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Review

Use of Red Cell Function Testing in Sickle Cell Disease Clinical Trials

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Abstract

Sickle cell disease (SCD) is characterized by hemoglobin S polymerization and diverse red blood cell (RBC) abnormalities that impair microvascular blood flow. The endpoints of the majority of SCD clinical trials include acute pain complications, hemoglobin levels, and markers of hemolysis. Unfortunately, these outcomes do not directly establish whether a therapy normalizes RBC function. We review the role of hemorheological testing in evaluating established, investigational, and potentially curative SCD therapies, and describe the major determinants of abnormal blood flow in SCD, including whole blood viscosity (WBV), RBC deformability, hypoxia-induced sickling, cellular adhesion, and RBC density. Experiences with senicapoc, crizanlizumab, and voxelotor demonstrate how improvement in a single biological or hematologic parameter may fail to establish overall clinical benefit and underscore the need for multimodal functional assessment. Rheological testing may also permit more informative comparisons across hematopoietic stem cell transplantation and gene therapy approaches, for which conventional definitions of a cure remain incomplete. With further analytical standardization and prospective clinical validation, integrated RBC functional profiles could support mechanism-based patient selection, assess treatment response, and identify deterioration before overt acute pain complications or progressive organ injury. Predictive and early detection of clinical deterioration remain investigational. Incorporating rheology into SCD trials may therefore strengthen therapeutic evaluation and support investigation of more individualized clinical care.

Keywords: sickle cell disease; rheology; gene therapy; hemorheology; red blood cell deformability; blood viscosity; oxygen-gradient ektacytometry; cellular adhesion

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1. Introduction

Sickle cell disease (SCD) comprises a group of inherited hemoglobin disorders characterized by the production of sickle hemoglobin (HbS); as of 2021, SCD is estimated to affect 7.74 million people worldwide [1]. HbS results from a missense variant in the hemoglobin subunit beta (HBB) gene that replaces glutamic acid with valine at the sixth position of the β-globin chain [2]. Under deoxygenated conditions, HbS polymerizes into rigid intracellular fibers that distort red blood cell (RBC) morphology, increase cellular rigidity, and reduce deformability [3]. This polymerization-driven change, together with increased RBC adhesion, hemolysis, and altered whole blood viscosity (WBV), impairs microvascular flow and contributes to acute pain complications [3,4]. Because sickling and deformability vary dynamically with oxygen tension and cellular composition, rheological measurements provide mechanistically relevant approaches for characterizing disease severity and therapeutic

response [4,5]. Sickle RBCs also exhibit abnormal adhesion to the vascular endothelium, further impairing microvascular blood flow [6]. Hypoxia promotes HbS polymerization and RBC adhesion, compounding the risk of microvascular obstruction [7]. Together, these biophysical abnormalities contribute to acute pain complications, ischemic injury, and progressive organ damage.

Acute pain complications are therefore not simply a consequence of the characteristic sickle shape but reflect the interaction of multiple molecular, cellular, and hemodynamic processes. Blood rheology, the study of the deformation and flow of blood and its cellular components, is influenced by hematocrit, plasma viscosity, RBC deformability, aggregation, and cellular adhesion [4,8]. Rheological measurements, including WBV and the hematocrit-to-viscosity ratio (HVR), oxygen-dependent RBC deformability, RBC adhesion, and cell density, capture complementary aspects of SCD pathophysiology [4,5,8]. Recent studies have demonstrated that RBC deformability can be quantified across oxygen conditions, and that impaired deformability is associated with hemolysis, acute pain events, and treatment response [5,9,10].

The therapeutic landscape of SCD has expanded from supportive care, transfusion, and pharmacologic disease modification to potentially curative allogeneic hematopoietic stem cell transplantation and autologous gene therapies [11–13]. Gene-addition and gene-editing trials have demonstrated sustained production of anti-sickling hemoglobin and substantial reductions in severe vaso-occlusive outcomes [12,13]. However, clinical event rates and routine laboratory measurements, including total hemoglobin, fetal hemoglobin, and markers of hemolysis, do not directly establish whether RBC function and microvascular blood flow have normalized [8]. Incorporating validated rheological biomarkers alongside clinical and hematologic outcomes may provide a more comprehensive assessment of therapeutic response, residual disease biology, and the extent to which a treatment achieves functional correction [4,8,10]. The expanding therapeutic landscape creates a need for endpoints that distinguish improvement in isolated laboratory parameters from meaningful restoration of RBC function. Integrating analytically robust and clinically validated rheologic measures with conventional hematologic and clinical outcomes may provide a more comprehensive framework for assessing therapeutic efficacy and determining whether a functional cure has been achieved by gene therapy and allogeneic transplantation.

Literature Identification and Selection

Literature for this review was identified through PubMed without publication date restrictions, with an emphasis on more recent articles. Articles were selected for their relevance to RBC functional testing, its application in SCD treatment studies, and the interpretation of treatment-associated changes. PubMed searches combined “sickle cell disease” or “sickle cell anemia” with terms related to red cell function, including “blood rheology”, “whole blood viscosity”, “red blood cell deformability”, “oxygen-gradient ektacytometry”, “point of sickling”, and “red blood cell adhesion”. Additional searches focused on clinical trials and individual therapies. Earlier studies were retained when they described foundational methods or provided relevant clinical evidence. Peer-reviewed trials and observational studies were considered separately from ancillary analyses, conference abstracts, regulatory documents, company reports, and descriptions of ongoing work. The comparative synthesis considered study design, participant and sample counts, endpoint status, principal findings, and limitations. Related reports were assessed together to avoid treating potentially overlapping cohorts as independent evidence.

2. RBC Functional Assays and Their Interpretation

2.1. Rheological Mechanisms in SCD

Blood rheology describes the deformation and flow behavior of blood and its cellular components. Although WBV is its most familiar macroscopic measure, viscosity varies with shear rate and is influenced by hematocrit, plasma viscosity, red cell aggregation, and red cell deformability. At the microvascular level, the ability of red blood cells to deform while traversing narrow capillaries and avoid abnormal adhesive interactions with the vascular wall influences blood flow. Therefore, rheological measurements provide a functional link between HbS polymerization, cellular injury, impaired blood flow, and tissue perfusion that conventional measures such as hemoglobin concentration, reticulocyte count, and markers of hemolysis capture only in part [8,14].

Rheological impairment in SCD is dynamic and multidimensional. Deoxygenation initiates HbS polymerization, causing RBCs to change shape and become progressively less deformable. Repeated cycles of sickling and unsickling damage the membrane, while cellular dehydration increases intracellular HbS concentration and produces dense RBCs that are particularly susceptible to polymerization [8,14,15]. Impaired deformability occurs alongside abnormal RBC aggregation and adhesion to endothelial or subendothelial ligands, and these abnormalities can interact to restrict microvascular perfusion. Hematocrit further complicates the interpretation of WBV. At native hematocrit, blood from individuals with sickle cell anemia (HbSS) may have lower viscosity than blood from controls with the normal adult hemoglobin genotype (HbAA). When both groups were tested at a hematocrit of 0.50, viscosity was higher in the HbSS samples than in HbAA samples. Hypoxia also increased viscosity in the HbSS samples, with no increase in HbAA samples [4]. Consequently, a low native-hematocrit viscosity should not automatically be interpreted as favorable or normalized blood flow. Viscosity should be interpreted together with hematocrit, RBC deformability, density, and adhesion.

These dependencies explain why results can vary between assays and patients. At low shear, aggregation contributes more to viscosity, whereas at higher shear, the ability of RBCs to deform becomes more influential. Oxygen-dependent polymerization and dehydration increase cellular rigidity, but anemia may lower native hematocrit viscosity despite these abnormalities. Measurements at native and adjusted hematocrit therefore address different questions and should be reported separately [4,8,14]. The HVR relates RBC volume fraction to flow resistance. Interpreting tissue oxygen delivery also requires information on oxygen affinity, saturation, regional extraction, or cardiac output [16].

2.2. Measured Outputs and Interpretive Limits

Conventional rotational cone-and-plate viscometers measure WBV, generally reported in centipoise (cP), at one or more defined shear rates. Because blood is shear-thinning, results cannot be interpreted without specifying the shear rate, hematocrit, temperature, oxygenation state, and interval between collection and measurement [8,14]. The ADVIA 2120i hematology analyzer (Siemens Healthcare Diagnostics Inc., Tarrytown, NY, USA) quantify hyperchromia, while density-gradient methods quantify cellular density. Both measures inform the interpretation of sickling and deformability. Density-gradient fractionation traditionally defines dense RBCs as cells with a density greater than 1.11 g/mL, whereas the ADVIA 2120i estimates the dense cell fraction by identifying hyperchromatic RBCs with a measured mean corpuscular hemoglobin concentration greater than 41 g/dL [15,17].

Ektacytometry and microfluidic impedance assays provide more direct measurements of RBC mechanical behavior. The Laser Optical Rotational Red Cell Analyzer (LoRRca, RR Mechatronics Manufacturing B.V., Zwaag, The Netherlands) measures the elongation of RBCs exposed to shear stress from the shape of their laser diffraction pattern. Dur-

ing oxygen-gradient ektacytometry (Figure 1), deformability is measured continuously as the sample is deoxygenated and subsequently reoxygenated. The principal parameters include the maximum elongation index (EI) under normoxia (EImax), the minimum elongation index reached under hypoxia (EImin), and the point of sickling (PoS). The PoS is the oxygen tension at which deformability decreases by a specified amount, commonly 5% from EImax [18–20]. The microfluidic impedance red cell assay (MIRCA, Figure 2), by contrast, mechanically challenges RBCs in a micropillar network and uses electrical impedance to quantify normoxic and hypoxic occlusion indices. The normoxic occlusion index reflects baseline mechanical deformability, whereas the hypoxic occlusion index captures the additional impairment produced by deoxygenation [21]. LoRRca generates a population-averaged deformability curve, whereas MIRCA is sensitive to cells that physically obstruct small passages and may therefore detect poorly deformable subpopulations within a heterogeneous sample [21].

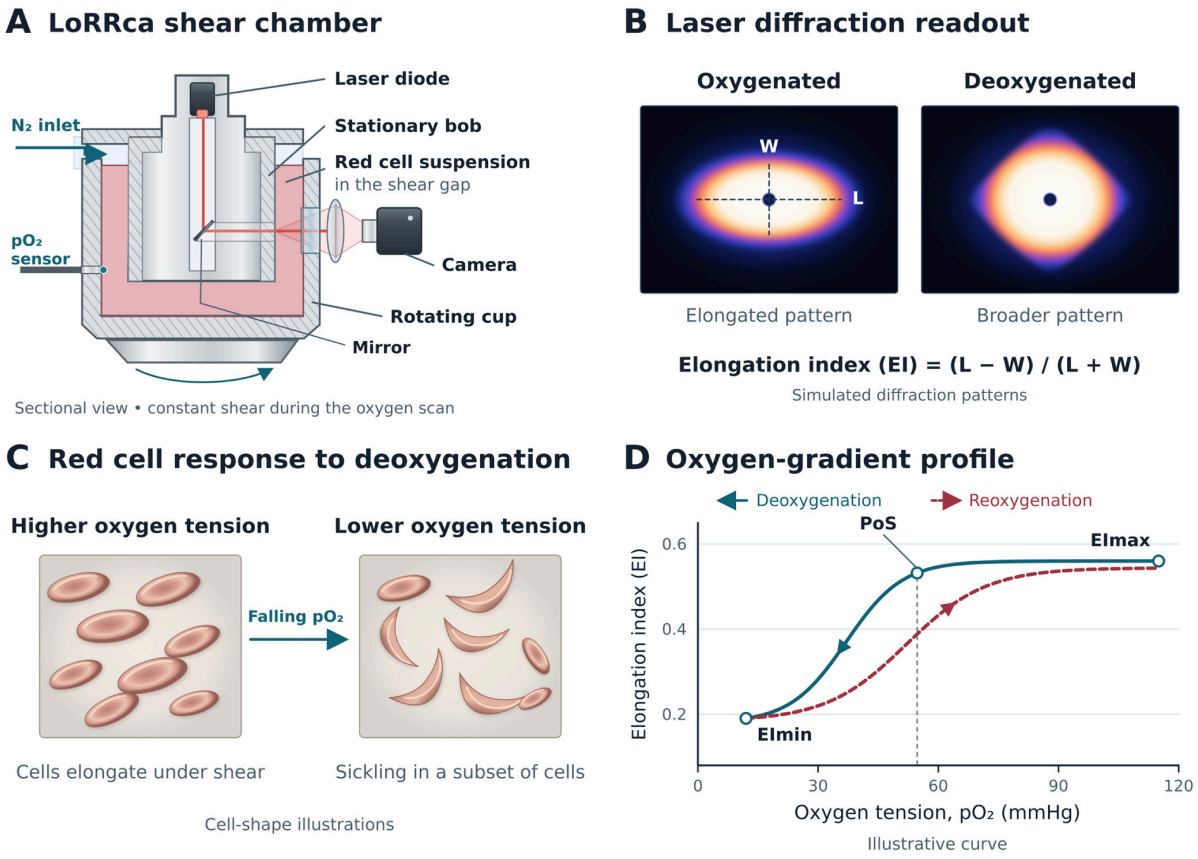


Figure 1. Oxygen-gradient ektacytometry using the Laser Optical Rotational Red Cell Analyzer (LoRRca). (**A**) A red cell suspension is subjected to constant shear between a stationary inner cylinder (bob) and a rotating outer cylinder (cup). Nitrogen lowers the oxygen tension (pO_2), which is monitored by an oxygen sensor. Laser light scattered by the suspension produces a diffraction pattern recorded by the camera. (**B**) Simulated diffraction patterns show an elongated pattern under oxygenated conditions and a broader pattern after deoxygenation. The elongation index (EI) is calculated from the long (L) and short (W) axes of the pattern as $(L - W)/(L + W)$. (**C**) Red cells elongate under shear at higher oxygen tension. As oxygen tension falls, a subset of cells undergo sickling. (**D**) An illustrative oxygen-gradient curve shows EI during deoxygenation (solid line) and reoxygenation (dashed line). EImax is the maximum EI before deoxygenation, EImin is the lowest EI reached during deoxygenation, and the point of sickling (PoS) is the pO_2 at which EI falls by 5% from EImax during deoxygenation [18–20]. The cell shapes, diffraction patterns, and curve are illustrative.

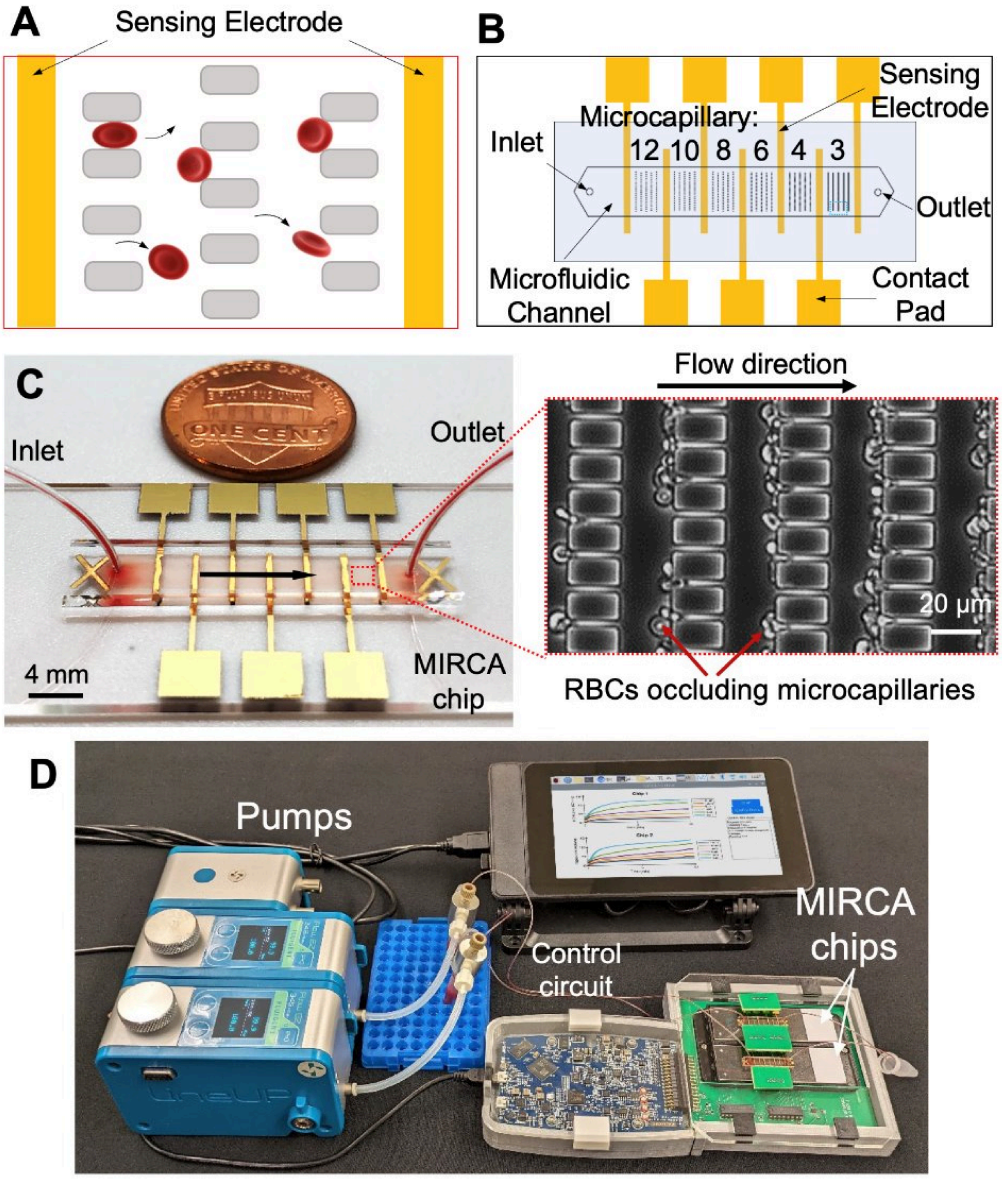


Figure 2. MIRCA Schematic. (**A**) Paired sensing electrodes detect changes in electrical impedance as red blood cells (RBCs) obstruct openings between micropillars. (**B**) The chip contains six micropillar arrays with openings that narrow from 12 to 3 μm along the direction of flow. (**C**) Photograph of the MIRCA chip and magnified micrograph showing RBCs obstructing micropillar openings (red arrows). Scale bars are 4 mm for the chip photograph and 20 μm for the micrograph. (**D**) The MIRCA system setup is compact and modular, including two FlowEZ pressure controllers (Fluigent, Le Kremlin-Bicetre, France) with an automated pressure control system, a control circuit that interfaces with two MIRCA chips, and a 7-inch touchscreen display [21].

Microfluidic adhesion assays assess another component of rheology by perfusing whole blood or isolated RBCs through channels coated with an adhesive ligand such as P-selectin, vascular cell adhesion molecule 1 (VCAM-1), or laminin. In imaging-based flow adhesion assays (Figure 3), investigators image retained cells and report the number of adherent cells per unit as a measure of adhesive burden. Whole-blood adhesion to P-selectin measured under flow has been prospectively associated with self-reported vaso-occlusive crises (VOCs) [22]. Other devices use laminin to model adhesion. An adhesive artificial microvascular network combines laminin-coated, capillary-sized channels with a perfusion measurement, assessing the combined effects of deformability and adhesion rather than evaluating either property in isolation [23,24]. Imaging flow cytometry represents an additional

functional approach, although it is not a direct measurement of deformability or viscosity. The sickle imaging flow cytometry assay (SIFCA) quantifies the proportion of RBCs that undergo morphologic sickling after controlled hypoxic exposure and has demonstrated sensitivity to fetal hemoglobin (HbF), pH, the proportion of HbS-containing blood, and ex vivo anti-sickling treatment [25].

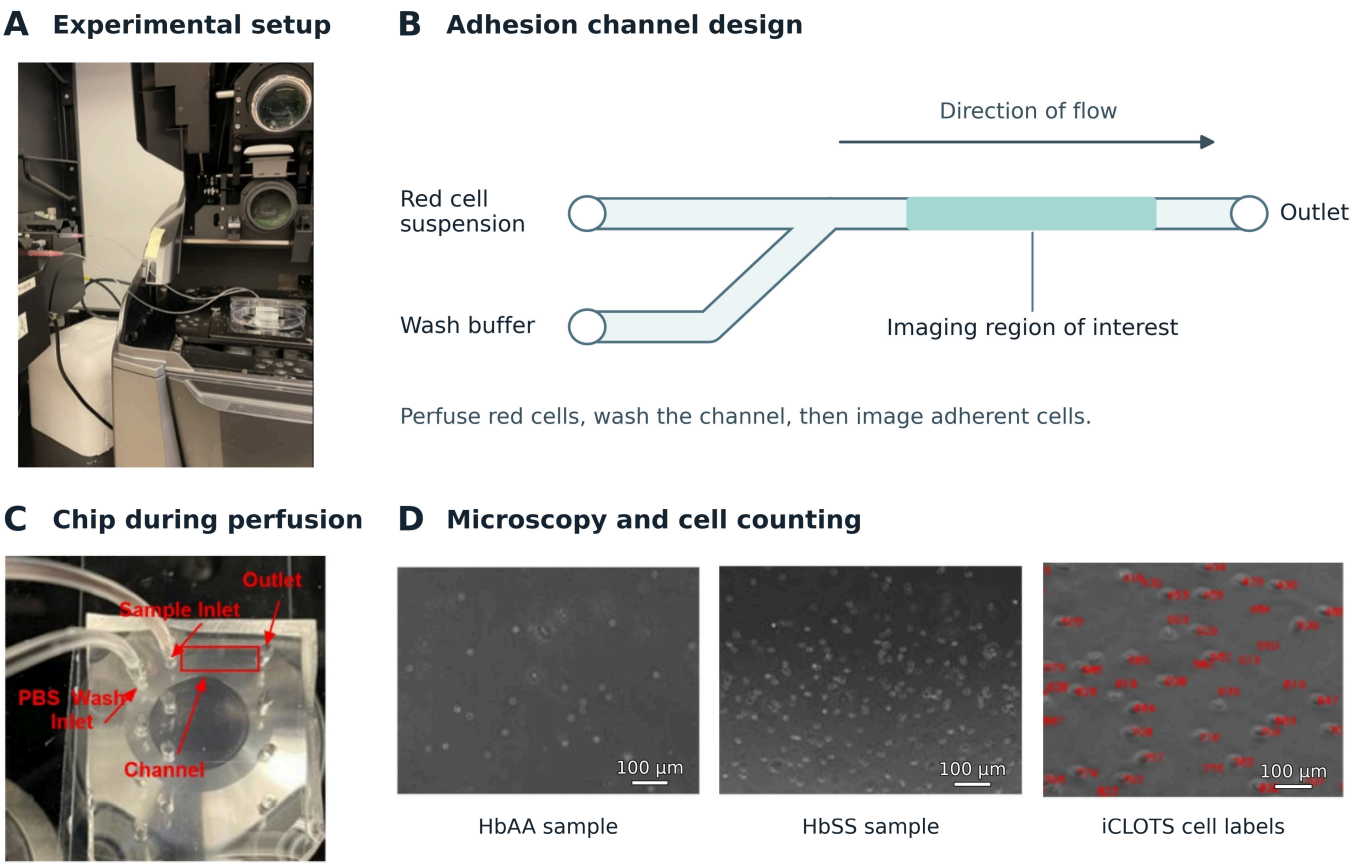


Figure 3. Microfluidic assay for red blood cell adhesion under flow. (**A**) Experimental setup showing the perfusion tubing and BZ-X810 microscope (KEYENCE Corporation of America, Itasca, IL, USA). (**B**) Channel schematic showing the red cell suspension inlet, wash buffer inlet, outlet, and imaging region of interest. (**C**) Photograph of the microfluidic chip during perfusion. (**D**) Representative images of adherent cells from normal adult hemoglobin (HbAA) and homozygous sickle hemoglobin (HbSS) samples and an example of automated cell labeling with iCLOTS (v0.1.1) [26].

These assays capture different aspects of red cell function. LoRRca measures the mechanical response of the red cell population, whereas MIRCA measures occlusion under defined conditions in a microfluidic device. Adhesion assays assess interactions with specific ligands, and the use of whole blood or isolated red cells affects which cell types can contribute to the measured response. Density, hyperchromia, and cell morphology provide complementary information about red cell properties [15,17,18,21,22,24,25]. Table 1 summarizes what each assay measures and the factors that affect its interpretation and use in clinical trials.

2.3. Reproducibility and Clinical Validation

Before these measurements can serve as trial endpoints, both analytical and clinical validation are required. Analytical validation establishes that an assay measures its intended property with adequate precision. It includes assessing within-run and between-run variability, coefficients of variation, operator and instrument effects, interlaboratory repro-

ducibility, linearity, sample stability, and sensitivity to preanalytical variables. For example, sample storage, temperature, hematocrit, shear conditions, and the rate of deoxygenation can materially alter rheological results. The acceptable coefficient of variation depends on the magnitude of the biological or treatment-related change the assay is expected to detect rather than treated as a universal cutoff. In an initial study of the adhesive artificial microvascular network, repeated measurements of the same sample across three devices had an average coefficient of variation below 5%, supporting device-level reproducibility, although this small study did not establish clinical validity [23].

Clinical validation requires evidence that an assay result is associated with a clinically meaningful phenotype or outcome and, ideally, that it adds information about prospectively ascertained outcomes. Dense cell percentage has been associated with leg ulcers, priapism, and renal dysfunction [15]. PoS and EImin have been associated with acute SCD complications [19]. Whole-blood adhesion to P-selectin has been prospectively associated with self-reported VOCs [22], and MIRCA occlusion indices have been associated with the frequency of acute complications [21]. These findings support the clinical relevance of rheological biomarkers, but no single measurement captures the full complexity of SCD blood flow.

A change in red cell function after treatment can provide evidence of pharmacodynamic activity, while an association with subsequent complications may support prognostic use. Predictive use requires evidence that the effect of a specific treatment differs according to the biomarker. For an assay measure to serve as a validated surrogate endpoint, treatment effects on that measure must reliably predict effects on a clinical outcome in the intended setting [27].

In multicenter trials, consistent sample collection, handling, instrument calibration, and quality control help make results comparable across sites. Evaluating assay performance across operators, instruments, and sites can identify sources of variation. A central laboratory can reduce variation related to testing at multiple sites when samples remain stable under the planned shipping conditions. Changes over time should be interpreted in light of both assay variability and natural variation within the same participant. Custom devices and methods that use chemically induced hypoxia can help explore biological responses to treatments. The validation needed for these methods depends on how the results will be used in the trial [18,20,23,28].

Table 1. Comparison of red cell functional assays in sickle cell disease. Major assays are compared by sample preparation, measured output, principal confounders and limitations, reproducibility, standardization, clinical validation, and feasibility in multicenter trials.

Assay	Sample Preparation and Output	Main Confounders and Limits	Reproducibility and Standardization	Clinical Validation	Multicenter Feasibility
Viscosity/HVR [4,8,14,16]	Whole blood at native or specified HCT. Viscosity at defined shear; derived HVR.	HCT, shear, oxygenation, temperature, and storage. Bulk-flow measurement.	Calibrate instruments; harmonize shear, temperature, and oxygenation.	Clinical associations and treatment-response studies.	Routine viscometry feasible; hypoxic protocols need qualification.
Oxygen-gradient ektacytometry [18–20,29,30]	Standard RBC input in viscous medium. EImax, EImin, and PoS.	Cell input, storage, and oxygen ramp. Population average; affinity affects PoS.	Repeatability reported; standardize preparation and instrument settings.	Treatment response and clinical associations; pain findings vary.	Specialized instrument; central or qualified-site testing.

Table 1. *Cont.*

Assay	Sample Preparation and Output	Main Confounders and Limits	Reproducibility and Standardization	Clinical Validation	Multicenter Feasibility
MIRCA [21]	Isolated RBCs at defined HCT. Normoxic and hypoxic occlusion indices.	Preparation, HCT, pressure, and chip geometry. Device-level occlusion.	Variability assessed across three devices; broader site validation needed.	Clinical associations; treatment-guidance algorithm proposed.	Portable instrument; chip preparation and sample handling need harmonization.
Flow adhesion [22,24]	Whole blood or isolated RBCs over a specified ligand. Adherent cells/index.	Ligand, shear, cell mixture, and imaging. Results are ligand-specific.	Cross-site precision NR; standardize coating and image analysis.	Prospective P-selectin risk association; laminin feasibility reports.	Platform-dependent; qualify storage and shipping.
Density/ hyperchromia [15,17]	Whole-blood analysis or density separation. Dense/hyperchromic-cell fraction.	Platform, threshold, hydration, and transfusion. Outputs differ by method.	Automated measurement; use consistent platform and thresholds.	Clinical associations and hydroxyurea response.	Analyzer testing is accessible; density gradients are more labor-intensive.
Sickling imaging [25]	Deoxygenated, fixed RBCs. Proportion of morphologically sickled cells.	Oxygen, pH, fixation, and classifier. Morphologic endpoint.	Automated scoring; multicenter precision NR.	Analytical and mechanistic studies.	Specialized imaging; standardized acquisition and classification.
Adhesive microvascular network [23]	RBC suspension through coated microchannels. Perfusion rate.	Coating, geometry, pressure, and HCT. Combined adhesion/mechanics.	Device characterization reported; multicenter precision NR.	Proof-of-concept functional studies.	Custom devices and specialist expertise.
Oxygen dissociation curve [31–34]	Blood/RBCs under controlled conditions. Saturation curve and p50.	pH, temperature, and Hb composition. Oxygen delivery needs complementary assessment.	Calibrate gas/saturation measurements; standardize assay conditions.	Mechanistic and pharmacody-namic use.	Specialized equipment; qualify specimen stability.

Note: Clinical validation refers to the use studied. Associations and treatment responses support biological relevance. Treatment-selection thresholds and surrogate endpoints require additional validation. Multicenter testing also depends on documented specimen stability. RBC, red blood cell; Hb, hemoglobin; HCT, hematocrit; HVR, hematocrit-to-viscosity ratio; EImax/EImin, maximum/minimum elongation index; PoS, point of sickling; MIRCA, microfluidic impedance red cell assay; p50, oxygen tension at 50% Hb saturation; NR, not reported in the cited source.

3. Heterogeneity of RBC Function in SCD

Although HbS arises from a single β-globin substitution, it does not produce a uniform clinical course. Even among individuals with the same genotype, the frequency of acute pain events, degree of anemia and hemolysis, development of chronic organ damage, and overall disease severity vary substantially. This heterogeneity is influenced by HbF concentration and distribution, coinheritance of alpha-thalassemia, additional genetic modifiers, age, treatment exposure, environmental factors, and access to comprehensive care [35,36]. Consequently, genotype and conventional hematologic measurements alone cannot fully predict which complications will predominate in an individual patient.

To organize this clinical heterogeneity, Kato and colleagues proposed two partially overlapping SCD subphenotypes [37]. The viscosity-vaso-occlusion phenotype is characterized by relatively higher hemoglobin, lower hemolytic activity, and a greater tendency toward complications such as recurrent acute pain complications, acute chest syndrome, and os-

teonecrosis. In contrast, the hemolysis-endothelial dysfunction phenotype is characterized by more severe hemolytic anemia and higher levels of hemolysis markers and has been associated with pulmonary hypertension, leg ulcers, priapism, and large-vessel stroke [37]. This framework provides a useful connection between underlying pathophysiology and clinical presentation, but it should not be interpreted as dividing patients into two mutually exclusive groups. The strength and causal interpretation of some proposed associations, particularly the relationship between hemolysis and pulmonary hypertension, have been debated, and many patients develop complications attributed to both subphenotypes [38]. Furthermore, hemolysis, inflammation, cellular adhesion, endothelial dysfunction, impaired RBC deformability, and increased viscosity interact dynamically rather than functioning as independent pathways [14].

Heterogeneity occurs among patients and among RBCs within the same sample. Single-cell microfluidic studies have identified deformable and nondeformable sickle RBC populations with different adhesion strengths [39]. Recent oxygen-gradient ektacytometry data from a large multicenter cohort similarly demonstrated that detailed functional parameters could distinguish differences in genotype, hemolytic profile, inflammation, and hydroxyurea exposure that may not be captured by conventional measurements alone [29]. These findings support investigating whether the degree of impairment across WBV, deformability, adhesion, and density varies within individuals. Future trials could test whether these profiles help identify subgroups with different functional responses to treatment.

4. Pharmacological Therapy and Transfusion

4.1. Hydroxyurea

Hydroxyurea is the first disease-modifying pharmacologic therapy approved for the treatment of SCD and remains a cornerstone of clinical management. The therapeutic benefits of hydroxyurea are primarily attributed to its ability to increase fetal hemoglobin (HbF) levels. HbF interferes with HbS polymer formation and reduces RBC sickling [40–42]. The percentage of HbF has been shown to be a powerful indicator of clinical severity in SCD. In a pediatric hydroxyurea cohort, HbF above 20% was associated with fewer hospitalizations, but this is not a universal threshold preventing recurrent pain [43]. Protection from recurrent pain varies among patients and depends in part on how HbF is distributed across RBCs and how much HbF individual cells contain [35,41]. Pharmacological induction of HbF helps prevent intracellular sickling, thereby decreasing acute pain complications and reducing hemolysis. Beyond HbF induction, the cytotoxic effects of hydroxyurea also reduce marrow production of neutrophils, reticulocytes, and platelets, which contribute to inflammation [42]. Both neutrophils and reticulocytes promote acute pain complications through vascular adhesion; hydroxyurea lowers their absolute numbers and reduces surface expression of adhesion receptors, which may improve blood flow and reduce the frequency of acute pain complications.

A randomized trial by the Multicenter Study of Hydroxyurea (MSH) showed that hydroxyurea reduced the median annual rate of painful crises from 4.5 to 2.5 and reduced the number of participants who developed acute chest syndrome [40]. In long-term observational follow-up of the MSH cohort, hydroxyurea use was associated with reduced mortality [44].

4.2. Transfusions

Red blood cell transfusion therapy is a well-established treatment strategy for SCD that aims to both increase the oxygen-carrying capacity of blood and to treat selected complications, such as stroke, severe symptomatic anemia, and acute chest syndrome, by lowering the proportion of circulating HbS [45]. Clinical studies have demonstrated that chronic

transfusion programs significantly reduce the incidence of ischemic stroke in children, establishing transfusion therapy as a standard of care in specific patient populations, such as children who have had abnormal results on transcranial Doppler ultrasonography [46].

Transfusions can be administered as a simple transfusion or as an exchange transfusion. Transfusion is not routinely indicated for uncomplicated acute vaso-occlusive pain alone. In an acute situation, a simple transfusion will increase the oxygen-carrying capacity, but there is a risk of hyperviscosity if the hemoglobin (Hb) is increased significantly above the patient's baseline. A hemoglobin target near 10 g/dL is often used for simple transfusion in patients with homozygous sickle hemoglobin (HbSS). Targets depend on the indication, baseline hemoglobin, HbS fraction, and transfusion modality [45,47]. Red cell exchange (RCE) can improve oxygen-carrying capacity and allow greater control of hematocrit while lowering the HbS fraction. The target HbS fraction is indication-specific [45,47]. Despite the clinical benefits of transfusion therapy for selected indications, long-term transfusion therapy is associated with significant complications such as iron overload and alloimmunization.

Alloimmunization, a major complication of RBC transfusion therapy, occurs when the recipient develops antibodies against non-self RBC antigens present on transfused donor cells. The development of these antibodies often results in clinical complications, delayed transfusions, and additional problems in crossmatching [48,49]. Alloimmunization can lead to delayed hemolytic transfusion reactions (DHTRs). A DHTR can be accompanied by hyperhemolysis syndrome, in which the patient's hemoglobin falls below the pretransfusion level, suggesting destruction of both patient and donor RBCs. Hyperhemolysis can occur without detectable alloantibodies and with a negative direct antiglobulin test. Its mechanisms remain incompletely understood [47,49].

4.3. Endari

Endari (L-glutamine oral powder) may reduce oxidative stress in sickle RBCs by increasing the proportion of reduced nicotinamide adenine dinucleotide. In the phase 3 trial, the median number of sickle cell pain crises over 48 weeks was three with L-glutamine and four with placebo. Hemoglobin concentrations and reticulocyte counts did not differ significantly between groups [50].

Additional preclinical findings suggested that Endari may influence the intestinal barrier function. Intestinal dysbiosis and increased permeability have been reported in patients with SCD. In a study using Townes sickle cell mice, Haroun et al. [51] found that Endari treatment reduced intestinal permeability and circulating lipopolysaccharide (LPS) concentrations. The authors discussed how increased intestinal permeability can allow luminal pathogen-associated molecular patterns (PAMPs), such as LPS, to enter the circulation. Circulating LPS can promote inflammation through neutrophil activation and aging. Haroun et al. proposed that improved barrier integrity could reduce this inflammatory stimulus [51]. Whether this mechanism contributes to the clinical effects of Endari remains to be established.

5. Potentially Curative Therapies

5.1. Allogeneic Stem Cell Transplantation

Allogeneic hematopoietic stem cell transplantation (HSCT) is the longest established curative therapy for SCD. HSCT replaces the patient's diseased hematopoietic cells with donor stem cells capable of producing normal hemoglobin. In patients who achieve stable donor engraftment, transplantation results in sustained production of adult hemoglobin (HbA) from donors with the normal adult hemoglobin genotype (HbAA) or sickle cell trait (HbAS) and can reduce hemolysis, acute pain events, and other disease-related complications. In a cohort of 234 patients younger than 30 years of age who underwent matched-

sibling transplantation, the overall five-year event-free survival rate was 93.9%; a later estimate applied to a cohort of 151 patients reached 97.9%. Patients with stable mixed chimerism experienced no recurrent SCD-related events, although evidence of hemolytic anemia persisted when donor chimerism was below 50% [52]. Other studies suggest that approximately 20% stable donor myeloid chimerism may be sufficient to prevent recurrence of the sickle phenotype [53]. Although haploidentical and unrelated donor approaches have expanded access for patients without matched sibling donors, donor type remains an important determinant of graft failure, transplant-related complications, and long-term survival [54].

Current guidelines support consideration of HSCT in selected patients with severe SCD complications such as stroke, acute chest syndrome, recurrent pain crises, nephropathy, retinopathy, osteonecrosis of multiple joints, and priapism [55,56]. Transplantation is only advisable when the benefits outweigh the risks of infection, graft-versus-host disease, and organ injury from pretransplant conditioning. HSCT can stabilize and even improve organ function by preventing further SCD-related damage [55,57]. Access depends on donor availability and eligibility for conditioning. Age and preexisting organ dysfunction can also affect outcomes. These limitations have driven the development of gene-based therapies that aim to achieve curative outcomes without donor availability constraints or the risk of graft-versus-host disease.

5.2. Gene Therapy and Assessment of Functional Cure

Autologous hematopoietic stem cell-based gene therapy has emerged as another potentially curative treatment for SCD. In contrast to allogeneic transplantation, gene therapy uses the patient's own CD34+ hematopoietic stem and progenitor cells, eliminating the need for a compatible donor and avoiding graft rejection and graft-versus-host disease [58]. Treatment nevertheless remains a complex, multistep process requiring assessment of organ function and the patient's ability to tolerate myeloablative conditioning [58].

Two autologous gene therapies are currently approved by the U.S. Food and Drug Administration (FDA) for SCD. Exagamglogene autotemcel (exa-cel; CASGEVY), developed by Vertex Pharmaceuticals, uses CRISPR-Cas9 editing to disrupt the erythroid-specific enhancer of BCL11A, a transcriptional repressor of γ-globin expression. Reduced BCL11A activity reactivates fetal hemoglobin production in developing erythrocytes. HbF interferes with HbS polymerization, thereby reducing sickling without directly correcting the disease-causing HBB mutation [13,59]. CASGEVY is currently FDA-approved for patients aged 2 years or older with SCD and recurrent vaso-occlusive crises (VOCs) [59]. In the phase 3 CLIMB SCD-121 trial, 44 patients received exa-cel. Among the 30 patients with sufficient follow-up for the primary efficacy analysis, 29 (97%) remained free from protocol-defined severe VOCs for at least 12 consecutive months, and all 30 remained free from hospitalization for severe VOCs for at least 12 consecutive months [13].

Lovotibeglogene autotemcel (lovo-cel; LYFGENIA), developed by bluebird bio (now Genetix Biotherapeutics), uses a self-inactivating lentiviral vector to insert a modified β-globin gene into autologous hematopoietic stem cells. The introduced gene produces HbA^{T87Q}, an anti-sickling hemoglobin that functions similarly to normal adult hemoglobin and reduces the relative intracellular concentration of HbS [12,60]. LYFGENIA is FDA-approved for patients 12 years or older with SCD and a history of vaso-occlusive events (VOEs) [60]. In the HGB-206 cohort, treatment produced sustained HbA^{T87Q} expression, increased total hemoglobin, and reduced markers of hemolysis. All 25 evaluable patients experienced complete resolution of severe VOEs during the primary evaluation period, compared with a median of 3.5 severe events per year before treatment [12].

Additional investigational strategies are expanding the range of possible genetic modifications. Renizgamglogene autogedtemcel (reni-cel), developed by Editas, uses CRISPR-Cas12a to disrupt BCL11A-binding sites within the HBG1 and HBG2 promoters and reactivates HbF production. In an early-phase study, 27 of 28 treated patients experienced no protocol-defined severe VOEs after infusion, although median follow-up was 9.5 months, and the study was terminated early following a reassessment of the sponsor's development priorities [61]. Base-editing approaches such as ristoglogene autogetemcel (risto-cel), developed by BEAM Therapeutics, similarly modify HBG1 and HBG2 promoters to inhibit BCL11A binding and increase γ-globin expression, thereby mimicking the protective effects of naturally occurring hereditary persistence of fetal hemoglobin, while avoiding the double-stranded DNA breaks generated by conventional CRISPR nucleases [62,63]. A distinct strategy involves direct correction of the pathogenic HBB variant. Nulabeglogene autogedtemcel (nula-cel), developed by Kamau Therapeutics, uses CRISPR–Cas9-mediated homology-directed repair (HDR) to convert the pathogenic sickle allele in the endogenous HBB gene to a normal HBB sequence, thereby restoring adult hemoglobin (HbA) and reducing sickle hemoglobin (HbS) [64,65]. In contrast, a separate lentiviral gene-addition strategy uses the GbG^{M} vector to introduce a modified γ-globin gene encoding the anti-sickling hemoglobin HbF^{G16D} without correcting the endogenous HBB variant. This approach has been evaluated with reduced-intensity melphalan conditioning with the aim of reducing conditioning toxicity [66].

Although autologous gene therapy avoids alloimmune complications, it does not eliminate the risks associated with stem-cell transplantation. Myeloablative conditioning can cause cytopenias, infections, mucositis, and organ injury [12,13,58–60]. Gonadal toxicity and infertility are potential long-term consequences of myeloablative conditioning [58–60]. Gene-editing approaches carry a potential risk of unintended off-target editing, whereas lentiviral gene-addition therapies carry a potential risk of vector-mediated insertional oncogenesis, necessitating long-term safety monitoring [58–60]. Lovo-cel has an FDA boxed warning for hematologic malignancy, and recipients require lifelong monitoring, including regular complete blood counts and evaluation for clonal or malignant hematologic abnormalities [60].

A cure for SCD cannot be defined solely by successful hematopoietic engraftment or the detection of genetically modified cells. Because current gene-addition and HbF-reactivation strategies do not eliminate endogenous HbS production, and because established organ injury may be irreversible, these therapies are more appropriately evaluated according to whether they produce a functional cure. A proposed functional cure would include sustained freedom from acute SCD complications without continued transfusion or disease-modifying therapy, durable correction of anemia and hemolysis, stabilization of end-organ function, clinically meaningful improvement in health-related quality of life, and the persistence of the intended genetic modification [67,68]. Prevention of additional organ damage may therefore indicate successful treatment even when complications such as stroke, osteonecrosis, or chronic kidney disease do not fully resolve [67].

Pivotal gene-therapy trials have evaluated freedom from severe vaso-occlusive events over defined periods, but the event definitions and evaluation rules differ between products and should be retained when comparing trials [12,13,67]. Additional clinical endpoints include hospitalization and transfusion requirements, acute chest syndrome and other SCD complications, overall survival, stabilization of cardiovascular, pulmonary, renal, and cerebrovascular function, and improvements in patient-reported pain, physical functioning, fatigue, and quality of life [67–70]. Clinical laboratory endpoints include total hemoglobin, HbS, HbF, or the therapeutic vector-derived globin, F-cell percentage, and markers of hemolysis such as lactate dehydrogenase, indirect bilirubin, haptoglobin, and reticulocyte

count. Investigators also measure persistence of allelic editing and vector copy numbers to establish the durability of genetically modified hematopoiesis [67,69].

Each of these endpoints has important limitations. Acute pain endpoints depend on trial-specific definitions and health care utilization and may therefore fail to capture pain treated at home, chronic pain, or less severe events [67,68,70]. Furthermore, freedom from severe events for 12 months demonstrates substantial clinical benefit but does not establish lifelong disease resolution or exclude continued progression of chronic organ damage [67–69]. Conventional hematologic measurements also do not directly assess red-cell deformability, hypoxia-induced sickling, adhesion, density, oxygen-delivery efficiency, or whole-blood viscosity. Consequently, laboratory improvement alone cannot establish complete normalization of red-cell function [4,15,16,18,67,69]. Recent Cure Sickle Cell Initiative recommendations emphasize that defining a cure will require genetic, clinical, functional, organ-based, and patient-reported assessments collected over many years and that no single laboratory or genetic assay can currently establish a cure [69].

6. Lessons from Discordant Biological and Clinical Responses

Crizanlizumab, voxelotor, and senicapoc illustrate how evidence of target engagement or improvement in laboratory endpoints does not necessarily translate into consistent clinical benefit and can diverge from clinical efficacy or safety outcomes.

6.1. Crizanlizumab

Crizanlizumab (Adakveo) is a monoclonal antibody that binds P-selectin and blocks its interactions with P-selectin glycoprotein ligand 1, thereby disrupting adhesive interactions among sickle erythrocytes, leukocytes, platelets, and the vascular endothelium [71]. In the phase 2 SUSTAIN trial, crizanlizumab at 5 mg/kg reduced the median annual rate of SCD-related pain crises from 2.98 with placebo to 1.63, representing a 45.3% reduction [71]. However, these results were not reproduced in the phase 3 STAND trial. The adjusted annualized rates of VOCs leading to a health care visit were 2.49 with 5 mg/kg of crizanlizumab, 2.04 with 7.5 mg/kg, and 2.30 with placebo. Neither dose produced a statistically significant benefit compared with placebo [72]. Based on the STAND trial results, the European Medicines Agency (EMA) recommended revocation of the European marketing authorization in 2023 [73]. Crizanlizumab nevertheless remains FDA-approved in the United States for reducing the frequency of VOCs in patients aged 16 years and older [74]. The STAND investigators noted that the COVID-19 pandemic, geographical differences in health care utilization and crisis management, and commercial availability of crizanlizumab during the study may have influenced the results [72]. These considerations are especially relevant because a clinical endpoint restricted to crises resulting in health care visits does not capture episodes managed at home. Whole-blood adhesion to P-selectin varies considerably among patients and has been prospectively associated with prospective risk of self-reported VOCs [22]. Whether baseline P-selectin adhesion identifies patients more likely to benefit from crizanlizumab remains unresolved. Section 9 discusses how a prospective study could test this question.

6.2. Voxelotor and Oxygen Affinity

Voxelotor (Oxbryta) preferentially bound and stabilized the nonpolymerizing, high oxygen-affinity R conformation of hemoglobin, thereby reducing the amount of deoxygenated HbS available for polymerization [31,75]. In the phase 3 HOPE trial, 51% of patients receiving voxelotor 1500 mg achieved an increase in hemoglobin greater than 1 g/dL at week 24, compared with approximately 7% of patients receiving placebo. Reductions in reticulocyte count and indirect bilirubin also indicated decreased hemolysis [75]. Improvements in

hemoglobin and hemolysis markers were maintained throughout 72 weeks [76]. The FDA granted accelerated approval in 2019 specifically on the basis of increased hemoglobin, with continued approval contingent on confirmation of clinical benefit [77]. In September 2024, Pfizer voluntarily withdrew voxelotor from the global market following postmarketing safety findings [78]. In HOPE Kids 2, eight children receiving voxelotor died, compared with two receiving placebo. The deaths occurred at sites in sub-Saharan Africa, and several involved malaria, sepsis, or other infections. The treating investigators considered all deaths to be unrelated to the study drug [78,79]. In translational studies of voxelotor, oxygen-gradient ektacytometry showed greater red deformability and a lower point of sickling during voxelotor treatment. Thus, the voxelotor experience should not be interpreted as evidence that these functional measurements were uninformative. Rather, it illustrates that improvements in hemoglobin and selected red cell properties must be interpreted alongside clinical outcomes and context-specific risks [79,80]. Because the mortality imbalance occurred in sub-Saharan Africa and several fatal events involved malaria, regional infectious exposures and the possibility that anti-sickling therapies alter interactions between host erythrocytes and malaria parasites warrant further investigation. However, this mechanism was not evaluated in HOPE Kids 2, and the cause of the mortality imbalance remains unresolved [79].

During voxelotor treatment, a lower point of sickling indicates that RBC deformability begins to decline at a lower oxygen tension in the assay. Voxelotor increases hemoglobin oxygen affinity, shifting the oxygen dissociation curve to the left. At a given oxygen tension, this leaves less deoxygenated HbS available to polymerize and can also reduce the amount of oxygen released from hemoglobin. Experimental and modeling studies illustrate this trade-off [31] (Figure 2). The net effect on tissue oxygenation in vivo remains uncertain because hemoglobin concentration, blood flow, and oxygen extraction also influence oxygen availability. Reporting the oxygen dissociation curve and p50 (the oxygen tension at 50% hemoglobin saturation) alongside ektacytometry would help explain the observed change in sickling. Measurements of tissue oxygenation or oxygen extraction could help determine whether that change translates into improved oxygen availability in vivo.

6.3. Senicapoc and Other Trial Lessons

Senicapoc provides an especially direct cautionary example for rheological endpoint selection. Senicapoc inhibited the erythrocyte Gardos channel, limiting potassium and water loss, and thereby reducing sickle red-cell dehydration. In a phase 2 study, high-dose senicapoc increased hemoglobin by 0.68 g/dL compared with 0.01 g/dL with placebo and significantly reduced the percentage of dense red cells, reticulocyte counts, lactate dehydrogenase, and indirect bilirubin [81]. Nevertheless, its phase 3 trial was terminated early for lack of efficacy because pain crises did not decrease significantly despite similar changes in hemoglobin, hematocrit, dense RBCs, and reticulocyte counts did not produce a significant reduction in pain crises. The observed crisis rates were 0.38 with senicapoc and 0.31 with placebo [82]. Red cell hydration and increased red cell mass can exert opposing effects on blood flow. Improved hydration and deformability may reduce flow resistance, whereas a higher hematocrit may increase WBV. Because the trial emphasized dense-cell and hematologic measurements without evaluating the net effect on WBV, the relationship between the observed laboratory improvements and blood flow remained unresolved [8,81,82]. The senicapoc experience demonstrates that improvement in a single RBC characteristic does not necessarily improve the overall microvascular blood flow. It supports selecting complementary functional measurements according to the mechanisms and the potential for countervailing effects, rather than assuming that improvement in dense-cell percentage establishes net benefit.

Other unsuccessful trials reinforce the importance of aligning the timing of treatment and choice of endpoint with the therapeutic mechanism, treatment timing, and endpoint. Sevuparin, an anti-adhesive heparinoid administered after hospitalization for an established acute pain complication, did not shorten the time to resolution of the vaso-occlusive crisis. Median resolution times were 100.4 h with sevuparin and 86.4 h with placebo [83]. The investigators proposed that selectin-mediated adhesion may be more important during crisis initiation than during maintenance, suggesting that a negative acute-treatment trial does not necessarily invalidate the biological target [83]. Poloxamer 188, which was intended to reduce WBV and adhesive or frictional interactions, did not shorten the time to the final dose of parenteral opioids in a phase 3 trial [84]. These examples demonstrate that future SCD trials require a multidimensional endpoint strategy. Clinical events and patient-reported outcomes remain necessary to establish benefit, while mechanism-appropriate rheological assays can confirm target engagement, identify heterogeneous biological responses, and help explain why a therapy succeeds or fails.

These examples motivate the mechanism-specific selection of functional assays proposed in Section 8.

7. Use of Rheology in Clinical Trials

Table 2 summarizes the treatment-associated evidence. Randomized clinical findings carry different inferential weight from uncontrolled functional studies, ancillary analyses, conference abstracts, and sponsor announcements. Endpoint status is reported when identifiable. An assay may be reported as exploratory even when it was included in the study protocol. Serial samples are not additional participants, and updates from the same trial are not independent replication.

The senicapoc trials left open whether improved red cell hydration offset the viscosity effects of increased hemoglobin and hematocrit [8,82]. A hydroxyurea study that measured these properties together found that whole-blood viscosity remained stable despite higher hemoglobin, while deformability improved and red cell aggregates became less robust [85]. The contrast illustrates how measuring viscosity alongside cellular properties can help interpret the net rheological response.

A more comprehensive strategy was used in the phase 2 study of epeleuton conducted by Afimmune. The study required elevated RBC adhesion to laminin at screening, defined as greater than 400 adherent cells/mm^2 in a microfluidic assay, using a functional RBC abnormality as an eligibility criterion. Its prospective endpoints included the point of sickling, RBC deformability, the percentage of dense cells, cellular adhesion, and annualized rates of VOCs leading to a health care visit [86]. Encouraging results from this study have since been reported, supporting the continued clinical development of epeleuton [87]. Thus, by combining function-based participant selection with complementary measures of RBC behavior and clinical outcomes, the epeleuton study represents one of the broadest applications of complementary RBC functional measurements in an SCD drug trial.

Hemoglobin S polymerization inhibitors provide another example of the distinction between clinical trial endpoints and supporting rheological studies. Direct rheological evidence for voxelotor came primarily from ex vivo experiments and an ancillary clinical study rather than from the principal efficacy endpoints of the pivotal trial. Ex vivo treatment of sickle blood with voxelotor preserved RBC deformability and reduced blood viscosity during deoxygenation [9]. Subsequently, an ancillary study of ten children enrolled in HOPE-KIDS 1 used osmotic and oxygen-gradient ektacytometry and showed increases in EImin and EImax elongation indices and a reduction in the point of sickling during voxelotor treatment [80]. The first in vivo studies of osivelotor, the pharmacologic successor to voxelotor, incorporated an even broader pharmacodynamic assessment, measuring oxygen

affinity, EImax, EImin, the point of sickling, the percentage of dense cells, and circulating sickled cells measured alongside hemoglobin and markers of hemolysis [32]. By demonstrating improved RBC deformability and delayed sickling alongside increased hemoglobin in six participants with SCD, these measurements provided a more complete assessment of osivelotor's effects on RBC function than conventional hematologic biomarkers alone.

These exploratory studies illustrate how red cell functional testing can complement clinical efficacy analyses by characterizing biological responses to treatment. For therapies that alter oxygen affinity, the point of sickling should be interpreted alongside the oxygen dissociation curve and oxygen unloading, as discussed in Section 6.2.

Studies of pyruvate kinase activators have also used oxygen-gradient ektacytometry to examine treatment effects beyond conventional hematologic measurements. In the phase 2 ESTIMATE study, the evaluation of mitapivat included hemoglobin oxygen affinity and the point of sickling, with an anti-sickling response defined as at least a 10% improvement in the point of sickling [33]. Although four of eight evaluable patients met this response criterion during the fixed-dose extension, the mean change in point of sickling was not statistically significant in the small cohort. In a phase 1 study of etavopivat, treatment increased RBC adenosine triphosphate (ATP), decreased 2,3-diphosphoglycerate, and increased hemoglobin while shifting both p50 and the point of sickling to lower oxygen tensions. Oxygen-gradient ektacytometry directly measured the change in the point of sickling [34]. These studies demonstrate the usefulness of rheological measurements as pharmacodynamic indicators of altered RBC physiology, while also emphasizing the need to determine whether rheological changes are prospectively associated with subsequent clinical outcomes.

Rheology testing is extensively incorporated into the BEACON study of ristoglogene autogetemcel, or risto-cel, formerly BEAM-101. Exploratory analyses assessed whole-blood adhesion to VCAM-1, P-selectin, and laminin under flow, real-time sickling kinetics, hemoglobin oxygen affinity, RBC deformability, RBC density, and cellular morphology [88,89]. Initial results from three participants demonstrated reduced adhesion and sickling, with several hemorheological parameters approaching values observed in sickle cell trait [88]. The subsequent clinical report of 31 risto-cel recipients established robust HbF induction and a reduction in HbS [62]. An updated exploratory analysis from the same BEACON trial, presented at the 2026 European Hematology Association (EHA) Congress, included data from 43 participants and reported broader improvement in RBC function [89]. Rates of sickling, maximum induced sickling, and whole-blood adhesion decreased to levels comparable to sickle cell trait, while the percentage of dense RBCs and point of sickling decreased. The report also described changes in RBC deformability, oxygen affinity, RBC count, and cellular morphology. Measurements of WBV under hypoxia complement these cellular assays by providing an integrated assessment of how hemoglobin concentration and RBC function interact under conditions that promote HbS polymerization.

Our group developed a hypoxia-based WBV assay using a DVNext cone-and-plate viscometer (AMETEK Brookfield, Middleboro, MS, USA) to address safety concerns associated with an increase in hemoglobin on blood viscosity when HbS polymerizes under hypoxia. For the study described in our conference report, we analyzed the data using GraphPad Prism (v10.5.0). Chemically induced hypoxia increased median WBV in HbSS samples from 3.23 to 6.30 cP, whereas the change in HbAA samples was not statistically significant. The hypoxia-induced change was smaller when HbSS samples were treated ex vivo with osivelotor than when they were untreated, and a single post-treatment gene therapy sample demonstrated a change comparable to that observed in HbAA samples [28]. Together, these findings suggest that hypoxic WBV can detect treatment-associated changes in the integrated rheological response to deoxygenation and may provide a complementary functional biomarker for

evaluating pharmacologic and gene-based therapies. The assay enables assessment of blood viscosity under controlled hypoxic conditions. Chemical deoxygenation and sample dilution should be considered when relating these findings to blood in vivo.

Other gene therapy programs illustrate that rheological testing is still not used consistently. Although the initial single-patient nula-cel report focused on gene correction, hemoglobin fractions, hematologic parameters, hemolysis, and vaso-occlusive events [65], a subsequent cross-program analysis included the nula-cel trial in its methods and assessed dense cell percentage and oxygen-gradient ektacytometry parameters across treatment groups [90]. The report did not provide a separate nula-cel functional result. Rheological characterization is now being incorporated directly into the ongoing nula-cel trial. Our laboratory is evaluating trial specimens using complementary measures of RBC physiology, including hemoglobin oxygen affinity, RBC deformability and sickling during deoxygenation, and measures of RBC hydration and density. When interpreted alongside measures of genetic correction and hemoglobin composition, these functional measurements will help determine whether correction of the sickle mutation translates into normalization of the post-treatment RBC phenotype.

Similarly, early ARU-1801 reports described engraftment, vector copy number, anti-sickling hemoglobin expression, and clinical events but did not include formal rheological endpoints [91]. More recently, a nonrandomized comparison analyzed 123 samples from 69 patients. The cohort included 43 HSCT recipients (32 matched-related and 11 haploidentical), 11 gene therapy recipients, and 15 hydroxyurea-treated patients with an optimal HbF response. In the gene therapy group, four patients received OTQ923, two received exa-cel, four received lovo-cel, and one received risto-cel. The specimen count includes serial samples from the same participants. Gene therapy recipients had heterogeneous RBC function and, as a group, less favorable values for hypoxic deformability and the point of sickling than recipients of HSCT from HbAS donors [92]. These small, heterogeneous, nonrandomized groups and differing follow-up preclude conclusions about comparative clinical benefit.

Table 2. (**a**) Treatment-associated red cell functional findings in sickle cell disease. Selected clinical and ex vivo studies are summarized by study design, functional measures, endpoint status, and principal findings. (**b**) Treatment-associated red cell functional findings.

(a) Peer-Reviewed Pharmacologic Studies

Treatment/Source	Study Design	Participants or Specimens	Assay	Endpoint Status	Principal Finding	Main Limitations
Hydroxyurea [85]	Prospective uncontrolled cohort	24 adults; serial visits over 12 months	Viscosity, deformability, aggregation	Rheological study objective; prespecification NR	Viscosity stable despite higher Hb/HCT	No control group. Testing under normoxia.
Senicapoc phase 2 [81]	Randomized, placebo-controlled	90 randomized; 88 efficacy-evaluable	Dense cells, Hb, hemolysis	Hb primary; dense cells/hemolysis secondary	Hb increased; dense cells and hemolysis decreased	12-week follow-up; clinical benefit unresolved
Senicapoc phase 3 [82]	Randomized, placebo-controlled	289: 145 active, 144 placebo	Dense cells, hematology	Painful crises primary; laboratory hierarchy NR	Laboratory improvement with no significant reduction in painful crises	Stopped early for lack of efficacy
Voxelotor HOPE-KIDS 1 [80]	Single-center ancillary study	10 children; repeated visits	Osmotic and oxygen-gradient ektacytometry	Ancillary functional assessment; prespecification NR	Deformability increased; PoS decreased	Small, uncontrolled subgroup; affinity affects interpretation

Table 2. *Cont.*

(a) Peer-Reviewed Pharmacologic Studies						
Treatment/Source	**Study Design**	**Participants or Specimens**	**Assay**	**Endpoint Status**	**Principal Finding**	**Main Limitations**
Osivelotor [32]	Phase 1, open-label SCD cohort	6 with SCD; serial samples; separate healthy cohort 129	Affinity, deformability, dense/ sickled cells	Planned PD assessments; exploratory/ descriptive analysis	Deformability increased; dense and sickled cells decreased	Small cohort; varying doses and exposure
Mitapivat ESTIMATE [33]	Phase 2, open-label extension	10 initial; 9 extension; 8 PoS-evaluable	PoS, p50	Anti-sickling response primary; p50 secondary	4/8 met ≥ 10% PoS reduction; mean PoS change nonsignificant	Small, selected extension cohort; missing assay data
Etavopivat [34]	Phase 1. Blinded cohort followed by an open-label phase.	36 unique; 15 open-label, including 6 rollovers; 14 completed	ATP, 2,3-DPG, p50, ektacytometry	PD assessments; RBC functional analyses exploratory	ATP increased; 2,3-DPG, p50, and PoS decreased	Short exposure; functional results mainly open-label
(b) Conference Abstracts, Sponsor Reports, and Treatment Comparisons						
Treatment/Source	**Study Design**	**Participants or Specimens**	**Assay**	**Endpoint Status**	**Principal Finding**	**Main Limitations**
Epeleuton [86,87]	Phase 2, open-label; abstract and sponsor reports	27 at abstract cutoff; 31 later enrolled; assay n NR	Laminin adhesion; sickling, density, deformability	Adhesion eligibility criterion; assay hierarchy NR	Sponsor reported improved adhesion, sickling, and hydration	No concurrent control; sponsor-reported results
Risto-cel BEACON [88,89]	Phase 1/2, single-arm; conference updates	3 initially; 43 with biomarker data in update; assay n varies	Adhesion, sickling, deformability, density, affinity	Exploratory	Several measures approached HbAS reference values	Variable assay/time point coverage; ongoing follow-up
Hypoxic viscosity/ ex vivo osivelotor [28]	Assay development; conference abstract	27 HbSS, 5 HbAA, 1 post-GT; paired-treatment n NR	Cone-and-plate viscosity with chemical hypoxia	Exploratory	Viscosity rose under hypoxia; osivelotor reduced the rise	Chemical hypoxia; dilution; one post-GT observation
Early HSCT/GT/HU comparison [90]	Observational comparison; conference abstract	Reported groups: GT 8, haplo-HSCT 4, HU 15	Ektacytometry, dense cells	Exploratory comparison	Higher hypoxic deformability after haplo-HSCT; GT variable	Small, heterogeneous groups; nula-cel results not separately reported
HSCT/GT/HU comparison [92]	Nonrandomized comparison; journal article	69 people, 123 specimens: HSCT 43, GT 11, HU 15	Ektacytometry, dense cells, viscosity/HVR	Observational comparison	HbAS-donor HSCT group had higher EImin and lower PoS than the GT group	Serial samples; mixed GT approaches; unequal follow-up

Note: (a) Counts refer to participants; repeated visits are not additional participants. HOPE-KIDS 1 is an ancillary subgroup of its parent trial. ESTIMATE extension participants overlap the initial cohort; etavopivat rollovers are included once. Endpoint hierarchy or prespecification is marked NR when not established in the cited report. SCD, sickle cell disease; Hb, hemoglobin; HCT, hematocrit; PoS, point of sickling; p50, oxygen tension at 50% Hb saturation; PD, pharmacodynamic; RBC, red blood cell; ATP, adenosine triphosphate; 2,3-DPG, 2,3-diphosphoglycerate; NR, not reported. (b) The BEACON reports describe one trial, and the epeleuton reports describe another. Denominators vary by assay and time point. The cohorts in the conference report [90] and the full comparison [92] may overlap. In the full comparison [92], HSCT comprised 32 matched-related and 11 haploidentical recipients. Gene therapy comprised four OTQ923 recipients, two exa-cel recipients, four lovo-cel recipients, and one risto-cel recipient. These laboratory comparisons describe functional differences. Comparative clinical benefit requires clinical outcome data. GT, gene therapy; HSCT, hematopoietic stem cell transplantation; HU, hydroxyurea; HbAS, sickle cell trait; HbSS, sickle cell anemia genotype; HbAA, normal adult hemoglobin genotype; EImin, minimum elongation index; HVR, hematocrit-to-viscosity ratio. The nula-cel trial is mentioned in the methods of [90], with no separately reported functional result.

8. Mechanism-Specific Assessment Framework

Investigators should select assays according to the therapeutic mechanism, trial phase, and the question being tested (Table 3). A feasible core retains clinical outcomes, patient-reported outcomes, safety assessments, a complete blood count with reticulocytes, hemoglobin fractions, and hemolysis markers. One analytically qualified functional assay, or a paired assessment when the mechanism requires it, should address the principal biological effect. A complementary measurement should address a plausible countervailing effect, such as increased viscosity when hemoglobin rises or altered unloading when oxygen affinity increases. Broader profiling can be restricted to exploratory substudies rather than required at every visit.

Table 3. Proposed mechanism-specific framework for selection of functional assessments in clinical trials. Suggested core assessments are matched to treatment mechanism, with optional exploratory measurements selected according to the biological question, assay availability, and trial resources. A pragmatic starting point is routine blood counts, reticulocytes, Hb fractions, and hemolysis markers, alongside baseline and follow-up sampling and assessment of trial-defined clinical outcomes. Select one functional assessment aligned with the mechanism and available laboratory expertise. Add exploratory tests for a specific biological question.

Treatment Mechanism	Feasible Functional Core	Optional Tests and When to Add Them
HbF induction/ polymerization inhibition	Oxygen-dependent deformability or a qualified sickling assay	Cellular HbF/HbS distribution for heterogeneous responses. Viscosity at native HCT when Hb/HCT rises.
Oxygen-affinity modification/pyruvate kinase activation	Pair a sickling or deformability assay with an oxygen dissociation curve, including p50	ATP/2,3-DPG for metabolic effects. Oxygen unloading or perfusion substudy to examine tissue implications.
Adhesion inhibition	Flow adhesion using the ligand targeted by the therapy	Other ligands or occlusion assays for broader effects. Identify contributing cell types in whole blood.
Hydration/ ion-channel modulation	Measure the dense or hyperchromic red cell fraction using a consistent method	Deformability and viscosity at native HCT to assess the combined effects of hydration and cell concentration.
Transfusion/exchange	Measure WBV at native HCT before and after transfusion. Record Hb, HCT, and HbS fraction.	Viscosity for Hb-target comparisons. Oxygen-gradient ektacytometry to assess residual sickling or impaired deformability.
HSCT/gene therapy	Measure oxygen-dependent deformability and Hb fractions alongside measures of engraftment or editing, as applicable.	Density, adhesion, and cellular Hb distribution for residual dysfunction. Viscosity when Hb/HCT rises.

Note: This framework is proposed for trial design. Choose methods with documented analytical performance and specimen stability for the planned setting. Interpret oxygen-affinity effects with oxygen unloading and evaluate clinical benefit through the trial outcomes. Sources informing assay selection [17,20–22,27,31–34,45,66,67,69,92]. Hb, hemoglobin; HbF, fetal hemoglobin; HbS, sickle hemoglobin; WBV, whole blood viscosity; HCT, hematocrit; p50, oxygen tension at 50% Hb saturation; ATP, adenosine triphosphate; 2,3-DPG, 2,3-diphosphoglycerate; HSCT, hematopoietic stem cell transplantation.

Sampling should include pretreatment and prespecified follow-up measurements, with the same preparation and assay conditions where possible. Record recent transfusion, treatment exposure, acute illness, sample age, hematocrit, temperature, and oxygenation. Prespecify the endpoint hierarchy, assay-specific denominators, handling of missing data, and analysis of repeated samples within patients. This framework is proposed for trial

design. Using it for treatment selection or as a substitute for clinical endpoints would require validation [27].

9. Lessons Learned and Future Clinical Use

The divergence between crizanlizumab's SUSTAIN and STAND trials raises the question of whether patient selection contributed to their different results [71,72]. Both trials selected participants primarily by clinical history. A SUSTAIN subgroup analysis examined genotype, hydroxyurea use, and previous crisis frequency [93]. Because crizanlizumab inhibits P-selectin-mediated cellular adhesion, a future controlled trial could test whether baseline P-selectin-dependent adhesion modifies the effect of crizanlizumab on clinical outcomes and measure whether treatment-related changes in adhesion accompany changes in deformability or viscosity.

Functional testing may be particularly important when comparing gene therapies and determining how closely each approach achieves functional correction. Gene-addition, gene-editing, and gene-correction therapies generate different mixtures of HbS-containing, HbF-rich, anti-sickling, or genetically corrected RBCs. Functional measurements can characterize residual RBC abnormalities after these therapies [92]. Their interpretation should account for durable engraftment or editing, hemoglobin composition, clinical events, transfusion needs, patient-reported outcomes, and organ function.

With prospective validation, functional profiles could be evaluated for treatment selection. For example, trials could test whether pronounced hypoxia-induced sickling identifies patients more likely to respond to an intervention that inhibits HbS polymerization or increases HbF. They could also examine whether baseline viscosity affects the response to treatments that raise hemoglobin.

Beyond investigational treatment selection, longitudinal rheological testing could establish an individualized measure of disease stability. Repeated assessment of hypoxic viscosity, deformability, sickling propensity, adhesion, and RBC density may reveal changes from a patient's usual functional profile that are not captured by hemoglobin concentration or hemolysis markers alone. Oxygen-gradient ektacytometry parameters have been associated with acute complications and have differed between steady state and vaso-occlusive crises [19,94], although a single steady-state measurement did not independently predict pain in one pediatric cohort [30]. These findings justify testing whether changes within an individual over time are more informative than a single measurement or population-wide cutoff. If worsening rheological profiles are shown to precede acute pain events, acute chest syndrome, hospitalization, or progressive organ injury, longitudinal testing could provide an early indication of clinical deterioration and create an opportunity to modify treatment before overt complications develop.

Ultimately, the principal lesson from previous SCD drug development programs is that improvement in a single hematologic or rheological parameter cannot establish net clinical benefit. Mechanism-specific RBC functional testing can characterize treatment effects and residual abnormalities across complementary domains. Its value in SCD trials depends on analytical standardization, prespecified interpretation, and evaluation alongside clinical outcomes. Prospective studies are needed to determine whether functional profiles can guide treatment selection or detect clinical deterioration earlier. RBC function testing can strengthen biological comparisons among potentially curative therapies and, with further validation, may support earlier and more individualized intervention before irreversible complications develop. The goal would be to identify the dominant functional abnormality in each patient, select the therapy most likely to correct it, and then determine whether the expected improvement in blood flow is achieved.

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Abbreviations

The following abbreviations are used in this manuscript:

SCD	Sickle cell disease
RBC	Red blood cell
HbS	Hemoglobin S
HbF	Fetal hemoglobin
HBB	Hemoglobin subunit beta
MCV	Mean corpuscular volume
WBC	White blood cell
LDH	Lactate dehydrogenase
RCE	Red-cell exchange
DHTR	Delayed hemolytic transfusion reaction
HSCT	Hematopoietic stem cell transplantation
HVR	Hematocrit-to-viscosity ratio
WBV	Whole blood viscosity
HbA	Adult hemoglobin
HbAA	Normal adult hemoglobin genotype
Hb	Hemoglobin
HCT	Hematocrit
FDA	U.S. Food and Drug Administration
MSH	Multicenter Study of Hydroxyurea
VOC	Vaso-occlusive crisis
VOE	Vaso-occlusive event
EI	Elongation index
PoS	Point of sickling
LoRRca	Laser Optical Rotational Red Cell Analyzer
MIRCA	Microfluidic impedance red cell assay
VCAM-1	Vascular cell adhesion molecule 1
SIFCA	Sickle imaging flow cytometry assay
ATP	Adenosine triphosphate
2,3-DPG	2,3-diphosphoglycerate
EHA	European Hematology Association
MBS	Sodium metabisulfite
PK	Pyruvate kinase
NR	Not reported

References

1. Thomson, A.M.; McHugh, T.A.; Oron, A.P.; Teply, C.; Lonberg, N.; Vilchis Tella, V.; Wilner, L.B.; Fuller, K.; Hagins, H.; Aboagye, R.G.; et al. Global, regional, and national prevalence and mortality burden of sickle cell disease, 2000–2021: A systematic analysis from the Global Burden of Disease Study 2021. *Lancet Haematol.* **2023**, *10*, e585–e599. [CrossRef] [PubMed]
2. Sundd, P.; Gladwin, M.T.; Novelli, E.M. Pathophysiology of Sickle Cell Disease. *Annu. Rev. Pathol. Mech. Dis.* **2019**, *14*, 263–292. [CrossRef] [PubMed]
3. Williams, D.C.; Szafraniec, H.M.; Wood, D.K. Sticking together: Polymerization of sickle hemoglobin drives the multiscale pathophysiology of sickle cell disease. *Biophys. Rev.* **2025**, *6*, 011309. [CrossRef] [PubMed]

4. Kucukal, E.; Man, Y.; Hill, A.; Liu, S.; Bode, A.; An, R.; Kadambi, J.; Little, J.A.; Gurkan, U.A. Whole blood viscosity and red blood cell adhesion: Potential biomarkers for targeted and curative therapies in sickle cell disease. *Am. J. Hematol.* **2020**, *95*, 1246–1256. [CrossRef] [PubMed]
5. Williams, D.C.; Wood, D.K. High-throughput quantification of red blood cell deformability and oxygen saturation to probe mechanisms of sickle cell disease. *Proc. Natl. Acad. Sci. USA* **2023**, *120*, e2313755120. [CrossRef] [PubMed]
6. Hebbel, R.P.; Yamada, O.; Moldow, C.F.; Jacob, H.S.; White, J.G.; Eaton, J.W. Abnormal Adherence of Sickle Erythrocytes to Cultured Vascular Endothelium. *J. Clin. Investig.* **1980**, *65*, 154–160. [CrossRef] [PubMed]
7. Papageorgiou, D.P.; Abidi, S.Z.; Chang, H.-Y.; Li, X.; Kato, G.J.; Karniadakis, G.E.; Suresh, S.; Dao, M. Simultaneous polymerization and adhesion under hypoxia in sickle cell disease. *Proc. Natl. Acad. Sci. USA* **2018**, *115*, 9473–9478. [CrossRef] [PubMed]
8. Lu, M.; Rab, M.A.; Shevkoplyas, S.S.; Sheehan, V.A. Blood rheology biomarkers in sickle cell disease. *Exp. Biol. Med.* **2020**, *245*, 155–165. [CrossRef] [PubMed]
9. Dufu, K.; Patel, M.; Oksenberg, D.; Cabrales, P. GBT440 improves red blood cell deformability and reduces viscosity of sickle cell blood under deoxygenated conditions. *Clin. Hemorheol. Microcirc.* **2018**, *70*, 95–105. [CrossRef] [PubMed]
10. Sahun, M.; Bernit, E.; Atwell, S.; Hornung, A.; Charrier, A.M.; Agouti, I.; Bonello-Palot, N.; Cerino, M.; Helfer, E.; Badens, C.; et al. A novel red blood cell deformability biomarker is associated with hemolysis and vaso-occlusive crises in sickle cell disease. *Sci. Rep.* **2025**, *15*, 15864. [CrossRef] [PubMed]
11. Kavanagh, P.L.; Fasipe, T.A.; Wun, T. Sickle Cell Disease: A Review. *JAMA* **2022**, *328*, 57. [CrossRef] [PubMed]
12. Kanter, J.; Walters, M.C.; Krishnamurti, L.; Mapara, M.Y.; Kwiatkowski, J.L.; Rifkin-Zenenberg, S.; Aygun, B.; Kasow, K.A.; Pierciey, F.J.; Bonner, M.; et al. Biologic and Clinical Efficacy of LentiGlobin for Sickle Cell Disease. *N. Engl. J. Med.* **2022**, *386*, 617–628. [CrossRef] [PubMed]
13. Frangoul, H.; Locatelli, F.; Sharma, A.; Bhatia, M.; Mapara, M.; Molinari, L.; Wall, D.; Liem, R.I.; Telfer, P.; Shah, A.J.; et al. Exagamglogene Autotemcel for Severe Sickle Cell Disease. *N. Engl. J. Med.* **2024**, *390*, 1649–1662. [CrossRef] [PubMed]
14. Connes, P.; Alexy, T.; Detterich, J.; Romana, M.; Hardy-Dessources, M.-D.; Ballas, S.K. The role of blood rheology in sickle cell disease. *Blood Rev.* **2016**, *30*, 111–118. [CrossRef] [PubMed]
15. Bartolucci, P.; Brugnara, C.; Teixeira-Pinto, A.; Pissard, S.; Moradkhani, K.; Jouault, H.; Galacteros, F. Erythrocyte density in sickle cell syndromes is associated with specific clinical manifestations and hemolysis. *Blood* **2012**, *120*, 3136–3141. [CrossRef] [PubMed]
16. Waltz, X.; Hardy-Dessources, M.-D.; Lemonne, N.; Mougenel, D.; Lalanne-Mistrih, M.-L.; Lamarre, Y.; Tarer, V.; Tressières, B.; Etienne-Julan, M.; Hue, O.; et al. Is there a relationship between the hematocrit-to-viscosity ratio and microvascular oxygenation in brain and muscle? *Clin. Hemorheol. Microcirc.* **2015**, *59*, 37–43. [CrossRef] [PubMed]
17. Sadaf, A.; Quinn, C.T.; Korpik, J.B.; Pfeiffer, A.; Reynaud, M.; Niss, O.; Malik, P.; Ware, R.E.; Kalfa, T.A.; McGann, P.T. Rapid and automated quantitation of dense red blood cells: A robust biomarker of hydroxyurea treatment response. *Blood Cells Mol. Dis.* **2021**, *90*, 102576. [CrossRef] [PubMed]
18. Rab, M.A.E.; Van Oirschot, B.A.; Bos, J.; Merkx, T.H.; Van Wesel, A.C.W.; Abdulmalik, O.; Safo, M.K.; Versluijs, B.A.; Houwing, M.E.; Cnossen, M.H.; et al. Rapid and reproducible characterization of sickling during automated deoxygenation in sickle cell disease patients. *Am. J. Hematol.* **2019**, *94*, 575–584. [CrossRef] [PubMed]
19. Rab, M.A.E.; Kanne, C.K.; Boisson, C.; Bos, J.; Van Oirschot, B.A.; Houwing, M.E.; Renoux, C.; Bartels, M.; Rijneveld, A.W.; Nur, E.; et al. Oxygen gradient ektacytometry–derived biomarkers are associated with acute complications in sickle cell disease. *Blood Adv.* **2024**, *8*, 276–286. [CrossRef] [PubMed]
20. Rab, M.A.E.; Kanne, C.K.; Bos, J.; Boisson, C.; Van Oirschot, B.A.; Nader, E.; Renoux, C.; Joly, P.; Fort, R.; Van Beers, E.J.; et al. Methodological aspects of the oxygenscan in sickle cell disease: A need for standardization. *Am. J. Hematol.* **2020**, *95*, E5–E8. [CrossRef] [PubMed]
21. Patwardhan, A.A.; Patel, A.; Pasupuleti, A.; Oshabaheebwa, S.; Delianides, C.A.; Sekyonda, Z.; Yoo, J.J.; Wade, J.; Evans, E.; Suster, M.A.; et al. Use of the microfluidic impedance red cell assay in sickle cell disease. *Blood Adv.* **2025**, *9*, 4004–4015. [CrossRef] [PubMed]
22. Hines, P.C.; Callaghan, M.U.; Zaidi, A.U.; Gao, X.; Liu, K.; White, J.; Tarasev, M. Flow adhesion of whole blood to P-selectin: A prognostic biomarker for vaso-occlusive crisis in sickle cell disease. *Br. J. Haematol.* **2021**, *194*, 1074–1082. [CrossRef] [PubMed]
23. Lu, M.; Kanne, C.K.; Reddington, R.C.; Lezzar, D.L.; Sheehan, V.A.; Shevkoplyas, S.S. Concurrent Assessment of Deformability and Adhesiveness of Sickle Red Blood Cells by Measuring Perfusion of an Adhesive Artificial Microvascular Network. *Front. Physiol.* **2021**, *12*, 633080. [CrossRef] [PubMed]
24. Evans, E.N.; Williams, E.K.; Kanne, C.K.; Fibben, K.A.; Patel, A.P.; Abel, L.; Fay, M.E.; Patwardhan, A.A.; Darazim, J.; Kostamo, Z.; et al. Radchip: A Laminin-Lined Red Blood Cell (RBC) Adhesion Device for Functional Characterization and Clinical Evaluation of Sickle RBCs at Steady-State. *Blood* **2023**, *142*, 3657. [CrossRef]

25. Van Beers, E.J.; Samsel, L.; Mendelsohn, L.; Saiyed, R.; Fertrin, K.Y.; Brantner, C.A.; Daniels, M.P.; Nichols, J.; McCoy, J.P.; Kato, G.J. Imaging flow cytometry for automated detection of hypoxia-induced erythrocyte shape change in sickle cell disease. *Am. J. Hematol.* **2014**, *89*, 598–603. [CrossRef] [PubMed]
26. Fay, M.E.; Oshinowo, O.; Iffrig, E.; Fibben, K.S.; Caruso, C.; Hansen, S.; Musick, J.O.; Valdez, J.M.; Azer, S.S.; Mannino, R.G.; et al. iCLOTS: Open-source, artificial intelligence-enabled software for analyses of blood cells in microfluidic and microscopy-based assays. *Nat. Commun.* **2023**, *14*, 5022. [CrossRef] [PubMed]
27. FDA-NIH Biomarker Working Group. *Glossary. BEST (Biomarkers, EndpointS, and Other Tools) Resource [Internet]*; Food and Drug Administration and National Institutes of Health: Silver Spring, MD, USA; Bethesda, MD, USA, 2016. Available online: https://www.ncbi.nlm.nih.gov/books/NBK338448/ (accessed on 2 November 2026).
28. Aliche, M.; Patel, A.; Sheehan, V. Development of whole blood viscosity measurements under hypoxia for use in sickle cell disease. *Blood* **2025**, *146*, 6120. [CrossRef]
29. Idrizovic, A.; Traets, M.J.M.; Gimbert, A.C.; Reidel, S.; Van Der Veen, S.; Pham Hung D'Alexandry D'Orengiani, A.-L.; D'Agnolo, M.; Adu, E.; Marin, M.; Bourdelier, E.; et al. Novel oxygenscan parameters differentiate unique hemolytic and inflammatory profiles associated with sickle cell disease genotypes. *Sci. Rep.* **2026**. [CrossRef]
30. Nardo-Marino, A.; Petersen, J.; Brewin, J.N.; Birgens, H.; Williams, T.N.; Kurtzhals, J.A.L.; Rees, D.C.; Glenthøj, A. Oxygen gradient ektacytometry does not predict pain in children with sickle cell anaemia. *Br. J. Haematol.* **2022**, *197*, 609–617. [CrossRef] [PubMed]
31. Henry, E.R.; Metaferia, B.; Li, Q.; Harper, J.; Best, R.B.; Glass, K.E.; Cellmer, T.; Dunkelberger, E.B.; Conrey, A.; Thein, S.L.; et al. Treatment of sickle cell disease by increasing oxygen affinity of hemoglobin. *Blood* **2021**, *138*, 1172–1181. [CrossRef] [PubMed]
32. Lisbon, E.A.; Brown, R.C.; Redfern, A.; Duchin, K.; Barth, A.; Roberts, E.S.; Gaputan, P.; Dufu, K.; Lo, A.; Pochron, M.P.; et al. Safety, Pharmacokinetics, and Pharmacodynamics of Osivelotor for Sickle Cell Disease: First-in-Human Studies in Healthy Participants and Patients. *Clin. Pharma Ther.* **2026**, *119*, 120–130. [CrossRef] [PubMed]
33. Van Dijk, M.J.; Rab, M.A.E.; Van Oirschot, B.A.; Bos, J.; Derichs, C.; Rijneveld, A.W.; Cnossen, M.H.; Nur, E.; Biemond, B.J.; Bartels, M.; et al. One-year safety and efficacy of mitapivat in sickle cell disease: Follow-up results of a phase 2, open-label study. *Blood Adv.* **2023**, *7*, 7539–7550. [CrossRef] [PubMed]
34. Saraf, S.L.; Hagar, R.; Idowu, M.; Osunkwo, I.; Cruz, K.; Kuypers, F.A.; Brown, R.C.; Geib, J.; Ribadeneira, M.; Schroeder, P.; et al. Multicenter, phase 1 study of etavopivat (FT-4202) treatment for up to 12 weeks in patients with sickle cell disease. *Blood Adv.* **2024**, *8*, 4459–4475. [CrossRef] [PubMed]
35. Steinberg, M.H.; Sebastiani, P. Genetic modifiers of sickle cell disease. *Am. J. Hematol.* **2012**, *87*, 795–803. [CrossRef] [PubMed]
36. Williams, T.N.; Thein, S.L. Sickle Cell Anemia and Its Phenotypes. *Annu. Rev. Genom. Hum. Genet.* **2018**, *19*, 113–147. [CrossRef] [PubMed]
37. Kato, G.J.; Gladwin, M.T.; Steinberg, M.H. Deconstructing sickle cell disease: Reappraisal of the role of hemolysis in the development of clinical subphenotypes. *Blood Rev.* **2007**, *21*, 37–47. [CrossRef] [PubMed]
38. Hebbel, R.P. Reconstructing sickle cell disease: A data-based analysis of the "hyperhemolysis paradigm" for pulmonary hypertension from the perspective of evidence-based medicine. *Am. J. Hematol.* **2011**, *86*, 123–154. [CrossRef] [PubMed]
39. Alapan, Y.; Little, J.A.; Gurkan, U.A. Heterogeneous Red Blood Cell Adhesion and Deformability in Sickle Cell Disease. *Sci. Rep.* **2014**, *4*, 7173. [CrossRef] [PubMed]
40. Charache, S.; Terrin, M.L.; Moore, R.D.; Dover, G.J.; Barton, F.B.; Eckert, S.V.; McMahon, R.P.; Bonds, D.R. Effect of Hydroxyurea on the Frequency of Painful Crises in Sickle Cell Anemia. *N. Engl. J. Med.* **1995**, *332*, 1317–1322. [CrossRef] [PubMed]
41. Ware, R.; Aygun, B. Advances in the use of hydroxyurea. *Hematol. Am. Soc. Hematol. Educ. Program* **2009**, *2009*, 62–69. [CrossRef] [PubMed]
42. Agrawal, R.K.; Patel, R.K.; Shah, V.; Nainiwal, L.; Trivedi, B. Hydroxyurea in Sickle Cell Disease: Drug Review. *Indian J. Hematol. Blood Transfus.* **2014**, *30*, 91–96. [CrossRef] [PubMed]
43. Estepp, J.H.; Smeltzer, M.P.; Kang, G.; Li, C.; Wang, W.C.; Abrams, C.; Aygun, B.; Ware, R.E.; Nottage, K.; Hankins, J.S. A clinically meaningful fetal hemoglobin threshold for children with sickle cell anemia during hydroxyurea therapy. *Am. J. Hematol.* **2017**, *92*, 1333–1339. [CrossRef] [PubMed]
44. Steinberg, M.H.; Barton, F.; Castro, O.; Pegelow, C.H.; Ballas, S.K.; Kutlar, A.; Orringer, E.; Bellevue, R.; Olivieri, N.; Eckman, J.; et al. Effect of Hydroxyurea on Mortality and Morbidity in Adult Sickle Cell Anemia: Risks and Benefits up to 9 Years of Treatment. *JAMA* **2003**, *289*, 1645–1651. [CrossRef]
45. Howard, J. Sickle cell disease: When and how to transfuse. *Hematol. Am. Soc. Hematol. Educ. Program* **2016**, *2016*, 625–631. [CrossRef] [PubMed]
46. Adams, R.J.; McKie, V.C.; Hsu, L.; Files, B.; Vichinsky, E.; Pegelow, C.; Abboud, M.; Gallagher, D.; Kutlar, A.; Nichols, F.T.; et al. Prevention of a First Stroke by Transfusions in Children with Sickle Cell Anemia and Abnormal Results on Transcranial Doppler Ultrasonography. *N. Engl. J. Med.* **1998**, *339*, 5–11. [CrossRef] [PubMed]

47. Chou, S.T.; Alsawas, M.; Fasano, R.M.; Field, J.J.; Hendrickson, J.E.; Howard, J.; Kameka, M.; Kwiatkowski, J.L.; Pirenne, F.; Shi, P.A.; et al. American Society of Hematology 2020 guidelines for sickle cell disease: Transfusion support. *Blood Adv.* **2020**, *4*, 327–355. [CrossRef] [PubMed]
48. Vichinsky, E.P.; Earles, A.; Johnson, R.A.; Hoag, M.S.; Williams, A.; Lubin, B. Alloimmunization in Sickle Cell Anemia and Transfusion of Racially Unmatched Blood. *N. Engl. J. Med.* **1990**, *322*, 1617–1621. [CrossRef] [PubMed]
49. Yazdanbakhsh, K.; Ware, R.; Noizat-Pirenne, F. Red blood cell alloimmunization in sickle cell disease: Pathophysiology, risk factors, and transfusion management. *Blood* **2012**, *120*, 528–537. [CrossRef] [PubMed]
50. Niihara, Y.; Miller, S.T.; Kanter, J.; Lanzkron, S.; Smith, W.R.; Hsu, L.L.; Gordeuk, V.R.; Viswanathan, K.; Sarnaik, S.; Osunkwo, I.; et al. A Phase 3 Trial of l -Glutamine in Sickle Cell Disease. *N. Engl. J. Med.* **2018**, *379*, 226–235. [CrossRef] [PubMed]
51. Haroun, E.; Lim, S.H.; Dutta, D. Endari treatment ameliorates sickle cell-related disruption in intestinal barrier functions and is associated with prolonged survival in sickle cell mice. *Hematology* **2024**, *29*, 2331940. [CrossRef] [PubMed]
52. Bernaudin, F.; Dalle, J.-H.; Bories, D.; De Latour, R.P.; Robin, M.; Bertrand, Y.; Pondarre, C.; Vannier, J.-P.; Neven, B.; Kuentz, M.; et al. Long-term event-free survival, chimerism and fertility outcomes in 234 patients with sickle-cell anemia younger than 30 years after myeloablative conditioning and matched-sibling transplantation in France. *Haematologica* **2020**, *105*, 91–101. [CrossRef] [PubMed]
53. Fitzhugh, C.D.; Cordes, S.; Taylor, T.; Coles, W.; Roskom, K.; Link, M.; Hsieh, M.M.; Tisdale, J.F. At least 20% donor myeloid chimerism is necessary to reverse the sickle phenotype after allogeneic HSCT. *Blood* **2017**, *130*, 1946–1948. [CrossRef] [PubMed]
54. Eapen, M.; Brazauskas, R.; Walters, M.C.; Bernaudin, F.; Bo-Subait, K.; Fitzhugh, C.D.; Hankins, J.S.; Kanter, J.; Meerpohl, J.J.; Bolaños-Meade, J.; et al. Effect of donor type and conditioning regimen intensity on allogeneic transplantation outcomes in patients with sickle cell disease: A retrospective multicentre, cohort study. *Lancet Haematol.* **2019**, *6*, e585–e596. [CrossRef] [PubMed]
55. Ashorobi, D.; Naha, K.; Bhatt, R. Hematopoietic Stem Cell Transplantation in Sickle Cell Disease. In *StatPearls [Internet]*; StatPearls Publishing: Treasure Island, FL, USA, 2025. Available online: http://www.ncbi.nlm.nih.gov/books/NBK538515/ (accessed on 26 January 2026).
56. Kanter, J.; Liem, R.I.; Bernaudin, F.; Bolaños-Meade, J.; Fitzhugh, C.D.; Hankins, J.S.; Murad, M.H.; Panepinto, J.A.; Rondelli, D.; Shenoy, S.; et al. American Society of Hematology 2021 guidelines for sickle cell disease: Stem cell transplantation. *Blood Adv.* **2021**, *5*, 3668–3689. [CrossRef] [PubMed]
57. Bernaudin, F.; Socie, G.; Kuentz, M.; Chevret, S.; Duval, M.; Bertrand, Y.; Vannier, J.-P.; Yakouben, K.; Thuret, I.; Bordigoni, P.; et al. Long-term results of related myeloablative stem-cell transplantation to cure sickle cell disease. *Blood* **2007**, *110*, 2749–2756. [CrossRef] [PubMed]
58. Sharma, A.; Kassim, A.; Thompson, A.; Williams, D.A.; Liu, H.D.; Boelens, J.J.; LaBelle, J.L.; Tisdale, J.; De La Fuente, J.; Kanter, J.; et al. Gene Therapy for Sickle Cell Disease: Practice Recommendations from the American Society for Transplantation and Cellular Therapy and the International Society for Cell & Gene Therapy. *Transplant. Cell. Ther.* **2026**, *32*, 778–791. [CrossRef] [PubMed]
59. Vertex Pharmaceuticals Incorporated. *CASGEVY (Exagamglogene Autotemcel) Prescribing Information [Internet]*; Vertex Pharmaceuticals Incorporated: Boston, MA, USA, 2026. Available online: https://www.fda.gov/media/174615/download (accessed on 29 August 2026).
60. bluebird bio, Inc. *LYFGENIA (Lovotibeglogene Autotemcel) Prescribing Information [Internet]*; bluebird bio, Inc.: Somerville, MA, USA, 2023. Available online: https://www.fda.gov/media/174610/download (accessed on 29 August 2026).
61. Hanna, R.; Frangoul, H.; Pineiro, L.; McKinney, C.; Mapara, M.; Dalal, J.; Rangarajan, H.G.; Atkins, H.; Sharma, A.; Chang, K.-H.; et al. CRISPR-Cas12a Gene Editing of *HBG1* and *HBG2* Promoters to Treat Sickle Cell Disease. *N. Engl. J. Med.* **2026**, *394*, 1281–1291. [CrossRef] [PubMed]
62. Gupta, A.O.; Sharma, A.; Frangoul, H.; Kanter, J.; Mapara, M.Y.; Dalal, J.; Alavi, A.; Jaroscak, J.J.; Ayala, E.; DiPersio, J.F.; et al. Base Editing of *HBG1* and *HBG2* Promoters for Sickle Cell Disease. *N. Engl. J. Med.* **2026**, *394*, 1824–1835. [CrossRef] [PubMed]
63. Ravi, N.S.; Wienert, B.; Wyman, S.K.; Bell, H.W.; George, A.; Mahalingam, G.; Vu, J.T.; Prasad, K.; Bandlamudi, B.P.; Devaraju, N.; et al. Identification of novel HPFH-like mutations by CRISPR base editing that elevate the expression of fetal hemoglobin. *eLife* **2022**, *11*, e65421. [CrossRef] [PubMed]
64. Lattanzi, A.; Camarena, J.; Lahiri, P.; Segal, H.; Srifa, W.; Vakulskas, C.A.; Frock, R.L.; Kenrick, J.; Lee, C.; Talbott, N.; et al. Development of β-globin gene correction in human hematopoietic stem cells as a potential durable treatment for sickle cell disease. *Sci. Transl. Med.* **2021**, *13*, eabf2444. [CrossRef] [PubMed]
65. Shyr, D.C.; Lowsky, R.; Miller, W.; Schroeder, M.A.; Buchholz, T.; Dougall, K.; Intondi, A.; Charles, A.; Lehrer, J.; Bouge, A.; et al. One Year Follow-up on the First Patient Treated with Nula-Cel: An Autologous CRISPR/Cas9 Gene Corrected CD34+ Cell Product to Treat Sickle Cell Disease. *Blood* **2023**, *142*, 5000. [CrossRef]

66. Grimley, M.; Davies, S.M.; Shrestha, A.; Shova, A.; Asnani, M.; Kent, M.; Sayani, F.; Quinn, C.T.; Niss, O.; Lutzko, C.; et al. Lentiviral gene therapy with reduced-intensity conditioning for sickle cell disease: A phase 1/2 trial. *Nat. Med.* **2025**, *31*, 2204–2212. [CrossRef] [PubMed]
67. Locatelli, F.; Corbacioglu, S.; Hobbs, W.; Frangoul, H.; Walters, M.C. Defining curative endpoints for sickle cell disease in the era of gene therapy and gene editing. *Am. J. Hematol.* **2024**, *99*, 430–438. [CrossRef] [PubMed]
68. Farrell, A.T.; Panepinto, J.; Desai, A.A.; Kassim, A.A.; Lebensburger, J.; Walters, M.C.; Bauer, D.E.; Blaylark, R.M.; DiMichele, D.M.; Gladwin, M.T.; et al. End points for sickle cell disease clinical trials: Renal and cardiopulmonary, cure, and low-resource settings. *Blood Adv.* **2019**, *3*, 4002–4020. [CrossRef] [PubMed]
69. Lanzkron, S.; Coleman-Cowger, V.H.; Thompson, A.A.; Carroll, C.P.; Clemons, T.; DeBaun, M.; Kanter, J.; Malik, P.; Manwani, D.; Pierciey, F.J.; et al. Cure Sickle Cell Initiative recommendations on common data elements for sickle cell disease gene therapy trials. *Blood Adv.* **2026**, *10*, 3960–3965. [CrossRef] [PubMed]
70. Farrell, A.T.; Panepinto, J.; Carroll, C.P.; Darbari, D.S.; Desai, A.A.; King, A.A.; Adams, R.J.; Barber, T.D.; Brandow, A.M.; DeBaun, M.R.; et al. End points for sickle cell disease clinical trials: Patient-reported outcomes, pain, and the brain. *Blood Adv.* **2019**, *3*, 3982–4001. [CrossRef] [PubMed]
71. Ataga, K.I.; Kutlar, A.; Kanter, J.; Liles, D.; Cancado, R.; Friedrisch, J.; Guthrie, T.H.; Knight-Madden, J.; Alvarez, O.A.; Gordeuk, V.R.; et al. Crizanlizumab for the Prevention of Pain Crises in Sickle Cell Disease. *N. Engl. J. Med.* **2017**, *376*, 429–439. [CrossRef] [PubMed]
72. Abboud, M.R.; Cançado, R.D.; De Montalembert, M.; Smith, W.R.; Rimawi, H.; Voskaridou, E.; Güvenç, B.; Ataga, K.I.; Keefe, D.; Grosch, K.; et al. Crizanlizumab with or without hydroxyurea in patients with sickle cell disease (STAND): Primary analyses from a placebo-controlled, randomised, double-blind, phase 3 trial. *Lancet Haematol.* **2025**, *12*, e248–e257. [CrossRef] [PubMed]
73. European Medicines Agency. *Adakveo: Referral [Internet]*; European Medicines Agency: Amsterdam, The Netherlands, 2023. Available online: https://www.ema.europa.eu/en/medicines/human/referrals/adakveo (accessed on 2 September 2026).
74. U.S. Food and Drug Administration. *Search Orphan Drug Designations and Approvals: Adakveo*; U.S. Food and Drug Administration: Silver Spring, MD, USA, 2008.
75. Vichinsky, E.; Hoppe, C.C.; Ataga, K.I.; Ware, R.E.; Nduba, V.; El-Beshlawy, A.; Hassab, H.; Achebe, M.M.; Alkindi, S.; Brown, R.C.; et al. A Phase 3 Randomized Trial of Voxelotor in Sickle Cell Disease. *N. Engl. J. Med.* **2019**, *381*, 509–519. [CrossRef] [PubMed]
76. Howard, J.; Ataga, K.I.; Brown, R.C.; Achebe, M.; Nduba, V.; El-Beshlawy, A.; Hassab, H.; Agodoa, I.; Tonda, M.; Gray, S.; et al. Voxelotor in adolescents and adults with sickle cell disease (HOPE): Long-term follow-up results of an international, randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet Haematol.* **2021**, *8*, e323–e333. [CrossRef] [PubMed]
77. U.S. Food and Drug Administration. *FDA Approves Voxelotor for Sickle Cell Disease [Internet]*; U.S. Food and Drug Administration: Silver Spring, MD, USA, 2019. Available online: https://www.fda.gov/drugs/resources-information-approved-drugs/fda-approves-voxelotor-sickle-cell-disease (accessed on 2 September 2026).
78. Karkoska, K.; Jacob, S.A.; McGann, P.T. Accelerated drug approvals and patient trust: Impact of voxelotor and crizanlizumab for sickle cell disease. *Blood Adv.* **2025**, *9*, 2857–2862. [CrossRef] [PubMed]
79. Bello-Manga, H.; Abdullahi, S.U.; Hassab, H.; Brown, B.; Nduba, V.; Githanga, J.; Akinsete, A.M.; El-Beshlawy, A.; Hanna, D.; Segbefia, C.; et al. HOPE Kids 2: Phase 3, Randomized Trial of Voxelotor in Children with SCD and Conditional Cerebral Blood Flow Velocities. *Am. J. Hematol.* **2026**, *101*, 2799–2811. [CrossRef] [PubMed]
80. Chonat, S.; Fields, E.; Baratz, H.; Watt, A.; Pochron, M.; Dixon, S.; Tonda, M.; Brown, C.; Archer, D. Voxelotor improves red blood cell functionality in children with sickle cell anaemia: An ancillary study of the HOPE-KIDS 1 trial. *EJHaem* **2024**, *5*, 125–130. [CrossRef] [PubMed]
81. Ataga, K.I.; Smith, W.R.; De Castro, L.M.; Swerdlow, P.; Saunthararajah, Y.; Castro, O.; Vichinsky, E.; Kutlar, A.; Orringer, E.P.; Rigdon, G.C.; et al. Efficacy and safety of the Gardos channel blocker, senicapoc (ICA-17043), in patients with sickle cell anemia. *Blood* **2008**, *111*, 3991–3997. [CrossRef] [PubMed]
82. Ataga, K.I.; Reid, M.; Ballas, S.K.; Yasin, Z.; Bigelow, C.; James, L.S.; Smith, W.R.; Galacteros, F.; Kutlar, A.; Hull, J.H.; et al. Improvements in haemolysis and indicators of erythrocyte survival do not correlate with acute vaso-occlusive crises in patients with sickle cell disease: A phase III randomized, placebo-controlled, double-blind study of the gardos channel blocker senicapoc (ICA-17043). *Br. J. Haematol.* **2011**, *153*, 92–104. [CrossRef] [PubMed]
83. Biemond, B.J.; Tombak, A.; Kilinc, Y.; Al-Khabori, M.; Abboud, M.; Nafea, M.; Inati, A.; Wali, Y.; Kristensen, J.; Kowalski, J.; et al. Sevuparin for the treatment of acute pain crisis in patients with sickle cell disease: A multicentre, randomised, double-blind, placebo-controlled, phase 2 trial. *Lancet Haematol.* **2021**, *8*, e334–e343. [CrossRef] [PubMed]
84. Casella, J.F.; Barton, B.A.; Kanter, J.; Black, L.V.; Majumdar, S.; Inati, A.; Wali, Y.; Drachtman, R.A.; Abboud, M.R.; Kilinc, Y.; et al. Effect of Poloxamer 188 vs Placebo on Painful Vaso-Occlusive Episodes in Children and Adults with Sickle Cell Disease: A Randomized Clinical Trial. *JAMA* **2021**, *325*, 1513–1523. [CrossRef] [PubMed]

85. Lemonne, N.; Charlot, K.; Waltz, X.; Ballas, S.K.; Lamarre, Y.; Lee, K.; Hierso, R.; Connes, C.; Etienne-Julan, M.; Romana, M.; et al. Hydroxyurea treatment does not increase blood viscosity and improves red blood cell rheology in sickle cell anemia. *Haematologica* **2015**, *100*, e383–e386. [CrossRef] [PubMed]
86. Andemariam, B.; Sheehan, V.; El Rassi, F.; Hamza, M.; Kelly, K.; Climax, J. A phase 2 open-label study of epeleuton in patients with sickle cell disease. *Blood* **2025**, *146*, 4754. [CrossRef]
87. Afimmune Ltd. *Afimmune Reports Positive Phase 2 Results for Epeleuton in Sickle Cell Disease, Reducing Vaso-Occlusive Crisis Rate by 57.4% [Internet]*; Afimmune: Dublin, Ireland, 2026. Available online: https://www.afimmune.com/2026/06/02/afimmune-reports-positive-phase-2-results-for-epeleuton-in-sickle-cell-disease-reducing-vaso-occlusive-crisis-rate-by-57-4/ (accessed on 27 September 2026).
88. Chockalingam, P.S.; Lin, L.; Chen, G.; Minella, A.C.; Chen, Y.; Sheehan, V.; Zhang, N.; Armant, M.; Zaidi, A.U.; Goodrich, R.; et al. Impact of BEAM-101 Treatment on Red Blood Cell Hemoglobin Expression, Rheology and Sickling Properties: Initial Data from the BEACON Phase 1/2 Study of Autologous CD34+ Base Edited Hematopoietic Stem Cells in Sickle Cell Disease. *Blood* **2024**, *144*, 4957. [CrossRef]
89. Chockalingam, P.S.; Lin, L.; Chen, Y.; Habtemariam, B.; Rutecki, J.; Ender, K.L.; Ambati, S.; Sheehan, V.; Zhang, N.; Vinçon, H.; et al. Ristoglogene autogetemcel treatment restored red blood cell health and function in patients with sickle cell disease, with sickling and rheology parameters comparable to sickle cell trait. *HemaSphere* **2026**, *10*, PS2332.
90. Hernandez, B.; Patel, A.P.; Evans, E.N.; Zgodny, J.; Gottschalk, S.; Triplett, B.M.; Vita, S.D.; Wallace, K.; Shyr, D.C.; Stenger, E.O.; et al. Rheological Comparison of Red Cells from Individuals with SCD Treated with Gene Therapy, Haploidentical Transplantation or Hydroxyurea. *Blood* **2024**, *144*, 4956. [CrossRef]
91. Grimley, M.; Asnani, M.; Shrestha, A.; Felker, S.; Lutzko, C.; Arumugam, P.I.; Witting, S.; Knight-Madden, J.; Niss, O.; Quinn, C.T.; et al. Safety and Efficacy of Aru-1801 in Patients with Sickle Cell Disease: Early Results from the Phase 1/2 Momentum Study of a Modified Gamma Globin Gene Therapy and Reduced Intensity Conditioning. *Blood* **2021**, *138*, 3970. [CrossRef]
92. Patel, A.P.; John, T.D.; Gottschalk, S.; Triplett, B.M.; De Vita, S.; Stenger, E.O.; Sharma, A.; Sheehan, V.A. Blood Rheology After Allogeneic Hematopoietic Stem Cell Transplantation or Gene Therapy in Sickle Cell Disease. *Blood Adv.* **2026**, *10*, 6170–6177. [CrossRef] [PubMed]
93. Kutlar, A.; Kanter, J.; Liles, D.K.; Alvarez, O.A.; Cançado, R.D.; Friedrisch, J.R.; Knight-Madden, J.M.; Bruederle, A.; Shi, M.; Zhu, Z.; et al. Effect of crizanlizumab on pain crises in subgroups of patients with sickle cell disease: A SUSTAIN study analysis. *Am. J. Hematol.* **2019**, *94*, 55–61. [CrossRef] [PubMed]
94. Boisson, C.; Nader, E.; Renoux, C.; Gauthier, A.; Poutrel, S.; Bertrand, Y.; Stauffer, E.; Virot, E.; Hot, A.; Fort, R.; et al. Shear-Stress-Gradient and Oxygen-Gradient Ektacytometry in Sickle Cell Patients at Steady State and during Vaso-Occlusive Crises. *Cells* **2022**, *11*, 585. [CrossRef] [PubMed]

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