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CNS gene therapy: present developments and emerging trends accelerating industry-academia pathways

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ABSTRACT

The recent success of first central nervous system gene therapies has reinvigorated the growing community of gene therapy researchers and strengthened the field's market position. We are witnessing an increase of clinical trials with long-term efficiency mainly for neurometabolic, neurodegenerative and neurodevelopmental diseases caused by loss-of-function mutations. The ever-expanding knowledge and accessibility to the most advanced tools allow enrichment of applications to more complex diseases. This gradually contributes towards sealing the gap between top diseases impacting current global health and those towards which gene therapy development is currently aimed. Here, we highlight innovative therapeutic approaches that have reached the clinics and outline the latest improvements of vector design and targeting. Finally, we address the pressing challenges faced by clinical trials and the direction they are heading.

CURRENT STATUS OF GENE THERAPY

The present level of gene therapy development offers unprecedented opportunities for central nervous system (CNS) diseases. Strategies inspired by several decades of knowledge are mainly focusing on genetic diseases caused by the loss-of-function mutations, where symptom management is often the sole treatment option. Orphan drug and rare paediatric disease fast track designation have contributed to the development of strategies for neurodegenerative, neurometabolic and neurodevelopmental disorders [1]. Though, the application spectrum is being increasingly enriched by more complex disorders, including Alzheimer's disease [2], Parkinson's disease [3], and epilepsy [4]. Academic laboratories have initially been at the forefront of the translational research work, paving the way toward gene therapy products that successfully reached the market [5]. The pioneering gene therapies that were approved in Europe and/or USA include Glybera (alipogene tiparvovec) for lipoprotein lipase deficiency [6], later withdrawn from the market for commercial reasons [7], Strimvelis® (*ex vivo* hematopoietic stem and progenitor cell (HSPC) gene therapy) for adenosine deaminase deficiency-induced severe combined immunodeficiency (ADA-SCID) [8], Zynteglo® for β-thalassemia [9] and Luxturna® (voretigene neparvovec) for inherited retinal dystrophy [10]. For CNS indications, the first gene therapy drugs to receive marketing authorization were Zolgensma® (onasemnogene abeparvovec) for spinal muscular atrophy (SMA) in 2019 [11], and Libmeldy® (*ex vivo* HSPC gene therapy) for metachromatic leukodystrophy (MLD) in 2020 [12].The most recent marketing approval (August 2022) was granted to Upstaza™ (eladocagene exuparvovec) for aromatic L-amino acid decarboxylase (AADC) deficiency. Commercialization of these products and the ever-expanding portfolio of diseases targeted by gene therapy initiated a wave of interest of pharmacological companies. This has been reflected by both Zolgensma® and Libmeldy®, originally developed in academic environment, being later acquired by pharmaceutical companies. In 2020, the global gene therapy market size was valued at $ 2.26 billion, where SMA applications represented 41 % of revenue shares. By 2027, this market is estimated to bridge $ 35 billion globally [13].

CNS GENE THERAPY: CLINICAL TRIALS

The abovementioned success was preceded by valuable lessons learned from the clinical trials conducted over time. For the CNS, early gene therapy trials applied *ex vivo* approach for leukodystrophy diseases [14,15]. Lentivirally (LV) transduced CD34+ haematopoietic stem cells showed therapeutic

benefit in a safe and efficient manner, comparable to the allogenic stem-cell transplantation, formerly the only available treatment choice. Subsequently, LV-based *in vivo* strategies emerged, including dopamine replacement drug Prosavin-LV, for Parkinson's disease (PD), developed by Oxford Biomedica. Prosavin-LV, directed on symptom management, achieved moderate improvements in motor behaviour at 6 and 12 months, lasting for up to four years in most patients [16]. The time between 2000 and 2010 was marked by the influx of adeno-associated vectors (AAV) based approaches with AAV2-GAD and AAV2-neurturin for PD, AAV2-ASPA for Canavan disease, and AAV2/5-NAGLU for Sanfilippo type B syndrome (MPSIIIB) [17].

The recent years have offered growing market opportunities for CNS gene therapy, with an escalating launch of new clinical-stage biotech companies. Presently, rare disorders targeted by AAV are the predominant pipeline runners and would also be the central focus in the following sections.

The gradually occurring shift of gene therapy interest by industry and young biotech firms, though often stemming from academic ground, may bring new solutions to issues that were not previously tackled.

Neurometabolic diseases

Lysosomal storage disorders (LSDs) are the major focus of current gene therapy pipeline for inherited neurometabolic diseases, including gangliosidoses, mucopolysaccharidoses (MPS) and metachromatic leukodystrophy. With enzymatic deficiency being their cause, this approach takes advantage of the fact that functional enzyme secreted by the transduced cells may be taken up by distal non-transduced cells through cross-correction [18]. This way, therapeutic benefit may be reached by only modifying certain proportion of the CNS cells.

At the moment, extensive efforts are flowing into tackling GM1 and GM2 gangliosidoses. The AXO-AAV-GM1 and AXO-AAV-GM2 of the Sio Gene Therapies Inc. pipeline are targeting GM1 gangliosidoses and Tay-Sachs/Sandhoff disease, respectively. So far, ten patients have been intravenously administered with AAV9-based AXO-AAV-GM1 gene therapy in Phase 1/2 clinical study (NCT03952637), with encouraging risk: benefit outcomes. To reduce immune response to the viral capsid and/or the β-galactosidase protein following IV administration, immunosuppression was given prior to vector delivery, maintained for six months afterwards. The low- and high-dose patient cohorts presented with amended disease biomarkers such as GM1 ganglioside activity in cerebrospinal fluid (CSF) and β-galactosidase activity in the serum. In another Phase 1/2 clinical trial (NCT04669535), four

Tay-Sachs and Sandhoff disease patients received AXO-AAV-GM2 treatment. Two neurotrophic AAV8 vectors delivering HEXA and HEXB genes in 1:1 ratio were co-administered into thalamus and cisterna magna. To our best knowledge, this is the first double vector CNS trial targeting thalamus, to ensure broad diffusion in the CNS. Both transduction of thalamus and diffusion in the CSF would lead to widespread coverage via axonal transport with connected brain structures [19].

Passage Bio Inc., is striving to lead its GM1 gangliosidosis AAV-therapy through Phase 1/2 clinical trial. It employs the AAVhu68 serotype, constructed from the natural isolate carrying the beta-galactosidase (GLB1) gene. Improved spread in the brain is predicted by being administered directly into the cisterna magna. The safety and biomarker data of Imagine-1 trial (NCT04713475), for early infantile, low dose and late infantile, high dose cohorts are expected to be released later this year.

Lysogen is also advancing its pipeline with GM1 gangliosidosis and MPS IIIA therapies. The LYSGM101 candidate is now in the Phase I/II clinical trial (NCT04273269), in which AAVrh10 with *GLB1* gene cDNA is injected at a dose of 2 x 10^{12} vg/mL of CSF into cisterna magna of two early onset and two late onset GM1 child patients [20].

For the MPS IIIA, also known as the Sanfilippo A Syndrome, following on promising safety and efficacy outcomes from Lysogen's MPS IIIA Phase I/II trial [21], the AAVrh-10-based LYS-SAF302 (olenasufligene relduparvovec), carrying the *SGSH* gene cDNA is presently in Phase II/III testing (NCT03612869). Nineteen patients were dosed between February 2019 and March 2020 and improvement or stabilization of neurodevelopmental status in around half of them was confirmed after up to two-year follow-up. The complete results are underway and the company is now in discussion of the next steps [22].

Other MPS conditions are mainly being tackled by Lysogen and Regenxbio. The Regenxbio has a Phase I/II clinical study (NCT03580083) underway, assessing the safety and tolerability of RGX-111. This is an AAV9- α-L-iduronidase (IDUA) gene therapy administered directly into the CNS via intracisternal injection of patients with MPS type I. In the trial for severe MPS II (NCT03566043) the RGX-121 agent with AAV9-based iduronate-2-sulfatase (I2S) expression cassette was administered into the CNS of patients (4 months - 5 years of age). The RGX-121 was well tolerated in all dose cohorts (1.3 x 10^{10}, 6.5 x 10^{10}, 2.0 x 10^{11}), each containing three patients. No drug-related serious adverse events were reported for up to 2 years post-treatment. There was gradual reduction of heparan sulfate

CSF levels, which are increased in MPS II. Normal neurodevelopment was also demonstrated by continuous gain of skills in various areas [23].

There is s continuous development and clinical testing for different types of Batten disease, also regarded as neuronal ceroid lipofuscinoses (CLNs), on both academic and industrials grounds [24].

A bold approach was adapted by Sondhi et al., where CLN2 gene was intraparenchymally delivered by AAVrh.10h to treat late infantile Batten disease in paediatric patients (NCT01161576). There was a 1.3 - 2.6-fold increase of CLN gene product (TPP1) in cerebrospinal fluid post-therapy. Up to 47.5 % lowering of decline rate of motor and language function was recorded, compared to the European natural history cohort. Four out of seven children also showed reduced grey matter loss, detected by magnetic resonance imaging (MRI). However, this strategy did not outperform the conventional recombinant TPP1 therapy. With a more optimized vector design and possibly multiple sites of administration, gene therapy could present a one-and-done solution, as recombinant TPP1 therapy is currently required bi-weekly [25].

At the industry level, Amicus Therapeutics released encouraging data with its PhaseI/II AAV9-based drug AT-GTX-502 (NCT03770572) for CLN3 Batten disease (17th Annual WORLDSymposium™ 2021). The intrathecally-administered therapy was safe and well tolerated in children patients for up to 15 months post-surgery, with early indications of disease stabilization. This program was advanced following discontinuation of the CLN6 Batten disease Phase I/II trial. The intrathecally-delivered AAV9 therapeutic AT-GTX-501 (NCT04273243) showed disease stabilization at early timepoint of the trial, which was not sustained at the 24-months mark.

Neurogene has freshly initiated its PhaseI/II trial for CLN5 Batten disease (NCT05228145) in which AAV9 therapeutic NGN-101 is administered via both intravitreal (IVT) and intracerebroventricular (ICV) injection. This is the first trial to investigate treatment efficacy on both ocular and neurodegenerative disease aspects.

Neurodegenerative diseases

Gene therapy approaches for neurodegenerative diseases have witnessed their own evolution over time. For the PD, the treatment was initially relying on AAV vectors, focused on enhanced conversion of orally-taken levodopa into dopamine. This was achieved by delivering the Aromatic L-Amino Acid Decarboxylase (AADC) gene to express the AADC enzyme that facilitates this conversion. Such

treatment targeted to brain putamen was shown to be well tolerated, while restoring AADC expression in PD patients [26,27].

Lately, a clinical trial (NCT01973543) with AAV2-VY-AADC agent developed by Voyager Therapeutics, exhibited stable or improved motor function in the three-year follow-up in patients with moderately advanced PD [28]. Here, the treatment was administered via intraoperative magnetic resonance imaging (iMRI) guidance, allowing visualization of the virus spread and thus efficient target coverage. Combined with the convection enhanced delivery (CED), this trial instigated the new era of intraparenchymal virus delivery [29].

AADC deficiency disease itself also benefited from the AADC gene delivery. Promising outcomes from the earlier studies prompted AADC utilization to compensate for its loss-of function. In the clinical studies (NCT01395641 NCT02926066), the intraputaminal AAV2-hAADC- based eladocagene exuparvovec demonstrated durable safety profile, with notable motor and cognitive improvements persisting during the >5 years follow-up [30]. Built on this success, the newly approved AADC drug Upstaza™ by PTC Therapeutics, Inc., is the first gene therapy on the market directly administered into the brain, available for paediatric patients over 18 months old.

Taysha Gene Therapies is moving forward with two programs for giant axonal neuropathy (GAN) and Rett syndrome. The AAV9-based TSHA-120 candidate is currently in a Phase I study (NCT02362438) to treat GAN, conducted by National Institute of Health (NIH). This program is the first to intrathecally (IT) dose a gene therapy in clinical setting.

To target peripheral and autonomic CNS manifestations, Taysha is currently investigating drug delivery via the vagus nerve. In its study, GAN rats were administered AAV9/GAN via IT or IT plus vagus nerve injection [31]. Twenty months post injection, IT plus vagus nerve AAV9/GAN was found to be more efficient than IT alone, based on the heart rate, blood pressure and respirations measurements comparable to the wild-type (WT) rats. Nerve fibre loss in dorsal columns of the spinal cord was shown to be prevented to greater extent than IT route only. These results were in agreement with subsequent study in dogs, where direct vagus nerve delivery of AAV9 CBh-GFP mediated robust transduction of neurons critical for autonomic nervous system function. Also, no sign of neuroinflammation or significant chronic inflammatory infiltrates were detected, supporting high safety profile of this approach. Assessment of the possibility of AAV9 re-dosing via vagus nerve is presently underway.

The company Passage Bio Inc. partnered with the University of Pennsylvania's Gene Therapy program to run Phase 1/2 upliFT-D trial for Frontotemporal dementia (NCT04747431) and GALax-C trial for Krabbe disease (Globoid cell leukodystrophy) (NCT04771416). The Cohort 1 interim safety and biomarker data of the latter should be available by the end of the year.

Finally, disease-modifying therapy termed AMT-130 for Huntington's disease has lately seen encouraging progress amid the uniQure's update on the ongoing U.S. Phase I/II clinical trial (NCT04120493). Following direct delivery of rAAV5-miHTT into the brain striatum, 53.8 % mean decrease of mutant Huntingtin was recorded in low dose-treated patients 12 months post-surgery. At this time point, the nerofilament light chain (NfL), a neuronal damage biomarker, also reached close to baseline levels. Successively, the AMT-130 European cohort PhaseI/II trial (NCT05243017) is currently enrolling new patients to follow up on the demonstrated safety in the previous trial.

Most recently, AskbBio received a green light for Phase I/II trial with an AAV-based BV-101 drug, directly administered to the brain of early-stage HD patients [32]. Unlike other strategies for HD, it is designed to restore cholesterol pathway in affected neurons by delivering CYP46A1, which shows lower expression in HD patients [33]. This should allegedly lead to neuroprotection and improved mutant Huntingtin clearance and physical performance. The trial will begin in the last quarter of 2022. Interestingly, CYP46A1 was previously implicated in Phase I trial (NCT03706885), where it was pharmacologically stimulated in AD patients, with results underway.

Although there is a dynamic clinical assessment of the mentioned diseases, the CNS gene therapy field has also observed halting of several other trials.

Voyager Therapeutics recently announced moving its (mi)RNA HTT candidate VY-HTT01 for Huntington's disease (HD) treatment into the clinics in the Phase I trial (NCT04885114). However, the study of this AAV1-base intraparenchymal drug was withdrawn before patient enrolment in the summer of 2021.

Interestingly, in March 2021, Phase III study (NCT03842969) of ASO drug tominersen, conducted by Roche was also discontinued, as no clinical benefit was achieved compared to placebo. At frequent doses, tominersen even resulted in worsened condition. In the same month, Wave Life Sciences also discontinued Phase I/II trial of its two ASOs for HD (NCT04617847 and NCT04617860), due to lack of efficacy.

Also, the Phase I/II trial for GM2 gangliosidosis with AAV9-TSHA-101 candidate conducted by Taysha Therapeutics has been suspended while regulatory information is being required. These results have revealed safety concerns and technological bottlenecks that will have to be acted upon for successful clinical outcomes.

ONGOING DEVELOPMENTS

As gene therapy treatment becomes available for more and more patients, there is a pressing urge to identify novel vector variants for targeted gene delivery, optimize manufacturing process at large scale, address delivery method efficiency and evade immune responses.

AAV variants to improve transduction

At present, majority of AAV capsids utilized in the clinics are in most cases natural serotypes [34]. These AAV serotypes vary in their capsid protein sequences which affects their ability to transduce specific organs or cell types. Clinical data indicate that one of the limiting factors remains weak *in vivo* transduction or sub-optimal cell-type specific targeting [35]. In recent years, novel viral vector variant generation, primarily to improve organ targeting, has been observed at high rate. The custom-designed capsids hold the promise of greatly improving delivery efficiency, which would allow administration of lower virus dose. This could help reduce side effects, that appear to be dose-dependent [36]. Moreover, batches accounted for more doses could be manufactured, thus treating larger patient cohorts more economically. Availability of such capsids would positively impact patient eligibility, safety and efficacy of the treatment.

Rational design and directed evolution have originally been at the forefront of novel capsid discovery. The rational design harnesses prior knowledge about AAV biology and structure, to generate capsid variants with desired properties by systematic assessment and refinement. The new variants are engineered via genetic mutation of capsid residues, insertion of non-viral parts or chemical modifications [37]. In directed evolution, processes such as capsid shuffling of known serotypes, peptide insertion or error-prone PCR are employed to produce highly diverse capsid libraries. Most potent functional variants are recovered following multi-round selection process [38]. Today, the state-of-art AAV capsid design is the focus of several laboratories and biotech start-ups. Machine learning complemented by high throughput measurement and characterization methods are progressively becoming the new standard [3940]. Here, automatic learning is facilitated by a collection of advanced

algorithms. The input data are readily used to predict possible outcomes of complex processes such as new AAV capsid design, based on the learned and integrated rules. The accuracy of the outcomes is in proportion to the amount of the input datasets. On top of this, integration of biological knowledge would produce robust results with smaller data size, considering the sequence-to-function correlation. Altogether, the typical outcome would deliver possible new capsids with their predicted function and efficiency [35].

These applications drove, for example, the formation of Dyno Therapeutics, for the discovery and optimization of AAV vectors through artificial intelligence. The company has entered CNS gene therapy space through collaboration with Roche. Dyno employs its CapsidMap™ platform, employing machine learning combined with experimental data, for next-generation AAV vector development. *In vivo* delivery properties of new synthetic AAV capsids are measured in high throughput, harnessing the synthesis of DNA library and next-generation DNA sequencing.

In the novel capsid identification, Voyager is advancing its RNA-driven TRACER (Tropism Redirection of AAV by Cell-type-specific Expression of RNA) platform. Cell-specific RNA expression is harnessed for capsid libraries, as it might pose a more realistic and reliable assessment of functional transduction than DNA-based screening. The technology is applied on AAV5 serotype, as there is low occurrence of pre-existing neutralizing antibodies in general population, which are the eligibility determinant for patients in clinical trials. The newly identified variant, VCAP-100 has outperformed the conventionally used AAV5 in brain transduction in rats and NHPs with 40-fold and 60-fold, respectively [41]. Upon intravenous administration, (5×10^{13} viral genomes per kg), in cynomolgus monkeys, 20-fold greater brain transduction and 5-fold greater spinal cord transduction was recorded, compared to the AAV9. Both neuronal and glial cells were potently transduced across the whole brain region, but mainly in the thalamus, hippocampus, caudate, putamen, cerebellar cortex and deep cerebellar nuclei, suggesting applicability of VCAP-100 in various CNS diseases.

Affinia Therapeutics and Taysha Gene Therapies are pursuing similar strategies. Harnessing the AAV evolutionary path, novel AAV capsid libraries are devised by advanced computational algorithms termed ancestral sequence reconstruction, or ASR [42]. It enables characterization of variants with enhanced properties, by reconstructing ancestral AAVs to the known natural capsids. The newly designed capsids are then manufactured and individually evaluated in experiments by the use of specific barcodes.

Employing this workflow, a highly efficient gene therapy vector, Anc80, has been previously identified in the academic setting, from the AAV 1,2, 8 and 9 ancestry line. Initially showing robust targeting of muscle and liver, the synthetic Anc80L65 sub-variant was shown to be especially potent in mouse retina, following the sub retinal delivery [43]. Encouraging outcomes were replicated in the same study in Rhesus macaques, proposing the vector for further clinical use in eye retina.

In the murine brain, the Anc80L65 was characterized by Hudrey et al., where it reached transduction efficiency of neurons and astrocytes comparable to the conventional AAV9 after intravenous and intraparenchymal delivery [44]. Via the intraceberoventricular route, Anc80L65 reached broader diffusion than AAV9, with expression extending to the cerebellum. This vector might be of particular interest for application to certain neurologic diseases, including mucopolysaccharidosis type IIIA [45], Batten disease [46] or metachromatic leukodystrophy (MLD) [47] for its strong tropism for ependymal cells and choroid plexus. Indeed, the Anc80L65 capsid used for MLD therapy is currently in preclinical development at Affinia. Anc80L65 was also shown to have superior expression and targeting properties over AAV9 in CNS in adult cynomolgus monkeys following the lumbar puncture injection and cisternal magna injection. Furthermore, four-fold increase in the yield of this candidate carrying the ARSA gene was reached in collaboration with Lonza. Through a multi-year, non-exclusive contract, Lonza provides development and manufacturing services of Affinia's lead candidates.

Improving transgene expression: promoters

Apart from the lawful ownership for the company, new promoters designed *in silico* are being extensively considered to direct enhanced gene expression and cell-type specificity. There is an urgent need for such promoters, as limited treatment efficiency with low transgene expression and toxicity are still being observed due to unspecific transduction. Ubiquitous promoters are actually implemented in 67% of clinical trials for CNS disorders, with CMV and CAG promoters being the most frequent [34]. These two promoters are also the principal choice in clinical trials overall. This might pose an issue in the long-term as it has been established that CMV enhancer, present in both CMV and CAG is often gradually silenced both *in vitro* and *in vivo*, due to CpG dinucleotide methylation [48,49].

In July 2021, Affinia has partnered with the Institute of Molecular and Clinical Ophthalmology Basel (IOB) to tackle efficient gene expression, by identifying new rationally-designed next-generation promoters.

Transgene clearance is another concern observed with robust synthetic promoters. It usually occurs due to cellular stress caused by transgene overexpression and thus imbalance in proper expression of other genes. Remarkably, the CMV and CAG promoters were outperformed by mouse PGK and hSYN within the AAV1 construct in brain and spinal cord of the *in vivo* models, though their usage has not yet been translated into the clinical setting [50].

Cell type-specificity: miRNA detargeting strategy

To induce optimal transgene expression, Taysha therapeutics introduces a miRNA target component in its TSHA-102 candidate for treatment of Rett syndrome, presently in preclinical testing[51]. This allows controlled expression of the MECP2 transgene, which has previously shown dose-dependent toxicity. The system comprises AAV9-miniMECP2-miRARE vector, harnessing the miR-Responsive Autoregulatory Element (miRARE), for miRNA targeting. It serves to minimize possible overexpression of exogenous miniMECP2 in transduced cells by using CNS-relevant miRNAs, whose expression rises in correlation with MECP2. Therefore, overexpression of the transgene would increase expression of miRNA whose non-coding targets are comprised in the 3' untranslated region of the transgene transcript. Following the binding of these miRNAs in the exogenous MECP2 mRNA, its expression is conditionally downregulated via endogenous RNAi machinery, creating a negative feedback loop.

Preclinical efficacy of TSHA-102 was demonstrated in the knock-out (KO) mouse dose escalation study by intrathecal (IT) delivery. Here, over 50 % life extension of KO mice was observed following the maximum dose at P28 (8.8×10^{11} vg/mouse; human equivalent dose 2.86×10^{15} vg). At earlier administration points of P7 and P14, lifespan was extended with 10-fold lower dose. The apnoea frequency was reduced by over 50 % in the maximum dose KO group, while earlier administration points resulted in lowered apnoea frequency with 10-fold lower dose [52]. This is a significant translational factor, as the respiratory health of Rett syndrome patients is often heavily compromised [53].

CHALLENGES FOR CLINICAL TRIALS

As highlighted clinical trials for gene replacement therapies are beginning to produce a pipeline from identification of genetic cause through testing, manufacturing and delivery. The success of these trials has generated strategies around dosing, delivery and study design, although concerns remain – particularly about the permanent nature of many of the treatments [54]. The rapid growth of gene therapies

and the fast-increasing populations of patients that could benefit, means the one of the biggest challenges may become simply obtaining clinical grade gene therapy products to bring to trials, a growing (and frustrating) barrier to new studies (**Figure 1**) [55]. This is combined with the challenges of increase in scale as the number and range of treatments begins to grow exponentially (reviewed for AAV in [56]). However, there are potentially valuable lessons from the COVID vaccine manufacture, which may be translatable to large scale GMP manufacture of other gene and cell therapy treatments [57]. Even with potential improvements, the costs of development and treatment remain a concern, with one estimate that by 2034, 1.09 million patients will be treated by gene therapy with a total cost of $306 billion [58].

The real challenge

On a positive note, emerging manufacturing shortages and regulatory delays are symptoms of success in gene replacement therapies, which has offered hope to thousands of patients. However, this success has also introduced an understandable bias in the field of gene therapy for neurological disorders. As successes in delivering gene therapy treatments to rare genetic diseases stack up, more research groups and industrial partners have joined the field.

But is this approach at risk of diminishing returns, as more companies and researchers chase increasingly rare diseases? Is there a more strategic way to capture the promise of gene therapy for improving global health and well-being?

An uncomfortable truth for researchers in gene therapy is that these treatments are expensive, and may not be fairly available to all patients [59]. One issue is that the focus on rare diseases means that currently the expense of R&D for many rare disease gene therapies areas orphan treatments, which are subject to higher costs per patient [60].

Researchers interested in developing expensive new treatments may wish to focus on those with the largest impact on global health, and this may require shifting away from more gene replacement therapies for rare genetic disorders to industrial partners, and refocussing high risk research funding on diseases with less clear gene therapy avenues.

The Parkinson's field has led this effort, with mixed results (reviewed above). However, compared to industrial efforts, fundamental research is more robust to high risk approaches, and new approaches to treating Parkinson's continue. Forays into Alzheimer's Disease have also begun, in spite of enormous

challenges around identifying the mechanism of this common disease [61]. Indeed, one treatment focusses on the first identified risk factor APOE4 homozygosity, by supplementing with the protective APOE2 variant (NCT03634007). Thus, in spite of the lack of clarity around how APOE variants increase risk of the disease, there is a potential 'gene supplementation therapy' for the approach.

Taking on the big challenge

One possible way forward may be a re-alignment of fundamental gene therapy research in neurology to refocus on the global burden of diseases. The global impact of different neurological diseases is systematically reviewed in the Global Burden of Diseases Study [62]. A concern is the mismatch between the top diseases impacting global health and those towards which gene therapy development is currently aimed. Globally, stroke and migraine are the leading cause of age-standardised DALY rates, but currently there are no clinical trials for genetic therapies for either of these disorders. We must descend to the third cause of DALYs, Alzheimer's and other Dementias, to reach the first possible hope for a gene therapy treatment, which is receiving increasing interest [2]. For epilepsy (5th) there is a single trial in the US ClinicalTrials database. Parkinson's is 11th, and 'Other neurological disorders' for which so many gene therapy trials are targeted, comes in at the 12th even as a total.

Stroke is an acute change in blood flow, but current treatment have recently extended the window for treatment from 4.5 to up to 24 hours [63] meaning that some genetic treatments, may be effective if delivered soon enough. What microRNA, siRNA or other targets may be possible to protect neurons? Migraine presents a different set of problems, here the challenge is less about the speed of intervention, and more about the route of delivery – are there non-invasive ways of delivering treatments that could lead to long term reduction in migraine severity? Treatments for migraine are rapidly changing with the introduction of novel monoclonal antibiotics, and there is potential for gene delivery [64] if research is guided in this direction.

There are a growing number of research teams with hard-earned expertise in design and delivery of gene and genetic therapies, but they have traditionally mainly emerged from studies of rare genetic diseases where their expertise lies and the therapeutic approach is more straightforward. Collaborations bringing this gene therapy expertise with groups leading in mechanisms of complex diseases as stroke and migraine could open the doors for gene therapy to address leading global burdens of disease – if manufacturing can keep up.

Conclusion

The recent months have witnessed significant clinical efforts in rare CNS disease treatment, both academia and industry-driven. There are still outstanding challenges, such as up-scaling the vector production and downstream processing, to be most likely tackled by the industry sector. However, we have endorsed substantial recession in biotechnology companies' investments following the clinical trial underperformance of several therapeutics accompanied by the public market downturn. Although all drug research areas have been touched by this downfall, publicly traded gene therapy sector seemed to be especially susceptible, reflected in extremely decreased and volatile companies' shares. The current financial situation is clearly pushing companies into tough capital conservation, leading to prioritisation of only highly promising activities further down their pipeline, ideally, with lower competitive dynamics. This may have notable future implications, like facing decelerating development process, as many research programs haven't yet reached the clinic and might require several more years to prove their strategies efficient, provided that they will have enough financial means to do so. Despite this, new gene therapy approvals still emerged, maintaining the momentum, crucial for accelerating more therapies through clinical trials to help the patients suffering from these incurable diseases.

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FIGURE LEGEND

Figure 1. The route towards a gene therapy for a complex disease.

The diseases that impact a major fraction of the general population are complex, so their aetiology is a combination of multiple and diverse genetic and environmental factors. The symptomatology affects different aspects of the nervous system physiology, which requires a careful selection of disease models to study and dissect the pathophysiology of the disease. The elements affected will range from the microscopic to the organic level and safety concerns must be taken into account when selecting what to target. Furthermore, the therapeutic approach will depend in whether treating the most pressing symptomatology or restoring low/high genetic expression to rescue part of the homeostasis. Depending on the therapeutic approach, the most convenient delivery route will also need to be tested. Reached this point, the testing through clinical trials of our gene therapy will be necessarily subjected to a close assessment of reliable biomarkers. The selection of biomarkers will be crucial to be able to assess the effectiveness of a gene therapy among an heterogeneous patient cohort in the most objective way possible.

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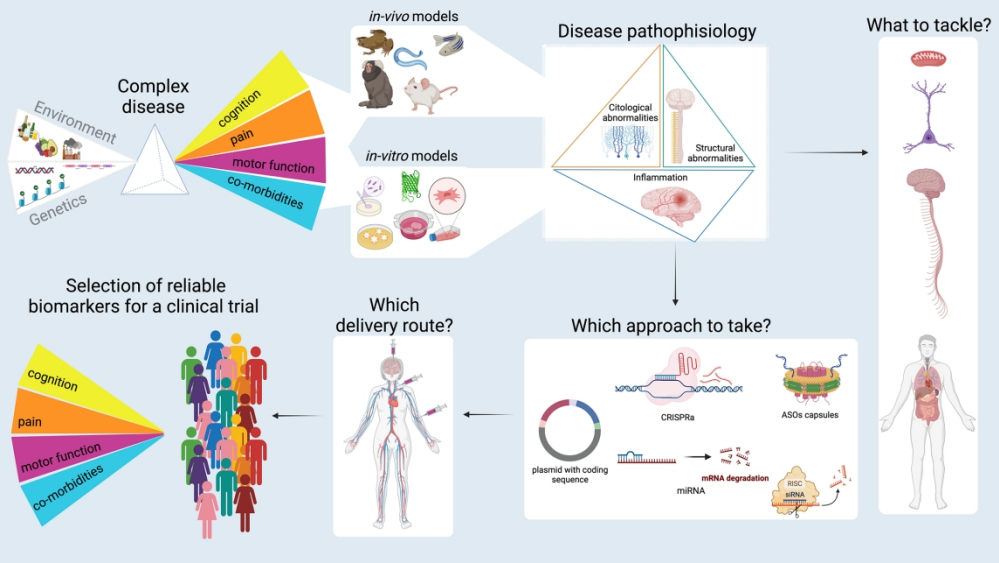
Complex disease
Environment
Genetics
cognition
pain
motor function
co-morbidities
in-vivo models
in-vitro models
Disease pathophisiology
Citological abnormalities
Structural abnormalities
Inflammation
What to tackle?
Which approach to take?
CRISPRa
ASOs capsules
plasmid with coding sequence
miRNA
mRNA degradation
RISC
siRNA
Which delivery route?
Selection of reliable biomarkers for a clinical trial
cognition
pain
motor function
co-morbidities

Figure 1