October 9, 2020

Martha Kruhm, MS RAC
Head, Protocol and Information Office
Quality Assurance Section
CTEP, DCT, NCI
6130 Executive Blvd, EPN Room 7000
Bethesda, MD 20892

Dear Ms. Kruhm:

Enclosed is Addendum #26 to EAY131, *Molecular Analysis for Therapy Choice (MATCH).*

Please replace your current copy of the protocol and Informed Consent document with these updated versions. We recommend that each institution maintain a file containing the original protocol, Informed Consent, and all subsequent revisions/versions.

IRB Review Requirements:

This addendum has been reviewed and approved by the Central IRB, which is the sole IRB of record for this study. Local IRB review and approval is unnecessary.

Implementation of this addendum must occur on the activation date. Sites are not permitted to conduct the study utilizing outdated versions of any MATCH protocol documents after the activation date of this addendum.

The following revisions to EAY131 protocol have been made in this addendum:

	Section	Change
1.	Cover Page	Updated Version Date and addendum number.
2.	Appendix IX	Table 2: Updated addendum component version dates for the MATCH Master Protocol and all affected sub protocols.

The following revisions to EAY131 Screening Protocol Consent Document have been made in this addendum:

	Section	Change
1.	Page 1	Updated Version Date.

The following revisions to EAY131 Research Biopsy Consent Document have been made in this addendum:

	Section	Change
2.	Page 1	Updated Version Date.

If you have any questions regarding this addendum, please contact aagu@ecog-acrin.org or 857-504-2900.

28 State Street, Suite 1100, Boston, MA 02109 p: 857.504.2900 f: 617. 589.0914 ecog-acrin.org

We request review and approval of this addendum to EAY131 so ECOG-ACRIN may activate it promptly.

Thank you.

Sincerely,

Pamela Cogliano

Senior Director of Protocol Development

Molecular Analysis for Therapy Choice (MATCH)

	NCI STUDY CHAIR:	Alice Chen, MD
Rev. Add13	ECOG-ACRIN STUDY CHAIR:	Keith Thomas Flaherty, MD
Rev. Add25	NCI STUDY CO-CHAIR:	Lyndsay N. Harris, MD
	ECOG-ACRIN STUDY CO-CHAIR:	Peter O'Dwyer, MD
	NCI LABORATORY LEAD:	Mickey Williams, PhD
	NCI LABORATORY CO-LEAD:	James V. Tricoli, PhD
	ECOG-ACRIN LABORATORY LEAD:	Stanley Hamilton, MD
	NCI STUDY STATISTICIANS:	Lisa McShane, PhD
		Larry Rubinstein, PhD
	ECOG-ACRIN STUDY STATISTICIANS:	Robert Gray, PhD
		Shuli Li, PhD
	NCI IMAGING CO-CHAIR:	Lalitha Shankar, MD, PhD
Rev.5/16	ECOG-ACRIN IMAGING CO-CHAIR:	Susanna Lee MD, PhD
Rev. 3/17	ECOG-ACRIN IMAGING STATISTICIAN:	Constantine Gatsonis, PhD
	LYMPHOMA CO-CHAIR:	Paolo Caimi, MD
	MYELOMA CO-CHAIR:	Shaji Kumar, MD
	PATHOLOGY CO-CHAIR:	Stanley Hamilton, MD
Rev.2/16	ECOG-ACRIN SAFETY CO-CHAIR:	Edith Mitchell, MD
	NCI SAFETY CO-CHAIR:	John J. Wright, MD
Rev.5/16	ECOG-ACRIN SCIENTIFIC CO-CHAIR:	Carlos L. Arteaga, MD

Version Date: October 9, 2020
NCI Update Date: August 12, 2015

STUDY PARTICIPANTS

US Sites Only

SWOG / SWOG
NRG / NRG Oncology
ALLIANCE / Alliance for Clinical Trials in Oncology
ECOG-ACRIN / ECOG-ACRIN Cancer Research Group
Rev.8/15 **COG** / Children's Oncology Group

Rev. Add12 Rev. Add19 **NOTE: As of 11/17, all protocol changes will be noted by addendum number.**

ACTIVATION DATE

August 12, 2015

PRE-ACTIVATION DATE

May 29, 2015
Update #1 – Incorporated Prior to Activation

Addendum #1 – 8/15	Addendum #14
Update #2 – 8/15	Addendum #15
Addendum #2 – 2/16	Addendum #16
Addendum #3 – 5/16	Addendum #17
Addendum #4 – 7/16	Addendum #18
Addendum #5 – 12/16	Addendum #19
Addendum #6 – 1/17	Addendum #20
Addendum #7 – 3/17	Addendum #21
Addendum #8 – 3/17	Addendum #22
Addendum #9 – 3/17	Addendum #23
Addendum #10 – 5/17	Addendum #24
Addendum #11 – 8/17	Addendum #25
Addendum #12	Addendum #26
Addendum #13	

MATCH (EAY131) IND#: [redacted]
IND Sponsor: Division of Cancer Treatment and Diagnosis, NCI

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ECOG-ACRIN STUDY CHAIR

Keith Flaherty, MD
Massachusetts General Hospital
55 Fruit Street, LH 202
Boston, MA 02114
Phone: 617-724-4800
Fax: 617-724-6898
Email: kflaherty@partners.org

NCI STUDY CHAIR

Alice Chen, MD
Early Clinical Trials Development Program
DCTD/NCI
Suite 31/3A44
31 Center Drive
Bethesda MD 20892
Phone: 301-496-4291
Fax: 240-276-7894
Email: chenali@mail.nih.gov

ECOG-ACRIN STUDY CO-CHAIR

Peter O'Dwyer, MD
University of Pennsylvania, Abramson Cancer Center
16 Penn Tower
3400 Spruce Street
Philadelphia, PA 19104
Phone: 215-662-7606
Fax: 215-243-3268
Email: peter.odwyer@uphs.upenn.edu

NCI STUDY CO-CHAIR

Lyndsay N. Harris, MD
Cancer Diagnosis Program
9609 Medical Center Drive MSC 9730
Bethesda, MD 20892
Phone: 240-276-6505
Fax: 240-276-7889
Email: lyndsay.harris@nih.gov

Rev.2/16

ECOG-ACRIN SAFETY CO-CHAIR

Edith Mitchell, MD, FACP
Thomas Jefferson University Hospital
233 South 10th Street
BLSB, Suite 502
Philadelphia, PA 19107
Phone: 215-955-4652
Fax: 215-503-4103
Email: edith.mitchell@jefferson.edu

NCI LABORATORY CO-LEAD

James V. Tricoli, Ph.D.
Chief Diagnostic Biomarkers and Technology Branch, Cancer Diagnosis Program
9609 Medical Center Drive
3 West, Room 526, MSC 9728
Rockville, MD 20892
Phone: (240) 276-5725
Fax: (240) 276-7889
Email: tricolij@mail.nih.gov

MATCH TEAM ALIAS

MATCH@jimmy.harvard.edu

NCI SAFETY CO-CHAIR

John J. Wright, MD, Ph.D.
Investigational Drug Branch
CTEP, DCTD, NCI, NIH
9609 Medical Center Drive
Room 5-W-454, MSC 9739
Rockville, MD 20850
Phone: 240-276-6105
Fax: 240-276-7894
Email: wrightj@ctep.nci.nih.gov

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LABORATORY SITES:

Molecular Characterization Laboratory at the NCI Frederick National Laboratory for Cancer Research

LABORATORY PRINCIPAL INVESTIGATOR

Mickey Williams, PhD
Director of the Molecular Characterization Laboratory
Leidos Biomedical Research Inc.
Frederick National Laboratory for Cancer Research
PO Box B
Frederick, MD 21702
Phone: 301-228-4654
Fax: 301-846-5923
Email: mickey.williams@nih.gov

Massachusetts General Center for Integrated Diagnostics

LABORATORY PRINCIPAL INVESTIGATOR

John Anthony Iafrate MD PhD
Translational Research Laboratory, Mass General Hospital
70 Blossom Street, GRJ-1008
Boston, MA 02114
Phone: 617-726-0503
Fax: 617-726-6974
Email: aiafrate@partners.org

MD Anderson Molecular Diagnostics NGS Laboratory

LABORATORY PRINCIPAL INVESTIGATOR

Stanley R. Hamilton MD
M.D. Anderson Cancer Center
Department of Pathology
Unit 85 - Room G1.3540
1515 Holcombe Boulevard
Houston, TX 77030
Phone: 713-792-2040
Fax: 713-792-4094
Email: shamilto@mdanderson.org

Yale University, Clinical Molecular Pathology

LABORATORY PRINCIPAL INVESTIGATOR

Jeffrey Sklar MD, PhD
Department of Pathology
310 Cedar Street
214A Lauder Hall PO Box 208023
New Haven, CT 06520-8023
Phone: 203-785-5439
Fax: 203-785-3103
Email: jeffrey.sklar@yale.edu

IMAGING CORE LABORATORY:

IMAGING PRINCIPAL INVESTIGATOR

Susanna I. Lee, MD, PhD
Massachusetts General Hospital
Department of Radiology
55 Fruit St, White 270
Boston, MA 02114
Phone: 617-643-2009
Fax: 617-726-4891
Email: slee0@mgh.harvard.edu

ECOG-ACRIN IMAGING STATISTICIAN

Dr. Constantine Gatsonis PhD
E-A Biostatistical Center – Providence
Brown University
Box G-121-7
121 South Main Street, 7th Floor
Providence, RI 02912
Phone: 401-863-9183
Fax: 401-863-9182
Email: gatsonis@stat.brown.edu

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CANCER TRIALS SUPPORT UNIT (CTSU) ADDRESS AND CONTACT INFORMATION

CONTACT INFORMATION		
For regulatory requirements:	**For patient enrollments:**	**Submit study data**
Regulatory documentation must be submitted to the CTSU via the Regulatory Submission Portal. Regulatory Submission Portal: (Sign in at www.ctsu.org, and select the Regulatory Submission sub-tab under the Regulatory tab.) Institutions with patients waiting that are unable to use the Portal should alert the CTSU Regulatory Office immediately at 1-866-651-2878 to receive further instruction and support. Contact the CTSU Regulatory Help Desk at 1-866-651-2878 for regulatory assistance.	Please refer to the patient enrollment section of the protocol for instructions on using the Oncology Patient Enrollment Network (OPEN) which can be accessed at https://www.ctsu.org/OPEN_SYSTEM/ or https://OPEN.ctsu.org. Contact the CTSU Help Desk with any OPEN-related questions at ctsucontact@westat.com.	Data collection for this study will be done exclusively through Medidata Rave. Please see the data submission section of the protocol for further instructions.
The most current version of the **study protocol and all supporting documents** must be downloaded from the protocol-specific Web page of the CTSU Member Web site located at https://www.ctsu.org. Access to the CTSU members' website is managed through the Cancer Therapy and Evaluation Program - Identity and Access Management (CTEP-IAM) registration system and requires user log on with CTEP-IAM username and password. 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 CTSU RSS.		
For clinical questions (i.e. patient eligibility or treatment-related) Contact the MATCH Treatment Subprotocol PI.		
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.		

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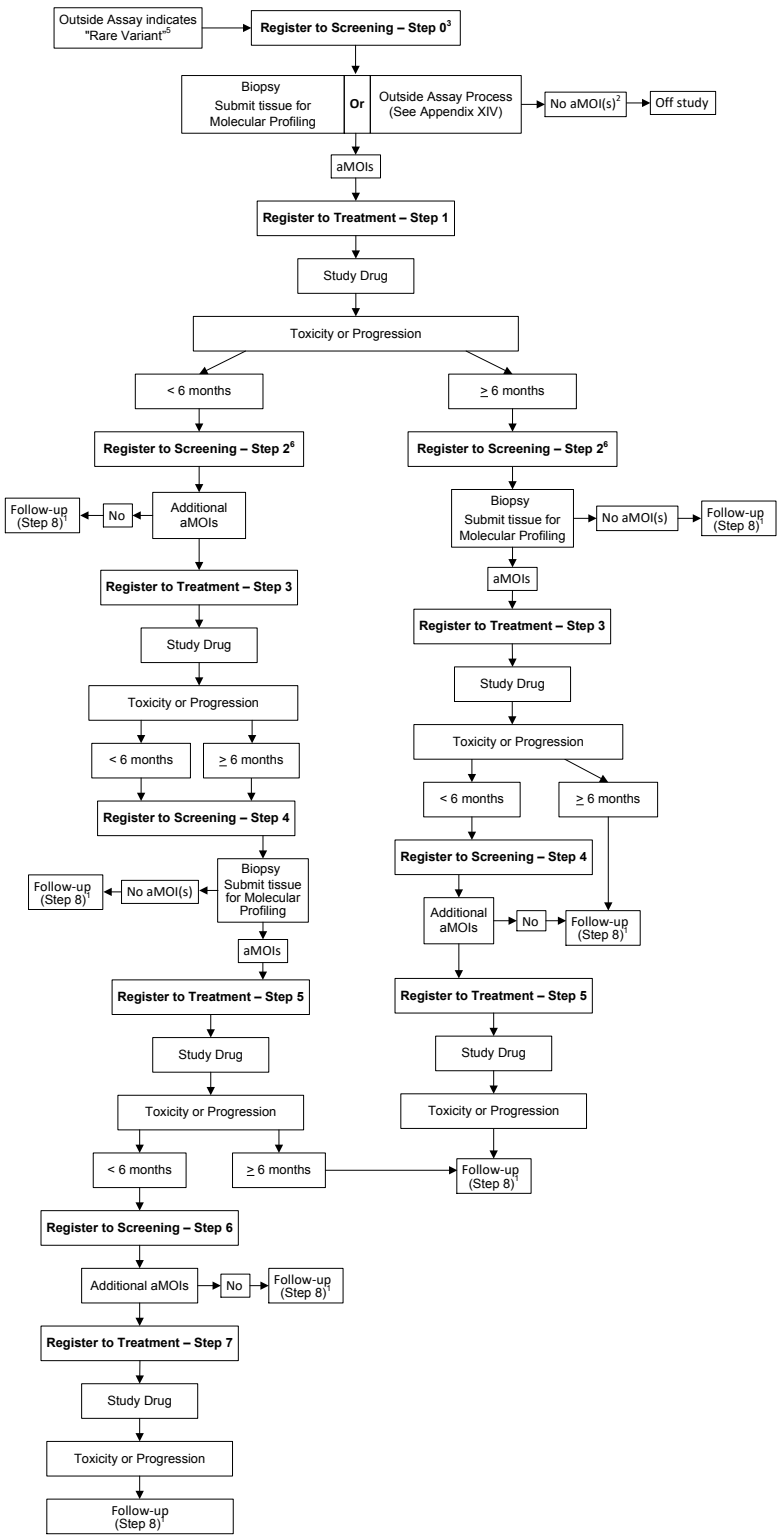
Schema



1. For patients that have consented to undergo the research biopsy and additional optional blood collections, register to Step 8 (see Section 3.9).
2. Patients may be eligible to be reconsidered for treatment. Please see Section 3.2.2 and Section 5.1.
3. Image Submission Requirements see Section 11 for submission instructions.
4. For multiple myeloma patients (excluding plasmacytoma), a bone marrow aspirate is required.
5. Outside assay sequencing results from designated laboratory indicate an applicable 'rare variant' aMOI and the ordering physician received a specific notification of potential e igibility from the designated lab (see Appendix XIV). Tissue is to be submitted for central confirmation testing per Section 9.3.2.2.
6. Patients assigned to Step 1 using a rare variant determined by an 'Outside Assay" go to follow-up and are not eligible for registration to Step 2. Patients are eligible for Step 8.

1. Introduction

This trial aims to establish whether patients with tumor mutations, amplifications or translocations in one of the genetic pathways of interest are likely to derive clinical benefit (primary objective: objective response; secondary objective: progression-free survival of at least 6 months) if treated with agents targeting that specific pathway in a single-arm design.

The targets being evaluated in this study are listed in Appendix VII.

The study design was developed in conjunction with experts in developmental therapeutics, clinical trial design, genetic sequencing, molecular oncology, cell biology, and statistics, who developed an algorithm that defines clinical action based on genetic variants reported in the genes of interest.

Molecular profiling will be performed in a study-specific network of approved Clinical Laboratory Improvement (CLIA)-certified Molecular Characterization Laboratories.

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1.1 Description of Trial

This study is a type of basket trial designed with the flexibility to open and close subprotocols. The study drugs included in this trial are specified in Appendix VII and by subprotocol in Appendix IX, and include single agents and combinations that have at least a recommended phase 2 dose. Patients enrolled on study will have a tumor biopsy for molecular characterization and those with molecular variants addressed by treatments included in the trial will be offered treatment on MATCH.

All profiling will be performed in specific CLIA-certified laboratories. The MATCH laboratories are MD Anderson Cancer Center Molecular Diagnostics NGS Laboratory, Massachusetts General Hospital Center for Integrated Diagnostics, Molecular Characterization Laboratory at the Frederick National Laboratory for Cancer Research and Yale University Clinical Molecular Pathology lab. The MATCH laboratories will perform profiling of fresh biopsies and of archived specimens of patients entering with a “rare variant” from an assay performed in a CLIA-certified laboratory outside of the MATCH laboratory group. The molecular profiling assays in the MATCH laboratories will include massively parallel tumor sequencing (next generation sequencing) using a targeted Ampliseq panel, and certain other molecular assays e.g. immunohistochemistry (IHC). The genes interrogated on the NGS assay are listed in Appendix X.

After Step 0 registration, the “MATCH screening assessment” will differ depending on the method of study entry. For patients that previously entered the study via the original screening process (i.e. to submit tissue for testing by a MATCH laboratory), this assessment will consist of both molecular profiling by the MATCH assay and the subsequent process of assigning treatment. Please note, after 5/22/17, new patients may no longer be enrolled on the study through this process. For patients entering the study through testing performed by one of the designated labs outside of the MATCH laboratory group (i.e. the Outside Assay process; see Appendix XIV), the assessment will only consist of the study's determination of treatment assignment. This is the only method of study entry moving forward.

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1.2 Study Diseases

Patients with histologically documented solid tumors, lymphomas and multiple myeloma whose disease has progressed following at least one line of standard systemic therapy or for whom no standard therapy exists will be registered on the screening step to undergo tumor biopsy and analysis

For each agent (treatment), patients with tumors for which there is a U.S. Food and Drug Administration (FDA) approved indication, or with histologies that have been shown not to respond or not to have prolonged PFS will be ineligible.

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1.3 Genomic Sequencing Research and Results Return

This trial will collect somatic (tumor) genomic data from all patients enrolled to determine whether or not their tumors have a molecular abnormality in one of the pathways that can be addressed by the treatments in the MATCH trial (an actionable mutation of interest, or aMOI). We will screen for pre-defined variations in genes in a MATCH CLIA-certified laboratory and other designated laboratories (see below) for assignment of patient treatment on study. Only malignant tissue will be screened, not normal tissue, so definitive abnormalities in germline tissues (heritable diseases) cannot be identified with any certainty. Clinicians will receive the results of the molecular studies done in MATCH CLIA approved laboratories, along with a laboratory report detailing the genes/translocations interrogated. (See Appendix X)

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The protocol includes separate informed consent forms/processes for the screening versus treatment steps of the study. A screening informed consent form will be presented at the time of registration to Step 0. An additional informed consent form will be provided at the registration to each treatment step. Patients will know (by whether or not they are offered the option to participate in the treatment part of the study) whether their tumor has a variation in a gene of interest – a variant that is addressed by one of the subprotocols of MATCH (aMOI). The appended aMOI tables in each subprotocol are annotated using methods consistent with the NCI-MATCH assay, but there are potentially other annotations that represent the exact genomic mutations either at the protein or genomic level. All identical mutations will be considered as inclusion aMOIs for the purposes of NCI-MATCH enrollment. Information on tumor gene variants and other molecular assays (e.g. immunohistochemistry results) that form the basis of treatment assignment as well as those on the targeted testing that may not lead to treatment assignment will be sent to clinicians (via separate CLIA lab reports for IHC and for tumor sequencing), who will share them with the patient (example report in Appendix X). ECOG-ACRIN has a Certificate of Confidentiality from the NIH to help protect the privacy of all study participants.

Some of the genes tested in the MATCH assay on the patient's tumor could be tumor specific, but could also be in the patient's germline (and may be passed to subsequent generations). We have discussed this possibility with a committee of multidisciplinary experts (genetics, oncology, laboratorians, bioethicists). There is no consensus on what type of findings on a tumor sequencing assay would imply the presence/absence of germline mutation. The tumor findings will be communicated to the treating clinician with advice to consider germline testing if clinical and/or family history is consistent with the presence of such an abnormality. In many cases we do not know the medical significance of genetic

variants that are beyond the aims of this study (e.g., for which a targeted treatment is not available) (4, 5). The gene panel and the policy of result return will continue to be evaluated by an interdisciplinary study team throughout the course of the trial to maintain the rigor and integrity of the study and the wellbeing of our patients.

We are seeking consent from patients for optional research including whole-exome sequencing of tumor and blood, tumor mRNA sequencing and potentially other tests that will explore potential correlations between molecular features and the patient's disease, response, or course. Research on biopsies will only be done with excess samples, except at the end of the patient's participation in the study when we will request a biopsy solely for research purposes after the patient's tumor has progressed on the last MATCH treatment. These samples and results will be de-identified and are only for research to generate hypotheses concerning response or resistance to a particular treatment. We currently have no plans to validate or disclose for clinical use any whole exome sequencing or other sequencing conducted with de-identified samples collected in this study and no clinical decisions should be made based on the results of such research testing given the uncertain and/or unknown medical significance of some of these variants. To guard against the possibility of unintentional and potentially injurious disclosure of unvalidated research results, we will perform the optional whole exome or other sequencing as noted above on de-identified samples only. No genomic data will be shared with the patient other than the data from the targeted sequencing panel and other clinical tests performed for assessment of eligibility in one of the CLIA accredited laboratories. All other analyses will be performed on de-identified samples.

This study affords a unique opportunity to collect information about the prevalence of mutations, translocations and amplifications in genes associated with cancer, as well as how patients' tumors with such abnormalities respond to targeted therapy in the refractory tumor setting; DNA variants and changes in RNA expression from tumors collected when patients progress on study drugs is anticipated to illuminate resistance mechanisms that will inform subsequent studies and improve patient outcomes. The NCI Central IRB will be consulted and kept informed of progress of this study and other trials conducted with the study agents that may affect study design and conduct.

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For certain rare variants, described below, patients may enter a subprotocol using results from an assay performed in a CLIA certified laboratory outside of those laboratories engaged in the MATCH study. For these patients, we will request archived tissue for performing the MATCH assays, to judge concordance with results of the outside laboratory. Similar to the MATCH assays done with a fresh biopsy, the MATCH results on the archived tissue will be returned to the patient's provider for sharing with the patient.

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1.4 Rationale for the Study

Since the first validation of the molecular, oncogene-targeted therapy with imatinib, the abl kinase inhibitor, in chronic myelogenous leukemia, it has been appreciated that treating the principal driver oncogene can have a powerful impact. However, in subsequent successful applications of molecularly targeted therapy, such as EGFR and ALK inhibitors in non-small cell lung cancer and BRAF inhibitors in melanoma, the presence of the target oncogene in a subset of

patients requires genomic pre-screening of patient's tumor. Most recently, efforts to catalog driver mutations across the entire cancer population have identified very small fractions of patients with any given driver within any particular cancer type based on site of origin (see TCGA table below). To effectively investigate the efficacy of molecularly targeted treatment for such targets, and to understand the relative efficacy of the same therapy applied to oncogene-defined subsets across different tumor histologies, we propose to initiate a broad-based genomic pre-screening study to assign patients whose tumors harbor specific molecular abnormalities to relevant targeted treatments, regardless of tumor histology type.

The study is designed to assign targeted treatment based on a biopsy obtained just before study entry. This biopsy is requested because tumors sometimes accrue additional mutations after various treatments or with continued growth and metastasis. In addition, the preanalytic procedures of biopsy handling, shipping, review and isolation of nucleic acids will be uniform, with minimal variation in suitability that can sometimes be seen with archived tissues. However, in certain cases, we will be able to accept tissue obtained relatively recently prior to enrollment in screening (see Section 3.1.6.3 in Appendix XV)

Mutation Frequency in TCGA (%): Table courtesy of MD Anderson investigators

Gene	Bladder	Breast	Cervical	Colorectal	GBM	Glioma	Head and Neck	Lung (Adeno)	Lung (Squam)	Melanoma	Ovarian	Pancreas	Prostate	Stomach	Thyroid	Uterine
AKT	0	2.4	0	0.9	0.4	0	0.7	0.9	0.6	1.4	0	3.5	0.4	1.4	0.7	1.6
BRAF	0.8	0.6	2.6	9.9	2.1	1	1.4	9.6	4.5	50.2	0.6	1.8	2.4	4.1	61.3	2.8
EGFR	1.5	0.8	2.6	4.5	26.1	5.9	4.7	14.3	3.9	6.5	2.2	1.8	1.2	5	0	3.2
FGFR1	3.1	0.3	0	1.3	1.1	0	0.4	1.3	1.7	3.6	0	0	0	4.5	0	3.2
FGFR2	2.3	1.1	0	1.3	1.1	0.3	0.7	3.1	3.9	9.3	0	1.8	1.2	3.6	0.2	12.5
FGFR3	14.6	0.4	0	0.9	1.1	0	2.2	0.9	2.3	2.9	0.3	1.8	0.4	1.4	0	2
HRAS	4.6	0.2	0	0	0	0	3.9	0.4	2.8	1.1	0	1.8	0.8	0	3.5	0.4
IDH1	3.6	0.4	0	1.3	4.9	76.1	0.7	1.3	1.1	5.7	0	1.8	1.6	0.5	0	1.6
IDH2	0	0	0	3.1	0	4.2	0	0.9	0.6	0.4	0	1.8	0	0.5	0	1.6
KIT	2.3	1	0	2.7	1.1	0.7	1.3	2.2	3.9	3.9	2.2	1.8	0	4.1	0	6.9
KRAS	0	0.8	2.6	43	1.1	0.3	0.4	32.6	1.1	1.4	0.6	57.9	0	11.4	1	21.4
NF1	9.2	2.8	7.7	3.6	14.3	6.6	2.9	11.8	11.9	13.3	4.4	1.8	0.4	5.9	0.5	8.1
NF2	3.6	0.4	5.1	1.3	0	0.3	1.4	0.9	1.1	1.1	0.3	1.8	0.8	1.4	0.2	2.4
NRAS	0	3.6	0	9	1.1	0.3	0.4	1.7	0.6	30.8	0.6	0	0	0.9	8.5	3.6
PIK3CA	20	35.1	23.1	20.1	10.6	0	20.9	6.5	15.7	3.6	0.6	1.8	3.2	21.8	0.5	53.2
PIK3R1	3.6	2.6	2.6	4	11.3	4.5	2	1.3	1.1	0.7	0.3	1.8	0.8	3.6	0.2	33.1
PTCH	7.1	1.2	2.6	4	1.1	0.3	3.6	4.8	2.8	2.5	1.9	1.8	0.8	5	0.5	7.7
PTEN	3.1	3.6	7.7	3.6	31.9	4.5	2	3.1	7.9	8.2	0.6	1.8	5.2	6.4	0.5	64.9
SMO	3.6	0.5	0	0.4	0.4	0	0.3	2.6	0.6	3.6	0	3.5	0	4.1	0	2
TSC1	10.7	0.6	2.6	2.2	1.1	0.3	0.7	2.6	3.4	3.2	0.9	1.8	0	1.4	0	4
TSC2	2.3	0.6	2.6	0.9	2.2	0.7	1.1	2.2	3.4	3.9	0.6	2	0.8	3.2	0	4.8

Amplification Frequency in TCGA (%)

Gene	Bladder	Breast	Cervical	Colorectal	GBM	Glioma	Head and Neck	Lung (Adeno)	Lung (Squam)	Melanoma	Ovarian	pancreas	Prostate	Stomach	Thyroid	Uterine
HER2	6.3	12.9	2.3	3.1			2.6	2.6	2.2	0.6	2.6			13	0.6	5.5
FGFR1	9.4	12	1.2	3.6		0.4	8.5	3.5	16.9	0.3	3.5	4	2	1		2.5
FGFR2	0.5	1.8		0.3	0.6		0.7	0.9			3.5			5.1		
FGFR3	5.5	0.5		0.4	1.8	0.8	0.7	1.3	0.6	1.5	7.9	2	0.8	0.7	0.2	2.2
MET	0.5	0.8	1.8	0.4	8.9	0.8	0.7	3.5	1.1	3.9	6.3		1.2	4.1		0.3
PIK3CA	5.5	4.9	19.3		2.9	1.1	21.1	2.2	38.2		28.8	4	2.8	5.5		14.3

In recent years, targeted agents have been shown to have benefit in many malignant diseases. Some of these agents have obtained FDA approval for different diseases with the same and/or different target. For example, imatinib was FDA approved for chronic myelogenous leukemia with *BCR/ABL* translocation, but is also approved for gastrointestinal stromal tumor carrying mutations in *KIT*, and for adult patients with relapsed or refractory Philadelphia chromosome positive acute lymphoblastic leukemia. Trastuzumab is approved for HER2 *(ERBB2)* overexpressing breast, gastric or gastroesophageal cancer.

The current proposal is designed to address whether patients with underlying abnormalities (e.g. mutations, amplifications or translocations) in a given pathway in their tumor could achieve tumor shrinkage or prolonged time to progression with agents that target their tumor's particular molecular abnormality.

1.4.1 Levels of evidence for MATCH treatments

We will include targeted agents in NCI-MATCH if one of the following 3 criteria is met:

1. The drug is FDA approved for a malignant indication and there is a molecular abnormality that can serve as a valid predictive marker. The subprotocol will not enroll patients with conditions for which the drug is approved, or patients with conditions for which the drug has been shown not to have benefit.
2. The drug is investigational, but met a clinical endpoint (PFS, response) in any malignancy, has evidence of target inhibition and has evidence of a predictive molecular marker.
3. The drug is investigational but has demonstrated clinical activity in any malignancy and evidence of target inhibition, and has evidence of a predictive molecular marker.

In addition, targeted drugs or drug combinations must have at least a recommended phase 2 dose.

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1.4.2 Levels of Evidence for Actionable Variants Within a Gene

In order to use a given variant (mutation, insertion, deletion, amplification, translocation, protein expression) as a potential predictor of effect of a targeted treatment, the variant must meet one of the levels of evidence below:

- Level 1: Gene variant credentialed for selection of an approved drug (e.g. BRAF V600E and vemurafenib in melanoma); such a variant will be considered level 1 in any tissue

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- Level 2: Gene variant is eligibility criterion for an ongoing clinical trial for that treatment OR gene variant has been identified as etiology of N of 1 response
- Level 3: Preclinical inferential data (in vitro or in vivo models) providing biological evidence to support use of the variant for treatment selection. E.g.:
  - Models with variant respond to the drug while models without the variant do not
  - Gain of function mutations demonstrated in a preclinical model; e.g. D769H variant of ERBB2 results in increased

tyrosine kinase-specific activity and upregulates pathway signaling (clinical evidence not required)

- Loss of function genes, tumor suppressor or pathway inhibitor (e.g. NF1) – variant produces a stop codon including frameshift or demonstrated loss of function of resultant protein in preclinical model (clinical evidence not required)

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NCI-MATCH now uses external "designated laboratories" (DL) to identify eligible patients (see Section 1.6 below). The DLs have identified several variants which are not currently designated as aMOIs, but which they consider potentially eligible for the study. NCI-MATCH has articulated that additional aMOIs can be considered actionable (used to assign a treatment in NCI-MATCH), if they meet NCI-MATCH's levels of evidence. We have classified these types of variants into six categories described in a study tool posted on the E-A and CTSU websites. These new aMOIs will be assessed, confirmed and included as actionable variants for their associated subprotocols in a 'dynamic' fashion. This means they can be used immediately for patient treatment assignment, without the need for an amendment, provided they are shown by the DL, and confirmed by the study team, to meet LOE 3 or higher. Please refer to Appendix XVII for additional information.

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1.5 Rationale for Image Collection

1.5.1 Noninvasive Biomarkers of Drug Resistance

MATCH trial [15] tests whether tumors sharing common somatic genetic alterations will be variably responsive to therapies targeting the given oncogenic pathway. We hypothesize that measured changes during targeted therapy in quantitative radiomic tumor phenotypes, computationally extracted by mining conventional CT and MR image datasets at baseline and early in the course of treatment will yield candidate biomarkers to predict subsequent patient outcome and predict development of drug resistance. We also hypothesize that specific associations between radiomic features and tumor gene mutation patterns can be demonstrated.

Computational advances in the past decade now allow for high-throughput extraction of quantitative features that convert digital medical images into mineable data and subsequent analysis of these data to support clinical decision-making. This is called radiomics. The first step is acquisition of tomographic imaging, CT or MRI, typically acquired in the course of standard care. Next, macroscopic tumor is defined on these images, usually using automated segmentation method in combination with manual editing. A large number of quantitative features are then extracted from the defined tumor volumes to generate a phenotype database using first- and higher-order statistics. This, when integrated with clinical, biochemical, pathologic, and genomic information, allows for data mining to develop diagnostic, prognostic or predictive models relevant to patient care.

Large-scale sequencing analyses of tumor genomes over the past decade have demonstrated extensive heterogeneity that can be classified as intra-tumoral, inter-metastatic, intra-metastatic, and inter-patient [26]. This heterogeneity allows for tumor evolution and has been shown to contribute to treatment failure [27]. An approach to molecularly targeted therapy that relies solely on biopsy samples is subject to sampling error and could underestimate the potential of the tumor for genomic adaptation to drug resistance. In contrast, imaging enables whole-body evaluation repeatedly during therapy and allows for quantitative assessment of heterogeneity at the pixel or voxel level [28]. Based on these features, we expect that imaging parameters may better distinguish patients with deep and durable responses from those with minimal and short-lived disease control.

Radiomics has been successful at identifying CT or MRI phenotypes that describe tumor gene expression patterns and predict therapeutic outcome in a number of cancers. [29-34]. More recent radiomic studies have demonstrated tumor heterogeneity to be an independent predictor of patient survival in a number of cancers. Lung cancer heterogeneity has been captured with semantic (e.g., spiculation) and agnostic (e.g., entropy ratio) radiomic features. Several investigations have demonstrated these signatures of tumor heterogeneity to be predictors of patient survival in multivariate models [35]. Aerts and colleagues extracted 440 features quantifying tumor radiomic features from computed tomography data of 1019 patients with lung or head-and-neck cancer [36]. They found significant associations between the signature features and gene-expression patterns. Signatures of tumor heterogeneity were strongly correlated with cell cycling pathways driving proliferation. The investigators demonstrated that these radiomic signatures could be used to predict outcome in completely independent cohorts with lung cancer from two separate institutions and could be applied to head and neck cancer patients with equivalent prognostic power. These studies demonstrate the potential of radiomics for the identification of prognostic imaging phenotypes generalizable to several forms of cancer.

The analyses of the predictive performance of imaging features and their correlation with genomic profiles will primarily aim at discovery. The quality of the datasets significantly impacts on the numbers needed for modeling. As MATCH images will represent a collection exclusively enriched for specific genomic profiles, sample numbers required for successful radiomic modeling will likely prove lower than in analyses to date which have all been performed on datasets retrospectively acquired for other purposes of treatment or screening. Moreover, as oncologic molecular profiling increases in prevalence both in clinical practice and trials, we anticipate that other datasets with imaging, genomic, and clinical outcome data will be available to test the radiomic features modeled on the MATCH dataset.

1.5.2 Optimize Biopsy Yield for Molecular Profiling

Collection of images from the biopsy procedure itself will address two goals. First, for the purposes of the scientific aims of the treatment

trial, it will enable central quality assurance of the biopsy procedure (i.e., target selection, needle placement) to optimize the yield of the molecular profiling. With current practice standards, 10-15% of patients do not have sufficient material for Next Generation Sequencing molecular profiling [37]. The literature is silent on which variables of the biopsy technique (e.g. fine needle aspiration vs. core, number of passes, needle gauge), target lesion choice (e.g. size, length of needle throw, anatomic location) or needle placement (e.g. central, peripheral, tangential, etc.) impact on this yield. Collection of a pre-biopsy lesion assessment by the interventional radiologist, biopsy reports and images will enable identification of factors that impact on the yield of biopsies performed for genomic profiling. Second, for the purposes of radiomic biomarker analysis, biopsy images will enable precise localization of the tissue targeted for profiling and comparison of its imaging features with the phenotype of the overall tumor burden and assess its' evolution under targeted on serial imaging.

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1.6 Rules for treatment selection

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Patients will be assigned by consistent and locked rules to the agents/regimens targeted to their tumor's identified molecular abnormality. If more than one molecular abnormality is identified as actionable for treatment in MATCH, the one with the greater level of evidence (see Section 1.4.2) that tumors harboring the variant will respond will be used to assign treatment. The molecular profiling assays have 4 "parts" (point mutations or indels; amplification; translocation; IHC); levels of evidence from each of these parts will be compared, and the treatment will be assigned based on the highest (e.g. Level 1 or 2 or 3) level of evidence. Additional molecular assays (e.g., FISH) when used, will also be considered "equal" (see below). For SNV/indel, if more than one aMOI is present with equivalent level of evidence, then the abnormality with the higher allele frequency (must be > 15% higher than the next lower allele frequency) will be used to assign treatment. If two variants are equivalent using these 2 parameters, the patient will be assigned to the subprotocol with fewer patients, or randomized between subprotocols if numbers of patients on the relevant subprotocols are equal.

The following are considered equal: single nucleotide variants (SNV), insertions/deletions (indels), copy number variants (CNV) translocation/fusions, IHC, or other assay.

For each component SNV, indel, CNV and translocation/fusion, IHC or other assay

- Ascertain level of evidence (LOE) within each category of variant as above (levels 1 > 2 >3).
- If > 1 SNV/indel with best LOE (1 > 2 > 3), and the allele frequency of one of the aMOIs is more than 15% greater than the next lower allele frequency, select aMOI with greater allele frequency. If the allele frequency of the 2 variants is within 15% of each other, assign patient to subprotocol with fewer enrollees (or randomize if number of enrollees is equal)
- If > 1 aMOI with best LOE (1 > 2 > 3) and allele frequency of each is within 15% of the other then assign patient to subprotocol with fewer enrollees

- Compare “winner” of each assay component SNV/indel, CNV, translocation/fusion, IHC, or other molecular assay:
- LOE 1 "wins" over level 2, which “wins” over level 3
- If > 1 best LOE, assign patient to subprotocol with fewer enrollees (or randomize if number of enrollees is equal).

These rules will be applied by a rigorously validated informatics system to derive a tentative treatment assignment. If a patient is ineligible for an assigned treatment but their tumor harbors additional abnormalities for which treatments are available on the study, the system will continue to provide assignments until all potentially available options are exhausted. Subprotocol assignments will be monitored by a study steering committee throughout the course of the study.

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1.6.1 “Rare Variant” Arm Enrichment

Although NCI-MATCH will sequence biopsies from 6000 patients, certain variants are so rare that subprotocols with such eligibility are not expected to accrue adequate patients. As these treatments have evidence that they may be effective in patients whose tumors have these rare variants, NCI-MATCH plans to enhance the ability to recruit such patients with the help of several CLIA accredited laboratories that sequence large numbers of patient tumors. Additionally, on May 22, 2017, accrual to central screening by the MATCH laboratories closed and NCI-MATCH will recruit patients for selected arms with assistance from designated and vetted outside laboratories (MATCH-designated outside laboratories). **Those arms open for recruitment via results determined by an outside laboratory and the applicable variants will be termed “rare variant” for the purposes of this trial**.

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MATCH-designated outside laboratories will notify the ordering physician that the patient with the “rare variant” may be eligible for NCI-MATCH. The designated outside laboratories will be eligible to notify the ordering physician on any of the “rare variant” arms for which their assay reports all aMOIs, including the exclusionary variants, as defined prior to the addition of any novel variants (inclusion or exclusion) of the trial arm. Designated laboratories with assays that detect the novel variants (inclusionary and/or exclusionary) are to include those variants when determining applicable referrals. These laboratories have agreed to participate with NCI-MATCH and will communicate to the medical provider the potential opportunity for patients with rare variants to enroll in NCI-MATCH, but are not obligated to limit communication about potential trials to NCI-MATCH. It will be the provider’s and patient’s decision whether or not to enroll in NCI-MATCH, and the outside lab report will only be transmitted to NCI-MATCH if the patient decides to enroll in Step 0.

Outside laboratories will identify patients whose tumors have been sequenced with the sequencing assay used by the outside laboratories and have rare variants that may be eligible for NCI-MATCH. The outside laboratories will then notify the ordering clinician that the patient may be eligible for a treatment in NCI-MATCH and will

provide information on how to contact the MATCH trial. Clinicians and patients will decide whether to use that information to enroll into NCI-MATCH. The form of notification will be at the discretion of the outside laboratory (e.g. by telephone, fax, electronic medical record, lab report, etc). Outside laboratories will not transfer any patient information directly to the NCI-MATCH study but will keep records as to the number of patients that were notified.

A NCI-MATCH Trial Arm that has been designated as a “rare variant” arm, based on either NCI-MATCH results to date or other similar data, will be considered a “rare variant” arm. Once it is deemed a “rare variant” arm, it will remain a “rare variant” arm. As additional arms are activated, other arms may be added into the “rare variant” arm group.

Patients who have a “rare variant” by a designated “outside” assay, have specifically been notified of such by a NCI- MATCH designated outside laboratory, and are otherwise eligible will be permitted to enroll on the arm with the results of the “outside” assay. However, these results will be verified by the NCI-MATCH NGS assay, and NCI-MATCH IHC assays. Only patients whose tumor samples have aMOIs that have been verified with the NCI-MATCH assay(s) will be included in the primary analysis for outcome, though all patients treated on the arm will be evaluable for toxicity. Patients without such verification may continue treatment, but will not be included in the primary outcome analyses (see Section 8.1).

2. Objectives

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2.1 Primary Objective

2.1.1 To evaluate the proportion of patients with objective response (OR) to targeted study agent(s) in patients with advanced refractory cancers/lymphomas/multiple myeloma.

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2.2 Secondary Objectives

2.2.1 To evaluate the proportion of patients alive and progression free at 6 months of treatment with targeted study agent in patients with advanced refractory cancers/lymphomas/multiple myeloma.

2.2.2 To evaluate time until death or disease progression.

2.2.3 To identify potential predictive biomarkers beyond the genomic alteration by which treatment is assigned or resistance mechanisms using additional genomic, RNA, protein and imaging-based assessment platforms.

2.2.4 To assess whether radiomic phenotypes obtained from pre-treatment imaging and changes from pre- through post-therapy imaging can predict Objective Response and Progression Free Survival and to evaluate the association between pre-treatment radiomic phenotypes and targeted gene mutation patterns of tumor biopsy specimens.

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3. Selection of Patients

Each of the criteria in the checklist that follows must be met in order for a patient to be considered eligible for this study. Use the checklist to confirm a patient's eligibility. For each patient, this checklist must be photocopied, completed and maintained in the patient's chart.

NOTE: This checklist is for eligibility for the Master Screening Protocol only; each subprotocol will have additional eligibility criteria that will be outlined in Section 2.1 of the agent-specific subprotocol.

In calculating days of tests and measurements, the day a test or measurement is done is considered Day 0. Therefore, if a test is done on a Monday, the Monday four weeks later would be considered Day 28.

ECOG-ACRIN Patient No. _____

Patient's Initials (L, F, M) _____

Physician Signature and Date _____

NOTE: Policy does not allow for the issuance of waivers to any protocol specified criteria (http://ctep.cancer.gov/protocolDevelopment/policies_deviations.htm). Therefore, all eligibility criteria listed in Section 3 must be met, without exception. The registration of individuals who do not meet all criteria listed in Section 3 can result in the participant being censored from the analysis of the study, and the citation of a major protocol violation during an audit. All questions regarding clarification of eligibility criteria must be directed to the Group's Executive Officer (EA.Execofficer@jimmy.harvard.edu) or the Group's Regulatory Officer (EA.RegOfficer@jimmy.harvard.edu).

NOTE: Institutions may use the eligibility checklist as source documentation if it has been reviewed, signed, and dated prior to registration/randomization by the treating physician.

NOTE: Please refer to the Schema for details on the registration process.

NOTE: Patients may not receive more than two rounds of treatment (e.g. may only be assigned 2 different treatments based on aMOIs) between biopsies.

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3.1 Eligibility Criteria for Screening Biopsy (Step 0)

_____ 3.1.1 Patients must be ≥ 18 years of age. Because no dosing or adverse event data are currently available on the use of study investigational agents in patients < 18 years of age, children are excluded from this study.

_____ 3.1.2 Women of childbearing potential must have a negative serum pregnancy test within 2 weeks prior to registration. Patients that are pregnant or breast feeding are excluded.

A female of childbearing potential is any woman, regardless of sexual orientation or whether they have undergone tubal ligation, who meets the following criteria: 1) has not undergone a hysterectomy or bilateral oophorectomy; or 2) has not been naturally postmenopausal for at least 24 consecutive months (i.e., has had menses at any time in the preceding 24 consecutive months).

Female of childbearing potential? _____ (Yes or No)

Date of serum pregnancy test: _____

_____ 3.1.3 Women of childbearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry, for the duration of study participation, and for 4 months after completion of study.

Should a woman become pregnant or suspect while she or her partner is participating in this study, she should inform her treating physician immediately.

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_____ 3.1.4 Patients must have histologically documented solid tumors or histologically confirmed diagnosis of lymphoma or multiple myeloma requiring therapy and meet one of the following criteria:

- Patients must have progressed following at least one line of standard systemic therapy and there must not be other approved/standard therapy available that has been shown to prolong overall survival (i.e. in a randomized trial against another standard treatment or by comparison to historical controls). Patients who cannot receive other standard therapy that has been shown to prolong overall survival due to medical issues will be eligible, if other eligibility criteria are met. If the patient is currently receiving therapy, the clinician must have assessed that the current therapy is no longer benefitting the patient prior to enrolling on MATCH, regardless of whether it is considered standard.

  OR

- Patients for whose disease no standard treatment exists that has been shown to prolong overall survival.

NOTE: No other prior malignancy is allowed except for the following:

a) adequately treated basal cell or squamous cell skin cancer
b) in situ cervical cancer
c) adequately treated Stage I or II cancer from which the patient is currently in complete remission
d) any other cancer from which the patient has been disease-free for 5 years.

_____ 3.1.5 Patients must have measurable disease as defined in Section 6.

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_____ 3.1.6 Patients must meet the criteria below and have received results from one of the designated outside laboratories indicating a “rare variant” that is an actionable Mutation of Interest (aMOI) for specific select subprotocols. See Appendix XIV for more information on the designated laboratories and applicable arms.

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The following requirements apply:

- The outside laboratory specifically notified the site that patient may be a potential candidate for MATCH due to a detected “rare

variant." The outside lab reports are **NOT** sufficient for this purpose.

NOTE: The content and format of these specific notifications for the Outside Assay process will vary depending on the designated outside lab in question, as they are each responsible for their own outreach efforts. It is strongly recommended that the designated outside laboratory be contacted to confirm the format and receipt of this notification prior to registering any patients to Step 0.

- Patients with an applicable "rare variant" must be able to meet the eligibility criteria for the appropriate subprotocols within 4 weeks following notification of treatment assignment, per Section 4.2.

  NOTE: The receipt of this notification (and the start of the associated deadline for Step 1 registration) may occur shortly after Step 0 registration, since these patients will not be submitting tissue for screening purposes. However, for certain "rare variant" arms, submission of archival tissue for central IHC testing may be required.

- Registration to Step 0 must occur after stopping prior systemic anti-cancer therapy. There is no specific duration for which patients must be off treatment prior to registration to Step 0, as long as all eligibility criteria are met.
- There is no particular window of time after notification of potential eligibility from an outside lab in which the patient must be registered to Step 0, but treatment slots will be assigned on a first come, first serve basis to those who do register to Step 0, and are not held for those notified of potential eligibility who do not register to Step 0.
- Patients may have received other non-targeted, immunotherapy or targeted treatment between the prior genetic testing at the outside lab and registration to Step 0. The decision to stop such treatment in favor of participation in MATCH, if no further clinical benefit is expected, is per the treating physician's discretion. Documentation of a lack of response to the prior treatment is not required in these cases. The requirements in Section 3.1.4 still apply.

  NOTE: Other potential aMOIs that would be eligibility criteria for "NON RARE" arms, as determined by the designated laboratories, are not applicable for this process in MATCH. See additional requirements outlined in Section 4.1.5.4.

  NOTE: Tumor tissue for the confirmation of "rare variant" by the MATCH assay is to be submitted, preferably from the same time of collection as that evaluated by the designated outside laboratory, per Section 4.1.5, Section 7.2 and Section 9.

Rev.2/16, 5/16 _____ 3.1.7 Patient must not require the use of full dose coumarin-derivative anticoagulants such as warfarin. Low molecular weight heparin is permitted for prophylactic or therapeutic use. Factor X inhibitors are permitted.

NOTE: Warfarin may not be started while enrolled in the EAY131 study.

Stopping the anticoagulation for biopsy should be per site SOP.

Rev.5/16 _____ 3.1.8 Patients must have ECOG performance status ≤ 1 (see Appendix V) and a life expectancy of at least 3 months.

_____ 3.1.9 Patients must not currently be receiving any other investigational agents.

Rev.3/17 _____ 3.1.10 Patients must not have any uncontrolled intercurrent illness including, but not limited to:

- Symptomatic congestive heart failure (NYHA classification of III/IV)
- Unstable angina pectoris or coronary angioplasty, or stenting within 6 months prior to registration to Step 0, 2, 4, 6
- Cardiac arrhythmia (ongoing cardiac dysrhythmias of NCI CTCAE v4 Grade ≥ 2)
- Psychiatric illness/social situations that would limit compliance with study requirements
- Intra-cardiac defibrillators
- Known cardiac metastases
- Rev.5/16 Abnormal cardiac valve morphology (≥ grade 2) documented by ECHO (as clinically indicated); (subjects with grade 1 abnormalities [i.e., mild regurgitation/stenosis] can be entered on study). Subjects with moderate valvular thickening should not be entered on study

NOTE: To receive an agent, patient must not have any uncontrolled intercurrent illness such as ongoing or active infection. Patients with infections unlikely to be resolved within 2 weeks following screening should not be considered for the trial.

_____ 3.1.11 Patients must be able to swallow tablets or capsules. A patient with any gastrointestinal disease that would impair ability to swallow, retain, or absorb drug is not eligible.

_____ 3.1.12 Patients who are HIV-positive are eligible if:

___ CD4+ cell count greater or equal to 250 cells/mm^3

___ If patient is on antiretroviral therapy, there must be minimal interactions or overlapping toxicity of the antiretroviral therapy with the experimental cancer treatment; for experimental cancer therapeutics with CYP3A/4 interactions, protease inhibitor therapy is disallowed; suggested regimens to replace protease inhibitor therapy include dolutegravir given with tenofovir/emtracitabine; raltegravir given with

tenofovir and emtracitabine. Once daily combinations that use pharmacologic boosters may not be used.

___ No history of non-malignancy AIDS-defining conditions other than historical low CD4+ cell counts

___ Probable long-term survival with HIV if cancer were not present.

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_____ 3.1.13 Any prior therapy, radiotherapy (except palliative radiation therapy of 30 Gy or less), or major surgery must have been completed ≥ 4 weeks prior to start of treatment. All adverse events due to prior therapy have resolved to a grade 1 or better (except alopecia and lymphopenia) by start of treatment. Palliative radiation therapy must have been completed at least 2 weeks prior to start of treatment. The radiotherapy must not be to a lesion that is included as measurable disease.

NOTE: Prostate cancer patients may continue their LHRH agonist.

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NOTE: For patients entering the study via the original screening process, patients may receive non-protocol treatment after biopsy (if clinically indicated) until they receive notification of results; however, lack of response (per Section 6) must be documented prior to registration to Step 1. New non-protocol treatment will NOT be permitted as intervening therapy after registration to Step 0. The only intervening treatment permitted is prior therapy that the patient already received prior to Step 0 registration.

The decision to stop the intervening non-protocol treatment will be left up to the treating physician if patient has an aMOI. However, patients will need to be off such therapy for at least 4 weeks before receiving any MATCH protocol treatment. Please refer to Section 4.2 for additional relevant time restrictions.

NOTE: For patients entering the study via a designated outside laboratory, no intervening systemic non-protocol treatment is permitted after Step 0 registration. All other eligibility requirements still apply to these patients, including the washouts for prior therapy noted above in this section, the time restrictions outlined in Section 4.2, and the eligibility criteria for the intended subprotocol.

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_____ 3.1.14 Patients with brain metastases or primary brain tumors must have completed treatment, surgery or radiation therapy ≥ 4 weeks prior to start of treatment.

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_____ 3.1.15 Patients must have discontinued steroids ≥ 1 week prior to registration to Step 0 and remain off steroids thereafter, except as permitted (see below). Patients with glioblastoma (GBM) must have been on stable dose of steroids, or be off steroids, for one week prior to registration to treatment step (Step 1, 3, 5, 7).

NOTE: The following steroids are permitted (in the list below, low dose steroid use is defined as prednisone 10 mg daily or less, or bioequivalent dose of other corticosteroid):

- Temporary steroid use: e.g. for CT imaging in setting of contrast allergy
- Low dose steroid use for appetite
- Chronic inhaled steroid use
- Steroid injections for joint disease
- Stable dose of replacement steroid for adrenal insufficiency or low doses for non-malignant disease

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- Topical steroid
- Steroids required to manage toxicity related to study treatment, as described in the subprotocols
- Steroids required as pre- or post-chemotherapy medication for acceptable intervening chemotherapy

NOTE: Steroids must be completed alongside last dose of chemotherapy.

_____ 3.1.16 Patients must have adequate organ and marrow function as defined below within 2 weeks prior to screening step registration and within 4 weeks prior to treatment step registration:

- Leukocytes ≥ 3,000/ mcL*

  Leukocyte Count: _____

  Date of Test: _____
- Absolute neutrophil count ≥ 1,500/ mcL*

  ANC_____

  Date of Test_____
- Platelets ≥ 100,000/ mcL*

  Platelet Count_____

  Date of Test_____

  NOTE: *Patients with documented bone marrow involvement by lymphoma are not required to meet the above hematologic parameters, but must have a platelet count of at least 75,000/mcL and neutrophil count of at least 1000/mcL.
- Total bilirubin ≤1.5 X institutional ULN (unless documented Gilbert's Syndrome, for which bilirubin ≤ 3 x institutional ULN is permitted)

  Total bilirubin_____

  Institutional ULN _____ Date of Test _____
- AST(SGOT)/ALT(SGPT) ≤ 2.5 X institutional upper limit of normal (ULN) (up to 5 times ULN in presence of liver metastases)

AST_____ ALT_____

Institutional ULN _____

Date of Test _____

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- Creatinine clearance ≥ 45 mL/min/1.73 m^2 for patients with creatinine levels above institutional normal

  Creatinine clearance _____

  As defined by the Cockcroft-Gault Equation

  $$\text{CrCl (ml/min)} = \frac{(140 - \text{age in years}) \times \text{actual wt (in kg)}}{72 \times \text{serum creatinine (mg/dl)}} \times 0.85 \text{ (for female pts)}$$

  Date of Test_____

_____ 3.1.17 Patients must have an electrocardiogram (ECG) within 8 weeks prior to registration to screening step and must meet the following cardiac criteria:

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_____ 3.1.17.1 Resting corrected QT interval (QTc) ≤ 480 msec.

NOTE: If the first recorded QTc exceeds 480 msec, two additional, consecutive ECGs are required and must result in a mean resting QTc ≤ 480 msec. It is recommended that there are 10-minute (± 5 minutes) breaks between the ECGs.

The following only need to be assessed if the mean QTc > 480 msec.

- Check potassium and magnesium serum levels
- Correct any identified hypokalemia and/or hypomagnesemia and may repeat ECG to confirm exclusion of patient due to QTc
- For patients with HR 60-100 bpm, no manual read of QTc is required.
- For patients with baseline HR < 60 or > 100 bpm, manual read of QT by trained personnel is required, with Fridericia correction applied to determine QTc.
- Patient must not have hypokalemia (value < institutional lower limit of normal).

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_____ 3.1.17.2 No factors that increase the risk of QTc prolongation or risk of arrhythmic events such as heart failure, congenital long QT syndrome, family history of long QT syndrome or unexplained sudden death under 40 years of age or any concomitant medication known to prolong the QT interval (For a list of these medications, please see Appendix XIII)

Date of ECG: _____

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NOTE: Patient must be taken off prohibited medication prior to registration to the screening Step (Step 0, 2, 4, 6), and remain off these medications

thereafter, unless permitted on a subprotocol for the management of treatment related toxicity. Patient must be off the drug for at least 5 half lives prior to registration to the treatment step (Step 1, 3, 5, 7). The medication half life can be found in the package insert for FDA approved drugs.

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3.2 Eligibility Criteria for First Treatment (Step 1)

3.2.1 Eligibility requirements for registration onto Step 1 are outlined in Section 2.1 of the agent-specific subprotocol.

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NOTE: If patients have been biopsied or submitted archived tumor tissue obtained within the last 6 months for assessment with the MATCH assays, patients may receive non-protocol treatment after biopsy/tissue submission (if clinically indicated) until they receive notification of results however, lack of response (per Section 6) must be documented prior to registration to Step 1. New non-protocol treatment will NOT be permitted as intervening therapy after registration to Step 0.

For patients entering Step 0 with assay results from outside laboratories, no systemic treatment is allowed after Step 0 registration.

The decision to stop the intervening nonprotocol treatment will be left up to the treating physician if patient has an aMOI. Waiting periods as described in Section 3.1.13 will apply.

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3.2.2 As MATCH is designed to add additional subprotocols, implement limited expansions of accrual for certain subprotocols, and/or amend existing arm-specific eligibility criteria, some patients entering under the original screening method may be eligible to have their results rerun in MATCHbox, even if they did not match to a treatment initially or did not receive a treatment assignment due to a lack of available assignment slots. Patients whose sequence results will be rerun through MATCHbox must also meet the following criteria:

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3.2.2.1 Samples must have been collected within 5 months of the activation of the addendum, as there is an additional month needed to get the patients on trial.

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3.2.2.2 Patient has not had treatment within the 5 months that resulted in a PR or better after the performance of the screening assessment.

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3.2.2.3 Patient must meet eligibility criteria, including performance status 1 or better and life expectancy of at least 3 months.

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3.2.2.4 Patients must meet the eligibility requirements found in Section 3.1.13, with the following exceptions:

- Patients may have received other non-targeted, immunotherapy or targeted treatment, which could be stopped in favor of returning to MATCH, if no response to the interim treatment has occurred and no further benefit is expected from this interim treatment, per the treating physician's discretion. Documentation of a lack of response to the interim treatment is not required in these cases.

  However, the following restrictions apply:

  - Enrollment onto another investigational therapeutic study is not permitted.
  - Patient cannot be responding to interim treatment, since the benefit of the MATCH treatment is unknown and may deprive patient of an effective treatment if it were given when a patient is responding to another treatment.

NOTE: Patients meeting these criteria will NOT be biopsied at this time point. Instead, their Step 0 results will be re-interrogated to determine if another treatment is available.

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3.3 Eligibility Criteria for Second Screening (Step 2)

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_____ 3.3.1 Patient's disease has progressed on Step 1 treatment (per Section 6) or patient could not tolerate assigned treatment.

NOTE: PATIENTS ENTERING STEP 1 WITH A "RARE VARIANT" FROM AN "OUTSIDE" LAB ARE NOT ELIGIBLE FOR STEP 2. SEE SECTION 3.3.3 BELOW.

3.3.1.1 Patients must meet one of the following criteria:

_____ 3.3.1.1.1 No response and progression (or inability to tolerate further treatment) occurred < 6 months from start of Step 1 treatment.

NOTE: Patients meeting these criteria will NOT be biopsied at this time point. Instead, their Step 0 MATCH assay results will be re-interrogated to determine if another treatment is available upon registration to this study step. It is not necessary to confirm the availability of another potential treatment assignment in advance. Only aMOIs detected by the MATCH assay may be used for the determination of eligibility to a relevant subprotocol.

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OR

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_____ 3.3.1.1.2 Progression (or inability to tolerate further treatment) occurred after a (1) response **OR** (2) after ≥ 6 months from start of Step 1 treatment. Patient must have tumor amenable to percutaneous biopsy and be willing and able to undergo a tumor biopsy or bone marrow aspirate for collection and submission of tumor tissue OR patient will be undergoing a procedure due to medical necessity during which the tissue may be collected for the central determination of the presence of one or more of the specific "actionable" mutations/amplifications of interest (aMOI) (defined in Appendix VII). See Section 9. Archived specimens cannot be accepted.

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_____ 3.3.2 Patients must meet eligibility criteria as defined in Section 3.1 (excluding Section 3.1.6).

_____ 3.3.3 Patient must not have been assigned to Step 1 treatment based on a "rare variant" determined by a designated outside laboratory (See Appendix XIV).

3.4 Eligibility Criteria for Second Treatment (Step 3)

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3.4.1 Eligibility requirements for registration onto Step 3 are outlined in Section 2.1 of the agent-specific subprotocol.

Rev.5/16

NOTE: **If screening biopsy samples were submitted during Step 2,** patients may receive non-protocol treatment after biopsy (if clinically indicated) until they receive notification of results however, lack of response (per Section 6) must be documented prior to registration to Step 3. New non-protocol treatment will NOT be permitted as intervening therapy after registration to Step 2.

The decision to stop the intervening nonprotocol treatment will be left up to the treating physician if patient has an aMOI. Waiting periods as described in Section 3.1.13 will apply.

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3.5 Eligibility Criteria for Third Screening (Step 4)

Rev.5/16

_____ 3.5.1 Patient's disease has progressed on Step 3 treatment or patient could not tolerate assigned treatment.

3.5.1.1 Patient must meet one of the following criteria:

_____ 3.5.1.1.1 No response and progression (or inability to tolerate further treatment) occurred < 6 months from start of Step 3 (second) treatment **AND** a biopsy was performed at Step 2 screening.

NOTE: Patients meeting these criteria will NOT be biopsied at this time point. Instead, their latest MATCH assay results will be re-interrogated to determine if another treatment is available upon registration to this study step. It is not necessary to confirm the availability of another potential treatment assignment in advance.

OR

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_____ 3.5.1.1.2 Progression (or inability to tolerate further treatment) occurred on Step 3 treatment and a biopsy was not performed at Step 2 screening (due to presence of additional aMOIs at that stage). Patient must have tumor amenable to percutaneous biopsy and be willing and able to undergo a tumor biopsy or bone marrow aspiration for collection and submission of tumor tissue OR patient will be undergoing a procedure due to medical necessity during which the tissue may be collected for the central determination of the presence of one or more of the specific "actionable" mutations/amplifications of interest (defined in Appendix VII). Biopsy must not be considered to be more than minimal risk to the patient. See Section 9. Archived specimens cannot be accepted.

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_____ 3.5.2 Patients must meet eligibility criteria as defined in Section 3.1 (excluding Section 3.1.6).

NOTE: A patient may have a maximum of 2 screening biopsies (not including re-biopsy due to assay failure), and 2 MATCH treatments per biopsy (if > 1 aMOI).

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See Section 4.12 for further information on biopsies.

3.6 Eligibility Criteria for Third Treatment (Step 5)

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3.6.1 Eligibility requirements for registration onto Step 5 are outlined in Section 2.1 of the agent-specific subprotocol.

Rev.5/16

NOTE: **If screening biopsy was submitted on Step 4,** patients may receive non-protocol treatment after biopsy (if clinically indicated) until they receive notification of results however, lack of response (per Section 6) must be documented prior to registration to Step 5. New non-protocol treatment will NOT be permitted as intervening therapy after registration to Step 4.

The therapy cannot be an arm in the MATCH trial. The decision to stop the intervening nonprotocol treatment will be left up to the treating physician if patient has an aMOI. Waiting periods as described in Section 3.1.13 will apply.

3.7 Eligibility Criteria for Fourth Screening (Step 6)

_____ 3.7.1 Patient's disease has progressed on Step 5 protocol treatment or patient could not tolerate assigned treatment.

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_____ 3.7.2 Patient must have had no response, and progression (or inability to tolerate further treatment) occurred < 6 months from start of Step 5 treatment **AND** a biopsy was performed at Step 4 screening.

NOTE: Patients meeting these criteria will NOT be biopsied at this time point. Instead, based on their 2nd biopsy, their results will be interrogated to determine if another treatment is available upon registration to this study step. It is not necessary to confirm the availability of another potential treatment assignment in advance.

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_____ 3.7.3 Patients must meet eligibility criteria as defined in Section 3.1 (excluding Section 3.1.6).

Rev.8/15

NOTE: For Step 6, biopsies to collect material for screening assay will NOT be performed. A patient may have a maximum of 2 successful screening assessments of submitted tissue (Steps 0 and 2 OR Steps 0 and 4), and 2 MATCH treatments per biopsy (if > 1 aMOI). See Section 4.12 for further information on biopsies.

3.8 Eligibility Criteria for Fourth Treatment (Step 7)

Eligibility requirements for registration onto Step 7 are outlined in Section 2.1 of the agent-specific subprotocol.

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3.9 Eligibility Criteria for Research Biopsy and Circulating Tumor DNA Collection (Step 8)

NOTE: **ALL PATIENTS SHOULD BE ENCOURAGED TO PARTICIPATE IN STEP 8, IF SAFE; IF BIOPSY IS REFUSED OR INADVISABLE, ENCOURAGE COLLECTION OF WHOLE BLOOD FOR CIRCULATING TUMOR DNA, PER SECTION 9.3.2.**

As a reminder, the submission of the Step 8 samples requires additional patient consent, as well as registration to Step 8.

NOTE: Patients who entered the study (Step 0) with a "rare variant" determined by an outside assay ARE potentially eligible and encouraged to participate in Step 8.

_____ 3.9.1 Patient has completed their most recent MATCH study treatment, will not undergo an additional screening biopsies, and will receive no additional treatment on MATCH.

NOTE: If a 2nd Screening Biopsy was performed and showed no study-actionable abnormalities, and blood samples were

submitted with the screening biopsy, no additional end of treatment specimens are requested.

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_____ 3.9.2 For patients who consent to undergo the optional research biopsy, patient's disease responded to the most recent MATCH study treatment and then progressed (or unable to tolerate further treatment) OR the disease progression (or inability to tolerate further treatment) occurs > 6 months since the last screening biopsy.

NOTE: Performance of a biopsy to collect tumor tissue for research is strongly encouraged but not required. It is acceptable to submit only the optional blood specimens.

NOTE: If a biopsy will be performed solely to collect tumor specimens for research, the patient must be willing and able to undergo a tumor biopsy or bone marrow aspirate (for applicable multiple myeloma), per Section 9, for the collection and submission of tissue for research.

_____ _____

Physician Signature Date

OPTIONAL: This signature line is provided for use by institutions wishing to use the eligibility checklist as source documentation.

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4. Registration Procedures

CTEP Registration Procedures

Food and Drug Administration (FDA) regulations and National Cancer Institute (NCI) policy require all individuals contributing to NCI-sponsored trials to register and to renew their registration annually. To register, all individuals must obtain a Cancer Therapy Evaluation Program (CTEP) Identity and Access Management (IAM) account (https://ctepcore.nci.nih.gov/iam). In addition, persons with a registration type of Investigator (IVR), Non-Physician Investigator (NPIVR), or Associate Plus (AP) (i.e., clinical site staff requiring write access to OPEN, RAVE, or TRIAD or acting as a primary site contact) must complete their annual registration using CTEP's web-based Registration and Credential Repository (RCR) (https://ctepcore.nci.nih.gov/rcr). Documentation requirements per registration type are outlined in the table below.

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

An active CTEP-IAM user account and appropriate RCR registration is required to access all CTEP and CTSU (Cancer Trials Support Unit) websites and applications. In addition, IVRs and NPIVRs must list all clinical practice sites and IRBs covering their practice sites on the FDA Form 1572 in RCR to allow the following:

- Added to a site roster
- Assigned the treating, credit, consenting, or drug shipment (IVR only) tasks in OPEN
- Act as the site-protocol PI on the IRB approval

Additional information can be found on the CTEP website at https://ctep.cancer.gov/investigatorResources/default.htm. 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).

IRB Approval:

NOTE: Sites must utilize the CIRB as their IRB of record to participate in EAY131.

Sites participating on the NCI CIRB initiative that are approved by the CIRB for the study are not required to submit separate IRB approval documentation to the CTSU Regulatory Office for initial, continuing or amendment review. However, sites must submit a Study Specific Worksheet for Local Context (SSW) to the CIRB (via IRB Manager) to indicate their intention to open the study locally. The CIRB's approval of the

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SSW is then communicated to the CTSU Regulatory Office for compliance in the RSS. The Signatory site must provide the CTSU Regulatory Office the participating institutions on the study. Other site registration requirements (i.e., laboratory certifications, protocol-specific training certifications, or modality credentialing) must be submitted to the CTSU Regulatory Office or compliance communicated per protocol instructions.

For sites under the CIRB initiative, IRB data will automatically load to RSS.

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Downloading Site Registration Documents:

Site registration forms may be downloaded from the EAY131 protocol page located on the CTSU members' website. 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 CTSU RSS.

- Go to https://www.ctsu.org and log in to the members' area using your CTEP-IAM username and password
- Click on the Protocols tab in the upper left of your screen
- Either enter the protocol # in the search field at the top of the protocol tree, or
- Click on the By Lead Organization folder to expand
- Click on the ECOG-ACRIN link to expand, then select trial protocol EAY131
- Click on LPO Documents, select the Site Registration documents link, and download and complete the forms provided.

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Checking Your Site's Registration Status:

Check the status of your site's registration packets by querying the RSS site registration status page of the members' section of the CTSU website.

- Go to https://www.ctsu.org and log in to the members' area using your CTEP-IAM username and password
- Click on the Regulatory tab
- Click on the Site Registration tab
- Enter your 5-character CTEP Institution Code and click on Go

NOTE: The status given only reflects compliance with IRB documentation and institutional compliance with protocol-specific requirements outlined by the Lead Network. It does not reflect compliance with protocol requirements for individuals participating on the protocol or the enrolling investigator's status with the NCI or their affiliated networks.

The CTSU encourages you to go to the following CTSU RSS webpage so that more information on RSS2.0 as well as the submission forms can be accessed. Log in to http://www.ctsu.org and click on the Regulatory tab to access the RSS webpage. If you have questions regarding regulatory document submission, please telephone the CTSU Help Desk at 1-888-823-5923 or E-mail CTSUContact@westat.com.

Patient registration can occur only after pre-treatment evaluation is complete, eligibility criteria have been met, and the study site is listed as 'approved' in the CTSU RSS. Patients must have signed and dated all applicable consents and authorization forms.

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. To access OPEN, the site user must have an active CTEP-IAM account (check at

https://ctepcore.nci.nih.gov/iam) and a 'Registrar' role on either the LPO or participating organization roster. Registrars must hold a minimum of an AP registration type.

All site staff will use OPEN to enroll patients to this study. It is integrated with the CTSU Enterprise System for regulatory and roster data and, upon enrollment, initializes the patient in the Rave database. OPEN can be accessed at https://open.ctsu.org or from the OPEN tab on the CTSU members' side of the website at https://www.ctsu.org. To assign an IVR or NPIVR as the treating, crediting, consenting, drug shipment (IVR only), or investigator receiving a transfer in OPEN, the IVR or NPIVR must list on their Form FDA 1572 in RCR the IRB number used on the site's IRB approval.

Prior to accessing OPEN site staff should verify the following:

- All eligibility criteria have been met within the protocol stated timeframes. Site staff should use the registration forms provided on the group or CTSU web site as a tool to verify eligibility.
- 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. Please print this confirmation for your records.

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

4.1 Pre-Registration to First Biopsy (Step 0)

The following information will be requested:

4.1.1 Protocol Number

4.1.2 Investigator Identification

- Institution and affiliate name
- Investigator's name

4.1.3 Patient Identification

- Patient's initials (first and last)
- Patient's Hospital ID and/or Social Security number
- Patient demographics
  - Gender
  - Birth date (mm/yyyy)
  - Race
  - Ethnicity
  - Nine-digit ZIP code
  - Method of payment
  - Country of residence

4.1.4 Eligibility Verification

Patients must meet all of the eligibility requirements listed in Section 3.1

4.1.5 Additional Requirements

4.1.5.1 Sites must utilize the CIRB as their IRB of record to participate in EAY131.

4.1.5.2 Patients must provide a signed and dated, written informed consent form.

NOTE: Copies of the consent are not collected by the ECOG-ACRIN Operations Office-Boston.

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4.1.5.3 Archived specimens are to be submitted to the ECOG-ACRIN CBPF (MD Anderson – MATCH Laboratory) for central testing, per Section 7.2 and Section 9, as follows:

- It is requested that the samples be representative of what was submitted to the designated outside laboratory for assessment per Section 9.2.2.
- If the site receives notification that archived tissue is required for additional central testing (e.g. IHC) to determine patient eligibility for treatment, submit tissue using the kit for submission of confirmation tissue within 2 weeks. It is requested that adequate tissue for these additional central tests AND confirmation testing be submitted. Treatment assignment cannot be determined until this assessment is complete.
- For all other patients, tissue is to be submitted within 8 weeks after registration to Step 1, for confirmation of the “rare variant” by the MATCH assay. Tissue not submitted within 8 weeks following registration to Step 1 will be considered delinquent.
- Log sample in the ECOG-ACRIN Sample Tracking System (STS) and include STS shipment manifest in the package. LOGGING THE SUBMISSION IN STS IS MANDATORY, as STS is an integral aspect for the informatics system for this trial.

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- **Include paper copy of original diagnostic pathology report in submission.**

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- Include completed CLIA Laboratory Sample Submission Form. The contact information for the treating physician must include FAX number as the CLIA report will be distributed only to the designated physician. Information on the CLIA submission form must be clearly legible. Do not use cursive.
- Pathology reports submitted with specimens for confirmation testing are NOT to be redacted to ensure the appropriate materials have been submitted as the

results of the CLIA assessment will be returned to the site and will impact patient care.

- **A hard copy of the applicable sequencing report from the designated outside lab should be included inside the kit.**

NOTE: Notify the central MATCH laboratory (MD Anderson) of any delays pertaining to the submission of the pathology materials. In the event that archived tissue is not available, pathology reports and CLIA reports must still be submitted to the CBPF and nucleic acids from the designated outside laboratory may be requested for the confirmation assessments. The site will be informed of the receipt of nucleic acids by the ECOG-ACRIN CBPF, but results of the assays will not be returned to the site.

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4.1.5.4 For patients for whom an applicable "rare variant" was identified by one of the designated laboratories per Appendix XIV, following registration to Step 0:

NOTE: ALL OF THE FOLLOWING MUST BE UPLOADED FOR ACCURATE ASSIGNMENT.

NOTE: This will be the only method of study entry for patients registered to Step 0 after May 22nd, 2017 at 5:00 PM ET.

- Upload the following to the relevant folders in Medidata Rave:
  - An electronic copy of the applicable sequencing report with "EAY131" and patient case ID written on the form, and the following information **appropriately redacted** from every page of the report: **patient name** and **date of birth**. A copy of the full report must be uploaded. If the size of the report is restrictive, separate the report into two parts and upload each part separately.

    AND

  - Upload an electronic copies of the pathology reports associated with the analyzed tumor tissue and the original diagnostic pathology (if different).

    AND

  - Notification of potential MATCH eligibility from the outside laboratory (if separate from the sequencing report).

NOTE: Failure to upload the required outside assay and pathology reports will result in the inability to assign patient to treatment.

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4.2 Registration to First Treatment (Step 1)

Patient registration can occur only after pre-treatment evaluation is complete, eligibility criteria have been met, and the patient has provided signed and dated informed consent (pertaining to the selected targeted treatment agent(s)).

Patients must not start protocol treatment prior to registration to step 1.

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Treatment must start within seven days from registration to Step 1 and not before repeat pre-treatment imaging, if necessary, is completed. Study agent must be ordered after patient is registered to the treatment subprotocol as no starter supplies are available for this study. If expedited shipment is required, sites should provide an express courier account through the Online Agent Order Processing (OAOP) application.

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NOTE: If registration to Step 1 is not completed within 28 days of treatment assignment, the patient's slot will be released, and the patient will no longer be eligible for that treatment and will be taken off study.

The following information will be requested:

4.2.1 The information requested in Section 4.1.1-Section 4.1.3.

4.2.2 Eligibility Verification

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Patients must meet all of the eligibility requirements listed in Section 3.1 (excluding Section 3.1.6) and 3.2.

4.2.3 Classification Factors

4.2.3.1 Patient's simplified disease code

4.2.3.2 1 versus > 1 applicable targeted treatment

4.2.4 Additional Requirements

4.2.4.1 Sites must utilize the CIRB as their IRB of record to participate in EAY131.

4.2.4.2 Patients must provide a signed and dated, written informed consent form associated with the applicable targeted agent(s).

NOTE: Copies of the consent are not collected by the ECOG-ACRIN Operations Office-Boston.

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4.2.4.3 Specimens for research are to be submitted, per patient consent, as defined within the EAY131 Master Protocol or MATCH treatment subprotocol, if applicable. Please refer to Section **Error! Reference source not found.**.

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4.3 Registration to Second Screening (Step 2)

NOTE: PATIENTS ENTERING STEP 1 WITH A "RARE VARIANT" FROM AN "OUTSIDE" LAB ARE NOT ELIGIBLE FOR STEP 2. SEE SECTION 3.3.3.

4.3.1 The information requested in Section 4.1.1-Section 4.1.3.

4.3.2 Eligibility Verification

Patients must meet all of the eligibility requirements listed in Section 3.3

4.3.3 Additional Requirements

4.3.3.1 Sites must utilize the CIRB as their IRB of record to participate in EAY131.

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4.3.3.2 To proceed to Step 3, either of the following must be done:

- Tumor tissue from the re-biopsy is to be submitted as indicated in Section 9 for central assessment. See Section 4.1.5.3 in Appendix XV for submission requirements.

  NOTE: It may take up to 6 weeks for results to be returned to the site on a second biopsy and potential treatment assignments made available via Medidata Rave. Registration cannot proceed until the central determination is complete.

  OR

- Patient's prior Biopsy Analysis indicated >1 targeted therapies.

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4.3.3.3 Specimens for research are to be submitted, per patient consent, as defined within the EAY131 Master Protocol. Please refer to Section **Error! Reference source not found.**.

4.3.4 Classification Factors

Time from start of Step 1 treatment to toxicity or progression:

- < 6 months
- ≥ 6 months

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4.4 Registration to Second Treatment (Step 3)

Patient registration can occur only after the Step 2 evaluation is complete, eligibility criteria have been met, and the patients have signed and dated the relevant agent-specific informed consent document.

Patients must not start protocol treatment prior to registration to Step 3

Treatment must start within seven days from registration to Step 3 and not before repeat pre-treatment imaging, if necessary, is completed. Study agent must be ordered after patient is assigned to the treatment subprotocol as no starter supplies are available for this study. If expedited shipment is required, sites should provide an express courier account through the Online Agent Order Processing (OAOP) application.

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NOTE: If registration to Step 3 is not completed within 28 days of treatment assignment, the patient's slot will be released, and the patient will no longer be eligible for that treatment and will be taken off study.

The following information will be requested:

4.4.1 The information requested in Section 4.1.1-Section 4.1.3.

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4.4.2 Eligibility Verification

Patients must meet all of the eligibility requirements listed in Section 3.1 (excluding Section 3.1.6) and Section 3.4

4.4.3 Additional Requirements

4.4.3.1 Sites must utilize the CIRB as their IRB of record to participate in EAY131.

4.4.3.2 Patients must provide a signed and dated, written informed consent form for targeted treatments not previously consented for.

NOTE: Copies of the consent are not collected by the ECOG-ACRIN Operations Office-Boston.

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4.4.3.3 Specimens for research are to be submitted, per patient consent, as defined within the EAY131 Master Protocol or MATCH treatment subprotocol, if applicable. Please refer to Section **Error! Reference source not found.**.

4.5 Registration to Third Screening (Step 4)

4.5.1 The information requested in Section 4.1.1-Section 4.1.3.

4.5.2 Eligibility Verification

Patients must meet all of the eligibility requirements listed in Section 3.5.

4.5.3 Classification Factors

Time from start of Step 3 treatment to toxicity or progression:

- < 6 months
- ≥ 6 months

Number of screening biopsies

- 0
- 1

4.5.4 Additional Requirements

4.5.4.1 Sites must utilize the CIRB as their IRB of record to participate in EAY131.

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4.5.4.2 To proceed to Step 4, either of the following must be done:

- Tumor tissue from the second screening biopsy is to be submitted as indicated in Section 9 for central assessment. See Section 4.1.5.3 in Appendix XV for submission requirements.

NOTE: It may take up to 6 weeks for results to be returned to the site on a second biopsy and potential treatment assignments made

available via Medidata Rave. Registration cannot proceed until the central determination is complete.

OR

- Patient's prior second screening Biopsy Analysis indicated >1 targeted treatments.

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4.5.4.3 Specimens for research are to be submitted, per patient consent, as defined within the EAY131 Master Protocol. Please refer to Section **Error! Reference source not found.**.

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4.6 Registration to Third Treatment (Step 5)

Patient registration can occur only after the Step 4 evaluation is complete, eligibility criteria have been met, and the patients have signed and dated the relevant agent-specific informed consent document.

Patients must not start protocol treatment prior to registration to Step 5

Treatment must start within seven days from registration to Step 5 and not before repeat pre-treatment imaging, if necessary, is completed. Study agent must be ordered after patient is assigned to the treatment subprotocol as no starter supplies are available for this study. If expedited shipment is required, sites should provide an express courier account through the Online Agent Order Processing (OAOP) application.

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NOTE: If registration to Step 5 is not completed within 28 days of treatment assignment, the patient's slot will be released, and the patient will no longer be eligible for that treatment and will be taken off study.

The following information will be requested:

4.6.1 The information requested in Section 4.1.1-Section 4.1.3.

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4.6.2 Eligibility Verification

Patients must meet all of the eligibility requirements listed in Section 3.1 (excluding Section 3.1.6) and Section 3.6

4.6.3 Additional Requirements

4.6.3.1 Sites must utilize the CIRB as their IRB of record to participate in EAY131.

4.6.3.2 Patients must provide a signed and dated, written informed consent form for targeted treatments not previously consented for.

NOTE: Copies of the consent are not collected by the ECOG-ACRIN Operations Office-Boston.

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4.6.3.3 Specimens for research are to be submitted, per patient consent, as defined within the EAY131 Master Protocol or MATCH treatment subprotocol, if applicable. Please refer to Section **Error! Reference source not found.**.

4.7 Registration to Fourth Screening (Step 6)

4.7.1 The information requested in Section 4.1.1-Section 4.1.3.

4.7.2 Eligibility Verification

Patients must meet all of the eligibility requirements listed in Section 3.7.

4.7.3 Additional Requirements

4.7.3.1 Sites must utilize the CIRB as their IRB of record to participate in EAY131.

4.7.3.2 To proceed to Step 6, the patient's second screening Biopsy Analysis indicated >1 unaddressed targeted treatments.

Rev.5/16 **NOTE:** It may take up to 6 weeks for results to be returned and potential treatment assignments made available via Medidata Rave. Registration cannot proceed until the central determination is complete.

Rev. 2/16 4.7.4 Classification Factors

Time from start of Step 5 treatment to toxicity or progression:

- < 6 months
- ≥ 6 months

Rev. 8/15 4.8 Registration to Fourth Treatment (Step 7)

Patient registration can occur only after the Step 6 evaluation is complete, eligibility criteria have been met, and the patients have signed and dated the relevant agent-specific informed consent document.

Patients must not start protocol treatment prior to registration to Step 7

Treatment must start within seven days from registration to Step 7 and not before repeat pre-treatment imaging is completed, if necessary. Study agent must be ordered after patient is assigned to the treatment subprotocol as no starter supplies are available for this study. If expedited shipment is required, sites should provide an express courier account through the Online Agent Order Processing (OAOP) application.

Rev. 12/16 **NOTE:** If registration to Step 7 is not completed within 28 days of treatment assignment, the patient's slot will be released, and the patient will no longer be eligible for that treatment and will be taken off study.

The following information will be requested:

4.8.1 The information requested in Section 4.1.1-Section 4.1.3.

Rev. 2/16 4.8.2 Eligibility Verification

Patients must meet all of the eligibility requirements listed in Section 3.1 (excluding Section 3.1.6) and Section 3.8

4.8.3 Additional Requirements

4.8.3.1 Sites must utilize the CIRB as their IRB of record to participate in EAY131.

4.8.3.2 Patients must provide a signed and dated, written informed consent form for targeted treatments not previously consented for.

NOTE: Copies of the consent are not collected by the ECOG-ACRIN Operations Office-Boston.

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4.8.3.3 Specimens for research are to be submitted, per patient consent, as defined within the EAY131 Master Protocol or MATCH treatment subprotocol, if applicable. Please refer to Section **Error! Reference source not found.**.

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4.9 Registration to Research Biopsy AND Blood for Circulating Tumor DNA (Step 8)

NOTE: Patients who entered the study (Step 0) with a "rare variant" determined by an outside assay ARE potentially eligible and encouraged to participate in Step 8.

Patient registration can occur only after the eligibility criteria outlined in Section 3.9 have been met, and the patients have signed and dated the relevant Research Biopsy informed consent document.

The following information will be requested:

4.9.1 The information requested in Section 4.1.1-Section 4.1.3.

4.9.2 Eligibility Verification

Patients must meet all of the eligibility requirements listed in Section 3.9.

4.9.3 Additional Requirements

4.9.3.1 Sites must utilize the CIRB as their IRB of record to participate in EAY131.

4.9.3.2 Patients must provide a signed and dated, written informed consent form for the performance of a biopsy and submission of the tissue and blood for research.

NOTE: Copies of the consent are not collected by the ECOG-ACRIN Operations Office-Boston.

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4.9.3.3 Specimens for research are to be submitted (research biopsy and whole blood collection). If the patient does not agree to the additional research biopsy, **the site should still request the collection of the blood**. These specimens should be obtained PRIOR to the start of any future treatment. Please refer to Section **Error! Reference source not found.**.

4.10 Instructions for Patients who Do Not Start Assigned Protocol Treatment

If a patient is not assigned to a Step 1 treatment subprotocol, no follow-up data is collected.

If a patient registers to Step 1, but does not receive any assigned protocol treatment, baseline and follow-up data will still be collected and must be submitted through Medidata Rave according to the schedule in the MATCH Forms Completion Guidelines.

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4.11 Data collection

Data collection for this study will be done exclusively through Medidata Rave. Access to the trial in Rave is granted through the iMedidata application to all persons with the appropriate roles in RSS after IRB approval is obtained. To access iMedidata/Rave the site user must have an active CTEP IAM account (https://ctepcore.nci.nih.gov/iam). In addition, site users that are a member of ECOG-ACRIN must have the mapped ECOG-ACRIN roles or explicit Rave roles (Rave CRA, Read-Only, CRA, Lab Admin, SLA or Site Investigator) in RSS at the enrolling site. Site users that are not members of ECOG-ACRIN must have the Rave roles on the CTSU roster at the enrolling sites. The Site Administrator or Data Administrator at the enrolling site may assign the appropriate roles from the Site Roles tab on the CTSU website. To the hold Rave CRA role or CRA Lab Admin role, the user must hold a minimum of an AP registration type. To hold the Rave Site Investigator role, the individual must be registered as an NPIVR or IVR. Associates can hold read-only roles in Rave.

Upon initial site registration approval for the study in RSS, all persons with Rave roles assigned on the appropriate roster will be sent study invitation e-mail from iMedidata. To accept the invitation, site users must log into the Select Login (https://login.imedidata.com/selectlogin) using their CTEP-IAM user name and password, and click on the “accept” link in the upper right-corner of the iMedidata page. Please note, site users 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 link in the upper right pane of the iMedidata screen.

Users that have not previously activated their iMedidata/Rave accounts will also receive a separate invitation from iMedidata to activate their account. Account activation instructions are located on the CTSU website, Rave tab under the Rave resource materials (Medidata Account Activation and Study Invitation Acceptance). Additional information on iMedidata/Rave is available on the CTSU website under the Rave tab at http://www.ctsu.org/RAVE/ or by contacting the CTSU Help Desk at 1-888-823-5923 or by e-mail at ctsucontact@westat.com.

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4.12 Biopsy Reimbursements

Reimbursements are applicable to procedures performed to collect the samples for purposes of this trial only and thus are not billed to insurance. Acceptable procedures are those that are low risk, as detailed in Section 9, and do not include any open or laparoscopic surgery or endoscopic biopsies. Procedures which are performed as part of patient’s actual care and billed to insurance are not eligible to receive these reimbursements.

For patients registered as of May 11, 2017 with treatment assignment via the “Rare Variant” determined by a designated outside laboratory: Biopsy

reimbursement for an on-trial biopsy is applicable only for those collected following registration to Step 8 after completion or discontinuation of treatment on Step 1. There are no reimbursements available for biopsies performed for the purpose of testing by a designated outside laboratory.

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For patients registered prior to May 23, 2017 with tissue collected following Step 0 registration for screening assessments performed by the MATCH LABORATORIES: Tumor samples will be obtained for patients enrolled on the EAY131 study up to a maximum of 5 biopsies (including bone marrow aspirations for applicable multiple myeloma) as indicated below (2 maximum screening biopsies [each with 1 potential re-biopsy due to assay failure] and 1 potential end of treatment biopsy):

NOTE: The submission of tumor tissue for Step 0 Screening, collected prior to pre-registration (FFPE) or biopsy following pre-registration, is mandatory and all subsequent biopsies are part of the protocol, but performed based on study requirements and safety considerations.

Summary of biopsy reimbursements:

- Step 0 Screening: Biopsy to obtain tissue for the mandatory screening

  NOTE: If FFPE collected prior to pre-registration to Step 0 is used for the screening assessment, this reimbursement is not applicable.
- Step 0 Screening: Re-biopsy if the initial tumor tissue submission was found inadequate for the screening assessments
- Potential 2nd Screening Biopsy (Step 2 or 4, as applicable- see flow diagram): Biopsy to obtain tissue for screening for consideration of an additional treatment with different study drug
- 2nd Screening Biopsy: Re-biopsy if the 2nd Screening Biopsy was found inadequate for the screening assessments
- Potential End of treatment Biopsy (Step 8): Biopsy to obtain tissue for research studies. This biopsy may or may not be performed depending on the patient's status and continued agreement at the time all treatment has been completed on the MATCH study. Also, if a 2nd Screening Biopsy shows no study-actionable abnormalities, no additional end of treatment biopsy is needed.

Reimbursements for these research biopsies will be made according to current NCTN processes. Please see the EAY131 funding sheet on the CTSU web site for current reimbursement information.

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5. Treatment Plan

5.1 Study Design

After Step 0 registration, the "MATCH screening assessment" will differ depending on the method of study entry. For patients that previously entered the study via the original screening process (i.e. to submit tissue for testing by a MATCH laboratory), this assessment will consist of both molecular profiling by the MATCH assay and the subsequent process of assigning treatment. Please note, after 5/22/17, new patients may no longer be enrolled on the study through this process. For patients entering the study through testing performed by one of the designated labs outside of the MATCH laboratory group (i.e. the Outside Assay process; see Appendix XIV), the assessment will only consist of the study's determination of treatment assignment. This is the only method of study entry moving forward.

Determination of aMOIs by the MATCH assay:

A pre-treatment tumor biopsy and blood draw will be obtained from patients who sign the informed consent document to enroll in the study. The results of the evaluation of the biopsy specimens will determine if the patient's tumor has an aMOI (actionable mutation of interest) for which a MATCH treatment subprotocol is available. Additionally, subsequent tumor biopsies may be taken if the patient has response and/or stable disease for more than 6 months or at the time of disease progression and the patient wishes to be re-evaluated for additional molecular variants. Information about the gene mutations/alterations in pathways of interest is included in Appendix VII.

No more than ONE mandatory biopsy and four conditional tumor biopsies will be obtained through Interventional Radiology by a percutaneous (bone marrow aspirate for multiple myeloma, radiology services not required) approach (see Section 9):

- Step 0 Screening: Biopsy to obtain tissue for the mandatory screening OR submission of FFPE (metastatic tissue preferred) from a prior procedure. If previously collected tissue is submitted, the tissue must meet the criteria specified in Section 9 and Section 3.1.6

  NOTE: For patients for whom an applicable "rare variant" was identified by one of the designated outside laboratories (See Appendix XIV), archived tumor tissue is to be submitted within 8 weeks following registration to Step 1 for assessment by the MATCH assay to confirm the "rare variant." Please refer to Section 4.1.5 for additional information and exceptions.
- Step 0 Screening: Re-biopsy if the initial tissue submission was found inadequate for the screening assessments
- 2nd Screening Biopsy (Step 2 or 4, as applicable- see flow diagram): Biopsy to obtain tissue for screening for consideration of additional rounds of treatment with different study drug
- 2nd Screening Biopsy: Re-biopsy if the 2nd Screening Biopsy was found inadequate for the screening assessments

- End of treatment: Biopsy to obtain tissue for research studies per patient consent. If a 2nd Screening Biopsy shows no study-actionable abnormalities, no additional End of treatment biopsy is requested.

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Percutaneous biopsies for accrual on the trial will be performed on patients with solid tumors or lymphomas or myeloma plasmacytomas. Bone marrow aspirates are to be used for obtaining tumor cells in patients with other myelomas. Permitted Biopsy procedures should entail no more than minimal risk and are detailed in Section 9.

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Tissue specimens will be sent for analyses as defined in Section 9. Blood samples, per patient consent, will also be collected and submitted for research with the biopsy samples and either during or at the end of each MATCH treatment.

If eligible, a patient may undergo up to two rounds of biopsy with up to two different MATCH treatments following each biopsy if additional variants of interest eligible for treatment on MATCH exist. Patient consent will occur in sequential steps.

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NOTE: Patients deemed ineligible for Step 1 treatment or who did not receive a treatment assignment due to a lack of available assignment slots may be eligible to have their MATCH assay screening results rerun through MATCHbox. Sites will be notified via e-mail if their patient is eligible to be rerun through the MATCHbox. Upon receipt of this notification, the site will be required to complete the appropriate form in Medidata Rave. This form may be completed within 6 months from the collection of specimen. If not completed by this date, the patient will not be reconsidered for treatment. Please refer to Section 3.2 to review the eligibility criteria.

Determination of 'Rare Variant" aMOIs by a designated Outside Laboratory:

For patients for whom an applicable "rare variant" was identified by one of the designated laboratories (see Appendix XIV), notification of potential MATCH eligibility from the outside laboratory and an electronic copy of the applicable sequencing report is to be uploaded to the *Outside Assay* folder in Medidata Rave. A copy of the pathology reports associated with the analyzed tumor tissue and the original diagnostic pathology (if different) are also to be uploaded in Medidata Rave.

Patients who enter screening for determination of treatment assignment based on an outside assay result are eligible for only one treatment assignment on MATCH. If the patient is determined not to be eligible for a subprotocol open to outside assay "rare variant" results, the patient is off-study.

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Screening (Step 0 procedures):

- Patients consent to the screening aspects of the study – biopsy and molecular characterization of their biopsy material and collection and submission of blood for research.

  NOTE: Step 0 screening consent includes consent for all screening steps (Steps 0, 2, 4, and 6). If treatment is to be determined based on a "rare variant" detected by an Outside Laboratory, a tissue biopsy

to collect screening materials will not be applicable. However, collection of blood is encouraged (see Sections 7.2 and 9).

- Patients are registered to Step 0 in the OPEN Registration system. This "pre-registration" step will capture information relevant to potential treatment assignment (TA), including but not limited to: site of disease and baseline eligibility characteristics.
- Patients will undergo the mandatory tumor biopsy (or bone marrow aspirate for myeloma patients, if applicable) OR have FFPE tumor tissue (metastatic tissue preferred) available for analysis following a clinically indicated diagnostic or therapeutic procedure (e.g. craniotomy and tumor resection for brain tumors, lung wedge resection, endoscopic procedure) as described in Section 9 OR outside "rare variant" sequencing results will be uploaded to RAVE.

NOTE: For patients for whom an applicable "rare variant" was identified by one of the *outside* designated laboratories as described in Appendix XIV, FFPE tumor tissue may be required to be submitted to MD Anderson for additional central assessments (e.g. IHC) prior to final treatment arm assignment based on the Outside Laboratory results. The collection and submission of archived tumor tissue for the confirmation of the "rare variant" must follow the guidelines for the submission of tissue for screening at Step 0 with the following changes:

- FFPE tumor tissue may be submitted after but no later than 8 weeks following registration to Step 1
- FFPE tissue should be representative of the materials used by the designated outside laboratory and may have been collected more than 6 months prior to registration to Step 0. Patients may have had treatment between the collection of the biopsy and registration to Step 0.
- Treatment arm assignment will be based on the *Outside Laboratory* results. The MATCH assay assessments will be reported to the site but will not, per protocol, impact patient's eligibility for or continued treatment on the relevant Subprotocol on Step 1. However, for certain "rare variant" arms, submission of FFPE tumor tissue may be required for additional central assessments (e.g. IHC) prior to final treatment arm assignment based on the Outside Laboratory results.
- Patients are potentially eligible for only one treatment assignment. Patients not assigned to treatment are off study. Patients not assigned to treatment are not eligible to undergo a biopsy for screening purposes.

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- The biopsy/tumor sample, blood, and fine needle aspiration specimens in the collection kit are submitted to the ECOG-ACRIN Central Biorepository and Pathology Facility in the Division of Pathology and Laboratory Medicine at the University of Texas MD Anderson Cancer Center for pre-analytic processing and quality control.

NOTE: Previous clinical pathology report MUST be submitted with the specimen, or the sample will not be processed.

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- Immunohistochemistry analytes, if applicable, are evaluated in the CLIA-accredited clinical Immunohistochemistry and Image Analysis Laboratories in the Department of Pathology at MD Anderson.
- The nucleic acid analytes will be forwarded to one of the four CLIA-certified laboratories in the study-specific network for molecular profiling to assess for the presence of specific, pre-defined mutations, amplifications or translocations of interest.
- The laboratories will return notification of whether or not a biomarker for patient assignment to one of the clinical trial subprotocols has been detected to the accruing site, to the ECOG-ACRIN Operations Office-Boston, and to the NCI-EA informatics pipeline in approximately 2-4 weeks.

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NOTE: For patients pre-registered to the trial based on a "rare variant" referral from a designated Outside Laboratory,it is estimated that the treatment assignment would be available in 2 weeks or less.

- The automated rules engine will generate a list of potential treatment assignment (TA).
- Prioritized TAs will be sent to the ECOG-ACRIN Operations Office. The highest priority **TA will then be made available to the registering site via Medidata Rave.**
- Patient is assessed for eligibility and consents to treatment with an agent in the trial subprotocol.

Rev. Add24

- If the patient is ineligible for highest priority TA, the treatment assignment process may be repeated, in order of priority, until either all TAs are exhausted or the patient is confirmed eligible and is registered to a treatment subprotocol.
- In cases where insufficient tumor is obtained for biomarker analysis, the patient will be given the option to undergo a repeat PERCUTANEOUS or excisional qualifying biopsy of tumor to obtain more tissue unless the primary tumor tissue was obtained secondary to a clinically indicated procedure. Patients in whom sufficient tumor DNA material cannot be obtained for targeted analysis will be taken off-study.

Register to First Treatment

Treat until patient experiences either progression OR unacceptable toxicity OR voluntary discontinuation of treatment.

The following options are available at treatment failure:

- Discontinue treatment and enter follow-up,
- Register to Step 2 for re-screening and the opportunity to receive a different targeted study agent.

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NOTE: From consenting patients, research blood samples are to be collected and submitted after cycle 2 or end of treatment, whichever occurs first (and before any subsequent investigational or other systemic treatment, such as the next cycle of treatment). Please refer to Section **Error! Reference source not found.**.

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Register to Subsequent Screening (Steps 2, 4, 6)

- Patients with Step 1 treatments assigned based on "rare variants" identified by designated outside laboratories are not eligible to proceed to Step 2.
- Step 2 or 4: If progression (or inability to tolerate further treatment) occurred after a (1) response **OR** (2) after ≥ 6 months from start of Step 1 treatment, patients will undergo a second tumor biopsy using the collection and shipping kit, as described in Section 9.1. Patients with tumor in non-minimal risk locations who were initially screened using tumor tissue obtained during a necessary clinical procedure (surgery, endoscopy) would not undergo a biopsy or second procedure unless clinically indicated or a new tumor site in a location of minimal risk develops.

  The biopsy, blood, and optional fine needle aspiration specimens are forwarded in the shipping kit to the ECOG-ACRIN Central Biorepository and Pathology Facility at MD Anderson for processing (protocol Section **Error! Reference source not found.**). Analytes will be prepared and distributed to the study-specific network of approved CLIA-certified Laboratories for molecular profiling to assess for the presence of specific, pre-defined mutations, amplifications, translocations, and altered expression of genes of interest and notification of results done as in Step 0.

  NOTE: Submission of prior pathology reports with the screening samples are required, as they were in Step 0.

  The laboratory will return notification of whether an alteration of interest has been detected to the site and the ECOG-ACRIN Operations Office-Boston. Details about potential treatment subprotocol, assigned based on predefined criteria, will be made available via Medidata Rave.

  OR

- Step 2, 4 or 6: Patients who progressed OR had unacceptable toxicity OR voluntarily discontinued treatment within 6 months AND who had additional actionable mutations, amplifications or translocations, as determined by the MATCH assay for which a treatment subprotocol is currently available will not undergo an additional biopsy, but may be offered additional TAs as below based on their Step 0 (or Step 2/4) results.

  NOTE: A single MATCH assay screening may be used to assign no more than two consecutive treatments regardless of the number of aMOIs detected. Further participation/treatment assignment requires submission and analysis of fresh tumor tissue. See Study Schema.

Treatment Consent (prior to registration to Steps 3, 5, 7):

Treatment assignment will occur in the same manner as that for the first round (Prior to Step 1)

If patients experiences stable disease, complete or partial response, he or she will continue on study until disease progression or inability to tolerate treatment, at which time s/he will proceed to the next registration step or long term follow-up per Section 3.

Rev. 3/17

NOTE: From consenting patients, research blood samples are to be collected and submitted after cycle 2 or end of treatment, whichever occurs first (and before any subsequent investigational or other systemic treatment, such as the next cycle of treatment). Please refer to Section **Error! Reference source not found.**.

Consent for Collection of End of Treatment Research Tumor and Blood Specimens (prior to registration to Step 8):

If patients meet the criteria outlined in Section 3.9 and consent is obtained, proceed to Step 8 registration and submit samples as outlined in Section 9.

5.1.1 Specimen qualification

For a tumor biopsy sample to be considered adequate for sequencing a minimum of 70% of the biopsy should have non-necrotic tumor content; biopsy samples with lower tumor content will undergo macrodissection to enrich for non-necrotic tumor cells. In cases where sufficient tumor DNA is not obtained for analysis in the initial screening biopsy, the patient will be given the option to undergo a repeat tumor biopsy to obtain more tissue. Patients in whom sufficient tumor DNA material cannot be obtained for targeted analysis will be taken off-study. However, the assays will be attempted on biopsies that have at least 20% non-necrotic tumor cells present.

5.1.2 Immunohistochemistry alterations

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Changes in gene product expression in tumor cells will be reported as required by the individual analytes based upon evidence of association with agents of interest. These reports will be sent to the site separately from the sequencing results.

5.1.3 Detection of mutations, amplifications or translocations

Nucleic acid alterations will be reported based upon their characteristics. Mutations will be reported based on their presence in tumor cells. For single nucleotide variants (SNV)/insertions and deletions (indels), the report will include percent of reads demonstrating the predefined mutation compared to reference sequence. Copy number variations will be reported as level of fold change. Re-arrangements will be reported based upon genes involved. If mutations/alterations are detected in more than one pathway, treatment will be assigned based on levels of evidence (Section 1.4.2). If the subprotocols have equal number of patients, patients will be randomized between those subprotocols. If the patient stops treatment, and the patient still meets eligibility criteria, the patient will have the option to receive the other treatment regimen. For the purposes of assessing the primary endpoint of this trial, response to any assigned treatment will be used.

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5.1.4 Treatment Assignment

If mutations are detected in more than one pathway, treatment will be assigned based on levels of evidence as detailed above (Section 1.4.2).

NOTE: Each treatment subprotocol will attempt to recruit only a limited number of patients from more common disease sites (e.g., lung, breast, prostate, colorectal). If the disease site cohort has been completed, either (1) the patient will be assigned the treatment based on another actionable mutation (if any), or (2) if the patient is only eligible for a single targeted agent, the patient will not be assigned to treatment and will not be followed. A completed subprotocol may be expanded to permit accrual of sufficient patients in a disease of interest (e.g., colorectal cancer) to permit a more accurate assessment of activity in the disease. Please refer to Section 8.1 for additional information concerning the treatment expansions.

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5.2 Administration Schedule Summary

All Administration instructions are contained in Section 3 of the corresponding treatment-specific subprotocol document.

5.3 Adverse Event Reporting Requirements

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The guidelines outlined here for EAY131 Adverse Event (AE) Reporting apply to all EAY131 subprotocols. However, each subprotocol contains its own AE Reporting section with agent specific stipulations and must be referenced to ensure all reporting requirements are met.

NOTE: Starting April 1, 2018, CTEP-AERS reporting will use CTCAE v5. Please refer to each subprotocol to verify which version of the CTCAE should be used for dose modifications. Please refer to the instructions on the Adverse Event Case Report Form in Rave for instructions on which version of the CTCAE must be used for routine reporting.

5.3.1 Purpose

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Adverse event (AE) data collection and reporting, which are a required part of every clinical trial, are done so investigators and regulatory agencies can detect and analyze adverse events and risk situations to ensure the safety of the patients enrolled, as well as those who will enroll in future studies using similar agents.

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- **Routine reporting:** Adverse events are reported in a routine manner at scheduled times during a trial using the Medidata Rave clinical data management system. Please refer to Section 4 of this protocol for more information on how to access the Medidata Rave system and the subprotocol forms packet for instructions on where, when and what adverse events are to be reported routinely.
- **Expedited reporting**: In addition to routine reporting, certain adverse events must be reported in an expedited manner for timelier monitoring of patient safety and care. The following sections provide information and instructions regarding expedited adverse event reporting.

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5.3.2 Terminology

- **Adverse Event (AE):** Any untoward medical occurrence associated with the use of an agent or intervention (biopsy) in humans, whether or not considered agent or intervention (biopsy) related. Therefore, an AE can be **ANY** unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.
- **Attribution:** An assessment of the relationship between the adverse event and the protocol treatment, using the following categories.

ATTRIBUTION	DESCRIPTION
Unrelated	The AE is *clearly NOT related* to treatment.
Unlikely	The AE is *doubtfully related* to treatment.
Possible	The AE *may be related* to treatment.
Probable	The AE is *likely related* to treatment.
Definite	The AE is *clearly related* to treatment.

- **CAEPR (Comprehensive Adverse Events and Potential Risks List):** An NCI generated list of reported and/or potential AEs associated with an agent currently under an NCI IND. Information contained in the CAEPR is compiled from the Investigator's Brochure, the Package Insert, as well as company safety reports.
- **CTCAE:** The NCI Common Terminology Criteria for Adverse Events provides a descriptive terminology that is to be utilized for AE reporting. A grade (severity) is provided for each AE term.
- **Hospitalization (or prolongation of hospitalization):** For AE reporting purposes, a hospitalization is defined as an inpatient hospital stay equal to or greater than 24 hours.
- **Life Threatening Adverse Event:** Any AE that places the subject at immediate risk of death from the AE as it occurred.
- **Serious Adverse Event (SAE):** Any adverse event occurring at any dose that results in **ANY** of the following outcomes:
  - Death
  - A life-threatening adverse event
  - Inpatient hospitalization or prolongation of existing hospitalization (for ≥ 24 hours).
  - A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
  - A congenital anomaly/birth defect.
  - Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon medical judgment, they may jeopardize the patient or subject and may require medical

or surgical intervention to prevent one of the outcomes listed in this definition.

- **SPEER (Specific Protocol Exceptions to Expedited Reporting):** A subset of AEs within the CAEPR that contains list of events that are protocol specific exceptions to expedited reporting. If an AE meets the reporting requirements of the protocol, and it is listed on the SPEER, it should ***ONLY*** be reported via CTEP-AERS if the grade being reported exceeds the grade listed in the parentheses next to the event.

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5.3.3 Reporting Procedure

This study requires that expedited adverse event reporting use CTEP's Adverse Event Reporting System (CTEP-AERS). The CTEP's guidelines for CTEP-AERS can be found at http://ctep.cancer.gov. A CTEP-AERS report must be submitted electronically to ECOG-ACRIN and the appropriate regulatory agencies via the CTEP-AERS Web-based application located at http://ctep.cancer.gov.

In the rare event when Internet connectivity is disrupted a 24-hour notification is to be made by telephone to

- the AE Team at ECOG-ACRIN (857-504-2900)
- the NCI (301-897-7497)

An electronic report MUST be submitted immediately upon re-establishment of internet connection.

Supporting and follow up data: Any supporting or follow up documentation must be uploaded to the Supplemental Data Folder in Medidata Rave within 48-72 hours. In addition, supporting or follow up documentation must be faxed to the NCI (301-897-7404).

NCI Technical Help Desk: For any technical questions or system problems regarding the use of the CTEP-AERS application, please contact the NCI Technical Help Desk at ncictephelp@ctep.nci.nih.gov or by phone at 1-888-283-7457

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5.3.4 Determination of Reporting Requirements

Many factors determine the reporting requirements of each individual protocol, and which events are reportable in an expeditious manner, including:

- the phase (0, 1, 2, or 3) of the trial
- whether the patient has received an investigational or commercial agent or both
- the seriousness of the event
- the Common Terminology Criteria for Adverse Events (CTCAE) grade
- whether or not hospitalization or prolongation of hospitalization was associated with the event

- when the adverse event occurred (within 30 days of the last administration of investigational agent vs. > 30 days after the last administration of investigational agent)
- the relationship to the study treatment (attribution)

Using these factors, please follow the instructions and use the tables in the following section, as well as refer to the AE section in each subprotocol, to comply with the adverse event reporting requirements for study EAY131.

5.3.5 Steps to determine if an adverse event is to be reported in an expedited manner

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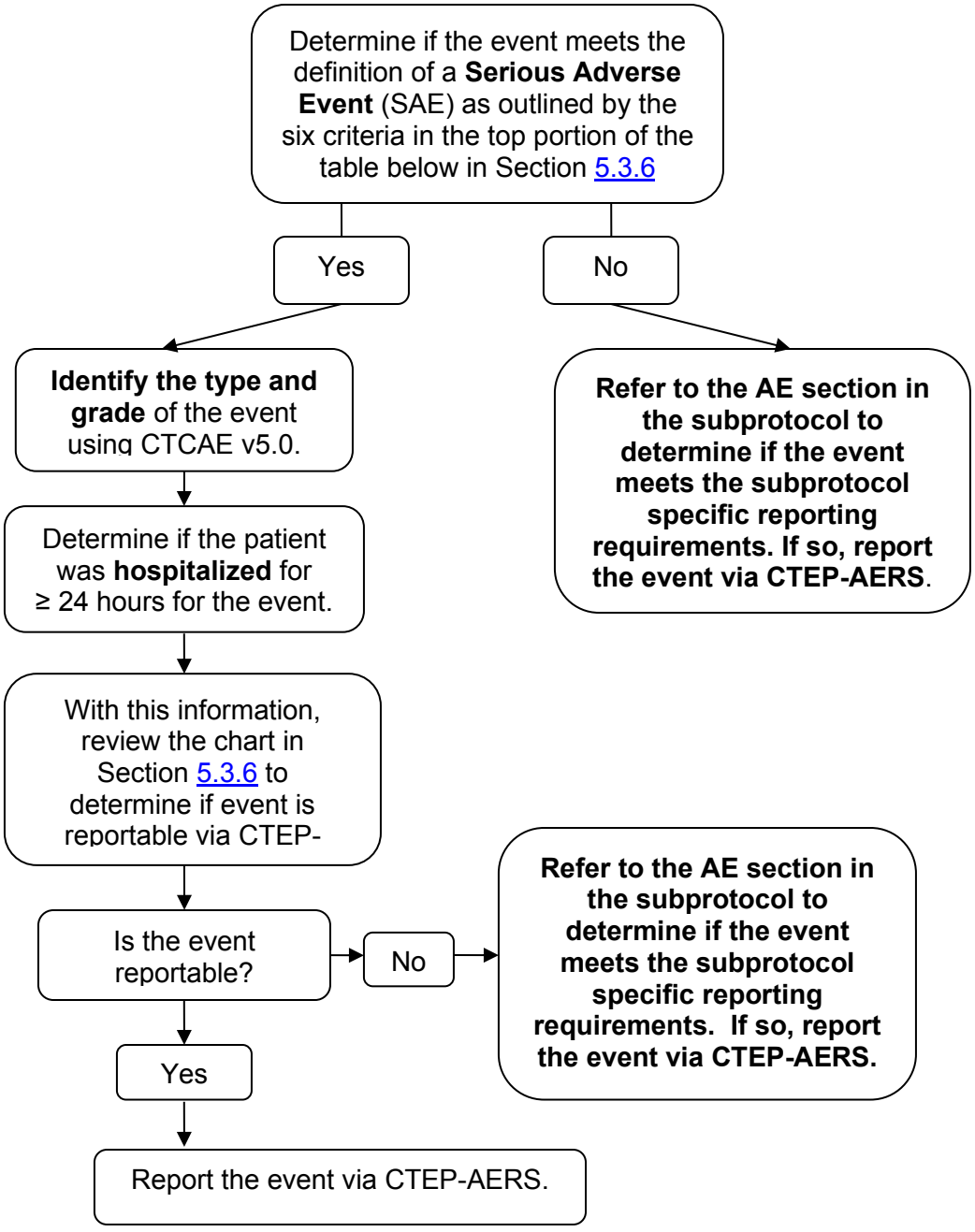
5.3.5.1 Guidelines for reporting adverse events **OCCURRING WHILE ON PROTOCOL TREATMENT AND WITHIN 30 DAYS** of the last administration of the investigational agent(s) /intervention (biopsy).



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5.3.5.2 Guidelines for reporting adverse events **OCCURRING GREATER THAN 30 DAYS** after the last administration of the investigational agent(s) /intervention (biopsy).

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If the adverse event meets the definition of a **Serious Adverse Event (SAE)** as outlined by the six criteria in the top portion of the table below in Section 5.3.6 OR any agent specific requirements (refer to specific stipulations in each subprotocol) AND has an attribution of possible, probably or definite, the following events require reporting as follows:

Expedited 24-hour notification followed by complete report within 5 calendar days for:

- All Grade 3, 4 and Grade 5 AEs

NOTE: Any death occurring greater than 30 days after the last dose of investigational agent/intervention (biopsy) with an attribution of possible, probable or definite must be reported via CTEP-AERS even if the patient is off study.

Expedited 10 calendar day reports for:

- Grade 2 adverse events resulting in hospitalization or prolongation of hospitalization

5.3.6 Expedited Reporting Requirements for protocol EAY131

Phase 1 and Early Phase 2 Studies

Expedited Reporting Requirements for Adverse Events that Occur on Studies under an IND *within 30 Days of the Last Administration of the Investigational Agent/Intervention.*[1]

NOTE: Footnote 1 instructs how to report serious adverse events that occur more than 30 days after the last administration of investigational agent/intervention.

Rev. 8/15

NOTE: The following reporting requirements apply to all steps (both treatment steps and biopsy (screening) steps). If an event occurs that meets the reporting requirements below, it is a reportable event, regardless of the step.

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NOTE: In order to complete the CTEP-AERS reporting properly, if the event being reported is related to a biopsy, please be sure to add 'Screening Step Biopsy' to the 'Other Contributing Causes' section of the CTEP-AERS report and add any other pertinent information to the 'Description of Events' section.

Rev. 2/16

NOTE: For SAEs reported on a screening step (Step 0, 2, 4, 6) please always indicate if the event being reported is biopsy related or not

Rev. 12/16

NOTE: If a patient is registered to Step 0 and has a biopsy performed, the patient must be followed for 30 days from

biopsy for possible SAEs, even if they are not subsequently assigned to a MATCH treatment.

FDA REPORTING REQUIREMENTS FOR SERIOUS ADVERSE EVENTS (21 CFR Part 312)

NOTE: Investigators **MUST** immediately report to the sponsor (NCI) **ANY** Serious Adverse Events, whether or not they are considered related to the investigational agent(s)/intervention (21 CFR 312.64)

An adverse event is considered serious if it results in **ANY** of the following outcomes:

1. Death
2. A life-threatening adverse event
3. An adverse event that results in inpatient hospitalization or prolongation of existing hospitalization for ≥ 24 hours
4. A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
5. A congenital anomaly/birth defect.
6. Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. (FDA, 21 CFR 312.32; ICH E2A and ICH E6).

ALL SERIOUS adverse events that meet the above criteria MUST be immediately reported to the NCI via CTEP-AERS within the timeframes detailed in the table below.

Hospitalization	Grade 1 and Grade 2 Timeframes	Grade 3-5 Timeframes
Resulting in Hospitalization ≥ 24 hrs	10 Calendar Days	24-Hour 5 Calendar Days
Not resulting in Hospitalization ≥ 24 hrs	Not required	

NOTE: Protocol-specific exceptions to expedited reporting of serious adverse events are found in the Specific Protocol Exceptions to Expedited Reporting (SPEER) portion of the CAEPR.

Expedited AE reporting timelines are defined as:

- "24-Hour; 5 Calendar Days" – The AE must initially be reported via CTEP-AERS within 24 hours of learning of the AE, followed by a complete expedited report within 5 calendar days of the initial 24-hour report.
- "10 Calendar Days" – A complete expedited report on the AE must be submitted within 10 calendar days of learning of the AE.

[1] Serious adverse events that occur more than 30 days after the last administration of investigational agent/intervention and have an attribution of possible, probable, or definite require reporting as follows:

Expedited 24-hour notification followed by complete report within 5 calendar days for:

- All Grade 3, 4, and Grade 5 AEs

Expedited 10 calendar day reports for:

- Grade 2 AEs resulting in hospitalization or prolongation of hospitalization

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5.3.7 Additional adverse event reporting instructions, requirements and instructions for protocol EAY131

Reporting a death on study: A death occurring while on study or within 30 days of the last dose of treatment requires both routine and expedited reporting, regardless of causality. Attribution to treatment or other cause must be provided.

NOTE: A death due to progressive disease should be reported as a Grade 5 "Disease progression" under the System Organ Class (SOC) "General disorder and administration site conditions". Evidence that the death was a manifestation of underlying disease (e.g. radiological changes suggesting tumor growth or progression: clinical deterioration associated with a disease process) should be submitted.

Additional adverse event reporting instructions are contained in the treatment-specific subprotocol document. Each subprotocol must be referenced to ensure all reporting requirements are met.

5.3.8 Other recipients of adverse event reports and supplemental data

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DCTD/NCI will notify the pharmaceutical collaborator(s)of AE/SAEs in accordance with the contract with the company. Any additional written AE information requested MUST be submitted to BOTH the NCI and ECOG-ACRIN.

Adverse events determined to be reportable via CTEP-AERS must also be reported by the institution, according to the local policy and procedures, to the Institutional Review Board responsible for oversight of the patient.

5.3.9 Second Primary Cancer Reporting Requirements

NOTE: The following are the standard requirements and instructions for reporting second primary cancers. However, some subprotocols have additional second primary reporting requirements and it is imperative the AE section of each subprotocol be carefully read to ensure all requirements are being met.

All cases of second primary cancers, including acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS), that occur following treatment on NCI-sponsored trials must be reported to ECOG-ACRIN using Medidata Rave

- **A <u>second malignancy</u> is a cancer that is UNRELATED to any prior anti-cancer treatment (including the treatment on this protocol). Second malignancies require ONLY routine reporting as follows:**
  1. Complete a Second Primary Form in Medidata Rave within 14 days.
  2. Upload a copy of the pathology report to ECOG-ACRIN via Medidata Rave confirming the diagnosis.
  3. If the patient has been diagnosed with AML/MDS, upload a copy of the cytogenetics report (if available) to ECOG-ACRIN via Medidata Rave.
- **A <u>secondary malignancy</u> is a cancer CAUSED BY any prior anti-cancer treatment (including the treatment on this protocol). Secondary malignancies require both routine and expedited reporting as follows:**

1. Complete a Second Primary Form in Medidata Rave within 14 days
2. Report the diagnosis via CTEP-AERS at http://ctep.cancer.gov

   Report under a.) leukemia secondary to oncology chemotherapy, b.) myelodysplastic syndrome, or c.) treatment related secondary malignancy
3. Upload a copy of the pathology report to ECOG-ACRIN via Medidata Rave and submit a copy to NCI/CTEP confirming the diagnosis.
4. If the patient has been diagnosed with AML/MDS, upload a copy of the cytogenetics report (if available) to ECOG-ACRIN via Medidata Rave and submit a copy to NCI/CTEP.

NOTE: The Second Primary Form and the CTEP-AERS report should not be used to report recurrence or development of metastatic disease.

NOTE: If a patient has been enrolled in more than one NCI-sponsored study, the Second Primary Form must be submitted for the most recent trial. ECOG-ACRIN must be provided with a copy of the form and the associated pathology report and cytogenetics report (if available) even if ECOG-ACRIN was not the patient's most recent trial.

NOTE: Once data regarding survival and remission status are no longer required by the protocol, no follow-up data should be submitted via CTEP-AERS or by the Second Primary Form.

5.4 Dose Modifications

Please refer to treatment-specific subprotocol document.

5.5 Duration of Therapy

Patients who were pre-registered (Step 0) only are not considered to be on-study and will not be followed.

For all other patients who begin a treatment and progress, patients will be offered a biopsy unless:

- Patients whose disease progresses (without objective response) within 6 months of entry will be offered the opportunity to go through two different treatment regimens, if additional actionable variants were found.
- Patients who respond to initial assigned treatment or progress after 6 months on study will be offered a 2nd biopsy and molecular profiling assay(s) and may be assigned new treatments if additional actionable molecular abnormalities are found.
- All treatment regimens for the patient's actionable molecular variants of interest have been exhausted (see Section 5.1)
- Intercurrent illness prevents further administration of treatment or safe biopsies.

5.6 Duration of Follow-Up

For this protocol**,** all patients who enroll on a treatment subprotocol, including those who discontinue protocol therapy early, will be followed for response until progression, even if non-protocol therapy is initiated, and for survival for 3 years from the date of registration (every 3 months for ≤ 2 years and every 6 months for year 3). All patients must be followed through completion of all MATCH protocol therapy.

6. Measurement of Effect

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6.1 Solid Tumor Response

For the purposes of this study, patients should be re-evaluated for response:

- For treatments given in 21 day (3 week) cycles: every 3 cycles (9 weeks) for the first 33 cycles, and every 4 cycles thereafter (12 weeks)
- For treatments given in 28 day (4 week) cycles: every 2 cycles (8 weeks) for the first 26 cycles, and every three cycles thereafter (12 weeks)
- For treatments given in 42 day (6 week) cycles: every 2 cycles (12 weeks)

Solid Tumors: Response and progression will be evaluated in this study using the international criteria proposed by the revised Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1) [Eur J Ca 45:228-247, 2009]. Changes in the largest diameter (unidimensional measurement) of the tumor lesions and the shortest diameter in the case of malignant lymph nodes are used in RECIST.

The following general principles must be followed:

1. To assess objective response, it is necessary to estimate the overall tumor burden at baseline of each treatment step to which subsequent measurements will be compared. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than six weeks before registration to each treatment step.
2. Measurable disease is defined by the presence of at least one measurable lesion.
3. All measurements should be recorded in metric notation by use of a ruler or calipers.
4. The same method of assessment and the same technique must be used to characterize each identified lesion at baseline and during follow-up.

6.1.1 Definitions

<u>Evaluable for Objective Response</u>

Only those patients who have measurable disease present at the baseline of each treatment step, have received protocol therapy, and have had their disease re-evaluated will be considered evaluable for response. These patients will have their response classified according to the definitions stated below.

(**NOTE:** Patients who exhibit objective disease progression prior to the end of cycle 1 will also be considered evaluable.)

<u>Evaluable Non-Target Disease Response</u>

Patients who, in addition to measurable disease, have lesions present at the baseline of each treatment step that are evaluable but do not meet the definitions of measurable disease, have received protocol therapy, and have had their disease re-evaluable will be considered evaluable for non-target lesion assessment. The response assessment is based on the presence, absence, or unequivocal progression of the lesions.

6.1.2 Disease Parameters

Measurable Disease

Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as ≥ 20 mm by chest x-ray, as

≥ 10 mm with CT scan, or ≥ 10 mm with calipers by clinical exam. All tumor measurements must be recorded in millimeters.

NOTE: Measurable or non-measurable disease must be present outside the previous radiation field or a new or progressing lesion inside the port must be present.

Malignant Lymph Nodes

To be considered pathologically enlarged and measurable, a lymph node must be ≥ 15 mm in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm). At baseline and in follow-up, only the short axis will be measured and followed.

Non-measurable Disease

All other lesions (or sites of disease), including small lesions (longest diameter < 10 mm or pathological lymph nodes with ≥ 10 to < 15 mm short axis), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonitis, inflammatory breast disease, and abdominal masses (not followed by CT or MRI), are considered as non-measurable. Non-measurable also includes lesions that are < 20 mm by chest x-ray.

NOTE: Cystic lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable nor non-measurable) since they are, by definition, simple cysts.

'Cystic lesions' thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above. However, if non-cystic lesions are present in the same patient, these are preferred for selection as target lesions.

Target Lesions

All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total, representative of all involved organs, should be identified as target lesions and recorded and measured at baseline of each treatment step. Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, but in addition should be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement in which circumstance the next largest lesion which can be measured reproducibly should be selected.

A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum diameters. If lymph nodes are to be included in the sum, then only the short axis is added into the sum. The baseline sum of the diameters will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease.

Non-target Lesions

All other lesions (or sites of disease) including any measurable lesions over and above the 5 target lesions should be identified as non-target lesions and should also be recorded at baseline of each treatment step. Measurements of these lesions are not required, but the presence or absence of unequivocal progression of each should be noted throughout follow-up.

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6.1.3 Methods for Evaluation of Disease

All measurements should be taken and recorded in metric notation using a ruler or calipers. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than six weeks before registration to each treatment step.

The same method of assessment and the same technique must be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam.

Clinical Lesions

Clinical lesions will only be considered measurable when they are superficial (e.g., skin nodules and palpable lymph nodes) and ≥ 10 mm in diameter as assessed using calipers (e.g., skin nodules). In the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

Chest X-ray

Lesions on chest x-ray are acceptable as measurable lesions when they are clearly defined and surrounded by aerated lung. However, CT is preferable.

Conventional CT and MRI

This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5 mm or less. If CT scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (e.g. for body scans).

Use of MRI remains a complex issue. MRI has excellent contrast, spatial, and temporal resolution; however, there are many image acquisition variables involved in MRI which greatly impact image quality, lesion conspicuity, and measurement. Furthermore, the availability of MRI is variable globally. As with CT, if an MRI is

performed, the technical specifications of the scanning sequences used should be optimized for the evaluation of the type and site of disease. Furthermore, as with CT, the modality used at follow-up must be the same as was used at baseline and the lesions should be measured/assessed on the same pulse sequence. It is beyond the scope of the RECIST guidelines to prescribe specific MRI pulse sequence parameters for all scanners, body parts, and diseases. Ideally, the same type of scanner should be used and the image acquisition protocol should be followed as closely as possible to prior scans. Body scans should be performed with breath-hold scanning techniques, if possible.

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PET-CT

At present, the low dose or attenuation correction CT portion of a combined PET-CT is not always of optimal diagnostic CT quality for use with RECIST measurements. However, if the site can document that the CT performed as part of a PET-CT provides image quality equivalent to a diagnostic CT then the CT portion of the PET-CT can be used for RECIST measurements and can be used interchangeably with conventional CT in accurately measuring cancer lesions over time. Note, however, that the PET portion of the CT introduces additional data which may bias an investigator if it is not routinely or serially performed.

Ultrasound

Ultrasound is not useful in assessment of lesion size and should not be used as a method of measurement. Ultrasound examinations cannot be reproduced in their entirety for independent review at a later date and, because they are operator dependent, it cannot be guaranteed that the same technique and measurements will be taken from one assessment to the next. If new lesions are identified by ultrasound in the course of the study, confirmation by CT or MRI is advised. If there is concern about radiation exposure at CT, MRI may be used instead of CT in selected instances.

Endoscopy, Laparoscopy

The utilization of these techniques for objective tumor evaluation is not advised. However, such techniques may be useful to confirm complete pathological response when biopsies are obtained or to determine relapse in trials where recurrence following complete response (CR) or surgical resection is an endpoint.

Tumor Markers

Tumor markers alone cannot be used to assess response. If markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response. Specific guidelines for both CA-125 response (in recurrent ovarian cancer) and PSA response (in recurrent prostate cancer) have been published [JNCI 96:487-488, 2004; J Clin Oncol 17, 3461-3467, 1999; J Clin Oncol 26:1148-1159, 2008]. In addition, the Gynecologic Cancer Intergroup has developed CA-125 progression criteria which are to be

integrated with objective tumor assessment for use in first-line trials in ovarian cancer [JNCI 92:1534-1535, 2000].

Cytology, Histology

These techniques can be used to differentiate between partial responses (PR) and complete responses (CR) in rare cases (e.g., residual lesions in tumor types, such as germ cell tumors, where known residual benign tumors can remain).

The cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment when the measurable tumor has met criteria for response or stable disease is mandatory to differentiate between response or stable disease (an effusion may be a side effect of the treatment) and progressive disease.

6.1.4 Response Criteria for solid tumors (RECIST)

6.1.4.1 Evaluation of Target Lesions

Complete Response (CR)

Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to < 10 mm.

Partial Response (PR)

At least a 30% decrease in the sum of the diameters of target lesions, taking as reference the baseline of each treatment step sum diameters.

Progressive Disease (PD)

At least a 20% increase in the sum of the diameters of target lesions, taking as reference the smallest sum on that step (this includes the baseline sum if that is the smallest on that step). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm.

NOTE: The appearance of one or more new lesions is also considered progression, See Section 6.1.4.3

NOTE: Clinical Progressive Disease can be determined using the following criteria:

1. ECOG PS of at least 3
2. Patient unable to have follow-up radiologic assessment due to performance status decline
3. Symptomatic decline deemed related to patient's disease (not toxicity from therapy or concurrent illness)

Stable Disease (SD)

Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest

sum diameters while on that step. (Note: a change of 20% or more that does not increase the sum of the diameters by 5 mm or more is coded as stable disease)

To be assigned a status of stable disease, measurements must have met the stable disease criteria at least once after registration to that treatment step at a minimum interval of 2 cycles (for cycles of 28 or 42 days length) of the treatment OR a minimum interval of 3 cycles (for cycles of 21 day length).

6.1.4.2 Evaluation of Non-Target Lesions

Complete Response (CR)

Disappearance of all non-target lesions. All lymph nodes must be non-pathological in size (< 10 mm short axis)

Non-CR/Non-PD

Persistence of one or more non-target lesions.

Progressive Disease (PD)

Appearance of one or more new lesions and/or unequivocal progression of existing non-target lesions (see Section 6.1.4.3). Unequivocal progression should not normally trump target lesion status. It must be representative of overall disease status change, not a single lesion increase.

When the patient also has measurable disease, there must be an overall level of substantial worsening in non-target disease such that, even in the presence of SD or PR in target disease, the overall tumor burden has increased sufficiently to merit discontinuation of therapy. A modest "increase" in the size of one or more non-target lesions is usually not sufficient to qualify for unequivocal progression status. The designation of overall progression solely on the basis of change in non-target disease in the face of SD or PR of target disease will therefore be extremely rare.

When the patient only has non-measurable disease, the increase in overall disease burden should be comparable in magnitude to the increase that would be required to declare PD for measurable disease: i.e., an increase in tumor burden from "trace" to "large", an increase in nodal disease from "localized" to "widespread", or an increase sufficient to require a change in therapy.

Although a clear progression of "non-target" lesions only is exceptional, the opinion of the treating physician should prevail in such circumstances, and the progression status should be confirmed at a later time by the review panel (or Principal Investigator).

6.1.4.3 Evaluation of New Lesions

The appearance of new lesions constitutes Progressive Disease (PD).

A growing lymph node that did not meet the criteria for reporting as a measurable or non-measurable lymph node at baseline should only be reported as a new lesion (and therefore progressive disease) if it:

a) increases in size to ≥ 15 mm in the short axis, or

b) there is new pathological confirmation that it is disease (regardless of size).

New effusion or ascites that appears during treatment should only be reported as a new lesion (and therefore progressive disease) if it has cytological confirmation of malignancy.

6.1.4.4 Evaluation of Best Overall Response

The best overall response is the best response recorded from the start of the treatment step until disease progression/recurrence or non-protocol therapy taking as reference for progressive disease the smallest measurements recorded on that step (this includes the baseline sum, if that is the smallest on that step). The patient's best response assignment will depend on the achievement of both measurement and confirmation criteria.

For Patients with Measurable Disease (i.e., Target Disease)

Target Lesions	Non-Target Lesions	New Lesions*	Overall Response	Best Overall Response determination
CR	CR	No	CR	Confirmation needed: ≥ 4 weeks confirmation****
CR	Non-CR***/Non-PD	No	PR	Confirmation needed: ≥ 4 weeks confirmation****
CR	Not evaluated	No	PR	
PR	Non-PD***/not evaluated	No	PR	
SD	Non-PD***/not evaluated	No	SD	Documented at least once at a minimum interval of 2 cycles (for cycles of 28 or 42 days) or 3 cycles (for cycles of 21 days) from registration to the treatment step
PD	Any	Yes or No	PD	No prior SD, PR or CR
Any	PD***	Yes or No	PD***	
Any	Any	Yes	PD	

* See RECIST 1.1 manuscript for further details on what is evidence of a new lesion.

** In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.

*** PD in non-target lesions should not normally trump target lesion status. It must be representative of overall disease status change, not a single lesion increase. Please refer to the Evaluation of Non-Target Lesions – Progressive Disease section for further explanation.

**** It is recommended that the confirmation be obtained at the next scheduled evaluation.

NOTE: Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as "*symptomatic deterioration."* Every effort should be made to document the objective progression even after discontinuation of treatment.

6.1.4.5 Duration of Response

Duration of Overall Response

The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started).

The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that progressive disease is objectively documented.

Duration of Stable Disease

Stable disease is measured from the baseline of each treatment step until the criteria for progression are met, taking as reference the smallest measurements recorded

since the treatment started, including the baseline measurements.

To be assigned a status of stable disease, measurements must have met the stable disease criteria at least once after entry to that step at a minimum interval 2 cycles (for cycles of 28 or 42 days), or 3 cycles (for cycles of 21 days).

6.2 Lymphoma Response Criteria

NOTE: These criteria are based upon the criteria from the Revised Response Criteria for Malignant Lymphoma, (Cheson et al.), Journal of Clinical Oncology, 2014: 32: 3059-3067.

The criteria use the following categories of response: Complete Response (CR), Partial Response (PR), Stable Disease (SD), Relapse and Progression (PD). In the case of stable disease, follow-up assessments must have met the SD criteria at least once after entry to that step at a minimum interval of eight weeks.

The following guidelines are to be used for establishing tumor measurements at the baseline of each treatment step and for subsequent comparison:

- The six largest measurable nodes or extranodal masses must be identified as Target Lesions at baseline.
- If there are 6 or fewer measurable nodes and extranodal masses, all must be listed as Target Lesions
- If there are more than 6 involved measurable nodes or extranodal masses, the 6 largest nodes or extranodal masses should be selected as Target Lesions according to the following features: a) they should be clearly measurable in at least two perpendicular measurements; b) they should be from as disparate regions of the body as possible; and c) they should include mediastinal and retroperitoneal areas of disease whenever these sites are involved. When there are more than 6 involved measurable nodes or extranodal masses, any lesions that are not included within these 6 Target Lesions will be considered non-measured lesions.
- Nonmeasured lesions: Any disease not selected as measured, dominant disease and truly assessable disease should be considered not measured. These sites include any nodes, nodal masses, and extranodal sites not selected as dominant or measurable or that do not meet the requirements for measurability but are still considered abnormal, as well as any site of suspected disease that would be difficult to follow quantitatively with measurement, including pleural effusions, ascites, bone lesions, leptomeningeal disease, abdominal masses, and other lesions that cannot be confirmed, measured or followed by imaging.
- Measurements for all Target Lesions will be reported at baseline of each treatment step. Measurements for non-measured lesions are not required.
- The lymph nodes or extranodal masses selected as Target Lesions for measurement should be measured in two perpendicular diameters, one of which is the longest perpendicular diameter. The lymph nodes should be measured in centimeters to the nearest one tenth of a centimeter (e.g. 2.0 cm, 2.1cm, 2.2 cm, etc.). A measurable node must have an longest diameter

(LDi) greater than 1.5 cm. Measurable extranodal disease (eg, hepatic nodules) may be included in the six representative, measured lesions. A measurable extranodal lesion should have an LDi greater than 1.0 cm.

- The two measured diameters of each Target Lesion should be multiplied giving a product for each nodal site or extranodal mass. The product of each site should be added, yielding the sum of products of the diameters (SPD). The SPD will be used in determining the definition of response for those who have less than a complete response.
- In clinical trials where PET is unavailable to the vast majority of participants, or where PET is not deemed necessary or appropriate for use (e.g. a trial in patients with MALT lymphoma), response should be assessed as above, but only using CT scans. However, residual masses should not be assigned CRu status, but should be considered partial responses.
- PET-based response should use the following 5 point scale: PET 5 point scale: 1, no uptake above background; 2, uptake ≤ mediastinum; 3, uptake > mediastinum but ≤ liver; 4, uptake moderately > liver; 5, uptake markedly higher than liver and/or new lesions; X, new areas of uptake unlikely to be related to lymphoma.

6.2.1 Complete Response

Complete disappearance of all detectable clinical evidence of disease, and disease-related symptoms if present prior to therapy.

6.2.1.1 PET-CT Based Criteria

Complete metabolic response with a 5 – point scale score of 1, 2 or 3, with or without a residual mass.

In patients with bone marrow involvement before treatment there must be no residual FDG uptake in the marrow.

In patients with a typically FDG-avid lymphoma with no pre-treatment PET scan, or for lymphomas for which the PET scan was positive prior to therapy: a post-treatment residual mass of any size is permitted as long as it is PET-negative.

6.2.1.2 CT Based Criteria

For variably FDG-avid lymphomas without a pretreatment PET scan, or if a pretreatment PET scan was negative: all lymph nodes and extranodal masses must have regressed on CT to normal size (≤ 1.5 cm in their greatest transverse diameter for nodes > 1.5 cm prior to therapy). Previously involved nodes that were 1.1-1.5 cm in their long axis and > 1.0 cm in their short axis prior to treatment must have decreased to ≤ 1 cm in their short axis after treatment.

The spleen and/or liver, if considered enlarged prior to therapy on the basis of a physical examination or CT scan, should not be palpable on physical examination, and nodules related to lymphoma should disappear. However, no normal size can be specified because of the difficulties

in accurately evaluating splenic and hepatic size and involvement. For instance, a spleen considered normal size may contain lymphoma, whereas an enlarged spleen may not necessarily reflect the presence of lymphoma, but variations in anatomy, blood volume, the use of hematopoietic growth factors, or other causes.

If the bone marrow was involved by lymphoma prior to treatment, the infiltrate must have cleared on repeat bone marrow biopsy. The biopsy sample on which this determination is made must be adequate (with a goal of > 20 mm unilateral core). If the sample is indeterminate by morphology, it should be negative by immunohistochemistry. A sample that is negative by immunohistochemistry but demonstrating a small population of clonal lymphocytes by flow cytometry will be considered a CR until data become available demonstrating a clear difference in patient outcome.

NOTE: Complete Remission/unconfirmed (CRu): Using the above definition for CR and that below for PR eliminates the category of CRu.

6.2.2 Partial Response (PR)

The designation of PR requires all of the following:

6.2.2.1 PET-CT Based Criteria

Partial metabolic response with reduced uptake compared with baseline AND a 5 point scale score of 4 or 5.

For a typically FDG-avid lymphoma with no pretreatment PET scan or one that was PET-positive prior to therapy, the post-treatment PET should be positive at any previously involved sites.

In patients with bone marrow involvement before treatment, Residual uptake higher than uptake in normal marrow but reduced compared with baseline (diffuse uptake compatible with reactive changes from chemotherapy allowed). If there are persistent focal changes in the marrow in the context of a nodal response, consideration should be given to further evaluation with MRI or biopsy or an interval scan.

6.2.2.2 CT Based Criteria

For variably FDG-avid lymphomas/FDG-avidity unknown, without a pretreatment PET scan, or if a pretreatment PET scan was negative, CT scan criteria should be used.

A ≥ 50% decrease in sum of the product of the diameters (SPD) of up to 6 of the largest Target Lesions. These nodes or masses should be selected according to the following: (a) they should be clearly measurable in at least

2 perpendicular dimensions; if possible, they should be from disparate regions of the body; (b) they should include mediastinal and retroperitoneal areas of disease whenever these sites are involved.

No increase in the size of other nodes, liver or spleen.

Bone marrow assessment is irrelevant for determination of a PR if the sample was positive prior to treatment. However, if positive, the cell type should be specified, e.g., large-cell lymphoma or small cleaved cell lymphoma.

No new sites of disease.

Patients who achieve a CR by the above criteria, but who have persistent morphologic bone marrow involvement will be considered partial responders.

When the bone marrow was involved before therapy and a clinical CR was achieved, but with no bone marrow assessment after treatment, patients should be considered partial responders.

6.2.3 Stable Disease (SD)

6.2.3.1 PET-CT Based Criteria:

Absence of metabolic response, with a score of 4 or 5 AND no significant change from baseline at interim or end of treatment.

In patients with bone marrow involvement before treatment, there must be no change from pre-treatment PET scan.

No new areas of FDG uptake.

6.2.3.2 CT Based Criteria

For variably FDG-avid lymphomas/FDG-avidity unknown: For patients without a pretreatment PET scan or if the pre-treatment PET was negative, there must be no change in the size of the previous lesions on the post-treatment CT scan.

Less than 50% decrease from baseline in SPD of up to 6 Target Lesions.

No increase in organ enlargement and non-measurable lesions compatible with progressive disease.

6.2.4 Progression (PD) and Relapse

6.2.4.1 PET-CT Based Criteria

Progressive metabolic disease:

- Individual target nodes and nodal masses must present increase intensity of uptake from baseline, with a 5 point score of 4 or 5, or

- Extranodal lesions with new FDG-avid foci consistent with lymphoma at interim or end of treatment assessment, or
- New FDG-avid foci consistent with lymphoma rather than another etiology (e.g. infection, inflammation). If uncertain regarding the etiology of new lesions, a biopsy or repeat imaging scan should be considered.

6.2.4.2 CT Based Criteria

For determination of relapsed and progressive disease, lymph nodes should be considered abnormal if the long axis is more than 1.5 cm, regardless of the short axis. If a lymph node has a long axis of 1.1 to 1.5 cm, it should only be considered abnormal if the short axis is more than 1 cm. Lymph nodes ≤ 1 x ≤ 1 cm will not be considered as abnormal for relapse or progressive disease.

- At least a 50% increase from nadir in the SPD of any previously involved Target Lesions, or in a single involved node or extranodal mass, or the size of other lesions (e.g. splenic or hepatic nodules).
- To be considered progressive disease, a lymph node or extranodal mass with a diameter of the long or short axis axis of ≤ 2.0 cm must have increased by at least 0.5 cm; lesions larger than 2.0 cm must have increased by at least 1.0 cm.
- In the setting of splenomegaly, the splenic length must increase by >50% of the extent of its prior increase from baseline. If no prior splenomegaly, must increase by at least 2.0cm from baseline.
- New lesions: Regrowth of previously resolved lesions; or a new lymph node > 1.5cm in any axis; or a new extranodal site > 1.0 cm in any axis (new extranodal disease < 1.0 cm in any axis, can be considered progressive disease if its presence is unequivocal and attributable to lymphoma).
- New or recurrent bone marrow involvement

6.2.4.3 Clinical Progressive Disease can be determined using the following criteria:

- ECOG PS of at least 3
- Patient unable to have follow-up radiologic Assessment due to performance status decline
- Symptomatic decline deemed related to metastatic disease or disseminated disease (not toxicity from therapy or concurrent illness)

6.3 RANO Criteria for GBM Patients

This trial will utilize the criteria recently proposed by the Response Assessment in Neuro-Oncology (RANO) working group (Wen, 2010). The RANO Criteria updates its established predecessor, the modified Macdonald Criteria, by adding assessment of non enhancing lesions.

6.3.1 Antitumor Effect – Definitions

Evaluable for toxicity: All participants who receive at least one dose of study treatment will be evaluable for toxicity from the time of their first treatment.

Evaluable for objective response: Only those participants who have measurable disease present at baseline of each treatment step and have received at least one dose of study treatment will be considered evaluable for response. These participants will have their response classified according to the definitions stated below.

(**NOTE:** Participants who exhibit objective disease progression or die prior to the end of cycle 1 will also be considered evaluable.)

Measurable disease: Bidimensionally, contrast-enhancing, measurable lesions with clearly defined margins by CT or MRI scan, with a minimal diameter of 1 cm, and visible on 2 axial slices which are at least 5 mm apart with 0 mm skip. Measurement of tumor around a cyst or surgical cavity, if necessary, requires a minimum thickness of 3 mm. If there are too many measurable lesions to measure at each evaluation, the investigator must choose the largest two to be followed before a participant is entered on study. The remaining lesions will be considered non-measureable for the purpose of objective response determination. Unless progression is observed, objective response can only be determined when all measurable and non-measurable lesions are assessed.

Non measurable evaluable disease: Unidimensionally measurable lesions, masses with margins not clearly defined, lesions with maximal diameter < 1cm.

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6.3.2 Response/Progression Categories

NOTE: To be eligible, all patients must have measurable disease

Complete response (CR). All of the following criteria must be met:

a) Complete disappearance of all enhancing measurable and non-measurable disease sustained for at least 4 weeks (for cycles of 28 day length) or 3 weeks (for cycles of 21 day length). In the absence of a confirming scan 8-9 weeks later, this scan will be considered only stable disease.

b) No new lesions.

c) All measurable and non-measurable lesions must be assessed using the same techniques as baseline.

d) Participants must be on no steroids or on physiologic replacement doses only.

e) Stable or improved non-enhancing (T2/FLAIR) lesions

f) Stable or improved clinically, for clinical signs and symptoms present at baseline and recorded to be disease related

Participants with any non-measurable disease cannot have a complete response. The best response possible is stable disease.

Partial response (PR). All of the following criteria must be met:

a) Greater than or equal to 50% decrease compared to baseline in the sum of products of perpendicular diameters of all measurable enhancing lesions sustained for at least 4 weeks. In the absence of a confirming scan 4 weeks later, this scan will be considered only stable disease.

b) No progression of non-measurable disease.

c) No new lesions.

d) All measurable and non-measurable lesions must be assessed using the same techniques as baseline.

e) The steroid dose at the time of the scan evaluation should be no greater than the dose at time of baseline scan.

f) Stable or improved non-enhancing (T2/FLAIR) lesions on same or lower dose of corticosteroids compared to baseline scan.

g) Stable or improved, for clinical signs and symptoms present at baseline and recorded to be disease related clinically.

Participants with non-measurable disease cannot have a partial response. The best response possible is stable disease.

Progressive disease (PD). The following criterion must be met:

a) 25% increase in sum of the products of perpendicular diameters of enhancing lesions (over best response or baseline if no decrease) on stable or increasing doses of corticosteroids and/or one or more of the of the following:

b) Significant increase in T2/FLAIR non-enhancing lesion on stable or increasing doses of corticosteroids steroids compared to baseline scan or best response following initiation of therapy, not due to co-morbid events (radiation therapy, demyelination, ischemic injury, infection, seizures, post-operative changes, or other treatment effects).

c) Any new lesion

d) Clear clinical deterioration not attributable to other causes apart from the tumor (e.g. seizures, medication side effects, complications of therapy, cerebrovascular events, infection, etc.). The definition of clinical deterioration is left to the discretion of the investigator but it is recommended that a decline in the Karnofsky Performance Score (KPS) from 100 or 90 to 70 or less, a decline in KPS of at least 20 from 80 or less, or a decline in KPS from any baseline to 50 or less, for at least 7 days, be considered neurologic deterioration, unless attributable to co-morbid events or changes in corticosteroid dose.

e) Failure to return for evaluation due to death or deteriorating condition

Stable disease (SD). All of the following criteria must be met:

a) Does not qualify for CR, PR, or progression.

b) All measurable and non-measurable sites must be assessed using the same techniques as baseline.

c) Stable non-enhancing (T2/FLAIR) lesions on same or lower dose of corticosteroids compared to baseline scan. In the event that the corticosteroid dose has been increased, the last scan considered to show stable disease will be the scan obtained when the corticosteroid dose was equivalent to the baseline dose.

d) Stable clinically.

These RANO Response Criteria are also summarized in the following table:

	CR	PR	SD	PD#
T1-Gd +	None	≥50% decrease	<50% decrease- <25% increase	≥25% increase*
T2/FLAIR	Stable or decrease	Stable or decrease	Stable or decrease	Increase*
New Lesion	None	None	None	Present*
Corticosteroids	None	Stable or decrease	Stable or decrease	NA
Clinical Status	Stable or increase	Stable or increase	Stable or increase	Decrease*
Requirement for Response	All	All	All	Any*

CR=complete response; PR=partial response; SD=stable disease; PD=progressive disease
#: Progression occurs when any of the criteria with * is present
NA: Increase in corticosteroids alone will not be taken into account in determining progression in the absence of persistent clinical deterioration

6.3.3 Methods for Evaluation of Measurable Disease

All measurements should be taken and recorded in metric notation, using a ruler, calipers, or digital measurement tool. All baseline evaluations should be performed as closely as possible to the beginning of each treatment step and never more than 14 days before the beginning of the treatment.

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up.

6.3.4 Evaluation of Best Response

The best overall response is the best response recorded from the baseline of each treatment step until disease progression (taking as reference for progressive disease the smallest measurements recorded since the treatment started). If a response recorded at one scheduled MRI does not persist at the next regular scheduled MRI, the response will still be recorded based on the prior scan, but will be designated as a non-sustained response. If the response is sustained, i.e. still present on the subsequent MRI, it will be recorded as a sustained response, lasting until the time of tumor progression.

Participants without measurable disease may only achieve SD or PD as their best "response."

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6.4 Response criteria for multiple myeloma

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6.4.1 Terms and definitions

- **M-protein:** synonyms include M-spike, monoclonal protein and myeloma protein, paraprotein, M-component. Serum M-protein level is quantitated using densitometry on SPEP except in cases where the SPEP is felt to be unreliable, such as:
  - M-proteins migrating in the β-region (usually IgA M-proteins)
  - Cases in which the M-spike is so large and narrow on agarose (some specimens >4 g/dL) that they underestimate the actual immunoglobulin level (by greater than 1500 mg/dL) due to technical staining properties of the agarose gel
  - Cases in which there are multiple peaks of same monoclonal protein (aggregates or dimers)

  If SPEP is not available or felt to be unreliable (above examples) for routine M-protein quantitation, then quantitative immunoglobulin levels derived from nephelometry or turbidometry can be accepted. However, this must be explicitly reported at baseline, and only nephelometry can be used for that patient to assess response. SPEP derived M-spike values and quantitative nephelometric immunoglobulin values cannot be used interchangeably.

  Urine M-protein measurement is estimated using 24-hr UPEP only. Random or 24 hr urine tests measuring kappa and lambda light chain levels are not reliable and are not recommended.
- **FLC estimation** is currently carried out using the serum FLC assay (Freelite, The Binding Site Limited, UK). Patients with kappa/lambda FLC ratio < 0.26 are defined as having monoclonal lambda FLC and those with ratios >1.65 as having a monoclonal kappa FLC. The monoclonal light chain isotype is considered the involved FLC isotype, and the opposite light chain type as the uninvolved FLC type.
- **Imaging studies for plasmacytoma:** For patients with plasmacytoma alone (and no measurable serum or urine M protein or serum free light chain) who are followed by imaging studies, the same imaging modality should be used for follow up assessment. For patients who are **not being followed** by plasmacytoma(s), serial imaging is not required.
- **Response terms:** The following response terms will be used to define M protein response: stringent Complete Response (sCR), complete response (CR), very good partial response (VGPR), partial response (PR), Minimal Response (MR), stable disease (SD), and progressive disease (PD).
- **Measurable disease:** Patients who have a measurable serum or urine M-protein.

- Serum M-protein ≥ 1 g/dl
- Urine M-protein ≥ 200 mg/24 h
- Serum FLC assay: Involved FLC level ≥10 mg/dl provided serum FLC ratio is abnormal
- For patients with only plasmacytoma, at least one lesion that has a single diameter of ≥ 2 cm

The serum free light chain (FLC) assay is of particular use in monitoring response to therapy in patients who have oligo-secretory or non-secretory disease and **should be used in assessing response only if the baseline serum and/or urine M proteins are not "measurable" as above, and the baseline level of the involved FLC is "measurable."** When using this assay, it is important to note that the FLC levels vary considerably with changes in renal function and in patients with renal insufficiency, the levels of both the kappa and lambda may remain elevated, but the ratio normalizes with achievement of CR. Thus, both the level of the involved and the uninvolved FLC isotype (i.e., the involved/uninvolved ratio or involved-uninvolved difference) should be considered in assessing response. **Patients included on the study on the basis of FLC alone (i.e., no measurable serum/urine m-spike) should be the only ones who are evaluated using FLC response criteria. The others should follow usual criteria and ignore FLC results** with the exception of defining stringent complete response.

6.4.2 **Clarification of test indications:** Listed below are the minimal required tests required to assess response based on the characteristics of their disease at on study.

Table 6.1

Tests Required To Assess M protein Response (Must Be Done At Each Disease Measurement Visit except as indicated[1,2])

On Study Baseline Value	SPEP	24 hr UPEP	Ig FLC	BM Bx[1,2]
Serum M-spike ≥ 1 g/dl, and urine M-spike ≥ 200 mg/24 hrs	X	X		
Serum M-spike ≥1 g/dl, but urine M-spike < 200 mg/24 hrs	X			
Serum M-spike < 1 g/dl, and urine M-spike ≥ 200 mg/24 hrs		X		
Serum M-spike < 1 g/dl, urine M-spike < 200 mg/24 hrs, but involved Ig FLC is ≥ 10 mg/dL			X	

[1] **Immunofixation studies of both serum and urine** are required to document CR regardless of registration values, and in addition **FLC** measurement and **bone marrow immunophenotyping** is required to document sCR. SPEP and UPEP are required to document VGPR regardless of registration values.

[2] Bone marrow biopsy results do not need to be confirmed (i.e. repeated after documented response).

6.4.3 Confirmed response

In order to be classified as an M protein response, confirmation of serum monoclonal protein, serum immunoglobulin free light chain (when primary determinant of response) and urine monoclonal protein (when primary determinant of response) results must be made by verification on two consecutive determinations. Bone marrow aspirate and biopsy are **only** required to document CR or sCR, except for patients with evaluable disease **only**, where a bone marrow is required to document all response categories including progression. However, a second confirmatory bone marrow is **not** required to confirm response in any case. Radiographic studies are not required to satisfy these response requirements; however, if radiographic studies were performed there should be no evidence of progressive or new bone lesions.

Appropriate tests required to document and confirm response are listed in Table 6.1.

6.4.4 Bone progression

Caution must be exercised to avoid rating progression or relapse on the basis of variation of radiologic technique alone. Compression fracture does not exclude continued response and may not indicate progression. When progression is based on skeletal disease alone, it should be discussed with the Study Chair before removing the patient from the study.

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6.4.5 Response and Progression

Criteria for response and progression are listed in Table 6.2. Progressive disease for all patients as defined in Table 6.2. Although the definition for “relapse from CR (or sCR) is listed, this will be documented as a response category in ONLY those protocols evaluating disease free survival.

Table 6.2

CATEGORY	RESPONSE CRITERIA [a]
Stringent complete response (sCR)[e]	• CR as defined below plus all of the following: • Normal serum FLC ratio • Absence of clonal cells in bone marrow by immunohistochemistry or immunofluorescence [b] • Not applicable for those patients who do not have a “measurable” serum M-spike, serum free light chain, or urine M-spike at baseline
Complete response (CR)	• Negative immunofixation of the serum and urine • <5% plasma cells in bone marrow • If at on study, the only measurable non-bone marrow parameter was FLC, normalization of FLC ratio

Table 6.2	
CATEGORY	**RESPONSE CRITERIA [a]**
Very good partial response (VGPR)[e]	• PR as defined below plus all of the following: • Serum and urine M-component detectable by immunofixation but not on electrophoresis or • If at on study, serum measurable, ≥ 90% or greater reduction in serum M-component plus urine M-component < 100 mg per 24 hr • If at on study, the only measurable non-bone marrow parameter was FLC, ≥90% or greater reduction in the difference between involved and uninvolved free light chain levels • Not applicable for those patients who do not have a "measurable" serum M-spike, serum free light chain, or urine M-spike at baseline

Partial Response (PR)	• One of the following:
	  • If at on study, serum and urine measurable, a ≥ 50% reduction of serum M-protein and reduction in 24-h urinary M-protein by ≥ 90% or to < 200 mg per 24 hr
	  • If at on study, only serum measurable (but urine not), a ≥ 50% reduction of serum M-protein
	  • If at on study, urine measurable (but serum not), a reduction in 24-h urinary M-protein by ≥ 90% or to < 200 mg per 24 hr
	  • If at on study, the only measurable parameter was FLC, a ≥ 50% decrease in the difference between involved and uninvolved FLC levels
	  • If plasmacytoma was present at baseline, a ≥ 50% reduction in the size (SPD = sum of the products of the maximal perpendicular diameters of measured lesions) of soft tissue plasmacytomas is also required.

Stable disease (SD)	Not meeting criteria for sCR, CR, VGPR, PR, or progressive disease

Progressive disease (PD) [d]	Any one or more of the following:
	• Increase of 25% from lowest value in [f]
	  • Serum M-component (absolute increase must be ≥ 0.5 g/dl)[c]
	  • Serum M-component increase ≥ 1 g/dl, if lowest M component was ≥ 5 g/dl
	  • Urine M-component (absolute increase must be ≥ 200 mg/24 h)
	  • If at on study, the only measurable parameter was FLC, the difference between involved and uninvolved FLC levels (absolute increase must be >10 mg/dl) [c]
	  • Bone marrow plasma cell percentage (absolute % must be ≥10%)[c]
	  • Appearance of a new lesion(s), ≥ 50% increase from nadir in SPD of >1 lesion, or ≥50% increase in the longest diameter of a previous lesion >1 cm in short axis
	Or any one or more of the following felt related to the underlying clonal plasma cell proliferative disorder
	• Hypercalcemia (≥11.5 mg/dl) if considered related to myeloma
	• Decrease in hemoglobin of ≥2 g/dl if considered related to myeloma
	• Serum creatinine level ≥2 mg/dl if considered related to myeloma

[a] All response categories require two consecutive assessments made at anytime before the institution of any new therapy; sCR, CR, VGPR, PR, MR and SD categories also require no known evidence of

progressive or new bone lesions if radiographic studies were performed. Radiographic studies are not required to satisfy these response requirements. Bone marrow assessments need not be confirmed. Each category, except for stable disease, will have a working subcategory of "unconfirmed" [prefix 'u"] to designate first time point at which response category MAY have been achieved if confirmed.

[b] Presence/absence of clonal cells is based upon the k/λ ratio. An abnormal k/λ ratio by immunohistochemistry and/or immunofluorescence requires a minimum of 100 plasma cells for analysis. An abnormal ratio reflecting presence of an abnormal clone is k/λ of >4:1 or <1;2.

[c] Positive immunofixation alone in a patient previously classified as CR will not be considered progression.

[d] Progressive disease should be confirmed. However, treatment may be discontinued for progressive disease that is unconfirmed per physician discretion. In this case, an objective status of PD should be entered on the measurement form and progressive disease should be reported on the event monitoring form.

[e] Does not apply to EMD or PCL

[f] In the case where a value is felt to be a spurious result per physician discretion (for example, a possible lab error), that value will not be considered when determining the lowest value.

	CR	VGPR	PR	MR	SD	PD
SPEP	Absent M spike	90% decrease	>=50% decrease	>=25% decrease	< 50% decrease	>=25% increase
Serum IFE	Negative	Positive	Positive	Positive	Positive	Positive
UPEP[1]	Absent M spike	< 100 mg	<200 mg or >=90% decrease	>=50% decrease	< 50% decrease	>=25% increase
Urine IFE	Negative	Positive	Positive	Positive	Positive	Positive
Serum FLC (only if serum and urine are not measurable)	Normal ratio[2]	>=90% decrease in dFLC[3]	>=50% decrease in dFLC	NA	< 50% decrease	>=25% increase
Bone marrow (only for CR)	< 5% PC	NA	NA	NA	NA	NA

[1]Refers to 24-hour urine
[2]Normal FLC ratio is needed for a CR only in those patients with FLC as the measurable disease.
[3]dFLC is the absolute difference between the kappa and lambda light chains.

6.5 Duration of Response

This is measured from the documented beginning of response (CR or PR) to the time of relapse. This is measured in responders.

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6.6 Overall Survival

Overall survival (OS) is evaluated specifically for each drug (or step). Specifically, OS is defined as the time from start of treatment that step until death, or censored at the date of last contact.

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6.7 Progression-Free Survival

Progression-free Survival (PFS) is defined as the time from start of treatment on that step until determination of disease progression or death from any cause, censored at the date of last disease assessment for patients who have not progressed (regardless of whether non-protocol therapy has been given). In the case of initiation of alternative therapy prior to disease progression, the following

sensitivity analyses will be performed: 1) censoring follow-up at start of alternative therapy; 2) counting treatment switches as events.

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6.8 Time to Progression

Time to progression (TTP) is defined as the time from start of treatment on that step until determination of disease progression or death due to disease, censored at the date of last disease assessment for patients who have not progressed. TTP is not as useful as PFS unless the majority of deaths on a study are unrelated to the malignancy due to the efficacy of the treatment and/or prolonged follow-up.

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7. Study Parameters

7.1 Step 0, 2, 4 and 6 Parameters (Registration to Screening Steps)

NOTE: Refer to the Study Schema for information on screening steps.

1. On treatment Study Parameters will be detailed in the agent-specific subprotocol.

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2. Prestudy scans (CT, MRI, and PET) and x-rays used to assess all measurable or non-measurable sites of disease must be done within 3 months prior to registration to Step 0, 2, 4, 6.

   NOTE: For patients who register to treatment steps (Step 1, 3, 5, 7), scans must be done within 6 weeks prior to registration to treatment.

   NOTE: Patients with glioblastoma multiforme are required to have baseline imaging performed within 2 weeks of the start of treatment.

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   NOTE: For patients with myeloma who register to treatment steps (Step 1, 3, 5, 7), labs must be performed within 2 weeks prior to registration to treatment.

3. Vital signs, physical exams and all required prestudy tests, with the exception of the electrocardiogram (ECG) and as outlined in Section 3, should be done ≤ 2 weeks prior to registration to Step 0, 2, 4, 6 – unless specifically required on Day 1 as per protocol.

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Test/Assessment	Prior to Registration to Steps 0, 2, 4 and 6	During Step 0, 2, 4, 6	During Step 1, 3, 5, 7	Step 8[7]
Tumor biopsy for genetic analysis[2]		X[6]		X[5]
H&P, weight, vital signs	X			
Performance status	X			
CBC w/diff, plts	X			
Serum chemistry[1]	X			
EKG	X			
Radiologic evaluation[4]	X			
β-HCG[3]	X			
Serum Protein electrophoresis (myeloma only)	X			
24 hour urine protein electrophoresis (myeloma only)	X			
Serum Free light chain assay (sFLC) (myeloma only)	X			

Test/Assessment	Prior to Registration to Steps 0, 2, 4 and 6	During Step 0, 2, 4, 6	During Step 1, 3, 5, 7	Step 8[7]
Quantitative immunoglobulins (myeloma only)	X			
Blood Sample (EDTA and Streck vacutainers)		X[5]	X[5]	X[5]

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1. Albumin, alkaline phosphatase, total bilirubin (if total bilirubin is elevated, direct bilirubin should be done), bicarbonate, BUN, calcium, creatinine, glucose, LDH, phosphorus, potassium, SGOT[AST], SGPT[ALT], sodium, magnesium. For eligibility purposes, participants with creatinine levels above institutional normal, Cockcroft-Gault will be used to calculate creatinine clearance.

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2. See Sections 7.2, 9 and 10. Pre-registration screening is MANDATORY. All specimen submissions must be logged into the ECOG-ACRIN Sample Tracking System (STS). See Section 7.2 and Section **Error! Reference source not found.**.
   a. FFPE from patients who qualify for "rare variant" arms, unless requested for additional central assessments prior to treatment assignment, is to be sent within 8 weeks following registration to Step 1 treatment as described in Sections 4.1.5.4, 7.2 and **Error! Reference source not found.**. If tissue is not available for confirmation testing, the CBPF must be notified and the pathology and CLIA reports are still required to be submitted.

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   b. For patients with treatment assignment determined by the MATCH assay (i.e. original screening process; not via outside assay results), submission of tumor tissue is mandatory for all Step 0 patients, collected via tumor biopsy or bone marrow aspiration/biopsy (multiple myeloma, if applicable) during Step 0 OR FFPE tumor tissue available for analysis following a clinically indicated diagnostic or therapeutic procedure as described in Section 9. This is no longer applicable for new patients, since the original screening process has closed.
   c. For patients previously assigned to a study treatment via the MATCH assay, a second screening biopsy may be required either at Step 2 or Step 4 for consideration of additional MATCH study treatments, as applicable per protocol.
3. Blood pregnancy test (women of childbearing potential only) should be done within 2 weeks prior to registration to Step 0, 2, 4, 6.
4. For treatments given in 21 day (3 week) cycles: every 3 cycles (9 weeks) for the first two years, and every 4 cycles thereafter (12 weeks); for treatments given in 28 day (4 week) cycles: every 2 cycles (8 weeks) for the first two years, and every three cycles thereafter (12 weeks); for treatments given in 42 day (6 week) cycles: every 2 cycles (12 weeks).

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5. OPTIONAL (but strongly encouraged for all patients, including patients entering a "rare variant" arm with outside assays). See Sections 7.2 and 9. Submit from patients who have provided signed and dated, written informed consent form for the collection and submission of the specimens for research. Blood samples are collected and submitted with the biopsies (1 EDTA and 2 Streck tubes) AND either after the first 2 cycles of each MATCH treatment (2 Streck tubes only) or the end of each MATCH treatment (2 Streck tubes only), whichever comes first. End of treatment biopsy tissue and blood (Step 8) are provided from patients who meet the criteria specified in Section 3.9. Archived FFPE materials are also requested.
6. Imaging from the performance of the biopsy are to be submitted as outlined in Section 11. Include image before needle placement, image with FNA or trocar needle in lesion, image with core biopsy or trocar needle in lesion, biopsy report describing target lesion location (organ, laterality or segment if applicable and image number on pre-biopsy imaging if available) and size.
7. Registration to Step 8 and the submission of *end of treatment* blood and tumor tissue require additional consent for the collection and submission of these samples. Blood specimens are required to be submitted from all patients registered to Step 8. Tumor specimens collected from SOC procedures of via optional biopsy are to be submitted. Biopsies to collect tumor tissue for research purposes must be minimal or less than minimal risk.

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7.2 Biospecimen Submissions

Failure to update Sample Tracking System (STS) appropriately may result in delays in the central assessments and reporting results to the site. See Section 9 for specific instructions regarding the collection and submission of specimens.

The pathology and related available reports, including reports associated with general histological and sequencing, are to be submitted with every submission of tissue:

- **Reports associated with submitted tissue,**
- **Original diagnostic reports,**
- **The pathology reports associated with the tissue analyzed by the designated Outside Laboratory**

Pathology reports submitted with tissue specimens should NOT be redacted. Results from screening and confirmation MATCH-assay testing are returned to the site and may impact patient care. Adequate identification must be provided to ensure that the appropriate materials have been submitted and reporting is correct.

Snapshot user's guide for STS is provided in Section **Error! Reference source not found.**. Most information, if entered incorrectly, may be corrected by the site. To correct mistakes for *sample type* or *timepoint*, contact the ECOG-ACRIN Lab Team (ecog.tst@jimmy.havard.edu or 857-504-2900). Please do not delete samples and re-log to correct these errors, especially after samples have already been shipped.

7.2.1 Overall Summary of Options for Sample Submissions for Screening as of May 15, 2017:

Step	Disease	Options for submissions	Biopsy 1	Biopsy Repeat
Step 0	"Rare Variant" report per Outside Laboratory	Timepoint: "Confirmation of Pre-Registration Outside Results" Submit: Pre-Trial FFPE[3]		
Screening 2[2] (Step 2 or 4)	MATCH assay screens		Fresh Biopsy[1]	Fresh biopsy (#2)[1]
Step 8	All		Blood Specimens Fresh Biopsy[1], if performed	

1. MULTIPLE MYELOMA REQUIRING FRESH BONE MARROW ASPIRATE ONLY: For all screenings and end of treatment, use 1 drop to make 4 – 5 smears, put 1 – 1.5 ml in one EDTA tube, and put remainder (5 - 10 ml) of bone marrow aspirate in the other EDTA tube. SUBMIT ASPIRATES FROM THE FIRST PULL OF AN ASPIRATION SITE FOR TESTING. The submitted smears should also be made from the same aspirate.
2. Open only to patients assigned and accrued to Step 1 treatment based on the original MATCH-assay screening process. Patients assigned to treatment based on a referral from a designated laboratory (see Section 3.1.6 and Appendix XIV) are eligible for only one treatment on MATCH and are not to undergo a biopsy to collect material for

screening. Patients who progress on Step 1 are eligible for subsequent registration to Step 8 and the subsequent collection and submission of research samples.

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3. For patients entering MATCH via a referral from a designated outside laboratory, submit previously collected FFPE tumor tissue representative (if possible) of that analyzed by the designated outside laboratory for central confirmation of the outside assay results, as follows:
   - Within 2 weeks following notification that archived FFPE specimens are required for central assessments (e.g. IHC) for purposes of treatment assignment.

     OR
   - Within 8 weeks following registration to treatment (Step 1), if not required for mandatory assessments (e.g. IHC).

   Match assay results of the assessment on tissue submitted by the sites will be returned to the site, but will not impact patient participation in the assigned treatment arm (unless required in advance for treatment assignment purposes).

   NOTE: If tissue is not available, it must be noted in STS and the CBPF/MD Anderson TQL must be notified. The CBPF/MD Anderson TQL will outreach directly to the referring designated Outside Laboratory to request residual nucleic acids for confirmation assessments. Note, confirmation results from submitted nucleic acids will not be returned to the sites.

7.2.2 Specimen Submission Requirements:

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Time Point	Sample Type	Alternatives In STS, Click "SHOW" to view Alternatives	Comment
FORMALIN FIXED PARAFFIN-EMBEDDED (FFPE) SUBMISSIONS – "RARE VARIANT" CONFIRMATION: Tissue representative of that analyzed by the designated outside laboratory. See Error! Reference source not found.			
Pre-trial Diagnostic Material[1]	FFPE Tumor Block	• H&E • Unstained slides (20+)	Tissue representative of that analyzed by the designated outside laboratory preferred. STS timepoint = "Confirmation of Pre-Registration Outside Results"
FRESH BIOPSY SUBMISSIONS[1]			
Screening 2 (Step 2 or 4)	Fresh Tissue (4 Fresh Tissue Cores in Formalin)	If Multiple Myeloma Requiring Aspirate[4] -BM aspirate, EDTA	**Archived FFPE specimens may be requested for confirmation of MLH1 or MSH2 testing.**
	Cells, Fine Needle Aspirate (FNA cells in Cytolyte)		
	UNSTAINED SLIDE_FNA (FNA smear)	Stained FNA smear OR	

Time Point	Sample Type	Alternatives In STS, Click "SHOW" to view Alternatives	Comment
		If Multiple Myeloma requiring aspirate[4] - BM aspirate smear (4)	
SUBMISSION OF ADDITIONAL SUBMISSIONS PER PATIENT CONSENT – SubProtocol specific submissions are outlined in the specifc subprotocol.			
Pre-trial Diagnostic Material[3]	FFPE Tumor Block	• H&E • Unstained slides (20+)	On EAY131 Master Protocol Consent Form, patient answered "Yes" to "I agree to provide additional specimens for research"
Each Screening Submission	• Periph Bld K2-ETDA (1) • Peripheral blood, Streck Cell-Free DNA Tube (2)		On EAY131 Master Protocol Consent Form, patient answered "Yes" to "I agree to provide additional specimens for research"
Step 1, 3, 4, 6: End of Cycle 2 or End of Treatment[5]	Peripheral blood, Streck Cell-Free DNA Tube (2)		On EAY131 Master Protocol Consent Form, patient answered "Yes" to "I agree to provide additional specimens for research"
Progression, End of Treatment Biopsy	• Periph Bld K2-ETDA (1) • Peripheral blood, Streck Cell-Free DNA Tube (2)		Consent to submission of progression samples (Step 8) AND registered to Step 8
	• Fresh Tissue Cores in Formalin • Cells, Fine Needle Aspirate in Cytolyte • FNA smears	If Multiple Myeloma requiring aspirate[4] - BM aspirate, EDTA - BM aspirate smear (4)	

1. If previously collected FFPE will be submitted for Step 0 screening, the following criteria must be met:
   - The optimal block is 70% TUMOR CELLULARITY. Specimen size requirement is as follows:
     - Surface area: 25mm^2 is optimal. Minimum is 5mm^2
     - Volume: 1mm^3 optimal. Minimum volume is 0.2mm^3, however the success of DNA extraction decreases at suboptimal tissue volume
     - Tissue, if available, is to be submitted within 8 weeks of accrual to step 1. The results of the confirmation will be reported to the site. This additional assessment will not impact the screening assessment timeline and treatment assignment will not be held pending the outcome of the confirmation assessments. If the results are complete prior to treatment assignment, the confirmation results will be used by MATCHbox.

2. NOT APPLICABLE TO MYELOMA PATIENTS WITH ASPIRATE SUBMITTED FOR SCREENING.
3. MULTIPLE MYELOMA REQUIRING FRESH BONE MARROW ASPIRATE ONLY. SUBMIT ASPIRATES FROM THE FIRST PULL OF AN ASPIRATION SITE FOR SCREENING.
4. For Step 8, submission of blood specimens are mandatory. Biopsy for collection of the research fresh tumor specimens (or bone marrow from myeloma patients) is to be performed only if patient is amenable and biopsy is minimal risk or less OR if the procedure performed is standard of care. If the fresh biopsy will not be submitted, indicate “Can’t Submit” in STS and provide the reason why.
5. Collect blood end of Cycle 2 (prior to Cycle 3 treatment) or at end/discontinuation of MATCH treatment, whichever is earlier. Please refer to Section **Error! Reference source not found.**.

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7.2.3 Sample Tracking System (STS) Timepoint Summary:

Timepoint	Sample Submission Purpose
Pre-trial Diagnostic Material	Tissue from procedures performed prior to Step 0 registration which are submitted for research studies from patients who, on EAY131 Master Protocol Consent Form, answered "Yes" to "I agree to provide additional specimens for research"
Confirmation of Pre-Registration Outside Results	MANDATORY: Archived formalin fixed tumor tissue (FFPE) submitted from patients assigned and subsequently registered to Step 1 treatment based on a referral from a designated outside laboratory ("rare variant" results). Sample is submitted AFTER registration to Step 1 treatment unless it is requested to be submitted for MANDATORY CENTRAL IHC for determination of treatment assignment.
Step 0, Screening 1, Biopsy 1	Accrual CLOSED May 22, 2017– Original MATCH-assay screening patients
Step 0, Screening 1, Biopsy Repeat	Accrual CLOSED May 22, 2017– Original MATCH-assay screening patients
Screening 2, Biopsy 1	NOT OPEN TO PATIENTS REFERRED BY A DESIGNATED OUTSIDE LABORATORY • Patients assigned and registered to treatment based original MATCH-assay screening • Patients registered to Step 2 or Step 4 • Specimens obtained from a fresh biopsy and submitted for screening. • Peripheral blood collected for research per patient consent
Screening 2, Biopsy Repeat	NOT OPEN TO PATIENTS REFERRED BY A DESIGNATED OUTSIDE LABORATORY • Patients assigned and registered to treatment based original MATCH-assay screening • Patients registered to Step 2 or Step 4 • Specimens obtained and submitted for screening after failure of "Screening 2, Biopsy 1" assessment.
Step 1, End of Cycle 2 Step 3, End of Cycle 2 Step 5, End of Cycle 2 Step 7, End of Cycle 2	Peripheral blood samples collected for research per patient consent at End of Cycle 2 (prior to start of Cycle 3 treatment) or upon discontinuation of treatment, whichever comes first.
Progression (Step 8)	Sample submissions for research from patients who signed **the "Consent for additional sample collection for research studies" AND registered to Step 8.** Submission of peripheral blood (required) and fresh biopsy (from standard of care procedure OR if patient willing to undergo a "minimal risk" biopsy).

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8. Statistical Considerations

This is a multi-arm molecular profile-driven non-randomized phase II trial. Consenting patients who are eligible for the screening will have their tumors molecularly characterized and treatment will be directed by the tumor's molecular feature profile. All patients in a particular molecularly-defined subgroup will receive the treatment matched to that tumor alteration. Molecular characteristics to be assessed for each tumor include SNVs, indels, CNVs, translocations, and IHC-based markers, which will be quantified and recorded as described in Section 5.1. In cases for which more than one molecular abnormality is identified in the tumor, treatment assignment will be prioritized by the rules specified in Section 1.6. As many as 6452 patients will be screened and additional patients with outside assays sufficient to accrue approximately 35 patients (31 eligible, treated) in each biomarker-defined subgroup.

The primary endpoint is objective response rate (ORR), defined as a complete or partial response, which is defined consistent with RECIST version 1.1 criteria for solid tumors, the Cheson (2014) criteria for lymphoma patients, the criteria listed in Section 6.4 for multiple myeloma and the RANO criteria for GBM patients (See Section 6). After completing participation in a given treatment subprotocol, patients may enroll on additional sub-studies if they have another actionable mutation and meet the eligibility requirements. Such patients will be counted toward the accrual, and their data will be included in the primary analysis, for any sub-studies in which they participated and were confirmed eligible. Patients who are determined to be ineligible for a subprotocol on which they are enrolled, or who do not start treatment on that subprotocol, will be excluded from the primary analysis for the subprotocol. The OR rate will be compared against a null benchmark value of 5%. If the observed objective response rate is ≥ 5/31 (16%), it will then be concluded that the agent is promising and worthy of further testing. Allowing for 10% ineligibility rate, 35 patients will need to be accrued to each treatment subprotocol in order to obtain 31 eligible patients for each arm.

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The proposed design has the following operating characteristics:

Power is 91.8% to conclude an agent is promising if its true OR rate is 0.25;

Type 1 error rate (one-sided) is 1.8% (this is the probability to conclude that a given agent is promising if its true OR rate is 0.05).

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If accrual to an arm is allowed to continue beyond 35 patients (see below) and more than 31 eligible, treated patients are available for analysis, the overall test of whether the response rate is > 5% will be conducted at the same type I error (1.8%).

8.1 Accrual

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The target screening accrual for this study is approximately 6452 patients, with the goal of obtaining samples for analysis for ~6000 patients and accruing 35 patients in each treatment subprotocol. Assuming approximately 5000 patients will be screened per year, the following table gives the number of patients that needed to have samples submitted and the approximate accrual duration needed, for a given subprotocol with alteration rate p:

p	# of patients with samples submitted	Time (yr)
0.86%	5994	1.29

p	# of patients with samples submitted	Time (yr)
2%	2578	0.55
5%	1032	0.22
10%	516	0.115

If after screening 500 patients, the total number of patients with actionable tumor alteration (therefore qualifying for treatment) is below 50 (overall actionable alteration rate of 10%), results will be presented to the steering committee for consideration of terminating the trial. Within any given subprotocol, if rate of enrollment is such that it is unlikely accrual can reach 25 patients by the time the overall study closes, and if 13 patients have been treated and no responses have been observed, then the steering committee may consider terminating accrual in that subgroup due to lack of feasibility. This rule is based on the fact that the exact binomial upper 97.5% confidence limit on the response rate will then be less than 25%. The eligibility rate will continue to be monitored periodically and presented to the steering committee for additional consideration if it remains below 15% once at least 22 arms are activated.

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If 35 patients have been enrolled on an arm and a replacement arm for the target is not ready for activation, and if the pharmaceutical collaborator agrees to support the expansion according to the terms of its agreement with CTEP, then accrual can continue until either the response data are available on the first 31 eligible patients or for 6 additional months, whichever comes first, at which time accrual to the arm will be suspended. Also, based on the amounts of drug supplies that are currently approved by the pharmaceutical collaborators, accrual to any expansion cohort will not exceed 35 patients (up to a total of 70 patients). The arm will remain suspended until the response analysis of the first 31 eligible patients has been performed. The results will then be discussed with CTEP, and CTEP approval will be required for any further accrual to that arm to occur.

Enrollment past 35 patients will take into account disease histology. There will be no restriction on the number of patients with any eligible histology during the first 35 patients, but additional accrual beyond 35 will only be allowed for cancer types with less than 10 patients enrolled. During the additional accrual, once 10 patients with a particular cancer type have been enrolled on the arm, that cancer type will be excluded from future enrollment on that arm. Because of the time lag between assignment to an arm and enrollment on the arm, patients who have already been assigned to the arm at the time the 10th patient with a particular cancer type is enrolled would still be allowed to enroll on the arm.

As stated above, several MATCH subprotocols have as eligibility criteria molecular variants that are so rare that we do not expect to recruit 31 eligible patients even after sequencing 6000 patient tumors. NCI-MATCH has entered into collaboration with certain CLIA certified laboratories that screen over 6000 patients per year, such that the laboratories will notify clinical sites when a patient appears to be eligible for a subprotocol in MATCH that concerns “rare variants.” We will attempt to confirm the molecular eligibility with archived tumor tissue from patients who were entered into a subprotocol of MATCH with results from an “outside” assay. Eligible and treated patients who are entered based on the MATCH assay or who are entered based on outside assays and whose molecular abnormalities are confirmed by the MATCH assays will be included in

the primary analyses. As secondary analyses, outcome may also be reported by combining all patients, or separately for those patients with results from outside assays that were not confirmed by the MATCH assay. All treated patients will be evaluable for toxicity assessment. Patients enrolled based on outside assay results do not count towards the ~6452 screening accrual goal, and accrual from this cohort will be tracked separately. Patients enrolled on treatment arms based on outside assays whose molecular abnormalities are confirmed by the MATCH assay will count towards the accrual goals for the treatment arms.

After Step 0 registration, the "MATCH screening assessment" will differ depending on the method of study entry. For patients that previously entered the study via the original screening process (i.e. to submit tissue for testing by a MATCH laboratory), this assessment will consist of both molecular profiling by the MATCH assay and the subsequent process of assigning treatment. Please note, after 5/22/17, new patients may no longer be enrolled on the study through this process. For patients entering the study through testing performed by one of the designated labs outside of the MATCH laboratory group (i.e. the Outside Assay process; see Appendix XIV), the assessment will only consist of the study's determination of treatment assignment. This is the only method of study entry moving forward.

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8.2 Interim Monitoring

An analysis of the distributions of actionable alterations and of tumor types, both overall and within treatment subprotocols, will be performed after 1000 additional patients have completed screening following the reactivation of the study in May 2016, and the steering committee will be consulted regarding whether modifications, such as restrictions on tumor types, might be needed. Accrual will not be suspended for the additional interim analyses. For publication purposes, updated results may be reported.

Given a number of unknown aspects about this study (e.g., prevalence of specific alterations), after 500 patients are screened the design will be reassessed to assure its appropriateness. An analysis of the distributions of actionable alterations and of tumor types, both overall and within treatment subprotocols, will continue to be performed after 1000 and 2000 additional patients have completed screening following the reactivation of the study in May 2016, and the steering committee will be consulted regarding whether modifications, such as restrictions on tumor types, might be needed. Accrual will not be suspended for these additional interim analyses.

Patients who participate in multiple treatment sub-studies will be counted towards the accrual of each subprotocol in which they enroll, and they will be included in the primary analysis for each of those sub-studies. However, the proportion of patients who participate in multiple sub-studies will be monitored. If a situation occurs where this proportion becomes too high (>25%), then a discussion with CTEP will take place to determine whether the study should be amended to reduce repeat participation.

8.3 Safety Monitoring

Safety monitoring is the responsibility of the MATCH Steering Committee, the MATCH subprotocol study chairs, the study statisticians, and the study Safety Co-Chairs and toxicity monitors. Adverse events are reported in a routine

manner at scheduled times using Medidata Rave. In addition, expedited reporting of certain adverse events through the CTEP-AERS mechanism is required, as described in each treatment-specific subprotocol document. The ECOG-ACRIN data managers monitor the routine and expedited data to ensure that all required AEs have had expedited reports submitted.

8.3.1 MATCH Steering Committee

The MATCH Steering Committee will meet regularly. At least one safety chair will be required to participate in each meeting and adverse events across all subprotocols will be routinely monitored.

8.3.2 Semiannual Reports

Comprehensive analyses of interim toxicity data are routinely performed at least twice yearly. Reports of these analyses will be reviewed by the Steering Committee and the Chairs of the subprotocols. These reports are also sent to the ECOG-ACRIN Principal Investigator or Senior Investigator at the participating institutions.

8.3.3 Monthly Toxicity Monitoring

The study's Safety Co-Chair(s) or designees will serve as toxicity monitors for this aspect of the study. As is true for all ECOG-ACRIN studies, adverse events reported through the CTEP-AERS mechanism for a subprotocol will be reviewed by the chair(s) of the subprotocol, the toxicity monitor and statistician in order to identify untoward or severe toxicities. In a given month, if the following trigger occurs, a report will be sent to the toxicity monitor, chair of the subprotocol and the statistician, asking them to review the events and determine if a more in-depth review is necessary.

- one or more CTEP-AERS reports containing grade 3 or higher adverse events in the previous month.

If any of the reviewers feel that further review is necessary, the reports are distributed and then discussed on a conference call with the Steering Committee. If the reviewers feel it necessary, the subprotocol can then be amended or suspended. In the event of a very serious situation, one of the ECOG-ACRIN Co-Chairs can suspend the study prior to the detailed review.

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8.4 Endpoints/Analysis Plan

Primary analyses will be based on the group of eligible and treated patients excluding those who were entered into a subprotocol of MATCH with results from an "outside" assay that could not be confirmed by the MATCH assays or a validated orthogonal assay. For each treatment subprotocol, 90% two-sided confidence intervals will be calculated for the following two endpoints: OR rate (the primary endpoint, defined earlier in Section 8); proportion of patients alive and progression free at 6 months from start of treatment on that treatment step. For arms that enroll less than 31 eligible, treated patients, overall activity will be assessed using 5% one-sided exact binomial tests of the null hypothesis that the OR is ≤ 5%, regardless of the total number enrolled.

Descriptive statistics will be reported for the subgroup of patients who entered into a subprotocol of MATCH with results from an "outside" assay that could not be confirmed by the MATCH assay or a validated orthogonal assay. Secondary analyses combining these cases with the primary analysis set may also be reported.

Given the potential heterogeneity of cancer types within each treatment subprotocol, separate exploratory analysis may be performed within other specific tumor types to evaluate whether certain tumor types might be worthy of further evaluation of the targeted therapy. There will be no automatic intention to pursue such promising restricted tumor subsets as part of this study. These signals may be followed up in separate phase II studies, and would only be included within the MATCH study if an amendment was submitted and it was reviewed and approved by CTEP and CIRB.

For each subprotocol, survival outcomes (OS, PFS, TTP, as defined in Sections 6.6 - 6.8) will be estimated using the Kaplan-Meier method. Analysis population for survival outcomes will be defined as the population of patients who are eligible and receive any protocol treatment. For toxicity analysis, patients who receive any protocol treatment will be considered evaluable.

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8.5 Correlative Study

Within a given biomarker-defined subgroup, it may be possible to identify additional variants that are associated with differential activity of the experimental agent. Biomarkers measured prior to treatment could be predictive of response or non-response to the agent; biomarkers measured at time of progression might provide clues for mechanisms of acquired resistance. We plan to perform whole exome and RNAseq and potentially other assays in patients who entered a treatment arm and consented to use of their tissue for research (Section 10.2.1). Analyses conducted to identify these types of biomarkers will be primarily exploratory and will consist of simple comparisons of biomarkers levels, either using binomial-based tests (for binary biomarkers), t-tests (continuous biomarkers, possibly transformed), or non-parametric tests, as appropriate depending on the distribution of the biomarker values. Changes in biomarker levels from baseline (prior to receiving the treatment) to progression within patients will be assessed using paired tests appropriate for the type of biomarker measurement (e.g., paired t-tests for normally distributed biomarker values or McNemar tests for paired binary data). It will also be assessed whether patterns of changes in biomarkers are different depending on length of time to progression. Given the exploratory nature of these analyses, p-values will be reported unadjusted.

Specimens remaining after use for eligibility for MATCH and for whole exome and other sequencing (Section 10.2.1) will be prioritized for use: 1) to use as "bridge" specimens for a companion diagnostic assay in subprotocols that have a positive signal of activity, if requested by the pharmaceutical company along with a plan of analysis approved by appropriate review in NCTN/NCI; 2) additional correlative studies requested by investigators along with a plan of analysis approved by appropriate standard review in NCTN/NCI.

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8.6 Image Analysis Plan

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CT, MRI and nuclear medicine scans will be collected on MATCH participants and archived centrally for analyses addressing the corresponding protocol aims. Separate analyses will be performed within each arm of the trial, when adequate imaging, genomic and outcome data are available. In addition, cross-arm analyses will be performed, using data from multiple arms of the trial. For quantitative imaging markers already defined, such as measures of tumor heterogeneity, the analytic approaches will be similar to those described in Section 8.5. In further exploratory analyses, regularized regression will be used to study the predictive strength of sets of imaging-derived features (16). The association of imaging-derived features and gene expression features will be examined using methods such as unsupervised clustering and association map construction (17, 18, 19).

8.7 Gender and Ethnicity

Assuming number of treated patients will be approximately 700, based on the past 2 years of accrual to ECOG-ACRIN coordinated therapeutic studies by any participating group, excluding leukemia, the anticipated accrual in subgroups by gender and race is as follows:

Racial Categories	Ethnic Categories				Total
	Not Hispanic/Latino		Hispanic/Latino		
	Female	Male	Female	Male	
American Indian or Alaskan Native	2	1	0	0	3
Asian	15	6	0	0	21
Native Hawaiian or other Pacific Islander	1	0	0	0	1
Black or African American	40	23	1	0	64
White	320	255	27	9	611
Total	378	285	28	9	700

The accrual targets in individual cells are not large enough for definitive subgroup analyses. Therefore, overall accrual to the study will not be extended to meet individual subgroup accrual targets.

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9. Biopsy Target Selection, Performance and Submission of Specimens

Please review the study schema, Section 5.1 and Section 7.2 for a summary of submissions. Specimens to be submitted during screening steps or from all consenting patients on any treatment arm are outlined in the Master protocol. Specimen submissions associated with only a specific study subprotocol are be outlined in the specific subprotocol.

As of May 22, 2017, patients are referred by designated Outside Laboratories to EAY131 based on standard of care genetic assessments performed by the designated laboratories. The historical information for the prior Step 0 MATCH-assay screening process is located in Appendix XV.

The date and time of collection and shipping date must be entered into the ECOG-ACRIN Samples Tracking System (STS) for all submitted specimens. ***Failure to update STS appropriately may result in delays in the applicable central assessments and reporting results to the site.***

Results from central MATCH-assay assessments will be reported only for the following:

- Original MATCH-assay screening on fresh biopsy on Step 0 (closed May 22, 2017), Step 2, or Step 4.
- Original MATCH-assay screening on archived tissue obtained within 6 months prior to registration to Step 0 (closed May 22, 2017).
- Central “Confirmation of pre-registration outside results” assessments performed on diagnostic tissue submitted directly from the treating sites to MD Anderson during Step 0 or Step 1.

Results of assessments performed on derivatives generated by non-MATCH laboratories, even for purposes of confirmation testing, will not be returned to the treating site. Additionally, results of research only studies will not be returned to the treating site.

LABELING: All samples are to be labeled with the ECOG-ACRIN protocol number EAY131, the assigned ECOG-ACRIN patient sequence number, **date and time of collection**, and type of material.

For results and specimen submission inquiries:

MATCHTRIAL@mdanderson.org & MATCH@jimmy.harvard.edu

Phone: 1-844-744-2420 (OPTION 4) Fax: 713-745-4925

For all kit related inquiries:

EACBPF@mdanderson.org

Phone: 1-844-744-2420 (OPTION 5) Fax: 713-563-6506

To order kits, email or fax the kit order form, located in Appendix XII, to the CBPF. See Section 9.4

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9.1 Schedule of Collection and Submission

Summary of submissions and schedules are provided in table format in Section 7.2.

1. Step 0:

FFPE tumor tissue is to be submitted only if requested for central screening IHC assessments where results are required for treatment assignment. Otherwise, submit after registration to Step 1.

2. Step 1:
   - MANDATORY: Pre-trial FFPE tumor tissue submitted for "Confirmation of Pre-Registration Outside Results" submitted within 8 weeks following registration to Step 1. Materials are to be submitted utilizing the provided kit.

     NOTE: In the event that FFPE tissue is not available from patients assigned to treatment based on a referral from a designated outside laboratory, residual nucleic acids from the designated laboratory may be requested by the CBPF/MD Anderson TQL for the required confirmation sequencing.
   - From patients who answered "Yes" to "I agree to provide additional specimens for research" on the EAY131 MASTER Screening Protocol Consent
     - Additional pre-trial diagnostic material (FFPE tumor tissues) from procedures separate from that required for confirmation testing. Primary tumor tissue is preferred if material from only one procedure can be submitted.
     - Peripheral blood prior to start of treatment
     - Peripheral blood at end of Cycle 2, prior to Cycle 3 treatment or end of treatment if treatment discontinued prior to Cycle 3

   NOTE: *Additional On-Treatment* blood draws outlined within the applicable subprotocols require additional consent as dictated by the relevant subprotocol and subprotocol consent document and specimen collection and submission guidelines are outlined within that subprotocol.
3. Step 2 (or Step 4): Original MATCH-assay screened patients ONLY
   - MANDATORY: Fresh tumor tissue biopsy must be submitted for MATCH-assay central assessment if the original MATCH-assay screening results do not indicate an additional aMOI which may be used to assign a patient to treatment. Materials are to be submitted utilizing the provided kit.
4. Step 3 (or Step 5): From patients who answered "Yes" to "I agree to provide additional specimens for research" on the EAY131 MASTER Screening Protocol Consent
   - Peripheral blood at Prior to start of treatment (can be collected and submitted at time of Step 2 (or Step 4) fresh biopsy
   - Peripheral blood at end of Cycle 2, prior to Cycle 3 treatment or end of treatment if discontinued prior to Cycle 3

   NOTE: *Additional On-Treatment* blood draws outlined within the applicable subprotocols require additional consent as dictated by the relevant subprotocol and subprotocol consent document and specimen collection and submission guidelines are outlined within that subprotocol.

5. Step 8, progression: From patients who signed the "Consent for additional sample collection for research studies" AND registered to Step 8.
   - MANDATORY: Peripheral blood
   - Fresh tissue biopsy or tissue from standard of procedure

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9.2 Pathology Submissions

The submitting pathologist and clinical research associate should refer to Appendix II (Pathology Submission Guidelines) for guidelines and summary of submission requirements.

9.2.1 Documentation Submissions

Do NOT redact the reports. Adequate identification must be provided to retain "chain-of-custody", ensure that the appropriate materials have been submitted and allow appropriate and correct reporting. Results from MATCH-assay screening and confirmation assessments on tissue submitted by the site are returned to the site and will impact patient care.

NOTE: In the event that FFPE tissue is not available from patients assigned to treatment based on a referral from a designated outside laboratory, the pathology, clinical and sequencing reports outlined in Section A below are still required to be submitted.

A. The following must be submitted with every submission of pathology materials:
   - Previous clinical pathology reports, including the original diagnostic pathology report for the primary, MUST be submitted with every pathology submission.
   - Related immunological, immunohistochemical, and external sequencing reports.
   - ECOG-ACRIN Sample Tracking System shipping manifest.

B. Required additional documentation from sites which submitted materials for "Confirmation of outside results" or for "Screening 2, biopsy 1" (or "Biopsy Repeat"):
   - Completed patient CLIA Laboratory Sample Submission Form.

     Information on the CLIA submission form must be clearly legible. Do not use cursive. This form is **not** to be submitted with tissue collected for research only (registration to Step 8). FAX numbers must be provided for those individuals who are required to obtain the results; to prevent reporting errors, please double check these numbers are correct.

9.2.2 **Formalin-fixed Paraffin-embedded Tumor Tissue Submissions**:

MANDATORY: For patients registered to Step 0 based on a referral from a designated outside laboratory for confirmation of the outside sequencing results per the MATCH-assay. It is preferred that the material be representative of that analyzed by the outside laboratory or a specimen obtained as close to the collection date of that one

analyzed. Tissue is to be submitted within 8 weeks following registration to Step 1 unless requested for mandatory Step 0 central screening IHC assessments for selected candidate arms only.

Optional from patients who answer "Yes" to "I agree to provide additional specimens for research" on the EAY131 MASTER Screening Protocol Consent: Additional archived tissue from procedures performed prior to patient entry to Step 0. Primary tumor tissue is preferred if material from only one procedure can be submitted.

A. Formalin-fixed paraffin-embedded tumor tissue block(s) must be submitted. The optimal block is 70% tumor tissue. Specimen size requirement is as follows:
   - Surface area: 25mm^2 is optimal. Minimum is 5mm^2
   - Volume: 1mm^3 optimal. Minimum volume is 0.2mm^3, however the success of DNA extraction decreases at suboptimal tissue volume

   Specimens of suboptimal size, cellularity, or tumor content will require submission of more than one tumor block

   If an existing block is not available, the following is to be submitted:
   - One (1) H&E slide, **AND**
   - Twenty (20) or more recently cut 4 – 5 micron unstained sections on positively charged slides to meet the surface area and volume requirements outlined above.

NOTE: In the event that FFPE tissue is not available for confirmation testing from patients assigned to treatment based on a referral from a designated outside laboratory, residual nucleic acids from the designated laboratory may be requested by the CBPF/MD Anderson TQL. Sites will be informed of the receipt of nucleic acids as the submissions will be logged into STS by the CBPF, but results from assessment of the nucleic acids will NOT be reported to the site.

9.2.3 Fresh Tumor Biopsy Submissions:

NOTE: **For Step 0 patients registered after May 22nd, 2017, fresh biopsy submissions for Step 0 screening are not allowed. Assignment to treatment is based on a referral from a designated outside laboratory only and tissue submissions are outlined in Section 9.2.2.**

Material must be submitted in the appropriate MATCH-screening or Progression Kit. Guidelines for the performance of the biopsy are outlined in Section 9.6.

Submit for:

- Screening 2, Biopsy 1 (or 2) at Step 2 (or Step 4) – patient must have been screened and assigned to Step 1 treatment based on

the original MATCH-assay and registered to the appropriate registration step prior to submission of the biopsy materials.

Tumor biopsy sample must be considered adequate for evaluation for sequencing to be performed; assays will be attempted on tissue specimens that have at least 20% non-necrotic tumor cells present if deemed potentially adequate. If insufficient tumor is obtained for sequencing analysis, patients will be given the option to undergo repeat tumor biopsy to attempt to obtain more tissue. Patients in whom sufficient tumor material cannot be obtained for targeted sequencing analysis will not proceed to be treatment assignment for Step 3 (or Step 5).

- Patients on Step 8, patients must be consented to the "Consent for additional sample collection for research studies" and registered to Step 8. If patient does not have a lesion amenable to a minimal risk biopsy or is not willing to undergo a biopsy, submit blood samples as outlined in Section 9.3 only.

Specimen collection days: Specimen collection can be done Monday through Friday with overnight shipping for arrival on Tuesday through Saturday at the ECOG-ACRIN Central Biorepository and Pathology Facility (EA CBPF). To order kits, email or fax the kit order form, located in Appendix XII, to the CBPF.

1. For patients with solid tumors: Submission of fresh tumor tissue cores in formalin and FNA cells in cytolyte is required. See Section 9.6.

   For the collection of biopsy specimens, fine needle aspiration followed by core needle biopsy, with submission of the FNA and the biopsy cores, is required. The tumor cellularity yielded by FNA may be superior to core needle biopsy in some patients, particularly when the lesion is comprised predominantly of fibrosis, bone, or other non-tumor tissue.

   - The FNA sampling with at least 2 passes should be sufficient to provide a specimen for preparation of smears and a cell block.
     - Immediately fix one slide in 95% ethanol (provided by enrolling institution) and air dry another slide for each FNA pass. The air dried slide will be stained by Diff-Quik method to assess tumor cell quantity and quality
     - Rinse FNA needle into the 30 ml CytoLyt tube, secure the tube cap, and place the tube into the shipping kit.
   - At least four (preferred) core biopsies 16-18 gauge in diameter and at least 1 cm in length after a fine needle aspiration specimen are obtained. Place two cores between sponges in each of the two cassettes (total of four cores), snap the cassette lids in place, place the two cassettes into the formalin-filled container, secure the container lid, and place the container into the shipping kit.

NOTE: If touch preparation slides of cores are made, place them in the dry slide container. Secure the slide container lid and place it in the shipping kit.

Screening samples with loss of MLH1 or MSH2, as determined by IHC, will result in a request for the submission of pre-trial diagnostic tumor tissue as outlined in Section 9.2.2 for central confirmation of this result. The results of the confirmation will be reported to the site. This additional assessment will not impact the Step 2 (or 4) screening assessment timeline and treatment assignment will not be held pending the outcome of the confirmation assessments. If the results are completed prior to treatment assignment, the confirmation results will be used by MATCHbox.

OR

2. MULTIPLE MYELOMA ONLY: SUBMIT ASPIRATES FROM THE FIRST PULL.

   For patients with plasmacytomas, follow the instructions for the submission of solid tumors. If aspirate will be submitted for screening, draw the bone marrow aspirate materials into a plain syringe, then:

   1. Use 1 drop to make 4 – 5 smears.
   2. Put 1 – 1.5 ml in one EDTA tube.
   3. Put remainder (5 - 10 ml) of bone marrow aspirate in the other EDTA tube.

9.3 Optional Whole Blood Collections for Research

Whole blood is to be collected and submitted:

- From those who answer “Yes” to “I agree to provide additional specimens for research” on the EAY131 MASTER Screening Protocol Consent
  - Prior to start treatment
  - End of cycle 2, prior to cycle 3 treatment (or end of treatment if discontinued prior to cycle 3)
- MANDATORY from those who signed the “Consent for additional sample collection for research studies” AND registered to Step 8

Additional sub-protocol specific submissions are outlined within the relevant subprotocol and consented to at the time of completing consent to participate in the subprotocol.

Samples are to be submitted at ambient temperatures on the day of collection. To order kits for these collections, please see Appendix XII.

9.3.1 Collection and submission guidelines.

Blood draws are to occur in the following order: EDTA, Streck

1. 7-10 mL of whole blood into one (1) EDTA vacutainer collected at
   - Prior to start of protocol treatment

- Following Step 8, registration

2. 18-20 mL of whole blood into two (2) Streck vacutainers collected at:
   - Prior to start of protocol treatment
   - End of Cycle 2, prior to treatment on Cycle 3 (or end of treatment, if applicable)
   - Following Step 8, registration

9.4 NCI MATCH EAY131 Clinical Trial Specimen Kit Instructions for Specimen Collection and Shipping

For all kit related inquiries:

EACBPF@mdanderson.org

Phone: 1-844-744-2420 (OPTION 5) Fax: 713-563-6506

Kits to submit the tissue for confirmation testing and the pre-treatment and on-study blood specimens will be provided automatically upon registration to Step 1. To order follow-up kits, email or fax the kit order form, located in Appendix XII, to the CBPF.

9.4.1 Contents of "Rare Variant" shipping **kit for Formalin Fixed Paraffin-Embedded Tumor Sample for Confirmation of "Rare Variant" Results Determined by Designated Outside Laboratory.**

This kit may be ordered after either receiving notification to submit tissue for mandatory central IHC required to determine treatment assignment OR after receiving treatment assignment. Kits contain all materials to ship tissue and blood samples separately.

1. One patient CLIA Laboratory Sample Submission Form.
2. Two EDTA venipuncture blood collection tube and four Streck venipuncture blood collection tubes with labels and absorbent protective packing material for the two time points.
3. Materials to submit the required tissue
4. One pre-printed FedEx shipping label sticker pre-addressed to the EA Central Biorepository and Pathology Facility. Contact the EA-CBPF to discuss additional shipment airbills for the separate shipments.
5. **Neon green address labels – to be used with submission of the mandatory tissue**
6. Cold pack, warm pack, and temperature monitor device.
7. Strip of specimen labels.
8. Kit instructions.

9.4.2 Instructions for use of screening or Step 8 shipping kit:

Reminder: Patients must be consented for the collection and submission of these additional specimens, and subsequently registered to Step 8 prior to ordering the kit.

A: Fresh tissue and research blood submission

If a research or clinical biopsy or other clinical procedure will be performed to obtain the fresh tissue (or bone marrow for multiple myeloma) follow the instructions outlined in Section 9.2.3 and 9.6.

Procedure checklist:

1. Label blood collection tubes and, if applicable, relevant supplies for the tissue submission, including the frosted-tip slides, cassettes and all collection containers.
2. If tissue will be submitted:
   a. Complete CLIA Laboratory Sample Submission Form. Information on the CLIA submission form must be clearly legible. Do not use cursive.
   b. Perform the biopsy or relevant clinical procedure to obtain the fresh tissue (or bone marrow)
3. Perform venipuncture for collection of blood into labeled EDTA and Streck tubes and place filled labeled tubes into biohazard bag with absorbent protective packing material.
4. Log the samples into the EA Sample Tracking System and print the STS generated shipping manifest.
5. Place the following forms into the shipping kit:

   ___ This checklist

   ___ Prior clinical pathology reports, and any immunologic, immunohistochemical, or molecular study reports performed on pre-trial diagnostic tumor specimen. **Do NOT redact the reports.**

   ___ CLIA Laboratory Sample Submission Form completely filled out. Information on the CLIA submission form must be clearly legible. Do not use cursive.

   ___ The Sample Tracking System (STS) generated shipping manifest.
6. Activate the temperature monitor by pressing and holding the start button for 10-15 seconds. The green LED light will be solid, and then both red and green LED's will be solid. At this time, release the start button, and the green LED will blink rapidly for 3 seconds. Place the unit in the kit inside the blood tube packaging – it will flash red and green LED for 15 minutes. For temperature control, add cold pack or warm pack, dependent on time of year specimen is shipped. Use warm pack if outside temperature at your site or along transit route will be below freezing.
7. Use one kit per patient. Do not place specimens from more than one patient into a kit. Secure the packing, seal the kit, and apply the following shipping labels: Three neon green address labels, FedEx airbill, and UN3373 IATA label. Refer to

separate shipping guide document for pictures of label placement and further shipping guidelines. Contact FedEx for pickup. **Package must be picked up the same day specimen is collected.**

9.5 Shipping Procedures

Specimen collection can be done Monday through Friday with overnight shipping for arrival on Tuesday through Saturday at the ECOG-ACRIN Central Biorepository and Pathology Facility (EA CBPF).

Use one kit per patient. Do not place specimens from more than one patient into a kit. Use the shipping kit provided. See Appendix XII for the EAY131 Collection and Shipping Kit Order Form and instructions for ordering. Line the styrofoam containers with enough absorbent material to absorb any contents that may leak.

Follow directions enclosed in the shipping kit. Ship to the following address:

ECOG-ACRIN Central Biorepository and Pathology Facility
MD Anderson Cancer Center
Department of Pathology, Unit 085
Tissue Qualification Laboratory for ECOG-ACRIN, Room G1.3598
1515 Holcombe Blvd
Houston, TX 77030
Phone: Toll Free 1-844-744-2420 (713-745-4440 Local or International Sites)
Fax: 713-563-6506
Email: eacbpf@mdanderson.org

Sites must use the pre-printed, prepaid FedEx airbill within the specimen collection kit. If the kit does not include an airbill or additional airbills are required, please contact the CBPF at 1-844-744-2420 (OPTION 5).

9.6 Biopsy Instructions

A pre-treatment tumor biopsy or bone marrow aspirate (myeloma patients only) on Step 0 [closed May 22, 2017] and Step 2 (or 4) [Step 0 MATCH-assay screened patients only] will be obtained from patients who sign the consent to enroll in the study as detailed in Section 5. Step 8 end-of-treatment biopsies are requested from patients who have provided additional consent and meet the criteria outlined in Section 3.9.

Brain biopsies will be permitted if the patient has medical necessity for craniotomy for clinical care. Mediastinal, laparoscopic, gastrointestinal, or bronchial endoscopic biopsies can be obtained incidentally to a clinically necessary procedure and not for the sole purpose of the clinical trial. Acceptable biopsy procedures are:

- Percutaneous biopsy with local anesthetic and/or sedation with an expected risk of severe complications < 2%
- Excisional cutaneous biopsy with local anesthetic and/or sedation with an expected risk of severe complications < 2%
- Biopsy with removal of additional tumor tissue during a medically necessary mediastinoscopy, laparoscopy, gastrointestinal endoscopy, bronchoscopy or craniotomy

- Removal of additional tumor tissue during a medically necessary surgical procedure
- Bone marrow aspirate (myeloma patients only, if applicable)

No open surgical, laparoscopic endoscopic procedure will be performed solely to obtain a biopsy for this protocol.

9.6.1 Contraindications to percutaneous biopsy:

1. Significant coagulopathy that cannot be adequately corrected.
2. Severely compromised cardiopulmonary function or hemodynamic instability.
3. Lack of a safe pathway to the lesion.
4. Inability of the patient to cooperate with, or to be positioned for, the procedure.

9.6.2 Tissue specimens will be sent for analyses as defined in Section **Error! Reference source not found.**. Specimens are generally expected to provide 20 nanograms of nucleic acids. If a site is deemed appropriate for biopsy with minimal risk (no more than 2% risk of serious complication requiring hospitalization) to the participant by agreement between the investigators and Interventional Radiology, an attempt at biopsy will be made.

The biopsy procedure to be used in this protocol is described below; local anesthesia will be administered as needed. Sedation may be used for comfort if considered safe for the patient. Such biopsies can be safely performed as evidenced by literature reports [12,13, 20], as well as, experience at the NIH Clinical Center, where, among 244 research tumor biopsies (18G needle) in liver (126), subcutaneous/chest wall/abdominal wall (36), intramuscular (18), lung and lymph nodes, there were 8 minor cases of bleeding, and one pneumothorax requiring 48h hospitalization (21) and MD Anderson Cancer Center (22). Most biopsies will probably be in liver. The complication rate at Massachusetts General Hospital (23) of percutaneous liver biopsy similar to those that will be done for this trial is 1.2 % of 732 cases. Risks of the procedure include, but are not limited to, bleeding, infection, pain, and scarring.

9.6.3 Complications associated with any screening biopsy or end-of-treatment biopsy will be reported and tracked as protocol-related AEs within Medidata Rave.

9.6.3.1 Severe or major complications are considered to be (13):

- Requires therapy, minor hospitalization (more than overnight only but < 48 h)
- Requires major therapy; unplanned increase in level of care, prolonged hospitalization> 48h
- Permanent adverse sequelae
- Death

9.6.3.2 Minor complications

- No therapy, no consequence
- Nominal therapy, no consequence - includes overnight admission for observation only

9.6.4 Pre-biopsy Lesion Assessment (for those undergoing biopsy by Interventional Radiologist)

The Interventional Radiologist should provide the investigator with an assessment of whether a lesion that is likely to yield enough material for molecular profiling can be found and biopsied with acceptable risk. Three main factors are considered:

i. whether a suitable lesion (viable tumor) for biopsy is present (yield)
ii. whether the lesion selected for biopsy can be sampled aggressively (yield)
iii. the expected level of risk of major complication to the patient (< 2% major complication to be eligible for biopsy)

9.6.4.1 A pre-biopsy lesion scoring system (scale from 1-3) adapted from the one used by Interventional Radiology at the MD Anderson Cancer Center (courtesy of Dr. Michael Wallace, Chair Ad-Interim of the Department of Interventional Radiology) will be used to assign a value to the interventional radiologist's assessment of the lesion. An analogous 5-point subjective scale has been internally studied at MD Anderson Cancer Center and lesions given a score of ≤ 2 were found to have a lower yield rate for CMS-46 testing when compared to lesions that were scored ≥ 4 (24).

9.6.4.2 The radiologist will refer to the Radiology Biopsy Manual for case examples and detailed criteria for scoring and assign a pre-biopsy lesion score to the patient. Briefly, the qualitative assessment criteria for the 3-point scale is as follows:

Pre-Biopsy Lesion Scoring Scale (courtesy MD Anderson Cancer Center)

Score Assigned	Likelihood of Yield	Reason for Score & Biopsy Disposition
1	Low (< 25%)	Reason: no target amenable to biopsy, high risk procedure. Biopsy disposition: should not be performed.

Score Assigned	Likelihood of Yield	Reason for Score & Biopsy Disposition
2	Uncertain (25-75%)	Reason: Uncertainty about success either due to technical challenges or lesion characteristics (e.g., small size, necrotic, sub-solid lesion, sclerotic, not FDG–avid, technically difficult biopsy). Sclerotic bone lesion usually low yield. Biopsy disposition: Communication with investigator for these types of lesions should occur to determine whether to proceed with biopsy.
3	High (> 75%)	Reason: viable tumor demonstrated on diagnostic imaging (enhancing lesion, growing lesion) that can be sampled aggressively. Disposition: Proceed with biopsy.

9.6.4.3 Record the pre-biopsy lesion score and the name of the interventional radiologist on the screening eCRF in Rave. If a lesion is rated 1 or 2, the reason should be denoted on the CRF and this suspicion for low likelihood of success should be communicated to the investigator to discuss whether or not to proceed with the biopsy. Final disposition of the discussion (to proceed or not to proceed) should be documented on the CRF.

9.6.4.4 Pre-biopsy imaging and CRF with lesions score assessment and interventional radiologist's name are submitted in all cases, including those where decision is made to not proceed with biopsy.

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9.6.5 Biopsy Procedure

This section applies to patients who have not had tumor tissue acquired through normal clinical care for either diagnostic or therapeutic procedures. Tumor tissue acquired during regular clinical care would be harvested by the appropriate physician (e.g., neurosurgeon during craniotomy).

9.6.5.1 If a non-cutaneous lesion site is deemed appropriate for biopsy with minimal risk to the participant by agreement between the investigators, patient and Interventional Radiologist an attempt at biopsy will be made. All internal organ biopsies will be done by the Interventional Radiologist with a percutaneous approach, or during a clinically necessary surgical/endoscopic procedure, including craniotomy for brain metastasis or tumor.

- No endoscopic, laparoscopic, or surgical procedure will be done solely to obtain a biopsy for this protocol.

9.6.5.2 The choice of imaging modality to be used to facilitate tissue acquisition during the biopsy procedure will be decided by members of the Interventional Radiology team at the clinical site and may include ultrasound, CT scan, or MRI. Should CT scan be needed for biopsy, the number of scans for each procedure will be limited to the minimum number needed to safely obtain a biopsy. Tumor biopsies will be performed only if they are considered to be of low risk (< 2% major complication rate) to the participant as determined by the investigators and Interventional Radiologist. Biopsies will be performed under local anesthesia and/or sedation.

9.6.5.3 For the collection of biopsy specimens, fine needle aspiration followed by core needle biopsy, with submission of the FNA and the biopsy cores, is required. For all specimen types, the tumor cellularity yielded by FNA may be superior to core needle biopsy in some patients, particularly when the lesion is comprised predominantly of fibrosis, bone, or other non-tumor tissue. The FNA sampling with at least 2 passes should be sufficient to provide a specimen for preparation of smears and a cell block. It is preferred that at least four core biopsies 16-18 gauge in diameter and at least 1 cm in length after a fine needle aspiration specimen are obtained as described below. Biopsies will be sent for analyses as defined in the protocol.

9.6.5.4 Biopsy images should be submitted to IROC at the ACR Core Laboratory (see Section 11) and include image before needle placement, image with FNA or trocar needle in lesion, image with core biopsy or trocar needle in lesion, biopsy report describing target lesion location (organ, laterality or segment if applicable and image number on pre-biopsy imaging if available) and size.

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9.7 ECOG-ACRIN Sample Tracking System

It is **required** that all samples submitted on this trial be entered and tracked using the ECOG-ACRIN Sample Tracking System (STS). The software will allow the use of either 1) an ECOG-ACRIN user-name and password previously assigned (for those already using STS), or 2) a CTSU username and password.

When you are ready to log the collection and/or shipment of the samples required for this study, please access the Sample Tracking System software by clicking https://webapps.ecog.org/Tst

Important: **Failure to complete required fields in STS may result in assay result reporting delays.** Any case reimbursements associated with sample submissions will not be credited if samples are not logged into STS.

Additionally, please note that the STS software creates pop-up windows, so you will need to enable pop-ups within your web browser while using the software. A user manual and interactive demo are available by clicking this link: http://www.ecog.org/general/stsinfo.html Please take a moment to familiarize yourself with the software prior to using the system.

A shipping manifest form must be generated and shipped with all sample submissions.

Please direct your questions or comments pertaining to the STS to ecog.tst@jimmy.harvard.edu

Study Specific Notes

A time point summary (for STS) is provided in Section 7.2.3.

A Generic Specimen Submission Form (#2981) will be required only if STS is unavailable at time of sample submission. Indicate the appropriate Lab on the submission form:

- ECOG-ACRIN Central Biorepository and Pathology Facility

Retroactively enter all collection and shipping information when STS is available

Note: If the CBPF/MD Anderson TQL requests and receive nucleic acids from a designated outside laboratory, the receipt of materials will be logged into STS by the EA-CBPF.

Snapshot STS users guide for NCI-MATCH EAY131:

Most information, if entered incorrectly, may be corrected by the site. To correct mistakes for sample type or timepoint, contact the ECOG-ACRIN Lab Team (ecog.tst@jimmy.havard.edu or 857-504-2900) or the CBPF (eacbpf@mdanderson.org or (844) 744-2420). Please do not delete samples and re-log to correct these errors, especially after samples have already been shipped.

1. For protocol, enter “EAY131”
2. For Patient, enter the ECOG-ACRIN patient ID assigned upon pre-registration to step 0
3. Click “Log Samples”
4. Select the relevant time point (s) (see table below) and click “Select These Timepoints”
5. Select the sample types to be submitted. If the sample type is not listed, click on the blue word “Show” next to the preferred sample type and the alternative sample options will appear. If a sample type will not be submitted, it is requested that “Can’t submit” be indicated and a simple reason provided.
6. Click on “Ship Selected Samples”
7. Complete all information for the samples. Modify Pathology Status indicating if the tissue is “Tumor, Primary” or “Metastasis” and select the anatomical site from which the specimen was collected. Please provide specifics for anatomical location in the “Comments” section for the sample

8. Click “Create New Shipment”. NOTE: For EAY131, please do not pack samples from other trials or patients within the kit. One set of patient samples for one patient per kit.
9. Complete the shipping date, courier, and tracking number information. Indicate if the required documents are included in the shipment.
10. Click “Save” at the bottom of the page.

Print two copies of the shipping manifest, one to provide in the Box, the other for your own records.

9.8 Use of Specimens in Research

Specimens will be distributed to investigators for the laboratory research studies defined in Section 10.

Specimens from patients who consented to allow their specimens to be used for future approved research studies, including residuals from the currently defined research studies, will be retained in an ECOG-ACRIN-designated central repository. For this trial, specimens will be retained at the ECOG-ACRIN Central Biorepository and Pathology Facility at the University of Texas MD Anderson Cancer Center.

Samples will be retained, if at all possible, to allow “bridging” to an assay intended to be a companion diagnostic by the company providing the treatment drug(s).

For studies in which the ORR or PFS at 6 months is promising, every effort will be made to preserve tissue samples for "bridging" diagnostic studies. Specimens submitted will be processed to maximize their utility for current and future research projects and may include, but not be limited to, extraction of plasma, serum, peripheral blood leukocytes, serum, DNA, and RNA, and proteins.

If future use is denied or withdrawn by the patient, the samples will be removed from consideration for use in any future study.

9.9 Sample Inventory Submission Guidelines

Inventories of all samples submitted from institutions will be tracked via the ECOG-ACRIN STS. Receipt and usability will be verified by the receiving laboratory. Inventories of specimens forwarded and utilized for approved laboratory research studies will be submitted by the investigating laboratories to the ECOG-ACRIN Operations Office-Boston upon request in an electronic format defined by the ECOG-ACRIN Operations Office-Boston.

9.10 Institutional Biopsy Reimbursements

Research biopsies will be obtained from the primary tumor or metastasis at the time of screening biopsy or the time of end-of-treatment biopsy. Information on reimbursement for these research biopsies is provided in Section 4.12.

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10. Laboratory Clinical Research and Non-clinical Research Studies

The molecular profiling assays performed by the MATCH-assay laboratories will include massively parallel tumor sequencing (next generation sequencing) using a targeted Ampliseq panel, and certain other molecular assays e.g. immunohistochemistry (IHC). The genes interrogated on the NGS assay are listed in Appendix X .

All MATCH-assay laboratories are CLIA or CAP/CLIA certified laboratories with experience in clinical next generation sequencing on the Ion Torrent PGM or S5. Analytical validation studies assessing reproducibility, accuracy, precision, lower limit of detection, false positive and false negative rates have been completed; proficiency assessments are planned every 6 months. Analytical validation and proficiency assessments will be performed any time the assay is "versioned" (gene variant added or deleted), and the assay will be locked for the updated version. Specific ongoing QA/QC measures are noted below.

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MD Anderson Cancer Center Molecular Diagnostics NGS Laboratory will also serve as the receiving laboratory for all specimens, will be responsible for the preanalytic procedures and will also perform all mandatory immunohistochemical (IHC) assessments required to be conducted centrally. This laboratory will lead the performance of procedures and documentation required, as necessary, for New York State Department of Health licensure.

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The Molecular Characterization Laboratory at the Frederick National Laboratory for Cancer Research will lead the validation planning and procedures required for the U.S Food and Drug Administration Center for Diagnostics and Radiologic Health.

The Massachusetts General Hospital Center for Integrated Diagnostics, and the Yale University Clinical Molecular Pathology lab will participate with all the labs in carrying out assay validation and performance proficiency procedures. Additionally, a new CLIA certified/CAP accredited laboratory, the Dartmouth-Hitchcock Laboratory for Clinical Genomics and Advanced Technologies, will join the NCI-MATCH laboratory network and begin NGS testing of MATCH FFPE specimens in 2017. All the labs will participate as NGS sequencing centers for patient diagnostics in the NCI-MATCH trial.

For confirmation testing, some tumors may undergo additional orthogonal assessments on different platforms to confirm variants that may not be analyzed by the MATCH-assay. Results from orthogonal assessments are separate from the MATCH-assay and will not be reported to the site or the patient.

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10.1 Targeted Genetic Analysis for MATCH eligibility

The tumor biopsy materials will be processed as follows (See Appendix I for work flow):

10.1.1 DNA extraction and RNA Extraction: The H&E section will serve to confirm original diagnosis and also estimate tumor nuclei content. The remaining biopsy and whole blood will be extracted for nucleic acids using the Qiagen FFPET All-Prep procedure.

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10.1.2 Genomic Analysis

Molecular profiling will be performed in a study-specific network of approved CLIA-certified Molecular Characterization Laboratories noted above.

DNA will be assessed for quantity and quality by spectroscopy (OD 260/280) and a PCR-based amplification quality assessment test. All specimens that meet necessary quantity and quality will be sequenced using a targeted sequencing assay. This assay will identify those mutations that are used for treatment assignment.

Sequencing will be done on the Ion Torrent PGM or S5 using a targeted ampliseq panel of about 161 genes, (see Appendix X). The assay will report SNV, indel, amplifications and translocations; a subset of these will be actionable based on treatments in MATCH.

Mutations will be reported based on their presence in tumor cells and the number of reads demonstrating the pre-defined mutation as indicated. Amplifications will be reported as fold amplified; translocations will be reported by genes involved. If actionable mutations are detected in more than one pathway, treatment will be assigned based on the level of evidence and treatment assignment rules as stated in Section 1.5

The laboratory will return MATCH assay results (whether a mutation/amplification/translocation of interest has been detected and the results of any additional molecular diagnostic tests), including details about the specific mutation/pathways for assignment to a treatment subprotocol based on predefined criteria. Patients in whom actionable mutations, amplifications or translocations are not detected during Step 0 screening will not continue to Step 1 registration and will not be followed. The analysis could take up to 4 weeks, or longer if additional procedures are required to obtain enough tissue of appropriate quality to run the assay.

For the original screening process (i.e. submission of tissue for testing by a MATCH laboratory), all specimens will be assessed for expression or no expression of PTEN, MLH1 and MSH2. Rb by IHC will only be done in patients who are positive for CCND1, 2, or 3 amplification or CDK4 or CDK6 amplification. Additional tumor tissue specimens may be requested from selected patients for the performance of repeat MLH1 and MSH2 IHC assessments for confirmation of eligibility for relevant treatment arms.

Patients who enter MATCH subprotocols with an approved “rare variant” via an outside assay will have archived tissue (as similar as possible to the tissue that was sequenced by the outside assay) assessed by the NCI-MATCH molecular profiling assays, as above. No IHC assessments will be performed on these specimens, unless necessary for eligibility on the applicable arms. Results of these assays will be returned to the clinical site for sharing with the patient.

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10.1.3 Site Notification of Pre-defined Mutations

If adequate materials are submitted, the mutation status will be completed within 4 weeks of receipt of materials. Results will be forwarded from the laboratory directly to the site contact. Only MATCH-assay assessment results on tissue or bone marrow submitted directly by the participating site will be returned to sites.

Results will not be distributed to sites for confirmation sequencing assessments performed on residual nucleic acids received from any referring Designated Outside laboratory.

For patients who will be evaluated for treatment assignment based on a MATCH-assay screening assessment, it may take a few days from the receipt of biopsy results for potential treatment assignments to be made available via Medidata Rave. For these patients, registration to treatment cannot occur until the central review determination is complete.

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10.1.4 Turnaround Time Monitoring

We will monitor turnaround time on at least a monthly basis, as it is tracked in MATCHbox. If turnaround time extends beyond 21 days median, we will bring this to the steering committee for consideration of remedies, which may include increasing laboratory or pathology capacity.

Because there are a number of reasons why results may be delayed, a dedicated call center has been created at MD Anderson that will monitor specimen progress and alert the clinical site when there are expected to be delays or when a specimen is not adequate and may need to be repeated.

10.2 Research Studies

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10.2.1 Whole Exome and other Sequencing

This assessment will be performed on samples from patients who agree to allow their samples to be used for optional research studies (not for treatment assignment). These assays will not be done in a clinical laboratory (e.g. CLIA certified) and results will not be returned to the clinical site, but will be used to understand response or non-response in patients who have been treated on one of the MATCH treatment arms.

De-identification of patient biospecimens will be performed in a secure manner prior to use for research purposes. The de-identified data will be stored on a controlled access database for use by other researchers.

If sufficient DNA is available, whole-exome, mRNA and other sequencing will be performed retrospectively for research purposes on samples with identifiers de-linked. RNA sequence will be performed if sufficient RNA is available. These studies will be exploratory, and will assess other molecular features that may have influence on response or lack of response to the treatment, as well as potential resistance mechanisms.

Whole blood and plasma will also be sequenced to compare to tumor sequencing (deidentified).

Other researchers may apply for remaining specimens for MATCH using standard mechanisms developed by CTEP and the NCTN. Proposals will be reviewed for scientific merit. Funding will not be

provided for these studies, but will need to be provided by the applicants. Such studies may include bridging studies for companion diagnostics proposed by pharmaceutical companies participating in MATCH.

10.2.2 Sample Inventory Submission Guidelines

Inventories of all samples submitted from institutions will be tracked via the ECOG-ACRIN STS and receipt and usability verified by the receiving laboratory. Inventories of specimens forwarded and utilized for approved laboratory research studies will be submitted by the investigating laboratories to the ECOG-ACRIN Operations Office-Boston on a monthly basis in an electronic format defined by the ECOG-ACRIN Operations Office-Boston.

10.2.3 Lab Data Transfer Guidelines

The data collected on the above mentioned laboratory research studies will be submitted electronically using a secured data transfer to a central NCI database by the investigating laboratories on a quarterly basis.

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11. Imaging Submissions

ACR Imaging Core Laboratory, through funding provided under IROC, will perform image collection via TRIAD® software v4. TRIAD can be installed on one or several computers of choice within the institutional "firewall" and on the institutional network. The TRIAD application can then be configured as a DICOM destination on a scanner and/or PACS system for direct network transfer of study related images into the TRIAD directory. Once configured, TRIAD software de-identifies, encrypts, and performs a lossless compression of the images before they are transferred to the ACR Imaging Core Laboratory and image archive in Philadelphia. Subjects enrolled at screening/registration (Step 0) via RSS / OPEN will be selectable in TRIAD following registration by the participating institution. CTEP IAM credentials will be used to access TRIAD software for image submission via CD/DVD, PACS or scanner. Image submission will require a 'TRIAD site user' role, a designation that can be obtained through the CTSU Administrator.

The following categories of imaging will be collected:

1. Pre-enrollment imaging – all subjects including those who do not go forward with biopsy nor with treatment.
2. Biopsy imaging – all subjects
   a. Image before needle placement
   b. Image with FNA or trocar needle in lesion
   c. Image with core biopsy or trocar needle in lesion
   d. Biopsy report describing target lesion location (organ, laterality or segment if applicable and image number on pre-biopsy imaging if available) and size.
3. Followup imaging on targeted therapy – all subjects on all therapy arms and all timepoints
4. Imaging documenting progression on targeted therapy – all subjects in therapy arms
5. Imaging at re-biopsy – all subjects who undergo re-biopsy

For categories 1, 3, and 4, CT, MRI and nuclear medicine (including PET-CT) of all body parts will be collected. Plain film X-ray, ultrasound, and fluoroscopy images will not be collected. For categories 2 and 5, images of any modality used for biopsy will be collected. The images and time points to be collected are represented in the schema below:

	All Subjects				
Time Point Name	Pre-enrollment[1]	Biopsy[2]	Follow-up[1]	Progression[1]	Re-biopsy[3]
Types of Imaging to Be Collected	CT, MRI, nuclear medicine (including PET/CT)	Any imaging completed should be submitted	CT, MRI, nuclear medicine (including PET/CT)	CT, MRI, nuclear medicine (including PET/CT)	Any imaging completed should be submitted
	Submit to ACR Core Laboratory via TRIAD				

[1] Imaging collected for each subject may include CT, MRI, or nuclear medicine scans. Plain film X-ray, ultrasound, and fluoroscopy images will not be collected.
[2] See above for required imaging.
[3] If applicable.

A separate Image Submission Manual will describe the specifics of the image collection procedures. A correlative proposal will detail the plan for image analysis.

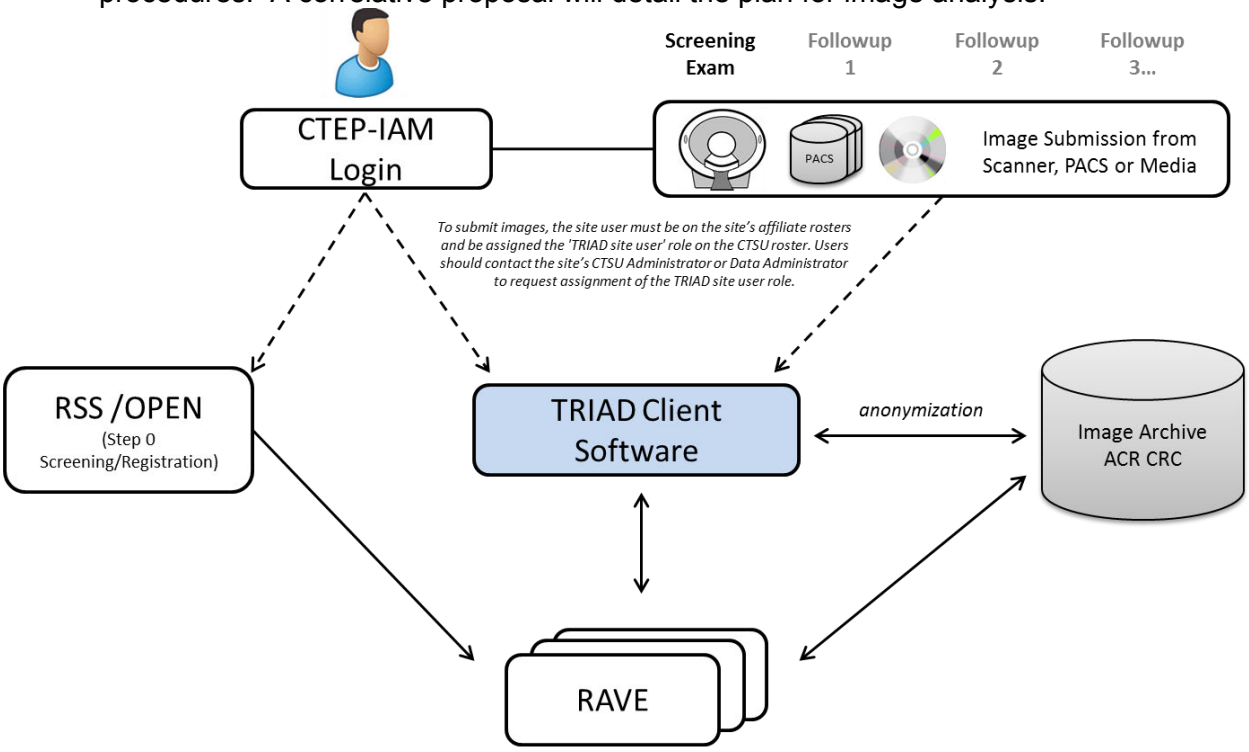

Image submission overview: All imaging will be de-identified and submitted via ACR TRIAD software. Subjects enrolled at Step 0 Screening/Registration via RSS / OPEN will be selectable in TRIAD following registration by the participating institution. CTEP IAM credentials will be used to access TRIAD software for image submission via CD/DVD, PACS or scanner.
TRIAD installation and Support information: triad-support@acr.org

12. Electronic Data Capture

Please refer to the MATCH Forms Completion Guidelines for the forms submission schedule. Data collection will be performed exclusively in Medidata Rave.

This study will be monitored by the CTEP Data Update System (CDUS) version 3.0. Cumulative CDUS data will be submitted quarterly from the ECOG-ACRIN Operations Office-Boston to CTEP by electronic means.

12.1 Records Retention

FDA regulations (21 CFR 312.62) require clinical investigators to retain all trial-related documentation, including source documents, long enough to allow the sponsor to use the data to support marketing applications.

All records pertaining to the trial (including source documents) must be maintained for:

- two years after the FDA approves the marketing application, or
- two years after the FDA disapproves the application for the indication being studied, or
- two years after the FDA is notified by the sponsor of the discontinuation of trials and that an application will not be submitted.

Please contact the ECOG-ACRIN Operations Office-Boston prior to destroying any source documents.

13. Patient Consent and Peer Judgment

Current FDA, NCI, state, federal and institutional regulations concerning informed consent will be followed.

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14. References

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Appendix I

Biopsy Workflow Schematic

Rev. Add13 **NOTE:** The below workflow schematic applies to the original screening process (i.e. for patients submitting tissue to the MATCH trial laboratories for testing/screening purposes after Step 0 registration) and will remain in the protocol as a historical reference. As of May 22nd, 2017 new patients can longer be registered to the study for this original screening process, and can only enter the study via the Outside Assay process.

<table>
<tr><th colspan="5">Prior to specimen receipt by MDACC</th></tr>
<tr><td rowspan="2">Site</td><td colspan="4">Registered to Screening
(Prior to biopsy)</td></tr>
<tr><td colspan="4">Request Specimen Kit
Step 0, automatic [MATCH-ASSAY BIOPSY SCREENING CLOSED MAY 22, 2017]
Steps 2, 4 or 8 via form</td></tr>
<tr><td>MD Anderson CBPF</td><td colspan="4">Ships kit to site within 24 hours</td></tr>
<tr><td rowspan="3">Site</td><td colspan="4">Collects specimens per protocol requirements</td></tr>
<tr><td colspan="4">Logs samples into the EA Sample Tracking System (STS)</td></tr>
<tr><td colspan="4">Ship samples to TQL</td></tr>
<tr><th colspan="5">MDACC – Day 1 (Note: Workflow for residual nucleic acids from designated laboratories, jump to MDACC-Day 2)</th></tr>
<tr><td>Shipper/Receiving Dock</td><td colspan="4">Delivery of shipment to MD Anderson</td></tr>
<tr><td rowspan="13">TQL</td><td colspan="4">Retrieval and transport of shipment from Receiving Dock to TQL</td></tr>
<tr><td colspan="4">Opening of shipping kit and examination of specimen containers condition</td></tr>
<tr><td colspan="4">Receipt logged into EA STS and relevant MD Anderson database</td></tr>
<tr><td colspan="4">Accessioning with Seq#, recording of specimen conditions</td></tr>
<tr><th colspan="3">TISSUE</th><th>BLOOD</th></tr>
<tr><td colspan="3">Gross examination & dictation of specimens into PowerPath, comparison with description from enrolling institution</td><td>Centrifuge</td></tr>
<tr><td rowspan="2">For fresh biopsy submissions</td><td colspan="2">Transfer into barcoded cassettes with one core in each, and loading of processor</td><td>Processed buffy coat for DNA and freezing</td></tr>
<tr><td colspan="2">Removal of tissue from processor and embedding</td><td>Aliquoting and freezing of plasma</td></tr>
<tr><td colspan="3">Sectioning for required number and type of slides, and H&E staining of scout slide</td><td>Storage of frozen specimens and documentation</td></tr>
<tr><td colspan="3">Histopathologic examination, confirmation and demarcation of sufficient tumor</td><td>DNA and RNA extraction</td></tr>
<tr><td colspan="3">For screening and “rare-variant” confirmation assessments only</td><td rowspan="2">DNA and RNA QA/AC, quantitation, and documentation</td></tr>
<tr><td colspan="2">Decision on micro dissection or laser capture micro dissection (LCM)</td><td>TQL: Delivery of unstained slides to IHC and FISH labs</td></tr>
<tr><td colspan="2">Microdissection (or LCM if required) of demarcated tumor</td><td>IHC/FISH Labs: performance of IHC</td><td>Storage of frozen RNA and DNA</td></tr>
</table>

			and FISH, generation of reports	
	Image capture of scout slides and of microdissected slide(s)		Results reported to MATCHbox	
Clincial Image Analysis Lab	Image analysis for % of tumor cell nuclei in mocrodissected areas			
TQL	QA/QC check of microdissected slides and images for copy number variation-level purity and T/N quantity by imaging			
	DNA and RNA extraction over night	Storage of blocks and documentation		
MDACC – Day 2				
TQL	DNA and RNA QA/QC, quantitation, and documentation			
	Recording for 100ng each of DNA and RNA, packaging, documentation			
	Notification of destination MATCH Sequencing Lab and E-A Operations Office of analyte package overnight shipment.			
Courier	OVERNIGHT Shipping			
Day 3				
Sequencing Lab	Receipt of analysis package for sequencing.			

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Appendix II

Pathology Submission Guidelines

The following items are included in Appendix II:

1. List of Required Materials for MATCH
2. Instructional memo to submitting pathologists
3. ECOG-ACRIN Generic Specimen Submission Form (#2981)
4. CLIA Laboratory Sample Submission Form

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List of Required Material

MATCH: Molecular Analysis for Therapy Choice (MATCH)

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REQUIRED PATHOLOGY MATERIALS:

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Please review the study schema for a summary on when tumor tissue for screening assessments (Step 0 and Step 2 or 4) and progression (Step 8 after completion of all treatment on MATCH) is to be submitted.

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NOTE: Performance of biopsies during Step 0 for screening was closed to enrollment on May 22, 2017. Step 2 (or 4) screening is open only for patients who were originally assigned and registered to Step 1 treatment based on MATCH-assay screening results.

A repeat biopsy procedure, or bone marrow aspirate for multiple myeloma (if applicable), may be requested if submitted screening biopsy materials are inadequate for the first attempted screening assessments for Step 0 or Step 2 (or 4). Blood samples, from consenting patients, will also be collected and submitted with each biopsy (see Section 9), end of cycle 2 or treatment on each MATCH subprotocol, and at Step 8.

For a tumor sample to be considered adequate for copy number variation evaluation, a minimum of 70% tissue must be tumor after manual microdissection for tumor cell enrichment at the MD Anderson Tissue Qualification Laboratory. However, the assays will be attempted on tissue specimens that have at least 20% non-necrotic tumor cells present, which have been shown to be adequate for sequencing.

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Prior clinical pathology and molecular studies reports, including prior sequencing assessments, from the original diagnostic procedures and/or most recent procedure performed prior to step 0 registration, are required for central confirmation of histology and MUST be submitted with all screening specimens, or the sample may not be processed (see Section Error! Reference source not found.). This information is required for treatment assignment and WILL RESULT IN DELAYS if not provided in a timely manner.

Pathology reports submitted with tissue specimens are NOT to be redacted. Results from screening and confirmation testing are returned to the site and will impact patient care. Adequate identification must be provided to ensure that the appropriate materials have been submitted and reporting is correct and to maintain "chain-of-custody".

All sample submissions MUST be logged and tracked in the ECOG-ACRIN Sample Tracking System (STS). If STS is unavailable at time of submission, a completed ECOG-ACRIN Generic Specimen Submission Form (#2981) may be submitted with the biopsy materials with STS updated retroactively as soon as possible. Failure to update STS may result in delays in the central assessments and reporting results to the site.

Biopsy guidelines and blood submission guidelines are provided in Section 9.

NOTE: The order form for sample submission Kits is located in Appendix XII.

NOTE: For the collection of biopsy specimens, fine needle aspiration followed by core needle biopsy is the preferred technique.

Rev.5/16

A. Pre-Registration (Step 0)

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1. Screening by the MATCH-assay **– CLOSED MAY 22, 2017**

Tumor tissue specimens are to be submitted following pre-registration to Step 0. If tumor tissue is collected for the trial by a procedure following pre-registration to step 0, tissue is to be sent the day of collection.

For multiple myeloma, not plasmacytomas, fresh bone marrow aspirates collected in an EDTA vacutainer and smears must be submitted for all screenings. For plasmacytomas, follow the instructions for the solid tumor tissue submission requirements.

For all other disease sites, FFPE blocks which meet the criteria below may submitted in lieu of fresh biopsy material for the first or repeat (if performed) screening attempt on Step 0, but not both.

Options for sample submissions:

Step 0	Biopsy 1	Biopsy Repeat
Option 1	Fresh Biopsy	Fresh biopsy (#2)
Option 2	Fresh Biopsy	Pre-Trial FFPE[1]
Option 3	Pre-Trial FFPE[1]	Fresh Biopsy
Outside Assay	Pre-Trial FFPE[2]	N/A

1 If archived FFPE is submitted for analysis and additional FFPE is requested to meet the needs for the screening, the additional materials are considered to be for the same assessment and are logged under the same time point as the initial FFPE submission in STS.

2. For patients for whom an applicable "rare variant" was identified by one of the outside designated laboratories (see Appendix XIV), the collection and submission of tumor tissue for the confirmation of the "rare variant" must follow the guidelines for the submission of tissue for screening at Step 0, Biopsy 1 options with the following changes:
   - FFPE tissue may be submitted after but no later than 8 weeks following registration to Step 1.
   - Submission should be logged under the STS time point named "Confirmation of Pre-Registration Outside Results"

Submission Requirements

- Screening tissue submissions:
  a) Four fresh core biopsies in 10% NBF. FNA slide and FNA cells in cytolyte also requested to be submitted. FNA, with cyto-pathologist real-time review, is to be performed to verify tumor tissue is being collected. For alternative acceptable submissions, see Section 9.

  OR

  b) FFPE Block(s): Metastatic tissue preferred. Tissue must have been collected within 6 months prior to pre-registration to Step 0 and patient must not have received any intervening therapy as excluded in Section 3.1.6.3 (see Appendix XV). FFPE tumor tissue block(s) must be submitted. The optimal block is 70% tumor tissue. Specimen size requirement is as follows:
     - Surface area of 25mm^2 is optimal. Minimum is 5mm^2 and
     - Volume: 1mm^3 optimal. Minimum volume is 0.2mm^3, however the success of DNA extraction decreases at suboptimal tissue volume

Specimens of suboptimal size, cellularity, or tumor content will require submission of more than one tumor block

OR

c) MULTIPLE MYELOMA ONLY: SUBMIT ASPIRATES FROM THE FIRST PULL. For plasmacytomas, see (a) and (b) above.

Draw the bone marrow aspirate materials into a plain syringe, then:

1. Use 1 drop to make 4 – 5 smears.
2. Put 1 – 1.5 ml in one EDTA tube.
3. Put remainder (5 - 10 ml) of bone marrow aspirate in the other EDTA tube.

- Forms and Reports
  a) STS-generated shipping manifest
  b) Completed CLIA Laboratory Sample Submission Form
  c) Pertaining to original diagnostic primary and most recent biopsies:
     - Pathology report
     - All molecular, histological, and cytological reports, including prior sequencing studies
     - Do NOT redact the reports.
  d) Upon completion of review, the Institutional pathology report is to be submitted via Medidata Rave.

NOTE: If the submitted material is inadequate for testing, a repeat biopsy for the screening assessment will be requested if the patient agrees.

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2. Pre-trial representative tumor tissue

Tissue requirements: The optimal block is 70% tumor tissue. Specimen size requirement is as follows:

- Surface area of 25mm^2 is optimal. Minimum is 5mm^2 and
- Volume: 1mm^3 optimal. Minimum volume is 0.2mm^3, however the success of DNA extraction decreases at suboptimal tissue volume

If an existing block cannot be provided, the number of slides needed are dictated by the surface area and volume requirements above. It is important that slides be sent as soon as possible after cutting to be adequate for nucleic acid extraction.

a. Tissue submitted for confirmation of “rare variant” assessments, as determined by a laboratory outside of the MATCH trial, tissue from the same specimen and timepoint (if possible) to that assayed by the outside laboratory is to be submitted either (a) upon request for mandatory central IHC required to determine treatment assignment or (b) within 8 weeks following registration to Step 1 treatment. If not available, submit any available archived tumor tissue. Tissue requirements in terms of tumor content are equivalent to that outlined above for screening submissions.

   It is essential that the tissue submitted meet the guidelines indicated above. If tissue is unavailable for submission, the UNREDACTED pathology report and a statement justifying reason for inability to submit tissue must still be provided to

the MD Anderson TQL. Additionally, the inability to submit the material must be documented in the EA Sample Tracking System.

NOTE: For these types of submissions, **PLEASE STATE ON THE SHIPPING MANIFEST IF ENCLOSED SUBMITTED TISSUE WAS COLLECTED AT THE SAME TIME AS THE TISSUE SENT TO THE OUTSIDE LABORATORY OR IF IT IS FROM A DIFFERENT COLLECTION DATE THAN THE TISSUE SENT TO THE OUTSIDE LABORATORY.** This information may be important for assessment of discordant results.

NOTE: If adequate tissue is not available for confirmation testing, the MD Anderson TQL may reach out to the referring designated outside laboratory to request residual nucleic acids to complete these assessments. Results from residual nucleic acids will not be distributed to the site as sequencing results were already provided to the site by the original referring designated laboratory.

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b. Additional tissue submitted from patients who consented to allow additional tissue to be submitted for research. available tissue is requested from any procedure performed, but if materials are limited the primary tumor tissue is preferred.

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B. Progression following Treatment – Submission of material for SECOND SCREENING

If progression occurs after a CR/PR or after 6 months on assigned study treatment or the first screening biopsy occurred > 6 months, to be considered for additional treatment a biopsy is required. Tissue is to be sent day of collection. Delays of submission should be reported to the CBPF.

Patients who are to be considered for a second round of treatment on this trial MUST be registered to a screening step (STEP 2 or STEP 4) prior to submission of the biopsy material in order for the submitted tissue to be tagged as SCREENING materials.

- Tissue submissions:
  a) Four fresh core biopsies in 10% NBF. FNA slide and FNA cells in cytolyte also requested to be submitted. FNA, with cyto-pathologist real-time review, is to be performed to verify tumor tissue is being collected. Fresh excisional biopsy or tumor captured during a clinical procedure may be submitted in lieu of the core biopsy and FNAs. For alternative acceptable submissions, see Section 9.

  OR

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  b) MULTIPLE MYELOMA ONLY: IF BM ASPIRATE WILL BE SUBMITTED, SUBMIT ASPIRATES FROM THE FIRST PULL.

  NOTE: For plasmacytomas, follow (a) above.

  Draw the bone marrow aspirate materials into a plain syringe, then:

  1. Use 1 drop to make 4 – 5 smears.
  2. Put 1 – 1.5 ml in one EDTA tube.
  3. Put remainder (5 - 10 ml) of bone marrow aspirate in the other EDTA tube.

- Forms and Reports
  a) STS-generated shipping manifest
  b) Completed CLIA Laboratory Sample Submission Form

c) Pertaining to original diagnostic primary and most recent biopsies:
- Pathology report
- All molecular, histological, and cytological reports
- Do NOT redact the reports.

d) Upon completion of review, if performed, the Institutional pathology report is to be submitted via Medidata Rave.

NOTE: If the submitted material is inadequate for testing, a repeat biopsy for the screening assessment will be requested if the patient agrees.

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C. Progression after completion of all EAY131 Protocol Treatment

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NOTE: For plasmacytomas, follow the instructions in (a) above.

A research biopsy will be requested upon progression once all treatment has been completed on the MATCH study for research purposes only in order to determine mechanisms of resistance. No results of the research tests performed to determine potential mechanisms of resistance will be provided to the physician or patients. This biopsy may or may not be performed depending on the patient's status, signed patient consent and registration to Step 8. If disease progression on the last treatment that the patient receives on the MATCH study occurs within 6 months of the most recent screening biopsy, no "end of treatment" biopsy material is needed/requested. If this research biopsy is performed, the tissue is to be sent day of collection. Delays of submission should be reported to the CBPF.

- Tissue submissions:

a) Four fresh core biopsies in 10% NBF. FNA slide and FNA cells in cytolyte also requested to be submitted. FNA, with cyto-pathologist real-time review, is to be performed to verify tumor tissue is being collected. For alternative acceptable submissions, see Section 9.

OR

b) MULTIPLE MYELOMA ONLY: SUBMIT ASPIRATES FROM THE FIRST PULL.

Draw the bone marrow aspirate materials into a plain syringe, then:

1. Use 1 drop to make 4 – 5 smears.
2. Put 1 – 1.5 ml in one EDTA tube.
3. Put remainder (5 - 10 ml) of bone marrow aspirate in the other EDTA tube.

- Forms and Reports

a) STS-generated shipping manifest

b) Completed CLIA Laboratory Sample Submission Form

c) Pertaining to original diagnostic primary and most recent biopsies:
- Pathology report
- All molecular, histological, and cytological reports, including prior sequencing studies.
- Do NOT redact the reports.

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d) Upon completion of review, if performed, the Institutional pathology report is to be submitted via Medidata Rave.

NOTE: If the submitted material is inadequate for testing, a repeat biopsy for the screening assessment will be requested if the patient agrees.

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D. SUBMISSION REQUIREMENTS:

Samples must be labeled with

Patient's name (last, first)
Protocol number (EAY131)
The patient's EAY131 ECOG-ACRIN case ID
Date and time of sample collection
Institution name

Reminder: Prior clinical pathology and molecular studies reports, from the original diagnostic procedures and/or most recent procedure performed prior to step 0 registration, are required for central confirmation of histology and MUST be submitted with all screening specimens, or the sample may not be processed.

Ship to the following address:

ECOG-ACRIN Central Biorepository and Pathology Facility
MD Anderson Cancer Center
Department of Pathology, Unit 085
Tissue Qualification Laboratory for ECOG-ACRIN, Room G1.3598
1515 Holcombe Blvd
Houston, TX 77030

For results and general inquiries:

MATCHTRIAL@mdanderson.org & MATCH@jimmy.harvard.edu

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Phone: 1-844-744-2420 (OPTION 4) Fax: 713-745-4925

For all kit related inquiries:

EACBPF@mdanderson.org

Phone: 1-844-744-2420 Fax: 713-563-6506

Robert L. Comis, MD, and Mitchell D. Schnall, MD, PhD
Group Co-Chairs

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MEMORANDUM

TO: _____
(Submitting Pathologist)

FROM: Stanley Hamilton, M.D., Chair
ECOG-ACRIN Laboratory Science and Pathology Committee

DATE: _____

SUBJECT: Submission of Pathology Materials for EAY131: Molecular Analysis for Therapy Choice (MATCH)

The patient named on the attached request has been entered onto an ECOG-ACRIN protocol by _____ (ECOG-ACRIN Investigator). This protocol requires the submission of pathology materials for screening assessments and research.

Keep copies of all submitted forms for your records and return the completed Requisition Form, the surgical pathology report(s), the slides and/or blocks and any other required material (see List of Required Material) to the Clinical Research Associate (CRA). The CRA will forward all required pathology material to MD Anderson Pathology Laboratory.

Guidelines for performance of the biopsies are provided in Section 9 of the protocol.

Reports submitted with the tissue are NOT to be redacted, as adequate identification is required to ensure the appropriate materials have been received and to maintain “chain-of-custody” of the materials.

A report of the screening results or confirmation testing results from the evaluation of tissue or bone marrow provided by the site, including the identification of the trial-specific actionable targets, will be returned to the site and may impact patient care.

Residual materials from specimens submitted for this study will be retained at the ECOG-ACRIN Central Biorepository for future studies. If you have any questions regarding this request, please call 1-844-744-2420 (713-745-4440 Local or International Sites), Fax: 713-745-4925, or Email: MATCHTRIAL@mdanderson.org.

The ECOG-ACRIN CRA at your institution is:

Name:_____

Address:_____

Phone: _____

Thank you.

ECOG-ACRIN Generic Specimen Submission Form

Form No. 2981v3

Institution Instructions: This form is to be completed and submitted with **all specimens** ONLY if the Sample Tracking System (STS) is not available. **Use one form per patient, per time- point.** All specimens shipped to the laboratory must be listed on this form. Enter all dates as MM/DD/YY. Keep a copy for your files. Retroactively log all specimens into STS once the system is available. **Contact the receiving lab to inform them of shipments that will be sent with this form.**

Protocol Number _____ **Patient ID** _____ **Patient Initials** Last _____ First _____

Date Shipped _____ **Courier** _____ **Courier Tracking Number** _____

Shipped To (Laboratory Name) _____ **Date CRA will log into STS** _____

FORMS AND REPORTS: Include all forms and reports as directed per protocol, e.g., pathology, cytogenetics, flow cytometry, patient consult, etc.

Required fields for all samples				**Additional fields for tissue submissions**				**Completed by Receiving Lab**
Protocol Specified Timepoint:								
Sample Type (fluid or fresh tissue, include collection tube type)	**Quantity**	**Collection Date and Time 24 HR**		**Surgical or Sample ID**	**Anatomic Site**	**Disease Status** (e.g., primary, mets, normal)	**Stain or Fixative**	**Lab ID**

Fields to be completed if requested per protocol. Refer to the protocol-specific sample submissions for additional fields that may be required.					
Leukemia/Myeloma Studies:	**Diagnosis**	**Intended Treatment Trial**	**Peripheral WBC Count (x1000)**	**Peripheral Blasts %**	**Lymphocytes %**
Study Drug Information:	**Therapy Drug Name**	**Date Drug Administered**	**Start Time 24 HR**	**Stop Time 24HR**	
Caloric Intake:	**Date of Last Caloric Intake**		**Time of Last Caloric Intake 24HR**		

CRA Name _____ **CRA Phone** _____ **CRA Email** _____

Comments

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THE UNIVERSITY OF TEXAS
MD Anderson Cancer Center
Making Cancer History®

Department of Pathology
Tissue Qualification Laboratory
T 713-745-4901 F 713-745-4925
T 844-744-2420 (Toll Free)
Unit 0085
1515 Holcombe Blvd
Room G1.3598
Houston, Texas 77030

CLIA Laboratory Sample Submission Form

The information on this form is required for results reporting from the CLIA laboratory. This form MUST be completed and submitted with all specimens. Failure to submit this form will result in a delay in tissue processing and a delay in the reporting of results to the ordering physician.

Section I. TRIAL INFORMATION

ECOG-ACRIN Trial Number: ☐ EAY131 (NCI-MATCH) ☐ EA1131 (PAM50)
☐ Other ECOG-ACRIN TRIAL NUMBER: _____

ECOG-ACRIN Patient ID: _____

Section II. SITE'S TIME ZONE INFORMATION

Time Zone: ☐ Atlantic ☐ Eastern ☐ Central ☐ Mountain ☐ Pacific ☐ Alaska ☐ Hawaii-Aleutian
☐ Other: _____ Is Daylight Saving Time observed: ☐ Yes ☐ No

Section III. PATIENT INFORMATION

Last Name: _____ First Name: _____
Date of Birth (MM/DD/YYYY): _____ Gender: ☐ Female ☐ Male

Section IV. SITE CONTACTS: Sections A and B must be completed.

If applicable, has or will the tissue submitted be reviewed by a local pathologist? ☐ Yes ☐ No
If yes, Section C must be completed and a copy of the local pathology report must be uploaded into Medidata Rave within 5 days of submission of the tissue or per protocol instructions.

Section A Ordering Physician	Section B Site CRA or Research Contact	Section C Pathology Group
Last Name:	Last Name:	Last Name (optional):
First Name:	First Name:	First Name (optional):
NPI:		
Phone:	Phone:	Phone:
Fax:	Fax:	Fax (optional):
Institution:	Institution:	Institution:
Department:	Department:	Department:
Street Address Line 1:	Street Address Line 1:	Street Address Line 1:
Street Address Line 2:	Street Address Line 2:	Street Address Line 2:
City:	City:	City:
State: Zip Code:	State: Zip Code:	State: Zip Code:
Physician E-mail (optional):	Research Contact E-mail (optional):	Pathologist E-mail (optional):
Office Contact Name (optional):	Office Contact Name (optional):	Office Contact Name (optional):
Office Contact E-mail (optional):	Office Contact E-mail (optional):	Office Contact E-mail (optional):

Molecular Analysis for Therapy Choice (MATCH)

Appendix III

Patient Thank You Letter

We ask that the physician use the template contained in this appendix to prepare a letter thanking the patient for enrolling in this trial. The template is intended as a guide and can be downloaded from the ECOG-ACRIN web site at http://www.ecog.org. As this is a personal letter, physicians may elect to further tailor the text to their situation.

This small gesture is a part of a broader program being undertaken by ECOG-ACRIN and the NCI to increase awareness of the importance of clinical trials and improve accrual and follow-through. We appreciate your help in this effort.

[PATIENT NAME] [DATE]

[PATIENT ADDRESS]

Dear [PATIENT SALUTATION],

Thank you for agreeing to take part in this important research study. Many questions remain unanswered in cancer. With the participation of people like you in clinical trials, we will improve treatment and quality of life for those with your type of cancer.

We believe you will receive high quality, complete care. I and my research staff will maintain very close contact with you. This will allow me to provide you with the best care while learning as much as possible to help you and other patients.

On behalf of ***[INSTITUTION]*** and the ECOG-ACRIN Cancer Research Group, we thank you again and look forward to helping you.

Sincerely,

[PHYSICIAN NAME]

Molecular Analysis for Therapy Choice (MATCH)

Appendix IV

CRADA/CTA

The agent(s) supplied by CTEP, DCTD, NCI used in this protocol is/are provided to the NCI under a Collaborative Agreement (CRADA, CTA, CSA) between the Pharmaceutical Company(ies) (hereinafter referred to as "Collaborator(s)") and the NCI Division of Cancer Treatment and Diagnosis. Therefore, the following obligations/guidelines, in addition to the provisions in the "Intellectual Property Option to Collaborator" (http://ctep.cancer.gov/industryCollaborations2/intellectual_property.htm) contained within the terms of award, apply to the use of the Agent(s) in this study:

1. Agent(s) may not be used for any purpose outside the scope of this protocol, nor can Agent(s) be transferred or licensed to any party not participating in the clinical study. Collaborator(s) data for Agent(s) are confidential and proprietary to Collaborator(s) and shall be maintained as such by the investigators. The protocol documents for studies utilizing Agents contain confidential information and should not be shared or distributed without the permission of the NCI. If a copy of this protocol is requested by a patient or patient's family member participating on the study, the individual should sign a confidentiality agreement. A suitable model agreement can be downloaded from: http://ctep.cancer.gov.
2. For a clinical protocol where there is an Agent used in combination with (an)other investigational Agent(s), each the subject of different Collaborative Agreements, the access to and use of data by each Collaborator shall be as follows (data pertaining to such combination use shall hereinafter be referred to as "Multi-Party Data"):
   a. NCI will provide all Collaborators with prior written notice regarding the existence and nature of any agreements governing their collaboration with NCI, the design of the proposed combination protocol, and the existence of any obligations that would tend to restrict NCI's participation in the proposed combination protocol.
   b. Each Collaborator shall agree to permit use of the Multi-Party Data from the clinical trial by any other Collaborator solely to the extent necessary to allow said other Collaborator to develop, obtain regulatory approval or commercialize its own Agent.
   c. Any Collaborator having the right to use the Multi-Party Data from these trials must agree in writing prior to the commencement of the trials that it will use the Multi-Party Data solely for development, regulatory approval, and commercialization of its own Agent.
3. Clinical Trial Data and Results and Raw Data developed under a Collaborative Agreement will be made available to Collaborator(s), the NCI, and the FDA, as appropriate and unless additional disclosure is required by law or court order, as described in the IP Option to Collaborator (http://ctep.cancer.gov/industryCollaborations2/intellectual_property.htm). Additionally, all Clinical Data and Results and Raw Data will be collected, used and disclosed consistent with all applicable federal statutes and regulations for the protection of human subjects, including, if applicable, the *Standards for Privacy of Individually Identifiable Health Information* set forth in 45 C.F.R. Part 164.
4. When a Collaborator wishes to initiate a data request, the request should first be sent to the NCI, who will then notify the appropriate investigators (Group Chair for Cooperative Group studies, or PI for other studies) of Collaborator's wish to contact them.

5. Any data provided to Collaborator(s) for Phase 3 studies must be in accordance with the guidelines and policies of the responsible Data Monitoring Committee (DMC), if there is a DMC for this clinical trial.
6. Any manuscripts reporting the results of this clinical trial must be provided to CTEP by the Group office for Cooperative Group studies or by the principal investigator for non-Cooperative Group studies for immediate delivery to Collaborator(s) for advisory review and comment prior to submission for publication. Collaborator(s) will have 30 days from the date of receipt for review. Collaborator shall have the right to request that publication be delayed for up to an additional 30 days in order to ensure that Collaborator's confidential and proprietary data, in addition to Collaborator(s)'s intellectual property rights, are protected. Copies of abstracts must be provided to CTEP for forwarding to Collaborator(s) for courtesy review as soon as possible and preferably at least three (3) days prior to submission, but in any case, prior to presentation at the meeting or publication in the proceedings. Press releases and other media presentations must also be forwarded to CTEP prior to release. Copies of any manuscript, abstract and/or press release/ media presentation should be sent to:

   Email: ncicteppubs@mail.nih.gov

   The Regulatory Affairs Branch will then distribute them to Collaborator(s). No publication, manuscript or other form of public disclosure shall contain any of Collaborator's confidential/ proprietary information.

Molecular Analysis for Therapy Choice (MATCH)

Appendix V

ECOG Performance Status

PS 0	Fully active, able to carry on all pre-disease performance without restriction
PS 1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature e.g., light house work, office work.
PS 2	Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours.
PS 3	Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
PS 4	Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.

Molecular Analysis for Therapy Choice (MATCH)

Appendix VI

CYP3A4 Inducers and Inhibitors

Because the lists of these agents are constantly changing, it is important to regularly consult a frequently-updated list such as Facts and Comparisons or Lexicomp; medical reference texts such as the Physicians' Desk Reference may also provide this information. The Principal Investigator should be alerted if the subject is taking any agent on these lists.

List of CYP3A4 Inducing Agents:

Carbamazepine
Dexamethasone
Ethosuximide
Glucocorticoids
Griseofulvin
Modafinil
Naficillin
Nelfinavir
Nevirapine
Oxcarbazepine
Phenobarbitol
Phenylbutazone
Phenytoin
Primidone
Progesterone
Rifabutin
Rifampin
Rifapentine
Rofecoxib
St. John's Wort
Sulfadimidine
Sulfinpyrazone
Tipranavir
Troglitazone

List of CYP3A4 Inhibitors:

Amiodarone
Cimetidine
Ciprofloxacin
Clarithromycin
Delavirdine
Diethyl-dithiocarbamate
Diltiazem
Erythromycin
Fluconazole
Fluvoxamine
Gestodene
Grapefruit or Grapefruit juice
Indanvir
Itraconazole
Ketoconazole
Mibefradil
Mifepristone
Nefazodone
Nelfinavir
Norfloxacin
Norfluoxetine
Ritonavir
Roxithromycin
Saquinavir
Troleandomycin
Voriconazole
Warfarin
Amprenavir
Atazanavir
Miconazole
Telithromycin
Verapamil

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Appendix VII

MATCH Actionable Mutations of Interest

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Cell survival

PI3K pathway

- mTOR : MLN0128/TAK-228
- TSC1/2: MLN0128/TAK-228
- PTEN Mutation or Deletion:
  - GSK2636771 (arm completed/closed);
  - copanlisib
- PTEN loss:
  - GSK2636771 (arm completed/closed);
  - copanlisib
- PIK3CA:
  - taselisib (arm completed/closed);
  - copanlisib (arm completed/closed)
- AKT mutations/amplifications:
  - AZD5363 (arm completed/closed)
  - ipatasertib

MAP Kinase pathway

- BRAF mutations:
  - BRAF nonV600 mutations/fusions:
    - trametinib (arm completed/closed)
    - BVD-523FB (ulixertinib)
  - BRAF V600E or K mutations: trametinib/dabrafenib (arm completed/closed)
- NRAS mutations: binimetinib (arm completed/closed)
- NF1 mutations: trametinib (arm completed/closed)
- GNAQ, GNA11 mutations: trametinib
- NF2 loss: VS6063 (defactinib) (arm completed/closed)

Growth Factors

- EGFR:
  - Rare activating mutations: afatinib
  - T790M mutations, certain rare activating mutations: AZD9291
- HER2 mutation: afatinib, (arm completed/closed)
- HER2 amplification:
  - TDM1 (arm completed/closed);
  - trastuzumab/pertuzumab
- FGFR translocation/mutation:

  - AZD4547 (arm completed/closed)
  - JNJ-42756493 (erdafitinib)
- C-KIT: sunitinib
- ALK rearrangements: crizotinib
- ROS translocation: crizotinib
- MET amplification: crizotinib (arm completed/closed)
- MET exon 14 skipping: crizotinib
- Hedgehog pathway: vismodegib
- NTRK fusions: LOXO-101

Cell cycle inhibitors:

- CCND1, 2, 3 amplification: palbociclib (arm completed/closed)
- CDK4, CDK6 amplification: palbociclib (arm completed/closed)

DNA Repair:

- BRCA1, BRCA2: AZD1775 (arm completed/closed)
- Mismatch repair: nivolumab (arm completed/closed)

Other

- DDR2 mutation: dasatinib (arm closed)

Please refer to Section 1.4.2 and Appendix XVII for information on the definitions of the study's Levels of Evidence (LOEs) for agents and variants, and the process by which certain types of specific aMOIs (within the categories noted above) may be added dynamically to the study.

NOTE: The information above does not reflect the accrual status of the subprotocols in real time. Please refer to the following website for information on the accrual status of each subprotocol: http://ecog-acrin.org/trials/nci-match-eay131

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Appendix VIII

Instructions for Reporting Pregnancies on a Clinical Trial

What needs to be reported?

All pregnancies and suspected pregnancies (including a positive or inconclusive pregnancy test regardless of age or disease state) of a female patient while she is on the MATCH subprotocol treatment or within 28 days of the female patient's last dose of the MATCH subprotocol treatment must be reported in an expeditious manner. The outcome of the pregnancy and neonatal status must also be reported.

How should the pregnancy be reported?

The pregnancy, suspected pregnancy, or positive/inconclusive pregnancy test must be reported via CTEP's Adverse Event Reporting System (CTEP-AERs)

(http://ctep.cancer.gov/protocolDevelopment/electronic_applications/adverse_events.htm)

When does a pregnancy, suspected pregnancy or positive/inconclusive pregnancy test need to be reported?

An initial report must be done within 24 hours of the Investigator's learning of the event, followed by a complete expedited CTEP-AERs report within 5 calendar days of the initial 24-hour report.

What other information do I need in order to complete the CTEP-AERs report for a pregnancy?

- The pregnancy (fetal exposure) must be reported as a Grade 3 "Pregnancy, puerperium and perinatal conditions – Other (pregnancy)" under the System Organ Class (SOC) "Pregnancy, puerperium and perinatal conditions"
- The pregnancy must be reported within the timeframe specified in the Adverse Event Reporting section of the protocol for a grade 3 event.
- The start date of the pregnancy should be reported as the calculated date of conception.
- The potential risk of exposure of the fetus to the investigational agent(s) or chemotherapy agent(s) should be documented in the "Description of Event" section of the CTEP-AERs report.

What else do I need to know when a pregnancy occurs to a patient?

- The Investigator must follow the female patient until completion of the pregnancy and must report the outcome of the pregnancy and neonatal status via CTEP-AERs.
- The decision on whether an individual female patient can continue protocol treatment will be made by the site physician in collaboration with the study chair and ECOG-ACRIN Operations Office – Boston. Please contact the ECOG-ACRIN Operations Office – Boston to ask for a conference call to be set up with the appropriate individuals.
- *It is recommended the female subject be referred to an obstetrician-gynecologist, preferably one experienced in reproductive toxicity for further evaluation and counseling.*

How should the outcome of a pregnancy be reported?

The outcome of a pregnancy should be reported as an *amendment* to the initial CTEP-AERs report if the outcome occurs on the same cycle of treatment as the pregnancy itself. However, if the outcome of the pregnancy occurred on a subsequent cycle, a *new* and separate CTEP-AERs report should be initiated reporting the outcome of the pregnancy.

What constitutes an abnormal outcome?

An abnormal outcome is defined as any pregnancy that results in the birth of a child with persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions (formerly referred to as disabilities), congenital anomalies, or birth defects. For assistance in recording the grade or category of these events, please contact the CTEP AEMD Help Desk at 301-897-7497 or aemd@tech-res.com, for it will need to be discussed on a case by case basis.

Reporting a Pregnancy Loss

A pregnancy loss is defined in CTCAE as "*A death in utero.*"

It must be reported via CTEP-AERs as Grade 4 "*Pregnancy Loss*" under the System Organ Class (SOC) "*Pregnancy, puerperium and perinatal conditions"*.

A pregnancy loss should **NOT** be reported as a Grade 5 event as currently CTEP-AERs recognizes this event as a patient's death.

Reporting a Neonatal Death

A neonatal death is defined in CTCAE as "*A newborn death occurring during the first 28 days after birth*" that is felt by the investigator to be at least possibly due to the investigational agent/intervention. However, for this protocol, any neonatal death that occurs within 28 days of birth, without regard to causality, must be reported via CTEP-AERs AND any infant death after 28 days that is suspected of being related to the *in utero* exposure to the MATCH subprotocol treatment must also be reported via CTEP-AERs.

It must be reported via CTEP-AERs as Grade 4 "*Death neonatal*" under the System Organ Class (SOC) "General disorder and administration site conditions".

A neonatal death should **NOT** be reported as a Grade 5 event as currently CTEP-AERs recognizes this event as a patient's death.

Additional Required Forms:

When submitting CTEP-AERs reports for pregnancy, pregnancy loss, or neonatal loss, the **CTEP *'Pregnancy Information Form'*** must be completed and faxed along with any additional medical information to CTEP (301-897-7404). This form is available on CTEP's website (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/PregnancyReportForm.pdf)

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Appendix IX

List of MATCH Subprotocols and Associated Subprotocol Version Dates

Table 1. List of MATCH Subprotocols

EAY131-A	Molecular Analysis for Therapy Choice (MATCH) - MATCH Treatment Subprotocol A: Phase II Trial of Afatinib in Patients with Solid Tumors (Other than Small Cell and Non-Small Cell Lung Cancer) or Lymphomas, That Have Activating Mutations of EGFR and Have Progressed After Standard Treatment
EAY131-B	Molecular Analysis for Therapy Choice (MATCH) - MATCH Treatment Subprotocol B: Phase II Study of Afatinib in Patients with Tumors with HER2 Activating Mutations
EAY131-C1	Molecular Analysis for Therapy Choice (MATCH) - MATCH Treatment Subprotocol C1: Crizotinib in Patients with Tumors with MET Amplification
EAY131-C2	Molecular Analysis for Therapy Choice (MATCH) - MATCH Treatment Subprotocol C2: Crizotinib in Patients with Tumors with MET Exon 14 Deletion
EAY131-E	Molecular Analysis for Therapy Choice (MATCH) - MATCH Treatment Subprotocol E: AZD9291 in Patients with Tumors Having EGFR T790M Mutations (Except Non-Small Cell Lung Cancer) or Rare Activating Mutations of EGFR
EAY131-F	Molecular Analysis for Therapy Choice (MATCH) MATCH Treatment Subprotocol F: Crizotinib in Patients with Tumors (Other Than Adenocarcinoma of Lung or ALCL) with ALK Rearrangements
EAY131-G	Molecular Analysis for Therapy Choice (MATCH) - MATCH Treatment Subprotocol G: Phase II Study of Crizotinib in Patients with ROS1 Translocations (Other Than Patients with Non-Small Cell Lung Cancer)
EAY131-H	Molecular Analysis for Therapy Choice (MATCH) - MATCH Treatment Subprotocol H: Phase II Study of Dabrafenib and Trametinib in Patients with Tumors with BRAF V600E or V600K Mutations (Excluding Melanoma, Thyroid Cancer, and Colorectal Adenocarcinoma)
EAY131-I	Molecular Analysis for Therapy Choice (MATCH) - MATCH Treatment Subprotocol I: GDC-0032 (taselisib) in Patients with Tumors (other than breast cancer) with PIK3CA Mutation but without KRAS Mutation or PTEN Loss
EAY131-J	Molecular Analysis for Therapy Choice (MATCH) - MATCH Treatment Subprotocol J: Trastuzumab and Pertuzumab (HP) in Patients with Non-Breast, Non-Gastric/GEJ Cancers with HER2 Amplification
EAY131-K1	Molecular Analysis for Therapy Choice (MATCH) - MATCH Treatment Subprotocol K1: Phase 2 Study of JNJ-42756493 (Erdafitinib) in Patients with Tumors with FGFR Amplifications
EAY131-K2	Molecular Analysis for Therapy Choice (MATCH) - MATCH Treatment Subprotocol K2: Phase 2 Study of JNJ-42756493 (erdafitinib) in Patients with Tumors with FGFR Mutations or Fusions
EAY131-L	Molecular Analysis for Therapy Choice (MATCH) - MATCH Treatment Subprotocol L: Phase II Study of MLN0128 (TAK-228) in Patients with Tumors with mTOR Mutations

EAY131-M	Molecular Analysis for Therapy Choice (MATCH) - MATCH Treatment Subprotocol M: Phase II Study of MLN0128 (TAK-228) in Patients with Tumors with TSC1 or TSC2 Mutations
EAY131-N	Molecular Analysis for Therapy Choice (MATCH) - MATCH Treatment Subprotocol N: Phase II Study of PI3K Beta Specific Inhibitor, GSK2636771, in Patients with Tumors with PTEN Mutation or Deletion, with PTEN Expression on IHC
EAY131-P	Molecular Analysis for Therapy Choice (MATCH) - MATCH Treatment Subprotocol P: Phase II Study of PI3K Beta Specific Inhibitor, GSK2636771, in Patients with Tumors with PTEN Loss by IHC
EAY131-Q	Molecular Analysis for Therapy Choice (MATCH) - MATCH Treatment Subprotocol Q: Ado-trastuzumab Emtansine in Patients with Tumors with HER2 Amplification (Except Breast and Gastric/Gastro-Esophageal Junction (GEJ) Adenocarcinomas)
EAY131-R	Molecular Analysis for Therapy Choice (MATCH) - MATCH Treatment Subprotocol R: Phase II Study of Trametinib in Patients with BRAF Fusions, or with Non-V600E, Non-V600K BRAF Mutations
EAY131-S1	Molecular Analysis for Therapy Choice (MATCH) - MATCH Treatment Subprotocol S1: Phase II Study of Trametinib in Patients with Tumors with NF1 mutations
EAY131-S2	Molecular Analysis for Therapy Choice (MATCH) - MATCH Treatment Subprotocol S2: Phase II Study of Trametinib in Patients with Tumors with GNAQ or GNA11 mutations
EAY131-T	Molecular Analysis for Therapy Choice (MATCH) - MATCH Treatment Subprotocol T: GDC-0449 (vismodegib) in Patients with Tumors (except basal cell skin carcinoma) with Smoothened (SMO) or Patched 1 (PTCH1) Mutations
EAY131-U	Molecular Analysis for Therapy Choice (MATCH) - MATCH Treatment Subprotocol U: VS-6063 (Defactinib) in Patients with Tumors with NF2 Loss
EAY131-V	Molecular Analysis for Therapy Choice (MATCH) - MATCH Treatment Subprotocol V: Phase II Study of Sunitinib in Patients with Tumors with cKIT Mutations (Excluding GIST, Renal Cell Carcinoma or Pancreatic Neuroendocrine Tumor)
EAY131-W	Molecular Analysis for Therapy Choice (MATCH) - MATCH Treatment Subprotocol W: Phase II Study of AZD4547 in Patients with Tumors with Aberrations in the FGFR Pathway
EAY131-X	Molecular Analysis for Therapy Choice (MATCH) - MATCH Treatment Subprotocol X: Phase II Study of Dasatinib in Patients with Tumors with DDR2 Mutations
EAY131-Y	Molecular Analysis for Therapy Choice (MATCH) - MATCH Treatment Subprotocol Y: AZD5363 in Patients with Tumors with AKT Mutations
EAY131-Z1A	Molecular Analysis for Therapy Choice (MATCH) - MATCH Treatment Subprotocol Z1A: Binimetinib in Patients with Tumors (Other Than Melanoma) with NRAS Mutations
EAY131-Z1B	Molecular Analysis for Therapy Choice (MATCH) - MATCH Treatment Subprotocol Z1B: Phase II Study of Palbociclib in Patients with Tumors with CCND1, 2, 3 Amplification
EAY131-Z1C	Molecular Analysis for Therapy Choice (MATCH) - MATCH Treatment Subprotocol Z1C: Phase II Study of Palbociclib (PD-0332991) in Patients with Tumors with CDK4 or CDK6 Amplification and Rb Protein Expression by IHC
EAY131-Z1D	Molecular Analysis for Therapy Choice (MATCH) - MATCH Treatment Subprotocol Z1D: Nivolumab in Patients with Tumors with Mismatch Repair Deficiency (Excluding Colorectal Cancer)

EAY131-Z1E	Molecular Analysis for Therapy Choice (MATCH) - MATCH Treatment Subprotocol Z1E: LOXO-101 in Patients with Tumors with NTRK Fusions
EAY131-Z1F	Molecular Analysis for Therapy Choice (MATCH) - MATCH Treatment Subprotocol Z1F: Phase II Study of Copanlisib in Patients with Tumors with PIK3CA Mutations (PTEN Loss Allowed)
EAY131-Z1G	Molecular Analysis for Therapy Choice (MATCH) - MATCH Treatment Subprotocol Z1G: Phase II Study of Copanlisib in Patients with Tumors with PTEN Loss by IHC and any PTEN Sequencing Result
EAY131-Z1H	Molecular Analysis for Therapy Choice (MATCH) - MATCH Treatment Subprotocol Z1H: Phase II Study of Copanlisib in Patients with Tumors with Deleterious PTEN Sequencing Result and PTEN Expression by IHC
EAY131-Z1I	Molecular Analysis for Therapy Choice (MATCH) - MATCH Treatment Subprotocol Z1E: Phase II Study of AZD1775 in Patients with Tumors Containing BRCA1 and BRCA2 Mutations
EAY131-Z1K	Molecular Analysis for Therapy Choice (MATCH) - MATCH Treatment Subprotocol Z1K: Ipatasertib in Patients with Tumors with AKT Mutations
EAY131-Z1L	Molecular Analysis for Therapy Choice (MATCH) - MATCH Treatment Subprotocol Z1L: BVD-523FB (Ulixertinib) in Patients with Tumors with BRAF Fusions, or with Non-V600E, Non-V600K BRAF Mutations

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Table 2. Index of MATCH Subprotocol Version Dates

The EAY131 study is considered one study under one IND consisting of a Master Protocol component and multiple subprotocol components, with each component being contained in its own separate document. **Each "component" consists of the protocol document and its associated informed consent document.** Since each subprotocol component operates independently from the other subprotocol components contained in EAY131, each has its own version date. As changes are made in the various components of the EAY131 study over time via addendum/amendment or update, the version date for a particular subprotocol component (i.e., protocol document and associated informed consent document) will change ONLY IF there are changes made in that subprotocol. In order to keep track of changes in the various component documents over the course of the study, the index below will track each addendum/amendment or update made and list the version date of each component of EAY131 associated with that addendum/amendment or update. The Master Protocol component of EAY131 will change if there are ANY changes in ANY of the subprotocols as the Master Protocol component will always need to include the updated version dates for all subprotocol components in the index below.

The index will allow the sites enrolling patients on the EAY131 study or any stakeholder overseeing various aspects of the study (e.g., Network Group, NCI/DCTD, NCI CIRB, FDA) to know if changes have been made in any of the EAY131 study components over time, and if so, in relation to which addendum/amendment or update and the date of that change.

EAY131 Component	EAY131 Component Version Date										
	Pre-Activation	For Add #1/ Activation	For Add #2	For Add #3	For Add #4	For Add #5	For Add #6	For Add #7	For Add #8	For Add #9	For Add #10
EAY131 (Master Protocol)	5/7/2015	8/6/2015	1/20/2016	5/25/2016	7/1/2016	11/9/2016	12/6/2016	2/6/2017	2/15/2017	3/8/2017	4/10/2017

EAY131 Component	EAY131 Component Version Date										
	Pre-Activation	For Add #1/ Activation	For Add #2	For Add #3	For Add #4	For Add #5	For Add #6	For Add #7	For Add #8	For Add #9	For Add #10
EAY131-A	N/A	8/6/2015	1/20/2016	3/3/2016	3/3/2016	11/9/2016	11/9/2016	2/6/2017	2/15/2017	2/15/2017	2/15/2017
EAY131-B	N/A	8/6/2015	1/20/2016	3/3/2016	3/3/2016	11/9/2016	11/9/2016	2/6/2017	2/15/2017	2/15/2017	2/15/2017
EAY131-C1	N/A	N/A	N/A	5/3/2016	5/3/2016	11/9/2016	11/9/2016	2/6/2017	2/6/2017	2/6/2017	2/6/2017
EAY131-C2	N/A	N/A	N/A	5/3/2016	5/3/2016	11/9/2016	11/9/2016	2/6/2017	2/6/2017	2/6/2017	2/6/2017
EAY131-E	N/A	8/6/2015	1/20/2016	3/3/2016	7/1/2016	11/9/2016	11/9/2016	2/6/2017	2/6/2017	2/6/2017	2/6/2017
EAY131-F	5/7/2015	8/6/2015	1/20/2016	5/3/2016	5/3/2016	11/9/2016	11/9/2016	2/6/2017	2/6/2017	2/6/2017	2/6/2017
EAY131-G	5/7/2015	8/6/2015	1/20/2016	5/3/2016	5/3/2016	11/9/2016	11/9/2016	2/6/2017	2/6/2017	2/6/2017	2/6/2017
EAY131-H	5/7/2015	8/6/2015	1/20/2016	5/3/2016	5/3/2016	11/9/2016	12/6/2016	2/6/2017	2/6/2017	2/6/2017	2/6/2017
EAY131-I	N/A	N/A	1/20/2016	3/3/2016	7/1/2016	11/9/2016	11/9/2016	2/6/2017	2/6/2017	2/6/2017	2/6/2017
EAY131-J	N/A	N/A	N/A	N/A	N/A	N/A	N/A	2/6/2017	2/6/2017	2/6/2017	4/10/2017
EAY131-K1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
EAY131-K2	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
EAY131-L	N/A	N/A	N/A	N/A	N/A	N/A	N/A	2/6/2017	2/6/2017	3/8/2017	3/8/2017
EAY131-M	N/A	N/A	N/A	N/A	N/A	N/A	N/A	2/6/2017	2/6/2017	3/8/2017	3/8/2017
EAY131-N	N/A	N/A	1/20/2016	3/3/2016	3/3/2016	11/9/2016	11/9/2016	2/6/2017	2/6/2017	2/6/2017	2/6/2017
EAY131-P	N/A	N/A	1/20/2016	1/20/2016	1/20/2016	11/9/2016	11/9/2016	2/6/2017	2/6/2017	2/6/2017	2/6/2017
EAY131-Q	N/A	8/6/2015	1/20/2016	3/3/2016	3/3/2016	11/9/2016	12/6/2016	2/6/2017	2/6/2017	2/6/2017	2/6/2017
EAY131-R	5/7/2015	8/6/2015	1/20/2016	5/3/2016	5/3/2016	11/9/2016	12/6/2016	2/6/2017	2/6/2017	2/6/2017	2/6/2017
EAY131-S1	N/A	N/A	1/20/2016	5/3/2016	5/3/2016	11/9/2016	12/6/2016	2/6/2017	2/6/2017	2/6/2017	2/6/2017
EAY131-S2	N/A	N/A	1/20/2016	5/3/2016	5/3/2016	11/9/2016	12/6/2016	2/6/2017	2/6/2017	2/6/2017	2/6/2017
EAY131-T	N/A	N/A	1/20/2016	5/3/2016	5/3/2016	11/9/2016	11/9/2016	2/6/2017	2/6/2017	2/6/2017	4/10/2017
EAY131-U	N/A	8/6/2015	1/20/2016	3/3/2016	7/1/2016	11/9/2016	11/9/2016	2/6/2017	2/6/2017	2/6/2017	2/6/2017
EAY131-V	N/A	8/6/2015	1/20/2016	3/3/2016	3/3/2016	11/9/2016	11/9/2016	2/6/2017	2/6/2017	2/6/2017	2/6/2017
EAY131-W	N/A	N/A	N/A	5/25/2016	7/1/2016	11/9/2016	11/9/2016	2/6/2017	2/6/2017	2/6/2017	2/6/2017
EAY131-X	N/A	N/A	1/20/2016	1/20/2016	1/20/2016	11/9/2016	11/9/2016	2/6/2017	2/6/2017	2/6/2017	2/6/2017
EAY131-Y	N/A	N/A	N/A	5/3/2016	7/1/2016	11/9/2016	11/9/2016	2/6/2017	2/6/2017	2/6/2017	2/6/2017
EAY131-Z1A	N/A	N/A	N/A	5/3/2016	5/3/2016	11/9/2016	12/6/2016	2/6/2017	2/6/2017	2/6/2017	2/6/2017

EAY131 Component	EAY131 Component Version Date										
	Pre-Activation	For Add #1/ Activation	For Add #2	For Add #3	For Add #4	For Add #5	For Add #6	For Add #7	For Add #8	For Add #9	For Add #10
EAY131-Z1B	N/A	N/A	N/A	5/3/2016	5/3/2016	11/9/2016	11/9/2016	2/6/2017	2/6/2017	2/6/2017	2/6/2017
EAY131-Z1C	N/A	N/A	N/A	N/A	N/A	N/A	N/A	2/6/2017	2/6/2017	2/6/2017	2/6/2017
EAY131-Z1D	N/A	N/A	N/A	5/3/2016	7/1/2016	11/9/2016	12/6/2016	2/6/2017	2/6/2017	2/6/2017	2/6/2017
EAY131-Z1E	N/A	N/A	N/A	N/A	N/A	N/A	N/A	2/6/2017	2/6/2017	2/6/2017	2/6/2017
EAY131-Z1F	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
EAY131-Z1G	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
EAY131-Z1H	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
EAY131-Z1I	N/A	N/A	N/A	N/A	N/A	N/A	N/A	2/6/2017	2/6/2017	2/6/2017	2/6/2017
EAY131-Z1K	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
EAY131-Z1L	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

EAY131 Component	EAY131 Component Version Date										
	For Add #11	For Add #12	For Add #13	For Add #14	For Add #15	For Add #16	For Add #17	For Add #18	For Add #19	For Add #20	For Add #21
EAY131 (Master Protocol)	7/19/2017	1/29/2018	5/16/2018	6/21/2018	7/26/2018	12/11//2018	1/8/2019	1/25/2019	3/11/2019	5/14/2019	7/10/2019
EAY131-A	2/15/2017	2/15/2017	5/16/2018	5/16/2018	5/16/2018	5/16/2018	5/16/2018	5/16/2018	3/11/2019	3/11/2019	7/10/2019
EAY131-B	2/15/2017	2/15/2017	5/16/2018	5/16/2018	5/16/2018	5/16/2018	5/16/2018	5/16/2018	5/16/2018	5/16/2018	7/10/2019
EAY131-C1	7/19/2017	7/19/2017	5/16/2018	5/16/2018	5/16/2018	5/16/2018	5/16/2018	1/25/2019	1/25/2019	1/25/2019	1/25/2019
EAY131-C2	7/19/2017	7/19/2017	5/16/2018	5/16/2018	5/16/2018	12/11/2018	12/11/2018	1/25/2019	3/11/2019	3/11/2019	3/11/2019
EAY131-E	2/6/2017	1/29/2018	5/16/2018	6/21/2018	6/21/2018	6/21/2018	6/21/2018	6/21/2018	6/21/2018	6/21/2018	6/21/2018
EAY131-F	7/19/2017	7/19/2017	5/16/2018	5/16/2018	5/16/2018	5/16/2018	5/16/2018	1/25/2019	3/11/2019	3/11/2019	3/11/2019
EAY131-G	7/19/2017	7/19/2017	5/16/2018	5/16/2018	5/16/2018	5/16/2018	5/16/2018	1/25/2019	1/25/2019	1/25/2019	1/25/2019
EAY131-H	2/6/2017	2/6/2017	5/16/2018	5/16/2018	5/16/2018	5/16/2018	5/16/2018	5/16/2018	3/11/2019	3/11/2019	7/10/2019
EAY131-I	2/6/2017	2/6/2017	5/16/2018	5/16/2018	5/16/2018	5/16/2018	5/16/2018	1/25/2019	1/25/2019	1/25/2019	1/25/2019
EAY131-J	4/10/2017	4/10/2017	5/16/2018	5/16/2018	5/16/2018	5/16/2018	5/16/2018	1/25/2019	1/25/2019	1/25/2019	7/10/2019
EAY131-K1	N/A	N/A	5/16/2018	5/16/2018	5/16/2018	12/11/2018	12/11/2018	12/11/2018	3/11/2019	3/11/2019	3/11/2019

EAY131 Component	EAY131 Component Version Date										
	For Add #11	For Add #12	For Add #13	For Add #14	For Add #15	For Add #16	For Add #17	For Add #18	For Add #19	For Add #20	For Add #21
EAY131-K2	N/A	N/A	5/16/2018	5/16/2018	5/16/2018	12/11/2018	12/11/2018	12/11/2018	3/11/2019	3/11/2019	3/11/2019
EAY131-L	3/8/2017	3/8/2017	5/16/2018	5/16/2018	5/16/2018	12/11/2018	12/11/2018	12/11/2018	12/11/2018	12/11/2018	12/11/2018
EAY131-M	3/8/2017	3/8/2017	5/16/2018	5/16/2018	5/16/2018	12/11/2018	12/11/2018	12/11/2018	12/11/2018	12/11/2018	12/11/2018
EAY131-N	2/6/2017	2/6/2017	2/6/2017	2/6/2017	2/6/2017	2/6/2017	2/6/2017	2/6/2017	2/6/2017	2/6/2017	2/6/2017
EAY131-P	2/6/2017	2/6/2017	2/6/2017	2/6/2017	2/6/2017	2/6/2017	2/6/2017	2/6/2017	2/6/2017	2/6/2017	2/6/2017
EAY131-Q	2/6/2017	2/6/2017	2/6/2017	2/6/2017	2/6/2017	12/11/2018	1/8/2019	1/8/2019	1/8/2019	1/8/2019	1/8/2019
EAY131-R	2/6/2017	2/6/2017	5/16/2018	5/16/2018	5/16/2018	5/16/2018	5/16/2018	5/16/2018	3/11/2019	3/11/2019	3/11/2019
EAY131-S1	2/6/2017	2/6/2017	5/16/2018	5/16/2018	5/16/2018	5/16/2018	5/16/2018	5/16/2018	3/11/2019	3/11/2019	3/11/2019
EAY131-S2	2/6/2017	2/6/2017	5/16/2018	5/16/2018	5/16/2018	5/16/2018	5/16/2018	5/16/2018	3/11/2019	3/11/2019	3/11/2019
EAY131-T	4/10/2017	4/10/2017	5/16/2018	5/16/2018	5/16/2018	5/16/2018	5/16/2018	5/16/2018	5/16/2018	5/16/2018	7/10/2019
EAY131-U	7/19/2017	7/19/2017	7/19/2017	7/19/2017	7/19/2017	7/19/2017	7/19/2017	7/19/2017	7/19/2017	7/19/2017	7/10/2019
EAY131-V	2/6/2017	2/6/2017	5/16/2018	5/16/2018	5/16/2018	5/16/2018	5/16/2018	5/16/2018	3/11/2019	3/11/2019	7/10/2019
EAY131-W	2/6/2017	2/6/2017	5/16/2018	5/16/2018	5/16/2018	5/16/2018	5/16/2018	5/16/2018	5/16/2018	5/16/2018	5/16/2018
EAY131-X	2/6/2017	2/6/2017	2/6/2017	2/6/2017	2/6/2017	2/6/2017	2/6/2017	2/6/2017	2/6/2017	2/6/2017	2/6/2017
EAY131-Y	7/19/2017	7/19/2017	5/16/2018	5/16/2018	5/16/2018	5/16/2018	5/16/2018	5/16/2018	5/16/2018	5/16/2018	7/10/2019
EAY131-Z1A	2/6/2017	2/6/2017	5/16/2018	5/16/2018	5/16/2018	5/16/2018	5/16/2018	5/16/2018	5/16/2018	5/16/2018	5/16/2018
EAY131-Z1B	2/6/2017	2/6/2017	5/16/2018	6/21/2018	6/21/2018	6/21/2018	6/21/2018	6/21/2018	6/21/2018	6/21/2018	6/21/2018
EAY131-Z1C	2/6/2017	2/6/2017	5/16/2018	6/21/2018	6/21/2018	6/21/2018	6/21/2018	6/21/2018	6/21/2018	6/21/2018	6/21/2018
EAY131-Z1D	2/6/2017	2/6/2017	5/16/2018	5/16/2018	7/26/2018	12/11/2018	12/11/2018	12/11/2018	12/11/2018	12/11/2018	12/11/2018
EAY131-Z1E	2/6/2017	1/29/2018	5/16/2018	6/21/2018	6/21/2018	6/21/2018	1/8/2019	1/8/2019	1/8/2019	1/8/2019	1/8/2019
EAY131-Z1F	N/A	N/A	5/16/2018	5/16/2018	5/16/2018	12/11/2018	12/11/2018	12/11/2018	12/11/2018	12/11/2018	12/11/2018
EAY131-Z1G	N/A	N/A	5/16/2018	5/16/2018	5/16/2018	12/11/2018	12/11/2018	12/11/2018	12/11/2018	12/11/2018	12/11/2018
EAY131-Z1H	N/A	N/A	5/16/2018	5/16/2018	5/16/2018	12/11/2018	12/11/2018	12/11/2018	12/11/2018	12/11/2018	12/11/2018
EAY131-Z1I	2/6/2017	2/6/2017	5/16/2018	6/21/2018	6/21/2018	6/21/2018	1/8/2019	1/8/2019	1/8/2019	1/8/2019	7/10/2019
EAY131-Z1K	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	5/14/2019	5/14/2019
EAY131-Z1L	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	5/14/2019	5/14/2019

EAY131 Component	EAY131 Component Version Date	EAY131 Component Version Date	EAY131 Component Version Date	EAY131 Component Version Date	EAY131 Component Version Date
	For Add #22	For Add #23	For Add #24	For Add25	For Add26
EAY131 (Master Protocol)	8/12/2019	10/03/2019	2/28/2020	9/15/2020	10/09/2020
EAY131-A	7/10/2019	7/10/2019	7/10/2019	9/15/2020	9/15/2020
EAY131-B	7/10/2019	7/10/2019	7/10/2019	7/10/2019	7/10/2019
EAY131-C1	1/25/2019	1/25/2019	2/28/2020	8/19/2020	8/19/2020
EAY131-C2	3/11/2019	3/11/2019	2/28/2020	8/19/2020	8/19/2020
EAY131-E	6/21/2019	10/03/2019	10/03/2019	8/19/2020	8/19/2020
EAY131-F	3/11/2019	3/11/2019	3/11/2019	3/11/2019	3/11/2019
EAY131-G	1/25/2019	1/25/2019	1/25/2019	1/25/2019	1/25/2019
EAY131-H	7/10/2019	7/10/2019	7/10/2019	8/19/2020	8/19/2020
EAY131-I	7/10/2019	7/10/2019	7/10/2019	7/10/2019	7/10/2019
EAY131-J	8/12/2019	8/12/2019	8/12/2019	8/19/2020	8/19/2020
EAY131-K1	3/11/2019	3/11/2019	3/11/2019	3/11/2019	3/11/2019
EAY131-K2	3/11/2019	3/11/2019	3/11/2019	8/19/2020	8/19/2020
EAY131-L	12/11/2018	10/03/2019	10/03/2019	8/19/2020	8/19/2020
EAY131-M	12/11/2018	10/03/2019	2/28/2020	8/19/2020	8/19/2020
EAY131-N	2/6/2017	2/6/2017	2/6/2017	2/6/2017	2/6/2017
EAY131-P	2/6/2017	2/6/2017	2/6/2017	2/6/2017	2/6/2017
EAY131-Q	1/8/2019	1/8/2019	2/28/2020	8/19/2020	8/19/2020
EAY131-R	3/11/2019	3/11/2019	3/11/2019	8/19/2020	8/19/2020
EAY131-S1	3/11/2019	3/11/2019	3/11/2019	8/19/2020	8/19/2020
EAY131-S2	3/11/2019	3/11/2019	3/11/2019	8/19/2020	8/19/2020
EAY131-T	7/10/2019	7/10/2019	7/10/2019	8/19/2020	8/19/2020
EAY131-U	7/10/2019	7/10/2019	7/10/2019	7/10/2019	7/10/2019
EAY131-V	7/10/2019	7/10/2019	7/10/2019	8/19/2020	8/19/2020
EAY131-W	5/16/2018	5/16/2018	5/16/2018	5/16/2018	5/16/2018

EAY131 Component	EAY131 Component Version Date	EAY131 Component Version Date	EAY131 Component Version Date	EAY131 Component Version Date	EAY131 Component Version Date
	For Add #22	For Add #23	For Add #24	For Add25	For Add26
EAY131-X	2/6/2017	2/6/2017	2/6/2017	2/6/2017	2/6/2017
EAY131-Y	7/10/2019	7/10/2019	7/10/2019	8/19/2020	8/19/2020
EAY131-Z1A	5/16/2018	5/16/2018	5/16/2018	5/16/2018	5/16/2018
EAY131-Z1B	6/21/2018	6/21/2018	6/21/2018	6/21/2018	6/21/2018
EAY131-Z1C	6/21/2018	6/21/2018	2/28/2020	8/19/2020	10/9/2020
EAY131-Z1D	12/11/2018	12/11/2018	12/11/2018	12/11/2018	12/11/2018
EAY131-Z1E	1/8/2019	10/03/2019	10/03/2019	8/19/2020	8/19/2020
EAY131-Z1F	8/12/2019	8/12/2019	8/12/2019	8/12/2019	8/12/2019
EAY131-Z1G	8/12/2019	8/12/2019	8/12/2019	8/19/2020	8/19/2020
EAY131-Z1H	8/12/2019	8/12/2019	8/12/2019	8/19/2020	8/19/2020
EAY131-Z1I	7/10/2019	7/10/2019	7/10/2019	7/10/2019	7/10/2019
EAY131-Z1K	5/14/2019	5/14/2019	5/14/2019	8/19/2020	8/19/2020
EAY131-Z1L	5/14/2019	5/14/2019	5/14/2019	8/19/2020	8/19/2020

Molecular Analysis for Therapy Choice (MATCH)

Appendix X

Rev. 8/15
Rev. 2/16
Rev. Add13
Rev. Add24
Rev. Add25

Example MATCH-Assay Laboratory Reports

This appendix provides templates of reports that may be distributed from a MATCH-assay laboratory providing the results of the MATCH-assay screening or confirmation assessments performed by the MATCH-assay laboratories. These results are used to assign patients to treatment if they entered the screening step of the trial prior to May 21, 2017 and to confirm the evaluability of patients who were assigned treatment via a referral from a Designated Laboratory. Reminder, the MATCH-assay confirmation assessments are performed only after a patient is enrolled on an assigned treatment arm and will not affect treatment assignment or patient participation in EAY131. When the sequencing results arrive, please read the notes especially regarding potential germline findings.

NOTE: For templates of reports distributed by designated Laboratories that provide referrals for MATCH, please contact the specific designated Laboratory.

NOTE: Results of additional central assessments, including IHC and histology confirmation reports, are provided separately. For patients enrolled on EAY131 based on a referral from a Designated Laboratory, results of any central assessments which impact treatment assignment, such as IHC, will be performed and reported prior to treatment assignment.

Results of the NCI-MATCH NGS Assay for the NCI-MATCH Clinical Trial (CTEP-EAY131)
Molecular Sequencing No. _____

Report Date: mm/dd/yyyy

INVESTIGATIONAL USE ONLY

The NCI-MATCH (Molecular Analysis for Therapy Choice) Next Generation Sequencing (NGS) assay is for investigational use only. This report is intended to provide background information to the referring physician and the patient about mutations detected by the assay within the patient's tumor. It does not replace or alter the assignment of a patient to a clinical trial arm. The assay identifies greater than 3,000 annotated mutations of interest (MOIs) characterized into 5 mutation types: single nucleotide variants (SNV), small insertions/deletions (Indels), large (> 3 bases) insertions/deletions (Large Indels), copy number variants (CNV), and gene fusions. This report summarizes annotated mutations identified in the tumor specimen identified below.

Patient Name:	Patient Sequence No.:	Biopsy Sequence No.:	Molecular Sequence No.:

DOB: (mm/dd/yyyy)	Sex: ☐ Male ☐ Female	Primary Diagnosis:	
Referring Physic Email:		Tel:	Fax:
Biopsy Site:	Date Collected:	DNA/cDNA Block Source:	Tumor Content (%):

Type	MOIs (Mutations of Interest) Detected							
SNV/ Indel	Gene[1]	HGVS	ID Code	VAF[2]	Protein Change	Transcript ID	Oncomine Variant Class[3]	Function

[1]*Some genes (*) identified by this assay may be associated with an inherited cancer syndrome. See Attachment 1.*

[2]*Treatment threshold established at ≥ 0.05 Variant Allele Frequency (VAF) for variants annotated as MOIs and at ≥ 0.10 VAF for nonsense SNV and frame-shift indels in tumor suppressor genes.*

[3]*Oncomine Variant Classes: Hotspot, Deleterious, Amplification, Fusion (see Key to Variant Classes)*

CNV[4]	Gene	Chr:Position	Copy Number	[4]*Treatment threshold established at ≥ 7 copies/cell as identified by the MATCH assay, or the MATCH equivalent of 7 or greater as identified by a designated laboratory assay*

Gene Fusion	Driver Gene	Partner Gene	Annotation (Exon Junction)

Comments:	
SIGNATURE APPROVAL: The signature below attests that the signee has reviewed the data and results reported and concurs with the stated conclusions. _____ *Laboratory Director* *Date*	**DISCLAIMER:** This assay was developed specifically for the NCI-MATCH clinical trial and is considered an investigational device limited by Federal (United States) law to investigational use. Its performance characteristics have been determined through extensive testing by the NCI-MATCH network laboratories. It has not been cleared by the US Food and Drug Administration and such approval is not required for clinical implementation. Furthermore, any Comments included in this report are strictly interpretive and the opinion of the reviewer and may not be specifically relevant to the NCI-MATCH clinical trial. The _____is registered under Clinical Laboratory Improvement Amendments (CLIA) for the performance of high-complexity molecular testing for clinical purposes.

Comments:	
SIGNATURE APPROVAL: The signature below attests that the signee has reviewed the data and results reported and concurs with the stated conclusions. _____ *Laboratory Director* *Date*	**DISCLAIMER:** This assay was developed specifically for the NCI-MATCH clinical trial and is considered an investigational device limited by Federal (United States) law to investigational use. Its performance characteristics have been determined through extensive testing by the NCI-MATCH network laboratories. It has not been cleared by the US Food and Drug Administration and such approval is not required for clinical implementation. Furthermore, any Comments included in this report are strictly interpretive and the opinion of the reviewer and may not be specifically relevant to the NCI-MATCH clinical trial. The _____is registered under Clinical Laboratory Improvement Amendments (CLIA) for the performance of high-complexity molecular testing for clinical purposes.

The NCI-MATCH NGS Assay

Methodology: The NCI-MATCH NGS Assay is a next-generation sequencing (NGS) assay that utilizes a multiplex polymerase chain reaction (PCR) with DNA and RNA extracted from formalin-fixed tissue for sequencing on the Ion Torrent S5 XL platform and analyzed by Torrent Suite Software and Ion Reporter. The NCI-MATCH NGS Assay currently can reliably identify the presence or absence of greater than 3,000 known MOIs in the 161 unique genes listed below, with results compared to the Human Reference Genome hg19. The NCI-MATCH assay utilizes the Thermo Fisher Scientific Oncomine® Comprehensive Assay (formerly the Oncomine Cancer Panel).

Rev. Add24 **Analytical Sensitivity and Specificity:** The NCI-MATCH NGS Assay has been determined to be suitably analytically sensitive and specific for the various types of actionable abnormalities (aMOIs) within its reportable range. Details are found in the NCI-MATCH NGS Assay version 3 Validation Report. Treatment threshold has been established at ≥ 0.05 Variant Allele Frequency (VAF) for variants annotated as MOIs, at ≥ 0.10 VAF for novel nonsense SNV and frame-shift indel variants in tumor suppressor genes, and ≥ 7 copies/cell as identified by the MATCH assay, or the MATCH equivalent of 7 or greater as identified by a designated laboratory assay for CNVs. All quality measures for this assay were within defined assay parameters.

Scope and Application: The NCI-MATCH NGS Assay is a research test classified as an investigational device and limited by Federal law for investigational use only. This assay was not designed for routine clinical use. It was developed to determine eligibility for assignment to investigational treatment in the NCI-MATCH clinical trial and is not intended for any other use. This test was designed to find gene mutations within tumors (somatic mutations). It was not designed to find gene mutations that are passed down in families (hereditary or germline mutations).

Hereditary and germline mutations: This assay examines tumor tissue only and does not examine normal (non-tumor) tissue. Mutations detected by the assay may be present only in the tumor, or in every cell of the body (including non-tumor cells). This test also cannot tell whether a potential germline mutation causes or will cause a hereditary cancer syndrome. If the patient's personal and/or family history are suggestive of a hereditary cancer predisposition, it is recommended that the physician arrange for the patient to meet with a genetic counselor and, if warranted, undergo the appropriate genetic test on normal (i.e. non-tumor) tissue (blood or cells brushed from the oral surface of the cheek) to check for a germline abnormality, regardless of the results of this research study.

Mutations that are tested for by the NCI-MATCH NGS Assay and that, when present in normal tissue, are associated with hereditary cancer conditions (a predisposition to certain types of cancer) are listed in Attachment 1. Genes on the list were selected based on available published data about known associations with certain health issues. Results demonstrating a mutation in one of these genes may or may not be compatible with a germline mutation. Please refer to the publication "American Society of Clinical Oncology Expert Statement: collection and use of a cancer family history for oncology providers" [Lu KF et al. J Clin Oncol. 2014; 32(8):833-841] for further information.

Hotspot Genes			
AKT1	FGFR1	MAP2K1	RAF1
AKT2	FGFR2	MAP2K2	RET
AKT3	FGFR3	MAP2K4	RHEB
ALK	FGFR4	MAPK1	RHOA
AR	FLT3	MAX	ROS1
ARAF	FOXL2	MDM4	SF3B1
AXL	GATA2	MED12	SMAD4
BRAF	GNA11	MET	SMO
BTK	GNAQ	MTOR	SPOP
CBL	GNAS	MYC	SRC
CCND1	H3F3A	MYCN	STAT3
CDK4	HIST1H3B	MYD88	TERT
CDK6	HNF1A	NFE2L2	TOP1
CHEK2	HRAS	NRAS	U2AF1
CSF1R	IDH1	NTRK1	XPO1
CTNNB1	IDH2	NTRK2	
DDR2	JAK1	NTRK3	
EGFR	JAK2	PDGFRA	
ERBB2	JAK3	PDGFRB	
ERBB3	KDR	PIK3CA	
ERBB4	KIT	PIK3CB	
ERCC2	KNSTRN	PPP2R1A	
ESR1	KRAS	PTPN11	
EZH2	MAGOH	RAC1	

Full-Length Genes	
ARID1A	NOTCH3
ATM	PALB2
ATR	PIK3R1
ATRX	PMS2
BAP1	POLE
BRCA1	PTCH1
BRCA2	PTEN
CDK12	RAD50
CDKN1B	RAD51
CDKN2A	RAD51B
CDKN2B	RAD51C
CHEK1	RAD51D
CREBBP	RB1
FANCA	RNF43
FANCD2	SETD2
FANCI	SLX4
FBXW7	SMARCA4
MLH1	SMARCB1
MRE11A	STK11
MSH2	TP53
MSH6	TSC1
NBN	TSC2
NF1	
NF2	
NOTCH1	
NOTCH2	

Copy Number Genes	
AKT1	IGF1R
AKT2	KIT
AKT3	KRAS
ALK	MDM2
AR	MDM4
AXL	MET
BRAF	MYC
CCND1	MYCL
CCND2	MYCN
CCND3	NTRK1
CCNE1	NTRK2
CDK2	NTRK3
CDK4	PDGFRA
CDK6	PDGFRB
CDKN2A	PIK3CA
CDKN2B	PIK3CB
EGFR	PPARG
ERBB2	RICTOR
ESR1	TERT
FGF19	TSC1
FGF3	TSC2
FGFR1	
FGFR2	
FGFR3	
FGFR4	
FLT3	

Gene Fusions (Inter and Intragenic)	
AKT2	MYBL1
ALK	NF1
AR	NOTCH1
AXL	NOTCH4
BRAF	NRG1
BRCA1	NTRK1
BRCA2	NTRK2
CDKN2A	NTRK3
EGFR	NUTM1
ERBB2	PDGFRA
ERBB4	PDGFRB
ERG	PIK3CA
ESR1	PPARG
ETV1	PRKACA
ETV4	PRKACB
ETV5	PTEN
FGFR1	RAD51B
FGFR2	RAF1
FGFR3	RB1
FGR	RELA
FLT3	RET
JAK2	ROS1
KRAS	RSPO2
MDM4	RSPO3
MET	TERT
MYB	

Assay	Configuration	Unique Genes	DNA	RNA
OCAv3	OCAv3 DNA + OCAv3 RNA	161	146	51

Key to Variant Types:

Hotspot:	Recurrent missense or indel mutation in one of the identified oncogenes or one of the identified tumor suppressor genes
Deleterious:	Nonsense or frameshift mutation in one of the tumor suppressor genes
Amplification:	Increased copy number in one of the copy number identified genes
Fusion:	Gene fusion Involving one of the fusion driver genes

THE UNIVERSITY OF TEXAS
MD Anderson Cancer Center
1515 HOLCOMBE BOULEVARD
HOUSTON, TX 77030-4095

Clinical Trial Report

Department of Pathology, Box 85
Tel: 713-792-3205 Fax: 713-794-4630

T-EAY131-306

Doe, John
DOB: 1/1/1969 Sex: M
Physician: Referring Physician, MD

Collected: 11/30/2014
Received: 12/01/2014 11:55 Case type: MATCH

Accession: **T-14-000025**

DETAILS

Immunohistochemistry Results

PTEN Immunohistochemistry: Positive (retained expression)

"Some tests reported here may have been developed and performance characteristics determined by UT MD Anderson Pathology and Laboratory Medicine. These tests have not been specifically cleared or approved by the U.S. Food and Drug Administration."

Entire report and diagnosis completed by: Mark J Routbort MD, PhD 11133 Dec 01, 2014

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Surgical Pathology Report
File under: Pathology

The MATCH sequencing assay is performed on somatic tissue only with no testing on germline material. During study development, most available literature and guidance documents on the topics of Incidental Findings (IFs) and return of individual research results that were consulted[1-4] were predicated on the assumption that germline material was the subject of the research test. Although this is not the case for this study, they formed the framework of an Incidental Findings return strategy for MATCH. It is assumed that this area will continue to evolve and the MATCH Incidental Findings policy will be periodically reviewed by the study Steering Committee and revised as necessary during the conduct of the study. For purposes of this study, the term Incidental Finding (IF) indicates any detected variant which may be compatible with heritable risk for cancer if present in germline tissues.

The MATCH NGS assay is a research test developed to serve as an investigational assay for eligibility for assignment to investigational treatments in the MATCH protocol. It is not intended for any other use. It was not designed to find hereditary (germline) gene mutations. This assay examines tumor tissue only and does not examine normal (non-tumor) tissue. Mutations detected by the assay may be present only in the tumor, or in every cell of the body (including non-tumor cells). Furthermore, this test also cannot tell whether a potential germline mutation is pathogenic.

However, some variants on the assay panel, when present in normal tissue, have published associations with hereditary cancer conditions. A patient's assay result showing a mutation in one of these genes may or may not be compatible with a germline mutation.

Rev. Add24 All variant (mutation) results, including those that are actionable (have a study-available treatment) as well as study-defined IFs (including variants of unknown significance as well as variants which are significant but not matched to an active study subprotocol treatment), will be reported as present or not on a report sent from the sequencing lab to the treating physician. An incidental findings coversheet and gene list will accompany these results. The coversheet will explain the assay and its use (as summarized above) followed by a list of genes that have hereditary cancer implications, annotated with associated conditions and a reference. The genes were selected for the list based on the guiding principles of the ACMG[1] (Is penetrance high? Are there confirmatory approaches to medical diagnosis available? Are preventive measures and/or treatment available? Might individuals with pathogenic mutations be asymptomatic for long periods of time?).

It is expected that in most cases physicians will share the MATCH assay report and the IF documents with the patient as part of their care or provide these materials upon request. Patients may also directly request their study results under current CLIA and HIPAA regulations[5]. It is hoped that caregivers will be available to review results with patients and answer questions, but the coversheets were written to be readable outside the clinic setting with attempts to simply define concepts and rely less on medical terminology.

As stated, the MATCH assay was designed to find gene mutations within tumors, and was not designed to find hereditary (germline) gene mutations.

A patient's MATCH assay result may or may not be compatible with a germline mutation. If the patient's personal and/or family history are suggestive of a hereditary cancer predisposition, it is recommended that the physician arrange for the patient meet with a genetic counselor and, if warranted, undergo the appropriate genetic test on normal (i.e. non-tumor) tissue to assess for a germline abnormality, regardless of the results of this research study[6].

References:

1. Green RC1, Berg JS, Grody WW, et al. ACMG recommendations for reporting of incidental findings in clinical exome and genome sequencing. Genet Med 2013; 15(7):565-74.
2. Wolf SM, Lawrenz FP, Nelson CA, et al. Managing incidental findings in human subjects research: analysis and recommendations. J Law Med Ethics 2008; 36:219–248
3. Presidential Commission for the Study of Bioethical Issues. Anticipate and Communicate Ethical Management of Incidental and Secondary Findings in the Clinical, Research, and Direct-to-Consumer Contexts. 2013; Dec
4. Williams Parsons D, Roy A, Plon SE, et al. Clinical Tumor Sequencing: An Incidental Casualty of the American College of Medical Genetics and Genomics Recommendations for Reporting of Incidental Findings. J Clin Oncol 2014; 32(21):2203-2205
5. https://www.federalregister.gov/articles/2014/02/06/2014-02280/clia-program-and-hipaa-privacy-rule-patients-access-to-test-reports
6. Lu, KF, Wood ME, Daniels M, et al. American Society of Clinical Oncology Expert Statement: collection and use of a cancer family history for oncology providers. J Clin Oncol 2014; 32(8):833-841

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Appendix XI

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Rev. Add13

ECOG-ACRIN/NCI MATCH Working Groups

	ECOG-ACRIN REPRESENTATIVES	NCI REPRESENTATIVES
PROTOCOL LOGISTICS (includes STATISTICAL DESIGN INPUT AND DATA CAPTURE)	*Keith Flaherty (PI)	*Alice Chen (PI)
	Stan Hamilton (co-PI)	Mickey Williams
	Peter O'Dwyer (co-PI)	Lisa McShane
	Joseph Sparano	Lyndsay Harris
	Donna Marinucci	Dave Patton
	Bob Gray	Carol Weil
	Bob Catalano	Tali Johnson
	Jean Macdonald	Mike Montello
	Shuli Li	Cayden Maican
	Becky Fillingham	Rodney Howells
	Pamela Cogliano	Charles Hall
	Jeffrey Zhang	Lalitha Shankar
	Mary Lou Smith	Larry Rubenstein
	Paolo Caimi	Ashleigh Fredlock
	Lynne Wagner	James Tricoli
	Mike Fisch	
	Charles Cleeland	
	Matthias Weiss	
	John P. Leonard	
	Gary Schwartz	
	Karla Ballman	
	Steve Adler	
	Edith Mitchell	
	Robert L. Comis, MD	
SITE PARTICIPATION and EDUCATION	Jean Macdonald	Margo Cavenagh
	Ruth Lambersky	Andrea Denicoff
	Lauren DiFerdinando	Holly Massett
	Diane Dragaud	Lorna Patrick
	Mary Lou Smith	Lynn Cave
	Marissa Six	Grace Schroer
	Donna Marinucci	Rhonda DeJoice
	Ann Kolker	Claudia Menashe
	Pamela Cogliano	John Horrigan

IP/CONTRACTS	*Donna Marinucci	*Sherry Ansher
	Stan Hamilton	Jason Cristofaro
		John Wright
SAMPLE COLLECTION AND SEQUENCING CENTERS	*Stan Hamilton	*Mickey Williams
	Peter O'Dwyer	Larry Rubinstein (optional)
	Joseph Sparano	Lyndsay Harris
	Donna Marinucci	Lisa McShane
	Becky Fillingham	Dave Patton
	Karen Ronski (Yale)	Irina Lubensky
	John Anthony Iafrate (MGH)	Sequencing Center PIs
	Hayley Robinson (MGH)	Ashleigh Fredlock
	Kathy Vernovsky (MGH)	Vivekananda Datta
	Amelia (MGH)	David Sims
	Raja Luthra (MDA)	Ben Kim
	KP Patel (MDA)	Lori Keisling
	Jeffrey Sklar (Yale)	Robert Shirley
	Zenta Walther (Yale)	Mary Anderson
	Karen Ronski (YNHH)	Hala Makhlouf
	Sandar Canosa (Yale)	Julie Rhie
	Mark Routbort (MDA)	Brent Coffey
	D. Jones (MDA)	James Tricoli
	Karen Friery (Yale)	Sean McDermott
	Leslie Evans (Lifetech)	
	Gabriella Armin (Lifetech)	
	Peter Wyngaard (Lifetech)	
	Savitri Krishnamurthy	
	Geeta Mantha	
	Neelima Reddy	
IMAGING	Dave Mankoff	*Lalitha Shankar
	*Susanna Lee	Lyndsay Harris
	Keith Flaherty	Alice Chen
	Peter O'Dwyer	
	Paolo Caimi	
	Alda Tam	
	Michael Soulen	
	Donna Hartfeil	
INFORMATICS	* Rick Magnan	*Dave Patton
	Shuli Li	Mickey Williams (or sub.)
	Robert Comis	Rich Simon
	Donna Marinucci	Tali Johnson (Rodney Howells and Skip Charles are alternates)
	Pamela Cogliano	Mike Montello
	Ruth Lambersky	Lyndsay Harris
	Jean MacDonald	James Tricoli
	Jeffrey Zhang	

AGENT and GENE SELECTION	*Carlos Arteaga	*Lyndsay Harris
	Keith Flaherty	Alice Chen
	Peter O'Dwyer	John Wright
	Stan Hamilton	Naoko Takebe
	Paolo Caimi	Jim Tricoli
	Debu Tripathy	Mickey Williams
	Nilofer Azad	IDB drug monitor (ad hoc)
	Gary Schwartz	Vivekananda Datta
	Funda Meric -Bernstam	Sherry Ansher
	Rich Carvajal	David Sims
	Pamela Cogliano	Margo Cavenagh
	Jeffrey Zhang	James Doroshow
	David Hyman	Biswajit Das
	Mac Cheever	
INCIDENTAL FINDINGS	John Iafrate	*Carol Weil
	Hayley Robinson	Lyndsay Harris
	Bob Catalano	Mickey Williams
	Mary Lou Smith	Dave Kaufman
	Jeffrey Sklar	Naoko Takebe
	Molly Daniels	Jim Tricoli
	Leslie Evans	
INFORMED CONSENT	*Jeffrey Zhang	Lyndsay Harris
	Keith Flaherty	Alice Chen
	Bob Catalano	Lorna Patrick
	Mary Lou Smith	Andrea Denicoff
	Peggy Anthony	Holly Massett
	Becky Fillingham	Lynn Cave
		Carol Weil

PATIENT ADVOCATES	* Mary Lou Smith	Nancy Roach
	Peggy Anthony	Holly Massett
	Charles Florsheim	Lyndsay Harris
	Pat Gavin	
	Lisa Tichenor	
	Tom Simon	
	Rick Bangs	
	Peter O'Dwyer	
	Donna Marinucci	
	Bob Catalano	
	Ruth Lambersky	
	Lauren DiFerdinando	
	Pamela Cogliano	
	Diane Dragaud	
	Edith Mitchell	
PUBLIC RELATIONS	*Diane Dragaud	Mike Miller
	Robert Comis	Frank Blanchard
	Donna Marinucci	Alice Chen
	Keith Flaherty	Lyndsay Harris
	Stan Hamilton	Rhonda DeJoice
	Peter O'Dwyer	Andrea Denicoff
	Mary Lou Smith	Shannon Hatch
	Danielle Preston	Sona Thakkar
	Deborah Deal	Mickey Williams
	Pamela Cogliano	Eileen Resnick
	Jeffrey Zhang	LaTonya Hendricks
		Holly Massett
		Margo Cavenagh
CORRELATIVE PROPOSAL	*Stan Hamilton	*Irina Lubensky
	Keith Flaherty	Alice Chen
	Peter O'Dwyer	Lyndsay Harris
	Shuli Li	Lisa McShane
	Robert Gray	Mickey Williams
	Becky Fillingham	Naoko Takebe
	Michael Balco	Sherry Ansher
	Carlos Arteaga	Jason Denner
		Rebecca Enos
		Cayden Maican
		James Tricoli

MATCH STEERING COMMITTEE	Keith Flaherty	Alice Chen
	Peter O'Dwyer	Lyndsay Harris
	Stanley Hamilton	Mickey Williams
	Robert Gray	Lisa McShane
	Bruce Giantonio	Larry Rubinstein
	Donna Marinucci	John Wright
	Shuli Li	Warren Kibbe
	Edith Mitchell	Lalitha Shankar
	Rick Magnan	Melissa von Kerczek
	Susanna Lee	Worta McCaskill-Stevens
	Pamela Cogliano	James Tricoli
	Jeffrey Zhang	
	Shaji Kumar	
	Carlos Arteaga	
	Mark Routbort	

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Appendix XII

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EAY131 Collection and Shipping Kit Order Form

Rev. 3/17

Use this form to request kits for the EAY131 specimen kits for screening and Step 8 as indicated below. The first kit for each patient will automatically be ordered upon pre-registration to STEP 0.

On study (end of cycle 2 or treatment) blood collection and submission kits will be distributed automatically upon registration to subprotocol treatment.

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FAX Completed form **to ECOG-ACRIN Central Biorepository and Pathology Facility at (713) 563-6506.**

Date: _____

ECOG-ACRIN Case ID of the Patient: _____

Kit to be ordered:

	Confirmation of Pre-Registration Outside Results	AUTOMATIC. Do not use form.
	Screening 2, Biopsy 1 (Step 2 or 4)	
	Screening 2, Biopsy Repeat (if Screening 2, Biopsy 1 inadequate)	
	On Treatment (End of Cycle 2 or treatment) - AUTOMATIC	
	Step 8, Progression (END OF ALL TREATMENT ON EAY131)	

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Is this a MULTIPLE MYELOMA patient who will have a bone marrow aspirate for screening collected? ☐ Yes ☐ No

Kit is to be shipped to:

Institution Contact: _____

Phone number for contact: _____

E-mail for contact: _____

Institution Address:

_____

_____

_____

NOTE: Questions are to be directed to the ECOG-ACRIN CBPF

Phone: Toll Free 1-844-744-2420 (713-745-4440 Local or International Sites), OPTION 5
Fax: 713-563-6506
Email: eacbpf@mdanderson.org

Comments:

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Appendix XIII

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Medications Known to Prolong the QT Interval

Generic Name	Brand Names (Partial List)	Drug Class	Therapeutic Use	Route
Amiodarone	Cordarone®, Pacerone®, Nexterone®	Anti-arrhythmic	Abnormal heart rhythm	oral, injection
Anagrelide	Agrylin®, Xagrid®	Phosphodiesterase 3 inhibitor	Thrombocythemia	oral
Arsenic trioxide	Trisenox®	Anti-cancer	Cancer (leukemia)	injection
Astemizole (Removed from US Market)	Hismanal®	Antihistamine	Allergic rhinitis	oral
Azithromycin	Zithromax®, Zmax®	Antibiotic	Bacterial infection	oral, injection
Bepridil (Removed from US Market)	Vascor®	Anti-anginal	Angina Pectoris (heart pain)	oral
Chloroquine	Aralen®	Anti-malarial	Malaria	oral
Chlorpromazine	Thorazine®, Largactil®, Megaphen®	Anti-psychotic / Anti-emetic	Schizophrenia, nausea, many others	oral, injection, suppository
Cilostazol	Pletal®	Phosphodiesterase 3 inhibitor	Intermittent claudication	oral
Ciprofloxacin	Cipro®, Cipro-XR®, Neofloxin®	Antibiotic	Bacterial infection	oral, injection
Cisapride (Removed from US Market)	Propulsid®	GI stimulant	Increase GI motility	oral
Citalopram	Celexa®, Cipramil®	Anti-depressant, SSRI	Depression	oral
Clarithromycin	Biaxin®, Prevpac®	Antibiotic	Bacterial infection	oral
Cocaine	Cocaine	Local anesthetic	Anesthesia (topical)	topical
Disopyramide	Norpace®	Anti-arrhythmic	Abnormal heart rhythm	oral
Dofetilide	Tikosyn®	Anti-arrhythmic	Abnormal heart rhythm	oral
Domperidone (On non US Market)	Motilium®, Motillium®, Motinorm Costi®, Nomit®	Anti-nausea	Nausea, vomiting	oral, injection, suppository
Donepezil	Aricept®	Cholinesterase inhibitor	Dementia (Alzheimer's Disease)	oral

Generic Name	Brand Names (Partial List)	Drug Class	Therapeutic Use	Route
Dronedarone	Multaq®	Anti-arrhythmic	Abnormal heart rhythm	oral
Droperidol	Inapsine®, Droleptan®, Dridol®, Xomolix®	Anti-psychotic / Anti-emetic	Anesthesia (adjunct), nausea	injection
Erythromycin	E.E.S.®, Robimycin®, EMycin®, Erymax®, Ery-Tab®, Eryc Ranbaxy®, Erypar®, Eryped®, Erythrocin Stearate Filmtab®, Erythrocot®, E-Base®, Erythroped®, Ilosone®, MY-E®, Pediamycin®, Zineryt®, Abboticin®, Abboticin-ES®, Erycin®, PCE Dispertab®, Stiemycine®, Acnasol®, Tiloryth®	Antibiotic	Bacterial infection, increase GI motility	oral, injection
Escitalopram	Cipralex®, Lexapro®, Nexito®, Anxiset-E® (India), Exodus® (Brazil), Esto® (Israel), Seroplex®, Elicea®, Lexamil®, Lexam®, Entact® (Greece), Losita® (Bangladesh), Reposil® (Chile), Animaxen® (Colombia), Esitalo® (Australia), Lexamil® (South Africa)	Anti-depressant, SSRI	Depression (major), anxiety disorders	oral
Flecainide	Tambocor®, Almarytm®, Apocard®, Ecrinal®, Flécaine®	Anti-arrhythmic	Abnormal heart rhythm	oral
Fluconazole	Diflucan®, Trican®	Anti-fungal	Fungal infection	oral, injection
Gatifloxacin (Removed from US Market)	Tequin®	Antibiotic	Bacterial infection	oral, injection
Grepafloxacin (Off market worldwide)	Raxar®	Antibiotic	Bacterial infection	oral
Halofantrine	Halfan®	Anti-malarial	Malaria	oral
Haloperidol	Haldol® (US & UK), Aloperidin®,	Anti-psychotic	Schizophrenia, agitation	oral, injection

Generic Name	Brand Names (Partial List)	Drug Class	Therapeutic Use	Route
	Bioperidolo®, Brotopon®, Dozic®, Duraperidol® (Germany), Einalon S®, Eukystol®, Halosten®, Keselan®, Linton®, Peluces®, Serenace®, Serenase®, Sigaperidol®			
Ibutilide	Corvert®	Anti-arrhythmic	Abnormal heart rhythm	injection
Levofloxacin	Levaquin®, Tavanic®	Antibiotic	Bacterial infection	oral, injection
Levomethadyl (Removed from US Market)	Orlaam®	Opiate	Narcotic dependence	oral
Mesoridazine (Removed from US Market)	Serentil®	Anti-psychotic	Schizophrenia	oral
Methadone	Dolophine®, Symoron®, Amidone®, Methadose®, Physeptone®, Heptadon®	Opiate	Narcotic dependence, pain	oral, injection
Moxifloxacin	Avelox®, Avalox®, Avelon®	Antibiotic	Bacterial infection	oral, injection
Ondansetron	Zofran®, Anset®, Ondemet®, Zuplenz®, Emetron®, Ondavell®, Emeset®, Ondisolv®, Setronax®	Anti-emetic	Nausea, vomiting	oral, injection
Pentamidine	Pentam®	Antifungal	Fungal infection (Pneumocystis pneumonia)	injection
Pimozide	Orap®	Anti-psychotic	Tourette's Disorder	oral
Probucol (Removed from US Market)	Lorelco®	Antilipemic	Hypercholesterolemia	oral
Procainamide (Oral off US mkt)	Pronestyl®, Procan®	Anti-arrhythmic	Abnormal heart rhythm	injection
Propofol	Diprivan®, Propoven®	Anesthetic, general	Anesthesia	injection
Quinidine	Quinaglute®, Duraquin®, Quinact®,	Anti-arrhythmic	Abnormal heart rhythm	oral, injection

Generic Name	Brand Names (Partial List)	Drug Class	Therapeutic Use	Route
	Quinidex®, Cin-Quin®, Quinora®			
Sevoflurane	Ulane®, Sojourn®	Anesthetic, general	Anesthesia	inhaled
Sotalol	Betapace®, Sotalex®, Sotacor®	Anti-arrhythmic	Abnormal heart rhythm	oral
Sparfloxacin (Removed from US Market)	Zagam®	Antibiotic	Bacterial infection	oral
Sulpiride (On non US Market)	Dogmatil®, Dolmatil®, Eglonyl®, Espiride®, Modal®, Sulpor®	Anti-psychotic, atypical	Schizophrenia	oral
Terfenadine (Removed from US Market)	Seldane®	Antihistamine	Allergic rhinitis	oral
Thioridazine	Mellaril®, Novoridazine®, Thioril®	Anti-psychotic	Schizophrenia	oral
Vandetanib	Caprelsa®	Anti-cancer	Cancer (thyroid)	oral

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Appendix XIV

Screening Assessments for Designated Outside Assays

NOTE: "RARE VARIANT" is defined as variants which are associated with selected subprotocols ("rare variant" arms) for which screening of aMOIs and exclusion variants are performed by MATCH-designated "outside" laboratories prior to entry on the screening protocol (Step 0).

For patients for whom an applicable "rare variant" was identified by one of the designated laboratories (as noted below), the following requirements apply for the patient to be eligible for use of the outside assessment for possible treatment assignment on MATCH:

- Patient must meet the eligibility criteria in Section 3.1
- ***The outside laboratory notified the site that the patient may be a potential candidate for MATCH due to a detected "rare variant."*** The testing reports from the designated labs are **NOT** sufficient for this purpose. MATCH is **NOT** an option for a patient until this study-specific notification/referral has been received.
- Patients with an applicable "rare variant" must be able to meet the eligibility criteria for the appropriate subprotocols within 4 weeks following notification of treatment assignment, per Section 4.2. Please note, the receipt of this notification (and the start of the associated deadline for Step 1 registration) may occur shortly after Step 0 registration, since these patients will not be submitting tissue for screening purposes.

Patients who enter screening for determination of treatment assignment based on an outside assay result are eligible for only one treatment assignment on MATCH. If the patient is determined not to be eligible for a subprotocol open to outside assay "rare variant" results, the patient is off-study.

It is important that the designated laboratory has indicated that the patient is a potential patient for a MATCH subprotocol based on results of their genetic assay. Only a subset of the screened alterations may be considered aMOIs for this trial.

The designated outside laboratories may only act (i.e. refer patients) on any of the "rare variant" arms for which their assay reports all aMOIs, including the exclusionary variants, as defined prior to the addition of any novel variants (inclusion or exclusion) of the trial arm. Designated laboratories with assays that also detect any of the novel variants (inclusionary and/or exclusionary) are to include those variants when determining applicable referrals.

NOTE: The content and format of these specific notifications/study referrals for the Outside Assay process will vary depending on the designated outside lab in question, as they are each responsible for their own outreach efforts. It is strongly recommended that the designated outside laboratory be contacted to confirm the format and receipt of this notification of patient eligibility prior to registering any patients to Step 0.

NOTE: Please refer to Section 1.4.2 for information on the definitions of the study's Levels of Evidence (LOEs) for agents and variants, and the process by which certain types of specific aMOIs may be added dynamically to the study.

The list of designated laboratories and applicable subprotocols can be found on the following webpages:

Designated Laboratories: http://ecog-acrin.org/nci-match-eay131-designated-labs

Applicable Subprotocols: http://ecog-acrin.org/trials/nci-match-eay131

New designated laboratories may be considered for addition to the outside laboratory network, and will be incorporated into the webpage noted above on a rolling basis. Outside laboratories submit an application for review and approval by the NCI-MATCH Steering Committee and have undergone vetting to ensure referrals are consistent with the requirements of the MATCH trial. Laboratories added to the outside laboratory network will have met the following general requirements:

- Testing is performed in a CLIA-certified laboratory
- Outside laboratory NGS panels are analytically and clinically validated, with performance characteristics that meet NCI-MATCH outside laboratory requirements as defined by the Steering Committee.
- Outside laboratory NGS test panels interrogate all actionable mutations of interest (aMOIs) required for enrollment into the "Rare Variant" Arms (please refer to the webpage noted above)
- Outside laboratories agreed to use the existing workflow established by the core outside laboratories to identify patients for the "Rare Variant" Arms. This includes use of the MATCH "Rare Variant" template to identify aMOIs for submission to MATCHBox.
- The outside laboratory NGS test underwent central qualification review, during which the performance of the outside laboratory test may be compared with the NCI-MATCH central laboratory test to ensure good agreement with that assay. Any additional assay or platform that will be used by the outside laboratory to refer patients must also undergo a similar concordance assessment prior to implementation in their referral process.
- Outside laboratories must agree to formally collaborate with NCI (e.g. signed a collaboration agreement).

Summary of Requirements:

Below is a brief summary of requirements following patient registration to Step 0:

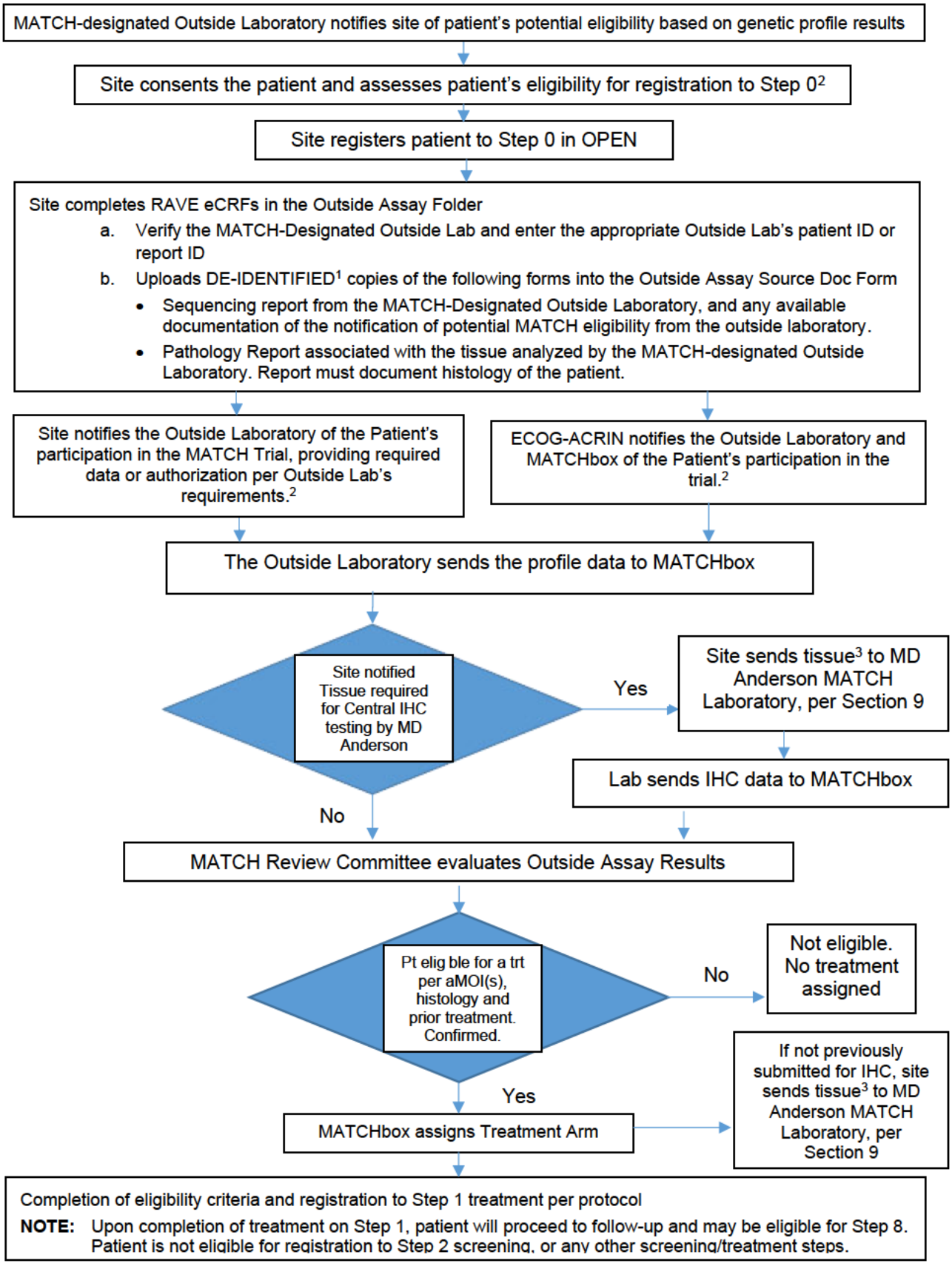
- For the copy of the report uploaded to Medidata Rave, modify a COPY of the sequencing report as follows:
  - Write "EAY131" and the patient's EAY131 case ID on each page of the report.
  - **REDACT** patient **NAME** and **DATE OF BIRTH** from every page of the report.
- Complete all required eCRFs in Medidata Rave including uploading electronic copies of:
  - The applicable sequencing report. A copy of the full report must be uploaded. If the size of the report is restrictive, separate the report into two parts and upload each part separately. Any available documentation of the notification of potential MATCH eligibility from the outside laboratory is also requested.
  - An electronic copy of the pathology reports indicating disease histology associated with the analyzed tumor tissue and the original diagnostic pathology (if different).

- Notify the MATCH-designated Outside Laboratory of the patient's participation in the trial. At a minimum, the site will be required to provide the trial information (EAY131) and the patient's EAY131 Case ID. The Outside Laboratory may require additional documentation authorizing the Outside Laboratory to release the data directly to MATCHbox and ECOG-ACRIN. Contact the outside lab directly to obtain guidance regarding their required documentation requirements.
- Archived tumor specimens are to be submitted to the MD Anderson MATCH trial laboratory as follows:
  - Copies of pathology reports and the report from the outside lab should be submitted with the tissue samples. Do NOT redact the reports. Write "EAY131" and the patient's EAY131 patient number on every page of the documents. Submission of these reports are required even if tissue is not available for the confirmation assessments.
  - If the site receives notification that archived tissue is required for central immunohistochemical (IHC) testing to determine patient eligibility for treatment, submit tissue using the kit for submission of confirmation tissue within 2 weeks. It is requested that adequate tissue for the IHC and confirmation testing be submitted. Treatment assignment cannot be determined until this assessment is complete.
  - For all other patients, tissue is to be submitted within 8 weeks following registration to Step 1. Tissue not submitted within 8 weeks following registration to Step 1 will be considered delinquent.
- If possible, submit tissue from the same specimen and time point as analyzed by the outside laboratory. This tissue can have been from more than 6 months prior to registration to MATCH if necessary and the patient can have received intervening cancer treatment between collection of the tissue and Step 0 registration. See Section 9 for tissue submission guidelines. Section **Error! Reference source not found.** describes the specifications for the submitted tissue.
- Notify the MD Anderson laboratory of any delays pertaining to the submission of the required IHC or central confirmation pathology materials. **A hard copy of the applicable sequencing report from the designated outside lab should be included with the tissue samples submitted to MD Anderson.**

  Log sample in the ECOG-ACRIN Sample Tracking System (STS) and include STS shipment manifest in the package. LOGGING THE SUBMISSION IN STS IS MANDATORY, as STS is an integral aspect for the informatics system for this trial.

NOTE: Failure to submit tissue for central IHC testing (if applicable) will result in the inability to assign patient to treatment.

SITE PROCESS FLOWCHART:



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1) **Deidentification for RAVE upload:** Redact Patient Name and Date of Birth ONLY. Write in patient Initials, "EAY131", and the EAY131 Patient Case ID on each page of the documents. Do not redact any other pathology or report identification numbers.

2) ECOG-ACRIN will provide the protocol number (EAY131), the EAY131 Case ID, and the Outside Laboratory's patient or report identifier (provided by the site in RAVE) to the Outside Laboratory. Verification of the patient's participation must be provided by the Site directly to the Outside Laboratory. At a minimum, the site will be required to provide the trial information (EAY131) and the patient's EAY131 Case ID. The Outside Laboratory may require additional documentation authorizing the Outside Laboratory to release the data directly to MATCHbox and ECOG-ACRIN. Contact the Outside Lab directly to obtain guidance regarding their required documentation requirements.

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3) Archival tissue is to be representative of that analyzed by the MATCH-designated Outside Laboratory. Do not redact reports submitted to the MD Anderson TQL to allow the laboratory to verify that the tissue received is from the appropriate patient, to retain "chain-of-custody" of the tissue submitted for evaluation, and to ensure proper reporting of results to the site.

Molecular Analysis for Therapy Choice (MATCH)

Appendix XV

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Historical Record of the Original Screening Process

Please see below for the eligibility criteria that were originally in place for patients submitting tissue to the MATCH trial laboratories for testing/screening purposes after Step 0 registration (i.e. the original screening process). As of 5:00 PM ET on May 22nd, 2017, patients can longer be registered to the study for this original screening process.

3.1.6.1 Patients must have tumor amenable to image guided or direct vision biopsy and be willing and able to undergo a tumor biopsy for molecular profiling. Patients with multiple myeloma other than plasmacytomas are to have a bone marrow aspirate to obtain tumor cells. Biopsy must not be considered to be more than minimal risk to the patient. See Section 9.

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NOTE: Registration to screening steps (Step 0, 2, 4, 6) must occur after stopping prior therapy. There is no specific duration for which patients must be off treatment prior to registration to the relevant screening step (and subsequently, the biopsy), as long as all other eligibility criteria are met.

OR

3.1.6.2 Patient will be undergoing a procedure due to medical necessity during which the tissue may be collected.

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NOTE: Registration to screening steps (Step 0, 2, 4, 6) must occur after stopping prior therapy. There is no specific duration for which patients must be off treatment prior to registration to the relevant screening step (and subsequently, the biopsy), as long as all other eligibility criteria are met.

OR

3.1.6.3 Formalin-fixed paraffin-embedded tumor tissue block(s) are available for submission following pre-registration (not applicable for bone marrow aspirate specimens). Criteria for the submission of FFPE tissue are:

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- Tissue must have been collected within 6 months prior to pre-registration to Step 0
  - Patient may receive treatment after tissue collection; however, lack of response (per Section 6) must be documented prior to Step 1. The following restrictions apply:
  - Enrollment onto another investigational study is not permitted.

- Intervening therapy that constitutes a new, molecularly targeted therapy is not permitted. Please note, immunotherapy is not considered molecularly targeted.
  - Continuation of an agent/regimen for which disease progression has been observed prior to biopsy is permitted, including targeted therapy.
- A new immunotherapy regimen is permitted; but, lack of response must also be documented prior to registration to Step 0.

- Formalin-fixed paraffin-embedded tumor tissue block(s) must meet the minimum requirements outlined in Section **Error! Reference source not found.**

Please see below for the registration procedures section that was previously in place for specimen submission in the original screening process (Step 0):

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4.1.5.3 Specimens must be submitted to the ECOG-ACRIN CBPF for central testing. Notify the laboratory of any delays pertaining to the submission of the pathology materials.

- Log sample in the ECOG-ACRIN Sample Tracking System (STS) and include STS shipment manifest in the package. LOGGING THE SUBMISSION IN STS IS MANDATORY, as STS is an integral aspect for the informatics system for this trial.

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- Include **paper copy of original diagnostic pathology report in submission**, and upload electronically in Medidata Rave.
- Include completed CLIA Laboratory Sample Submission Form. The contact information for the treating physician must include FAX number as the CLIA report will be distributed only to the designated physician. Information on the CLIA submission form must be clearly legible. Do not use cursive.

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- Upload electronic copy of pathology report for the submitted biopsy tissue (if performed) in Medidata Rave when available (within 5 days of the performance of the biopsy)

Failure to complete these steps may result in assay result reporting delays

It may take up to 14 days from the time the treatment assignment is made available in RAVE for results to be returned by fax to the physician designated on the CLIA Laboratory Sample Submission and potential treatment assignments made available via Medidata Rave. Registration cannot proceed until the central determination is complete, unless treatment assignment is based on results from a designated outside laboratory.

Please see below for the specimen collection and submission procedures section that was previously in place for specimen submission in the original screening process (Step 0) from Sections 7.2 and Section 9:

7.2 Biospecimen Submissions

Failure to update Sample Tracking System (STS) appropriately may result in delays in the central assessments and reporting results to the site. See Section 9 for specific instructions regarding the collection and submission of specimens.

Pathology reports from original diagnostic and/or most recent biopsy prior to registration to Step 0 MUST be submitted with all screening specimen submissions, regardless of the screening step. Failure to provide the reports, and thus, the inability to confirm histology, will result in delay of notification of treatment assignment.

Pathology reports submitted with tissue specimens should NOT be redacted. Results from screening and confirmation testing are returned to the site and will impact patient care. Adequate identification must be provided to ensure that the appropriate materials have been submitted and reporting is correct.

Snapshot user's guide for STS is provided in Section 9.6. Most information, if entered incorrectly, may be corrected by the site. To correct mistakes for sample type or timepoint, contact the ECOG-ACRIN Lab Team (ecog.tst@jimmy.havard.edu or 857-504-2900). Please do not delete samples and re-log to correct these errors, especially after samples have already been shipped.

i. Overall Summary of Options for Sample Submissions for Screening:

Step	Disease	Options for submissions	Biopsy 1	Biopsy Repeat
Step 0[1]	Solid Tumor Lymphoma Plasmacytoma	Option 1	Fresh Biopsy	Fresh biopsy (#2)
		Option 2	Fresh Biopsy	Pre-Trial FFPE[1]
		Option 3	Pre-Trial FFPE[1]	Fresh Biopsy
Step 0	Myeloma requiring bone marrow aspirate[2]		Fresh Biopsy	Fresh biopsy (#2)
Step 0	"Rare Variant" report per Outside Laboratory	Timepoint: "Confirmation of Pre-Registration Outside Results" Submit: Pre-Trial FFPE[3]		
Screening 2 (Step 2 or 4)	All[3]		Fresh Biopsy	Fresh biopsy (#2)
Step 8	All		Blood Specimens Fresh Biopsy, if performed	

1. Archived FFPE tissue meeting the requirements in Section **Error! Reference source not found.** may be submitted for the Step 0 Biopsy 1 OR the Step 0 Biopsy Repeat, but not BOTH. If archived FFPE is submitted for analysis and additional FFPE is requested to meet the needs for the screening, the additional materials are considered to be for the same assessment and are logged under the same time point as the initial FFPE submission in STS.
2. MULTIPLE MYELOMA REQUIRING FRESH BONE MARROW ASPIRATE ONLY: For all screenings and end of treatment, use 1 drop to make 4 – 5 smears, put 1 – 1.5 ml in one EDTA tube, and put remainder (5 - 10 ml) of bone marrow aspirate in the other EDTA tube. SUBMIT ASPIRATES FROM THE FIRST PULL OF AN ASPIRATION SITE FOR TESTING. The submitted smears should also be made from the same aspirate.

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3. For patients entering MATCH via a referral from a designated outside laboratory, submit previously collected FFPE tumor tissue representative (if possible) of that analyzed by the designated outside laboratory for central confirmation of the outside assay results, as follows:
   - Within 2 weeks following notification that archived FFPE specimens are required for central assessments (e.g. IHC) for purposes of treatment assignment.

     OR
   - Within 8 weeks following registration to treatment (Step 1), if not required for mandatory assessments (e.g. IHC).

Results of the assessment will be returned to the site, but will not impact patient participation in the assigned treatment arm (unless required in advance for treatment assignment purposes).

NOTE: Patients assigned to treatment based on a "rare variant" determined by a designated laboratory (see Section 3.1.6 and Appendix XIV) are eligible for only one treatment on MATCH and are not to undergo a biopsy to collect material for screening. Patients who progress on Step 1 are eligible for subsequent registration to Step 8 and the subsequent collection and submission of research samples.

7.2.2 Specimen Submission Requirements:

Time Point	Sample Type	Alternatives In STS, Click "SHOW" to view Alternatives	Comment
FRESH BIOPSY SUBMISSIONS[1]			
Screening	Fresh Tissue (4 Fresh Tissue Cores in Formalin)	If Multiple Myeloma Requiring Aspirate[4] -BM aspirate, EDTA	**Archived FFPE specimens may be requested for confirmation of MLH1 or MSH2 testing.** See "Pre-trial diagnostic material"[3]
	Cells, Fine Needle Aspirate (FNA cells in Cytolyte)		
	UNSTAINED SLIDE_FNA (FNA smear)	Stained FNA smear OR If Multiple Myeloma requiring aspirate[4] - BM aspirate smear (4)	

Time Point	Sample Type	Alternatives In STS, Click "SHOW" to view Alternatives	Comment
FORMALIN FIXED PARAFFIN-EMBEDDED (FFPE) SUBMISSIONS[2,3] – Alternative to Fresh Biopsy Submission, with collection following registration to screening step or within 6 months prior to registration to Step 0 (See Section 7.2.1). To log FFPE blocks or slides, as alternatives to Fresh Tissue Cores, or Cells in cytolyte (if submitting cell blocks generated from pleural or peritoneal effusions), click "SHOW" in STS to view sample type.			
Screening	FFPE Tumor Block	• H&E • Unstained slides (20+)	**Archived FFPE specimens may be requested for confirmation of MLH1 or MSH2 testing.** See "Pre-trial diagnostic material"[3]
FORMALIN FIXED PARAFFIN-EMBEDDED (FFPE) SUBMISSIONS – "RARE VARIANT" CONFIRMATION: Tissue representative of that analyzed by the designated outside laboratory. See **Error! Reference source not found.**			
Confirmation of Pre-Registration Outside Results	FFPE Tumor Block	H&E Unstained slides (20+)	**Submit following notification that archived FFPE specimens are required for central IHC assessments for purposes of treatment assignment** **OR** **After treatment assignment, if not required for mandatory IHC assessments.**
SUBMISSION OF ADDITIONAL SUBMISSIONS PER PATIENT CONSENT – SubProtocol specific submissions are outlined in the specifc subprotocol.			
Pre-trial Diagnostic Material[3]	FFPE Tumor Block	• H&E • Unstained slides (20+)	Tissue collected PRIOR to registration to Step 0 and... a) On EAY131 Master Protocol Consent Form, patient answered "Yes" to "I agree to provide additional specimens for research" OR b) **Specimens requested for confirmation of MLH1 or MSH2 testing**[3]

Time Point	Sample Type	Alternatives In STS, Click "SHOW" to view Alternatives	Comment
Each Screening Submission	• Periph Bld K2-ETDA (1) • Peripheral blood, Streck Cell-Free DNA Tube (2)		On EAY131 Master Protocol Consent Form, patient answered "Yes" to "I agree to provide additional specimens for research"
Step 1, 3, 4, 6: End of Cycle 2 or End of Treatment[5]	Peripheral blood, Streck Cell-Free DNA Tube (2)		On EAY131 Master Protocol Consent Form, patient answered "Yes" to "I agree to provide additional specimens for research"
Progression, End of Treatment Biopsy	• Periph Bld K2-ETDA (1) • Peripheral blood, Streck Cell-Free DNA Tube (2)		Consent to submission of progression samples (Step 8)
	• Fresh Tissue Cores in Formalin • Cells, Fine Needle Aspirate in Cytolyte • FNA smears	If Multiple Myeloma requiring aspirate[4] - BM aspirate, EDTA - BM aspirate smear (4)	

1. Exceptions to submission requirements must receive approval from the MATCH pathologist.
2. If previously collected FFPE will be submitted for Step 0 screening, the following criteria must be met:
   - Tissue must have been collected within 6 months prior to pre-registration to Step 0 and patient must not have received any targeted intervening therapy (see Section 3.1.6.3 in Appendix XV) for their cancer since the collection of the tumor sample. This does not apply for patients entering a "rare variant" arm via an outside assay.
   - Formalin-fixed paraffin-embedded tumor tissue block(s) must be submitted. The optimal block is 70% TUMOR CELLULARITY. Specimen size requirement is as follows:
3. NOT APPLICABLE TO MYELOMA PATIENTS WITH ASPIRATE SUBMITTED FOR SCREENING. Screening tumor samples with loss of MLH1 or MSH2, as determined by IHC, will result in a request for the submission of pre-trial diagnostic tumor tissue for central confirmation of this result. Tissue, if available, is to be submitted within 8 weeks of request or within 8 weeks of assignment to treatment subprotocol EAY131-Z1D. The results of the confirmation will be reported to the site. This additional assessment will not impact the screening assessment timeline and treatment assignment will not be held pending the outcome of the confirmation assessments. If the results are complete prior to treatment assignment, the confirmation results will be used by MATCHbox.
4. MULTIPLE MYELOMA REQUIRING FRESH BONE MARROW ASPIRATE ONLY. SUBMIT ASPIRATES FROM THE FIRST PULL OF AN ASPIRATION SITE FOR SCREENING. Step 8 submissions may be from first or second pull. See Section 7.2.1 (footnote 2).

   For Step 8, submission of blood specimens are mandatory. Biopsy for collection of the research fresh tumor specimens is to be performed only if patient is amenable and biopsy is minimal risk or less OR if the procedure performed is standard of care. If the fresh tumor will not be submitted, indicate "Can't Submit" in STS and provide the reason why
5. Collect blood end of Cycle 2 (prior to Cycle 3 treatment) or at end/discontinuation of MATCH treatment, whichever is earlier. Please refer to Section **Error! Reference source not found.**.

7.2.2 Sample Tracking System (STS) Timepoint Summary:

Timepoint	Sample Submission Purpose
Pre-trial Diagnostic Material	Archived Formalin fixed tumor tissue (FFPE) collected prior to registration to Step 0 and submitted only for banking (future research) or if requested for MHL1/MSH2 confirmation
Confirmation of Pre-Registration Outside Results	Submitted from patients screened for treatment assignment based on "rare variant" results determined by designated outside laboratories. Archived Formalin fixed tumor tissue (FFPE) collected prior to registration to Step 0 representative of tissue analyzed by designated outside laboratories. Indicate if sample submitted for MANDATORY IHC.
Step 0, Screening 1, Biopsy 1	• Specimens submitted for screening, see table in Section 7.2.1 for sample type options. • Peripheral blood collected for research per patient consent.
Step 0, Screening 1, Biopsy Repeat	• Specimens submitted for screening after failure of "Screening 1, Biopsy 1" assessment. See table in Section 7.2.1 for sample type options. Specimens submitted for screening, see table in Section 7.2.1 for sample type options. • Peripheral blood collected for research per patient consent.
Screening 2, Biopsy 1	• Specimens obtained from a fresh biopsy and submitted for screening. • Peripheral blood collected for research per patient consent.
Screening 2, Biopsy Repeat	• Specimens obtained and submitted for screening after failure of "Screening 2, Biopsy 1" assessment. • Peripheral blood collected for research per patient consent.
Step 1, End of Cycle 2 Step 3, End of Cycle 2 Step 5, End of Cycle 2 Step 7, End of Cycle 2	Peripheral blood samples collected for research per patient consent at End of Cycle 2 (prior to start of Cycle 3 treatment) or upon discontinuation of treatment, whichever comes first.
Step 8	Sample submissions for research only: Fresh biopsies and peripheral blood.

9. Biopsy Target Selection, Performance and Submission of Specimens

Please review the study schema, Section 5.1 and Section 7.2 for a summary on when a biopsy for screening assessments (Step 0 and Step 2 or 4) and progression (Step 8 after completion of all treatment on MATCH) is to be performed. A repeat biopsy may be requested if submitted screening biopsy materials are inadequate for the screening assessments. Blood samples, from consenting patients, will also be collected and submitted with each biopsy (1 EDTA tube and 2 Streck tubes) and EITHER at end of cycle 2 or discontinuation of treatment on each MATCH subprotocol treatment (2 Streck tubes only), per Section **Error! Reference source not found.**.

NOTE: For the collection of fresh biopsy specimens, fine needle aspiration followed by core needle biopsy, with submission of the FNA and the biopsy cores, is required.

NOTE: If a report of a "rare variant" determined by one of the designated outside laboratories (Appendix XIV) is used for Step 0 screening, representative (from the same tissue analyzed by the outside lab, if possible) archived tumor tissue is to be submitted as outlined in Section **Error! Reference source not found.** for confirmation of the "rare variant" by the MATCH assay. The MATCH assay results for these patients will be returned to the site but will not

impact assignment to or continuation of treatment on MATCH. However, for certain "rare variant" arms, submission of FFPE tumor tissue may be required for additional central assessments (e.g. IHC) prior to final treatment arm assignment based on the Outside Laboratory results. FNA aspirations and fresh biopsies should not be submitted for these patients. However, submission of optional blood specimens as described below (2 Streck tubes and one EDTA tube) is encouraged.

Specimen submissions associated with a specific study subprotocol will be outlined in the specific subprotocol.

The date and time of collection and shipping date must be entered into the ECOG-ACRIN Samples Tracking System (STS) for all submitted specimens. ***Failure to update STS appropriately may result in delays in the central assessments and reporting results to the site.***

KIT for specimen collection and shipping: order form is located in Appendix XII.

LABELING: All samples are to be labeled with the ECOG-ACRIN protocol number EAY131, the assigned ECOG-ACRIN patient sequence number, **date and time of collection**, and type of material.

The submitting pathologist and clinical research associate should refer to Appendix II (Pathology Submission Guidelines) for guidelines and summary of submission requirements.

NOTE: Previous clinical pathology report MUST be submitted with the specimen, or the sample will not be processed. Do NOT redact the pathology reports.

9.1 Biopsy Instructions

NOTE: For Multiple Myeloma, bone marrow aspirate must be submitted. See Section **Error! Reference source not found.**

A pre-treatment tumor biopsy or bone marrow aspirate (myeloma patients only) (Step 0 and Step 2 or 4) will be obtained from patients who sign the consent to enroll in the study as detailed in Section 5. End-of-treatment biopsies are requested from patients who have provided additional consent and meet the criteria outlined in Section 3.9.

Brain biopsies will be permitted if the patient has medical necessity for craniotomy for clinical care. Mediastinal, laparoscopic, gastrointestinal, or bronchial endoscopic biopsies can be obtained incidentally to a clinically necessary procedure and not for the sole purpose of the clinical trial. Acceptable biopsy procedures are:

- Percutaneous biopsy with local anesthetic and/or sedation with an expected risk of severe complications < 2%
- Excisional cutaneous biopsy with local anesthetic and/or sedation with an expected risk of severe complications < 2%
- Biopsy with removal of additional tumor tissue during a medically necessary mediastinoscopy, laparoscopy, gastrointestinal endoscopy, bronchoscopy or craniotomy
- Removal of additional tumor tissue during a medically necessary surgical procedure

- Bone marrow aspirate (myeloma patients only, if applicable)

No open surgical, laparoscopic endoscopic procedure will be performed solely to obtain a biopsy for this protocol.

NOTE: For Step 0, biopsy 1, pre-trial FFPE tumor tissue that meets the criteria in Section **Error! Reference source not found.** may be submitted for analysis in lieu of a fresh biopsy sample for Biopsy 1 or the repeat biopsy (if performed) but not both.

9.1.1 Contraindications to percutaneous biopsy:

1. Significant coagulopathy that cannot be adequately corrected.
2. Severely compromised cardiopulmonary function or hemodynamic instability.
3. Lack of a safe pathway to the lesion.
4. Inability of the patient to cooperate with, or to be positioned for, the procedure.

9.1.2 Tissue specimens will be sent for analyses as defined in Section **Error! Reference source not found.**. Specimens are generally expected to provide 20 nanograms of nucleic acids. If a site is deemed appropriate for biopsy with minimal risk (no more than 2% risk of serious complication requiring hospitalization) to the participant by agreement between the investigators and Interventional Radiology, an attempt at biopsy will be made.

The biopsy procedure to be used in this protocol is described below; local anesthesia will be administered as needed. Sedation may be used for comfort if considered safe for the patient. Such biopsies can be safely performed as evidenced by literature reports [12,13, 20], as well as, experience at the NIH Clinical Center, where, among 244 research tumor biopsies (18G needle) in liver (126), subcutaneous/chest wall/abdominal wall (36), intramuscular (18), lung and lymph nodes, there were 8 minor cases of bleeding, and one pneumothorax requiring 48h hospitalization (21) and MD Anderson Cancer Center (22). Most biopsies will probably be in liver. The complication rate at Massachusetts General Hospital (23) of percutaneous liver biopsy similar to those that will be done for this trial is 1.2 % of 732 cases. Risks of the procedure include, but are not limited to, bleeding, infection, pain, and scarring.

9.1.3.1 Complications associated with any screening biopsy or end-of-treatment biopsy will be reported and tracked as protocol-related AEs within Medidata Rave.

9.1.3.2 Severe or major complications are considered to be (13):

- Requires therapy, minor hospitalization (more than overnight only but < 48 h)
- Requires major therapy; unplanned increase in level of care, prolonged hospitalization> 48h
- Permanent adverse sequelae

- Death

9.1.3.3 Minor complications

- No therapy, no consequence
- Nominal therapy, no consequence – includes overnight admission for observation only

9.1.4 Pre-biopsy Lesion Assessment (for those undergoing biopsy by Interventional Radiologist)

The Interventional Radiologist should provide the investigator with an assessment of whether a lesion that is likely to yield enough material for molecular profiling can be found and biopsied with acceptable risk. Three main factors are considered:

iv. whether a suitable lesion (viable tumor) for biopsy is present (yield)

v. whether the lesion selected for biopsy can be sampled aggressively (yield)

vi. the expected level of risk of major complication to the patient (< 2% major complication to be eligible for biopsy)

9.1.4.1. A pre-biopsy lesion scoring system (scale from 1-3) adapted from the one used by Interventional Radiology at the MD Anderson Cancer Center (courtesy of Dr. Michael Wallace, Chair Ad-Interim of the Department of Interventional Radiology) will be used to assign a value to the interventional radiologist's assessment of the lesion. An analogous 5-point subjective scale has been internally studied at MD Anderson Cancer Center and lesions given a score of ≤ 2 were found to have a lower yield rate for CMS-46 testing when compared to lesions that were scored ≥ 4 (24).

9.1.4.2. The radiologist will refer to the Radiology Biopsy Manual for case examples and detailed criteria for scoring and assign a pre-biopsy lesion score to the patient. Briefly, the qualitative assessment criteria for the 3-point scale is as follows:

Pre-Biopsy Lesion Scoring Scale (courtesy MD Anderson Cancer Center)

Score Assigned	Likelihood of Yield	Reason for Score & Biopsy Disposition
1	Low (< 25%)	Reason: no target amenable to biopsy, high risk procedure. Biopsy disposition: should not be performed.
2	Uncertain (25-75%)	Reason: Uncertainty about success either due to technical challenges or lesion characteristics (e.g., small size, necrotic, sub-solid lesion, sclerotic, not FDG–avid, technically difficult biopsy). Sclerotic bone lesion usually low yield. Biopsy disposition: Communication with investigator for these types of lesions should occur to determine whether to proceed with biopsy.
3	High (> 75%)	Reason: viable tumor demonstrated on diagnostic imaging (enhancing lesion, growing lesion) that can be sampled aggressively. Disposition: Proceed with biopsy.

9.1.4.3. Record the pre-biopsy lesion score and the name of the interventional radiologist on the screening eCRF in Rave. If a lesion is rated 1 or 2, the reason should be denoted on the CRF and this suspicion for low likelihood of success should be communicated to the investigator to discuss whether or not to proceed with the biopsy. Final disposition of the discussion (to proceed or not to proceed) should be documented on the CRF.

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9.1.4.4. Pre-biopsy imaging and CRF with lesions score assessment and interventional radiologist's name are submitted in all cases, including those where decision is made to not proceed with biopsy.

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9.1.5. Biopsy Procedure

This section applies to patients who have not had tumor tissue acquired through normal clinical care for either diagnostic or therapeutic procedures. Tumor tissue acquired during regular clinical care would be harvested by the appropriate physician (e.g., neurosurgeon during craniotomy).

9.1.5.1. If a non-cutaneous lesion site is deemed appropriate for biopsy with minimal risk to the participant by agreement between the investigators, patient and Interventional Radiologist an attempt at biopsy will be made. All internal organ biopsies will be done by the Interventional Radiologist with a percutaneous approach, or during a clinically necessary surgical/endoscopic procedure, including craniotomy for brain metastasis or tumor.

- No endoscopic, laparoscopic, or surgical procedure will be done solely to obtain a biopsy for this protocol.

9.1.5.2. The choice of imaging modality to be used to facilitate tissue acquisition during the biopsy procedure will be decided by members of the Interventional Radiology team at the clinical site and may include ultrasound, CT scan, or MRI. Should CT scan be needed for biopsy, the number of scans for each procedure will be limited to the minimum number needed to safely obtain a biopsy. Tumor biopsies will be performed only if they are considered to be of low risk (< 2% major complication rate) to the participant as determined by the investigators and Interventional Radiologist. Biopsies will be performed under local anesthesia and/or sedation.

9.1.5.3. For the collection of biopsy specimens, fine needle aspiration followed by core needle biopsy, with submission of the FNA and the biopsy cores, is required. For all specimen types, the tumor cellularity yielded by FNA may be superior to core needle biopsy in some patients, particularly when the lesion is comprised predominantly of fibrosis, bone, or other non-tumor tissue. The FNA sampling with at least 2 passes should be sufficient to provide a specimen for preparation of smears and a cell block. It is preferred that at least four core biopsies 16-18 gauge in diameter and at least 1 cm in length after a fine needle aspiration specimen are obtained as described below. Biopsies will be sent for analyses as defined in the protocol.

9.1.5.4. Biopsy images should be submitted to IROC at the ACR Core Laboratory (see Section 11) and include image before needle placement, image with FNA or trocar needle in lesion, image with core biopsy or trocar needle in lesion, biopsy report describing target lesion location (organ, laterality or segment if applicable and image number on pre-biopsy imaging if available) and size.

9.1.5.5. The date and time of collection and shipping date must be entered into the ECOG-ACRIN Sample Tracking System (STS) for all submitted specimens.

9.1.5.6. LABELING: All samples are to be labeled with the ECOG-ACRIN protocol number EAY131, the assigned ECOG-ACRIN patient sequence number, date and time of collection, and type of material.

9.1.5.7. The submitting pathologist and clinical research associate may refer to Appendix II (Pathology Submission Guidelines) for guidelines and summary of submission requirements.

9.2. NCI MATCH EAY131 Clinical Trial Specimen Kit Instructions for Specimen Collection and Shipping

For results and specimen submission inquiries:

MATCHTRIAL@mdanderson.org & MATCH@jimmy.harvard.edu

Phone: 1-844-744-2420 (OPTION 4) Fax: 713-745-4925

For all kit related inquiries:

EACBPF@mdanderson.org

Phone: 1-844-744-2420 (OPTION 5) Fax: 713-563-6506

Specimen collection days:

Specimen collection can be done Monday through Friday with overnight shipping for arrival on Tuesday through Saturday at the ECOG-ACRIN Central Biorepository and Pathology Facility (EA CBPF). For Step 0, kit requests are automatically sent to the CBPF upon patient registration. For subsequent steps (including Step 0 repeat biopsy), email or fax the kit order form, located in Appendix XII, to the CBPF.

9.2.1 Acceptable specimen types

- Core needle biopsy, fine needle aspiration smears and rinses. Submission of FNA alone is not acceptable
- Excisional cutaneous biopsy tissue
- Additional endoscopic, laparoscopic, or surgical tissue acquired during medically necessary procedure for clinical care
- Pleural biopsy with effusions (contact MD Anderson prior to performance of the biopsy and sample collection to confirm adequacy)
- Bone marrow aspirate (myeloma patients only, if applicable)
- Archived FFPE tissue or unstained slide alternative

9.2.2. Contents of screening or Step 8 shipping **kit for solid tumor patients (for multiple myeloma patients for whom bone marrow aspirates will be submitted, see Section** Error! Reference source not found.**)**:

1. One patient CLIA Laboratory Sample Submission Form.
2. One EDTA venipuncture blood collection tube and two Streck venipuncture blood collection tubes with labels and absorbent protective packing material.
3. Five frosted-tip slides in container, for use in preparation of FNA smears.
4. One 30 mL container of Cytolyt for rinsing of FNA needle.
5. Two tissue specimen cassettes and four sponges for core needle biopsy specimens.
6. One neutral-buffered formalin-filled container for fixing the collected core biopsies.
7. One pre-printed FedEx shipping label sticker pre-addressed to the EA Central Biorepository and Pathology Facility.
8. Three neon green address labels.
9. Cold pack, warm pack, and temperature monitor device.
10. Strip of specimen labels.

1. UN3373 IATA labels
2. LockMailer
3. Shipping guide document
4. Image-guided biopsy manual
5. Kit instructions.

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9.2.3. Contents of screening or Step 8 shipping **kit for Myeloma patients only who will have a bone marrow aspirate collected:**

1. One patient CLIA Laboratory Sample Submission Form.
2. One EDTA venipuncture blood collection tube and two Streck venipuncture blood collection tubes with labels and absorbent protective packing material
3. Two EDTA collection tubes for Bone Marrow Aspirate collection.
4. Five frosted-tip slides in containers.
5. Two plain syringes.
6. One pre-printed FedEx shipping label sticker pre-addressed to the EA Central Biorepository and Pathology Facility.
7. Three neon green address labels.
8. Cold pack, warm pack, and temperature monitor device.
9. Strip of specimen labels.
10. IATA labels (UN3373).
11. Shipping guide document
12. Kit instructions.

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9.2.4. Contents of "Rare Variant" shipping **kit for Formalin Fixed Paraffin-Embedded Tumor Sample for Confirmation of "Rare Variant" Results Determined by Designated Outside Laboratory.**

1. One patient CLIA Laboratory Sample Submission Form.
2. One EDTA venipuncture blood collection tube and two Streck venipuncture blood collection tubes with labels and absorbent protective packing material.
3. Five slide containers.
4. One pre-printed FedEx shipping label sticker pre-addressed to the EA Central Biorepository and Pathology Facility.
5. Three neon green address labels.
6. Cold pack, warm pack, and temperature monitor device.
7. Strip of specimen labels.
8. Kit instructions.

9.2.5. Instructions for use of screening or Step 8 shipping kit (select procedure type A, B, C, D, E or F):

A: Fine needle aspiration and core needle biopsy procedure:

For the collection of biopsy specimens, fine needle aspiration followed by core needle biopsy, with submission of the FNA and the biopsy cores, is required. For all specimen types, the tumor

cellularity yielded by FNA may be superior to core needle biopsy in some patients, particularly when the lesion is comprised predominantly of fibrosis, bone, or other non-tumor tissue. The FNA sampling with at least 2 passes should be sufficient to provide a specimen for preparation of smears and a cell block. It is preferred that at least four core biopsies 16-18 gauge in diameter and at least 1 cm in length after a fine needle aspiration specimen are obtained. Biopsies will be sent for analyses as defined in the protocol.

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FNA sampling should provide sufficient material for preparation of smears and a cell block.

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Preferred procedure checklist:

_____ 1. Verify patient identification, enrollment in OPEN, and signed consent for EAY131.

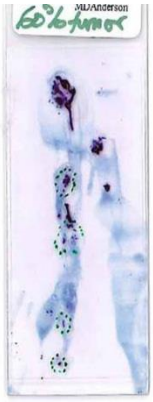
_____ 2. Complete CLIA Laboratory Sample Submission Form and label blood collection tubes, frosted-tip slides, cassettes and all specimen collection containers. Information on the CLIA submission form must be clearly legible. Do not use cursive.

_____ 3. Perform venipuncture for collection of blood into labeled EDTA and Streck tubes and place filled labeled tubes into biohazard bag with absorbent protective packing material.

_____ 4. Perform image-guided or visually-guided fine needle aspiration (FNA) of lesion. The FNA sampling with at least 2 passes should be sufficient to provide a specimen for preparation of smears and a cell block.

_____ 5. If a cytopathologist and/or cytologic technologist is available on site, immediately fix one slide in 95% ethanol (provided by enrolling institution) and air dry another slide for each FNA pass. The air dried slide will be stained by Diff-Quik method to assess tumor cell quantity and quality (see examples below)

Optimal specimen



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Satisfactory specimen

If a cytopathologist and/or cytologic technologist is unavailable for immediate assessment, please skip Step 6 and proceed to Step 7 below.

____ 6. If the first FNA specimen has low tumor cellularity (see illustrations above for examples of satisfactory FNA smears), repeat the FNA in a different location in larger sized lesions (> 2cm) or in a different lesion if one is accessible.

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_____ 7. If on-site cytopathologic assessment is not available, collect tumor sample by FNA. Label slides and prepare smears for each FNA pass. Immediately fix one slide in 95% ethanol (provided by enrolling institution) in a LockMailer and air dry another slide. Place the air dried slide in the dry slide container provided. Secure the lid of the LockMailer and place it inside the biohazard bag containing an adsorbent. Place this in the kit. Secure the lid of the slide container and place it in the shipping kit.

_____ 8. Rinse FNA needle into the 30 ml CytoLyt tube, secure the tube cap, and place the tube into the shipping kit.

_____ 9. It is preferred that at least four core biopsies 16-18 gauge in diameter and at least 1 cm in length after a fine needle aspiration specimen are obtained. Place two cores between sponges in each of the two cassettes (total of four cores), snap the cassette lids in place, place the two cassettes into the formalin-filled container, secure the container lid, and place the container into the shipping kit. If touch preparation slides of cores are made, place them in the dry slide container. Secure the slide container lid and place it in the shipping kit.

_____ 10. If your institution requires pathologic examination of removed tissue, place a fifth core in a labeled

specimen container with fixative for transport to your Pathology Department. (This specimen container is not provided in the shipping kit.) Results of this review must be uploaded to Medidata Rave as soon as available.

_____ 11. Log the samples into the EA Sample Tracking System and print the STS generated shipping manifest.

_____ 12. Place the following forms into the shipping kit:

____ This checklist

____ Prior clinical pathology reports, and any immunologic, immunohistochemical, or molecular study reports performed on pre-trial diagnostic tumor specimen. **Do NOT redact the reports.**

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____ CLIA Laboratory Sample Submission Form completely filled out. Information on the CLIA submission form must be clearly legible. Do not use cursive.

____ The Sample Tracking System (STS) generated shipping manifest.

_____ 13. Activate the temperature monitor by pressing and holding the start button for 10-15 seconds. The green LED light will be solid, and then both red and green LED's will be solid. At this time, release the start button, and the green LED will blink rapidly for 3 seconds. Place the unit in the kit inside the blood tube packaging – it will flash red and green LED for 15 minutes. For temperature control, add cold pack or warm pack, dependent on time of year specimen is shipped. Use warm pack if outside temperature at your site or along transit route will be below freezing.

_____ 14. Use one kit per patient. Do not place specimens from more than one patient into a kit. Secure the packing, seal the kit, and apply the following shipping labels: Three neon green address labels, FedEx airbill, and UN3373 IATA label. Refer to separate shipping guide document for pictures of label placement and further shipping guidelines. Contact FedEx for pickup. **Package must be picked up the same day specimen is collected.**

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B. Excisional cutaneous biopsy specimens checklist:

_____ 1. Verify patient identification, enrollment in OPEN, and signed consent for EAY131.

_____ 2. Complete CLIA Laboratory Sample Submission Form and label blood collection tubes, cassettes and all

specimen collection containers. Information on the CLIA submission form must be clearly legible. Do not use cursive.

_____ 3. Perform venipuncture for collection of blood into labeled EDTA and Streck tubes and place filled labeled tubes into biohazard bag with absorbent protective packing material.

_____ 4. Obtain tumor biopsy tissue, place between the sponges in one or two of the cassettes as needed depending upon the size of the tissue, place the cassette(s) in the formalin-filled container, secure the container lid, and place the container into the specimen kit.

_____ 5. If your institution requires pathologic examination of removed tissue, place a portion of the biopsy specimen in a labeled specimen container with fixative for transport to your Pathology Department. (This specimen container is not provided in the shipping kit.) Results of this review must be uploaded to Medidata Rave as soon as available.

_____ 6. Log the samples into the EA Sample Tracking System and print the STS generated shipping manifest.

_____ 7. Place the following forms into the shipping kit:

____ This checklist

____ Prior clinical pathology reports, and any immunologic, immunohistochemical, or molecular study reports performed on pre-trial diagnostic tumor specimen. **Do NOT redact the reports.**

____ CLIA Laboratory Sample Submission Form completely filled out. Information on the CLIA submission form must be clearly legible. Do not use cursive.

____ The Sample Tracking System (STS) generated shipping manifest.

_____ 8. Activate the temperature monitor by pressing and holding the start button for 10-15 seconds. The green LED light will be solid, and then both red and green LED's will be solid. At this time, release the start button, and the green LED will blink rapidly for 3 seconds. Place the unit in the kit inside the blood tube packaging – it will flash red and green LED for 15 minutes. For temperature control, add cold pack or warm pack, dependent on time of year specimen is shipped. Use warm pack if outside temperature at your site or along transit route will be below freezing.

_____ 9. Use one kit per patient. Do not place specimens from more than one patient into a kit. Secure the packing, seal the kit, and apply the following shipping labels: Three neon green address labels, FedEx airbill, and UN3373 IATA label. Refer to separate shipping guide document for pictures of label placement and further shipping guidelines. Contact FedEx for pickup. **Package must be picked up the same day specimen is collected.**

C. Additional specimens from medically necessary endoscopic, laparoscopic, or surgical procedures performed for clinical care

See instructions and use checklist for excisional cutaneous biopsy specimens in Section B above.

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D. Multiple Myeloma – If bone marrow aspirate to be submitted. See Section **Error! Reference source not found.**

For patients with plasmacytomas, follow the instructions outlined above for solid tumors. FOR MULTIPLE MYELOMA WHICH REQUIRE BM ASPIRATES ONLY: SUBMIT ASPIRATE FROM THE FIRST PULL.

Perform venipuncture for collection of blood into labeled EDTA and Streck tubes and place filled labeled tubes into shipping kit with protective packing material.

Draw the bone marrow aspirate materials into a plain syringe, then:

1. Use 1 drop to make 4 – 5 smears.
2. Put 1 – 1.5 ml in one EDTA tube.
3. Put remainder (5 - 10 ml) of bone marrow aspirate in the other EDTA tube.

E. Archival FFPE tissue or unstained slides alternative:

_____ 1. Verify patient identification, enrollment in OPEN, and signed consent for EAY131.

_____ 2. Complete CLIA Laboratory Sample Submission Form and label blood collection tubes.

_____ 3. Perform venipuncture for collection of blood into labeled EDTA and Streck tubes and place filled labeled tubes into biohazard bag with absorbent protective packing material.

_____ 4. Log the samples into the EA Sample Tracking System and print the STS generated shipping manifest. Log screening tissue under time point “Step 0, Screening 1, Biopsy 1”. The time point “Pre-trial Diagnostic Material” is for research tissue submission only.

_____ 5. Place the following forms into the shipping kit (archival FFPE submission for screening must be sent in the provided specimen collection kit):

- ____ This checklist
- ____ Prior pathology reports, and any immunologic, immunohistochemical, or molecular study reports performed on pre-trial diagnostic tumor specimen. **Do NOT redact the reports.**
- ____ CLIA Laboratory Sample Submission Form completely filled out.
- ____ The Sample Tracking System (STS) generated shipping manifest.

_____ 6. Discard any unused supplies from the kit prior to shipping.

_____ 7. Activate the temperature monitor by pressing and holding the start button for 10-15 seconds. The green LED light will be solid, and then both red and green LED's will be solid. At this time, release the start button, and the green LED will blink rapidly for 3 seconds. Place the unit in the kit inside the blood tube packaging – it will flash red and green LED for 15 minutes. For temperature control, add cold pack or warm pack, dependent on time of year specimen is shipped. (Use warm pack if outside temperature at your site or along transit route will be below freezing.)

_____ 8. Use one kit per patient. Do not place specimens from more than one patient into a kit. Secure the packing, seal the kit, and apply the following shipping labels: Three neon green address labels, FedEx air bill, and UN3373 IATA label. Refer to separate shipping guide document for pictures of label placement and further shipping guidelines. Contact FedEx for pickup. **Package must be picked up the same day specimen is collected.**

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F. "Rare variant" confirmation testing (submit archival FFPE or unstained slides alternative):

_____ 9. Verify patient identification, enrollment in OPEN, and signed consent for EAY131.

_____ 10. Complete CLIA Laboratory Sample Submission Form and label blood collection tubes.

_____ 11. Perform venipuncture for collection of blood into labeled EDTA and Streck tubes and place filled labeled tubes into biohazard bag with absorbent protective packing material.

_____ 12. Log the samples into the EA Sample Tracking System and print the STS generated shipping manifest. **Log it**

under the time point "Confirmation of Pre-Registration Outside Results". Do not select the time point "Step 0, Screening 1, Biopsy 1" or "Pre-trial Diagnostic Material" for submission of "rare variant" material.

_____ 13. Place the following forms into the shipping kit (archival FFPE submission for screening must be sent in the provided specimen collection kit):

INCLUSION OF ALL REQUESTED MATERIALS IS VERY IMPORTANT.

____ This checklist

____ Prior pathology reports, and any immunologic, immunohistochemical, or molecular study reports performed on pre-trial diagnostic tumor specimen. **Do NOT redact the reports.**

____ **The molecular study report with the "rare variant" result is required for submission of "rare variant" material.**

____ CLIA Laboratory Sample Submission Form completely filled out.

____ The Sample Tracking System (STS) generated shipping manifest.

_____ 14. Discard any unused supplies from the kit prior to shipping.

_____ 15. Activate the temperature monitor by pressing and holding the start button for 10-15 seconds. The green LED light will be solid, and then both red and green LED's will be solid. At this time, release the start button, and the green LED will blink rapidly for 3 seconds. Place the unit in the kit inside the blood tube packaging – it will flash red and green LED for 15 minutes. For temperature control, add cold pack or warm pack, dependent on time of year specimen is shipped. (Use warm pack if outside temperature at your site or along transit route will be below freezing.)

_____ 16. Use one kit per patient. Do not place specimens from more than one patient into a kit. Secure the packing, seal the kit, and apply the following shipping labels: Three neon green address labels, FedEx air bill, and UN3373 IATA label. Refer to separate shipping guide document for pictures of label placement and further shipping guidelines. Contact FedEx for pickup. **Package must be picked up the same day specimen is collected.**

14.1.1 Submission information

The date and time of collection and shipping date must be entered into the ECOG-ACRIN Samples Tracking System (STS) for all submitted specimens. STS shipment manifest must be included in the package, along with CLIA Laboratory Sample Submission Form.

NOTE: Previous clinical pathology report MUST be submitted with the specimen, or the sample will not be processed. **Pathology reports submitted with tissue specimens should NOT be redacted. Results from screening and confirmation testing are returned to the site and will impact patient care. Adequate identification must be provided to ensure that the appropriate materials have been submitted and reporting is correct.**

Specimen collection and shipping kit order form is located in Appendix XII.

LABELING: All samples are to be labeled with the ECOG-ACRIN protocol number EAY131, the ECOG-ACRIN patient sequence number, patient's name (last, first), **date and time of collection**, and type of material.

The submitting pathologist and clinical research associate should refer to Appendix II (Pathology Submission Guidelines) for guidelines and summary of submission requirements.

14.2 Materials Required For This Protocol

14.2.1 Forms

Copies of the following forms and reports are required to be included with all submissions of pathology materials:

- The pathology report(s) on tumor from original, if available, and the most recent pre-trial diagnostic biopsy or surgery specimen(s)

NOTE: **Pathology reports submitted with tissue specimens should NOT be redacted. Results from screening and confirmation testing are returned to the site and will impact patient care. Adequate identification must be provided to ensure that the appropriate materials have been submitted and reporting is correct.**

NOTE: The prior diagnostic clinical pathology reports are required for central confirmation of histology and MUST be submitted with all screening specimen submissions. **Failure to provide the reports,** and thus, the inability to confirm histology, **will result in the delay of notification of treatment assignment.**

- Reports on immunologic, immunohistochemical, or molecular studies, including prior sequencing studies, performed on pre-trial diagnostic and most recent metastatic tumor specimens
- A completed CLIA Laboratory Sample Submission Form (available at end of Appendix II) - Submit with MATCH screening specimens only. Information on the CLIA submission form must be clearly

legible. Do not use cursive. This form is not to be submitted with tissue collected for research only (registration to Step 8). FAX numbers must be for those individuals who are required to obtain the results.

- The Sample Tracking System (STS) generated shipping manifest

NOTE: The pathology report for the submitted biopsy material, if pathology review was performed locally, is required to be uploaded into RAVE as soon as it is available.

NOTE: For patients for whom an applicable "rare variant" was identified by one of the outside designated laboratories (see Appendix XIV), the appropriate documentation must be uploaded to Medidata Rave as outlined in Section 4.1.5.4. Time frame for treatment assignment is dependent on upload of the reports and MATCH committee review of the reports.

For the submission of archived tumor tissue for confirmatory testing, a hard copy of the applicable sequencing report from the designated outside lab should be included inside the kit.

14.2.2 Biological Material

14.2.2.1 Tumor Sample for Screening

NOTE: For patients registered after May 22nd, 2017 at 5:00 PM ET for the Outside Assay process, only the Step 8 collections noted in this section apply.

ALL TISSUE SAMPLES SUBMITTED FOR SCREENING MUST BE LOGGED UNDER THE APPROPRIATE SCREENING TIMEPOINT (e.g. Step 0, Screening 1, Biopsy 1, OR Screening 2, Biopsy 1).

The schema for the trial should be consulted to determine time frames and rules for the collection and submission of specimens for MATCH screening versus final progression biopsies.

All tumor tissue cores collected during biopsy procedures performed at Steps 0, 2 or 4, and 8 are fixed in the formalin provided in the kit and submitted. FNA specimens (slides and cells in cytolyte) from procedures performed as outlined in Section **Error! Reference source not found.** are also required.

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For a tumor biopsy sample to be considered adequate for copy number variation evaluation, a minimum of 70% tissue must be tumor after manual microdissection for tumor cell enrichment at the MD Anderson Tissue Qualification Laboratory. However, the assays will be attempted on tissue specimens that have at least 20%

non-necrotic tumor cells present, which have been shown to be adequate for sequencing. In cases where insufficient tumor is obtained for sequencing analysis, patients will be given the option to undergo repeat tumor biopsy to attempt to obtain more tissue. Patients in whom sufficient tumor material cannot be obtained for targeted sequencing analysis will not proceed to be registered/randomized to treatment.

For patients for whom an applicable "rare variant" was identified by one of the outside designated laboratories (see Appendix XIV): the collection and submission of tumor tissue for the confirmation of the "rare variant" must follow the guidelines for the submission of tissue for screening at Step 0, Biopsy 1 options with the following changes:

- FFPE tissue may be submitted after, but no later than 8 weeks following registration to Step 1.

1) MANDATORY TUMOR SPECIMEN: Step 0, Screening 1, Biopsy 1. Tissue is collected either:

1. During the mandatory tumor biopsy following pre-registration. Submission of fresh tumor tissue cores in formalin are required. The additional submission of FNA cells in cytolyte is required. See Sections 9.1-**Error! Reference source not found.**.

   OR

2. Tumor tissue is already available for analysis following a clinically indicated diagnostic or therapeutic procedure performed prior to pre-registration. Requirements for existing FFPE submission are outlined below.

   OR

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3. MULTIPLE MYELOMA ONLY: SUBMIT ASPIRATES FROM THE FIRST PULL.

   For patients with plasmacytomas, follow the instructions for the submission of solid tumors. If aspirate will be submitted for screening, draw the bone marrow aspirate materials into a plain syringe, then

   1. Use 1 drop to make 4 – 5 smears.
   2. Put 1 – 1.5 ml in one EDTA tube.
   3. Put remainder (5 - 10 ml) of bone marrow aspirate in the other EDTA tube.

If the submitted material results in a failed screening assay, due to inadequate materials or assay failure, a second tumor tissue specimen may be submitted for a repeat screening.

Options for sample submissions for Step 0:

Step 0	Biopsy 1	Biopsy Repeat
Option 1	Fresh Biopsy	Fresh biopsy (#2)
Option 2	Fresh Biopsy	Pre-Trial FFPE[1]
Option 3	Pre-Trial FFPE[1]	Fresh Biopsy

1. If archived FFPE is submitted for analysis and additional FFPE is requested to meet the needs for the screening, the additional materials are considered to be for the same assessment and are logged under the same time point as the initial FFPE submission in STS.

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Fresh biopsy materials are to be collected and submitted as outlined in Section 9.1-**Error! Reference source not found.** above.

If previously collected FFPE will be submitted, the following criteria must be met (see Section 3.1.6.3 in Appendix XV):

Tissue must have been collected within 6 months prior to pre-registration to Step 0.

Patient may receive treatment after tissue collection; however, lack of response (per Section 6) must be documented prior to Step 1. The following restrictions apply:

- Enrollment onto another investigational study is not permitted.
- Intervening therapy that constitutes a new, molecularly targeted therapy is not permitted. Please note, immunotherapy is not considered molecularly targeted.
- Continuation of an agent/regimen for which disease progression has been observed prior to biopsy is permitted, including targeted therapy.
- A new immunotherapy regimen is permitted; but, lack of response must also be documented prior to registration to Step 0.
- Formalin-fixed paraffin-embedded tumor tissue block(s) must be submitted. The optimal block is 70% tumor tissue. Specimen size requirement is as follows:
- Surface area: 25mm^2 is optimal. Minimum is 5mm^2
- Volume: 1mm^3 optimal. Minimum volume is 0.2mm^3, however the success of DNA extraction decreases at suboptimal tissue volume

Specimens of suboptimal size, cellularity, or tumor content will require submission of more than one tumor block

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If an existing block cannot be submitted, the following is requested:

- One (1) H&E slide, AND

- Twenty (20) or more recently cut 4 - 5 micron unstained sections on positively charged slides.

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NOTE: Screening tumor samples with loss of MLH1 or MSH2, as determined by IHC, will result in a request for the submission of pre-trial diagnostic tumor tissue (See Section **Error! Reference source not found.**) for central confirmation of this result. The results of the confirmation will be reported to the site. This additional assessment will not impact the screening assessment timeline and treatment assignment will not be held pending the outcome of the confirmation assessments. If the results are complete prior to treatment assignment, the confirmation results will be used by MATCHbox.

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2) Biopsy at progression for second screening assessment (Screening 2, Biopsy 1 at Step 2 or Step 4) – patient must be registered to the appropriate registration step prior to submission of the biopsy materials:

1. Submission of fresh tumor tissue cores in formalin is required. The additional submission of FNA cells in cytolyte is required. See Sections 9.1-**Error! Reference source not found.**.

   OR

2. MULTIPLE MYELOMA ONLY: SUBMIT ASPIRATES FROM THE FIRST PULL.

   For patients with plasmacytomas, follow the instructions for the submission of solid tumors. If aspirate will be submitted for screening, draw the bone marrow aspirate materials into a plain syringe, then:

   4. Use 1 drop to make 4 – 5 smears.
   5. Put 1 – 1.5 ml in one EDTA tube.
   6. Put remainder (5 - 10 ml) of bone marrow aspirate in the other EDTA tube.

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3) Biopsy and blood collection at progression upon discontinuation of all EAY131 protocol treatment (Step 8) from patients who provide additional consent for the collection. Submission of this tissue is for research purposes only.

Submission of blood samples from patients registered to Step 8 is mandatory. Performance of biopsy to collect tumor tissue for research purposes only from patients willing to undergo the research biopsy, is to be

performed only if the procedure will be minimal or less than minimal risk as defined in Section 9.1.

NOTE: If a SOC procedure or research biopsy will not be performed, indicate in STS that the tumor tissue will not be submitted.

1. During the tumor biopsy, if performed, following registration to Step 8. Submission of fresh tumor tissue cores in formalin is requested. The additional submission of FNA cells in cytolyte is strongly encouraged, but not required. See Sections 9.1-**Error! Reference source not found.**.

   OR

2. MULTIPLE MYELOMA ONLY: SUBMIT ASPIRATES FROM THE FIRST PULL (PREFERRED) OR SECOND PULL (ACCEPTABLE) OF AN ASPIRATION SITE FOR TESTING. Do not submit samples beyond the second pull of the same aspiration site.

   NOTE: For plasmacytomas, follow the directions above for submission of solid tumor tissue specimens.

   Draw the bone marrow aspirate materials into a plain syringe, then:

   7. Use 1 drop to make 4 – 5 smears.
   8. Put 1 – 1.5 ml in one EDTA tube.
   9. Put remainder (5 - 10 ml) of bone marrow aspirate in the other EDTA tube.

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14.1.1.1 Formalin Fixed Paraffin-Embedded Tumor Sample for Confirmation of "Rare Variant" Results Determined by Designated Outside Laboratory

Submit:

- Within 2 weeks following notification that archived FFPE specimens are required for central IHC assessments for purposes of treatment assignment

  OR

- Within 8 weeks following Step 1 registration, if not required for mandatory IHC assessments.

FFPE to be submitted using regular Screening Kit.

Log Samples in STS under time point: *Confirmation of Pre-Registration Outside Results*

Submit archived (previously collected) FFPE tumor tissue representative (from the same tissue that was sequenced by the outside lab if possible, otherwise any prior tumor tissue) of that analyzed by the designated outside laboratory for central confirmation of the outside assay

results. **PLEASE STATE ON THE SHIPPING MANIFEST IF ENCLOSED SUBMITTED TISSUE WAS COLLECTED AT THE SAME TIME AS THE TISSUE SENT TO THE OUTSIDE LABORATORY OR IF IT IS FROM A DIFFERENT COLLECTION DATE THAN THE TISSUE SENT TO THE OUTSIDE LABORATORY. This information may be important for assessment of discordant results.**

The optimal block is 70% tumor tissue. Specimen size requirement is as follows:

- Surface area: 25mm^2 is optimal. Minimum is 5mm^2
- Volume: 1mm^3 optimal. Minimum volume is 0.2mm^3; however the success of DNA extraction decreases at suboptimal tissue volume

If an existing block cannot be submitted, the following is requested:

- One (1) H&E slide, **AND**
- Twenty (20) or more recently cut 4 – 5 micron unstained sections on positively charged slides.

NOTE: Failure to submit the requested material will not impact patient treatment on the trial, but will impact the evaluability of the clinical data from the patient during data analysis for the trial endpoints. If tissue is unavailable for submission, the UNREDACTED pathology report and a statement justifying reason for inability to submit tissue must be provided to the MD Anderson TQL. Additionally, indicate in STS the reason for inability to submit the required materials.

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14.1.1.2 Formalin Fixed Paraffin-Embedded Tumor Sample for Research

In STS, please log these samples under "Pre-trial Diagnostic Material". **DO NOT USE THIS TIMEPOINT IN STS FOR THE SUBMISSION OF SCREENING SAMPLES OR THE SUBMISSION OF SAMPLES FOR CONFIRMATION OF A "RARE VARIANT" DETERMINED BY AN ASSAY OUTSIDE OF MATCH.**

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NOTE: Kits are not available for specimens submitted separately from the screening submission.

NOTE: Screening tumor samples with loss of MLH1 or MSH2, as determined by IHC, will result in a request for the submission of pre-trial diagnostic tumor tissue for central confirmation of this result.

Additionally, a pre-trial representative tumor tissue block is requested from patients who consent "Yes" to "I agree to provide additional samples for research." Tissue may be submitted with the Step 0 Screening biopsy material, or separately upon Registration to Step 1. If FFPE tissue is submitted as the screening sample on Step 0, additional materials may be requested, if the remaining material from the archival screening sample is insufficient for banking purposes.

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If a block cannot be submitted, the following is requested:

- One (1) H&E slide, and
- Twenty (20) or more recently cut 4 – 5 micron unstained sections on positively charged slides.

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14.1.1.3 Optional Whole Blood Collections for Research

Whole blood is to be collected and submitted from patients who consent to allow the submission of blood for research. Samples are to be submitted at ambient temperatures on the day of collection. To order kits for these collections, please see Appendix XII.

Screening and *Step 8* Kits – Kit descriptions are provided above in Section 9.2.

On-Treatment Kits – At time of registration to subprotocol treatment, provide the receiving address for collection/shipping kit, which will automatically be shipped to the site upon registration. If the patient did not consent to the optional blood submissions, answer question with "*Not Consented".* Kit contents include:

i. Two Streck venipuncture blood collection tubes with label and protective packing material.
ii. One pre-printed FedEx shipping label pre-addressed to the EA Central Biorepository and Pathology Facility.
iii. Cold pack, warm pack, and temperature monitor device.
iv. Strip of specimen labels.
v. Kit instructions.

Collection and submission guidelines.

A. From patients who answer "YES" to "I agree to provide additional samples for research" on the EAY131 MASTER Screening Protocol Consent
    1. 7-10 mL of whole blood into one (1) EDTA vacutainer collected at:
        a. For patients enrolled on the study via the original screening process (tissue analyzed by central MATCH labs):
            - Step 0, screening 1, biopsy 1

- Step 0, screening 1, biopsy repeat
- Screening 2 (Step 2 or 4), biopsy 1
- Screening 2 (Step 2 or 4), biopsy repeat

b. For patients enrolled on the study via the outside assay process ("rare variants" identified by a designated lab):

- Step 1, prior to treatment

2. 18-20 mL of whole blood into two (2) Streck vacutainers collected at:

a. For patients enrolled on the study via the original screening process (tissue analyzed by central MATCH labs):

- Step 0, screening 1, biopsy 1
- Step 0, screening 1, biopsy repeat
- Screening 2 (Step 2 or 4), biopsy 1
- Screening 2 (Step 2 or 4), biopsy repeat

b. For patients enrolled on the study via the outside assay process ("rare variants" identified by a designated lab):

- Step 1, prior to treatment

c. For all patients:

- *On Treatment*, End of Cycle 2 (Cycle 3, day 1 prior to start of treatment) of each MATCH subprotocol treatment. If treatment is discontinued prior to completion of cycle 2 treatment, samples are requested to be collected and submitted at the time of the patient's next clinical blood draw.

B. Step 8: From patients who consented to and were registered to Step 8. Disease status is progression and completion of all MATCH subprotocol treatments (patients will not be screened for consideration for another treatment on MATCH). Collect:

- 7-10 mL of whole blood into one (1) EDTA vacutainer
- 18-20 mL of whole blood into two (2) Streck vacutainers

NOTE: If the patient does not agree to the additional research biopsy, the site should still request the patient's participation on Step 8 for the collection of the blood samples alone.

If *On Treatment* blood samples were already submitted for the patient's progression on the last MATCH subprotocol

treatment they received, as outlined above, and a biopsy for research will not be performed, registration to Step 8 is not requested.

Molecular Analysis for Therapy Choice (MATCH)

Appendix XVI

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CS-MATCH-0007

Comprehensive Genomic Analysis of NCI-MATCH

Principal Investigators:

NCI PI:	Lyndsay Harris, MD, FRCP(C)
NCI SCIENTIFIC LEAD:	Lou Staudt, MD, PhD
ECOG-ACRIN PI	Peter O'Dwyer, MD, PhD

Principal Investigators for Translational and Clinical Oncology:

Stanley Hamilton, MD	Alice Chen, MD
James V. Tricoli, PhD	Keith Flaherty, MD
P. Mickey Williams, PhD	Carlos Arteaga, MD

Co-Investigators:

BIOSTATISTICS	Lisa McShane, PhD; BRB DCTD, NCI
BIOSTATISTICS	Laura Yee PhD; BRB DCTD, NCI
BIOSTATISTICS	Robert J. Gray, PhD; ECOG/ACRIN
BIOSTATISTICS	Shuli Li PhD; ECOG/ACRIN
BIOINFORMATICS	Lou Staudt, MD, PhD; CCG, NCI
BIOINFORMATICS	Jean-Claude Zenklusen, MS, PhD; CCG, NCI David Patton, PhD, CBITT, NCI
GENOMIC SEQUENCING AND CHARACTERIZATION CENTER	Roy Tarnuzzer, PhD. CCG, NCI
BIOETHICS	Carol Weil, JD, DEB, CDP, DCTD, NCI
IMAGING	Lalitha K. Shankar, MD, PhD; CIP, DCTD, NCI
IMAGING	Susanna I. Lee, MD, PhD; ECOG/ACRIN
IMAGING	Constantine Gatsonis, PhD; ECOG/ACRIN Imaging Statistician
SUB-PROTOCOL PRINCIPAL INVESTIGATORS	*See Subprotocols PI list in the full CS-0007 protocol document (archived)*
DCTD STUDY RESEARCH COORDINATORS	Margaret Cavenagh
DCTD STUDY RESEARCH COORDINATORS	Ben Kim
CTSU PROTOCOL COORDINATOR	Ben Kim

Precis

This companion biomarker study to NCI-MATCH, "Comprehensive Genomic Analysis of NCI-MATCH", aims to investigate additional underlying molecular factors associated with responses or lack of responses to a drug or class of drugs within the parent protocol. Despite the fact that all patients on a given treatment have the required molecular abnormality in their tumor, not all patients will respond to treatment and some may even respond exceptionally well to treatment. Each patient's tumor was originally characterized with the NCI-MATCH next generation sequencing (NGS) assay that interrogates targets from about 161 genes. A fuller genomic landscape of tumors from NCI-MATCH patients will be ascertained using comprehensive molecular analysis, such as whole exome sequencing (WES) and mRNA sequencing (RNA-seq) in an effort to discover additional features that are predictive of response to treatment.

This project will characterize tumor tissue DNA and RNA and matched germline DNA from all patients on any treatment subprotocol of NCI-MATCH who consented to allow their specimens to be used for future research. DNA and RNA samples will undergo WES and RNA-seq respectively. Those with sufficient nucleic acid quantities may undergo additional analyses (e.g., whole genome sequencing, microRNA (miRNA) sequencing, promoter methylation analysis, single nucleotide polymorphism (SNP) analysis, etc.). Each case will be annotated with demographic and clinical information, along with follow-up information sufficient to enable correlations between molecular profiles and drug response. Patient information and associated data will be de-identified prior to sending nucleic acid for sequencing, and this data will be placed in a controlled-access database for use by other investigators.

Clinically-annotated tissue specimens will be provided by ECOG-ACRIN Central Biorepository and Pathology Facility (EA-CBPF) at MD Anderson Cancer Center (MDACC). The samples will be in the form of nucleic acids extracted at this biorepository from FFPE tissue collected on the MATCH trial. If existing nucleic acid was found to be insufficient for a given case, an attempt may be made to extract FFPE tissue for that case if enough material is available. This will be decided on an arm by arm basis if the sample size is inadequate for meaningful analysis.

Nucleic acids that pass QC will be shipped to a sequencing and characterization center designated by the NCI Center for Cancer Genomics to perform designated sequencing and analysis. The Analysis Working Group (AWG), composed of investigators on this protocol and the sub-protocol principal investigators (PIs) of NCI-MATCH, will analyze the findings in relation to treatment and clinical outcome. Once published, the genomic and clinical data will be made available to the broader cancer research community through a controlled-access database (dbGAP).

I. OBJECTIVES

The main objective of this study is to explore the molecular basis of why patients enrolled in a treatment subprotocol of NCI-MATCH, with molecular abnormalities deemed predictive of response by NCI-MATCH 'Levels of Evidence' (LOE) as defined within the master protocol EAY131, do or do not respond to the drug or drug combination with which they were treated.

Findings from this study may lead to confirmatory non-clinical or clinical studies to demonstrate the predictive qualities of one or more molecular features. Specifically, the objectives of this study are to:

1.1. Identify molecular indicators of response or primary resistance in malignant tissues from patients who received therapy on NCI-MATCH subprotocols, using WES, RNA-seq, and potentially other sequencing and molecular methods.

1.2. Explore associations between the identified molecular indicators and the putative mechanism of action of the treatment received by the patient.

1.3. Compare results from WES and RNA-seq studies to the results of the eligibility assays for NCI-MATCH.

II. BACKGROUND AND RATIONALE

The feasibility of this approach is supported by reports in the literature discovering mutations in patients who experienced an exceptional response to an agent that did not meet a phase 2 endpoint. In one of these studies, loss of function mutations in genes Tuberous Sclerosis 1 (TSC1) and in Neurofibromin 2 (NF2) were detected in a patient with urothelial cancer who had a multiyear complete response to everolimus in a phase 2 trial (Iyer et al., 2012). Alterations in these genes had been associated with mammalian target of rapamycin complex (mTORC) dependence in preclinical studies, and everolimus inhibits this pathway. The investigators sequenced tumors from 96 other individuals with high-grade bladder cancer and found 5 additional patients with TSC1 mutations. They then sequenced 13 patients with bladder cancer who had received everolimus on the trial and found that 4 of 5 patients with TSC1 mutations had some tumor shrinkage to everolimus, while those without such a mutation did not have tumor shrinkage. Of note, not all patients with the putative response biomarker (TSC1 mutation) had a response. In a second report, Wagle et al. report finding novel mechanistic target of rapamycin (mTOR) mutations by WES in a patient with urothelial cancer who had a complete response to the experimental combination of everolimus and pazopanib (Wagle et al., 2014). This work demonstrated that mutations in mTOR are likely to predict clinical sensitivity to treatment with the mTOR inhibitor everolimus and laid the groundwork for the principles behind NCI-MATCH where specific molecular characteristics are required to select patients for a targeted therapy associated with that pathway. NCI-MATCH identifies LOE that need to be met to allow a drug into the study and includes other LOE to determine if a particular molecular abnormality can be used as an eligibility criterion. Unfortunately, even when patients' tumors possess a molecular abnormality targeted by a drug that is FDA approved for use in patients with that abnormality, 100% response rates are rare. Studying the tumors of patients enrolled in an NCI-MATCH subprotocol could identify novel or unknown molecular abnormalities that may be predictive of response or resistance to agents targeting the relevant pathway. The ultimate goal of NCI-MATCH is to prospectively identify patients who may respond to particular targeted agents, and as importantly, identify those who will not respond and for whom other treatments should be employed. However, the technology used to screen for NCI-MATCH is a targeted sequencing panel of only about 161 genes, which limits the discovery of mechanisms of response and resistance to the targeted therapy in question. For example, if a group of patients with various AKT mutations were treated with an AKT inhibitor, and some patients respond while others do not respond, it may be important to investigate additional molecular abnormalities in these patients.

With only 161 genes interrogated in the initial eligibility panel, the ability to find the molecular lesion in question is limited - a broader molecular analysis may be needed to help identify abnormalities responsible for the response and/or resistance to treatments. To address this need, the study team will perform WES and RNA-seq in tumors from patients who received therapy on NCI-MATCH subprotocols. The ability to identify molecular features that predict a clinical response in these subsets of patients will provide the tools to advance the ability to conduct precision medicine studies. It will also

provide a better biological understanding of the complete or partial responses observed in this cohort of patients, perhaps leading to new and more effective treatments.

For each patient that entered the NCI-MATCH trial, 4 cores of tissue or a recent archival specimen were requested at screening and profiled with the NCI-MATCH assays, which include the customized Oncomine NGS assay, as well as immunohistochemistry (IHC) assays for PTEN, MLH1 and MSH2. These initial NCI-MATCH tumor profiling assays will be used as a source of nucleic acid for WES and RNAseq. Of note, no additional tumor biopsy tissue will be collected for this study.

An important aspect of this study is that it is not expected to satisfy the expectations of statistical power that are often associated with drug studies (i.e., large numbers of events that allow sufficient occurrences to not be explained by chance alone), even though patients will have been treated and followed in a uniform manner. The findings from this study will be the basis of further hypotheses that will then need to be tested in either larger, statistically well powered studies or by functional tests and investigations. It is also possible that the type of tumor and the state of the host will have a significant effect on response or non-response. Enrollment in a NCI-MATCH subprotocol arm is not defined by tumor type, and thus each arm will be composed of several different tumor histologies, each having the subprotocol's specific molecular eligibility criteria, in some cases with several genes used for eligibility.

This exploratory correlative study to NCI-MATCH seeks to generate a database intended to be shared, built upon, and examined further. It will also explore the feasibility of conducting such a study as there is a limited amount of available tissue for additional sequencing analysis. The output of the study might include a list of plausible mutations, putative mutations, or simply all the mutations found in the responder and non-responder cases – passenger, drivers, etc. This study will not involve downstream analyses, such as functional validation studies. Other investigators can seek to build on the data generated by this study by, for example, comparing their own dataset with the shared data.

III. SELECTION OF PATIENTS AND TUMORS

The following criteria are required to be met for patient and specimen inclusion:

3.1. Patient started treatment on a sub-protocol of NCI-MATCH;

3.2. Sub-protocol treatment was assigned using NCI-MATCH Oncomine® assay results;

3.3 Patient consented to use specimens for research;

3.4. The amount of nucleic acid available for WES and/or RNAseq must meet minimum specimen requirements as described below in section IV; and

3.5. Normal, germline DNA will be provided, if available.

IV. BIOSPECIMEN REQUIREMENTS

4.1. *TISSUE REQUIREMENTS*

Samples collected most recently will be given the highest priority for molecular characterization to minimize chances of degradation during prolonged storage.

4.1.1. General Specimen Characteristics and Requirements:

- Tissue samples from the initial screening trial will be utilized as a source of nucleic acids extracted for WES and RNAseq. If existing nucleic acid is found to be insufficient for a given case, an attempt may be made to extract FFPE tissue for that case if material is available.
- Tumor specimens collected prior to the initiation of treatment on NCI-MATCH will be given priority if they are representative of specimens evaluated by the MATCH NGS assay (i.e., adjacent tumor tissue blocks, scrolls and/or sections).
- Specimens should be accompanied by documentation of diagnosis (de-identified pathology report).

4.1.2. Solid Tumor Tissue:

All tumor samples underwent a pathology review in the MDACC pathology laboratory prior to nucleic acid extraction. The criteria for an acceptable sample is ultimately based on the presence of tumor in the specimen and amount of nucleic acid obtained, which can vary considerably by tumor histology and biopsy size.

4.2. NORMAL SPECIMENS

Peripheral blood, blood cells, purified DNA from blood, or a FFPE sample of normal tissue will be used to obtain germline DNA for this study.

4.3. SAMPLE PROCESSING AND MOLECULAR CHARACTERIZATION

4.3.1. Nucleic Acid Extraction from FFPE:

Extraction from FFPE is performed at MD Anderson Cancer Center per the protocol utilized to extract nucleic acids for screening of tumor samples by the MATCH-assay.

The analysis of a progression sample and its comparison to pre-treatment samples might identify molecular lesions that would point to a molecular pathway that has relevant sensitivity or resistance to the targeted agent. If such progression biopsies are available, we may attempt to study those using similar methods.

4.3.2. Molecular Characterization:

Quality Metrics

All DNA and/or RNA from tumor and germline control tissues must also meet quality metrics prior to molecular characterization.

Sequencing

Tumor characterization by platform will proceed in a step-wise manner to identify molecular features potentially predictive of response to therapy. Finding mutations in known cancer genes (oncogenes or tumor suppressors) will be the most readily interpretable and will be primarily determined by WES. RNA-seq will be performed, which can capture some mutations that are missed by exome sequencing if suitable RNA can be isolated from the tumor tissues. Whole genome

sequencing will be considered if adequate amounts of material are available. The amount and integrity of tumor tissue may ultimately be the limiting factor in what methodologies will be employed. WES and RNA-seq have been successfully performed using nucleic acids purified from FFPE tissue. If the quantity and quality of DNA and RNA are sufficient, DNA methylation and miRNA characterization can also be performed.

In general, the analysis will first focus attention first on clonal heterozygous or homozygous mutations. There are standard mechanisms to identify these (Carter et al., 2012). Subclonal mutations can be indicative that a particular signaling pathway is active in a tumor, which could be the reason for an observed therapeutic response. For this study, whole exomes will be sequenced to provide approximately 10 Gb of raw data per sample using 1/4 of an Illumina HiSeq 2000 lane (Illumina; San Diego, CA) and to achieve ~150X coverage to afford sensitivity down to .05 or less.

Batching

Upon receipt at the molecular characterization centers, samples will be sequenced in batches using a common library protocol and sequencing strategy, such that sequencing results can be expeditiously reported every 3 months.

Samples will be extracted and molecular characterization performed by treatment subprotocol arm, wherever possible, to reduce batch effect.

Prioritization

Tumor and normal tissue DNA will be characterized by WES, and tumor RNA will be characterized by transcriptome sequencing. The NCI-MATCH NGS Oncomine® assay used for eligibility and IHC for PTEN, MLH1, and MSH2 are available for analysis. Whole genome and other sequencing (e.g., miRNA) may be performed if enough tumor DNA is available. If tumor tissue is limiting, sequencing would be performed as prioritized and noted in Figure 1. If multiple independent samples are available for a case, more than one may be analyzed. Figure 1 demonstrates the flow of materials for the DNA sequencing and analysis.

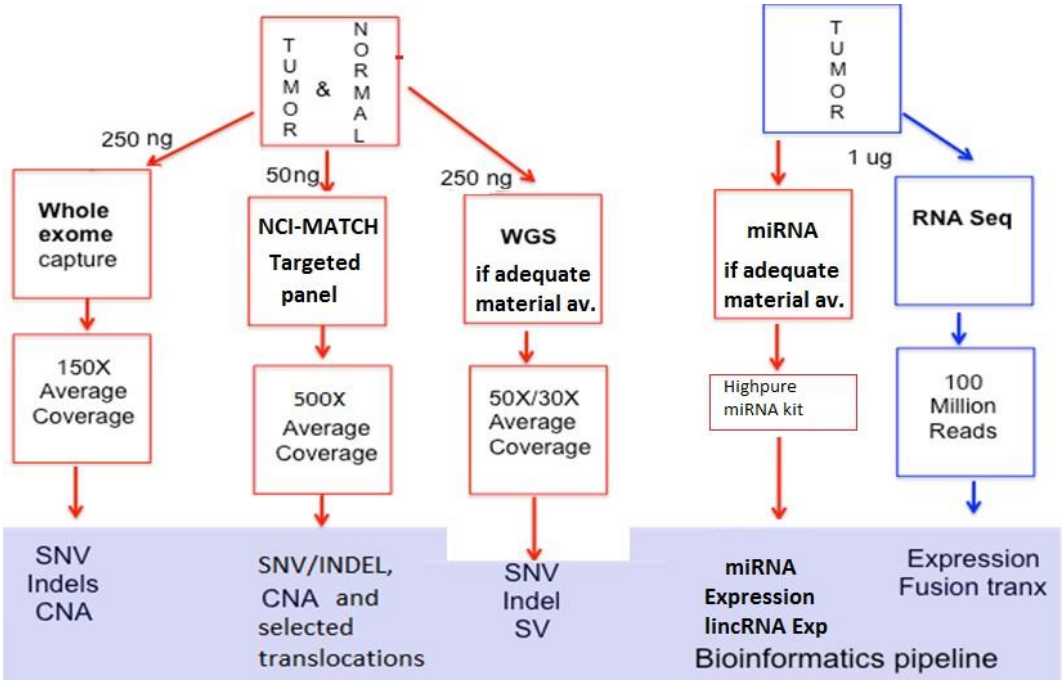

Figure 1. DNA sequencing and analysis of tumor/normal pairs. Red boxes, genomic DNA; blue boxes, RNA. The DNA and RNA sequencing data flow is illustrated for tumor and normal tissue pairs. The amount of DNA or RNA (in µg or ng) required for every sequencing methodology is listed. RNA-seq utilizes tumor RNA. The types of mutations discovered by each sequencing platform are included in blue shaded area at the bottom of the figure (see Tables 1-3 for bioinformatics analysis).

Sequencing Platforms

1. Whole exomes will be sequenced to provide approximately 10 Gb of raw data per sample using 1/4 of an Illumina HiSeq 2000 lane (Illumina; San Diego, CA) and to achieve ~150X mean coverage to afford sensitivity down to 5% or less. If DNA amounts are limiting, we will use the ThruPLEX® DNA-seq Kit (Rubicon Genomics; Ann Arbor, MI) to obtain libraries from 20 to 50 ng of DNA.
2. Whole genome sequencing will be performed to generate approximately 150 Gb per tumor for a depth of 80X, and the matched normal specimens will generate approximately 90 Gb for a depth of 30X. The data will be analyzed to identify point mutations, copy number changes, and translocations. Illumina paired end libraries using 300 to 400 bp fragments will be utilized. If DNA amounts are limiting, we will use the ThruPLEX® DNA-seq Kit (Rubicon Genomics; Ann Arbor, MI) to obtain libraries from 20 to 50 ng of DNA.
3. Omni Express SNP arrays (Illumina; San Diego, CA) will be used as a quality control for sample identity and contamination. It will also provide orthogonal validation for copy number alterations.
5. RNA-seq using a poly-A selection and a stranded protocol will be used to ascertain expression levels and search for fusion

transcripts. **Please Note**: New RNA capture protocols using both Illumina (San Diego, CA) and Roche NimbleGen (Madison, WI) reagents are actively being evaluated. We plan to use these approaches as the preferred alternative to the current RNA-seq pipelines.

4.3.3. Other Molecular Characterization:

Techniques are rapidly evolving for improved molecular characterization of FFPE specimens, tissue storage, and analyte isolation. If enough material tumor is available, other characterization methods, such as miRNA sequencing, and epigenetic sequencing may be performed. If it is not possible to perform RNA-seq on FFPE samples, expression array analyses will be considered.

V. BIOINFORMATICS

It is conceivable that a response or lack of response could be correlated with somatic variants affecting gene expression directly related to the mechanism of action of the drug associated with response or resistance. It is also conceivable that SNPs of genes involved in metabolism of the drug with which the response/resistance is associated could have played a role in the outcome. Once available, the data will be analyzed with these questions in mind.

5.1. *BIOINFORMATIC DATA PROCESSING*

The data from each platform will be analyzed for mutations with an impact on known cancer genes and genes involved in known cancer pathways. Genes will be ranked and reported based on their known or potential involvement in cancer. Genes of unknown significance will be reported as passengers.

The DNA sequencing data flow is outlined in Figure 1 for tumor and normal tissue pairs. Sequencing will be prioritized from left to right based on available DNA and RNA. The types of mutations discovered by each sequencing platform are included at the bottom in the blue shaded area. The bioinformatics of sequence data includes alignment of DNA sequencing reads to the reference sequence (Table 1) generating Binary Alignment/Map format (BAM) files for storing the alignments, variant discovery and somatic mutation calling (Table 2), and annotation of the variants using a variety of normal and mutation databases (Table 3). Since the variant discovery is part of well-defined bioinformatics pipelines, its functions are presented in tabular format.

The analyses in Tables 1 to 3 will be performed as a naive starting point for all patients. However, details of each patient's disease, the drugs under study, and specific responses might draw attention to particular types of analyses, pathways, or types of mutations, ultimately varying the actual analyses performed.

A BAM file, a compressed, indexed file format with the sequence aligned to a reference human genome, will be generated for each sample in this project. This format contains a basic DNA sequence alignment containing both germline and somatic information. BAM files are access controlled and will be deposited in NCI's Genomic Data Commons (GDC). Further analysis for sequence variants and mutations will be performed, germline mutations will be filtered out, and the resulting annotated, tab-delimited mutation data will be stored in a Mutation

Annotation Format (MAF) file. MAFs can be either "protected" and still contain unfiltered germline mutations or "somatic" with all validated/known germline mutations filtered out. Sequence variants will also be deposited in Variant Call Format (VCF) files containing annotated calls in text format containing somatic and germline variant information.

Both the MAF and VCF files will have controlled access and will be deposited in the GDC or other repositories as determined by the Center for Cancer Genomics. For information on data access and sharing, see Section 5.2 below.

Table 1. Alignment of DNA sequencing reads

Data type	Software
Illumina DNA	BWA,[1] GATK[2]
Illumina RNA Seq	STAR[3]

1. *Burrows-Wheeler Aligner for short read alignment (Li H. and Durbin R. (2009) Fast and accurate short read alignment with Burrows-Wheeler Transform. Bioinformatics, 25:1754-60. PMID: 19451168) and for long read alignment (Li H. and Durbin R. (2010) Fast and accurate long-read alignment with Burrows-Wheeler Transform. Bioinformatics, Epub. PMID: 20080505); http://bio-bwa.sourceforge.net*
2. *GATK realignment as implemented in the HGSC read processing pipeline "Mercury", Reid JR, et al. submitted.*
3. *Dobin A, Davis CA, Schlesinger F, Drenkow J, Zaleski C, Jha S, Batut P, Chaisson M, Gingeras TR. (2013). STAR: ultrafast universal RNA-seq aligner. Bioinformatics 29:15-21. PMID: 23104886*

Table 2. Detection of variation in tumor and normal genomes

Mutation Type	Data type	Software
SNV	MATCH NGS Assay	See Appendix[5]
	Illumina Whole Exome	HGSC-SNV-Pipeline[1,2,3]
Small Indels	Illumina Whole Exome MATCH NGS Assay	HGSC-Indel Pipeline,[1,2,3] Pindel[4]
Copy Number Alteration	Illumina Whole Exome	VarScan2[5]
	Illumina SNP Array	Nexus[6]
	MATCH NGS Assay	
Loss of heterozygosity	Illumina Whole Exome	LOHcate[7]
Structural variation	Illumina Whole Genome	BreakDancer,[8] Svstat[9]
	MATCH NGS Assay	
Gene Expression	Illumina RNA Seq	Cufflinks[10] & TopHat[10]
Fusion Transcripts	Illumina RNA Seq MATCH NGS Assay	TopHat-Fusion[10]

1. *Cancer Genome Atlas Research Network. (2013). Comprehensive molecular characterization of clear cell renal cell carcinoma. Nature 499:43-9. PMID: 23792563*
2. *Biankin AV, Waddell N, Kassahn KS, et al. (2012). Pancreatic cancer genomes reveal aberrations in axon guidance pathway genes. Nature 491:399-405. PMID: 23103869*
3. *Cancer Genome Atlas Network. Comprehensive molecular characterization of human colon and rectal cancer. (2012). Nature 487:330-7 PMID: 22810696*
4. *Ye K, Schulz MH, Long Q, Apweiler R, Ning Z. (2009). Pindel: a pattern growth approach to detect break points of large deletions and medium sized insertions from paired-end short reads. Bioinformatics 25:2865-71. PMID: 19561018*
5. *Koboldt DC, Zhang Q, Larson DE, Shen D, McLellan MD, Lin L, Miller CA, Mardis ER, Ding L, Wilson RK. (2012). VarScan 2: somatic mutation and copy number alteration discovery in cancer by exome sequencing. Genome Res. 22:568-76. PMID: 22300766*
6. *Nexus Copy Number: http://www.biodiscovery.com/software/nexus-copy-number/*
7. *Dewal N, Reddy S, Trevino L, Wang L, and Wheeler DA (2013). LOHcate: A novel approach for detection and evaluation of loss of heterozygosity in cancer samples. In preparation.*
8. *Chen K, Wallis JW, McLellan MD, Larson DE, Kalicki JM, Pohl CS, McGrath SD, Wendl MC, Zhang Q, Locke DP, Shi X, Fulton RS, Ley TJ, Wilson RK, Ding L, Mardis ER. (2009). BreakDancer: an algorithm for high-resolution mapping of genomic structural variation. Nat Methods. 6:677-81. PMID: 19668202*
9. *Davis C, and Lau C. SVStat: Detection of large-scale structural variants in short-read whole genome sequencing data. In preparation.*
10. *Trapnell C, Roberts A, Goff L, Pertea G, Kim D, Kelley DR, Pimentel H, Salzberg SL, Rinn JL, Pachter L. (2012). Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and Cufflinks. Nat Protoc.n7:562-78. PMID: 22383036*

Table 3. Annotation of variants

Transcript set	Gencode
Known polymorphism	dbSNP[1] validated by frequency
Known mutations	COSMIC[2]
Known structural variation	Database of Genomic Variants[3]
Known genotype-phenotype correlations	ClinVar[4]
Druggable gene targets	DrugBank[5]

1. *dbSNP: http://www.ncbi.nlm.nih.gov/snp/*
2. *Forbes SA, Tang G, Bindal N, Bamford S, Dawson E, Cole C, Kok CY, Jia M, Ewing R, Menzies A, Teague JW, Stratton MR, Futreal PA. (2010). COSMIC*

(the Catalogue of Somatic Mutations in Cancer): a resource to investigate acquired mutations in human cancer. Nucleic Acids Res.38:D652-7. PMID: 19906727

3. *MacDonald JR, Ziman R, Yuen RK, Feuk L, Scherer SW. (2013). The database of genomic variants: a curated collection of structural variation in the human genome. Nucleic Acids Res. E pub. PMID: 24174537*
4. *ClinVar: http://www.ncbi.nlm.nih.gov/clinvar/*
5. *Knox C, Law V, Jewison T, Liu P, Ly S, Frolkis A, Pon A, Banco K, Mak C, Neveu V, Djoumbou Y, Eisner R, Guo AC, Wishart DS. (2011). DrugBank 3.0: a comprehensive resource for 'omics' research on drugs. Nucleic Acids Res. 39:D1035-41. PMID: 21059682*

5.2. *DATA SHARING*

All data will be shared utilizing infrastructure already established by NCI. All molecular data will be shared in a controlled access database with raw sequence data (BAM files) being shared via GDC, and the remainder of the data, including clinical data, being coordinated through The Cancer Genome Atlas Project (TCGA) data coordinating center. Procedures for controlled access will be implemented through the NIH dbGAP system (https://dbgap.ncbi.nlm.nih.gov/aa/wga.cgi?page=login).

VI. STATISTICAL CONSIDERATIONS

The study goal is to collect interpretable molecular results (WES, RNAseq and targeted deep sequencing) on patients who were treated on NCI-MATCH subprotocols. We anticipate approximately 700 patients will match to a MATCH treatment. However, not all these patients will have an adequate amount of residual DNA and/or RNA available for study assays. We anticipate that at least 50% of these patients will have available nucleic acid (DNA and/or RNA) for the genomic analyses proposed in this study.

This biomarker study is hypothesis-generating. The hypotheses that we will try to generate will relate to genetic alterations in cancer cells. The plausibility of the hypothesis will be based on evidence that a certain mutation affects a component of a regulatory pathway that is relevant to the mechanism of action of the drug.

The hypotheses generated by this study will need to be tested in larger patient cohorts with the same histology and treatment. Examples of the success of this methodology using patient tumor specimens have been published (Iyer et al., 2012). Importantly, all the data will be made publicly available (controlled access), so that other researchers who have specific knowledge of drug response predictors (e.g., from the DTP drug response database) will be able to generate and publish hypotheses and devise studies to test the hypotheses.

As noted above, this study is not expected to satisfy the expectations of internal (i.e., within the data collection of the study) statistical power that are often associated with other drug studies. Here, even single instances will be considered and will be the basis of further hypotheses that will then need to be tested in either larger, statistically well powered studies or by functional tests.

6.1. *STATISTICAL ANALYSIS APPROACH*

The statistical plan centers around the following primary aims:

Aim 1: Identify molecular indicators of response or primary resistance in malignant tissues from patients who were treated on the NCI-MATCH clinical trial using WES, RNA-seq, as well as, potentially other sequencing and molecular characterization methods.

The molecular features of patients' tumors will be discernible as arising from the tumor by comparison to (i) samples from normal tissue in the same patient, if available, and (ii) databases of similar data for normal and other tumor types. The mode of (i) is the standard that has been used in most tumor studies to date and results in a low 'noise' per sample. In other words, the presence of the normal tissue allows definitive testing of the presence of the tumor and specific molecular changes. The mode of (ii) relies on deep database representations and imposes a higher burden on the rate of occurrence and the need for follow-up testing of hypotheses generated by these studies.

Aim 2: Explore associations between identified molecular features and the putative mechanisms of action of the treatments that the patients received when they experienced response or lack of response.

Statistical analyses will be primarily descriptive. Feasibility and success in discovering molecular features with potential therapeutic relevance ("promising discoveries") will be assessed.

Aim 3: To compare results from WES and RNA-seq studies to the results of the eligibility NGS assays for NCI-MATCH.

Again, statistical analyses will be primarily descriptive. To characterize differences between the MATCH NGS assay used for eligibility and WES/RNA-seq, we will describe discordances within the same sample between the NGS assay and WES/RNA-seq for all studied mutations. We will also summarize any discordance between WES and RNA-seq for samples that have both available.

Feasibility will be assessed by calculation of the following statistics:

1. Percentage of patients for which adequate nucleic acid material with appropriate informed consent is available.
2. Percentage of acquired cases with nucleic acid material for which the minimum molecular characterization (i.e. WES, RNA-seq) is successfully obtained.

Promising discoveries will be summarized for the group of cases for which the minimum molecular characterization was successfully obtained. For each such case, molecular data provided by the sequencing and characterization center, the NCI-MATCH NGS assay, and the data analysis center, will comprise a list of potentially relevant molecular abnormalities (e.g., mutations or amplifications from WES or the MATCH NGS assay, and if possible, mRNA expression data for pathway analysis), which will be reviewed by an expert panel, the AWG, to identify interesting features. The AWG will be composed of the investigators on this protocol, members of the data analysis center, as well as ad hoc panel members from the drug development and scientific community as necessary, as well as the PIs of the subprotocol on which the patient was treated. For each treatment, this AWG will be responsible for listing genetic abnormalities in pathways that would be affected by the treatment that produced the response or resistance. This will be done while the molecular characterization is ongoing. The notion of a molecular feature will be interpreted broadly. For example, a

molecular feature might refer to a specific mutation in a gene predicted to be the target of the NCI-MATCH treatment, or it could refer to activation or inactivation of an entire genetic pathway thought to be related to a mechanism of action of a drug. The AWG's review will proceed in two steps for each evaluated case.

The AWG will receive an initial report that lists all features of the molecular profile. These features will be categorized according to involvement in known pathways relevant to cancer (e.g., growth factor receptor, MAP kinase pathway, DNA repair pathways, PI3kinase pathway, cell cycle pathway, etc.) that have potential therapeutic relevance (either responsiveness or resistance to an investigational or approved drug or drug combination). After the AWG has reviewed and classified the molecular data into relevant pathways, the AWG will correlate this information with the putative mechanism of action of the drug that the patient received.

We will summarize the outcome of this study by calculating the following statistics:

1. Percentage of molecularly characterized cases for which the Molecular Characterization report identified at least one feature judged to have potential therapeutic relevance when compared to the putative mechanism of action of the specific treatment that was associated with the outcome. In addition to providing a potential molecular reason for the response or resistance, such cases will provide clinical evidence that molecular profiling may be useful in assigning a particular treatment for a specific molecular abnormality (even in the expected circumstance that more than one molecular abnormality will coexist in any given tumor).
2. Percentage of cases with at least one additional feature from the targeted panel, WES or RNA-seq, in addition to molecular eligibility criteria for the MATCH subprotocol, that was judged to have potential therapeutic relevance to the specific class of drug that the patient received when the outcome was experienced. The additional features not found by the MATCH assay will be divided into two groups, with percentages calculated for both groups: a) Additional features that were not found by the MATCH assay but are included in the MATCH assay (discordances with WES or RNA-seq), and b) additional features that were not found by the MATCH assay because they are not included in the MATCH assay (other potential targets as defined by WES or RNA-seq).
3. Percentage of cases with at least one molecular feature that correlates with the mechanism of action or resistance to the specific agent or class of agents to which the outcome occurred, found after further analyzing WES or RNA-seq and not identified in the original Molecular Characterization report. These analyses will again be split into groups—discordances and other potential targets.

In the event that a patient experienced responses to multiple investigational agents, the multiplicity will be noted in study reports and publications, but each episode will be treated as though it was a distinct case for purposes of statistical summary calculation.

All estimated proportions will be accompanied by exact 95% confidence intervals (Korn & Graubard, 1999). As this is an exploratory discovery study, no statistical

adjustment will be made for the fact that multiple confidence intervals will be computed.

6.2. *SAMPLE SIZE RATIONALE*

The targeted sample size for this study is based on achieving the goal of successful molecular characterization of tumor samples from patients enrolled on subprotocols of NCI-MATCH (EAY131). The number of tumor samples that will be screened and have molecular characterization attempted will depend on the success rates for remaining tissues adequate to perform WES and RNA-seq. If, after molecular assays have been attempted on samples from 100 cases, the number of cases for which WES or RNA-seq was successful (i.e., interpretable results) is less than 20, then shipment of samples from the ECOG/ACRIN Repository to the sequencing laboratories will be suspended for re-evaluation of study feasibility. If at least 20 but fewer than 49 of the first 100 samples yield useable WES results, the study investigators' feasibility assessment will consider the number of molecular features with potential therapeutic relevance that were identified among the cases that yielded useable exome or transcriptome sequencing results. The study will be terminated when interpretable molecular results (minimum WES) have been obtained on at most 700 cases or when a total of 1400 (i.e. 700 each tumor/normal) molecular characterization assays have been attempted, whichever occurs first.

The probability that the study will be suspended after assays have been attempted on the first 100 cases due to observed number of successes less than 20 is at least 89% if the true assay success rate is at most 15%. The probability that the observed number of successful assays among the first 100 attempted will be 20 or more is at least 90% if the true assay success rate is at least 25%. Observed success percentages and sample size will determine the width of 2-sided 95% confidence intervals calculated to estimate the true percentages, as demonstrated in the following table:

	Exact 2-sided 95% confidence interval for percentage		
Observed percentage	N=200	N=600	N=1000
10	6.2, 15.0	7.7, 12.7	8.2, 12.0
20	14.7, 26.2	16.9, 23.4	17.6, 22.6
30	23.7, 36.9	26.4, 33.8	27.2, 32.9
40	33.2, 47.1	36.1, 44.0	36.9, 43.1
50	42.9, 57.1	45.9, 54.1	46.9, 53.1
60	52.9, 66.8	56.0, 63.9	56.9, 63.1
70	63.1, 76.3	66.2, 73.6	67.1, 72.8
80	73.8, 85.3	76.6, 83.1	77.4, 82.4
90	85.0, 93.8	87.3, 92.3	88.0, 91.8

If a total of 600 assays are performed, and 50% of those assays yield interpretable molecular results, the 95% confidence interval for the overall assay success rate is 45.9-54.1%. If 20 of the 200 cases yielding interpretable molecular results (10%) are judged to have at least one promising discovery, a

95% confidence for the promising discovery rate (among successfully performed assays) is 6.2-15.0 %.

6.3. *RESULTS INTERPRETATION AND NEXT STEPS*

This study is designed as a signal finding and hypothesis generating study. It is intended that interesting molecular features identified in this study will be investigated in other studies conducted outside of this protocol. These additional studies might include, for example, preclinical studies based on cell lines or xenografts, or retrospective studies using more clinical samples to examine whether the molecular features of interest for a particular drug can be found repeatedly in responders to that drug and not found in non-responders. Finally, the promising discoveries might lead to prospective clinical trials evaluating the drugs of interest in patients having the indicated molecular characteristics.

VII. ETHICAL AND PRIVACY CONSIDERATIONS

7.1. *DATA ACCESS AND SHARING PLANS:*

This study proposes to conduct whole exome and mRNA sequencing, and if possible whole genome sequencing with previously collected tissue, including germline tissue, if available.

It is imperative to know that this data will have been generated in a research setting, not in a CLIA certified laboratory, and no clinical decisions should be taken in response to data obtained in a facility that has not been CLIA certified.

In addition, de-identified genomic data generated by the project will, in accordance with NIH policy, be made available to other researchers in a controlled access database, raising privacy considerations for subjects as well as genetic relatives, given evolving technological capabilities for re-identification.

7.2. *LINKED PROTOCOL AND "CODED" IDENTIFIERS*

This study operates as a "linked" protocol, with each participant ID being doubly de-referenced (i.e., "coded" twice) before data are distributed to sequencing sites. The first linking key is retained by ECOG-ACRIN and CBPF for participants enrolled in NCI-MATCH. Access to this key is under the purview of ECOG-ACRIN institutional policies. The second linking key is retained by the CDC and is only made available within the program for quality control purposes upon approval by the PI(s). The second key will also be provided to ECOG-ACRIN. The NCI-MATCH subprotocol PIs may obtain sequencing results, if desired, for the patients enrolled onto this study. However, the results are not conducted in a CLIA certified laboratory, will not be returned to sites, and will not be used for clinical decision making.

7.3. *GENETIC DATA: CONTROLLED-ACCESS TIERS*

This study will generate genetic data unique to an individual ("genetic fingerprints" or genotypes). These data are not directly tied to an identified individual, and the clinical information associated with these data are de-identified as described above. Nevertheless, a risk exists that the genetic data could lead to the re-identification of a participant or relative. Consequently, NIH policy is that individual genetic data from the characterization studies are kept in a restricted-access tier of the database.

In accordance with the NIH Genome Data Sharing policy data, de-identified exome and RNA-seq data will be stored in an NIH controlled access database and made available to qualified researchers upon request. To be authorized to access the restricted tier of data, investigators must submit an application to a Data Access Committee (DAC) of the National Institutes of Health designated to review applications for the NCI-MATCH protocol. Upon approval by the DAC that the access request is for bona fide research purposes and consistent with any consent limitations under which the data were collected, the Investigator, scientists under their control, and their institution must subscribe to a Data Use Certification (DUC) that controls their ability to access the data, redistribute the data, prohibits the re- identification of participants, and includes requirements for data security.

7.4. *DISPOSITION OF SAMPLES AT THE END OF THE STUDY*

Residual samples and analytes will be distributed, retained, or destroyed at the discretion of the National Cancer Institute Center for Cancer Genomics.

VIII. INTELLECTUAL PROPERTY CONSIDERATIONS

8.1. *PROPRIETARY INTELLECTUAL PROPERTY GENERATION*

This study is intended as a hypothesis generating study. Neither the sequencing centers, the Clinical Data Center, nor the NCI intend to generate proprietary intellectual property from this study.

IX. REFERENCES

1. Carter, S. L., Cibulskis, K., Helman, E., McKenna, A., Shen, H., Zack, T., et.al. (2012). Absolute quantification of somatic DNA alterations in human cancer. *Nat Biotechnol, 30*(5), 413-421. doi:10.1038/nbt.2203
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Appendix XVII

Rev. Add13

Dynamic aMOI Process

As of Addendum #10, NCI-MATCH uses external "designated laboratories" (DL) to identify eligible patients. The DL have identified several variants which are not currently listed in the MATCH Huddle spreadsheet, but which the DL consider potentially eligible for MATCH. These include variants classified as actionable mutations of interest (aMOIs) by a DL but not specified as such by the MATCH protocol, as well as non-hotspot variants not identified by the current MATCH assay.

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NCI-MATCH has articulated that additional aMOIs (inclusionary OR exclusionary) can be considered actionable (used to assign a treatment in NCI-MATCH) if they meet the NCI-MATCH study's levels of evidence. This document elaborates on the process by which aMOIs not currently considered to be actionable may be deemed actionable. Designated laboratories' are to utilize novel aMOIs to determine an applicable referral for a specific arm if their assay are able to report the variants.

We have classified variants identified by DL assays but not listed in the MATCH Huddle spreadsheet into six categories as below, grouped by the workflow required for enrolling the patient. The MATCH Huddle spreadsheet is a direct representation of actionable variants listed in the subprotocol appendices.

Category 1. A novel oncogene alteration that is classified by the DL as gain-of-function.

This alteration would not meet existing levels of evidence (LOE) for MATCH and would therefore require that the designated laboratory submit either published or unpublished data that meet our current levels of evidence for variants (Level 1, 2 or 3) to support the gain-of-function claim (see EAY131 Section 1.4.2).

Examples:

a. A mutation in the kinase domain of a receptor tyrosine kinase (RTK) which the DL predicts will result in constitutive activation of the RTK.

b. An intragenic in-frame fusion event in an oncogene (e.g. novel MET ex14 event that additionally removes codons in ex13) that the DL predicts will be oncogenic.

Category 2. A tumor suppressor gene alteration that is classified by the DL as loss-of-function and which does not result in the introduction of a stop codon or frameshift at the protein level.

This alteration would not meet existing LOE and would require that the designated laboratory submit either published or other data to support the loss-of-function claim.

Examples:

a. A missense mutation in a tumor suppressor gene

b. A splice site alteration in a tumor suppressor gene.

c. An intragenic deletion which preserves the open reading frame in a tumor suppressor gene.

Category 3. A loss of function alteration in a tumor suppressor gene where the alteration results in the introduction of a stop codon, a frameshift alteration or a 3' deletion of the gene that effectively introduces a stop codon in the predicted protein product.

This alteration would meet existing Level 3 evidence in MATCH and would not require additional documentation from the designated laboratory.

Examples:

a. A 4 bp deletion that introduces a stop codon into the predicted protein of a gene.
b. A large deletion of a tumor suppressor gene that removes the carboxy terminus of the predicted protein (e.g. deletion of exons 17-42 in TSC2, a 42-exon gene).
c. A homozygous deletion that results in the complete loss of a tumor suppressor gene (e.g. TSC1, TSC2).

Category 4. A novel fusion event of the kinase domain of an oncogene that the DL predicts will be oncogenic.

Functional and clinical data from the literature suggest a novel fusion event that leaves the kinase domain of the oncogene partner intact will be activating. Therefore, this alteration would meet existing LOE for MATCH and would not require additional documentation from the designated laboratory.

Examples:

a. A novel BRAF fusion

Category 5. A gain or loss-of-function alteration that is not identified by the MATCH assay at the DNA level, but which results in an alteration at the protein level that is identified in the MATCH Huddle spreadsheet.

This alteration would meet existing LOE for MATCH and would not require additional documentation from the designated laboratory.

Examples:

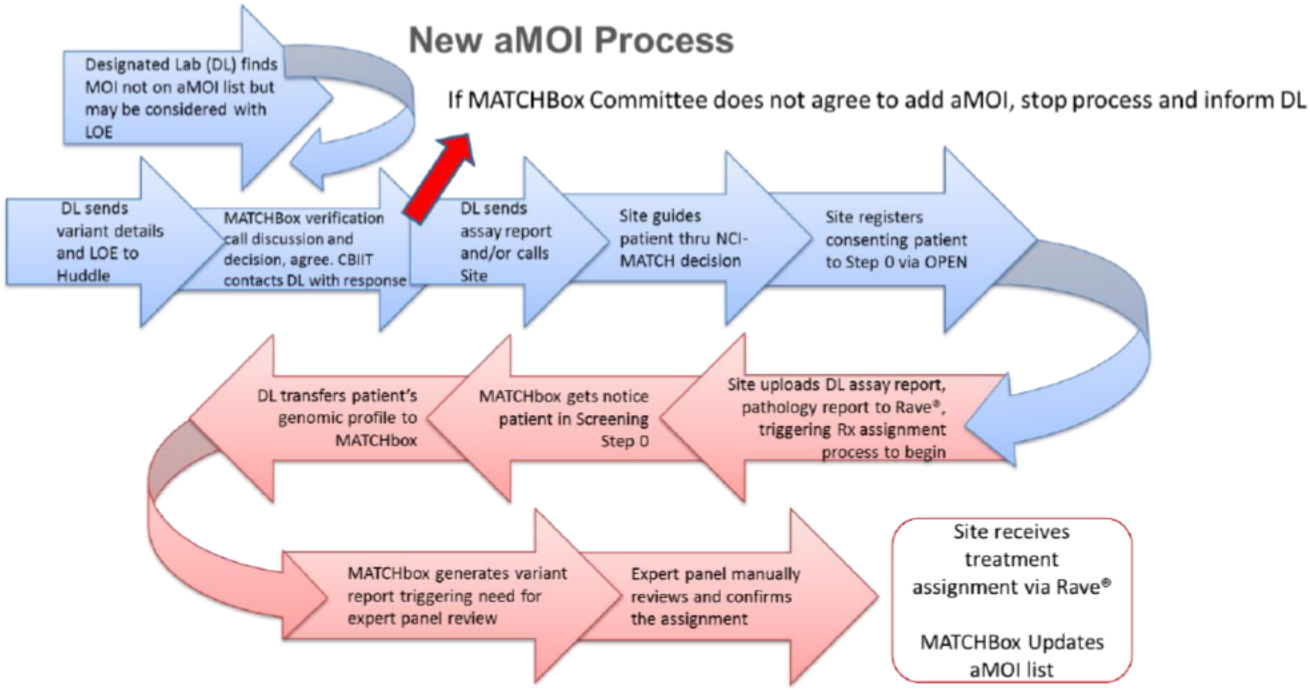
a. EGFR, L858R T->G COSM6224 (listed in Matchbox)
b. EGFR, L858R CT->AG (not listed in Matchbox but found by the DL)

Category 6. All other alterations.

These would be evaluated on a case-by-case basis and may or may not require supporting data, depending on the nature of the alteration. The MATCHBox verification team will consider the supporting data, if any has been submitted by the DL, and render a decision regarding patient eligibility for MATCH for these cases. Clinical sites should not be notified of potential eligibility until this determination is made.

Moving forward, where a documented LOE is required from the DL (categories 1 and 2), the workflow below will be followed. For category 3, the rationale is that these cases

currently meet MATCH LOE and require no additional documentation from the DL. Variant categories 4 and 5 are also considered to meet the current MATCH LOE.



The following table lists the members of the MATCHBOX verification team involved in these determinations, though others (e.g. relevant MATCH Subprotocol PIs) may be called upon to provide input on an ad-hoc basis.

Clinical Oncologist	Laboratorian	CBIIT
Alice Chen	Jeff Sklar	David Patton
Edith Mitchell	John Iafrate	Brent Coffey
Funda Meric-Bernstam	Mark Routbort	
John Wright	Mickey Williams	
Keith Flaherty	Stan Hamilton	
Lyndsay Harris	David Sims	
Naoko Takebe	Anand Datta	
Nilo Azad	Chris Karlovich	
John Wright		

Molecular Analysis for Therapy Choice (MATCH)

Appendix XVIII

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CS-MATCH-0009

Longitudinal Evaluation of Mutation Profile in NCI-MATCH Patients on All Treatment Arms through Evaluation of Archival Specimens

PRINCIPAL INVESTIGATOR: Stanley Hamilton, MD
CO-INVESTIGATORS: P. Mickey Williams, PhD
John Iafrate, MD
Jeffrey Sklar, MD PhD
Lyndsay Harris, MD

BIOSTATISTICS Lisa McShane, PhD; BRB DCTD, NCI
Laura Yee, PhD; BRB DCTD, NCI
Robert J. Gray, PhD; ECOG/ACRIN
Shuli Li, PhD; ECOG/ACRIN

ABSTRACT:

This project will characterize archival diagnostic specimens by next generation sequencing (NGS) assays, including but not limited to the NCI-MATCH Oncomine NGS assay, whole genome sequencing (WGS), whole exome sequencing (WES), and transcriptomic RNA sequencing (RNAseq) and compare the results to those obtained from similar genomic analyses of the matched pre-treatment NCI-MATCH tumor tissue. The project will initially focus on the approximately 700 patients assigned to one of the treatment arms. Sequencing will proceed in two phases: 1) pilot analysis of 100 samples, followed by a planned data analysis pause; and 2) sequencing of the remaining approximately 600 samples. Tumor biospecimens analyzed will be the archival diagnostic tissue and any intervening biopsy/resected tissues. Biospecimens will be sent to the NCI-MATCH biorepository within the MD Anderson Cancer Center (MDACC) Molecular Pathology Laboratory for pre-analytical processing, including pathology review, immunohistochemistry (IHC), and nucleic acid extraction. The NCI-MATCH Oncomine NGS assay will be performed within one of the five MATCH molecular characterization centers, MDACC, NCI-Frederick Molecular Characterization Laboratory (MoCha), Yale University, Massachusetts General Hospital (MGH), and Dartmouth College. Additional NGS assays (WES, WGS, RNAseq) will be performed at MoCha depending on funding. Data analysis will be performed by MDACC, MoCha, Yale, MGH, Dartmouth, and ECOG-ACRIN in conjunction with input from the Division of Cancer Treatment and Diagnosis (DCTD) at NCI.

1. OBJECTIVES AND HYPOTHESES

The main objective of this effort will be to determine if the actionable mutation of interest (aMOI) for which the patient was treated on NCI-MATCH exists in the archival, diagnostic and/or intervening tissues. The answer to this question will help drive full understanding of the NCI-MATCH clinical trial results. If a high concordance of aMOIs is observed between archival diagnostic specimens and the MATCH biopsies, this would suggest “fresh” pretreatment biopsies are not required for genomic analysis and treatment assignment. This result would minimize trial costs and risk to patients, and therefore likely increase enrollment in future precision-medicine studies. In addition, if patient response is linked with concordant specimens, this would support the notion that the aMOI was a driver/truncal mutation.

1.1. Objectives:

- To determine if “fresh” biopsies are required for precision medicine studies.
- To determine how the archival diagnostic mutation profile compares to the MATCH pretreatment biospecimen mutation profile.
- To determine if a treatment-assignable aMOI was concordant between archival and MATCH pretreatment specimens in a particular patient and if better clinical outcomes were seen in those with discordant results.
- To assess how the archival diagnostic mutation profile compares to the mutation profile in ctDNA (an additionally planned activity for NCI-MATCH)

1.2. Hypotheses

A true targetable oncogenic driver mutation will be present in all biopsies sampled over time, and patient response will correlate with the targetable oncogenic driver mutation in the archived diagnostic specimen.

2. BACKGROUND AND SIGNIFICANCE

The NCI-MATCH clinical trial required a pretreatment "freshly" collected tumor for use in the NCI-MATCH screening assays (IHC and Oncomine NGS). The reason for requiring a fresh pretreatment tissue was to permit screening of the tumor just prior to MATCH trial treatment and after all previous therapies. Previous treatments have been demonstrated to alter the genomic landscape of the disease, with acquisition of newly acquired mutations, some leading to resistance to prior therapies and others resulting from rapid evolution and error prone cell division. Although new mutations can occur with treatment or with cancer progression, it is essential to know whether true drivers differ with time, and to correlate the presence of a driver from malignant tissue acquired earlier in the course of a patient's malignancy with the IHC and NGS results obtained just prior to entry onto a NCI-MATCH treatment arm. Comparison of assay results from the archival diagnostic specimen and MATCH pretreatment specimen with response to the targeted treatment(s) may show whether the driver mutation is correlated with response. If so, then the need for a biopsy collection just prior to targeted treatment would be obviated.

To date, there are limited studies comparing NGS (WES, WGS, RNAseq) results from different biopsies over time from the same patient, especially for solid cancers. The Cancer Genome Atlas (TCGA) initiative primarily examined primary tumors, as opposed to metastatic lesions that are typically refractory or relapsed. The TCGA only studied secondary tumors if they matched to a primary tumor as part of a series (germline, primary and metastasis). Recent work in paired primary and metastatic ovarian cancers showed an increase in copy number alterations in the metastatic lesions (Brodsky et al, PLoS One. 2014).

EGFR-mutant lung adenocarcinoma is one area where several studies comparing matched pre- and post-treatment tumor molecular profiles have been performed. These studies show that while resistance can arise by multiple means, the primary EGFR sensitizing mutation (i.e., Ex19del or L858R) continues to be present in all patients who progress on an EGFR-targeted therapy. This result is consistent with the hypothesis that a true oncogenic driver mutation present at diagnosis will continue to be present after one or more rounds of therapy (Sequist et al, Sci. Transl. Med. 2011).

3. RESEARCH DESIGN AND METHODS

3.1. Biospecimens to be analyzed

Archived FFPE specimens collected prior to screening on NCI-MATCH from patients assigned to a MATCH treatment and consented to allow their tissue to be submitted and used for research will be analyzed. The optimal tissue requirements are the same as those defined in Section 9.3.2.1 of the EAY131 Master Protocol.

3.2. Laboratory methods

The primary laboratory method will be targeted deep sequencing using the NCI-MATCH Oncomine version 3 NGS assay. This assay has been validated in three labs to date and will be validated in all 5 MATCH network sequencing labs. The 3 network laboratories (MoCha, MDACC, and Dartmouth), performing all experiments as designed in the validation plan and using harmonized and locked SOPs, were able to demonstrate an overall sensitivity of 97.63%, overall specificity of 100%, overall accuracy of 99.99%, and an overall positive percent agreement of 99.99%.

The samples will undergo review, quality assurance/quality control evaluation, and processing in the same manner as the screening samples on NCI-MATCH, including:

- Pathology review in the MDACC pathology laboratory (Stan Hamilton, PI) to: 1) confirm pathologic diagnosis; 2) confirm presence of tumor cells and specimen adequacy; and 3) perform any immunohistochemical assessments required.
- DNA and RNA will be extracted and checked for sufficient quantity and quality. After processing, qualified cases will be shipped to one of the five MATCH molecular characterization centers (MCC) [MoCha, MDACC, Yale, MGH, Dartmouth]. All DNA and RNA from tumor and germline control tissues must also meet quality metrics before shipping to a MCC.
- Tumor characterization by platform will proceed in a step-wise manner to identify molecular features potentially predictive of response to therapy. Finding mutations in known cancer genes (oncogenes or tumor suppressors) will be the most readily interpretable and will be primarily determined by the NCI-MATCH Oncomine NGS assay. Sequencing will be performed on Ion Torrent S5XL systems at the five MCC labs. NOTE: The amount and integrity of tumor tissue may ultimately be the limiting factor in what sequencing methodologies will be employed.
- WES and RNAseq may be performed, depending on funding. WES and RNAseq have been successfully performed using FFPE tissue if quantity and quality of DNA and RNA are sufficient. If performed, WES and RNAseq will be done on Illumina machines, such as HiSeq 2500 or NovaSeq.

3.3. Prioritization

Tumor tissue will be characterized by targeted deep sequencing (NCI-MATCH Oncomine version 3 NGS assay). WES and RNAseq may be done if enough tumor DNA and RNA is available and sufficient funding exists. If tumor sample is limiting, the NCI-MATCH Oncomine NGS assay will be prioritized.

4. DATA ANALYSIS AND STATISTICAL CONSIDERATONS

Data from the NCI-MATCH Oncomine platform will be analyzed for concordance between mutations in the archival diagnostic and pretreatment biopsy specimens. The BAM files will be subjected to analysis through NCI-MATCHBox, the rules-based engine devoted to determining patient assignment. If data from other platforms (WES and RNAseq) is available, the data will be analyzed for mutations with an impact on known cancer genes and genes involved in known cancer pathways.

Genes will be ranked and reported based on their known or potential involvement in cancer. Genes of unknown significance will be reported as passengers. MDACC, MoCha, Yale, MGH, Dartmouth, NCI, and ECOG-ACRIN will be responsible for the statistical and bioinformatic analyses of the data.

4.1. Endpoints (outcomes)

The primary endpoint is the NCI-MATCH Oncomine version 3 NGS assay. A secondary endpoint is concordance of immunohistochemistry, as appropriate, with that required by the screening assay. Tertiary endpoints may be WES and RNAseq.

4.2. Case selection

The study goal is to collect NCI-MATCH Oncomine version 3 NGS molecular results on patients who were assigned to a MATCH treatment arm and who consented to

allow their tissue to be submitted and used for possible research studies. To date, there are about 700 patients who were assigned to a MATCH treatment. After May 2017, additional patients will be assigned based on outside assay results. We anticipate that at least 50% of assigned patients will have available and suitable archival and/or diagnostic tissue. Cases must meet the criteria for consent approval and response documentation, and each case must have sufficient tumor.

The targeted sample size for this study is based on achieving the goal of successful molecular characterization of tumor samples from patients enrolled on treatment arms of NCI-MATCH. The number of tumor samples that will be screened and have molecular characterization attempted will depend on the success rates for remaining tissues adequate to perform the NCI-MATCH Oncomine NGS assay. If, after molecular assays have been attempted on samples from 100 cases, the number of cases for which Oncomine was successful (i.e., interpretable results) is less than 20, then sample processing and sequencing will be suspended for re-evaluation of study feasibility.

4.3. Statistical analysis plan:

4.3.1. Aim 1: To determine if “fresh” pretreatment biopsies are required for precision medicine studies.

Statistical analyses will be primarily descriptive. To characterize differences between the archival diagnostic biopsy, intervening samples, and MATCH pretreatment biopsy, we will describe discordances in samples from the same patient using the MATCH targeted panel. We will provide summary statistics for concordance observed for putative oncogenic driver mutations. Analyses could potentially be stratified by driver mutation and by histology. Of note, the NCI-MATCH Oncomine panel has been versioned with additions and subtractions of various genes. It is conceivable the current targeted panel version will identify aMOIs in the archival diagnostic sample that were not originally tested in the MATCH pretreatment biopsy sample.

4.3.2. Aim 2: To determine how the archival diagnostic biopsy mutation profile compares to the MATCH pretreatment biopsy mutation profile.

Statistical analyses will be primarily descriptive. We will describe discordances in the NCI-MATCH Oncomine NGS assay between the archival diagnostic sample and MATCH pretreatment biopsy sample (Step 0).

4.3.3. Aim 3: To determine if a treatment-assignable aMOI was concordant between the archival and MATCH pretreatment specimens in a particular patient and if better clinical outcomes were seen than in those with discordant results.

Statistical analyses will be primarily descriptive. Feasibility and success in discovering molecular features with potential therapeutic relevance (“promising discoveries”) will be assessed.

4.3.4. Aim 4: To assess how the archival diagnostic mutation profile compares to the mutation profile in ctDNA (an additionally planned activity for NCI-MATCH).

Statistical analyses will be primarily descriptive. We will describe discordances between the MATCH targeted panel and mutational profile in the baseline ctDNA sample (Step 0). The analysis of ctDNA samples have not been finalized and may include a targeted panel similar to the NCI-MATCH Oncomine NGS assay.

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Appendix XIX

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List of Subprotocol-Specific Correlative Studies

Please see below for a listing of all correlative studies specific to the subprotocols.

Additional information regarding these correlative studies can be found in an appendix of the associated subprotocol.

Proposal #	Subprotocol	Title
CS-MATCH-0005	EAY131-Z1D	Tissue / blood biomarker analysis of samples collected from patients with mismatch repair deficient tumors (non-colorectal cancers) treated with nivolumab (EAY131-Z1D)
CS-MATCH-0008	EAY131-C2	Confirmation of low-level MET exon 14 skipping (Arm C2)
CS-MATCH-0012	EAY131-Q	An Explorative Analysis of the Roles of Tumor Intrinsic and Micro-Environmental Factors in the Clinical Response to TDM1 in the NCI-MATCH Q Arm

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Appendix XX

CS-MATCH-0014ct
Genomic landscape of plasma-derived ctDNA from patients enrolled on the NCI-MATCH trial

PRINCIPAL INVESTIGATOR: Filip Janku, MD PhD
CO-INVESTIGATORS: Funda Meric-Bernstam, PhD
Stanley Hamilton, MD
Rajyalakshmi Luthra, PhD
Mark Routbort, MD PhD
Richard Lanman, MD
Victoria Raymond, MS
Justin Odegaard, MD PhD
BIOSTATISTICS: Kenneth Hess, PhD
Lisa McShane, PhD; BRB DCTD, NCI
Laura Yee, PhD; BRB DCTD, NCI
Robert J. Gray, PhD; ECOG/ACRIN
Shuli Li, PhD; ECOG/ACRIN

5. INTRODUCTION

Obtaining blood-based liquid biopsies is a minimally invasive approach, which allows to collect material such as plasma-derived cell-free DNA, which can contain fragments of ctDNA.1 ctDNA is released to the circulation from tumor cells undergoing apoptosis or necrosis from primary or metastatic cancer lesions and can be identified in the blood and other body fluids of patients with cancer (1). ctDNA can be detected and quantified in majority of patients with metastatic cancers and some patients with early disease (1). In metastatic cancers detection of molecular aberrations in ctDNA using PCR-based methods have acceptable concordance with the molecular profile of tumor tissue and molecular testing of liquid biopsies is being increasingly accepted in the clinical care (2,3). However, PCR-based molecular testing of liquid biopsies can probe only limited number of hot spot mutations in common cancer genes of known clinical relevance detected in small subsets of patients, which limits potential utility.(4-6) This can be overcome by introduction of technologies such as targeted NGS, which allow to test for large number of cancer genes; however, concordance with molecular testing of tumor tissue across multiple metastatic cancers has not been established except for small studies.(3,7,8) In addition, plasma-derived ctDNA can be used to assess cancer genotype at different time points and provide valuable information about genetic changes that occur during the disease trajectory reflecting the tumor clonal evolution, as this is not a static process (1) Also, dynamic changes in VAF for specific alterations in ctDNA isolated from blood or urine were found to correspond with response and progression-free survival to systemic targeted and non-targeted therapies in patients with advanced cancers and malignant histiocytosis.(9,10)

The assay, Guardant360®, utilizes NGS-based digital sequencing to comprehensively profile 73 genes commonly altered in human cancer.(11) Of these 73 genes, 65 genes were also included in the assay, Oncomine, which was used for molecular profiling of pretreatment tumor biopsies in patients participating in the NCI-MATCH clinical trial. Guardant360®, is offered by Guardant Health, which is a CLIA certified, CAP-accredited, NYSDOH - approved molecular diagnostic laboratory, as a clinical test compliant with

the CAP and CLIA laboratory standards. The digital sequencing Guardant360® method was initially analytically and clinically validated across the panel of > 50 cancer-related genes with analytic sensitivity down to 0.1% VAF in serial dilution studies and analytic specificity of > 99.9999%.(11) Clinical sensitivity of plasma-derived ctDNA NGS was 85.0%, comparable to 80.7% sensitivity for the tissue. The assay success rate on 1,000 consecutive samples in clinical practice was 99.8%. More recently, expanded panel of 73 cancer genes (Table 1) was analytically and clinically validated and demonstrated variant detection down to 0.02% to 0.04% VAF/2.(12) copies with ≤0.3%/2.24-2.76 copies 95% limits of detection while maintaining high specificity (prevalence-adjusted positive predictive values >98%). Clinical validation using orthogonal plasma- and tissue-based clinical genotyping across >750 patients demonstrated high accuracy and specificity (positive percent agreement and negative percent agreement >99% and adjusted positive predictive values 92%-100%). (7) Clinical use in 10,593 advanced adult solid tumor patients demonstrated high feasibility (>99.6% technical success rate) and clinical sensitivity of 85.9%. Finally, in a retrospective analysis of molecular testing of plasma-derived ctDNA from 21,807 patients with advanced cancers demonstrated patterns and prevalence of ctDNA alterations in major driver genes for non-small cell lung, breast, and colorectal cancer similar to the tumor tissue sequencing in compendia such as The Cancer Genome Atlas and COSMIC (r = 0.90-0.99). (8)

Table 1. Guardant360® reports single nucleotide variants and splice site mutations in all clinically relevant exons in 73 genes and reports other variant types in select genes as indicated below. Genes not included in the MATCH-assay Oncomine panel are in bold and italicized.

AKT1	ALK	*APC*	AR	ARAF	ARID1A	ATM	BRAF	BRCA1	BRCA2
BRCA2	CCND1	CCND2	CCNE1	*CDH1*	CDK4	CDK6	CDKN2A	CTNNB1	DDR2
EGFR	ERBB2	ESR1	EZH2	FBXW7	FGFR1	FGFR2	FGFR3	*GATA3*	GNA11
GNAQ	GNAS	HNF1A	HRAS	IDH1	IDH2	JAK2	JAK3	KIT	KRAS
MAP2K1	MAP2K2	*MAPK3*	MET	MLH1	*MPL*	MTOR	MYC	NF1	NFE2L2
NOTCH1	*NPM1*	NRAS	NTRK1	NTRK3	PDGFRA	PIK3CA	PTEN	PRPN11	RAF1
RB1	RET	RHEB	RHOA	*RIT1*	ROS1	SMAD4	SMO	STK11	TERT
TP53	TSC1	*VHL*							

6. OBJECTIVES AND HYPOTHESES

6.1. Objectives

6.1.1.
To demonstrate concordance between molecular testing of plasma-derived ctDNA and simultaneously obtained tumor tissue collected from patients before treatment on selected arms of the NCI-MATCH protocol.

6.1.2.
To demonstrate that molecular profile of plasma-derived ctDNA such as presence or absence of molecular alterations, number of simultaneous alterations and mutation burden as reflected by variant allele frequency (VAF) in ctDNA and its dynamic changes in serially collected samples correspond with treatment outcomes.

6.1.3.
To demonstrate that molecular changes in serially collected samples of plasma-derived ctDNA from treated patients can provide

information about clonal evolution to understand mechanisms of intrinsic and adaptive resistance.

6.1.4. To demonstrate that molecular testing of plasma-derived ctDNA can detect actionable alterations in patients with insufficient tissue or failed results from the molecular testing of tumor tissue.

6.2 Hypotheses

It is hypothesized that plasma-derived ctDNA reflects to molecular profile of the tumor tissue and that there is concordance between molecular testing of plasma-derived ctDNA and molecular testing of tumor tissue DNA. We also hypothesize that dynamic changes in tumor genomic landscape in plasma-derived ctDNA can reflect therapeutic responses to cancer therapy.

7. RESEARCH DESIGN AND METHODS

7.1 Biospecimens to be analyzed

2 mLs of plasma to be analyzed were isolated from Streck tubes submitted:

a. At baseline, the end of cycle 2, and at end of treatment from patient on the selected arms indicated in Table 2 below. The treatment arms were selected based on:

- Therapeutically relevant molecular alteration, which is included in our Guardant360® NGS liquid biopsy assay.
- Meaningful number of patients enrolled.
- Inclusion of samples from consenting patients who enrolled onto any of the indicated treatment arms which are still actively accruing, including those from expansion cohorts, if applicable.

A minimum of 600 plasma samples from 500 patients accrued to treatment will be analyzed.

Table 2. Selected Treatment Arms

Arm	Drug	Variant	Rationale
C1	crizotinib	MET amplification	Detection of *MET* amplifications and other simultaneous alterations
C2	crizotinib	MET exon 14 deletion	Detection of *MET exon 14* mutations and other simultaneous alterations
E	AZD9291	EGFR (T790M)	Detection of $EGFR^{T790M}$ mutations and other simultaneous alterations
G	crizotinib	ROS1	Detection of *ROS1* rearrangement and other simultaneous alterations
H	dabrafenib and trametinib	BRAF (V600E, V600K)	Detection of *BRAF* mutations and other simultaneous alterations
I	Taselisib	PIK3CA	Detection of *PIK3CA* mutations and other simultaneous alterations

Arm	Drug	Variant	Rationale
J	trastuzumab and pertuzumab	HER2 (amplification)	Detection of *HER2* amplification and other simultaneous alterations
L	TAK228	mTOR	Detection of *mTOR* mutations and other simultaneous alterations
Q	ado-trastuzumab emtansine	HER2 (amplification)	Detection of *HER2* amplification and other simultaneous alterations
S1	trametinib	NF1	Detection of *NF1* mutations and other simultaneous alterations
V	sunitinib	KIT	Detection of *KIT* mutations and other simultaneous alterations
W	AZD4547	FGFR	Detection of *FGFR* alterations and other simultaneous alterations
Y	AZD5363	AKT	Detection of *AKT* mutations and other simultaneous alterations
Z1A	binimetinib	NRAS	Detection of *NRAS* mutations and other simultaneous alterations
Z1E	larotrectinib	NTRK	Detection of *NTRK* fusions and other simultaneous alterations
Z1I	AZD1775	BRCA1, BRCA2	Detection of *BRCA1* and *BRCA2* mutations and other simultaneous alterations

b. Baseline plasma samples from approximately 200 patients, if available, who were enrolled, but did not have tumor samples tested by the MATCH-assay because of insufficient biopsy sample or, who had attempted tissue sequencing, which failed to produce results.

7.2 Laboratory methods

Cell-free DNA extraction, processing, and sequencing will be performed in a CLIA-certified, CAP accredited laboratory (Guardant Health, Inc.) as previously described. (7,11) Total of 5-30 ng of extracted cell-free DNA will be labeled with non-random oligonucleotide barcodes (IDT, Inc.) and used to prepare sequencing libraries, which will be then enriched by hybrid capture (Agilent Technologies, Inc.), pooled, and sequenced by paired-end synthesis (NextSeq 500 and/or HiSeq 2500, Illumina, Inc.).

Bioinformatics analysis and variant detection: All variant detection analyses will be performed using the locked clinical Guardant360® bioinformatics pipeline and reported unaltered by post-hoc analyses. All decision thresholds were determined using independent training cohorts, locked, and applied prospectively to all validation and clinical samples as previously described.7,8,11 Expected detection limit for single nucleotide variants (SNVs) is ~0.04% VAF, for indels ~0.02% VAF and for fusions ~0.04% VAF (Table 3).7

Assay validation procedure and data: The Guardant360® assay was initially analytically and clinically validated for >50 cancer related genes and later on of 73 cancer related genes as described in Section 1.(7,8,11). Validation of a

plasma-based comprehensive cancer genotyping assay (73 genes) utilizing orthogonal tissue- and plasma-based methodologies: In a validation study the test demonstrated exceptional sensitivity (LOD95 ≤0.3% for SNVs, indels, and fusions and near 2.2 copies for CNAs), while maintaining high PPV (>98%) even in sample cohorts enriched for alterations at or below the applicable LOD.7 Specificity and precision are similarly high, allowing accurate variant identification as low as 0.02%-0.04%, which is critical for clinical ctDNA analysis as many relevant alterations are present at very low levels. Performance was verified in 349 clinical samples using internal and blinded external comparison to clinical ddPCR, which demonstrated very high qualitative and quantitative concordance. Analysis of 543 matched tissue genotyping results demonstrated high PPVs relative to standard-of-care tissue diagnostics. Assay performance is summarized in Tables 3 and 4.

Table 3. Analytical performance of Guardant360® (73 genes)

Variant type	Reporting threshold	LOD_{95}	Sensitivity	PPV	Specificity	
					Per sample	Per call
SNVs	0.04%/2 molecules	>0.25%	100%	99.2%	97%	>99.9999%
		0.05-0.25%	63.8%	96.3%		
Indels	0.02%/1 molecule	>0.02%	100%	98.2%	100%	100%
		0.05-.2%	67.8%	98.2%		
Fusions	0.04%/2 molecules	>0.02%	95%	100%	100%	100%
		0.05-.2%	83%	100%		
Amplifications	2.12 copies	2.24-2.76 copies	95%	100%	100%	100%

Table 4. Clinical validation of Guardant360® (73 genes)

Positive % agreement	>99%
Negative % agreement	>99%
Positive predictive value	92%-100%
Technical success rate	>99.6%
Clinical sensitivity	85.9%

8. DATA ANALYSIS AND STATISTICAL CONSIDERATONS

8.1. Aim 1: We will assess concordance between molecular testing of plasma-derived ctDNA and simultaneously collected tumor tissue on a gene-by-gene basis using the kappa coefficient. The inventory indicates 393 baseline samples. Therefore, we performed power calculations for 375 samples assuming that some samples might not be adequate for analysis. With 375 patients we will have > 90% power to detect a kappa of 0.8 (very good agreement) as significantly different from 0.4 (poor agreement) with a 2-sided 5% alpha and assuming true prevalence of 5%. For low prevalence aberrations, we will use exact methods of inference. Only genes and regions covered by both Guardant360 and Oncomine assays will be included for the concordance analysis. For a gene alteration with 10% prevalence, if sensitivity equals 89% (33/37) then a 95% Wilson confidence interval would extend from 75% to 96% and if specificity equals 90% (304/338)

then a 95% Wilson confidence interval would extend from 86% to 93%.Aim 2: To determine how the archival diagnostic biopsy mutation profile compares to the MATCH pretreatment biopsy mutation profile.

8.2. Aim 2: (a) We will use logistic regression analysis to assess the association of presence of matched ctDNA alterations, number of simultaneous ctDNA alterations, and mutation burden with clinical benefit defined by RECIST 1.1 (complete response/partial response/stable disease > 6 months).12 We will use Cox proportional hazards regression analysis to assess the association of presence of matched alterations, number of simultaneous alterations, and mutation burden with progression-free survival (PFS, defined as time from the start of study medication to progression or death). We will only look at the gene alterations associated with each arm of the study. A patient will be evaluated as "positive" if their ctDNA had the targeted alteration for their arm of the study and "negative" otherwise. The logistic regression model with have clinical benefit as the response variable and have 3 predictor variables: matched alteration status, number of simultaneous ctDNA alterations, and mutation burden. The Cox proportional hazard model with have PFS as the outcome and have 3 predictor variables: matched alteration status, number of simultaneous ctDNA alterations, and mutation burden. With 375 patients we will have > 80% power to detect an odds ratio of 2.2 as significantly different from 1.0 (the null value) with a 2-sided 5% alpha if the clinical benefit rate is 30% and the prevalence of the alteration is 20%. For a 15% prevalence, the detectable odds ratio is 2.4; for 10% it is 2.8 and for 5% it is 3.9. With 100 PFS events (in 375 patients) we will have > 90% power to detect a hazard ratio of 2.3 as significantly different from 1.0 (the null value) with a 2-sided 5% alpha if the prevalence of the alteration is 20%. For a 15% prevalence, the detectable hazard ratio is 2.5; for 10% it is 3.0 and for 5% it is 4.5. (b) We will conduct exploratory analyses assessing the association between dynamic changes in ctDNA profile in serially collected samples using logistic regression analysis for clinical benefit and Cox proportional hazards regression analysis for PFS. We will create an indicator variable that is "yes" if the cycle 2 sample lacked the targeted alternation for the given treatment arm while the baseline sample had the targeted alteration and "no" otherwise and correlate this indicator with outcome. Graphical analyses will also be used. With 25 PFS events out of 90 patients with baseline and cycle 2 samples, we will have >80% power to detect a hazard ratio of 3.1 as significantly different from 1.0 (the null value) with a 2-sided 5% alpha if the prevalence of the indicator is 50% and 3.7 for an indicator prevalence of 75%.

8.3. Aim 3: This aim is descriptive not necessitating formal statistical analysis.

8.4. Aim 4: For patients whose tumor tissue testing yielded no molecular aberrations, we will estimate the proportion of patients in which ctDNA molecular testing detected actionable alterations. We will randomly sample 25 patients from each of the 8 most common tumor types. With 200 patients, an exact 95% confidence interval for an estimate of 5% extends from 2.4% to 9.0% and for an estimate of 2.5% extends from 0.8% to 5.7%.

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Molecular Analysis for Therapy Choice (MATCH)

Appendix XXI

CS-MATCH-0015ct

Rev. Add24

A Retrospective Study of Liquid Biopsies Using a Pan-Cancer NGS Assay in NCI-MATCH Patients with Less Common Tumor Histologies or Who Enrolled in Subprotocol Arms Z1D, T or M

PRINCIPAL INVESTIGATOR: P Mickey Williams, PhD
CO-INVESTIGATORS: Alice P Chen, MD
Naoko Takebe, MD PhD
Phillip Febbo, MD
Traci Pawlowski, PhD
James V. Tricoli, PhD
Chris Karlovich, PhD
Biswajit Das, PhD
BIOSTATISTICS Lisa McShane, PhD

1. INTRODUCTION

The analysis of circulating tumor DNA (ctDNA) from cancer patient plasma is emerging as a powerful complement to tumor biopsies with the recent incorporation of plasma testing into the CAP/IASLC/AMP guideline for patients with non-small cell lung cancer1. While the potential clinical utility of liquid biopsies has now been extensively explored in more common tumor types (2) such as lung (3), colorectal (3), breast (3) and prostate (4) a comprehensive study in rare tumor histologies has not to our knowledge been published. Using a 523-gene ctDNA NGS assay platform, TSO500, we propose to test the plasma from ~2500 patients with less common/rare tumor types who were screened for NCI-MATCH but never underwent treatment. Results will be compared to tumor sequence data, and a comprehensive profile of the plasma mutational and biological landscape for each histology will be generated. As a secondary objective, we propose to test the plasma of patients who enrolled to arms T, M and Z1D to investigate mechanisms of resistance. A tertiary objective will be to test plasma from patients with "common" histologies (i.e. lung, breast, colon and prostate) who were never treated, and compare the results to tumor molecular profiles.

The TSO500 assay is uniquely positioned to address the study objectives. This large ctDNA panel interrogates all but three of the 2445 SNV/Indel mutations of interest (MOIs) and actionable MOIs (aMOIs) interrogated by the 161-gene MATCH tumor panel (OCA), thus enabling a comprehensive comparison between plasma and OCA tumor results. Additionally, the TSO500 panel can be used to assess tumor mutational burden (TMB) and microsatellite instability (MSI) status (including Lynch syndrome loci) as well as known checkpoint inhibitor resistance mutations in JAK2 and B2M. Collectively, our proposed studies may provide novel translational insights and further advance the clinical potential of ctDNA.

Our highest priority request and focus of our proposal (Workstream 1) is for plasma from patients with less common/rare cancer histologies who were never treated on NCI-MATCH. As a second priority (Workstream 2), we propose to test plasma from patients treated on arms Z1D, T and M. Our Workstream 3 request is for plasma from patients with common histologies (i.e. lung, breast, colon and prostate) who never were treated on NCI-MATCH.

1.1. Workstream 1 - Testing of ctDNA from patients with less common/rare cancer histologies in the NCI-MATCH cohort screened but not enrolled on a treatment arm [~60% of cases (~2500) among 4289 cases with pretreatment plasma]

Approximately 60% of all screened NCI-MATCH patients had a less common (e.g. ovarian, head and neck) or rare cancer (e.g. cholangiocarcinoma, pancreatic endocrine), defined as a cancer type other than breast, lung, colorectal or prostate (6). To our knowledge, a comprehensive assessment of matched tumor and plasma molecular profiles in patients with rare histologies has not been conducted. We therefore propose to test the ctDNA of all patients with less common/rare histologies who provided a biopsy but did not enroll on a treatment arm of NCI-MATCH. We will compare TSO500 ctDNA results to matched OCA or whole exome sequencing (WES) tumor data (if available).

When completed, we expect these assessments will provide new translational insights for the field of liquid biopsies. Specifically, data from our study will provide a comprehensive understanding of how ctDNA molecular profiles compare to and complement matched tumor molecular profiles in patients with rare histologies. Cases may even be identified where NCI-MATCH "mutations of

interest" (MOIs; defined as mutations interrogated by OCA for which evidence of oncogenicity exists in the literature and/or public databases such as COSMIC or ClinVar), and "actionable mutations of interest" (aMOIs; defined as mutations deemed clinically actionable because they are targeted by an NCI-MATCH study drug) are seen in plasma but not tumor because of tumor heterogeneity. Our study will additionally establish the fraction of patients in each less common/rare histology with ctDNA of sufficient quantity for evaluation. In aggregate, this information will help inform whether these patient populations would be good candidates for a liquid biopsy in future clinical trials and in clinical practice.

A critical and distinguishing feature of the TSO500 ctDNA platform is its comprehensive panel design. TSO500 is a 523-gene panel (~2 megabases total, ~1.3 megabases of which are protein-coding sequence) that covers the full exonic regions of all genes from FoundationOne, MSK IMPACT, TruSight170 and TruSight Myeloid tumor panels, with additional custom designs. It is capable of detecting small variants in 523 genes, copy number events in 59 genes and fusions in 23 genes. The panel also includes HLA Class I loci to inform neoantigen prediction (Appendix B). Most notably, the panel interrogates all but three of the 2445 SNV and indel MOIs and aMOIs interrogated by the NCI-MATCH OCA tumor test. It can assess MSI status and TMB in ctDNA more accurately than smaller gene panels, which would require at least 0.5 Mb of gene content for consistently accurate determination of clinically relevant levels of TMB (7). We therefore plan to characterize MSI status and TMB in untreated patients with rare histologies, and to compare the results to tumor assessments of MSI status and TMB from WES data, if available. Given the recent demonstration of MSI status and TMB as clinically validated biomarkers for checkpoint inhibitors (7,8,9), a successful retrospective demonstration that a blood test can accurately call both genomic features would be of great potential clinical utility. The bioinformatics pipeline associated with TSO500 can also provide information on percentage of ctDNA and clonal hematopoiesis, providing further information on the biology of malignancies.

1.2. Workstream 2 - Treated patient arms Z1D, T and M (pre-treatment plasma, and where available paired pre-, on-treatment and/or post-treatment plasma)

Circulating tumor DNA has shown great promise as a tool to identify putative mechanisms of primary and acquired resistance to targeted therapies in several tumor types (10) including lung (EGFR inhibitors) (11), prostate (PARP inhibitors) (12), ovarian (PARP inhibitors) (13), breast (PARP inhibitors) (13) and colorectal cancer (anti-EGFR antibodies) (14). Our workstream 2 proposal will hopefully extend these findings to other therapeutic areas. Specifically, we propose to test pre-treatment plasma, as well as matched pre-, on- treatment and/or post-treatment plasma where available, to identify mechanisms of resistance in patients treated on arms Z1D (nivolumab for MMR deficiency), T (vismodegib for SMO and PTCH1 mutations) and M (TAK-228 for TSC1 and TSC2 mutations). We are requesting plasma from these arms because we believe our assay is best positioned to investigate mechanisms of resistance in them. Most commercial ctDNA assays do not interrogate all actionable mutations from arms T and M. Further, we do not know of any commercial assay that can assess TMB, MSI status and B2M resistance mutations, which would be highly desirable for any proposed analysis of Z1D plasma specimens. As mentioned previously,

a successful retrospective demonstration that a blood test can accurately call MSI and TMB would be of great potential clinical utility.

Patients with matched baseline and progression plasma will be most informative and of primary interest for this proposal. This is because a putative mechanism of resistance should be present at a higher level (i.e. allele fraction) in post-treatment ctDNA, reflecting clonal expansion of resistant tumor cell populations under the selection pressure of therapy. Given the numbers of patients with matched pre-treatment and progression blood samples will be small in these arms (Arm Z1D has 7 cases of matched pre- and on- treatment or post-treatment samples; Arm T has 3 cases of pre and on-treatment or post-treatment samples; Arm M has 11 cases of matched pre- and on-treatment or post-treatment samples), power for statistical tests is expected to be low and the analyses should be considered exploratory and hypothesis- generating.

In most instances, only a pre-treatment sample will be available. While we propose to analyze all of these cases as well, our emphasis will be on the subset of patients who rapidly progressed. We hypothesize that the rapid progressors will be the ones mostly likely to have an identifiable candidate resistance mechanism present in pretreatment plasma.

1.3. Workstream 3 -Testing of ctDNA from patients with “common” cancer histologies in the NCI- MATCH untreated cohort [40% of cases (~1700) among 4289 cases with pretreatment plasma]

Our workstream 3 proposal is to test the ctDNA of all patients who provided a biopsy representing a “common” histology, but who never received treatment on NCI-MATCH. Similarly, as for rare histologies, we will compare TSO500 ctDNA results to matched Oncomine tumor results and to tumor WES data, if available. Compared with rare tumor histologies, more is known about plasma molecular profiles in common histologies (2,3,4). However, matched tumor and plasma data from NCI-MATCH patients with common histologies would be a meaningful addition to the body of literature for liquid biopsies in these patient populations. As discussed previously, we are well-positioned to perform a comprehensive comparison of matched ctDNA and tumor data because the TSO500 panel interrogates virtually all MOIs and aMOIs interrogated by the NCI-MATCH tumor test.

2. OBJECTIVES AND HYPOTHESES

2.1. Primary Objective

To characterize the molecular landscape of each less common/rare tumor histology as profiled in ctDNA and compare it to the molecular landscape as assessed by the OCA tumor test used during patient selection

2.2. Secondary Objectives

2.2.1. To establish for each less common/rare tumor histology evaluated the distribution of ctDNA burden (i.e., percentage of ctDNA of all cfDNA) in patients, and the fraction of patients without any detectable ctDNA

2.2.2. To determine the fraction of patients with an actionable mutation by OCA tumor test who also have the same actionable mutation by TSO500 plasma test [i.e., the “Positive Percent Agreement” (PPAref_tumor) of the

TSO500 plasma test, with OCA tumor test as reference] in the selected untreated patient cohort, Arm Z1D, Arm T and Arm M.

2.2.3. To determine the fraction of patients with an actionable mutation by TSO500 plasma test who have the same actionable mutation by OCA tumor test [i.e., the “Positive Percent Agreement” (PPAref_plasma) of the OCA tumor test, with TSO500 plasma test as reference] in the selected untreated patient cohort, Arm Z1D, Arm T and Arm M

2.2.4. To identify, using the TSO500 plasma test, candidate molecular predictors of response and resistance in patients treated on Arms Z1D, T and M

2.2.5. To characterize tumor heterogeneity using the TSO500 pan-cancer liquid biopsy assay and compare the results and degree of heterogeneity seen to that observed in matched tumor biopsies that had been assayed using the OCA tumor test

2.3. Hypotheses

2.3.1. The molecular landscape of rare tumor histologies profiled by plasma and tumor tests will be similar, except that more tumor heterogeneity will be observed in the ctDNA molecular profile assessed by the TSO500 plasma test.

2.3.2. For patients for whom plasma is assessed by the TSO500 test, the positive percent agreement (PPAref_tumor) of the biomarker results from TSO500 with the OCA tumor test (as non-reference standard) will be >=85% as reported previously in similar comparisons (5). Conversely, the positive percent agreement (PPAref_plasma) of OCA tumor test to the TSO500 results will be >=85%.

2.3.3. At least 85% of patients assigned to NCI-MATCH Arms Z1D, T and M would have been assigned the same treatment arm using an appropriate pan-cancer liquid biopsy assay (5).

2.3.4. Prospective selection based on the TSO500 pan-cancer liquid biopsy assay with broad capabilities (e.g., MSI status, TMB) would have changed the clinical actionability of molecular finding for certain NCIMATCH patients screened by the OCA tumor test, especially those not assigned to treatment. Some of the patients not assigned to any treatment arms would have been eligible for other therapeutic options, including immunotherapy.

2.3.5. The molecular landscape of common tumor histologies profiled by plasma and tumor tests will be similar, except that more tumor heterogeneity will be observed in the ctDNA molecular profile.

3. RESEARCH DESIGN AND METHODS

3.1. Biospecimens to be analyzed

a. From NCI-MATCH treatment arms per Workstream #2:

Plasma to be analyzed was isolated from 1-2 Streck tubes collected at baseline, on treatment, and progression from the following NCI-MATCH trial sub-protocol arms in decreasing order of priority:

- Arm Z1D: nivolumab in MMR-deficient tumors
- Arm T: Smoothened (SMO) or Patched1 (PTCH1) mutations
- Arm M: TSC1 or TSC2

b. From patients screened by the MATCH-assay but not enrolled to NCI-MATCH treatment arms as described for Workstreams #1 and #3:

Plasma to be analyzed was isolated from 1-2 Streck tubes collected at baseline

3.2 Laboratory methods

Testing will be completed using the Illumina ctDNA TruSight Oncology 500 (TSO500) ctDNA assay on the Illumina NovaSeq instrument and S4 flow cells. After having reviewed various methods of assessing ctDNA available on the market and evaluating a number of these assays at MoCha, including both ddPCR and NGS assays, this TSO500 panel is unique not only because of its large size, but because of the ability to report somatic variants, tumor mutational burden (TMB), and microsatellite instability (MSI) status. The TSO500 assay uses reagents that are already on market in other research use only (RUO) kits, with the exception of the newly developed gene probe panel. The RUO assay including the 500-gene panel has undergone significant testing at Illumina to ensure the assay meets expectations for reproducibility, sensitivity and specificity. It will first be marketed as a “Research Use Only” assay and then submitted for CE-IVD and FDA approval (formulations for RUO and IVD are identical). The in vitro diagnostic (IVD) tissue assay is being developed in partnership with Bristol Myers Squibb (BMS) to identify patients who may benefit from BMS immunotherapeutics. Illumina has partnered with the MoCha laboratory to allow early access to this assay.

The assay interrogates 1.3 megabases in coding regions in 523 genes to calculate TMB. The full assay size is 2.0 megabases, enabling the detection of single nucleotide variants (SNVs), insertions/deletions (indels), copy number variants (CNVs, amplifications only), and translocations from ctDNA. A subset of variants detected will be considered MOIs as described in the Definitions appendix. Through collaboration between MoCha and Illumina, raw variant results are available and detailed analysis is possible above and beyond the MOIs that are reported to further delve into the data as needed.

This assay incorporates unique molecular identifiers (UMIs) to remove artifactual errors introduced during DNA extraction and storage, library preparation, and sequencing. With the use of UMIs, PCR duplicates belonging to the same DNA template are identified as families. PCR duplicates are first collapsed into one consensus read pair by template strand to remove random sequencing errors. When both strands of the same template are present, they are further collapsed to remove DNA damage introduced during DNA extraction and storage. After being collapsed into one consensus sequence, read pairs are stitched together as unique fragments.

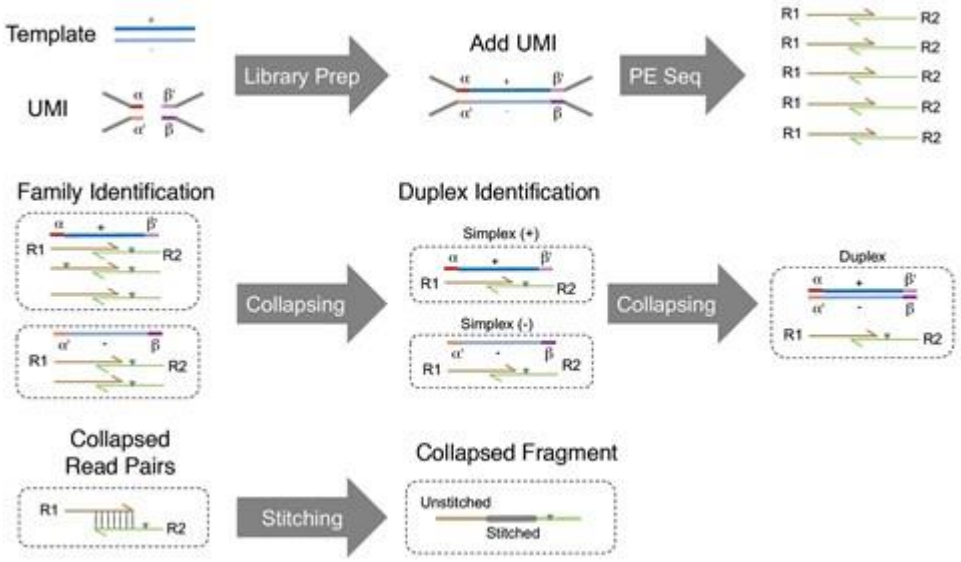

Figure 1. Error suppression workflow.

Using UMI technology and read collapsing algorithm, the overall error rate can be reduced from 0.1% to 0.002%, with the fraction of error free positions above 96% in ctDNA. Additional proprietary bioinformatic features of the assay can assess tumor fraction, clonal hematopoiesis, copy number variation, ctDNA fusions and detect SNVs and indels to 0.1% VAF. It is well known that DNA is susceptible to different types of damage such as oxidation and deamination which is a major source of false positives (22). The degree of damage usually varies from sample to sample. One unique aspect of the proprietary variant calling algorithm is that it measures sample-specific error profile and incorporates it into variant calling to achieve robust and uniform performance across a broad collection of samples collected over time and different conditions.

Since this assay is an RUO assay, it requires a full analytical validation at the MoCha laboratory prior to implementation, which is ongoing. Preliminary feasibility data are provided in section 16 of this proposal. An extensive MoCha analytical validation of the assay is expected to be complete in the fall of this year, and the plan is described in section 16 of the proposal. If granted approval for the use of these valuable specimens, we would agree to submit the final analytical validation report for the TSO500 assay to the committee reviewing these proposals for its final review and approval prior to receiving any specimens.

Cell-free DNA will be extracted and quantified. The recommended input is 30ng cfDNA, but the protocol allows for lower inputs as needed. Sensitivity may be affected by lower input, which will be determined as part of the full validation of the assay and a minimum input requirement will be established.

Library preparation will follow the TruSight Oncology 500 cfDNA User Guide and use the associated reagents from Illumina. In summary, end repair and A-tailing will be done, followed by ligation of adapters and clean-up of reaction, indexing PCR, two rounds of hybridization and capture using TSO500 probes, amplification and clean-up of the enriched library. Library size will be evaluated

using the Agilent TapeStation 4200. Library size must be between 275 and 425 bp. Libraries will be quantified and sufficient library must be obtained to pool for sequencing. A total of 16 libraries will be pooled for multiplexed sequencing per S4 standard flow cell.

In addition to the library requirements, additional quality control metrics will be used to evaluate the quality of data obtained from each library. The Median Target Coverage (MTC), which reflects the median number of molecular families for each target region, should be at least 954. Samples with MTC between 700 and 954 will be flagged, as the data can be used but there will be a decrease in sensitivity. Samples with an MTC below 700 will fail and not be analyzed. The Mean Family Depth (MFD), which reflects the average number of sequence reads for a molecular family, must be at least 7. If the MFD is less than 7, the sample will fail and not be analyzed. The UMI error correction is measured using the noiseAF. The noiseAF is the average allele fraction of all reported variant frequencies <5% and is an indicator of artifactual base changes that may be introduced during sample preparation or processing. The noiseAF must be less than 7 x 10-5. If the noiseAF is greater than 7 x 10-5, the sample will fail and not be analyzed.

Additionally, Illumina is developing a method that determines if there is an indication of contamination in a sample, which will be included as part of the full analytical validation. Note that the noiseAF and contamination metrics are not applied to contrived samples, because they often are created by mixing multiple cell lines or other sources. Since these metrics analyze low level SNP changes in the specimen, these QC metrics are inappropriate for contrived samples due to the way the samples are created.

Using these metrics, the sample failure rate at MoCha is being tracked and compiled. As an example of assay performance that is anticipated to reflect the sample failure rate for MoCha, the sample failure rate for 154 clinical samples tested at Illumina is shown below (Table1). The “Fail” category below includes both sample failures as well as samples flagged due to MTC 700 - 954.

Table 1:

Input [ng]	N pass	N fail	Failure rate (95% CI)
0 — 10	6	12	66.7% (41.2% - 85.6%)
10 — 20	38	3	7.3% (1.9% - 21%)
20 — 30	12	1	7.7% (4% - 37.9%)
30 — 40	81	0	0% (0% - 5.6%)
40 — 50	1	0	0% (NA)

A nucleosomal preparation of a reference hapmap (NA12878) will be included with each batch of library preparation and will be sequenced as a control. Nucleosomal preparations of cell lines have been demonstrated by Illumina (23) and others in the field to closely mimic plasma cfDNA, and are therefore appropriate control material24. The QC metrics of this sample will be evaluated to ensure no issues occurred during library preparation or sequencing.

To demonstrate within-lab assay precision, initial testing has included two operators assaying SeraSeq ctDNA Mutation Mix v2 in two replicates each and results are presented in section 16. Two intra-run replicates by operator one were completed using the SeraSeq ctDNA Mutation Mix v2 purchased from Seracare as plasma, while two intra-run replicates by operator two were completed using the SeraSeq ctDNA Mutation Mix v2 purchased from Seracare as nucleic acid. This demonstrates precision between operators and run, even when considering the pre-analytical handling of the samples. As part of the full assay validation, precision will be further evaluated by multiple operators on different days using a larger sample set.

If multiple laboratories will perform any testing, cross-lab reproducibility will first be thoroughly evaluated as part of the validation. The SOPs to be followed will be standardized and harmonized, so that each laboratory performs the procedures the same way.

4. DATA ANALYSIS AND STATISTICAL CONSIDERATONS

4.1. Primary objective: To characterize the molecular landscape of each less common/rare tumor histology as profiled in ctDNA and compare it to the molecular landscape as assessed by the OCA tumor test used during patient selection

The molecular variants that TSO500 plasma and OCA tumor panels are capable of detecting have been compared to identify the overlapping (intersection) set to be used for cross-platform comparisons. As noted in section 11, TSO500 is a 523-gene panel which interrogates all but three of the 2445 SNV and indel MOIs and aMOIs interrogated by the OCA gene panels which include OCAv1, v2 and v3 (most NCI- MATCH patients were screened with OCAv2). Based on information provided in the RFP and public presentations describing the distribution of tumor types observed in the study, we expect that approximately 2500 baseline plasma samples (approximately 60% of the 4289 (4886-597) screened patients not enrolled on a treatment arm) will be available from patients with non-common tumors who were screened but did not enroll on a treatment arm in the trial.

For each molecular variant detectable by each test (TSO500, OCA), we will tabulate the proportion of samples (n »2500) in which it was detected accompanied by an exact binomial two-sided 95% confidence interval (without correction for multiple comparisons). To assess for differences in detection rates for variants in the overlap of variants detectable by both tests, we will calculate a p-value associated with a

Fisher's exact test for difference in proportions detected with control of the false discovery rate (FDR) at 10% using the method of Benjamini and Hochberg26.

4.2 Secondary objectives:

a) To establish for each less common/rare tumor histology evaluated the distribution of ctDNA burden (i.e., percentage of ctDNA of all cfDNA) in patients, and the fraction of patients without any detectable ctDNA

For each sample, the proportion of ctDNA present in the cfDNA will be calculated and these proportions will be summarized by tumor type with graphical displays (e.g., violin plot or boxplot) and summary statistics including mean, median,

standard deviation (SD), minimum, maximum, and inter-quartile range (IQR). The proportion of patients with no detectable ctDNA within detectable cfDNA will be reported for each tumor type along with exact binomial two-sided 95% confidence intervals (CIs), If there are any patients with no detectable cfDNA, those proportions (with exact binomial two-sided 95% CIs) will be reported by tumor type as well.

b) To determine the fraction of patients with an actionable mutation by OCA tumor test who also have the same actionable mutation by TSO500 plasma test (i.e., the "Positive Percent Agreement (PPAref_tumor)) of the TSO500 plasma test, with OCA tumor test as reference) in the untreated patient cohort, Arm Z1D, Arm T and Arm M

Based on the existing tumor sequencing results obtained by OCA, the set of aMOIs detected at baseline in each patient's tumor will be identified. For each of these cases we will tabulate which of the aMOIs detected by the OCA tumor test were also detected in the baseline plasma sample using the TSO500 test (restricted to the overlap of the OCA and TSO500 panels) to calculate PPAref_tumor. The primary PPAs will be calculated separately by variant class (SNVs, Indels, CNVs, and fusions) across all patients and aMOIs as the proportion of aMOIs detected at baseline in tumor (OCA) that were also detected in baseline plasma (TSO500). These proportions will be accompanied by exact binomial two-sided 95% CIs. If there are sufficient numbers of patients whose tumors had the same aMOIs (OCA) we will also calculate PPAref_tumor (with exact two-sided 95% CIs) for those specific aMOIs. We will separately tabulate any variant level discordant results (aMOIs detected by OCA test in tumor but the same aMOI not detected by the TSO500 test in baseline plasma). These calculations will be performed separately for the untreated cohort, and for Arm Z1D, T, and M cohorts. Patients who tested positive in tumor by OCA for a particular aMOI that would have made them eligible for arm Z1D, T, or M, but did not enroll on the treatment arm for any reason, will be considered part of the treatment arm cohort rather than the untreated cohort for purposes of these PPAref_tumor calculations. These calculations will be repeated separately by tumor type, but the arm-specific PPAs within tumor type might be based on very small numbers of cases, thus limiting their interpretability. If access is granted to all patient samples (common and less common/rare tumors), then these analyses will be performed for all tumor types; otherwise the analyses will be restricted to non-common tumor types.

c) To determine the fraction of patients with an actionable mutation by TSO500 plasma test who have the same actionable mutation by OCA tumor test (i.e., the "positive percent agreement" of the OCA tumor test, i.e., PPAref_plasma, with TSO500 plasma test as reference) in the untreated patient cohort, Arm Z1D, Arm T and Arm M

Based on the TSO500 tests that will be performed, the set of aMOIs identified at baseline in each patient's plasma will be identified. For each of these cases we will tabulate which of the aMOIs were also identified in the baseline tumor sample using the OCA test (restricted to the overlap of the OCA and TSO500 panels) to calculate PPAref_plasma (TSO500 as reference). The primary PPAref_plasma values will be calculated separately by variant class (SNVs, Indels, CNVs, and fusions) across all patients and aMOIs as the proportion of aMOIs detected at

baseline in plasma (TSO500) that were also detected in baseline tumor (OCA). If there are sufficient numbers of patients whose plasma samples had the same aMOIs (TSO500) we will also calculate PPAref_plasma (with exact binomial two-sided 95% CIs) for those specific aMOIs. We will separately tabulate any variant level discordant results (aMOIs detected by the TSO500 test in baseline plasma but not detected by the OCA test in tumor). These calculations will be performed separately for the untreated cohort, and for Arm Z1D, T, and M cohorts. Patients who tested positive in tumor by OCA for particular aMOIs that would have made them eligible for arm Z1D, T, or M, but who did not enroll on the treatment arm for any reason (including that the variant was not detected in tumor), will be considered part of the treatment arm cohort rather than the untreated cohort for purposes of these PPA calculations. These calculations will be repeated separately by tumor type, but the arm-specific PPAref_plasma values within tumor type might be based on very small numbers of cases, thus limiting their interpretability. Reported proportions will be accompanied by exact binomial two-sided 95% CIs. If access is granted to all patient samples (common and less common/rare tumors), then these analyses will be performed for all tumor types; otherwise the analyses will be restricted to non-common tumor types. Any matched tumor/plasma pairs where a valid NGS result could not be obtained in tumor (e.g. because no tumor cells were present in the biopsy) will be deemed unevaluable for this analysis. Any matched tumor/plasma pairs where a valid NGS result could not be obtained in plasma (e.g. because the cfDNA tested failed to meet appropriate quality control metrics) will be deemed unevaluable for this analysis.

d) To identify, using the TSO500 plasma test, candidate molecular predictors of response and resistance in patients treated on Arms Z1D, T and M

This objective is considered exploratory because of the relatively small numbers of patients enrolled to these arms, and the fewer still who are likely to have the matched pre- and post-treatment blood samples that will be most informative for these analyses. The first analysis will be to compare, for each treatment arm (Z1D, T, and M) and MOI, the proportion of patients with a positive variant call in plasma (TSO500) at baseline for the responders (CR+PR) versus nonresponders. We will calculate for each variant a p-value associated with a Fisher's exact test for each difference in proportions with control of the false discovery rate (FDR) at 10% using the method of Benjamini and Hochberg26. We will also examine serial plasma ctDNA results (TSO500) to identify which patients in these three arms had changes in detection calls over time for any MOIs. For each MOI for each patient, the MOI result will be scored as -1, 0, or +1 to signify whether the change (from baseline to latest follow-up point with plasma sample available) was present-to- absent, no change, or absent-to-present. The distribution of these scores will then be compared visually between responders and nonresponders within each treatment arm. For select MOIs deemed a priori potentially relevant to the therapy, or found interesting by visual inspection using a criterion of greater than 30% difference in proportion of -1 or +1 scores between responders and nonresponders, we will additionally explore the data using graphical displays. For these select MOIs, spaghetti plots will be constructed showing allele frequencies over time for each patient, separately for responders and nonresponders. Additionally, scatterplots will be constructed for these MOIs showing the relationship between change in allele frequency (x-axis)

versus time on treatment (plotting symbol formatted to denote alive and free of progression versus death, progression or off treatment for any reason). Due to the anticipated very small numbers of patients in any arm who have paired samples and the large numbers of comparisons, all of these "change analyses" will be considered exploratory, and no formal statistical testing will be performed. We note also that these analyses must be restricted to patients who were positive for the qualifying variants in tumor and other eligibility criteria for the treatment arms, as patients who might have been eligible for enrollment on the arm based on plasma (TSO500) but not based on tumor assessment (OCA) would not have been identified as eligible for the treatment arms in the trial.

Using baseline plasma ctDNA results as described in "Tumor Mutational Burden, Microsatellite Instability, and Tissue Concordance" from Section 16, we will determine the proportion of patients enrolled on arm Z1D who would have been confirmed as MMR-deficient based on plasma results. The proportion of responders will be compared for the group of patients who were confirmed MMR-deficient versus not MMR-deficient according to the plasma-based assessments. The difference in response proportions will be assessed for statistical significance using a two-sided 0.05 level Fisher's exact test. This test may have low statistical power, so statistical significance may be unlikely, but an estimate of difference in response proportion (with two-sided 95% confidence interval) will be reported regardless. Similarly, in further exploratory analyses the difference in response proportions between two groups of patients defined by a cutoff applied to the plasma TMB score and between two groups defined by a cutoff applied to the plasma MSI score will be assessed. Optimal cutoffs for TMB score and MSI score in plasma have not yet been established. As the samples size will be small and the cohort enrolled onto arm Z1D represents a mixture of histologies, any suggested optimized cut-off will require verification in independent studies with suitable numbers of specific tumor types to confidently draw conclusions.

As a supplementary analysis for this objective, we can also determine TMB and MSI status from plasma samples for any patients not enrolled on arm Z1D to describe the overall distribution of these measures across the range of tumor types (not restricted to patients determined eligible for arm Z1D based on tumor mutation profile). This will give a sense for how many more patients might be considered for immunotherapy based on TMB and MSI status determined from plasma in a future clinical trial or in clinical practice (in settings where immunotherapies are already approved).

e) To characterize tumor heterogeneity using the TSO500 pan-cancer liquid biopsy assay and compare the results and degree of heterogeneity seen to that observed in matched tumor biopsies that had been assayed using the OCA tumor test

Heterogeneity in detected MOIs will be characterized at both the trial level and the patient level. Separately for each tumor type, the different MOIs detected across the screened patients (regardless of enrollment on a treatment arm) will be tabulated and, for each MOI, expressed as the number and proportion of patients deemed positive based on tumor results (OCA). A similar tabulation will be produced for plasma results (TSO500). For patient-level analysis, we will score the comparison between tumor and plasma results (restricted to the overlap of the OCA and TSO500 panels) for each patient as follows:

-3 = Sets of MOIs detected in plasma (SO500) compared to tumor (OCA) are completely nonoverlapping, with number detected in plasma (TSO500) smaller than in tumor (OCA)

-2 = Fewer MOIs detected in plasma (TSO500) compared to tumor (OCA); they overlap but MOIs detected in plasma are not a subset of those detected in tumor

-1 = MOIs detected in plasma (TSO500) wholly contained within and strictly smaller than the set of MOIs detected in tumor (OCA)

0 = Identical set of MOIs detected in plasma and tumor

+1 = MOIs detected in tumor (OCA) wholly contained within (and not identical to) set of MOIs detected in plasma (TSO500)

+2 = At least as many MOIs detected in plasma (TSO500) compared to tumor (OCA); they overlap but MOIs detected in tumor are not a subset of those detected in plasma

+3 = Sets of MOIs detected in tumor (OCA) compared to plasma (TSO500) are completely nonoverlapping, with number detected in tumor (OCA) no greater than in plasma (OCA)

For each possible score, we will tabulate the number and proportion of patients classified into that category and provide exact binomial two-sided 95% CIs for each proportion (with no adjustment for multiple comparisons). Initially these analyses will be performed considering agreement at the MOI level, but secondary analyses may be conducted that take into consideration whether differences in detected MOIs would have led to differences in treatment recommendations at the patient level. If access is granted to all patient samples (common and less common/rare tumors), then these analyses will be performed for all tumor types; otherwise the analyses will be restricted to non-common tumor types.

4.3 Analyses evaluating specific hypotheses

i) The molecular landscape of rare tumor histologies profiled by plasma and tumor tests will be similar, except that more tumor heterogeneity will be observed in the ctDNA molecular profile assessed by the TSO500 plasma test.

This hypothesis will be addressed by the analyses described above for the primary objective and secondary objectives b, c, and e.

ii) For patients for whom plasma is assessed by the TSO500 test, the positive percent agreement (PPAref_tumor) of the biomarker results from TSO500 with the OCA tumor test (as non-reference standard) will be >=85% as reported previously in similar comparisons. Conversely, the positive percent agreement (PPAref_plasma) of OCA tumor test to the TSO500 results will be >=85%.

This hypothesis will be addressed by the analyses described above for secondary objective b.

iii) At least 85% of patients assigned to NCI-MATCH Arms Z1D, T and M would have been assigned the same treatment arm using the TSO500 pan-cancer liquid biopsy assay.

This hypothesis will be addressed by the analyses described above for secondary objective c, noting whether the reported exact binomial two-sided 95% CI for PPA for each arm-eligible cohort falls above 85%.

iv) Prospective selection based on the TSO500 pan-cancer liquid biopsy assay with broad capabilities (e.g. MSI status, TMB) would have changed the clinical actionability of molecular finding for certain NCI-MATCH patients screened by the OCA tumor test, especially those not assigned to treatment. Some not assigned would have been eligible for other therapeutic options, including immunotherapy.

This hypothesis will be addressed by the analyses described above for secondary objective d, including the suggested supplementary analysis.

v) A pan-cancer liquid biopsy test samples ctDNA originating from multiple lesions and thus captures tumor heterogeneity that may not be present in a tumor biopsy.

This hypothesis will be addressed by the analyses described above for the primary objective and secondary objectives b, c, and e. This hypothesis will be addressed by the analyses described above for secondary objectives b, c, and e.

4.4 Statistical justification for sample size

The analyses proposed in this study are largely descriptive with the primary goal to comprehensively characterize the molecular landscape of each less common/rare tumor histology as profiled in ctDNA and compare it to the molecular landscape as assessed by the OCA tumor test used during patient selection. Based on the information available about patient accrual and plasma samples available, we estimate that the number of patients from whom we will be able to obtain baseline plasma ctDNA results (accounting for a small percentage of nonevaluable assays results due to inadequate samples or failed assays) will be approximately 2500 for the set of non-common tumors. Once these results are obtained, the same data can be used to address all other objectives and hypotheses; therefore, we base our sample size rationale on this primary objective (see below). If we are approved to receive plasma sample from all screened patients we assume that we would have baseline plasma ctDNA results on approximately 5000 patients.

Plasma ctDNA results for 2500 patients will effectively ensure (100% probability) we will detect a MOI that is truly present in the population at prevalence 0.5%, in at least one patient sample. Table10 below shows additional examples of the probability of detecting a MOI in at least one patient sample for a range of values for true MOI prevalence and sample size. The smaller sample sizes are applicable to detection of MOIs in subgroups, for example a particular tumor histology or treatment arm.

Table2. Probability of detecting a MOI in at least one patient sample

	Prevalence of MOI in population (%)									
Number of patient samples	0.1	0.25	0.5	0.75	1.0	2.0	3.0	4.0	5.0	10.0
5000	99	100	100	100	100	100	100	100	100	100
2500	92	100	100	100	100	100	100	100	100	100
1000	63	92	99	100	100	100	100	100	100	100
500	39	71	92	98	99	100	100	100	100	100
200	18	39	63	78	87	98	100	100	100	100
100	10	22	39	53	63	87	95	98	99	100
75	7	17	31	43	53	78	90	95	98	100
50	5	12	22	31	39	64	78	87	92	99
30	3	7	14	20	26	45	60	71	79	96
20	2	5	10	14	18	33	46	56	64	88
15	1	4	7	11	14	26	37	46	54	79
10	1	2	5	7	10	18	26	34	40	65

For analyses reporting exact binomial two-sided 95% CIs, the width of the CI will depend on the sample size (i.e., the denominator of the proportion) and the observed successes (i.e., the numerator of the proportion). Tables11.A – 11.H below provide example CIs for a range of scenarios. Example proportions of interest could include PPA, or prevalence of a particular MOI in the full group of patient samples or in subsets.

A sample size of 2500 patients will provide a confidence interval width less then 4 percentage points for any observed proportion. (Note: from Table11.B the confidence interval width is largest for an observed proportion equal to 50%, with a CI width of 51.98-48.02=3.96).

Table3.A Exact binomial two-sided 95% confidence intervals for sample size n=5000

Observed proportion expressed as %	Lower CI limit (as %)	Upper CI limit (as %)
0/5000 = 0%	0	0.07
5/5000 = 0.1%	0.03	0.23
12/5000 = 0.24%	0.12	0.42
25/5000 = 0.5%	0.32	0.74
37/5000 = 0.74%	0.52	1.02
50/5000 = 1.0%	0.74	1.32
250/5000 = 5.0%	4.41	5.64
500/5000 = 10.0%	9.18	10.87

Observed proportion expressed as %	Lower CI limit (as %)	Upper CI limit (as %)
1250/5000 = 25.0%	23.8	26.22
2500/5000 = 50.0%	48.6	51.4
3750/5000 = 75.0%	73.78	76.2
4000/5000 = 80.0%	78.86	81.1
4500/5000 = 90.0%	89.13	90.82
4750/5000 = 95.0%	94.36	95.59
4850/5000 = 97.0%	96.49	97.46
4900/5000 = 98.0%	97.57	98.37
4950/5000 = 99.0%	98.68	99.26
5000/5000 = 100%	99.93	100

Table3.B Exact binomial two-sided 95% confidence intervals for sample size n=2500

Observed proportion expressed as %	Lower CI limit (as %)	Upper CI limit (as %)
0/2500 = 0%	0	0.15
2/2500 = 0.08%	0.01	0.29
6/2500 = 0.24%	0.09	0.52
12/2500 = 0.48%	0.25	0.84
19/2500 = 0.76%	0.46	1.18
25/2500 = 1.0%	0.65	1.47
125/2500 = 5.0%	4.18	5.93
250/2500 = 10.0%	8.85	11.24
625/2500 = 25.0%	23.31	26.75
1250/2500 = 50.0%	48.02	51.98
1875/2500 = 75.0%	73.25	76.69
2000/2500 = 80.0%	78.38	81.55
2250/2500 = 90.0%	88.76	91.15
2375/2500 = 95.0%	94.07	95.82
2425/2500 = 97.0%	96.25	97.63
2450/2500 = 98.0%	97.37	98.51
2475/2500 = 99.0%	98.53	99.35
2500/2500 = 100%	99.85	100

Table3.C Exact binomial two-sided 95% confidence intervals for sample size n=500

Observed proportion expressed as %	Lower CI limit (as %)	Upper CI limit (as %)
0/500 = 0%	0	0.74
1/500 = 0.2%	0.01	1.11
3/500 = 0.6%	0.12	1.74
5/500 = 1.0%	0.33	2.32
25/500 = 5.0%	3.26	7.29
50/500 = 10.0%	7.51	12.97
125/500 = 25.0%	21.26	29.04
250/500 = 50.0%	45.53	54.47
375/500 = 75.0%	70.96	78.74
400/500 = 80.0%	76.22	83.42
425/500 = 85.0%	81.56	88.02
450/500 = 90.0%	87.03	92.49
475/500 = 95.0%	92.71	96.74
485/500 = 97.0%	95.1	98.31
495/500 = 99.0%	97.68	99.67
500/500 = 100%	99.26	100

Table3.D Exact binomial two-sided 95% confidence intervals for sample size n=250

Observed proportion expressed as %	Lower CI limit (as %)	Upper CI limit (as %)
0/250 = 0%	0	1.46
1/250 = 0.4%	0.01	2.21
2/250 = 0.8%	0.1	2.86
3/250 = 1.2%	0.25	3.47
13/250 = 5.2%	2.8	8.73
25/250 = 10.0%	6.58	14.41
63/250 = 25.2%	19.94	31.06
125/250 = 50.0%	43.63	56.37
187/250 = 74.8%	68.94	80.06
200/250 = 80.0%	74.5	84.78
212/250 = 84.8%	79.74	89.01
225/250 = 90.0%	85.59	93.42

Observed proportion expressed as %	Lower CI limit (as %)	Upper CI limit (as %)
237/250 = 94.8%	91.27	97.2
244/250 = 97.6%	94.85	99.11
248/250 = 99.2%	97.14	99.9
250/250 = 100%	98.54	100

Table3.E Exact binomial two-sided 95% confidence intervals for sample size n=100

Observed proportion expressed as %	Lower CI limit (as %)	Upper CI limit (as %)
0/100 = 0%	0	3.62
1/100 = 1.0%	0.03	5.45
2/100 = 2.0%	0.24	7.04
5/100 = 5.0%	1.64	11.28
10/100 = 10.0%	4.9	17.62
25/100 = 25.0%	16.88	34.66
50/100 = 50.0%	39.83	60.17
75/100 = 75.0%	65.34	83.12
80/100 = 80.0%	70.82	87.33
85/100 = 85.0%	76.47	91.35
90/100 = 90.0%	82.38	95.1
95/100 = 95.0%	88.72	98.36
97/100 = 97.0%	91.48	99.38
98/100 = 98.0%	92.96	99.76
99/100 = 99.0%	94.55	99.97
100/100 = 100%	96.38	100

Table3.F Exact binomial two-sided 95% confidence intervals for sample size n=50

Observed proportion expressed as %	Lower CI limit (as %)	Upper CI limit (as %)
0/50 = 0%	0	7.11
1/50 = 1.0%	0.05	10.65
2/50 = 4.0%	0.49	13.71
5/50 = 10.0%	3.33	21.81
12/50 = 24.0%	13.06	38.17
25/50 = 50.0%	35.53	64.47

Observed proportion expressed as %	Lower CI limit (as %)	Upper CI limit (as %)
37/50 = 74.0%	59.66	85.37
40/50 = 80.0%	66.28	89.97
43/50 = 86.0%	73.26	94.18
45/50 = 90.0%	78.19	96.67
48/50 = 96.0%	86.29	99.51
49/50 = 98.0%	89.35	99.95
50/50 = 100%	92.89	100

Table3.G Exact binomial two-sided 95% confidence intervals for sample size n=30

Observed proportion expressed as %	Lower CI limit (as %)	Upper CI limit (as %)
0/30 = 0%	0	11.57
1/30 = 3.3%	0.08	17.22
2/30 = 6.7%	0.82	22.07
3/30 = 10.0%	2.11	26.53
8/30 = 26.7%	12.28	45.89
15/30 = 50.0%	31.3	68.7
23/30 = 76.7%	57.72	90.07
24/30 = 80.0%	61.43	92.29
26/30 = 86.7%	69.28	96.24
27/30 = 90.0%	73.47	97.89
29/30 = 96.7%	82.78	99.92
30/30 = 100%	88.43	100

Table3.H Exact binomial two-sided 95% confidence intervals for sample size n=15

Observed proportion expressed as %	Lower CI limit (as %)	Upper CI limit (as %)
0/15 = 0%	0	21.8
1/15 = 6.7%	0.17	31.95
2/15 = 13.0%	1.66	40.46
3/15 = 20.0%	4.33	48.09
6/15 = 40.0%	16.34	67.71
9/15 = 60.0%	32.29	83.66
12/15 = 80.0%	51.91	95.67
13/15 = 86.7%	59.54	98.34

Observed proportion expressed as %	Lower CI limit (as %)	Upper CI limit (as %)
14/15 = 93.3%	68.05	99.83
15/15 = 99.0%	78.2	100

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Molecular Analysis for Therapy Choice (MATCH)

Appendix XXII

CS-MATCH-0020

Rev. Add25

Discovery of Driver Fusions in MOI-Negative NCI-MATCH Screening Samples

PRINCIPAL INVESTIGATOR: John Iafrate, MD PhD
BIOSTATISTICS: Lisa McShane, PhD
CO-INVESTIGATORS: Chris Karlovich, PhD
Mickey Williams, PhD
David Sims, PhD
Jeffrey Sklar, MD PhD

1. INTRODUCTION

Anchored Multiplex PCR is a method developed in the MGH Molecular Diagnostics Laboratory to allow unbiased fusion detection when only one partner is known. The most recent version of AMP at MGH termed the Solid Fusion Assay (SFA) interrogates over 100 known fusion partners using RNA as input.

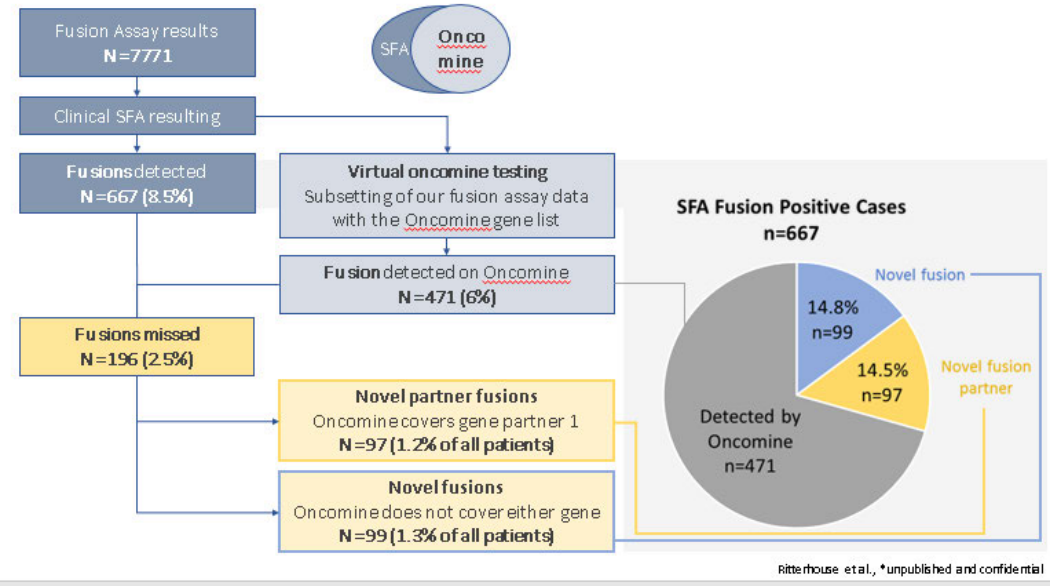

Figure 1.

Using this assay, 7,771 clinical advanced cancer samples have been genotyped and fusions were detected in 667 or 8.5% (Figure 1). The majority of the fusions were mutually exclusive of other driver mutations. By cross-referencing the identified fusions with the Oncomine primer designs we calculate that Oncomine would have missed 30% of these fusions, including completely novel fusions, or a novel fusion partner. This strongly suggests that we would detect 30% more fusions in the NCI-MATCH cohort.

Recent data from Memorial Sloan Kettering Cancer Center has revealed that their FDA-approved MSK-Impact assay also failed to detect gene fusions. By using AMP in their driver-negative NSCLC cases they found 15% harbored gene fusions, many of which were actionable (1). This suggests that focusing on the MOI-negative subset of the NCI-MATCH screened population will provide the highest likelihood of identifying novel fusion driver cases missed by Oncomine.

2. OBJECTIVES AND HYPOTHESES

We hypothesize that gene fusions may be more prevalent as driver events in the NCI-MATCH screened population than detected by Oncomine.

The primary objective is to analyze the MOI-negative tumors from the screening cohort of 5,540 patients for driver gene fusion events using Anchored Multiplex PCR. Driver gene fusions are defined as in-frame fusions with at least 50 supporting reads, where at least one of the genes is a known fusion partner. We will identify the prevalence of fusions in the overall cohort, and in individual diseases where fusions may be particularly important (e.g. FGFR2 fusions in cholangiocarcinoma).

3. RESEARCH DESIGN AND METHODS

1. Patient and Biospecimen Selection
   - Residual banked RNA from tissue utilized for screening from patients screened by the MATCH-assay.
   - No markers of interest were identified by the Oncomime MATCH-assay assessment.
   - Patient were not enrolled to NCI-MATCH treatment arms.
   - Patients consented to allow their specimens to be used for research.
2. Laboratory methods

   The Solid Fusion Assay is a CLIA Next Generation Sequencing- based methodology for the clinical detection of gene fusions using a laboratory-developed test version of Anchored Multiplex PCR (2). Over 10,000 samples have been analyzed in the MGH clinical lab with this assay. The assay first involves conversion of tumor RNA to cDNA using random priming, following by end repair and sequencing adapter ligation. Two rounds of nested-PCR occurs next, with one primer anchored in the gene target of interest and the second primer pair annealing to sequences in the adapter. The Illumina NextSeq is used for sequencing the libraries.

   Validation Methods

   We validated the ability of the Fusion assay to detect translocations in 72 cases with known fusion status. Findings were confirmed by FISH using the established protocols. Samples were interpreted as positive or negative for translocations according to the analysis guidelines in the Clinical Fusion Assay SOP and the Clinical Fusion Bioinformatics Analysis Pipeline. Greater than 90% concordance was expected between the established FISH protocol and the AMP Fusion protocol.

   Validation Results

   71 out of 72 samples have concordant results between FISH and sequencing assays. Concordance is measured at 98% and is considered acceptable.

   The following criteria are used to evaluate Fusion sequencing results:

   a. For known, non-novel fusions to be considered positive: ≥ 50 uniquely mapped positions on potential partners

   b. For novel fusions to be considered positive: ≥ 50 uniquely mapping positions on potential partners with in-frame fusion

   Sensitivity and Specificity

   Based on the Fusion and FISH results, sensitivity and specificity were calculated as:

   Sensitivity = 96% Specificity = 100%

   The lowest % tumor content of positive case for which a translocation was detected is 10%. For formal analytical sensitivity assessment, three translocation-positive cases (determined by FISH) were serially diluted with

a translocation-negative lung FFPE total nucleic acid (TNA) pool in order to obtain solutions between 50% and 3.125% (ALK and RET) and between 60% and 3.75% (ROS1).

The lowest level of analytical sensitivity at which translocations could be detected was determined by the original tumor percentage divided by the highest dilution at which a positive result was detected and was 3%. All MATCH samples will have a percentage substantially higher than this number since the cellularity is above 60% in the vast majority of samples.

Precision

Three negative and three positive clinical samples were assayed in triplicate (intra-assay) and three separate runs (inter-assay) to establish precision and reproducibility, 100% for both.

QC

We rely on two quality metrics to determine the quality of results for any given sample tested with our SFA Fusion assay. First, we have extensive experience with using a commercial qualitative reverse transcription real-time PCR assay which amplifies the GAPDH gene after the second strand synthesis step of the protocol. We have found that for the SFA Fusion assay, CT values below 28 tend to produce good quality sequencing results to confidently pass a case. Values between 28 and 30 tend to be borderline for quality. Samples with CT values greater than 30 tend to fail. Passing a case for signout requires that the sample first pass this real-time PCR check.

In addition to the GAPDH check, we also review the coverage from the sequencing data to have confidence in reporting our test results. Our extensive clinical experience with our targeted RNA-Seq has shown that it is difficult to maintain sequence coverage consistency for any one of the genes in our panel, particularly at the RNA (cDNA) level. There is great variability in tumors, tissue types, normal cell contamination in the samples that we test, and sequencing throughput in our assay, which may lead to unpredictable expression levels for any of the targeted genes. Because of this great variability, we rely on a global RNA abundance measure from the sequence analysis to qualify the performance of a sample. This measure is based on alignment annotation to determine whether a read is RNA or DNA, and also the percentage of split-reads (highly likely to be RNA). Our current quality metric thresholds for good, passable sequencing results are: $\geq$30% split reads, $\geq$100,000 uniquely mapped reads, and $\geq$1.4 RNA:DNA ratio.

QA

The lab actively participates in proficiency testing, including CAP surveys, with no failed surveys since the SFA assay has been launched. The assay has received New York State approval and the Lab is accredited and regularly inspected by the Joint Commission. The MGH Laboratory will perform a pilot to ensure that the Yale Laboratory meets quality standards for running this assay prior to testing samples for this project.

4. DATA ANALYSIS AND STATISTICAL CONSIDERATONS

1. Primary analysis:

   We will compute the number and proportion of all cases analyzed by the SFA that harbor driver gene fusion events. The proportion will be accompanied by an exact binomial 2-sided 95% confidence interval (CI).

2. Secondary analyses:

   Secondary analyses will be largely descriptive. We will compute the distribution of number and proportion of driver gene fusion events by tumor histology and by patient age at time of screening for the NCI-MATCH trial. It is expected that tumors of certain histologies such as cholangiocarcinoma and sarcoma, and from younger patients, might have a higher likelihood of harboring driver gene fusion events. For each of the histologic subgroups represented in the case set, we will also compute exact binomial 2-sided 95% CIs for the proportion positive for driver gene fusion events unless the number of cases in the subgroup is too small for sensible CI calculation. Similarly, we will report numbers, proportions, and CIs for the AYA patients (age < 40 yrs) and older adults (age $\geq$ 40 yrs). A Fisher's exact test comparing proportion of cases positive for driver gene fusion events between AYA patients and older adults will be conducted and p-value reported. Given the potentially large number of histologic subtypes, formal statistical testing will not be attempted to assess differences in proportion of driver gene fusion events across histologies. Given the exploratory nature of these secondary analyses, p-values and confidence levels (for CIs) will not be adjusted for multiple testing.

3. Sample size estimate:

   Given that the number of cases with driver fusion events is expected to be fairly low, we are requesting RNA from all cases from the NCI-MATCH screening cohort for which no MOIs were identified and for which sufficient amount of RNA is available. According to information available to us, there are 849 cases from the NCI-MATCH screening cohort for which no MOIs were identified. We anticipate that approximately 15% of these cases will have insufficient RNA available for assay. Further, we will conservatively assume that up to 5% of assays will not successfully yield results (although in truth we expect that the assay failure rate will be much lower than 5%). Therefore, we will assume that we will have assay results for 685 cases, which we will round down to n=650 for purposes of sample size justification.

   For subset analyses in cholangiocarcinoma and sarcoma, there are 41 and 44 cases, respectively, among the cases from the NCI-MATCH screening cohort for which no MOIs were identified.

4. Rationale for the sample size estimate:

   Sample size justification is based on the width of the exact binomial 2-sided 95% confidence interval for the proportion of all cases with assay results that harbor driver gene fusion events. Example calculations are shown in Table 1. Based on the MGH and MSK data cited in above, we anticipate that the true proportions, for the full cohort and for the cholangiocarcinoma and sarcoma subgroups, could lie between 5% and 20%. Table 1 below provides confidence intervals based on observed proportions in a similar range and for sample sizes of 650 (full cohort)

and 30 (cholangiocarcinoma and sarcoma subgroups). In addition, we note that the probability of observing at least one case with a driver gene fusion event is at least 95% when the true proportion is 0.005 in the full cohort of 650 cases and 0.095 in each of the cholangiocarcinoma and sarcoma subgroups (assuming 30 cases in each).

Table 1. Example exact binomial 2-sided 95% confidence intervals for an observed proportion

N=650 (Full cohort)		
Observed proportion positive for driver gene fusion event	Lower limit of exact binomial 2-sided 95% CI	Upper limit of exact binomial 2-sided 95% CI
32/650 = 0.049	0.034	0.069
65/650 = 0.100	0.078	0.126
97/650 = 0.149	0.123	0.179
130/650 = 0.200	0.170	0.233
N=30 (Histology subgroup)		
Observed proportion positive for driver gene fusion event	Lower limit of exact binomial 2-sided 95% CI	Upper limit of exact binomial 2-sided 95% CI
1/30 = 0.033	0.001	0.172
2/30 = 0.067	0.008	0.221
3/30 = 0.100	0.021	0.265
4/30 = 0.133	0.038	0.307
5/30 = 0.167	0.056	0.347
6/30 = 0.200	0.077	0.386

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