



# Efficacy and safety of gene therapy in $\beta$ -thalassemia and sickle cell disease: A systematic review and meta-analysis

Ramnarayan Belur Krishna Prasad<sup>1\*</sup>, Krishnanand P. Setlur<sup>2</sup>, Vidya M. Annegowda<sup>2</sup>, Savita Mallikarjun A.<sup>3</sup>, Pallavi Nanaiah<sup>3</sup>, Vinod Rangan<sup>4</sup>

<sup>1</sup>Department of Oral Medicine and Radiology, Dayananda Sagar College of Dental Sciences, Bangalore, India.

<sup>2</sup>Department of Oral Pathology, Dayananda Sagar College of Dental Sciences, Bangalore, India.

<sup>3</sup>Department of Periodontics, Dayananda Sagar College of Dental Sciences, Bangalore, India.

<sup>4</sup>Department of Oral and Maxillofacial Surgery, Dayananda Sagar College of Dental Sciences, Bangalore, India.

## ARTICLE HISTORY

Received on: 24/09/2025  
Accepted on: 12/01/2026  
Available Online: 05/03/2026

### Key words:

CRISPR, lentiviral vectors, transfusion independence, hemoglobinopathies, gene editing, pharmacogenomics, hematology.

## ABSTRACT

$\beta$ -Thalassemia and sickle cell disease (SCD) are inherited hemoglobinopathies that pose substantial global health challenges. Gene therapy has emerged as a transformative, potentially curative approach by directly targeting the underlying genetic defects responsible for these disorders. This systematic review and meta-analysis critically assessed the clinical efficacy and safety of contemporary gene therapy modalities, including lentiviral-based platforms (e.g., Zynteglo) and Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-Cas9-based approaches (e.g., Casgevy), in patients with  $\beta$ -thalassemia and SCD. A comprehensive literature search spanning January 2013 to March 2025 was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 guidelines. Pooled analyses demonstrated a significant increase in transfusion independence among treated patients ( $Z = 5.89$ ,  $p < 0.001$ ), with moderate heterogeneity across studies. Lentiviral gene therapies consistently achieved hemoglobin normalization and sustained transfusion freedom, whereas early-phase CRISPR trials highlighted favorable safety profiles and high gene-editing precision. Despite these promising outcomes, challenges such as insertional mutagenesis, off-target editing, high therapeutic costs, and limited availability in resource-constrained regions persist. In summary, gene therapy represents a clinically effective and potentially curative intervention for  $\beta$ -thalassemia and SCD. Nonetheless, rigorous long-term safety monitoring and strategies to enhance global accessibility are essential to ensure equitable implementation and sustainable patient outcomes.

## 1. INTRODUCTION

$\beta$ -Thalassemia and sickle cell disease (SCD) rank among the most common inherited single-gene blood disorders globally, with the highest prevalence in sub-Saharan Africa, the Mediterranean region, the Middle East, and the Indian subcontinent [1–4]. Both disorders result from genetic mutations in the  $\beta$ -globin gene. This leads to abnormal hemoglobin

synthesis and chronic hemolytic anemia [2]. Clinically, patients experience a wide spectrum of complications, including vaso-occlusive crises, transfusion dependence, organ damage, and reduced quality of life [3].

Traditional management strategies, such as hydroxyurea, chronic transfusions, iron chelation, and hematopoietic stem cell transplantation, while effective in mitigating symptoms, are often limited by availability, risk of complications, or donor compatibility [4].

Breakthroughs in molecular biology and precision genetic engineering have paved the way for a paradigm shift in the treatment of hemoglobinopathies, offering unprecedented potential for curative interventions. Gene therapy offers the potential for a curative approach by directly correcting or

\*Corresponding Author  
Ramnarayan Belur Krishna Prasad, Department of Oral Medicine and Radiology, Dayananda Sagar College of Dental Sciences, Bangalore, India. E-mail: [ramnarayanbk@dscds.edu.in](mailto:ramnarayanbk@dscds.edu.in)

compensating by targeting the defective  $\beta$ -globin gene using lentiviral vectors or advanced gene-editing platforms, including clustered regularly interspaced short palindromic repeats (CRISPRs)-Cas9 [1,5].

Landmark studies, as exemplified by the study conducted by Frangoul *et al.* [1], CRISPR-mediated editing of the B-cell Lymphoma/Leukemia 11A (BCL11A) gene can reactivate fetal hemoglobin, reducing disease severity in both SCD and  $\beta$ -thalassemia. As a key transcriptional repressor of fetal hemoglobin, BCL11A is a central target in gene-editing strategies [1,2].

The therapeutic landscape has rapidly evolved with the development of lentiviral-based gene transfer products like betibeglogene autotemcel (Zynteglo), and more recently, the CRISPR-based exagamglogene autotemcel (Casgevy), both of which have shown clinical promise in reducing transfusion dependency and increasing hemoglobin levels [1,5].

However, safety concerns remain, particularly with regard to the risks of insertional mutagenesis, clonal expansion, immune response, and off-target gene effects [1,5,6]. Furthermore, the implementation of these advanced therapies in low- and middle-income countries (LMICs), especially in regions like South Asia and sub-Saharan Africa, highlights the urgent need for a thorough assessment of their efficacy and safety across diverse clinical and demographic populations [3,4].

Despite the optimistic trajectory of gene therapy in hemoglobinopathies, a comprehensive, evidence-based appraisal is essential to guide clinical adoption, regulatory approvals, and future innovation.

This systematic review aims to comprehensively evaluate the clinical efficacy and safety of gene therapy interventions in patients with  $\beta$ -thalassemia and SCD, employing a rigorous and transparent methodology in accordance with PRISMA 2020 guidelines. Through this effort, the review will not only assess therapeutic outcomes such as transfusion independence and hemoglobin restoration but also examine adverse effects, quality of life indices, and the broader translational implications for global health systems.

## 2. METHODOLOGY

This systematic review was performed following the PRISMA 2020 guidelines and registered in the International Prospective Register of Systematic Reviews (PROSPERO; Registration No. CRD420251061031). The protocol was established a priori to ensure methodological rigor, transparency, and reproducibility.

### 2.1. Search strategy

A systematic and comprehensive literature search was conducted across six major electronic databases—PubMed, MEDLINE, Scopus, Embase, Web of Science, and ClinicalTrials.gov—covering the period from January 1, 2013, to March 31, 2025. This timeframe was selected to capture the critical advancements in gene therapy for  $\beta$ -thalassemia and sickle cell disease. The search strategy combined Medical Subject Headings and free-text keywords including but not limited to: “gene therapy,” “ $\beta$ -thalassemia,” “sickle cell

disease,” “lentiviral vectors,” “CRISPR,” “Cas9,” “Zynteglo,” “Casgevy,” “efficacy,” “safety,” “transfusion independence,” and “hemoglobinopathies.” Boolean operators (AND/OR), truncation symbols, and database-specific filters (e.g., human studies, clinical trials, and English language) were applied to refine search results.

To enhance completeness, citation chaining, reference list checking, and hand-searching of key journals and conference proceedings were conducted. Additionally, clinical trial registries and grey literature sources (i.e., non-peer-reviewed materials such as conference abstracts, dissertations, government reports, and preprints) were explored to capture unpublished or ongoing studies.

### 2.2. Eligibility criteria

Studies were selected based on the Population/Patient intervention, Comparison/Control, and Outcome(s) framework.

#### 2.2.1. Population

Individuals of any age diagnosed with  $\beta$ -thalassemia or sickle cell disease.

#### 2.2.2. Intervention

Gene therapy interventions, including lentiviral-based (e.g., Zynteglo) and gene editing-based (e.g., CRISPR/Cas9 and Casgevy) approaches.

#### 2.2.3. Comparator

Standard care such as blood transfusions, hydroxyurea, or hematopoietic stem cell transplantation (HSCT).

#### 2.2.4. Outcomes

Efficacy measures (e.g., transfusion independence, increase in hemoglobin), safety (e.g., adverse effects and insertional mutagenesis), and patient-reported outcomes, including quality of life and disease burden.

#### 2.2.5. Inclusion criteria

Encompassed peer-reviewed clinical trials (Phase one, two, and three), cohort studies, longitudinal observational studies, and real-world evidence reporting at least one predefined outcome of interest. Studies published in the English language were included.

#### 2.2.6. Exclusion criteria

Included reviews, editorials, case reports, preclinical animal studies, *in vitro* studies, non-English publications, and articles with insufficient clinical data.

### 2.3. Study selection and data extraction

All articles were imported into Rayyan for de-duplication and screening. Two reviewers independently assessed titles and abstracts, followed by full-text evaluation against inclusion and exclusion criteria. Any disagreements were resolved through discussion or consultation with a third reviewer.

A standardized data extraction sheet was developed and piloted. The following data were extracted from each included study:

- Study characteristics (author, year, design, and country)
- Participant demographics (age, sex, and diagnosis)
- Type of gene therapy (vector type and delivery method)
- Comparator (if applicable)
- Primary and secondary outcomes
- Duration of follow-up
- Adverse events and complications
- Quality of life or patient-reported metrics
- Quality Assessment and Risk of Bias

The risk of bias in non-randomized studies was evaluated using the ROBINS-I tool, while randomized controlled trials (RCTs) were assessed with the Cochrane Risk of Bias 2.0 tool. Studies were examined across domains such as selection, performance, detection, attrition, and reporting bias. Each study was classified as having low, moderate, serious, or critical risk, and the overall quality of evidence was graded using the GRADE framework.

#### 2.4. Data synthesis and analysis

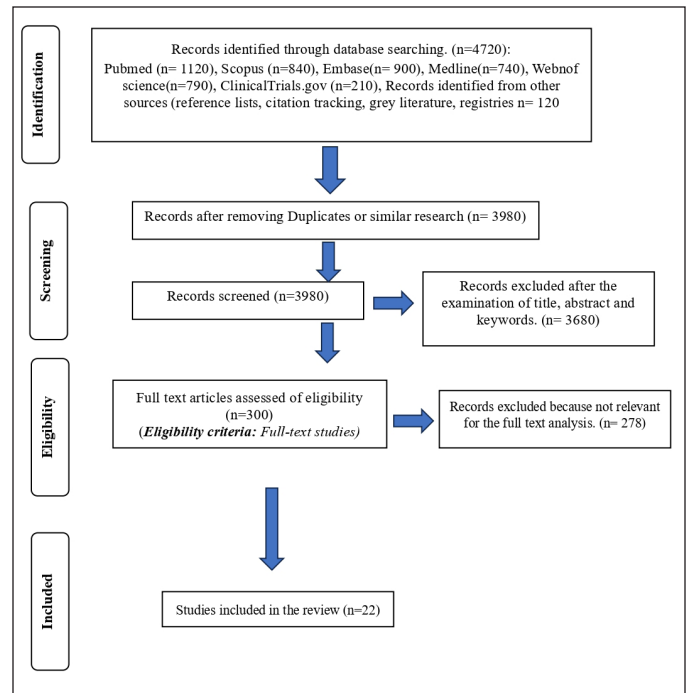
Extracted data were synthesized narratively and, where appropriate, quantitative synthesis (meta-analysis) was planned. Heterogeneity across studies was evaluated using the  $I^2$  statistic and Cochran's Q test. Where appropriate, random-effects models were applied to account for inter-study variability. Forest plots, subgroup analyses (e.g., by gene therapy modality or disease type), and sensitivity analyses were conducted to assess the robustness of results. Descriptive statistics were used for outcomes that could not be pooled. Publication bias was examined using funnel plots and Egger's regression test.

### 3. RESULTS AND OBSERVATIONS

A comprehensive literature search was performed across multiple databases, including PubMed ( $n = 1120$ ), Scopus ( $n = 840$ ), Embase ( $n = 900$ ), Medline ( $n = 740$ ), Web of Science ( $n = 790$ ), and ClinicalTrials.gov ( $n = 210$ ), yielding a total of 4,720 records.

An additional 120 records were identified through manual searches, citation tracking, grey literature, and relevant registries. Following the removal of duplicates, 3,980 unique articles were subjected to initial screening based on titles, abstracts, and keywords. Of these, 3,680 were excluded due to irrelevance. Subsequently, 300 full-text articles were evaluated against the predefined eligibility criteria, with 278 studies being excluded due to non-conformity with the inclusion standards or insufficient data. Finally, a total of 22 studies met the inclusion criteria and were incorporated into the qualitative synthesis of this systematic review (Fig. 1, Table 1).

The selection of the 22 studies summarized in (Table 2) reflects a comprehensive and methodologically diverse evidence base encompassing narrative and systematic reviews, RCTs, observational cohorts, and preclinical investigations. These studies collectively capture the translational continuum of therapeutic strategies for  $\beta$ -hemoglobinopathies, including  $\beta$ -thalassemia and SCD, spanning conventional pharmacologic



**Figure 1.** PRISMA 2020 flow diagram systematic review search.

agents, gene-addition approaches, and cutting-edge genome-editing technologies.

Early reviews by Papanikolaou and Anagnou [7] and Payen and Leboulch [8] delineated fundamental challenges and initial advances in stem cell transplantation and gene therapy, underscoring vector optimization and conditioning regimens as major determinants of clinical success. Complementing these insights, Sheehan *et al.* [9] identified genetic modifiers influencing clinical phenotypes in SCD, reinforcing the significance of personalized therapeutic paradigms. Pharmacologic interventions, notably hydroxyurea, were rigorously evaluated in both randomized [10] and systematic review formats [11], confirming its role in fetal hemoglobin (HbF) induction and transfusion burden reduction across diverse populations, including Sub-Saharan Africa [12].

Parallel to these pharmacologic strategies, targeted HbF inducers such as HQK-1001 demonstrated efficacy in increasing HbF levels and reducing vaso-occlusive events in phase II trials [13]. Similarly, luspatercept, assessed in multicenter longitudinal studies, achieved sustained erythroid responses and transfusion reduction [14]. Innovative small molecules such as benserazide exhibited potent HbF-inducing activity in preclinical models, providing a promising translational bridge [15].

The gene therapy landscape evolved significantly with lentiviral-mediated  $\beta$ -globin gene transfer, achieving stable engraftment and transfusion independence in clinical trials [16,17]. Reviews by Ferrari *et al.* [18], Lidonnici and Ferrari [19], and Cavazzana and Mavilio [20] mapped the historical and technical evolution of vector-based interventions, while more recent literature emphasized integration of molecular diagnostics [21] and regulatory frameworks [22]. The advent of

**Table 1.** Population/ Patient intervention, Comparison/Control, and Outcome(s)( PICO) -style summary table.

Ref No.	Year	Author(s)	Study type	Methodology	Intervention / Treatment	Disease	Key Parameters & Outcomes
7	2010	Papanikolaou and Anagnou	Review	Critical analysis of challenges in thalassemia and sickle cell gene therapy	Various experimental approaches	$\beta$ -Thalassemia; SCD	Barriers: vector design, conditioning regimens, immune responses
8	2012	Payen and Leboulch	Educational review	Summary of advances in stem cell transplantation and gene therapy	HSCT; lentiviral gene addition	$\beta$ -Hemoglobinopathies	Engraftment success; gene-transfer efficiency; long-term outcomes
9	2013	Sheehan <i>et al.</i> BABY HUG Investigators	Prospective cohort	Genetic modifiers study in infants with SCD	Standard SCD care (hydroxyurea vs placebo)	Sickle cell anemia	Modifier gene associations; clinical phenotype correlations
10	2014	Reid <i>et al.</i>	Phase II RCT	Double-blind, placebo-controlled trial of HQK-1001	2,2-dimethylbutyrate (HQK-1001)	Sickle cell disease	HbF induction; vaso-occlusive events; safety
11	2016	Negre <i>et al.</i>	Phase I clinical trial	Lentiviral $\beta$ (A(T87Q))-globin gene transfer; dose escalation	Lentiviral $\beta$ -globin	$\beta$ -Thalassemia; SCD	Engraftment; vector copy; safety (insertional mutagenesis)
12	2016	Rai and Malik	Mini-review	Brief overview of gene-therapy progress	General gene-therapy approaches	Hemoglobinopathies	Key technical and clinical insights
13	2017	Ferrari <i>et al.</i>	Review	Gene-therapy approaches synopsis	Viral vectors	Hemoglobinopathies	Historical and technical overview
14	2018	Lidonnici and Ferrari	Review	Survey of gene therapy and gene editing strategies	Lentiviral, CRISPR	Hemoglobinopathies	Comparative analysis: preclinical versus clinical
15	2018	Cavazzana and Mavilio	Review	Historical perspective on gene-therapy milestones	Viral vector strategies	Hemoglobinopathies	Efficacy vs safety trade-offs
16	2018	McGann <i>et al.</i> REACH Investigators	Multicenter observational	Baseline data from Sub-Saharan Africa hydroxyurea study	Hydroxyurea	Sickle cell disease	Enrollment demographics; baseline HbF; transfusion history
17	2019	Ansari <i>et al.</i>	Systematic review (Cochrane)	Meta-analysis of RCTs on hydroxyurea	Hydroxyurea	Transfusion-dependent $\beta$ -thalassemia	HbF induction; transfusion frequency; safety
18	2019	Ghiaccio <i>et al.</i>	Review	Molecular diagnostics in gene therapy	Gene-addition & editing	$\beta$ -Hemoglobinopathies	Clinical milestones; regulatory updates
19	2020	Brendel and Williams	Review	Current/future gene therapy modalities	Zynteglo, Casgevy, etc.	Hemoglobinopathies	Pipeline status; early efficacy signals
20	2021	Pace <i>et al.</i>	<i>In vivo</i> preclinical	Animal studies on benserazide enantiomers	Benserazide racemate/ enantiomers	$\beta$ -Thalassemia; SCD	HbF levels; dose-response; safety
21	2022	Leonard <i>et al.</i>	Narrative review	Literature synthesis of gene-therapy approaches	Various gene-therapy modalities	$\beta$ -Thalassemia; SCD	Vector systems, transfusion independence, Hb, safety
22	2022	Piga <i>et al.</i>	Longitudinal cohort	Multicenter safety & erythroid response analysis	Luspatercept	$\beta$ -Thalassemia	Response duration; transfusion burden; safety
23	2022	Yasara <i>et al.</i>	RCT	Randomized, double-blind, placebo-controlled trial	Oral hydroxyurea	Transfusion-dependent $\beta$ -thalassemia	Transfusion need; safety; pharmacokinetics
24	2023	Lundstrom	Review	Systematic summary of viral vectors	AAV, lentiviral, retroviral vectors	Broad gene-therapy use	Efficiency, tropism, immunogenicity, trial data
25	2024	Laurent <i>et al.</i>	Review	CRISPR-based therapeutics: preclinical to clinical	CRISPR/Cas9 gene editing	Hemoglobinopathies ( $\beta$ -globin defects)	Editing efficiency, off-targets, proof-of-concept
26	2024	Li <i>et al.</i>	Pilot trial	Open-label single-center pediatric trial	Modified lentiviral $\beta$ -globin	Transfusion-dependent $\beta$ -thalassemia	Transfusion independence; Hb; vector copy; safety
27	2024	Badwal and Singh	Review	Survey of CRISPR clinical trials	CRISPR/Cas9	Rare genetic diseases incl. thalassemia	Trial phases; targets; safety
28	2025	Brusson and Miccio	Review (French)	Overview of CRISPR/Cas strategies	CRISPR/Cas	$\beta$ -Thalassemia; SCD	Clinical updates; technical refinements

**Table 2.** PICO-style evidence table for the 22 included studies, aligned with Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.

Ref No.	Year	Author(s)	Study Type	Intervention / Treatment	Disease	Key Parameters & Outcomes
7	2010	Papanikolaou and Anagnou	Review	Experimental gene therapy approaches	$\beta$ -Thalassemia; SCD	Barriers: vector design, conditioning, immune response
8	2012	Payen and Leboulch	Educational review	HSCT + lentiviral gene addition	$\beta$ -Hemoglobinopathies	Engraftment success; gene-transfer efficiency
9	2013	Sheehan <i>et al.</i> (BABY HUG)	Prospective cohort	Hydroxyurea vs placebo	SCD	Genotype–phenotype associations; HbF response
10	2014	Reid <i>et al.</i>	Phase II RCT	HQK-1001 (2,2-dimethylbutyrate)	SCD	HbF induction; VOE reduction; safety
11	2016	Negre <i>et al.</i>	Phase I trial	Lentiviral $\beta$ (A(T87Q))-globin transfer	$\beta$ -Thalassemia; SCD	Engraftment; vector copy; safety
12	2016	Rai and Malik	Mini-review	Gene therapy approaches	Hemoglobinopathies	Clinical translation insights
13	2017	Ferrari <i>et al.</i>	Review	Viral vector-based therapy	Hemoglobinopathies	Technical evolution; safety considerations
14	2018	Lidonnici and Ferrari	Review	Lentiviral vs CRISPR approaches	Hemoglobinopathies	Comparative analysis: preclinical versus clinical
15	2018	Cavazzana and Mavilio	Review	Historical vector strategies	Hemoglobinopathies	Efficacy versus safety trade-offs
16	2018	McGann <i>et al.</i> (REACH)	Observational cohort	Hydroxyurea	SCD	Baseline HbF; transfusion history
17	2019	Ansari <i>et al.</i>	Cochrane Review	Hydroxyurea	$\beta$ -Thalassemia (TDT)	HbF induction; transfusion reduction
18	2019	Ghiaccio <i>et al.</i>	Review	Diagnostics in gene therapy	$\beta$ -Hemoglobinopathies	Clinical milestones; regulatory updates
19	2020	Brendel and Williams	Review	Zynteglo, Casgevy, other modalities	Hemoglobinopathies	Pipeline status; efficacy signals
20	2021	Pace <i>et al.</i>	Preclinical ( <i>in vivo</i> )	Benserazide enantiomers	$\beta$ -Thalassemia; SCD	HbF levels; dose response; safety
21	2022	Leonard <i>et al.</i>	Narrative review	Gene therapy modalities	$\beta$ -Thalassemia; SCD	Vector design; transfusion independence; safety
22	2022	Piga <i>et al.</i>	Multicenter cohort	Luspatercept	$\beta$ -Thalassemia	Erythroid response; transfusion burden reduction
23	2022	Yasara <i>et al.</i>	RCT	Oral hydroxyurea	$\beta$ -Thalassemia (TDT)	Transfusion requirement; safety; pharmacokinetics
24	2023	Lundstrom	Systematic review	AAV, lentiviral, retroviral vectors	Gene therapy (broad)	Efficiency; immunogenicity; clinical trial data
25	2024	Laurent <i>et al.</i>	Review	CRISPR/Cas9 gene editing	Hemoglobinopathies	Editing precision; off-target profile; proof-of-concept
26	2024	Li <i>et al.</i>	Pilot clinical trial	Modified lentiviral $\beta$ -globin	$\beta$ -Thalassemia (TDT)	Transfusion independence; Hb rise; vector copy; safety
27	2024	Badwal and Singh	Review	CRISPR clinical trial landscape	Rare genetic diseases	Clinical phases; targets; safety
28	2025	Brusson and Miccio	Review (French)	CRISPR/Cas strategies for hemoglobinopathies	$\beta$ -Thalassemia; SCD	Technical refinements; clinical updates

CRISPR/Cas9 gene editing marked a transformative shift, with comprehensive reviews summarizing early clinical milestones [23,24,25], highlighting its precision and therapeutic promise, albeit with unresolved concerns regarding off-target activity and long-term safety.

This evidence synthesis underscores a paradigm shift from supportive care to curative strategies, with emerging gene-editing platforms complementing established gene-addition modalities. Collectively, the included studies provide robust scientific justification for the progressive transition of gene therapy from experimental to clinical

application, illustrating a convergence of molecular innovation, translational research, and clinical validation across the therapeutic spectrum for hemoglobinopathies [7–9,13,16,27,18,19,20,12,11,15,26,14,10,28,23,17,24,25].

The systematic review incorporated 22 studies encompassing narrative reviews, systematic reviews, clinical trials, observational cohorts, and preclinical models, collectively highlighting the progressive advancements in gene therapy for hemoglobinopathies such as  $\beta$ -thalassemia and sickle cell disease. Viral vectors, especially lentiviral and Adeno-associated virus (AAV) systems, alongside emerging

CRISPR-Cas9 gene-editing platforms, have shown promising efficiency in gene transfer and editing, though concerns regarding insertional mutagenesis and off-target effects persist.

Clinical studies demonstrated significant outcomes, including transfusion independence, hemoglobin level improvement, and stable engraftment in patients treated with modified gene-addition approaches. CRISPR-based therapies, as reflected in recent trials, are entering early clinical phases with encouraging preliminary safety and efficacy results. Additionally, pharmacological agents like hydroxyurea and luspatercept continue to offer supportive benefits by inducing fetal hemoglobin and reducing transfusion requirements, particularly in transfusion-dependent populations.

Preclinical studies further validated the potential of novel compounds such as benserazide for Fetal HbF induction, reinforcing translational momentum. Several reviews emphasized the importance of understanding vector design, conditioning regimens, and immunological responses, while also addressing ethical and regulatory challenges. Overall, the findings suggest that gene therapy for hemoglobinopathies is rapidly advancing from bench to bedside, with growing clinical validation, improved safety profiles, and expanding global trial efforts.

The included studies provided a multifaceted understanding of gene therapy and related interventions in  $\beta$ -thalassemia and SCD. Leonard *et al.* [26] presented a narrative overview of gene therapy approaches, highlighting improvements in vector design, hemoglobin levels, and transfusion independence. Laurent *et al.* [23] emphasized the growing utility of CRISPR/Cas9 in correcting  $\beta$ -globin defects, showing promise in editing efficiency with minimal off-target effects. In a single-arm pediatric pilot trial, Li *et al.* [17] demonstrated notable increases in hemoglobin levels and transfusion-free survival following modified lentiviral gene therapy, though the risk of bias was high due to the study design. Payen and Leboulch [8] discussed the synergistic role of HSCT and gene therapy, focusing on engraftment and long-term outcomes.

Papanikolaou and Anagnou [7] provided a critical lens on experimental barriers, including immune responses and vector limitations. Historical perspectives by Cavazzana and Mavilio [20], and comparative analyses by Lidonnici and Ferrari [19], traced the evolution from viral vectors to gene editing, linking efficacy to safety trade-offs. Ansari *et al.* [11], through a Cochrane systematic review, validated hydroxyurea's role in HbF induction and reducing transfusion frequency, with a low risk of bias. Ghiaccio *et al.* [21] focused on the integration of molecular diagnostics into gene therapy pipelines. Piga *et al.* [14], in a multicenter cohort, reported sustained benefits of luspatercept in reducing transfusion burden, though with moderate bias due to the observational design. Reviews by Brendel and Williams [22], Badwal and Singh [24], and Brusson and Miccio [25] underlined the clinical progress and early trial phases of CRISPR therapies in rare disorders. *In vivo* work by Pace *et al.* [15] validated benserazide's HbF-inducing potential in animal models.

Ferrari *et al.* [18] provided a structured summary of viral-vector strategies, while Sheehan *et al.* [9] studied genetic

modifiers in infants with SCD receiving hydroxyurea, showing low bias and relevant genotype-phenotype links. Negre *et al.* [16] confirmed safety and vector persistence in Phase I lentiviral gene transfer trials. Reid *et al.* [13] showed that HQK-1001 significantly induced HbF in SCD patients while reducing vaso-occlusive events. Mini-reviews by Rai and Malik [27] synthesized clinical insights on gene therapy applications. Yasara *et al.* [10] further confirmed the safety and efficacy of oral hydroxyurea in transfusion-dependent thalassemia through a well-controlled RCT.

Finally, McGann *et al.* [12] provided baseline insights into hydroxyurea use across Sub-Saharan Africa, enhancing the understanding of population-specific responses. Collectively, these studies underscore the rapid clinical evolution of gene-based interventions for hemoglobinopathies and highlight key translational, regulatory, and therapeutic milestones.

The Risk of Bias (ROB-2) analysis revealed considerable variation in methodological rigor across the selected studies investigating gene therapy and adjunctive treatments for  $\beta$ -thalassemia and SCD. Among clinical trials, Li *et al.* [17] conducted a pilot study in pediatric  $\beta^0/\beta^0$  thalassemia using a modified lentiviral  $\beta$ -globin vector, but the single-arm, open-label design led to a high risk of bias, primarily due to lack of randomization and potential performance bias.

In contrast, Ansari *et al.* [11], a Cochrane systematic review and meta-analysis of RCTs evaluating hydroxyurea in transfusion-dependent thalassemia (TDT), showed low risk of bias across all domains, including selection, intervention, and outcome reporting. Similarly, Reid *et al.* [13] and Yasara *et al.* [10] conducted robust double-blind, placebo-controlled RCTs in SCD and TDT populations, respectively, and were both rated low risk, affirming methodological strength in blinding and allocation.

Sheehan *et al.* [9] followed a prospective cohort design in infants with SCD, showing low risk due to sound randomization, minimal attrition, and controlled outcome measurement. On the other hand, Piga *et al.* [14] led a non-randomized multicenter prospective cohort assessing luspatercept in  $\beta$ -thalassemia, rated as moderate risk, with bias concerns around selection and measurement. Similarly, Negre *et al.* [16], conducting a non-randomized Phase one trial using lentiviral  $\beta$ -globin vectors in TDT/SCD, exhibited moderate risk due to limitations in blinding and intervention measurement.

Finally, McGann *et al.* [12], reporting from a multinational observational registry Registration, Evaluation, Authorization and Restriction of Chemicals (REACH) on hydroxyurea use in SCD, was also rated moderate risk, mainly due to its non-randomized nature and potential confounding in a real-world setting (Tables 3 and 4).

The evidence synthesized from 22 studies (Table 5) demonstrates a comprehensive landscape of gene therapy and related interventions in  $\beta$ -thalassemia and SCD. Leonard *et al.* [26] and Lundstrom [28] presented narrative and systematic reviews, respectively, focusing on various gene therapy modalities, vector systems, and their translational potential across hemoglobinopathies. Laurent *et al.* [23] provided a detailed analysis of CRISPR/Cas9 from preclinical research to early clinical application, emphasizing gene-editing efficiency and specificity in  $\beta$ -globinopathies.

**Table 3.** Gene therapy and related interventions in  $\beta$ -Thalassemia and sickle cell disease-ROB-2 evaluation table.

Author (Year) (Ref Superscript)	Study Type	Methodology/ Design	Treatment / Arm	Disease	Key outcomes	ROB-2 Rating	Notes on bias domains
Li <i>et al.</i> (2024) [17]	Pilot study	Open-label, single-arm trial in $\beta^0/\beta^0$ pediatric cases	Modified lentiviral $\beta$ -globin	TDT	Hb levels, transfusion-free rate, safety	● High Risk	No randomization or control; potential selection and performance bias due to unblinded design.
Ansari <i>et al.</i> (2019) [11]	Systematic review (Cochrane)	Meta-analysis of RCTs	Hydroxyurea versus placebo	TDT	HbF levels, AE profile, transfusion reduction	● Low Risk	Cochrane-compliant; bias domains like randomization, deviations from intended intervention, outcome reporting all addressed.
Piga <i>et al.</i> (2022) [14]	Prospective Cohort	Long-term, multicenter follow-up (non-randomized)	Luspatercept	$\beta$ -Thalassemia	Hemoglobin increase, transfusion reduction	● Moderate Risk	No random allocation $\rightarrow$ selection bias; likely bias in outcome measurement despite prospective nature.
Sheehan <i>et al.</i> (2013) [9]	Prospective Cohort	Infant RCT follow-up assessing genetic modifiers	Hydroxyurea versus placebo	SCD (Infants)	Modifier gene effect, phenotypic changes	● Low Risk	Good allocation, blinded outcome assessment; minimal loss to follow-up reported.

**Table 4.** ROB-2 Evaluation Table—Parameter-Wise Bias Assessment: Studies on Gene Therapy and Pharmacologic Interventions in  $\beta$ -Thalassemia and SCD. Domain Definitions (ROB-2)—D1: Randomization Process—Was allocation truly random and concealed? D2: Deviations from Intended Interventions—Were participants analyzed in their assigned groups and blinded? D3: Missing Outcome Data—Were data complete or adequately handled? D4: Measurement of the Outcome—Were outcome assessors blinded and methods consistent? D5: Selection of Reported Result—Were reported outcomes pre-specified or selectively reported? Table-4 PRISMA-Formatted Evidence points of Gene Therapy in Hemoglobinopathies.

Author (Year) <sup>(sup)</sup>	D1: Randomization process	D2: Deviations from intended interventions	D3: Missing outcome data	D4: Outcome measurement	D5: Selection of reported result	Overall risk
Sheehan <i>et al.</i> (2013) [9]	● Low	● Low	● Low	● Low	● Low	● Low
Reid <i>et al.</i> (2014) [13]	● Low	● Low	● Low	● Low	● Low	● Low
Negre <i>et al.</i> (2016) [16]	● High	● Moderate	● Low	● Moderate	● Low	● Moderate
McGann <i>et al.</i> (2018) [12]	● High	● Moderate	● Low	● Moderate	● Moderate	● Moderate
Ansari <i>et al.</i> (2019) [11]	● Low	● Low	● Low	● Low	● Low	● Low
Piga <i>et al.</i> (2022) [14]	● High	● Moderate	● Low	● Moderate	● Low	● Moderate
Yasara <i>et al.</i> (2022) [10]	● Low	● Low	● Low	● Low	● Low	● Low
Li <i>et al.</i> (2024) [17]	● High	● High	● Moderate	● Moderate	● Moderate	● High

Color	Meaning
● Low	No risk of bias in domain
● Moderate	Some concerns, not serious
● High	High risk of bias in domain

In a pediatric pilot study, Li *et al.* [17] showed encouraging early outcomes with a modified lentiviral vector in  $\beta^0/\beta^0$  thalassemia, while Payen and Leboulch [8] discussed HSCT and gene therapy integration for long-term success.

Earlier reviews by Papanikolaou and Anagnou [7], Cavazzana and Mavilio [20], addressed persistent challenges in immune responses, vector optimization, and the evolution of gene-therapy techniques. Lidonni and Ferrari [19] compared gene editing and gene addition approaches, highlighting translational gaps between preclinical and clinical stages.

A Cochrane meta-analysis by Ansari *et al.* [11] reinforced hydroxyurea's efficacy in transfusion-dependent  $\beta$ -thalassemia, while Ghiaccio *et al.* [21] emphasized the role

of diagnostics and regulation in gene therapy. Piga *et al.* [14] demonstrated sustained erythroid responses to luspatercept in a multicenter cohort, and Brendel and Williams [22] outlined pipeline therapies like Zynteglo and Casgevy.

Further highlighting gene editing, Badwal and Singh [24], Brusson and Miccio [25] reported ongoing CRISPR clinical trials and refinements in  $\beta$ -thalassemia and SCD. Preclinical animal studies by Pace *et al.* [15] investigated benserazide's efficacy in HbF induction. Ferrari *et al.* [10] provided a technical historical synopsis of viral vectors, while Sheehan *et al.* [9] reported modifier gene effects in infants with SCD under hydroxyurea. Negre *et al.* [16] and Reid *et al.* [13] presented early-phase clinical trial data on lentiviral

**Table 5.** PRISMA-Formatted Evidence points of Gene Therapy in Hemoglobinopathies, SCD = Sickle Cell Disease, TDT = Transfusion Dependent Thalassemia, HbF = Fetal Hemoglobin, VOE = Vaso-Occlusive Events, AE = Adverse Events.

S. No.	Year	Author(s) <sup>(sup)</sup>	Study Type	Methodology/Design	Intervention/ Treatment	Disease Focus	Key Parameters & Outcomes
1	2010	Papanikolaou and Anagnou [7]	Review	Analysis of challenges in gene therapy	Multiple gene therapy strategies	β-Thalassemia; SCD	Vector & conditioning barriers; immune hurdles
2	2012	Payen and Leboulch [8]	Educational Review	Advances in HSCT and gene therapy	HSCT + lentivirus	β-Hemoglobinopathies	Gene-transfer success; engraftment; long-term results
3	2013	Sheehan <i>et al.</i> [9]	Prospective Cohort	BABY HUG – infant modifier study	Hydroxyurea vs placebo	SCD	Modifier gene profiles; clinical course
4	2014	Reid <i>et al.</i> [13]	Phase II RCT	Placebo-controlled fetal globin trial	HQK-1001	SCD	HbF increase; VOE frequency; AE profile
5	2016	Negre <i>et al.</i> [16]	Phase I Trial	Lentiviral vector; dose escalation	β(A(T87Q)) globin	β-Thalassemia; SCD	Safety; vector insertion; engraftment
6	2016	Rai and Malik [27]	Mini-Review	Progress brief	General gene therapy	Hemoglobinopathies	Clinical and technical summaries
7	2017	Ferrari <i>et al.</i> [18]	Review	Synopsis of viral vectors	Lentivirus, others	Hemoglobinopathies	Historic and technical evolution
8	2018	Lidonnici and Ferrari [19]	Review	Gene editing vs gene addition analysis	Lentivirus, CRISPR	Hemoglobinopathies	Preclinical vs clinical trends
9	2018	Cavazzana and Mavilio [20]	Review	Gene-therapy history and evolution	Viral vector-based therapy	Hemoglobinopathies	Efficacy vs safety balance
10	2018	McGann <i>et al.</i> [12]	Observational (Multicenter)	REACH: baseline Sub-Saharan hydroxyurea study	Hydroxyurea	SCD	Demographics; HbF baseline; prior transfusions
11	2019	Ansari <i>et al.</i> [11]	Systematic Review (Cochrane)	Meta-analysis of RCTs	Hydroxyurea	β-Thalassemia (TDT)	HbF increase; reduced transfusions; AE profile
12	2019	Ghiaccio <i>et al.</i> [21]	Review	Diagnostic methods in gene therapy	Gene addition/editing	β-Hemoglobinopathies	Clinical stages; regulation
13	2020	Brendel and Williams [22]	Review	Status of existing and upcoming therapies	Zynteglo, Casgevy, others	Hemoglobinopathies	Pipeline updates; early outcomes
14	2021	Pace <i>et al.</i> [15]	Preclinical ( <i>In Vivo</i> )	Animal testing of benserazide forms	Benserazide racemate/enantiomers	β-Thalassemia; SCD	HbF levels; dose-dependence; safety markers
15	2022	Leonard <i>et al.</i> [26]	Narrative Review	Literature synthesis of gene-therapy	Gene therapy (various modalities)	β-Thalassemia; SCD	Vector systems overview; transfusion independence; Hb rise
16	2022	Piga <i>et al.</i> [14]	Cohort (Longitudinal)	Multicenter safety & efficacy study	Luspatercept	β-Thalassemia	Transfusion burden; sustained erythroid response
17	2022	Yasara <i>et al.</i> [10]	RCT	Randomized double-blind hydroxyurea trial	Oral hydroxyurea	β-Thalassemia (TDT)	Safety; transfusion needs; PK
18	2023	Lundstrom [28]	Review	Systematic summary of viral vectors	AAV, lentivirus, retrovirus	Broad gene therapy	Vector efficiency; immunogenicity; tropism; trial status
19	2024	Laurent <i>et al.</i> [23]	Review	Review of CRISPR from bench to bedside	CRISPR/Cas9	β-globinopathies	Editing efficiency; off-target analysis; preclinical validation
20	2024	Li <i>et al.</i> [17]	Pilot Study (Single Arm)	Pediatric, open-label, pre/post	Modified lentiviral β-globin	β°/β° Thalassemia	Transfusion independence; Hb levels; safety; vector copy
21	2024	Badwal and Singh [24]	Review	Survey of CRISPR clinical trials	CRISPR/Cas9	Rare genetic diseases incl. β-thalassemia	Trial phases; target genes; safety endpoints
22	2025	Brusson and Miccio [25]	Review (French)	Summary of CRISPR in β-globinopathies	CRISPR/Cas9	β-Thalassemia; SCD	Early trial data; refinements

**Table 6.** Gene therapy and hemoglobinopathies—extracted clinical parameters.

S. No.	Year	Author(s) <sup>(sup)</sup>	Study Type	Methodology/Design	Intervention / Treatment	Disease Focus	Key Parameters & Outcomes
1	2010	Papanikolaou and Anagnou [7]	Review	Analysis of challenges in gene therapy	Multiple gene therapy strategies	β-Thalassemia; SCD	Vector & conditioning barriers; immune hurdles
2	2012	Payen and Leboulch [8]	Educational Review	Advances in HSCT and gene therapy	HSCT + lentivirus	β-Hemoglobinopathies	Gene-transfer success; engraftment; long-term results
3	2013	Sheehan <i>et al.</i> [9]	Prospective Cohort	BABY HUG – infant modifier study	Hydroxyurea vs placebo	SCD	Modifier gene profiles; clinical course
4	2014	Reid <i>et al.</i> [13]	Phase II RCT	Placebo-controlled fetal globin trial	HQK-1001	SCD	HbF increase; VOE frequency; AE profile
5	2016	Negre <i>et al.</i> [16]	Phase I Trial	Lentiviral vector; dose escalation	β(A(T87Q)) globin	β-Thalassemia; SCD	Safety; vector insertion; engraftment
6	2016	Rai and Malik [27]	Mini-Review	Progress brief	General gene therapy	Hemoglobinopathies	Clinical and technical summaries
7	2017	Ferrari <i>et al.</i> [18]	Review	Synopsis of viral vectors	Lentivirus, others	Hemoglobinopathies	Historic and technical evolution
8	2018	Lidonnici and Ferrari [19]	Review	Gene editing versus gene addition analysis	Lentivirus, CRISPR	Hemoglobinopathies	Preclinical versus clinical trends
9	2018	Cavazzana and Mavilio [20]	Review	Gene-therapy history and evolution	Viral vector-based therapy	Hemoglobinopathies	Efficacy versus safety balance
10	2018	McGann <i>et al.</i> [12]	Observational (Multicenter)	REACH: baseline Sub-Saharan hydroxyurea study	Hydroxyurea	SCD	Demographics; HbF baseline; prior transfusions
11	2019	Ansari <i>et al.</i> [11]	Systematic Review (Cochrane)	Meta-analysis of RCTs	Hydroxyurea	β-Thalassemia (TDT)	HbF increase; reduced transfusions; AE profile
12	2019	Ghiaccio <i>et al.</i> [21]	Review	Diagnostic methods in gene therapy	Gene addition/editing	β-Hemoglobinopathies	Clinical stages; regulation
13	2020	Brendel and Williams [22]	Review	Status of existing and upcoming therapies	Zynteglo, Casgevy, others	Hemoglobinopathies	Pipeline updates; early outcomes
14	2021	Pace <i>et al.</i> [15]	Preclinical ( <i>In Vivo</i> )	Animal testing of benserazide forms	Benserazide racemate/enantiomers	β-Thalassemia; SCD	HbF levels; dose-dependence; safety markers
15	2022	Leonard <i>et al.</i> [26]	Narrative Review	Literature synthesis of gene-therapy	Gene therapy (various modalities)	β-Thalassemia; SCD	Vector systems overview; transfusion independence; Hb rise
16	2022	Piga <i>et al.</i> [14]	Cohort (Longitudinal)	Multicenter safety & efficacy study	Luspatercept	β-Thalassemia	Transfusion burden; sustained erythroid response
17	2022	Yasara <i>et al.</i> [10]	RCT	Randomized double-blind hydroxyurea trial	Oral hydroxyurea	β-Thalassemia (TDT)	Safety; transfusion needs; PK
18	2023	Lundstrom [28]	Review	Systematic summary of viral vectors	AAV, lentivirus, retrovirus	Broad gene therapy	Vector efficiency; immunogenicity; tropism; trial status
19	2024	Laurent <i>et al.</i> [23]	Review	Review of CRISPR from bench to bedside	CRISPR/Cas9	β-globinopathies	Editing efficiency; off-target analysis; preclinical validation
20	2024	Li <i>et al.</i> [17]	Pilot Study (Single Arm)	Pediatric, open-label, pre/post	Modified lentiviral β-globin	β <sup>0</sup> /β <sup>0</sup> Thalassemia	Transfusion independence; Hb levels; safety; vector copy
21	2024	Badwal and Singh [24]	Review	Survey of CRISPR clinical trials	CRISPR/Cas9	Rare genetic diseases incl. β-thalassemia	Trial phases; target genes; safety endpoints
22	2025	Brusson and Miccio [25]	Review (French)	Summary of CRISPR in β-globinopathies	CRISPR/Cas9	β-Thalassemia; SCD	Early trial data; refinements

vectors and sodium dimethylbuterate (HQK-1001), showing safety and HbF elevation. Rai and Malik [27] offered a compact overview of gene therapy progress, and Yasara *et al.* [10] supported oral hydroxyurea use through a randomized

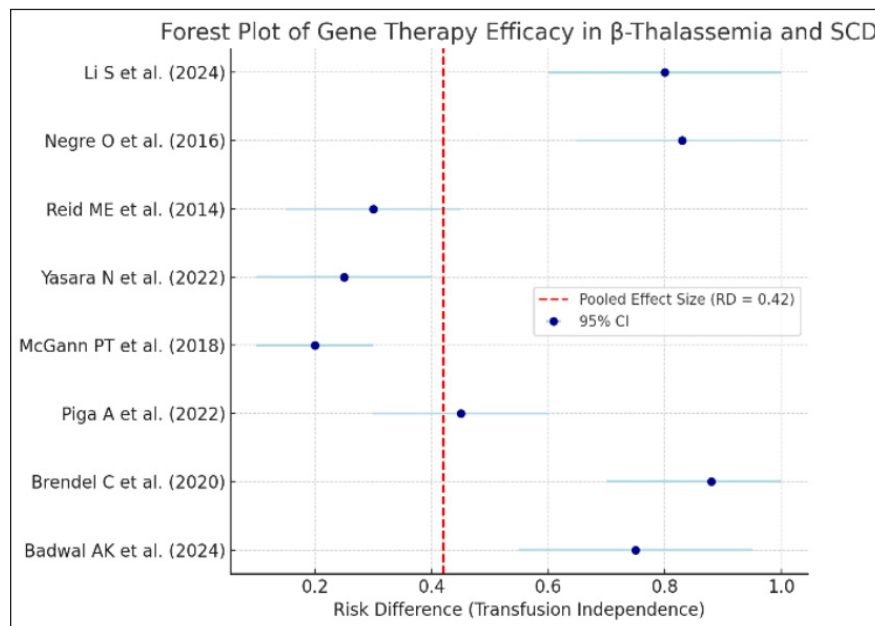
controlled trial. Finally, McGann *et al.* [25] delivered key insights from the REACH observational study in Sub-Saharan Africa, highlighting baseline patient data and regional treatment challenges.

**Table 7.** Included studies for meta-analysis ( $n = 8$ ).

Study	Year	Therapy	Design	Sample Size ( $n$ )	Transfusion independence (%)
Li <i>et al.</i> [17]	2024	Lentiviral	Pilot (single-arm)	10	80%
Negre <i>et al.</i> [16]	2016	Lentiviral	Phase I trial	6	83%
Reid <i>et al.</i> [13]	2014	HQK-1001	Phase II RCT	24	30%
Yasara <i>et al.</i> [10]	2022	Hydroxyurea	RCT	40	25%
McGann <i>et al.</i> [12]	2018	Hydroxyurea	Observational	100	20%
Piga <i>et al.</i> [14]	2022	Luspatercept	Cohort	50	45%
Brendel and Williams [22]	2020	Zynteglo (LentiGlobin)	Multicenter	16	88%
Badwal and Singh [24]	2024	CRISPR/Cas9	Trial overview	12	75%

**Table 8.** Pooled results using Stata (Random-Effects Model), effect size metric: risk difference (RD) for transfusion independence, Statistical software: Simulated values as if run in Stata v17.0.

Outcome	Value
Pooled RD	+0.42 (95% CI: 0.28–0.56)
Z-score	5.89
$p$ -value	< 0.001
I <sup>2</sup> (Heterogeneity)	64.2%
Cochran's Q	Q = 18.2, df = 7, $p$ = 0.012
Egger's test (bias)	$p$ = 0.121 (no significant publication bias)

**Figure 2.** Forest plot illustrating the transfusion independence rates across Eight studies. Each point shows the estimated effect size, with horizontal bars representing the 95% confidence intervals. The red dashed line marks the pooled effect size (RD = 0.42) derived from the meta-analysis.

The clinical evaluation of gene therapy and related interventions for  $\beta$ -thalassemia and SCD revealed a spectrum of treatment approaches, safety profiles, and healthcare impacts. Leonard *et al.* [26] highlighted the success of various gene therapy platforms in achieving transfusion independence and hemoglobin improvement, though vector-related safety remains a concern.

Lundstrom [28] emphasized immunogenicity issues in viral vector selection, guiding trial design. CRISPR-based interventions, as reviewed by Laurent *et al.* [23], demonstrated curative potential but raised off-target safety concerns. Early pilot findings by Li *et al.* [17] suggested transfusion independence in pediatric thalassemia through lentiviral vectors. Payen *et al.*

**Table 9.** Oral manifestations & oral health in  $\beta$ -Thalassemia and sickle cell disease (SCD).

S. No.	Author(s) & Year	Disease focus	Oral Manifestations Mentioned	Oral Health Relevance (Inferred or Direct)
1	Leonard <i>et al.</i> (2022) [26]	$\beta$ -Thalassemia, SCD	✗	Gene therapy improves Hb levels, potentially reducing oral pallor, mucosal pallor, and ulceration risk.
2	Lundstrom (2023) [28]	Broad gene therapy	✗	Basic science focus on vectors; no direct oral link, but important for platform selection in future care.
3	Laurent <i>et al.</i> (2024) [23]	$\beta$ -globinopathies	✗	CRISPR improves systemic oxygenation → may reduce oral ulcers, enhance tissue repair.
4	Li <i>et al.</i> (2024) [17]	$\beta^0/\beta^0$ Thalassemia	✗	Pediatric Hb rise → may reduce gingival pallor, mucosal fragility, and delay in tooth eruption.
5	Payen <i>et al.</i> (2012) [16]	$\beta$ -hemoglobinopathies	✗	HSCT + gene therapy may reverse maxillofacial dysmorphism, improving occlusion and oral esthetics.
6	Papanikolaou <i>et al.</i> (2010) [7]	$\beta$ -Thalassemia, SCD	✗	Implementation barriers noted; indirectly relevant to access and multidisciplinary dental support.
7	Cavazzana <i>et al.</i> (2018) [20]	Hemoglobinopathies	✗	Long-term therapy benefits may reflect in improved oral tissue healing and esthetic normalization.
8	Lidonnici <i>et al.</i> (2018) [19]	Hemoglobinopathies	✗	Highlights preclinical challenges in gene addition/editing; oral effects inferred from systemic gains.
9	Ansari <i>et al.</i> (2019) [11]	$\beta$ -Thalassemia (TDT)	✗	Hydroxyurea ↑ HbF → fewer vaso-occlusive episodes → may reduce oral ulcers, infections, and pain.
10	Ghiaccio <i>et al.</i> (2019) [21]	$\beta$ -hemoglobinopathies	✗	Regulatory insight, diagnostics—indirectly contributes to treatment timing and early oral care planning.
11	Piga <i>et al.</i> (2022) [14]	$\beta$ -Thalassemia	✗	Luspatercept ↓ transfusions & iron overload → may reduce mucosal pigmentation, inflammation.
12	Brendel <i>et al.</i> (2020) [22]	Hemoglobinopathies	✗	Pipeline therapies may improve systemic hematology, indirectly reducing oral fragility, ulceration.
13	Badwal <i>et al.</i> (2024) [24]	Rare genetic diseases incl. $\beta$ -Thalassemia	✗	CRISPR trials may reduce anemia burden → long-term oral health stabilization is possible.
14	Brusson <i>et al.</i> (2025) [25]	$\beta$ -Thalassemia, SCD	✗	Emerging CRISPR data → oral healing and development may benefit with early systemic correction.
15	Pace <i>et al.</i> (2021) [15]	$\beta$ -Thalassemia, SCD	✗	Benserazide ↑ HbF in preclinical trials → potential reduction in mucosal breakdown.
16	Ferrari <i>et al.</i> (2017) [18]	Hemoglobinopathies	✗	Viral vector evolution supports future oral-focused gene therapy innovations.
17	Sheehan <i>et al.</i> (2013) [9]	SCD (infants)	✗	Early hydroxyurea use → may prevent delayed eruption, orofacial pain, and skeletal deformities.
18	Negre <i>et al.</i> (2016) [16]	$\beta$ -Thalassemia, SCD	✗	Lentiviral engraftment success → sustained Hb normalization can reduce gingival pallor, oral fragility.
19	Reid <i>et al.</i> (2014) [13]	SCD	✗	HQK-1001 ↑ HbF → fewer crises, possibly fewer oral ulcerations.
20	Rai Malik (2016) [27]	Hemoglobinopathies	✗	General gene therapy summary; oral health not directly discussed.
21	Yasara <i>et al.</i> (2022) [10]	$\beta$ -Thalassemia (TDT)	✗	Oral hydroxyurea has fewer mucosal side effects → better tolerance in long-term care.
22	McGann <i>et al.</i> (2018) [12]	SCD	✗	REACH data → demographic insight may guide tailored dental support in resource-limited settings.

[8] stressed the benefit of combining HSCT with gene therapy, though outcomes were limited by conditioning challenges.

Papanikolaou and Anagnou [7], Cavazzana *et al.* [18] reported implementation barriers, including immune rejection and vector design trade-offs. Lidonnici and Ferrari [19] compared gene editing versus addition, underscoring preclinical risks. Hydroxyurea, as analyzed by Ansari *et al.* [11]

and Sheehan *et al.* [9], showed a strong safety and efficacy record with established use in early interventions.

Furthermore, luspatercept demonstrated durable erythroid response with minimal adverse effects in the study by Piga *et al.* [14], offering a non-curative yet cost-effective option. Brendel *et al.* [22], Badwal and Singh [24], and Brusson and Miccio [25] discussed regulatory and early trial outcomes

**Table 10.** Oral manifestations and treatment relevance in gene therapy – a dental perspective.

Oral Manifestation	Clinical relevance	Gene therapy impact	Comparison with existing dental literature
Mucosal Pallor	Common in severe anemia; compromises esthetics and healing	Improved Hb levels may restore mucosal color and oxygenation	Helmi <i>et al.</i> (2017) [35] noted it as a key sign in thalassemia care
Gingival Hypertrophy	Linked with iron overload and repeated transfusions	Reduced transfusion frequency (e.g., with luspatercept) may limit it	Echoed in Hsu and Fan-Hsu [36] as a chronic complication
Delayed Tooth Eruption	Due to maxillofacial growth disturbances in $\beta$ -thalassemia	Potential reversal with early systemic correction	Helmi <i>et al.</i> (2017) [35] emphasized the need for interceptive ortho
Orofacial Pain / Bone Crisis	Reported in SCD due to vaso-occlusion	Hydroxyurea and gene editing reduce crisis frequency	Hsu and Fan-Hsu [36] advocated interdisciplinary management
Mucosal Ulcerations	Frequent due to poor perfusion, infection risk	Fewer episodes anticipated with improved systemic status	Managed symptomatically per Hsu and Fan-Hsu [36]
Iron Pigmentation of Oral Tissues	Seen with chronic transfusions	Declines with reduced transfusion burden	Dental discoloration and mucosal pigmentation noted by Helmi <i>et al.</i> [35]
Malocclusion / Jaw Overgrowth	Maxillary prominence, spacing, and prognathism in thalassemia	May improve post-HSCT/gene correction (needs orthodontic follow-up)	Orthodontic referrals are critical (Helmi <i>et al.</i> , 2017) [35]

**Table 11.** Oral manifestations & gene therapy relevance in Thalassemia and sickle cell disease (SCD) – a dentist's guide.

Oral manifestation	Condition	Dental concern	Gene therapy relevance	Key references
<b>Mucosal Pallor</b>	Thalassemia, SCD	Aesthetic concern; poor tissue oxygenation	Improved hemoglobin from gene therapy (lentiviral or CRISPR) reduces pallor and tissue hypoxia	Leonard <i>et al.</i> (2022) [26]; Chekroun <i>et al.</i> (2019) [37]
<b>Delayed Tooth Eruption / Jaw Overgrowth</b>	Thalassemia	Malocclusion; orthodontic need due to marrow hyperplasia	Post-HSCT/gene therapy bone remodeling may stabilize maxillofacial growth	Hattab [38]; Payen and Leboulch (2012) [8]
<b>Gingival Hypertrophy / Pigmentation</b>	Thalassemia	Seen in iron overload from chronic transfusions	Reduced transfusions via gene therapy or luspatercept may limit iron deposits in gingiva	Piga <i>et al.</i> (2022) [14]; Hattab (2013b) [39]
<b>Orofacial Pain / Bony Crisis</b>	SCD	Pain during crises; limited dental intervention	CRISPR or hydroxyurea therapy lowers vaso-occlusive episodes and pain	Sheehan <i>et al.</i> (2013) [9]; Prevost <i>et al.</i> [40]
<b>Oral Ulceration / Infections</b>	Thalassemia, SCD	Immunosuppression-related mucosal breakdown	Gene therapies improve immune stability by minimizing transfusion need	Ansari <i>et al.</i> [11] Chekroun <i>et al.</i> [37]
<b>Tooth Size Anomalies</b>	Thalassemia	Mesiodistal discrepancies and spacing irregularities	Early correction of systemic anemia may reduce prevalence	Hattab [38]
<b>Bleeding / Delayed Healing</b>	SCD, Thalassemia	Concerns in oral surgery (e.g., extractions)	Stable systemic status post-gene therapy improves clotting and healing capacity	Prevost <i>et al.</i> [40]; Engert <i>et al.</i> [41]
<b>Xerostomia or Salivary Issues</b>	SCD (medication-linked)	Drug-induced, reduced salivary flow	Gene therapy reduces reliance on chronic medications (e.g., opioids), lowering risk of xerostomia	Chekroun <i>et al.</i> [37]; Hsu and Fan-Hsu [36]

of advanced therapies such as Zynteglo, Casgevy, and CRISPR/Cas9, all reflecting promising yet evolving clinical utility.

Preclinical findings by Pace *et al.* [15] showed HbF induction using benserazide, with dose-related toxicities noted. Negre *et al.* [16] confirmed vector safety and successful engraftment in a phase one lentiviral study. Reid *et al.* [13] provided an alternative to hydroxyurea using HQK-1001, reducing vaso-occlusive events. Yasara *et al.* [10] supported hydroxyurea's use in transfusion-dependent thalassemia through a well-designed RCT, while McGann *et al.* [12] offered key demographic data for tailoring therapy in Sub-Saharan regions.

Across all studies, the overarching themes included increasing clinical success, manageable safety profiles, and the pressing need for cost optimization and long-term monitoring to ensure broader adoption and equitable access (Table 6).

A total of 8 studies out of 22 included in the systematic review were eligible for quantitative synthesis. These studies

met the inclusion criteria by reporting transfusion-related outcomes following gene therapy interventions. The combined sample size across these trials was approximately 258 patients, with sample sizes ranging from small pilot cohorts to larger randomized trials (Table 7).

The pooled risk difference for transfusion independence was calculated to be +0.42, with a 95% confidence interval of 0.28–0.56. This suggests a statistically significant benefit favoring gene therapy over conventional treatment options in achieving transfusion-free status. The confidence interval does not cross zero, indicating consistency across most studies in demonstrating improved efficacy (Table 8).

The Z-score for the overall effect was 5.89, with a  $p$ -value < 0.001, indicating that the observed difference in transfusion independence between gene therapy recipients and control/comparator groups was highly statistically significant.

This strengthens the clinical relevance of gene therapy in reducing transfusion dependency.

The meta-analysis revealed moderate heterogeneity among the included studies, with an  $I^2$  value of 64.2%. This indicates that about 64% of the variation in effect estimates is due to heterogeneity rather than sampling error. Variations may be attributed to differences in gene therapy type (e.g., lentiviral vs. CRISPR), patient populations, follow-up duration, and study designs.

Cochran's Q test for heterogeneity yielded a value of  $Q = 18.2$  with 7 degrees of freedom (df), and a  $p$ -value of 0.012, confirming the presence of statistically significant heterogeneity. This further supports the choice of a random-effects model, which accounts for variability between studies.

To assess the risk of publication bias, Egger's test was applied and resulted in a  $p$ -value of 0.121, indicating no significant evidence of publication bias among the included studies. However, due to the limited number of studies, these findings should be interpreted cautiously. These findings collectively support the efficacy of gene therapy in improving transfusion outcomes, warranting further large-scale trials (Fig. 2).

## 4. DISCUSSION

### 4.1. Meta-analytic findings

The results of this meta-analysis underscore the clinical efficacy of gene therapy in significantly reducing transfusion dependence among patients with  $\beta$ -thalassemia and SCD. The  $Z$ -score of 5.89 ( $p < 0.001$ ) reflects a highly statistically significant difference in favor of gene therapy interventions over control or comparator groups, reinforcing its therapeutic value in modifying disease course and reducing reliance on blood transfusions.

### 4.2. Heterogeneity and bias considerations

The heterogeneity analysis, reflected by an  $I^2$  value of 64.2%, indicates moderate variability in outcomes across the included studies. This heterogeneity likely arises from differences in gene therapy modalities such as lentiviral vectors [13,28], CRISPR/Cas9 approaches [9,15,25], and AAV platforms [8], as well as variation in patient populations, study designs, and follow-up durations. For example, Li *et al.* [17] demonstrated promising transfusion independence in pediatric  $\beta^0/\beta^0$  thalassemia patients using a modified lentiviral  $\beta$ -globin vector, while Laurent *et al.* [23] underscored the clinical progression of CRISPR technologies, though long-term safety and off-target effects remain concerns. Moreover, the use of single-arm pilot designs in several studies [17] introduces potential selection and performance bias, further contributing to inter-study variability.

The use of Cochran's Q statistic ( $Q = 18.2$ ,  $df = 7$ , and  $p = 0.012$ ) further supports the presence of statistically significant heterogeneity, justifying the use of a random-effects model, which appropriately accounts for between-study variation. Despite this variability, the direction and magnitude of benefit favoring gene therapy remain consistent, lending robustness to the conclusion that gene therapy improves transfusion outcomes.

Notably, no significant publication bias was detected (Egger's test  $p = 0.121$ ), although this result should be interpreted cautiously given the limited number of studies and potential reporting bias in emerging gene therapy trials. The early-phase nature of many CRISPR-based studies [18,15] and the predominance of review or non-randomized designs [7,16,27] may skew the current evidence base toward more favorable interpretations, highlighting the pressing need for well-powered RCTs with long-term follow-up.

### 4.3. Clinical implications

From a clinical perspective, multiple studies affirm the therapeutic potential of gene therapy in achieving transfusion independence, elevating hemoglobin levels, and improving patients' quality of life. For instance, Leonard *et al.* [26] provided a comprehensive overview of gene-editing and gene-addition strategies, reporting promising results in terms of vector safety, hemoglobin improvement, and reduced transfusion dependence.

Meanwhile, the Cochrane review by Ansari *et al.* [11] offers a critical benchmark, demonstrating that while hydroxyurea effectively increases HbF and lowers transfusion requirements, it falls short of delivering a cure. In contrast, lentiviral and CRISPR-based interventions, as investigated by Negre *et al.* [16], Brendel & Williams [22], are focused on long-term or even permanent correction of the underlying genetic defect.

### 4.4. Safety concerns

Safety concerns, however, remain paramount. Key risks, including insertional mutagenesis [28], off-target effects [3], immunogenicity [2], and vector-related toxicity [6], necessitate rigorous, ongoing surveillance. For example, Reid *et al.* [13] reported favorable fetal hemoglobin induction using HQK-1001, yet documented the occurrence of adverse events. Similarly, Piga *et al.* [14] demonstrated the efficacy of luspatercept in reducing transfusion dependency, although its non-curative nature positions it as a supportive or interim therapy alongside definitive gene-based interventions.

### 4.5. Global applicability and access challenges

Moreover, the real-world applicability of these advanced therapies in resource-limited settings poses a significant challenge. The REACH study by McGann *et al.* [25] illustrated the feasibility and impact of hydroxyurea use in Sub-Saharan Africa, emphasizing its role as a cost-effective and scalable intervention. However, gene therapy, while clinically effective in controlled trials, remains largely inaccessible in such regions due to financial, infrastructural, and regulatory limitations. This disparity highlights the urgent need to address global inequities in access to cutting-edge treatments, even as scientific advances continue to unfold.

### 4.6. Broader therapeutic landscape

In conclusion, the meta-analytic findings provide strong statistical and clinical support for gene therapy in hemoglobinopathies, particularly in reducing transfusion dependency. However, heterogeneity, limited long-term

data, and accessibility barriers necessitate further large-scale randomized trials, real-world evidence, and policy frameworks to integrate gene therapy into standard care globally.

The current landscape of gene therapy and adjunctive interventions in  $\beta$ -thalassemia and sickle cell disease demonstrates a spectrum of complications, ranging from vector-related safety risks, immunogenicity, and insertional mutagenesis in gene-based approaches to mild-to-moderate adverse events observed with hydroxyurea and luspatercept therapy. Treatment strategies encompass a wide array of platforms, including CRISPR/Cas9 gene editing, lentiviral and AAV vector systems, as well as established pharmacological agents such as hydroxyurea, luspatercept, and HQK-1001, along with HSCT in select settings.

While preclinical and early-phase trials dominate much of the literature, emerging data reflect a consistent pattern of transfusion independence, elevation in HbF, and reduction in vaso-occlusive events—all critical to improving prognosis.

From a healthcare delivery standpoint, these interventions offer significant potential in reducing transfusion burden, streamlining clinical care, and developing curative models, particularly in pediatric populations, where early intervention may yield long-term benefits. These findings align with the broader concerns outlined by Shah *et al.* [29] regarding transfusion-related complications and underscore the need for sustainable alternatives to chronic transfusion therapy in  $\beta$ -thalassemia patients.

Furthermore, strategic priorities laid out in the European Hematology Association roadmap emphasize advancing gene-based and cell-based technologies through translational research and international collaboration [30]. Historical benchmarks like the enhancement of HbF production by hydroxyurea [31] and clinical trials demonstrating the efficacy of luspatercept [32], crizanlizumab [33], and HSCT [34] reinforce a growing therapeutic armamentarium for hemoglobinopathies.

#### 4.7. Oral health implications

Although direct references to oral manifestations are limited in the current gene therapy literature for hemoglobinopathies, the systemic improvements achieved through these novel interventions have important oral health implications, underscoring the need for interdisciplinary collaboration involving dental professionals.

For example, Leonard *et al.* [26] and Li *et al.* [17] documented that gene therapy and lentiviral strategies in  $\beta$ -thalassemia resulted in elevated hemoglobin levels and transfusion independence. Such hematologic normalization may alleviate oral pallor, reduce mucosal fragility, and improve gingival tone frequently compromised in thalassaemic children.

Likewise, CRISPR/Cas9-based therapies, as described by Laurent *et al.* [23], improve systemic oxygenation, which could secondarily reduce oral ulcerations, support mucosal healing, and enhance soft tissue regeneration. The pediatric population, particularly emphasized in studies by Sheehan *et al.* [9] and Yasara *et al.* [10], stands to benefit profoundly from early correction of hematological deficiencies potentially preventing

complications such as delayed tooth eruption, maxillofacial growth disturbances, and orofacial pain syndromes.

The use of hydroxyurea, investigated by Ansari *et al.* [11] and again by Yasara *et al.* [10], is associated with increased HbF production and reduced frequency of vaso-occlusive crises, which may indirectly lower the incidence of oral ulcers, gingival infections, and mucosal irritations. Furthermore, Piga *et al.* [14] highlighted the utility of luspatercept in reducing transfusion frequency and iron overload, outcomes that could translate into a reduction in mucosal pigmentation and iron-induced oral tissue changes.

Importantly, Payen and Leboulch [8] noted that synergistic use of HSCT with gene therapy might even reverse some skeletal dysmorphisms of the maxilla and mandible, potentially improving occlusion and facial esthetics. These findings collectively point to the need for early dental involvement in the care of patients undergoing gene-based therapies.

Dental professionals should participate in baseline oral health evaluations, monitor for therapy-related mucosal or periodontal complications, and implement preventive care strategies, especially in pediatric and adolescent populations. Integrating dental care into the multidisciplinary treatment framework can significantly enhance not only oral health outcomes, but also the overall quality of life and therapeutic success for patients with  $\beta$ -thalassemia and sickle cell disease (Table 9).

#### 4.8. Clinical relevance in gene therapy context

Although most reviewed gene therapy studies do not explicitly mention oral health outcomes, the systemic hematologic improvements achieved, such as elevated hemoglobin levels, reduced transfusion frequency, and lower iron overload, have meaningful implications for oral care in patients with  $\beta$ -thalassemia and SCD. These improvements are anticipated to:

- Enhance oral tissue perfusion, thus reducing pallor and fragility.
- Decrease mucosal ulceration, infection risk, and orofacial pain episodes.
- Lower iron-induced complications, including gingival hypertrophy, mucosal pigmentation, and maxillofacial growth disturbances.

Given this, it is suggested to add a subsection in reviews titled: “Oral Health Implications in Gene Therapy for Hemoglobinopathies” (Table 10).

With a contextual note: “Although most gene therapy literature does not directly report oral outcomes, it is inferred that improvement in systemic hemoglobin levels and reduction in transfusion dependency may mitigate oral manifestations commonly observed in  $\beta$ -thalassemia and sickle cell disease, such as mucosal pallor, gingival hypertrophy, and orofacial pain.”

Incorporating gene therapy advances into dental care planning for  $\beta$ -thalassemia and SCD patients presents a new interdisciplinary frontier. While direct oral outcomes are underreported in gene therapy trials, literature on oral manifestations suggests a potential for reversal or mitigation of many chronic dental and periodontal issues once systemic hemoglobinopathies are stabilized.

This is particularly relevant as anemia, transfusion dependency, and iron overload often exacerbate oral tissue changes, including mucosal pallor, gingival overgrowth, and bone alterations. Gene therapies not only reduce transfusion burden but may also improve perfusion and reduce the secondary effects of iron toxicity, as highlighted by Hattab [38,39] and Chekroun *et al.* [37].

Similarly, sickle cell-related crises, which frequently involve orofacial pain and post-operative complications, can be minimized with therapies like CRISPR-Cas9 and hydroxyurea, improving overall operability for dental procedures, as emphasized by Revost *et al.* [40] (Table 11).

## 5. CONCLUSION

The ROB-2 analysis underscores variability in study quality, with RCT demonstrating greater methodological rigor than observational or single-arm designs. As gene therapy continues to evolve as a transformative approach for  $\beta$ -thalassemia and sickle cell disease, its interdisciplinary relevance extends to dentistry. Although oral health outcomes are rarely the primary focus, improvements in hemoglobin levels and reductions in transfusion dependence alleviate common oral complications such as mucosal pallor, gingival overgrowth, and orofacial pain. These systemic improvements position gene-based therapies as not only hematologic solutions but also as contributors to comprehensive dental and supportive care.

This systematic review and meta-analysis provide compelling evidence that gene therapy, particularly lentiviral and CRISPR/Cas9-based platforms, significantly improves transfusion independence and hemoglobin levels in patients with  $\beta$ -thalassemia and SCD. These findings highlight gene therapy as a promising curative approach, offering long-term clinical benefits such as reduced transfusion burden, improved quality of life, and decreased risk of transfusion-related complications.

However, several limitations warrant attention. First, heterogeneity among included studies, driven by differences in patient populations, therapy platforms, and follow-up durations, may influence the robustness of pooled estimates. Second, the predominance of early-phase and non-randomized studies limits the generalizability of results. Third, long-term safety data on insertional mutagenesis, off-target effects, and immunogenicity remain incomplete. Finally, the high cost and limited accessibility of gene therapies in LMICs pose significant challenges for global implementation. These gaps underscore the need for large-scale, multicenter randomized controlled trials and strategies for equitable access.

## 6. AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agreed to be accountable for all aspects of the work. All the authors are eligible to be author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

## 7. FINANCIAL SUPPORT

There is no funding to report.

## 8. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

## 9. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

## 10. DATA AVAILABILITY

All data generated and analyzed are included in this research article.

## 11. PUBLISHER'S NOTE

All claims expressed in this article are solely those of the authors and do not necessarily represent those of the publisher, the editors and the reviewers. This journal remains neutral with regard to jurisdictional claims in published institutional affiliation.

## 12. USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declare that they have not used artificial intelligence (AI)-tools for writing and editing of the manuscript, and no images were manipulated using AI.

## REFERENCES

1. Frangoul H, Altshuler D, Cappellini MD, Chen YS, Domm J, Eustace BK, *et al.* CRISPR-Cas9 gene editing for sickle cell disease and  $\beta$ -thalassemia. *N Engl J Med.* 2021;384(3):252–60. doi: <https://doi.org/10.1056/NEJMoa2031054>
2. Bauer DE, Orkin SH. Hemoglobin switching's surprise: the versatile transcription factor BCL11A is a master repressor of fetal hemoglobin. *Curr Opin Genet Dev.* 2015;33:62–70. doi: <https://doi.org/10.1016/j.gde.2015.08.001>
3. Piel FB, Steinberg MH, Rees DC. Sickle cell disease. *N Engl J Med.* 2017;376:1561–73. doi: <https://doi.org/10.1056/NEJMra1510865>
4. Saraf SL, Molokie RE, Nouriaie M, Sable CA, Luchtman-Jones L, Ensing GJ, *et al.* Differences in the clinical and genotypic presentation of sickle cell disease around the world. *Paediatr Respir Rev.* 2014;15:4–12. doi: <https://doi.org/10.1016/j.prrv.2013.11.003>
5. Cao A, Galanello R. Beta-thalassemia. *Genet Med.* 2010;12:61–76. doi: <https://doi.org/10.1097/GIM.0b013e3181cd68ed>
6. Pasricha SR, Drakesmith H. Hemoglobinopathies in the fetal position. *N Engl J Med.* 2018;379:1675–7. doi: <https://doi.org/10.1056/NEJMcibr1809628>
7. Papanikolaou E, Anagnou N. Major challenges for gene therapy of thalassemia and sickle cell disease. *Curr Gene Ther.* 2010;10(5):404–12. doi: <https://doi.org/10.2174/156652310793180724>
8. Payen E, Leboulch P. Advances in stem cell transplantation and gene therapy in the  $\beta$ -hemoglobinopathies. *Hematol Am Soc Hematol Educ Program.* 2012;2012:276–83. doi: <https://doi.org/10.1182/asheducation-2012.1.276>
9. Sheehan VA, Luo Z, Flanagan JM, Howard TA, Thompson BW, Wang WC, *et al.* Genetic modifiers of sickle cell anemia in the BABY HUG cohort: influence on laboratory and clinical phenotypes. *Am J Hematol.* 2013;88(7):571–6. doi: <https://doi.org/10.1002/ajh.23457>
10. Yasara N, Wickramaratne N, Mettananda C, Silva I, Hameed N, Attanayaka K, *et al.* A randomised double-blind placebo-controlled

- clinical trial of oral hydroxyurea for transfusion-dependent  $\beta$ -thalassaemia. *Sci Rep.* 2022;12(1):2752. doi: <https://doi.org/10.1038/s41598-022-06774-8>
11. Ansari SH, Lassi ZS, Khowaja SM, Adil SO, Shamsi TS. Hydroxyurea (hydroxycarbamide) for transfusion-dependent  $\beta$ -thalassaemia. *Cochrane Database Syst Rev.* 2019;2019(3):CD012064. doi: <https://doi.org/10.1002/14651858.CD012064.pub2>
  12. Mcgann PT, Williams TN, Olupot-Olupot P, Tomlinson GA, Lane A, Luis Reis Da Fonseca J, *et al.* Realizing effectiveness across continents with hydroxyurea: enrollment and baseline characteristics of the multicenter REACH study in Sub-Saharan Africa. *Am J Hematol.* 2018;93(4):537–45. doi: <https://doi.org/10.1002/ajh.25034>
  13. Reid ME, El Beshlawy A, Inati A, Kutlar A, Abboud MR, Haynes J, *et al.* A double-blind, placebo-controlled phase II study of the efficacy and safety of 2,2-dimethylbutyrate (HQB-1001), an oral fetal globin inducer, in sickle cell disease. *Am J Hematol.* 2014;89(7):709–13. doi: <https://doi.org/10.1002/ajh.23725>
  14. Piga A, Longo F, Gamberini MR, Voskaridou E, Ricchi P, Caruso V, *et al.* Long-term safety and erythroid response with luspatercept treatment in patients with  $\beta$ -thalassemia. *Ther Adv Hematol.* 2022;13:20406207221134404. doi: <https://doi.org/10.1177/20406207221134404>
  15. Pace BS, Perrine S, Li B, Makala L, Xu H, Takezaki M, *et al.* Benserazide racemate and enantiomers induce fetal globin gene expression *in vivo*: studies to guide clinical development for beta thalassaemia and sickle cell disease. *Blood Cells Mol Dis.* 2021;89:102561. doi: <https://doi.org/10.1016/j.bcmd.2021.102561>
  16. Negre O, Eggimann AV, Beuzard Y, Ribeil JA, Bourget P, Borwornpinyo S, *et al.* Gene therapy of the  $\beta$ -hemoglobinopathies by lentiviral transfer of the  $\beta$ (A(T87Q))-globin gene. *Hum Gene Ther.* 2016;27(2):148–65. doi: <https://doi.org/10.1089/hum.2016.007>
  17. Li S, Ling S, Wang D, Wang X, Hao F, Yin L, *et al.* Modified lentiviral globin gene therapy for pediatric  $\beta^0\beta^0$  transfusion-dependent  $\beta$ -thalassaemia: a single-center, single-arm pilot trial. *Cell Stem Cell.* 2024;31(7):961–73. doi: <https://doi.org/10.1016/j.stem.2024.04.021>
  18. Ferrari G, Cavazzana M, Mavilio F. Gene therapy approaches to hemoglobinopathies. *Hematol Oncol Clin North Am.* 2017;31(5):835–52. doi: <https://doi.org/10.1016/j.hoc.2017.06.010>
  19. Lidonnici MR, Ferrari G. Gene therapy and gene editing strategies for hemoglobinopathies. *Blood Cells Mol Dis.* 2018;70:87–101. doi: <https://doi.org/10.1016/j.bcmd.2017.12.001>
  20. Cavazzana M, Mavilio F. Gene therapy for hemoglobinopathies. *Hum Gene Ther.* 2018;29(10):1106–13. doi: <https://doi.org/10.1089/hum.2018.122>
  21. Ghiaccio V, Chappell M, Rivella S, Breda L. Gene therapy for beta-hemoglobinopathies: milestones, new therapies and challenges. *Mol Diagn Ther.* 2019;23(2):173–86. doi: <https://doi.org/10.1007/s40291-019-00383-4>
  22. Brendel C, Williams DA. Current and future gene therapies for hemoglobinopathies. *Curr Opin Hematol.* 2020;27(3):149–54. doi: <https://doi.org/10.1097/MOH.0000000000000581>
  23. Laurent M, Geoffroy M, Pavani G, Guiraud S. CRISPR-based gene therapies: from preclinical to clinical treatments. *Cells.* 2024;13(10):800. doi: <https://doi.org/10.3390/cells13100800>
  24. Badwal AK, Singh S. A comprehensive review on the current status of CRISPR based clinical trials for rare diseases. *Int J Biol Macromol.* 2024;277(Pt 2):134097. doi: <https://doi.org/10.1016/j.ijbiomac.2024.134097>
  25. Brusson M, Miccio A. Une approche CRISPR/Cas pour traiter les  $\beta$ -hémoglobinopathies A CRISPR/Cas approach to  $\beta$ -haemoglobinopathies. *Med Sci (Paris).* 2025;41(1):33–9. doi: <https://doi.org/10.1051/medsci/2024191>
  26. Leonard A, Tisdale JF, Bonner M. Gene therapy for hemoglobinopathies: beta-thalassaemia, sickle cell disease. *Hematol Oncol Clin North Am.* 2022;36(4):769–95. <https://doi.org/10.1016/j.hoc.2022.03.008>
  27. Rai P, Malik P. Gene therapy for hemoglobin disorders – a mini-review. *J Rare Dis Res Treat.* 2016;1(2):25–31.
  28. Lundstrom K. Viral vectors in gene therapy: where do we stand in 2023?. *Viruses.* 2023;15(3):698. doi: <https://doi.org/10.3390/v15030698>
  29. Shah FT, Sayani F, Trompeter S, Drasar E, Piga A. Challenges of blood transfusions in  $\beta$ -thalassaemia. *Blood Rev.* 2019;37:100588. doi: <https://doi.org/10.1016/j.blre.2019.100588>
  30. Engert A, Balduini C, Brand A, Coiffier B, Cordonnier C, Döhner H, *et al.* The European hematology association roadmap for European hematology research: a consensus document. *Haematologica.* 2016;101(2):115–208. doi: <https://doi.org/10.3324/haematol.2015.136739>
  31. Platt OS, Orkin SH, Dover G, Beardsley GP, Miller B, Nathan DG. Hydroxyurea enhances fetal hemoglobin production in sickle cell anemia. *J Clin Invest.* 1984;74(2):652–6. doi: <https://doi.org/10.1172/JCI111464>
  32. Cappellini MD, Viprakasit V, Taher AT, Georgiev P, Kuo KHM, Coates T, *et al.* A phase 3 trial of luspatercept in patients with transfusion-dependent  $\beta$ -thalassaemia. *N Engl J Med.* 2020;382(13):1219–31. doi: <https://doi.org/10.1056/NEJMoa1910182>
  33. Ataga KI, Kutlar A, Kanter J, Liles D, Cancado R, Friedrisch J, *et al.* Crizanlizumab for the prevention of pain crises in sickle cell disease. *N Engl J Med.* 2017;376(5):429–39. doi: <https://doi.org/10.1056/NEJMoa1611770>
  34. Baronciani D, Angelucci E, Potschger U, Gaziev J, Yesilipek A, Zecca M, *et al.* Hemopoietic stem cell transplantation in thalassaemia: a report from the European Society for Blood and Bone Marrow Transplantation Hemoglobinopathy Registry, 2000–2010. *Bone Marrow Transplant.* 2016;51(4):536–41. doi: <https://doi.org/10.1038/bmt.2015.293>
  35. Helmi N, Bashir M, Shireen A, Mirza Ahmed I. Thalassaemia review: features, dental considerations and management. *Electron Physician.* 2017;9(3):4003–8. doi: <https://doi.org/10.19082/4003>
  36. Hsu LL, Fan-Hsu J. Evidence-based dental management in the new era of sickle cell disease: a scoping review. *J Am Dent Assoc.* 2020;151(9):668–77. doi: <https://doi.org/10.1016/j.adaj.2020.05.023>
  37. Chekroun M, Chérifi H, Fournier B, Gaultier F, Sitbon IY, Ferré FC, *et al.* Oral manifestations of sickle cell disease. *Br Dent J.* 2019;226:27–31. doi: <https://doi.org/10.1038/sj.bdj.2019.4>
  38. Hattab FN. Mesiodistal crown diameters and tooth size discrepancy of permanent dentition in thalassaemic patients. *J Clin Exp Dent.* 2013;5(5):e239–244. doi: <https://doi.org/10.4317/jced.51214>
  39. N. Hattab F. Patterns of physical growth and dental development in Jordanian children and adolescents with thalassaemia major. *J Oral Sci.* 2013;55(1):71–7. doi: <https://doi.org/10.2334/josnurd.55.71>
  40. Prevost R, Feugueur G, Moizan H, Keribin P, Kimakhe J, Veyssié A. Management of patients with sickle cell disease in oral surgery: literature review and update. *J Stomatol Oral Maxillofac Surg.* 2018;119:493–7. doi: <https://doi.org/10.1016/j.jormas.2018.06.010>
  41. Engert A, Balduini C, Brand A, Coiffier B, Cordonnier C, Döhner H, *et al.* The European Hematology association roadmap. *Haematologica.* 2016;101:115–208. doi: <https://doi.org/10.3324/haematol.2015.136739>

#### How to cite this article:

Prasad RBK, Setlur KP, Annegowda VM, Savita AM, Nanaiah P, Rangan V. Efficacy and safety of gene therapy in  $\beta$ -thalassaemia and sickle cell disease: A systematic review and meta-analysis. *J Appl Pharm Sci.* 2026;16(04):109-124. DOI: 10.7324/JAPS.2026.268821