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Ann T. Farrell

Allison A. King

et al.

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End points for sickle cell disease clinical trials: patient-reported outcomes, pain, and the brain

Ann T. Farrell,^{1,*} Julie Panepinto,^{2,*} C. Patrick Carroll,^{3,*} Deepika S. Darbari,^{4,*} Ankit A. Desai,^{5,*} Allison A. King,^{6,*} Robert J. Adams,^{7,†} Tabitha D. Barber,^{8,†} Amanda M. Brandow,^{2,†} Michael R. DeBaun,^{9,†} Manus J. Donahue,^{10-12,†} Kalpna Gupta,^{13,†} Jane S. Hankins,^{14,†} Michelle Kameka,^{15,†} Fenella J. Kirkham,^{16,17,†} Harvey Luksenburg,^{18,†} Shirley Miller,^{19,†} Patricia Ann Oneal,^{1,†} David C. Rees,^{20,21,†} Rosanna Setse,^{1,†} Vivien A. Sheehan,^{22,†} John Strouse,^{23,24,†} Cheryl L. Stucky,^{25,†} Ellen M. Werner,^{18,†} John C. Wood,^{26,†} and William T. Zempsky^{27,†}

¹US Food and Drug Administration, White Oak, MD; ²Pediatric Hematology, Medical College of Wisconsin/Children's Wisconsin, Milwaukee, WI; ³Department of Psychiatry and Behavioral Sciences, Johns Hopkins School of Medicine, Baltimore, MD; ⁴Children's National Medical Center, Washington, DC; ⁵Krannert Institute of Cardiology, Indiana University, Bloomington, IN; ⁶Division of Hematology and Oncology in Pediatrics and Medicine, Washington University School of Medicine, St. Louis, MO; ⁷Department of Neurology, Medical University of South Carolina, Charleston, SC; ⁸Patient Advocate, Rochester, NY; ⁹Vanderbilt-Meharry Center of Excellence in Sickle Cell Disease, Vanderbilt University Medical Center, Nashville, TN; ¹⁰Department of Radiology and Radiological Sciences, ¹¹Department of Neurology, and ¹²Department of Psychiatry, School of Medicine, Vanderbilt University, Nashville, TN; ¹³Division of Hematology, Oncology, and Transplantation, Department of Medicine, Medical School, University of Minnesota, Minneapolis, MN; ¹⁴Department of Hematology, St. Jude Children's Research Hospital, Memphis, TN; ¹⁵Nicole Wertheim College of Nursing and Health Sciences, Florida International University, Miami, FL; ¹⁶Developmental Neurosciences Unit and ¹⁷Biomedical Research Unit, UCL Great Ormond Street Institute of Child Health, London, United Kingdom; ¹⁸National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD; ¹⁹Atrium Healthcare, Charlotte, NC; ²⁰Department of Haematological Medicine, King's College Hospital, London, United Kingdom; ²¹School of Cancer and Pharmaceutical Sciences, King's College London, London, United Kingdom; ²²Division of Hematology/Oncology, Department of Pediatrics, Baylor College of Medicine, Houston, TX; ²³Division of Hematology, Department of Medicine, and ²⁴Division of Pediatric Hematology/Oncology, Department of Pediatrics, Duke University School of Medicine, Durham, NC; ²⁵Department of Cell Biology, Neurobiology, and Anatomy, Medical College of Wisconsin, Milwaukee, WI; ²⁶Children's Hospital Los Angeles, Los Angeles, CA; and ²⁷Department of Pediatrics, Connecticut Children's/School of Medicine, University of Connecticut, Hartford, CT

To address the global burden of sickle cell disease (SCD) 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 outcomes (PROs), pain (non-PROs), the brain, end-organ considerations, biomarkers, measurement of cure, and low-resource settings. This article presents the findings and recommendations of the PROs, pain, and brain panels, as well as relevant findings and recommendations from the biomarkers panel. The panels identify end points, where there were supporting data, to use in clinical trials of SCD. In addition, the panels discuss where further research is needed to support the development and validation of additional clinical trial end points.

Introduction

Sickle cell disease (SCD) is the most common inherited red blood cell 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 effective therapies. To improve therapeutic options, clinical trials using 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.

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*A.T.F., J.P., C.P.C., D.S.D., A.A.D., and A.A.K. share first authorship.

†R.J.A., T.D.B., A.M.B., M.R.D., M.J.D., K.G., J.S.H., M.K., F.J.K., H.L., S.M., P.A.O., D.C.R., R.S., V.A.S., J.S., C.L.S., E.M.W., J.C.W., and W.T.Z. contributed equally to this study.

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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 outcomes (PROs), pain; nonpatient-reported measures of pain; the brain; other 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 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). Ideal 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 the research team. Furthermore, it should be interpretable, 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 online attendees via livestream 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 PROs, pain, and brain panels, as well as relevant findings and recommendations from the biomarkers panel. Findings and recommendations from the other panels are reported separately.²

The workshop recognized significant differences between definition of end points and biomarkers applied as end points. Building off of the Biomarkers, End pointS, and other Tools (BEST) resource,³ the panels concurred with the definition of a biomarker as a defined characteristic(s) 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 characteristic(s), 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. Although 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, 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 measurability, sensitivity, specificity, and reliability, as well as 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 points for PROs in SCD

The FDA and the National Institutes of Health define a PRO as "any report of the status of a patient's health condition that comes

directly from the patient, without interpretation of the patient's response by a clinician or anyone else."^{4(p2)} Instruments to measure PROs in a quantitative manner and capture the patient's voice complement traditional measures of efficacy such as survival and frequency as well as duration of hospitalization.⁵

The most common SCD symptoms include outcomes that are subjective, such as pain and fatigue. Therefore, it is imperative to use PROs as a clinical end point to measure efficacy in therapeutic trials. The PROs panel determined that 3 key PRO domains are particularly salient in SCD: pain (acute and chronic), affect (emotional impact, sleep quality, and fatigue), and functioning (social, physical, and cognitive function, as well as self-efficacy for disease management and occupational function). Although other PRO end points are important and valuable for patients with SCD, in choosing these 3 domains the PROs panel considered whether the outcomes would be relevant within a regulatory framework for assessing therapeutic products or devices. The panel then determined whether there were existing tools to measure the most relevant facets of those domains and evaluated the evidence supporting their use in SCD.

Extensive work over the past 2 decades has resulted in reliable and valid methods of measuring PROs that incorporate prior research, are valid across multiple conditions, use the most advanced current knowledge of psychometrics and testing, and minimize patient/participant burden in data collection. To capitalize on this work and further the goal of developing a common core set of PROs, the PROs panel focused on 2 core families of PROs, each with significant prior work in SCD. Overall, the panel considered the ages of the trial population to be an important criterion in selecting appropriate measures.

The first of these groups, the Patient-Reported Outcomes Measurement Information System (PROMIS), encapsulates a multidisciplinary effort to develop measures calibrated to patient health domains of greatest health impact.⁶ PROMIS measures are available in broad age ranges and are closely related to the Adult Sickle Cell Quality of Life Measurement Information System (ASCQ-ME) adult SCD-specific measures that were developed alongside them. The second is the Pediatric Quality of Life Inventory (PedsQL), which contains both general and SCD-specific modules of PROs for children.

An important consideration in measure selection is developmental level. The panel recognized the advantage of using PROMIS when both children and adults are the target population as it is a well-accepted multidimensional PRO set that allows for assessment and comparison across the lifespan. ASCQ-ME, although developed specifically for SCD, is validated only for adults, whereas PedsQL is validated for children only. In addition, very young children may require the use of proxy reports because many PRO measures require abstract or complex cognitive processes that are not appropriate for younger children. Parent or proxy measures can complement the use of child self-reports in settings in which the child is too young or not able cognitively to self-report. However, parent and child reports are not interchangeable and would need to be analyzed separately. Thus, child self-report is the best option for capturing PROs when possible.

In keeping with the accepted psychometric standards expected for PROs, the PROs panel evaluated measures of their validity, reliability, and responsiveness to change in clinical status, both

natural history of the illness and treatment response, when such evidence was available.^{7,8} Participant burden and an understanding of the clinical interpretation of the score and of a change in score were also considered. The acceptability of these PRO end points as coprimary, secondary, or “composite” end points for regulatory purposes will likely depend on the mechanism of action and anticipated therapeutic effect(s) of a product/device. PROs and means for measuring them were considered for the domains of acute and chronic pain, affect, and functioning. A separate article by this group discusses use of PROs in low-resource settings.²

Measuring patient-reported pain outcomes

Pain is the most common symptom of SCD^{9,10} but its etiology is complex and multifactorial.¹¹ An interplay of disease, genetic, psychological, and environmental factors contributes to development of a pain phenotype. Pain in SCD has been categorized as acute, chronic, or acute in the presence of chronic pain; and recent expert consensus suggests subdividing chronic pain in SCD into that associated with another identifiable cause and that which is not. Multiple pain mechanisms may be active at a given time. Furthermore, the pain phenotype among individuals with SCD can exhibit remarkable diversity, for reasons that have not been completely elucidated.

For example, painful crises vary in duration and intensity from person to person and over time for an individual person, but have a definable beginning and end. The most appropriate composite measure of the response of crisis to treatment may depend on the time period being observed (eg, at baseline, beginning to end of crisis, duration of an acute care visit, duration of a hospitalization), the initial level of pain, and appropriate measures of the significance of the reduction, such as patients' or clinicians' judgement of improvement or readiness for discharge. Whether an intervention improves the responsiveness of pain to standard therapy, such as opioid analgesics (an “opioid sparing effect”), requires simultaneous measurement of patient-reported pain intensity and analgesic use. The measurement of a therapeutic response of an intervention targeted at chronic pain will require different methods. Thus, unidimensional measures of pain intensity must be integrated with other relevant measures, such as multidimensional PRO measures of pain, time, and analgesic use, and used in a manner suitable for the setting and expected natural history of the pain phenomenon being studied.

Reduction in the frequency of acute painful episodes (vaso-occlusive crises [VOCs]) is accepted by the FDA as an important clinical outcome and an established surrogate end point of clinical benefit for SCD. Approval of the currently available agents (hydroxyurea and L-glutamine) were based on demonstrated improvement in the rate of painful crises among patients with SCD in randomized placebo-controlled trials. As a clinical trial end point (primary or key secondary end point), measures of pain intensity and interference should be patient-reported. Such PROs can be integrated into a composite end point that includes non-PRO pain measures (discussed in “Pain intensity”). Future studies should better define how non-PRO pain measures correlate with PROs. Nonetheless, non-PRO end points, many of which are in the early stages of validation, may be useful as correlative/secondary end points and drive development of mechanism-targeted therapies for pain in SCD.

Changes in acute pain are a vital end point for many interventions for SCD. In addition to changes in pain intensity, clinically important improvements in acute pain have been operationalized in a number of ways, including cessation of parenteral opioids for at least 5 hours, reported pain relief, ability to walk if previously ambulatory, or a decision that pain was low enough for discharge from acute care.^{12,13} In studies of preventive interventions, reduction in the rates of acute crises and utilization of opioid analgesics have also been used.¹⁴⁻¹⁶ In many cases, acute care utilization has been used as a proxy for crisis frequency, which likely is an underestimate of overall crisis pain.¹³

In the future, pain-related outcome measures should capture what patients want from a therapy, such as reductions in pain frequency, pain intensity, suffering, and interference with daily activities and life goals. Additional challenges lie in the inability to predict or classify patients at high risk for chronic pain through the use of biomarkers. The PROs panel considered measurements for pain intensity, interference, and behavior, applicable to both acute and chronic pain associated with SCD.

Pain intensity. In children, ordinal categorical scales, visual analog scales (VASs), and numeric rating scales (NRSs) are generally accepted as valid and reliable for measuring pain intensity, although this must be tempered by developmental considerations.¹⁷⁻¹⁹ Measurement of pain intensity in children below the ages of 7 to 8 years is particularly complicated. NRSs,²⁰⁻²⁴ VASs,²²⁻²⁴ and the Faces Pain Scale–Revised (FPS-R)^{22,25,26} have all been used in SCD in patients at least as young as 8 years of age, mainly for measuring acute pain, and have high convergent validity in general. To the extent that a study sample includes very young children and no adults, the FPS-R may be appropriate, although it may be unreliable in children younger than 7 years.^{23,27} For older children and particularly mixed populations with adults, an NRS or VAS is likely most appropriate.

For adults and children 8 years and older,²⁷ pain intensity should be measured using a VAS or an 11-point, 0 to 10 NRS with anchoring prompts at the low end (“none”) and the high end (“as bad as you can imagine”). An NRS scale is implemented in PROMIS 1a and as part of other multidimensional pain measures. It has been validated in SCD and is sensitive to chronic pain, improvements in acute pain, and therapeutic effects.^{12,13,23} VASs are less often used in clinical practice and require adequate cognitive and visuospatial abilities; they also require that the patient be able to see and manipulate the scale. In participants at least 8 years old, the PROs panel recommends use of an 11-point NRS or an appropriately reproduced and administered VAS as a measure of pain intensity.

With respect to measuring change in pain as a clinical outcome, patient-reported clinically important differences tend to be proportional to initial pain intensity (such as 30% reduction) rather than an absolute change and both should be reported.²⁸ For pain outcomes during hospitalizations or acute care visits, methods should include consideration of confounding of pain intensity with other factors affecting admission, discharge, and length of stay. For acute/crisis pain managed at home, there is little evidence regarding assessment to guide recommendations for pain evaluation.^{14,21} Electronic diaries,²⁹ or possibly ecological momentary assessment,^{30,31} may be reasonable options. However, the performance of these measures needs further investigation. Reductions in chronic pain intensity or interference as an end point

should account for the long duration of this symptom, such that longer-term follow-up and sustained response should be preferred in defining relevant end points. For chronic pain, established instruments use time anchors to evaluate average, least, and worst pain in a reasonable time period (such as 24 hours for the Brief Pain Inventory [BPI]). However, no standard is established for the use of time anchors. Anchors reflecting longer time periods are likely to be more influenced by recall bias, and there tend to be strong correlations among different time measures.^{32,33}

Pain interference/impact and behavior. Pain interference (often used synonymously with "impact") is an important clinical end point in addition to pain intensity.³⁴ Although it is strongly correlated with intensity, there is evidence that interference and intensity are differentially responsive to intervention.^{35,36} The relationship between self-reported pain interference scales and functional outcomes may be indirect and attenuated,^{37,38} which argues for more specific measures of function as an outcome.

In children 8 years and older, PedsQL, Pain Interference,³⁹ and PROMIS Pain Interference⁴⁰ have established validity and reliability and are responsive to changes in acute pain.⁴¹⁻⁴³ Proxy reported versions are available and also validated for use in SCD.^{44,45} In adults, PROMIS Pain Interference was validated in adults with SCD in a comparative study with ASCQ-ME and was found to be sensitive to differences in the presence of clinical complications.⁴⁶ ASCQ-ME Pain Impact was shown to be valid⁴⁷ with regard to overall SCD severity, but its longitudinal validity is not yet established.

There is some evidence that the BPI Pain Interference scale is superior to PROMIS measures on sensitivity to change, though whether this is true in SCD is unknown⁴⁸ and future research is needed regarding its usefulness in SCD. The BPI's inclusion of validated pain intensity NRS and a similarly well-validated pain interference scale allows for measurement of both pain intensity and interference.

To assess pain interference and pain impact, the PROs panel recommends the PROMIS Pain Interference measures for studies of adult, pediatric, or mixed age groups; ASCQ-ME Pain Impact for adults; and PedsQL, Pain Impact, and Pain and Hurt for children. Alternatively, the BPI may be used for studies limited to adult participants.

Pain behavior. PROMIS includes domains for Pain Behavior and Pain Quality for use in children and adults. The PROMIS pediatric Pain Behavior domain is a valid and reliable measure.^{48,49} Pain Behavior and Pain Quality domains have been used in adults with SCD.^{46,47} For assessment of pain behavior, the PROs panel recommends using the PROMIS Pain Behavior domain for children and adults with SCD.

Measuring PROs related to affect

The PROs panel identified depression/negative emotional impact and fatigue as the primary PROs in the affective domain.

Depression/negative emotional impact. For children, the specific impact of SCD on emotional well-being is measured by the Peds-QL, SCD Module Emotions measure, although the SCD Module Worry measures (I and II) may be of secondary interest. The Emotions module distinguished mild from severe disease as operationalized in a validation study.^{41,50} In adults, the

ASCQ-ME Emotional Impact tool was developed specifically for use in SCD, which shows some relationships to SCD disease activity.^{46,47}

The PROMIS Pediatric Depression and Anxiety tool may provide reasonable measures of low mood and anxiety, which have some advantages in comparison across conditions and in relation to other measures of depressive symptoms. PROMIS Pediatric Depression, Pediatric Anxiety, and Pediatric Anger scales have shown relationships both to disease severity and changes in disease severity.^{40,42} The adult PROMIS Depression and Anxiety scales have not been specifically evaluated in SCD. Although some work has been done to compare it to older instruments, the Depression measure may be less sensitive to the neurovegetative symptoms of Major Depressive Disorder (MDD) than some other measures.⁵¹ Great caution must be exercised in equating the "depression" measured by these instruments with a diagnosis of MDD or generalizing from changes in these scales in SCD to conclusions about the efficacy of treatments for MDD in SCD.

To measure specific impact of SCD on emotional health, the PROs panel recommends the PedsQL SCD Module Emotions measure for children and the ASCQ-ME Emotional Impact measure for adults. For more general measures of negative affect, and particularly when mixed age samples are used, the panel recommends the PROMIS Depression and Anxiety measures.

Fatigue. The PROMIS Pediatric Fatigue Scale has shown preliminary evidence of validity and sensitivity to differences in disease severity in children as well as in changes in the process of crisis recovery.^{40,42} The PedsQL, Multidimensional Fatigue Scale was validated in children with SCD.⁵² In adults, the PROMIS Fatigue Short Form was examined in SCD and performed adequately, with the advantage that it is a shorter and more participant/patient-friendly measure than most against which it was validated. PROMIS measures overall, including fatigue, were sensitive to disease severity, though they explained less unique variance in SCD severity than ASCQ-ME. However, there is no SCD-specific fatigue measure included in ASCQ-ME⁴⁷ because the PROMIS fatigue measures were validated in field studies. To assess fatigue, the PROs panel recommends using PROMIS or PedsQL, Fatigue measures in children and the PROMIS Fatigue measure in adults.

Measuring PROs related to function

The PROs panel identified social, physical, and cognitive functioning, occupational functioning, and self-efficacy as primary functional PROs associated with SCD.

Emotional/social, physical, cognitive domains. Both the PROMIS measures and the PedsQL measures have been shown to be valid and reliable. Currently, there are limited published data on PROs over time in patients with SCD. There are data supporting responsiveness of PedsQL, broadly in children with acute painful crises,^{53,54} and its broad sensitivity to SCD disease severity,^{50,55-57} but no additional longitudinal data have been found using PROMIS in adults or children.

Of note, the brain outcomes panel (see "End points for assessing brain outcomes in SCD") identified The Canadian Occupational Performance Measure (COPM) as a valid functional outcome measure for the age range 6 to 65 years, designed for use by

occupational therapists to assess client outcomes in the areas of self-care, functional mobility, productivity, and leisure.⁵⁸ Using a semistructured interview, the COPM is a 5-step process that measures individual, client-identified problem areas in daily function. Two scores for performance and satisfaction with performance are obtained. The measure is sensitive to change from an intervention. It requires clients to self-report problem areas in daily life but is not entirely patient-reported. A clinically significant change is 2 points from time 1 to time 2.⁵⁹ Participants may change their perspective on priorities of daily function over a prolonged period of time.

The PROs panel recommends using the relevant domains of the PROMIS and ASCQ-ME in adults, and the PROMIS and PedsQL in children, to measure emotional/social, physical, and cognitive function PRO domains. The COPM may be used as an outcome measure for functional capacity that integrates self-report and expert interview.

Occupational status assessment. Assessment of functional outcomes that includes occupational status is important and could be directly influenced by therapeutics or devices. Two occupational surveys were examined: (1) the World Health Organization (WHO) Health and Work Performance Questionnaire (HPQ) designed to assess indirect workplace cost secondary to health-related poor work performance and work absence and (2) a single question addressing current employment status that is part of the PhenX toolkit and publicly available for use. The group recommends using the 1-item PhenX question for assessment of employment status and the more detailed HPQ to assess multiple factors related to work performance.

Self-efficacy. Self-efficacy is the perceived ability to execute courses of action and behaviors to achieve desired goals, such as to care for oneself or manage an illness. Self-efficacy can potentially be improved by medical interventions, such as medications that increase patients' cognitive abilities or reduce symptom burden. Self-efficacy is 1 of the most potent predictors of behavior change and disease self-management, positively related to adherence to medication and treatment in general.⁶⁰ The Sickle Cell Self-Efficacy Scale (SCSES) is a measure of self-efficacy that has been validated in adolescents and adults with SCD.⁶¹ There is preliminary evidence that it is sensitive to interventions.^{62,63} Thus, the PRO panel recommends using the SCSES to measure self-efficacy in adolescents and adults with SCD.

Future directions in PROs in SCD

PROs allow the quantification of aspects of subjective experience, facilitating incorporation of subjective improvements into rigorous measures of treatment effect. It is likely that particular aspects of disease pathophysiology relate more specifically to some such experiences than others. One possible refinement of PROs is developing rational combinations of PROs (symptoms) and more objective measures (signs) that may then be linked to pathophysiology, thereby facilitating rational treatment development. Pain is an area ripe for such developments. Among other reasons, certain pain qualities have classically been linked to "neuropathic" as opposed to "nociceptive" mechanisms and some instruments have been developed that attempt to quantify the subjective correlates of these mechanisms.⁶⁴ Because of the causal complexity of the experience of pain and the relatively small effect sizes usually noted for

individual interventions, complexes of outcome measures that allow evaluation of additive or synergistic effects of targeting multiple mechanisms would be highly desirable.

Table 1 summarizes the PROs panel's recommended approaches to measuring PROs in patients with SCD.

End points for assessing pain in patients with SCD (non-PROs)

The experience of pain is always subjective and thus the gold standard of pain assessment is patient self-report, as described in the previous sections. Individuals may also experience pain in the absence of identifiable tissue damage or a known pathophysiological cause.

To accurately measure the pain experience of people with SCD and thereby measure its response to therapeutic interventions, multidimensional assessments are necessary.

Although the PROs panel focused on measures of pain intensity, interference, and pain behaviors, the non-PRO pain panel focused on measures of pain control and function, pain endophenotype, underlying pain mechanisms, and promising measures in animal models with potential for translation to humans.

Measures of pain control and function

A diverse set of measures of pain control and function were considered. These included health care utilization (HCU), analgesic use, missed days school/work, physical activity, and facial expression analysis.

HCU. HCU has been a standard surrogate end point for evaluating pain in SCD and is easily obtained from the medical record (unless care is sought at multiple sites). Total number of emergency department visits and hospitalizations, and where available, acute care clinic visits are the primary end points cited in the literature. These may be considered as proxies for pain.^{11,65-67} Additionally, hospital length of stay has been used to measure HCU. Given the current state of care in SCD, HCU may not be able to differentiate between acute and chronic pain and may be influenced by a number of other factors, such as mental health, socioeconomic status, and proximity to care. Ideally, acute HCU could serve as an end point to measure acute pain. However, given the difficulty in differentiating acute pain crises and exacerbations of chronic pain in SCD, it is likely that there is considerable mixing of the 2 phenomena, calling into question the accuracy of this end point. HCU may more accurately represent acute pain in children as they are less likely to have chronic pain, especially in the preteen age group.

Analgesic use. Analgesic use, or more typically opioid sparing, has been a common surrogate end point for pain trials whether SCD-related or not.^{7,15,68} This end point can be used in pharmacologic and nonpharmacologic trials. Given the prevalence of opioids for treating both acute and chronic pain in SCD, analgesic use could likely be used in acute and/or chronic pain. However, data on nonopioid medication and its efficacy in SCD are mixed; thus, it may be premature to consider use of other analgesics as an end point.

The number of opioid doses or total opioid dose can be used to measure acute pain; however, this may be affected by patient use of home opioids. Home opioid use could be tracked using an e-cap or electronic delivery system. Opioid delivery through patient-controlled analgesia may be the best metric for opioid use in the acute setting for hospitalized patients. Opioid utilization measured

Table 1. Recommended end points and measures for PROs in SCD

Outcome/End point	Recommended measurement/tool(s)
Pain	
Intensity	For patients at least 8 y of age, an 11-point NRS or appropriately reproduced and administered VAS
Interference/impact	PROMIS Pain Interference measures for studies of adult, pediatric, or mixed age groups ASCO-ME Pain Impact for adults or the Brief Pain Impact for adults PedsQL Pain Impact and Pain and Hurt for children
Behavior	PROMIS Pain Behavior domain for children and adults
Affect	
Depression/negative emotional impact	PedsQL SCD Module Emotions measure for children ASCO-ME Emotional Impact measure for adults PROMIS Depression and Anxiety measures for more general measures of negative affect, particularly when mixed age samples are used
Fatigue	PROMIS or PedsQL Fatigue measures in children PROMIS Fatigue measure in adults
Function	
Emotional/social, physical, cognitive domains	Relevant domains of the PROMIS and ASCO-ME in adults and PROMIS and PedsQL in children to measure emotional/social, physical, and cognitive function PRO domains The Canadian Occupational Performance Measure may be used to measure functional capacity that integrates self-report and expert interview
Occupational status	One item in PhenX for assessment of employment status and the more detailed WHO's Health and Work Performance Questionnaire to assess multiple factors related to work performance
Self-efficacy	Sickle Cell Efficiency Scale to measure self-efficacy in adolescents and adults with SCD

in morphine equivalent units would be most appropriate for measuring chronic pain.

Missed days at school or work. School attendance has been used as a surrogate end point in studies of adolescents with chronic pain and can be an objective measure of functional outcomes.^{69,70} Collected objectively, it can also be used to measure response to a multidisciplinary function-based therapy for chronic pain.⁷¹ However, both school and work attendance are influenced by multiple other psychosocial factors and there is limited evaluation of these measures in the SCD population.⁷² School or work records would be needed to make this an accurate non-PRO measure, requiring cooperation of schools and/or employers. Furthermore, the high rate of unemployment in adults with SCD may make work attendance less relevant for some patients.⁷³

Physical activity. Physical function measured by actigraphy has been used as a surrogate end point in studies of chronic pain.⁷⁴ A composite measure of pain and activity may be better than other measures to evaluate outcomes in individuals with bone/joint involvement. However, there is no available literature in SCD and physical activity measures do not inform much about pain mechanisms.

Facial expression analysis. Alterations in facial expressions in response to pain occur in animals and humans.^{75,76} Mouse grimace scale scores using facial images of sickle and control mice have been used.⁷⁷ In humans, several studies have demonstrated the validity of using facial expressions for quantifying pain.^{76,78,79} Pain can be quantified objectively from remote access using the subject's images communicated through cell phones or other media, which is most effective for pediatric settings and to assess patients remotely. However, this area requires significant research and development of algorithms to accurately quantify

pain without interference from other factors to eliminate false-positives or false-negatives.

Measures of pain endophenotype

Pain endophenotype appears to be an important contributor to pain experience. Preexisting vulnerabilities along with disease-related factors contribute to differences in pain-related peripheral and central nervous system (CNS) structure and function that leads to individual differences in pain endophenotype.⁸⁰ Quantitative sensory testing (QST) and functional neuroimaging with or without electrophysiological studies have been used in SCD and are the most commonly used methodologies.

QST. QST encompasses several psychophysical testing modalities to evaluate the sensitivity of the somatosensory system. Sensitivity assessed includes both stimuli detection and pain produced by the stimuli. Stimuli include thermal (heat, cold) and mechanical (pressure) modalities.^{68,81} QST is a psychophysical tool that allows for the assessment of alterations, differences, or variations in pain sensitivity between patients and controls or within a patient over time or between disease states (ie, baseline vs acute pain). These differences can suggest altered pain processing at the level of the peripheral and/or CNS.

It has been used to assess patients during their baseline state of health and in both acute and chronic pain states. It has been validated in children as young as 7 years of age. QST has been used as an indirect end point in children and adults with SCD⁸²⁻⁸⁶ and in other conditions with pain.⁸⁷⁻⁸⁹ A variety of QST protocols exist that attempt to differentiate alterations in peripheral vs central pain pathways; however, it can be difficult to determine clear-cut differences.

Multiple examples in which QST has been used as an outcome/end point in clinical trials have been published.⁹⁰⁻⁹⁹ To date, the majority of QST data for patients with SCD are derived from descriptive

studies. There are no published data in which QST has been used as an end point in a clinical trial targeted at pain treatment. Studies have correlated QST outcomes with fetal hemoglobin levels,¹⁰⁰ evaluated the reactivity of cytokines in patients with SCD after the induction of experimental pain using QST,¹⁰¹ and evaluated the vasoconstriction response to the application of heat pain.¹⁰² All studies have been conducted in patients during their baseline “healthy” state or immediately following an acute painful event.^{82-86,103,104} Murine studies have also been conducted using similar QST assessments and show similar findings to results in humans with increased heat, cold, and mechanical sensitivity in SCD mice as compared with control mice.¹⁰⁵⁻¹⁰⁷

Several challenges exist when operationalizing QST as an end point in a clinical trial. These include: (1) costly equipment; (2) time-intensive protocols; (3) variability in methods across sites; (4) the fact that it is not an assessment of pain intensity or morbidity from pain and cannot replace PROs of pain and function; (5) lack of clarity of the clinically meaningful QST threshold or change in QST threshold/outcomes¹⁰²; (6) outcomes that are associated with psychological comorbidities; and (7) lack of longitudinal data for QST assessments in patients with SCD.

Imaging and electrophysiological studies. Brain imaging and electrophysiological studies such as functional magnetic resonance imaging (MRI; fMRI), scalp electroencephalography (EEG), and positron emission tomography have been used to delineate cerebral signatures of pain and analgesia. Other imaging-based measures such as diffusion tensor imaging, spectroscopy, and brain volumetric imaging are also being used to understand changes in brain related to pain, especially chronic pain. Experience in SCD pain with these methodologies has been limited and relatively recent.

Imaging and electrophysiological studies have improved our understanding of the pathways involved in pain perception and modulation.^{108,109} Some features such as connectivity patterns have been used to identify patients with certain pain endophenotypes and some of these specific end points have been used as end points for drugs in managing pain in non-SCD conditions.^{96,110-112} Pilot studies and comparative studies have been completed in SCD comparing SCD patients with healthy controls.¹¹³ Associations between clinical pain burden and evoked pain have been shown in pilot studies.^{114,115} Although these approaches can objectively assess pain processing at the central level, nociceptive stimuli trigger a variety of responses that are part of the multidimensional experience of pain; thus many, if not all, features of brain activity that have been associated with pain are nonspecific. On the basis of current brain-imaging techniques, pain cannot be quantified although efforts are under way to further decode this information.^{116,117}

EEG and magnetoencephalography are direct and noninvasive measures of brain function and can provide novel insight into nociceptive pathways. EEG has been used to assess the evoked potential in response to a brief stimulus, which yields typical responses that mainly originate from somatosensory, insular, and cingulate cortices. Changes in the nociceptive pathways affect the amplitudes of these responses and thus could serve as a clinically useful measure of the integrity of nociceptive pathways to the brain.¹¹⁸⁻¹²⁰ The most commonly used EEG approach to pain has been the assessment of evoked potentials in responses to brief noxious stimuli of milliseconds in duration. This approach yields

a typical sequence of response and changes in the amplitude of these responses suggest damage to nociceptive pathways.¹²¹ Studies have assessed anticipation-evoked potential in patients with fibromyalgia and osteoarthritis.¹²²

Furthermore, in some diseases with chronic pain (eg, fibromyalgia, chronic back pain), a disinhibition or lack of habituation of evoked responses to a noxious stimulus can be seen.^{123,124} A study simultaneously recording fMRI and EEG showed that SCD patients had increased activity in pain-processing regions.^{125,126} The major strength of these methodologies is high temporal resolution in the range of milliseconds. Thus, these methodologies complement imaging methods, such as fMRI, which has temporal resolution on the order of 1 to 2 seconds. However, a major challenge for EEG in pain research is limited understanding of the processes by which sensory and contextual information translate into pain experience.

Measures of underlying pain mechanisms: pain/ vaso-occlusive crisis biomarkers

Given the subjective nature of pain and the difficulty distinguishing between acute and chronic pain, laboratory-based, objective, and quantitative biomarkers for acute VOCs are urgently needed. These would be especially useful for clinical trials and genomic studies.

To identify possible biomarkers for VOCs, the biomarkers panel conducted a PubMed query for “biomarker,” “pain,” and “sickle cell” including any articles in which potential biomarkers were assessed in human subjects with SCD during an acute pain crisis. Potential biomarkers assessed in patients with SCD at steady state with high and low rates of pain events were excluded. No biomarkers had been validated. Although some laboratory values and genetic modifiers are associated with higher or lower incidence of pain events in patients with SCD, such as fetal hemoglobin levels and coinheritance of α thalassemia,¹²⁷ these do not change during a pain event.

Some biomarkers linked to known aspects of SCD pathophysiology may be increased during a pain event: elevated C-reactive protein¹²⁸ or substance P (SP)¹²⁹ as markers of inflammation; microparticles¹³⁰ or cell-free DNA¹³¹ as markers of increased tissue infarction; or plasma-free heme,¹³² plasma arginine,^{133,134} and exhaled nitric oxide¹³⁵ as markers of hemolysis. Nitric oxide, endothelial progenitor cells,¹³⁶ soluble VCAM,¹³⁷ and vascular endothelial growth factor^{138,139} may also capture vascular damage and adhesion aspects of a pain crisis. None of these have been shown to be clinically useful or are suitable as important end points in studies of pain.

Other biomarkers address red cell or blood rheological abnormalities,¹⁴⁰⁻¹⁴⁴ such as deformability or whole-blood viscosity. Emerging technology also allows the measurement of red cell deformability under a range of oxygen concentrations, although as yet there is nothing to suggest that changes in this deformability are in some way related to pain. Dense cells are another related value, but the relationship between the percentage of dense red blood cells and pain is inconsistent, showing different results at different points in the pain cycle,^{140,145-149} possibly due to destruction of dense cells during a pain event. Measuring only 1 aspect of rheology can be misleading, for example, the senicapoc trial,¹⁵⁰ dense cells were reduced but pain events increased, likely due to increases in whole-blood viscosity. A more global picture

using a microfluidic system may provide multiple measures of blood rheology simultaneously: adhesion, deformability, and viscosity,^{151,152} although there is no evidence as yet that microfluidic measurements are useful biomarkers.

Another challenge is the clinical variability of SCD patients.¹⁵³ Some patients have increased baseline hemolysis,^{154,155} inflammation, or abnormal rheology. These values also vary by genotype, with hemoglobin SC disease (HbSC) patients exhibiting higher whole-blood viscosity than most homozygous SCD (HbSS) patients, and differing in some fundamental aspects of pathophysiology.¹⁵⁵ Although challenging, longitudinal monitoring of values for each patient to determine their steady-state values may allow for identification of significant worsening, predictive or diagnostic of an acute pain event.

In summary, although there is significant literature and potential for biomarker discovery in an acute VOC, further research is needed to validate a biomarker for this complication and bring it to the bedside.

Measures of underlying pain mechanisms: circulating biomarkers. Circulating biomarkers and their potential association with pain should be assessed with regard to their measurability, sensitivity, specificity, and reproducibility. The biomarkers panel considered the value of mast cell-associated biomarkers and leukocyte-associated biomarkers.

Mast cell activation has been associated with both acute and chronic pain in sickle mice.¹⁵⁶ Tryptase (a marker of mast cell activation) is elevated in the blood of sickle mice and SCD patients with pain.^{156,157} Mast cells reside in tissues, cohabiting with nerves and vasculature, promoting direct neurovascular and neuroinflammatory interactions.¹⁵⁸⁻¹⁶⁰ Inhibition of mast cells with imatinib significantly reduced circulating SP and calcitonin gene-related peptide (CGRP) and release of several cytokines from the skin of BERK sickle mice. *c-kit*/mast cell inhibitor imatinib also prevented VOCs in patients with SCD in 2 separate case reports.^{161,162} Mast cell activation markers, tryptase and chymase, and substances released from mast cells, SP, CGRP, histamine, interleukin 6, monocyte chemoattractant protein 1, tumor necrosis factor α , granulocyte-macrophage colony-stimulating factor, and RANTES may have potential as biomarkers for SCD pain.

Activation of neutrophils in sickle mice and patients with SCD has been reported.^{163,164} Release of neutrophil extracellular traps containing elastase, histones, and nucleosomes/DNA has been observed in BERK sickle mice and patients with SCD.¹⁶²⁻¹⁶⁶ Elastase, a proteolytic enzyme released from activated leukocytes mediates inflammation in the peripheral tissues and the dorsal root ganglion, was demonstrated to contribute to neuropathic pain in rodent models.^{167,168} Significantly increased circulating free DNA by quantitative polymerase chain reaction has been demonstrated in SCD patients in VOC compared with steady state.¹³¹ Therefore, circulating elastase, nucleosomal DNA, and A1AT may be biomarkers of chronic and acute pain in SCD.

Physiological measures of pain-sensing neurons in pain pathways. Primary sensory neurons convey stimuli about touch and pain to the CNS. Electrophysiological-recording studies have shown that both nociceptive (C fiber and A δ fiber) and low threshold tactile (A β fiber) cutaneous primary afferent neurons in sickle mice exhibit significantly increased ongoing "spontaneous" activity, and increased responses to mechanical stimuli and cold

stimuli applied to the skin.^{106,107,169} Cellular patch-clamp and calcium-imaging studies have shown that the somata of sensory neurons also show sensitization to mechanical and cold stimuli.^{106,107} These data indicate that in the murine model of SCD, multiple types of sensory afferent neurons exhibit spontaneous activity, which is a correlate of spontaneous pain behavior that is also reported by patients with SCD.

Electrophysiological recordings indicate action: potential firing in sensory neurons. Patch-clamp studies measure ion channel function in sensory neurons. Calcium-imaging studies indirectly show activation of the sensory neurons via increased free intracellular calcium levels. Electrophysiological recordings of C fibers have been performed in humans via microneurography¹⁷⁰ in complex regional pain syndrome,¹⁷¹ erythromelalgia-like symptoms,^{172,173} and other neuropathies. Microneurography has been suggested for potential use as a biomarker for pain, and these recordings could be performed in patients with SCD.

Molecules that may drive activation and/or sensitization of peripheral or central neurons within the pain pathway in SCD. Other potential mechanisms that may drive or contribute to acute or chronic pain in SCD have been demonstrated in murine sickle models but remain to be validated in SCD patients. These include neurogenic inflammatory mediators including: CGRP, tachykinin receptor 1, endothelin 1, endothelin type A receptor, and Nav1.8 channels¹⁷⁴; chemo chemokine receptor-5 and μ opioid receptors¹⁷⁵; ion channels including transient receptor potential vanilloid 1¹⁰⁶; and chemokine ligand 2 receptors,¹⁷⁶ Ca/calmodulin-dependent protein kinase II,¹⁷⁷ protein kinase C δ ,¹⁷⁸ and cannabinoid receptors.¹⁷⁹ None have yet been validated in human studies.

Skin biopsies. Skin biopsy has provided information on the activation of vascular, inflammatory, and neural systems in BERK sickle mice.¹⁵⁶ Skin biopsies could be performed in patients with SCD to examine potential pain markers and mechanisms such as elevation of neurogenic markers (eg, CGRP and SP), and elevation of receptors for endothelin 1, chemo chemokine receptor-2, and transient receptor potential vanilloid 1, and signaling molecules Ca/calmodulin-dependent protein kinase II α and protein kinase C δ . A limitation of this approach is the risk of impaired skin healing in patients with SCD.

Grip force strength. Grip force strength can be measured as a nonevoked quantitative measure and has been used to assess sustained muscle pain in experimental muscle hyperalgesia in healthy subjects induced by nerve growth factor¹⁸⁰; however, weak muscle performance, myalgia, and motivation of the patient may influence outcomes.

Summary of pain recommendations

Table 2 presents the pain panel's recommendations for prioritization, recognizing that the gold standard for pain assessment is patient report. End points proposed here must be used in conjunction with PROs of pain severity and interference as composite end points.

End points for assessing brain outcomes in SCD

The brain is very vulnerable to injury in SCD. Prior to the Stroke Prevention Trial in Sickle Cell Anemia (STOP) trial, ~10% of children with SCD experienced an overt stroke.¹⁸¹ Approximately

Table 2. Priority end points and measures for pain (non-PRO) in SCD

Tier	Prioritization	Domains	End point/outcome	Direct/indirect	Pros	Cons
I	End points currently being used and recommended by the panel	Measure of pain control/function	Health care utilization: ED/hospitalization Length of stay Analgesic use (opioid) Missing work/school Activity/function	Indirect	Experience with its use in SCD and other pain conditions	Can be affected by many other factors ¹
II	End points with experience in SCD in pipeline and available for use	Measures of pain endophenotype	QST Functional neuroimaging	Indirect Not an assessment of pain High potential for being secondary or correlative end point	Experience with its use in SCD and other pain conditions	Cost Expertise Evoked stimuli (QST) Operator dependence (QST) Possible impact of SCD biology (imaging)
		Measures indicating mechanisms: circulating biomarkers/rheology	Variable based on underlying mechanism (eg, selectins) and proposed interventions	Indirect Select based on the mechanism/target	Useful for targeted therapies	Need validation with clinical improvement
III	Promising end points in animal model/need further exploration	Measures indicating mechanism/function/degree of pain	Grip force	Indirect	Can be translated to humans for further investigation	Needs further validation with clinical pain
			Facial expression analysis	Direct		
			Skin biopsy	Indirect/pathological changes		
			Circulating biomarkers	Indirect/mechanism based		

QST, quantitative sensory testing.

40% of teens and 50% of 30-year-old adults with SCD experienced a silent cerebral infarct (SCI).^{182,183} STOP was a randomized trial to evaluate whether chronic transfusion could prevent initial stroke in children with sickle cell anemia at high risk as determined by transcranial Doppler (TCD). In addition to stroke, cognitive deficits are common in SCD patients,¹⁸³⁻¹⁸⁵ as are educational challenges.¹⁸⁶⁻¹⁸⁹ In considering new therapeutic options, efforts are critically needed to ameliorate the burden of SCD on the CNS. The brain panel identified 4 categories of end points and measures that can be used to assess brain outcomes in SCD patients: TCD, MRI, cognition, and education attainment. In addition, the biomarkers panel identified key neurological biomarkers for consideration.

TCD

TCD is used to assess the middle cerebral artery (MCA) and internal carotid artery (ICA) by determining the highest time averaged mean of the maximum velocity (TAMMV), a measure used to stratify patients as to stroke risk. Prevention of the first stroke (primary prevention) was made possible through the demonstration in the United States and Europe that high TAMMV found through TCD predicted stroke in HbSS and HbS/ β^0 thalassemia and that chronic transfusion prevented stroke. TCD results were classified as normal (<170 cm/s), conditional (≥ 170 to <200 cm/s), abnormal (≥ 200 cm/s on 2 occasions) or inadequate based on TAMMV readings in specific arterial segments. Those with abnormal TCDs received either monthly blood transfusions or no transfusions, and those randomized to transfusion had a much better outcome in terms of stroke (1 stroke, vs 10 in the control group) and also fewer other medical problems while transfused. TCD screening is recommended for children with HbSS and HbS/ β^0 thalassemia beginning at 24 months of age and repeated every 12 months through 16 years of age.¹⁹⁰

The suggested threshold for treatment should be based on TAMMV (not peak systolic velocity) and, using nonimaging TCD techniques, is TAMMV ≥ 200 cm/s whereas for imaging the equivalent is time-averaged mean maximum velocity ≥ 185 cm/s. Abnormal TCD is defined as 2 TCD measurements > 200 cm/s or a single measure of > 220 cm/s using the nonimaging technique, and 2 > 185 cm/sec or 1 > 205 cm/s using the imaging technique.

Measurements should be done at the terminal portions of ICA and the proximal portion of MCA. The predictive values of the TCD measurements in the other intracranial arteries has not been rigorously addressed and should not be used to stratify into high- and low-risk groups for future strokes. The greatest benefit would be to determine whether a participant decreased from the abnormal (meeting the threshold for transfusion) to the normal range. However, in children receiving therapy, blood transfusion therapy, or observation, the rate of strokes is too low (<1 event per 100 patient-years¹⁹⁰) to determine whether persistent abnormal TCD velocities or normal TCD velocities after therapy are associated with higher or lower incidence rates of strokes, respectively. There are no data to suggest that simply decreasing the TAMMV from a normal to slightly lower velocity is an added benefit.

In children receiving regular blood transfusion therapy with a goal to keep maximum hemoglobin S level <30% or hydroxyurea at maximum tolerated dose for abnormal TCD measurements, there are limited data suggesting that TCD measurements after starting therapy predict strokes.¹⁹¹ Interpretation of change in TCD measurements after starting treatment should be done cautiously because an absence of a decline in TCD measurements after starting therapy does not translate into an absence of a treatment benefit¹⁹¹ and may be indicative of stenosis of the interrogated arterial vessel that will not improve with therapy. In STOP, for all patients who had stroke including those who had

Table 3. Recommended anatomic measures for MRI of brain in SCD

3 Tesla MRI method: anatomical (basic)	Outcome measure	Rationale	Duration, min
2D T2w FLAIR (2 planes: axial and coronal) or 3D T2w FLAIR (reconstructed to 3 orthogonal planes)	1. Infarct (count) 2. White matter lesion (count) 3. Alternative pathology (Dx)	Evaluate presence of prior and new overt strokes or silent cerebral and cerebellar infarcts (SCIs); prior SCI is a risk factor for future SCI	5-7 (cumulative)
3D T1w MPRAGE	1. Infarct (count) 2. Tissue volume (volume; mm ³)	Required with FLAIR to characterize infarct (FLAIR hyperintense, T1 hypointense); progressive tissue atrophy may be associated with cognitive decline	5
2D T2w	1. Infarct (count) 2. Lesion (count)	Adds clarity for temporal lobe lesion identification	3

An adjudication committee is strongly recommended for imaging outcomes.

2D, 2-dimensional; Dx, diagnosis; MPRAGE, Magnetization Prepared-Rapid Gradient Echo.

stroke after starting transfusion, the last evaluable TCD was abnormal in all cases, suggesting that response to transfusion may be less effective long-term with advanced disease and persistent abnormal TCD.¹⁹²

Limitations include ultrasonographer variability, a large coefficient of variation of TCD measurement in the same child with HbSS measured only 3 hours apart (7.6% to 12%), and the high standard deviation of the TCD measurement in children with HbSS.^{193,194}

MRI of the brain

Given the high prevalence of SCI, at least 35% and 50% in children and adults with HbSS, and evidence that SCIs are associated with infarct recurrence, detection of SCI is an important component of care. MRI of the brain and a neurological examination are the only means to detect an SCI. Some individuals with an SCI may elect not to be treated because of the relative high number of treatments (13) needed to prevent 1 cerebral infarct recurrence.¹⁹⁵ Furthermore, many providers will not even screen for SCIs because of the high prevalence and the perception that the benefits of the transfusions do not outweigh the risks. However, based on the high prevalence, the association with cognitive morbidity, and the increased risk of future neurological recurrence, individuals at risk should be informed of their risk.

Table 3 presents recommended anatomical MRI measures available now to assess the number of infarcts/lesions. Of note, the definition of an SCI is based on work by DeBaun and colleagues in the Silent Infarct Transfusion Trial: an infarct-like lesion was defined as an MRI signal abnormality that was at least 3 mm in 1 dimension and that was visible in 2 planes on fluid-attenuated inversion recovery (FLAIR) T2-weighted images, as determined by agreement of 2 of the 3 study neuroradiologists.¹⁹⁵ The members of a neurology committee adjudicated a lesion as an SCI if the study participant had either a normal neurologic examination or an abnormality on examination that could not be explained by the location of the brain lesion or lesions. An enlarged SCI was defined as a previously identified SCI that increased by at least 3 mm along any linear dimension in any plane on MRI. In clinical trials, an adjudication process is needed to objectively confirm neurologic and imaging findings. As is the standard in all National Institutes of Health stroke trials, an adjudication committee is needed to objectively confirm neurologic and CNS-imaging findings. In the Silent Cerebral

Infarct Multi-Center Clinical Trial, ~7% of all children believed to have SCI actually had strokes when evaluated by a local pediatric neurologist and later reviewed by a panel of pediatric neurologists.¹⁹⁵

The neuroimaging recommendations represent the technological evolution in imaging. The recommendation to establish an infarct as a 3-mm dimension in 2 planes or 3-dimensional (3D) T2w FLAIR (reconstructed to 3 orthogonal planes) is currently in use by a neurology-hematology consortium and multiple clinical centers with expertise in neurological complications of SCD. However, the 3D T2w FLAIR definition has not been validated in a clinical trial setting. Although there is interest in hemometabolic measures and diffusion tensor imaging, these measures would benefit from additional research to validate the specific measures in order to quantify change.¹⁹⁶ Table 4 suggests additional imaging measures requiring additional research.

Cognition

The cerebral infarcts associated with SCD most commonly occur in the frontal, temporal, and parietal lobes. Lesions in these areas are associated with cognitive deficits in executive function, processing speed, working memory, and attention. These are all measures of fluid cognition and are associated with abilities to organize one's health care, succeed in school, and maintain a job. These cognitive deficits exist with or without a history of cerebral infarcts.

Table 5 presents recommended global and domain-specific measures of cognition based on the age of the person being assessed. These measures were based on those recommended by the PhenX panel¹⁹⁷ and previous SCD clinical trials.

Based on previous studies, the brain panel also recommends that the following 3 types of measures be completed to interpret cognition:

1. A measure of the home or social environment,¹⁹⁸ such as the Home Observation for Measurement of the Environment (HOME), a semistructured interview and observation tool for assessing parent-child interaction as well as the quantity and quality of stimuli present in the home environment. The HOME has been shown to be a reliable tool that can screen for developmental delay and is predictive of later academic achievement.
2. The head of household's level of educational attainment, which is also significantly related to a child's cognition.

Table 4. Additional MRI measures of brain in SCD requiring further research as potential end points in clinical trials

3 Tesla MRI method	Outcome measure	Rationale	Duration, min
Head time-of-flight magnetic resonance angiography	1. Vasculopathy (percent; categorical) 2. Associated pathology (eg, moya-moya)	Noninvasive alternative to head CTA/DSA; categorical grading (use 0-4) ²⁰⁵	5
Neck time-of-flight magnetic resonance angiography	1. Vasculopathy (percent; categorical)	Noninvasive alternative to neck CTA; presence of cervical vasculopathy extent remains debated in SCD	6
Diffusion tensor imaging	1. White matter structural connectivity 2. Tract-based spatial statistics 3. Fractional anisotropy, mean diffusivity, etc	Fiber tracking and related parameters (anisotropy, diffusivity) may indicate white matter damage and describe symptomatology	6
Susceptibility weighted imaging	1. Microbleeds (count; volume) 2. Quantitative susceptibility (iron) 3. Venous density	Characterize microvascular disease and iron deposition	4
Diffusion-weighted imaging if acute CNS event	1. Acute infarct (count)	Inform presence of recent infarcts	1
MR venography if acute CNS event	Thrombosis, stenosis	Unlikely to be abnormal in asymptomatic	
Hemometabolic			
Arterial spin labeling	1. Regional cerebral blood flow (mL/100g/min)	Inform extent of hypo- or hyperperfusion; hypoperfusion indicative of tissue-level impairment from vasculopathy; hyperperfusion marker of how well parenchyma is responding to anemia and reduced blood delivery; may also provide indicator of arterial-venous shunting	4
T2-relaxation-under-spin-tagging	1. OEF (ratio of oxygen consumed to oxygen delivered) 2. CMRO ₂ ; mL O ₂ /100 g/min; requires CBF measurement	Inform extent to which total oxygen delivery is meeting requirements; elevated OEF may be indicator of new or recurrent infarct; reduced CMRO ₂ may indicate suppressed neuronal activity and new lesion risk	2
Phase contrast angiography (head and neck)	1. Quantitative velocity assessment of major intracranial (eg, first segment MCA) and cervical vessels (ICA, BA) (mm/s)	Allows for whole-brain CBF assessment (with tissue volume information), which is not possible with arterial spin labeling; evaluate elevated flow velocity (provide comparison for TCD)	
Blood oxygenation level-dependent or arterial spin labeling cerebrovascular reactivity (requires respiratory stimulus such as hypercapnic or IV/oral vasodilatory stimulus such as acetazolamide)	1. Cerebrovascular reactivity, an indicator of microvascular reserve capacity (signal change)	Cerebrovascular reserve will be exhausted when CBF can no longer increase to compensate for anemia and/or vasculopathy	8

An adjudication committee is strongly recommended for imaging outcomes. Vasculopathy is a surrogate marker and difficult to measure as an outcome. BA, basilar artery; CBF, cerebral blood flow; CMRO₂, cerebral metabolic rate of O₂ consumption; CTA, computed tomographic angiography; DSA, digital subtraction angiography; MR, magnetic resonance; OEF, oxygen extraction fraction.

3. Recording of average daily morphine equivalent dose, based on a meta-analysis that found association of deficits with chronic opioid use,¹⁹⁹ to include verbal working memory, cognitive impulsivity (risk-taking), and cognitive flexibility (verbal fluency).

Educational attainment

A child's primary occupation is to attend school. Complications from SCD result in children missing, on average, 15 to 22 days of school per year.¹⁸⁷ New therapies could be considered successful if children were able to attend more school days. For adults, the process of attending more days of work would also be a positive change. Higher levels of educational attainment are associated with better health and greater wealth. The brain panel recommends that the following questions be asked to assess short-term benefit over the course of 1 school year:

For missed school days, how many were due to (a) scheduled (medical appointments) and (b) unpredictable hospitalizations?

For a longer-term study, the following example questions have been used in BABY HUG and the Silent Cerebral Infarct Transfusion (SIT) trial to assess educational outcomes in the United States:

- a. What is your child's current grade?
- b. Has your child ever been held back or repeated a grade?
 - i. If yes, how many grades? (1, 2, 3, or more)
- c. Does your child have any accommodations because of learning differences?
- d. Check all that apply
 - i. Special Education Services
 - ii. 504 plan
 - iii. IEP-individualized education plan
 - iv. Special tutoring or classes not available to regular students
 - v. Other
 1. Describe: _____
 - vi. My child does not receive any accommodation for learning differences

As a measure of educational attainment for adolescents and adults, questions can be asked about highest-grade level completed,

Table 5. Global and domain-specific measures to assess cognitive skills in SCD

Measure	Age range	Domains	Additional considerations
Global measures			
Bayley-III	0-3.5 y	Cognitive, motor, language, social-emotional, and adaptive behavior	
WPPSI-IV	2 y, 6 mo to 7 y, 7 mo	Working memory, processing speed, fluid reasoning, and visual, spatial, and verbal comprehension	Consider WPPSI cancellation (attention/processing speed)
WISC-V	6-16 y 11 mo	Verbal comprehension, processing speed, visual spatial, working memory, and fluid reasoning	Consider WPPSI cancellation (attention/processing speed)
Domain-specific measures			
Trails A and B; Trail Making Test, Part A	9 y and older	Executive function	D-KEFS tower Wisconsin card sorting
Children's Memory Scale	5-16 y	Working memory	
The Digit Span Test (Forward and Backward)	16 y and older	Working memory	Part of the WAIS ²³
NIH Toolbox DCCS Test	3-85 y	Executive function–cognitive flexibility	Takes 4 min
NIH Toolbox Flanker Inhibitory Control and Attention Test	3-85 y	Executive function and attention	Takes 3 min
NIH Toolbox Pattern Comparison Processing Speed Test	7-85 y	Processing speed	Takes 3 min

These tests have not been demonstrated to be superior to several other well-recognized age-specific tests for people with SCD in national and international assessments.

Bayley-III, Bayley Scales of Infant and Toddler Development, Third Edition; DCCS, Dimensional Change Card Sort Test; D-KEFS, Delis-Kaplan Executive Function System; NIH, National Institutes of Health; WAIS, Wechsler Adult Intelligence Scale; WISC-V, Wechsler Intelligence Scale for Children Fifth Edition; WPPSI-IV, Wechsler Preschool and Primary Scale of Intelligence Fourth Edition.

graduation status from high school, and dropout from high school. These measures would be used to assess changes over at least 1 year to balance the variation in seasons of weather and longer-term benefit. With global studies, regional differences in educational systems will necessitate different measures of educational attainment.

Neurological biomarkers

Biomarkers for neurological disorders are potentially valuable for early identification of children at increased risk of life-changing complication and to allow aggressive disease-modifying treatments as appropriate. Additionally, some neurological biomarkers are likely to be valuable as surrogate end points for therapeutic trials.

As with many complications in SCD, the genotype underlying SCD is particularly important in determining the risk of cerebrovascular disease, with overt cerebral infarction, SCI, and vasculopathy all significantly more common in HbSS than HbSC disease, and probably the many different types of HbS/thalassemia.¹⁸¹ Nearly all biomarker studies have been undertaken on patients with HbSS, with relatively little known about HbSC disease. Similarly, nearly all studies have been undertaken in Europe and the United States, with little known about predicting severity in Africa where the disease is most common.

Biomarkers for overt infarctive stroke. As described in "TCD," the measurement of TCD velocities in children with SCD (HbSS and HbS β^0 thalassemia) between the ages of 2 and 16 years is 1 of the most established biomarkers in SCD in general. TCD velocities have also been used as end points in clinical trials, most notably in a comparison of blood transfusion and hydroxyurea.²⁰⁰ Other established biomarkers of overt stroke include low hemoglobin, high systolic blood pressure, and low overnight oxygen saturation. Numerous studies have tried to identify genetic biomarkers associated with increased risk of overt stroke, including

genome-wide association studies, although no consistent, validated markers emerge other than the absence of thalassemia. Two genetic markers in tumor necrosis factor and ENPP1 genes have been confirmed in >1 study, although the effect of each of these markers appears to be small and they have not yet been clinically useful.²⁰¹ Other future biomarkers for stroke in a pediatric SCD population include circulating plasma levels of soluble receptor for advanced glycation end products²⁰² and CD34⁺ hematopoietic stem cell counts (sensitivity, 53%; specificity, 84% for a threshold of 8675 cells per milliliter).²⁰³

Biomarkers for SCI. As referenced previously, SCIs are identified by brain MRI, which is difficult and expensive to perform, particularly in young children who require general anesthesia for the procedure. Reliable biomarkers would therefore be particularly useful, although none are well established either in clinical trials or practice. Low hemoglobin, particularly in young children, is associated with increased risk of SCI, and 1 study suggested that regular transfusion reduces the risk of further SCIs developing.¹⁹⁵ Other identified biomarkers include increased systolic blood pressure, male sex, and extracranial stenosis of the internal carotid artery. Future biomarkers for SCI in children with SCD have also been reported in circulating plasma via a proteomic analysis, identifying proteins involved in hypercoagulability (α 2-antiplasmin, fibrinogen- γ chain, thrombospondin-4), inflammation (α 2-macroglobulin, complement C1s and C3), and atherosclerosis (apolipoprotein B-100) as well as higher levels of gelsolin and retinol-binding protein 4 in a population with silent infarcts, both of which have been previously linked to stroke.²⁰⁴

SCD is associated with an increased risk of various other neurological conditions, including intracranial hemorrhage, seizures, cognitive impairment (reduced IQ and processing speed), retinopathy, deafness and psychiatric disorders although very little information is available on biomarkers for these complications.

Table 6. Recommended end points for brain outcomes in SCD trials

Timeframe for outcome assessment	Physiology	Imaging	Function
Immediate <1 d	TCD , cerebral blood flow, oxygen extraction fraction, cerebrovascular reactivity, MR velocity		
Short-term <1 y	TCD , cerebral blood flow, oxygen extraction fraction, cerebrovascular reactivity, MR velocity	Thrombosis, infarction	School or job absences, functional outcome measures (COPM)
1-2 y	TCD , cerebral blood flow, oxygen extraction fraction, cerebrovascular reactivity, MR velocity	Thrombosis, infarction	School or job absences, cognition, functional outcome measures
Long-term 3 y or more	TCD , cerebral blood flow, oxygen extraction fraction, cerebrovascular reactivity, MR velocity	Thrombosis, infarction, iron deposition, white matter structural connectivity	School or job absences, cognition, functional outcome measures
Irreversible/degenerative		Stroke, SCI, microbleeds , atrophy, iron deposition, white matter structural connectivity, stenosis, vasculopathy including moyo-moya	Held back a grade level in school, cognition, functional outcome measures

Outcomes in bold can be measured with standard measures. As a reminder, for TCD, the greatest benefit would be to determine whether a participant decreased from the abnormal (meeting the threshold for transfusion) to the normal range. There are no data to suggest that simply decreasing the TAMMV from a normal to slightly lower velocity is an added benefit. COPM, Canadian Occupational Performance Measure.

Summary of brain outcome measures

Table 6 provides a summary of the brain panel's recommended end points to consider in SCD disease.

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 recommendations, the exact clinical context must be considered before clinical trial end point adoption.

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This article summarizes topics addressed at the US Food and Drug Administration (FDA)–American Society of Hematology (ASH) Sickle Cell Disease Clinical Endpoints 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 articles that present the findings of the panels. The Contribution section details how the authors were involved in the development of the actual manuscripts. Kathi E. Hanna, the contracted science writer, provided summaries based on discussions at the

workshop and initial summaries submitted by panels; prepared drafts of the manuscripts; managed review of the papers; and prepared the manuscript 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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Authorship

Contribution: A.T.F. and J.P. were both chairs, wrote the introductory and concluding manuscript sections, consolidated all sections of the manuscript, and revised the overall manuscript based on feedback from all other authors; C.P.C., D.S.D., A.A.D., and A.A.K. were panel chairs, wrote the first draft of their respective manuscript sections, and revised the manuscript based on feedback received by additional authors (panelists); R.J.A., T.D.B., A.M.B., M.R.D., M.J.D., K.G., J.S.H., M.K., F.J.K., H.L., S.M., P.A.O., D.C.R., R.S., V.A.S., J.S., C.L.S., E.M.W., J.C.W., and W.T.Z. critically reviewed the manuscript and provided input based on their expertise and panel topic; and all authors contributed to content development and subsequent recommendations and have approved the manuscript and corresponding tables.

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Correspondence: Julie Panepinto, Medical College of Wisconsin, 8701 Watertown Plank Rd, MS 756, Milwaukee, WI 53226; e-mail: jpanepin@mcw.edu.

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