End points for sickle cell disease clinical trials: renal and cardiopulmonary, cure, and low-resource settings


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To address the global burden of sickle cell disease and the need for novel therapies, the American Society of Hematology partnered with the US Food and Drug Administration to engage the work of 7 panels of clinicians, investigators, and patients to develop consensus recommendations for clinical trial end points. The panels conducted their work through literature reviews, assessment of available evidence, and expert judgment focusing on end points related to patient-reported outcome, pain (non-patient-reported outcomes), the brain, end-organ considerations, biomarkers, measurement of cure, and low-resource settings. This article presents the findings and recommendations of the end-organ considerations, measurement of cure, and low-resource settings panels as well as relevant findings and recommendations from the biomarkers panel.

Introduction

Sickle cell disease (SCD) is the most common inherited red blood cell (RBC) disorder in the United States, affecting 70 000 to 100 000 Americans.1 Although the molecular basis of SCD was established decades ago, it has been challenging to translate this knowledge into the development of novel therapies. To improve therapeutic options, clinical trials employing carefully defined and appropriately chosen end points are needed that can capture patient benefit. These end points will enable scientific advancement, improvements in patient care, and product approvals.
End-organ diseases: renal and cardiopulmonary end points in SCD

Adult patients with SCD have evidence of end-organ disease, and patients with multiple end-organ complications are at high risk for mortality. Conducting clinical trials aimed at preventing end-organ damage is vital to reduce morbidity and mortality. The end-organ considerations panel focused on renal and cardiopulmonary end points (Tables 1 and 2) while recognizing that other complications exist. Relevant recommendations of the biomarkers panel are also included here.

Renal end points

SCD patients face multiple renal morbidities, including chronic kidney disease (CKD), hypertension, hyposthenia, and acute kidney injury (see Table 1 for a summary).

Albuminuria and CKD The prevalence of albuminuria increases from early adolescence into adulthood. Risk factors for the development of albuminuria include increasing age, hypertension, elevated blood pressure, leukocytosis, anemia, and genetic variants. Moderate and severe albuminuria are highly prevalent, and understanding the natural history of progression, including the role of genetic variants, will facilitate targeting the prevention or treatment of albuminuria. Therefore, therapeutic development should use biomarkers and end points that capture progression to end-stage renal disease.

Preclinical data suggest that moderate albuminuria may be due to competitive inhibition of albumin uptake in the proximal tubular due to underlying hemolysis rather than progressive glomerular injury. Novel agents preventing hemolysis may improve mild albuminuria due to tubular reuptake of albumin rather than improving glomerular filtration. Therefore, higher importance should be placed on clinical trial end points for severe albuminuria as compared with moderate albuminuria. Clinical trials should consider the impact of genetic variants on progression to albuminuria possibly stratifying patients based on known risk factors for disease.

The Kidney Disease Improving Global Outcomes (KDIGO) 2012 Clinical Practice Guidelines for the Evaluation and Management of Chronic Kidney Disease define CKD by 3 albuminuria categories: normal to mildly increased (<30 mg/g), moderately increased (30-300 mg/g), and severely increased (>300 mg/g). KDIGO guidelines or similar appropriate clinical guidelines should be used for defining albuminuria end points in SCD clinical trials.

Ideally, an albumin creatinine ratio should be used to quantitate albuminuria, but a urine protein creatinine ratio is also acceptable. During eligibility screening, an early-morning urine sample should be used to differentiate postural or orthostatic proteinuria from recumbent proteinuria. As an alternative, an untimed albuminuria sample can be used but must be confirmed with either a positive early morning void result or a repeat untimed urine sample. During a trial, early morning samples should be used for albuminuria end points rather than untimed urine samples if feasible.

Assessing albuminuria by use of a 30% decrease in albumin creatinine ratio is recommended, although lower or higher percent decreases (10% to 50%) have been used. In sum, prevention of severe albuminuria and development of end-stage renal disease represents a benefit. Natural history studies are needed to understand...
disease progression in patients with low to moderate albuminuria (near 30 mg/g albumin creatinine ratio) prior to considering improvement in low to moderate albuminuria as an end point.

**CKD and GFR** Pediatric patients develop an elevated glomerular filtration rate (GFR) in early childhood (hyperfiltration) that persists during adolescence and young adulthood. In adulthood, patients begin to experience an annual GFR decline. Patients who progress to end-stage renal disease suffer increased mortality rates and may be less likely to receive a renal transplant.

Recent murine and human SCD studies suggest that hyperfiltration precedes the development of albuminuria. Prospective longitudinal studies must further define hyperfiltration and determine the temporal association of hyperfiltration with renal pathology in adults prior to recommending hyperfiltration prevention as a pediatric trial end point. If hyperfiltration is pathologic, preventing hyperfiltration development in the pediatric population is an important end point.

Although direct measures of GFR are preferred, the practicability of direct measurements can challenge trial feasibility. In these cases,

**Table 1. End points for renal complications associated with SCD**

<table>
<thead>
<tr>
<th>Condition</th>
<th>End point</th>
<th>Test</th>
<th>Outcome/outcome measure</th>
<th>Type of end point</th>
<th>Ages</th>
</tr>
</thead>
<tbody>
<tr>
<td>CKD, albuminuria</td>
<td>Albuminuria or proteinuria</td>
<td>First morning void or 2 consecutive untimed albumin/creatinine ratio or protein/creatinine ratio</td>
<td>Percent decline in albuminuria</td>
<td>Direct: severe albuminuria; surrogate: moderate albuminuria</td>
<td>All age groups</td>
</tr>
<tr>
<td>CKD, GFR</td>
<td>Renal replacement therapy</td>
<td>Initiation of renal replacement therapy (may differ based on clinical practice)</td>
<td>Percentage of patients who require initiation of renal replacement therapy</td>
<td>Direct</td>
<td>All age groups</td>
</tr>
<tr>
<td>CKD, GFR</td>
<td>GFR</td>
<td>Measured GFR and estimated GFR</td>
<td>Percentage of patients who progress in stage of CKD or percentage of decline in GFR</td>
<td>Surrogate</td>
<td>Adults</td>
</tr>
<tr>
<td>Hypertension</td>
<td>Cardiovascular end point</td>
<td>Clinically defined</td>
<td>Number of patients who develop stroke, myocardial infarction, death</td>
<td>Direct</td>
<td>All age groups</td>
</tr>
<tr>
<td>Hypertension</td>
<td>Systolic or diastolic blood pressure; pediatric studies should consider use of ambulatory blood pressure monitoring</td>
<td>Blood pressure measurement</td>
<td>Change in systolic or diastolic blood pressure (5 mm Hg)</td>
<td>Future</td>
<td>All age groups</td>
</tr>
<tr>
<td>Hyposthenuria</td>
<td>Urine concentration availability</td>
<td>Water deprivation test; not &gt;10 h in children; meaningful tests are those concentrating urine &gt;500 mOsm/kg H2O</td>
<td>Percentage of patients with preservation of urine concentration ability</td>
<td>Surrogate</td>
<td>Infants or toddlers</td>
</tr>
<tr>
<td>Kidney injury</td>
<td>Acute kidney injury defined by serum creatinine or urine output; novel biomarkers of kidney injury</td>
<td>Serum creatinine or urine output; (eg, NGAL, KIM-1)</td>
<td>Percentage of patients who develop acute kidney injury; reduction in urine biomarkers (25% change)</td>
<td>Future</td>
<td>All age groups</td>
</tr>
</tbody>
</table>

KIM-1, kidney injury molecule 1; NGAL, neutrophil gelatinase-associated lipoprotein.

**Table 2. End points for cardiovascular complications associated with SCD**

<table>
<thead>
<tr>
<th>Condition</th>
<th>End point</th>
<th>Test</th>
<th>Outcome/outcome measure</th>
<th>Type of end point</th>
<th>Ages</th>
</tr>
</thead>
<tbody>
<tr>
<td>PH and risk of death</td>
<td>Right heart catheter defined PH</td>
<td>RAP, CI, mPAP, and PVR</td>
<td>Percent change in RAP, CI, mPAP, TPG, or PVR</td>
<td>Direct: RAP, mPAP, CI; surrogate: PVR</td>
<td>Adults</td>
</tr>
<tr>
<td>PH and risk of death</td>
<td>TRV</td>
<td>Doppler echocardiography</td>
<td>TRV ≥3.0 m/s or TRV ≥2.5 m/s and NT-pro-BNP level &gt;160 pg/mL</td>
<td>Surrogate</td>
<td>Adults</td>
</tr>
<tr>
<td>PH and risk of death</td>
<td>Composite model</td>
<td>Doppler echocardiography, NT-pro-BNP, and 6-min walk test</td>
<td>TRV ≥2.5 m/s, NT-pro-BNP level ≥160 pg/mL, and 6-min walk test &lt;330 m</td>
<td>Surrogate</td>
<td>Adults</td>
</tr>
<tr>
<td>Diastolic dysfunction</td>
<td>E/e′ and E/A; myocardial fibrosis</td>
<td>Echocardiogram; cardiac MRI</td>
<td>Percent improvement toward normal</td>
<td>Future</td>
<td>All age groups</td>
</tr>
<tr>
<td>Functional exercise capacity</td>
<td>Walk distance</td>
<td>6-min walk test</td>
<td>Change in distance walked (30-40 m)</td>
<td>Direct</td>
<td>Adults</td>
</tr>
<tr>
<td>ACS</td>
<td>Incidence using an accepted definition; progression using an accepted definition</td>
<td>Clinically defined</td>
<td>Percentage of patients who develop ACS; percent of patients that clinically worsen; respiratory support, O2 requirement, transfusion therapy</td>
<td>Direct</td>
<td>All age groups</td>
</tr>
<tr>
<td>Asthma</td>
<td>Development of ACS, pain event, or asthma exacerbation</td>
<td>Clinically defined</td>
<td>Time to event in incidence of ACS, pain crisis, or use of rescue inhaler (number of events/days using rescue inhaler) low FEV1 at baseline</td>
<td>Direct</td>
<td>All age groups</td>
</tr>
<tr>
<td>Thromboembolism</td>
<td>Pulmonary embolism, VTE</td>
<td>Clinically defined thrombus</td>
<td>Percentage of patients with either pulmonary or VTE</td>
<td>Direct</td>
<td>All age groups</td>
</tr>
</tbody>
</table>

FEV1, forced expiratory volume in 1 second; MRI, magnetic resonance imaging.
from Doppler echocardiography, diastolic dysfunction, functional exercise capacity, acute chest syndrome (ACS), asthma, and thromboembolism (see Table 2 for a summary).

**PH** PH is newly defined hemodynamically as a mean pulmonary artery pressure (mPAP) > 20 mm Hg. Based on the prior definition of mPAP ≥ 25 mm Hg, PH occurs in 6% to 11% of SCD adults and carries with it a 40% 6-year mortality.57-59 Pulmonary arterial (PA) hypertension (PAH) is also newly defined by an mPAP of > 20 mm Hg, with a PA wedge pressure of ≤ 15 mm Hg and a pulmonary vascular resistance (PVR) of ≥ 5 Wood units, indicating an increase in the precapillary pulmonary pressures.50 However, an mPAP ≥ 25 mm Hg threshold as abnormal is not based on experimental data. The mean (standard deviation) mPAP in healthy patients is 14.0 (3.3) mm Hg.51 A mean PAP between 21 and 24 mm Hg has been identified as associated with high risk of death and has now been proposed to represent “mild” PH, likely significantly increasing the prevalence of hemodynamic PH in the SCD population.52 In patients with SCD, this pathophysiology is driven in large part by the intravascular injurious effects of hemolysis.53 PAH leads to progressive right heart failure and reduced exercise capacity. Pulmonary venous hypertension is often due to increases in left-sided filling pressures, related to systolic or, more commonly, diastolic dysfunction of the left ventricle or left-sided valvular disease. Both hemodynamic forms of PH are independent predictors of death in the adult SCD population.59

### Cardiopulmonary hemodynamic biomarkers as end points

RHC is the most accurate diagnostic test to establish PH. Hemodynamic variables, including PA systolic and diastolic pressures, mean PA pressures (mPAP), the transpulmonary gradient (TPG; which is the mPAP–PA wedge pressure), and PVR directly correlate with mortality risk in SCD.54 RHC hemodynamics have been used as clinical trial end points for PAH medications in non-SCD populations, and reductions in these hemodynamic end points could be employed. In non-SCD PAH, the 2 hemodynamic variables with the greatest associated mortality risk are an elevated mean right atrial pressure (RAP) (> 15–20 mm Hg) and a reduced cardiac index (≤ 2.0 L/min/m²).54 RAP reduction to < 8 mm Hg and cardiac index increase to > 2.5 L/min/m² after 6 months of macitentan treatment was observed to increase survival in patients with non-SCD PAH.55 In SCD, hemodynamic variables, including PA systolic and diastolic pressures, mPAP, and PVR, directly correlate with mortality risk. Moreover, precapillary PH, as evidenced by an elevated TPG and an elevated PA diastolic pressure to wedge pressure gradient, appears to confer higher mortality risk than postcapillary PH.56,57 This suggests that hemodynamic goals could be used as clinical trials end points. It is important to remember that in SCD, some of these hemodynamic threshold goals need modification, reflecting anemia-related consequences such as elevated cardiac index at baseline, which results in a lower “normal” PVR value. A value ≥ 160 dynes/s/cm² is 5 or 2 Wood units has been proposed as abnormal for adult patients with SCD.57

A 2017 report suggested a PVR of ≥ 160 dynes/s/cm² was more specific for PAH than PH in SCD.57,68 With adjustments, RAP, cardiac index, and PVR could serve as clinical end points for PAH in SCD. Additional clinical end points such as hospitalization related to PH and/or right heart failure, or initiation of additional PAH medications or dose modification, could also be
considered. Currently, our understanding of hemodynamics and PH-associated mortality in SCD is limited to adults ≥18 years of age. Clinical end points along with changes in cardiac index and PVR could be considered direct hemodynamic end points, while RAP (a reflection of right heart function) is a surrogate end point.

**TRV and NT-pro-BNP levels** RHC remains the gold standard test for PH diagnosis. In settings where RHC may not be available, or for initial screening of patients, the use of surrogate markers for PH, including elevated tricuspid regurgitation velocity (TRV) and N-terminal pro-brain natriuretic peptide (NT-pro-BNP) levels, can be considered. Forty-five screening studies for PH using Doppler echocardiographic determination of TRV values, including >6000 patients, have been analyzed. The average prevalence of elevated TRV ≥2.5 m/s in this meta-analysis was 21% (range, 17% to 26%) in children and 30% (range, 26% to 35%) in adults.66 A high TRV (≥2.5 m/s) is associated with a 30.4 m (range, 6.9-53.9) reduction in 6-minute walk distance and a mortality hazard ratio of 4.9 (range, 2.4-9.7).66 In a large single-institution cohort study, a TRV ≥2.5 m/s was associated with a hazard ratio of death of 6.81 in multivariate analysis (P < .001).67 In this study, a value of 2.5 m/s was identified by receiver operator analysis as the best cutoff value to predict mortality. Elevated TRV values also identify patients at higher risk of having PH by RHC; ~40% of patients with a TRV value of 2.5 to 2.9 m/s have PH, while 75% of patients with a value ≥3.0 m/s have PH.59

The observational cohort of the Treatment of Pulmonary Hypertension and Sickle Cell Disease with Sildenafil Therapy (Walk-PHaSST) study followed 632 patients with SCD for 24 months.68 Sixty-four patients (10.1%) had TRV measurements ≥3.0 m/s or higher, and 140 (22.2%) had NT-pro-BNP measurements ≥160 pg/mL; 39 patients (6.2%) had both TRV ≥3.0 m/s and NT-pro-BNP ≥160 pg/mL. At 24 months, the cumulative survival was 83% with TRV ≥3.0 m/s and 98% with TRV <3.0 m/s (P < .0001). The hazard ratios for death were 11.1 (95% confidence interval [CI], 4.1-30.1; P < .0001) for TRV ≥3.0 m/s, 4.6 (95% CI, 1.8-11.3; P = .001) for NT-proBNP ≥160 pg/mL, and 14.9 (95% CI, 5.5-39.9; P < .0001) for both TRV ≥3.0 m/s and NT-pro-BNP ≥160 pg/mL. Adjustment for aspartate aminotransferase and creatinine had little effect on the significance of the association between the combined TRV and NT-proBNP variable and mortality.

This composite biomarker yields both a hazard ratio of 14.86 (95% CI, 5.5-39.9) for mortality50,68 and improved PPV for the PH diagnosis.58 While patients with severe renal disease were excluded during the evaluation of association of the composite biomarker with PH, it has a positive predictive value for PH of >60%.58 Therefore, the panel suggests using a TRV ≥3.0 m/s alone or TRV value of 2.5 to 2.9 m/s in combination with a NT-pro-BNP level ≥160 pg/mL or 6-minute walk <330 m to identify patients at high risk of PH or death (excluding patients with severe renal disease). Reduction of the composite biomarker, however, has not been closely evaluated for predicting improved clinical outcome and is not generally accepted as a PAH clinical trial end point. The mortality risk of an elevated TRV and NT-pro-BNP level in SCD is limited to adults ≥18 years of age. Additional studies are needed to accurately define a composite model in the pediatric population. In 2014, National Heart, Lung, and Blood Institute guidelines were unable to make a recommendation for or against the use of echo alone as a screening PH test for mortality due to insufficient evidence (no comment on mortality).69 American Thoracic Society guidelines in the same year recommended that adult SCD patients undergo screening echocardiography and/or NT-pro-BNP testing to assess the risk of having PH and of death for the purposes of diagnosis and intensification of therapies while recognizing the absence of studies on the use of hydroxyurea and chronic transfusions in placebo-controlled trials in patients with SCD with PH.57

**Diastolic dysfunction** While “normal” ranges have not been rigorously defined for diastolic function in SCD, diastolic dysfunction is present in ≤60% of patients using non-SCD population as a reference.70-72 In the Walk-PHASST screening study, the ratio of mitral peak velocity of early filling (E) to early diastolic mitral annular velocity (e’), E/e’ was an independent predictor of a shorter 6-minute walk distance.73 In the National Institutes of Health PH screening study, diastolic dysfunction as reflected by a low E/A ratio (which is the ratio of peak velocity flow in early to late diastole caused by atrial contraction, the A wave) was associated with excess mortality, even after adjustment for TRV.74 The presence of both diastolic dysfunction and an elevated TRV conferred a risk ratio for death of 12.0 (95% CI, 3.8-38.1; P < .001).74 The panel determined that measurements of Doppler E/e’ and E/A are surrogate markers of diastolic function in SCD while invasively measured (via RHC) pulmonary artery wedge pressures are one of several direct measures of diastolic function. Because of associated mortality risk, further study is needed into the utility of the E/A velocity ratio. Cardiac MRI utility to assess myocardial fibrosis,73,74 while interesting mechanistically and clinically, is a future target for clinical trials design as well. Clinical studies suggest utility of these end points in pediatric and adult populations.

**Exercise capacity** The 6-minute walk test has been used in adults and pediatric patients to capture exercise capacity, as it predicts mortality and response to treatment in a diverse group of lung diseases.70,75,76 Both adult and pediatric patients with SCD can have abnormal 6-minute walk test results, which may be associated with anemia or low fetal hemoglobin (Hb) or cardiopulmonary dysfunction.77,78 Mild-moderate dyspnea with exertion is extremely common in SCD, occurring in 50% of HbSS and 40% of HbSC adults.79 Cardiopulmonary disease has been associated with poor exercise capacity in patients with SCD.74,80 The panel suggests performing a 6-minute walk test as a direct end point for PAH, using a change in distance walked (meters) as the outcome. In PAH, the minimal clinically important distance on this test is between 30 and 40 m; however, a minimally clinically important change has not been established in SCD patients.77 The panel identified more literature on cardiopulmonary outcomes in adults associated with abnormal 6-minute walk test results.70,74,81 Therefore, the panel recommends using the 6-minute walk test as an outcome in adult SCD trials but recognizes that in the future, this may be expanded to pediatric trials.82

**ACS** ACS is a frequent complication in both children and adults living with SCD.21,72,83,84 Patients diagnosed with ACS can deteriorate and SCD is a leading cause of disease-related mortality for patients with SCD.5,68-82 Hydroxyurea can decrease the incidence of ACS but does not eliminate the risk.83,80 Therefore, additional novel therapeutic strategies should be evaluated, alone or in combination with hydroxyurea, to further reduce the incidence and prevent progression of disease. Direct end points for clinical trials include (1) ACS using the currently accepted ACS definition83,84,91-93.

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and (2) additional therapeutic interventions for ACS (eg, assisted respiratory support, oxygen requirement, and/or RBC transfusion). C-reactive protein, sPLA2, thrombospordin-1, and pentraxin 3 are possible biomarkers for predicting occurrence of ACS.

**Asthma/reactive airway disease** Asthma is a comorbid factor in SCD. Studies confirm that asthma predisposes to complications such as vaso-occlusive crises (VOCs), ACS, and stroke and is associated with increased mortality. The panel recommends that studies use time to first sickle cell event (pain or ACS) or change in incidence for developing an event as direct end points in all ages.

Patients with SCD often have abnormal pulmonary function and low forced expiratory volume in 1 second at baseline, so it would be difficult to determine the meaning of this end point. However, it may be considered an indirect end point, because it has been demonstrated to be an independent predictor of early death in adults with SCD. "Symptom-free days" (no wheeze, no cough, no clinical need for albuterol, and no nighttime waking from cough) is a commonly used end point in asthma studies that may also be used in SCD.

**Venous thromboembolism** Clinical trial venous thromboembolism (VTE) end points in patients with SCD do not need to differ from other current thrombosis trials; guidance is available regarding end points and outcomes measures. The panel suggests trials that evaluate the percentage of patients that develop pulmonary embolism or VTE as direct end points as well as future research to assess VTE prevention strategies and the risks and benefits of sustained anticoagulation for VTE. Trials should include all age groups, though the risk of VTE in children with SCD may be primarily associated with indwelling catheters.

### End points for assessing therapies with curative intent

Curative intent approaches involve hematopoietic stem cell (HSC) transplant (HSCT), either using an unaffected donor or a donor with sickle cell trait (alloimmune transplant) or autologous hematopoietic stem and progenitor cells after gene modification (gene addition or gene editing). A precise correlation between efficacy parameters after allogeneic HSCT (eg, lineage-specific donor chimerism) or autologous/gene therapy (eg, vector copy number [VCN], percentage of alleles with homology-directed repair, and homology-directed repair edited) and outcome has not been standardized between clinical trials as the pace of disease modification can be variable depending on age, intervention, and disease burden. In addition, treatment-related adverse effects need to be tracked and taken into consideration in descriptions of the risk-benefit ratio.

Outcome measurements after transplant overlap with outcome descriptions from organ-specific panels. To reach a common goal of uniformity across trials, it is recommended that the end points discussed below be measured and reported across all HSCT studies in SCD. The panel recommends that data be collected at least at 1-, 2-, 5-, 10-, 15-, and subsequently 5-year intervals, even if the latter time points are through remote questionnaire or phone. It is important that mechanisms be established for long-term follow-up and for public reporting of these data. An overview of outcomes tracking following curative therapy interventions with suggestions regarding frequency of follow-up have been published recently based on a consensus conference held by the Pediatric Blood and Marrow Transplant Consortium.

### Biomarkers for therapies with curative intent

The effect of HSCT on the hematological expression of SCD has been reported. End points that capture primary hematological markers of the disease (see "Hematological biomarkers" and Table 3) must be obtained. These end points will not be useful for assessing a curative outcome until a period of time without concomitant medications and without disease-related complications has elapsed. Posttransplant complications and mortality, if present, should be tracked (eg, infections, graft-versus-host disease [GVHD] or antierythrocyte antibodies).

Multiple technologic developments are needed for assays such as F cells, where HbF is a potential therapeutic Hb. Improvements and standardization for F-cell assays are needed as well as assays to detect cellular distribution of antisickling Hb, such as T87Q and AS3 Hb, and assays to measure the cellular distribution of HbA/HbS.

Quantitative RBC physiology measures are needed to demonstrate recovery from abnormal properties after therapy. These assays may demonstrate the cellular impact of therapy on a critical pathophysiologic molecular event. Rheologic assays that might quantify hypoxia-induced rheologic changes in SCD and can distinguish SCD from changes after HSCT or RBC transfusions have been reported recently. For any of these assays, the panel encourages using untreated SCD, SCD after transfusions, and assays obtained after HSCT with healthy donor blood to benchmark the assays.

Traditional laboratory biomarkers of SCD amelioration or cure in HSCT clinical trials include those in Table 4, such as transfusion requirements and VOCs, organ function, and PROs identified in those specific sections. HSCT complications such as acute and


#### Table 3. Measures of normal or genetically corrected RBCs proposed to assess for cure in SCD

<table>
<thead>
<tr>
<th>End point</th>
<th>Measures</th>
<th>Grade of cure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anemia and hemolysis</td>
<td>Hb; reticulocyte counts; normal Epo; sTfR, LDH, haptoglobin; hemopexin; cell free Hb; RBC microparticles</td>
<td>1: All parameters normal</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2: Compensated anemia (Hb near normal, high reticulocyte counts, sTfR, Epo)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3: Improved anemia with ongoing hemolysis (high reticulocyte count, bilirubin, LDH)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4: Mildly improved Hb and persistent intra- and extravascular hemolysis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5: No improvement</td>
</tr>
<tr>
<td>RBCs, erythropoiesis, and genetically modified RBCs</td>
<td>RBC count; erythropoiesis; sTfR, Epo; Hb electrophoresis/HPLC, for HbS, anti-sickling Hb%; cellular distribution of anti-sickling Hb (eg, % F cells); research; deformability, osmoscan</td>
<td>1: Normal</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2: 75% improvement</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3: 50% improvement</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4: 25% improvement</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5: No improvement</td>
</tr>
</tbody>
</table>

Epo, erythropoietin; HPLC, high-performance liquid chromatography; LDH, lactate dehydrogenase; sTfR, soluble transferrin receptor.
Assess the success of SCD curative therapies aim to measure the laboratory biomarker panels and clinical outcome parameters and interrogate nuclease-specific off-target mutagenesis. Stem and progenitor cells, will be required to more precisely identify chromosomal translocations in primary human hematopoietic stem/progenitor cells, and the development of appropriate biomarkers to identify remain off-target insertional mutagenesis and transgene silencing will be similar to those outlined for HSCT (Table 4). The primary safety concerns if additive gene therapy using viral vectors will remain off-target insertional mutagenesis and transgene silencing, and the development of appropriate biomarkers to identify and monitor these effects will be critical (Table 6). With gene editing, further advances in methods for high-fidelity sequencing and genome-wide assessment of off-target cutting, off-target integrations of the homology donor template, and effects on genome integrity, including chromosomal translocations in primary human hematopoietic stem and progenitor cells, will be required to more precisely identify and interrogate nuclease-specific off-target mutagenesis.

The laboratory biomarker panels and clinical outcome parameters assess the success of SCD curative therapies aim to measure the amelioration of the overt SCD phenotype. They do not measure several important underlying biochemical, metabolic, and transcriptomic determinants of SCD pathophysiology such as endothelial cell activation and microvascular flow, ischemia and reperfusion, tissue oxygenation, and perturbations in the SCD patient’s epigenome, proteome, and metabolome (Table 7). Understanding these and other underlying mechanisms will permit deeper phenotyping of the SCD patient and better inform the short- and long-term phenotypic impact of the type/timing of the various curative SCD strategies.

While the hallmark of a successful outcome after allogeneic HSCT for SCD is the full replacement of recipient HSCs in the marrow by healthy donor HSCs, termed full donor engraftment, this is not necessary for a curative outcome. The observation of stable mixed hematopoietic chimerism after HSCT for thalassemia and SCD demonstrated that even a minority of donor cells that have stable engraftment are sufficient for eliminating anemia, hemolysis, and the clinical symptoms of SCD. Because the donor chimerism threshold sufficient for a curative effect is uncertain and influenced by a number of host factors such as the genetic makeup of the donor (unaffected or with trait) more research is needed for these end points (percent chimerism and HbS level). Current data from mixed chimerism following HSCT suggests that a percentage of normal donor chimerism may correct the disease and the degree of chimerism may be dependent on the sickle RBC half-life. Currently, data are lacking for the degree of chimerism of gene-modified grafts that correct the clinical phenotype and the laboratory parameters. However, engraftment in the range of 20% for erythroid or myeloid chimerism and an HbS level <50% with transfusion support has been suggested. For any genetic modification or stem cell transplant approach, morbidity and mortality from conditioning, including acute and long-term and durability of the efficacy outcomes, should be monitored and reported. Data on long-term side effects such as second malignancies, endocrine effects, and effects on fertility hormones, fertility, growth, and development should be collected. Clonal diversity may also be a correlate for efficacy and safety. Increasing evidence shows that clonal hematopoiesis is common after HSCT, with outcomes ranging from clinically silent oligoclonal hematopoiesis to myelodysplasia or leukemia. Assessment methods for clonality may provide useful information regarding the long-term effects and could provide an

| Table 4. Laboratory and clinical biomarkers proposed for therapeutic monitoring |
|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| Therapeutic monitoring biomarker | SCT or GT/E | Direct or surrogate biomarker | Biomarker value validation |
| Laboratory biomarkers | | | |
| Donor chimerism (%) | SCT/GT/E | Surrogate | Strong |
| Hb, reticulocyte (target; time to target) | SCT/GT/E | Surrogate | Strong |
| Increase in HbF (x2y2) | GT/E | Surrogate | Strong |
| Increase in or HbA (x2y2) | SCT/GT/E | Surrogate | Strong |
| Clinical biomarkers | | | |
| Change in TF requirements | SCT/GT/E | Direct | Intermediate* |
| Increase in VOC | SCT/GT/E | Direct | Intermediate* |
| Change in all pain levels | SCT/GT/E | Direct | Intermediate* |
| Change in HRQoL | SCT/GT/E | Direct | Intermediate* |
| Change in organ-specific function | SCT/GT/E | Direct | Intermediate* |

GT/E, gene therapy/editing; SCT, stem cell therapy.
*Could be strengthened by choosing targets that meet the FDA guidance for rare disease trial end points (https://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm613026.htm).

| Table 5. Laboratory and clinical biomarkers for adverse event monitoring |
|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| Adverse event monitoring biomarker | SCT or GT/E | Direct or surrogate biomarker | Biomarker value validation |
| Laboratory biomarkers | | | |
| Vector integration sites | GT/E | Surrogate | Strong |
| Transgene variegation and silencing | GT/E | Surrogate | Strong |
| Off-target mutagenesis | GT/E | Possibly predictive | Strong |
| Clinical biomarkers | | | |
| Early vs late graft rejection | SCT | Direct | Intermediate |
| alloantibody development | SCT | Direct | Intermediate |
| Acute vs chronic GVHD biomarker panels | SCT | Surrogate | Research gap |

For any genetic modification or stem cell transplant approach, morbidity and mortality from conditioning, including acute and long-term and durability of the efficacy outcomes, should be monitored and reported. Data on long-term side effects such as second malignancies, endocrine effects, and effects on fertility hormones, fertility, growth, and development should be collected. Clonal diversity may also be a correlate for efficacy and safety. Increasing evidence shows that clonal hematopoiesis is common after HSCT, with outcomes ranging from clinically silent oligoclonal hematopoiesis to myelodysplasia or leukemia. Assessment methods for clonality may provide useful information regarding the long-term effects and could provide an
undertaken. Established organ damage is not expected to return to normalcy. In certain situations, the intervention may be deemed beneficial in the absence of further deterioration as would be expected with the natural history of the disease. The Panel on Measurement of Cure recommends that organ function baselines be established before treatment and continue to be tracked 1, 2, 5, 10, and 15 years following intervention.

### Gonadal function and fertility preservation

The age at which curative treatment is undertaken and exposure to toxicities of conditioning therapy can influence gonadal outcomes. Two forms of gonadal insufficiency can occur after myeloablative therapy in childhood: hypogonadotropic hypogonadism due to pituitary iron overload and hypergonadotropic hypogonadism due to gonadal damage from conditioning chemotherapy. Hormonal function in prepubertal boys is usually better preserved than fertility, as Leydig cells are less sensitive to conditioning agents than spermatogonial cells. Hormonal function and fertility are equally impaired in prepubertal girls, and ovarian insufficiency is very common following HCT after puberty, particularly after exposure to myeloablative busulfan. Reduced-intensity conditioning has shown preservation of hormonal function and/or fertility in some of females, although further evidence on effects on male fertility is needed. Baseline gonadal function (Tanner staging and gonadotropin and hormone levels) should be assessed in all patients ≥11 years of age before myeloablation if a busulfan-based or other alklyator therapy is used. Following the curative therapy, annual assessments of pubertal development, sexual, and reproductive function is recommended based on accelerated impairment following exposure to conditioning regimens. This includes luteinizing hormone, follicle-stimulating hormone, anti-Mullerian hormone, and estradiol levels in females and follicle-stimulating hormone, luteinizing hormone, and early morning testosterone levels in males when recipients are ≥11 years of age.

### Liver

Liver function can be affected by prior vaso-occlusive infarction, iron deposition caused by chronic transfusion therapy, and conditioning therapy such as busulfan. Liver iron content can

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### Table 6. Potential biomarkers for postcurative monitoring

<table>
<thead>
<tr>
<th>Therapeutic monitoring laboratory biomarkers</th>
<th>SCT or GT/E</th>
<th>Direct or surrogate biomarker</th>
<th>Biomarker value validation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Change in deep tissue oxygenation steady state* (SpO₂, pCO₂, pO₂; bicarbonate, lactate; VO₂; VCO₂)</td>
<td>SCT/GT/E</td>
<td>Surrogate/direct</td>
<td>Research gap</td>
</tr>
<tr>
<td>Steady-state ischemia/reperfusion* (TNF, NF-kB, IL-1β; WBC adhesion)</td>
<td>SCT/GT/E</td>
<td>Surrogate/direct</td>
<td>Research gap</td>
</tr>
<tr>
<td>Change in endothelial activation* (P-selectin expression)</td>
<td>SCT/GT/E</td>
<td>Surrogate/direct</td>
<td>Research gap</td>
</tr>
<tr>
<td>Change in proteomics/epigenetics/metabolomics*</td>
<td>SCT/GT/E</td>
<td>Surrogate/direct</td>
<td>Research gap</td>
</tr>
</tbody>
</table>

*Potential biomarkers that meet the FDA guidance for rare disease trial end points (https://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm613026.htm).

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### Table 7. Quantification/surrogates of engrafted corrected HSCs proposed for use in measuring cure in SCD

<table>
<thead>
<tr>
<th>End point</th>
<th>Measures</th>
<th>Grade of Cure</th>
</tr>
</thead>
<tbody>
<tr>
<td>GT/E</td>
<td>VCN or edited cells and alleles in infusion product and 0.5, 1, 2, 5, 10, and 15 y after genetic modification (%); genetically modified and allele modified CFUc (%)</td>
<td>VCN in infused product and up to 6 mo after GT/E can be higher; stable VCN (at year 1 onward) that results in:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1: 100% genetically corrected RBCs</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2: 75%-100% corrected RBCs</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3: 50%-75% corrected RBCs</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4: 25%-50% corrected RBCs</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5: 0%-25% corrected RBCs</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Corrected RBCs may be defined as anti-sickling Hb containing RBCs and/or RBCs that resist sickling (%)</td>
</tr>
<tr>
<td>Engraftment</td>
<td>Normal or genetically modified HSC chimerism (%)</td>
<td>1: &gt;50%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2: &gt;20%</td>
</tr>
</tbody>
</table>

CFUc, colony-forming units in culture.
be assessed by T2* MRI scans. A liver biopsy in patients who have long-term transfusion exposure might be useful to identify hepatic fibrosis and the risk of cirrhosis after treatment.

**Specific clinical end points of cure related to SCD phenotype**

**Pain/VOCs** Tracking the frequency and severity of VOCs in the manner previously described (hospitalization and opioid use) is important after curative therapy interventions to determine the effects of the therapy on this important component of the disease. PROs can be determined with patient and caregiver input using validated measures as previously described.

**Cardiovascular and pulmonary end points** Although the change in markers of cardiovascular and pulmonary function would be predicted to change slowly over time, resolution of an underlying abnormality is not generally observed. Thus, these end points might be used to establish stability of function after the curative therapy.

(See "End-organ diseases: renal and cardiopulmonary end points in SCD" for further details.)

**Neurological end points** Neurological end points are provided by the brain panel and are discussed in detail in “End points for assessing brain outcomes in SCD” of the companion end point paper by Farrell et al.

**CKD end points** As noted for other end organs, end point stabilization in lieu of improvement is expected (see “Renal end points” for further details).

**Assessment of PROs**

PRO assessments following a curative therapy are valuable for complex decision making. They quantify broad non–organ-specific impacts of a disease-free state and are influenced by transplant complications such as chronic GVHD. Assessments before HCT and at 6 months, 12 months, 2 years, and yearly beyond if indicated can provide global functional outcomes. Detailed information on PRO assessments as clinical trial end points are provided in the PRO section of the companion end point paper by Farrell et al.

In summary, the panel recommends long-term multifaceted outcome assessment and analysis following curative therapies as applicable, including (1) disease-related complications related to SCD phenotype; (2) disease-related organ functions; (3) transplant-related complications; (4) generalized psychosocial performance, societal integration, and health-related quality of life (PRO); and (5) impact of cure-directed interventions such as genetic manipulation.

**Summary: defining the degree of “cure”**

After an allogeneic transplant when there is full “healthy” donor chimerism, a cure from SCD can be obtained; however, these patients still need to be assessed for acute and late adverse event end points. Partial chimerism is likely after gene transfer or gene editing and autologous transplantation, where the fraction of genetically modified or edited HSCs that engraft a patient will vary (depending on gene transfer/gene editing efficiency, HSC dose, conditioning regimen). Hence, there will be varying amount of chimerism of corrected versus disease HSCs. In this setting, the degree of cure may also vary.

A “scorecard” for judging the effect of the curative therapy becomes imperative when so many different curative options are in trials or in preparation. A graded end-point system will allow comparisons of different vectors, conditioning regimens, gene editing modalities, and transplant regimens, as well as the toxicities associated with each, so that providers and patients can make an informed decision and chose a curative modality that is acceptable, feasible, and safe for a particular individual.

The curative end points and associated toxicities could be graded. An initial grading proposal is suggested in Table 8, which could be refined iteratively as data become available. PROs could be integrated into this grading system.

**Adherence end points**

The biomarkers panel stratified adherence biomarkers for clinically indicated medical therapies in SCD based on those viewed as clinically validated and in wide use in children and adults. While much of the data and measures evaluate hydroxyurea use, many of the markers may be partly applicable for evaluating adherence to other treatments (eg, immunosuppressive agents posttransplant).

These include blood cell standard clinical measurements (eg, MCV, Hb, and absolute reticulocyte count) and pharmacy refill databases (eg, MCV, Hb, and绝对reticulocyte count). HbF levels (percentage or absolute amount, average untreated HbF) and Hb above the median (“Hb augmentation,” the benefit of which is improved physiology through suggestive markers such as energy and oxygen flow); and markers of hemolysis, which reflect improved RBC stabilization (lower reticulocytes by absolute reticulocyte count, reduced LDH to more normal levels, and reduced indirect bilirubin). Physiological biomarkers that are useful in young children but are poorly quantitative (except over the long term) include growth curve in young children, and early splenomegaly.

Promising but not rigorously validated biomarkers include (and are not limited to): sickle RBC rheology (from whole blood or microfluidic); RBC ekctometry; oxygen affinity by whole blood oximetry (eg, reduced 2,3 DPG); in vitro sickling (by bright field microscopy); microparticles (derived from PRB, platelets, and/or monocytes); and proteomic markers of cellular activation or sickle pathology.

Widespread poor adherence for therapeutic hydroxyurea in SCD has been documented by pharmacy refill databases and by reduction from peak HbF levels, a sensitive biomarker for dose-dependent hydroxyurea use. A validated measure for adherence is the highest historical hydroxyurea-induced HbF as a minimum target for an individualized personal best self-management goal.

For that reason, a personalized biomarker, “personal-best HbF,” can add to other adherence measures employed, both biological (eg, MCV, Hb, and absolute reticulocyte count) and pharmacy refill data. Most studies use more than one method to assess medication adherence, often a physiological and another. Importantly, while self-reporting is consistently the weakest marker of adherence, many studies continue to use self-reported adherence, often using a version of a “Moritsy” scale, which is not recommended.

**Hematological biomarkers** Hematological biomarkers can be divided as either “pure” characteristics of hematological manifestations in SCD, such as severity in anemia or hemolysis, or as...
characteristics that refer to changes in other extrahematological organ systems (such as creatinine or GFR) but found in the circulation. Pure hematological biomarkers include blood cell standard clinical measurements:\textsuperscript{133-135} Hb above the median (Hb augmentation, the benefit of which is improved physiology through better energy [soft] and oxygen flow [soft]); increased MCV as a marker of hydroxyurea effect; reduced WBC (a useful marker of reduced inflammation, cytokine production, and endothelial/cellular activation through receptor activation); reduced platelets (sudden decrease in platelet counts is often a marker of increase severity and impending worsening of ACS or multiorgan failure); HbF levels (percentage or absolute amount, average untreated HbF, intracellular concentration, and distribution)\textsuperscript{133,136} and markers of hemolysis, which reflect improved RBC stabilization (lower reticulocytes by absolute reticulocyte count,\textsuperscript{133} reduced LDH to more normal levels, and reduced indirect bilirubin and aspartate aminotransferase).

The bone marrow (BM) and the BM niche can be seen as an organ in itself, with potential for progressive end-organ damage, for which no marker has been suggested to date in SCD. In contrast, the process of BM aging in the general population has been well described, and markers such as mean telomerase length and the BM environment is exposed to stressors (eg, hydroxyurea). Conventional biomarkers, such as Hb, WBC, platelets and platelet activation markers, plasma free Hb, reticulocyte count, Hb F percentage, and \(\alpha\) thalassemia have all been reported within specific complications such as stroke, leg ulcers, priapism, and VOCs. Ferritin has been found to be a marker of early mortality in several case series, reflecting a probable biomarker of severe disease and inflammation/oxidative stress.\textsuperscript{149} WBC transcriptomes and microRNAs are newcomers to this field and deserve more rigorous scrutiny. Rheology assessment with microfluidics, as mentioned earlier, offers the promise to measure blood flow and is especially useful when evaluating a single patient under different clinical conditions. These assessments can be done in an endothelialized system or looking at P-selectin without the endothelial lining.

Hb is a useful clinical end point, and degree of anemia has been correlated with higher transcranial Doppler velocities, increased risk of stroke, renal and cardiopulmonary complications, priapism, leg ulcers, and cognitive dysfunction. While composite end points such as the hemolytic index have been validated as indicators of hemolysis and have been associated with certain complications, they need further studies for prognostic validation and clinical applications (ie, changes in the hemolytic index leading to improved survival or reduced complications). Markers of inflammation, such as C-reactive protein and erythrocyte sedimentation rate, which are not strictly hematological markers, may have value in assessing hematological response to a therapeutic intervention that targets the inflammatory pathway.

### End points for trials in low-resource settings

An estimated 300 000 affected children born every year in sub-Saharan Africa (SSA), representing 80% of the global burden of SCD.\textsuperscript{150} Approval of existing therapies for SCD have been based on clinical trials conducted in high-resource settings. Advantages of conducting trials in low-resource settings can include rapid enrollment, answering questions on disease heterogeneity, disparate outcomes in individuals of similar phenotypes, and uncovering the natural history of SCD and its specific complications in addition to a resource for experimental clinical trials. SCD clinical trials with primary and secondary outcome measures conducted in high- and low-resource settings often differ in their intent, with trials in low-resource settings more focused on dosing, effectiveness, and optimizing or expanding existing treatments than on testing new drugs.

The main drivers of SCD-related outcomes in individuals living in low-resource settings include early childhood mortality (<5 years old),\textsuperscript{151} perioperative mortality,\textsuperscript{192,193} and poor pregnancy-related outcomes (increased maternal and perinatal mortality).\textsuperscript{154} Based on the disparate disease-related outcomes of individuals with SCD in high- vs low-resource settings, research studies must develop relevant clinical end points that take into consideration unique factors that determine disease-related outcomes applicable to each region that are pivotal for investigating SCD biology, phenotypes, and new therapies.
Implications for clinical trials design

Health care delivery systems in SSA have implications for clinical trial design and focus (safety, dosing and effectiveness, lifesaving interventions, and primary vs secondary prevention). Most individuals with SCD in SSA do not have access to comprehensive care, and research outcomes from comprehensive care centers in urban areas may not be applicable to the rural settings where individuals have limited access to SCD care. Individuals with SCD living in rural areas who must travel long distances to SCD centers would face challenges with regular visits for tests and clinical examinations mandated by study requirements.

Further, the nutritional status of patients may pose challenges with dosing and bioavailability of therapeutic agents. Children with SCD, similar to children with other chronic diseases, are at risk for delayed growth and pubertal maturity, which may be exacerbated in the low socioeconomic environment of low-resource settings. This could result in exclusion of patients with very low z-scores based on body mass index measurements, leaving questions about application to malnourished/stunted patients unanswerable.

Clinical trial design process should involve practitioners managing individuals with SCD in SSA. Their local expertise and insights are crucial in contextualizing research design to achieve the aims and objectives. Safety reporting in SSA could be variable depending on the skillset and experience of research coordinators.

Meaningful PROs that are context specific for SSA communities are needed as end points. For example, growth and development are more meaningful in the region. In addition, assessment of physical activity and school attendance as a measure of health-related quality of life (HRQoL) among patients in the United States or Europe may not be as meaningful or applicable in SSA. Neuropsychological assessments would have to consider cultural sensitivities, including language barriers, and tools for assessments would have to align with what has been validated for use in clinical practice. End points that rely on hospitalization or utilization of health facilities would not be reliable in the SSA context, where resource constraints place barriers to utilization of health-care resources.

Overt stroke as an end point is feasible in SSA, as validated tools for stroke diagnosis based on neurological examination are feasible. However, resources for MRI are limited in SSA. Silent stroke, the diagnosis of which is imaging dependent, would not be feasible; however, neurocognitive assessment, a surrogate end point of SCD-related cerebrovascular disease, is possible using validated tools if adapted to the SSA setting. Where measurable biomarkers are needed as end points, the equipment and methodology for such measures need to be monitored to ensure accuracy of such measurements. Ease of safety reporting could also be variable depending on the skills and experience of research coordinators.

Reducing SCD mortality in low-resource settings

SCD is associated with a very high rate of childhood mortality at 50% to 90% in SSA. The Garki study provided valuable information on childhood survival and other aspects of the natural history of SCD, although current information on the burden of mortality from SCD in Africa among populations based on access to currently available treatments and preventive interventions is lacking. In Tanzania, 10,313 children with SCD <5 years of age are estimated to die every year, an estimated 7% of overall deaths in children <5 years.

In a prospective study evaluating posthospital mortality in children aged 2 to 12 years, SCD alone accounted for 21% (10/47) of the deaths in the first year after hospital discharge. Attributable factors included severe malnutrition, neurologic diseases, heart disease, cancer, and septic shock. Other risk factors significantly associated with mortality included older age, lower Hb level, lower Glasgow Coma Scale, history of decreased urine output, higher respiratory rate, estimated GFR <60 mL/min/1.73 m² (binary), and lower oxygen saturation. These determinants of outcome are risk factors that frequently complicate SCD, especially in low-resource settings, with implications for clinical trial design and feasible end points.

Though there has been increased childhood survival for patients with SCD in high-income countries, early mortality in young adulthood is still the norm. Individuals in low-income settings who survive to adulthood face the challenge of living with a chronic morbidity that requires comprehensive care in a region where this does not exist. The median survival for men and women with SCD remains dismal low, at ~42 and 48 years, respectively, in high-income settings but remains unknown in low-resource settings.

Understanding the patterns of morbidity, mortality, HRQoL, and health care utilization in individuals with SCD living in low-resource settings will be relevant in designing clinical trials with clinical trial end points that are impactful in these settings. Table 9 summarizes suggested clinical trial end points that focus on reducing mortality in low-resource settings.

Age and organ-specific SCD morbidity and mortality: relevance for clinical trial end point considerations in low-resource settings

Patients with SCD are at high risk for age-dependent morbidity and mortality from end-organ complications. While strokes, infection and organ sequestration are the leading causes of death in childhood, chronic organ failure, including CKD and cardiopulmonary disease become two of the most common causes of death or findings at the time of death in adults with SCD.

Increased childhood survival has also resulted in more females with SCD reaching reproductive age in low-resource settings, with poor pregnancy-related outcomes (increased maternal and perinatal mortality). SCD-related chronic impairment in multiple organs and its association with mortality highlight the need to understand the common mechanisms underlying chronic end-organ damage in SCD. In low-resource settings, there is an urgent need to develop simple interventions to prevent irreversible end-organ complications and poor pregnancy-related outcomes associated with SCD. Table 9 summarizes clinical trial end points appropriate for low-resource settings for PH, ACS, CKD, and primary and secondary stroke prevention, recognizing that traditional end points might not be feasible or pragmatic in these settings.

Therapeutic trials: relevant clinic trial end points in low-resource settings

Multiple ongoing clinical trials are investigating the use of novel agents in SCD. A review of the website www.clinicaltrials.gov reveals approximately >30 agents that are presently being tested in SCD, mainly in high-resource countries. These drugs include HbF inducers, anti-oxidants, antiadhesive agents,
The renewed focus on SCD specific clinical trials in SSA is long overdue. Identifying appropriate clinical trials with interventions that could affect the disparate mortality of affected individuals in low-resource settings are urgently needed. A renewed focus is needed on clinical trial designs with end points for primary (such as primary stroke prevention), secondary (such as secondary stroke prevention or reducing progression of CKD), and tertiary prevention (improving quality of life, symptom reduction, and disease amelioration) that can be implemented within existing health care frameworks in specific low-resource settings. Early identification of affected children through establishment of newborn screening programs, point-of-care testing, and capacity building for laboratory diagnosis of SCD, coupled with SCD comprehensive care programs, will help set the stage for opportunities for prospective studies with relevant clinical trial end points in these settings.

The large population of affected individuals in low-resource settings provides opportunities to improve the understanding of disease heterogeneity, disparate outcomes in individuals of similar

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### Table 9. Clinical trial end points focused on reducing mortality and morbidity in SCD in low-resource settings

<table>
<thead>
<tr>
<th>Research focus</th>
<th>Attributable risk factors</th>
<th>Clinical trial end points for low-resource settings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reducing &lt;5-y-old mortality</td>
<td>Unknown natural history of SCD in SSA; infections (malaria and invasive bacteria); splenic sequestration and severe anemia; severe malnutrition and diarrheal illnesses</td>
<td>Newborn screening programs to better estimate number of patients affected, survival, and complication rates. This needs to be coupled with comprehensive care programs. Estimate impact of antimalarial prophylaxis; vaccination for common bacterial infections and penicillin prophylaxis until at least 5 y; and improved nutrition, and interventions against diarrheal illnesses.</td>
</tr>
<tr>
<td>Reducing pregnancy-associated mortality</td>
<td>Increased risks of both SCD-specific and pregnancy-related complications (maternal death rate of SCD is 7%-12%)</td>
<td>Multidisciplinary interventions to reduce and manage hypertensive disorders of pregnancy (preeclampsia, eclampsia), severe anemia and urinary tract infections, antenatal and postnatal acute pain episodes, and pregnancy-associated VTE</td>
</tr>
<tr>
<td>Reducing perioperative mortality</td>
<td>Poor transfusion practices; perioperative supportive care; postoperative infections</td>
<td>Reducing risk of transmission of transfusion associated infections; local interventions to reduce ACS postoperative complications; reduction in postoperative VTE</td>
</tr>
</tbody>
</table>

### Organ-specific clinical trial end points in low-resource setting

| PH | Composite model: TRV >2.5 m/s and either NT-pro-BNP ≥160 pg/mL or 6-min <330 m | Mortality from PH exercise capacity (6-min walk test; change in distance walked) |
| ACS | Define ACS based on current accepted definition (definition of a new pulmonary infiltrate (excluding atelectasis) and chest pain, fever, tachypnea, wheezing, or cough | Percentage of patients who develop ACS based on modified criteria and adjudicated |
| CKD | Severe albuminuria (≥300 mg/g); moderate albuminuria (30-300 mg/g); CKD (stage >3 by GFR; stage >1) | Urine albumin/creatinine (% decrease in proteinuria); measured or estimated GFR (percentage of patients who require initiation of renal replacement therapy); progression in stage of CKD |
| Primary stroke prevention | Increased risk of stroke in children in low-resource setting (10%-11%); increased incidence of causes of severe anemia (malaria, iron deficiency anemia, severe malnutrition) | Reduction in TCD velocity in children; clinical and imaging evidence of new stroke, TIA, or death; neurocognitive testing, locally adapted Pediatric NIH Stroke Scale |
| Secondary stroke prevention | Increased risk of stroke in children in low-resource settings (10%-11%); increased incidence of causes of severe anemia (malaria, iron deficiency anemia, severe malnutrition); lack of and availability of safe transfusion practices; unavailability of disease-modifying therapy (hydroxyurea) | Prevalence of recurrent stroke: NIH stroke scale (questionnaire); clinical and imaging evidence of recurrent stroke, TIA, or death; neurocognitive testing (NIH scale) |

### Therapeutic trials: relevant clinic trial end points in low-resource settings

| Phase 2 study of novel agents | Pharmacokinetics and pharmacodynamics studies; safety and efficacy of new agents; effect on splenic and renal function; biological and hematologic correlates (HbF level, reduction of intracellular Hb concentration, increasing nitrous oxide bioavailability); antiplatelet, anti-inflammatory, anticoagulation, and antiadhesion properties | Improvement in baseline Hgb, acute pain episodes rate, hematologic toxicities or serious adverse events, biological and hematologic correlates, other clinical end points: ACS, ischemic stroke, CKD, priapism, PH, leg ulcers, avascular necrosis |

NIH, National Institutes of Health; TCD, transcranial Doppler; TIA, transient ischemic attack.
phenotypes, and uncovering of the natural history of SCD and its specific complications. In addition, this presents unique opportunities to help validate the efficacy of approved therapies, using larger patient cohorts. To succeed, ongoing research needs to establish a foundation of evidence within SSA, which involves establishing international research partnerships and capacity building, while simultaneously strengthening local SCD knowledge, clinical experience, and care delivery.169

Conclusions

Patients with SCD have significant complications due to complex pathophysiology. Identifying optimal end points for current use and future development was the goal of the ASH-FDA Sickle Cell Disease Clinical Endpoints Workshop. This report, along with the companion report, noted where data exist to support including clinical trial end points as a direct benefit, surrogate, or biomarker. In addition, the report identifies where future work is needed to develop additional end points in SCD. The results of this work provide an exhaustive list of suggested direct end points, surrogate end points, and biomarkers, along with future development recommendations. As with any recommendation, the exact clinical context must be considered before clinical trial end point adoption.

Acknowledgments

This article summarizes topics addressed at the US Food and Drug Administration (FDA)–American Society of Hematology (ASH) Sickle Cell Disease Clinical End Points Workshop. ASH and FDA engaged the work of 7 panels of clinicians, investigators, and patients to develop consensus recommendations for clinical trial end points. The panels conducted their work through literature reviews, assessment of available evidence, and expert judgment focusing on end points. This work, plus >30 preparatory calls with the panels and engaging discussions at the workshop, contributed to the development of 2 workshop articles that present the findings of the panels. The Contribution section details how the authors were involved in the development of the actual manuscripts. The authors thank Kathi E. Hanna, the contracted science writer who provided summaries based on discussions at workshop and initial summaries submitted by panels, prepared drafts of the manuscript, managed its review, and prepared it for submission. The authors acknowledge Peter Marks (Center for Biologics Evaluation and Research, FDA) and 2018 ASH President Alexis A. Thompson (Ann & Robert H. Lurie Children’s Hospital of Chicago, Feinberg School of Medicine, Northwestern University) for their support and involvement with the 2018 FDA-ASH Sickle Cell Disease Clinical Endpoints Workshop.

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The views of the authors represent their own and should not be interpreted to reflect the official policy of the US FDA.

Authorship

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