence of one in 14000–19000 live births [59, 60]. A third have an infantile-onset with a life expectancy of less than one year. Identification of newborns with infantile Pompe disease was the primary goal behind NBS for this disorder. However, the majority of cases have late-onset disease, and a positive test does not guarantee it will occur. Therefore, NBS for Pompe disease has created a number of 'patients in waiting'" [61]. Treatment with enzyme replacement therapy (ERT) helps slow progression, with an improved outcome with earlier treatment, but 20% may not be eligible and ERT is not a cure [62, 63]. There is also some question as to when to treat patients with late-onset disease [64]. Interviews with patients with late-onset disease prior to NBS showed that 63% had spent over five years searching for a diagnosis, 41% would have made different life decisions had they known their diagnosis sooner, and 78% agreed NBS would have improved their lives. The main concerns related to whether diagnosis would limit ability to obtain insurance, with less concern about removal of a child's autonomy and right to not know [65].

NBS for Pompe Disease was first studied in Taiwan, where it was added to their NBS platform in 2008. In the United States, further data was requested before being added to the Recommended Uniform Screening Panel (RUSP) in 2015, five years after the second enzyme replacement therapy was FDA-approved. A variety of strategies are under investigation to treat Pompe disease including gene therapy. While NBS may aid in identification of patients for studies, NBS is only considered justifiable by its immediate benefit to newborns [66].

Spinal muscular atrophy

SMA was initially recommended to the ACHDNC as early as 2010, and accepted as part of the RUSP until 2018, two years after the FDA-approved nusinersen in 2016 [67]. During this time, severe combined immunodeficiency (SCID) was added to NBS, paving the way for using polymerase chain reaction (PCR) technology for NBS. In addition, pilot studies for SMA were published, showing two-tier PCR testing for exon 7 deletions with a 100% positive predictive value (PPV), although 5% of cases have a different mutation and may be missed [68, 69]. The evidence review for SMA's nomination to the RUSP at that time stated that screening four million neonates 'compared with clinical detection ... could avert death or the need for mechanical ventilation in 48 (range: 16–100) infants by one year of life,' although cost and economic impact of treatment were not considered in that paper [70].

As of September 2020, 32 states (covering about 68% of newborns in the United States) currently include SMA on NBS [71]. Some concerns raised include whether to report carriers and whether it should be the responsibility of each US state's NBS lab to measure the *SMN2* copy number. While *SMN2* is the most important modifier of the natural history of SMA, it is not the only one [72] and reporting currently varies by state. How a positive NBS result is delivered to a family is also an important step. In one case series, strong emotional reactions to the testing led to loss of follow-up due to apparent disinterest, intense psychological distress, or family insistence of early initiation of treatment [72].

The opinions related to NBS of patients with SMA tend to depend on age of onset. Those with an earlier age of onset tend to view the condition less negatively and feel less strongly that NBS is warranted, while those with a later age of onset tend to view the condition more negatively and support screening. Most said a positive NBS would not detract from pre-symptomatic life [73].

Duchenne muscular dystrophy

NBS for DMD was first trialed in New Zealand [74], followed by Wales in the UK, from 1990–2011 with 94% participation [75, 76]. One trial in Ohio in the USA was a four-phase pilot study with over 30,000 infants to establish a CK range and cut-offs for abnormal values. It found that all DMD cases had a CK which was more than 2,000 u/l. This study also identified seven boys and two girls with significantly elevated CK but without a DMD mutation. Of the nine infants, three (33%) were found to carry mutations in genes related to LGMD [77]. Given that some forms of LGMD are also under clinical investigation for GTT, this may be an additional positive consideration.

NBS for DMD is not currently on the RUSP in the United States. A major limitation in being on the RUSP at this time is the lack of effective pre-symptomatic treatment options. Some additional concerns raised include whether to screen only boys when checking for DMD, or all children, to include other muscular dystrophies, as well as how to manage patients with an elevated CK who do not have positive genetic testing.

The families of patients with DMD tend to support NBS and would be more likely to participate in neonatal screening than screening at a later point in childhood [78, 79]. However, due to the potential for psychological distress and current lack of curative therapy, many experts and families advocate for informed consent [80] and careful timing of the result disclosure [75].

Conclusions

The above examples of neuromuscular disease that are or may soon be treated through GTT illustrate advances which now also benefit children on a population level. Families and physicians can impact NBS policy and implementation by becoming involved in advocacy at state and federal levels.

Conclusion

GTT is a rapidly emerging field of therapeutics to treat and potentially cure genetic disorders. While much is left to learn, ongoing clinical research shows promising results for neuromuscular disorders, including for SMA, DMD, and XLMTM, as reviewed here. While the number treated in most studies is small, all have shown significant improvement with children achieving milestones never seen with the natural course of the disease. Questions remain regarding whether and how these effective but extremely expensive therapies will be made available equitably, although pathways to doing so have been proposed [81]. It is also not yet known to what extent the promise of GTT will extend to children with other disorders. Finally, as more conditions move from fatal to curable, NBS brings promise of early identification and treatment.

Abbreviations

AAV – adeno-associated viruses
AAVrh – AAV rhesus, referring to AAVs isolated from the rhesus macaque
ACHDNC – American Committee on Heritable Disorders in Newborns and Children
AT132 – Audentes Therapeutics 132
CHOP INTEND Score – Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders
CK – creatine kinase
CK8 – creatinine kinase 8
CMT1A – Charcot-Marie-Tooth disease type 1A
DMD – Duchenne Muscular Dystrophy
DMD – Duchenne Muscular Dystrophy gene (encodes dystrophin)
DUX4 – double homeobox 4 gene
ERT – enzyme replacement therapy
FDA – United States Food and Drug Administration
FSHD – facioscapulohumeral muscular dystrophy
GGT – gamma-glutamyl transpeptidase
GTT – gene transfer therapy
kb – kilobases (1000 base pairs)
MECP2 – methyl CpG-binding protein 2 gene
MHCK7 – muscle hybrid creatine kinase 7
NBS – Newborn screening
NSAA – North Star Ambulatory Assessment
LGMD – limb-girdle muscular dystrophy
PCR – polymerase chain reaction
PMP22 – peripheral myelin protein 22
PPV – positive predictive value
rAAV8 – recombinant adeno-associated virus serotype 8
RUSP – Recommended Universal Screening Panel
SMA – spinal muscular atrophy
SMN1 – survival motor neuron-1 gene
SMN2 – survival motor neuron-2 gene
TEAE – treatment-emergent adverse events

vg/kg – viral genomes per kilogram
XLMTM – X-linked myotubular myopathy

Competing interests

Matthew J. Martin, MD has declared that he has no competing interests. Margie A. Ream, MD is a member of the Evidence Review Group for the Advisory Committee on Heritable Disorders in Newborns and Children and a member of the Ohio Newborn Screening Advisory Council.

Nancy L. Kuntz, MD serves on advisory boards for Audentes, Argenx, AveXis, Biogen, Cytokinetics, and Sarepta. She also serves as site Principal Investigator for clinical trials sponsored by AveXis, Audentes, Biogen and Sarepta.

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Anne M. Connolly, MD serves on advisory boards for AveXis Therapeutics, Sarepta Therapeutics, Genentech-Roche, Edgewise, Dyne, and Scholar Rock. She is a sub-investigator of the Avexis SMA gene therapy trial as well as the Sarepta Eteplirsen clinical trial and gene therapy trials.

Author contributions

Matthew J. Martin, MD – Conversion of symposium presentations into prose. Drafting and editing the symposium review article and giving final approval of the version to be published.

Margie A. Ream, MD, PhD – Created and presented 'The promise of gene therapy and newborn screening' for the CNS/ICNA 2020 Conference, as well as editing the symposium review article and giving final approval of the version to be published.

Nancy L. Kuntz, MD – Created and presented 'The ASPIRO Gene Therapy Trial in XLMTM: Update on Safety and Efficacy Findings' for the CNS/ICNA 2020 Conference, as well as editing the symposium review article and giving final approval of the version to be published.

Katherine D. Matthews, MD – Created and presented 'Is Gene Replacement a Viable Option for My Favorite disease?' for the CNS/ICNA 2020 Conference, as well as editing the symposium review article and giving final approval of the version to be published.

Anne M. Connolly, MD – Created and presented 'Gene Transfer Therapy for SMA and DMD: Where are we now?' for the CNS/ICNA 2020 Conference, as well as editing the symposium review article and giving final approval of the version to be published.

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