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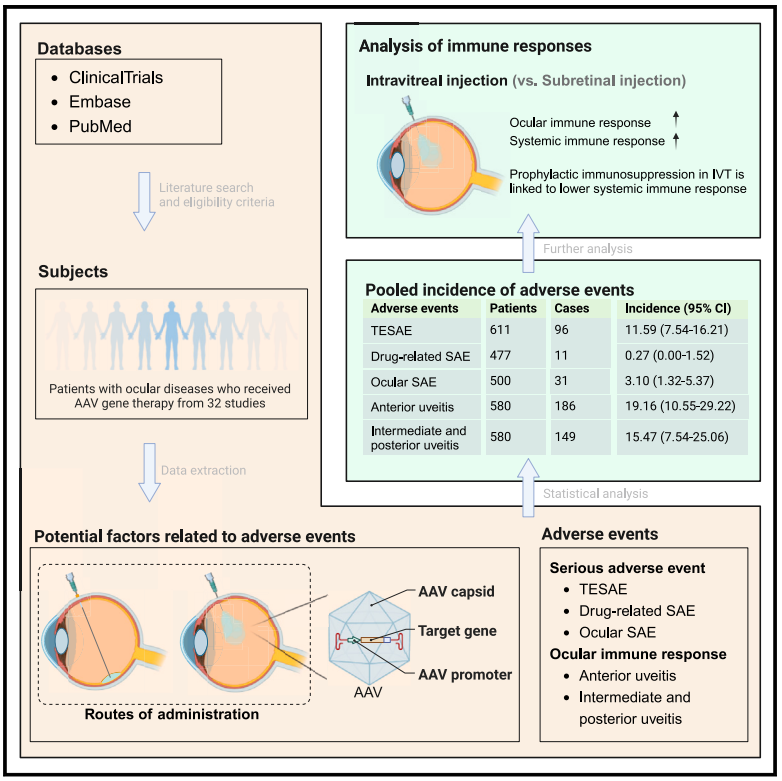
The clinical safety landscape for ocular AAV gene therapies: A systematic review and meta-analysis

Graphical abstract



Highlights

- Intravitreal administration shows higher immune responses compared to the subretinal route
- Prophylactic immunosupression did not affect ocular immune response
- Prophylactic immunosupression reduced systemic immune response in intravitreal administration

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In brief

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The clinical safety landscape for ocular AAV gene therapies: A systematic review and meta-analysis

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SUMMARY

Adeno-associated virus (AAV) gene therapy is a promising approach for treating ocular monogenic or acquired diseases, though immunogenicity and safety remain critical considerations. We conducted a systematic review of 120 trials and 32 publications to assess immune responses across different delivery routes. Intravitreal administration was associated with higher rates of anterior uveitis (43.06% vs. 10.22%) and intermediate/posterior uveitis (40.36% vs. 6.18%) compared to subretinal delivery. Engineered AAV capsids, used exclusively in intravitreal studies, showed no significant difference in either type of uveitis incidence compared to natural serotypes. Prophylactic immunosuppression (PI) did not affect ocular or systemic immune responses in subretinal delivery, but significantly reduced systemic immune responses in intravitreal administration. These findings underscore the potential of PI to mitigate systemic immune responses in intravitreal AAV therapy. This review should help guide the choice of routes of administration and immunosuppression strategies, and highlights current trends in ocular AAV gene therapy.

INTRODUCTION

Gene therapy using adeno-associated virus (AAV) as a vector represents a novel therapeutic modality to treat a wide variety of diseases by transferring engineered genetic material into target cells for the purpose of preventing, halting, or reversing pathological processes.[1,2] The engineered genetic material can be delivered as protein-coding or non-coding nucleic acids to implement gene augmentation or gene editing, respectively.[2] It was not until the 1990s that the first-in-human use of recombinant AAV (rAAV) gene therapy was reported treating patients with lung cystic fibrosis.[3,4] Given the distinctive attributes of their broad tissue tropism, non-pathogenic nature, favorable safety profile, and durable transgene expression, AAV vectors have become the preferred delivery system in clinical trials and US Food and Drug Administration (FDA)-approved applications.[5–7] With the advancement of rAAV engineered for enhanced specificity and transduction efficacy, more than 300 clinical trials have been initiated in treating a number of major human diseases in the past two decades, including ophthalmological, neurological, metabolic, hematologic, muscular diseases as well as oncogenic and infectious disorders.[7]

Among the broad spectrum of clinical applications, the eye is one of the most suitable target organs of gene therapy for several reasons. First, the eye is easily accessible and allows for the local application of therapeutic agents, with the intraocular dose of virus is about 1/1,000 of that used for systemic diseases.[8] Second, both the blood-ocular barriers and immune suppressive microenvironment protect the eye from external and internal insults, thereby greatly reducing ocular immune responses and suppressing potential systemic side effects.[9,10] Finally, with the continuous advancement of real-time imaging technologies, such as optical coherence tomography, the therapeutic effect can be assessed in a timely manner by various noninvasive methods.[11] Ocular AAV gene therapy has initially focused on inherited retinal degenerations (IRDs), a group of phenotypically and genotypically heterogeneous disorders that in the past were considered incurable, with a prevalence of monogenic IRD of approximately 1 in 2,000 individuals, affecting more than two million people worldwide.[12] A phase 3 clinical trial of *RPE65* gene

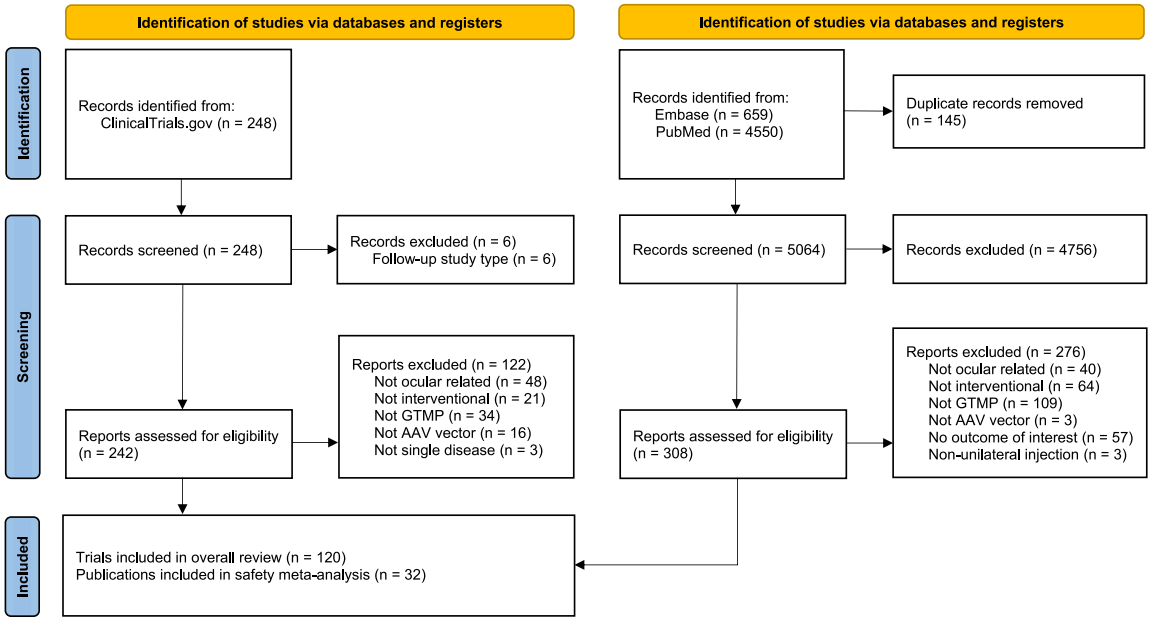

Figure 1. PRISMA flow diagram of searching qualified studies

The flow diagram displays the process of study identification, screening, and included in the analysis. GTMP, gene therapy medicinal product.

augmentation therapy, which demonstrated well-tolerated safety and promising efficacy in patients with *RPE65*-mediated IRD, boosted the approval of first ocular rAAV-based gene therapies by the US FDA and European Medicines Agency.[13] With the efficacy and safety of rAAV-based gene therapy have been established in clinical trials, the indications are gradually expanding to acquired diseases such as age-related macular degeneration and diabetic retinopathy.

Despite the enormous advantages of rAAV-based gene therapy for ocular diseases, there are still concerns about long-term efficacy and safety issues, including immunogenicity and adverse events (AEs) such as conjunctival hyperemia or hemorrhage, eye pain or irritation, blurred vision or diplopia, and transient changes in intraocular pressure.[14] The challenges posed by immune responses against rAAV-based agents have drawn clinically significant attention in ocular diseases, along with the recent discovery of compartmentalized lymphatic system within the eye.[15] Given the rising of this groundbreaking approach, we conducted a systematic review of the trends and safety landscape of ocular rAAV-based gene therapies. Here, we highlight the key trends and conduct a meta-analysis evaluating the safety outcomes of ocular AAV-based gene therapy, as well as discuss the implications for future practice.

RESULTS

Study selection

The search strategy identified 248 trials and 5,064 unique publications, of which 548 were reviewed in full-text, 120 trials were included in the overall systematic review (Table S1), and 32 publications with available safety data and classified safety events were included for further meta-analysis (Figure 1).[16–47]

Trial characteristics

The characteristics of the included trials are summarized in Table 1. The number of ocular rAAV-based gene therapy trials initiated rapidly increase from five (4%) between 2007 and 2009 to 49 (41%) between 2022 and July 2024. Ninety-one trials (76%) were open label, and most trials (96 [81%]) involved single nation with more than half of the trials (77 [66%]) conducted in the US and China (Figure S1). Thirty-one trials (26%) were randomized allocation. Eighty-nine trials (74%) were sponsored by a profit entity, while 31 (26%) by a nonprofit entity. Eighty-three trials (73%) were at the early stages prior to phase 2, while 34 (29%) had been completed. Of the 120 trials, most targeted a monogenic retinal disease by gene augmentation (110 [92%]), with a small portion opting for gene editing (3 [2%]) and optogenetics (7 [6%]).[48] The routes of administration (ROAs) were mainly intravitreal (42 [35%]) and subretinal (75 [63%]) injections. Trials enrolled exclusively adults accounted for 57% of the 120 trials, and 43% of the trials included children. In addition, 22 trials (20%) included only males due to X-linked recessive inheritance.

Historical trends of ocular AAV gene therapy

Compared with 22 trials identified before 2016, 98 trials conducted between 2016 and July 2024 had higher proportion of indications for acquired diseases (35 of 98 [36%] vs. 2 of 22 [9%]). Eighty-three trials (69%) were indicated for inherited diseases, predominantly retinitis pigmentosa and Leber congenital amaurosis (43 of 83 [52%]); and 37 (31%) were indicated for acquired diseases, mainly neovascular age-related macular degeneration (26 of 37 [70%]) (Figures 2A and S2). Proportion of completed trials gradually decreased from 80% (4 of 5) between 2007 and 2009 to 2% (1 of 48) between 2022 and July 2024; while recruiting trials gradually increased from 0% (0 of 5) between 2007 and 2009 to 79% (38 of 48) between 2022 and July 2024. It is noted

Table 1. Summary of trials' characteristics by setting

Characteristic	Trials, No. (%)[a]
Basic information	
Year of start (*n* = 120)	
2007–2009	5 (4)
2010–2012	8 (7)
2013–2015	9 (8)
2016–2018	24 (20)
2019–2021	25 (21)
2022-Jul 2024	49 (41)
Region (*n* = 118)	
Single nation	96 (81)
US	42 (36)
European nation	11 (9)
China	35 (30)
Others[b]	8 (7)
Multiple nations[c]	22 (19)
Funder type (*n* = 120)	
Profit	89 (74)
Nonprofit	31 (26)
Phase (*n* = 114)	
Early phase 1	11 (10)
Phase 1	19 (17)
Phase 1/2	53 (46)
Phase 2	18 (16)
Phase 2/3	4 (4)
Phase 3	9 (8)
Status (*n* = 119)	
Completed	34 (29)
Recruiting	48 (40)
Active, not recruiting	29 (24)
Not yet recruiting	6 (5)
Withdrawn/Unknown	2 (2)
Design details	
Therapeutic strategy (*n* = 120)	
Gene augmentation	110 (92)
Gene editing	3 (2)
Optogenetics	7 (6)
Allocation (*n* = 120)	
Randomized	31 (26)
Non-randomized	52 (43)
NA	37 (31)
Masking (*n* = 115)	
Open-label	92 (80)
Single blind	12 (10)
Double blind	5 (4)
Triple blind	1 (1)
Quadruple blind	5 (4)
Route of administration (*n* = 120)	
Intravitreal	42 (35)
Subretinal	75 (63)
Suprachoroidal	3 (3)
Information of subjects enrolled in trials	
Age (*n* = 120)	
Child, adult	14 (12)
Child, adult, older Adult	37 (31)
Adult, older adult	68 (57)
Older adult	1 (1)
Sex (*n* = 120)	
All	96 (80)
Male	24 (20)

[a]Descriptive data are provided for trials with available information, hence the varying denominators.
[b]Other single nations included Australia, Canada, India, Israel, Japan and Saudi Arabia.
[c]The trials were conducted simultaneously in different nations.

that the status of a trial is variable unless it has been completed. The proportion of trial status at different phases is relatively consistent (Figures 2B and 2C). Proportion of trials sponsored by profit organization gradually increased from 40% (2 of 5) between 2007 and 2009 to 78% (38 of 50) between 2022 and July 2024; and similarly, from 45% (5 of 11) in early phase 1 to 100% (9 of 9) in phase 3 (Figures 2D and 2E).

The analysis of drug design trends included 59 individual drugs with capsid and promoter disclosures from 120 clinical trials (Figure S3A). Choice of AAV capsids have diversified over time, from 76% (13 of 17) drug using AAV2 before 2016, to an almost equal use between AAV2, AAV5, AAV8, and engineered AAV variants during 2016–July 2024 (Figure 2F). The ubiquitous promoter (26 of 45 [58%]) is the most commonly used promoter. The retina-specific promoters had shifted from retinal pigment epithelium cell (RPE)-specific promoter dominating during 2007–2012 to photoreceptors-specific promoter thereafter (Figure 2G). In drugs with AAV2, AAV5, AAV8, and engineered AAV variants, ubiquitous and photoreceptors-specific promoters are both used (Figure S3B). Among the 64 gene augmentation drugs, the expression products of drugs used for acquired diseases were located extracellularly except for OCU410 (NCT06018558), whereas the expression products of the drugs used for inherited diseases were located intracellularly except for the five drugs targeting the *RS1* gene (Figure S3C). The AAV capsids used for intravitreal injection (IVT) of drugs are mainly engineered AAV variants and AAV2, while drugs with subretinal injection (SR) cover all types of AAV (Figure S3D).

Pooled and stratified incidence of adverse events

The characteristics of the 32 publications are summarized in Table 2. Table 3 gives the results of overall and subgroup meta-analyses. The pooled incidence of different AEs after ocular rAAV-based gene therapy was estimated using random-effects or fixed-effects models according to whether the heterogeneity was significant: 11.59% (95% confidence interval [CI], 7.54%–16.21%) for treatment-emergent serious adverse event

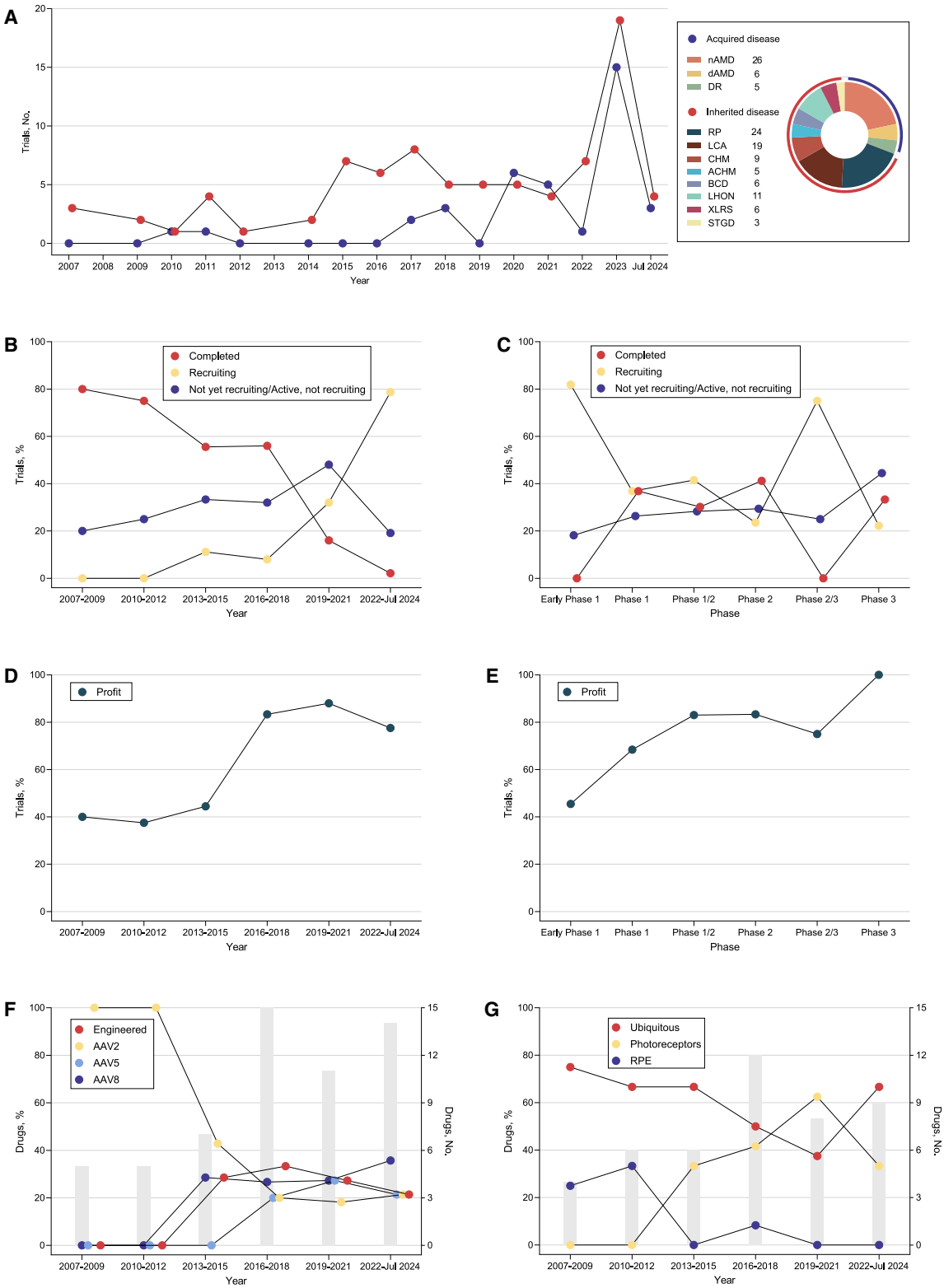

Figure 2. Historical trends of ocular AAV gene therapy

(A) Number of trials per indication by start year. The donut chart that on the right displays the proportions of indications for trials.

(B and C) Proportion of trials per status by start year (B) and phase (C). Trials with withdrawn/suspended/terminated/unknown status were excluded.

(legend continued on next page)

(TESAE), 0.27% (95% CI, 0.00%–1.52%) for drug-related serious AE (drug-related SAE), 3.10% (95% CI, 1.32%–5.37%) for ocular serious AE (ocular SAE), 19.16% (95% CI, 10.55%–29.22%) for anterior uveitis (AU), and 15.47% (95% CI, 7.54%–25.06%) for intermediate and posterior uveitis (IPU); the sensitivity analysis showed that no single study had an excessive influence on the pooled incidence of AEs after removing a single study at one time (Figures S4–S13). No publication bias was found based on the funnel plot, Egger test, and Begg test (Table 3, Table S9 and Figure S14).

The incidence of different AEs according to AAV capsid, AAV promoter, expression product location, and ROA is also given in Table 3. Based on all enrolled publications, the incidence of TESAE was higher when expression product located extracellularly (24.67%; 95% CI, 17.71%–32.25%) than when it was located intracellularly (8.31%; 95% CI, 4.57%–12.76%). The incidence of AU was higher when using engineered AAV capsids (54.31%; 95% CI, 27.91%–79.54%) than those using natural AAV capsids (15.40%; 95% CI, 7.53%–24.91%). Studies using IVT showed higher incidence rates among patients (AU: 43.06%; 95% CI, 23.05%–64.22%; IPU: 40.36%; 95% CI, 20.43%–61.91%) than those using SR (AU: 10.22%; 95% CI, 4.29%–17.68%; IPU: 6.18%; 95% CI, 1.84%–12.06%). The subgroup meta-analyses indicated no statistically significant difference in incidence of drug-related SAE and ocular SAE among studies by AAV capsid (natural vs. engineered), AAV promoter (retina-specific vs. ubiquitous), expression product location (intracellular vs. extracellular), and ROA (subretinal vs. intravitreal). No publication bias was found based on Egger test and Begg test (Table 3 and Table S9).

Further, considering that all three studies utilizing engineered capsids were administered via IVT injection,[28,32,44] we performed a subsequent subgroup meta-analysis focusing on trials employing IVT delivery. Our analysis revealed no statistically significant difference in the incidence of AU or IPU between engineered AAV serotypes and natural serotypes, with no evidence of publication bias (Table 4 and Table S9).

Immune responses and prophylactic immunosuppression

Given the significant differences in ocular immune response showed between ROAs, twenty-nine studies with disclosed application of prophylactic immunosuppression (PI) or not was performed subsequently by using a random-effects meta-analysis to generate stratified incidence of AU and IPU (Figures 3 and 4). Incidence of AU following SR with PI, SR without PI, IVT with PI, and IVT without PI were 8.76% (95% CI, 2.92%–16.46%), 11.31% (95% CI, 1.55%–26.10%), 24.28% (95% CI, 0.00%–92.14%), and 43.14% (95% CI, 21.19%–66.47%), respectively, with significant heterogeneity except SR with PI (I^2 = 29%; I^2 = 82%; I^2 = 93%; I^2 = 86%). Incidence of IPU following SR with PI, SR without PI, IVT with PI and IVT without PI were 8.15% (95% CI, 2.22%–16.24%), 4.18% (95% CI, 0.00%–13.23%), 14.85% (95% CI, 0.00%–63.08%), and 43.90% (95% CI, 20.07%–69.19%), respectively, with heterogeneity except SR with PI (I^2 = 36%; I^2 = 80%; I^2 = 86%; I^2 = 89%). There is no statistically significant difference in incidence of AU and IPU with either ROAs between patients receiving PI or not.

Seventeen studies with disclosed systemic immune response were included for subsequent analyses (Table S2). Figure 5 demonstrates the relationship of immune responses between ROAs (with PI or not) and doses. The dose of SR (range: 1E10–1E12 vg/eye) is generally higher than that of IVT (range: 1.2E8–1.8E11 vg/eye). Notably, ocular immune responses occurred across the dose range of IVT, whereas in SR it occurred only at a dose of 4.8E10 and above. A Fisher exact test was performed to assess potential correlations between systemic immune response and ROAs. Among patients receiving IVT AAV-based agents without PI, a significantly higher incidence of systemic immune responses was observed compared to those receiving SR administration (42% vs. 25%, p = 0.04) (Table S3). In contrast, among patients with SR administration, no significant difference in the incidence of systemic immune responses was found between those who received PI and those who did not (23% vs. 25%, p = 0.84) (Table 5). However, for patients receiving IVT injections, PI treatment was associated with a significant lower incidence of systemic immune responses compared to those not receiving PI (0% vs. 42%, p = 0.02) (Table 5). A list of all PI regimes included in the study is provided in Table S4.

DISCUSSION

This systematic review and meta-analysis, including studies from 120 clinical trials and 32 publications with comprehensive safety data from 2007 to 2024, indicate that ocular AAV gene therapy is a safe, well-tolerated modality. With recent advances in high-throughput sequencing methodologies, a trend of higher diversity of the AAV capsids was observed, while ubiquitous and photoreceptor-specific promoter remained dominant. Patients were more likely to have TESAE than their counterparts when the drug expression product located extracellularly. The incidence of AU and IPU varied significantly between different ROAs. Specifically, IVT administration exhibiting a higher incidence of both ocular and systemic immune responses compared to SR administration. Notably, no statistical differences were found between patients that received PI and those that did not, for either IVT or SR ROA. However, among patients receiving IVT administration, those treated with PI demonstrated a significantly lower incidence of systemic immune responses compared to those not receiving PI, suggesting the potential benefit of PI in managing systemic immune-related AEs.

(D and E) Proportion of profit trials by start year (D) and phase (E).
(F and G) Proportion of drugs per capsid (F) or promoter (G) by start year. The gray columns in (F) and (G) represent the number of drugs. (F) exclude drugs used AAV4 or AAV9 as capsids and (G) exclude drugs used bipolar cell-specific promoter or Müller cell-specific promoter because there are minimal numbers of them (only one or two drugs respectively). nAMD, neovascular age-related macular degeneration; dAMD, dry age-related macular degeneration; DR, diabetic retinopathy; RP, retinitis pigmentosa; LCA, Leber congenital amaurosis; CHM, choroideremia; ACHM, achromatopsia; BCD, Bietti's crystalline dystrophy; LHON, Leber hereditary optic neuropathy; XLRS, X-linked retinoschisis; STGD, Stargardt disease; RPE, retinal pigment epithelium cell.

Table 2. Characteristics of 32 included publications

NCT identifier number	Cited	Design	Condition	Sex (F/M)	Age: range (mean, median)	ROA	Agent (AAV): Capsid; Promoter; Expression product location	Prophylactic immunosuppression	Follow-up (M)	N[a]	N of TESAE	N of Drug-related SAE	N of Ocular SAE
NCT00643747	Bainbridge et al. 2015[16]	SAT Open-label	LCA	NR	6-23 (14.4, 15)	SR	Natural; Retina-Specific; Intracellular	Yes	36	12	2	NR	NR
NCT00481546	Jacobson et al. 2012[17]	SAT Open-label	LCA	8/7	11-30 (20, 19.6)	SR	Natural; Ubiquitous; Intracellular	No	16.1 ± 11.4 (1–36)	15	NR	NR	NR
NCT00516477	Maguire et al. 2009[18]	SAT Open-label	LCA	5/7	8-44 (20.8, 19.5)	SR	Natural; Ubiquitous; Intracellular	Yes	24	12	0	0	0
NCT00749957	Weleber et al. 2016[19]	SAT Open-label	LCA	6/6	6-39 (24.8, 31)	SR	Natural; Ubiquitous; Intracellular	No	24	12	1	0	0
NCT01024998	Heier et al. 2017[20]	SAT Open-label	nAMD	9/10	57-89 (60.2,/)	IVT	Natural; Ubiquitous; Extracellular	No	40.1 ± 13.5 (12–48)	19	5	0	1
NCT01267422	Wan et al. 2016[21]	SAT Open-label	LHON	2/7	9-46 (19.2, 17)	IVT	Natural; Ubiquitous; Intracellular	Yes	9	9	0	0	0
NCT01482195	Ghazi et al. 2016[22]	SAT Open-label	RP	1/5	14-54 (33.3, 32.5)	SR	Natural; Retina-Specific; Intracellular	No	24	6	0	0	0
NCT01496040	Le Meur et al. 2018[23]	SAT Open-label	LCA	NR	9-42 (24.1, 22)	SR	Natural; Retina-Specific; Intracellular	Yes	25.7 ± 10.9 (12–41)	9	2	0	0
NCT01461213	Xue et al. 2018[24]	SAT Open-label	CHM	0/14	25-73 (48.2, 45.5)	SR	Natural; Ubiquitous; Intracellular	Yes	24	14	1	0	1
NCT01494805[b]	Rakoczy et al. 2015[25]	RCT Single blind	nAMD	3/3	74-86 (81.2, 82)	SR	Natural; Ubiquitous; Extracellular	No	12	6	2	0	00
NCT01494805[b]	Constable et al. 2016[26]	RCT Single blind	nAMD	NR	/(80,/)	SR	Natural; Ubiquitous; Extracellular	No	12	21	5	0	0
NCT02064569	Bouquet et al. 2019[27]	SAT Open-label	LHON	2/13	/(47.9,/)	IVT	Natural; Ubiquitous; Intracellular	No	24	15	1	1	1
NCT02161380	Lam et al. 2022[28]	SAT Open-label	LHON	5/23	16-56 (31.1, 28.5)	IVT	Engineered; Ubiquitous; Intracellular	No	34.9 ± 5.6 (6–36)	28	2	0	2

(*Continued on next page*)

Table 2. *Continued*

NCT identifier number	Cited	Design	Condition	Sex (F/M)	Age: range (mean, median)	ROA	Agent (AAV): Capsid; Promoter; Expression product location	Prophylactic immunosuppression	Follow-up (M)	N[a]	N of TESAE	N of Drug-related SAE	N of Ocular SAE
NCT02341807	Aleman et al. 2022[29]	SAT Open-label	CHM	NR	20-57 (34.5, 33)	SR	Natural; Ubiquitous; Intracellular	No	24	15	2	0	2
NCT02317887	Cukras et al. 2018[30]	SAT Open-label	XLRS	0/9	23-72 (47.6, 52)	IVT	Natural; Retina-Specific; Extracellular	No	18	9	0	0	0
NCT02077361	Dimopoulos et al. 2018[31]	SAT Open-label	CHM	0/6	29-42 (34.3, 33.5)	SR	Natural; Ubiquitous; Intracellular	Yes	24	6	1	1	1
NCT02416622	Pennesi et al. 2022[32]	SAT Open-label	XLRS	0/27	10-79 (33.4, 33)	IVT	Engineered; Ubiquitous; Extracellular	UTD	12	27	6	0	3
NCT02553135	Lam et al. 2019[33]	SAT Open-label	CHM	0/6	32-72 (51, 50)	SR	Natural; Ubiquitous; Intracellular	Yes	24	6	0	0	0
NCT02610582	Fischer et al. 2020[34]	SAT Open-label	ACHM	1/8	24-59 (39.6,/)	SR	Natural; Retina-Specific; Intracellular	Yes	9	9	1	0	0
NCT02652780[c]	Yu-Wai-Man et al. 2020[35]	SAT Quadruple blind	LHON	8/29	15-67 (34.2,/)	IVT	Natural; Ubiquitous; Intracellular	No	24	37	1	0	0
NCT02671539	Fischer et al. 2019[36]	SAT Open-label	CHM	0/6	51-60 (54.9, 52.5)	SR	Natural; Ubiquitous; Intracellular	Yes	24	6	0	0	0
NCT02652767[d]	Newman et al. 2021[37]	SAT Quadruple blind	LHON	7/32	/(36.8,/)	IVT	Natural; Ubiquitous; Intracellular	No	24	39	2	0	0
NCT02407678	Cehajic-Kapetanovic et al. 2024[38]	SAT Open-label	CHM	0/30	/(32.1,/)	SR	Natural; Ubiquitous; Intracellular	Yes	24	30	6	4	4
NCT03001310	Michaelides et al. 2023[39]	SAT Open-label	ACHM	18/5	5-33 (17.0,/)	SR	Natural; Retina-Specific; Intracellular	Yes	6	23	2	2	2
NCT03116113[e]	Cehajic-Kapetanovic et al. 2020[40]	SAT NA	RP	0/18	20-50 (32.2, 28.5)	SR	Natural; Retina-Specific; Intracellular	Yes	6	18	0	0	0
NCT03116113[e]	Lam et al. 2024[41]	RCT NA	RP	0/23	/(28.9,/)	SR	Natural; Retina-Specific; Intracellular	Yes	12	23	8	NR	6

(*Continued on next page*)

Table 2. ***Continued***

NCT identifier number	Cited	Design	Condition	Sex (F/M)	Age: range (mean, median)	ROA	Agent (AAV): Capsid; Promoter; Expression product location	Prophylactic immunosuppression	Follow-up (M)	N[a]	N of TESAE	N of Drug-related SAE	N of Ocular SAE
NCT03066258	Campochiaro et al. 2024[42]	SAT Open-label	nAMD	22/20	50-89 (80,/)	SR	Natural; Ubiquitous; Extracellular	No	24	42	12	1	2
NCT03496012	MacLaren et al. 2023[43]	RCT Single blind	CHM	0/99	/(48.3,/)	SR	Natural; Ubiquitous; Intracellular	No	12	99	20	NR	NR
NCT03748784	Khanani et al. 2023[44]	SAT Open-label	nAMD	15/15	62-90 (78.9,/)	IVT	Engineered; Ubiquitous; Extracellular	Yes	24	30	10	2	4
NCT03920007	Jacobson et al. 2021[45]	SAT Open-label	LCA	2/1	23-45 (34.3, 35)	SR	Natural; Retina-Specific; Intracellular	Yes	9	3	0	0	0
NCT03872479	Pierce et al. 2024[46]	SAT Open-label	LCA	9/5	9-63 (34.6, 35.5)	SR	Natural; Retina-Specific; Intracellular	Yes	12.6 ± 6.0 (6–24)	14	2	0	0
NCT04722107	Wang et al. 2024[47]	SAT Open-label	BCD	6/6	27-50 (38.3, 40)	SR	Natural; Ubiquitous; Intracellular	Yes	9.0 ± 3.0 (6–12)	12	2	0	2

ACHM, achromatopsia; BCD, Bietti's crystalline dystrophy; CHM, choroideremia; Drug-related SAE, drug-related serious adverse event; IVT, intravitreal injection; LCA, Leber congenital amaurosis; LHON, Leber hereditary optic neuropathy; NA, not available; nAMD, neovascular age-related macular degeneration; Ocular SAE, ocular serious adverse event; RP, retinitis pigmentosa; RCT, randomized controlled trial, ROA: route of administration; SAT, single arm trial; SR, subretinal injection; TESAE, treatment-emergent serious adverse event; UTD: unable to determine; XLRS, X-linked retinoschisis.

[a]The number of participants in clinical trials included in the publications; we only counted patients who received AAV gene therapy.

[b]The two studies were based on the same clinical trial (NCT01494805), which was designed as two parts, Phase 1 and Phase 2a, so we collected the data of Phase 1 and Phase 2a respectively according to their published articles.

[c]The two studies are randomized, sham-controlled trials (NCT02652780, NCT02652767).

[d]The control group was the contralateral eye which received sham injection of the same patient, and we considered the trial to be single arm trial and collected only the treated eye data.

[e]The two studies were based on the same clinical trial (NCT03116113), which was designed as two parts, Part1 and Part 2, so we collected the data of Part 1 and Part 2 respectively according to their publications.

Table 3. Incidence of safety outcomes using meta-analysis and subgroup meta-analysis

Variable	No. of articles	No. of participants	No. of cases	Incidence (95% CI)	I^2 %	*p* value			Subgroup difference	
						Q test	Egger test	Begg test	*p* value	P_{fdr}
Analysis for serious adverse event										
TESAE	31	611	96	11.59 (7.54–16.21)	45.7	<0.01	0.13	0.27	NA	NA
Drug-related SAE	28	477	11	0.27 (0.00–1.52)	0.0	0.88	0.50	<0.01	NA	NA
Ocular SAE	29	500	31	3.10 (1.32–5.37)	22.4	0.14	0.92	0.92	NA	NA
Analysis for ocular immune response										
AU	30	580	186	19.16 (10.55–29.22)	84.2	<0.01	<0.01	0.12	NA	NA
IPU	30	580	149	15.47 (7.54–25.06)	85.7	<0.01	0.12	0.45	NA	NA
Subgroup analysis for TESAE										
AAV capsid										
Natural	28	526	78	10.52 (6.39–15.30)	42.3	0.01	0.16	0.37	0.33	0.48
Engineered	3	85	18	19.86 (6.65–37.27)	68.3	0.04	0.64	0.60		
AAV promoter										
Retina-specific	10	126	17	8.91 (2.03–18.58)	42.6	0.07	0.47	0.65	0.61	0.61
Ubiquitous	21	485	79	12.68 (7.97–18.10)	48.6	<0.01	0.26	0.32		
Expression product location										
Intracellular	24	457	56	8.31 (4.57–12.76)	31.4	0.07	0.19	0.80	<0.01	<0.01
Extracellular	7	154	40	24.67 (17.71–32.25)	12.4	0.33	0.31	0.65		
Route of administration										
Subretinal	22	398	69	13.04 (8.27–18.47)	27.6	0.11	0.06	0.14	0.36	0.48
Intravitreal	9	213	27	9.85 (3.30–18.66)	65.0	<0.01	0.78	0.92		
Subgroup analysis for drug-related SAE										
AAV capsid										
Natural	25	392	9	0.23 (0.00–1.62)	0.0	0.90	0.49	<0.01	0.81	0.87
Engineered	3	85	2	1.21 (0.00–5.56)	31.6	0.23	0.22	0.60		
AAV promoter										
Retina-specific	9	91	2	0.23 (0.00–4.19)	0.0	0.92	0.60	0.15	0.65	0.87
Ubiquitous	20	386	9	0.38 (0.00–1.88)	0.0	0.66	0.45	0.01		
Expression product location										
Intracellular	21	323	8	0.21 (0.00–1.78)	0.0	0.73	0.39	<0.01	0.87	0.87
Extracellular	7	154	3	0.55 (0.00–3.40)	0.0	0.82	0.70	0.88		
Route of administration										
Subretinal	19	264	8	0.63 (0.00–2.88)	0.0	0.90	0.65	0.01	0.17	0.68
Intravitreal	9	213	3	0.19 (0.00–2.03)	0.0	0.65	0.36	0.05		
Subgroup analysis for ocular SAE										
AAV capsid										
Natural	26	415	22	2.07 (0.49–4.34)	21.2	0.17	0.73	0.46	0.05	0.20
Engineered	3	85	9	10.46 (4.43–18.29)	0.0	0.76	0.67	0.60		
AAV promoter										
Retina-specific	9	114	8	2.85 (0.04–8.18)	32.8	0.16	0.23	0.66	0.80	0.80
Ubiquitous	20	386	23	3.32 (1.30–5.93)	21.2	0.19	0.54	0.97		
Expression product location										
Intracellular	22	346	21	2.58 (0.68–5.26)	30.4	0.09	0.73	0.65	0.63	0.80
Extracellular	7	154	10	4.58 (1.11–9.41)	0.0	0.46	0.41	0.45		
Route of administration										
Subretinal	20	287	20	3.61 (1.16–6.91)	12.7	0.30	0.41	0.79	0.31	0.62
Intravitreal	9	213	11	2.96 (0.65–6.32)	39.8	0.10	0.66	0.92		

(*Continued on next page*)

Table 3. *Continued*

Variable	No. of articles	No. of participants	No. of cases	Incidence (95% CI)	I^2 %	*p* value			Subgroup difference	
						Q test	Egger test	Begg test	*p* value	P_{fdr}
Subgroup analysis for AU										
AAV capsid										
Natural	27	495	140	15.40 (7.53–24.91)	82.2	<0.01	<0.01	0.39	<0.01	0.01
Engineered	3	85	46	54.31 (27.91–79.54)	83.6	<0.01	0.39	0.60		
AAV promoter										
Retina-specific	8	80	15	16.37 (7.88–26.57)	0.0	0.56	0.29	0.37	0.75	0.75
Ubiquitous	22	500	171	20.29 (9.50–33.35)	87.9	<0.01	0.01	0.08		
Expression product location										
Intracellular	23	426	127	16.09 (7.21–27.00)	83.3	<0.01	<0.01	0.31	0.30	0.40
Extracellular	7	154	59	29.38 (9.61–53.65)	87.2	<0.01	0.31	0.29		
Route of administration										
Subretinal	21	367	77	10.22 (4.29–17.68)	69.1	<0.01	<0.01	0.78	<0.01	0.01
Intravitreal	9	213	109	43.06 (23.05–64.22)	86.5	<0.01	0.11	0.25		
Subgroup analysis for IPU										
AAV capsid										
Natural	27	495	114	12.69 (5.19–22.16)	84.8	<0.01	0.15	0.95	0.08	0.16
Engineered	3	85	35	40.90 (13.33–71.78)	87.8	<0.01	0.70	0.60		
AAV promoter										
Retina-specific	8	80	16	13.87 (2.68–29.59)	55.3	0.03	0.52	0.52	0.95	0.95
Ubiquitous	22	500	133	15.99 (6.54–27.90)	88.7	<0.01	0.18	0.28		
Expression product location										
Intracellular	23	426	112	14.02(5.59–24.69)	83.4	<0.01	0.03	0.26	0.64	0.85
Extracellular	7	154	37	20.40 (3.28–44.89)	91.3	<0.01	0.61	0.65		
Route of administration										
Subretinal	21	367	48	6.18 (1.84–12.06)	62.5	<0.01	0.20	0.37	<0.01	<0.01
Intravitreal	9	213	101	40.36 (20.43–61.91)	87.5	<0.01	0.24	0.17		

AU, anterior uveitis; Drug-related SAE, drug-related serious adverse event; IPU, intermediate and posterior uveitis; Ocular SAE, ocular serious adverse event; TESAE, treatment-emergent serious adverse event.

AAV-associated AEs (e.g., immune responses) present substantial challenges for the advancement of ocular gene therapy in this immune-privileged organ, influencing patient enrollment, long-term efficacy, as well as transduction efficiency following subsequent vector applications to the fellow eye.[49] Previous systematic reviews have summarized the safety evidence related to ocular AAV gene therapy.[14,50,51] However, none of those studies provided a trend description of the trials or drug design; pooled and stratified incidence of different safety outcomes were also lacking. This study was the first systematic review and meta-analysis to explore the incidence of various AEs of ocular AAV gene therapy based on multiple intervention factors. The safety profile

Table 4. Incidence of AU using subgroup meta-analysis in trials using IVT

Variable	No. of articles	No. of participants	No. of cases	Incidence (95% CI)	I^2 %	*p* value			Subgroup difference
						Q test	Egger test	Begg test	*p* value
Subgroup analysis for anterior uveitis									
AAV capsid									
Natural	6	128	63	36.63 (11.04–66.53)	89.1	<0.01	0.18	0.44	0.39
Engineered	3	85	46	54.31 (27.91–79.54)	83.6	<0.01	0.92	0.60	
Subgroup analysis for intermediate and posterior uveitis									
AAV capsid									
Natural	6	128	66	39.73 (12.65–70.29)	89.2	<0.01	0.23	0.25	0.97
Engineered	3	85	35	40.90 (13.33–71.78)	87.8	<0.01	0.70	0.60	

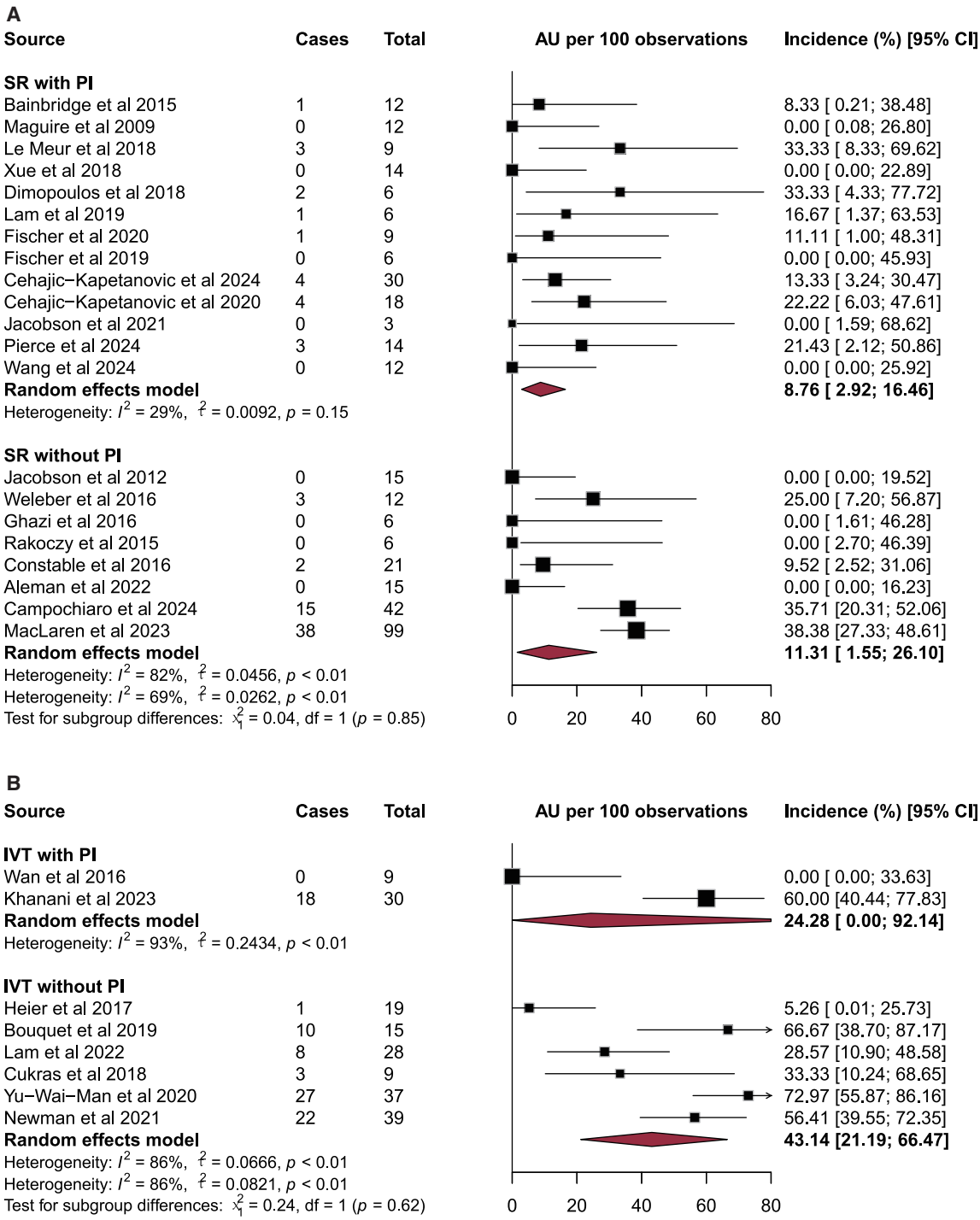

Figure 3. Forest plot of pooled incidence of anterior uveitis following either routes of administration with prophylactic immunosuppression or not

(A) Forest plot of pooled incidence of anterior uveitis following SR with PI or not.

(B) Forest plot of pooled incidence of anterior uveitis following IVT with PI or not. IVT, intravitreal injection; SR, subretinal injection; PI, prophylactic immunosuppression. Data are represented as pooled incidence and 95% CI.

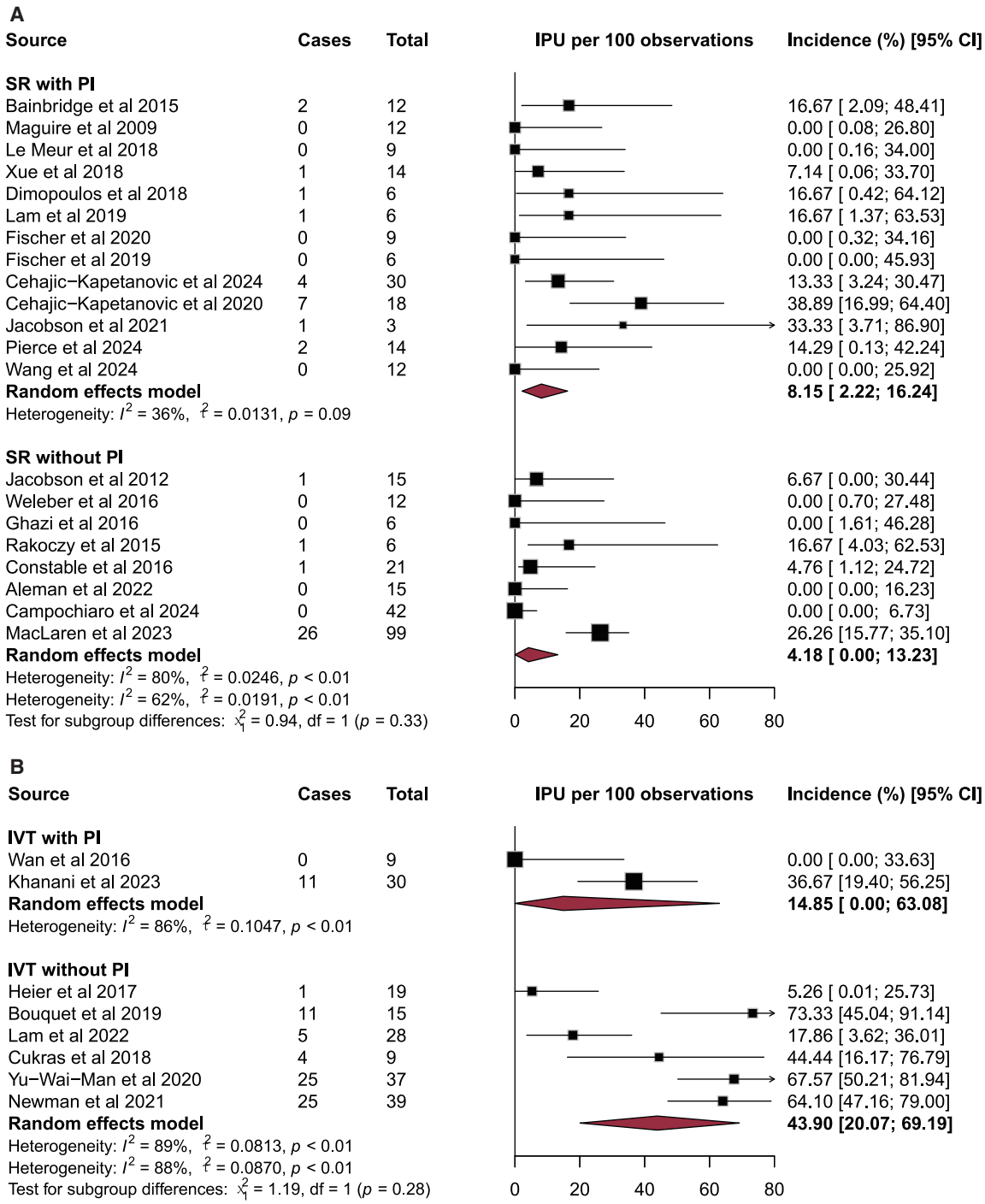

Figure 4. Forest plot of pooled incidence of intermediate and posterior uveitis following either routes of administration with prophylactic immunosuppression or not

(A) Forest plot of pooled incidence of intermediate and posterior uveitis following SR with PI or not.

(B) Forest plot of pooled incidence of intermediate and posterior uveitis following IVT with PI or not. IVT, intravitreal injection; SR, subretinal injection; PI, prophylactic immunosuppression. Data are represented as pooled incidence and 95% CI.

of rAAV gene therapy has been reported to be tightly connected to the disease indications, vector components (including the capsid, promoter, and target gene), vector dosage, and its ROAs,[7,52] while immunosuppression regimen is commonly administered to mitigate immune response caused by rAAV immunogenicity, these safety-related interventions were involved in our study.

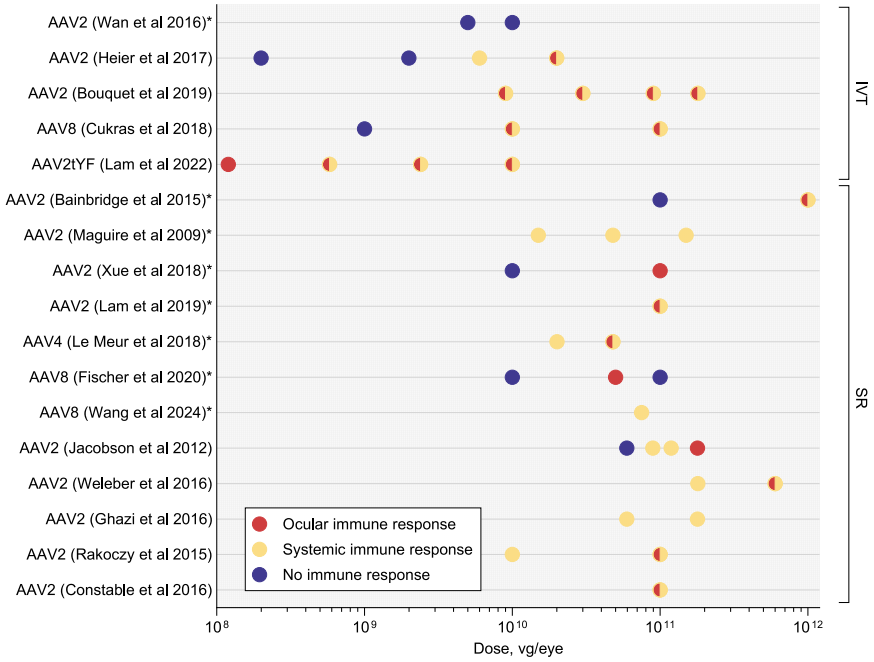

Figure 5. Immune responses following either routes of administration with prophylactic immunosuppression or not

Data are shown for the ocular and systemic immune responses in 17 ocular AAV gene therapy studies. Plotted points represent different dose groups divided according to the study protocol. Asterisk: the study was conducted with prophylactic immunosuppression. IVT, intravitreal injection; SR, subretinal injection.

Originally envisioned as a treatment solely for inherited diseases, AAV gene therapy is now being applied to acquired diseases.[53,54] Different indications may be an important reason for difference incidence of TESAE between drugs that express products extracellularly (24.67%; 95% CI, 17.71%–32.25%) and those that express products intracellularly (8.31%; 95% CI, 4.57%–12.76%). The indication for most drugs with expression products located extracellularly is acquired disease (Figure S3C). Among our studies, the mean age range of patients in the five studies related to acquired disease was 60.2–81.2 years, in contrast to 14.4–54.9 years in the 27 studies related to inherited disease studies (Table 2). The advancing age is intuitively associated with increased AEs due to complications from various organ systems. This may also explain why patients were more likely to experience TESAE when the drug product was expressed extracellularly.

Vector design plays a crucial role in the efficacy and safety of the treatment. Engineered capsids are commonly used for IVT, as they present stronger capacity of neuroretina penetration following IVT compared to natural capsids, such as AAV2, thus avoiding higher surgical risk related to SR (Figure S3D).[55] However, our study shows that there is no statistically significant difference in the incidence of AU or IPU among IVT studies by AAV capsid. Variants as AAV2tYF, AAV2.7m8, and 4D-R100 were modified to drive stronger and more widespread transgene expression, of which AAV2tYF was generated by rational design, while AAV2.7m8 and 4D-R100 were generated by directed evolution.[56] In the AAV2tYF variant, mutations to phenylalanine (Y444F/Y500F/Y730F) were generated to prevent ubiquitination and proteasome-mediated degradation of the AAV.[57–59] AAV2.7m8 variant is an AAV2 variant containing the insertion LALGETTRP after amino acid 587, attempting to disrupt the heparan sulfate proteoglycan binding of AAV2, therefore facilitating the spread of the vector in the neuroretina.[60] Similarly, 4D-R100 variant is also an AAV2 variant containing the insertion LAISDQTKHA after amino acid 587 and a point mutation (P34A) in the VP1 region.[61] Further research into how engineered AAV capsids interact with the host is vital, as it can be tailored based on the characteristics of the disease, showing more personalized advantages over natural AAV capsids.

In general, IVT is more surgically convenient and less invasive than SR, thus considering the preferred drug delivery method in treating retinal disease. However, studies in both humans and non-human primates have consistently demonstrated that IVT induces stronger immune responses compared to SR, which triggers relatively weaker humoral immune responses.[62–66] A recent study demonstrated that the anterior and posterior compartments of the eye have separate lymphatic drainage systems, with the IVT administration induced more AAV-specific $CD8^+$ T cells in deep cervical lymph nodes and retina, exacerbating

Table 5. Association of systemic immune responses with PI across different ROA

	Subretinal administration (n = 134)		Intravitreal administration (n = 80)	
Systemic immune responses	With PI	Without PI	With PI	Without PI
Yes	17	15	0	30
No	57	45	9	41
Total	74	60	9	71
p value	0.84 ^		0.02[a]	

PI, prophylactic immunosuppression.
[a]Fisher exact test.

Table 6. Definition of AEs related to the study

Adverse event	Definition	Example[a]
TESAE	A serious adverse event that happens during a clinical study or within a certain amount of time after the treatment.	1) Drug-related SAE 2) Ocular SAE 3) All other SAEs, such as ischemic stroke
Drug-related SAE	A serious adverse event that caused by the drug.	1) All SAEs that are considered to be drug-induced by the investigator
Ocular SAE	A serious adverse event that occurred in the eye.	1) Reduction in VA by $\geq$ 15 ETDRS letters 2) Acute foveal thinning
AU	An adverse event with the inflammation of the anterior uveal tract, characterized by the presence of leukocytes in the anterior chamber of the eye.	1) Iritis 2) Iridocyclitis 3) Anterior chamber cell
IPU	An adverse event with the inflammation of the vitreous, retina or choroid, presence of leukocytes in the vitreous humor and evidence of active chorioretinal inflammation are diagnostic of intermediate uveitis and posterior uveitis, respectively.	1) Vitritis 2) Retinitis 3) Choroiditis 4) Vitreous cell

AU, anterior uveitis; Drug-related SAE, drug-related serious adverse event; ETDRS, early treatment of diabetic retinopathy study; IPU, intermediate and posterior uveitis; Ocular SAE, ocular serious adverse event; TESAE, treatment-emergent serious adverse event; VA, visual acuity.
[a]The list of possible AEs we are looking for (not exhaustive).

the innate immune response to AAV.[15] It is possible that special drainage mechanism and organizational proximity to the vascular system may contribute to greater possibility for intravitreal delivery to present vector capsid antigens to the immune system.[64] This is in line with our findings that patients are more likely to have ocular and systemic immune responses when receiving IVT administration than SR administration. Although no differences were found in the incidence of ocular immune response with or without PI for either ROA, a lower frequency of systemic immune response was observed in patients receiving IVT with PI compared to those receiving IVT without PI. Thus, PI may be suggested to provide greater potentially safety benefits in patients treated with intravitreal delivery of AAV-based agents. The immunological processes in the eye that are triggered by gene therapy and its potential relationship to ROAs, as well as other elements of the drugs require further investigation for better knowledge and improvement of overall safety and efficacy of ocular AAV gene therapy.

AAV vectors are known to activate innate pattern recognition receptors (PRRs), such as Toll-like receptor 2 (TLR-2) and TLR-9, leading to the release of inflammatory cytokines and type I interferons.[49,67,68] Additionally, AAV vectors can elicit immune responses, including the generation of capsid-specific and transgene-specific T cells as well as neutralizing anti-AAV antibodies, which collectively constrain the therapeutic efficacy of AAV-based gene therapy.[49,63] However, the eye, as the target organ for retinal gene therapy, is an immune-privileged site. This immune privilege is characterized by reduced inflammation and enhanced immune tolerance, which may mitigate AAV-induced immune responses.[69,70] Our findings revealed no significant differences in the incidence of ocular immune response between patients receiving PI and those who did not, regardless of whether AAV was administered via IVT or SR injection. Ocular immune privilege and the suppression of immune responses to foreign antigens may contribute to the tolerance toward antigens derived from retinal gene therapy.[71] Meanwhile, retinal gene therapy requires relatively low vector titers, which might generally reduce the risk of deleterious responses and thus trimming the demand for systemic steroid. In addition, our results suggested that PI treatment was associated with a significantly lower incidence of systemic immune responses in patients receiving IVT delivery of AAV vectors. The ocular immune system's unique "immune privilege" has been redefined by intravital imaging and transcriptomics, uncovering specialized immune cell populations across eye tissues. Notably, while IVT delivery results in significant systemic biodistribution of the vector, SR injection may facilitate compartmentalization, potentially shielding the injection site from immune surveillance and promoting localized immunological benefits.[15,72]

The prophylactic immunosuppressive regimens used in clinical studies varied but generally followed a similar framework. As summarized in Table S4, oral prednisone was commonly administered at doses of 0.5–1.0 mg/kg/day, initiated 1–7 days prior to injection, followed by 1.0 mg/kg/day post-injection. Tapering of corticosteroids typically began after 1–2 weeks, with the total duration of treatment ranging from 13 to 63 days. This regimen aligns with the understanding that the risk of inflammation is highest during the early postoperative period, prior to the degradation of the vector capsids.[62,73] Further analyses stratified by the duration and intensity of corticosteroid treatment are necessary to establish standardized regimens, along with a comprehensive investigation into the immune responses elicited in diverse patient populations, ROA, and drug components.

Effective ocular gene therapies have the potential to deliver significant value beyond narrowly defined patient health and cost offsets to the health system but with an upfront high treatment cost. Although challenges remained in valuing long-lasting

benefits in ultrarare conditions, reports from economic analyses of LUXTURNA suggested that it is cost-effective compared with standard care using a lifetime horizon, even considering the indirect costs, including estimates based on productivity loss, caregiver burden and government program loss.[74] More evidence needs to be collected on long-term efficacy and safety outcomes, societal preference, and carer data, while considering broader societal value elements for health economic evaluation of these innovative therapies.[75,76]

The immunogenicity and safety concerns of rAAV pose a significant challenge to the clinical development of ocular gene therapies. Our findings indicate a trend toward greater diversity in AAV capsid selection since 2007, when the first ocular gene therapy was initiated. However, ubiquitous and photoreceptor-specific promoter has remained dominant. Systemic and ocular immune responses were generally more common in IVT (vs. SR). While no differences in ocular inflammation were observed with or without PI for either ROA, PI significantly reduced the incidence of systemic immune responses in patients receiving IVT. It is essential to establish standardized regimens and conduct a thorough investigation of immune responses across diverse patient populations, ROAs, and drug components. The potential systemic and ocular immune responses associated with various intervention factors should be carefully considered in future trial design. This review should help direct the choice of ROA and inform the consideration of immunosuppression in future clinical studies, while providing insights into the current trends in ocular rAAV-based gene therapy.

Limitations of the study

This review has several limitations. First, the limited patients and studies for AEs after gene therapy increased the uncertainty of our pooled incidence estimates especially in subgroup analysis. Second, with gene therapy currently in the early stages of development, the design is mostly small-sample studies with single-arm trials, and there is the potential for bias. Third, our investigation into the relationship between dose and immune responses were constrained by the high variability in drug dosage, and patient populations. This highlights the critical need for selection of dose and ROAs, as well as patient characteristics, including genetic background, disease progression, and treatment window.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources should be directed to and will be fulfilled by the lead contact, Prof Tong Li (litong_linda@outlook.com).

Materials availability

This study did not generate unique reagents.

Data and code availability

- The data used for the present meta-analysis were obtained from published studies and have been deposited at the Mendeley. DOI is listed in the key resources table.
- This study did not report original code.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

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AUTHOR CONTRIBUTIONS

Z.L., H.J., X.Z., T.L., and X.S. conceived and designed the study. Z.L., H.Z., H.J., and T.L. assisted with the data acquisition. Z.L., H.Z., H.J., Z.H., H.W., and T.L. performed the statistical analyses. Z.L., H.Z., H.J., Z.H., H.W., T.L., and X.S. interpreted the data. Z.L., Z.W., Z.H., and T.L. wrote the first draft of the paper. Z.L., H.J., X.Z., Y.T., J.H., T.L., and X.S. revised the paper. All of the authors approved the final version. All authors had full access to all of the study data and bear final responsibility for the decision to submit the paper for publication.

DECLARATION OF INTERESTS

X.S. reported being a consultant of Novartis, Roche, Alcon, Allergan, Bayer Healthcare, Innovent Biologics Inc., Kanghong Biotech Inc., and Carl Zeiss Meditec Inc. T.L. reported receiving grants from the National Nature Science Foundation of China and the Science and Technology Commission of Shanghai Municipality. X.S. reported receiving grants from the National Key R&D Program of China and the National Nature Science Foundation of China.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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- QUANTIFICATION AND STATISTICAL ANALYSIS

SUPPLEMENTAL INFORMATION

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Deposited data		
International prospective register of systematic reviews	PROSPERO	https://www.crd.york.ac.uk/PROSPERO/
Studies for meta-analysis	ClinicalTrials.gov, Embase and PubMed	The studies included are referenced in Table S5.
Raw data	This article	Mendeley Data, https://doi.org/10.17632/tkkh3styfy.1
Software and algorithms		
R Software version 4.3.0	Downloaded R software	https://cran.r-project.org/mirrors.html
GraphPad Prism version 9.5.1	Downloaded GraphPad Prism software	https://www.graphpad.com/updates
EndNote X9.1	Downloaded EndNote software	https://endnote.com/downloads

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Our study does not use experimental models typical in the life sciences.

METHOD DETAILS

Literature search

Studies on interventional trials of ocular rAAV-based gene therapy were retrieved from the following databases from inception to July 1, 2024, using a predefined search strategy (Table S6): ClinicalTrials.gov, Embase and PubMed. The search strategy was not limited by blinding, study size, and language.

Eligibility criteria

A trial was eligible for inclusion if the following criteria was met: (1) the study focused on patients with ocular diseases who received rAAV-based gene therapy; for publications, additional criteria were (2) study eyes for treatment are unilateral; (3) there was a clearly disclosed drug information and route of administration (ROA); and (4) the article reported the safety outcomes following gene therapy. If results from one trial were reported in several publications, data included in the meta-analysis were extracted from the publication that was considered the most appropriate to avoid using duplicate data. If a trial consisted of several phases and was reported separately in the publication, we included the publication from different phases separately. For trial with multiple publications, we list our reason for selection (Table S7). After removing duplicates, two reviewers (Z.L. and T.L.) independently screened titles, abstracts, and, if potentially eligible, full texts for inclusion. Disagreement was resolved by a third reviewer (H.J.).

Data extraction

Two reviewers (Z.L. and T.L.) independently extracted data from each eligible clinical trial protocol and publication; discrepancies were resolved by consensus arbitration with a third reviewer (H.J.). For each study, extracted data included: (1) study characteristics (condition, age, sex, start date, region, phase, status, funder type, study design and enrollment); (2) interventions (study eye, dose, ROA, drug information, and prophylactic immunosuppression regimen); and (3) details of adverse event (AE) and/or a specific safety outcome and/or immune response. A full list of data items abstracted is included in the data dictionary (Table S8). All the raw data are available in an Excel spreadsheet (Table S5).

Outcome measures

Safety outcomes of this study included incidence of AEs included treatment-emergent serious adverse event (TESAE), drug-related serious adverse event (drug-related SAE), ocular serious adverse event (ocular SAE), anterior uveitis (AU), and intermediate and posterior uveitis (IPU), if possible, the factors potentially associated with AEs were also explored. Definitions of AEs are provided in Table 6. In serious cases, uveitis is also considered an ocular SAE.

Quality assessment

Two reviewers (Z.L. and T.L.) independently assessed the quality of included publications for rating the risk of bias using the revised Cochrane risk-of-bias tool[77] (for randomized controlled trials) and the Newcastle-Ottawa Scale[78] (for single arm trials), respectively; Downs and Black Checklist[79] are used to uniformly assess the quality of all included publications (Tables S9 and S10).

ADDITIONAL RESOURCES

This review was pre-registered to PROSPERO (CRD42024591763) and followed the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) reporting guidelines.

QUANTIFICATION AND STATISTICAL ANALYSIS

The analysis of pooled incidence estimates takes into account the number of patients treated in each study. Before estimating, the variance of the raw incidence for each included study was stabilized by using the Freeman-Tukey double arc-sine transformation.[80] All estimates were presented after back transformation. We assessed heterogeneity of incidence estimates among studies using the Cochran Q test and I^2 index.[81,82] Heterogeneity was considered significant when $P < .05$ for Q test or values > 50% for I^2 index.[81–83] A Random-effects (DerSimonian and Laird method) or fixed-effects (Inverse variance method) meta-analysis was performed according to whether the heterogeneity was significant, and the overall pooled incidence of adverse events with 95% CIs was calculated throughout this study.[84] A leave-1-out sensitivity analysis for each meta-analysis was applied to examine whether single studies had a disproportionally excessive influence.[85] Publication bias was assessed by the funnel plot, Egger test, and Begg test when three or more estimates were available in a single analysis.[86–88] For TESAE, drug-related SAE, ocular SAE, AU and IPU, we conducted subgroup meta-analyses to determine the potential sources of heterogeneity. The false discovery rate (FDR) correction was used to ensure the reliability of the statistical significance.[89] Fisher exact test was performed to determine if there is a significant difference in systemic immune responses in trials that use different ROAs with PI or not. All raw data were collected using Excel 2021 (Microsoft, Seattle, WA, USA). All analyses and figures were generated using R version 4.3.0 (R Software, Vienna, Austria) and GraphPad Prism version 9.5.1 (GraphPad Software, San Diego, California, USA). Two-sided P values less than .05 were considered statistically significant.