Molecular Therapy
Nucleic Acids
Review

The evolving landscape of NF gene therapy: Hurdles and opportunities

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Neurofibromatosis type 1 (NF1)- and NF2-related schwannomatosis are rare autosomal dominant monogenic disorders characterized by a predisposition for nerve-associated tumors. Current treatments focus on symptomatic management, but advancements in the gene therapy field present unique opportunities to treat the genetic underpinnings and develop curative therapies for NF. Approaches such as nonsense suppression agents and oligonucleotide therapies are becoming more mature and have emerging preclinical data in the context of NF. Furthermore, there has been progress in developing gene therapy vectors that can be delivered locally into tumors to ablate or shrink their size. While still a nascent research area, gene addition and gene repair strategies hold tremendous promise for the prevention and treatment of NF-related tumors. These technologies will also require parallel development of delivery vectors able to target the Schwann cells from which tumors most commonly arise. This review seeks to contextualize these advancements and which hurdles remain for their clinical adoption.

INTRODUCTION

Neurofibromatoses (NFs) are a family of genetic disorders that harbor a predisposition for nerve-associated tumors, particularly neurofibromas and schwannomas.[1,2]

NF1 (neurofibromatosis type 1) is the most common of these conditions, occurring in 1 in 3,000–4,000 individuals.[3,4] NF1 is associated with cutaneous, subcutaneous, and plexiform neurofibromas that are initiated by somatic second-hit mutations of the *NF1* gene in Schwann cells but incorporate other cell types, including fibroblasts and perineural cells.[3] Malignant peripheral nerve sheath tumors and optic gliomas are less frequent but can require more aggressive intervention.[4] Individuals with NF1 can present with a constellation of other manifestations, including Lisch nodules, skinfold freckling, skeletal dysplasias, neurocognitive issues, and muscle hypotonia.[3] Loss of neurofibromin (the protein encoded for by the *NF1* gene) promotes Ras activity, resulting in the dysregulation of a variety of downstream cell signaling pathways, including the mitogen-activated protein kinase pathway.[5] Clinical data support the use of MEK inhibitors in managing the growth of some NF1 tumors; however, they are indicated for only some tumor types, have limited and variable efficacy, require ongoing dosing, and can produce adverse side effects.[6,7] In practice, NF1 tumor management involves monitoring alongside surgical resection, laser ablation, radiation therapy, chemotherapy, and pharmacotherapy.[4]

Schwannomatosis is far less frequent than NF1, yet it presents significant challenges for clinical management.[3] Schwannomas arise specifically from Schwann cells, forming well-encapsulated tumors that grow along the outside of the nerve sheath rather than becoming interwoven with the nerve fibers themselves. They are less likely to become malignant than plexiform neurofibromas and can remain asymptomatic for a long time; however, they can cause significant complications for patients through disruption and compression of affected and adjacent nerves, causing pain, numbness, or weakness.[8] *NF2*-related schwannomatosis characteristically features unilateral or bilateral vestibular schwannomas that can affect hearing and balance and, in some instances, cause facial paralysis. Other common tumor types include brain/spinal tumors (meningiomas and ependymomas) and cranial nerve tumors; the latter can lead to facial paralysis, difficulties swallowing, and vision problems. Like NF1, *NF2*-related schwannomatosis is caused by heterozygous germline mutations (in the *NF2* gene), and tumors are associated with sporadic double inactivation of *NF2*.[9] *NF2* encodes for merlin, a protein with a range of functions that include tumor suppression, cell signaling, and cytoskeletal modulation.[9–11] Clinical treatment aims to limit the impact of vestibular schwannomas and spinal tumors via surgeries and/or targeted radiation therapy. Pharmacotherapies, such as the vascular

https://doi.org/10.1016/j.omtn.2025.102475.

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endothelial growth factor-antibody bevacizumab[12] and the tyrosine kinase inhibitor brigatinib,[13] have shown clinical utility in managing schwannoma growth. Sporadic schwannomas can also arise outside the context of *NF2*-related schwannomatosis, but these are rare and often present as genetically undefined. These tumors can be caused by mosaic or somatic *NF2* mutations as well as genes such as *SMARCB1* and *LZTR1* (which have been linked to benign but painful schwannomas[14]); the genetic causes of many sporadic schwannomas remain unknown.[15]

There is growing interest in the potential of gene therapies—techniques aimed at preventing or treating disease by modifying or manipulating an individual's genetic material—to treat both NF1 and *NF2*-related Schwannomatosis. This has been highlighted in several prior reviews,[16–21] but since their publication, there have been many technical advancements in the field. Notably, both conditions share a common key cellular target; thus, any delivery system that aims to replace or correct gene function is precancerous or cancerous, and Schwann cells could be adapted to treat either disorder. Such therapies, which neutralize a mutation prior to tumorigenesis, could be prophylactically effective for preventing later-onset tumors.[21] With the increased accessibility of inexpensive sequencing, early genetic diagnosis could offer an opportunity for early intervention during childhood to minimize new tumor formation and/or tumor growth.

APPROACHES TO GENE THERAPY FOR NF1 AND *NF2*-RELATED SCHWANNOMATOSIS

This review describes a range of gene-focused approaches in the context of therapy for NF1 and *NF2*-related Schwannomatosis where we (1) describe the emerging technology, (2) discuss its applicability to NFs and any relevant topical reports, and (3) examine any outstanding hurdles, particularly those specific to that technology. Cui et al. previously speculated on the potential for isolated domains of neurofibromin to restore function in a gene-additional approach, noting the limited packaging capacity of current adeno-associated virus (AAV) vector systems.[18] Leier et al. gave a more general perspective on emerging technologies, recognizing that few had been trialed for NF at the time of review.[19] Staedtke et al. provided a broader educational viewpoint, tying together the preclinical and clinical challenges that need to be addressed prior to early-stage clinical trials.[20] Gene therapy for schwannomatosis associated with germline *SMARCB1* and *LZTR1* mutations (which rarely involve vestibular schwannomas and chiefly manifest in adulthood[22]) was deemed outside the scope of this review, primarily due to a lack of published research.

This review aims to provide a technical update of the therapies in this rapidly advancing field while concentrating on *in vivo* therapeutic options. It must be acknowledged that *ex vivo* gene therapy, which involves the removal and repair of cells and their subsequent reintroduction, is emerging as a powerful strategy for some genetic diseases. However, this approach is more applicable to treat conditions where repair of a small number of cells can produce a strong biological effect, such as liver enzyme deficiencies, including fumarylacetoacetate hydrolase or ornithine transcarbamylase deficiency,[23] or hematological disorders, including sickle cell disease.[24] The scope and complexity of *in vivo* interventions remains broad, from small-molecule and oligonucleotide drugs to more sophisticated advanced therapeutics. Cost and generality of application are clear considerations when it comes to the future applicability of any therapy; however, the price of manufacturing and quality control for currently high-cost vector systems are likely to become more affordable with continued innovation.[25]

Readthrough agents

Technology

Nonsense suppression therapy with small-molecule drugs is an appealing approach to deal with mutations that lead to nonsense-mediated decay (reviewed broadly in Morais et al.[26]). The archetypical drug is ataluren, an agent that binds to the ribosome and can allow readthrough of premature stop codons. Preclinical studies have shown efficacy in numerous models of nonsense mutation disease, and it has been clinically utilized for Duchenne muscular dystrophy.[27]

NF applications

Ataluren has been trialed in a range of murine and mammalian cell lines harboring NF1 patient mutations. In models of the *NF1* c.2041 C>T, p.Arg681X patient mutation, ataluren treatment achieved modest improvements in neurofibromin protein production in cortical neurons differentiated from murine embryonic stem cells[28] and led to minor but significant reductions in neurofibromas in a preclinical *Nf1* mouse model.[29] Notably, the tumor burden and impact was higher in female *Dhh-Cre;Nf1*$^{R683X/4F}$ mice, and females were less responsive than males to ataluren rescue. Another group evaluating ataluren in primary cells cultured from cutaneous neurofibromas of minipigs with the *NF1* p.Arg1947X mutation was less successful.[30] Only when combined with other readthrough agents were they able to restore neurofibromin to detectable levels.

Hurdles

While ataluren can positively enhance neurofibromin production for some nonsense mutations, the impacts on tumor burden have been minimal in preclinical animal models. It is likely that greater improvements would be required to justify progression to human trials. Moreover, this approach would also be limited to individuals with nonsense mutations. Nonsense mutations account for approximately 19% of likely pathogenic or pathogenic mutations in *NF1*- and *NF2*-related Schwannomatosis (NCBI ClinVar, August 2024).

Oligonucleotide therapeutics

Technology

Nucleic acid therapeutics is a broad field adjacent to or overlapping with conventional gene therapy (reviewed in Morais et al.[26]). The typical mechanism of action of many agents, such as antisense oligos (ASOs), small interfering RNA (siRNA), and microRNA (miRNA) is to promote mRNA degradation, gene suppression, and splice

modulation. In many respects, the need for frequent dosing mimics that of conventional pharmacotherapies. Of the oligonucleotide therapeutics, phosphorodiamidate morpholino oligomers (PMOs) are emerging as a preferred option due to their higher solubility, greater binding affinity, and resistance to metabolic degradation.[31]

NF applications

PMOs have been trialed in both NF1 patient-derived and engineered human induced pluripotent stem cells with cryptic splice site mutations.[31,32] Restoration of the normal splicing of *NF1* was heavily site dependent. The most favorable studies have been able to increase NF1 protein expression levels to 30% of wild-type fibroblasts and restore the functionality of NF1 as a Ras signaling inhibitor.[33] Such an approach would be applicable to only a subset of patient mutations; e.g., for NF1, 7% of variants are presumed to affect splicing, though this increases to 15% of likely pathogenic and pathogenic variants (ClinVar, November 2024). PMOs have shown similar efficacy in NF2 patient fibroblasts, restoring merlin levels and improving cytoskeletal organization.[34]

In addition to correcting aberrant gene splicing, PMOs can be conversely used to induce exon skipping; i.e., to remove exons containing disease-causing mutations from the final mRNA transcript. Leier et al. successfully used PMOs to skip *NF1* exon 17 in cells with patient-specific pathogenic variants in the affected exon.[35] A comprehensive study by Catasús et al. explored the utility of ASOs for *NF2* that covered truncating variants, exon-skipping variants, and splicing variants leading to intronic sequence inclusion.[36] Their most successful strategy resulted in expression of a hypomorphic merlin allele that slowed proliferation of targeted fibroblasts by 51%.

Hurdles

To date, testing of PMO therapies for *NF1* and *NF2* have been limited to *in vitro* studies.[33–36] While it is unclear how successful PMO-based therapies for NF would be *in vivo*, similar therapies have been utilized in other Schwann cell based diseases, such as Charcot-Marie-Tooth disease type 1A (CMT-1A) in rodent models[37] and, more recently, in human cells.[38] Delivery by subcutaneous injection has been sufficient for preclinical models, but the efficacy of the orphan CMT-1A drug VCA-892A has yet to be verified in human trials.

PMO-based approaches are limited to *NF1* and *NF2* mutations that affect splice sites or skipping exons that maintain the open reading frame. Splice site mutations account for 15% of *NF1* and 21% of *NF2* likely pathogenic or pathogenic mutations (NCBI ClinVar, August 2024). While exon skipping could be used to treat groups of mutations in the same exon, the open reading frame must be maintained following their deletion, and their exclusion must not significantly impact protein function, limiting their utility in many cases.[35]

Tumor-targeting/suicide genes

Technology

Localized gene therapy has the potential to disrupt individual tumors, including but not limited to the forced expression of suicide genes. This has been suggested to be applicable for the shrinkage of schwannomas. An advantage of this approach is that it would be gene agnostic and, thus, applicable for both *NF2*-related schwannomatosis and sporadic schwannomas. Oncolytic virotherapy and expression of suicide and tumor suppressor genes may be broadly applicable for many discrete brain tumors.[39] This includes glioblastoma multiforme, a rare but often fatal manifestation of NF1.

NF applications

One of the earliest studies used direct injection of AAV-1 to express caspase-1 (ICE) under the control of the P0 Schwann cell-specific promoter. This treatment achieved tumor regression in a xenograft model (human tumor cells in athymic nude mice).[40] Following this, another study induced tumor cell pyroptosis, a form of inflammatory programmed cell death, by expression of the pore-forming protein Gasdermin-D.[41] Progressing further, an analogous AAV1-P0 vector expressing an inflammasome adaptor protein, apoptosis-associated speck-like protein containing a caspase recruitment domain, resulted in cell death when locally delivered to schwannomas.[42] This all culminated in a preclinical study that examined minimum effective dose, neurotoxicity, and pre-existing AAV-1 immunity.[43]

Hurdles

While these results are promising and feature human cells as the therapeutic target, there may be risks associated with off-target gene expression in healthy Schwann cells, tumor resistance, and relapse. These factors may limit clinical translation and scalability.

Gene addition

Technology

Gene addition focuses on the functional restoration of a deficient or absent genetic factor linked to disease. This can involve allelic replacement or provision of one or new copies of the gene or a minigene with similar biological effects. Many approaches involve delivery of a transgene by means of viral vectors.[44] Such strategies are of limited use when a pathological mutation or "poison protein" negatively impacts function even in the presence of a normal allele. Examples of this include Ras mutations that are gain of function (G12V, G12D, and G61L) or dominant negative (S17N, T35S, and Y40C) and whose presence overrides the influence of the wild-type protein.[45]

Gene addition has spearheaded the gene therapy field, with many of the *in vivo* market-approved gene therapies relying on this concept. This includes Zolgensma (AAV9-SMN1, spinal muscular atrophy[46]), Luxturna (AAV2-RPE65, Leber congenital amaurosis type 2[47]), Roctavian (AAV5-BDD FVIII, hemophilia A[48]), and Hemgenix (AAV5-FIX Padua, hemophilia B[49]). The suitability of a gene addition approach is highly disease specific. Considerations include the rate of cellular turnover, the availability of tissue-specific vectors (which can be either contained/restricted to the target tissue), and the required expression levels for functional restoration.

Table 1. Advantages and drawbacks of different CRISPR gene editing approaches

CRISPR technology	Advantages	Drawbacks
HDR-mediated repair	precise insertion of a short DNA segment in a locus of interest	low efficiency risk of off-target integration genomic rearrangement, large deletions due to DNA double-stranded breaks
Base Editing	high efficiency in nucleotide conversion	promiscuous editing off target editing
Prime Editing	moderate efficiency in small nucleotide replacement versatility in replacing any nucleotides/small sequences	low efficiency for large fragment insertion
Homology-Indepdent Targeted Insertion	high efficiency for fragment insertions	risk of off-target (indels at the CRISPR cut site)
Serine-recombinases/ transposons	precise insertion of a large DNA sequence	low efficiency

NF applications and hurdles

There are intrinsic challenges associated with expressing the sizable transcript of *NF1* (cDNA, 12.4 kb; open reading frame, 8.5 kb) necessary to produce the 320 kDa protein. This is above the normal packaging size for single-vector AAV systems. To overcome this restriction, the NF1 GTPase-activating protein-related domain has been explored as an alternative approach to mitigate overactive Ras while constraining construct size. While initially explored using plasmid vectors in cultured cells,[50,51] more recent studies have tested malignant peripheral nerve sheath tumor (MPNST) cells *ex vivo* using a range of AAV vectors.[52] The key issue with this is delivery and relocation of the neurofibromin to the inner cell membrane, whereby it is dimerized and can activate the intrinsic Ras-GTPase pathway to downregulate signal propagation.

Gene addition is a key example where gene therapy approaches differ between NF1- and *NF2*-related schwannomatosis. In contrast to neurofibromin, the protein affected in NF2, merlin, is only 75 kDa (1.8 kb sequence), enabling it to be flanked by a synthetic promoter and polyadenylation sequence and packaged into an AAV vector. This approach was tested in human Schwann cells engineered using CRISPR-mediated genome editing to be *NF2* null. AAV1-merlin vectors have shown the capacity to rescue production of merlin in *NF2*-null cells and cause tumor regression in 7of 9 mice engrafted with these cells.[53] This study is encouraging for the future of gene addition strategies for *NF2*-related schwannomatosis.

One significant challenge in gene addition approaches is the potential for adverse effects caused by overexpression of neurofibromin or merlin. This concern extends beyond total protein levels to the precise dosage within individual cells, which can vary due to heterogeneous vector uptake.

Gene editing

Technology

The current gene editing field is driven by CRISPR-Cas technology. Mammalian CRISPR-Cas gene editing systems are adapted from bacterial nucleases capable of inducing double-stranded breaks at a specific DNA target as specified by a guide RNA (gRNA).[54] Several variations of the core CRISPR-Cas9 nuclease system have been engineered that extend the technology beyond targeted gene disruption.

To repair small point mutations and insertions or deletions (indels), a homology-directed repair (HDR) approach can be applied using a oligonucleotide repair template.[55] However, CRISPR-HDR editing of post-mitotic cells generally exhibits lower repair efficiency; this is noteworthy in the context of NF because Schwann and neuronal cells are post-mitotic.[56] Additionally, HDR editing of post-mitotic cells can result in an increased risk of large deletions as well as chromosomal re-arrangement at the target site due to formation of DNA double-strand breaks.[57] Other strategies to edit point mutations or small indels include base and prime editing systems. Both strategies retain the Cas/gRNA system but tether an additional enzyme to the complex.[58] This is either a deaminase capable of editing a base (base editing [BE]; currently limited to C → T, A → G, and C → G transversions[59–62]) or a reverse transcriptase that builds upon an exposed DNA flap to form small indels (prime editing [PE][63]).

Alternative CRISPR approaches have been developed to replace larger segments of DNA, including homology-independent targeted insertion (HITI).[64] HITI delivers a donor template (framed by the gRNA target sites) alongside a Cas9 enzyme and a self-targeting gRNA. Cas9 cleaves both at the insertion site and within the HITI donor template and then coerces the cell's repair mechanisms to insert the desired sequence. HITI has been shown to have a ∼5- to-10-fold higher repair efficiency and more precision than HDR, making it more attractive for gene replacement therapy for large DNA segments in post-mitotic cells.[65,66]

A critical factor in treating heterozygous conditions is designing strategies that target/repair the deleterious allele without disruption of the wild-type allele.[67] This can be achieved by either designing the gRNA to bind only to the diseased allele (mismatch between the disease and normal allele at the cleavage site[68]) or utilizing a diseased allele-specific protospacer adjacent motif sequence.[69]

NF applications

To date, CRISPR approaches have been primarily used to model NF disease states.[70–73] However, with an expansion of gene repair strategies (summarized in Table 1), combined with improvements in gene delivery, we are poised to see increased attempts to develop new therapeutic strategies that can be validated in preclinical models. It is likely that prototypical patient mutations will be used as exemplars in research, noting that some types of patient mutations (e.g., single-base changes and small indels) will be more amenable to certain therapeutic CRISPR applications.

Hurdles
Each CRISPR editing strategy has its own unique set of limitations that are not specific to NF. For example, while previous studies have demonstrated high repair efficiencies for both BE and PE strategies, they both have issues of bystander editing at the target site and off-target effects.[58,74] HITI and other large insertion systems often suffer from indel formation at the CRISPR cut site.[75] More general and persistent concerns regarding CRISPR editing strategies are risks associated with off-target edits.[76] These are hurdles that are not limited to NF and may be tackled by innovations in the underlying technology.

A major translational challenge for NF is the wide array of *NF1* and *NF2* mutations that necessitate precision medicine (patient mutation-specific) CRISPR/gRNA approaches. Currently, BE/PE strategies need to be designed *ad hoc* for each mutation and may need to be applied without prior clinical validation. HITI approaches focused on exon replacement could treat a range of mutations with a single strategy but generally have a lower efficiency. Based on the likely clinical need to target a majority of pre-tumor or tumor cells, high efficiency will be paramount. Ultimately, this technology is emergent and dynamic, and the applicability and scalability of each strategy will become more apparent with time.

AAV VECTOR TECHNOLOGY FOR TARGETING NF TUMORS
Technology
AAV vectors have emerged as powerful and versatile tools for therapeutic gene delivery. Their efficacy, lack of pathogenicity, favorable safety profile, and ability to effect long-term gene expression, combined with capsid-dependent selectivity (tropism) for a wide variety of cell types, has led to their rapidly increasing use in pre-clinical studies and clinical trials. Notably, they are smaller than some other viral vectors and can only package approximately 4.5 kb of DNA.

Over the past several years, the number of clinical trials initiated using AAV vectors has grown considerably. A critical discovery was that the prototypical AAV type 2 (AAV2) genome can be cross-packaged into the capsids of other AAV variants (pseudo-serotyping), which dramatically alters the vector tropism.[77] This has facilitated the generation of synthetic capsids with unprecedented efficacy for a wide range of tissues, dramatically increasing the therapeutic reach of this vector system.

NF applications
A major requirement for NF gene therapies is an ability to efficiently target Schwann cells. When targeting primary human Schwann cells, AAV6 and AAV-DJ have been found to be the most efficient, with AAV1 and AAV2 also showing limited transduction.[52] In human nerve explants, AAV2 has been shown to be the most effective in a comparison of the naturally occurring AAV serotypes 1–9.[78] In mice, AAV9 has been used to target Schwann cells to treat CMT disease using a single lumbar intrathecal injection.[79] More recently, a capsid derived from library selection in mice, AAV-F, transduced Schwann cells in the sciatic nerve of non-human primates following intrathecal delivery. This contrasted with the lack of gene delivery observed with the AAV9 capsid from which AAV-F was derived.[80] The potential for clinical translation of these capsids is nonetheless limited by their broad effects on multiple tissue types.

In a recent study, Drouyer et al. bioengineered novel AAV capsid variants, with two candidates (termed Pep2hSC1 and Pep2hSC2) showing high transduction efficiency and specificity for primary human Schwann cells.[81] The Pep2hSC1 and Pep2hSC2 capsids are derived from AAV2, differing only in a 9-peptide insertion in their cap sequence. This modification decreased binding to heparin sulfate proteoglycan, a key cellular receptor of AAV2.[82] These capsids also display reduced *in vivo* gene delivery to primary human hepatocytes in chimeric mouse-human livers. Notably, the capsids were sufficiently different to affect immune escape from pre-existing anti-AAV neutralizing antibodies. These are lead candidate capsids for AAV-based therapies targeting Schwann cells.

NF1 and schwannomatosis gene therapy is chiefly focused on prevention of neurofibromas and other nerve cell-associated tumors; however, these genetic disorders have distinct features affecting a range of other organs and cell types. For example, growth impairment,[83] low bone density,[84] as well as muscle weakness[85] are likely associated with *NF1* gene haploinsufficiency and may be improved by restoration of normal gene function in the affected tissues. A wide range of AAV serotypes and variants have been reported that can target tissues affected in NF1 (Table 2). It is possible that therapeutic gene addition or gene editing products could be adapted to target multiple tissues using combinations of AAV vector packaging strategies. For schwannomatosis, there is less need to target alternate cell types, with Schwann cells and arachnoid cells (the target cells for meningiomas) being the key cellular targets.[53]

Hurdles
The increasing number of clinical trials demonstrating clear therapeutic benefits has refocused attention on safety considerations related to the use of recombinant AAV vectors. These include the potential toxicity associated with the use of high vector doses and immune responses that could limit efficacy. For example, hepatotoxicity is a known risk associated with the use of Zolgensma to treat infants with spinal muscular atrophy (SMA), which can largely be mitigated with the use of prophylactic prednisolone treatment.[86] In rare cases, patients have died because of acute liver failure.[87] Thrombotic microangiopathy has also been reported in a small number of patients.[88] Notably, biodistribution analysis of tissues from SMA patients demonstrates that, after systemic delivery of Zolgensma, vector genomes are widely distributed throughout the body, with the liver having between 300- to 1,000-fold more copies than neural tissue, the desired target.[89]

This has led to considerable effort to develop AAV capsids that can not only cross the blood-brain barrier with greater efficiency but that are also de-targeted from the liver.[90,91] Strategies to prevent

Table 2. AAV variants reported to target cells and tissues relevant to NF1 in mice and humans

Cell/Tissue	Clinical manifestations	AAV capsids	References
Schwann cells	dermal and plexiform neurofibroma	AAV1, AAV2, AAV5, AAV6, AAV7, AAV8, AAV9, DJ, *ShH10*, *ShH19*, Pep2hSC1, Pep2hSC2	Bai et al.[52]; Hoyng et al.[78]; Kagiava et al.[79]; Beharry et al.[80]; O'Carroll et al.[112]
Oligodendrocytes	optic glioma	AAV8, AAV9, PhP.B, Rh10, Olig001	O'Carroll et al.[112]
Astrocytes	astrocytoma	AAV1, AAV2, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, 9P1, PHP.A, PHP.B, Rh8, Rh10, Rh43, Anc80L65, *ShH19*, L1-12	O'Carroll et al.[112]
Eye/retinal cells	glaucoma, Lisch nodules	AAV1, AAV2, AAV7, PHP.eB[a] (intravitreal or subretinal delivery)	Wu et al.[113]; Palfi et al.[114]
Keratinocytes (skin)	café au lait spots, freckling	AAVDJK2, AAV-Kera1, AAV-Kera2, AAV-Kera3, and HSV-1	Shen et al.[115]; Sallach et al.[116]; Gurevich et al.[117]
Osteoblasts (bone)	osteopenia, tibial dysplasia and/or pseudarthrosis, scoliosis	AAV2, AAV5, DJ, 2i8, LK03, and 8[a]	Lee et al.[118]
Myofibers (muscle)	muscle weakness and fatigue	AAV9, AAVMYO, myoAAV	Weinmann et al.[119]; Tabebordbar et al.[120]
Vascular endothelial cells (vasculature)	aneurysm, hypertension, Moyamoya disease	AAVsig	Nicklin et al.[121]
Neurons (central nervous system)	cognitive/learning difficulties	AAV9, PHP.eB	Mathiesen et al.[122]

[a]AAV variant reported as effective specifically in murine cells or models

patients from being excluded from clinical trials because of the presence of pre-existing anti-capsid antibodies are also actively being investigated.[92] Additionally, participation in early trials would lead to the formation of AAV antibodies that could preclude inclusion in future trials. This, in turn, could be a barrier to recruitment, as the benefits of early adoption would need to be balanced against future, more effective therapies.

AAV vectors are lead candidates not only for suicide gene delivery[43] but also for gene addition and CRISPR editing strategies. Perhaps the most significant challenge moving forward with AAV vectors will not be Schwann cell targeting but rather the packaging limitations of these vectors. AAV vector size is limiting for *NF1* gene replacement therapies but may be achievable for delivery of *NF2*. *NF1* replacement may be overcome using dual-vector therapies, which are increasingly being adapted to deliver both large transgenes and Cas9-based systems.[93]

Alternative viral and non-viral delivery methods

Although recombinant AAV is the current virus-based system of choice for direct *in vivo* delivery, other virus-based vector systems have also been investigated for NF. These systems include oncolytic herpes simplex virus (HSV) and HSV-based amplicons, adenoviral and retroviral vectors both in primary human tumor cells and *in vivo* models of NF2.[94–97] These viral delivery methods share similar safety concerns in terms of toxicity as well as persistence and/or integration into the genome.[98]

The use of nanoparticles as an alternative to virus-based gene delivery systems have also been investigated for NF. To examine the feasibility of using silver nanoparticles to treat NF1-associated MPNSTs, Alewine et al. assessed their cytotoxicity in mutant NF1-associated MPNSTs, NF1 wild type sporadic MPNST, and normal Schwann cells.[99] Although these nanoparticles were found to be selectively cytotoxic to NF1-associated MPNSTs, long-term data and their efficiency *in vivo* has yet to be examined. Nanoparticles have also been adapted to deliver siRNA therapeutics to human vestibular schwannoma cells in *NF2*-related schwannomatosis, although similar questions of long-term efficacy and i*n vivo* efficiency remain.[100] Experiences in treating other diseases has also shown that even intracerebroventricularly injected nanoparticles will internalize in off-target sites and cells.[101]

CONCLUSION

Despite the surge in technical advancements, gene addition or gene editing treatments require considerable development to be put into clinical practice. It would not be unreasonable to assume a hesitancy regarding the timelines for human NF treatments as the prospects of cancer gene therapy has been discussed for over two decades.[102] Nevertheless, recent advances in AAV vectors, increased versatility and efficiency of gene-editing enzymes, and a broader acceptance and clinical use of gene therapy suggest that clinical gene therapy trials for NF could be possible within the next 5–10 years.

It is likely that initial trials will focus on (1) therapeutic treatment of tumors (e.g., by disruption with suicide genes), (2) prevention of future tumors by repair of the mutant allele, or (3) *NF2* gene addition (whether it is the *NF2* open reading frame or an *NF1* Ras-GTPase-activating domain). While appealing, gene editing technologies are challenging in terms of obtaining sufficient efficiency, and gene repair

would need to optimally occur in a high percentage of cells. This contrasts with other conditions, such as hemophilia and certain metabolic disorders, where functional rescue in a small proportion of cells is sufficient to yield phenotypic effects.[103,104] While *NF2* gene replacement may be more straightforward to achieve, it also has challenges associated with regulating expression levels, and overexpression may be as physiologically detrimental as deficiency. It is important to note that most symptomatic tumors under NF conditions are benign rather than malignant, so the risk/benefit of intervention must be carefully considered.

The aforementioned therapies focus on childhood or adult intervention, but prenatal or neonatal treatments are theoretically possible. NF1- and *NF2*-related Schwannomatosis may not serve as ideal hereditary disease targets for fetal repair based on practical and ethical criteria outlined in the International Fetal Transplantation and Immunology Society Consensus Statement.[105–107] While many tumors arise from second hits later in life, there is evidence that structural changes occur in the NF1 brain during both development and growth that are unlikely to be improved by subsequent gene therapy.[108,109]

Finally, it must be acknowledged that gene technology and resultant cell and animal models of disease may have broad utility in NF research. For instance, CRISPR gene editing has allowed specific patient mutations to be recapitulated with relative ease, avoiding the need to harvest patient cells and allowing examination of a mutation's effects in comparison to controls with an identical genetic background. This benefit was highlighted in a recent review focused on creating cancer models with CRISPR-Cas9 and specifically highlights NF.[110] The authors note that this will need to be accompanied by advancements in cell culture modeling, particularly the conversion of primary human Schwann cells to MPNST cells. Stem cells may be of utility, as reflected by recent efforts to create induced pluripotent stem cell-derived neurons and neural organoids with specific NF1 patient mutations.[70] For *NF2*-related schwannomatosis, CRISPR-Cas9 editing has been used to model NF2 loss in meningioma cells, and these cells were subsequently used as a model for therapeutic drug testing.[71] CRISPR-Cas9 editing has also been used in the context of creating rat[111] and porcine[72] models of NF1 disruption and will likely streamline the creation of mice with specific patient mutations.[73]

In conclusion, the last decade has brought remarkable advancements in gene therapy, particularly through CRISPR and improved vector delivery systems. These technologies are already being applied to conditions such as liver and hematopoietic disorders, where even low levels of gene replacement can restore function. These disorders were always the most accessible starting point for gene therapy, representing "low-hanging fruit." Addressing more complex disorders, such as NF1- and NF2-related schwannomatosis, presents greater challenges. These conditions involve tumor suppressor genes, which pose unique technical and biological hurdles. Nevertheless, ongoing innovations in gene editing and vector technologies, coupled with insights from clinical trials, are steadily expanding the possibilities for treating these conditions. NF1- and NF2-related Schwannomatosis differ in their underlying disease mechanisms while also presenting with a wide range of mutations. These differences may necessitate tailored therapeutic strategies. It is likely that a combination of approaches, each targeting specific mutations, will ultimately be required. As research progresses, these combined strategies could advance into clinical trials and, eventually, into practical treatments.

ACKNOWLEDGMENTS

A.S., G.B., and S.L.G. currently oversee an NF gene therapy research program that receives philanthropic funding from the Flicker of Hope Foundation, Australia. The researchers currently receive NF research funding from the Children's Tumor Foundation, USA (A.S., S.L.G., and Y.B.); the National Health and Medical Research Council, Australia (2027987; A.S., G.B., and S.L.G.); and the Gilbert Family Foundation, USA (521005; S.L.G.).

AUTHOR CONTRIBUTIONS

All authors contributed to writing (original draft) and writing (review & editing) of this manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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