Effect Of Whole Genome Sequencing On The Clinical Management Of Acutely Ill Infants With Suspected Genetic Disease: the NICUSeq Randomized Time-Delayed Trial

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Clinical Study Protocol: NICU-R001

Document Title: NICUSeq: A Prospective Trial to Evaluate the Clinical Utility of Human Whole Genome Sequencing (WGS) Compared to Standard of Care in Acute Care Neonates and Infants

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PROTOCOL APPROVAL FORM

Document Title: NICUSeq: A Prospective Trial to Evaluate the Clinical Utility of Human Whole Genome Sequencing (WGS) Compared to Standard of Care in Acute Care Neonates and Infants

Protocol Number: NICU-R001

Version: 1.1

Effective Date: May 16, 2017

Approval Signatures:

Function	Name	Signature	Date
Clinical Affairs	Kimmy Dimalanta		
Biostatistics /Data Management	Jennifer Scott (Agility Clinical)		
Regulatory Affairs	Dave Kern		
Medical Monitor	John Belmont		
Scientific Research	Ryan Taft		

TABLE OF CONTENTS

1. PROTOCOL SYNOPSIS

Sponsor	Illumina, Inc.
Protocol Title	NICUSeq: A Prospective Trial to Evaluate the Clinical Utility of Human Whole Genome Sequencing (WGS) Compared to Standard of Care in Acute Care Neonates and Infants
Protocol Number	NICU-R001
Primary Objective	Evaluate the clinical utility of cWGS compared to standard of care testing in an acutely ill neonates or infants ("proband") in intensive care units who are suspected to have a genetic condition.
Study Design	Prospective, multi-site, study to evaluate the clinical utility of cWGS in a proband. One group will receive cWGS and a clinical report approximately 15 days after blood samples are received, while the other group will continue to receive standard of care until Day 60. The standard of care group will receive cWGS and a clinical report at Day 60 as part of secondary and tertiary analyses. Both groups will be followed for a total of 90 days.
Study Population	An evaluable proband (minimum of 300) and their biological parent(s) will be enrolled.
Primary Endpoint	A difference in Change of Management between the 15 day cWGS and standard of care groups at Day 60. Change of Management is a binary (yes or no) based on assignments made by the PI or designee at each site using the following domains: • Condition specific management • Condition specific supportive interventions • Palliative care/End of Life Care A change in any of these domains will be considered a change of management.
Study Duration	The study enrollment period will last up to 12 months per participating site.
Study Setting	Each proband will be recruited from participating medical centers in the United States.

2. LIST OF TERMS AND ABBREVIATIONS

Abbreviation	Term
ACMG	American College of Medical Genetics and Genomics
CLIA	Clinical Laboratory Improvement Amendments
CAP	College of American Pathologists
CM	Change of Management between the 15 day cWGS and standard of care groups. Change of Management is a binary (yes or no) assignment made by the PI
cWGS	Clinical whole genome sequencing
CNV	Copy number variants are structural gene variants that manifest as deletions or duplications
eCRF	Electronic case report forms
ICF	Informed Consent Form. A formal agreement that an individual signs to give permission to participate in research
ICSL	Illumina Clinical Services Laboratory
IRB	Institutional Review Board
NICU/ICU	Neonatal Intensive Care Unit/Intensive Care Unit
Participant	A biological parent or affected family member that is enrolled in this study (up to a maximum of three participants per proband)
PI	Principal Investigator. Qualified person responsible for conducting the clinical investigation at an investigation site
Proband	A neonate or infant affected with a disorder who is enrolled in this study
SAP	A Statistical Analysis Plan defines analysis guidelines for the study
SIN	Study Identification Number. A unique SIN generated by the study database that is assigned to each proband and participant enrolled in the study
SNV	A single nucleotide variant is a DNA sequence in which the purine or pyrimidine base of a single nucleotide has been replaced by another base.
SOC	Standard of Care - the management of the proband's care under the same or similar conditions as if the proband was not enrolled in this study
VUS	Variant of uncertain significance is a variation in genetic sequence whose association with disease risk is unknown
WES	Whole exome sequencing

3. INVESTIGATOR AGREEMENT

To be signed prior to the initiation of testing.

Document Title:	NICUSeq: A Prospective Controlled Trial to Evaluate the Clinical Utility of Human Whole Genome Sequencing (WGS) Compared to Standard of Care in Neonates and Infants
Protocol Number:	NICU-R001
Version:	1.1
Effective Date:	May 16, 2017

I have read, understood and agree to conduct and supervise the activities described in the NICU-R001 protocol titled, "NICUSeq: A Prospective Trial to Evaluate the Clinical Utility of Human Whole Genome Sequencing (WGS) Compared to Standard of Care in Acute Care Neonates and Infants".

I will ensure that all individuals to whom I delegate study-related responsibility are qualified and fully informed regarding the protocol and applicable study-related procedures.

I agree to maintain adequate and accurate study records, and to make these records available for inspection in accordance with state and federal regulations. I agree to adhere to FDA, local, and state regulations, as applicable.

_____ _____

Signature of Principal Investigator Date

_____

Name of Principal Investigator

4. INTRODUCTION

Background

Genetic disorders are one of the leading causes of neonate mortality in the United States [1], and affected children disproportionally account for the utilization of health care resources and costs [2, 3]. Despite the fact that early and accurate diagnosis is imperative for appropriate care, children with a genetic disorder will remain undiagnosed for an average of 7 years in the United States [4]. Current standard of care (SOC) is a testing odyssey that can include laboratory and imaging tests and, pertinent to this study, chromosome microarray testing and serial single gene sequencing. These approaches have cumulative diagnostic yields less than 10% [5]. Diagnostic yields from whole exome sequencing (WES) have varied from 17.5% [Posey 2016] to 31% [Retterer 2016] with differences based on age of the patients, clinical indication, and availability of parental samples. Whole genome sequencing (WGS) is a single platform test that enables testing across genetic disorders, including those traditionally detected by single gene, exome and chromosomal microarray testing.

Multiple independent studies support WGS diagnostic yields that are a minimum 2-fold higher than current SOC. For example, Gilissen et al. and Taylor et al. have recently reported diagnostic yields of 62% and 57% using WGS trio analysis, respectively, in neurodevelopmental disorder populations [1, 2]. Additionally, four recent reports have shown that WGS has improved exonic coverage, sensitivity, specificity, allele ratio calls and a systematic reduction in assay bias compared to whole exome sequencing (WES) [3-6]. WGS therefore captures the advantages of WES while performing better on medically relevant exons, and utilizing a workflow which can be performed without amplification or sequencing bias.

To date there has not yet been a systematic evaluation of the clinical utility of WGS to assess how receiving molecular diagnostic results across, theoretically, the vast majority of known human genetic conditions impacts the patient and the treatment plan. Several smaller studies have demonstrated pronounced clinical impact in patients with early-onset neurological and neurodevelopmental presentations who received whole exome sequencing [8-11]. For example, Tarailo-Graovac et al. [10] reported on a cohort of 47 children with biochemical and neurological

phenotypes assessed by exome sequencing. A diagnosis was obtained in 28 of 41 neonates (68%), 44% of which had a change in treatment beyond genetic counseling. The American College of Medical Genetics and Genomics (ACMG) has released a document stating that clinical utility in the context of genetic testing should take into account effects on diagnostic or therapeutic management, implications for prognosis, health and psychological benefits to patients and their relatives, and economic impact on health-care systems [7];a comprehensive assessment of WGS clinical utility, however, has not yet been completed

The NICU-R001 study described here will evaluate the clinical utility of cWGS compared to standard of care in acutely ill neonates and infants in intensive care units who are suspected to have a genetic condition. This study will assess utility measures, with diagnostic yield versus SOC as a secondary assessment metric. This study will also evaluate costs before and after cWGS results are reported, and personal utility for the parents (e.g., allowing for precise genetic counseling or improved estimates of recurrence risk) and the perceptions of clinicians.

5. DEVICE DESCRIPTION

The TruGenome Undiagnosed Disease Test ("TruGenome Test") is intended to provide information to physicians to aid in the diagnosis of inherited diseases with high penetrance (Mendelian disorders). The analysis and interpretation are designed to detect and report on single nucleotide variants (SNVs), small insertion/deletion events and copy number variants that impact genes that have established association to genetic disease [as found in the national Genetic Testing Registry (http://www.ncbi.nlm.nih.gov/gtr) and Online Mendelian Inheritance in Man (http://www.ncbi.nlm.nih.gov/omim)]. The analysis is typically done as a family-based analysis (e.g. a "trio" of the proband and his or her biological parents), but may be performed on a proband only. Family-based analyses may be comprised of a duo (parent and child), trio, or other higher order family structure. The analysis considers inheritance patterns consistent with the reported family history. In addition, the analysis considers clinical presentation, family history and peer-reviewed literature to contextualize resulting variants from the analyses.

The TruGenome Test consists of the following components:

- Consumable Kits
    - Paxgene or EDTA sample collection tubes
    - Test requisition form
- Analysis Software (the software version may change after validation studies are conducted)
    - ISAS version 6 – Variant calling and alignment
    - Nussetti version 2 – Variant filtering
    - Variant Interpretation Tool (VIT) version 3 – Variant curation and reporting
    - Wasabi version 3.2.3.41 – Project/sample/analysis management

Principles of the Test

The TruGenome Test detects the sequence of DNA fragments by using a DNA polymerase to catalyze the incorporation of fluorescently labeled deoxyribonucleotide triphosphates (dNTPs) into a DNA template strand during sequential cycles of DNA synthesis (sequencing-by-synthesis). The process is extended across millions of fragments in a massively parallel fashion. The TruGenome Test measures the fluorescence signals through the use of instrument specific reagents, flow cells, imaging hardware, and data analysis software.

6. STUDY OBJECTIVES

Primary Study Objective (Day 1 - 60 Measurement Period)

The primary objective is to evaluate Change of Management between 15 day cWGS and standard of care (SOC) groups at Day 60. Change of Management is a binary (yes or no) based on assignments made by the PI or designee at each site using the following domains:

- Condition specific management
- Condition specific supportive interventions
- Palliative care/End of Life Care

A change in any of these domains will be considered a change of management.

Secondary Study Objectives (Day 1 - 60 Measurement Period)

Data collected from the 15 Day cWGS and SOC groups will be used to evaluate:

- Diagnostic yield (# positive diagnoses/ total # of each proband expressed as a percentage)
- Diagnostic accuracy (percent positive agreement between test outcome classified by the medical monitor and the site PI or designee)
- % diagnoses returned before discharge or death
- Pre-test costs of hospital care by utilizing benchmark data (including biochemical tests, biomarker tests, gene sequencing, and DNA copy number testing, as applicable) and post-test costs of care when genomic data is integrated into the medical care plan.
- Average time (in days) to diagnose between cWGS and SOC based on the comparison of the (a) cWGS results and the (b) current clinical diagnoses informed by cWGS as designated by the PI.
- Clinical services utilization including laboratory and imaging tests, subspecialty consultations, care settings, length of stay, and discharge to home.
- Physician satisfaction and clinical utility
- Parent satisfaction and personal utility
- Change in care setting
- Time to diagnosis (in days of life)
- Length of stay

Tertiary Study Objectives (Exploratory)

Comparison of each proband with Positive and Negative test outcomes within cWGS and SOC with regard to:

- Change of management
- Condition-specific management or condition-specific supportive management
- Palliative care

Within subject (proband) comparison of SOC v cWGS for the relevant study group will be obtained with regard to:

- Diagnostic yield (within arm cWGS vs SOC)
- Change of Management

Descriptive statistics will be obtained for:

- Positive test outcome by allele/variant types
- Clinical services utilization and pre/post-test costs

7. STUDY DESIGN

Description

This is a prospective, multi-site, randomized study to evaluate the clinical utility of cWGS in each proband. Throughout this study, each proband will receive SOC testing as determined by the site clinical team. Upon enrollment in the study, each proband will be randomly assigned to the 15 day cWGS group or the SOC group. SOC is defined as the management of the proband's care under the same or similar conditions as if the proband was not enrolled in this study. A blood sample from each enrolled proband will be collected and shipped to the Illumina Clinical Services Laboratory ("ICSL"), which is Clinical Laboratory Improvement Amendments (CLIA)-certified and College of American Pathologists (CAP)-accredited. ICSL will conduct cWGS testing with the TruGenome Undiagnosed Disease Test ("TruGenome Test"). The TruGenome Test cWGS results will be provided to the Principal Investigator (PI) or designee who will evaluate each proband test outcome based on the aggregate medical information, informed by the cWGS or SOC results.

Study Population

This study will enroll a proband (minimum of 300 with at least one biological parent). One biological parent must provide a sample for each enrolled proband.

In addition to at least one biologic parent, other affected family members may be asked to participate by signing an informed consent form (ICF) and providing a blood sample.

Participant sequence data will be used for the interpretation and analysis of the proband's genetic data. Study/research data will not otherwise be collected on family members. The sample size justification may be found in Statistical Considerations.

Study Duration

The study will conclude for the proband at death or approximately ninety days following study enrollment.

Study Centers

Each proband will be recruited prospectively from qualified medical centers. All PIs and designated personnel recruiting each proband will be trained on the inclusion and exclusion criteria, study conduct and procedures by Illumina personnel or their designees.

Screening and Enrollment

Participating sites will screen each potential proband against the inclusion criteria and exclusion criteria. Each proband that meet the inclusion criteria and do not meet any exclusion criteria will be considered eligible for enrollment. Each eligible proband will have a genetic counseling before the consent process as described below as well as a post genetic counseling visit also described below.

Obtaining Informed Consent

Proband and participant (biological parents and other affected family members) informed consent will be documented by the use of a language-appropriate written ICF that has been approved by an Institutional Review Board (IRB). One parent will sign the ICF on behalf of the proband, the second parent's signature is not required. Each participant will sign an ICF prior to sample collection. The original signed and dated copies will be kept in a secure area at the clinical

site and will be available for verification by the Sponsor or Sponsor representative at any time. A copy of the signed and dated ICF will be provided for each proband and participant.

The informed consent discussion may be conducted by telephone according to the Site's institutional SOPs. Documentation of the process for each telephonic consent will be kept in a secure area at the clinical site and will be available for verification by the Sponsor or Sponsor representative at any time.

Assignment of Study Identification Number

Each proband and participant will be assigned a unique study identification number (SIN), which de-identifies the individual and contains no protected health information (PHI). The site number is assigned by the Sponsor, Illumina, Inc., and will be provided to the site once the site is selected. Each clinical site will maintain a screening and enrollment log that links the SIN to the proband and participant's PHI. The log will be stored in the investigator's secure study file.

Inclusion and Exclusion Criteria

Eligibility to take part in this study will be determined by whether the proband meets the inclusion criteria **AND** does not meet any of the exclusion criteria listed below. Each proband will be screened according to the inclusion and exclusion criteria. Each proband who meets the inclusion criteria and does not meet the exclusion criteria will be offered enrollment.

Proband Inclusion Criteria

1. Current admission in a Neonatal Intensive Care Unit/Intensive Care Unit at a participating clinical site at the time of enrollment from day of life 0 to 120 days
2. A suspected genetic etiology of disease, based on objective clinical findings or other phenotypic defects for which a genetic test would be considered
3. Must be able to have 1 - 1.25 ml tube of whole blood drawn for testing

4. One parent of the proband must be able to provide written informed consent
5. At least one biological parent must agree to participate and provide at least 4 ml of whole blood for testing

Proband Exclusion Criteria

1. Known non-genetic cause(s) of disease, disorder, or phenotypic defect
2. The phenotype is fully explained by complications of prematurity
3. Trisomy 13, 18 or 21 or Turner Syndrome is the likely diagnosis; such a proband will be eligible if a diagnostic karyotype is normal
4. Blood transfusion within 48 hours (each proband will be re-eligible 48 hours after the most recent transfusion)
5. The PI decides that the study is not in the best interest of the proband (for example, the neonate or infant is at a high risk of severe morbidity or mortality within the next 7 days and these risks could be mitigated by alternative testing). Subsequent eligibility for enrollment of each proband is at the discretion of the site PI.

After proband enrollment, biological parents and other affected family members will be screened according to the participant inclusion and exclusion criteria. Participants who meet the inclusion criteria and do not meet the exclusion criteria will be eligible for sample collection.

Each Proband Parent Participant Inclusion Criteria

1. Biological parent of the enrolled proband
2. Must be able to have 4 - 6 ml tubes of whole blood collected within 3 working days (Monday-Friday) of the proband
3. Must be able to provide informed consent as defined below

Each Proband Parent Participant Exclusion Criteria

1. Blood transfusion within 48 hours (participants will be re-eligible 48 hours after the most recent transfusion)

2. The PI decides that the study is not in the best interest of the participant

Other Affected Family Member Participant Inclusion Criteria

1. Must have objective clinical findings or other phenotypic defects that are consistent with those observed in the proband and for which a genetic test would be considered.
2. Must be able to have whole blood collected within 3 working days of the proband (1-1.25 ml < 2 years; 2 ml age 2-18 years; 4-6 ml ≥18 years).
3. Must able to provide written informed consent or parental consent and affected family member assent if applicable.

Other Affected Family Member Participant Exclusion Criteria

1. Not a biological relative of the enrolled proband
2. Affected family member has had a blood transfusion within 48 hours (participants will be re-eligible 48 hours after the most recent transfusion)
3. The PI decides that the study is not in the best interest of the participant

Group Assignment

Each proband will be randomly assigned to one of the two study groups by performing a 1:1 random assignment into cWGS or SOC group at each site. The cWGS group will receive a 15 Day WGS report while the SOC group will receive SOC testing until day 60 following enrollment according to the randomization scheme provided in a computer-generated randomization code by a biostatistician. Interactive Response Technology (IRT) will be utilized for randomization in this study.

VISIT PROCEDURES

The study visits are conducted at the sites as described in this section. Visit activities and timing are further detailed in **Figure 1** and **18. Appendix B – Schedule of Study Events** and **Appendix C – Visit Timing**.

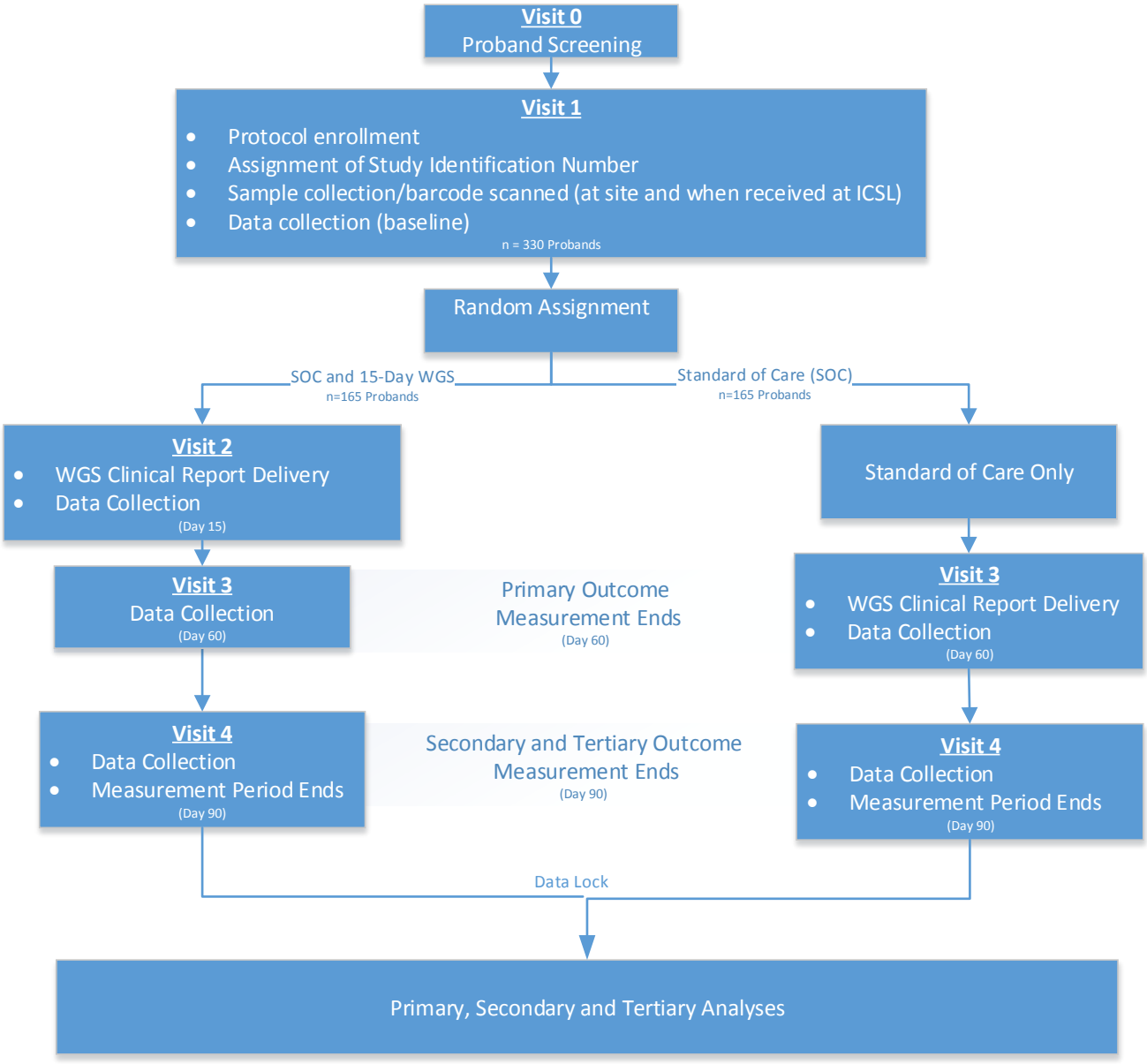

Figure 1: Description of cWGS and SOC groups

Visit 0 (Screening)

A member of the site study team or designee will identify each proband who meets the study criteria (see Inclusion and Exclusion Criteria). The study team member will contact the family to discuss the research project and ask if there is interest in learning more about the study. This process will be documented in the Study Screening and Enrollment Logs. A screening visit may be conducted if there is interest expressed by the family.

Visit 1 (Enrollment, Sample and Baseline Data Collection)

1. Following genetic counseling, the ICF will be reviewed with the proband's parent(s) and all questions and concerns will be addressed by the PI or designee. One parent will sign and date the ICF on behalf of their proband, the second parent's signature is not required. A copy of the signed and dated ICF will be provided for each proband.
2. In addition to the proband consent, each participant willing to provide a sample will sign their own ICF independently. A copy of the signed and dated ICF will be provided to each participant.
3. The PI or designee will assign a Study Identification Number (SIN) from the labels provided by the Sponsor and add the proband in the study database.
4. The IRT will randomly assign the proband 1:1 into the 15 day WGS group or SOC, stratified by site. The proband blood sample will be collected. A minimum of 1 – 1.25 ml EDTA or PaxGene whole blood tube will be needed for WGS testing. Sample collection and shipment details are discussed in Section 0. A test requisition form (provided by Illumina) will be completed by the PI or designee.
5. The participant blood sample(s) will be collected. EDTA or PaxGene whole blood tubes for each participant will be needed for WGS testing (1-1.25 ml < 2 years; 2 ml age 2-18 years; 4-6 ml ≥18 years). The participant blood samples may be collected during or immediately following Visit 1, but they must be received by ICSL within 3 business days of the proband's Visit 1. Sample collection and shipment details are discussed in COLLECTION AND SHIPMENT OF BLOOD SAMPLES.

6. The samples and completed test requisition form will be shipped to ICSL as discussed in COLLECTION AND SHIPMENT OF BLOOD SAMPLES.
7. The PI or designee will complete the Visit 1 electronic case report forms (eCRFs) in the study database.

Visit 2 (cWGS Results for 15 Day WGS Group Only)

1. The WGS results will be delivered to the PI for the 15 Day cWGS group ~15 days after samples are received by ICSL and meet quality metrics.
2. The PI or designee will review the cWGS results, assess the risk classification, plan further evaluation, if necessary, and provide an assessment of the results to the proband's family for the 15 Day cWGS group. Genetic counseling will also be provided during this time.
3. The PI or designee will complete the Visit 2 eCRFs in the study database.

Visit 3 (End of Measurement Period for **Primary Outcome**/cWGS Results for SOC Group)

1. This concludes the measurement period for the Primary Outcome for both the 15 day cWGS group and the SOC group
2. The cWGS results will be delivered to the PI for the SOC group ~60 days after samples are received by ICSL and meet quality metrics.
3. The PI or designee will review the cWGS results and then provide the results to the proband's family for the SOC group. Genetic counseling will also be provided during this time.
4. The PI or designee will complete the Visit 3 eCRFs in the study database.

Visit 4 (End of Measurement Period for **Secondary and Tertiary Outcomes**)

1. The visit timing is described in detail in Appendix C – Visit Timing.
2. The PI or designee will complete the Visit 4 eCRFs in the study database.
3. The PI or designee will contact the parents to complete a Parental Satisfaction Questionnaire.

Proband Withdrawal

Each proband may withdraw from the study at any time. If a proband withdraws prior to study completion, their data will not be included in the study analyses.

Each proband can also be withdrawn if:

1. Participant sample(s) not received in ICSL within three business days of proband sample.
2. Proband sample volume is below minimum requirement of 1.0 ml.
3. Parental sample(s) volume is below the minimum requirement of 4 ml (this only applies if one parental sample is received. If a second parental sample is received **and** it meets the volume requirements, then the proband is not withdrawn from the study).
4. Parental sample(s) are received but it is determined that they are not the proband's biological parent(s) (this only applies if one parental sample is received. If a second parental sample is received, then the proband is not withdrawn from the study).
5. Either at investigator's or the proband's legal guardian's request for any reason.
6. When the requirements of the protocol are not followed.

Once a proband is withdrawn from the study, they will continue to receive SOC at the site. Protocol withdraws and deviations will be reported in the annual report to the IRB.

8. cWGS Test Description and Methodology

Samples will be labelled with a SIN, date of birth, and barcode and will be shipped to ICSL. ICSL will conduct cWGS testing and deliver the results to the PI according to the TruGenome Test established laboratory procedures. The overview of cWGS testing and ICSL processes are described in

Figure 2.

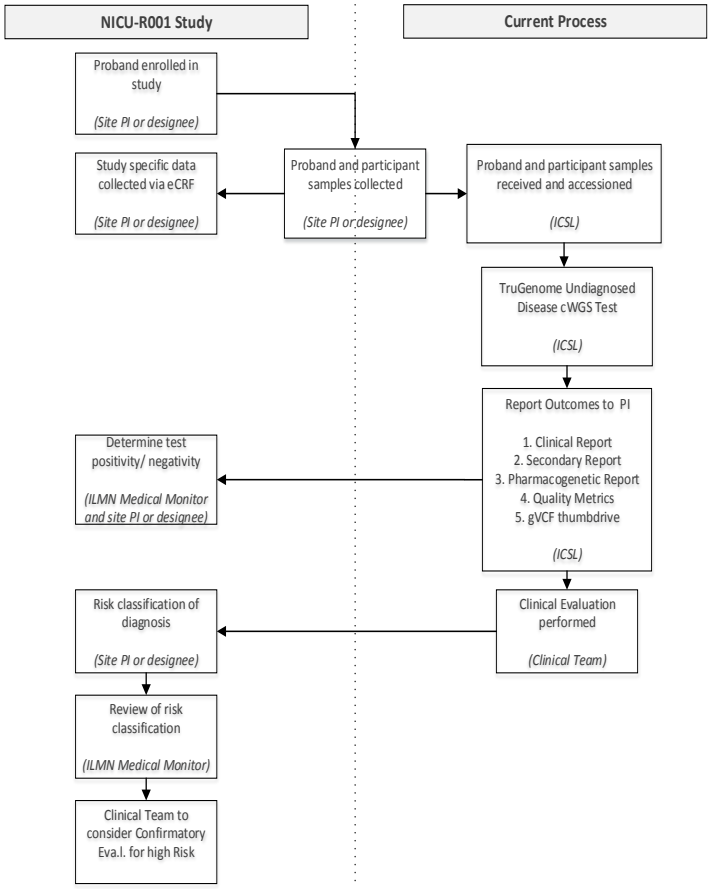

Figure 2: cWGS Testing Overview

Sample Receipt and Accessioning

Proband and participant samples will be shipped to ICSL and will be identified by the SIN, date of birth and bar code. ICSL will not accept proband or affected family member samples that contain less than 1 ml of whole blood and parental samples with less than 4 ml of whole blood. ICSL may request replacement samples if any fail quality criteria or <1.25 mL is received. A new SIN will not be provided for replacement samples.

cWGS testing will begin once acceptable samples have been received for the proband and at least one parent.

cWGS Testing

ICSL will conduct cWGS testing and deliver the results to the PI or designee according to the TruGenome Test established laboratory procedures. The TruGenome Test methodology, reporting, and limitations are described in Section 17. Appendix A - Test Description.

cWGS Reporting

The TruGenome Test results will be delivered to the PI at Day 15 for the cWGS group and Day 60 for the SOC group. The TruGenome results include the following, as described in section 17. Appendix A - Test Description.

1. A Clinical Report
2. A Secondary Findings Report
3. A Pharmacogenomics Report
4. Quality metrics
5. A password-protected BAM file recorded on electronic media (external USB solid state drive). The specifications of the BAM file is provided in the appended documents.

Participant specific cWGS results will not be delivered to the participants.

Test Outcome & Risk Classification

The PI or designee will provide the cWGS test result to the Clinical Team who will review the results then evaluate the findings based on the aggregate medical information, informed by the cWGS or SOC results. Once cWGS test results are received, the PI or designee will make a risk classification (high, moderate, or low risk) of the test result based on the potential clinical decision making and using the criteria contained in the Risk Classification eCRF. The PI risk classification will be recorded on the eCRF.

Confirmatory Evaluation

Following the risk classification, the Medical Monitor will review each classification and make a determination of "agree" or "disagree" on an eCRF. Determinations that "disagree" will be reevaluated by the PI or designee. Risk classification discrepancies between the PI or designee and medical monitor will be resolved by selecting the higher risk classification. Testing results that lead to a "high risk" designation will require a discussion of the risk classification between the PI and the clinical team for consideration of a confirmatory evaluation. The confirmatory evaluation can include additional functional or genetic testing at the discretion of the clinical team. Clinicians are advised to begin confirmatory testing with the lowest-risk assessment available (with potential escalation to other investigations as necessary) in accordance with best practices standard-of-care. The PI will utilize a Clinical Evaluation eCRF to capture confirmatory evaluation efforts for each proband with a high risk classification.

1. Report Review	The TrueGenome Undiagnosed Disease test results are returned to the principal investigator (PI).		
2. Risk Classification	**High**	**Moderate**	**Low**
	• Invasive laboratory, imaging or physiological testing especially procedures requiring sedation and anesthesia • Surgical or invasive procedure • Use of a specific therapeutic that has significant risk. This could include nutritional management, drugs, or biologicals • A determination of medical futility is made	• Use of specific therapeutic that has non-significant risk but no benefit • Use a disease specific supportive management that has no benefit	• Informational risk such as incorrect assessment of pattern of inheritance and risk of disease recurrence in future offspring • Unnecessary testing for close relatives such as for pre-symptomatic diagnosis and carrier testing • Psychosocial harms from genetic test results are non-significant for risk of physical injury but significant for life and well-being of the patient and close relatives
3. Mitigation	• PI's will advise the site clinical team of the Risk Classification as determined by the PI and Medical Monitor. PI's will utilize an eCRF to report each high risk evaluation.	No further confirmatory evaluation.	

Figure 3: Risk mitigation process and test result risk classifications

Determination of Test Outcomes

For the purposes of statistical analysis, the TruGenome results will be reviewed by the Medical Monitor to determine test outcome which can be Positive, Likely Positive, Inconclusive, or Negative.

The cWGS test will be considered Positive if a Pathogenic or Likely Pathogenic variant has been identified in the proband in a gene known to cause a human disease and there is clinical congruence. Congruence in this case is defined as: (1) variants in the gene are known to cause a single gene disorder; and (2) sufficient signs, symptoms and other objective clinical features are present in the proband as to be consistent with the suspected clinical diagnosis. A Negative result indicates that no variants were identified of likely relevance to the diagnostic indication.

ICSL will report a variant of uncertain significance (VUS) detected in an established disease-associated gene when there is clinical congruence with the suspected genetic disease, an allele frequency consistent with the known epidemiology of the suspected genetic disease (ACMG PM2), and one or more additional ACMG Moderate or Supporting evidence for pathogenicity. These include: (1) located in a mutational hot spot and/or critical and well-established functional domain (e.g., active site of an enzyme) without benign variation (ACMG PM1); (2) for recessive disorders, a VUS detected in trans with a pathogenic variant (ACMG PM3); (3) protein length changes as a result of in-frame deletions/insertions in a non-repeat region or stop-loss variants (ACMG PM4); (4) novel missense change at an amino acid residue where a different missense change determined to be pathogenic has been seen before (ACMG PM5); (5) assumed de novo, but without confirmation of paternity and maternity (ACMG PM6); (6) cosegregation of the variant (or variants in the case of suspected recessive inheritance) in a pattern consistent with the occurrence of the disease in other family members (ACMG PP1); (7) the VUS is predicted to cause an amino acid substitution in a protein domain either known to mediate the suspected disease, occurring in a gene with a low rate of benign missense variation and in which missense variants are a common mechanism of disease (ACMG PP2); (8) consistent algorithmic predictions of a deleterious effect (based on conservation, structure, etc.) due to a VUS (ACMG PP3); or (9) a

single heterozygous pathogenic or likely pathogenic variant is identified for a candidate recessive condition.

For the purposes of this research study cWGS will be considered **Likely Positive** when there is a VUS in a known disease gene, clinical congruence with the suspected genetic disease, an allele frequency consistent with the known epidemiology of the suspected genetic disease (ACMG PM2), and when one of the criteria from 1-6 above is present. If there is a VUS in a known disease gene, there is clinical congruence with the suspected genetic disease (ACMG PM2), and only a single criterion from 7-9 are met without other supporting evidence, for the purposes of this research, the test will be considered **Inconclusive**.

The medical monitor will communicate with the PI or designee to determine whether additional evidence supporting or refuting a specific molecular diagnosis has been obtained. Relevant post-test information could include observation of clinical course, confirmatory genetic or biochemical testing in the proband and/or other relatives, or other clinical testing. Such clinical information will be used to assess the diagnostic accuracy. Post hoc variant reclassification will be reported as a study outcome.

9. STATISTICAL CONSIDERATIONS

A formal statistical analysis plan (SAP) will provide full technical details of the statistical methods and analysis. The SAP will be finalized and approved prior to conducting any analyses. Details of any interim analyses will also be described in the SAP. Any changes to the SAP-specified planned analyses that are made after the database lock will be described in the clinical study report.

For the primary outcome, a $\chi 2$ test for independence will be used to compare change of management results between the rapid cWGS group and the SOC group at Day 60. In this analysis, each proband with any change of management will be compared regardless of the test results i.e., there is a proband with a change of management who has a negative or inconclusive genomic test. With a fixed total sample size of 300 at an alpha level of 0.05, there is 90% power

to detect a significant increase in proportion of cases with change of management when there is approximately a 20% improvement in diagnostic yield.

To compare the length of time to diagnosis, a survival analysis using a Kaplan-Meier estimator and/or a Cox proportional hazard model to account for the right censoring of the data (i.e., not all patients in the study will have reached final diagnosis during the study period) will be used. In all analyses, an alpha level of 0.05 will be used.

10. ETHICAL CONDUCT OF THE STUDY

Risk Determination

A risk determination was conducted by applying the study components to the FDA guidance on the IDE Determination and significant risk (SR)/non-significant risk (NSR) determination. The risk determination demonstrated that the study is exempt from the IDE regulation 21 CFR 812.2(c) and is also determined to be of non-significant risk with a direct potential for benefit for the proband.

Institutional Review Board

The protocol will be submitted to an IRB for approval prior to initiation of study conduct.

Potential Risks to Each Study Proband and Participant

The potential risks to each study proband and/ or participant are listed below:

1. Sample collection: Each proband and participant who provide a blood sample may have pain, bruising, lightheadedness, or, very rarely, infection at the blood draw site. There is also an increased risk of infection from accessing lines to obtain blood.
2. Psychosocial risks: There is potential risk in genetic testing for uncovering and conveying unwanted information regarding parentage or specific risk of disease. This potential risk is not specific to this protocol, but exists for any proband undergoing genetic testing.

Genetic results consistent with misattributed parentage will be reported confidentially to the PI but will not be placed in the laboratory report. Genetic sequences are inherently unique to an individual and there are, therefore, unique risks to privacy and confidentiality. If there is a breach in the database security, there is a small chance that someone could trace the information back to the proband.

3. Inherent test risks: There is a well-established framework through guidelines of professional organizations, including the American College of Medical Genetics and Genomics, for interpretation of genomics results. Even when these guidelines are followed, risks may include:
    - Sample identification error during sequencing, bioinformatics analysis, test interpretation and reporting. Use of laboratory best practices as defined by CLIA will minimize this risk
    - False positive and negative results due to errors in sequencing, variant calling and genotype inference
    - False positive and negative test interpretation due to errors in databases and published literature on the association between genes and diseases, as well as the analysis of said databases and literature
    - Results may not change the clinical diagnosis or affect clinical decisions

False negative results: If a false-negative result is given, the proband might not receive effective treatment (thereby missing benefits that treatment would confer), or might not be diagnosed with the correct disease or condition.

False positive results: If cWGS gives a false positive result, the proband might be exposed to risks associated with unnecessary additional procedures and tests, or potentially unnecessary treatment, as well as ramifications of falsely identifying a disease.

For risk mitigation see *Risk Classification* and *Confirmatory Evaluation*.

Benefits

Current SOC testing, which includes microarray, single gene, and targeted panel testing, yields less than 10% cumulative confirmed diagnoses of genetic diseases (diagnostic yield) [5]. Diagnostic yield from WES testing have varied from 17.5% [Posey 2016] to 31% [Retterer 2016] with differences based on age of the patients, clinical indication, and availability of parental samples. The cWGS testing conducted in this study may be used as an aid in the diagnosis for each proband, and there are data indicating that diagnostic yield can exceed 50% (Gilissen 2014, Taylor 2015), thereby improving the diagnostic yield as compared to SOC.

11. COLLECTION AND SHIPMENT OF BLOOD SAMPLES

Study Supplies

Illumina will provide required study identification numbers, blood collection tubes, shipping materials and study-specific documentation to the clinical site. The study site must provide all materials to collect blood samples (e.g., needles, prep pads etc.). The instructions and supplies provided will be adequate to help ensure that biologic samples are shipped in accordance with Federal regulations.

Blood Sample Collection

1. Blood samples for each proband and participant can be shipped Monday through Thursday. Blood samples collected on Thursday must be eligible for pick up for shipment on the same day.
2. Blood collection is performed by a qualified person at each participating site.
3. Whole blood collection tubes provided by Illumina will have a barcode label. Clinical sites will place the SIN (previously provided by the Sponsor) and date of birth on the tube once the participant is consented and prior to blood collection. Sites will perform a verification method of checking the tube label to ensure accuracy in the SIN and date of birth listed.

4. Sites will collect 1 - 1.25 ml of whole blood from each proband while affected family members and parents will have 1-1.25 ml < 2 years, 2 ml age 2-18 years and 4-6 ml ≥18 years collected. Samples should be collected by peripheral venipuncture or a pre-existing line according to each institution's guidelines into the blood collection tubes provided by Illumina.
5. Tubes containing collected blood should be shipped by the site the same day using shipping materials provided by Illumina and according to the shipping instructions provided by Illumina. Tubes must be maintained at ambient temperature.

Storage of Clinical Study Supplies

Blood sample collection kits should be stored at room temperature and in a secure location. Samples which have been frozen either before or during shipping will be not be accepted. The blood sample collection kits have expiration dates documented on the outside of the kit and on the blood collection tubes; these should be checked prior to use. Expired blood sample collection kits must be discarded.

12. STUDY CONDUCT

Good Clinical Practice, Regulatory Compliance, and Obligations of Investigators

The study shall be conducted in accordance with ICH E6 Guidelines, as well as the provisions specified in Title 21 Parts 11, 50, 54, 56, and 812 of the U.S. Code of Federal Regulations, and where applicable, all federal, provincial, state, and local laws applicable to the conduct of clinical studies [14]. The Investigator and all key personnel shall be thoroughly familiar with the regulations and agree to abide by them and the study protocol. Essential clinical documents shall be maintained to demonstrate the validity of the study and the integrity of the data collected. Participant files will be created at the beginning of the study, maintained for the duration of the study, and retained according to the appropriate law.

Confidentiality

All information obtained during the conduct of the study with respect to the participant's health status is protected from disclosure under applicable law and will be regarded as confidential, unless disclosure is authorized and executed in writing by the legally authorized representative or guardian (as applicable).

Inspection of Records

PIs and institutions involved in the study will permit study-related monitoring, audits, IRB review, and regulatory inspection(s) by providing direct access to all study records as well as all primary sources. In the event of a monitoring visit or audit, the PI and institutions will allow the Sponsor, representatives of the Sponsor, or representative of governmental regulatory agencies acting within their legal authority to have access to all study records and primary source documents.

Data Transfer

Data collected throughout the study will be performed using a safe and secure encrypted database maintained by lllumina and/or its CRO designee that is only accessible by designated users.

Data Collected

The following data will be collected using eCRFs:

- Visit 1 eCRFs: This entry collects baseline management data on the proband. The data collected will also include eligibility criteria and limited protected health information data to include the maternal and infant date of birth.
- Visit 2 eCRFs **(15 Day cWGS Only)**: Includes risk classification, risk verification and test outcome data. The risk classification is completed by the PI or designee while the risk verification and test outcome data is completed by the Medical Monitor. See section on Risk Classification.

- Visit 3 eCRFs: Includes risk classification, risk verification and test outcome data completed for the **SOC group only**. Management data is completed for both the 15 day cWGS and SOC groups to assess any change from the baseline collection.
- Visit 4 eCRFs: Includes management data to assess any change of management from the prior collection periods as well as test outcome data is completed by the PI or designee. Physician satisfaction and clinical utility data is collected. Parent satisfaction data is also collected in the form of a questionnaire which is distributed to an email address that is collected from the parent at the time of the proband's consent. The questionnaire may be administered telephonically in the absence of an email address in the study database. The email entry is only visible to the site who enrolled the proband.

Data Storage

All information collected by the study team or designee will be coded with a SIN and entered into the study database. cWGS data will be stored in compliance with the ICSL standard operating procedure on data storage unless written withdrawal is requested by the proband(s), explicitly asking to be removed from the study.

Source documentation

The following documents will be considered source documents:

- Screening and enrollment log
- Signed and dated ICFs
- cWGS test results
- Proband medical records or redacted reports

Database Submission

Deidentified and curated variant and limited phenotype information will be submitted to ClinVar. ClinVar is a freely accessible, public archive of reports of the relationships among human

variations and phenotypes hosted by the National Center for Biotechnology Information (NCBI) and funded by Intramural National Institutes of Health (NIH) funding. No personal health information (PHI) or information identifying the participant or family will be submitted.

Final Disposition of Samples

After testing has been completed, any leftover study samples will be discarded as soon as 30 days after testing, per the ICSL SOP.

Bias Avoidance

Bias will be minimized by performing a 1:1 random assignment into cWGS or SOC group at each site.

Device and Reagent Accountability

A reagent accountability log will be maintained by the ICSL lab.

General Test System Labeling

The test kits will be labeled in accordance with regulations applicable to investigational use of an IVD device in the US. The clinical report will be labeled with the following statement "CAUTION Investigational device. Limited by Federal (or United States) law to investigational use. Confirmatory Evaluation of high risk cases should begin with the lowest-risk assessment available in accordance with best practices standard of care."

13. SAFETY ASSESSMENTS

General

As this is a data interpretation study, there is a non-significant risk as per the definition of significant risk (21 CFR 812. 3 (m)) with a potential direct benefit to the proband. Study participation does not introduce new significant physical risks to the participants and there is no guarantee that any of the further analyses will lead to additional variants detected.

Adverse Event Reporting

An adverse event (AE) is any unanticipated, untoward, undesired, or unplanned event in the form of signs, symptoms, disease, or laboratory or physiologic observations occurring in a person given a test article or in a clinical study. The event does not need to be related to the test article or clinical study; if the event occurs during the study, it shall be handled as an AE.

An AE includes, but is not limited to, the following:

- Adverse psychological reaction to unexpected familial identification of variants that are predictive of a disease; or
- A serious adverse event (SAE) is an adverse event that:
    - led to death,
    - led to serious deterioration in the health of the proband, that either resulted in
    - a life-threatening illness or injury, or
    - a permanent impairment of a body structure or a body function, or
    - in-patient or prolonged hospitalization, or
    - medical or surgical intervention to prevent life-threatening illness or injury or permanent impairment to a body structure or a body function,
- led to fetal distress, fetal death or a congenital abnormality or birth defect

PI's are asked to notify the Sponsor in the event that any AEs or SAEs occur. Unanticipated, unexpected related SAE's should be reported to the Sponsor within 24 hours of occurrence.

The PI will notify the IRB or designated committee of the IRB of all SAE's in accordance with policy guidelines. An SAE will be followed by the PI until either resolved or stabilized. A summary of AE and SAE's will be included in the annual report to the IRB.

This study does not involve the use of a drug or clinical treatment. It is unlikely that an SAE will occur related to the study.

14. RESPONSIBILITIES

Principal Investigator Responsibilities

The PI shall be responsible for the day-to-day conduct of the study. The PI will ensure that the study is conducted in compliance with Good Clinical Practices (GCP), the study protocol, and the provisions presented in the Clinical Trials Agreement (contract).

The PI shall be responsible for:

- Receipt and proper storage of study test materials;
- Ensuring all members of the study team are adequately trained on the protocol and procedures and are available to conduct this study in a timely manner;
- Returning to Illumina or destroying (per Illumina instructions) all unused Illumina provided sample collection tubes;
- Disposing of all other used test materials following sample testing;
- Maintaining accurate study records and making those records available for inspections by the study Sponsor, its representatives, and health or government authority representatives at any time.

Sponsor Responsibilities

As the Sponsor of this clinical study, Illumina has the overall responsibility for the conduct of the study, including assurance that the study meets regulatory requirements. As the Sponsor, Illumina (or its representative) shall be responsible for:

- Providing sufficient materials to support the study to include test kits;
- Finalizing the selection of qualified Investigators;
- Training study site personnel;
- Maintaining study records and ensuring proper study monitoring;
- Compiling and reviewing all study data using clinical databases and data management procedures according to GCP and standards established by Illumina;
- Analyzing all study data in accordance with well-established statistical techniques, as applicable; and
- Preparing any required reports.

15. ADMINISTRATIVE AND REGULATORY CONSIDERATIONS

The clinical study was designed and will be conducted, recorded, and reported in accordance with the appropriate regulatory requirements and guidelines for clinical studies. The study will be conducted in compliance with good clinical practice and regulatory requirements.

Prior to Study Initiation

The following will be provided to the Sponsor prior to initiation of the study:

- Copies of curriculum vitae and medical licenses for the Investigator and co-investigators;
- The “Investigator Agreement” page of the protocol, signed and dated by the Investigator;
- A duly executed Clinical Trial Agreement (contract); and

16. Financial disclosure certification for the Investigator and sub-investigators.

16. REFERENCES

1. Gilissen C, Hehir-Kwa JY, Thung DT, van de Vorst M, van Bon BW, Willemsen MH, Kwint M, Janssen IM, Hoischen A, Schenck A, et al: **Genome sequencing identifies major causes of severe intellectual disability.** *Nature* 2014, **511:**344-347.
2. Taylor JC, Martin HC, Lise S, Broxholme J, Cazier JB, Rimmer A, Kanapin A, Lunter G, Fiddy S, Allan C, et al: **Factors influencing success of clinical genome sequencing across a broad spectrum of disorders.** *Nat Genet* 2015, **47:**717-726.
3. Meienberg J, Bruggmann R, Oexle K, Matyas G: **Clinical sequencing: is WGS the better WES?** *Hum Genet* 2016, **135:**359-362.
4. Belkadi A, Bolze A, Itan Y, Cobat A, Vincent QB, Antipenko A, Shang L, Boisson B, Casanova JL, Abel L: **Whole-genome sequencing is more powerful than whole-exome sequencing for detecting exome variants.** *Proc Natl Acad Sci U S A* 2015, **112:**5473-5478.
5. Meynert AM, Ansari M, FitzPatrick DR, Taylor MS: **Variant detection sensitivity and biases in whole genome and exome sequencing.** *BMC Bioinformatics* 2014, **15:**247.
6. Lelieveld SH, Spielmann M, Mundlos S, Veltman JA, Gilissen C: **Comparison of Exome and Genome Sequencing Technologies for the Complete Capture of Protein-Coding Regions.** *Hum Mutat* 2015, **36:**815-822.
7. Directors ABo: **Clinical utility of genetic and genomic services: a position statement of the American College of Medical Genetics and Genomics.** *Genet Med* 2015, **17:**505-507.
8. Srivastava S, Cohen JS, Vernon H, Baranano K, McClellan R, Jamal L, Naidu S, Fatemi A: **Clinical whole exome sequencing in child neurology practice.** *Ann Neurol* 2014, **76:**473-483.
9. Taft RJ, Ajay A, Gross A, Perry D, Avecilla J, Chawla A, Khouzam A, Thorpe E, Chowdury S, Kruglyak S, et al: **Expanding the clinical sensitivity of shole genome sequencing – Validation of copy number variation.** *American Society of Human Genetics Annual Meeting* 2016.
10. Tarailo-Graovac M, Shyr C, Ross CJ, Horvath GA, Salvarinova R, Ye XC, Zhang LH, Bhavsar AP, Lee JJ, Drogemoller BI, et al: **Exome Sequencing and the Management of Neurometabolic Disorders.** *N Engl J Med* 2016, **374:**2246-2255.
11. Vanderver A, Simons C, Helman G, Crawford J, Wolf NI, Bernard G, Pizzino A, Schmidt JL, Takanohashi A, Miller D, et al: **Whole exome sequencing in patients with white matter abnormalities.** *Ann Neurol* 2016, **79:**1031-1037.
12. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, Grody WW, Hegde M, Lyon E, Spector E, et al: **Standards and guidelines for the interpretation of sequence variants: a**

joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med* 2015, **17:**405-424.

13. Kearney HM, Thorland EC, Brown KK, Quintero-Rivera F, South ST, Working Group of the American College of Medical Genetics Laboratory Quality Assurance C: **American College of Medical Genetics standards and guidelines for interpretation and reporting of postnatal constitutional copy number variants.** *Genet Med* 2011, **13:**680-685.
14. ICH Harmonised Tripartite Guideline for Good Clinical Practice. E6(R1) Current Step 4 version dated 10 June 1996

17. Appendix A - Test Description

TruGenome™ Undiagnosed Disease Test

Test Description

Test Indication

The TruGenome Undiagnosed Disease Test is intended to provide information to physicians to aid in the diagnosis of inherited diseases with high penetrance (Mendelian disorders). The analysis and interpretation are designed to detect and report on single nucleotide variants (SNVs), small insertion/deletion events and copy number variants that impact genes that have established association to genetic disease [as found in the national Genetic Testing Registry (http://www.ncbi.nlm.nih.gov/gtr) and Online Mendelian Inheritance in Man (http://www.ncbi.nlm.nih.gov/omim)]. The analysis is typically done as a family-based analysis (e.g. a "trio" of the proband and his or her biological parents), but may be performed on a proband only. Family-based analyses may be comprised of a duo (parent and child), trio, or other higher order family structure. The analysis considers inheritance patterns consistent with the reported family history. In addition, the analysis considers clinical presentation, family history and peer-reviewed literature to contextualize resulting variants from the analyses.

Reasons for referral

This test is appropriate for situations where there are a large number of candidate genes to evaluate, the evaluation of the genome may clarify or refine the diagnosis because the presenting set of signs, symptoms, imaging and laboratory tests are inconclusive, or the phenotype might indicate multiple genetic conditions.

Examples of conditions for which this test is not appropriate include those that are caused by multiple genes each with small effect or gene-environment interactions. This may include diseases that are common in the population such as diabetes, immune disorders, and disorders which are thought to be caused by gene-environment interactions. To assess if a patient's disorder is likely to have a Mendelian etiology, the referring physician should consider other lines of evidence such as increased severity, earlier than expected age of onset, multiple affected close family members, and unexpected phenotypic complexity.

Physicians ordering this testing should understand the intended use of and the performance characteristics of this test. Physicians should provide pre-test counseling to their patients and the family members being tested to review the potential benefits, risks, limitations and alternatives to this testing. Physicians ordering this test are responsible for obtaining informed consent from the persons being tested.

Deliverables

- A Clinical Report of genomic findings deemed clinically significant based on the patient's reported phenotype, including variant interpretation according to the ACMG guidelines. Literature references used to support the classifications will be provided.
- A Secondary Findings Report including variants classified as likely pathogenic or pathogenic within the 59 genes recommended by the ACMG for secondary/incidental findings.
    - Please note the following:
        - Each family member tested through the TruGenome Undiagnosed Disease Test has the option to opt in or opt out of this analysis.
        - Variants reported for the individuals in the family who opt in to this analysis may inform carrier status of that variant in family members who have chosen to opt out.
        - Incidental findings (variants discovered in genes other than these 59 genes deemed of clinical importance by the clinical laboratory director) may still be reported even if an individual opts out of the ACMG secondary findings analysis.
        - Incidental findings related to the ACMG guidelines may still be reported even if an individual opts out if finding lies within a large reportable copy number variant (CNV) that contain multiple genes including those on the ACMG list.
- A Pharmacogenomics Report including 11 medically actionable genes associated with response to 16 different drugs (as specified by the FDA or the Clinical Pharmacogenomics Implementation Consortia (CPIC). – check the number of genes on this report
- A gVCF file with all variants identified throughout the genome

For family-based testing, technical data files, Secondary Findings Reports and Pharmacogenomics Reports are made available for each family member tested.

Criteria for variant classification of single nucleotide variants (SNVs), small deletions and small insertions

We follow the ACMG guidelines for variant classification and reporting (Richards et al., 2015). The guidelines take into account the variant consequence, location and inheritance, presence of absence of functional data supportive of a damaging effect on the gene or gene product, prevalence of the variant in cases and controls, segregation data, computational evidence and patient phenotype and family history to classify variants into one of four categories: pathogenic, likely pathogenic, likely benign or benign. Variants which do not meet the criteria for one of these four categories, or for which the criteria for benign and pathogenic classifications are contradictory are classified as being of uncertain significance.

Criteria for classification for copy number variants (CNVs)

We follow ACMG guidelines for interpretation and reporting of postnatal copy number variants (Kearney et al., 2011; South et al., 2013)

- Pathogenic: Documented as clinically significant in multiple publications, even if penetrance and expressivity are variable. Includes large CNVs which may not described in the literature of the same size, but overlap with an interval with established clinical significance.
- Uncertain clinical significance-likely pathogenic: CNV described in single case report but with well-defined breakpoints and phenotype that overlap with patient, and/or a gene within CNV has a very compelling function relevant to phenotype.
- Uncertain clinical significance: CNV contains genes but unknown if genes are dose sensitive, and/or CNV is described in multiple contradictory publications or databases.
- Uncertain clinical significance-likely benign: CNV has no genes in interval but is identified because of size, and/or CNV is described in a small number of cases in databases for the general population but does not represent a common polymorphism.
- Benign: CNV has been reported in publications or curated databases as a benign variant. CNV is documented to represent a common polymorphism.

Methods and performance characteristics of test

Human whole genome sequencing is performed on DNA extracted from whole blood using sequencing-by-synthesis (SBS) next generation sequencing (NGS). The data are aligned and reported according to build 37.1 of the Human Reference Genome (http://www.ncbi.nlm.nih.gov/projects/genome/assembly/grc/human/). We sequence to an average of ≥30 fold coverage. Over 99% of the genome is covered at 10 fold coverage or more and 96.5% of the genome is callable (passes all quality filters). Based on the quality filters and through the analysis of an extended, multigeneration family set (Platinum Genomes, http://www.illumina.com/platinumgenomes/), for SNVs, sensitivity is 97.7% and the analytical Positive Predictive Value (PPV), i.e.TP/[TP+FP] is 99.9%. Small insertion and deletion events are detected and reported for this test. Insertions up to 28 bases and deletions up to 12 bases have a sensitivity and analytical PPV of approximately 80-85%, determined through Platinum Genomes. This test has the capability to detect copy number events greater than 10 kilobase pairs (kb), however sensitivity was only assessed for events greater than 20 kb and was found to be approximately 85%. Boundaries of the CNVs reported cannot be assessed accurately with boundaries estimated to lie within +/- 1 kb of the event depending on the genomic region.

Some regions of the human genome not covered by this test, including stretches of the human reference genome that have not been completely resolved, or regions where it is difficult to align fragments accurately. Additionally, genes that are associated with regions of high homology are difficult for this test to resolve. These include, but are not limited to, some immunoglobulin (HLA) genes, SMN associated with Spinal Muscular Atrophy, and telomeres. Please contact the laboratory for specifics regarding ability to make calls in regions of specific interest.

Limitations

It is not technically possible to sequence the entire human genome at present. Only known bases of the human reference genome will be assessed. Single nucleotide substitutions, small insertion and deletion events, and copy number variants greater than 10 kb are reported for this test. Other types of genetic variants that may also lead to genetic disease are not detected or reported for this test (e.g. mitochondrial genome variants and trinucleotide repeat variants). If clinically indicated, additional testing and analyses may be appropriate. Clinical sensitivity is unknown and may be dependent on the patient's phenotype.

Lab Statement

TruGenome Undiagnosed Disease Test and TruGenome Undiagnosed Disease Trio Test are Laboratory Developed Tests. They are developed and its performance characteristics determined by the Illumina Clinical Services Laboratory (CLIA #05D1092911). It has not been cleared or approved by the U.S. Food and Drug Administration. Pursuant to the requirements of CLIA '88, this laboratory test has established and verified the test's accuracy and precision. The laboratory is regulated under CLIA as qualified to perform high-complexity testing. This test is used for clinical purposes. We cannot accept orders from the state of New York at this time.

References

Kearney HM, Thorland EC, Brown KK, Quintero-Rivera F, South ST; Working Group of the American College of Medical Genetics Laboratory Quality Assurance Committee. American College of Medical Genetics standards and guidelines for interpretation and reporting of postnatal constitutional copy number variants. Genet Med. 2011 Jul;13(7):680-5.

Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J6 Grody WW, Hegde M, Lyon E, Spector E, Voelkerding K, Rehm HL; ACMG Laboratory Quality Assurance Committee. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015 May;17(5):405-24

South ST, Lee C, Lamb AN, Higgins AW, Kearney HM; Working Group for the American College of Medical Genetics and Genomics Laboratory Quality Assurance Committee. ACMG Standards and Guidelines for constitutional cytogenomic microarray analysis, including postnatal and prenatal applications: revision 2013. Genet Med. 2013 Nov;15(11):901-9.

18. Appendix B – Schedule of Study Events

An "X" denotes a PI/Designee activity unless otherwise specified by a superscript.

Study Events	Visit 0	Visit 1	Visit 2	Visit 3	Visit 4
Proband screening	X				
Proband informed consent		X			
Proband enrollment		X			
Random assignment in Study Database into 15 day cWGS or SOC groups		X			
Proband sample collection		X			
Visit 1 eCRF (Clinical Features) completed		X			
Visit 1 eCRF (Management - Baseline) completed		X			
Participant(s) informed consent		X[a]			
Participant(s) sample collection		X[a]			
cWGS results sent to PI			X[b]	X[b]	
Visit 2 eCRFs (Risk Classification/15 Day cWGS) completed for			X		
Visit 2 eCRF (Risk Verification/15 Day cWGS) completed			X[c]		
Visit 2 eCRF (Test Outcome/15 Day cWGS) completed			X[c]		
Visit 3 eCRF (Management/ALL) completed				X	
Visit 3 eCRF (Risk Classification/SOC) completed				X	
Visit 3 eCRF (Risk Verification/SOC) completed				X[c]	
Visit 3 eCRF (Test Outcome/SOC) completed				X[c]	
Visit 4 eCRF (Management/ALL) completed					X
Visit 4 eCRF (Test Outcome/ALL) completed					X
Visit 4 eCRF (Physician Satisfaction/ALL) completed					X
Visit 4 Questionnaire (Parent Satisfaction/ALL) completed					X

[a] Participant consent and sample collection may occur after Visit 1, but the PI/designee must send samples to ICSL so that they are received within 3 business days of the proband's sample receipt in the lab.
[b] cWGS results for the 15 day cWGS group sent to PI on Visit 2 by the ICSL. cWGS results for the SOC group sent to PI by the ICSL on Visit 3.
[c] Completed by the Medical Monitor
ALL = both 15 Day cWGS and SOC groups

17. Appendix C – Visit Timing

	15 day cWGS group	SOC group
Visit 0 (Proband screening)	Prior to Visit 1	Prior to Visit 1
Visit 1 (Proband enrollment, sample collection and randomization)	Day 1	Day 1
Visit 1 (Clinical Features and Management – Baseline) Completion	Day 1	Day 1
Visit 1 (+3) (Participant informed consent and sample collection)	Day 1-4[a]	Day 1-4[a]
Visit 2 (15 Day cWGS results)	Day 12-18 [b]	N/A
Visit 2 - eCRF (Risk Classification, Risk Verification and Test Outcome) Completion	Day 12-18 [b]	N/A
Visit 3 - eCRF (Management) Completion	Day 57-63	Day 57-63
Visit 3 (SOC cWGS results)	N/A	Day 57-63 [b]
Visit 3 - eCRF (Risk Classification, Risk Verification and Test Outcome) Completion	N/A	Day 57-63 [b]
Visit 4 - eCRF Completion	Day 87-93	Day 87-93
Visit 4 Parent Satisfaction Questionnaire Completion	Day 87-93	Day 87-93

[a] Participant consent and sample collection may occur after Visit 1, but samples must be received within 3 business days of the proband's sample receipt in the lab and pass quality metrics.

[b] CWGS results are delivered to the PI (+/- 3 days) by ICSL after each proband and participant samples are received by ICSL and after passing quality metrics.

12. Appendix D – Parent Satisfaction Questionnaire

	Strongly Agree	Agree	Uncertain	Disagree	Strongly disagree
The whole genome sequence test was explained in a way that I understood	1	2	3	4	5
The reason WGS was offered for my baby was clear to me	1	2	3	4	5
WGS aided in the diagnosis of my baby	1	2	3	4	5
WGS aided care decisions	1	2	3	4	5
WGS led to targeted therapy	1	2	3	4	5
WGS helped me make decisions for my baby's medical care	1	2	3	4	5
My family benefited from WGS	1	2	3	4	5
I will share the WGS result with other family members	1	2	3	4	5
Other Comments					

Completed by:_____

STATISTICAL ANALYSIS PLAN

Illumina, Inc.

NICU-R001

Protocol Title:	NICUSeq: A Prospective Trial to Evaluate the Clinical Utility of Human Whole Genome Sequencing (WGS) Compared to Standard of Care in Acute Care Neonates and Infants
Protocol Version and Date:	Version 1.0; 28 March 2017
Sponsor:	Illumina, Inc. 5200 Illumina Way San Diego, CA 92122
Prepared By:	Jennifer R. Scott Agility Clinical, Inc. 6005 Hidden Valley Road, Suite 170 Carlsbad, CA 92011
Document Version and Date:	Version 1.0; 25 September 2017

1 STATISTICAL ANALYSIS PLAN APPROVAL

Sponsor:	Illumina, Inc.
Clinical Protocol Number:	NICU-R001
Protocol Title:	NICUSeq: A Prospective Trial to Evaluate the Clinical Utility of Human Whole Genome Sequencing (WGS) Compared to Standard of Care in Acute Care Neonates and Infants
Document File Name:	Illumina_NICU-R001_SAP_v1.0_25SEP2017.pdf
Document Version and Effective Date:	Version 1.0; 25 September 2017

Approved By:

Julia McEachern, PA MHS **Project Manager** **Illumina, Inc.**	**Date**
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Ryan Taft, PhD **Senior Director, Scientific Research** **Illumina, Inc.**	**Date**
Jennifer R. Scott, PhD MPH **Manager, Biostatistics** **Agility Clinical, Inc.**	**Date**

2 TABLE OF CONTENTS

TABLE OF TABLES

3 LIST OF ABBREVIATIONS

Table 1 List of Abbreviations

Abbreviation	Definition
AE	adverse event
CAP	College of American Pathologists
CI	confidence interval
CLIA	Clinical Laboratory Improvement Amendments
CNV	copy number variant
CSR	clinical study report
cWGS	clinical whole genome sequencing
eCRF	electronic case report form
ICF	informed consent form
ICH	International Conference on Harmonization
ICSL	Illumina Clinical Services Laboratory
indel	small insertion deletion
IRT	Interactive Response Technology
ITT	Intent-to-Treat
MedDRA	Medical Dictionary for Regulatory Activities
MNV	multinucleotide variant
mSNV	mitochondrial single nucleotide variants
OR	odds ratio
PI	principal investigator
PP	Per-Protocol
SAE	serious adverse event
SAP	statistical analysis plan
SE	standard error
SD	standard deviation
SNV	single nucleotide variants
SV	structural variant
SOC	standard of care
WGS	whole genome sequencing

4 INTRODUCTION

The purpose of this statistical analysis plan (SAP) is to provide comprehensive and detailed descriptions of the methods and presentation of data analyses proposed for the Illumina, Inc. Protocol NICU-R001 (NICUSeq: A Prospective Trial to Evaluate the Clinical Utility of Human Whole Genome Sequencing [WGS] Compared to Standard of Care in Acute Care Neonates and Infants). Descriptions of planned analyses are provided in order to avoid post hoc decisions that may affect the interpretation of the statistical analysis. The statistical methods applied in the design and planned analyses of this study are consistent with the International Conference on Harmonisation (ICH) guideline *Statistical Principals for Clinical Trials* (E9) (1998).

This SAP will be finalized prior to data analysis, and before database lock to provide full details to be presented in the clinical study report (CSR). Any changes between the statistical methods provided in the clinical study protocol and this SAP will be explained herein; any changes or deviations from this SAP relative to the final analysis will be fully documented in the CSR. Minor changes or deviations from the templates for tables and listings need not be documented in the CSR.

5 STUDY OBJECTIVES

5.1 Primary Study Objective

The primary objective of this study is to evaluate Change of Management between the 15 Day clinical whole genome sequencing (cWGS) and standard of care (SOC) groups at Visit 3 (Study Day 60).

5.2 Secondary Study Objectives

The secondary objectives of this study are to compare the following between cWGS and SOC study groups at Visit 3 (Study Day 60):

- Diagnostic yield
- Percent diagnoses returned before discharge or death
- Average time (in days) to diagnose between cWGS and SOC based on the comparison of the (a) cWGS results and the (b) current clinical diagnoses informed by cWGS as designated by the principal investigator (PI).
- Physician satisfaction and perceived clinical usefulness
- Parent satisfaction and personal utility
- Change in care setting
- Time to diagnosis (in days of life)

- Length of hospital stay

The following secondary objectives are described in the clinical study protocol however the analyses are outside the scope of this SAP:

- Pre-test costs of hospital care by utilizing benchmark data (including biochemical tests, biomarker tests, gene sequencing, and DNA copy number testing, as applicable) and post-test costs of care when genomic data is integrated into the medical care plan.

- Clinical services utilization including laboratory and imaging tests, subspecialty consultations, care settings, length of stay, and discharge to home.

5.3 Exploratory Study Objectives

Exploratory study objectives include comparison of each proband with Positive and Negative test outcomes within study group with regard to:

- Change of management

- Condition-specific management or condition-specific supportive management

- Palliative care

Within proband comparison in the SOC group only:

- Diagnostic yield before and after receiving cWGS report

- Change of management before and after receiving cWGS report

Diagnostic accuracy (percent positive agreement) of the cWGS results as determined by the medical monitor, compared with results determined by the site PIs.

Descriptive statistics will be obtained for:

- Positive diagnosis by allele/variant types.

- Clinical services utilization and pre/post-test costs. Analysis of these outcomes are outside the scope of this SAP.

6 INVESTIGATIONAL PLAN

6.1 Overall Study Design

6.1.1 Description

This is a prospective, multi-site, randomized study to evaluate the clinical utility of cWGS in each proband. Throughout this study, each proband will receive SOC testing as determined by the site clinical team. Upon enrollment in the study, each proband will be randomly assigned to the 15 Day cWGS group or the SOC group. A blood sample from each enrolled proband will be collected and shipped to the Illumina Clinical Services Laboratory (ICSL), which is Clinical Laboratory Improvement Amendments (CLIA)-certified and College of American Pathologists (CAP)-accredited. ICSL will conduct cWGS testing with the TruGenome Undiagnosed Disease Test ("TruGenome Test"). The TruGenome Test cWGS results will be provided to the PI or designee who will evaluate each proband diagnoses based on the aggregate medical information. Only the 15 Day cWGS group will receive their results during the 60 day study group comparison period. The SOC study group will receive their results at the end of the 60 day study group comparison period.

6.1.2 Study Population

This study will enroll a minimum of 300 probands with at least one biological parent participant. One biological parent must provide a sample for each enrolled proband. In addition to at least one biological parent, other affected family members may be asked to participate by signing an informed consent form (ICF) and providing a blood sample. An affected family member must have objective clinical findings or other phenotypic defects that are consistent with those observed in the proband and for which a genetic test would be considered. Participant sequence data will be used for the interpretation and analysis of the proband's genetic data. Study data will not otherwise be collected on family members. The sample size justification may be found in Section 7.6.

6.1.3 Study Duration

The study will conclude for the proband at death or approximately ninety days following study enrollment.

6.1.4 Study Centers

Each proband will be recruited prospectively from up to six qualified medical centers. All PIs and designated personnel recruiting each proband will be trained on the inclusion and exclusion criteria, study conduct, and procedures by Illumina personnel or their designees.

6.2 Schedule of Assessments

The schedule of assessments is presented in Table 2. An "X" denotes a PI/designee activity unless otherwise specified by a superscript.

Table 2 Schedule of Assessments

Study Events	Visit 0	Visit 1	Visit 2	Visit 3	Visit 4
Proband screening	X				
Proband inclusion/exclusion criteria		X			
Proband randomization		X			
Proband demographics		X			
Proband sample collection		X			
Clinical Features		X			
Management Plan		X			
Participant(s) informed consent		X[a]			
Participant(s) sample collection		X[a]			
cWGS results sent to PI			X[b]	X[b]	
Risk Classification for 15 Day cWGS group			X		
Risk Verification for 15 Day cWGS group			X[c]		
Test Outcome for 15 Day cWGS group			X[c]		
Management Plan				X	
Risk Classification for SOC group				X	
Risk Verification for SOC group				X[c]	
Test Outcome for SOC group				X[c]	
Management Plan					X
Test Outcome					X
Physician Satisfaction Questionnaire					X
Parent Satisfaction Questionnaire					X
Adverse Events		X	X	X	X

eCRF = electronic case report form

[a] Participant consent and sample collection may occur after Visit 1, but the PI/designee must send samples to ICSL so that they are received within 3 business days of the proband's sample receipt in the lab.

[b] cWGS results for the 15 Day cWGS group sent to PI on Visit 2 by the ICSL. cWGS results for the SOC group sent to PI by the ICSL on Visit 3.

[c] Completed by the Medical Monitor

Table 3 provides the visit timing for the study by study group:

Table 3 Visit Timing

	15 Day cWGS group	SOC group
Visit 0 (Proband screening)	Prior to Visit 1	Prior to Visit 1
Visit 1 (Proband enrollment, sample collection and randomization)	Day 1	Day 1
Visit 1 (Clinical Features and Management – Baseline) Completion	Day 1	Day 1
Visit 1 (+3) (Participant informed consent and sample collection)	Day 1-4[a]	Day 1-4[a]
Visit 2 (15 Day cWGS results)	Day 12-18 [b]	N/A
Visit 2 - eCRF (Risk Classification, Risk Verification and Test Outcome) Completion	Day 12-18 [b]	N/A
Visit 3 - eCRF (Management) Completion	Day 57-63	Day 57-63
Visit 3 (SOC cWGS results)	N/A	Day 57-63 [b]
Visit 3 - eCRF (Risk Classification, Risk Verification and Test Outcome) Completion	N/A	Day 57-63 [b]
Visit 4 - eCRF Completion	Day 87-93	Day 87-93
Visit 4 Parent Satisfaction Questionnaire Completion	Day 87-93	Day 87-93

eCRF = electronic case report form
[a] Participant consensaet and sample collection may occur after Visit 1, but samples must be received within 3 business days of the proband's sample receipt in the lab and pass quality metrics.
[b] cWGS results are delivered to the PI (+/- 3 days) by ICSL after each proband and participant samples are received by ICSL and after passing quality metrics.

6.3 Study Groups

6.3.1 Clinical Whole Genome Sequencing

The TruGenome Undiagnosed Disease Test ("TruGenome Test") is intended to provide information to physicians to aid in the diagnosis of inherited diseases with high penetrance (ie, Mendelian or single gene disorders, abnormalities of DNA copy number and mitochondrial mutations). The analysis is typically done as a family-based analysis, eg, proband and parent(s).

The cWGS study group will receive a 15 Day cWGS report in addition to standard of care (Section 6.3.2). For the purposes of statistical analysis, the TruGenome results will be reviewed by the Medical Monitor to determine test outcome which can be Positive, Likely Positive, Inconclusive, or Negative.

6.3.2 Standard of Care

The SOC group will receive only standard of care through Visit 3 (Study Day 60) following randomization. Standard of care may include laboratory and imaging studies, chromosome microarray testing, and serial single gene sequencing. The SOC group will receive cWGS results once the primary endpoint comparison period is completed, ie, at Visit 3 (Study Day 60).

6.3.3 Method of Assigning Probands to Study Groups

Each proband will be randomly assigned 1:1 according to the site stratified randomization scheme provided in a computer-generated randomization code by a biostatistician. Interactive Response Technology (IRT) will be utilized for randomization in this study. Due to the nature of the study groups, the study is not blinded.

6.4 Efficacy and Safety Variables

6.4.1 Efficacy Variables

Test outcome which can be Positive, Likely Positive, Inconclusive, or Negative will be summarized as follows:

- Positive: Includes Positive and Likely Positive outcomes
- Negative: Includes Negative and Inconclusive outcomes

6.4.1.1 Primary Efficacy Variable

The primary efficacy endpoint is Change of Management, which is a binary (Yes / No) outcome based on the management plan made by the PI or designee at each site using the following domains:

- Condition specific management
- Condition specific supportive interventions
- Palliative care/End of Life care

A change within any of these domains between Visit 1 (Study Day 1) and Visit 3 (Study Day 60) will be considered a Change of Management.

6.4.1.2 Secondary Efficacy Variables

Secondary efficacy endpoints include the following:

- Diagnostic yield (%)

$$\frac{\text{Number of positive diagnoses in study group}}{\text{Total \# of probands in study group}}$$

- Percent of diagnoses returned before discharge or death

$$\frac{\text{Number of diagnoses returned before discharge or death in study group}}{\text{Total \# of probands in study group}}$$

- Change in care setting (Yes / No)
    - Any change in care setting after the initial NICU hospitalization is considered a change in care setting (eg, discharge to home or transfer to new unit within hospital or new facility).
- Time to diagnosis (days of life)

$$\text{Date of Diagnosis} - \text{Date of Birth} + 1$$

- Length of hospital stay(s) (days)

$$\sum \text{Date of Discharge} - \text{Date of Admission} + 1 \text{ for each individual hospital stay}$$

- Physician satisfaction and clinical utility questionnaire (Likert scale)
- Parent satisfaction and personal utility (Likert Scale)

6.4.1.3 Exploratory Analysis Variables

Exploratory endpoints include:

- The impact of test outcome (positive or negative) within study group on:
    - Change of management (Yes / No)
    - Condition-specific management or condition-specific supportive management (genetic tests only) (Yes / No)
    - Palliative care (Yes / No)
- Within proband comparison before and after cWGS results provided for SOC study group only:
    - Change of management (Yes / No)
    - Diagnostic yield (%)

- Diagnostic accuracy (%): Percent positive agreement between test outcome classified by the medical monitor and the site PI or designee

- Positive diagnosis by allele/variant types
  - Single nucleotide variants (SNV)
  - Multinucleotide variant (MNV)
  - Small insertion deletion (indel)
  - Copy number variant (CNV)
  - Structural variant (SV)
  - Repeat Expansion
  - Mitochondrial single nucleotide variants (mSNVs)

Fractional contribution of allele type =

$$\frac{\text{Number of positive diagnoses for allele/variant type}}{\text{Total \# of alleles that contribute to the positive diagnoses}}$$

6.4.2 Description of Safety Variables

6.4.2.1 Adverse Events

An adverse event (AE) is any unanticipated, untoward, undesired, or unplanned event in the form of signs, symptoms, disease, laboratory, and/or physiologic observations occurring in a person given a test article or in a clinical study. The event does not need to be related to the test article or clinical study; if the event occurs during the study, it shall be handled as an AE. Adverse events will be collected for probands and family participants.

6.5 Data Quality Assurance

Report summaries will be generated using validated Base SAS® software, version 9.4 or higher, on a PC or server-based platform. Additional validated software may be used to generate analyses, as needed.

All SAS programs that create outputs or supporting analysis datasets will be validated by a second statistical programmer or biostatistician. At a minimum, validation of programs will consist of a review of the program log, review of output or dataset format and structure, and independent confirmatory programming to verify output results or dataset content. Additionally, all outputs will undergo a review by a senior level team member before finalization.

The content of the source data will be reviewed on an ongoing basis by project statistical programmers and statisticians. Data will be checked for missing values, invalid records, and extreme outliers through defensive programming applications, analysis-based edit checks, and other programmatic testing procedures. All findings will be forwarded to the project data manager for appropriate action and resolution.

7 STATISTICAL METHODS

7.1 General Methodology

Data will be analyzed by Agility Clinical biostatistics personnel. Statistical analyses will be reported with tables and listings, presented in rich text format, and using recommended ICH numbering. Output specifications for all tables, figures, and listings will be in conformance with guidelines specified by the ICH in Appendix 7 of the *Electronic Common Technical Document Specification* (Apr 2003).

7.1.1 Reporting Conventions

Tables will be summarized by study group. Tables summarizing demographics and other baseline characteristics will also include a column for all probands combined. In general, all data collected and any derived data will be presented in data listings, for all enrolled probands and their participating family members. Listings will be ordered by site, proband number, study group, and assessment or event date.

In general, continuous variables will be summarized to indicate the population sample size (N), number of probands/participants with available data (n), mean, standard deviation (SD), median, minimum, and maximum values. Categorical variables will be summarized by the population size (N), number of probands/participants with available data (n), number of probands/participants in each category, and the percentage of probands/participants in each category. Unless otherwise noted, the denominator to determine the percentage of probands/participants in each category will be based on the number of probands/participants with available data. Select ordinal data may be summarized using both descriptive statistics and counts and percentages of probands/participants in each category, as appropriate.

Non-zero percentages will be rounded to one decimal place. Rounding conventions for presentation of summary statistics will be based on the precision of the variable of summarization, as it is collected in its rawest form (ie, on the electronic case report form [eCRF] or as provided within an external file) and are outlined as follows:

- The mean and median will be rounded to one more decimal place than the precision of the variable of summarization;
- Measures of variability (eg, SD, standard error [SE]) will be rounded to two more decimal places than the precision of the variable of summarization; and
- Minimum and maximum values will be presented using the same precision as the variable of summarization.

Other statistics (eg, confidence intervals [CIs]) will be presented using the same general rules outlined above, or assessed for the most appropriate presentation based on the underlying data.

Statistical significance testing will be two-sided unless otherwise noted, and performed using α=0.05. P-values will be reported for all statistical tests, rounded to four decimal places. P-values less than 0.0001 will be displayed as "<0.0001"; p-values greater than 0.9999 will be displayed as ">0.9999".

7.1.2 Summarization by Visit

Data summarized by study visit will be based on the nominal, scheduled visit label as reported on the eCRF and will include a summary for both the scheduled study completion visit for those probands who completed the final scheduled study visit, per protocol, and the last visit completed to combine data collected for probands who completed the final scheduled study visit as well as the early termination visit for those probands who discontinue the study early. Visit timing for each study group is provided in Table 3.

7.1.3 Data Handling Rules

Unless otherwise noted, values reported as greater than or less than some quantifiable limit (eg, "< 1.0") will be summarized with the sign suppressed in summary tables, using the numeric value reported. Data will display on listings to include the sign.

7.1.4 Standard Calculations

Where appropriate, the calculated study day of each assessment or event will be presented with the assessment or event date on data listings, where study day will be determined as:

- The assessment/event date minus the date of Visit 1, if the assessment/event date is prior to the date of Visit 1; and
- The assessment/event date minus the date of Visit 1, plus one, if the assessment/event date is on or after the date of Visit 1.

Other variables requiring calculations will be derived using the following formulas:

- **Days:** A duration between two dates expressed in days will be calculated using the following conventions:
    - Later date – earlier date + 1, if the earlier date is on or after the date of Visit 1; or
    - Later date – earlier date, if the earlier date is prior to the date of Visit 1.
- **Months:** A duration expressed in months will be calculated by dividing the duration in days by (365.25 / 12);

- **Change from Baseline:** Change from baseline will be calculated as the post-baseline value minus the baseline value;

7.2 Analysis Populations

The analysis populations are defined as follows:

- Intent-to-Treat (ITT) Population: Includes all randomized probands
- Per-Protocol (PP) Population: Includes all randomized probands who have no major protocol violations.

7.3 Study Probands

7.3.1 Disposition of Probands

Proband disposition will be summarized for all enrolled probands by study group and overall probands combined. Summaries will include the number and percentage of probands in each analysis population, completing the study, and discontinuing the study early by the primary reason for discontinuation. Proband disposition will also be summarized separately for each study site.

7.3.2 Protocol Deviations

Deviations from the protocol and relevant details will be tracked throughout the study and summarized as part of the clinical study report; however, summarization is outside the scope of this SAP.

7.4 Efficacy

7.4.1 Datasets Analyzed

All efficacy summaries will be based on the ITT Population; select efficacy summaries will also be produced on the PP Population. A data listing of probands excluded from the PP Population, to include the reason for exclusion, will be presented.

7.4.2 Demographic and Other Baseline Characteristics

Demographic variables of the proband, including age (days), sex, ethnicity and race, will be summarized by treatment group and overall probands combined for the ITT and PP Populations.

Age will be summarized using descriptive statistics. Sex, ethnicity, and race will be summarized with the number and percentage of probands in each parameter category.

Demographic variables for the proband's mother and father, if collected, including age (years), sex, ethnicity and race, will be provided in a data listing only.

Age will be calculated relative to date of informed consent, as follows:

- Age (days) = date of informed consent – date of birth
- Age (years)
    - If the month and day portion of the informed consent date is prior to the month and day portion of the birthdate, age will be calculated as the year of informed consent minus the year of birth, minus one;
    - If the month and day portion of the informed consent date is on or after the month and day portion of the birthdate, age will be calculated as the year of informed consent minus the year of birth.

7.4.2.1 Proband Health Information

The proband's health information includes weight, height, length, and head circumference at birth, Apgar Scores, gestational age, karyotype and microarray results, family history of genetic disorders, congenital malformations, neurologic abnormalities, craniofacial dysmorphic facial features, and evaluations of the head, eyes, ears, and nose. This information will be summarized using descriptive statistics or frequency counts and percentages by study group and combined for both the ITT and PP Populations.

7.4.2.2 Mother's Health and Pregnancy Information

The mother's health and pregnancy information includes maternal weight (at first prenatal visit and at delivery), height, mode of delivery, prior pregnancies, alcohol and tobacco consumption during pregnancy, medication use during pregnancy, any maternal or fetal complications, and general pre-pregnancy health. This information will be summarized by proband study group and combined for the ITT and PP Populations.

7.4.3 Primary Efficacy Endpoint Analysis Methods

The primary analysis set is the ITT Population with no imputation of missing data. The primary efficacy endpoint is Change of Management from Visit 1 (Study Day 1) to Visit 3 (Study Day 60). The null hypothesis to be tested is there is no difference in Change of Management between the 15 Day cWGS and SOC study groups:

$$H_0: OR_{cwgs} = OR_{soc;}$$

Where OR_{cwgs} and OR_{soc} represent the Change of Management odds ratio (OR) for the cWGS and SOC study groups, respectively. The alternate hypothesis to be tested is that the study group odds differ:

$$H_1: OR_{cwgs} \neq OR_{soc;}$$

A Cochran-Mantel-Haenszel (CMH) test will be performed to compare the Change of Management in cWGS and SOC study groups, controlling for study site.

7.4.4 Secondary Efficacy Endpoint Analysis Methods

7.4.4.1 Diagnostic Yield and Percent Diagnoses Returned

Diagnostic yield and percent diagnoses returned for the cWGS and SOC group at Visit 3 (Study Day 60) will be compared using a t-test.

7.4.4.2 Diagnostic Accuracy

Diagnostic accuracy, ie, the percent positive agreement between test outcome classified by the medical monitor and the site PI or designee, will be compared using a t-test.

7.4.4.3 Change in Care Setting

Differences in the proportions of change in care setting for the cWGS and SOC groups will be compared using a $\chi 2$ or Fisher's exact test, as appropriate.

7.4.4.4 Time to Diagnosis

Time to diagnosis in days in the cWGS and SOC study groups will be compared using the Cox Proportional Hazards model.

7.4.4.5 Length of Hospital Stay

Length of combined hospital stay(s) of the cWGS and SOC study groups will be compared using a Wilcoxon rank-sum test.

7.4.4.6 Physician and Parent Questionnaires

The results of the Physician and Parent Questionnaires collected at Visit 4 (Study Day 90) will be summarized using descriptive statistics.

7.4.5 Exploratory Endpoint Analysis Methods

7.4.5.1 Impact of Test Outcome

The impact of test outcome (positive or negative) within a study group on Change of Management, condition-specific management or condition-specific supportive management (genetic tests only), and palliative care will be assessed using right-tailed t-tests.

7.4.5.2 Within Proband Comparison

Within proband comparisons before and after cWGS results are provided to the SOC study group will be conducted on Change of Management and diagnostic yield using McNemar's test for paired data.

7.4.5.3 Positive diagnosis by allele/variant types

The results of the positive diagnosis by allele/variant types will be summarized using descriptive statistics.

7.4.6 Statistical/Analytical Issues

7.4.6.1 Adjustments for Covariates

There are no planned applications of covariate adjustments; all statistical results are descriptive in nature.

7.4.6.2 Handling of Dropouts or Missing Data

No imputations will be performed on missing data; all analyses will be based on observed data only.

7.4.6.3 Interim Analyses and Data Monitoring

An interim analysis to support budget reports will be conducted after approximately 165 subjects are enrolled.

7.4.6.4 Multicenter Studies

This is a multicenter study, with approximately 3 sites expected to participate. The effect of site on the efficacy analysis will be assessed by including site as a stratification factor for analysis of the primary endpoint. Efficacy analyses may be run separately by individual site if there is evidence of a site impact on the data results.

7.4.6.5 Multiple Comparisons/Multiplicity

No adjustments for multiple comparisons will be made in this analysis. A single primary endpoint comparison has been identified.

7.4.6.6 Use of an "Efficacy Subset" of Probands

The primary efficacy analysis will be performed on the ITT population; the PP population will be utilized as a sensitivity analysis. The PP population will exclude probands with major protocol violations.

7.4.6.7 Examination of Subgroups

Select analyses, including diagnostic yield, will be conducted by allele type category:

- Single nucleotide variants (SNV)
- Multinucleotide variant (MNV)
- Small insertion deletion (indel)
- Copy number variant (CNV)
- Structural variant (SV)
- Repeat Expansion

Mitochondrial single nucleotide variants (mSNVs)

These select analyses will also be conducted by diagnosis class:

- Positive diagnosis
- Negative diagnosis

7.5 Safety Analysis

Safety analysis will be carried out for the ITT Population, to include all probands who are randomized in the study. Probands who do not complete the study, for whatever reason, will have all available data up until the time of termination included in the analysis.

7.5.1 Adverse Events

All proband AEs that occur during the study will be summarized by study group. Events reported with a partial onset date (eg, month and year are reported but the day is missing) will be considered to have occurred on study if it cannot be confirmed that the event onset was prior to the date of randomization based on the available date entries.

Verbatim terms on case report forms will be mapped to preferred terms and system organ classes using the Medical Dictionary for Regulatory Activities (MedDRA, version 19.1).

Summaries that are displayed by system organ class and preferred terms will be ordered by descending incidence of system organ class and preferred term within each system organ class. Summaries of the following types will be presented:

- Overall summary of number of unique AEs and serious adverse events (SAEs) and proband incidence of AEs meeting various criteria;
- Proband incidence of AEs by MedDRA system organ class and preferred term;
- Proband incidence of AEs by severity grade, MedDRA system organ class, and preferred term;
- Proband incidence of AEs by relationship to study procedure, MedDRA system organ class, and preferred term;

- Proband incidence of severe AEs related to study procedure by MedDRA system organ class and preferred term; and

- Proband incidence of SAEs by MedDRA system organ class and preferred term.

At each level of summarization (eg, any AE, system organ class, and preferred term), probands experiencing more than one AE will be counted only once. In the summary of AEs by severity grade, probands will be counted once at the highest severity reported at each level of summarization; in the summary of AEs by relationship, probands will be counted once at the closest relationship to study procedure.

Adverse event data will be presented in data listings by proband, study group, and event. Serious AEs and AEs leading to discontinuation of the study drug will be presented in separate data listings. Adverse event data from family participants will only be presented data listings.

7.5.2 Deaths, Other Serious Adverse Events, and Other Significant Adverse Events

All deaths during the study will be listed by proband, to include the primary cause of death. Serious AEs and other significant AEs, including those that led to withdrawal, will be provided in separate proband data listings.

7.6 Determination of Sample Size

The CMH test, with stratification by study site, will be applied to the ITT Population for the primary endpoint analysis. A sample size of 300 probands will provide approximately 90% power to detect a difference between the two study groups at an alpha-level of 0.05, assuming an effect size of 2.3. The following assumptions and simulations were used to estimate the effect size, with additional background provided in the clinical study protocol:

- All patients receive nonspecific supportive care regardless of test result or use of other interventions or management. This type of management is defined in the study, but not recorded or enumerated.
- Using the same Inclusion/Exclusion criteria, standard of care genetic testing gives an average diagnostic yield of 5-50% (investigated in sensitivity analysis).
- Clinical whole genome sequencing diagnostic yield is estimated to be 50% (or higher, but not investigated in sensitivity analysis).
- When an etiologic diagnosis is available, condition-specific management and condition-specific supportive care are possible in up to 40% of cases.
- When no etiologic diagnosis is available, condition-specific interventions are not employed.
- In the absence of a molecular diagnosis Palliative/End of Life Care is offered in 5% of cases.

- When a molecular diagnosis is available Palliative/End of Life Care is offered in 5-30% of cases (investigated in the sensitivity analysis).

Table 4 Estimated Change in Care by Test Type and Test Result*

Test Type	Test Result	Targeted Therapy	Palliative	Any Change in Care
SOC	Negative	0	0.05	0.05
SOC	Positive	0.10	0.075	0.175
cWGS	Negative	0	0.05	0.05
cWGS	Positive	0.2	0.15	0.35

* Estimated proportion of probands receiving targeted or palliative care when the SOC diagnostic yield is 25%.

Table 5 Estimated Proportions of Change by Test Type*

Test Type	Changed	Unchanged
SOC	0.225	0.775
cWGS	0.400	0.600

* Assuming SOC diagnostic yield is 25%

Effect Size = $\frac{0.400/0.600}{0.225/0.775} = 2.3$

7.7 Changes in the Conduct of the Study or Planned Analyses

- A χ^2 test was proposed in the clinical study protocol for the primary analysis. A CMH test, controlling for site, will be used instead.

- The diagnostic accuracy analysis is described as a secondary study objective in the clinical study protocol. The analysis is now considered exploratory.