Activated: 10-28-2021 Version Date: 4-22-2024
Closed: Amendment#: 1A

CHILDREN'S ONCOLOGY GROUP

APAL2020SC

Pediatric Acute Leukemia (PedAL) Screening Trial – Developing New Therapies for Relapsed Leukemias

A Leukemia & Lymphoma Society and COG Groupwide Screening Protocol

CONTACT INFORMATION		
For Regulatory Requirements	**For patient enrollments:**	**For Data Submission**
Regulatory documentation must be submitted to the Cancer Trials Support Unit (CTSU) via the Regulatory Submission Portal. (Sign in at https://www.ctsu.org, and select the Regulatory > Regulatory Submission.) Institutions with patients waiting that are unable to use the Portal should alert the CTSU Regulatory Office immediately by phone or email: 1-866-651-CTSU (2878), or CTSURegHelp@coccg.org to receive further instruction and support. Contact the CTSU Regulatory Help Desk at 1-866-651-CTSU (2878) or CTSURegHelp@coccg.org for regulatory assistance.	Refer to the patient enrollment section of the protocol for instructions on using the Oncology Patient Enrollment Network (OPEN). OPEN is accessed at https://www.ctsu.org/OPEN_SYSTEM/ or https://open.ctsu.org. Contact the CTSU Help Desk with any OPEN-related questions by phone or email: 1-888-823-5923, or ctsucontact@westat.com.	Data collection for this study will be done exclusively through Medidata Rave. Please see the Data Submission Schedule in the CRF packet for further instructions.
The most current version of the **study protocol and all supporting documents** must be downloaded from the protocol-specific page located on the CTSU members' website (https://www.ctsu.org). Permission to view and download this protocol and its supporting documents is restricted and is based on person and site roster assignment housed in the Roster Maintenance application and in most cases viewable and manageable via the Roster Update Management System (RUMS) on the CTSU members' website.		
For clinical questions (i.e., patient eligibility or treatment-related) Contact the Study PI of the Lead Protocol Organization.		
For non-clinical questions (i.e., unrelated to patient eligibility, treatment, or clinical data submission) Contact the CTSU Help Desk by phone or e-mail: CTSU General Information Line – 1-888-823-5923, or ctsucontact@westat.com. All calls and correspondence will be triaged to the appropriate CTSU representative.		
The CTSU Website is located at https://www.ctsu.org.		

TABLE OF CONTENTS

SECTION			PAGE
TABLE OF CONTENTS			**3**
STUDY COMMITTEE			**5**
ABSTRACT			**7**
EXPERIMENTAL DESIGN SCHEMA			**8**
1.0	**GOALS AND OBJECTIVES (SCIENTIFIC AIMS)**		**9**
	1.1	Primary Aims	9
2.0	**BACKGROUND**		**10**
	2.1	Rationale for a PedAL Screening Protocol	10
	2.2	Goals and Objectives of the PedAL Screening Trial (Scientific Aims)	11
	2.3	PedAL Treatment Sub-trials	12
3.0	**STUDY ENROLLMENT PROCEDURES AND PATIENT ELIGIBILITY**		**14**
	3.1	Study Enrollment	14
	3.2	Patient Eligibility Criteria	17
4.0	**SUBMISSION OF MATERIALS AND DATA FOR SCREENING**		**19**
	4.1	Required Biologic Materials for Initial Evaluation at Time of Suspected or Confirmed Relapse/Refractory Status	19
	4.2	Samples for Optional Banking	20
	4.3	Submission of Biologic Materials for Re-Screening	20
	4.4	Submission of Samples for Central Response Determination after Therapy for Relapse/Refractory Acute Leukemia (End of Cycle Disease Evaluations)	21
	4.5	Specimens for Correlative Studies on APAL2020SC Therapeutic Sub-trials	21
5.0	**SCREENING PROCEDURES FOR SUB-TRIAL ELIGIBILITY**		**23**
	5.1	Specimen Testing	24
	5.2	Biomarker Results	24
	5.3	Sub-trial Recommendation	25
	5.4	PedAL Sub-Trial Eligibility	25
6.0	**FOLLOW-UP DATA**		**26**
	6.1	Follow-up Time Points	26
	6.2	Data Collected During Follow-up	26
	6.3	Withdrawal of Consent Procedures	26
7.0	**OFF STUDY CRITERIA**		**27**
	7.1	Off Study Criteria	27
8.0	**STATISTICAL CONSIDERATIONS**		**28**
	8.1	Hypothesis	28
	8.2	Sample Size and Study Duration	28
	8.3	Statistical Analysis Methods	28
	8.4	Gender and Minority Accrual Estimates	28

9.0 EVALUATION CRITERIA 30
9.1 Common Terminology Criteria for Adverse Events (CTCAE) 30
9.2 APAL2020SC Pediatric Specific Response Criteria for Patients with AML 30
9.3 Relapse/Refractory Definitions for Patients with AML, ALL, and MPAL 33
10.0 PATHOLOGY GUIDELINES 36
10.1 Patients with AML and ALL 36
10.2 Patients with Myeloid Sarcoma (Chloroma): 37
10.3 Consultation Available 37
11.0 RECORDS AND REPORTING 38
11.1 Reporting 38
APPENDIX I: CTEP AND CTSU REGISTRATION PROCEDURES 39
INVESTIGATOR AND RESEARCH ASSOCIATE REGISTRATION WITH CTEP 39
APPENDIX II: YOUTH INFORMATION SHEETS 43
APPENDIX III: SAMPLES FOR USE IN PEDAL THERAPEUTIC SUB-TRIAL CORRELATIVE BIOLOGY STUDIES 45
APPENDIX IV: RESPONSE CRITERIA FOR MDS 47
APPENDIX V: RESPONSE CRITERIA FOR JJML 49
REFERENCES 52

The Children's Oncology Group has received a Certificate of Confidentiality from the federal government, which will help us protect the privacy of our research subjects. The Certificate protects against the involuntary release of information about your subjects collected during the course of our covered studies. The researchers involved in the studies cannot be forced to disclose the identity or any information collected in the study in any legal proceedings at the federal, state, or local level, regardless of whether they are criminal, administrative, or legislative proceedings. However, the subject or the researcher may choose to voluntarily disclose the protected information under certain circumstances. For example, if the subject or his/her guardian requests the release of information in writing, the Certificate does not protect against that voluntary disclosure. Furthermore, federal agencies may review our records under limited circumstances, such as a DHHS request for information for an audit or program evaluation or an FDA request under the Food, Drug and Cosmetics Act.
The Certificate of Confidentiality will not protect against mandatory disclosure by the researchers of information on suspected child abuse, reportable communicable diseases, and/or possible threat of harm to self or others.

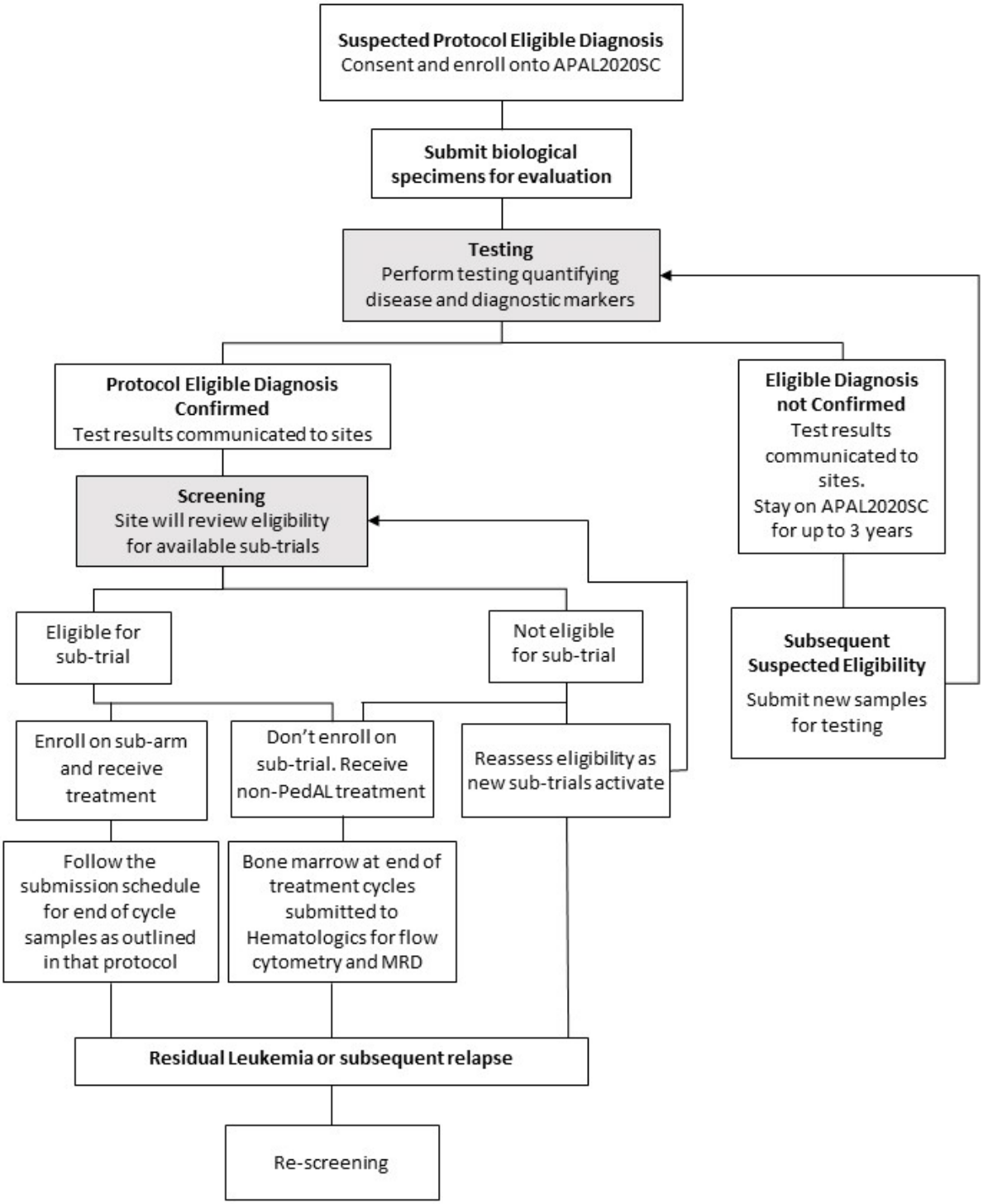
ABSTRACT

Therapeutic options for relapsed acute myeloid leukemia (AML) and some subsets of relapsed acute lymphoblastic leukemia (ALL) remain limited. Various re-induction regimens studied over the last decade have failed to improve our approach to relapsed AML.[1, 2] Conventional approaches to improving survival following relapse in AML, and in many patients with ALL, are failing.

Recent large-scale discovery efforts using next generation sequencing methods have identified recurrent mutational, signaling pathway and cell surface antigen targets for new therapies. Of those recently approved, some have strong rationale for testing in pediatric patients. However, given the clinical and biologic differences between adult and pediatric AML, significant improvement in survival of children with AML cannot be accomplished if we rely exclusively on approved adult AML drugs for pediatric development.[3, 4] The logistical barriers to studying new therapies for uncommon events in rare diseases in children are significant. Many of the high value targets in children (mesothelin, KMT2A, CD56, FLT3) are present in small subsets of patients, and this screening trial represents a mechanism by which we can efficiently interrogate agents in subsets of children with relapsed and refractory acute leukemias. While CAR-T therapy has shifted the paradigm for patients with ALL, relapse post CAR-T is challenging to treat. Identification of new targets in ALL will enable the development of specific therapies for relapse.

The Leukemia & Lymphoma Society (LLS) **Ped**iatric **A**cute **L**eukemia (PedAL) Screening Protocol will be conducted under a single IND sponsored by LLS and implemented through the Children's Oncology Group (COG). APAL2020SC will provide a single portal of entry with longitudinal collection of clinical, immunophenotype and molecular data for all children with relapsed/refractory leukemia. This trial will provide a mechanism for target detection and increased efficiency for sub-trial activation. This screening trial will improve new agent availability for children, as well provide efficient pathway for our pharma partners to fulfill regulatory requirements and drug approval.

EXPERIMENTAL DESIGN SCHEMA



1.0 GOALS AND OBJECTIVES (SCIENTIFIC AIMS)

1.1 Primary Aims

1.1.1 To utilize clinical and biological characteristics of acute leukemias to screen for patient eligibility for available Phase I/II PedAL sub-trials.

1.1.2 To maintain a longitudinal and comprehensive registry, as well as a specimen bank, from relapse in children and young adults with acute leukemias.

2.0 BACKGROUND

Therapeutic options for relapsed acute myeloid leukemia (AML) and some subsets of relapsed acute lymphoblastic leukemia (ALL) remain limited. Various re-induction regimens studied over the last decade have failed to improve our approach to relapsed AML.[1, 2] The median remission re-induction rate in first relapse is close to 60% with a range of 35% to 81% in studies that include as few as 11 patients and as many as 394 patients.[5] Children who relapse within a year from first remission have lower response rates to re-induction chemotherapy and overall survival rates are dismal.[2] Though anthracycline containing regimens generally induce better response rates, not all patients can tolerate additional anthracyclines at relapse. In ALL, overall survival of patients that experience first relapse is approximately 50% despite advances in salvage therapy including chimeric antigen receptor T-cell therapy (CAR-T) and allogeneic hematopoietic stem cell transplant (HSCT).[6] However, regardless of the regimens used, it is clear that conventional approaches to improving survival following relapsed leukemia in children are failing.

Recent large-scale discovery efforts using next generation sequencing (NGS) methods have identified recurrent mutational, signaling pathway and cell surface antigen targets for new therapies. Though several drugs have been approved for adults with AML (glasdigib, venetoclax, gilteritinib, CPX-351, enasidenib and ivosidenib), drug development for childhood AML lags far behind. Of those recently approved, some have strong rationale for testing in pediatric patients, but given the clinical and biologic differences between adult and pediatric AML, significant improvement in survival of children with AML cannot be accomplished if we rely exclusively on approved adult AML drugs for pediatric development.[3, 4] Immunotherapies, including cellular therapies, for ALL have advanced retrieval strategies and are currently being evaluated in COG de novo ALL studies (AALL1721, AALL1731 and AALL1732).[7-9] However, there are children with ALL that remain refractory to these therapies and additional targets and new agents need to be aggressively pursued. To this end, we must continue to identify therapeutic strategies for which there is both scientific rationale as well as regulatory incentives for development.

With the exception of FLT3, there is a paucity of 'druggable' targets in AML that are shared between children and adults. Many of the high value targets in children (mesothelin, KMT2A, CD56, FLT3) are present in small subsets of patients, and this screening trial represents a mechanism by which we can efficiently interrogate agents in subsets of children with relapsed acute leukemias. The most rapid and efficient way to evaluate the current leukemia pipeline of novel agents is through a PedAL screening trial (APAL2020SC) that provides a single portal of entry for all patients for target screening to inform sub-trial enrollments.

APAL2020SC will provide a single portal of entry with longitudinal collection of clinical, immunophenotype and molecular data for all children with relapsed/refractory leukemia. There are multiple development paths for relapse therapies in children with acute leukemia, yet few impactful trials and heterogeneous designs. Through the Pediatric Acute Leukemia (PedAL) initiative we intend to expand our trial portfolio and consolidate screening for eligible patients, while providing our Pharma partners an efficient path to fulfilling regulatory requirements.

2.1 Rationale for a PedAL Screening Protocol

To evaluate novel targeted therapies in children with relapsed leukemia, we must address that fact that many recurrent somatic mutations and antigen targets of interest in AML and ALL are expressed in a minority of patients. The logistical barriers to studying new therapies for uncommon events in rare disease in children are significant. There needs to be an available assay for target detection and screening as well as a mechanism that makes

it efficient for sites to activate a sub-trial for the eligible patient. The recent success of ProjectEveryChild (APEC14B1) and Beat AML confirm the role of screening trials as efficient approaches for the assignment of patients to targeted therapies. There is, however, currently no central screening mechanism that includes relapsed leukemias in children and young adults. In response to an increasing number of targeted leukemia therapies requiring molecular and immuno-flow cytometry screening for trial eligibility, the Leukemia & Lymphoma Society (LLS) is committed to supporting the PedAL initiative to facilitate the study of new therapies for children with relapsed leukemia.

Similar to ProjectEveryChild (APEC14B1), APAL2020SC will permit enrollment of patients with "known or suspected" relapsed AML or ALL that occurs in the pediatric, adolescent or young adult populations. This will permit early identification of patients for relapse trial eligibility determination, sub-trial activation and the collection of relapse specimens. All of these items have proven challenging and have limited the impact of past clinical trials at relapse through COG and other international cooperative groups. It is the intent of APAL2020SC to remove historical barriers to accrual on to relapse trials, to collection of vital clinical information, and to procurement of precious biologic samples that are vital to improving our understanding of relapsed acute leukemia in children. The PedAL screening trial will provide a mechanism for biospecimen collection, rapid central eligibility screening and longitudinal tracking of patients following relapse to address major barriers to the development of effective therapies in relapsed leukemias.

The LLS/COG **Ped**iatric **A**cute **L**eukemia (PedAL) Screening Protocol will be available to COG sites group-wide. Major European AML Cooperative Groups (DCOG, NOPHO, AIEOP, BFM, MRC as well as ANZCHOG) have agreed to participate in select PedAL sub-trials. Enrollment on APAL2020SC will be required for sub-trial enrollment for COG institutions and there will be a parallel screening process in the EU. The majority of patients in North America will have also enrolled on APEC14B1:ProjectEveryChild at diagnosis and will have had analytically validated comprehensive genomic profiling (CGP) through Foundation Medicine, Inc, as well as central immuno-flow cytometry for detection and quantification of cell surface targets performed at Hematologics, Inc. Although encouraged, participation in APEC14B1 is not required. Once enrolled on APAL2020SC, flow cytometry and CGP results from relapse will be provided to investigators to inform enrollment of patients onto PedAL sub-trials.

2.2 Goals and Objectives of the PedAL Screening Trial (Scientific Aims)

Building on the success of the Beat AML initiative launched in 2012, LLS has committed to a substantial investment in relapsed childhood acute leukemias. The intent is to create:

1. A trial that provides a mechanism for collecting tissue and screening for specific cell surface, molecular and genetic targets for all patients with relapsed AML and select subsets of ALL patients.

2. A screening mechanism that will incorporate standard diagnostic information (cytogenetics/FISH analysis), complete genomic profiling, molecular diagnostics, immunophenotyping and clinical data needed for sub-trial eligibility.

3. A data registry to capture treatment, response, toxicities, events and outcomes independent of trial assignment so that we may better understand and describe post-relapse survival in acute leukemia.

4. A mechanism for collecting, processing and banking biological specimens from patients at multiple time points, to enable concurrent and future research to better understand and treat relapsed leukemias.

2.3 PedAL Treatment Sub-trials

PedAL therapeutic sub-trials will be stand-alone clinical trials and enrollment on APAL2020SC screening will be required for enrollment onto all PedAL therapeutic sub-trials. The primary endpoints for PedAL sub-trials will be safety and/or efficacy. The PedAL sub-trials fall into 2 general categories: Registration trials with a primary efficacy objective and safety/feasibility trials with a signal finding efficacy objective. The registration trials will either be randomized or employ a well-designed historical comparator.

Most study drugs included in these sub-trials will include agents that have at least an adult recommended phase 2 dose (RP2D) and there will be an expectation of benefit for pediatric patients. If there are agents interrogated for pediatric-specific targets without adult data or without data in patients with leukemia, then safety assessments will be performed with industry and FDA guidance.

2.3.1 Agent Selection and Prioritization

Through PedAL, the LLS also funds a preclinical prioritization team led by Dr. Kim Stegmaier and a biomarker discovery team led by Dr. Soheil Meshinchi. The PedAL Clinical Trial Committee works with Pharma partners to secure those agents most likely to provide benefit. All sub-trials will include background data to justify use of the agent in children, target expression, prognostic data associated with target expression, and agent-specific toxicity data. Many of the highest value targets in pediatric AML are not mutation specific (e.g. CD123). Each sub-trial will include a rationale for use and an enrollment plan that accounts for other active studies.

Agents selected for use in PedAL sub-trials will be reviewed according to the following steps:

1. An Agent Prioritization Proposal will be presented to the PedAL Advisory Committee by the Concept Lead (Sub-trial study chair if approved). At minimum the proposal will review all available adult safety and efficacy data, target relevance and expression in children with leukemia, preclinical data, and a feasibility plan for testing in children.

2. Agent Prioritization will be reviewed by the PedAL Advisory Committee. Only agents receiving a majority vote will advance to clinical development.

2.3.2 Regulatory Elements of APAL2020SC and Sub-Trials

It will be the intent of PedAL to support development of therapies that are initiated with a goal to eventually obtain regulatory approval in children. The PedAL project team will seek FDA guidance and EMA Scientific Advice for therapeutic sub-trials funded by industry partners with an intent-to-file. Though APAL2020SC is a

Screening Trial, it is helpful to refer to recent published guidance. The FDA defined Master Protocols as "a protocol with multiple sub-studies, which may have different objectives and involves coordinated efforts to evaluate one or more investigational drugs in one or more disease subtypes within the overall trial structure." The guidance strongly recommends for pediatric trials that there be sufficient adult data to define a reasonable pediatric starting dose and that the drug provide the "prospect of benefit" for the patients. While this will be the goal, there are examples of targeted therapies appropriate for testing in children that have not been previously tested in adults with acute leukemia. In such instances, there will be detailed rationale presented and will incorporate a safety assessment.[10, 11]

3.0 STUDY ENROLLMENT PROCEDURES AND PATIENT ELIGIBILITY

3.1 Study Enrollment

3.1.1 Patient Registration

Prior to enrollment on this study, patients must be assigned a COG patient ID number. This number is obtained via the Patient Registry module in OPEN once authorization for the release of protected health information (PHI) has been obtained. The COG patient ID number is used to identify the patient in all future interactions with COG. If you have problems with the registration, please refer to the online help. For additional help or information, please contact the CTSU Help Desk at 1-888-823-5923 or ctsucontact@westat.com.

In order for an institution to maintain COG membership requirements, every patient with a known or suspected neoplasm needs to be offered participation in APEC14B1, *Project:EveryChild A Registry, Eligibility Screening, Biology and Outcome Study*. Enrollment on APEC14B1 is not required for enrollment on APAL2020SC.

A Biopathology Center (BPC) number will be assigned as part of the registration process. Each patient will be assigned only one BPC number per COG Patient ID. For additional information about the labeling of specimens please refer to the Pathology and/or Biology Guidelines in this protocol.

Please see Appendix I for detailed CTEP Registration Procedures for Investigators and Associates, and Cancer Trials Support Unit (CTSU) Registration Procedures including: how to download site registration documents; requirements for site registration, submission of regulatory documents and how to check your site's registration status.

3.1.2 IRB Approval

As of March 1, 2019, all U.S.-based sites must be members of the NCI Central Institutional Review Board (NCI CIRB) in order to participate in Cancer Therapy Evaluation Program (CTEP) and Division of Cancer Prevention (DCP) studies open to the National Clinical Trials Network (NCTN) and NCI Community Oncology Research Program (NCORP) Research Bases. In addition, U.S.-based sites must accept the NCI CIRB review to activate new studies at the site after March 1, 2019. Local IRB review will continue to be accepted for studies that are not reviewed by the CIRB, or if the study was previously open at the site under the local IRB. International sites should continue to submit Research Ethics Board (REB) approval to the CTSU Regulatory Office following country-specific regulations.

Sites participating through the NCI CIRB must submit the Study Specific Worksheet (SSW) for Local Context to the CIRB using IRBManager to indicate their intent to open the study locally. The NCI CIRB's approval of the SSW is automatically communicated to the CTSU Regulatory Office, but sites are required to contact the CTSU Regulatory Office at CTSURegPref@ctsu.coccg.org to establish site preferences for applying NCI CIRB approvals across their Signatory Network. Site preferences can be set at the network or protocol level. Questions

about establishing site preferences can be addressed to the CTSU Regulatory Office by email (CTSURegPref@ctsu.coccg.org) or by calling 1-888-651-CTSU (2878).

Sites using their local IRB or REB, must submit their approval to the CTSU Regulatory Office using the Regulatory Submission Portal located in the Regulatory section of the CTSU website. Acceptable documentation of local IRB/REB approval includes:

- Local IRB documentation;
- IRB-signed CTSU IRB Certification Form; and/or
- Protocol of Human Subjects Assurance Identification/IRB Certification/Declaration of Exemption Form.

In addition, the Site-Protocol Principal Investigator (PI) (i.e., the investigator on the IRB/REB approval) must meet the following criteria for the site to be able to have an Approved status following processing of the IRB/REB approval record:

- Have an active CTEP status;
- Have an active status at the site(s) on the IRB/REB approval (*applies to US and Canadian sites only*) and on at least one participating organization's roster;
- If using NCI CIRB, be active on the NCI CIRB roster under the applicable CIRB Signatory Institution(s) record;
- Include the IRB number of the IRB providing approval in the Form FDA 1572 in the RCR profile;
- List all sites on the IRB/REB approval as Practice Sites in the Form FDA 1572 in the RCR profile; and
- Have the appropriate CTEP registration type for the protocol.

Additional Requirements

Additional requirements to obtain an approved site registration status include:

- An active Federal Wide Assurance (FWA) number;
- An active roster affiliation with the Lead Protocol Organization (LPO) or a Participating Organization (PO)
- An active roster affiliation with the NCI CIRB roster under at least one CIRB Signatory Institution (US sites only); and
- Compliance with all applicable protocol-specific requirements (PSRs).

For information about the submission of IRB/REB approval documents and other regulatory documents as well as checking the status of study center registration packets, please see Appendix I.

Institutions with patients waiting that are unable to use the Portal should alert the CTSU Regulatory Office immediately at 1-866-651-2878 in order to receive further instruction and support. For general (non-regulatory) questions call the CTSU General Helpdesk at: 1-888-823-5923.

Note: Sites participating on the NCI CIRB initiative and accepting CIRB approval for the study are not required to submit separate IRB approval

documentation to the CTSU Regulatory Office for initial, continuing or amendment review.

3.1.3 Study Enrollment

Patients may be enrolled on APAL2020SC if they meet the eligibility criteria outlined in Section 3.2.

Patient enrollment will be facilitated using the Oncology Patient Enrollment Network (OPEN). OPEN is a web-based registration system available on a 24/7 basis. OPEN is integrated with CTSU regulatory and roster data and with the Lead Protocol Organization (LPOs) registration/randomization systems or the Theradex Interactive Web Response System (IWRS) for retrieval of patient registration/randomization assignment. OPEN will populate the patient enrollment data in NCI's clinical data management system, Medidata Rave.

Requirements for OPEN access:

- Active CTEP registration with the credentials necessary to access secure NCI/CTSU IT systems;
- To perform enrollments or request slot reservations: Must be on an LPO roster, ETCTN corresponding roster, or participating organization roster with the role of Registrar. Registrars must hold a minimum of an Associate Plus (AP) registration type;
- If a Delegation of Tasks Log (DTL) is required for the study, the registrars must hold the OPEN Registrar task on the DTL for the site; and
- Have an approved site registration for the protocol prior to patient enrollment.

To assign an Investigator (IVR) or Non-Physician Investigator (NPIVR) as the treating, crediting, consenting, or receiving investigator for a patient transfer in OPEN, the IVR or NPIVR must list the Institutional Review Board (IRB) number used on the site's IRB approval on their Form Food and Drug Administration (FDA) 1572 in Registration and Credential Repository (RCR). If a DTL is required for the study, the IVR or NPIVR must be assigned the appropriate OPEN-related tasks on the DTL.

To assign a Non-Investigational New Drug (IND)/Non-Treatment (NINT) as the treating, crediting, consenting, or receiving investigator for a patient transfer in OPEN, NINTs must list all clinical practice sites, Institutional Review Boards, and labs covering their practice sites on the NINT Investigator Acceptance Form in RCR. NINTs may only participate on studies flagged for NINT participation.

Prior to accessing OPEN, site staff should verify the following:

- Patient has met all eligibility criteria within the protocol stated timeframes; and
- All patients have signed an appropriate consent form and HIPAA authorization form (if applicable).

Note: The OPEN system will provide the site with a printable confirmation of registration and treatment information. You may print this confirmation for your records.

Access OPEN at https://open.ctsu.org or from the OPEN link on the CTSU members' website. Further instructional information is in the OPEN section of the CTSU website at https://www.ctsu.org or https://open.ctsu.org. For any additional questions, contact the CTSU Help Desk at 1-888-823-5923 or ctsucontact@westat.com.

3.1.4 Submission of Specimens
Submission of specimens for screening via APAL2020SC must only be done after obtaining written informed consent. See Section 4.0 for submission requirements.

3.2 Patient Eligibility Criteria

Important note: The eligibility criteria listed below are interpreted literally and cannot be waived. All clinical and laboratory data required for determining eligibility of a patient enrolled on this trial must be available in the patient's medical/research record which will serve as the source document for verification at the time of audit.

Inclusion Criteria

3.2.1 Age
Patients must be less than 22 years of age at the time of study enrollment.

3.2.2 Diagnosis
Patient must have one of the following **at the time of study enrollment**: (See Section 9.3 for definitions of relapse and refractory disease)

a. Patient has known or suspected relapsed/refractory (including primary refractory) AML as defined in Section 9.3.
   - This includes isolated myeloid sarcoma.

b. Patient has known or suspected relapsed/refractory (including primary refractory) myeloid leukemia of Down syndrome (ML-DS).

c. Patient has known or suspected relapsed ALL as defined in Section 9.3 that meets one of the following criteria:
   1. Second or greater B-ALL medullary relapse, excluding *KMT2A*r.
   2. Any first or greater B-ALL medullary relapse involving *KMT2A*r.
   3. Any first or greater T-ALL medullary relapse with or without *KMT2A*r.

d. Patient has known or suspected relapsed/refractory (including primary refractory) mixed phenotype acute leukemia (MPAL) as defined in Section 9.3.

e. Patient has known or suspected *de novo* or relapsed/refractory (including primary refractory) treatment-related AML (t-AML).

f. Patient has known or suspected *de novo* or relapsed/refractory (including primary refractory) Myelodysplastic Syndrome (MDS or treatment-related Myelodysplastic Syndrome (t-MDS). See Appendix IV for additional information.

Note: Relapsed/refractory disease includes Stable Disease, Progressive Disease, and Disease Relapse.

Patient has known or suspected *de novo* or relapsed/refractory (including primary refractory) Juvenile Myelomonocytic Leukemia (JMML). See Appendix V for additional information.

Note: Relapsed/refractory disease includes Stable Disease, Progressive Disease, and Disease Relapse.

3.2.3 Regulatory Requirements

3.2.3.1 All patients and/or their parents or legal guardians must sign a written informed consent.

3.2.3.2 All institutional, FDA, and NCI requirements for human studies must be met.

There are no Exclusion Criteria for APAL2020SC.

4.0 SUBMISSION OF MATERIALS AND DATA FOR SCREENING

4.1 Required Biologic Materials for Initial Evaluation at Time of Suspected or Confirmed Relapse/Refractory Status

The initial evaluation is the first evaluation after consent is signed and the patient is enrolled onto APAL2020SC. It is preferable, but not required, that the initial evaluation samples are obtained within 7 days of enrollment.

Samples should be shipped the same day they are collected.
It is of the utmost importance that samples be shipped fresh and the day they are collected. If possible, make arrangements to time diagnostic procedures so that they can be shipped the same day as collected. This is true even if the diagnosis of relapse is not confirmed. It is vital for proper molecular and genetic analysis that specimens be shipped immediately. This is especially important for specimens going to Foundation Medicine Inc. (FMI).

Obtaining bone marrow on Saturday or Sunday is strongly discouraged unless clinically indicated.

Please refer to the Manual of Procedures (MOP) for APAL2020SC for detailed instructions for collecting and shipping samples for initial evaluation of patients with relapsed/refractory disease. The manual can be found on the APAL2020SC web page on the COG members only site.

Summary of Specimens at Time of Initial Evaluation on APAL2020SC

Specimen	Volume	Tube Type	Laboratory	Consent
All Patients Enrolled				
Bone Marrow	2-4 mL	Sodium Heparin (Green Top)	Hematologics, Inc.	Covered under APAL2020SC consent
Peripheral Blood*	10 mL	Sodium Heparin (Green Top)		
Patients with AML, Treatment-Related AML, Myeloid Leukemia of Downs Syndrome, MDS, Treatment-Related MDS, and JMML For Foundation Medicine, only submit 1 type of specimen. DO NOT SEND BOTH BM AND PB. If both are available send the sample with the highest tumor content.				
Bone Marrow	1.5-2.5 mL	EDTA (purple top)	Foundation Medicine Inc.	Covered under APAL2020SC consent
Peripheral Blood*	2.5-10 mL	EDTA (purple top)		

Patients with Myeloid Sarcoma Submit FFPE slides or block as outlined in Appendix II of the APAL2020SC MOP.		

*If marrow cannot be obtained, see the specific sections of the APAL2020SC MOP for requirements for submitting peripheral blood. Do not send both marrow and blood.

4.2 Samples for Optional Banking

All patients enrolled may participate in the optional banking.

Specimen	Volume	Tube Type	Laboratory	Consent
Bone Marrow	4-8 mL	EDTA (purple top) **OR** COG Shipping Media	BPC	Patient must agree to optional banking in the APAL2020SC consent.
Peripheral Blood*	8-16 mL	EDTA (purple top) **OR** COG Shipping Media	BPC	

*If marrow cannot be obtained, see the specific sections of the APAL2020SC MOP for requirements for submitting peripheral blood.

4.3 Submission of Biologic Materials for Re-Screening

After patients are enrolled and initially evaluated for APAL2020SC, investigators with patients who did not have confirmed relapse/refractory disease, who relapse after remission was achieved subsequent to treatment for relapsed leukemia or whose patients have detectable leukemia after a re-induction attempt may submit new samples for re-screening and banking in the amounts outlined below. Samples will have flow cytometry for blast enumeration. Sites will get access to these results. If the patient agrees to optional biobanking, any leftover samples will be banked. Submission of a dedicated sample for biobanking at re-screening is encouraged for patients who consented.

A sample for next-generation sequencing should be submitted to FMI for re-screening only if sequencing of the initial APAL2020SC screening sample failed or if repeat FMI sequencing is specifically required as part of eligibility for a therapeutic sub-trial. See Section 4.1 for sample requirements.

Summary of Specimens for Re-Screening Table

Specimen	Volume	Tube Type	Laboratory
Bone Marrow	2-4 mL	Sodium Heparin (Green Top)	Hematologics, Inc.
Peripheral Blood*	10 mL	Sodium Heparin (Green Top)	Hematologics, Inc.
Bone Marrow	4-8 mL	EDTA (purple top) **OR** COG Shipping Media	BPC
Peripheral Blood*	8-16 mL	EDTA (purple top) **OR** COG Shipping Media	BPC

*Bone marrow is preferred. If marrow cannot be obtained, see the specific sections of the APAL2020SC MOP for requirements for submitting peripheral blood. Do not send both marrow and blood.

4.4 Submission of Samples for Central Response Determination after Therapy for Relapse/Refractory Acute Leukemia (End of Cycle Disease Evaluations)

4.4.1 Patients enrolled on a PedAL or COG therapeutic sub-trial

Patients enrolled on PedAL or COG therapeutic sub-trial should follow the submission schedule for end of cycle samples as outlined in those protocols.

4.4.2 Patients not enrolled on a PedAL or COG therapeutic sub-trial

If patients are not enrolled on a PedAL or COG therapeutic sub-trial, sites are strongly encouraged to submit bone marrow samples at the end of treatment cycles (or whenever a follow-up bone marrow evaluation is done for clinical purposes) to Hematologics for flow cytometry and MRD. Hematologics offers comprehensive panels and is a leader in residual disease testing by flow cytometry. This testing is a free resource for all patients on this study. Capturing central response to treatment is a critical component and a high priority for the APAL2020SC longitudinal data registry.

Typically, **peripheral blood cannot be evaluated** due to low levels of disease. But, if the patient has peripheral blasts and site does not plan to do a bone marrow, then peripheral blood may be submitted. If the patient does not have any peripheral blasts and the site does not plan to do a bone marrow, no specimens should be submitted. If the patient agrees to optional biobanking, any leftover samples will be banked. Submission of a dedicated sample for biobanking at re-screening is encouraged for patients who consented.

Specimen	Volume	Tube Type	Laboratory
Bone Marrow only	2-4 mL	Sodium Heparin (Green Top)	Hematologics, Inc.
Peripheral Blood*	10 mL	Sodium Heparin (Green Top)	Hematologics, Inc.
Bone Marrow	4-8 mL	EDTA (purple top) **OR** COG Shipping Media	BPC
Peripheral Blood*	8-16 mL	EDTA (purple top) **OR** COG Shipping Media	BPC

*Typically, **peripheral blood cannot be evaluated** due to low levels of disease. But, if the patient has peripheral blasts and site does not plan to do a bone marrow, then peripheral blood may be submitted. Do not send both marrow and blood.

4.5 Specimens for Correlative Studies on APAL2020SC Therapeutic Sub-trials

Some PedAL therapeutic sub-trials may require the use of a specimen submitted through APAL2020SC at the time of relapse for a correlative biology objective within the therapeutic sub-trial. The priority for specimens in these cases will always be the screening studies outlined in APAL2020SC. However if there is adequate sample available, samples

collected via APAL2020SC may be sent from the BPC to the laboratory coordinating the correlative biology study.

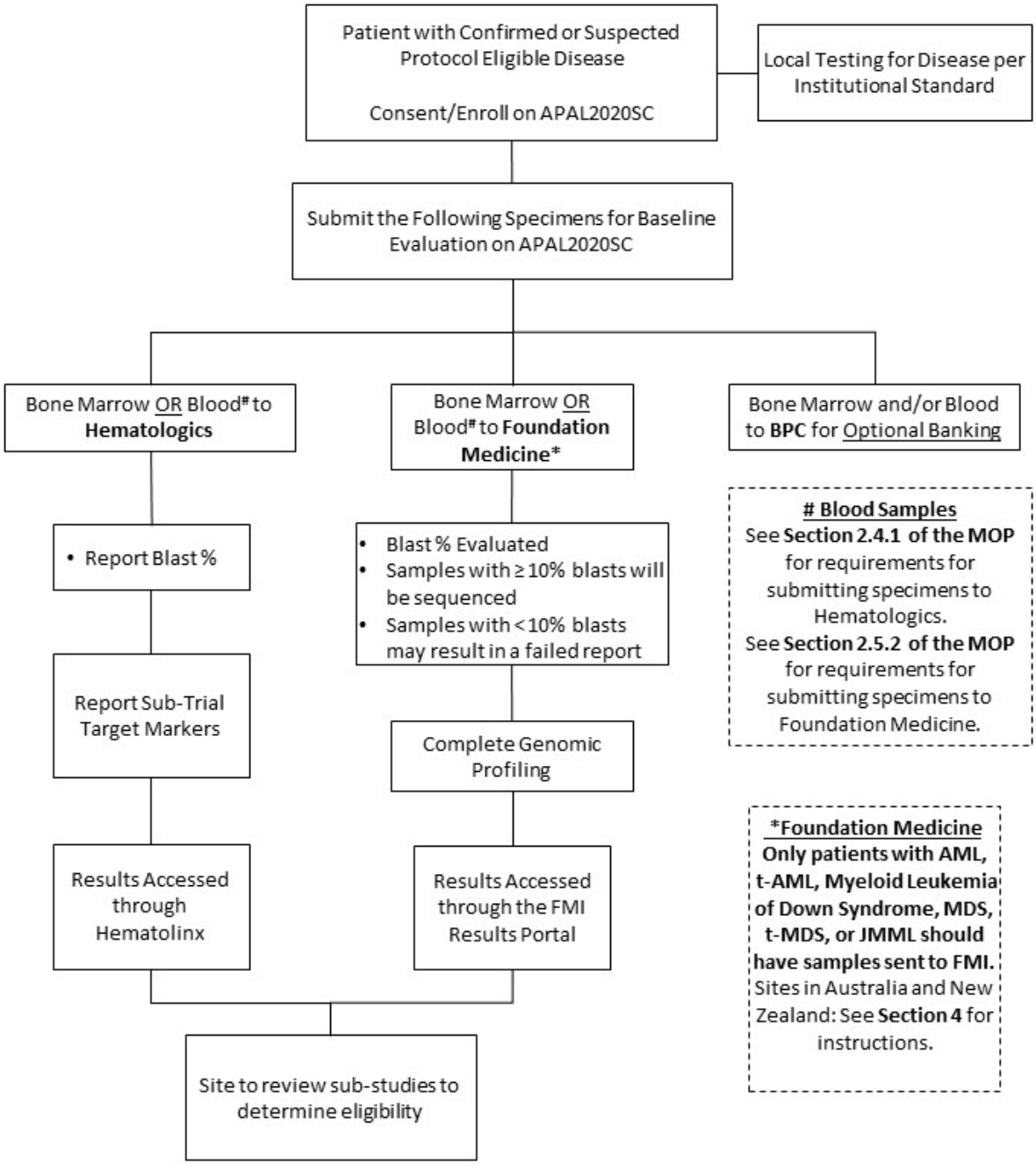
The correlative study objectives, sample requirements, collection, processing, and testing methods will be outlined in the APAL2020SC therapeutic sub-trial. The therapeutic sub-trial should specify that the diagnostic specimen submitted for APAL2020SC may also be used for objectives related to the sub-trial. Consent to direct these specimens for sub-trial studies will be obtained through the sub-trial consent. The sub-trial study chair will communicate directly with the BPC to confirm consent.

Appendix III includes a list of therapeutic sub-trials, for which specimens will be sent for correlative biology objectives. Within Appendix III the following are listed:

- Number/ Title of APAL2020SC therapeutic sub-trial.
- Correlative biology objectives for the sub-trial.
- Sample requested collected via APAL2020SC.
- Where the sample will be shipped.

5.0 SCREENING PROCEDURES FOR SUB-TRIAL ELIGIBILITY

The schema below provides and overview of sample distribution and testing at baseline evaluation.



5.1 Specimen Testing

5.1.1 Hematologics

Once specimens are received by Hematologics, the following will take place.

- Samples will be analyzed to determine the leukemic burden expressed as the percentage of leukemic blasts.
- Immunoflow cytometry will be performed to evaluate target markers to determine eligibility for PedAL sub-trials.

If the patient agrees during the consenting process, any specimen that is left-over after testing will be banked.

5.1.2 Foundation Medicine Inc.

Once specimens are received by FMI, the following will take place.

- Samples will be analyzed for tumor content.
  - Samples with ≥ 10% blasts will have a complete genomic profile performed.
  - AML samples with < 10% blasts may not be sequenced and a failure report may be issued.
  - MDS and JMML samples will be sequenced regardless of blast percentage.

5.1.3 BPC

Once specimens are received by the BPC, the following will take place.

- Bone marrow and/or blood will be banked for future research.
- For patients enrolled on a sub-trial, leftover samples may be sent from the BPC to the laboratory coordinating correlative biology on that trial. Consent to direct these specimens will be obtained through the sub-trial consent.

5.2 Biomarker Results

5.2.1 Results from Hematologics

Results from testing performed at Hematologics will be accessed through their results portal called Hematolinx. Site will need to request access to this portal after APAL2020SC activation. Please refer to the APAL2020SC MOP for instructions on accessing reports. Results will include the following:

- Confirmation of disease type and blast enumeration.
  - Results should be available within 3 days after sample received.
- Target markers for PedAL sub-trial eligibility.
  - Results should be available within 4 days after sample received.

- Central response assessment by flow cytometry for patients not enrolled on a PedAL or COG therapeutic sub-trial.
    - Sites are strongly encouraged to submit bone marrow samples at the end of treatment cycles (or whenever a follow-up bone marrow evaluation is done for clinical purposes).

5.2.2 Results from Foundation Medicine

Sites will be required to access the Foundation Medicine Results Portal. Each site will need to request an account for access to the portal. This is required even if you have an account for other COG protocols (AAML1831) or an account for clinical care. **Please refer to the APAL2020SC MOP for procedures regarding submitting the account access request**.

Sites will receive the full CLIA report via the portal. Results will be available a minimum of 14 days after being received by FMI. Sites will receive an email from the results portal when the results are available.

5.3 Sub-trial Recommendation

After results are obtained from the central labs, site investigators should review eligibility criteria to determine if a particular sub-trial is an option for their patient.

5.3.1 Patients Not Enrolling on a PedAL Sub-Trial

Patients who are either without a detectable target, are not eligible for a sub-trial, or who choose not to enroll in a sub-trial will continue to have post-relapse treatment, response, and outcome data collected for a maximum of 5 years. Sites are strongly encouraged to submit bone marrow samples at the end of treatment cycles (or whenever a follow-up bone marrow evaluation is done for clinical purposes) to Hematologics for flow cytometry and MRD (see section 4.4.2). Site investigators may continue to review eligibility for sub-trials at any time to see if there are sub-trials for which their patient may be eligible.

5.4 PedAL Sub-Trial Eligibility

PedAL sub-trials will be stand-alone clinical trials and APAL2020SC screening will be required for enrollment. Biomarker expression, prior therapy, organ function, and clinical features will be used to determine sub-trial eligibility.

Eligibility criteria for each sub-trial will be included in each individual PedAL sub-trial. Patients must meet all sub-trial inclusion and exclusion criteria in order to be enrolled onto the sub-trial. Samples collected via APAL2020SC may be sent to outside laboratories for additional biomarker screening using proprietary companion diagnostic testing if required for specific sub-trial eligibility. Example: A pharmaceutical company may use a specific commercial or private lab not outlined in APAL2020SC to evaluate eligibility for a specific sub-trial. These labs/tests will be included in the specific sub-trial.

6.0 FOLLOW-UP DATA

All patients enrolled onto APAL2020SC with disease confirmation will have follow-up submitted:

6.1 Follow-up Time Points

Follow-up will be required for all patients enrolled on APAL2020SC **regardless** of whether the patient enrolled onto a PedAL or COG therapeutic sub-trial.

- For patients **enrolled** onto a PedAL or COG therapeutic sub-trial.
  Sites should submit follow-up every 3 months for 2 years and then every 6 months for 3 years (total 5 years) from the **date of enrollment onto the most recent PedAL/COG therapeutic protocol**.

- For patients **not enrolled** onto a PedAL or COG therapeutic sub-trial.
  Sites should submit follow-up every 3 months for 2 years and then every 6 months for 3 years (total 5 years) from the date of enrollment on APAL2020SC.

6.2 Data Collected During Follow-up

Data to be collected during follow-up will include some or all of the following:

- PedAL/COG therapeutic sub-trial status.
- Vital status.
- Disease response evaluation/status.
- Anti-cancer therapy (including HSCT) received.
- Cardiac function data.
- Target toxicities (e.g. SOS, cardiac, infections).
- Other important medical data.
- Second malignant neoplasm.
- Off study status

* It is strongly recommended to utilize Hematologics for central evaluation for patients not enrolled on therapeutic clinical trials.

6.3 Withdrawal of Consent Procedures

If a patient should withdraw consent, sites should follow the SOP titled "Capturing & Tracking Withdrawal of Consents for COG Trials" found on the COG members only website. This SOP can be found under the "Administration" tab, under "Policies and Procedures", under "Study Conduct".

7.0 OFF STUDY CRITERIA

7.1 Off Study Criteria

a) Five years from the date of patient enrollment onto APAL2020SC if not enrolled onto a sub-trial.

b) Three years from the date of patient enrollment onto APAL2020SC if enrolling site failed to obtain an evaluable sample associated with APAL2020SC enrollment with no plans to submit subsequent samples.

c) Three years from the date of patient enrollment onto APAL2020SC if the diagnosis of protocol eligible disease (AML, ALL, t-AML, myeloid leukemia of Down Syndrome, MDS, t-MDS, JMML or MPAL) cannot be confirmed.

d) The fifth anniversary of the most recent date of patient enrollment onto a PedAL or COG therapeutic protocol.

e) Withdrawal of consent for any further data submission.

f) The results from eligibility studies are outside the parameters required for eligibility.

g) Death.

h) Lost to follow-up.

8.0 STATISTICAL CONSIDERATIONS

8.1 Hypothesis

Screening for genetic and immunophenotypic biomarkers in children with relapsed/refractory leukemia will improve and ensure enrollment on relevant targeted therapy sub-trials designed to produce clinically meaningful improvements in response rates.

8.2 Sample Size and Study Duration

Based on the relapse rate of patients with AML and ALL, we anticipate 16 patients per month for 60 months for a total of up to 960 patients in 5 years.

8.3 Statistical Analysis Methods

8.3.1 Study Endpoints

The primary goals of the APAL2020SC are:

- To identify *a priori* specified genomic and immunophenotypic targets to inform sub-trial eligibility.
- Maintain a longitudinal and comprehensive registry, as well as specimen bank, of children and young adults with acute leukemias.

Detailed statistical plans will be included in the individual sub-trials.

8.4 Gender and Minority Accrual Estimates

The gender and minority distribution of the study population is expected to be:

DOMESTIC PLANNED ENROLLMENT REPORT					
Racial Categories	**Ethnic Categories**				**Total**
	Not Hispanic or Latino		**Hispanic or Latino**		
	Female	**Male**	**Female**	**Male**	
American Indian / Alaska Native	1	2	2	1	6
Asian	13	29	1	1	44
Native Hawaiian or Other Pacific Islander	2	2	0	0	4
Black or African American	47	56	1	3	107
White	209	269	87	109	674
More Than One Race	1	3	0	1	5
Total	273	361	91	115	**840**

INTERNATIONAL (including Canadian participants) PLANNED ENROLLMENT REPORT					
Racial Categories	**Ethnic Categories**				**Total**
	Not Hispanic or Latino		**Hispanic or Latino**		
	Female	**Male**	**Female**	**Male**	
American Indian / Alaska Native	0	1	0	0	1
Asian	4	3	0	0	7
Native Hawaiian or Other Pacific Islander	0	0	0	0	0
Black or African American	4	8	0	0	12
White	43	34	9	12	98
More Than One Race	0	1	0	1	2
Total	51	47	9	13	**120**

This distribution was derived from AAML1031 and AALL1331.

9.0 EVALUATION CRITERIA

9.1 Common Terminology Criteria for Adverse Events (CTCAE)

This study will utilize version 5.0 of the CTCAE of the National Cancer Institute (NCI) for toxicity and performance reporting. A copy of the CTCAE version 5.0 can be downloaded from the CTEP website (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm). Additionally, toxicities are to be reported on the appropriate case report forms.

Please note: 'CTCAE v5.0' is understood to represent the most current version of CTCAE v5.0 as referenced on the CTEP website (i.e., v5.02 and all subsequent iterations prior to version 6.0).

9.2 APAL2020SC Pediatric Specific Response Criteria for Patients with AML

It is highly recommended to submit specimens to Hematologics for central response determination on this study (see Section 4.4.2). Hematologics offers comprehensive panels and is a leader in residual disease testing by flow cytometry. This testing is a free resource for all patients on this study. Capturing response to treatment is a critical component and a high priority for the APAL2020SC longitudinal data registry.

Individual PedAL therapeutic sub-trials will collect response data for that specific trial and should not be reported here.

The following response criteria refer to patients with known or suspected relapsed/refractory (including primary refractory) AML, patients with isolated myeloid sarcoma, patients with known or suspected relapsed/refractory ML-DS, and patients with known or suspected *de novo* or relapsed/refractory (including primary refractory) treatment-related AML (t-AML). These criteria are for patients who are not enrolled on PedAL sub-trials and should be used when completing baseline and follow-up CRFs.

AML patients who have 0.1 - 5% leukemic blasts at the time of enrollment must meet criteria for MRD negative response (< 0.1% leukemic blasts) to be considered to achieve a CR/CRp/CRi. For these cases, residual leukemic blasts > 0.1% will be considered Persistent Disease. The definition of Prolonged Aplasia is the same for this group of patients.

This study will collect response based on bone marrow flow cytometry AND bone marrow morphology. If flow cytometry evaluation is done by both a non-central lab and Hematologics, the Hematologics result will be used for response assessment.

9.2.1 Flow Cytometry Response Definitions

Flow Complete Response (Flow-CR):
Attainment of an M1 bone marrow (< 5% leukemic blasts) as determined by flow cytometry with no evidence of circulating blasts or extramedullary disease and with recovery of peripheral blood counts (ANC ≥ 500/µL and platelet count ≥ 50,000/µL). The CBC used for response assessment must be drawn on the day of the marrow or up to 14 days after bone marrow. There is no requirement for bone marrow cellularity.

MRD Negative Flow-CR:
Meets the above definition AND has < 0.1% leukemic blasts in the bone marrow by flow cytometry.

Flow Complete Response with Partial Recovery of Platelet Count (Flow-CRp):
Attainment of an M1 bone marrow (< 5% leukemic blasts) as determined by flow cytometry and no evidence of circulating blasts or extramedullary disease and with recovery of ANC ≥ 500/µL without platelet recovery to 50,000/µL. The CBC used for response assessment must be drawn on the day of the marrow or up to 14 days after bone marrow.

MRD Negative Flow-CRp:
Meets the above definition AND has < 0.1% leukemic blasts in the bone marrow by flow cytometry.

Flow Complete Response with Incomplete Blood Count Recovery (Flow-CRi):
Attainment of an M1 bone marrow (< 5% leukemic blasts) as determined by flow cytometry and no evidence of circulating blasts or extramedullary disease and with ANC > 200/µL and/or platelet count > 20,000/µL without dependence on platelet transfusions (defined as: a platelet count > 20,000/µL AND no platelet transfusions x 5 days). The CBC used for response assessment must be drawn on the day of the marrow or up to 14 days after bone marrow.

MRD Negative Flow-CRi:
Meets the above definition AND has < 0.1% leukemic blasts in the bone marrow by flow cytometry.

Flow Partial Response (Flow-PR):
M2 bone marrow (5% to 25% leukemic blasts) and at least a 50% decrease in bone marrow leukemic blast percent from pre-treatment baseline. A repeat bone marrow aspiration within 14 days may be required to distinguish between a PR and increased blasts caused by bone marrow regeneration and is left to the discretion of the investigator.

Flow Prolonged Aplasia:
Hypoplastic bone marrow for ≥ 60 days and failure to recover a peripheral ANC > 200/µL and a non-transfusion dependent platelet count > 20,000/µL not due to malignant infiltration or severe infection (defined as ≥ Grade 3).

Flow Persistent Disease (Flow-PD):
M3 bone marrow (> 25% leukemic blasts) or M2 bone marrow (5% to 25% leukemic blasts) with < 50% decrease in leukemic blast percent from pre-treatment baseline. Patients who had 0.1% to 5% leukemic blasts pre-treatment and do not achieve MRD negativity (< 0.1% leukemic blasts in the bone marrow) after treatment also are considered to have PD.

Flow Relapsed Disease:
A bone marrow evaluation that meets the definition of relapse in Sections 9.3.2, 9.3.3 or 9.3.4, in a patient who achieved CR, CRp or CRi after enrollment on APAL2020SC.

9.2.2 Morphological Response Definitions

Morphological Complete Response (Morph-CR):
Attainment of an M1 bone marrow (< 5% morphologic leukemic blasts) with no evidence of circulating leukemic blasts or extramedullary disease and with recovery of peripheral blood counts (ANC ≥ 500/µL and platelet count ≥ 50,000/µL). The CBC used for response assessment must be drawn on the day of the bone marrow evaluation or up to 14 days after bone marrow evaluation. There is no requirement for bone marrow cellularity.

Morphological Complete Response with Partial Recovery of Platelet Count (Morph-CRp):
Attainment of an M1 bone marrow (< 5% morphologic leukemic blasts) and no evidence of circulating leukemic blasts or extramedullary disease and with recovery of ANC ≥ 500/µL without platelet recovery to 50,000/µL. The CBC used for response assessment must be drawn either on the day of the bone marrow evaluation or up to 14 days after bone marrow evaluation.

Morphological Complete Response with Incomplete Blood Count recovery (ped-morph-CRi):
Attainment of an M1 bone marrow (< 5% morphologic leukemic blasts) and no evidence of circulating leukemic blasts or extramedullary disease and with ANC > 200/µL and/or platelet count > 20,000/µL without dependence on platelet transfusions (defined as: a platelet count > 20,000/µL AND no platelet transfusions x 5 days). The CBC used for response assessment must be drawn either on the day of the bone marrow evaluation or up to 14 days after bone marrow evaluation.

Morphological Partial Response (Morph-PR):
Bone marrow with 5-25% morphologic leukemic blasts and decrease of at least 50% from pre-treatment baseline. A repeat bone marrow evaluation within 14 days may be required to distinguish between a PR and increased blasts caused by bone marrow regeneration and is left to the discretion of the investigator.

Morphological Prolonged Aplasia:
Hypoplastic bone marrow for ≥ 60 days and failure to recover a peripheral ANC > 200/µL and a non-transfusion dependent platelet count > 20,000/µL not due to malignant infiltration or severe infection (defined as ≥ Grade 3).

Morphological Persistent Disease (Morph-PD):
M3 bone marrow (> 25% leukemic blasts) or M2 bone marrow (5% to 25% leukemic blasts) with < 50% decrease in leukemic blast percent from pre-treatment baseline.

Morphological Relapsed Disease:
Following achievement of Morph-CR/CRp/CRi while enrolled on APAL2020SC, emergence of > 5% morphologic leukemic blasts in the bone marrow OR absolute leukemic blast count greater than 1,000 per microliter in the peripheral blood OR biopsy or imaging (MRI/PET) proven extramedullary disease.

9.3 Relapse/Refractory Definitions for Patients with AML, ALL, and MPAL

Individual PedAL therapeutic sub-trials will include definitions of relapsed and refractory disease for that specific trial.

The following definitions for relapsed/refractory disease are used to determine eligibility for patients with AML, ALL, and MPAL for APAL2020SC. In addition, these definitions are for patients not enrolled on PedAL sub-trials and should be used when completing baseline and follow-up CRFs for APAL2020SC.

9.3.1 Refractory Disease

9.3.1.1 **AML**

- Patients with newly diagnosed AML refractory to ≥ 2 induction attempts as evidenced by ≥ 1% blasts confirmed by multidimensional flow cytometry (MDF). **OR**

- AML in first relapse or subsequent relapse refractory to ≥ 1 reinduction attempt as evidenced by ≥ 1% blasts MDF.

9.3.1.2 **MPAL**

- Patients with newly diagnosed MPAL refractory to ≥ 1 induction attempts as evidenced by ≥ 25% blasts identified by morphology and/or MDF **OR**

- MPAL in first relapse or subsequent relapse refractory to ≥ 1 reinduction attempt as evidenced ≥ 1% blasts confirmed by morphology and/or MDF.

9.3.2 Bone Marrow Relapse (AML, ALL, and MPAL)

Patient has one or more of the following after having achieved remission:

1. A single bone marrow sample showing ≥ 5% leukemic blasts by flow cytometry, FISH testing or other molecular method.

2. A single bone marrow sample with at least two tests showing ≥ 1% leukemic blasts. Examples of tests include:

- Flow cytometry showing leukemia ≥ 1% by multidimensional flow cytometry (MDF).
- Karyotypic abnormality.
- FISH abnormality identical to one present at diagnosis (must be above level of sensitivity of specific FISH probe.
- PCR or NGS-based demonstration of validated leukemogenic lesion (e.g., fusion, mutation) in a CLIA-approved laboratory that matches initial diagnosis and is quantifiable as ≥ 1%.

3. Two consecutive marrows (spaced at least 7 days apart) with at least two tests showing a one log increase of leukemic involvement with the second tests quantified as ≥ 0.1%. Examples of tests include:

- MDF in CLIA-approved laboratory.
- PCR or NGS-based demonstration of validated leukemogenic lesion (e.g., fusion, mutation) that matches diagnosis and is quantifiable where

the positive result is at or above the assay sensitivity and meets the criteria above (one log increase and second test quantified as ≥ 0.1%).

9.3.3 Extramedullary Relapse

Extramedullary relapse is defined as biopsy proven extramedullary disease after documented CR following initial therapy.

9.3.4 CNS Relapse

CNS relapse is defined as a patient having one of more of the following:

1. A single CSF sample with CNS3 status (see Section 9.3.4.2) after a previous status of CNS 1.

2. Clinical signs of CNS leukemia such as facial nerve palsy, brain/eye involvement, or hypothalamic syndrome (Rel-CNS3c).

9.3.4.1 **Equivocal CNS Relapse**

Patient has a single CSF sample with CNS2 status.

1. In the case of equivocal relapse, CSF evaluation should be repeated at least 1 week and at most 4 weeks later; for repeat CSF, flow cytometric testing and FISH (if a diagnostic FISH marker is available) should be sent.

2. To convert to definitive relapse, the repeat CSF or clinical status must:
   - Meet criteria for CNS relapse as defined in Section 9.3.4. **OR**
   - Re-demonstrate CNS2 status, but with blasts confirmed by flow cytometry and/or FISH (Rel-CNS2) on CSF samples from consecutive lumbar punctures.

9.3.4.2 **CNS Status Definitions**

CNS1 Disease

CNS1 (negative) at diagnosis is defined as no blasts in cytospin CSF regardless of CSF WBC or RBC counts AND does not meet CNS3c criteria.

CNS2 Disease

CNS2 disease at diagnosis is defined as any one of the following:

a) CNS2a: Blasts present in cytospin CSF with CSF WBC < 5/µL and CSF RBC < 10/µL (atraumatic tap)

b) CNS2b: Blasts present in cytospin CSF with CSF WBC < 5/µL and CSF RBC ≥ 10/µL (traumatic tap)

c) CNS2c: Blasts present in cytospin CSF with CSF WBC ≥ 5/µL and CSF RBC ≥ 10/µL (traumatic tap) in which the WBC/RBC ratio in the CSF is less than twice that in the peripheral blood (see calculation method below in Section 10.3.4.3).

CNS3 Disease

CNS3 disease at diagnosis is defined as any one of the following:

a) CNS3a: Blasts present in cytospin CSF with CSF WBC ≥ 5/uL and CSF RBC < 10/uL (atraumatic tap).

b) CNS3b: Blasts present in cytospin CSF with CSF WBC ≥ 5/uL in a traumatic tap (CSF RBC ≥ 10/uL) in which the WBC/RBC ratio in the CSF is twice or greater that in the peripheral blood (see calculation method below in Section 10.3.4.3).

c) CNS3c: Clinical signs of CNS leukemia (such as facial nerve palsy, brain/eye involvement or hypothalamic syndrome) ***OR*** radiographic evidence of an intracranial or intradural mass consistent with a chloroma. Retinal hemorrhage and extra-ocular orbital masses are not considered CNS leukemia.

9.3.4.3 **Method of Evaluating Initial Traumatic Lumbar Puncture**

If the patient has leukemic cells in the peripheral blood and the lumbar puncture is traumatic (CSF RBC ≥ 10/µL) and CSF contains WBC ≥5/uL and blasts on cytospin, the following algorithm should be used to distinguish CNS2c and CNS3b.

CNS2c:

$$\frac{\text{CSF WBC}}{\text{CSF RBC}} < 2 \times \frac{\text{Blood WBC}}{\text{Blood RBC}}$$

CNS3b

$$\frac{\text{CSF WBC}}{\text{CSF RBC}} \geq 2 \times \frac{\text{Blood WBC}}{\text{Blood RBC}}$$

Example of CNS3b: CSF WBC = 60/µL; CSF RBC = 1,500/µL; blood WBC = 46,000/µL; blood RBC = 3 X 10^6 /µL:

$$\frac{60}{1500} = 0.04 \text{ which is } \geq 2\text{x } \frac{46000}{3.0 \text{ X } 10^6} = 0.015$$

10.0 PATHOLOGY GUIDELINES

10.1 Patients with AML and ALL

Immunophenotyping Studies

Morphologic assessment and immunophenotyping studies are often done at the local institution as part of the initial diagnostic work-up of acute leukemia. Immunophenotyping studies are essential in confirming diagnosis and distinguishing AML from ALL.

All pertinent diagnostic information should be included in the bone marrow pathology report (to include the institutional morphologic assessment, immunophenotyping and cytochemistry). In addition to the central immunophenotyping, these local results will be captured in this study as they are contributory to the diagnosis and study of ALL.

The 2016 World Health Organization (WHO) classification requires assessment of a certain set of cytoplasmic and surface antigens, with or without use of cytochemistry to define myeloid, lymphoid or mixed/ambiguous lineage.

We strongly encourage the use of the following markers as a minimum, especially in difficult cases to further exclude the possibility of mixed phenotype acute leukemia (see Table 10.1):

1. MPO (by cytochemistry, flow cytometry or immunohistochemistry)
2. Cytoplasmic CD3 by flow cytometry
3. CD19 (by flow cytometry), and at least 2 additional B-cell markers such as CD10, CD79a or cytoplasmic CD22.

-At least 3 markers of monocytic lineage: CD11c, CD14, CD64, lysozyme (by flow or immunohistochemistry) or NSE cytochemistry.

- Additional markers as needed (example: CD56 will help exclude the rare Blastic Plasmacytoid Dendritic Cell Leukemia)

- If Early T-cell Precursor Lymphoblastic leukemia (ETP) is suspected, CD1a, CD4, CD8, CD117, CD33, CD13, CD65 and CD11b should be included. These markers should also be used for the work up of Mixed Phenotype Acute Leukemia (MPAL).

In this study, AML blasts will be classified based upon the 2016 WHO morphologic, immunophenotypic, and genetic criteria.[12]

Table 10.1

ASSIGNMENT OF LINEAGE IN ACUTE LEUKEMIA	
Lineage	**Criteria**
Myeloid lineage	•Myeloperoxidase (by flow cytometry, immunohistochemistry or cytochemistry) *OR* •Monocytic differentiation (express at least 2 of the following: CD11c, CD14, CD64, lysozyme or NSE)
T lymphoblastic	•Cytoplasmic CD3 by flow cytometry with antibodies to CD3 epsilon chain *OR* •Surface CD3 (rare)

B lymphoblastic	•Strong CD19 and strong expression of at least one of the following: CD79a, cytoplasmic CD22 or CD10 *OR* •Weak CD19 and strong expression of at least 2 of the following: CD79a, cytoplasmic CD22, CD10

10.2 Patients with Myeloid Sarcoma (Chloroma):

If touch preps are prepared from the tumor, the institutional pathologist should distribute/triage order(s) for FISH to be performed accordingly (e.g., KMT2A, RUNX1T1-RUNX1, CBFB).

10.3 Consultation Available

If a consultation for a difficult case is desired, this can be arranged on a case-by-case basis only. Please contact the study pathologist (Dr. Samir Kahwash) to request a consult and verify what is needed for the consultation prior to submitting pathology materials. The institution may send pathology materials (stained and unstained slides from bone marrow and peripheral blood and pathology, bone marrow, flow cytometry reports including copies of blast gate and dot plots of markers) and pertinent cytogenetics data to the study pathologist. A fee for this service may be charged. Copies of consult reports will be sent to the submitting institution.

Materials sent for consultation must be shipped using the institution's courier account to:

[redacted]

11.0 RECORDS AND REPORTING

See the Case Report Forms posted on the COG web site with each protocol under *"Data Collection/Specimens"*. A submission schedule is included.

11.1 Reporting

Demography Monitoring

Required submission of patient demographic data to NCI for this study will be submitted automatically via OPEN.

APPENDIX I: CTEP AND CTSU REGISTRATION PROCEDURES

INVESTIGATOR AND RESEARCH ASSOCIATE REGISTRATION WITH CTEP

Food and Drug Administration (FDA) regulations require sponsors to select qualified investigators. National Cancer Institute (NCI) policy requires all individuals contributing to NCI-sponsored trials to register with their qualifications and credentials and to renew their registration annually. To register, all individuals must obtain Cancer Therapy Evaluation Program (CTEP) credentials necessary to access secure NCI Clinical Oncology Research Enterprise (CORE) systems. Investigators and clinical site staff who are significant contributors to research must register in the Registration and Credential Repository (RCR). The RCR is a self-service online person registration application with electronic signature and document submission capability.

RCR utilizes four person registration types that are applicable to this study.

- Investigator (IVR) — MD, DO, or international equivalent;
- Non Physician Investigator (NPIVR) — advanced practice providers (e.g., NP or PA) or graduate level researchers (e.g., PhD);
- Associate Plus (AP) — clinical site staff (e.g., RN or CRA) with data entry access to CTSU applications such as the Roster Update Management System [RUMS], OPEN, Rave, acting as a primary site contact, or with consenting privileges; and
- Associate (A) — other clinical site staff involved in the conduct of NCI-sponsored trials.

RCR requires the following registration documents:

Documentation Required	IVR	NPIVR	AP	A
FDA Form 1572	✓	✓		
Financial Disclosure Form	✓	✓	✓	
NCI Biosketch (education, training, employment, licensure, and certification)	✓	✓	✓	
GCP training	✓	✓	✓	
Agent Shipment Form (if applicable)	✓			
CV (optional)	✓	✓	✓	

IVRs and NPIVRs must list all clinical practice sites and Institutional Review Boards (IRBs) covering their practice sites in RCR to allow the following:

- Addition to a site roster;
- Selection as the treating, credit, or consenting person in OPEN;
- Ability to be named as the site-protocol Principal Investigator (PI) on the IRB approval; and
- Assignment of the Clinical Investigator (CI) task on the Delegation of Tasks Log (DTL).

In addition, all investigators acting as the Site-Protocol PI (investigator listed on the IRB approval), consenting or treating investigator in OPEN, or as the CI on the DTL must be rostered at the enrolling site with a participating organization.

Refer to the NCI RCR page on the CTEP website for additional information. For questions, please contact the **RCR Help Desk** by email at RCRHelpDesk@nih.gov.

CTSU REGISTRATION PROCEDURES

This study is supported by the NCI Cancer Trials Support Unit (CTSU).

Downloading Site Registration Documents:
Download the site registration forms from the protocol-specific page located on the CTSU members' website. Permission to view and download this protocol and its supporting documents is restricted to institutions and their associated investigators and staff on a participating roster. To view/download site registration forms:

- Log in to the CTSU members' website (https://www.ctsu.org)
- Click on *Protocols* in the upper left of the screen
  - Enter the protocol number in the search field at the top of the protocol tree; or
  - Click on the By Lead Organization folder to expand, then select *COG*, and protocol number (*insert study number*).
- Click on *Documents*, *Protocol Related Documents,* and use the *Document Type* filter and select *Site Registration* to download and complete the forms provided. (Note: For sites under the CIRB, IRB data will load automatically to the CTSU.)

Protocol-Specific Requirements For APAL2020SC Site Registration:

- IRB approval (For sites not participating via the NCI CIRB; local IRB documentation, an IRB-signed CTSU IRB Certification Form, Protocol of Human Subjects Assurance Identification/IRB Certification/Declaration of Exemption Form, or combination is accepted)

Submitting Regulatory Documents
Submit required forms and documents to the CTSU Regulatory Office using the Regulatory Submission Portal on the CTSU website.

To access the Regulatory Submission Portal log in to the CTSU members' website, go to the Regulatory section and select Regulatory Submission.

Institutions with patients waiting that are unable to use the Regulatory Submission Portal should alert the CTSU Regulatory Office immediately by phone or email: 1-866-651-CTSU (2878), or CTSURegHelp@coccg.org to receive further instruction and support.

Checking Your Site's Registration Status
Site registration status may be verified on the CTSU members' website.

- Click on *Regulatory* at the top of the screen;
- Click on *Site Registration; and*
- Enter the site's 5-character CTEP Institution Code and click on Go.
  - Additional filters are available to sort by Protocol, Registration Status, Protocol Status, and/or IRB Type.

Note: The status shown only reflects institutional compliance with site registration requirements as outlined within the protocol. It does not reflect compliance with protocol requirements for individuals participating on the protocol or the enrolling investigator's status with NCI or their affiliated networks.

Delegation of Task Log (DTL)
Each site must complete a protocol-specific Delegation of Tasks Log (DTL) using the DTL application which is accessible via the Delegation Log link on the CTSU members' website or directly at https://dtl.ctsu.org. The Clinical Investigator (CI) is required to review and electronically sign the DTL prior to the site receiving an approved site registration status and enrolling patients to the study.

To maintain an approved site registration status the CI must re-sign the DTL at least annually and when a new version of the DTL is released; and to activate new task assignments requiring CI sign-off. Any individual at the enrolling site on a participating roster may initiate the site DTL. Once the DTL is submitted for CI approval, only the designated DTL Administrators or the CI may update the DTL. Instructions on completing the DTL are available in the Help Topics button in the DTL application and describes DTL task assignments, CI signature, and CTEP registration requirements, as well as include a Master Task List.

Data Submission / Data Reporting
Medidata Rave is the clinical data management system being used for data collection for this trial/study. Access to the trial in Rave is controlled through the CTEP-IAM system and role assignments.

Requirements to access Rave via iMedidata:

- Active CTEP registration with the credentials necessary to access secure NCI/CTSU IT systems; and
- Assigned a Rave role on the LPO or PO roster at the enrolling site of: Rave CRA, Rave Read Only, Rave CRA (LabAdmin), Rave SLA, or Rave Investigator.
-

Rave role requirements:

- Rave CRA or Rave CRA (Lab Admin) role must have a minimum of an Associate Plus (AP) registration type;
- Rave Investigator role must be registered as a Non-Investigational New Drug (IND)/Non-Treatment (NINT), Non-Physician Investigator (NPIVR), or Investigator (IVR); and
- Rave Read Only or RAVE SLA role must have at a minimum an Associates (A) registration type.

Refer to https://ctep.cancer.gov/investigatorResources/default.htm for registration types and documentation required. NINTs will only have write access in Rave for studies flagged for NINT participation.

This study has a Delegation of Tasks Log (DTL). Therefore, those requiring write access to Rave must also be assigned the appropriate Rave tasks on the DTL.

Upon initial site registration approval for the study in the Regulatory application, all persons with Rave roles assigned on the appropriate roster will be sent a study invitation email from iMedidata. No action will be required; each study invitation will be automatically accepted and study access to the study in Rave will be automatically granted. Site staff will not be able to access the study in Rave until all required Medidata and study specific trainings are completed. Trainings will be in the form of electronic learnings (eLearnings) and can be accessed by clicking on the eLearning link in the *Tasks* pane located in the upper right corner of the iMedidata screen. If an

eLearning is required for a study and has not yet been taken, the link to the eLearning will appear under the study name in the *Studies* pane located in the center of the iMedidata screen; once the successful completion of the eLearning has been recorded, access to the study in Rave will be granted, and a *Rave EDC* link will replace the eLearning link under the study name.

No action will be required by site staff (to activate their account) who have not previously activated their iMedidata/Rave account at the time of initial site registration approval for the study in the Regulatory application. Pending study invitations (previously sent but not accepted or declined by a site user) will be automatically accepted and study access in Rave will be automatically granted for the site user. Account activation instructions are located on the CTSU website in the Data Management section under Data Management Help Topics > Rave resource materials (*Medidata Account Activation and Study Invitation*). Additional information on iMedidata/Rave is available on the CTSU members' website in the *Data Management* > *Rave* section or by contacting the CTSU Help Desk at 1-888-823-5923 or by email at ctsucontact@westat.com.

Data Quality Portal

The Data Quality Portal (DQP) provides a central location for site staff to manage unanswered queries and form delinquencies, monitor data quality and timeliness, generate reports, and review metrics.

The DQP is located on the CTSU members' website under Data Management. The Rave Home section displays a table providing summary counts of Total Delinquencies and Total Queries. DQP Queries, DQP Delinquent Forms, DQP Form Status and the DQP Reports modules are available to access details and reports of unanswered queries, delinquent forms, forms with current status, and timeliness reports. Site staff should review the DQP modules on a regular basis to manage specified queries and delinquent forms.

The DQP is accessible by site staff who are rostered to a site and have access to the CTSU website. Staff who have Rave study access can access the Rave study data via direct links available in the DQP modules.

CTSU Delinquency Notification emails are sent to primary contacts at sites twice a month. These notifications serve as alerts that queries and/or delinquent forms require site review, providing a summary count of queries and delinquent forms for each Rave study that a site is participating in. Additional site staff can subscribe and unsubscribe to these notifications using the CTSU Report and Information Subscription Portal on the CTSU members' website.

To learn more about DQP use and access, click on the Help Topics button displayed on the Rave Home, DQP Queries, DQP Delinquent Forms, DQP Form Status, and DQP Reports modules.

This study does not use the Rave Calendaring functionality, and therefore the DQP Delinquent Forms module will not include details for this study, and the DQP Summary table on the Rave Home page will display *N/A* for the Total Delinquencies summary count.

APPENDIX II: YOUTH INFORMATION SHEETS

INFORMATION SHEET REGARDING RESEARCH STUDY APAL2020SC
(for children from 7 through 13 years of age)

We want to tell you all about this study. You and your family can decide if you want to be in it. Ask questions if you don't understand.

1. **What is the name of the study?** *A study to test bone marrow and blood in children with leukemia that has come back after treatment or is difficult to treat.*

2. **Who is in charge of the study?** The study is being done by *Children's Oncology Group* and is being done at other hospitals.

3. **What is the study about?** We are asking you to take part in a research study because you have leukemia other treatments did not get rid of the leukemia. A research study is when doctors work together to try out new ways to help people who are sick. In this study, we are trying to learn more about how to treat the kind of leukemia that you have.

4. **What will happen to me in the study?** Children who are part of this study will have their bone marrow and blood collected to see if the leukemia has certain features. Knowing that could help us decide what medicine might be best to treat the leukemia. If you are part of this study, we will test your bone marrow and blood to see if the leukemia has certain features that could help us decide what medicine might be best to treat the leukemia. The bone marrow and blood will be taken when you are having tests done for your regular medical care. You will not have extra bone marrow procedures or blood draws. The study will also collect data about your treatment for leukemia and how well it worked to get rid of the leukemia.

   Sometimes good things can happen to people when they are in a research study. These good things are called "benefits." We hope that a benefit to you is that your doctors can use the results of the tests to find a medicine that may cause your leukemia to stop growing. The information we collect for this study may help children with leukemia and other cancers. But we don't know for sure if there is any benefit of being part of this study.

   Sometimes bad things can happen to people when they are in a research study. These bad things are called "risks." A risk to you from being in this study is the very small chance that someone who does not have permission gets information about you, like your name, address or phone number. We will be working hard to make sure this does not happen. There may be risks that we don't know about.

5. **Do I have to be in the study?** Your family can choose to be part of this study or not. Your family can also decide to stop being in this study at any time once you start. Make sure to ask your doctors if you have any questions.

6. **Optional blood or bone marrow request.** We are asking your permission to keep your blood or bone marrow for optional banking. We want to see if there are ways to tell how the cancer will respond to treatment. These samples would be taken as leftovers from other standard tests, so there would be no extra procedures. You can still take part in this study even if you don't allow us to keep your samples for research.

INFORMATION SHEET REGARDING RESEARCH STUDY APAL2020SC
(for children from 14 through 17 years of age)

We want to tell you all about this study. You and your family can decide if you want to be in it. Ask questions if you don't understand.

1. **What is the name of the study?** *A study to test bone marrow and blood in children with leukemia that has come back after treatment or is difficult to treat.*

2. **Who is in charge of the study?** The study is being done by *Children's Oncology Group* and is being done at other hospitals.

3. **What is the study about?** We are asking you to take part in a research study because you have leukemia other treatments did not get rid of the leukemia. A research study is when doctors work together to try out new ways to help people who are sick. In this study, we are trying to learn more about how to treat the kind of leukemia that you have.

4. **What will happen to me in the study?** Children and teens who are part of this study will have their bone marrow and blood collected to see if the leukemia has certain features. Knowing that could help us decide what medicine might be best to treat the leukemia. If you are part of this study, we will test your bone marrow and blood to see if the leukemia has certain features that could help us decide what medicine might be best to treat the leukemia. The bone marrow and blood will be taken when you are having tests done for your regular medical care. You will not have extra bone marrow procedures or blood draws. The study will also collect data about your treatment for leukemia and how well it worked to get rid of the leukemia.

   Sometimes good things can happen to people when they are in a research study. These good things are called "benefits." We hope that a benefit to you is that your doctors can use the results of the tests to find a medicine that may cause your leukemia to stop growing. The information we collect for this study may help children with leukemia and other cancers. But we don't know for sure if there is any benefit of being part of this study.

   Sometimes bad things can happen to people when they are in a research study. These bad things are called "risks." A risk to you from being in this study is the very small chance that someone who does not have permission gets information about you, like your name, address or phone number. We will be working hard to make sure this does not happen. There may be risks that we don't know about.

5. **Do I have to be in the study?** Your family can choose to be part of this study or not. Your family can also decide to stop being in this study at any time once you start. Make sure to ask your doctors if you have any questions.

6. **Optional blood or bone marrow request.** We are asking your permission to keep your blood or bone marrow for optional banking. We want to see if there are ways to tell how the cancer will respond to treatment. These samples would be taken as leftovers from other standard tests, so there would be no extra procedures. You can still take part in this study even if you don't allow us to keep your samples for research.

APPENDIX III: SAMPLES FOR USE IN PEDAL THERAPEUTIC SUB-TRIAL CORRELATIVE BIOLOGY STUDIES

Specimens collected via APAL2020SC may be used for the correlative biology objectives contained within each sub-trial outlined below.

ITCC101-APAL2020D: A Randomized Phase 3 Trial Of Fludarabine/Cytarabine/Gemtuzumab Ozogamicin With Or Without Venetoclax In Children With Relapsed AML

Objective:
The correlative biology objective for the sub-trial is to describe biomarkers of response to FLA+GO with and without venetoclax combination including BH3 profiling, genetic data obtained by NGS, and in vitro drug sensitivity to venetoclax.

Sample requested to be collected via APAL2020SC:

- Ampule of 10 x 10^6 viable frozen blasts- St. Jude for BH3 protein dependency profiling
- 10^7 frozen mononuclear cells or 1 microg RNA- Erasmus for molecular MRD testing
- Ampule of 10 x 10^6 viable frozen blasts- Essen for BH3 gene expression profiling
- Ampule of 40 x 10^6 viable frozen blasts- PMC for drug sensitivity testing

Samples will be shipped from BPC to the following locations:

Molecular MRD (ISO certified lab)
Vincent van der Velden, immunologist,
Erasmus Universitair Medisch Centrum
Wytemaweg 80, 3015GN Rotterdam, Netherlands
E-mail: v.h.j.vandervelden@erasmusmc.nl
Phone: +31 10 704 0 704

Central bone marrow morphology review, BH3 family gene expression profiling
AML-BFM Reference Laboratories
171 Virchowstraße
45147 Essen Germany
E-mail: nils.vonneuhoff@uk-essen.de
Phone: +49 201 723 1055

BH3 dependency profiling
Paul Mead, PhD
St. Jude Children's Research Hospital
262 Danny Thomas Place, Memphis, Tennessee, 38105 USA
E-mail: paul.mead@stjude.org

***In vitro* drug sensitivity**
Pediatric Oncology Research lab
Prof dr ML den Boer
Princess Máxima Center, Utrecht, Netherlands
E-mail: m.l.denboer@prinsesmaximacentrum.nl

ITCC109-APAL2020K: A Phase 1 Trial of Menin-Inhibitor Ziftomenib in Combination with Chemotherapy for Children with Relapsed/Refractory KMT2A-r/NUP98-r/NPM1-m Acute Leukemia

Objective:
The correlative biology objective for the sub-trial is to perform pharmodynamic assays related to the activity of ziftomenib. Molecular MRD and in vitro drug sensitivity testing from baseline samples collected on APAL2020SC will be performed at the laboratories listed below.

Specimens collected via APAL2020SC may be used for the correlative biology objectives detailed below:

- One vial of 1 x 10^6 frozen nucleated white blood cells or 5 microg RNA- for molecular MRD testing to be performed at the University of Padova
- One vial of 2 x 10^7 frozen nucleated white blood cells or 2 vials of 1 x 10^7 – for in vitro drug sensitivity testing at the Princess Maxima Center

Samples will be shipped to the following locations:

FOR EUROPE/MÁXIMA TERRITORY

Molecular MRD (ISO certified lab)
Martina Pigazzi
University of Padova, Department for Woman and Child health
Onco-Hematology Clinic and Lab
Via Guistiniani, 3
35128 Padova PD, Italy
E-mail: martina.pigazzi@unipd.it

In vitro **drug sensitivity profiling and RNA sequencing**
Pediatric Oncology Research lab
Olaf Heidenreich
Princess Máxima Center, Utrecht, Netherlands
E-mail: o.t.heidenreich@prinsesmaximacentrum.nl

APPENDIX IV: RESPONSE CRITERIA FOR MDS

The MDS response criteria are derived from the revised MDS International Working Group (IWG) Criteria.[13]

Response	IWG 2023 Definitions
Complete Remission (CR)	BM: < 5% myeloblasts;* dysplasia may persist PB: Hb 10 g/dL, platelets 100 × 10^9/L; neutrophils 1.0 × 10^9/L; blasts 0%†
CR equivalent*	Patients with < 5% BM blasts at baseline BM: < 5% myeloblasts*; dysplasia may persist PB: Hb 10 g/dL, platelets 100 × 10^9/L; neutrophils 1.0 × 10^9/L; blasts 0%† Full cytogenetic clearance of baseline abnormalities (complete cytogenetic response)
Partial Response (PR)	All CR criteria except: BM blasts decreased by 50% over pretreatment but still 5% Cellularity and morphology not relevant
CR with limited count recovery (CRL - $CR_{unilinage}$ and $CR_{bilineage}$)	BM: < 5% myeloblasts;* dysplasia may persist PB: blasts 0%† $CR_{unlineage}$: PB, not meeting CR but only 1 of the following: Hb 10 g/dL; platelets 100 × 10^9/L; neutrophils 1.0 × 10^9/L $CR_{bilineage}$: PB, not meeting CR but only 2 of the following: Hb 10 g/dL; platelets 100 × 10^9/L; neutrophils 1.0 × 10^9/L
CR with partial hematologic recovery (CRh)	BM: < 5% myeloblasts;* dysplasia may persist PB: Not meeting criteria for CR or CRL, no Hb threshold required, platelets 50 × 10^9/L; neutrophils 0.5 × 10^9/L; blasts 0%†
Hematologic Improvement (HI)	HI defined according to IWG 2018 response criteria: Not meeting criteria for CR (or CR equivalent) or CRuni or CRL $HI_{erythroid}$ (HI-E) $HI_{platelets}$ (HI-P) $HI_{neutrophils}$ (HI-N)
Overall Response Rate (ORR)	ORR = CR (or CR equivalent)* + PR + CRL + CRh + HI
No response	Not meeting criteria for CR (or CR equivalent)*, PR, CRL, CRh, or HI‡
Not evaluable	All registered patients should be reported in the denominator of response assessment analyses in line with the intention-to-treat principle. This category may include patients yet to have a response assessment, suffering early death, exiting the study early, or those with a technically suboptimal BM sample precluding assessment
Cytogenetic response¶	Complete: disappearance of the chromosomal abnormality without appearance of new ones. Partial: 50% reduction of the chromosomal abnormality.
Progressive Disease (PD)	Fulfilling any of the criteria below:#,**,†† Disease progression by blasts 50% relative increase in blasts and absolute increase of blast percentage by at least 5% from pretreatment sample taken before current line of therapy. Disease progression by worsening cytopenia: new, repeated (more than once and

	intercurrent illness (eg, sepsis, gastrointestinal tract bleed) or treatment effect, in the absence of HI of at least one other blood lineage as defined above. Progression to AML: 50% increase in blasts from baseline assessment to 20% blasts.
Disease relapse	Fulfilling any of the criteria below:# Disease relapse by blasts: absolute and relative increase in BM blasts by at least 5% and 50%, respectively, from prior assessment, or reappearance of blasts in the blood, or development of extramedullary disease (myeloid sarcoma). Disease relapse by worsening cytopenias: decrement in one or more blood cell lineage counts by 50% from maximum remission/response levels for platelets or absolute neutrophil count or a reduction of Hb by 1.5 g/dL combined with an absolute reduction in the same lineage(s) as follows: Hb < 10 g/dL, platelets < 100×10^9/L, or absolute neutrophils < 1.0×10^9/L or repeated (more than once and separated by 7 days) need for RBC or platelet transfusions which are not related to acute intercurrent illness (eg, sepsis, gastrointestinal tract bleed) or treatment effect; in the absence of HI of at least one other blood lineage as defined above.

*Patients require 5% blasts before treatment initiation to be considered evaluable for CR, PR, CRh, or CR_L. For patients with < 5% blasts who have HR-MDS owing to adverse cytogenetics and/or severe cytopenias, full cytogenetic clearance (complete cytogenetic response) and blood counts that meet CR criteria are considered CR equivalent but should be reported separately. Full trilineage count recovery is defined as Hb 10 g/dL, platelets 100×10^9/L, and ANC 1.0×10^9/L independent of baseline PB. Given that molecular clearance has not been validated prospectively, it was not used for CR definition by the 2023 IWG.

¶If cytogenetic analyses fail, repeating cytogenetics during a subsequent response assessment is recommended. MRD assessment in MDS is insufficiently validated at this time as a surrogate for OS. MRD-negative response can be reported as a provisional response category, and clinical trial protocols should predefine what techniques are used to detect MRD and what cutoffs are considered to define an MRD response.

APPENDIX V: RESPONSE CRITERIA FOR JJML

The JMML response criteria were developed by an international consensus panel.[14]

Table 1: Variables for evaluation of response to therapy in JMML

Variables for Response		Definition of response			Definition of disease progression or relapse (applicable to all patients)
		Assessment of CR and PR is feasible if the following are present before therapy	**Requirement for CR for each variable (vCR)**	**Requirement for PR for each variable (vPR)**	**Requirement for PD for each variable (vPD)**
Clinical variables	**1) BM blasts**	≥ 5%	< 5%	Decreased by ≥50% over pre-treatment but still ≥5%	≥ 20%
	2) Spleen size#				
	a) Clinical evaluation or	≥2 cm under the costal margin	No splenomegaly	50% decrease by cm under the costal margin	Increase by ≥ 50% if baseline < 10cm from under the costa margin or > 30% if baseline >10 cm
	b) Sonography	Length of spleen ≥ 150% of upper limit of normal range	No splenomegaly	> 25% decrease by length, but still splenomegaly	Increase by ≥ 25% of length
	3) Extramedullary disease#	Extramedullary leukemic infiltration	No evidence of extramedullary leukemic infiltration		Worsening or new lesions of extramedullary leukemic infiltration
4) Cytogenetic response		Somatic cytogenetic abnormality detected	Normal karyotype	-	Reappearance or additional acquirement of cytogenetic abnormalities
5) Molecular response		Somatic genetic anomalies detected **	Absence of somatic genetic anomalies	-	Reappearance or additional acquirement of JMML-specific somatic gene abnormalities

CR: complete response, PR: partial response, PD: progressive disease, WBC: white blood cell, PB: peripheral blood, BM: bone marrow
*Myeloid precursors include promyelocytes, myelocytes, and metamyelocytes. The myeloid and erythroid precursors and blasts in PB are given as % of the total nucleated cells in PB (WBC including erythroblasts).

**in *NF-1, PTPN11, NRAS, KRAS, RRAS2,* or *CBL,* thus the mutation are thought to be initiating. In patients with germ-line *NF-1, PTPN11* or *CBL* mutation, only acquired mutations can be evaluated for response and relapse after therapy. The germ-line mutation remains even if patients achieved complete molecular response. The molecular response will be determined using the "JMML Mutant Allele Burden" assay.
Criteria for progression including WBC, platelet count and spleen size cannot be used in the setting of an active infection.
#Extramedullary disease includes infiltration of skin, lung, and very rarely cranial nerves or central nervous system.

Table 2: Definition of response following therapy other than HSCT in JMML

Clinical remission status: parameter 1-6 of table 1		**Genetic remission status: parameter 7-9 of table 1**	
Clinical complete remission (cCR)	Patient fulfills the criteria of CR of all applicable clinical variables 1-6 of table 1 The response parameters must be maintained for at least 4 weeks.	**Genetic complete remission (gCR)**	Defined if the patient shows a normal karyotype and absence of acquired mutations in *PTPN11*, *NF-1*, *NRAS*, *KRAS*, or *CBL*.
Clinical partial remission (cPR)	Defined if the patient does not fulfill the criteria of cCR, but vPR was achieved in at least one of clinical variables (1 – 6) and none of clinical variables showed vPD.	**-**	-
Clinical stable disease (cSD)	Defined if the patient does not fulfill the criteria of cCR and cPR, but none of the parameter showed vPD.	**Genetic stable disease (gSD)**	Defined if the patient does not fulfill the criteria of gCR, but none of the genetic parameters (7-9) showed vPD.
Clinical progressive disease (cPD)	Defined if any of the parameters 1-6 showed vPD**.**	**Genetic progressive disease (gPD)**	Defined if any of the parameters 7-9 showed vPD.
Clinical relapse (cRel)	Defined if any of the parameters 1 - 6 showed vPD after the achievement of cCR or cPR.	**Genetic relapse (gRel)**	Reappearance of an abnormal karyotype *and/or* mutation of genes related with JMML if previously undetected, *and/or* (only for patients after HSCT) increase in recipient chimerism with at least 10% of autologous cells and > 50% increase above the base line.

**In patients with germ-line *NF-1, PTPN11* or *CBL* mutation, the germ-line mutation remains even if patients achieved a genetic complete remission.

Table 3: Evaluation of the overall response to therapy based on the clinical (c) and genetic (g) remission status as defined in Table 2

		Genetic remission status			
		No genetic marker	gCR	gSD	gPD/cRel
Clinical	cCR	CR	CR	PR	PD/Rel

remission status	cPR	PR	CR*	PR	PD/Rel
	cSD	SD	CR*	SD	PD/Rel
	cPD/cRel	PD/Rel	(CR**)	PD/Rel	PD/Rel

CR: complete remission, PR: partial remission, SD: stable disease, PD: progressive disease, Rel: relapse.
*It is conceivable that **a patient with complete remission as indicated by genetic studies has persistent splenomegaly or leukocytosis from other causes such as infections despite of remission of JMML**
****It is unlikely that a patient has sign of progressive disease of JMML despite a genetic complete remission. In such a patient, any possible errors of genetic examinations, or other disorders which cause JMML like clinical features, should be excluded.**

REFERENCES

1. Gamis AS, Alonzo TA, Perentesis JP, et al.: Children's Oncology Group's 2013 blueprint for research: acute myeloid leukemia. Pediatr Blood Cancer 60:964-71, 2013
2. Rasche M, Zimmermann M, Borschel L, et al.: Successes and challenges in the treatment of pediatric acute myeloid leukemia: a retrospective analysis of the AML-BFM trials from 1987 to 2012. Leukemia 32:2167-2177, 2018
3. Tarlock K, Zhong S, He Y, et al.: Distinct age-associated molecular profiles in acute myeloid leukemia defined by comprehensive clinical genomic profiling. Oncotarget 9:26417-26430, 2018
4. Bolouri H, Farrar JE, Triche T, Jr., et al.: The molecular landscape of pediatric acute myeloid leukemia reveals recurrent structural alterations and age-specific mutational interactions. Nat Med 24:103-112, 2018
5. Zwaan CM, Kolb EA, Reinhardt D, et al.: Collaborative Efforts Driving Progress in Pediatric Acute Myeloid Leukemia. J Clin Oncol 33:2949-62, 2015
6. Hunger SP,Raetz EA: How I treat relapsed acute lymphoblastic leukemia in the pediatric population. Blood 136:1803-1812, 2020
7. Jen EY, Xu Q, Schetter A, et al.: FDA Approval: Blinatumomab for Patients with B-cell Precursor Acute Lymphoblastic Leukemia in Morphologic Remission with Minimal Residual Disease. Clinical Cancer Research 25:473-477, 2019
8. Maude SL, Laetsch TW, Buechner J, et al.: Tisagenlecleucel in Children and Young Adults with B-Cell Lymphoblastic Leukemia. New England Journal of Medicine 378:439-448, 2018
9. Kantarjian HM, DeAngelo DJ, Stelljes M, et al.: Inotuzumab Ozogamicin versus Standard Therapy for Acute Lymphoblastic Leukemia. New England Journal of Medicine 375:740-753, 2016
10. Woodcock J,LaVange LM: Master Protocols to Study Multiple Therapies, Multiple Diseases, or Both. New England Journal of Medicine 377:62-70, 2017
11. Renfro LA,Sargent DJ: Statistical controversies in clinical research: basket trials, umbrella trials, and other master protocols: a review and examples. Ann Oncol 28:34-43, 2017
12. Arber DA, Orazi A, Hasserjian R, et al.: The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood 127:2391-2405, 2016
13. Zeidan AM, Platzbecker U, Bewersdorf JP, et al.: Consensus proposal for revised International Working Group 2023 response criteria for higher-risk myelodysplastic syndromes. Blood 141:2047-2061, 2023
14. Niemeyer CM, Loh ML, Cseh A, et al.: Criteria for evaluating response and outcome in clinical trials for children with juvenile myelomonocytic leukemia. Haematologica 100:17-22, 2015