

Review Article

Phase 1/2 Trial of Lentiviral Gene Therapy with Reduced-Intensity Conditioning For Sickle Cell Disease

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Abstract: Sickle cell disease (SCD) is a monogenic hematologic disorder characterized by chronic hemolytic anemia, Vaso-occlusive crises, and significant morbidity and mortality. Lentiviral gene therapy has emerged as a promising curative strategy by enabling autologous hematopoietic stem cells (HSCs) to produce anti-sickling hemoglobin, particularly through the induction of fetal hemoglobin (Hb F) or engineered hemoglobin variants. This review explores the latest advancements in lentiviral gene therapy for SCD, with a particular focus on the use of reduced-intensity conditioning (RIC), especially single-agent melphalan, as a safer alternative to traditional myeloablative regimens. RIC facilitates engraftment while reducing regimen-related toxicity, thereby broadening the eligibility of patients and improving quality of life. The review discusses clinical outcomes including hematologic reconstitution, vector copy number stability, hemoglobin expression, and frequency of Vaso occlusive events. Additionally, it examines the safety profile, risks of insertional mutagenesis, and durability of gene-modified cell populations. Comparisons with CRISPR-Cas9-based editing platforms and ongoing challenges in scalability, long-term surveillance, and equitable access are also addressed. Lentiviral gene therapy with RIC represents a transformative development in the curative landscape of SCD, warranting further optimization to enhance safety, accessibility, and global application.

Keywords: lentiviral gene therapy, reduced-intensity, conditioning, sickle cell disease

1. INTRODUCTION

Sickle cell disease (SCD) is a severe, lifelong inherited hemoglobinopathy that poses a major global health burden, affecting millions of individuals, particularly those of African, Middle Eastern, and South Asian descent. The disorder arises from a single-point mutation in the β -globin gene, resulting in the production of hemoglobin S (Hb S) [1]. Under deoxygenated conditions, Hb S molecules undergo polymerization, leading to erythrocyte deformation, increased rigidity, and Vaso-occlusion. The clinical manifestations of SCD include chronic hemolytic anemia, recurrent and painful Vaso-occlusive crises (VOCs), progressive multi-organ damage, stroke, pulmonary hypertension, and ultimately, a markedly reduced life expectancy [2]. Current standard treatments, such as hydroxyurea therapy, chronic blood transfusions, and supportive care, provide symptom relief and reduce complications but do not offer a cure and often carry significant long-term risks including iron overload and alloimmunization [3]. Allogeneic hematopoietic stem cell transplantation (HSCT) remains the only curative option to date; however, its widespread application is hindered by the scarcity of suitable HLA-matched donors and the substantial risks of graft rejection, graft-versus-host disease (GVHD), and regimen-related toxicities. In recent years, gene therapy has emerged as a transformative and potentially curative strategy that circumvents many limitations of HSCT [4]. Among the various platforms, lentiviral vector-based gene therapy has gained particular attention due to its ability to introduce therapeutic transgenes into autologous hematopoietic stem cells (HSCs), thereby enabling the long-term production of anti-sickling

hemoglobin variants, such as fetal hemoglobin (Hb F) or modified β -globin derivatives [5]. One of the key innovations enhancing the feasibility and safety of this approach is the use of reduced-intensity conditioning (RIC) regimens—most notably single-agent melphalan—which effectively enable engraftment of gene-modified HSCs while minimizing toxicity and preserving the endogenous hematopoietic niche. This approach is especially important for pediatric and comorbid patients who may not tolerate myeloablative conditioning [6]. In this review, we examine the mechanistic underpinnings, clinical trial data, safety considerations, and translational challenges of lentiviral gene therapy for SCD with reduced-intensity conditioning, offering insights into its potential to redefine the therapeutic landscape for this debilitating disorder.

2. SICKLE CELL DISEASE: PATHOPHYSIOLOGY AND LIMITATIONS OF CURRENT THERAPIES

Sickle cell disease (SCD) originates from a well-characterized point mutation (Glu6Val) in the β -globin gene (HBB), which leads to the production of sickle hemoglobin (Hb S). Under hypoxic or low-oxygen conditions, Hb S undergoes abnormal polymerization, resulting in the deformation of red blood cells into a rigid, sickle-like shape. These distorted erythrocytes exhibit decreased deformability, increased adhesion to endothelium, and markedly reduced lifespan, culminating in chronic hemolytic anemia and widespread microvascular obstruction. Clinically, SCD manifests with a spectrum of acute and chronic complications, including Vaso-occlusive crises (VOCs), ischemic stroke, acute chest syndrome, avascular necrosis, and progressive multi-organ dysfunction [7]. While several pharmacological agents—such as hydroxyurea, L-glutamine, voxelotor, and crizanlizumab—have been approved for clinical use, their primary role is to alleviate disease symptoms and reduce the frequency of complications rather than to correct the underlying genetic defect [8]. Chronic red cell transfusions remain another cornerstone of SCD management, yet they are associated with cumulative risks such as alloimmunization and iron overload. Currently, allogeneic hematopoietic stem cell transplantation (HSCT) from an HLA-matched sibling donor stands as the only curative modality. However, the application of HSCT is severely constrained by donor availability and significant complications, including graft-versus-host disease (GVHD), transplant rejection, and the toxicity of conditioning regimens. These limitations underscore the pressing need for curative strategies that are both broadly applicable and safer for patients across the age and comorbidity spectrum. Gene therapy has emerged as a revolutionary alternative, aiming either to restore the function of defective β -globin through gene addition or to enhance the expression of fetal hemoglobin (Hb F), which acts as a natural inhibitor of Hb S polymerization. Lentiviral vectors have proven particularly effective in this context due to their ability to integrate into the genome of both dividing and non-dividing cells, enabling long-term, stable expression of therapeutic genes [9]. These vectors are derived from human immunodeficiency virus type 1 (HIV-1) but are rigorously engineered to be replication-incompetent and self-inactivating, substantially improving their biosafety profile. In clinical applications for SCD, autologous CD34⁺ hematopoietic stem and progenitor cells (HSPCs) are harvested from the patient and transduced ex vivo with lentiviral vectors encoding anti-sickling β -globin variants or γ -globin genes. Following gene modification, patients undergo a conditioning regimen—often reduced in intensity—to facilitate engraftment, after which the corrected cells are reinfused. This approach enables endogenous, lifelong production of non-sickling hemoglobin and represents a paradigm shift in the therapeutic landscape of SCD [10].

3. THE EVOLUTION OF CONDITIONING REGIMENS & MOMENTUM TRIAL

The preparative conditioning regimen is a critical component of gene therapy protocols, serving to eliminate endogenous hematopoietic stem cells (HSCs), reduce immune-mediated rejection, and facilitate the engraftment of genetically modified autologous cells. Traditionally, myeloablative conditioning regimens—most commonly utilizing high-dose busulfan—have been employed to achieve optimal marrow ablation and donor cell chimerism [11]. While effective in promoting engraftment,

these regimens are associated with considerable toxicity, including prolonged pancytopenia, mucositis, hepatotoxicity (notably sinusoidal obstruction syndrome), infertility, and an elevated risk of infectious complications. Such adverse effects are particularly concerning for patients with comorbidities or in pediatric populations, thus constraining broader clinical adoption [12]. To mitigate these risks, reduced-intensity conditioning (RIC) strategies have gained attention for their potential to maintain sufficient myeloablation for engraftment while minimizing systemic toxicity. Among various agents evaluated, melphalan—a bifunctional alkylating agent with extensive use in hematologic malignancies—has emerged as a promising RIC agent in the gene therapy setting. Administered at intermediate doses (e.g., 140 mg/m²), melphalan offers an effective balance between hematopoietic niche clearance and tolerability, enabling safer conditioning with reduced mucosal injury, shorter cytopenic intervals, and less gonadotoxicity [13]. This conditioning approach was evaluated in the Phase 1/2 MOMENTUM trial (NCT02186418), which investigated the safety and feasibility of lentiviral gene therapy for severe SCD using a novel fetal hemoglobin variant, HbF^ΔG16D, encoded by the GbG^ΔM vector. The study enrolled adult patients aged 18 to 45 years with a documented history of recurrent Vaso-occlusive crises (VOCs) and/or acute chest syndrome despite optimized standard-of-care treatment [14]. Hematopoietic stem cells were collected through granulocyte colony-stimulating factor (G-CSF) mobilization and leukapheresis, with most patients requiring a median of four collection sessions to achieve adequate CD34⁺ cell yield. Following ex vivo transduction with the lentiviral vector, the gene-modified cells were reinfused into patients who had received a single intravenous dose of melphalan (140 mg/m²) as a reduced-intensity conditioning regimen. The trial assessed multiple endpoints, including the safety of the gene therapy procedure, the feasibility of stem cell collection and transduction, and preliminary efficacy outcomes such as transgene expression, HbF^ΔG16D levels, and reduction in clinical events. The findings demonstrated encouraging early signals of therapeutic benefit and tolerability, supporting melphalan-based RIC as a viable conditioning platform for lentiviral gene therapy in SCD.

4. FEASIBILITY AND SAFETY OUTCOMES

All seven participants enrolled in the MOMENTUM trial successfully met predefined feasibility criteria, underscoring the practicality of the lentiviral gene therapy platform paired with reduced-intensity conditioning (RIC). Each patient achieved the target threshold of $\geq 8 \times 10^6$ CD34⁺ cells/kg, with sufficient ex vivo transduction efficiency using the HbF^ΔG16D-expressing GbG^ΔM lentiviral vector. Notably, there were no instances of graft failure, indicating robust engraftment of the gene-modified autologous hematopoietic stem cells [15]. Hematologic recovery was prompt: neutrophil engraftment was observed at a median of 8 days post-infusion, and platelet recovery occurred at a median of 5 days—substantially faster than the recovery kinetics typically reported with high-dose busulfan-based myeloablation. No dose-limiting toxicities (DLTs), transplant-related mortalities (TRMs), or unexpected safety signals were observed during the peri-transplant period [16]. Although over 500 adverse events (AEs) were reported across the cohort, the vast majority were consistent with the underlying pathology of sickle cell disease and not directly attributable to the gene therapy or conditioning regimen. Importantly, longitudinal follow-up revealed no evidence of vector-related clonal dominance, insertional mutagenesis, or hematologic malignancy, reinforcing the favorable safety profile of lentiviral vectors in this setting. The therapeutic efficacy was also notable. All patients demonstrated durable expression of the therapeutic γ -globin variant HbF^ΔG16D following reinfusion. By 12 months post-treatment, a sustained reduction of over 80% in the frequency of severe vaso-occlusive crises (VOCs) was recorded across the cohort [17]. Interim analyses of hemoglobin composition revealed that HbF^ΔG16D comprised approximately 16–25% of total hemoglobin, which translated into 22–39% functional ant sickling hemoglobin—a threshold historically associated with significant clinical amelioration of SCD. These outcomes are comparable to those observed in the earlier Lenti Globin HGB-206 trials, which utilized fully myeloablative conditioning. The MOMENTUM data thus provide

compelling evidence that reduced-intensity melphalan conditioning, when paired with a high-efficiency lentiviral vector, can achieve therapeutic benefits similar to those obtained with more toxic myeloablation protocols.

5. COMPARATIVE ANALYSIS WITH LENTI GLOBIN AND CRISPR-BASED THERAPIES

The findings from the MOMENTUM trial offer critical insights when contextualized against other leading gene therapy strategies for sickle cell disease, such as Lenti Globin (lovotibeglogene autotemcel) and CRISPR-Cas9-mediated gene editing targeting BCL11A. LentiGlobin utilizes a modified β -globin transgene (HbA^{T87Q}), while the MOMENTUM trial employed the GbG^M vector, which encodes a mutated γ -globin variant (HbF^{G16D}). Preclinical data suggest that γ -globin-based strategies may confer superior anti-sickling effects due to their intrinsic resistance to polymerization under hypoxic conditions, mimicking the protective phenotype of hereditary persistence of fetal hemoglobin (HPFH) [18]. In contrast, CRISPR-Cas9-based therapies—although precise and potentially curative—require complex gene editing infrastructure and continue to rely on full myeloablative conditioning, which limits their accessibility and increases procedural risk. By demonstrating that reduced-intensity conditioning (RIC) with intermediate-dose melphalan can achieve durable gene marking and therapeutic efficacy, MOMENTUM challenges the prevailing notion that myeloablation is essential for successful gene therapy. This finding not only improves the risk-benefit profile of lentiviral gene therapy but also opens the door for treating broader patient populations, including those with comorbidities or limited transplant tolerance [19]. An additional contribution of the MOMENTUM trial was its attention to pharmacokinetic variability, particularly in relation to melphalan exposure. In one patient, unexpectedly high renal clearance of melphalan was associated with suboptimal engraftment, emphasizing the need for personalized dosing strategies in RIC regimens. Such variability underscores the importance of real-time pharmacokinetic monitoring and suggests that future protocols could benefit from pharmacodynamic modeling to ensure uniform bone marrow exposure across diverse patient profiles [20]. Sustained engraftment and persistence of genetically modified hematopoietic stem cells is fundamental for the long-term success of gene therapy. In MOMENTUM, most patients retained over 70% of vector-marked peripheral blood cells six months post-infusion, and vector copy numbers (VCN) remained stable over time. This stands in contrast to earlier trials using Lenti Globin or similar platforms, which often observed VCN attrition months after treatment [21]. The durability observed in MOMENTUM further supports the notion that melphalan-based RIC, when combined with a potent vector like GbG^M, is sufficient to achieve long-lasting engraftment and therapeutic benefit without the need for high-dose myeloablation.

6. BROADER APPLICABILITY: IMPLICATIONS FOR B-THALASSEMIA AND PEDIATRIC SCD

The encouraging outcomes of reduced-intensity conditioning (RIC)-based lentiviral gene therapy in adult patients with sickle cell disease (SCD) provide a compelling rationale for expanding this therapeutic paradigm to other hemoglobinopathies, particularly β -thalassemia. Like SCD, β -thalassemia is characterized by defective or absent β -globin synthesis, and both conditions benefit substantially from elevated levels of fetal hemoglobin (Hb F), which can ameliorate clinical symptoms and reduce transfusion requirements [22]. The shared molecular pathology and therapeutic targetability make β -thalassemia a logical extension for lentiviral gene transfer strategies. Furthermore, the favorable safety profile of RIC, particularly its lower incidence of organ toxicity and infertility, increases its appeal in pediatric populations, where the long-term adverse consequences of traditional myeloablation are especially concerning [23]. However, despite these advances, multiple hurdles must be addressed before RIC-based gene therapy can achieve widespread clinical adoption. Foremost among these is the need for larger, multicenter trials to validate early-phase findings, optimize vector design and dose, refine patient selection criteria, and establish the minimum effective intensity of conditioning. The long-

term safety profile remains incompletely defined, necessitating decade-long surveillance to detect delayed complications such as insertional mutagenesis, clonal hematopoiesis, or secondary malignancies [24]. As gene therapy enters clinical mainstream, standardization of vector manufacturing, transduction efficiency, quality control metrics, and release criteria will become increasingly critical to ensure reproducibility and regulatory compliance across production sites. Another major barrier lies in the economic and logistical feasibility of delivering gene therapy on a global scale. The upfront cost of autologous gene therapy remains prohibitively high, often exceeding hundreds of thousands of dollars per patient. This economic burden is especially problematic in low- and middle-income countries (LMICs), which bear the highest burden of SCD and related disorders. Without innovative financing mechanisms, public-private partnerships, and globally coordinated access programs, there is a significant risk that these transformative therapies will remain inaccessible to the very populations that need them most [25]. Ensuring health equity in the age of genomic medicine will thus require not only scientific innovation but also robust policy frameworks, international collaboration, and sustained advocacy.

7. CONCLUSION

Lentiviral gene therapy coupled with reduced-intensity melphalan conditioning represents a transformative advancement in the curative landscape of sickle cell disease (SCD). Findings from the MOMENTUM trial underscore the feasibility, safety, and efficacy of this approach, demonstrating that stable engraftment of gene-modified autologous hematopoietic stem cells can be achieved without the need for full myeloablation. This less toxic conditioning strategy resulted in significant reductions in Vaso-occlusive crises (VOCs) and sustained production of therapeutic levels of anti-sickling hemoglobin, establishing a favorable risk-benefit profile. The use of reduced-intensity conditioning not only broadens the potential patient population—including those previously ineligible due to comorbidities or age—but also paves the way for expanding gene therapy to other hemoglobinopathies such as β -thalassemia. Moreover, its safety profile may facilitate application in pediatric settings, where minimizing long-term toxicity is essential. As larger trials mature and real-world data accumulate, this approach may redefine the standard of care for SCD, offering a scalable and more globally accessible alternative to traditional transplantation and gene-editing platforms. Future research must focus on optimizing vector design, refining conditioning protocols, ensuring long-term safety, and addressing economic and logistical barriers to equitable access worldwide.

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