Clinical Study Protocol

Protocol Title: Plasma-Based Next-Generation Sequencing for Pathogen Detection and Quantification in Children with Musculoskeletal Infections

Protocol Number: **KDG-002**

Study Sponsor: **Karius Inc.**

Protocol Version Version 0.9

CLINICAL STUDY PROTOCOL

Protocol Title: Plasma-Based Next-Generation Sequencing for Pathogen Detection and Quantification in Children with Musculoskeletal Infections

Protocol Number: KDG-002

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Karius Approvals:

_____ _____

Asim Ahmed, MD
Sr. Medical Director Date

_____ _____

Radha Duttagupta, PhD
Head of Clinical Operations Date

_____ _____

Carine Ho
Sr. Clinical Research Associate Date

Principal Investigator Protocol Acceptance Page

Protocol Title: Plasma-Based Next-Generation Sequencing for Pathogen Detection and Quantification in Children with Musculoskeletal Infections

Protocol Version: Version: **0.9**

By signing this protocol acceptance page, I confirm I have read, understood, and agree to the following:

The conduct of the study will be in accordance with the current protocol and in accordance with the ethical principles that have their origin in the Declaration of Helsinki, Title 21 of the Code of Federal Regulations§§50,56, the International Conference of Harmonization E6, and any applicable regulatory requirements.

The study protocol and any amendments are to be approved by the sponsor and reviewed and approved by an Institutional Review Board (IRB) before implementation.

Written informed consent is to be obtained from each study subject prior to the conduct of any study procedures that exceed or differ from standard practice at the study site.

Confidential information in this document will not be used for any purpose other than the evaluation or conduct of the clinical investigation without prior written consent of Karius.

_____
Principal Investigator (Print Name)

_____ _____
Principal Investigator (Signature) Date

Please return original form to Karius at the address provided below.

Attn: Clinical Operations
Karius Inc.
975 Island Drive
Redwood Shores, CA 94065

After signing, please place a copy of this protocol and signature page in your study files.

TABLE OF CONTENTS

ACRONYMS

AHO	Acute hematogenous osteomyelitis
CRP	C-reactive protein
cfDNA	Cell-free DNA
CFR	Code of Federal Regulations
CRF	Case Report Forms
DNA	Deoxyribonucleic Acid
DVT	Deep vein thrombosis
ESR	Erythrocyte sedimentation rate
GCP	Good Clinical Practice
ICF	Informed Consent Form
ICU	Intensive care unit
ID	Identification
IRB	Institutional Review Board
IU	Indiana University
LOT	Length of therapy
MPM	Molecules per microliter
MSKI	Musculoskeletal infection
NGS	Next-generation sequencing
PCT	Procalcitonin
PHI	Protected Health Information

RHC	Riley Hospital for Children
WBC	White blood cell count

STUDY SYNOPSIS

Protocol Number:	KDG-002
Protocol Title:	Plasma-Based Next-Generation Sequencing for Pathogen Detection and Quantification in Children with Musculoskeletal Infections
Study Objectives:	1. To evaluate and compare pathogen identification of children with musculoskeletal infections (MSKI) using Karius® plasma-based quantitative Next-Generation Sequencing Test (Karius Test) and standard culture methods. 2. To examine the kinetics of pathogen cell-free DNA (cfDNA) in the plasma of children with MSKI and compare the quantity of cfDNA to clinical symptoms and inflammatory markers, C-reactive protein (CRP), Erythrocyte sedimentation rate (ESR), and procalcitonin (PCT), a biomarker thought to be more specific for bacterial infections. 3. To evaluate whether samples quantitative cfDNA predicts severe disease in children with MSKI.
Study Design:	We will prospectively enroll children evaluated at Riley Hospital for Children (RHC) with musculoskeletal infections (osteomyelitis, septic arthritis, or pyomyositis) over a 12-24-month period. Eligible subjects will be identified by referral from the infectious diseases and orthopedic services at RHC. Subjects and their parent(s) or guardian(s) will be informed of the study and, upon providing informed consent, will be enrolled in the study. Study samples will be collected at time points that closely parallel clinical testing as performed during routine, standard care for treatment of MSKI. Immediately after enrollment, up to two detection blood samples will be obtained for real-time NGS testing at Karius Laboratory (Redwood City, CA) to determine if additional study samples will be obtained. Detection of a pathogen by NGS will be interpreted as a positive result that qualifies the collection of subsequent study samples. Pathogen identification by NGS will be compared to standard cultures methods, and quantitative cfDNA will be evaluated over time.
Duration of Study:	12-Month Period
Number of Sites:	1: Riley Hospital for Children
Sample Size:	38 Subjects

1 BACKGROUND INFORMATION AND RATIONALE

1.1 Background Information

Musculoskeletal infections (MSKI; osteomyelitis, septic arthritis, and pyomyositis) are a common cause for hospitalization and, in children, among the most frequent type of invasive infection. Due to the risk of local tissue destruction and metastatic bacterial spread, MSKI require prompt diagnosis and targeted treatment. Current diagnostic modalities require isolation of a causative organism by culture from a sterile site (e.g., blood, bone or joint fluid); however, nearly half of all MSKI in children remain culture-negative [1,2,3]. Several reasons for this exist, including antibiotic use prior to obtaining a culture; lack of a surgical or drainage procedure; and the fastidious nature of certain organisms in culture [4]. Absence of a microbiologic diagnosis has significant ramifications on the management of children with MSKI, as patients are ultimately treated with broad, often multi-agent antimicrobial regimens to cover all likely causative pathogens.

1.2 Rationale for the Study

A culture-independent molecular test, such as the Karius® plasma-based next-generation sequencing (Karius Test) test, would not be prone to the aforementioned barriers and has the potential to increase pathogen detection allowing for targeted antimicrobial therapy.

Additionally, quantitative NSG has the potential to improve another component of the complex management of children with MSKI—length of therapy (LOT). Typically, length of therapy for MSKI in children is 2-6 weeks depending on the infection type and severity, however, variability in this practice exists [5,6]. Ultimately LOT is typically determined by clinical symptoms and inflammatory makers (e.g., C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR)) at the end of the pre-determined timeframe. This approach, while often effective, is challenging in children given that the clinical exam can be unreliable and inflammatory markers are non-specific, often influenced by intercurrent illnesses or recent procedures. An objective assay with improved specificity, such as the Karius Test, which has the ability to quantitate cell-free pathogen DNA (cfDNA) and has been shown to correlate with clinical findings in other disease states [7], could allow for a tailored treatment approach and address an unmet need for children with MSKI.

Finally, the ability of the Karius Test to quantitate cfDNA has the potential to inform disease severity and potentially predict complications in children with MSKI. While numerous proposed strategies have been published to develop a prediction algorithm for disease severity in children with MSKI, each of these methods has significant limitations [8], and none involve quantification of cfDNA. If the Karius Test could be used to predict severe disease, it could have a significant impact on early management of this complex disease.

Taken together, the Karius Test has the potential to significantly impact antibiotic utilization and quality of care for children with MSKI. In this proposal, we seek to investigate the utility of this novel technology in children with MSKI.

2 STUDY OBJECTIVES AND ENDPOINTS

2.1 PRIMARY OBJECTIVES

1) To evaluate and compare pathogen identification of children with musculoskeletal infections using Karius plasma-based quantitative NGS test (Karius Test) and standard culture methods. We hypothesize that the Karius Test will have a high positive agreement for culture positive samples and increase pathogen detection in culture-negative infections.

2) To examine the kinetics of pathogen cell-free DNA (cfDNA) in the plasma of children with MSKI and compare the quantity of cfDNA to clinical symptoms and inflammatory markers, CRP, ESR, and procalcitonin (PCT), a biomarker thought to be more specific for bacterial infections. We hypothesize that the cfDNA quantity will correlate strongly with clinical symptoms as well as inflammatory markers (CRP, ESR and PCT).

3) To evaluate whether initial detection samples quantitative cfDNA predicts severe disease[1] in children with MSKI. We hypothesize that cfDNA quantity at admission will correlate with severe or complicated MSKI.

[1]Severe disease defined by any of the following: need for intensive care unit (ICU) care, infection in two or more non-contiguous anatomic sites (disseminated disease), complicated disease (need for more than 1 debridement procedure, deep vein thrombosis [DVT] or thromboembolic disease, pathologic fracture)

2.2 EXPLORATORY OBJECTIVE

1) To evaluate and compare pathogen identification from synovial fluid of children with septic arthritis using Karius Test and standard culture methods.

2.3 PRIMARY ENDPOINT MEASURES

1) To evaluate and compare pathogen identification of children with musculoskeletal infections using Karius Test NGS and standard culture methods:
   a. Describe pathogen identification by Karius Test vs. standard culture methods

2) To examine the kinetics of pathogen cell-free DNA (cfDNA) in the plasma of children with MSKI and compare the quantity of cfDNA to clinical symptoms and inflammatory markers, CRP, ESR, and procalcitonin (PCT), a biomarker thought to be more specific for bacterial infections.

a. Determine quantification of cf DNA in molecules per microliter (MPM) at each time point using the Karius Test.
b. Determine CRP, ESR, PCT at each timepoint and compare to cfDNA MPM.

3) To evaluate whether initial detection sample quantitative cfDNA predicts severe disease in
children with MSKI.
   a. Determine the correlation of cfDNA level in MPM to severity of infection (defined in SECTION 2.1)

2.4 EXPLORATORY ENDPOINT MEASURES

1) To evaluate and compare pathogen identification from synovial fluid of children with septic arthritis using the Karius Test and standard culture methods.
   a. Describe pathogen identification by NGS of the synovial fluid compared to standard culture methods.

3 STUDY DESIGN

This is a prospective study of children 6 months to 18 years of age (inclusive) with the diagnosis of MSKI at Riley Hospital for Children (Indianapolis, IN) over a 12-month period. Eligible subjects will be identified by referral from the infectious diseases and orthopedic services at RHC. Subjects will be identified and screened for eligibility (**Section 5.3**) by the study team. Subjects and their parent(s) or guardian(s) will be informed of the study and, upon providing informed consent, will be enrolled in the study.

Study samples will be collected to coincide with routine clinical care. Samples will be collected when sufficient sample is available after routine care samples are obtained. Additional venipunctures specific for collection of study samples will be performed only with participant's consent.

Collection of NGS samples for real-time testing:
Detection samples will be collected prior to any surgical procedures and will be sent to Karius® Laboratory (Redwood City, CA) for real-time testing to determine if the pathogen is detected by NGS. Karius will notify the study team of the result(s). A positive result for any detection sample will qualify the collection of subsequent study samples. Detection samples will be collected within 96 hours of enrollment, and if applicable, 12-72 hours after the first sample, upon any re-admission for MSKI, or when prior detection sample results are inconclusive.

Collection of subsequent study samples
If NGS testing identifies a pathogen in the detection sample(s), subsequent study samples will, will be collected ≥ 48 hours after previous sample collected. When the Detection NGS result(s)

is inconclusive or still pending, study samples may be collected at the appropriate timepoint(s). Collected sample(s) will be sent to Karius with qualifying result(s) from the Detection NGS sample(s). Up to 4 samples, including detection samples, will be collected per inpatient admission. (**Figure 1**)

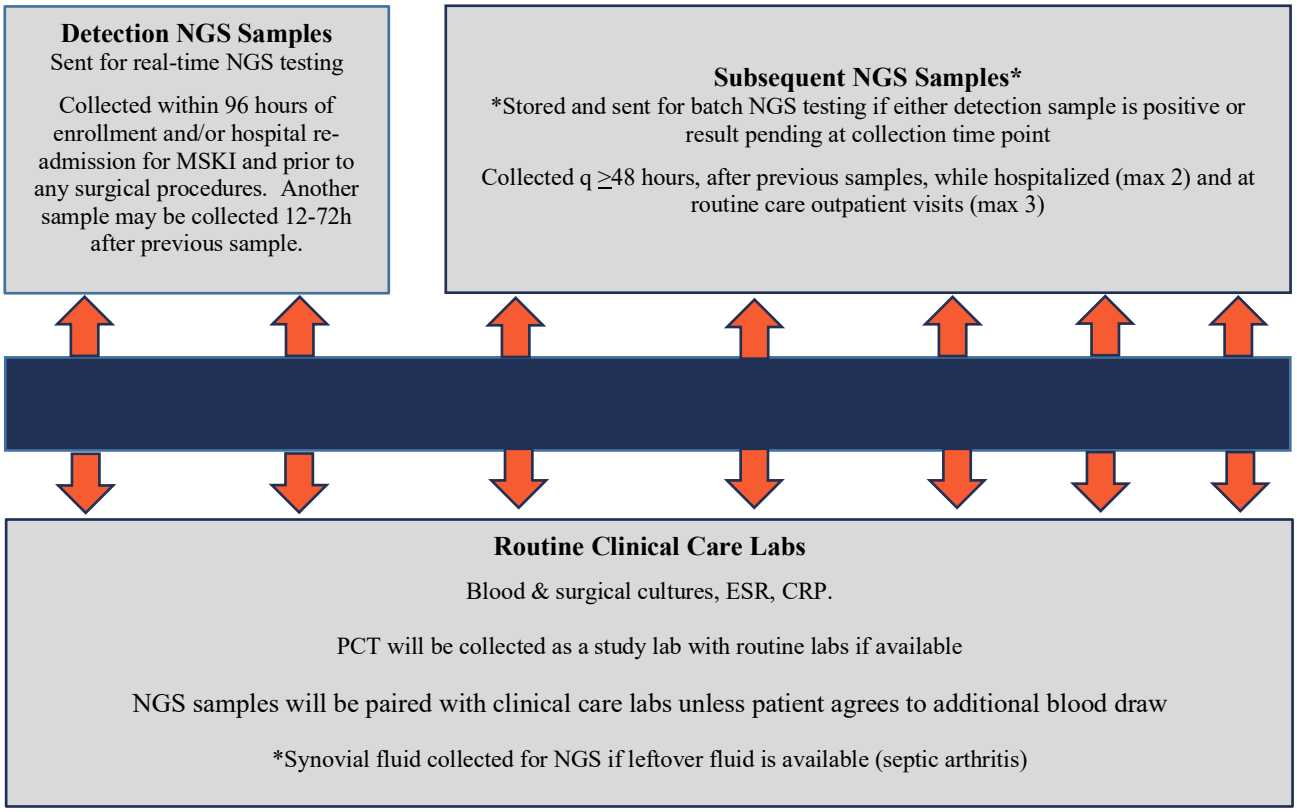

Figure 1. Study Design- *Samples for NGS testing will be collected to coincide with routine clinical care testing. Detection sample(s) sent for real-time NGS testing to determine if the Karius test identifies a pathogen. If a pathogen is detected by NGS, additional study samples will be collected throughout the treatment and follow up for MSKI.*

Collection of Synovial Fluid from patients with septic arthritis:

When synovial fluid is obtained as part of routine medical care, excess or leftover synovial fluid may be collected and sent to Karius for potential NGS testing of the synovial fluid.

Procalcitonin:

A procalcitonin (PCT) level will be obtained at each study sample time point, along with routine clinical labs when sufficient sample can be collected.

If the Karius Test identifies an infectious agent in a clinically meaningful timeframe that may be of interest for the treating physician, Dr. Wood, at his discretion, can contact the attending

physician and notify them. Dr. Wood will stress that these results are from a research-use only test but will guide the treating physician to confirm the diagnosis with a commercially available CLIA-approved test.

4 STUDY METHODS

Plasma samples will be obtained at the time points outlined in **Section 3**. Approx 2-4ml of whole blood will be collected in 2mL K2-EDTA tubes for NGS testing and centrifuged at 1,600 RCF for 10 minutes at room temperature, within 24 hours of blood draw. Plasma will be transferred into sterile polypropylene tubes. Detection samples will be sent at ambient temperature to Karius Laboratory within 96 hours of blood draw. Subsequent NGS samples will be frozen at -20^{o}C until shipping to Karius. For patients with septic arthritis, in which synovial fluid is collected as part of standard of care, left-over synovial fluid will be frozen at -20^{o}C until shipping to Karius. Blood and source specimen (bone biopsy, abscess aspiration, or synovial fluid) cultures for standard microbiologic diagnosis, will be collected as per the treating physicians. Cultures will be performed per standard of care procedures by the Indiana University clinical laboratory. Standard of care laboratory testing (e.g., ESR, CRP) will be obtained per the treating physicians and run in the Indiana University (IU) clinical laboratory. Additionally, procalcitonin (PCT), which is not part of current standard of care laboratory testing, will be drawn with NGS samples and run in the IU clinical laboratory for research purposes only.

5 SELECTION AND WITHDRAWAL OF SUBJECTS

5.1 Study Population

We will prospectively enroll children, 6 months to 18 years of age, evaluated at Riley Hospital for Children (RHC) with clinical presentation consistent with a musculoskeletal infection (osteomyelitis, septic arthritis, or pyomyositis; MSKI) over a 12-24-month period. Eligible subjects will be identified by referral from the infectious diseases and orthopedic services at RHC.

5.2 Medications

There are no medication restrictions or medication requirements for this study.

5.3 Inclusion Criteria

Eligible subjects will be identified by referral from the infectious diseases and orthopedic services at RHC. Subjects and their parent(s) or guardian(s) will be informed of the study and, upon providing informed consent, will be enrolled in the study if they meet the following inclusion criteria:

1. 6 months (to ensure adequate blood volume drawn) to 18 years of age.
2. Strong clinical suspicion of MSKI as evidenced by fever, osteoarticular pain (e.g. tenderness to palpation of a joint, bone pain, or refusal to bear weight); and elevated ESR or CRP.

5.4 Exclusion Criteria

1. Subjects will be excluded if they have clinical evidence suggesting an alternative diagnosis; PI discretion; inability or unwillingness to consent for the study.
2. Subjects will be excluded if they have a surgical procedure prior to obtaining NGS samples.

5.5 Subject Screening and Enrollment

The study team will be notified by the pediatric infectious diseases and/or orthopedic team, at admission/evaluation, of any child presenting with possible MSKI. A study team member will screen the patient for eligibility (**Section 5.3**), and if eligible will obtain informed consent/assent for study participation.

5.6 Subject Withdrawal

5.6.1 Withdrawal Criteria

The primary consideration in any determination to discontinue a subject's participation must be the health and welfare of the subject. Reasons for study withdrawal may include but are not limited to:

a) A serious medical complication or an unacceptable situation, whether attributed to study procedure or not.

b) Noncompliance with study procedures.

c) Subject's right to withdraw their consent at any time during the study with or without stated reason.

d) The PI's opinion is that it is not in the subject's best interest to continue study participation.

5.6.2 Documentation of Withdrawal of Subjects

Document the reasons for early withdrawal of any subject from the study on the appropriate Case Report Forms (CRF). If the reason for early withdrawal is an adverse event or an abnormal laboratory value, record the specific event or test result on the relevant CRF.

5.7 Study Termination

Sponsor has the right to terminate this study at any time. Reasons for terminating the study may include, but are not limited to, the following:

a) Subject enrollment is unsatisfactory.

b) Data recording is inaccurate or incomplete.

c) Investigators do not adhere to the protocol or applicable regulatory guidelines in conducting the study.

6 TREATMENT OF SUBJECTS

6.1 Treatment Regimen(s)

NA

7 STUDY PROCEDURES

7.1 Screening

Patients will be screened for eligibility (**Section 5.3**) by the study team at evaluation at RHC.

7.2 Study Visits

Study visits coincide with a patient's course of treatment for MSKI by Riley Pediatric Infectious Disease at RHC. Up to 4 visits may occur per inpatient admission and up to 4 visits may occur per outpatient course.

7.3 Sample Handling

Sample collection materials may be supplied by the sponsor. The main sample handling instructions are as below:

All samples should be collected using the institutional guidelines for sterile sample collection. The venous blood sample collection can be collected from either a peripheral vein or a central line catheter.

A) Detection Samples:

Specimen: Minimum 1.2 mL of plasma isolated from whole blood collected in K2-EDTA tube within 24 hours of draw by centrifugation of the specimen at 1,600 RCF for 10 minutes and transferred to a sterile polypropylene tube. Ship to Karius laboratory at ambient temperature. Must be received within 96 hours (4 days) of collection.

B) Subsequent study samples:

B1. Collect 2-4.0 mL of whole blood in a K2-EDTA 2.0 mL blood collection tube
B2. CRITICAL STEP: Immediately after collection, gently invert the filled tube 180º and back 8-10 times to adequately mix the blood and tube reagents together.
B3. Place tube upright in rack at room temperature. The tube may be stored upright at room temperature for no more than 24 hours prior to processing for plasma collection. Do not freeze or refrigerate the tube.
B4. For plasma collection, centrifuge the K2-EDTA blood tube at 1,600 rcf for 10 minutes at 20ºC. It is critical that tubes be centrifuged at the appropriate speed to ensure proper plasma separation.
B5. Using a sterile micropipette, transfer blood plasma (top layer) into labeled 1.0 mL aliquot tubes. Take care not to disturb the pellet at the bottom of the tube by tilting the tube and placing the pipette tip along the lower side of the collection tube wall.
B6. In order to ensure Karius receives sufficient sample for processing and to avoid tubes cracking prior to shipment, each aliquot tube should ideally be filled to 1.0 mL with plasma after processing. If there is plasma that won't fill a full tube, the remaining amount should still be sent to Karius.
B7. Freeze the plasma samples immediately following processing by transferring to 80°C freezer. Store all samples in -80ºC until transport to Karius.

7.4 Sample Banking

As agreed by the subject on the signed informed consent form (ICF), his or her blood (and blood products) will be kept in the Sponsor's lab or designated facility equipped with freezers (at -80 ºC) and may be used by the sponsor for research. The ICF will include the subject's right to release or restrict their sample for purpose of research by the sponsor.

Each sample will be identified by a unique number on a bar-coded label (sample ID) which will not have any personal information in order to maintain subject confidentiality. Only the study site staff will keep a matching list of sample barcode numbers, subject identifiers, subject names, and visit dates to fully identify these samples.

8 SAFETY MONITORING AND REPORTING

No unexpected adverse events or side effects related to the conduct of the protocol are anticipated. All venous samples will be collected by routine techniques performed by qualified phlebotomists and/or licensed health care personnel. All adverse events will be reported to the IRB and to the Medical Monitor.

Medical Monitor: Jack Schneider , M.D., 317-944-7265; jgschnei@iu.edu.

9 CLINICAL ADJUDICATION

9.2 Adjudication Process

NA

9.3 Data Review

Upon the study completion, the comparison between Karius NGS testing and standard culture methods for pathogen identification will be reviewed by Dr. Wood and Karius. The study results will be summarized in a peer-reviewed publication.

10 STATISTICAL CONSIDERATIONS

10.1 General Conditions for Statistical Analysis

Positive and negative agreement (percentage) will be used to compare culture and NGS for pathogen identification. Correlation between inflammatory markers and cfDNA quantification will be compared using Spearman's coefficient. Lastly, simple regression modeling will be used to assess the ability of cfDNA to predict severe disease.

10.2 Analysis Data Sets

MINIMAL CLINICAL DATA SET

** All of these data will be striped of protected health information (PHI). The Investigator will maintain a log that can link these subject ID and sample IDs to medical record numbers and sample-collection dates.

Variable	Acronym definition	Type of data
Subject ID	Subject Identifier	Numerical
Sample ID	Sample Identifier (i.e., IP1)	Numerical

Admission Time	Date and time of hospital admission	Date and time
Discharge Time	Date and time of hospital discharge	Date and time
Sample Time	Date and time of NGS sample collection	Date and time
Age	Age at the time of diagnosis of MSKI	Years
Sex		F/M
Race		Nominal
Infection Type	Acute hematogenous osteomyelitis (AHO), Septic Arthritis, Pyomyositis	Nominal
Infection Location	Name of bone/joint infected	Nominal
Days of Symptoms	Days of symptoms (number) related to MSKI (fever, pain) prior to hospital admission	Numerical
Antibiotic Prior Admit	Antibiotic given prior to admission (Y/N)	Yes/No
Antibiotic Name	Name of initial antibiotic(s) given for MSKI	Nominal
Antibiotic Time	Date and time initial antibiotic(s) given	Date and time
Surgical Procedure	Was a surgical procedure performed	Yes/No
Culture Pathogen ID	Name of pathogen by culture	Nominal
Culture source	Source of culture (i.e., blood, bone, synovial fluid)	Nominal
NGS Pathogen ID	Name of pathogen by NGS	Nominal
NGS Quantification	Molecule per microliter (MPM) of cfDNA	Numerical
WBC	White blood cell count	Numerical
WBC Timepoint	Time and Date of WBC collection	Date and time
CRP	C-reative protein	Numerical
CRP Timepoint	Time and Date of CRP collection	Date and time

ESR	Erythrocyte sedimentation rate	Numerical
ESR Timepoint	Time and Date of ESR collection	Date and time
PCT	Procalcitonin	Numerical
PCT Timepoint	Time and Date of PCT collection	Date and time

10.3 Study Endpoints for Clinical Samples and Data Collection

Study objectives and endpoint measures are listed in Protocol Section 2.

10.4 Determination of Sample Size

Based on current rates, it is estimated that at least 50 subjects will be admitted for MSKI at Riley Hospital for Children over a 12-month period. Based on an estimated >75% participation (consistent with similar previous studies) the target sample size of this study is 38.

10.5 Analysis Population(s)

Subject Disposition

Summary tables will be provided containing frequency counts for subject disposition (all enrolled subjects, all subjects with uncontaminated samples, all subjects who discontinued from study and reason for discontinuation). Disposition in terms of number of subjects excluded from the ITT analysis set will also be provided for the study.

Replacement of Subjects

No replacements for subjects withdrawing from the study are planned.

Procedures for Handling Missing, Unused, or Spurious Data

10.6 Demographics and Baseline Characteristics

As per above, baseline demographic information including age, race/ethnicity, and gender will be collected via review of the patient's medical record after enrollment.

10.7 Analysis

Statistical analysis is outlined in Section 10.1

11 ADMINISTRATIVE CONSIDERATIONS

This study will be conducted at one site in the United States. The investigator will submit this protocol, the informed consent, and any other relevant supporting information to the appropriate IRB for review and approval prior to study initiation. A letter confirming IRB approval of the protocol and informed consent**,** and a statement that the IRB is organized and operates according to Good Clinical Practice (GCP) and the applicable laws and regulations, and financial disclosures must be forwarded to the Sponsor prior to the enrollment of subjects into the study. Amendments to the protocol must also be approved by the IRB prior to the implementation of changes in this study.

11.1 Study Compliance

The study will be conducted in compliance with this protocol, principles of ICH-6, GCP and the Declaration of Helsinki, and all applicable national regulations governing clinical trials.

11.2 Informed Consent and Protected Subject Health Information Authorization

A copy of the IRB approved informed consent may be audited or requested by the Sponsor for regulatory purposes. The investigator or designee must explain to each subject the purpose and nature of the study, the study procedures, the possible adverse effects, and all other elements of consent as defined in 21CFR §50. Each subject must provide a signed and dated informed consent prior to enrollment into this study. Signed consent forms must remain in each subject's study file and be available for verification by study monitors at any time.

In accordance to individual local and national subject privacy regulations, the investigator or designee must explain to each subject prior to screening that for the evaluation of study results, the subject's protected health information obtained during the study may be shared with the Sponsor and its designees, regulatory agencies, and IRBs. The study sponsor will not use the subject's protected health information or disclose it to a third party without applicable subject authorization. It is the investigator's or designee's responsibility to obtain a written permission to use protected health information from each subject. If a subject withdraws permission to use protected health information, it is the investigator's responsibility to obtain a written request from the subject or subject's legal guardian and to ensure that no further data will be collected from the subject. Any data collected on the subject prior to withdrawal will be used in the analysis of study results.

11.3 Subject Screening Log

The Principal Investigators must keep a record that lists all subjects considered for screening and enrollment in the study. For those subjects subsequently excluded, record the reason(s) for exclusion.

Case Report Forms

Study site personnel will collect and record data on paper and/or electronically. If electronic data collection is not feasible, other forms of source document will be used to capture the data. A single set of standardized case report forms (CRFs), which primarily include the Inclusion/Exclusion Criteria, Medical History, Height and Weight, Concomitant Medications, will be required for every study subject who has undergone any amount of study procedures. Standardized CRFs will be completed for each study subject and monitored.

11.4 Study Monitoring

Representatives of the Sponsor or its designee will monitor for proper completion of study procedures until completion. Monitoring will be conducted through personal visits with the Principal Investigators and site staff as well as any appropriate communications by mail, fax, E-mail, or telephone. The purpose of monitoring is to ensure compliance with the protocol and the quality and integrity of the data. This study is also subject to Quality Assurance reviews and/or audits under the Sponsor's practices or procedures.

Every effort will be made to maintain the anonymity and confidentiality of all subjects during this clinical study. However, the Principal Investigators agrees to allow the IRB, representatives of the Sponsor or its designated agent, and authorized employees of the appropriate regulatory agencies to inspect the facilities used in this study and have direct access to the hospital or clinic record of all enrolled into the study, for purposes of verification. A statement to this effect will be included in the informed consent form authorizing the use of protected health information.

11.5 Retention of Records

The Principal Investigators must retain a copy of all documents for 5 years unless the Sponsor notifies the Principal Investigators in writing that the documents no longer need to be retained. The Principal Investigators must retain the documents for a longer period where so required by other applicable requirements. Essential documents shall be archived in a way that ensures that they are readily available, upon request, to the competent authorities. The medical files of study subjects shall be retained in accordance with national legislation and the maximum period of time permitted by the hospital, institution, or private practice. The Principal Investigators is responsible for contacting the Sponsor before the destruction of any study related documents and must wait for a written approval from the Sponsor before destruction proceeds.

11.6 Confidentiality Policy

By conducting this study, the Principal Investigators affirms to the Sponsor that all study results and information furnished by the Sponsor will be maintained in strict confidence. Such information will be communicated to the Principal Investigator's IRB under an appropriate understanding of confidentiality.

11.7 Principal Investigators Obligations in Conduct of Study and Protection of Human Subjects

The Principal Investigators must ensure that:

1. He or she will personally conduct or supervise the study.
2. His or her staff and all persons who assist in the conduct of the study clearly understand their responsibilities and have their names included in the Study Staff Signature and Delegation of Authority log. The Principal Investigators will sign the authorization log whenever it is updated with new responsibilities or staff membership.
3. The study is conducted according to the protocol and all applicable regulations.
4. The protection of each subject's rights and welfare is maintained.
5. Signed and dated informed consent and permission to use protected health information are obtained from each subject prior to conducting study procedures. If a subject withdraws permission to use protected health information, the investigators will obtain a written request from the subject and will ensure that no further data be collected from the subject.
6. The consent process is conducted in compliance with all applicable regulations and privacy acts.
7. The IRB complies with applicable regulations and conducts initial and ongoing reviews and approvals of the study.
8. Any amendment to the protocol is submitted promptly to the IRB.
9. Any significant protocol deviations are reported to the Sponsor and the IRB according to the guidelines at the study site.

12 APPENDIX A: SCHEDULE OF EVENTS

Procedure	Screening Visit	SHC ED Enrollment Visit (V1)

13 APPENDIX B: ID-DX SAMPLE COLLECTION INSTRUCTIONS

14 APPENDIX C. CASE REPORT FORM

Eligibility Evaluation

Inclusion Criteria	Yes	No
1. Is the patient 6 months to ≤ 18 years old?		
2. Is there a strong clinical suspicion of MSKI? ☐ Fever (temperature ≥38 degrees C ☐ Osteoarticular pain ☐ Elevated inflammatory markers CRP ⩾ 3 mg/dL or ESR ⩾ 40 mm/hr		
3. Is patient, or legal representative, capable and willing to give written informed consent prior to any screening procedures? The Informed Consent form must be signed before any study procedures are performed. Date of Informed Consent: _ _/ _ _/ _ _ _ _ (mm/dd/year) Time of Informed Consent: _____ (24-hr clock)		
4. Is there clinical evidence of an alternative diagnosis?		
5. Will the patient have a surgical procedure prior to obtaining NGS sample?		

In order to qualify for the study, the following inclusion criteria must be met:

Inclusion criteria questions 1-3, 4 must be marked "yes".

Exclusion criteria question 4 and 5 must be marked “no”

Does the patient qualify for the study? ☐ YES ☐ NO

Date of Enrollment	_____ (MM-DD-YYYY)
Study Number	
Admission Date and Time	(MM-DD-YYYY); 00:00
Discharge Date and Time	(MM-DD-YYYY); 00:00
Sex:	☐ Male ☐ Female
Age	Years
Sex	F/M
Race/Ethnicity (circle one)	White Non-Hispanic, White Hispanic, Black Non-Hispanic, Black-Hispanic, Asian, Other _____
Infection Type (circle one)	Acute hematogenous osteomyelitis (AHO), Septic Arthritis, Pyomyositis
Infection Location	Name of bone/joint infected
Days of Symptoms	Days of symptoms (number) related to MSKI (fever, pain) prior to hospital admission
Antibiotic Prior Admit	Antibiotic given prior to admission (Y/N)
Antibiotic (Initial) Name	Name of initial antibiotic(s) given for MSKI

Antibiotic (Initial) Time	(MM-DD-YYYY); 00:00
Surgical Procedure	Y/N
Culture Pathogen ID	Name of pathogen by culture
Culture source	Blood, bone/synovial fluid/tissue, both
NGS Pathogen ID- (IP1)	Name of pathogen by NGS
NGS Quantification- (IP1)	Molecule per microliter (MPM) of cfDNA
NGS Pathogen ID- (IP2)	Name of pathogen by NGS
NGS Quantification- (IP2)	Molecule per microliter (MPM) of cfDNA
NGS Pathogen ID- (IP3)	Name of pathogen by NGS
NGS Quantification- (IP3)	Molecule per microliter (MPM) of cfDNA
NGS Pathogen ID- (IP4)	Name of pathogen by NGS
NGS Quantification- (IP4)	Molecule per microliter (MPM) of cfDNA
NGS Pathogen ID- (OP1)	Name of pathogen by NGS
NGS Quantification- (OP1)	

	Molecule per microliter (MPM) of cfDNA
NGS Pathogen ID- (OP2)	Name of pathogen by NGS
NGS Quantification- (OP2)	Molecule per microliter (MPM) of cfDNA
NGS Pathogen ID- (OP3)	Name of pathogen by NGS
NGS Quantification- (OP3)	Molecule per microliter (MPM) of cfDNA
WBC x 10^3/uL	White blood cell count
WBC Timepoint	(MM-DD-YYYY); 00:00
CRP	mg/dL
CRP Timepoint	(MM-DD-YYYY); 00:00
ESR	mm/hr
ESR Timepoint	(MM-DD-YYYY); 00:00
PCT	ng/mL
PCT Timepoint	(MM-DD-YYYY); 00:00

15 APPENDIX D: REFERENCES

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