PREDICTION OF ADVERSE EVENTS IN CHILDREN AND ADOLESCENTS WITH CANCER AT HIGH RISK OF INFECTION (PREDSEQ)

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Protocol Summary

**Protocol:** PREDICTION OF ADVERSE CONSEQUENCES IN CHILDREN AND ADOLESCENTS WITH CANCER AT HIGH RISK OF INFECTION (PREDSEQ)

**Principal Investigator:** Joshua Wolf, MBBS

**IND Holder:** Not Applicable

**Brief Overview:** The majority of children and adolescents diagnosed with cancer will experience one or more episodes of fever or infection during their course of therapy. The most common microbiologically documented infection is bloodstream infection (BSI), which can be associated with severe sepsis or death.

Traditionally, diagnosis of BSI is made by culture of blood in growth media to determine whether pathogens are present. Although culture-based diagnostic techniques are important, they require a significant load of live bacteria in the blood, so in early bacteremia or focal infection preceding bacteremia, blood cultures are typically negative. The primary aim of this study is to determine whether metagenomic sequencing of cell free pathogen DNA could identify bacteremia prior to currently available clinical or laboratory approaches.

Delayed diagnosis and delayed optimal therapy of BSI is associated with increased morbidity and mortality, so novel diagnostic tools are being explored to speed up the identification and characterization of these infections. Diagnostic tools that enable rapid diagnosis of BSI on the first day of positive blood cultures are available or being explored.

However, even these rapid tests generally require active BSI to be positive, so severe sepsis may already be present before diagnosis. Ideally, a predictive test would identify patients with impending bloodstream infection to enable pre-emptive targeted therapy. Surveillance and pre-emptive therapy might be an alternative strategy to replace routine antibacterial prophylaxis. Although antibacterial prophylaxis is effective, it leads to high-density broad-spectrum antibiotic exposure and contributes to subsequent development of antibiotic resistance so a replacement would be of significant potential benefit.

One potential tool for this purpose is next generation sequencing (NGS) of pathogens to identify the presence of pathogen nucleic acids in sterile sites. In preliminary studies, NGS performed in parallel with blood culture appears to be sensitive and specific for diagnosis of BSI. However, testing prior to onset of bacteremia has not been assessed.

This study aims to determine whether next generation pathogen sequencing is able to identify the presence of pathogens in blood prior to the onset of clinical bloodstream infection.

Plasma samples collected but not required for clinical care (discarded samples) will be collected and stored. Results of NGS will be compared between patients who develop BSI immediately (within 72 hours) after sample collection, those who develop other infectious syndromes and those who remain well.
<table>
<thead>
<tr>
<th><strong>Protocol:</strong></th>
<th>PREDICTION OF ADVERSE CONSEQUENCES IN CHILDREN AND ADOLESCENTS WITH CANCER AT HIGH RISK OF INFECTION (PREDSEQ)</th>
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<tbody>
<tr>
<td><strong>Intervention:</strong></td>
<td>Next generation pathogen sequencing will be performed directly on plasma samples obtained for clinical care and results immediately preceding (within 72 hours) development of bloodstream infection will be compared against others.</td>
</tr>
<tr>
<td><strong>Study Design:</strong></td>
<td>Prospective cohort study; an exploratory phase will identify whether the test has potential utility, and a completion phase will estimate the sensitivity and specificity of the test.</td>
</tr>
<tr>
<td><strong>Sample Size:</strong></td>
<td>Exploratory Phase, approximately 50 participants; Completion Phase, up to 200 participants.</td>
</tr>
<tr>
<td><strong>Data Management:</strong></td>
<td>Data will be collected by staff in the Department of Infectious Diseases and maintained on a password-protected database with access limited to study staff. Only information required to perform the study will be collected.</td>
</tr>
<tr>
<td><strong>Human Subjects:</strong></td>
<td>Because this study involves collection of personal or private information there is the risk of accidental release of this information. No other serious risks have been identified. Only samples that would otherwise be discarded will be collected.</td>
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1.0 OBJECTIVES

1.1. **Primary Objective**

1.1 To estimate the sensitivity and specificity of next generation pathogen sequencing for prediction of bloodstream infection in children with cancer at high risk of infection.

1.2. **Secondary Objectives**

2.1 To describe the frequency and characteristics of infection episodes in a cohort of children with cancer at high risk of infection.

2.2 To estimate the sensitivity and specificity of next generation pathogen sequencing for prediction of any microbiologically documented infection in children with cancer at high risk of infection.

1.3. **Exploratory Objectives**

3.1 To explore the results of next generation pathogen sequencing during clinically or microbiologically documented infection or febrile neutropenia in children with cancer at high risk of infection.

3.2 To explore other alternative approaches to prediction, diagnosis or severity assessment for infectious syndromes in children with cancer at high risk of infection.

3.3 To use excess plasma from these studies to explore the frequency of somatic mutations in cell-free DNA from children with relapsed leukemia and correlate these with peripheral blood blast count and minimal residual disease levels.

2.0 BACKGROUND AND RATIONALE

2.1. **Background**

BSI is the most common microbiologically documented infectious complication of therapy for leukemia in children. [Fratino, 2005; Inaba, 2017] Microbial contamination of a central venous catheter (CVC) at the time of insertion, or during use, can lead to accumulation of organisms on the catheter, predominantly on the luminal surface, and eventually to development of symptomatic BSI. [Raad, 1993] Further, mucositis from cytotoxic chemotherapy can allow
translocation of bacteria across the oral or gastrointestinal mucosa. [Flagg, 2015; Epstein, 2016]

BSI Rates

Rates of BSI vary between institutions, CVC types and patient groups, but the problem does not appear to be eliminable. Recent studies in pediatric cancer patients show a rate of 1.6 – 2.8 BSI episodes per 1000 line days. [Allen, 2008; Henrickson, 2000; Hord, 2011] However, some patients are at much higher risk. Factors contributing to risk include profound neutropenia, external CVC, mucosal injury, bone marrow transplantation and tumor type. Patients with leukemia or relapsed malignancies are at highest risk. [Ammann, 2015] Further, organisms differ between patient populations, cancer therapy and prophylaxis regimens. Patients with relapsed leukemia are at especially high risk of viridans group streptococcal infection. [Nielsen, Med, 2015]

A number of groups of St. Jude patients have these high-risk features, including those with medulloblastoma or neuroblastoma, acute myeloid leukemia, relapsed leukemia and following bone marrow transplantation. For example, in patients with relapsed leukemia undergoing therapy for relapsed leukemia on the RELHEM, RELHEM2 and SELHEM trials, bacterial bloodstream infection was identified in 15 of 77 (19.5%) participants during their first course of experimental therapy. The initial recruitment will focus on patients with relapsed or refractory leukemia, and other populations will be accessed as needed to ensure adequate recruitment.

Mortality, Sepsis and Clinical Complications

In the pediatric oncology population, mortality attributable to BSI is rare (~2% of episodes), [Adler, 2006] but clinical sepsis occurs in 9 - 19% of episodes [Adler, 2006; Flynn, 2003; Aledo, 1998], and many patients require intensive care unit (ICU) admission. Many BSIs are related to central venous catheters, and the rate and proportion of catheter loss due to infection varies considerably between studies, depending on local salvage protocols and duration of follow-up, however rates of up to 37 - 46% are reported. [Stamou, 1999; Adler, 2006] During induction therapy for acute lymphoblastic leukemia, even patients with non-fatal bacteremia frequently have evidence of severe infection. Analysis of severity and complications of non-fatal bloodstream infections in children undergoing chemotherapy for acute lymphoblastic leukemia found that severe disease was common. [Wolf, Unpublished] Of 154 bacterial bloodstream infections identified in these participants, 34 (22.1%) had severe sepsis or septic shock, or required supplemental oxygen, fluid bolus or intensive care. Further, the risk of severe disease was highest during the reintensification phases (4/7 events, 57.1%), which most closely reflect the population that will be studied in this trial.
Length of Hospital Stay and Attributable Cost

In addition to clinical complications, the resource and financial costs of BSI in pediatric oncology patients is high. One study in pediatric oncology patients found that central-line related BSI was associated with a mean attributable cost of $69,332. [Wilson, 2014] The same study found that the mean attributable increase in length of stay was 21.2 days. This is consistent with another study of central-line related BSI in pediatric patients which did not focus on the oncology population. [Goudie, 2014] This study found a mean attributable length of stay of 19 days and cost of $55,646. Bloodstream infection is also the most expensive hospital acquired infection in adults, according to a large 2012 study, with a mean cost of $45,814. [Zimlichman, 2013]

2.2. Rationale

The most important modifiable risk factor for clinical complications and mortality in patients with sepsis is delay in administration of antibiotics. The odds of mortality from bloodstream infection rise with delay of appropriate antibiotic therapy. [Lodise, 2007] This is especially true in profoundly neutropenic patients, in whom a 24 hour delay in appropriate therapy for bloodstream infection was associated with an adjusted odds ratio of 17.2 for mortality, compared to an adjusted odds ratio of 1.75 for non-neutropenic patients. [Lin, 2008] A one hour delay in antibiotic administration for severe sepsis is associated with a 46% increase in the odds of mortality. [Sterling, 2015] An effective predictive test could eliminate delay in initiation of antibiotics by identification of patients before the onset of high-grade bacteremia and allowing pre-emptive treatment.

This study uses next generation pathogen sequencing to identify cell-free DNA in plasma samples collected as part of clinical care from children at high risk of infection. Next generation metagenomic sequencing (NGS) amplifies all available strands of pathogen genome to allow a more unbiased approach than competing technologies such as 16s rRNA or other PCR techniques. NGS from blood in patients with leukemia, using a process similar to that used in this study, has been shown to differentiate patients with fever from those without, but has not been used to diagnose undifferentiated bloodstream infection. [Gyarmati, 2016] Further, a similar technique was used to identify invasive astrovirus infection in an immunocompromised patient with encephalitis. [Naccache, 2015]

The NGS technique that will be used in this study was developed by Karius inc. by optimizing previously published methods for clinical use. [De Vlaminck, 2013, De Vlaminck 2015]. The procedure uses several parallel techniques to enrich pathogen DNA at the preparation, sequencing and bioinformatic steps of the process, allowing computational power to be focused on pathogen identification. The method has been tested for correlation with results of blood cultures and found to have reasonable sensitivity and specificity. One study of NGS using the
Karius platform showed that 80.0% of positive blood cultures were identified in samples collected on the same day. [Hong, 2016]. Specificity of the test in this setting was good (73.8%), and in patients with negative blood cultures but positive NGS testing, the same organism was often (26.3% of cases) identified from another site suggesting that ‘false positives’ are clinically relevant. Accounting for all culture-based information (including blood, respiratory tract, intraperitoneal and urinary samples) the positive and negative agreement for plasma NGS were 82.4% and 79.1%. Additional clinical information was not available to determine whether the other ‘false negatives’ were clinically consistent.

Table 1. Performance of the plasma-based next-generation sequencing assay in patients with suspected bacteremia [Hong, 2016]

<table>
<thead>
<tr>
<th>Plasma NGS</th>
<th>Blood culture</th>
<th>Any culture-based test</th>
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<tr>
<td>+</td>
<td>+</td>
<td>100</td>
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<tr>
<td></td>
<td>-</td>
<td>38</td>
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A number of clinical studies are underway to assess the performance of this assay in adult patients with cancer (NCT02912117) or undergoing hematopoietic stem cell transplantation (NCT02804464). These studies will all assess performance at the time of clinical or laboratory diagnosis of infection rather than prior to its onset.

The use of blood samples that are collected for clinical care but not required allows the study to obtain samples prior to the onset of a clinical event at regular intervals. Frequent sampling for research purposes would be an inappropriate burden, and less regular sampling would likely miss the 72 hour window period for many BSI events. However, because patients undergoing therapy for cancer have frequent collection of CBC for monitoring of hemoglobin, platelets and leukocyte counts and the majority of the sample is unused (Gawad C, personal communication) there is sufficient leftover sample on most days for testing. On average, eligible patients are expected to have blood collected on 70% of days, so missing a 72 hour window prior to a BSI event is expected to be relatively uncommon.

Bloodstream infection was chosen as the primary endpoint because it is the most common microbiologically proven infection in children with cancer, and because of the clinical ramifications described in the Background. [Inaba, 2017] Further, the sensitivity of the test might be highest for BSI because there are live bacteria in the blood at the time of diagnosis. However, because the test examines cell-free DNA, the effect of the presence of bacterial cells in the bloodstream on the sensitivity of the test is unknown.
As the performance of the test for this purpose is unknown, the exploratory phase will assess the alternate hypothesis that sensitivity is below the clinically useful range. Antibacterial prophylaxis typically reduces the risk of infection by around 50%, but risks are high. If this new test did not predict at least 30% of bloodstream infections to allow pre-emptive therapy, it would be unlikely to have a significant impact on clinical care, and we would like the test to be able to detect at least 50% of bloodstream infections. Therefore, we aim to determine whether the sensitivity of the test is within this clinically useful range. A two-stage Simon’s design was employed [Simon, 1989]

2.3. Background and Rationale for Ancillary and Exploratory Studies

A clinical description of infectious complications in a broad group of patients with relapsed leukemia is an important potential contribution. Although many studies have reported that relapsed leukemia is an important risk factor for serious infection, [Lai, 2003; Hale, 2010] there are few available data for risk stratification and determination of risk factors for infection. A Pubmed search on 11-22-16 using the search strategy “infection relapsed leukemia (Pediatric OR paediatric OR child OR infant)”, with exclusion of irrelevant, adult-only publications, HSCT-related publications and case reports, identified a number of individual trial results, but no multi-trial prospective studies aimed at identification of risk factors for serious infection in this population. The studies all showed that patients undergoing therapy for relapsed leukemia were at high risk of infection. [Ochs, 1990; Wells, 1994; Leblanc, 1994; Lockhart, 1994; Wells, 2003; Morland, 1996; Whitlock, 1997; McCarthy, 1999; Thomson, 2004; Berg, 2011; Lawson, 2000; Hijiya, 2008; Jeha, 2009; Hijiya, 2011; Messinger, 2012; Trioche, 2012] However, additional risk factors or time-periods were not reported. This study will provide information about absolute risk, risk reduction and risk stratification that can be used for clinical care and future research.

Estimation of the sensitivity and specificity of NGS for prediction of any infectious episodes in children with malignancies at high risk of infection will further assist in the determination of a possible clinical role for this test. Although some episodes of infection are associated with bloodstream infection, many are clinically or microbiologically diagnosed infection at a specific site, or fever without a clear source or etiology. In a study of children with acute lymphoblastic leukemia undergoing induction therapy, only 14.4% of episodes were associated with bloodstream infection, whereas 38.7% were other documented infections, and 48.0% were febrile neutropenia without a documented source. [Inaba, 2017] Therefore, the specificity of the test may appear very poor if accounting only for BSI. The potential value of this secondary analysis is to determine the maximum specificity of the test for infection-related episodes.

Circulating tumor DNA holds great promise as a noninvasive method for detecting and monitoring the treatment responses of malignancies [Bettegowda, 2014] Current flow cytometric and immune receptor-based methods for
monitoring minimal residual disease have suboptimal sensitivity and specificity. As high-risk and relapse leukemia patients are more likely to have high levels of residual disease during treatment, we will use this exploratory objective as a first measure of how the frequency of somatic variants in cell-free DNA correlate with standard minimal residual disease measurements and peripheral blast count. After 50 patients, if the exploratory studies provide data to support a larger study, a separate protocol will be submitted.

3.0 RESEARCH PARTICIPANT ELIGIBILITY CRITERIA AND STUDY ENROLLMENT

According to institutional and NIH policy, the study will accession research participants regardless of gender and ethnic background. Institutional experience confirms broad representation in this regard.

3.1. Inclusion Criteria

3.1.1. Under 25 years of age at time of study enrollment

3.1.2. Undergoing care for cancer at St. Jude

3.1.3. In a category of patients who are considered by the investigator to be at high risk of infection

3.1.4. Expected to receive care at St. Jude for at least 7 days

3.2. Exclusion Criteria

3.2.1. Any condition that would, in the opinion of the investigator, place the subject at an unacceptable risk of injury or render the subject unable to meet the requirements of the protocol

3.3. Research Participant Recruitment and Screening

Participants will be recruited from St. Jude Children’s Research Hospital. Potential participants will be identified by direct contact with primary clinicians, from acceptance and enrollment notices for new patients.

Based on data from 2015 – 16, it is expected that around 20 participants will be eligible for inclusion annually.

3.4. Enrollment on Study at St. Jude

A member of the study team will confirm potential participant eligibility as defined in Section 3.1-3.2, complete and sign the ‘Participant Eligibility...
Checklist’. The study team will enter the eligibility checklist information into the Patient Protocol Manager (PPM) system, and sign the completed checklist.

The CPDMO is staffed 7:30 am-5:00 pm CST, Monday through Friday. A staff member from the Milli helpline is on call Saturday, Sunday, and holidays from 8:00 am to 6:00 pm. If you have a prospective research enrollment and need assistance releasing your consent, please call the Milli helpline (901-338-0596) on call number.

3.5. Procedures for Identifying Research Participants

Potential study participants will be identified from contact with primary clinicians, protocol enrollment records and institution acceptance records. Local study staff will obtain written informed consent from the participant or their legal representative. Because of the observational nature of the trial re-consent will not be obtained at age of majority in children and adolescents.

4.0 DESIGN AND METHODS

4.1. Design and Study Overview

This pilot study is a single arm observational cohort study. Clinical data describing baseline information about the patient and malignancy, antibiotic and chemotherapy exposure, microbiology testing, hematology results and infection-related events will be collected prospectively from the electronic medical record, pharmacy and laboratory databases into a password protected database maintained by the Department of Infectious Diseases according to usual departmental protocol.

Leftover clinical blood samples collected in EDTA will be collected up to daily from the clinical hematology laboratory and processed using standard plasma collection procedures.

The blood will be spun at low speed for separation using Ficoll-Paque PLUS. This will be followed by transferring the plasma to a new tube and a high-speed spin to pellet any remaining cells. The supernatant that contains the plasma and cell-free DNA will then be transferred into aliquots that contain a maximum of 750ul prior to freezing at -80 degrees Celsius in the Gawad lab. The cells from the mononuclear cell layer will also be removed, resuspended in freezing media, and frozen in liquid nitrogen.

After identification of relevant episodes, plasma samples will be transferred to Karius for testing by sending a 750ul aliquot for each patient to be tested on dry ice. The samples will be blinded during testing to reduce the risk of bias in interpretation.
A group of samples that contain greater than 750ul of plasma or are determined by the PI not to be required for the primary infectious aims of the study will undergo standard cell-free DNA isolation, followed by library preparation and exome sequencing to identify persistent circulating somatic mutations. This will only be performed for participants who have provided prior authorization for genomic sequencing in a research context and have already undergone germline and tumor sequencing by Clinical Genomics. Thus, it is not expected that previously unidentified germline variants, necessitating reporting to participants, would be identified. Any such variants would have already been identified through sequencing performed in a CLIA setting through the Clinical Genomics Laboratory. The clinical significance of new somatic variants identified by circulating tumor DNA during treatment has not been established, so somatic variants that were not found in the diagnostic clinical sample would not be reported to participants. The allele frequency of those mutations will be correlated with persistent circulating blasts in the patient's blood and minimal residual disease levels.

We will apply Simon’s two-stage design for the primary objective. Our minimal acceptable sensitivity is 30% and favorable sensitivity is 50%. The exploratory stage is designed to ensure early trial discontinuation if the sensitivity of the test is unlikely to reach 50%. Setting the minimal acceptable sensitivity at 30% provides a 72% probability of early trial discontinuation if the true sensitivity is lower than 30%.

In this exploratory stage, we will obtain 15 BSI episodes, and if 5 or fewer correct positive diagnoses are made by NGS, we would stop the study early for futility. The completion stage is designed initially (for the first 46 participants, including the 15 from the exploratory stage) to assess whether the sensitivity of the test is greater than 50%, and then subsequently to precisely estimate the sensitivity and specificity of the test. The value of 50% as the favorable sensitivity was chosen because it represents the approximate efficacy of antibacterial prophylaxis, which has been implemented in this population. [Gafter-Gvili, 2012]

During the completion stage, we will aim to achieve a total of 100 BSI episodes. If out of the first 46 events, NGS correctly diagnoses 18 or fewer positive cultures, the NGS test does not achieve satisfactory sensitivity performance and would not warrant further investigation for this purpose. The overall target sample size is 100 BSI events with the aim of estimating the sensitivity and specificity of the test to a 95% confidence interval of ±10%, assuming a sensitivity of ~50% and specificity of 80%.

Data collection forms for infection-related complications are provided in Appendix 2.
4.2. Human Genetic Studies

Prior to cell-free DNA (cfDNA) isolation, we will examine each patient's clinical chart to insure they have been consented by the Tissue Bank, and have already consented to undergo Clinical Sequencing. Patient samples will then be anonymized using the study ID, as well as the patient and sample numbers. We will make standard sequencing libraries on cell-free DNA using protocols established in the Gawad lab. Those libraries will then undergo standard exome capture, also using protocols in the Gawad lab that are optimized for cfDNA. Sequencing will be done to 70-100X coverage by the Gawad Lab on a NextSeq instrument located in the Computational Biology Department. Sequencing reads will be aligned, followed by variant calling using the standard computational biology pipelines.

Cell-free exomes will be compared to the germline exome from the same patient using the germline exome BAM file, which will be requested through the Sequencing Data Access Committee. The standard somatic variant calling pipeline does not identify variants that are present in both the leukemia cells and germline sample. Consequently, we will not examine germline variants. It is possible we will identify somatic variants that were selected for during treatment. However, the clinical significance of those variants are not known.

4.3. Return of Incidental and Secondary Genetic Findings:

Because testing for pathogens and somatic mutations will be batched and is likely to be delayed beyond clinical usefulness, and because cell-free DNA testing has not been shown to be clinically predictive of infection or cancer outcomes in this setting, there is no plan to return the results of cell-free testing for either pathogens or somatic mutations. Further, as a result of the variant calling pipeline and because only participants who have undergone germline sequencing will be eligible for somatic mutation identification, previously unidentified germline mutations will not be discovered. The clinical value of somatic mutations identified by cfDNA sequencing that had not been identified at diagnosis is unknown, so they will not be returned.

However, in the unforeseen event that a potentially clinically significant genetic finding was identified, the principal investigator would present the finding to the Institutional Review Board and invite guidance.

5.0 REQUIRED EVALUATIONS, TESTS, AND OBSERVATIONS

Data and plasma samples will be collected throughout the study.

Data to be collected:
Baseline information about demographics, past infection-related episodes, primary disease and past treatment will be collected at the time of enrollment.

Data about antimicrobial use, including drug, route and indication will be collected for 30 days prior to study enrollment and then throughout the study.

Data about infection-related complications, including febrile neutropenia and clinically or microbiologically documented infection, and cancer outcomes will be collected for 30 days prior to study enrollment, then throughout the study.

Plasma samples will be collected directly from the clinical pathology laboratories when leftover blood is available.

Samples to be collected:

- Plasma samples will be collected daily throughout the study if leftover blood is available. No samples will be collected specifically for the study.

6.0 CRITERIA FOR REMOVAL FROM PROTOCOL

6.1. Off Study Criteria

6.1.1. All protocol interventions are complete
6.1.2. Death
6.1.3. Lost to follow-up or no longer receiving care at St. Jude
6.1.4. Request of the Patient/Parent
6.1.5. Patient no longer requires treatment for cancer
6.1.6. Discretion of the Study PI, such as the following:
   - The researcher decides that continuing in the study would be harmful
   - Blood testing is not expected to be performed at least weekly for clinical care

7.0 SAFETY AND ADVERSE EVENT REPORTING REQUIREMENTS

7.1. Reporting Adverse Experiences and Deaths to St. Jude IRB

7.1.1. Only “unanticipated problems involving risks to participants or others” referred to hereafter as “unanticipated problems” are required to be reported to the St. Jude IRB promptly, but in no event later than 10 working days after the investigator first learns of the unanticipated problem. Regardless of whether the event is internal or external (for example, an IND safety report by the sponsor pursuant to 21 CFR 312.32), only adverse events that constitute unanticipated problems are reportable to the St. Jude IRB. As further described in the definition of unanticipated problem, this includes any event that in the PI’s opinion was:
   - Unexpected (in terms of nature, severity, or frequency) given (1) the research procedures that are described in the protocol-related
documents, such as the IRB-approved research protocol and informed consent document, as well as other relevant information available about the research; (2) the observed rate of occurrence (compared to a credible baseline for comparison); and (3) the characteristics of the subject population being studied; and

- Related or possibly related to participation in the research; and
- Serious; or if not serious suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

Unrelated, expected deaths do not require reporting to the IRB. Though death is “serious”, the event must meet the other two requirements of “related or possibly related” and “unexpected/unanticipated” to be considered reportable. Deaths meeting reporting requirements are to be reported immediately to the St. Jude IRB, but in no event later than 48 hours after the investigator first learns of the death.

7.1.2. The following definitions apply with respect to reporting adverse experiences:

7.1.2.1. **Serious Adverse Event**: Any adverse event temporally associated with the subject’s participation in research that meets any of the following criteria:
- results in death;
- is life-threatening (places the subject at immediate risk of death from the event as it occurred);
- requires inpatient hospitalization or prolongation of existing hospitalization;
- results in a persistent or significant disability/incapacity;
- results in a congenital anomaly/birth defect; or
- any other adverse event that, based upon appropriate medical judgment, may jeopardize the subject’s health and may require medical or surgical intervention to prevent one of the other outcomes listed in this definition (examples of such events include: any substantial disruption of the ability to conduct normal life functions, allergic bronchospasm requiring intensive treatment in the emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse), a congenital anomaly/birth defect, secondary or concurrent cancer, medication overdose, or is any medical event which requires treatment to prevent any of the medical outcomes previously listed.

7.1.2.2. **Unexpected Adverse Event**: 
• Any adverse event for which the specificity or severity is not consistent with the protocol-related documents, including the applicable investigator brochure, IRB approved consent form, Investigational New Drug (IND) or Investigational Device Exemption (IDE) application, or other relevant sources of information, such as product labeling and package inserts; or if it does appear in such documents, an event in which the specificity, severity or duration is not consistent with the risk information included therein; or
• The observed rate of occurrence is a clinically significant increase in the expected rate (based on a credible baseline rate for comparison); or
• The occurrence is not consistent with the expected natural progression of any underlying disease, disorder, or condition of the subject(s) experiencing the adverse event and the subject’s predisposing risk factor profile for the adverse event.

7.1.2.3. **Internal Events**: Events experienced by a research participant enrolled at a site under the jurisdiction of St. Jude IRB for either multicenter or single-center research projects.

7.1.2.4. **External Events**: Events experienced by participants enrolled at a site external to the jurisdiction of the St. Jude Institutional Review Board (IRB) or in a study for which St. Jude is not the coordinating center or the IRB of record.

7.1.2.5. **Unanticipated Problem Involving Risks to Subjects or Others**: An unanticipated problem involving risks to subjects or others is an event which was not expected to occur and which increases the degree of risk posed to research participants. Such events, in general, meet all of the following criteria:
• unexpected;
• related or possibly related to participation in the research; and
• suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized. An unanticipated problem involving risk to subjects or others may exist even when actual harm does not occur to any participant.

7.1.3. Consistent with FDA and OHRP guidance on reporting unanticipated problems and adverse events to IRBs, the St. Jude IRB does not require the submission of external events, for example IND safety reports, nor is a summary of such events/reports required; however, if an event giving rise to an IND safety or other external event report constitutes an “unanticipated problem involving risks to subjects or others” it must be reported in accordance with this policy. In general, to be reportable
external events need to have implications for the conduct of the study
(for example, requiring a significant and usually safety-related change in
the protocol and/or informed consent form).

7.1.4. Although some adverse events will qualify as unanticipated problems
involving risks to subjects or others, some will not; and there may be
other unanticipated problems that go beyond the definitions of serious
and/or unexpected adverse events. Examples of unanticipated problems
involving risks to subjects or others include:

- Improperly staging a participant’s tumor resulting in the participant
  being assigned to an incorrect arm of the research study;
- The theft of a research computer containing confidential subject
  information (breach of confidentiality); and
- The contamination of a study drug.

Unanticipated problems generally will warrant consideration of
substantive changes in the research protocol or informed consent
process/document or other corrective actions in order to protect the
safety, welfare, or rights of subjects or others.

8.0 DATA COLLECTION, STUDY MONITORING, AND
CONFIDENTIALITY

8.1. Data Collection

Clinical data will be collected by study staff from the Department of Infectious
Diseases from the electronic medical record and from protocol, pharmacy,
laboratory and other institutional databases. Data will be collected on paper case
report forms, and entered into a password-protected study database. The study
database will be accessible only to study staff.

8.2. Study Monitoring

The Principal Investigator and study team are responsible for ensuring protocol
compliance. 100% of eligibility checklists will be reviewed for accuracy and
completeness by the Eligibility Coordinators. Monitoring of timeliness of serious
adverse event reporting will be done as events are reported in TRACKS.

8.3. Confidentiality

Protected health information will be obtained from participants as part of this
study, including medical record number, date of birth, and details of clinical care.
To protect participants, the research database and any data collection forms
containing protected health information will be maintained in a locked room or
file-cabinet or in a password protected file. No research participant names will be
recorded on data collection forms. A unique patient identifier will be used instead
of the medical record number in all other documentation. The list containing the
link between study number and medical record number will be maintained in a locked file. All potentially identifying participant information will be accessible only by study staff. The medical records of study participants may be reviewed by the St. Jude IRB, FDA, clinical research monitors, etc.

8.4. Data Deposition

Anonymized cfDNA exome data (BAM or FASTQ files) will be deposited in accordance with practices established by the St. Jude Pediatric Cancer Genome Project and in accordance with the NIH Genomic Data Sharing (GDS) policy. Human exome data that would have only been generated from patients with a Tissue Bank consent will be deposited and maintained in the Short Read Archive at NCBI. To facilitate data sharing and because exome data is not identifiable, we will not place it in a database with more restricted access, such as dbGaP.

9.0 STATISTICAL CONSIDERATIONS

Sample size considerations are outlined in Section 4.1.

9.1. Primary Objective

To estimate the sensitivity and specificity of next generation pathogen sequencing for prediction of bloodstream infection in children with cancer at high risk of infection.

Sensitivity is defined as the proportion of NGS positive results in all positive BSI cultures. Specificity is defined as the proportion as NGS negative results in all negative BSI cultures. Proportions and 95% confidence intervals will be reported.

9.2. Secondary Objectives

9.2.1 To describe the frequency and characteristics of infection episodes in a cohort of children with cancer at high risk of infection.

Frequency of infection episodes will be summarized. Characteristics of those episodes will be reported with appropriate descriptive statistics (frequency and proportion for categorical variables and mean/median etc. for continuous variables) and risk factors for infection identified by univariate and multivariate testing as appropriate.

9.2.2 To estimate the sensitivity and specificity of next generation pathogen sequencing for prediction of any microbiologically documented infection in children with cancer at high risk of infection.
Sensitivity and specificity of NGS predicting any microbiologically documented infections will be reported as proportions along with 95% confidence intervals.

9.3. Exploratory Objectives

9.3.1 To explore the results of next generation pathogen sequencing during clinically or microbiologically documented infection or febrile neutropenia in children with cancer at high risk of infection.

Sensitivity and specificity of NGS predicting any clinically or microbiologically documented infections or febrile neutropenia will be reported as proportions along with 95% confidence intervals.

9.3.2 To explore other alternative approaches to prediction, diagnosis or severity assessment for infectious syndromes in children with cancer at high risk of infection.

Sensitivity and specificity of alternative approaches predicting, diagnosing or assessing severity for infectious syndromes will be reported as proportions along with 95% confidence intervals. Multivariate logistic regression may be explored to assess the impact of identified risk factors on prediction, diagnosis or severity of infectious syndromes.

9.3.3 To explore the frequency of somatic mutations in cell free DNA from children with high-risk leukemia and correlate these with peripheral blood blast count and minimal residual disease levels.

Frequency of somatic mutations will be summarized and correlation coefficient will be reported with peripheral blood blast count and minimal residual disease levels.

9.1. Anticipated Completion Dates

<table>
<thead>
<tr>
<th>Anticipated Primary Completion Date: December 31, 2020</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anticipated Study Completion Date: December 31, 2021</td>
</tr>
</tbody>
</table>
9.2. Summary of Primary and Secondary Objectives

Primary and Secondary Summary of Objectives

<table>
<thead>
<tr>
<th>Objective #</th>
<th>Objective Type</th>
<th>Analysis #</th>
<th>Resp Party</th>
<th>Stat</th>
<th>Safety</th>
<th>Analysis Measure</th>
<th>Analysis Title</th>
<th>Data Collection Time Frame</th>
<th># of Participants</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>P</td>
<td>3</td>
<td>JW</td>
<td>LT</td>
<td>N</td>
<td>Episode</td>
<td>Estimate the sensitivity and specificity of next generation pathogen sequencing for BSI</td>
<td>Throughout study</td>
<td>Up to 100</td>
</tr>
<tr>
<td>2.1</td>
<td>S</td>
<td>1</td>
<td>JW</td>
<td>LT</td>
<td>N</td>
<td>Episode</td>
<td>Describe frequency and characteristics of infection</td>
<td>Throughout study</td>
<td>Up to 200</td>
</tr>
<tr>
<td>2.2</td>
<td>S</td>
<td>1</td>
<td>JW</td>
<td>LT</td>
<td>N</td>
<td>Episode</td>
<td>Estimate sensitivity and specificity of next generation pathogen sequencing for prediction of any infection</td>
<td>Throughout study</td>
<td>Up to 200</td>
</tr>
<tr>
<td>3.1</td>
<td>S</td>
<td>1</td>
<td>JW</td>
<td>LT</td>
<td>N</td>
<td>Episode</td>
<td>Explore results of next generation pathogen sequencing during documented infection</td>
<td>Throughout study</td>
<td>Up to 200</td>
</tr>
<tr>
<td>3.2</td>
<td>S</td>
<td>As needed</td>
<td>JW</td>
<td>LT</td>
<td>N</td>
<td>Episode</td>
<td>Explore alternative approaches to prediction, diagnosis or severity assessment for infection</td>
<td>Throughout study</td>
<td>Up to 200</td>
</tr>
<tr>
<td>3.3</td>
<td>S</td>
<td>As needed</td>
<td>CG</td>
<td>LT</td>
<td>N</td>
<td>Episode</td>
<td>Explore frequency of resistance mutations in cell free DNA from children with high-risk leukemia</td>
<td>Throughout study</td>
<td>Up to 200</td>
</tr>
</tbody>
</table>
10.0 OBTAINING INFORMED CONSENT

Eligible subjects will first be approached by the patient’s primary physician or a member of the study team regarding the study purpose, methods and design details. Consent procedures will be completed in a private room and following St. Jude Children's Research Hospital institutional guidelines. Written assent will be obtained from participants 14 to less than 18 years old, but formal assent will not be sought from younger children. The consent/assent process will be documented in the medical record per institutional guidelines. Research participants and parents may decline participation without any negative repercussions. Declinations will be documented in the research records and examined for any possible patterns.

All research participants who meet eligibility criteria regardless of gender or minority status are fully eligible to participate in this study. All data will be kept confidential and stored in locked offices.

10.1 Consent When English is Not the Primary Language

When English is not the patient, parent, or legally authorized representative’s primary language, the Social Work department will determine the need for an interpreter. This information will be documented in the study database. Either a certified interpreter or the telephone interpreter’s service will be used to translate the consent information. The process for obtaining an interpreter and for the appropriate use of an interpreter is outlined on the Interpreter Services, OHSP, and CPDMO websites.
11.0 REFERENCES


APPENDICES

APPENDIX I: SCHEDULE OF EVALUATIONS

<table>
<thead>
<tr>
<th>Event</th>
<th>Study Entry</th>
<th>During study (Weekly)</th>
<th>Collection of final sample +7 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Data collection</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Demographic data</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Past history (30 days)</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infectious episodes</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Antibiotic exposure</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Sample collection</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma samples</td>
<td>X&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Up to daily when leftover samples available.
## APPENDIX II: RESEARCH TESTS

<table>
<thead>
<tr>
<th>Research Test</th>
<th>Course</th>
<th>Required</th>
</tr>
</thead>
<tbody>
<tr>
<td>Next generation plasma pathogen sequencing</td>
<td>Prior to and during each infectious episode</td>
<td>Required</td>
</tr>
<tr>
<td>Cell free DNA disease monitoring</td>
<td>Prior to and during each cancer-related episode</td>
<td>Required</td>
</tr>
</tbody>
</table>
APPENDIX IV: SAMPLE PROCESSING

A running list of participants in the study will be maintained by the study team.

At the end of each business day, a staff member from Gawad laboratory will go to the clinical lab to collect available samples for participants that were collected in EDTA or other heparin-free tubes.

The samples will be taken to the Gawad lab where they will first be centrifuged at 1,600 x g for 10 minutes. The plasma will then be transferred to a second tube which will be spun at 16,000 x g for another ten minutes. The supernatant will be removed and transferred in 750ul aliquots to new microcentrifuge tubes.

The samples will be deidentified at that time prior to placing a computer-generated label. The samples will be stored in a -80 freezer in the Gawad lab prior to shipping to Karius.

Dr. Gawad will keep the key for the samples in a locked desk in his office, and patient labels will be destroyed. Dr. Wolf will lead all clinical outcome analyses, so he will not have access to the patient key. Information on samples being included in the study will be given in batches of a minimum of ten samples to make sure those acquiring outcome data will remain blinded.

Samples for NGS analysis will be transferred to Karius inc. for testing. One 750ul aliquot of each sample will be sent to:

David Hong, MD
Karius Inc.
1505A Adams Drive
Menlo Park, CA 94025

Karius will perform nucleic acid isolation, sequencing, and data analysis using their CLIA-approved clinical pipeline. They will provide a list of microbes identified in each sample to the investigators.

Aliquots of plasma not used in the study will undergo standard cell-free DNA isolation, library preparation, exome capture, sequencing, and analysis in the Gawad lab. Funds for these experiments have been awarded with grants from the Hyundai Foundation for Pediatric Cancer Research and St. Jude Cancer Center Developmental Funds.

After completion of all study procedures and assessments, any unused samples will be placed in an institutional sample bank for use in future IRB approved studies.