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.

As part of a multifaceted initiative addressing the global burden of SCD, the American Society of Hematology (ASH) partnered with the US Food and Drug Administration (FDA) to engage the work of 7 panels of clinicians, investigators, and patients to develop consensus recommendations for SCD 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 (PROs), pain (non-PROs), the brain, end-organ considerations, biomarkers, measurement of cure, and those appropriate for low-resource settings. In conducting their reviews, the panels considered a broad range of end point definitions, including biomarkers as well as fully qualified clinical end points denoting clinical benefit that could be used for a regulatory approval. Clinical benefit was defined as "what a patient would want from a therapeutic procedure, such as improved survival, symptom improvement, or decreased risk of developing disease or morbidity (eg, stroke)." End points should reflect patient desires and integrate objective measurements to assess disease severity and progression. Ideally, an end point should be easy to measure accurately at low cost and at low burden for the patient and research team. Further, it should be interpretable and clinically relevant and available to be measured in all patients in a study facilitating complete data collection.

The results of the panels' work were presented and discussed at a public workshop in October 2018 attended by 188 in-person and 750 viewing online via live stream from 20 countries. Intra- and interpanel discussions as well as exchanges with attendees further informed the process. 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. Findings and recommendations from the other panels are reported separately (see accompanying article by Farrell et al[2]).

The workshop presenters noted significant differences between definition of end points and biomarkers applied as end points. Building off the BEST (Biomarkers, End pointS, and other Tools) resource,[3] the panels concurred with the definition of a biomarker as a defined characteristic measured as an indicator of normal biological or pathogenic processes or responses to an exposure or intervention. A biomarker is not an "end point" that evaluates how an individual feels, functions, or survives. A full biomarker description includes the biomarker name, the source/matrix, the measurable characteristics, and the analytic method used to measure the biomarker. Biomarkers can be further classified as those that, for example, stratify susceptibility/risk biomarker, diagnosis, disease/product monitoring, and prognosis. While many biomarkers associated with SCD complications represent findings from single and small study populations, the authors attempted to discriminate those biomarkers that are well established from those that are used for research purposes. Specifically, in to evaluate a biomarker in SCD, several pieces of information were evaluated and varied for each biomarker, including but not limited to evidence (quantity and quality of) on the measurability, sensitivity, specificity, reliability, and laboratory-to-laboratory reproducibility. These characteristics are defined as analytical validation for a given biomarker by the BEST document and helped guide committee views on defining the presence and value of biomarkers in SCD.

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.[4-9] 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.[10-12] Risk factors for the development of albuminuria include increasing age, hyperfiltration, elevated blood pressure, leukocytosis, anemia, hemoglobinuria and increased hemolytic markers.[10,11,13-15] Moderate and severe albuminuria are highly prevalent, and understanding the natural history of progression, including the role of genetic variants (eg, APOL1[16-18]), 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.[19] 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.[20] 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

Table 1. End points for renal complications associated with SCD

Condition	End point	Test	Outcome/outcome measure	Type of end point	Ages
CKD, albuminuria	Albuminuria or proteinuria	First morning void or 2 consecutive untimed albumin/creatinine ratio or protein/creatine ratio	Percent decline in albuminuria	Direct: severe albuminuria; surrogate: moderate albuminuria	All age groups
CKD, GFR	Renal replacement therapy	Initiation of renal replacement therapy (may differ based on clinical practice)	Percentage of patients who require initiation of renal replacement therapy	Direct	All age groups
CKD, GFR	GFR	Measured GFR and estimated GFR	Percentage of patients who progress in stage of CKD or percentage of decline in GFR	Surrogate	Adults
Hypertension	Cardiovascular end point	Clinically defined	Number of patients who develop stroke, myocardial infarction, death	Direct	All age groups
Hypertension	Systolic or diastolic blood pressure; pediatric studies should consider use of ambulatory blood pressure monitoring	Blood pressure measurement	Change in systolic or diastolic blood pressure (5 mm Hg)	Future	All age groups
Hyposthenuria	Urine concentration availability	Water deprivation test; not >10 h in children; meaningful tests are those concentrating urine >500 mOsm/kg H_2O	Percentage of patients with preservation of urine concentration ability	Surrogate	Infants or toddlers
Kidney injury	Acute kidney injury defined by serum creatinine or urine output; biomarker of kidney injury	Serum creatinine or urine output; novel biomarkers of kidney injury (eg, NGAL, KIM-1)	Percentage of patients who develop acute kidney injury; reduction in urine biomarkers (25% change)	Future	All age groups

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

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.[21-23] In adulthood, patients begin to experience an annual GFR decline.[24,25] Patients who progress to end-stage renal disease suffer increased mortality rates and may be less likely to receive a renal transplant.[26-28]

Recent murine and human SCD studies suggests that hyperfiltration precedes the development of albuminuria.[14,29] 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 2. End points for cardiovascular complications associated with SCD

Condition	End point	Test	Outcome/outcome measure	Type of end point	Ages
PH and risk of death	Right heart catheter defined PH	RAP, CI, mPAP, and PVR	Percent change in RAP, CI, mPAP, TPG, or PVR	Direct: RAP, mPAP, CI; surrogate: PVR	Adults
PH and risk of death	TRV	Doppler echocardiography	TRV ≥3.0 m/s or TRV ≥2.5 m/s and NT-pro-BNP level >160 pg/mL	Surrogate	Adults
PH and risk of death	Composite model	Doppler echocardiography, NT-pro-BNP, and 6-min walk test	TRV >2.5 m/s, NT-pro-BNP level ≥160 pg/mL, 6-min walk test <330 m	Surrogate	Adults
Diastolic dysfunction	E/e′ and E/A; myocardial fibrosis	Echocardiogram; cardiac MRI	Percent improvement toward normal	Future	All age groups
Functional exercise capacity	Walk distance	6-min walk test	Change in distance walked (30-40 m)	Direct	Adults
ACS	Incidence using an accepted definition; progression using an accepted definition	Clinically defined	Percentage of patients who develop ACS; percent of patients that clinically worsen; respiratory support, O_2 requirement, transfusion therapy	Direct	All age groups
Asthma	Development of ACS, pain event, or asthma exacerbation	Clinically defined	Time to event in incidence of ACS, pain crisis, or use of rescue inhaler (number of events/days using rescue inhaler) low FEV_1 at baseline	Direct / Indirect	All age groups
Thromboembolism	Pulmonary embolism, VTE	Clinically defined thrombus	Percentage of patients with either pulmonary or VTE	Direct	All age groups

FEV_1, forced expiratory volume in 1 second; MRI, magnetic resonance imaging.

studies should use an estimated GFR equation (serum creatinine and/or cystatin) with the least bias based on the current literature but future research is needed to determine a sickle cell–specific estimated GFR or more feasible measured GFR.[21,30,31] For clinical trials evaluating GFR decline, the end point could be either progression in CKD stages or percentage GFR decline. Future research is needed to improve our understanding of the natural history of annual decline in GFR on clinical outcomes. Caution is needed in enrolling patients with hyperfiltration and using the percent decrease in GFR as an end point, as these patients may return to a normal filtration if their overall SCD improves, which would not indicate disease progression.

For patients with CKD stages 4 or 5, trials using initiation of renal replacement therapy is a direct end point. For patients with CKD stages 1 to 3, trials using decline in stage or percent decline in GFR should be considered.

Hypertension Guidelines have been developed to diagnose and treat hypertension in the adult and pediatric populations.[32,33] Although patients may have lower blood pressure than the general population, hypertension in these patients is associated with significant morbidity and mortality.[34-38] Accepted end point measures of antihypertensive intervention can be used in clinical trials including clinical cardiovascular end points (death, myocardial infarction, and stroke) or change in systolic or diastolic blood pressure. The methodology for obtaining blood pressure in a clinical trial should follow current guideline statement recommendations.[32,33] Using 24-hour ambulatory blood pressure monitoring can allow for a more accurate assessment of hypertension and is superior to office assessments to predict long-term cardiovascular disease outcomes.[32,39]

Hyposthenuria Loss of renal concentrating ability (hyposthenuria) occurs early in life. Almost one-quarter of infants in the Baby HUG trial were unable to concentrate their urine after modest water deprivation.[22,40] Because urine-concentrating defects develop early in life, clinical trials aimed at reducing hyposthenuria development should occur in children with this manifestation. Hyposthenuria should be viewed as a surrogate biomarker; however, if future studies identify a link between hyposthenuria and kidney disease, hyposthenuria prevention could be considered a direct end point.

Biomarkers of kidney injury Acute kidney injury has a bidirectional association with CKD.[41] Patients develop acute kidney injury during a crisis.[42-44] KDIGO defines acute kidney injury as an increase in serum creatinine or a decrease in urine output. In SCD, serum creatinine may underestimate a change in GFR and urine output may be affected by hyposthenuria. Therefore, additional research in urine biomarkers of acute kidney injury are warranted, such as N-acetyl-b-D-glucosaminidase,[10] HMOX1 variants,[45] uric acid,[46] β2 microglobulin,[47-50] soluble FMS-like tyrosine kinase,[51] kidney injury molecule 1,[45,52,53] monocyte chemoattractant protein,[54] neutrophil gelatinase-associated lipoprotein,[45] transforming growth factor-β1,[10,55] endothelin-1,[56] and nephrin.[29]

Cardiopulmonary end points

Several direct end points involve cardiopulmonary complications that occur commonly in SCD, including pulmonary hypertension (PH), defined by right heart catheterization (RHC) or estimated 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 a mPAP of >20 mm Hg, with a PA wedge pressure of ≤15 mm Hg and a pulmonary vascular resistance (PVR) of ≥3 Wood units, indicating an increase in the precapillary pulmonary pressures.[60] 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.[61] 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.[62] In patients with SCD, this pathophysiology is driven in large part by the intravascular injurious effects of hemolysis.[63] 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.[59]

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).[64] 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.[65] 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.[59] 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/cm⁻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/cm⁻⁵ or 2 Wood units was more specific for PAH than PH in SCD.[57,65] 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 mortality[60,68] 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 pediatrics 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 PH, 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.[71,72,83,84] Patients diagnosed with ACS can deteriorate and it is a leading cause of disease-related mortality for patients with SCD.[5,85-89] Hydroxyurea can decrease the incidence of ACS but does not eliminate the risk.[83,90] 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 definition[83,84,91-93]

and (2) additional therapeutic interventions for ACS (eg, assisted respiratory support, oxygen requirement, and/or RBC transfusion).[84,94,95] C-reactive protein, sPLA2, thrombospondin-1, and pentraxin 3 are possible biomarkers for predicting occurrence of ACS.

Asthma/reactive airway disease Asthma is a comorbid factor in SCD.[96-103] Studies confirm that asthma predisposes to complications such as vaso-occlusive crises (VOCs),[82,104-107] ACS,[108,109] and stroke[109] and is associated with increased mortality.[110,111] 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.[112,113] "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.[114] 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 assesses 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.[115]

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 (allogeneic 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

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

End point	Measures	Grade of cure
Anemia and hemolysis	Hb; reticulocyte counts; normal Epo, sTfR, LDH, haptoglobin, hemopexin; cell free Hb; RBC microparticles	1: All parameters normal 2: Compensated anemia (Hb near normal, high reticulocyte counts, sTFR, Epo) 3: Improved anemia with ongoing hemolysis (high reticulocyte count, bilirubin, LDH) 4: Mildly improved Hb and persistent intra- and extravascular hemolysis 5: No improvement
RBCs, erythropoiesis, and genetically modified RBCs	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	1: Normal 2: 75% improvement 3: 50% improvement 4: 25% improvement 5: No improvement

Epo, erythropoietin; HPLC, high-performance liquid chromatography; LDH, lactate dehydrogenase; sTFR, soluble transferrin receptor.

published recently based on a consensus conference held by the Pediatric Blood and Marrow Transplant Consortium.[116]

Biomarkers for therapies with curative intent

The effect of HSCT on the hematological expression of SCD has been reported.[117-121] 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.[122] 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 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 ($\alpha2\gamma2$)	GT/E	Surrogate	Strong
Increase in or HbA ($\alpha2\beta2$)	SCT/GT/E	Surrogate	Strong
Clinical biomarkers			
Change in TF requirements	SCT/GT/E	Direct	Intermediate*
Change 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.html).

chronic GVHD, early and late graft rejection, immune constitution, and impact on organ functions need to be collected as previously described.[116] Tracking the novel predictive biomarkers for acute and chronic GVHD[123] severity, which are beginning to emerge in the allogeneic transplant setting, can be considered (Table 5).[124]

Gene therapy and gene editing

Despite the curative potential of HSCT, limitations and complications have led to development of transplantation of autologous HSCs genetically corrected through both gene addition and editing. These alternative approaches are targeted to patients for whom a medically suitable donor is not available or the risk of an unmodified HSCT is unacceptably high.[125,126] Determinants of successful gene therapy and editing will include (1) adequate HSCT dose of gene-modified CD34$^+$ cells and the ability to accurately ascertain the CD34$^+$ subpopulation of gene-targeted HSCs, (2) adequate stable engraftment of gene-modified HSC's and chimerism to ensure phenotype-ameliorating increases in HbA or HbF, and (3) optimization of recipient BM niche to enable adequate engraftment. Current laboratory and clinical biomarkers of successful amelioration of the SCD phenotype in the design of gene therapy/editing would 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).[125] 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

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

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.[119-125] 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.[117,126,127,128] 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.[127,128]

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.[129] Assessment methods for clonality may provide useful information regarding the long-term effects and could provide an

Table 6. Potential biomarkers for postcurative monitoring

Therapeutic monitoring laboratory biomarkers	SCT or GT/E	Direct or surrogate biomarker	Biomarker value validation
Change in deep tissue oxygenation steady state* (SpO$_2$, pCO$_2$, pO$_2$; bicarbonate, lactate; VO$_2$; VCO$_2$)	SCT/GT/E	Surrogate/direct	Research gap
Steady-state ischemia/reperfusion* (TNF, NF-κB, IL-β1; WBC adhesion)	SCT/GT/E	Surrogate/direct	Research gap
Change in endothelial activation* (P-selectin expression)	SCT/GT/E	Surrogate/direct	Research gap
Change in proteomics/epigenetics/ metabolomics*	SCT/GT/E	Surrogate/direct	Research gap

IL, interleukin; TNF, tumor necrosis factor.

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

early warning for complications (eg, onset of aplasia or progression to myelodysplasia).

The analysis and reporting of off-target modifications and effects on genome integrity after genomic editing is important, but standardization of this end point is not yet possible. The end point will be better defined as the clinical translation experience expands with better algorithms to design gene editing tool sets.

Clinical toxicity end points The incidence and severity of GVHD in the acute and chronic forms must be recorded and included in outcome measurements after allogeneic HSCT. There are standardized methods for documenting and reporting GVHD that should be followed.[130] Similarly, duration of immune suppression/modulation and its side effects (infection incidence) and immune reconstitution/response to immunization (postimmunization titers) should be recorded.

Clinical end points are necessary to document the influence of the treatment modality on the manifestations of the disease as it relates to organ functions. Change in organ function after treatment is expected to vary with the age at which the treatment (stem cell transplant or autologous genetically modified cell infusion) is undertaken. Established organ damage is not expected to recover back to a baseline normal.[24,131,132] Permanent organ

function impairment is likely in older patients and those with severe disease manifestations and may have predictive genotypic, phenotypic, or biochemical biomarkers. However, these are not established and remain speculative at the current time. In many patients, the clinical benefits of intervention may have to be "graded" to measure benefit as opposed to an expectation of 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

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

End point	Measures	Grade of Cure
GT/E	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 (%)	VCN in infused product and up to 6 mo after GT/E can be higher; stable VCN (at year 1 onward) that results in: 1: 100% genetically corrected RBCs 2: 75%-100% corrected RBCs 3: 50%-75% corrected RBCs 4: 25%-50% corrected RBCs 5: 0%-25% corrected RBCs. Corrected RBCs may be defined as antisickling Hb containing RBCs and/or RBCs that resist sickling (%)
Engraftment	Normal or genetically modified HSC chimerism (%)	1: >50% 2: >20%

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.[2]

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.[2]

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.[2] 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.[2]

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 pheno-type; (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[133-135]; increased mean corpuscular volume (MCV; uncertain physiological benefit vs marker of hydroxyurea effect); reduced white blood cell (WBC) count (useful for reduced inflammation, cytokine production, and endothelial/cellular activation through receptor activation); reduced platelets (similar to WBCs); HbF levels (percentage or absolute amount, average untreated HbF)[133,135] 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,[133] 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,[136,137] and early splenomegaly.[5]

Promising but not rigorously validated biomarkers include (and are not limited to): sickle RBC rheology (from whole blood[138] or microfluidic[122]); RBC ektacytometry[138]; oxygen affinity by whole blood oximetry[139] (eg, reduced 2,3 DPG[140]); in vitro sickling (by bright field microscopy[141]); microparticles (derived from PRB, platelets, and/or monocytes[142]); 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.[143,144] 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.[143] Most studies use more than one method to assess medication adherence, often a physiological and another.[145] Importantly, while self-reporting is consistently the weakest marker of adherence, many studies continue to use self-reported adherence,[146] often using a version of a "Moritsy" scale,[147] 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

Table 8. Potential grading schema for pain and patient-reported quality-of-life outcomes after curative therapy

End point	Measures	Grade of cure
Pain	Pain events; ain events requiring hospitalization; oral pain medications (narcotics and NSAIDs) per month and per year; parenteral opiate use per month and per year	1: Pain completely relieved 2: 75%-99% improvement 3: 55%-74% improvement 4: ≤50% improvement 5: No improvement
Patient-reported quality-of-life outcomes	Pain, affect functionality; PROMIS; ASCQ-ME; PedsQL; PHENX toolkit; HPQ; SCSES; COPM	Grade per measure to assess improvement before and after (%) 1: 100% 2: 75% improvement 3: 50% improvement 4: 25% improvement 5: No improvement

ASCQ-ME, Adult Sickle Cell Quality of Life Measurement Information System; CPOM, Composite Functional Outcome; HPQ, Health and Work Performance questionnaire; NSAID, nonsteroidal anti-inflammatory drugs; PedsQL, Pediatric Quality of Life Inventory; PHENX, phenotypes and exposures; PROMIS, Patient-Reported Outcomes Measurement Information System; SCSES, Sickle Cell Self-Efficacy Scale.

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[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)[133,135]; and markers of hemolysis, which reflect improved RBC stabilization (lower reticulocytes by absolute reticulocyte count,[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 genes such as DNMT3A, TET2, or ASXL1[148] have been reported. Such markers could be relevant in SCD as patient age increases 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 α 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.[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.[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),[151] perioperative mortality,[152,153] and poor pregnancy-related outcomes (increased maternal and perinatal mortality).[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,[155-157] 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,[158] 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.[159,160] 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.[161] 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.[162]

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.[163] 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 m^2 (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.[1] Individuals in low-income settings who survive to adulthood face the challenge of living with a chronic morbidities requires comprehensive care in a region where this does not exist. The median survival for men and women with SCD remains dismally low, at ~42 and 48 years, respectively, in high-income settings but remains unknown in low-resource settings.[87] 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.[164-166] Table 9 summarizes suggested clinic 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.[4-6] 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).[154] SCD-related chronic impairment in multiple organs and its association with mortality[167] 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.[168] 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,

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

Research focus	Attributable risk factors	Clinical trial end points for low-resource settings
Reducing mortality in SCD in low-resource settings		
Reducing <5-y-old mortality	Unknown natural history of SCD in SSA; infections (malaria and invasive bacteria); splenic sequestration and severe anemia; severe malnutrition and diarrheal illnesses	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.
Reducing pregnancy-associated mortality	Increased risks of both SCD-specific and pregnancy-related complications (maternal death rate of SCD is 7%-12%)	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
Reducing perioperative mortality	Poor transfusion practices; perioperative supportive care; postoperative infections	Reducing risk of transmission of transfusion associated infections; local interventions to reduce ACS postoperative complications; reduction in postoperative VTE
Organ-specific clinic 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.

anti-inflammatory agents, antisickling agents that modify Hb oxygen affinity, anticoagulants, and antiplatelet agents. To improve generalizability, some of these trials need to be conducted in low-resource settings. In addition to traditional clinical trial end points such as the evaluation of acute pain crisis, other surrogate end points such as SCD-related end-organ complications, exercise capacity, and locally applicable PROs in low-resource settings should be introduced. These are warranted and necessary in order to advance the development of these novel therapeutic agents. Table 10 reviews traditional clinical trial end points and suggested modifications in low-resource settings.

Summary: clinical trial end points for low-resource settings

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.[8] 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

Table 10. Traditional clinical trial end points and suggested modifications in low-resource settings

Disease-related factors	Traditional clinical end points	Clinical end points for low-resource settings
Recurrent pain episodes	Proportion of days with symptom exacerbation (SCDSM); annualized sickle cell crisis rate; annualized VOC events (number and length of hospital/ER visits, phone diary, home-health visits over time); time to first pain crisis; duration and severity of pain crisis	Electronic daily pain diary, phone tracking, plus hospitalization and emergency room visit rate; reduction in average visual analog score for pain
Disease amelioration	Measuring HRQoL; increase in Hb >1 g/dL from baseline; hemolysis biomarkers (total bilirubin, LDH, reticulocyte, total WBC, platelet count, CRP, plasma D-dimers laboratory values); frequency of SCD-related complications (VOC, ACS, hepatic and splenic sequestration, leg ulcers, priapism and stroke); changes in functional exercise capacity (6-min walk test)	Increase in baseline Hb >1 g/dL; decrease in frequency of SCD-related complications; changes in functional exercise capacity (6-min walk test); improved growth in children; locally relevant PROs
Hematologic responses	Increase in baseline Hb by 2 points; improvement in HbF from baseline	Increase in baseline Hb by 2 points, quite feasible; improvement in HbF from baseline, dependent on availability of HPLC machines, limits to tertiary centers
PROs	Symptom relief or HRQoL (multidimensional), including physical, functional, social, emotional, and spiritual QoL, as measured by QoL instruments	Lack of context-specific and culturally specific instruments that have demonstrated validity, reliability, and sensitivity to clinically important changes

CRP, C-reactive protein; QoL, quality of life.

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.

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Authorship

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