CURE SICKLE CELL.

CureSCi CDE Project
Genetics and Assays Working Group

The goals of the CureSCi CDE project are to support the NIH roadmap and address challenges of varied data collection standards and difficulties in comparing between studies and poor definitions around the specific data elements collected. CDEs are recommended by the NIH Strategic Plan for Data Science improving data quality, facilitating collection of data, data-sharing and comparison and reducing study start-up time and overall study cost.

The group first worked on ranking various CDEs (listed below) and then discussed methodology and protocol or instrument recommendations. The scope for this group focused on the following modules:

1. Genetic diagnostic testing for sickle cell
2. RBC, hemolysis, and, erythropoiesis
    a) Complete blood count, LDH
    b) Iron measurements
    c) Rheological/Morphological/Sickling assays
    d) Red cell lifespan span assays
3. Gene therapy specific assays
    a) More focused on VCN and editing but some overlap with monitoring side effects WG
    b) Includes recommendations on release testing to drug product
4. Other Biomarkers of interest:
    a) Inflammation-ischemia reperfusion assays
    b) Vasculopathy/endothelial activation assays
    c) Molecular assays (proteomics/epigenetics/metabolomics)
5. GMP-ready assessments

The members self-assign module(s) within your primary and secondary area of expertise and worked independently and then during smaller meeting to rank based on the following:

Ranking:
Core: Question/instrument/data element that collects essential/required information applicable to any sickle cell disease genetics study. It is anticipated that investigators will need to collect the disease Core CDEs. Only a few elements should be listed as Core. Team will consider availability/accessibility of tests as well as usefulness/feasibility for long-term follow up
Supplemental-Highly Recommended: This classification may be needed for important elements that are not required in all sickle cell disease genetics study.
Supplemental: A data element which is commonly collected in clinical research studies. Use depends upon the study design, protocol or type of research involved. These are recommended, but not required, for studies.
Exploratory: A data element that requires further validation but may fill current gaps in the questions/instruments (data elements) and/or substitute for an existing element once validation is complete. Such data elements show great promise but require further validation before they are ready

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for prime-time use in clinical research studies. They are reasonable to use with the understanding that it has limited validation in the target group.

The group discussed questions such as:

1. What is needed at baseline?
2. Should we have a field to designate if a parameter is related to therapy, disease or other complications?
3. Grading biologic cure

****Patient Advocates Only** (These questions are pending response but were asked in each working group from patient/advocate/caregiver.)**

1. What role did people with SCD/advocates/caregivers play in the drafting of recommendations?

2. How did the subgroup consider and factor in the experiences of people with SCD in making recommendations?

3. What consideration was given to the burden and/or acceptability of instruments to people with SCD (and family when applicable)?

4. Was the subgroup able to make recommendations that capture the diversity and complexity of the clinical presentations of SCD?

5. Can you identify specific needs or gaps? (e.g., specific need for an instrument to assess pain that is easier for patients to complete)

Summary recommendations (Full list on following document)
List of Select CDEs:

CDE	Definitions	Reference	Classifications	Do we have it?
Complete blood count with differentials	Quantification of major cellular blood components	Nivaggioni, V, Bouriche, L, Coito, S, et al. Use of Sysmex XN-10 red blood cell parameters for screening of hereditary red blood cell diseases and iron deficiency anaemia. Int J Lab Hematol. 2020; 00: 1– 8. https://doi.org/10.1111/ijlh.13278 Brown, W., Keeney, M. and Hedley, B.D. (2014), Initial performance evaluation of the UniCel® DxH slide maker/stainer Coulter® cellular analysis system. Int. Jnl. Lab. Hem., 36: 172-183. doi:10.1111/ijlh.12150 ERMENS, A.A.M., HOFFMANN, J.J.M.L., KROCKENBERGER, M. and Van WIJK, E.M. (2012), New erythrocyte and reticulocyte parameters on CELL-DYN	Core	Yes-Phenx

CDE	Definitions	Reference	Classifications	Do we have it?
		Sapphire: analytical and preanalytical aspects. International Journal of Laboratory Hematology, 34: 274-282. doi:10.1111/j.1751-553X.2011.01391.x		
Reticulocyte Absolute Count	Number of new red blood cells in production	Mauro Buttarello, MD, Pietro Bulian, MD, Giorgio Farina, MD, Valeria Temporin, MD, Lucia Toffolo, MD, Ernesto Trabuio, MD, Paolo Rizzotti, MD, Flow Cytometric Reticulocyte Counting: Parallel Evaluation of Five Fully Automated Analyzers: An NCCLS-ICSH Approach, American Journal of Clinical Pathology, Volume 115, Issue 1, January 2001, Pages 100–111, https://doi.org/10.1309/M26B-1YNQ-VNU8-M1CE	Core	Yes-Phenx
Reticulocyte %	% of erythrocytes that are reticulocytes	Phenx	Core	Yes-Phenx
Hemoglobin characterization	Proportion of globin chains in a whole blood sample	Phenx	Core	Yes-Phenx
Determination of the proportion of RBCs expressing non-βS globin chains	Proportion of individual RBCs expressing fetal hemoblobin or engineered globin chains	Hebert, N, Rakotoson, MG, Bodivit, G, et al. Individual red blood cell fetal hemoglobin quantification allows to determine protective thresholds in sickle cell disease. Am J Hematol. 2020; 1– 11. https://doi.org/10.1002/ajh.25937	Core	Gene Product CRF
LDH	Crude marker of cell turnover	Phenx	Core	Yes-Phenx
Total bilirubin		Phenx	Core	Yes-Phenx
Direct bilirubin		Phenx	Core	Yes-Phenx
Soluble transferrin receptor		Alan E Mast, Morey A Blinder, Ann M Gronowski, Cara Chumley, Mitchell G Scott, Clinical utility of the soluble transferrin receptor and comparison with serum ferritin in several populations, Clinical Chemistry, Volume 44, Issue 1, 1 January 1998, Pages 45–51, https://doi.org/10.1093/clinchem/44.1.45 Ilenia Infusino, Federica Braga, Alberto Dolci, MD, Mauro Panteghini, MD, Soluble Transferrin Receptor (sTfR) and sTfR/log Ferritin Index for the Diagnosis of Iron-Deficiency Anemia A Meta-Analysis, American	Supplemental	

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CDE	Definitions	Reference	Classifications	Do we have it?
		Journal of Clinical Pathology, Volume 138, Issue 5, November 2012, Pages 642–649, https://doi.org/10.1309/AJCP16NTXZLZFAIB		
D-dimers		Int J Hematol. 2013 Aug;98(2):158-63. doi: 10.1007/s12185-013-1392-y.; J Lab Clin Med . 1999 Oct;134(4):352-62.	Supplemental-Highly Recommended	Need
CRP		Phenx	Core	Yes-Phenx
Immunologic Reconstitution: Lymphocyte Mitogen, Antigen Screen	Measurement of human lymphocytes' proliferative responses to various stimuli is a fundamental technique used to assess their biological status and functions. Mitogens, such as plant lectins phytohemagglutinin (PHA), concanavalin A (Con A) and pokeweed mitogen (PWM), are able to nonspecifically stimulate lymphocyte proliferation and used to evaluate patient immune responsiveness. Lymphocyte proliferation response to antigens, such as Candida, tetanus toxoid and tuberculin purified protein derivative (PPD), are evaluated as a function of memory in cell-mediated immunity.	Balandya E, et al 2016	Supplemental	Need
Immunologic Reconstitution: Immunoglobulins Panels	serum IgG, IgA, IgM level	Balandya E, et al 2016	Core	Immune Reconstitution Core
Immunologic Reconstitution: Tetanus Titers	specific IgG to tetanous toxoid vaccine	Balandya E, et al 2016	Supplemental	
Immunologic Reconstitution: S. Typhi Titers	specific IgG to typhoid Virulence Antigen Polysaccharide accine	Bausch-Jurken MT, Verbsky JW, Gonzaga KA, Elms NP, Hintermeyer MK, Gauld SB, Routes JM. The Use of Salmonella Typhim Vaccine to Diagnose Antibody Deficiency. J Clin Immunol. 2017 Jul;37(5):427-433. doi:	Supplemental	

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CDE	Definitions	Reference	Classifications	Do we have it?
		10.1007/s10875-017-0406-6. Epub 2017 Jun 7. PMID: 28589420.		
Immunologic Reconstitution: Pneumococcal Titers	specific IgGs to nonconjugated 23-valent and conjugated 13-valent vaccine	Balandya E, et al 2016	Supplemental	
VCN, INDELS or percentage of correction in peripheral blood	Bulk VCN/Editing/Correction in a whole blood sample		Core	Gene Product CRF
Proportion of transduced, edited or corrected cells in peripheral blood	Single cell assessment of transduction/editing/correction		Core	Gene Product CRF
Purity	Proportion of CD34+ cells (of CD45+)	https://www.fda.gov/media/113760/download	Core	Drug Product CRF
Identity	Confirm/quantify production of globin chain of interest	https://www.fda.gov/media/113760/download	Core	Drug Product CRF
VCN, INDELS or Correction		https://www.fda.gov/media/113760/download	Core	Drug Product CRF
off-target editing frequency		https://www.fda.gov/media/113760/download	Core	Drug Product CRF
CFC Assay		https://www.fda.gov/media/113760/download	Core	Need
Potency Assay	Additional drug product potency assay to measure the "intended biological effect" as to be determined by sponsor based on MOA of product	https://www.fda.gov/media/79856/download	Core	Drug Product CRF

CDE	Definitions	Reference	Classifications	Do we have it?
Laboratory Procedure Hemoglobin Analysis Method Type for all types of Hb	The method use for hemoglobin analysis.	https://www.aphl.org/aboutAPHL/publications/Documents/NBS_HemoglobinopathyTesting_122015.pdf	Core	Phenx
Laboratory Procedure Hemoglobin Genetic Modified A Variant Result Percentage Value		https://www.aphl.org/aboutAPHL/publications/Documents/NBS_HemoglobinopathyTesting_122015.pdf	Core	Gene Product CRF

Exploratory assays not included:
Adhesion, Rheologic assays in exploratory CRF