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Review

GENE TARGET: A framework for evaluating Mendelian neurodevelopmental disorders for gene therapy

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Interest in gene-based therapies for neurodevelopmental disorders is increasing exponentially, driven by the rise in recognition of underlying genetic etiology, progress in genomic technology, and recent proof of concept in several disorders. The current prioritization of one genetic disorder over another for development of therapies is driven by competing interests of pharmaceutical companies, advocacy groups, and academic scientists. Although these are all valid perspectives, a consolidated framework will facilitate more efficient and rational gene therapy development. Here we outline features of Mendelian neurodevelopmental disorders that warrant consideration when determining suitability for gene therapy. These features fit into four broad domains: genetics, preclinical validation, clinical considerations, and ethics. We propose a simple mnemonic, GENE TARGET, to remember these features and illustrate how they could be scored using a preliminary scoring rubric. In this suggested rubric, for a given disorder, scores for each feature may be added up to a composite GENE TARGET suitability (GTS) score. In addition to proposing a systematic method to evaluate and compare disorders, our framework helps identify gaps in the translational pipeline for a given disorder, which can inform prioritization of future research efforts.

INTRODUCTION

Although individually rare, Mendelian neurodevelopmental disorders (NDDs) are a significant burden on individuals as well as the healthcare and public school systems supporting them.[1–3] Recent advances have enabled identification of the genetic basis of many NDDs. In parallel, technological advances have demonstrated the feasibility of a range of techniques to manipulate gene expression in the central nervous system (CNS). A notable example is Spinal Muscular Atrophy (SMA; OMIM: 25330), for which three US Food and Drug Administration (FDA)-approved therapies increase functional gene expression.[4,5] Although exciting and hopeful, this situation presents a challenge for the scientific community, which has neither the capacity nor the funding to address all Mendelian NDDs at once. We propose a framework for prioritization of research and development of gene-based therapies for NDDs that includes scientific and ethical considerations. The framework should serve as a reminder to the reader of the breadth of considerations that need to be evaluated when developing a gene therapy program, with each topic intended as an entry point for nuanced considerations for that specific field.

This paper is intended for clinicians and researchers building gene therapy programs, scientists in the field of gene therapy research, and rare disease advocacy organizations. There are two main ways to use this framework. Clinicians, scientists, and gene technology companies could use the framework to help focus gene therapy development efforts on conditions with a higher score for gene therapy suitability. Advocacy groups and funding agencies could utilize the framework to identify gaps in the body of research for a specific disorder and direct funding accordingly. Thus, there is utility as an evaluation tool (so that GENE TARGET suitability scores can be compared across disorders) and as a research guidance tool (so that low-scoring domains would direct future efforts for a given disorder).

Each letter of the GENE TARGET mnemonic represents an evaluation criterion that falls into one of four domains: genetics, preclinical validation, clinical considerations, and ethics (Table 1). These criteria comprise a set of talking points designed for comprehensive evaluation of distinct gene-disease pairs. A preliminary scoring framework is also proposed. For a given disorder, individual criteria are assigned a score to elucidate relative strength or weakness, with a higher score denoting strength. Criteria scores have been intentionally set so that the four broad domains (genetics, preclinical, clinical, and ethical considerations) are of equal weight, each yielding a maximum score of 10. The total attainable GENE TARGET Suitability (GTS) score for a gene-disease pair is 40. In Table 2, we evaluate six gene-disease

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Table 1. GENE TARGET framework and working model of GTS score guide

	Consideration	Scoring guide			
G	genetic mechanism is understood and amenable	genetic mechanism is unknown; recommend establishing before proceeding	multi-gene CNVs account for most cases of the disorder	loss of function or altered function of a single gene	in addition, gene harbors special characteristics that can be exploited
	maximum score = 6	0 points	2 points	4 points	6 points
E	early diagnosis is typical	age of diagnosis is highly variable	diagnosis in infantile to early childhood period	diagnosis typically made prenatally or neonatally	
	maximum score = 2	0 points	1 point	2 points	
N	natural history is understood	no natural history data available; recommend natural history studies before proceeding	cross-sectional data available	longitudinal data available	longitudinal data with standardized neurodevelopmental measures
	maximum score = 3	0 points	1 point	2 points	3 points
E	endpoints are validated and meaningful	no validated endpoints; recommend establishing endpoints before proceeding	neurodevelopmental endpoints (e.g., I.Q,) available	indirect measures/biomarkers associated with neurodevelopmental phenotype available	direct endpoints available; how patients feel, function, and survive
	maximum score = 3	0 points	1 point	2 points	3 points
T	tools deliver to target tissue at the right time	target tissue unknown; recommend establishing target before proceeding	potentially targetable but limited by properties of target or tissue	target tissue is known, appropriate tools are available	
	maximum score = 6	2 points	4 points	6 points	
A	availability of other safe and effective treatments is limited	disease-modifying treatments are available and approved by regulators	disease-modifying treatments are limited in population, symptomatic domain, or duration of use	no disease-modifying treatments are available	
	maximum score = 2	0 points	1 points	2 points	
R	reversibility has been demonstrated	reversibility not established; recommend establishing before proceeding	reversibility established but temporal window unknown	reversibility and temporal window for rescue established	
	maximum score = 4	0 points	2 points	4 points	
G	gene is tolerant to dosage changes	gene tolerance to dosage change is unknown; suggest establishing dosage window before proceeding	narrow therapeutic window	wide therapeutic window	
	maximum score = 4	0 points	2 points	4 points	
E	ethical principles have been considered	ethical principles have not been considered	favorable risk-benefit ratio OR treatment generalizable across population	favorable risk-benefit ratio AND treatment generalizable across population	
	maximum score = 6	0 points	3 points	6 points	

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Table 1. *Continued*

	Consideration	Scoring guide			
T	target populations are accessible and engaged	patient population not accessible or not engaged	engaged population accessible through community organizations	engaged populations accessible through clinical cohorts or registries	
	maximum score = 4	0 points	2 points	4 points	

The table shows the GENE TARGET framework with features for consideration and corresponding scores. Each of the four broad equally important domains can yield a maximum of 10 points: genetics (genetic mechanism is understood and amenable, gene is tolerant to dosage changes), preclinical (tools deliver to target tissues at the right time, reversibility has been demonstrated), clinical (early diagnosis is typical, natural history is understood, endpoints are validated and meaningful, availability of other treatments is limited), and ethics (ethical principles have been considered, target population is engaged and accessible). Points for a given gene-disease pair are added up to a GTS (gene target suitability) score that is out of 40 and only valid for a particular point in time. The scoring guide illustrates how the framework could be used to quantitively evaluate gene-disease pairs for suitability for gene therapy development but is subject to validation using real-world data and refinement.

entities and illustrate how scoring could work using our preliminary scoring framework.

Our analysis is most relevant for therapies that target expression of a single gene at a DNA level (principally virus-mediated gene delivery) and at an RNA level (principally expression modulators). Some aspects of our analysis could be helpful to assess pharmacological approaches and novel gene editing techniques currently in development.

GENETIC CONSIDERATIONS

Genetic mechanism is understood and amenable

The first step in evaluation of a given Mendelian disorder for gene-based therapy is a robust analysis of the underlying genetic mechanism, which informs feasibility and design approach. Critical components include gene function, genomic architecture, tolerance to induced haploinsufficiency/overexpression, and the mutational spectrum of the genetic condition.

In this section, we discuss isoform specificity and four categories of genetic mechanism: copy number variants, loss of function, altered function, and special genomic characteristics.

Isoform specificity

An evaluation of the abundance, localization and function of different human isoforms of a given gene is important when exploring therapeutic options to determine which protein isoform needs to be replaced or suppressed in the target tissue.[34] Examples of genes for which this is relevant include *UBEA3* (Angelman syndrome; OMIM: 105830),[34] for which there are three isoforms expressed in human neurons with different localization and neuronal function, and *TCF4* (Pitt-Hopkins syndrome; OMIM 610954), for which usage of alternative 5′ exons yields numerous isoforms that differ in subcellular localization and transcription activation efficiency.[35] The isoform affected by the disorder and the isoforms modulated by the therapeutic strategy should be understood for the gene within the context of the associated disorder.

Copy number variants (CNVs)

This framework is focused on monogenic disorders. Complex CNVs, particularly those that are variable in size and breakpoints, and those with multiple disease-critical genes would not be considered favorably for gene-based therapy development at the present time because of the challenges in addressing different spatial and temporal expression patterns in multiple genes. CNVs with highly recurrent breakpoints could be evaluated using our framework if there is a single established putative disease gene, such as, for example, *SHANK3* (Phelan McDermid syndrome; OMIM: 606232). Taking PMS as an example, although a single putative disease gene has been established, CNVs account for most cases,[6] and so this disorder would score lower in this category than disorders with single gene involvement (such as *MECP2*, Rett syndrome; OMIM: 312750) because of the potential role of other contiguous genes in variably modifying the phenotype

Table 2. The GENE TARGET framework with examples

Score	Gene-disease pair (OMIM) Description of scores for each category in text	Rett syndrome *MECP2* (OMIM: 213750)	Spinal muscular atrophy (SMA) *SMN1* (OMIM: 25330)	Tuberous sclerosis complex *TSC2* (OMIM: 613254)	Phelan Mcdermid syndrome (PMS) *SHANK3* (OMIM: 606232)	Schinzel-Giedion syndrome *SETBP1* (OMIM: 269150)	Sanfillippo syndrome *SGSH* (OMIM: 252900)
G	genetic mechanism is understood	X-linked dominant severe NDD caused by *MECP2* loss of function; special characteristic: XLD, X-reactivation could be exploited	autosomal recessive disorder caused by biallelic loss of *SMN1* function, resulting in loss of anterior horn cells; special characteristic: presence of paralog gene	autosomal dominant multisystem NDD caused by mono-allelic loss of function of *TSC2*	autosomal dominant NDD caused by mono-allelic loss of function of *SHANK3*; majority (>90%) arise from multi-gene deletions; larger deletions are associated with a more severe intellectual phenotype[6]	autosomal dominant neurodegenerative life-limiting disorder arising from gain-of-function variants; special characteristic: recurrent hotspot; variants are limited to a 12-hotspot domain[7]	autosomal recessive lysosomal storage disorder arising from biallelic loss of function
	Score (maximum 6)	6	6	4	4	6	4
E	early diagnosis is typical	mean age of diagnosis is published as 2.7 years but at present likely to be younger[8]	presentation of the most severe subtype is neonatal (type 1, most frequent subtype)[9]	those with severe disease are likely to be diagnosed in infancy; with improved prenatal imaging, rate of prenatal diagnosis increasing	typical presentation infantile hypotonia, delayed early-motor milestones[10]	the classic presentation is in the neonatal period	diagnosis is typically in late infancy/early childhood phase after symptom onset[11,12]
	score (maximum 2)	1	2	1	1	2	1
N	natural history is understood	longitudinal natural history studies with large populations have been carried out, and critical periods have been identified[13,14]	natural history demonstrates median survival 8–10 months	variability in natural history of disorder is observed, but possible to stratify based on epilepsy or NDD[15,16]	longitudinal natural history studies have been carried out, and critical periods have been identified[17]	cross-sectional data published[7]	well documented in prospective and retrospective cohorts
	score (maximum 3)	3	3	3	2	2	2
E	endpoints are validated and meaningful	Rett-specific severity scales have been developed and validated[18]	survival and ventilator dependence are direct endpoints	somatic and CNS endpoints have been established[19–21]	specific neuropsychiatric scales have been validated in the PMS population[10,22]	survival could be used as an endpoint because this is a life-limiting disorder, but death for systemic rather than neurological reasons	enzyme-based biomarkers and cognitive endpoints established for early interventional trials[23–25]
	score (maximum 3)	2	3	2	2	2	2
T	tools deliver to target tissues at the right time	target tissue is brain parenchyma, but currently CNS tools have low efficiency	target tissue is anterior horn cells of spinal cord, which can be targeted with multiple delivery vehicles	target tissue is CNS and somatic tissue, but *TSC2* exceeds carrying capacity of rAAV	target tissue is brain parenchyma, but currently CNS delivery tools have low efficiency	target tissue is brain parenchyma, but currently CNS delivery tools have low efficiency	target tissue is primarily brain parenchyma, and protein is secreted, so cross-correction is possible[26]
	score (maximum 6)	4	6	4	4	4	6

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Table 2. *Continued*

Score	Gene-disease pair (OMIM) Description of scores for each category in text	Rett syndrome *MECP2* (OMIM: 213750)	Spinal muscular atrophy (SMA) *SMN1* (OMIM: 25330)	Tuberous sclerosis complex *TSC2* (OMIM: 613254)	Phelan Mcdermid syndrome (PMS) *SHANK3* (OMIM: 606232)	Schinzel-Giedion syndrome *SETBP1* (OMIM: 269150)	Sanfillippo syndrome *SGSH* (OMIM: 252900)
A	availability of other safe and effective treatments is limited	no disease-modifying treatments are available	FDA-approved disease-altering treatments available	mTOR inhibitors FDA approved	no disease-modifying treatments are available	no disease-modifying treatments are available	no disease-modifying treatments are available[23]
	score (maximum 2)	2	0	0	2	2	2
R	reversibility/rescue has been demonstrated in a model system	phenotypic reversibility and prevention have been demonstrated in a mouse model[27]	enhanced survival has been demonstrated in a mouse model[18]	reversibility of distinct phenotypes has been demonstrated for specific phenotypes in animal models and clinical populations[28,29]	phenotypic reversibility and prevention have been demonstrated in mouse model of *Shank3* haploinsufficiency but not larger deletion[30]	reversibility has not been demonstrated in a model system	phenotypic reversibility and prevention have been demonstrated in a mouse model[31]
	score (maximum 4)	4	4	4	4	0	2
G	gene is tolerant to dosage changes	known bidirectional dosage sensitivity; *MECP2* duplication leads to a distinct syndrome (OMIM: 300260)	multiple copies within a natural experiment	tuberin is part of a protein complex, limiting the risk of overexpression	known bidirectional dosage sensitivity[32,33]	loss of function results in a distinct NDD	autosomal recessive but risk of competitive SUMF1 sequestration[26]
	score (maximum 4)	2	4	4	2	2	4
E	ethical principles have been considered	severe NDD; treatments translatable across affected individuals	life-limiting disorder; treatment translatable across affected individuals	highly variable disorder, warranting careful consideration of risks versus benefit; treatment would be translatable across affected individuals; the mTOR pathway could be informative for other disorders	characterized by a moderate to severe level of disability; risk of a proposed intervention versus benefit needs to be considered; treatment translatable across affected individuals	this is a progressive disorder with survival limited to childhood; a higher degree of risk would be considered acceptable for this life-limiting disorder; treatment translatable across affected individuals	this is a severe, neurodegenerative, pediatric disorder; a higher degree of risk would be considered acceptable for this life-limiting disorder; treatment translatable across affected individuals
	score (maximum 6)	6	6	6	6	6	6

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Table 2. *Continued*

Score	Gene-disease pair (OMIM)	Rett syndrome *MECP2* (OMIM: 213750)	Spinal muscular atrophy (SMA) *SMN1* (OMIM: 25330)	Tuberous sclerosis complex *TSC2* (OMIM: 613254)	Phelan Mcdermid syndrome (PMS) *SHANK3* (OMIM: 606232)	Schinzel-Giedion syndrome *SETBP1* (OMIM: 269150)	Sanfillippo syndrome *SGSH* (OMIM: 252900)
	Description of scores for each category in text	well-organized clinics and engaged affected individual/family community (https://reverserett.org/rett-syndrome-global-registry/; https://www.rettsyndrome.org/)	well-organized clinics and engaged affected individual/family community; RESTORE Registry (https://www.curesma.org/)	network of hospital centers of excellence; well-organized clinics and engaged affected individual/family community; TOSCA patient registry (https://www.tscalliance.org/)	well-organized clinics and engaged patient/family community (https://www.pcori.org/research-results/2015/phelan-mcdermid-syndrome-data-network-pmsdn; https://pmsf.org/)	well-organized advocacy group and registry (https://sgsfoundation.org/sgs-registry/; https://sgsfoundation.org/)	several hospital LSD clinics; well-organized advocacy groups and registries (https://mpssociety.org/; https://www.sanfilippo.org.au/about-sanfilippo/patient-registry)
T	target population is engaged and accessible	4	4	4	4	4	4
	score (maximum 4)	34	38	32	31	30	33
	GTS score						

The table shows the GENE TARGET framework with examples to illustrate the preliminary scoring rubric along with the composite GTS score.
mTOR Mammalian Target of Rapamycin; LSD Lysosomal storage disorder

and, potentially, the response to therapy. These two disorders are evaluated in Table 2.

Loss of function

Loss of function, whether complete or partial, may be corrected with restoration of gene expression in target cells with DNA or RNA re-expression. Another option for haploinsufficiency disorders is to boost expression of the wild-type allele; for example, by upregulation of a promotor,[36] an approach that would maintain the integrity of the cell-specific isoform. The therapeutic treatment window for biallelic loss of function (autosomal recessive disorders) is likely to be comfortably wide, with only up to 50% of physiological levels required for cellular function. Thus, potentially toxic levels are over 2-fold higher than therapeutic levels. In contrast, treatment of haploinsufficiency because of mono-allelic loss of function, either autosomal or X-linked dominant, has less margin for error. This is reflected in our scoring metric for "gene dosage."

Altered function

Altered functions include excessive activity of the mutant protein (gain of function) or a mutant protein that interferes with the wild type (dominant negative), although the distinction between these subcategories is not always clear.[37] In either case, the therapeutic objective is to knock down expression of the abnormal/overexpressed protein. This may be accomplished at the DNA or RNA level, and the biological consequences of induced haploinsufficiency are important to consider (see "Gene is tolerant to dosage changes").

Special genetic characteristics

Effective genetic therapies have been developed for several disorders by exploiting their unique genomic characteristics. In our framework, the presence of a "special characteristic" yields the highest ranking in the genetic mechanism category. Such special mechanisms, including recurrent mutational hotspots, the presence of non-productive transcripts, genomic imprinting, X reactivation for X-linked dominant disorders, and the presence of a paralog gene, are described in Table 3, along with examples of each.

Gene is tolerant to dosage changes

For each gene, identification of the therapeutic range of expression levels is of critical importance. In general, recessive disorders are more permissive than dominant disorders, as are those mediated through non-cell-autonomous mechanisms (i.e., secreted proteins). Treatments that change levels of gene expression necessitate a clear understanding of the tolerance of the gene to over- and underexpression. The tight regulation of gene dosage is critical for many "Goldilocks" genes in which over- and underexpression are pathogenic. Many dominant neurodevelopmental syndromes are caused by dosage imbalance within single genes in which deletion and duplication result in clinically distinct syndromes that often share core features.[44] Constitutive dosage sensitivity, though, is not equivalent to induction of a dosage change postnatally, which highlights the importance of understanding critical periods of temporal expression of a given gene.

Table 3. Special genetic mechanisms with examples

Mechanism	Examples
Recurrent hotspot mutations	• A feature in which a restricted mutational spectrum accounts for a significant proportion of affected individuals with a given condition, enabling mutation-specific rather than gene-specific therapies • For example, Schinzel-Gideon syndrome (OMIM: 269150) is characterized by recurrent gain-of-function mutations in the *SETBP1* gene, clustering to a 12-bp hotspot coding for residues 868–871.[38] Given the restricted mutational spectrum in this disorder with established gain-of-function mechanism, knockdown strategies (for example, with an allele-specific antisense oligonucleotide [ASO]) could be translatable across a large proportion of individuals with this condition.
Non-productive transcripts	• Transcripts that are subject to nonsense-mediated decay; for example, by the presence of an alternative cassette or "poison" exon[39,40] • The potential for ASOs to modulate pre-mRNA splicing of these naturally occurring alternative transcripts to generate productive mRNA and increase expression of full-length protein has been demonstrated in preclinical studies using several target genes, including *SYNGAP1* and *SCN1A*.[39] It is estimated that up to one-third of genes produce non-productive transcripts.[40]
Genomic imprinting	• A characteristic of a minority of genes in which expression of an allele is dependent on the parent of origin. • This mechanism is being harnessed in trials of ASO for Angelman syndrome (OMIM: 105830). The principle of activating intact but silenced alleles or, conversely, decreasing expression of improperly biallelically expressed genes could potentially be translated to other imprinting disorders.[41]
X-linked dominant disorders	• Such disorders present unique challenges and opportunities for gene-based therapeutic approaches based on reactivation of the transcriptionally silent allele. • Proof of concept has been demonstrated with reactivation of Xi *Mecp2* in adults.[42] The unique challenge is that X inactivation is random in each cell; that is, in affected females, each cell expresses the wild-type allele or the mutant allele, so a gene therapy approach necessitates cell-specific dosage titration.
Paralog gene	• The exploitation of a paralog gene to compensate for mutations in the primary gene has been a highly successful gene therapy approach. • ASO therapy in SMA, an autosomal recessive disorder caused by biallelic *SMN1* loss of function, harnesses the unique presence of the paralog gene *SMN2* to generate functional SMN protein through alternative splicing.[43]

On a disease level, expected tolerance of affected individuals to a range of dosage levels of the causative gene is considered. The first criterion is establishment of the window of dosage tolerance across development and across target tissues.[45] The association of biomarkers and phenotypes with haploinsufficiency or overexpression in *in vivo* models or in the natural variation of the human population should be ascertained to establish the target ranges for functional re-expression. For example, *MECP2* is implicated in disorders of overexpression and haploinsufficiency, and dosage is subject to cellular mosaicism because of localization on the X chromosome, requiring a high degree of precision in gene therapy strategies.[46] In parallel, the tolerance for variation in expression of the gene construct in non-target tissues should be considered in anticipation of off-target effects. Genes that are tolerant of a wide dosage range or strategies associated with endogenous regulatory constraints (reactivation of endogenously regulated genes) are prioritized over those with a narrow dosage range in this category.

PRECLINICAL CONSIDERATIONS

Tissue and temporal specificity are critical for ensuring that the therapy is delivered to the tissue mediating disease pathology at a developmentally appropriate time point[47] using precision tools. Currently available methods to deliver therapeutic genetic constructs to the CNS include virus- and non-virus-mediated strategies. The two main considerations are the suitability of the delivery mechanism and the design of the genetic payload.[48]

Tools deliver to target tissue at the right time

Virus-based vectors tools

Virus-mediated gene therapies harness the endogenous ability of viruses to infect cells and introduce and drive foreign transgene expression. Tissue expression is determined by the tropism of the strains of virus employed and the regulatory regions included in the genetic construct. Viral delivery is primarily achieved with recombinant adeno-associated viruses (rAAV) or lentiviral vectors, which introduce a DNA construct in an episomal or integrating fashion, respectively, that is read and translated by the cells to produce a therapeutic protein.

The routes of delivery for genetic technology for neurological disorders are summarized in Table 4. To achieve a therapeutic effect in the brain, a viral vector must cross the blood-brain barrier (BBB) or encode a protein that is able to cross the BBB. Otherwise, administration needs to be directly into the CNS. rAAV vectors infect cells and introduce a persistent episomal genome that can be stably translated but rarely propagated.[49] Several rAAV serotypes demonstrate tropism for the CNS and can be used to deliver transgenes to neurons and glial cells, although none exclusively target the CNS at high levels.[50] Systemic and cerebrospinal fluid (CSF) delivery of rAAV to reintroduce transgenes is best suited to gene targets in which cell non-autonomous effects are expected because the transduction efficiency is relatively low. Alternatively, direct intra-parenchymal injection of rAAV to a specific neural structure or nucleus is better suited for disorders in which the affected gene has a restricted pattern of

Table 4. Routes of genetic technology delivery for neurological disorders

Method of delivery	Comment
Intraparenchymal	Direct injection into brain nuclei bypasses the BBB and peripheral immune responses. Can achieve high levels of expression with low volumes of virus and avoid off-target effects. Requires surgical intervention.
Intra-CSF (IT,ICV, and ICM)	Targets the CNS with moderate systemic exposure. Limited permeation through brain parenchyma. Well suited for targets in the choroid plexus and spinal cord and for secreted proteins that act in regions close to CSF circulation.
Intramuscular	Allows targeting of spinal cord, brain stem, and sensory ganglia through retrograde transport from neuromuscular junctions but can be limited by axonal dysfunction. High risk for immunogenicity.
Intravenous	Non-invasive administration. Limited library for BBB-penetrating viral vectors. Typically requires high viral titers to achieve sufficient CNS exposure. Moderate risk for immunogenicity.

Shown are routes of genetic technology delivery for neurological disorders with a description and challenges of each method.
BBB, blood-brain barrier; CSF, cerebrospinal fluid; IT, intrathecal; ICV., intracerebroventricular; ICM, intra-cisterna magna; CNS, central nervous system.

expression or an anatomically discrete pathogenic locus.[51] The therapeutic applications of rAAV are limited by their relatively small cargo capacity (up to 4.8 kb), which is further reduced when self-complementary AAV is required to increase CNS transduction (up to 2.2 kb).[52] However, efforts are ongoing to circumvent this limitation through the packaging of truncated versions of genes and promoters that retain the function of the full-length version.[53] Nonetheless, the small carrying capacity of AAVs justifies the prioritization of disorders caused by loss of function of genes that can be re-expressed within this size limit.

Lentiviruses are engineered retroviral vectors with a higher cargo capacity (8–10 kb) than AAVs. Lentiviruses infect proliferating, postmitotic, and quiescent cells. Like AAV vectors, tissue selectivity can be manipulated through pseudotyping of the viral envelope glycoproteins.[54] Lentiviral vectors are integrating, allowing incorporation of the genetic construct into the host genome in a specified location (typically) for stable transcription from the infected cells and their cellular progeny. Although lentiviral tools have a theoretically broad applicability for NDDs and are widely used preclinically, use in the clinical setting for neurological indications has been stalled by limitations in CNS transduction efficiency and safety risks.[55] For this reason, lentiviral tools are currently primarily used for *ex vivo* applications. However, safe development of *in vivo* lentiviral therapeutic strategies will expand their applications for large-gene disorders and proliferative target tissues, such as those that expand during pediatric development.

For AAVs and lentiviruses, achieving sufficient vector transduction in the brain is challenging. Consequently, disorders that do not require a high percentage of corrected cells for phenotypic rescue and those that can be rescued through secreted, non-cell-autonomous effects are prime targets for gene therapies.

Non-viral gene therapy approaches

Endogenous gene expression can be therapeutically manipulated with non-virally delivered oligonucleotide-based tools, including lipid nanoparticles or other extracellular vesicle delivery vehicles of DNA or RNA and antisense oligonucleotides (ASOs). Non-viral delivery tools currently deliver regulatory genetic constructs that can alter the expression patterns by increasing expression of functional alleles and splice variants or decreasing expression of toxic transcripts. Recently, non-virally delivered ASOs, synthesized short, nucleic acid assemblies that bind to a unique RNA target, have been successfully used in the treatment of SMA.[43] Unlike virus-mediated gene therapies, nonviral therapies have discrete pharmacokinetics with half-lives ranging from 2–3 days to several weeks, minimal immunogenicity, and broad distribution through the bloodstream or CSF.[56] Although many nonviral modalities circumvent the limitations of virus-mediated therapies, only short-acting RNA- or ASO-based therapies have been successfully used clinically so far.[36] ASOs may be used for disorders in which the therapeutic strategy is to reduce expression of an altered/toxic protein or increase expression of an alternate allele splice variant. In haploinsufficiency disorders, ASO treatment has been used in preclinical studies to increase endogenous expression of full-length protein by preventing naturally occurring, non-productive alternative splicing and promoting generation of productive RNA.[39,57]

Reversibility has been demonstrated

NDDs that arise from genetic, developmental, and environmental interactions are not necessarily reversible by genetic interventions alone. Therefore, the demonstration of reversibility or prevention of a pathological phenotype in a validated preclinical model strengthens the justification for gene therapy development for a given disorder.

Genetically modified animal models, typically rodents but increasingly large animals, have been historically used to identify disease-associated phenotypes and demonstrate treatment response[58] because of the high construct validity of these model systems. In contrast, the more recent development of *in vitro* cellular models derived from induced pluripotent stem cells (iPSCs) from affected individuals allow modeling of reversibility in differentiated cells directly relevant to disease pathology and genetic background of affected individuals.[59] The model system selected to demonstrate reversibility should be amenable to recapitulate the genetic mechanism of the disorder. Reversibility of loss-of-function mutations can be modeled using traditional gene knockout strategies to identify treatment-responsive phenotypic domains and critical windows of treatment efficacy with gene reintroduction (using conditional genetics or gene transfer).[60]

Models recapitulating variants identified in affected populations are critical, though, for gain-of-function, dominant-negative, and isoform-specific pathological mechanisms to determine the effect of gene therapy in the context of diseased proteins.[61] Models that express defined point mutations are critical for diseases with mutational hotspots and to test the efficacy of sequence-specific rescue strategies (e.g., exon skipping and suppression of stop codons).[62]

Ultimately, demonstration of reversibility may require multiple complementary model systems. *In vivo* models facilitate identification of translational, treatment-responsive measures and biomarkers that can be used as endpoints in preclinical and clinical studies. *In vivo* assessment of the developmental window for therapeutic intervention may inform inclusion criteria for early clinical trials. For NDDs, however, rodent models may be limited in their recapitulation of the complexity of human brain structure; some aspects of neurological function, particularly language; and their lack of genetic diversity. The translatability of many mouse models to humans is hampered by inherent differences in the severity of specific mutations across organisms. For example, although the female heterozygous *Mecp2* $^{-/+}$ mouse has the best construct validity, most studies use the hemizygous null *Mecp2* male mouse because of the more severe and relevant phenotype.[63] The latter model, however, does not recapitulate the cell-to-cell mosaicism that poses a major challenge to therapeutic development for this condition in girls affected with this disorder.[63]

iPSC models derived from affected individuals can be used to assess the reversibility and gene dosage sensitivity of morphological and functional phenotypes at a disease- and individual-specific level given a high level of amenability to gene editing. Although these models capture the genetic background and diversity of the population of affected individuals and the complexity of human neuronal differentiation and cell-autonomous phenotypes,[64] they are limited in the ability to reflect the multi-system effects of gene changes and developmental time course. For this reason, investment in validated *in vivo* animal models and *in vitro* models derived from affected individuals builds confidence in translatability.

CLINICAL CONSIDERATIONS

Natural history is understood

Robust natural history data are necessary to understand the typical age of diagnosis, disease course and progression, severity and variability, and potential temporal windows for improving outcomes. Data that are longitudinal rather than cross-sectional and incorporate standardized measures of neurodevelopment best reveal the breadth and course of the phenotypic spectrum. Numerous challenges exist for collection of such data for rare NDDs, including the wide range of ages, developmental levels, co-morbidities of cohorts, and floor and ceiling effects of commonly used neurocognitive measures.[65] Natural history studies may evolve into clinical readiness programs,[66] and, increasingly, historical cohorts may be a substitute for a control group for clinical trials.[67] For ultra-rare genetic disorders, accumulating sufficient cohorts to obtain such data is particularly challenging, highlighting the need for international collaboration.[47] Monogenic disorders that have been very recently described would not score favorably in this category based on limited time to accumulate sufficient natural history data.

In our proposed framework, specific features of the natural history, like severity or symptomatic domains, are not standalone measures of suitability for a gene-based therapeutic disorder but, rather, are features to be understood in the context of natural history. Disease severity is considered when assessing in an ethical context, with weighing of potential risk versus benefit of a proposed treatment. Although disorders that are severe, life-limiting, and with no other treatment options would generally be considered favorable targets in this framework, the scientific rationale is that the potential for improvement, or "reversibility," does not necessarily correlate with the severity of a disorder, and, thus, a well-defined window of reversibility for a moderate disorder would render it equally prioritized given minimal risk (see "Ethical considerations").

Availability of other safe and effective treatments is limited

We prioritize gene-based therapy development for genetic conditions with no existing safe and effective treatments to rescue neurological/neurodevelopmental features. Although enzyme replacement therapy (ERT) is currently available for many genetic metabolic disorders, the BBB prevents improvement of neurodevelopmental features. In the case of severe Hunter syndrome (OMIM: 309900) (due to *IDS* loss of function), ERT results in somatic improvement but does not rescue cognition,[68] and, consequently, the disorder would merit higher scoring despite the presence of an approved therapy.

Early diagnosis is typical

Timely treatment maximizes benefits by increasing life years of recovery and the magnitude of treatment effect based on the "snowballing" of developmental consequences. In the phase I/II trial of intracerebral gene therapy for the lysosomal storage disorder mucopolysaccharidosis (MPS) IIIB, all four subjects showed cognitive improvement, but the greatest benefit has been shown in the youngest subject, the only child under the age of 2 years at enrollment.[69] These findings underscore the need for prompt genomic evaluation of infants and children with suspected NDDs. Unfortunately, the number of years to diagnosis for rare genetic disorders currently remains unnecessarily high.[70,71]

Disease characteristics that facilitate early diagnosis include seizures, significant motor features, and structural malformations that can be detected neonatally or even prenatally. A further consideration is the feasibility of identifying the disorder on newborn screening; for example, a metabolic biomarker (as for phenylketonuria) or genomic technology (as for SMA due to homozygous deletion of *SMN1*). Considerations for inclusion of a given disorder into newborn screening include clinical characteristics, the analytical validity of the screening platform itself, and, critically, the availability of an efficacious treatment for the condition.[72] A major obstacle for establishing an evidence base for treatment efficacy, however, is early diagnosis itself. The breath of disorders included in newborn screening is likely to

expand with advances in screening technology and treatment availability. The clinical utility and public health benefits of wide-scale genomic newborn sequencing remain under evaluation,[73–77] so the effect of such an initiative on gene therapy development for rare disorders remains to be seen.

Many rare monogenic NDDs, even those that significantly impair life quality, are currently diagnosed later in childhood,[78] and for such disorders, the question of whether diagnosis typically occurs within the temporal window of opportunity for phenotype rescue is critically important, ideally addressed by preclinical studies (see "Reversibility has been demonstrated"). The ideal gene target allows diagnosis to be made sufficiently early so that therapeutic manipulation is possible prior to critical developmental expression of the endogenous gene.

In the scoring schema (see Table 1), the categories within "early diagnosis" are considered in the context of the degree of neuronal maturation that occurs within each time frame, with earlier diagnosis increasing the probability of phenotype rescue prior to accumulation of irreversible pathology as the nervous system develops.

Endpoints are validated and meaningful

Well-designed clinical trials include clear evidence-based endpoints centered on the affected individuals. Such endpoints should be accurately measurable using validated instruments and reproducible over time and across observers.[79] Endpoints may be clinically meaningful direct measures of how affected individuals feel, function, and survive or indirect measures, such as biomarkers (for example, laboratory tests), considered "surrogates" for clinically meaningful endpoints.[80]

For children with NDDs, establishing such endpoints presents unique challenges. Although survival (such as in SMA trials) and motor endpoints (such as in Angelman syndrome) are highly reliable, the demonstration of reliability of cognitive endpoints is more challenging. Longitudinal data plotting cognitive outcomes at multiple time points to demonstrate the base rate of deficits and reproducibility is recommended,[81] but the selection of appropriate measures is challenging. Neurodevelopmental scales were designed to identify delay in a normative population, and it cannot be assumed that individuals with rare NDDs will respond to therapies by acquiring skills in this linear fashion.[65] Cognitive trials are vulnerable to weak study design because measures may be subject to retest effects or may not be sufficiently sensitive to the intervention, particularly within the duration of the study.[81,82] Increasingly, neurodevelopmental measures may be tailored to specific disorders. For example, several new disease-specific scales have been developed, adapted, and validated for Rett syndrome that highlight the core symptomatic profile of the disorder expected to be modified through therapeutic interventions.[83–85]

Given the challenges of using neurocognitive measures for trials, validated biomarkers are particularly important for rare NDDs. Biomarkers, such as those derived from electroencephalograms (EEGs), serve several roles in development of a clinical trial.[86] They may be used to stratify subjects and, thus, inform inclusion criteria. Target engagement during a trial may be demonstrated through use of biomarkers, ensuring that sufficient levels of the therapeutic agent are present in the target organ tissue. Finally, biomarkers play a role as early indicators of efficacy in demonstrating that therapy is exerting the expected biological effect.

In addition to being validated, endpoints should be meaningful to the population of affected individuals and their caregivers and families.[87] The importance of partnering with families and advocacy groups to establish priorities and select meaningful endpoints and to inform considerate trial design is becoming increasingly recognized and is central to ultimate regulatory approval.[88]

ETHICAL CONSIDERATIONS

Ethical principles have been considered

A detailed review of the ethical issues involving gene therapy for rare disorders is beyond the scope of this paper, and such important discussions have been published previously.[89–91] The very notion of prioritizing one gene-disease pair over another raises an ethical dilemma because it could be seen as deciding to help one group of people versus another. In this section, we focus on the two most relevant ethical issues for selection of gene targets, principally (1) an assessment of the risk-benefit ratio for genetic intervention within each disease and proposed treatment and (2) an assessment of how applicable the intervention is to the wider population of individuals affected by a particular monogenic disorder or a mechanistically defined class of disorders.

The risk-benefit ratio in the treatment of a disorder is rooted in assessment of quality and length of life for affected individuals versus the potential risks of the specific genetic intervention. Treatment trials that pose a significant risk are considered more favorably in an ethical framework and are more likely to be acceptable to the public for disorders that are severe and/or life limiting.[92] The burden of risk is particularly relevant for gene replacement therapy, for which there are safety concerns (known and unknown) not yet mitigated because of the nascency of the field. In addition, trialing a gene therapy treatment may preclude future trial eligibility. Judging quality of life is more nuanced than survival, raising the question of who is qualified and has the right to judge quality of life or disease severity, especially for individuals who cannot express themselves verbally.[93] In our scoring schema, the benefit of the proposed therapy needs to be significant in relation to the known and unknown risks of current technologies as judged by individual and community standards.

The second major ethical consideration centers around the concept of generalizability of a therapeutic approach to the wider population of affected individuals for a given disorder or closely related disorders. Under the key ethical principle of justice, therapies that are generalizable across affected subjects with a given disorder would be considered more favorably than individualized treatments. For example, although an individual may harbor a novel gain-of-function variant that is highly amenable to ASO therapy, we would favor treatment

development for a recurrent amenable variant, generalizable to a common mechanism and, therefore, a broader population base. Alternatively, a gene-based therapy for a Mendelian disorder may have functional consequences for downstream mechanistic pathways that could be leveraged for other genetic or idiopathic disorders, which would strengthen the argument for prioritizing such disorders.

Target populations are accessible and engaged

Accessibility to an engaged population of affected individuals with committed families, community stakeholders, and caregivers is vital for the success of clinical trials. For many rare and ultra-rare disorders, clinical trial cohorts are cultivated through specialty clinics or registries, often in collaboration with advocacy groups. Centers of expertise that not only establish and characterize clinical cohorts but also provide clinical trial infrastructure are of critical value. Engagement of affected individuals is considered alongside accessibility. The majority of the stakeholder population should be in favor of gene-based interventions for their disorder.

Disease prevalence per se is difficult to measure and, to some extent, less relevant than the existence of an accessible target population. Therefore, it is not a standalone consideration in our framework; we do not recommend necessarily prioritizing rare NDDs that are more prevalent. In the Decipher Developmental Disorders (DDD) study, *de novo* variants in the top five genes (*ARID1B*, *SATB2*, *SCN2A*, *ANKRD11*, and *MED13L*)[78] account for 5% of NDD presentations. It could be argued that these disorders should be prioritized for gene-based therapy development to maximize statistical power in clinical trials to translate into more collective benefits. Although economic considerations are not part of this framework, we acknowledge that financial sustainability of a treatment for a rare disease is closely tied to the disease prevalence. Population size alone, however, does not indicate the availability and preparedness of the population for clinical trials. We propose that it is not disease prevalence itself but the availability of rigorous high-quality natural history data, often related to disease prevalence and establishment of a well-organized support organization, that should be prioritized.

All disorders exemplified in Table 2 have a potentially favorable risk/benefit ratio by community standards, have generalizability to a common mechanism present in a significant subset of the population, and have an engaged and accessible community of affected individuals. This may be a reflection of the bias in selection of disorders that are well described and mature in the translational pipeline. The ethical considerations for all gene-disease pairs, particularly those that are less well understood, must be considered uniquely in the context of the risks of the proposed treatment together with input from the community.

DISCUSSION

The letters of our mnemonic GENE TARGET represent the key elements that we suggest should be considered in evaluation of a Mendelian NDD for suitability for gene-based therapy. In our suggested scoring framework, we define a score for each category, and these, in turn, may be added to yield a composite GTS score out of 40. Category scores have been intentionally weighted so that the overall equally important domains of genetic, preclinical, clinical, and ethical considerations are worth 10 points each. This proposed framework allows for (1) disorders to be evaluated and compared against each other for suitability for gene-based treatments and (2) identification of translational gaps for individual disorders, which could be the focus of future research efforts. An assessment is valid only for a particular point in time, and frequent re-evaluation is recommended as the knowledge and evidence base for a given disorder expands. Many components of this framework are likely to evolve over years (e.g., availability of natural history data and preclinical models). Others, however, are less likely to change (e.g., genetic mechanism and gene dosage tolerance). The intent of the scoring framework is to illustrate how scores could be applied to evaluation of NDDs for suitability for gene therapy. It is preliminary only and subject to validation and refinement over time.

In Table 2, six different monogenic disorders are evaluated under the GENE TARGET framework. We illustrate our preliminary scoring framework and assign a GTS to each disorder. Although the intention of this table is to illustrate use of GENE TARGET rather than to specifically compare these six disorders, SMA, the only disorder in this set with availability of an FDA-approved gene therapy, yields the highest GTS score of 38.

Figure 1 illustrates the flow of genetic mechanisms informing preclinical development and of clinical features of the disorder informing clinical development, all of which contribute to clinical trial readiness. Our framework favors NDD genes that tolerate a wide range of gene dosages. Although, on one hand, autosomal recessive disorders are considered attractive targets in view of their wider therapeutic window, the epidemiological burden argument, in a non-consanguineous population, would be that *de novo* variants account for a greater burden of disease in NDDs[78] and should be the focus of initial efforts of gene-based therapies. In this group, the approximately equal contribution of loss of function and altered function highlights the concept that a range of technologies will be needed to be able to address NDDs in a meaningful way. Disorders in which there is potential to harness special genomic mechanisms translatable to most or all affected individuals with that disorder also score well.

Understanding mechanism informs the treatment approach, and tools need to be available to deliver treatment to target tissues within the temporal window with a clear, reproducible demonstration of reversibility. Disorders that are diagnosed early, well within the critical window of reversibility, are favored. Disorders with accessible target populations, natural history data, and validated meaningful endpoints should also be prioritized, with engagement and leadership from rare disease advocacy groups likely to play a key role in these characteristics. The ethical considerations we have highlighted are weighing of risk versus benefit for a proposed treatment of a given disorder and translatability of the proposed treatment to affected individuals.

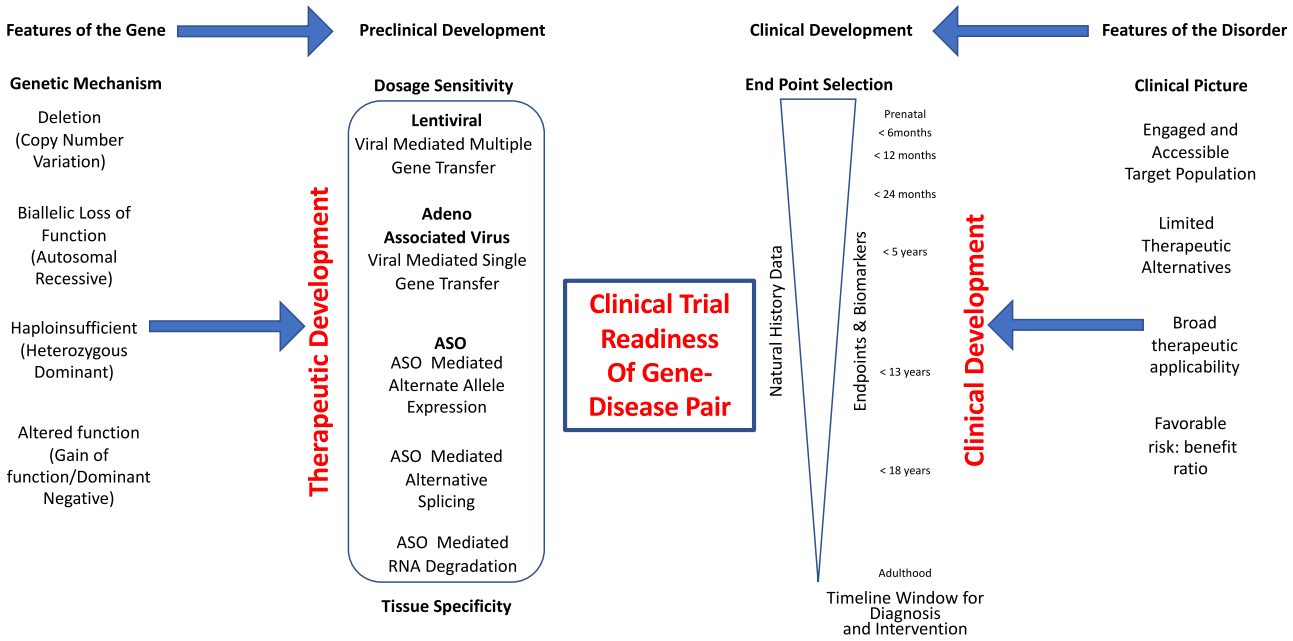

Figure 1. The gene-disease pair defines the path toward gene therapy

Preclinical development is rooted in the genetic mechanism. This is balanced **with** the features of the disorder, which drive selection of therapeutic endpoints. Preclinical development of the therapeutic construct and clinical development of the trial design must be completed before initiating a clinical trial for a gene therapy. Gaps on either side of development identified through the framework should serve as flags for areas of future research.

This framework could be used by scientists engaged in gene therapy research to aid evaluation of monogenic NDDs for inclusion into gene therapy programs. For those engaged in a gene therapy development program for a specific disorder, this framework could serve to highlight areas of relative weakness in the research strategy. In a clinical setting, this framework may serve as a guide for rare disease physicians orienting newly diagnosed families about prospective gene therapy options for that disorder. This schema may also be of interest to funding bodies to help guide resources toward disorders that are most amenable and clinical trial ready and toward domains of relative weakness for specific disorders. For the scientific advisory boards of advocacy organizations for single monogenic disorders, this framework could serve as a roadmap to identify translational gaps in the gene therapy pipeline to direct future research efforts.

DATA AVAILABILITY

No original data were generated in this review article.

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AUTHOR CONTRIBUTIONS

M.C. and M.M. were responsible for the conceptualization of the paper and writing of the original draft. K.A.D., S.J.B., L.P., E.D.B., and M.S. contributed to the methodology (the design of the framework and scoring metric) and review and editing of the manuscript. N.L.C. contributed to review and editing of the manuscript.

DECLARATION OF INTERESTS

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