Matched Targeted Therapy (MTT) Recommendation for Patients with Recurrent, Refractory, or High Risk Leukemias and Myelodysplastic Syndrome

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1.0 ABSTRACT

We hypothesize that it will be feasible to identify actionable genomic alterations and make targeted treatment recommendations for children, adolescents and young adults with relapsed/refractory/high-risk leukemia and myelodysplastic syndrome (MDS). This protocol will identify actionable mutations in two cohorts of patients using a cancer-focused sequencing panel for all patients and evaluation of a panel of fusion genes in patients with acute lymphoblastic leukemia (ALL). Cohort 1 will include patients with relapsed/refractory leukemia or MDS and cohort 2 will include patients with newly diagnosed "high-risk" leukemias, such as acute myeloid leukemia (AML), juvenile myelomonocytic leukemia JMML), leukemia of ambiguous lineage and subsets of patients with ALL with poor prognosis (e.g., young infants with MLL rearrangement, those with low hypodiploidy). We will 1) determine whether it is feasible to identify actionable genomic alterations and match these with a targeted therapy for pediatric patients with recurrent/refractory and high risk leukemia or MDS; 2) describe the range of somatic genomic alterations identified using a cancer-focused sequencing panel in pediatric patients with recurrent/refractory and high risk leukemia or MDS; 3) describe the time from sample receipt to results reporting and interpretation of genomic data when done in a research context and factors that impact this time interval; 4) explore whether patients are able to receive matched targeted therapy and the barriers that prevent them from receiving it; and 5) explore the ability to create xenograft models of leukemia, use these to measure response to the matched targeted therapy, and determine if these models can be used to potentially inform clinical decisions. Results from this study will inform the development of targeted therapy for pediatric and young adult patients with leukemia and the design of "basket" trials in the future.

2.0 BACKGROUND/RATIONALE

2.1 PROBLEM AND RATIONALE

Significant advances have been made in the treatment of pediatric acute lymphoblastic leukemia (ALL), with standard therapy resulting in cure in 80-90% of the patients. For pediatric patients with recurrent or refractory ALL, outcome remains dismal. Additionally, there are subsets of patients with high-risk ALL, such as patients with hypodiploidy and infants with MLL-rearranged ALL, for whom standard therapy is less likely to result in a long-term cure. Less progress has been made in the treatment of pediatric acute myeloid leukemia (AML), where long-term survival remains at about 50%. Myelodysplastic syndrome (MDS) accounts for less than 5% of pediatric hematological malignancies with stem cell transplant being the only curative option.[1,2]

The standard approach has been to use intensive cytotoxic chemotherapy to induce remission. Emergence of knowledge surrounding activating mutations in leukemia has slowly been changing this paradigm. For example, the BCR-ABL translocation resulting in a constitutively active ABL kinase, first discovered and successfully targeted in chronic myelogenous leukemia (CML), is also found in ALL and is considered to be a high-risk feature. Patients with ALL harboring the BCR-ABL translocation have, until very recently, been treated with chemotherapy followed by stem cell transplant. The COG study AALL0031 revealed that the addition of imatinib, a BCR-ABL inhibitor, to an aggressive chemotherapy regimen improved survival in pediatric patients and avoided the toxicity of stem cell transplant, changing the treatment paradigm for this high risk disease[3,4]. Recently, another subtype of ALL, Philadelphia-like ALL (Ph-like ALL), has been identified by a gene expression profile similar to that of BCR-ABL-positive ALL[5–7]. This subtype has been associated with an inferior prognosis[5,7,8]. Kinase activating mutations have been found in up to 91% of Ph-like ALL patients, raising the possibility that the integration of a tyrosine kinase inhibitor for treatment of these patients may improve their outcome.[8] Genomic analysis of hypodiploid ALL found mutations in genes activating the Ras pathway signaling pathway and also activation of the Ras/PI3K signaling pathway in the absence of known activating mutations[9]. Recent sequencing of pediatric MDS showed that Ras pathway mutations are very common in this subset of patients, with 45% of patients in the primary cohort with mutations in this pathway[2].

The increasing identification of targetable genomic alterations in cancer has spurred development of targeted therapy. Genotype selection of patients for therapy has changed treatment in many solid tumors, such as *EGFR* and *ALK*-mutated non-small cell lung cancer, HER2 positive breast cancer, or *KIT*-mutated Gastrointestinal Stromal Tumors (GIST) [10–16]. In leukemia, CML treatment with tyrosine kinase inhibitors (TKI) dramatically altered the approach to and treatment of this disease[17–19]. While targeted therapies are actively being integrated into the clinical arena, these drugs are generally much less available for the pediatric population.

With an increasing understanding of the genomic alterations in leukemia, it will be essential to match an individual alteration with a targeted therapy. This study is a first step into the rigorous characterization of the genomic alterations present in pediatric patients with rare, high risk or relapsed/refractory leukemias as a group, with the goal of matching targetable lesions with potentially available therapies. Functional assays of drug sensitivity in vitro will yield information regarding sensitivity of individual leukemia cells to particular targeted therapy, to correlate with known identified mutations or yield information regarding activating pathways where mutations are not identified. In addition, we will use murine xenograft models derived from an individual patient's leukemia to study the effects of matched targeted therapies in the mouse, particularly when these therapies are not yet available for testing in the patient. The results of this trial and the correlative laboratory studies, whether positive or negative, will inform our understanding of the genomic landscape of very high risk pediatric leukemias and the design of future clinical investigations using genomic characterization to personalize leukemia treatment for patients most affected.

2.2 BACKGROUND

2.2.1 Matching genomic alterations to targeted therapy: A treatment paradigm with demonstrated success in leukemias

The identification of BCR-ABL fusion in patients with CML and the subsequent identification of imatinib as an ABL inhibitor drastically altered the treatment of this disease, reducing toxicity of therapy and dramatically prolonging relapse-free survival. Similarly, identification of BCR-ABL fusions in patients with ALL and the ability to integrate imatinib, or the later generation TKIs, into a chemotherapy backbone has also altered treatment and prognosis of this high-risk leukemia. For pediatric Ph-like B-ALL, a subtype approximately three times larger than Ph+ ALL, with a particularly poor prognosis, a large proportion of patients have been identified as having mutations resulting in constitutively active tyrosine kinases, such as ABL1, ABL2, CSF1R, PDGRB, JAK2, that could be potentially targeted by TKIs[8]. More recently, identification of *IDH1/2* mutations in adult AML has led to the development of IDH mutant inhibitors, which are already showing efficacy in Phase 1 trials[20,21]. With constantly emerging leukemia genomic information, the rate of targeted therapy development is also increasing.

For pediatric patients with solid tumors, Dr. Katherine Janeway led and completed a multi-institutional feasibility study to identify potentially targetable variants and make an individualized cancer therapy (iCat) recommendation. Tumor profiling consisted of mutation detection with a targeted sequencing assay (OncoPanel), copy number assessment with array CGH (aCGH) and, in some cases, IHC and FISH validation of genetic findings. Tumor profiling results were reviewed by a panel of experts in pediatric oncology, pathology, genetics, molecular and cancer biology, and developmental therapeutics. iCat recommendations were made if a potentially targetable variant was present and a targeted drug was available via a clinical trial or as an FDA approved drug with an age-appropriate dose and formulation. Recommendations were tiered from 1 (strongest) to 5 (weakest) based upon strength of supporting evidence. With 4 participating institutions, 100 patients were enrolled in 14 months. The most common diagnoses were neuroblastoma, Ewing sarcoma, rhabdomyosarcoma and osteosarcoma though 35% of

patients had rare solid tumors. With 100 patients having completed testing and review to date, such a study in hematologic malignancies has not been done.

2.2.2 Technologies for identification of targetable alterations in individual leukemia cases.

Standard technologies utilized to characterize leukemia for diagnostic purposes and basic research include pathologist evaluation, flow cytometry, cytogenetics, fluorescence in situ hybridization (FISH) and candidate gene sequencing. Several newer technologies permit more extensive characterization than has previously been possible. Rapid Heme Panel (RHP) is a platform developed by Drs. Frank Kuo and Neal Lindeman at the Brigham and Women's Hospital. This platform uses next-generation sequencing technology to sequence regions in approximately 95 genes in which alterations have been previously associated with leukemia. RHP can be performed utilizing DNA derived from fresh or frozen leukemia cells or already extracted DNA. Goal turnaround time for this test is under 5 days, allowing for time-sensitive clinical decisions to be made based on this test. The rapid turnaround also allows for sequential assessments of patients receiving targeted therapy or with progression of disease. RHP was developed and is run in a CLIA (Clinical Laboratories Improvement Amendments) certified lab within the Center for Advanced Molecular Diagnostics (CAMD) at the Brigham and Women's Hospital. It is commercially available for patients and clinical decisions today.

Although the cure rate for childhood acute lymphoblastic leukemia (ALL) is 80-90%, a subset of patients were recently identified in Children's Oncology Group (COG) studies to have a much worse prognosis. These individuals have a Philadelphia chromosome-like (Ph-like or *BCR-ABL1*-like) gene expression profile, but lack the classical, Philadelphia t(9;22) chromosome translocation fusion. Many of the identified alternate fusion rearrangements involve genes encoding for kinases (e.g., *ABL1, JAK2,* and *PDGFRB*) that induce malignant transformation by fusing with novel translocation partner genes. Over the course of this study, several CLIA certified laboratories, such as Nationwide children's hospital, Boston Children's, Mass General, MSK and Children's Hospital of Philadelphia, have developed RNA-based targeted fusion assays for identification of oncogenic fusions. Although these assays are limited to the targets on the selected panels, these have greatly expanded our ability to detect targetable fusions. We are now in the early days of clinical RNASeq implementation, which is unbiased and can potentially identify other targets. However, RNAseq analysis for fusions can be difficult and further correlation between RNASeq and targeted fusions is to inform clinical implementation.

2.2.3 Need for and availability of pre-clinical biology studies in leukemia

Though specific genomic alterations often predict sensitivity to a matched targeted therapy, this is not always the case. For example, patients with chronic neutrophil leukemia or atypical CML harboring *CSF3R* mutations may have sensitivity to dasatanib or JAK inhibitors depending on the location of the mutation[22]. In the case of targeted therapy resistance in solid tumors, assessment of patient tissue often does not point to mutations that account for the resistance[23]. Leukemia samples offer a unique opportunity for functional assessment of sensitivity to a particular inhibitor. Assays are currently available to assess response of primary leukemia cells to a panel of drugs, by viability or ability to trigger an apoptotic program (BH3 profiling), or to a panel of targeted shRNAs. Leukemia blasts can be easily isolated from bone marrow or peripheral blood samples using Ficoll separation. Blasts can be incubated in media with various concentrations of a panel of drugs and viability assessed after 3 days of exposure by a cell proliferation assay[24–26]. Additionally, BH3 profiling may be used to assess the ability of a panel of drugs to prime leukemia cell for apoptosis. This property has been correlated with chemotherapy sensitivity *in vivo*[27,28]. These functional assessments can identify a potential therapy in the absence of an actionable genetic alteration and potentially bolster our ability to match individual biology to a targeted therapy. An understanding of the correlation between known genetic alterations and the response of the leukemia cells to the particular inhibitor is also critical.

2.2.4 Germline assessment in patients with leukemia: what is the proper sample in the pediatric population?

A variety of specimens have been used to extract germline DNA, including buccal swabs, saliva samples, fibroblasts derived from skin biopsies and bone marrow, remission bone marrow samples and fingernail clippings. In the pediatric population, the ability to obtain a specific sample, particularly at the time of diagnosis, may be complicated by the 1) ability to obtain consent for an additional procedure such as a skin biopsy or 2) patient ability to cooperate with the sample collection such as a saliva collection. At the time of leukemia diagnosis, buccal swabs and saliva may be contaminated with patient leukemia cells. Additionally, buccal swabs may be contaminated by non-human DNA[29]. Use of fibroblasts or remission marrow will delay the timing for getting the germline sample by 4-6 weeks. Thus, assessment of a variety of samples to provide for a timely evaluation, without contamination and avoiding additional procedures, is necessary.

2.2.5 Clonal evolution in leukemia

The advent of sequencing has allowed further appreciation of the genetic heterogeneity and clonal evolution of leukemia. Analysis of matched leukemia samples at diagnosis and relapse shows both acquisition of new mutations at the time of relapse, and loss of leukemia specific mutations from diagnosis[30–35]. Deep sequencing often reveals the presence of the predominant mutation from the relapse specimen to be present as a minor population in the diagnostic sample. Clonal evolution may also be influenced by selective pressure imparted by chemotherapy or targeted therapy. Mutations in *NT5C2*, encoding a 5'-nucleotidase, are found in ALL at the time of relapse and confer resistance to mercaptopurine and thioguanine[36,37]. Clonal architecture varies during leukemia progression and fitness of specific clones may also vary. For example, Klco et al. showed that AML subclones have variable ability to establish disease in a xenograft model[34]. Further understanding of this process, especially during the use of targeted therapy, will enhance our ability to deliver effective, curative therapy in the future.

2.2.6 Patient/parent understanding of and perspectives on participation in leukemia genomics research

Previous research has shown that patients and families often conflate the goals of clinical care and research and have unrealistic expectations of early phase clinical trials[38]. Genomic data and the inherent uncertainty associated with it only further complicate this issue[39–41]. As part of a recent study out of the Dana-Farber / Boston Children's Cancer Center (coined iCat1), patients and parents were queried on their perspectives following receipt of a treatment recommendation based on genomic profiling of pediatric solid tumors. This pilot survey addressed domains including: 1) understanding of genetics and genomic testing; 2) hopes / expectations for testing; 3) concerns about testing; 4) preferences regarding return of results; and 5) non-clinical impact of the testing on the subject and his/her family. Preliminary analysis has shown that, though significant variability exists, the majority of participants report a positive impact of participation, even in the absence of actionable results (communication from iCat study, Dr. K. Janeway and Dr. J. Marron). Reported positive effects include providing hope, the feeling of having done everything, and a sense of satisfaction from having helped other children with cancer. No data currently exists regarding these issues in pediatric patients with leukemia.

2.3 POTENTIAL BENEFITS TO SUBJECTS AND/OR SOCIETY

While technological advances are resulting in ever increasing possibilities for tumor profiling, research paradigms for studying the clinical utility of large-scale leukemia genomic profiling and a matched targeted therapy approach to treatment in the setting of high risk/recurrent/refractory leukemia or rare subtypes without a clear treatment, are lacking. If a reasonable proportion of participants can receive a matched targeted therapy recommendation within this study we will demonstrate that, using the profiling technologies currently available,

a personalized approach to treatment is feasible in recurrent / refractory / high-risk leukemia in pediatric participants.

The result of this trial would have implications for continued development and testing of targeted therapy in pediatric patients. If this study is feasible, future studies could then be designed to more rigorously evaluate the efficacy of a personalized approach to leukemia therapy. Systematic identification of genetic alterations and identification of activated pathways will also inform development of future "basket trials" where several different therapies are offered under the umbrella of a single clinical protocol.

This will be the first attempt to broadly characterize the range of actionable alterations present in recurrent / refractory / high-risk pediatric patients with leukemia. The actionable alterations identified in this study and the follow-up laboratory investigation promoted by those results have the potential to add greatly to our understanding of the oncogenic mechanisms in recurrent / refractory / high-risk leukemia. This may help to inform future drug development and future clinical trials.

This study has the potential to help individual children enrolled on the trial who may potentially receive matched therapy based on the identified genomic alteration. Because we plan to follow the outcome of participants who receive treatment based on the therapy recommendation, we will be able to explore whether the type of personalized cancer therapy studied here has the potential to impact the outcome of pediatric participants with recurrent / refractory leukemias.

Evaluation of germline sequence is critical for advanced sequencing efforts. For patients with leukemia, germline DNA sources used for patients with solid tumors, such as buccal swabs and saliva, may be contaminated with leukemia at the time of diagnosis. Exploration of the use of other sources of germline DNA, such as skin or bone marrow derived fibroblasts or fingernail clippings, will help guide future clinical sequencing efforts in patients with leukemia.

Given that tumor profiling is likely to only become more important in the diagnosis and management of recurrent / refractory / high-risk pediatric leukemia patients in the future, it is important that we learn how patients and parents understand this type of testing so that we can improve informed consent practices. This study will provide information from patients and parents themselves to enable us to better recognize gaps in understanding so that we can identify ways to address these and improve patient and parent assent / consent for future genomic tumor profiling and treatment protocols.

3.0 OBJECTIVES/STUDY AIMS

3.1 HYPOTHESIS

It will be feasible to identify an actionable alteration with a matched targeted therapy for pediatric participants with recurrent / refractory / high-risk de novo leukemia and myelodysplastic syndrome.

3.2 PRIMARY OBJECTIVE

To determine whether it is feasible to identify actionable alterations with a matched targeted therapy for pediatric participants with recurrent / refractory / high-risk de novo leukemia and myelodysplastic syndrome.

3.3 SECONDARY OBJECTIVES

3.3.1 Describe the range of somatic genomic alterations identified using a leukemia-focused sequencing panel in pediatric patients with recurrent / refractory / high-risk de novo leukemia and myelodysplastic syndrome.

3.3.2 Describe the time between receipt of sample and results reporting and interpretation of genomic data when performed in a research context and explore the factors that impact this time interval

3.3.3 Analyze the hopes and concerns of parents of children with recurrent / refractory / high- risk de novo leukemia or myelodysplastic syndrome regarding genomic testing of their child's leukemia as well as their understanding of the testing and evaluate whether the hopes and concerns were realized following return of results.

3.3.4 Study the feasibility and utility of pre-clinical studies including testing primary leukemia sensitivity testing to a panel of drugs or shRNA and the creation of xenograft models of leukemia and measure response to genomic-based recommendations in these models of leukemia.

3.4 EXPLORATORY OBJECTIVES

3.4.1 Explore whether patients received targeted therapy matched to an actionable alteration recommended by the expert review panel and explore reasons that a drug was not received.

3.4.2 Explore whether actionable alterations can be identified using technology beyond the targeted cancer gene panel(s).

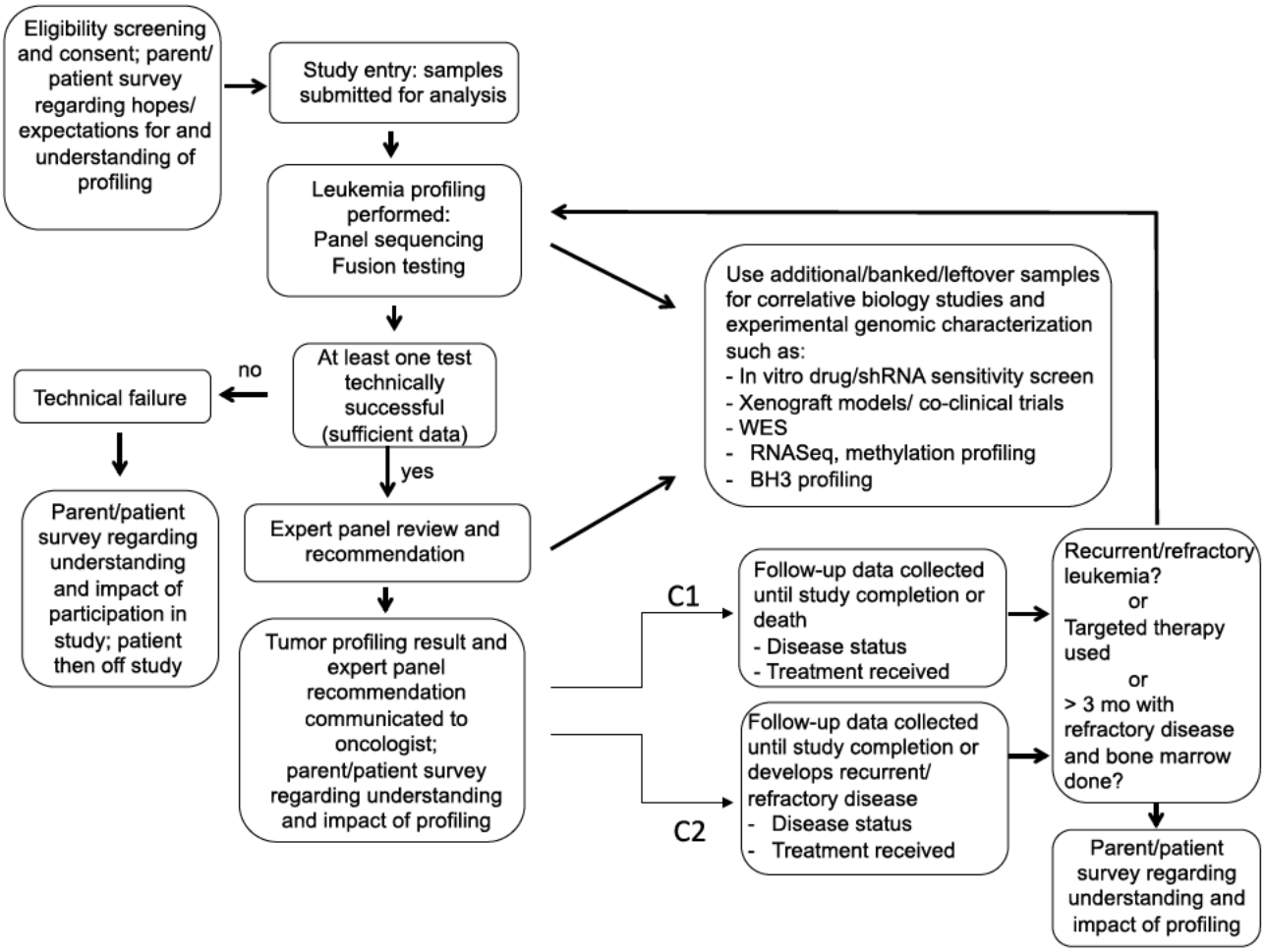
3.4.3 Explore the different sample types (e.g., buccal swab, saliva, nail clippings, bone marrow- and skin-derived fibroblasts, remission bone marrow) that may be used to assess germline status in patients with relapsed/refractory/high-risk leukemia or myelodysplastic syndrome.

3.4.4 Evaluate whether relapsed/refractory/high-risk pediatric leukemias or myelodysplastic syndrome acquire altered clonal predominance and additional actionable alterations during disease progression or during treatment with a matched therapy.

3.4.5 To store leukemia material, derived xenografts, DNA derived from buccal swabs/saliva/nail clippings/ bone marrow- and skin-derived fibroblasts, and remission bone marrow samples for potential future research.

3.5 STUDY DESIGN AND SCHEMA

This study is a multi-center, non-therapeutic trial to determine the feasibility of identifying an actionable genomic alteration and making a matched targeted therapy treatment recommendation.



C1 = Cohort 1:

- Acute lymphoblastic leukemia, first or greater relapse
- Acute myeloid leukemia, first or greater relapse
- Leukemia refractory to induction chemotherapy
- Other recurrent leukemia
- Myelodysplastic syndrome (MDS), first or greater relapse, or refractory to initial therapy

C2 = Cohort 2-closed to accrual as of 11/18/2019

- Acute myeloid leukemia, new diagnosis
- New diagnosis infant MLL-rearranged ALL or low hypodiploid ALL
- Rare leukemia- e.g., JMML, leukemia of ambiguous lineage
- Secondary leukemia
- Myelodysplastic syndrome (MDS)

4.0 ELIGIBILITY

4.1 INCLUSION CRITERIA

4.1.1 Age

Age birth to ≤ 30 years at study entry

4.1.2 Diagnosis: Patients will be enrolled in one of the two cohorts based on diagnosis:

Cohort 1: Relapsed/refractory leukemia

- Acute lymphoblastic leukemia, first or greater relapse
- Acute myeloid leukemia, first or greater relapse
- Leukemia refractory to induction chemotherapy
- Other recurrent leukemia
- Myelodysplastic syndrome (MDS), first or greater relapse, or refractory to initial therapy

Cohort 2: New diagnosis- closed to accrual as of 11/18/2019

- Acute myeloid leukemia, new diagnosis
- New diagnosis infant *MLL*-rearranged ALL or low hypodiploid (<40 chromosomes) ALL
- Rare leukemia- e.g., JMML, leukemia of ambiguous lineage
- Secondary leukemia
- Myelodysplastic syndrome (MDS)

4.1.3 Pathologic Criteria

Histologic confirmation of leukemia or myelodysplastic syndrome (MDS) at the time of diagnosis or recurrence

4.1.4 Specimen Samples

Sufficient leukemia or MDS specimen available for profiling from diagnosis or recurrence OR bone marrow aspirate or blood draw planned for clinical care anticipated to allow collection of minimum specimen for testing (See Sect.8.1 for description of specimen requirements); OR clinical tumor profiling using Rapid Heme Panel available in the medical record.

4.2 EXCLUSION CRITERIA

4.2.1 Insufficient leukemia or MDS specimen available for profiling from recurrence or bone marrow evaluations NOT planned for clinical care; or peripheral blast percentage <20% AND clinical blood draw not planned; or patient sample did NOT already complete Rapid Heme Panel leukemia profiling clinically (See Sect. 8.1)

5.0 SUBJECT ENROLLMENT

5.1 SUBJECT IDENTIFICATION

Potential subjects will be identified by the participant's treating physician during routine clinical care. In addition, potential subjects will also be identified through self-referral.

5.2 SUBJECT RECRUITMENT

Potential participants will be identified by their primary physician and referred to the study team at the appropriate participating institution for enrollment and education. In order to inform physicians about this project, the protocol will be presented at scientific and protocol conferences at the participating institutions in order to educate potential referring physicians of the protocol's existence. The protocol may be presented at scientific conferences and at grand rounds at regional institutions in order to educate potential referring physicians of the protocol's existence. The study will also be registered on clinicaltrials.gov. Patients who read this internet posting may self-refer. Self-referred patients will then be referred to the DFCI study team by their treating physician or through direct communication with the DFCI study team (contact information on clinicaltrials.gov). Remote consent will be conducted. If they are a patient at one of the participating institutions, they will be referred to the appropriate contact there for screening and enrollment. Otherwise, the protocol will not be advertised directly to patients and direct patient recruitment materials will not be created for this project.

5.3 ELIGIBILITY SCREENING

Eligibility screening will be conducted by qualified clinical research personnel. Every potentially eligible participant will be seen at one of the participating sites prior to enrollment unless the participant has self-referred or are referred by their physician and is not a patient at a participating institution. A complete assessment will be performed by qualified clinical research personnel to determine eligibility. This will be completed remotely by one of the participating sites if the patient is not a patient at one of those sites.

Screening will involve the following:

- Review of the participant's medical record
- If necessary, review of existing pathology slides documenting recurrent / refractory leukemia or MDS
- History and physical examination

Screening for referral participants will be the same above with the following modifications:

- The patient will not be required to have a history and physical exam at one of the participating sites. Review of the last clinical note of patient history and physical exam. The note needs to be dated within 90 days of screening.
- A full review of the eligibility documentation will be completed by an Investigator at one of the participating sites. That Investigator will be the enrolling physician for the participant.

5.4 SUBJECT CONSENT

The Principal Investigator, the Co-Investigators of this project, and/or the treating physician may offer eligible participants the opportunity to participate. Before issuing an invitation to participate, the Principal Investigator, the Co-Investigators, and/or the treating physician will provide a full explanation of this protocol to the potential subject and a parent or legal guardian (if subject less than 18), review the consent form with the potential subject and a parent or legal guardian (if subject less than 18), and answer any and all associated questions according to institutional and federal guidelines. Subjects and or their parents or legal guardian electing to participate in the study must sign the informed consent form following guidelines of the IRB at the enrolling Institution. At the DFCI and where local IRB guidelines do not specify, the procedures detailed in the remainder of this paragraph will be followed. Those subjects who elect to participate in the study must sign the informed consent form. For DFCI patients, Adobe Sign can be used as part of a remote consent process to sign the informed consent form for patients who are less than 10 years old or 18 years and older. For participants who are at an appropriate developmental age to provide assent, a parent or legal guardian must sign the consent form and the participant must assent to participation according to local institutional guidelines. For participants whose developmental age is not appropriate to provide assent, assent may be waived and a parent or legal

guardian must sign the consent form. Waiver of assent for participants whose developmental age is not appropriate for assent includes those patients who are of chronological age to assent but who have developmental delay or other neurologic condition. It should be determined by each investigator whether or not a waiver of consent is appropriate to the individual under consideration for entry onto the study.

Subjects who consent will be either given or mailed a signed copy of the form for their records. Subjects who request additional time to consider participation will be provided with a mailed or emailed copy of the consent form.
For potential participants who are not patients at a participating institution, Investigators and delegated staff who are genetic counselors who are trained and delegated to consent at trial study sites may consent the patient remotely via phone. Initially, the participant and/or their physician should express their interest in the study via email to one of the trial study sites after obtaining permission from the patient to do so. An intake questionnaire will then be sent to the patient in order to verify basic criteria of eligibility and provide a blank copy of the consent form and the patient-physician enrollment form (Appendix 18). Study staff may speak with, email, and/or send a letter to a physician directly for a patient who independently self-refers so that the primary physician listed on the intake form will be informed that their patient is interested in participating in this study (Appendix 22). Eligibility and availability and use of specimens for molecular profiling will be discussed. The primary physician will be asked to document their understanding of the project and agreement with receiving the MTT report if successful by completing the Physician section of the patient-physician enrollment form.

Once approval from the primary physician is confirmed, a time for a phone call consent discussion will be initiated with the participant. The consenting Investigator will initiate the consent discussion over the phone. Adobe Sign can be used as part of a remote consent process to sign the informed consent form for patients who are <10 years old or ≥18 years old. After the patient emails or mails the signed consent and fully completed patient-physician enrollment forms back to the enrolling study site, the patient will be assessed for eligibility as outlined above. If enrolled on the protocol, the patient will receive a letter of enrollment, a copy of the fully executed consent, and an enrollment specimen package if that is needed.

A participant's decision to participate or to not participate in this study will not affect the quality of care he or she receives.

5.4.1 Informed Consent Form

The informed consent forms will ensure that each participant/donor signatory understands and agrees to the following:

- The procurement of participant biospecimens through: (i) obtaining previously collected DNA and RNA extracted from a bone marrow sample; (ii) obtaining previously collected bone marrow samples where mononuclear cells have been frozen or DNA and RNA has been extracted and frozen; (iii) procedures that are called for by routine clinical care, after adequate material is collected for clinical diagnostic and treatment purposes.

- The use of biospecimens for tumor profiling with a targeted cancer gene panel, such as Rapid Heme Panel (RHP), and fusion testing in a CLIA lab when available and appropriate.

- The use of the tumor profiling information to match with a recommendation for a matched targeted therapy.

- The possibility that the recommendation may indicate a clinical course of action, or eligibility for a particular clinical trial. These profiling results and the treatment recommendation will be conveyed to them and their treating oncologist, who may not be a study investigator.

- The collection, storage and use of participant health information and linkage of participant health information with tumor profiling data by study staff at DFCI and BCH. This collection of data will be used for the purposes of creating a recommendation for a matched targeted therapy.

- The use of materials and clinical data for additional investigational tests and experiments some of which have not yet been designed. The clinical utility of such tests are unknown at this time and the results of these investigational tests will not be made available to participants or their physician(s). Some of the specific tests to be performed include whole genome sequencing, RNA sequencing, *in vitro* drug sensitivity testing and *in vivo* models. These additional investigations may be performed by researchers other than study investigators after approval of the study investigators. In this case, all specimens and data will be removed of identifying information.

- In some cases, specimens may be shared with for-profit companies that are working with researchers on a specific research project. Specimens will not be sold to any person or company for profit. Specimens shared with external companies will not contain identifying information. Subjects will not benefit from any financial gain to the institutions or their investigators based on these projects.

- Sharing or publication of de-identified genomic information in aggregate or individually. Subjects will be informed of the safeguards provided by de-identification but will also be informed that no one can predict how genomic information might be used in the future.

- There will be no costs to subjects for specimen contribution and no reimbursement to subjects for their participation.

- The optional activities for which consent is sought will be bundled into four individual requests for consent on the Consent Form: 1) return of germline result to the oncologist and this will prompt further testing; 2) willingness to complete questionnaires about perspectives on the matched targeted therapy study; 3) banking specimens for future use and investigational studies; 4) future contact by the study staff.

- Withdrawal of consent, as well as partial withdrawal from selected components, is possible at any time at participant discretion. Upon request by a participant, his or her specimens and derivative material will be removed from research specimen repositories. (Material collected for clinical purposes will not be removed from clinically relevant archives e.g., Departments of Pathology at individual institutions)

- Only study participants at sites participating in the survey portion of the study will be offered the opportunity to complete a questionnaire at the time of enrollment about their perspectives on genomic testing and asked to provide basic sociodemographic information including poverty measures. Participants who are not patients at participating sites will not be eligible for questionnaires. Those who complete this questionnaire will also be offered a follow-up questionnaire after the profiling results and interpretation are conveyed to the treating oncologist and again at the time of relapse/progression (if applicable). These questionnaires are optional and consent will be obtained for them. As of June 15, 2021, Questionnaire #1 is no longer being actively collected as part of this study. Questionnaires for timepoints 2 and 3 may be collected for patients who consented prior to this date, as is feasible for each site. Data from collected questionnaires continues to be analyzed by Dr. Jonathan Marron.

5.5 REGISTRATION AND WITHDRAWAL

All subjects, including those enrolled at collaborating sites and referred subjects, will be registered in the protocol registration database and assigned a study ID number in the Clinical Trials Management System (CTMS) Oncore. If collaborating sites require individual registration at institutional offices, the sites will be required to follow their own procedures as well as the central institution.

5.5.1 General Guidelines for DF/HCC Institutions

Institutions will register eligible participants in the CTMS OnCore.

A member of the study team will confirm eligibility criteria and complete the protocol-specific eligibility checklist.

5.5.2 Registration Process for DF/HCC Institutions

DF/HCC Standard Operating Procedure for Human Subject Research Titled *Subject Protocol Registration* (SOP #: REGIST-101) must be followed.

5.5.3 Registration Process for Participating Institutions

Eligible participants will be enrolled on study centrally at the DFCI by the Study Coordinator. Registration is only available during weekday business hours, between 9am-5pm EST. Once registration is confirmed, the DFCI study staff facilitating the registration will notify the participating site study staff regarding confirmation of registration. To register a participant, the following documents should be completed by the research nurse of data manager and faxed [redacted] or emailed to the Study Coordinator:

- Copy of pathology report confirming diagnosis
- Signed participant consent form
- HIPPA authorization form
- Eligibility Checklist

The coordinator will fax or email the participant study number to the participating site once the patient has been enrolled at DFCI.

5.5.4 Study Withdrawals

Participants may withdraw consent to participate in this study at any time. If a participant chooses to withdraw from the study, any remaining samples he/she contributed to research biorepositories will be discarded. However, data obtained prior to the participant's withdrawal from the study will be kept. Samples essential for routine clinical care e.g., archived tissues in institutional Departments of Pathology, will not be affected by study withdrawal. Every effort will be made to preserve the subject's privacy. An indication will be made in the database regarding this individual's desire to withdraw from the study to ensure that this individual is not contacted regarding this study in the future. Clinical data collected as part of other research studies in which a participant is participating and from which the participant does not withdraw consent will not be deleted or affected by withdrawal from this study. Additionally, a participant may withdraw selectively from particular components of this study.

6.0 CLINICAL AND SURVEY DATA COLLECTION

6.1 PURPOSE OF CLINICAL DATA COLLECTION

Clinical data will be collected at the following time points at study enrollment. For participants in Cohort 1 (relapsed/refractory leukemia or MDS), clinical data will be collected following a recommendation for a matched targeted therapy (every 4 months) until treatment initiation, progression or death. For participants in Cohort 2, clinical data will be collected every 6 months following study enrollment.

The purpose of clinical data collection at these time points is:

1. At study enrollment: Obtain the information required for a recommendation for a matched targeted therapy
2. Follow-up regarding the recommendation for a matched targeted therapy: Determine whether treatment based on the recommendation has been initiated. Follow up of patient clinical status, as outlined in the required data table below, will be done for patients without a matched targeted therapy recommendation.
3. Follow-up regarding the recommendation for a matched targeted therapy initiation: Determine response to treatment based on the treatment recommendation

In addition, participant identifiers will be collected by DFCI on participants from all study sites due to the need to perform testing on their leukemia in CLIA certified laboratories at DFCI and, in some cases, by an outside vendor (see Sect. 9.1.3). Participants will not be charged for this additional testing.

7 REQUIRED DATA

7.1.1 <u>At Study Enrollment:</u>

- Participant identifiers including name, medical record number, date of birth, gender, race, ethnicity.
- Diagnosis, including immunophenotype, cytogenetics, FISH studies
- Date of enrolling diagnosis
- Status (recurrent or refractory leukemia)
- Prior treatment and response to treatment (especially targeted therapy)
- Any molecular/sequencing data obtained prior to enrollment
- Treating oncologist's name and contact information

7.1.2 <u>Following matched therapy treatment recommendation:</u>

- For all patients enrolled with recurrent, or refractory disease (Cohort 1)
    - Every four months: vital status, disease status, any anti-leukemia treatment(s) received since the last assessment
- For all patients with newly diagnosed high-risk disease without recurrence (Cohort 2):
    - Every six months: vital status, disease status, any anti-leukemia treatment(s) received since the last assessment
- For participants who received a matched therapy recommendation:
    - If the recommendation was not used, a query of the site PI will be performed to assess barriers to receiving the matched therapy recommendation (e.g., Provider did not agree with recommendation, drug not available, insurance denied coverage, drug not available in correct formulation, patient unable to tolerate, progressive disease, etc). Site PI response may involve contacting the treating oncologist or a limited chart review.
- For participants who receive treatment according to the matched therapy recommendation:
    - Data from chart review of the medical participant's records at the participating institutions and query of primary site PI will document dates of treatment initiation, best response to treatment,

date of best response, and date of progression while receiving treatment based on the matched therapy recommendation.

- Participants will be followed until death or for 5 years from the time of study enrollment.
- Required data will be collected using case report forms.

REQUIRED DATA

	ALL PATIENTS		Cohort 1: recurrent or refractory leukemia or MDS	Cohort 2: newly-diagnosed high-risk leukemia/MDS	ALL PATIENTS
	Screening	**Enrollment**	**Every 4 Months after MTT Report**	**Every 6 Months after Enrollment**	**At Time of Disease Progression**
Baseline Demographics[A]	X				
Pathology Report	X				X
Molecular/sequencing data previously obtained or obtained outside of this study		X			X
Vital Status			X	X	
Disease status:		X	X	X	X
Interval anti-leukemia treatment received			X	X	X
Response to anti-leukemia treatment			X	X	
Interval progression or no interval progression [B]			X	X	X
Barriers to Receipt of Treatment			X		
Sample Submission		X			X[C]
Parent / Participant Survey (if consent)**		X			X

[A]Baseline demographics include: Participant identifiers; Age; Sex; Race; Ethnicity; Enrolling institution; Diagnosis; Disease status (refractory; recurrent; newly-diagnosed high risk); Prior anti-leukemia/MDS treatment; Name and contact information for primary oncologist.

[B]Sites will be queried every 4 months for Cohort 1 and every 6 months for Cohort 2 regarding clinical status determined by physical exam, laboratory evaluation and any interval bone marrow analysis.

[C]Each patient will have up to three samples profiled as part of the study. Additional samples from additional assessment of relapse/refractory disease (up to three per patient during protocol enrollment) may be submitted to inform exploratory objectives of the study. Additional bone marrow samples beyond the three total may be submitted, but limited to no more than three bone marrow samples over the course of three months

** for patients enrolled prior to June 15, 2021

7.2 DATA COLLECTION METHODS

At study enrollment every enrolled subject will have a baseline visit at one of the study sites with one of the study investigators. Baseline clinical data as outlined in Section 7.2.1 will be obtained both from the medical record and from history and physical examination. Study staff will input clinical data into a password protected, database constructed for the purposes of this study.

Clinical data following the matched therapy treatment recommendation will be collected via case report forms and directly from the participant's medical records at the participating institution by the site PI and transmitted to the study staff at DFCI.

For participants who are not patients at participating sites, information will be provided to the enrolling site for data entry. The participant and their primary treating physician(s) will be sent data collection forms based on the study schedule above. This case report form (CRF) Adobe Sign can be used as part of a remote consent process to sign the informed consent form. can be found in Appendix 21. The forms and information may be sent back via mail or email, whichever method is preferred. Source documentation will be requested for the data collected.

7.3 PATIENT / PARENT QUESTIONNAIRES

Administration and collection of Questionnaire 1 was halted on June 15, 2021. Surveys collected after this date may be submitted for analysis but are not required. Questionnaires for timepoints 2 and 3 (if applicable) should continue to be collected and submitted to Dr. Jonathan Marron for patients enrolled prior to June 15, 2021.

Optional questionnaires may be offered to study participants at three separate time points during this study for subjects enrolled at Institutions participating in the survey component of the study:

1) 0-60 days after the date of enrollment AND before the tumor profiling report and expert panel recommendation is discussed with participant/family
2) 30-90 days after the date on which the first the tumor profiling report and expert panel recommendation or first report of sample inadequacy/technical failure was provided to the treating oncologist
3) 30-90 days after the date of first relapse or progression post-enrollment, when such is applicable

The first questionnaire (Questionnaire 1) should have been offered to all consenting study enrollees at survey sub-sites, and follow-up questionnaires should be offered to all who completed the first questionnaire. Participants (or parents/legal guardians for those <18years) will be contacted by phone or in person to ascertain their willingness to complete a survey about their perspectives on the tumor profiling study (Appendix 17). Those who agree to complete a survey will be contacted in person in the oncology clinic or inpatient unit and/or mailed the paper questionnaire along with a postage-paid return envelope and an opt-out postcard (Appendix 16) if the study staff is unable to reach them by phone. Those who are contacted in person may take an electronic version of the questionnaire on a tablet device. Questionnaire 1 (Appendix 4 & 7) is a patient perspective questionnaire for participants age 18 or older at the time of enrollment or a parent questionnaire for participants younger than 18 was at the time of enrollment). Questionnaire 1 is offered to the individual who consented to take part in the tumor profiling research study, either the patient with cancer (if ≥18 years at the time of enrollment) or his/her parent or legal guardian.

Those who have not responded or returned the opt-out postcard will be called within two weeks of the initial contact mentioned above. At that time, study staff will offer to answer any questions and provide a second questionnaire, either through the mail or by bringing it to the patient in the clinic or inpatient setting. A final attempt will be made approximately two weeks after the second attempt for participants who have not responded or returned the opt-out postcard. These participants will be mailed a paper copy of the questionnaire, postage-paid return envelope, and an opt-out postcard. A similar follow-up procedure will be utilized for administration of Questionnaire 2 (Appendix 5 & 8) and 3 (Appendix 6 & 9) as outlined below.

The follow-up questionnaire (Questionnaire 2) will be offered to all study enrollees who completed the first questionnaire at the time of enrollment. Questionnaire 2 will be administered according to the same protocol as Questionnaire 1. Questionnaire 2 (Appendix 5 & 8) will be a patient perspective questionnaire for participants age 18 or older at the time of enrollment or a parent questionnaire for participants younger than 18 was at the time of enrollment. All study participants who completed the initial questionnaire will be given the opportunity

to take the survey (regardless of whether or not they received a tumor profiling report and expert panel treatment recommendation) once his/her treating oncologist has received the tumor profiling report and expert panel recommendation or the report that the tumor sample was inadequate or a technical failure.

A final questionnaire (Questionnaire 3) will be offered to those study enrollees who completed the enrollment questionnaire and then experience relapse or progression of disease during the study period. Questionnaire 3 will be administered according to the same protocol as the questionnaires above. Questionnaire 3 (Appendix 6 & 9) will be a patient perspective questionnaire for participants age 18 or older at the time of enrollment or a parent questionnaire for participants younger than 18 was at the time of enrollment.

A $10.00 gift card will be distributed with each of the questionnaires directly to the person who is completing the questionnaire. In the case of the assenting participant, the questionnaires will be directed to the consenting parent/guardian and therefore the gift card will be distributed to that person.

These questionnaires consist of a series of multiple-choice questions in various domains, as appropriate to the timing of the individual questionnaire's administration. Some domains will be queried in all three questionnaires and some only in one or two of the individual questionnaires. These domains and a brief description of the topics addressed by the questions in each domain are as follows:

1) *Experience with genetics and genetic testing* – several yes/no questions addressing the respondent's prior experiences with genetics and genetic testing
2) *Genetic knowledge* – several true/false questions addressing the respondent's knowledge of genetics and genetic testing
3) *Patient data* – several multiple choice questions inquiring about the patient's cancer, health status, treatment, and prognosis
4) *Understanding of the tumor profiling study and its purpose* – several multiple-choice questions inquiring about the respondent's understanding of the purpose of the tumor profiling study, its purpose, and its potential impact on participants
5) *Hopes for the tumor profiling study* – several multiple-choice questions addressing what hopes the respondent had for the tumor profiling and expert panel recommendation at the time that he/she enrolled in the study
6) *Concerns about the tumor profiling study* -- several multiple-choice questions addressing what concerns the respondent had about the tumor profiling and expert panel recommendation at the time that he/she enrolled in the study
7) *Return of results* – several yes/no and multiple-choice questions inquiring as to what types of genetic/genomic information families would like to receive from future studies about cancer genomics
8) *Research study requirements* – several yes/no questions inquiring as to what requirements would be reasonable for enrollment in future studies about cancer genomics
9) *Follow-up of results* – several multiple-choice questions inquiring as to the patient's current cancer treatment and how/if tumor profiling and expert panel recommendation played a role in this treatment choice
10) *Impact of results* – a series of multiple-choice or yes/no questions looking at the patient's current medical status, how their treatment might have changed based on the tumor profiling and expert panel recommendation, and what impact the tumor profiling study and its results have had on the participant and his/her family
11) *Participant demographics* – several multiple-choice questions ascertaining the participant's age, gender, education, socioeconomic status (including housing insecurity), race/ethnicity, and relationship to the patient with cancer.

Questionnaire items are based on previously validated questions and indices, when available. When such is not available, original questionnaire items have been created. A prior version of these questionnaires was administered as part of the iCat1 study. Modifications have been made based on cognitive interviews performed as part of iCat1 and analysis of the pilot data procured from the iCat1 questionnaires.

7.4 RESEARCH DATABASE ARCHITECTURE

Timely quality data management will be performed in conjunction with the DFCI Office of Data Quality (ODQ) via the web-based DFCI electronic data capture system for clinical trials. All participating institutions will be able to report data over the internet via the DFCI electronic data capture system screens.

The infrastructure for the conduct of the trial, data collection, and data analysis will be provided by the ODQ and the Biostatistics Program of the Division of Pediatric Hematology/Oncology of BCH and DFCI. The biostatistics faculty and staff and the ODQ will contribute expertise for study design and protocol development, assist with obtaining IRB approval, design and implement electronic data collection forms, monitor for adherence with regulatory requirements, facilitate specimen collection and data audit, perform statistical data analysis and interpretation, and participate in manuscript generation. The Biostatistics Program has dedicated computer servers, and SAS will be the primary analytic tool to address the study objectives.

We will build the primary study database for baseline, clinical and tumor profiling variables required to assess the primary objective using Phase Forward's InForm™ ITM (Integrated Trial Management) software application. InForm is a Web-based electronic data capture (EDC) and clinical data management system used by research teams to facilitate study data collection, monitoring and analysis. InForm™ is based on open data standards (CDISC ODM, XML, etc.), is designed to facilitate the integration of disparate clinical or bioinformatics services and supports enhanced reporting and integration capabilities. The application software employs Microsoft and Oracle technologies to deliver secure Web services and data storage. Sites will have the ability to submit data over the internet via a secure network connection, and data will be stored on the servers of the Dana-Farber / Harvard Cancer Center's (DF/HCC) Office of Data Quality behind the firewall maintained by the Partners Healthcare Information Systems.

8.0 BIOSPECIMEN COLLECTION AND PROCESSING

8.1 BIOSPECIMEN TYPES TO BE COLLECTED

8.1.1 Minimal Biospecimen Requirement

For patients with leukemia: The minimal requirements for enrollment are frozen mononuclear cells, DNA and RNA, or fresh bone marrow aspirate sample with at least 20% leukemia blasts in an EDTA tube collected from the most recent leukemia disease evaluation OR 3mL peripheral blood sample containing at least 20% blasts OR pheresis sample of at least 2 mL OR other fresh sample of patient leukemia cells in discussion with overall PI. The equivalent of 2 mL of liquid bone marrow aspirate is sufficient for RHP and fusion testing and is the minimal requirement for this study. Additional bone marrow/peripheral blood/DNA, etc, will be prioritized for profiling as detailed below. These requirements are not applicable if the patient has tumor profiling testing completed clinically using RHP.

For patients with MDS: The minimal requirements for enrollment are frozen mononuclear cells, DNA/RNA or fresh bone marrow aspirate sample in an EDTA tube collected from the most recent MDS disease evaluation. Blast percentage of 20% is not required for MDS samples. Additional bone marrow/peripheral blood/DNA, etc, will be prioritized for profiling as detailed below. These

requirements are not applicable if the patient has tumor profiling testing completed clinically using RHP.

8.1.2 Biospecimens to be Collected

- Leukemia samples* (one of the following is **required**, more than one sample type can be submitted if available)
    - Two tubes of 2-5 mL each of liquid bone marrow (minimum of one EDTA tube of 2 mL is required), with at least 20% leukemia involvement (blast percentage may be lower for patients with MDS or in consultation with overall PI)

      AND/OR

    - DNA and RNA extracted from mononuclear cells from most recent bone marrow aspirate
    - Apheresis product, four EDTA tubes of 2-4 mL each
    - Two EDTA tubes of peripheral blood, 3-5 mL each, if patient with at least 20% blasts (minimum of one tube of 3 mL is required)
    - Other sample type containing leukemia cells or DNA and RNA from leukemia may be accepted in consultation with overall PI

- Leukemia samples should be collected and submitted at a time when patient first enrolls onto protocol. Additional samples may be submitted with subsequent bone marrow assessments in patients with persistent or relapsed disease. Samples from three different timepoints per patient may be submitted during the trial participation period. Additional samples can be submitted after discussion with protocol PI.

- For patients able to provide more than 1 sample (as outlined above), the following tests will be prioritized:
    1. Rapid Heme Panel (RHP) testing (for all patients)
    2. Fusion screen (for all patients)
    3. RNA Sequencing (for all patients)
    4. Xenografting (may be done at local site institution if an IRB-approved protocol exists)
    5. Functional drug screening assay

- Sample for germline assessment (**highly recommended**)
    - Preferred:
      - One tube of 2 mL bone marrow obtained during clinical care at which remission was confirmed morphologically
    - If remission marrow is not available, the following will be accepted. These can also be submitted in addition to the remission marrow sample. Please see Section 8.4 below for details on the procurement of these samples and Appendix 3 for sample submission:
      - Saliva sample
      - Buccal swab
      - Fibroblasts from skin biopsy
      - Fingernail clippings

8.2 COLLECTION SITES

All biospecimens will have already been or will be collected as part of the participant's routine clinical care at the participant's treating institution.

For participants enrolled at participating sites other than Dana-Farber / Children's Hospital Cancer Center biospecimens will be shipped to the Dana-Farber / Children's Hospital Cancer Center and OHSU. Sample shipping will depend on specimen availability.
For participants who are not patients at participating sites, coordination for the required tissue and blood samples will be directly with the enrolling participant and through the primary physician. Samples will be collected at the time of a scheduled bone marrow assessment or blood draw at the enrolling patient's institution and sent to Dana-Farber Cancer Institute for testing as outlined above.

Materials Transfer and Data Use Agreements will be established with outside sites as is stipulated by each site if necessary.

8.3 SPECIMEN STORAGE/DISPOSAL

Frozen samples will be stored in secure freezers at BCH, Dana-Farber Cancer Institute, at the Boston Children's Hospital Biorepository or at the biorepository facility at OHSU. Storage and retrieval of DNA or banked bone marrow samples will be handled using routine procedures of the Pathology Department of the hospital at which the specimen was collected. Additional detail regarding specimen tracking and storage is provided in Sections 11.1 and 11.3.

Disposal of biospecimens will be considered under certain circumstances including but not limited to reduced specimen integrity, exhausted capacity or insufficient funds for long-term maintenance or storage of low priority biospecimens. Determination of the integrity and priority of biospecimens is at the discretion of Principal Investigator. Primary enrolling site will be notified if samples are to be disposed and may be returned to the primary enrolling institution.

8.4 BIOSPECIMEN COLLECTION RISKS TO PARTICIPANT

Participant blood or bone marrow used in this protocol will have been collected at the same time as the procedure being performed for clinical care purposes. Therefore, there is no incremental risk of participating in the study beyond the risk that may occur with routine bone marrow aspirations or blood draws. Thus, risks experienced by subjects would be the same as those consented to as part of their usual medical care.

The participants will have the option of submitting samples for germline assessment. These samples may be buccal swabs, saliva, fingernail clippings, or fibroblasts from clinical skin biopsies. Fibroblasts from skin biopsies will be submitted if they are obtained and available from clinical studies. The collection of fingernail clippings may result in physical side effects, described below.

- Buccal swab: Buccal swab will be collected using a DNA buccal swab kit. There is no additional risk of this procedure.
- Saliva samples: Saliva will be collected using a saliva collection kit in participants who are old enough to cooperate with this procedure. These kits will be funded by the study and provided to participating sites. For self-referred patients, these kits will be funded by the study and mailed directly to the patient. There is no additional risk to the patient with this procedure.
- Fingernail clipping: Fingernail clippings may be provided by the study participant for evaluation of germline DNA. Collection should be done at time of routine personal care. There is potential for minor trauma at the time of fingernail clipping such as small cut or pinch of skin. Since this would be done at the time of routine personal care, this is not additional risk.

9 TUMOR PROFILING AND MATCHED THERAPY RECOMMENDATION

9.1 TUMOR PROFILING

Initial profiling will be performed on the most recent bone marrow sample documenting relapsed/refractory, high risk or rare leukemia.

9.1.2 Rapid Heme Panel (RHP)

Leukemia/MDS sample will be profiled utilizing Rapid Heme Panel to determine the presence of oncogenic mutations at the Center for Advanced Molecular Diagnostics (CAMD) at the Brigham and Women's Hospital. Leukemia sample in an EDTA tube will be submitted directly to CAMD for profiling using the Rapid Heme Panel as specified in the Manual of Operations (Appendix 3).

The method of mutation analysis performed by the CAMD may change over time in order to permit analysis of a larger number of genes. In addition, potential CLIA-certified methods of mutation analysis that may be performed by CAMD in the future may include exome or whole genome sequencing.

If RHP testing fails and sequencing results from a comparable panel are available for a newly enrolled patient, these results may be used in the place of RHP results to inform a Matched Targeted Therapy recommendation. The decision to use this comparable sequencing data must be made by the overall study PI and after RHP testing has been attempted at least once.

9.1.3 Gene Fusion Analysis

Gene fusions analysis will be performed at Boston Children's Hospital, Children's Hospital of Philadelphia or in other CLIA-certified laboratories to allow for return of these results. With development and availability of other gene fusion assays the location of this assessment may change. The leukemia sample will be submitted to Dana-Farber Cancer Institute for nucleic acid extraction and will then be submitted for sequencing at Boston Children's Hospital or another CLIA certified laboratory as specified in the Manual of Operations (Appendix 3). With development and availability of other gene fusion assays the location of this assessment may change and may include other leukemia subtypes.

9.1.4 Other testing

Given the rapid development of new genomic technologies offered by CLIA-approved laboratories, additional tests may be performed as they are developed during the course of this protocol. These may include Nanostring assay for assessment of gene fusions and RNA Sequencing (RNASeq) among others.

Other leukemia profiling tests may be performed at the discretion of the Principal Investigator or as recommended by the expert panel if the clinical history or results of sequencing panel test indicate a particular test would have clinical utility. Any leukemia profiling test in which results will be returned to the primary oncologist will be performed in a CLIA- and / or CAP-certified laboratory. Examples of additional tests that may be performed and returned to the primary oncologist are as follows. If an enrolled participant has a leukemia for which there is published evidence supporting the occurrence of an oncogenic mutation in a proportion of participants with that leukemia type and the gene is not included in the sequencing panel test, then candidate gene sequencing may be performed. Confirmatory FISH (fluorescence in situ hybridization) or IHC (immunohistochemistry) may be performed to confirm findings on the RHP.

9.2 GENOMIC ANALYSES

RHP and Gene Fusion assay results will be analyzed in line with current protocol at Brigham and Women's Hospital/Boston Children's Hospital and Boston Children's Hospital or another CLIA certified laboratory where fusion testing is performed.

9.3 RECOMMENDATION FOR A MATCHED TARGETED THERAPY

9.3.1 Formulation of Recommendation

The Principal Investigator or one of the Pediatric Oncology Co-Investigators will utilize clinical data and the interpreted profiling data to create a treatment recommendation matched to the identified genomic alteration. As the basis for the recommendation, actionable alterations will be determined from analysis from RHP and fusion gene testing as well as any molecular/sequencing data obtained in a CLIA- and / or CAP-certified laboratory prior to enrollment. Considerations taken into account in determining the recommendation will include: IC_{50} of a particular drug against the particular mutation identified; available drug formulations; prior therapies received; participant age and availability of data regarding a pediatric dose; side effect profiles; availability of phase I/II trials. The recommendation will be tiered according to the strength of evidence for the specific genomic alteration in leukemia, or cancer in general (Appendix 1). Additional tiering will be applied for drug availability (Appendix 2).

9.3.2 Review and Approval of Genomic Alteration and Matched Treatment Recommendation by Expert Panel

The recommendation will be reviewed and approved by a panel composed of experts from pediatric oncology, developmental therapeutics, and cancer biology. The Expert Panel will meet twice a month either in person or by conference call. Conference call-in information will be provided to the Expert Panel members to allow for travel, scheduling conflicts and to include members from all participating sites. Members of the panel will be available to the investigators for consultation during creation of the recommendation as needed.

9.3.3 Communication of Genomic Alteration and Matched Treatment Recommendation to Study Participants and Their Physician

For participants with AML, MDS, rare or other high-risk leukemia who have not had a relapse, the leukemia profiling results will be released at the time of profiling. The expert panel will address in their letter communicating results and recommendation clearly distinguish recommendations which could be considered to be acted on during upfront therapy (which are expected to be few) and those that could be considered to be acted on only at the time of non-response to standard therapy or disease recurrence.

The profiling results and matched therapy recommendation will be provided to the participants' treating oncologist in the form of a letter containing an assessment of the technical performance of the profiling, the interpreted RHP and gene fusion results, a list of the actionable alteration(s) identified and the matched targeted therapy for treatment consideration. The letter will clearly state that treatment based on the recommendation is experimental and is not known to be better than standard therapy.

An information technology (IT) systems may be employed to assist in the curation process and / or report generation and delivery. An IT based curation tool would assist the curator in systematically accessing publicly available databases such as COSMIC, Pubmed, 1,000 genomes, and OMIM during their review of each variant. Study data such as patient's somatic variants including allelic fraction, diagnosis, age and study ID number would need to be entered into an IT based curation tool. An IT based report generation tool would assist the molecular pathologist and pediatric oncologist(s) in generating and, once finalized, delivering the molecular pathology interpretation and clinical review. Study data including patient identifiers such as name, MRN, diagnosis, pathology case number, prior molecular alterations identified in patient's leukemia, and prior therapy would need to be entered into an IT based reporting tool. Such information technology solutions would be HIPAA-compliant and approved by the DFCI Information Services, Information Security Office and the local site IRB.

10 FUTURE EXPLORATORY TUMOR PROFILING

Future exploratory leukemia evaluation and profiling will be performed only after sufficient material has been collected for the RHP/gene fusion profiling performed for evaluation of a matched therapy recommendation and any subsequent samples have been prioritized to for pre-clinical studies.

In those cases in which sufficient tumor material is available, deep sequencing of targeted exomes known to be involved in cancer, RNA sequencing or whole exome sequencing may be performed. Whether these techniques identify potentially actionable alterations in addition to those identified with RHP and gene fusion assay will be explored.

Results from these exploratory studies will not be released to participants or physicians unless they are performed in CLIA-certified laboratories. If mutations with significant clinical implications are identified such as germline TP53 mutation associated with the Li Fraumeni Syndrome via a non-CLIA-certified test, results will be released only after confirmation of the result is confirmed in a CLIA-certified laboratory by a newly collected clinical specimen and the case has been discussed by the expert panel. At the time of releasing germline mutation results, participants will be offered appropriate genetic counseling.

Additional research studies may be performed with de-identified tumor and fluid specimens acquired under this research protocol. These additional research studies may include derivation of in vitro testing for drug sensitivity, cell lines and xenograft models.

11 SPECIMEN AND DATA MANAGEMENT, ACCESS AND OVERSIGHT

11.1 SPECIMEN TRACKING

Specimens will be obtained and delivered to the appropriate clinical research staff at DFCI as specified in the Manual of Procedures (Appendix 3). If additional samples are available, they will be sent for additional testing in the priority order as follows as outlined in the Manual of Procedures: 1. Fusion panel testing (Boston Children's Hospital or another CLIA-certified laboratory as appropriate) 2. RNA Sequencing, 3. Xenografting at each participating institution, 4) Oregon Health & Science University.

Specimens will be tracked using a participant and sample database created by the study staff using Redcap database software on servers at Boston Children's Hospital. The database will be maintained as secure behind the firewall at Boston Children's Hospital. Access to the database will be restricted to the approved listed study staff members.

For all testing performed in CLIA-certified laboratories, each specimen will be uniquely identified by at least 2 participants identifiers (such as the participant's name, date of birth) and the study participant case number and a specimen ID number.

Clinical research personnel will hand deliver specimens to the respective DFCI, BCH and BWH Departments and labs for processing as specified in the Manual of Procedures (Appendix 3).

Participant-derived materials not depleted by local testing will be banked in the BCH Biorepository. Participant-derived materials not depleted by testing at OHSU will be banked in the biorepository at OHSU under the control of this research protocol. If specifically requested, participant-derived materials (DNA) not depleted by testing may be returned to the Pathology Department from which the original sample was submitted.

11.2 DATA CONFIDENTIALITY AND SECURITY

The confidentiality of each participant record will be rigorously maintained using existing DFCI standards. HIPAA and state/federal government regulations for protecting participant privacy and security will be strictly

observed. Printing study documents will be discouraged and avoided whenever possible. If it is necessary to print source documentation, it will be maintained and protected by clinical research personnel and kept in a locked file cabinet. Electronic study data will be encrypted both at rest and during transfer using current industry-grade practices. All computers used in the study will be encrypted according to intuitional policies. All of the databases used to support this project will be stored on the servers of the Dana-Farber / Harvard Cancer Center's (DF/HCC) Quality Assurance Clinical Trials Program and Dana-Farber Cancer Institute Research Computing. Investigators from collaborating sites will have the ability to access data containing identifiers from participants enrolled at their own site only with one exception as follows: study investigators participating in producing the identified matched targeted therapy report will be able to view identified laboratory reports of the patient's assigned to them for review. This will allow them to verify that the correct genes and variants are reviewed and reported for the correct patient. There will be granular user/group-based access control that restricts viewing of subject information.

No participant or subject-identifiable information will be given to third parties, including family members (except legal guardians of minors), unless that subject (or legal guardian of minors) has given written or witnessed consent to do so. The results of research studies may be published but all data will be de-identified prior to publication and individual subjects will not be identified.

If a participant contacts the study's project personnel, he or she will be informed of the status of the research without revealing specific findings.

11.3 ACCESS TO SPECIMENS AND DATA FOR RESEARCH PURPOSES

11.3.1 Access to Research Specimens

Access to research specimens or their derivative material by investigators other than those listed as Co-Investigators on the protocol will require, in all cases, approval by the Principal Investigator. The Principal Investigator will evaluate requests for access to these materials by balancing scientific merit and potential impact of the proposed study against the amount of remaining material. The specimens collected through this protocol may be shared with investigators other than those listed as Co-Investigators on the protocol through a Usage Agreement only if such investigators complete the following steps: i) obtain approval by the Principal Investigator; ii) Agree that samples will remain de-identified and that no attempt will be made to identify or contact study participants; iii) that any use of the material beyond that described in this protocol requires review and approval by the DFCI, and other applicable IRBs; and iv) that, when relevant, Material Transfer Agreements have been executed.

11.3.2 Data Safety Monitoring and Executive Committees

The activities governed by this protocol do not require oversight by a DSMC.

11.3.3 Data Access Oversight

In addition to the oversight provided by the DF/HCC IRB as described above, data access from the study database will be guided by institutional SOPs. All data access will occur through a secure access layer that authenticates the user. All access will be logged and periodically monitored following institutional SOPs. Investigators from collaborating sites will have the ability to access data containing identifiers from participants enrolled at their own site only.

11.3.4 Repository Oversight and Distribution

In some cases data and specimens may be shared with investigators other than those listed as Co-investigators on the protocol. Access to data by investigators other than those listed as Co-Investigators on the protocol will require, in all cases, approval by the Principal Investigator. The Principal

Investigator will serve as the Honest Broker of the specimens and data generated by this project. The Principal Investigator will evaluate requests for scientific merit and potential impact of the proposed study. Once reviewed and approved, the requested data and specimens may be released de-identified only if such investigators complete the following steps: i) obtain approval by the Principal Investigator; ii) agree that data will remain de-identified and that no attempt will be made to identify or contact study participants; iii) that any use of the data beyond that described in this protocol requires review and approval by the DFCI, and other applicable IRBs; and iv) that, when relevant, Material Transfer Agreements have been executed.

11.4 SPECIMEN PROPERTY RIGHTS

Specimens collected from participants registered at DFCI, BWH, or BCH are the property of those hospitals and will remain at those hospitals even if the staff members who obtained those specimens leave. Specimens submitted by outside institutions continue to be the property of that institution. Participant-derived materials not depleted by testing, including materials from non-BCH/DFCI/BWH sites, will be banked at BCH/DFCI. If requested, participant-derived materials (e.g., DNA) not depleted by testing may be returned to the Pathology Department from which the sample was submitted.

12 DATA ANALYSIS AND STATISTICAL CONSIDERATIONS

12.1 ACCRUAL

The feasibility rule described below requires at most 100 eligible Cohort 1 patients. After feasibility is established, an additional 100 Cohort 1 patients will be accrued to further describe the actionable mutations in disease type (specifically AML and ALL). Given increased access to CLIA fusion panel testing and research RNASeq, Cohort 1 accrual will be increased by 50 patients to further describe actionable mutations that can be found using fusion testing, and describe the alterations that are detected by RNASeq but may be missed in targeted panel assessment. Approximately 10-15 patients with recurrent / refractory leukemia, 5-10 patients with newly diagnosed AML (excluding APL), and 2-3 patients with rare leukemias are evaluated per year the DF/CHCC. Almost all of these patients receive ongoing care at the DF/CHCC. Because additional procedures are not required for enrollment onto the study and given our department previous success with enrolling patients on similar trials, we anticipate that the proportion of eligible subjects who consent to enrollment will be about 90% resulting in, on average, 11 eligible, enrolled subjects per year for Cohort 1 and 10 eligible patients for Cohort 2 at DF/CHCC. The collaborating sites, Columbia University Medical Center, Children's Hospital of Philadelphia, Children's Hospital at Montefiore, Johns Hopkins Children's Center, Seattle Children's Hospital, Children's Hospital Colorado, University of California, San Francisco, University of Chicago, Children's Hospital of Wisconsin, Memorial Sloan-Kettering Cancer Center, Children's Healthcare of Atlanta, and Children's Hospitals and Clinics of Minnesota anticipate a combined enrollment of at least 40 eligible patients per year for Cohort 1. If a patient on Cohort 2 develops refractory/relapsed disease, the patient will be changed to Cohort 1. Overall, the accrual rate will be approximately 50 Cohort 1 patients per year, and enrollment of 350 eligible patients can be completed in about 5 years. Patients will only be enrolled on Cohort 1 during this time, with patients already enrolled on Cohort 2 remaining in follow-up.

12.2 DEFINITION OF FEASIBILITY

The primary objective is to determine the feasibility of identifying actionable alterations (candidate to receive a matched therapy recommendation) in pediatric patients with recurrent/refractory leukemia (Cohort 1). A patient is defined to be a candidate to receive a matched therapy recommendation if (1) there is a sufficient leukemia/DNA specimen of acceptable quality for a complete RHP or gene fusion assay (profiling data), and (2) an actionable alteration is identified. It is estimated that 10% of enrolled patients are expected to lack sufficient profiling data and will be considered technical failures. Next generation sequencing for AML samples

identified mutations in approximately 30% of samples[33], however, large-scale assessment for oncogenic mutations for recurrent/refractory/high risk leukemias have not been performed. As a result, it is conservatively estimated that 15% of leukemia samples that are not technical failures will have an actionable alteration. The predicted proportion of enrolled patients who would have profile data and an actionable alteration is 13.5%. Therefore, this study will be considered feasible if at least 13% of participants have profile data and identifiable actionable alterations.

A total of 100 eligible patients will be enrolled to Cohort 1 for this feasibility study. With 100 eligible patients, the 90% exact binomial confidence interval (CI) will be within ±8.6%. The following table displays the exact 90% binomial CI for identifiable actionable alterations under various scenarios.

No. with identifiable actionable alterations	% (90% exact CI)
6	6.0% (2.6-11.5%)
7	7.0% (3.3-12.7%)
8	8.0% (4.0-13.9%)

With 100 patients, at least 8 patients need to have profiling data and an identifiable actionable alteration to have the upper bound of the 90% CI covering 13%. Therefore, identifying actionable alterations in pediatric patients with recurrent/refractory leukemia will be considered feasible if at least 8 of 100 patients have identifiable actionable alterations.

12.3 EFFICACY

Response will be assessed only in those participants receiving treatment according to the matched therapy recommendation. Bone marrow or other assessments to monitor response to the treatment will be performed at the discretion of the treating oncologist. Response will be determined by report from the participants' treating oncologists via data collection from chart review at the participant's institution and query of the treating oncologist/site PI. The proportion of participants receiving therapy according to the matched therapy recommendation with a partial or complete response while receiving therapy based on the recommendation will be determined. The proportion of responders will be compared to the 10% reported response rate in all pediatric phase I trials because the participant population is similar to that enrolled on phase I trials[42].

12.4 ASSESSMENT OF STUDY OBJECTIVES

12.4.1 Primary Objective

Feasibility of identifying an actionable alteration will be addressed by the feasibility definition outlined in section 12.2. The observed rate of patients with actionable alterations, out of all patients enrolled to cohort 1, will be reported with a 90% confidence interval. The rate of patients with actionable alterations in Cohort 2 will be similarly reported.

The observed rate of patients with actionable alteration for the ALL and AML subgroups in Cohort 1 will be estimated along with a 90% confidence interval. Based on previous enrollment, it is estimated that 94, 15, and 72 out of the planned 200 Cohort 1 patients will have relapsed/refractory B-ALL, T-ALL, and AML, respectively. The remaining 19 are expected to have other rare leukemias. The width of the 90% exact confidence interval will be no larger than 17.9%, and 20.5% for relapsed/refractory B-ALL and AML, respectively. The rate of actionable mutations in relapsed/refractory T-ALL and other rare leukemias will be described. Additionally, it is estimated that 67 newly diagnosed AML Cohort 2 patients will be enrolled by the time Cohort 1 completes accrual. The width of the 90% exact confidence interval will be no larger than 23.5% for newly diagnosed AML.

12.3.2 Secondary Objectives

Secondary Objective 1 will be addressed by describing the somatic genomic alterations in Cohort 1 and Cohort 2 that are discovered by genomic analyses.

Secondary Objective 2 will be addressed by a description of the time of results reporting. We will document time of sample receipt and processing, resources and personnel utilized at each step of the process, time to results reporting, interpretation and review by Expert panel and communication of results to the primary oncologist.

Secondary Objective 3 will be addressed by describing the hopes and concerns of parents of children with recurrent / refractory / high- risk de novo leukemia regarding genomic testing of their child's leukemia as well as their understanding of the testing and evaluate whether the hopes and concerns were realized following return of results.

Secondary Objective 4 will be addressed by completing analysis of the primary leukemia sensitivity testing to a panel of drugs or shRNA and establishing xenograft models in dedicated participating research laboratories and conducting co-clinical trials with the recommended matched therapy once the animal model is established.

12.3.3 Exploratory Objectives

Exploratory Objective 1: to explore whether patients received drugs that are matched to the genomic alteration and recommended by the expert review panel. The number of patients with an actionable alteration and a drug available that targets the alteration will be summarized. Exploration of reasons that a drug was not received, if available, will be addressed by review of the participants' medical record at the enrolling institution and by query of the primary oncologist/site PI.

Exploratory Objective 2: to perform whole exome sequencing, RNA sequencing, DNA methylation analyses, and primary leukemia sensitivity testing to a panel of drugs or shRNA, or other technology as it is developed. The results of this aim will not be used for a matched therapy recommendation by the expert panel but can be correlated with findings on the RHP/gene fusion assay. Concordance between the RHP/gene fusion assay and these exploratory technologies will be assessed to inform future clinical trials.

Exploratory Objective 3: to explore different sample types that can be used to assess germline status in pediatric patients with leukemia by extracting DNA from submitted samples from various germline sources (e.g., buccal swab, saliva, fingernail clippings). DNA quality will be assessed, and research sequencing may be performed to aid in interpretation of exploratory analyses described in Exploratory Objective 3. Exploration of whether DNA is contaminated with patient's leukemia will be explored for samples derived from buccal swabs and saliva at the time of diagnosis/protocol enrollment.

Exploratory Objective 4: to evaluate whether pediatric leukemias acquire altered clonal predominance and additional alterations during disease progression or during treatment with a matched therapy by assessing serial bone marrow analyses and repeat RHP if collected clinically. The Site PI may request to submit serial samples for one participant at study entry, relapse of disease or every three months if the participant is receiving a matched targeted therapy recommendation and the participant is getting serial bone marrow analyses for clinical purposes. Number of samples per patient is limited to no more than one sample per month, up to a total of three samples per patient. The total number can be increased with discussion with the overall PI.

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