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Bietti crystalline corneoretinal dystrophy: Advances in understanding and gene therapeutic approaches

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Bietti crystalline dystrophy (BCD), an autosomal recessive inherited retinal disorder caused by mutations in the *CYP4V2* gene, has long remained therapeutically challenging. Recent advances in adeno-associated virus–based gene therapy have emerged as promising therapeutic strategies for patients with BCD. This review synthesizes current knowledge regarding the molecular genetic mechanisms underlying BCD pathogenesis and examines recent developments in diagnostic approaches and gene therapeutic interventions. We specifically analyze the clinical outcomes of three investigational gene therapy products—ZVS101e, NGGT001, and VGR-R01—focusing on their preliminary efficacy, safety profiles, and tolerability. Key parameters evaluated include dosing strategies, routes of administration, adverse event profiles, and improvements in best-corrected visual acuity. The collective evidence suggests these therapeutic candidates show potential for decelerating disease progression and enhancing visual function. Future optimization of these approaches should carefully consider administration sites and modalities, injection volumes, and disease severity at intervention. With gene replacement therapy for BCD advancing through late-stage clinical development, regulatory approval and clinical implementation may be anticipated in the near future.

Bietti crystalline dystrophy (BCD) is a rare, progressive inherited retinal dystrophy characterized by distinctive crystalline deposits in the retinal posterior pole and corneal limbus, accompanied by progressive degeneration of the retinal pigment epithelium (RPE), choroidal capillaries, and photoreceptors [1,2]. First described by Italian ophthalmologist G.B. Bietti in 1937, BCD demonstrates variable prevalence across different populations, with an estimated global incidence of 1 per 57,600 and a notably higher frequency in East Asian populations, particularly in China, where it affects approximately 1 per 25,000 individuals [3,4].

The clinical course of BCD typically initiates between the second and fourth decades of life, manifesting with progressive night blindness (nyctalopia), deterioration of visual acuity, and constriction of visual fields. Disease progression leads to severe visual impairment, significantly impacting patients' quality of life [5]. Despite its devastating effects, therapeutic options for BCD have remained limited, with management strategies primarily focused on symptomatic relief and monitoring of disease progression.

Recent advances in molecular genetics have revolutionized our understanding of BCD pathogenesis, identifying *CYP4V2*, which encodes a human ω-hydroxylase, as the causative gene. This breakthrough has facilitated the development of targeted therapeutic approaches, particularly in the realm of gene therapy. The generation of Cyp4v3 knockout mouse models has provided crucial insights into disease mechanisms, notably revealing the role of ferroptosis in BCD pathophysiology [6]. Furthermore, innovative gene-editing technologies, including CRISPR/Cas9-based homology-independent targeted integration, have opened new therapeutic possibilities [7].

Significant progress in gene delivery platforms has positioned adeno-associated virus (AAV)–mediated gene therapy as a promising therapeutic strategy. Preclinical studies, including collaborative research between the Institute of Zoology, Chinese Academy of Sciences, and Peking Union Medical College Hospital, have demonstrated encouraging results using AAV-mediated *CYP4V2* gene delivery in high-fat-fed Cyp4v3 knockout mice [8]. These advances have paved the way for several clinical-stage gene therapy candidates, marking a new era in BCD treatment development.

METHODS

A systematic literature search was conducted in PubMed, Web of Science, Embase, and ClinicalTrials.gov databases from inception to July 2024. The following search terms were used in combination: ("Bietti crystalline Ddystrophy" OR "Bietti crystalline corne retinal dystrophy" OR "BCD") AND ("gene therapy" OR "genetic therapy" OR "CYP4V2" OR "adeno-associated virus" OR "AAV" OR "gene treatment" OR "genetic treatment"). Additionally, specific gene therapy

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product names ("ZVS101e" OR "NGGT001" OR "VGR-R01") were included in the search.

Articles were limited to those published in English. Original research articles, review articles, case reports, and clinical trials were included. Conference abstracts, letters, and articles without full text were excluded. The reference lists of included articles were manually reviewed to identify additional relevant studies.

Two independent reviewers screened titles and abstracts for relevance, followed by full-text reviews of potentially eligible articles. Any disagreements were resolved through discussion with a third reviewer. The final selection of articles was based on their relevance to BCD's molecular mechanisms, diagnostic approaches, and gene therapeutic strategies.

Molecular genetic mechanisms of BCD: The molecular pathogenesis of BCD is predominantly attributed to mutations in *CYP4V2*, an 11-exon gene encoding a cytochrome P450 family protein integral to fatty acid and steroid hormone metabolism [9]. CYP4V2 protein, predominantly expressed in RPE cells and photoreceptors, catalyzes the hydroxylation of saturated fatty acid C-terminals to dicarboxylic acids, facilitating their entry into mitochondrial β-oxidation and subsequent conversion to ω-3 polyunsaturated fatty acids [10]. Mutations in *CYP4V2* disrupt RPE lipid homeostasis, compromising RPE cell function and consequently impairing photoreceptor outer segment renewal and function, ultimately manifesting as retinopathy [11].

Numerous *CYP4V2* mutations have been documented in various databases, with a substantial portion classified as potentially pathogenic. Comprehensive genetic analyses have revealed diverse mutation patterns across different populations. In a seminal study of 92 unrelated Chinese patients with BCD, Meng et al. [12] identified several key mutations: p. Tyr343Asp missense mutation, p. Gln11X nonsense mutation, splice site alterations, and c.802_810del17insGC insertion-deletion mutation, with the latter being most prevalent. Subsequently, Yin et al. [13] characterized 17 distinct *CYP4V2* mutations, including four predominant variants (c.802-8_810del17bpinsGC, c.802-8_810del17bpinsGT, c.992A>C (p.H331P), and c.1091-2A>G), accounting for 71% of mutant alleles in the Chinese population. This study also identified four novel mutations: c.65T>A (p.L22H), c.681_4delTGAG (p. S227Rfs*1), c.802-8_810del17bpinsGT, and c.965_7delaag (p.321).

Further genetic screening by Jiao et al. [14] in 58 patients with BCD revealed 28 *CYP4V2* mutations, comprising 19 missense mutations (68%), 4 nonsense mutations (14%), 2 deletion mutations (7%), 2 splice-site mutations (7%), and 1 insertion-deletion mutation (4%). A comprehensive analysis by Guo et al. [15] of 234 patients from 173 families at Peking University Third Hospital (2010-2018) demonstrated a 93.1% CYP4V2 mutation rate, identifying eight novel and three known mutations. Notably, c.802-8_810del17bp, c.1091-2A>G, and c.992A>C emerged as mutational hotspots, collectively representing 73.5% of mutations in Chinese Han patients with BCD. A 2021 familial study revealed homozygous c.802-8_810del17insGC mutations in one proband and affected siblings, while another proband exhibited compound heterozygous mutations (c.219T>A (p.F73L) and c.802-8_810del17insGC), both disrupting normal *CYP4V2* gene expression [16]. Table 1 summarizes the common types of CYP4V2 mutations identified in these studies.

The diagnosis of BCD:

Clinical features and diagnostic imaging—BCD presents with a constellation of distinctive clinical manifestations that facilitate diagnosis. These manifestations encompass visual field abnormalities, widespread distribution of crystalline yellow-white intraretinal deposits, variable degrees of RPE atrophy, retinal pigment aggregation, choroidal vascular sclerosis, photoreceptor dysfunction affecting both rod and cone cells, and characteristic punctuation hyperreflectivity [17].

Multiple imaging modalities play crucial roles in diagnosis and disease monitoring. Standard retinal examination and widefield color fundus photography enable visualization of bilateral crystalline deposits concentrated in the posterior pole and mid-peripheral retina. Widefield autofluorescence imaging reveals distinctive patterns of extensive nummular RPE atrophy affecting both macular and peripheral regions. Spectral-domain optical coherence tomography demonstrates bilateral chorioretinal atrophy in the macula and, through selective segmentation analysis, allows precise localization of hyperreflective crystalline deposits within specific retinal layers [18].

Recent technological advances have introduced artificial intelligence–based diagnostic approaches. Zhang et al. [19] have pioneered the application of advanced deep learning architectures, including ResNeXt, Wide ResNet, and ResNeSt, for automated BCD diagnosis using ultra-widefield color fundus photographs. Their study, conducted on a Chinese cohort, demonstrated promising diagnostic accuracy, suggesting potential utility in clinical practice.

Gene detection—In advanced stages of BCD, widespread fundus atrophy can make it challenging to differentiate

Table 1. Gene therapy drugs and main clinical research results in BCD.

Characteristics	ZVS101e	NGGT001	VGR-R01
Basic Information			
Trail number	NCT04722107	NCT06302608	NCT05399069
Vector	rAAV8	rAAV2	rAAV8
CYP4V2 Gene	Wild-type	Codon optimized	Codon optimized
Administration route	Sub-retinal injection	Sub-retinal injection	Sub-retinal injection
Study design			
Number of patients	12	12	15
Dose groups	Single dose: 7.5×10^{10} vg (n=12)	Two doses: 1.5×10^{11} vg (n=6) 3.0×10^{11} vg (n=6)	Three doses: 6.0×10^{10} vg (n=3) 1.2×10^{11} vg (n=6) 2.0×10^{11} vg (n=6)
Follow-up duration	6–12 months	9 months	6–9 months
Efficacy outcomes			
Mean BCVA change[1]	Day 180: 9.0±10.8 Day 365: 11.0±10.6	Day 270: 12.8±3.7	Day 180: Low dose: 13.7±2.2 Medium dose: 21.1±7.5 High dose: 27.4±16.7 Day 270: Low dose: 13.3±1.9 Medium dose: 27.9±16.5
BCVA improvement Rate[2]	Day 180: 77.8% (7/9) Day 365: 80% (4/5) gained letters;40% (2/5) gained >15 letters	46% (5/11) gained >15 letters	75% (9/12) gained >10 letters; 66.7% (8/12) gained >15 letters
Safety profile			
Ocular adverse events[3]	Conjunctival edema Anterior chamber flare Posterior corneal deposits	Conjunctival edema Eyelid edema	Conjunctival edema Elevated IOP Posterior corneal deposits

1. BCVA (best corrected visual acuity) changes are presented as mean ± standard deviation in ETDRS letters. 2. BCVA improvement rates are shown as percentages, with the actual number of patients shown in parentheses (n/N, where n=number of patients showing improvement, n=total number of patients evaluated). 3. Adverse events are listed in order of frequency of occurrence across all treatment groups. Abbreviations: BCD=Bietti crystalline dystrophy; rAAV=recombinant adeno-associated virus; vg=vector genome; IOP=intraocular pressure; ETDRS=early treatment diabetic retinopathy study.

BCD from other serious retinal conditions such as retinitis pigmentosa and choroidopathy. Therefore, genetic testing for double allele mutations becomes essential for accurate diagnosis of patients and their family members, particularly when assessing genetic risks for offspring. Studies have shown that 28.3% of patients initially diagnosed with retinitis pigmentosa or choroidopathy were subsequently identified as having *CYP4V2* mutations and reclassified as having BCD following comprehensive genetic testing for hereditary ophthalmologic conditions [15].

Standard genetic sequencing protocols focus on analyzing exons and their intronic junction regions, with pathogenic mutations and their severity being evaluated through comparison with reference sequences. Various genetic testing methodologies have emerged, including high-throughput sequencing, which can accurately identify multiple hereditary retinal disorders, including BCD, with an accuracy rate exceeding 50% [20]. Targeted capture chip technology offers the advantages of enhanced accuracy, increased sensitivity, and cost-effective implementation [21]. Additionally, whole-exome sequencing has gained

widespread adoption in diagnosing hereditary retinal degeneration, particularly due to its capability to identify novel pathogenic genes in monogenic disorders while maintaining cost efficiency [22].

In cases where only single-allele mutations are identified, further investigation for large fragment deletions becomes necessary, requiring verification through real-time fluorescence quantitative PCR. Currently, approximately 20% to 30% of patients have not undergone genetic testing, highlighting the critical need for accumulating more comprehensive genetic data. This expanded genetic database will facilitate deeper understanding of genotype-phenotype correlations, natural disease progression, and pathogenic mechanisms, as well as help identify optimal timing for diagnostic and therapeutic interventions in BCD [23].

Gene treatment opportunity in BCD: Gene therapy represents a novel therapeutic approach that involves the introduction of exogenous genetic material into cells to ameliorate diseases arising from genetic defects or aberrant gene expression, offering significant therapeutic potential for hereditary disorders [24]. The retina presents unique characteristics that make it particularly amenable to gene therapy interventions. Its nonregenerative nature and sophisticated visual signal transduction system enable single-gene therapy administrations to exert sustained pharmacologic effects, potentially achieving therapeutic outcomes or disease progression delay when sufficient target cells remain viable.

The eye's anatomic and immunologic properties make it an optimal target organ for gene therapy applications. Its relative isolation from systemic circulation and immune-privileged status significantly mitigates risks associated with systemic genotoxicity and adverse immune responses. Furthermore, the selection of appropriate delivery modalities is crucial for optimizing the safety, efficacy, and accessibility of gene therapeutic interventions. While intravitreal administration primarily targets inner retinal cellular populations, subretinal delivery specifically addresses outer retinal cellular components, including RPE cells and photoreceptors, which represent the primary cellular targets in BCD pathology [25].

Historical evolution of gene therapy in hereditary diseases: The conceptual framework for gene therapy as a therapeutic approach for hereditary disorders was first proposed by Friedmann and Roblin [26] in 1972, marking a pivotal moment in molecular medicine. A significant breakthrough emerged in 2008 when Bennicelli and colleagues [27] demonstrated the safety and efficacy of AAV2.RPE65-mediated gene transfer in RPE65-deficient mouse models, establishing a promising preclinical foundation for retinal gene therapy. The field achieved a crucial milestone in 2017 when Russell [28] validated the therapeutic efficacy of gene therapy for RPE65-associated inherited retinal diseases through phase III clinical trials, which proved instrumental in advancing the treatment toward commercialization.

The field witnessed a transformative moment with the approval of LUXTURNA by the US Food and Drug Administration, the first ophthalmic gene therapy product specifically indicated for RPE65 mutation-induced inherited retinal diseases. This regulatory success catalyzed extensive research and development in the field, leading to numerous therapeutic candidates and expanded indications advancing through preclinical and clinical stages [29]. Notably, significant progress has been achieved in developing therapeutic strategies for BCD targeting *CYP4V2*, using both gene replacement and genome editing approaches.

The clinical application of gene therapy in BCD:

Preclinical studies—The foundational groundwork for BCD gene therapy was established in 2014 with Lockhart's development of a Cyp4v3 knockout mouse model, providing a crucial experimental platform for therapeutic investigation [30]. A significant advancement occurred in 2020 when Qu and colleagues [8] demonstrated a successful therapeutic intervention using AAV-mediated *CYP4V2* gene delivery via subretinal administration in a high-fat diet-induced BCD model, resulting in enhanced electroretinogram responses and improved retinal thickness.

Further progress was achieved in 2023 through the comprehensive study by Jia et al. [31], which revealed that AAV2/8-CYP4V2 treatment effectively enhancedinduced pluripotent stem cell (iPSC)-RPE cellular function, mitigated lipid accumulation, and demonstrated sustained improvements in electroretinogram responses and RPE lipid metabolism for up to 15 months, with observed dose-dependent therapeutic effects. A breakthrough study in 2024 by Yang and colleagues [7] explored CRISPR/Cas9-mediated homology-independent targeted integration technology, demonstrating precise and efficient target fragment integration, sustained improvement in retinal histologic and morphologic parameters, and, notably, no significant off-target effects. This research established gene editing as a viable complementary approach to conventional gene replacement therapy for BCD treatment.

Clinical trials—Gene therapy products for BCD are progressively advancing into clinical research phases. China, having the largest BCD patient population globally, has emerged as a leader in gene therapy drug development and clinical research. Currently, three Chinese-developed

products have reached the clinical trial stage worldwide (Table 2).

ZVS101e (rAAV-hCYP4V2), a gene replacement therapy targeting CYP4V2, utilizes a recombinant AAV serotype 8 vector expressing human CYP4V2 protein. This therapeutic approach is potentially applicable to all patients with BCD who have *CYP4V2* mutations. The world's first gene therapy clinical trial for BCD was initiated in 2021 (NCT04722107). In 2024, Wang et al. [32] published their findings in *Signal Transduction and Targeted Therapy*. This single-arm, open-label exploratory trial included 12 subjects (6 males, 6 females, mean age 40 years) who received single-eye subretinal ZVS101e injections. Six subjects completed a 365-day follow-up, while another six completed a 180-day follow-up. The study reported no unexpected serious adverse events (AEs) among 73 treatment-associated AEs (69 mild, 4 moderate). Common systemic AEs included COVID-19 infection (15%), hypercholesterolemia (13.7%), and leukocytosis (12.3%). The most frequent ophthalmic AEs were conjunctival edema (10%), anterior chamber flash (9.7%), and posterior corneal deposition (4.3%). Visual acuity outcomes showed promising results: visual acuity outcomes showed promising results: at 180 days (n = 9), patients could read a mean of 9.0 ± 10.8 additional letters on the vision chart, with improvement in seven subjects; at 365 days (n = 5), patients could read a mean of 11.0 ± 10.6 additional letters on the vision chart, with four subjects showing improvement. Visual improvements were observed as early as day 14 posttreatment, with sustained benefits in electroretinogram, visual field, and quality-of-life scores. Two additional studies of ZVS101e are ongoing: an exploratory study (NCT05714904) and a phase I/II registration study in China (NCT05832684).

VGR-R01's initial exploration trial results (NCT05399069) were reported in 2023 by Shanghai Vitalgen BioPharma Co., Ltd. Three subjects received escalating doses (2.01010 vg, 6.01010 vg, and 1.2×10^{11} vg), showing improvements in multibrightness mobility test scores and best-corrected visual acuity (BCVA), with no serious AEs. In 2024, updated phase I/II trial data (NCT05694598) presented at the 27th Annual Meeting of the American Society for Gene & Cell Therapy showed promising results across 12 patients in three dose groups. After six to nine months, nine subjects showed BCVA improvements ≥10, and eight subjects showed improvements ≥15. The treatment demonstrated a favorable safety profile with only mild to moderate AEs.

NGGT001, comprising rAAV2 and a codon-optimized *CYP4V2* gene sequence, has shown enhanced expression efficiency compared to the wild-type gene [33]. In 2024, researchers from Xiamen University Eye Center and Southwest Hospital reported preliminary results (NCT06302608) from 12 subjects (7 males, 5 females). After nine months, treated eyes showed a mean BCVA improvement of 12.8 ± 3.7, with contralateral eyes improving by 6.6 ± 1.8. The treatment demonstrated good tolerability with only six transient AEs in five subjects. A phase I/II trial (CTR20240103) is currently ongoing in China to further evaluate safety, tolerability, and preliminary efficacy of subretinal NGGT001 administration.

Recent advances in other hereditary retinal diseases:

Preclinical research—Recent breakthroughs in preclinical research have shown promising results for treating hereditary retinal diseases. In a groundbreaking study, Bi and colleagues [34] demonstrated successful gene correction in retinitis pigmentosa mouse models using precision base editing technology. Their approach used adenine base editors to specifically target pathogenic single nucleotide variations in retinal nerve cells. The treated mice showed significant preservation of outer nuclear layer thickness and rod photoreceptor cells, with approximately 70% restoration of visual function as measured by electroretinography responses. The study employed both rd10 and rd1 mouse models, which exhibit rapid photoreceptor degeneration similar to human retinitis pigmentosa, making them ideal for testing therapeutic interventions.

In another significant discovery, Wei and colleagues [35] uncovered a novel mechanism linking intestinal microbiota to retinal degeneration in CRB1-associated retinitis pigmentosa. Using the retinal degeneration 8 mouse model, which carries a naturally occurring Crb1 mutation (c.3481delC), they demonstrated that intestinal barrier dysfunction preceded retinal pathology. Through fluorescence imaging and bacterial culture analysis, they found that specific intestinal bacteria, primarily Enterobacteriaceae family members, could traverse compromised intestinal and blood-retina barriers. These bacteria triggered retinal inflammation through activation of the TLR4/NF-κB signaling pathway, leading to microglial activation and subsequent photoreceptor cell death. The researchers validated their findings through two therapeutic approaches: (1) systemic administration of broad-spectrum antibiotics, which reduced bacterial translocation and inflammation, resulting in a 40% reduction in photoreceptor loss, and (2) restoration of functional Crb1 expression, specifically in the lower digestive tract, using an AAV serotype 9 vector, which strengthened the intestinal barrier and prevented bacterial invasion.

Additional preclinical studies have focused on understanding the role of oxidative stress and inflammation in

Table 2. Summary of CYP4V2 mutation types in BCD patients.

Study	Mutation	Type	Population	Frequency	Clinical/Experimental Findings	Reference
Meng et al. (2014)	c.802_810del17insGC	Insertion-deletion	Chinese	Most prevalent	- Severe retinal dysfunction - Early onset crystalline deposits - Significant chorioretinal atrophy	[12]
Meng et al. (2014)	p.Tyr343Asp	Missense	Chinese	Common	- Moderate retinal dysfunction - Variable age of onset	[12]
Meng et al. (2014)	p.Gln11X	Nonsense	Chinese	Rare	- Severe phenotype - Early onset	[12]
Yin et al. (2016)	c.802–8_810del17bpinsGC	Complex	Chinese	0.375	- Progressive vision loss - Extensive RPE atrophy - Choroidal sclerosis	[13]
Yin et al. (2016)	c.992A>C (p.H331P)	Missense	Chinese	0.194	- Moderate disease progression - Variable crystalline deposits	[13]
Yin et al. (2016)	c.1091–2A>G	Splice-site	Chinese	0.141	- Abnormal splicing - Moderate to severe phenotype	[13]
Yin et al. (2016)	c.65T>A (p.L22H)	Missense (Novel)	Chinese	Single family	- Early onset - Rapid progression - Affects protein stability	[13]
Yin et al. (2016)	c.681_4delTGAG (p.S227Rfs*1)	Frameshift (Novel)	Chinese	Single family	- Severe phenotype - Protein truncation - Early onset	[13]
Jiao et al. (2017)	Multiple missense mutations	Missense (68%)	Mixed	68% of total	- Variable expressivity - Age-dependent penetrance	[14]
Guo et al. (2019)	c.802–8_810del17bp	Complex	Chinese Han	0.412	- Severe chorioretinal atrophy - Early crystalline deposits - Poor visual prognosis	[15]
Guo et al. (2019)	c.1091–2A>G	Splice-site	Chinese Han	0.178	- Moderate to severe phenotype - Variable age of onset	[15]
Guo et al. (2019)	c.992A>C	Missense	Chinese Han	0.145	- Moderate phenotype - Later onset - Slower progression	[15]
Yang (2021)	c.802–8_810del17insGC (Homozygous)	Insertion-deletion	Chinese	Family study	- Severe phenotype in siblings - Early onset - Rapid progression	[16]
Yang (2021)	c.219T>A (p.F73L) / c.802–8_810del17insGC	Compound heterozygous	Chinese	Family study	- Variable expressivity - Disrupted gene expression - Moderate phenotype	[16]

This table summarizes reported CYP4V2 mutations in Bietti crystalline dystrophy (BCD) patients, primarily from Chinese populations. The data includes mutation types, population frequencies, and associated clinical/experimental findings from various studies (2014–2021). RPE=retinal pigment epithelium. Novel mutations are specifically indicated. Frequencies are reported as percentages where available or described as occurrence patterns in family studies. Clinical findings encompass both phenotypic manifestations and functional impacts on protein structure/function.

retinal degeneration. The retinal degeneration 8 mouse model exhibits progressive retinal degeneration starting at postnatal day 14, characterized by the formation of retinal folds, photoreceptor displacement, and eventual vision loss. These features closely mirror the pathologic changes observed in human patients, making it an invaluable tool for testing therapeutic strategies. Current therapeutic approaches under investigation include antioxidant supplementation, anti-inflammatory agents, and various gene delivery methods using different AAV serotypes optimized for retinal cell tropism.

Clinical trials—Recent clinical trials have demonstrated promising advances in gene therapy for various inherited retinal disorders. A notable phase 1/2 open-label, single-dose escalation study investigated EDIT-101 in 12 adults with CEP290-associated inherited retinal degeneration. Mark and colleagues [36] reported significant improvements in multiple

outcome measures: 29% of patients showed enhanced BCVA or improved object and letter recognition, while 43% experienced better vision-related quality of life. Remarkably, 43% of patients improved in at least two evaluation metrics, and 79% showed improvement in at least one category. These results establish EDIT-101 as a safe therapeutic option for Leber congenital amaurosis, highlighting the potential of gene editing in treating retinal diseases.

A landmark Leber congenital amaurosis study published in *The Lancet* evaluated ATSN-101 in 15 patients with confirmed biallelic *GUCY2D* mutations [37]. The trial used unilateral subretinal injections across different dosage cohorts: three adult groups receiving escalating doses (1.0×10^{10}, 3.0×10^{10}, and 1.0×10^{11} vg/eye), as well as one adult group and one pediatric group both receiving the highest dose. Results showed rapid and sustained visual improvement throughout the one-year follow-up period. Light sensitivity tests revealed remarkable outcomes, with average visual function improving 100-fold and some patients achieving 10,000-fold improvement. Importantly, no serious drug-related adverse events were observed.

In another significant advancement, a three-year interim analysis of gene therapy for *RLBP1*-associated retinal dystrophy was conducted. André's research [38] demonstrated that subretinal administration of AAV8-RLBP1 was well tolerated, though accompanied by dose-dependent intraocular inflammation and focal retinal pigment epithelium atrophy. The treatment significantly improved dark adaptation kinetics across all dose cohorts and successfully resolved disease-related retinal deposits. These collective findings in hereditary retinal disease trials provide valuable insights for developing gene therapy approaches for BCD, particularly in areas such as target selection, drug design, clinical trial implementation, and delivery system optimization.

DISCUSSION

Gene therapy for BCD has recently advanced to confirmatory clinical research stages, offering potential for the first curative treatment, though published research remains limited. Analysis of hereditary retinal disease trials reveals a preference for AAV vectors, with dosages typically ranging from 2×10^8 to 1×10^{12} vg (averaging 1.1×10^{11} vg) and injection volumes of 0.03 to 1 ml. Most AEs occur within 30 days postadministration, primarily consisting of mild to moderate reactions such as conjunctival edema, bleeding, eye pain, blurred vision, and intraocular pressure changes. These AEs generally relate to administration methods rather than the therapeutic agents themselves [39]. The three BCD treatment products under development utilize similar dosage ranges and administration methods, suggesting comparable safety profiles.

Clinical trials typically use functional indicators like BCVA as primary endpoints, with regulatory authorities generally considering a 15-letter improvement as clinically significant. However, given the heterogeneous nature of hereditary retinal diseases and varying patient presentations, authorities now encourage alternative endpoints that can better represent clinical benefits across different patient populations (Food and Drug Administration. Human Gene Therapy for Retinal Disorders). Current BCD trials report BCVA improvements of 11 to 30 letters, which, compared to the natural disease progression of a 2- to 4.5-letter annual decline, demonstrate promising therapeutic potential for disease modification and vision improvement.

While current clinical studies demonstrate acceptable safety and therapeutic benefits of gene therapy for BCD, several complexities affect safety and efficacy evaluations. Despite the eye's immune privilege, advanced disease states can compromise ocular barriers, introducing risks of systemic and local immune responses. Research has shown that COVID-19 infection can impact vision and trigger nonspecific immune memory, potentially enhancing local immune responses and affecting AAV8 clearance [30]. Administration methods also significantly influence outcomes, with invasive injections risking complications such as ocular inflammation, retinal breaks, and hemorrhage [40]. Furthermore, injection site selection and volume affect drug-target cell interactions, influencing vector transfection and protein expression. Disease severity and resulting fundus structural changes impact drug distribution and efficacy through variations in residual target cell populations.

For future confirmatory clinical trials, considering the disease's rarity and typically small sample sizes, several key aspects warrant consideration. First, careful patient population selection should either focus on uniform disease severity or implement stratified analysis based on disease stage while balancing statistical requirements with practical recruitment constraints. Second, standardized drug delivery protocols with unified training and assessment procedures should be developed, particularly regarding injection site selection. Third, standardized treatment protocols for expected AEs, such as ocular inflammation and conjunctival edema, should be implemented based on clinical practice experience. Fourth, comprehensive risk assessment for unexpected adverse reactions should incorporate safety data from similar studies to develop appropriate countermeasures. Finally, pretrial design evaluation from the patient perspective should consider scientific validity, practicality, and risk-benefit profiles to ensure

reliable and complete trial outcomes through effective stakeholder cooperation.

Conclusion: Gene therapy for BCD has evolved significantly from its nascent stages to reach meaningful clinical development milestones. The progression of therapeutic products into late-stage development signals a transformative period, with potential market approvals on the horizon. This advancement represents more than incremental progress—it offers the first potential disease-modifying treatment for patients with BCD. The ongoing elucidation of disease mechanisms and natural progression continues to yield valuable insights that shape therapeutic strategies. These insights not only refine current approaches but also illuminate new pathways for intervention, suggesting that treatment modalities will likely continue to evolve and improve. As our understanding of BCD's molecular basis and clinical course deepens, we can anticipate further optimization of gene therapy approaches, potentially leading to more personalized and effective treatment strategies for patients affected by this rare but devastating condition.

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