

Non-human primate models and multimodal technologies synergistically drive translational breakthroughs in gene therapy for optic nerve diseases

Jian Wu^{1,*}

¹Henan Academy of Innovations in Medical Science, Zhengzhou Aviation Port Economic Zone, No. 1, Bio-Technology Street, Henan, 450008 China

*Author for correspondence:
Email: karenawu@foxmail.com

Received date: May 21, 2024
Accepted date: June 25, 2025

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Abstract

This commentary systematically examines the pivotal role of non-human primate (NHP) models in advancing gene therapy for optic nerve diseases, alongside the ethical and practical challenges they face. It highlights how synergistic innovations in artificial intelligence (AI), organoid technology, CRISPR-based gene editing, and novel delivery systems are reshaping translational paradigms. NHPs, with their anatomical and functional parallels to human visual systems, serve as a gold-standard models for evaluating therapeutic safety, delivery optimization, and long-term efficacy, enabling breakthroughs in treating conditions such as Leber congenital amaurosis (LCA) and non-arteritic anterior ischemic optic neuropathy (NAION). Despite ethical controversies, prolonged experimental timelines, and interspecies variability, AI-driven virtual screening, physiologically relevant organoid models, and non-viral nanocarriers are reducing reliance on NHPs while enhancing therapeutic precision. The review proposes future directions focusing on adeno-associated virus (AAV) vector refinement, multimodal data integration, and interdisciplinary collaboration to accelerate the translation of neuroregenerative therapies for irreversible vision loss.

Keywords: NHPs, Optic neuropathies, Gene therapy, AAV, Retinal organoids

Introduction

Clinical demand for optic nerve therapies and the translational value of NHPs

Gene therapy for optic nerve disorders has made remarkable strides in recent years, with NHPs have emerged as a critical translational bridge in preclinical research. Owing to their striking anatomical, physiological, and pathological similarities to humans, NHPs have become an indispensable model for studying disease mechanisms and therapeutic interventions [1,2]. The advantages of NHP models in optic nerve research are multifaceted. Their neuroanatomical and functional parallels to human vision systems enhance the clinical relevance of experimental outcomes [3]; they provide a robust platform for evaluating the safety and efficacy of novel gene therapies, with studies demonstrating promising neuroprotective effects and axonal regeneration potential in optic nerve disorders [4,5]; and they enable investigations into the genetic underpinnings of these diseases and the durability of therapeutic outcomes, which are crucial for developing personalized treatments tailored to specific genetic profiles [6]. Despite this potential, challenges persist. The long-term safety and sustained efficacy of gene therapies require validation through large-scale clinical trials [7].

NHP models, while invaluable for biomedical research due to their evolutionary and physiological proximity to humans, face substantial practical challenges. First, their high maintenance costs and prolonged reproductive cycles (3 - 5 years to sexual maturity) significantly extend experimental timelines [8,9]. Second, ethical concerns regarding primate cognitive capacities, exemplified by stringent regulations such as the EU Directive 2010/63/EU, have led to stricter oversight, resulting in a global decline in NHP use [10]. Notably, the convergence of emerging technologies is reshaping preclinical research paradigms. AI platforms, such as DeepMind's AlphaFold, enable high-fidelity simulations of drug-target interactions, improving traditional screening efficiency [11]. Cai *et al.* [12] first proposed a vascular network-inspired diffusible scaffold and validated its role in enhancing organoid physiological functions, reducing necrosis, and optimizing pharmacological responses. This innovation addresses critical challenges in organoid culture by improving nutrient diffusion and structural integrity, thereby providing a more reliable model for studying brain disorders and advancing drug discovery. The scaffold's success in recapitulating neuropathological features demonstrates exceptional translational potential. Future applications could extend this technology to developing organoids of other vital organs, accelerating breakthroughs in regenerative medicine and precision disease modeling. Meanwhile, CRISPR-Cas9-derived base-editing tools (e.g., Rad51DBD fusion) have demonstrated a significant enhancement in base-editing efficiency in zebrafish models [13]. These findings suggest potential for analogous breakthroughs in NHPs, offering promising avenues for refining gene-editing applications in complex mammalian systems. Collectively, these advancements underscore a paradigm shift: AI-driven virtual screening demonstrates potential to substantially reduce NHP utilization through enhanced target prediction accuracy and minimized experimental iterations; organoid-on-chip systems enable *in vitro* neurotoxicity testing, slashing evaluation periods [14]. Crucially, evolving ethical frameworks, such as the revised NIH Animal Welfare Guidelines, promote the 3R principles, ensuring alignment between technological progress and ethical standards.

Core role of NHPs in optic nerve gene therapy

NAION, as a prevalent ischemic optic neuropathy, is pathologically characterized by apoptosis of retinal ganglion cells resulting from microcirculatory dysfunction, leading to irreversible vision loss. While rodent models relying solely on optic nerve compression or ischemia-reperfusion injury fail to replicate human-specific pathological hallmarks such as ischemic disc edema and axonal transport deficits, NHP models employing laser-induced choroidal ischemia combined with AAV2-OSK factor delivery have successfully demonstrated, for the first time in primates, the therapeutic potential of epigenetic reprogramming [15]. Notably, the first FDA-approved ocular gene therapy, Luxturna®, derived its pivotal safety profile exclusively from NHP studies. Over a 2-year follow-up, subretinal AAV2-hRPE65v2 administration in macaques showed no retinal inflammation, vector integration-associated mutations, or intraocular pressure abnormalities, demonstrating long-term biosafety [16]. These findings directly informed the FDA's approval decision, underscoring the indispensable role of NHP models in advancing therapies for inherited optic neuropathies.

AAV has emerged as the cornerstone vector for ocular gene therapy due to its favorable safety profile, sustained transgene expression, and inherent retinal tropism. However, multiple

challenges persist in clinical translation, spanning immune responses, suboptimal transduction efficiency, limitations in capsid engineering for cell-type specificity, route-of-administration optimization, and scalable manufacturing bottlenecks [17]. Therefore, comprehensive optimization of AAV vectors—through rational engineering of capsid proteins [18,19], promoter selection [20], and delivery strategies [21,22]—is critical to enhance transduction efficiency and address current limitations in gene therapy applications.

Tissue tropism of AAV vectors is a pivotal determinant in gene therapy efficacy. In rodent models, AAV2 has been widely utilized for its high-efficiency transduction of retinal pigment epithelial (RPE) cells [23]. However, its targeting specificity for retinal ganglion cells (RGCs) in NHPs remains suboptimal [24]. A 2024 study by Wei Wenbin's team employed subretinal delivery of the AAV2/8 hybrid vector (rAAV2/8-hCYP4V2) in crab-eating macaques, which demonstrated sustained transgene expression in primate retinal tissues over a follow-up period of 365 days [25]. This NHP-guided serotype optimization establishes a strategic roadmap for refining delivery systems in RGC-targeted therapies for human ocular disorders such as glaucoma and LHON.

Synergistic Advances in Emerging Technologies

Non-viral delivery innovations

Mutations in the *MYOC* gene leading to abnormal aggregation of myocilin protein represent a key pathological mechanism underlying trabecular meshwork fibrosis. Mutant myocilin disrupts extracellular matrix metabolic homeostasis, activating TGF- β and Rho kinase signaling pathways, which ultimately elevates aqueous humor outflow resistance and intraocular pressure [26]. In glaucoma-targeted gene therapy, conventional AAV vectors exhibit suboptimal transduction efficiency in trabecular meshwork cells [17]. Tong *et al.* demonstrated enhanced brain-targeted siRNA delivery using Angiopep-2-modified lipid nanoparticles (C2 LNPs), extending survival in glioblastoma murine models [27]. In parallel, a reactive oxygen species-scavenging lipid-based nanocarrier (cLpT@siRNA) was developed to accelerate wound healing in diabetic mouse models [28]. Lou *et al.* reported intraocular pressure reduction and attenuated retinal ganglion cell degeneration following polydopamine nanoparticle administration in optic nerve injury models, with no detectable immunogenicity [29]. These findings collectively establish a non-viral vector-based strategy for primary open-angle glaucoma gene therapy.

AI-driven research paradigms

Gungor *et al.* [30] developed a deep learning diagnostic system using 961 fundus images from 802 confirmed patients. The system demonstrated superior performance in distinguishing between arteritic and NAION, achieving an accuracy rate exceeding 90% in clinical evaluations—significantly outperforming assessments by experienced neuro-ophthalmologists.

The research team [31] established the VisionFM system by integrating a global ophthalmic imaging dataset comprising over 3.4 million multimodal scans from 18 countries, spanning external eye images, anterior segment slit-lamp photographs, fundus photography, optical coherence tomography, fundus fluorescein angiography, ocular B-scan ultrasonography, ultrasound biomicroscopy, and magnetic resonance imaging. This comprehensive platform enables simultaneous execution of ophthalmic disease screening, pathological lesion segmentation, and progression prediction

across multiple ocular conditions. Evaluation results demonstrated VisionFM's high diagnostic accuracy in detecting 10 prevalent eye diseases, including glaucoma, through its unified multimodal analytical framework. Zhu *et al.* [32] constructed an AI model named "KeystoneFold" to predict the structural conformations of Trk and NT proteins. Through molecular dynamics modeling, the study revealed that several known TrkB agonists could dock onto the extracellular domain of TrkB, with 7,8-dihydroxyflavone (DHF) demonstrating the highest predicted binding affinity. Subsequent *in vivo* experiments validated DHF's neuroprotective efficacy. Those research highlights a novel AI-driven paradigm for identifying drug targets and streamlining drug discovery and evaluation processes, offering valuable insights for accelerating therapeutic development timelines and reducing associated costs. The findings indicate that this AI system could identify microscopic pathological features beyond human visual perception. Such functionality underscores its viability as a diagnostic adjunct for complex optic neuropathies, facilitating not only enhanced diagnostic accuracy but also enabling early and precise subtype differentiation to refine therapeutic intervention windows, ultimately improving patient prognoses.

Organoid and organ-on-chip systems

The formation of retinal organoids relies on the self-renewal and differentiation capacities of pluripotent stem cells. Through specific culture conditions and the addition of growth factors, these cells can be directed to differentiate into retinal progenitor cells, ultimately forming organoids with a layered retinal structure. This model system is used to study various retinal diseases, such as Stargardt disease—a hereditary neurodegenerative disorder caused by biallelic loss-of-function mutations in the *ABCA4* gene, leading to macular degeneration and blindness [33]. Müller *et al.* developed an AAV vector-based adenine base editing strategy to correct the *ABCA4* c.5882G>A mutation in therapeutically relevant cell types. This approach achieved efficient genetic repair in *ex vivo* retinal organoids, as well as *in vivo* mouse and non-human primate models [34], offering new therapeutic potential for inherited retinal disorders. Adult-onset hereditary optic atrophy (ADOA) is a common hereditary optic neuropathy primarily caused by heterozygous *OPA1* gene mutations [35], characterized by the loss of RGCs and degeneration of the optic nerve. Lei *et al.* [36] employed three-dimensional retinal organoids to investigate the impact of *OPA1* mutations on retinal development. The study utilized human induced pluripotent stem cells carrying specific *OPA1* mutations to generate retinal organoids with disease-relevant genetic backgrounds. The findings revealed that the mutation induced abnormal electrophysiological properties in organoid-derived RGCs, suppressed progenitor cell proliferation, and led to mitochondrial dysfunction. This research underscores the potential of retinal organoids for drug testing and screening, highlighting their utility in developing therapeutic agents relevant to ADOA.

Gene editing innovations

In NHP models, researchers developed species-specific delivery vectors tailored to the subjects, successfully achieving *CEP290* gene editing efficiency exceeding the therapeutic threshold of 10%. This pivotal finding not only confirmed the feasibility of CRISPR/Cas9-mediated *in vivo* somatic cell editing in primates but also underscored the unique value of NHP models in translational medicine [37]. Building on these breakthroughs, the world's first CRISPR-based gene editing therapy for LCA10 entered Phase I/II

clinical trials in 2020. Clinical assessments using four key efficacy metrics—Best Corrected Visual Acuity, Full-Field Stimulus Testing, Visual Navigation Challenge, and Visual Function Quality of Life—revealed that 11 participants demonstrated clinically meaningful improvements in at least one outcome measure. Notably, 6 participants showed improvements in two or more outcomes, while 4 exhibited clinically significant gains in BCVA [38]. This landmark trial, representing the first *in vivo* administration of CRISPR therapeutics in humans, highlights its potential to correct genetic defects underlying severe vision impairment. These findings provide promising insights for treating previously intractable diseases and further validate the irreplaceable role of NHP models in evaluating gene editing safety, efficacy, and delivery optimization strategies.

Perspectives

From epigenetic repair in NAION to gene replacement therapy for LCA [39-41], from AAV serotype screening to CRISPR-mediated base editing precision, NHP models remain the living bridge connecting foundational research to clinical translation. Their value has transcended the conventional role of preclinical validation tools, evolving into translational innovation engines that propel therapeutic paradigms for optic neuropathies from symptom management to neuronal regeneration. Despite persistent challenges—ethical constraints, interspecies variability, and technical limitations—each breakthrough in NHP research reshapes the therapeutic landscape for optic nerve disorders. With the convergence of gene editing, AI-driven optimization, and organoid technology, we stand at a historic inflection point in vision restoration. Conditions once deemed irreversible, such as progressive vision loss, may gradually transition into treatable entities under the guidance of NHP models. For 61 million patients worldwide at risk of blindness [42,43], this primate bridge bears not only the hope of scientific progress but also the tangible possibility of restored vision.

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