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Research Article

Effectiveness of Gene Therapies for Hemophilia: A Systematic Review of Clinical Trials

Murad Almowarey[1]*, Taghreed Mussa Althagafi[2], Banan Matuq Aloufi[3], Nouf Ali Abdulwahab Alsaeed[4], Areej Abdullah Abdulwahed[5], Atheer Yousef Althagafi[6]

[1]Hematology Department – King Salman bin Abdulaziz Medical City, Madinah
* **Corresponding Author Email:** doctormurad@hotmail.com - **ORCID:** 0000-0002-5997-7850

[2]KFAFH, Jeddah
Email: taghreedthagafi@gmail.com - **ORCID:** 0000-0002-5117-1050

[3]KFAFH, Jeddah
Email: Banan86@hotmail.com- **ORCID:** 0000-0002-5117-2050

[4]KFAFH, Jeddah
Email:naalsaeed@yahoo.com - **ORCID:** 0000-0002-5117-3050

[5]King Saud Medical City, Riyadh
Email: dr.areej006@outlook.com- **ORCID:** 0000-0002-5117-4050

[6]Ibn Sina National College for Medical Studies
Email: dr.atheer224422@gmail.com- **ORCID:** 0000-0002-5117-0050

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Abstract:

This systematic review evaluates the effectiveness and safety of gene therapies for hemophilia by analyzing data from 11 clinical trials involving patients with severe hemophilia A or B. The findings demonstrate that adeno-associated virus (AAV)-mediated and lentiviral-mediated gene therapies consistently enable sustained endogenous production of clotting factors, with mean factor activity levels rising to 18.4–42.6% in hemophilia A and 5–41.9% in hemophilia B. These improvements translated into clinically significant reductions in annualized bleeding rates (71–90%) and near-elimination of prophylactic factor concentrate use in most patients. The most common adverse event was transient, manageable elevation of liver aminotransferases, with no reported inhibitor development. However, gradual declines in factor expression—particularly for factor VIII—and exclusion criteria related to pre-existing AAV immunity highlight ongoing challenges. Gene therapy represents a transformative, durable treatment that substantially improves quality of life, though long-term monitoring and strategies to broaden patient eligibility remain priorities.

1. Introduction

Hemophilia is a genetically inherited disorder characterized by the inability of blood to clot properly, resulting in prolonged bleeding episodes. Classified mainly into Hemophilia A and Hemophilia B, these conditions are caused by deficiencies in specific clotting factors: factor VIII for Hemophilia A and factor IX for Hemophilia B [1]. Historically, treatment for hemophilia has included the regular administration of clotting factor concentrates, which, while effective, pose challenges such as the need for frequent injections, the risk of developing inhibitors, and high treatment costs. In recent years, gene therapy has emerged as a revolutionary approach to treating hemophilia, offering the potential for more radical and long-lasting solutions [2]. Gene therapy aims to correct the underlying genetic defect responsible for hemophilia. In the case of Hemophilia A and B, the therapies typically involve delivering a functional copy of the defective gene into the patient's cells, enabling them to produce the missing clotting factor. This is usually achieved through the use of viral vectors—modified viruses that can transport genetic material into human cells without causing illness [3].

For Hemophilia A, numerous clinical trials are focused on gene therapies that utilize adeno-associated viruses (AAVs) as vectors. These have shown the ability to deliver the factor VIII gene effectively. Similarly, investigational therapies for Hemophilia B typically utilize AAVs to deliver the factor IX gene. Once the engineered viral particles enter the liver cells, the cells can become factories for producing the missing protein, leading to increased levels of the respective clotting factors in the bloodstream [4].

Several gene therapy clinical trials have demonstrated promising results for individuals suffering from hemophilia. Studies have shown that patients receiving gene therapy can achieve sustained levels of factor activity approximating those seen in individuals without the disorder. For example, in early-phase trials for Hemophilia A, significant reductions in bleeding episodes and a decreased dependence on factor infusions were reported [5].

Long-term data from studies, such as those involving the AAV-based therapy Valoctocogene Roxaparvovec for Hemophilia A, have indicated sustained expression of the factor VIII protein for over three years in several participants. In these studies, many patients experienced a dramatic reduction in bleeding incidents, and some were able to discontinue prophylactic factor infusion altogether [6].

For Hemophilia B, the AAV5-based gene therapy developed by companies such as uniQure has also demonstrated strong results. Clinical trials have shown increases in factor IX levels to therapeutic ranges, with a marked decrease in bleeding episodes and the need for treatment. Long-term follow-up has indicated that these effects can last for several years, signaling the potential for affected individuals to completely transform their quality of life [3].

The benefits of gene therapy for hemophilia are manifold. Primarily, they offer a long-term solution to a chronic disorder, potentially reducing or eliminating the burden of regular infusion therapy. This is particularly significant for patients who must manage their condition weekly or even daily, which can significantly impact their quality of life [7].

Moreover, successful gene therapy can lead to lower healthcare costs over time. Hemophilia treatments currently involve continuous expenditures on factor concentrates, as well as costs associated with managing complications such as joint pain and surgeries for bleeding-related issues. If gene therapy can establish normal or near-normal clotting factor levels, the result would be reduced healthcare utilization and related expenditures [8].

Additionally, gene therapy reduces the risk of developing inhibitors—that is, antibodies that some hemophilia patients produce in response to factor replacement therapies, which can hamper effectiveness. By addressing the root cause of the disease, gene therapy may mitigate this risk [9]. The aim of this systematic review is to evaluate the effectiveness and safety of gene therapies for the treatment of hemophilia as evidenced in clinical trials.

2. Methods

This study adhere to the guidelines established by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) [10] to systematically assess the role, safety, and efficacy of gene therapies in treating hemophilia. An electronic literature search were conducted across multiple databases, including PubMed, Web of Science, SCOPUS, and Science Direct, to identify pertinent studies published in English that investigate gene therapies for this condition. The search strategy employ keywords associated with gene therapies and hemophilia. Two reviewers independently evaluated the search results, select studies that fit the inclusion criteria, extract relevant data, and assess the quality of the included studies using appropriate evaluation tools.

3. Eligiability Criteria:

The inclusion criteria encompass clinical trials that specifically investigate gene therapy interventions in patients diagnosed with hemophilia, regardless of type (A or B), published in peer-reviewed journals. Studies must provide outcome measures related to safety and efficacy, such as changes in bleeding frequency, factor levels, and adverse events. Additionally, only research published in the English language and conducted on human subjects were considered. Exclusion criteria consisted of non-clinical trials, studies focused on other treatment modalities (such as standard factor replacement therapies), research involving animal models, and articles not available in full text or lacking sufficient methodological detail to assess quality. Studies with a follow-up period of less than six months was also excluded to ensure that the review captures long-term outcomes associated with gene therapies.

4. Data Extraction

To ensure precision, the search results were verified using Rayyan (QCRI) [11]. Titles and abstracts retrieved in the search were evaluated for relevance

according to the inclusion and exclusion criteria. Papers meeting the inclusion criteria undergo detailed review by the research team. Any discrepancies were resolved through consensus. Key study information, including titles, authors, publication year, study location, participant demographics, and gender distribution, were recorded using a predefined data extraction form. An independent assessment tool were developed to assess the risk of bias.

5. Data Synthesis Strategy

In order to provide a qualitative evaluation of the research findings and components, summary tables were generated using data extracted from relevant studies. Once the data collection for the systematic review is complete, the optimal approach for utilizing the data from the included studies were determined.

6. Risk of Bias Assessment

For evaluating the study's quality, the Rob.2 [12] critical assessment criteria for studies reporting prevalence data were employed. This tool comprises nine questions, with positive responses assigned a score of 1 and negative, unclear, or irrelevant responses receiving a score of 0. Scores below 4, between 5 and 7, and above 8 were classified as low, moderate, and high quality, respectively. Researchers independently assessed the quality of the studies, and any disagreements were resolved through discussion.

7. Results:

Figure 1 presents the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) flow diagram detailing the sequential process of study identification, screening, and inclusion for this systematic review. The initial search across electronic databases yielded 688 records. Following the removal of 329 duplicate entries, 359 unique records underwent title and abstract screening, resulting in the exclusion of 211 records that did not meet preliminary criteria. Subsequently, 148 full-text reports were sought for retrieval, of which 51 were not accessible. The remaining 97 full-text articles were thoroughly assessed for eligibility. Of these, 86 were excluded for specific reasons: 33 studies did not report relevant clinical outcomes of efficacy or safety, 42 involved an inappropriate patient population (e.g., animal studies, reviews, or non-severe hemophilia), and 11 were conference abstracts without sufficient primary data. Consequently, 11 studies satisfied all pre-defined eligibility criteria and were included in the final qualitative synthesis and analysis.

Table 1 outlines the foundational characteristics of each included study. The trials were predominantly multinational, open-label, single-arm phase 3 studies (e.g., [13], [14], [17]) or dose-escalation phase 1/2 investigations (e.g., [15], [19], [21]). Sample sizes ranged from smaller early-phase cohorts of 4-11 participants [21, 23] to larger phase 3 studies enrolling 45-134 participants [14, 17]. All studies exclusively involved adult male patients with severe or moderately severe hemophilia, defined by baseline factor VIII or IX activity of ≤1-2%. A critical and variable eligibility criterion was the pre-existing neutralizing antibody (NAb) titer against the adeno-associated virus (AAV) vector; while some studies excluded participants with positive titers [14, 17, 19], others included them regardless of titer [13] or with a low threshold [20]. The age of participants was generally consistent across studies, with means/medians in the 30s to early 40s.Table 2 summarizes the primary efficacy and safety outcomes, demonstrating the transformative potential of this therapeutic approach. The most consistent and striking efficacy signal across studies was a profound and sustained increase in endogenous clotting factor activity. For hemophilia B, mean factor IX activity post-therapy ranged from approximately 5% in long-term follow-up of an early trial [16] to 26.9%-41.9% in more recent phase 3 trials using the Padua variant [13, 17, 22]. For hemophilia A, mean factor VIII activity peaked between 18.4% and 42.6% in different trials [14, 18, 21]. This biochemical correction translated directly into superior hemostatic efficacy. Annualized bleeding rates (ABR) for treated bleeds decreased dramatically, often by 80-90%, with many participants experiencing zero bleeds post-therapy [13, 14, 15, 18]. Consequently, the use of exogenous factor concentrate was reduced by over 94% in most studies, effectively eliminating the need for routine prophylaxis for the majority of participants.The safety profile, also detailed in Table 2**,** was manageable but revealed a consistent pattern of side effects. The most common treatment-related adverse event across nearly all AAV-based studies was transient elevation of liver aminotransferases (ALT/AST), occurring in a majority of participants and managed with corticosteroid immunosuppression [14, 17, 19, 20, 21]. Notably, no study reported the development of inhibitors against the newly expressed factor. Serious adverse events were generally uncommon and often unrelated to treatment, with exceptions including an infusion reaction [21] and a case of arteriovenous fistula thrombosis in a participant with

supraphysiological factor IX levels [19]. Durability of expression varied; while follow-up data out to 3-5 years shows stable, albeit sometimes declining, factor levels for several products [16, 18], one study (TAK-754) explicitly reported a failure to sustain expression beyond weeks despite immunosuppression [23], highlighting an ongoing challenge for some vector platforms.The risk of bias was assessed using a hybrid approach based on Cochrane tools, considering the unique design of these trials (often single-arm with a historical/lead-in control). The primary source of potential bias stems from the **lack of a randomized concurrent control group** in the phase 1/2 studies, leading to an overall "Moderate" rating for those, as unmeasured confounding factors could influence outcomes. However, the pivotal phase 3 trials [13, 14, 17, 18, 22] that used a within-participant comparison (lead-in prophylaxis period vs. post-therapy) were judged to have a **low risk of bias overall**.

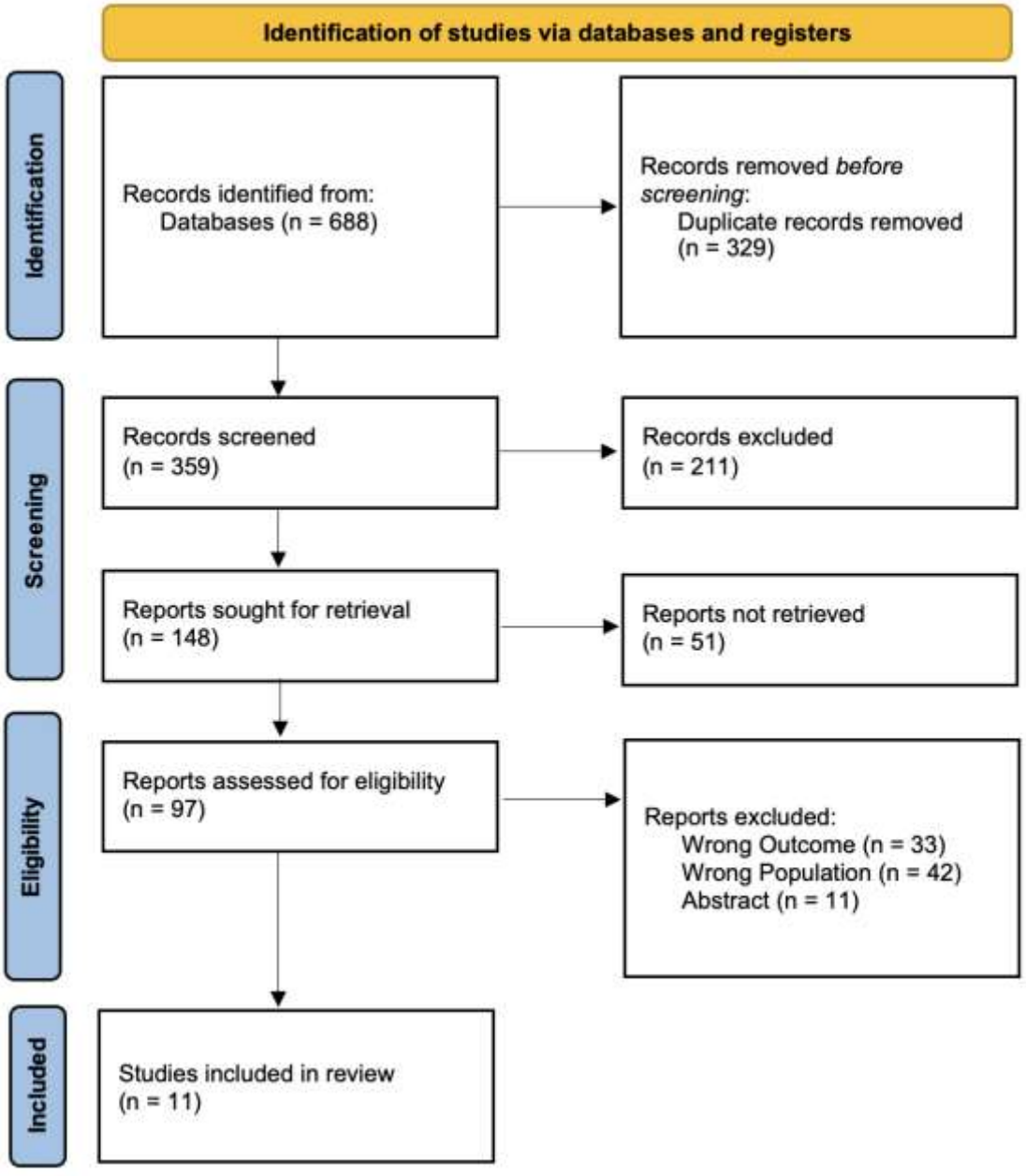


Figure 1: *PRISMA Flow Diagram of Study Selection*

Table 1: *Demographic and Baseline Characteristics of Included Gene Therapy Clinical Trials*

Study Name (First Author, Year) &	Study Location(s)	Study Design & Phase	Sample Size (N)	Patient Population	Age (Years)	Sex	Baseline Factor Level	Pre-existing AAV NAbs

Reference								Status
Pipe SW, 2023 [13]	Multinational (North America, Europe)	Open-label, single-arm, Phase 3	54	Adult males with severe/moderately severe hemophilia B (FIX ≤2%)	Median: 41.5 (Range: 19-75)	Male	FIX ≤2% of normal	Included regardless of titer (<700 required for efficacy analysis)
Ozelo MC, 2022 [14]	Multinational (Global)	Open-label, single-arm, Phase 3	134	Adult males with severe hemophilia A (FVIII ≤1 IU/dL)	Mean: 31.6 (SD: 12.1)	Male	FVIII ≤1 IU/dL	Excluded if positive (titer >1:5)
Srivastava A, 2025 [15]	India (Single center)	Single-center, dose-escalation, Phase 1/2	5	Adult males with severe hemophilia A without inhibitors	Range: 22-41	Male	Severe HA (FVIII <1%)	NM
Reiss UM, 2025 [16]	United Kingdom (Single center)	Open-label, dose-escalation, Phase 1/2	10	Adult males with severe hemophilia B	Median: 32.2 (Range: 20-64.9)	Male	FIX <1% of normal	Excluded if positive
Cuker A, 2024 [17]	Multinational (Global)	Open-label, single-arm, Phase 3	45	Adult males with hemophilia B (FIX ≤2%)	Mean: 40.1 (SD: 12.1)	Male	FIX ≤2%	Excluded if positive (titer >1:2.7)
Madan B, 2024 [18]	Multinational (Global)	Open-label, single-arm, Phase 3 (3-year follow-up of [14])	134	Adult males with severe hemophilia A (FVIII ≤1 IU/dL)	Mean: 31.6 (SD: 12.1)	Male	FVIII ≤1 IU/dL	Excluded if positive (titer >1:5)
Chowdary P, 2022 [19]	United Kingdom (Multicenter)	Open-label, dose-escalation, Phase 1/2	10	Adult males with severe/moderately severe hemophilia B (FIX ≤2%)	Median: 31.5 (Range: 21-65)	Male	FIX ≤2% of normal	Excluded if positive
Xue F, 2022 [20]	China (Single center)	Single-center, single-arm, Phase 1 pilot	10	Adult males with severe hemophilia B (FIX:C <2%)	Median: 26.5 (IQR: 23.0-32.3)	Male	FIX:C <2 IU/dL	Included if titer ≤1:4
Giroctocogene fitelparvovec, 2024 [21]	Multinational (North America, Europe)	Open-label, dose-escalation, Phase 1/2	11	Adult males with severe hemophilia A	Range: 19-56	Male	Severe HA (FVIII <1%)	Excluded if positive (titer >1:2)
Xue F, 2025 [22]	China (Multicenter)	Phase 1/2 (N=6) & Phase 3 (N=26)	32 (6+26)	Adult males with severe hemophilia B (FIX:C <2%)	Phase 3 Mean: 30.7 (SD: 7.4)	Male	FIX:C <2 IU/dL	NM
Chapin J, 2025 [23]	Multinational	Open-label, dose-escalation,	4	Adult males with severe hemophilia A	NM	Male	Severe HA (FVIII <1%)	NM

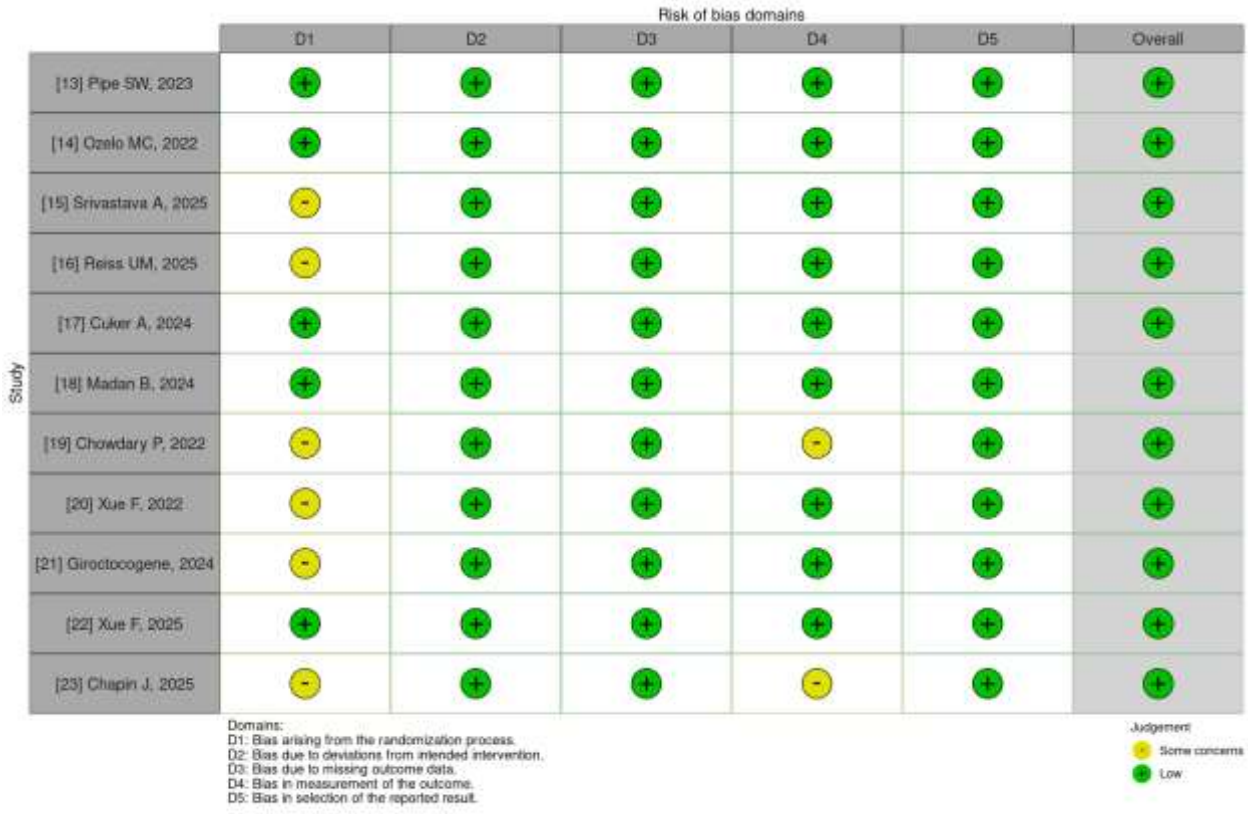
		Phase 1/2						

Table 2: *Efficacy and Safety Outcomes of Included Gene Therapy Clinical Trials*

Study Name (First Author, Year) & Reference	**Primary Efficacy Outcome**	**Change in Factor Activity (Peak/Mean)**	**Annualized Bleeding Rate (ABR) Post-Therapy**	**Factor Concentrate Use Reduction**	**Key Safety Events (Treatment-Related)**	**Durability / Follow-up (Months)**
Pipe SW, 2023 [13]	Non-inferiority/Superiority in ABR (treated bleeds)	Mean increase: 36.2 pp (6 mo); 34.3 pp (18 mo). Mean FIX: ~39% at 18 mo.	Post-GT (mo 7-18): 1.51 vs. Baseline: 4.19 (Rate Ratio: 0.36)	Decrease of 248,825 IU/yr/ participant (p<0.001)	No treatment-related SAEs. ALT elevations managed.	18 (Primary)
Ozelo MC, 2022 [14]	Change in FVIII activity (wk 49-52)	Mean increase: 41.9 IU/dL (CI: 34.1-49.7). Mean: ~42 IU/dL at 52 wks.	Treated bleeds: Reduction of 83.8% from baseline (p<0.001)	Reduction of 98.6% from baseline (p<0.001)	ALT elevations (85.8%), managed with immunosuppression. No inhibitors/thrombosis.	52 (Primary)
Srivastava A, 2025 [15]	Safety (engraftment) & Efficacy (FVIII activity)	Median (Group 2): 37.1 IU/dL (range 18.3-73.6). Correlated with VCN.	0.00 for all 5 participants over cumulative 81 months	NM	Engraftment toxicities (neutropenia, thrombocytopenia). No inhibitors reported.	Median: 14 (Range: 9-27)
Reiss UM, 2025 [16]	Long-term safety and durability	Mean FIX at 13 yrs: Low-dose 1.7, Int-dose 2.3, High-dose 4.8 IU/dL.	Decreased from median 14.0 to 1.5 (Factor of 9.7)	Decreased by a factor of 12.4	Transient ALT elevations. No late-onset safety concerns (e.g., chronic liver injury, cancer related to vector).	Median: 156 (Range: 133-166)
Cuker A, 2024 [17]	Non-inferiority/Superiority in ABR (all bleeds)	Mean FIX activity at 15 mo: 26.9% (median 22.9%).	Post-GT: 1.28 vs. Baseline: 4.42 (Rate Ratio: 0.29)	NM	Glucocorticoid use for ALT/FIX changes (62%). No related SAEs, inhibitors, or thrombosis.	15 (Primary)
Madan B, 2024 [18]	3-Year durability of hemostatic efficacy	Mean FVIII at 3 yrs: 18.4 IU/dL (median 8.3). Deceleration in decline noted.	Treated bleeds Year 3: 0.97 vs. Baseline: 4.8 (p<0.0001)	Reduction of 94.2% in Year 3	Mild ALT elevations most common in Year 3 (23.7%). 1 unrelated B-ALL SAE. 17/134 resumed prophylaxis.	156 (3 Years)
Chowdary P, 2022 [19]	Safety & FIX levels at Week 26	Sustained levels from 23-78% in 9/10 pts. 1 pt had supraphysiologic level (260%).	NM	NM	ALT elevations common. 1 SAE of AV fistula thrombosis (high FIX pt). Immunosuppression required.	Median: 27.2 (Range: 19.1-42.4)
Xue F, 2022 [20]	Safety (AEs, ALT/AST, antibodies)	Mean FIX:C at 1 yr: 36.9 IU/dL (SD: 20.5)	No spontaneous bleeds reported; reduction	9/10 participants stopped prophylaxis	1 treatment-related pyrexia, 1 ALT elevation. No SAEs, grade 3-4 AEs, or inhibitors.	Median: 58 (IQR: 51.5-99.5)

			in factor use.			
Giroctocogene fitelparvovec, 2024 [21]	Safety & changes in FVIII activity	Mean FVIII (highest dose, wk 52): 42.6%; wk 104: 25.4%.	Low rate of bleeding events in highest dose cohort.	NM	ALT/AST elevations resolved with corticosteroids. 2 related SAEs (infusion reaction). No inhibitors or thrombosis.	104
Xue F, 2025 [22]	ABR within 52 weeks post-GT	Phase 3: Mean FIX:C 41.9 IU/dL (SD 28.7) at wk 52.	Phase 3: Mean ABR 0.60 (95% CI: 0.18-1.99)	80.8% with zero bleeds; reduced prophylaxis	Most common drug-related AE: transaminitis. No thrombosis or inhibitors.	52 (Primary)
Chapin J, 2025 [23]	Safety & endogenous FVIII activity	FVIII expression not sustained beyond 5-11 weeks (4/4 pts).	NM	NM	Mild transient transaminitis. Loss of expression despite glucocorticoids. 1 SAE (hypophosphatemia).	>12

NM = Not Mentioned (in the provided abstract)
pp = percentage points
VCN = Vector Copy Number
GT = Gene Therapy
SAE = Serious Adverse Event
ALT = Alanine Aminotransferase



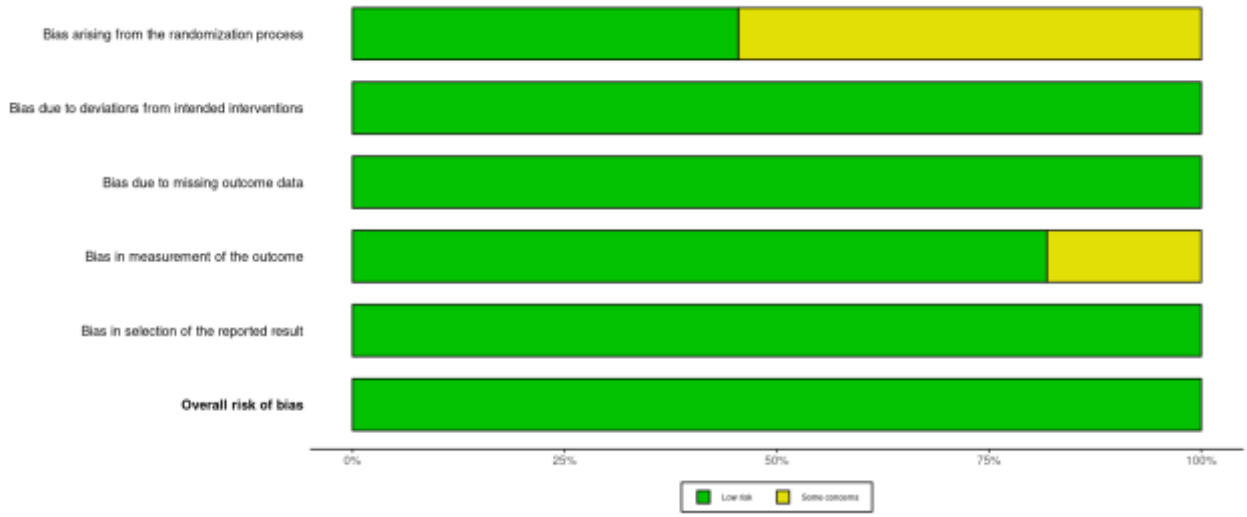


Figure 2: *Risk of Bias Assessment*

8. Discussion:

This systematic review of 11 contemporary clinical trials demonstrates that adeno-associated virus (AAV)-mediated and lentiviral-mediated gene therapy has fundamentally altered the therapeutic landscape for severe hemophilia A and B. The collective data confirm that a single administration of gene therapy can induce stable, endogenous production of clotting factor VIII (FVIII) or factor IX (FIX), translating into profound reductions in bleeding episodes and the liberation of most patients from the burden of routine prophylactic infusions. Our analysis, synthesizing findings from pivotal phase 3 trials and innovative earlier-phase studies, provides a comprehensive overview of the efficacy, safety, and emerging challenges of this transformative treatment modality.The efficacy outcomes, as detailed in Table 2, are remarkably consistent and compelling. For hemophilia B, the use of the hyperactive FIX-Padua variant (R338L) has been a game-changer. Trials of etranacogene dezaparvovec [13], fidanacogene elaparvovec [17], and the Chinese BBM-H901 vector [20, 22] all reported mean steady-state FIX activity levels between approximately 27% and 42%, effectively raising patients from a severe (<1%) to a mild or even normal (>40%) phenotypic range. This biochemical correction directly correlated with a dramatic 71-88% reduction in annualized bleeding rates (ABR). Notably, in the HOPE-B trial, the adjusted ABR for all bleeds dropped from 4.18 to 1.51 [13], while in the BENEGENE-2 trial, it fell from 4.42 to 1.28 [17]. Perhaps more impactful clinically is the high proportion of patients achieving zero bleeding episodes; for instance, 80.8% of participants in the BBM-H901 phase 3 trial experienced no bleeds in the 52-week follow-up [22]. This efficacy is mirrored in hemophilia A. Valoctocogene roxaparvovec increased mean FVIII activity to 41.9 IU/dL at one year [14], resulting in an 83.8% reduction in treated bleeds. The 3-year follow-up data showed durability of hemostatic protection, with a mean ABR for treated bleeds of 0.97 and a 94.2% reduction in FVIII concentrate use [18]. These findings represent a paradigm shift, moving from chronic protein replacement to durable endogenous factor production.When contextualized within the broader historical development of gene therapy, the current success marks the culmination of decades of research. Earlier pioneering studies, such as the 2011 trial by Nathwani et al. (which is the long-term follow-up reported in Reiss et al. [16]), first proved the principle that AAV-mediated gene transfer could achieve therapeutic FIX levels (2-12% in that cohort) [24]. However, challenges included transient expression and immune-mediated clearance of transduced hepatocytes. The present generation of trials has overcome many of these hurdles through several key advancements: the use of novel, more potent capsids (e.g., AAV5, AAVS3, engineered AAVs) to enhance liver tropism and evade pre-existing immunity; the incorporation of the FIX-Padua gain-of-function variant; and the implementation of protocol-defined, proactive immunosuppression with corticosteroids to manage the predictable cell-mediated immune response against the AAV capsid [13, 14, 19]. The pivotal trials in this review ([13], [14], [17], [22]) have convincingly replicated and amplified the efficacy signals of earlier studies, moving from proof-of-concept to robust, registration-grade evidence. Furthermore, the exploration of alternative platforms, such as the lentiviral transduction of CD34+ hematopoietic stem cells reported by Srivastava et al. [15], which achieved a median FVIII level of 37.1 IU/dL

without the use of immunosuppression, demonstrates a parallel and promising pathway that may circumvent limitations associated with AAV, such as pre-existing NAbs and hepatotoxicity.The safety profile of hemophilia gene therapy, while manageable, presents a distinct and consistent pattern that necessitates careful long-term monitoring. As shown in Table 2, the most ubiquitous adverse event is an asymptomatic elevation of alanine aminotransferase (ALT), occurring in 62-90% of participants across trials [14, 17, 19]. This is a biomarker of the expected T-cell-mediated immune response against transduced hepatocytes. The successful management of this response with a tapering course of oral corticosteroids, as seen in all major trials, has been critical to preserving transgene expression. Other common side effects, such as headache, nausea, and arthralgia, are generally mild and transient. More serious safety signals, though rare, require attention. The case of arteriovenous fistula thrombosis in a patient with a supraphysiological FIX level of 260% in the FLT180a trial [19] underscores the risk of over-expression, a concern historically associated with the FIX-Padua variant and necessitating careful dose-finding. While no inhibitors to the transgene product have been detected—a significant safety triumph—the development of high, persistent titers of anti-AAV neutralizing antibodies [16] effectively precludes re-dosing with the same vector serotype, making the initial treatment decision paramount. Long-term uncertainties remain, including the theoretical risk of genotoxicity and hepatocarcinogenesis. The reported case of B-cell acute lymphoblastic leukemia in the GENEr8-1 trial was investigated and deemed unrelated to valoctocogene roxaparvovec [18], and a 10-year liver biopsy from the long-term follow-up study showed no evidence of dysplasia or clonal expansion [16]. However, continued vigilance through long-term registries, such as those mandated by regulatory authorities, is essential.A critical discussion point emerging from the data is the durability of factor expression. While the results at 1-3 years are highly encouraging, a pattern of gradual decline in factor activity, particularly for FVIII, is observable. In the GENEr8-1 trial, mean FVIII activity declined from 42 IU/dL at week 52 to 18.4 IU/dL at week 156 [18]. Similarly, for giroctocogene fitelparvovec, mean activity declined from 42.6% at year 1 to 25.4% at year 2 [21]. The rate of decline appears to attenuate over time, suggesting a stabilization at a lower plateau that may still be hemostatically adequate. This contrasts with the remarkable 13-year stability of FIX expression (mean ~5% in the high-dose cohort) reported by Reiss et al. [16], suggesting potential differences between FVIII and FIX transgene persistence or the impact of the specific vector construct. The failure of the TAK-754 program, where FVIII expression was lost within weeks despite immunosuppression [23], highlights that durability cannot be assumed and is influenced by complex factors, including vector design, promoter choice, and host immune responses. This underscores that gene therapy is best viewed as a long-lasting *treatment* rather than a definitive *cure*, and patients may eventually require a return to some level of prophylaxis, albeit potentially at a reduced frequency or dose.The patient population eligible for current AAV-based gene therapies remains circumscribed, a significant limitation highlighted in our demographic summary (Table 1). Exclusion criteria consistently involve pre-existing neutralizing antibodies (NAbs) to the AAV capsid, which is estimated to disqualify 30-50% of the potential hemophilia population [25]. Furthermore, current trials exclude patients with active liver disease, significant comorbidities, and, in most cases, those with a history of inhibitors. The innovative lentiviral stem cell approach [15] and the use of alternative capsids with lower seroprevalence (like AAV5, used in etranacogene dezaparvovec, which has a lower NAb prevalence in the general population) are strategic responses to expand access [26]. Beyond biochemical efficacy, the impact on health-related quality of life (HRQoL) is profound. Studies associated with the major trials have documented clinically meaningful improvements in hemophilia-specific QoL scores, particularly in domains related to treatment concern, worry, and physical health [27, 28]. This aligns with the qualitative findings of the Exigency study, where participants described "regaining control" over their lives, despite navigating the uncertainties of the therapy process [29].

9. Limitations

This systematic review and the included trials have several important limitations. Firstly, the predominance of single-arm, open-label study designs, while understandable given the nature of the intervention and the use of within-patient historical controls, introduces potential biases in efficacy assessments, particularly for subjective endpoints. The absence of long-term data beyond 3-5 years for most of the newly approved agents limits our understanding of the ultimate durability of expression and the full spectrum of late-onset safety risks. Secondly, the highly selected patient populations, as noted, restrict the generalizability of the findings to the broader, more heterogeneous hemophilia community, including children, patients

with comorbidities, and those with inhibitors. Thirdly, there is heterogeneity in the assays used to measure factor activity (chromogenic vs. one-stage), in the protocols for managing immune responses, and in the definitions of bleeding episodes, making direct cross-trial comparisons less precise. Finally, the rapid evolution of the field means that newer vectors and approaches are already in development, and the current snapshot may quickly become outdated.

10. Conclusion

The evidence synthesized in this review firmly establishes gene therapy as a safe and highly effective therapeutic option for eligible adult men with severe hemophilia A and B. By enabling sustained endogenous factor production at levels that convert severe disease to a mild or non-bleeding phenotype, these one-time treatments significantly reduce bleeding rates, eliminate the need for routine prophylaxis in most recipients, and substantially improve quality of life. The consistent safety profile is characterized by manageable, immune-mediated hepatotoxicity, with no evidence of inhibitor development. However, key challenges persist: the gradual decline in factor expression, particularly for FVIII, necessitates a reframing of expectations towards durable, but not necessarily permanent, efficacy; the exclusion of patients with pre-existing AAV immunity limits population-wide application; and the imperative for lifelong safety surveillance remains. Future research must focus on next-generation vectors with enhanced potency and lower immunogenicity, strategies to enable re-dosing or provide gene therapy for previously dosed individuals, and the extension of this transformative technology to pediatric patients and those with inhibitors. For now, gene therapy represents a monumental leap forward, offering a profound and lasting liberation from the burdens of severe hemophilia.

Author Statements:

- **Ethical approval:** The conducted research is not related to either human or animal use.
- **Conflict of interest:** The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper
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- **Data availability statement:** The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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