Specimen Collection and Processing by Collection Site and Biorepository for CIMAC Studies

VERSION 7: MAY 16, 2019

1 SCOPE

The purpose of this Standard Operating Procedure (SOP) is to establish a consistent process for the sites and Biorepositories involved in CIMAC studies to collect and process tissue and blood samples for immune monitoring and profiling analyses to be performed by the CIMACs. This SOP defines ***the options to be selected for each correlative study protocol*** for collection schema, handling, processing, and freezing protocols of ***tissue, bone marrow aspirate, stool, plasma, and PBMCs.*** Protocol drafting suggestions are indicated as [xx].

2 SUMMARY OF SAMPLE COLLECTION AND PROCESSING

Table 1: Summary of Collection and Processing Activities Intended for Tier 1 and Tier 2 Assays

		Section 3	Section 4		Section 5
Specimen Type	**Timepoints***	**Collection and Processing at Site**	**Immediate Processing at Biobank**	**Processing for Distribution at Biobank****	**Intended Assay Use at CIMAC**
De Novo Core Needle Biopsy **OR** **Endoscopic/ Punch Biopsy** **OR** **De Novo Surgical Resection**	One or more	1-2 cores OR 1 segment Each protocol will select an option: • Fix and embed on-site%; • OR Fix in formalin and ship in EtOH	Embed fixed tissue Store blocks	• 5-15 Unstained slides + H&E (Imaging Assays) • DNA / RNA	**<u>FFPE Samples</u>** • IF • IHC • MIBI* • WES • RNA-Seq • TCR-Seq **<u>Fresh Frozen Samples</u>** • WES • RNA-Seq • TCR-Seq
		1-2 cores flash frozen^ OR 1 Segment flash frozen^	Store frozen	DNA / RNA	
Archival FFPE Material	Typically, one	Ship FFPE blocks OR • 15 unstained slides • Core punches, OR • 4/5 µm Scrolls	Store blocks OR • Vacuum-seal slides • Refrigerate punches + scrolls	• 5-15 Unstained slides + H&E (Imaging Assays) • DNA / RNA	

		Section 3	Section 4		Section 5
Specimen Type	**Timepoints***	**Collection and Processing at Site**	**Immediate Processing at Biobank**	**Processing for Distribution at Biobank****	**Intended Assay Use at CIMAC**
Sodium Heparin Green-Top Tubes	Multiple	30 mL Draw (Ship ambient)	Isolate plasma and PBMCs Freeze aliquots	Ship plasma or PBMC aliquots	Plasma (Olink and ELISA#) PBMCs (CyTOF, TCR-Seq)
Streck Cell-Free DNA Tubes	Multiple	10 mL Draw (Ship ambient)	Isolate plasma and Freeze aliquots	Ship plasma aliquots	Plasma (cfDNA)
EDTA Purple-Top Tubes	Baseline only for germline; Multiple	2 mL Draw (Ship ambient)	Freeze 0.5 mL aliquots	Ship whole blood aliquots	Germline DNA TCR-Seq RNA-Seq
Bone Marrow Aspirates	Multiple	• Liquid aspirate in blood tube Solid Aspirate in blood tube (FFPE)	• Process + aliquot liquid aspirates • Fix + embed solid aspirate • Store blocks	• Ship Aliquots • FFPE blocks • 5-15 Unstained slides + H&E (Imaging Assays) DNA / RNA	CyTOF Olink IF IHC MIBI* RNA-Seq
Stool Samples	Multiple	Self-collection (Ship ambient or frozen depending on kit)	Homogenize$ and Freeze aliquots	Ship frozen aliquots	16S rRNA Gene Amplicon Seq# Microbe Characterization#
Sample Data	All	Collection, processing, and shipping times Core number Path reports	Processing and storage details	• Path Review • Sample QC • Thawing and shipment details	All

* Detailed description of Timepoints will be given in the Specimen Collection table in each Protocol.
**Directions for distributing sample derivatives to CIMAC labs will be provided at a later time.
^ Flash-frozen are preferred over FFPE for genomic assays.
# Not a Tier-One assay at this time: Tier 1 assays are those broadly recommended for most trials collaborating with CIMAC.
% FFPE blocks will be fixed and embedded onsite for NCTN protocols.
$ Homogenize samples when appropriate

2.1.1 Biomarker Plan Suggestions

- Clearly indicate the specimen type and timepoint for each assay.
- An assay can be requested for multiple specimen types if low amount of biological material is anticipated (e.g. TCR-seq on blood could be prioritized for Heparin PBMCs but also indicated as alternative for EDTA whole blood).
- Blood timepoints should match when tissue is acquired with additional on-treatment and follow-up timepoints included. Blood collection beyond 6 months on-treatment should be considered judiciously to minimize total number of blood samples collected.
- If archival slides are requested, please indicate # of slides, section thickness, and tumor % required for genomic analysis.

3 COLLECTION SITE ACTIVITIES

3.1 Tissue Collection and Processing at Collection Site

3.1.1 Pre-Analytic Information

Collection site must record all preanalytical information **[Appendix VI]** and enter the following into a specimen tracking system (STS) used by each trial network or record and provide with shipping manifest:

- Ischemia start time (time when sample was devascularized OR estimated time of surgery)—***Tissue Collection Time/Date.***
- Ischemic end time ***for each tissue core and surgical segment*** (time when sample was moved to preservative such as formalin or dry ice)—***Tissue Processing (Formalin Start) Time/Date.***
- Completion of formalin fixation should be recorded as ***Formalin End Time/Date*** in the STS (or under "comments" if field is not available).
- Start of 70% Ethanol dehydration should be recorded as ***Ethanol Start Time/Date*** in STS (or under "comments" if field is not available)
- Completion of 70% Ethanol dehydration should be recorded as ***Ethanol End Time/Date*** in the STS (or under "comments" if field is not available).
- Core # for each core needle biopsy obtained. Each core should be recorded in the STS as a separate specimen with a unique Specimen ID that captures the chronological order in which the biopsy cores were obtained.
- Segment # for each surgical resection. These can be hand-labeled on the sample and ***captured electronically as separate specimens in the STS***.

3.1.2 Sample Labeling Recommendations

- [Tissue sample labeling procedures standard for each trial group will be followed].

3.1.3 Tissue Collection

NOTE: Cold ischemia time should be minimized as much as possible, optimally less than 20 min for formalin-fixed samples and <2 minutes for flash-frozen specimens (or as indicated by each study protocol). Ischemia time stamp should be documented for every tissue core, module or segment.

Core Needle Biopsy Tissue: for most trials, core needle biopsies will be collected using a 16-18-gauge needle (condition permitting), at [time points of collection].

- At least 4 cores (1 cm in length) should be obtained for CIMAC analysis. [Additional cores may be obtained if specified by study Intake Form]
- Alternating passes: First obtain a core for FFPE processing (core 1), followed by a core for flash freezing (core 2), followed by a core for FFPE (core 3), followed by a core for flash freezing (core 4). [Number of FFPE vs. frozen samples may vary for each correlative study based on assays requested]
- [Flash frozen cores are preferred for genomics assays, however FFPE blocks are acceptable]
- The number of specimens obtained will be affected by the patient's clinical condition at the time of biopsy and determined by the specialist performing the procedure.
- Each research sample must be placed in a prelabeled cassette dedicated to each study. Up to two cassettes may be used per jar.
- Record the core number for each core needle biopsy sample on the sample label.

Surgical Resection Tissue: for some trials, surgical resection will be obtained at [time points of collection]. From this resected tissue, harvest a part of the tumor measuring approximately 1x1x1 cm, avoiding necrotic areas, and divide this tissue ***into two almost equal segments***.

- One piece will be processed as an FFPE sample and the other as a flash frozen sample [refer to ***Section 3.1.4***].
- For some clinical trials, more than two segments may be obtained as described by the protocol.

Endoscopic/Punch Biopsy Tissue: for some trials, endoscopic or punch biopsies may be obtained at [time points of collection].

- Endoscopic/punch biopsy of at least 3 mm diameter should be obtained for CIMAC analysis.
- Endoscopic/punch biopsies should be processed as FFPE blocks or Freshly Frozen. [depending on need identified by the Correlative Study Intake Form (refer to ***Section 3.1.4)***]

Bone Marrow Aspirates: for some trials, bone marrow aspirates may be obtained at [time points of collection].

- [Number of draws and needle pulls will be selected for each trial].
- [Draw volumes and container types will be selected for each trial].
- [Bone marrow aspirates will be shipped to Biorepository for processing and embedding].

NOTE: Fine needle aspirations (FNAs) are <u>not an acceptable</u> replacement for tissue cores intended for CIMAC assays. However, a special request can be made for consideration to supplement frozen tissue with FNA material for genomics and flow cytometry-based assays (not for IHC).

3.1.4 Tissue Processing

Formalin Fixation of Tissue Samples

- ***The preferred method is to fix and embed the tissue in paraffin at the collection site <u>if all requirements can be followed</u>.*** If FFPE samples cannot be processed on-site as described, the clinical site should formalin-fix tissues as described below, and then transfer to in 70% Ethanol to send to the Biorepository for embedding.
- Neutral-buffered formalin ***must be used*** as fixative (no acid-based products).
- [Tissue will be embedded at the collection site for hybrid study protocols that indicate use of ETCTN samples for testing at the MoCha lab].
- [Ideally, each study should choose and implement ***one processing option to all samples*** if possible, otherwise allow collection sites to choose based on their clinical workflow].

Fixation Options

One of the following options should be selected by each protocol:

Option #1: Embedding Tissue at Collection Site [*required for NCTN*]:

- Samples must be fixed in formalin for ***12-24 hours*** and embedded directly at the collection site. Embedding must be completed ***within 72 hours*** of adding 70% ethanol to tissue.
- Sites must use automated tissue processors and ***not use*** microwave tissue processors.
- Sites should follow embedding protocols where the total processing time from 70% ethanol to block embedding ***exceeds 4 hours.*** [protocol should include table from ***Appendix I***]

Option #2: Shipping Formalin-Fixed Tissue to Biorepository in Ethanol: [not permitted for NCTN]

- Alternatively, samples can be fixed in formalin for a ***minimum of 12 hours but no more than 24 hours*** before being transferred to 70% ethanol. Tissue can be shipped in ethanol to the biorepository; processing and embedding must be completed ***within 72 hours*** of adding the fixed tissue to ethanol.

Tissue samples fixed in formalin for 24-36 hours ***will be collected and shipped*** but will be recorded as non-compliant by CIMAC labs based on the preanalytical data collected.

Flash Freezing of Core Needle Biopsy and Surgical Resection Samples

Surgical Resections

- Samples should be dissected soon after the specimen is released by the supervising physician and each module or segment should be placed in a separate prelabeled cryovial.
- Prefer a minimum of 25 mg (1x1x1 cm).

Core Needle Biopsies

- Each core sample should be placed directly into a separate prelabeled cryovial.

Flash Freezing on Dry Ice

- Each specimen contained in its cryovial should be flash frozen using a dry ice/alcohol slurry (freezing in liquid nitrogen is an acceptable alternative).
- Frozen specimens should be shipped (the day of collection) Priority Overnight on dry ice in an insulated shipper or a dual temperature-chambered kit.
- [For some correlative studies, flash-frozen samples will be shipped from the collection site directly to the assay lab (shipping directions will be specified by the clinical trial protocol)].

3.1.5 Archival FFPE Tissue

Even when patients are able to provide a biopsy/resection specimen, a prior (archived) representative tumor tissue block may be requested. If previously-collected FFPE will be submitted, then the following criteria must be met:

- [It is recommended that blocks be submitted for NCTN bank trials; otherwise unstained slides will be requested].
- Tissue should ideally have been collected within 6 months prior to registration. [Older archival material will be considered on a case-by-case basis]
- A copy of the original pathology report must be provided, and the tissue collection date must be recorded so the sample age can be derived.
- Formalin-fixed paraffin-embedded tumor tissue block(s) ***must be submitted or used to provide the specimens listed below.*** Preferred specimen requirement is as follows:
    - Block should contain at least 30% tumor, however less tumor content is acceptable for imaging studies.
    - Material requested ***exclusively for genomics*** should ideally contain at least 70% tumor although less is acceptable.
    - Surface area: 25 mm^2 is optimal. Minimum is 5 mm^2.
    - Volume: 1 mm^3 optimal. Minimum volume is 0.2 mm^3.

If the archival block cannot be submitted, the following can be provided [based on study need]:

- Two (first and last cuts in a series) sectioned H&E slides (minimum of one is required),
- Fifteen to twenty [or other number specified by each correlative study] 4 µm unstained air-dried (unbaked) plus slides, ***OR***;
- One (1) or more core punches (minimum of 1-2 mm diameter) from tumor block placed into a clean vial, ***OR***;

- **For nucleic acid extraction only**: Three to five 10µm FFPE scrolls or six to ten 4µm FFPE scrolls cut from blocks and placed into a clean vial. [number and thickness will depend on tumor size and sample need for each correlative study]

3.1.6 Tissue Shipment from Collection Site to Biorepository

Do not send samples the day before a national holiday or on Friday (unless Biorepository is able to process on Saturdays). ***FedEx Priority Overnight is mandatory for all samples***.

- An external sample label should be fixed to the shipping container to alert the Biorepository of ***Formalin-fixed*** sample ***time*** and ***date*** it was placed into ***Ethanol*** (this helps to identify and prioritize received samples that have processing time requirements—Option #2).
- [Archival material does not need to be shipped on the day of collection].
- [The Biorepository will provide sample kits based on contents selected in Table 2 OR what has been selected for the clinical trial].
- [The collection site must refer to each clinical trial protocol for all shipping addresses].

Table 2. Shipping Conditions for Tissue Samples

Tissue Sample	Collection Kit Contents	Shipping Schedule *	Shipment Conditions
Option 1 (select option 1 or 2 for a given protocol) **FFPE blocks, slides, core punches, or scrolls**	No kit provided, unless FFPE is submitted in Dual Chambered Kit with frozen materials	Monday through Thursday (FedEx Priority Overnight)	Ambient, include a gel-pack or cold-pack (NOT a frozen pack) on hot days and insulation on cold days
Option 2 (select option 1 or 2 for a given protocol) **Tissue fixed in formalin and shipped in 70% ethanol**	Formalin-prefilled jars and cassettes, Single or Dual Chambered Kit, depending on protocol-specific details	Fixed in formalin on site for ***12-24 hours*** and placed in ethanol for shipment to biorepository for embedding within 72 hours of Ethanol Tissue collected Monday through Thursday and shipped in ethanol (after fixation) overnight (FedEx Priority Overnight)	Ambient, include a gel-pack or cold-pack (NOT a frozen pack) on hot days and insulation on cold days
Snap-frozen specimens	Single or Dual Chambered Kit depending on protocol-specific details	Monday through Thursday (FedEx Priority Overnight)	Frozen, on dry ice
Bone Marrow Aspirates	Vacutainer or specialized tubes may be provided	Monday through Thursday (FedEx Priority Overnight)	To be selected

*For samples shipped late in the week, collection sites will work with the Biorepository to determine the most optimal sample processing conditions.

3.2 Blood Collection and Processing at Collection Site

3.2.1 Time Points of Collection

Blood will be collected at [timepoints of collection]. [Total volume may be less for pediatric trials based on maximal draw limits put in place by individual protocols]

3.2.2 Sodium Heparin Green-Top Tubes (30 mL Total Draw per timepoint)

- Label Sodium Heparin Green-Top Tubes (Vacutainer®), Becton Dickinson Cat No. 367874 (or equivalent). [***at minimum*** a generated patient ID, specimen ID, specimen type (blood), draw time and collection date]
- Collect a total of ***30 mL*** of peripheral blood in Sodium Heparin Green-Top Tubes (use 5- or 10-mL tubes). [Total draw volume may be adjusted according to study need]
- After collection, gently invert tube(s) 8-10 times to ensure adequate mixing of sodium heparin. Maintain specimens at ambient temperature (room temperature) during collection and transport.

3.2.3 Streck Cell-Free DNA Tubes (10 mL)

- Label one 10 mL Streck cfDNA BCT (Streck catalog # 218961, 218962, or 218992). [***at minimum*** a generated patient ID, specimen ID, specimen type (blood), draw time and collection date]
- Collect ***10 mL*** of blood into the pre-labeled tube and invert to mix. ***Note: Blood must be thoroughly mixed to ensure preservation of specimen.***
- After collection, blood in cfDNA Streck BCT should ***never be refrigerated***, as this will compromise the specimen. Blood collected in cfDNA Streck Tubes is stable at room temperature.

3.2.4 EDTA Purple-Top Vacutainer Tubes (2 mL)

- One tube should be collected at baseline for germline analysis if WES is requested.
- Label each EDTA Purple-Top Tube. [***at minimum*** a generated patient ID, specimen ID, specimen type (blood), draw time and collection date]
- Collect 2 mL of peripheral blood in EDTA Purple-Top Tube. [Additional blood may be collected based on need]
- After collection, gently invert tube(s) 8-10 times to ensure adequate mixing of EDTA. Maintain specimens at ambient temperature (room temperature) during collection and transport.

3.2.5 Whole Blood Shipment from Collection Site to Biorepository

Do not send samples the day before a national holiday or on Friday (unless Biorepository is able to process on Saturdays). ***FedEx Priority Overnight is mandatory for all samples***.

- An external sample label should be fixed to the shipping container to alert the Biorepository of ***blood*** sample collection ***time*** and ***date*** (this helps to identify and prioritize received samples that have processing time requirements).
- Blood should be shipped ambient FedEx Priority Overnight to the biorepository where it is processed the day of receipt within 24 hours of collection.
- Sodium Heparin blood samples should ***not be shipped*** if they cannot be processed by the Biorepository within 48 hours of collection.
- [The Biorepository will provide sample kits based on contents selected in Table 3 OR what has been selected for the clinical trial].
- [The collection site must refer to each clinical trial protocol for all shipping addresses].

Table 3. Shipping Conditions for Blood Samples

Blood Sample	Collection Kit Contents	Shipping Schedule	Shipment Conditions
Blood in Sodium Heparin Green-Top Tubes	Ambient shipper	Day of Collection (Samples collected and shipped ***together*** Monday through Thursday*; FedEx Priority Overnight)	Ambient
Blood in Streck Cell-Free DNA Tubes	Streck tubes provided with ambient shipper		
Blood in EDTA Purple-Top Tubes	Ambient shipper		

* Blood samples may be shipped Friday to Biorepositories (ETCTN, COG, NRG BB-Columbus, and SWOG) which are open and able to process samples on Saturdays.

3.3 Stool Collection and Processing at Collection Site

3.3.1 Stool Samples

Partial stool samples will be collected at [time points] using provided self-collection kits and written instructions. Clinical site staff will explain to patients how to use the kits at the clinic or in the privacy of their home.

- Stool collected at the baseline timepoint will employ a Cold Chain collection method (ALPCO Diagnostics; EasySampler Stool Collection Kit; #58EZSampler—shipped frozen).
- Baseline and subsequent timepoints will use OMNIgene GUT kits (OMR-200.100—shipped ambient) which include a DNA stabilizing solution.
- Collection kits will include directions and a Sample Collection Form to be completed by the patient/collection site to record selected pre-analytical details.
- Collection kits will contain a Bristol Stool Scale Form to be completed by patients to classify their sample.

3.3.2 Stool Sample Shipment from Collection Site to Biorepository or CIMAC lab

- Study participants will collect and return stool samples to the clinical site which will ship each specimen to the Biorepository or CIMAC lab where it will be homogenized, aliquoted, and stored frozen for distribution.
- It is recommended that patients return collection kits within 24 hours and the clinical site ships samples within 72 hours of sample collection.
- [The collection site must refer to each clinical trial protocol for all shipping addresses].

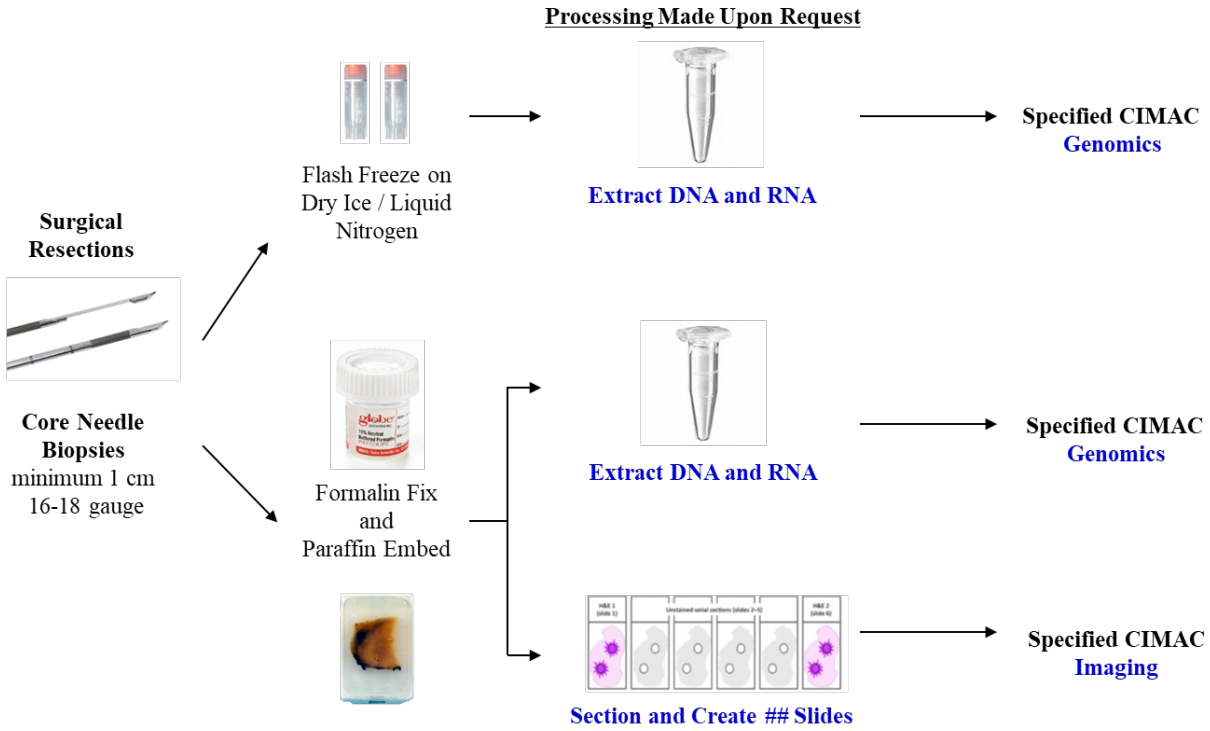
Table 4. Shipping Conditions for Stool Samples

Sample	Collection Kit Contents	Shipping Schedule	Shipment Conditions
Stool Samples	Collection container and bags, collection aids, DNA stabilizing solution, Bristol Stool Scale and collection forms, Instructions	Samples should be shipped Monday through Thursday only (FedEx Priority Overnight)	Frozen for Cold Chain Ambient for OMNIgene GUT

4 BIOREPOSITORY ACTIVITIES

4.1 Tissue Processing by Biorepository

Overall Tissue Processing Schema



4.1.1 Pre-Analytic Information

Biorepository will collect the following information for received specimens ***[Appendix VI]***:

- Time/date of sample receipt.
- Time/date blood processing was initiated.
- Time/date formalin-fixed tissue in Ethanol is moved into an automated processor—***recorded as Ethanol End Time.***
- Record if frozen tissue sample arrived with insufficient amount of dry ice.
- Collected pre-analytic information will be entered into the shipping manifest (NCI specimen tracking system).

4.1.2 Sample Labeling Recommendations

- Upon CIMAC sample request, Biorepository will work with NCI to generate CIMAC Network IDs for patients and their sample derivatives.
- Label samples ***to be banked*** with thermostable labels typically used by the Biorepository.
- [Each Biorepository may use their own labeling sample schema until a time when the CIMAC network provides custom-labeling instructions].
- Label sample derivatives (slides, extracted DNA etc.) ***to be shipped*** with a thermostable CIMAC network label.

4.1.3 Collection of Clinical Reports

Collect all relevant clinical pathology reports ***for each sample time point*** and upload to study data management system [or a copy of report sent with the sample for some NCTN groups]:

- [ETCTN: collect pathology verification forms and Path reports/Procedural forms].
- [NCTN: all standard-of-care pathology reports, pathology verification forms and procedural reports (for some NCTN groups)].
- [NRG: path reports may need to be obtained a Data Center for some sites (Pittsburgh)].
- [Research biopsies: collect procedural reports].
- [Archival samples: collect original diagnostic pathology reports].

4.1.4 Quality Control Activities (QC) by the Biorepository

Before distributing samples to CIMACs, the Biorepository will perform the following:

- A sample assessment may be requested by CIMAC to determine how many cases and specimens are available for each assay.
- Histology preparation such as H&E staining and mounting unstained whole sections for immunohistochemistry and immunofluorescence.
- ***Histology concordance confirmation and percent viable tumor evaluation*** of tissues. [Include ***Appendix V***]
- Quality assessment of extracted DNA and RNA (to ensure sufficient amount and quality of material is shipped to CIMAC labs for testing). ***For small biopsies with low nucleic acid content, please contact CIMAC lab if quantity or quality is sufficient.***
- ***Note the condition*** **of blood samples** for processing [refer to ***Appendix III and IV:*** Plasma Isolation sections].

BIOREPOSITORY WILL RECEIVE ONLY INFORMATION FOR IMMEDIATE PROCESSING AND LONG-TERM STORAGE OF CIMAC SPECIMENS. ADDITIONAL INFORMATION TO THAW, ALIQUOT AND DISTRIBUTE SAMPLE DERIVATIVES WILL BE ADDED AT A LATER TIME.

4.1.5 Formalin-Fixed Tissue Samples Arriving in Ethanol

Upon receiving a formalin-fixed sample shipped in ethanol, the Biorepository will process and embed each sample in paraffin to create separate formalin-fixed paraffin-embedded (FFPE) block(s):

- [Include ***Appendix I***, "Processing and Paraffin Embedding of Tissue" for details].
- For tissue arriving in 70% ethanol: Processing should occur within **72 hours** of the specimen having been placed in ethanol, otherwise record as non-compliant in STS.

4.1.6 Frozen Tissue

Frozen tissue specimens received from the collection site should be stored in liquid nitrogen vapor phase ***until a request*** for sample processing is made by CIMAC:

- [The clinical trial protocol should specify that DNA/RNA will be co-extracted by the Biorepository unless some other practice has been approved by NCI].
- **DNA/RNA will be co-extracted by the Biorepository for genomics assays** [refer to ***Appendix II*** for SOPs]

4.1.7 FFPE Tissue

FFPE blocks received from the collection site or blocks embedded by the Biorepository should be stored at room temperature ***until a request*** for sample processing is made by CIMAC:

- A preliminary H&E slide may be requested, as part of the initial sample assessment, and sent to the lead CIMAC for imaging to determine how much tissue material will be required based on TIL content and percentage of viable tumor.
- [The protocol should specify that DNA/RNA will be co-extracted by the Biorepository unless some other practice has been approved by NCI].
- **DNA/RNA will be co-extracted by the Biorepository for genomics assays** [refer to ***Appendix II*** for SOPs]
- **H&E slides:** create at least one H&E slide per block (preferably two; first and last cut in a series of multiple sections taken for unstained slides).
- **Unstained air-dried plus slides for imaging only:** cut 5-15 [or number requested by each correlative study] tissue sections of 4-5 [specify] microns per case, using a microtome, and mount on "plus" (charged) glass slides.
- **FFPE scrolls:** Cut fresh scrolls (4 µm or 10 µm thickness) for nucleic acid extraction to be performed by Biorepository.

4.1.8 Archival Tissue

Upon receiving FFPE blocks, slides, scrolls, or core punches, from the collection site, the Biorepository should perform the following until a request for shipment is made:

- Store each FFPE block at room temperature.
- Vacuum seal unstained slides and store refrigerated, otherwise distribute to CIMAC labs.
- FFPE core punches and scrolls should be stored refrigerated.

4.1.9 Bone Marrow Aspirates

Upon receiving bone marrow aspirates from the collection site, the Biorepository should:

- Fix and embed (FFPE) the solid tissue component of the bone marrow aspirate according to Biorepository practices and store at room temperature.
- Process the liquid component of the bone marrow aspirate according to Biorepository practices and store an appropriate number of frozen aliquots. [determined by study need]
- [Cellular fractions (plasma and PBMC phases) may be requested according to study need].

When a request for bone marrow material is made, the Biorepository will provide:

- Frozen aliquots of bone marrow cell fractions or FFPE blocks.
- **Unstained air-dried plus slides for imaging only:** cut from each FFPE block [number requested] tissue sections of 4-5 [specify] microns and mount on charged glass slides.
- DNA/RNA co-extracted from FFPE material or aspirate aliquots for genomics assays.

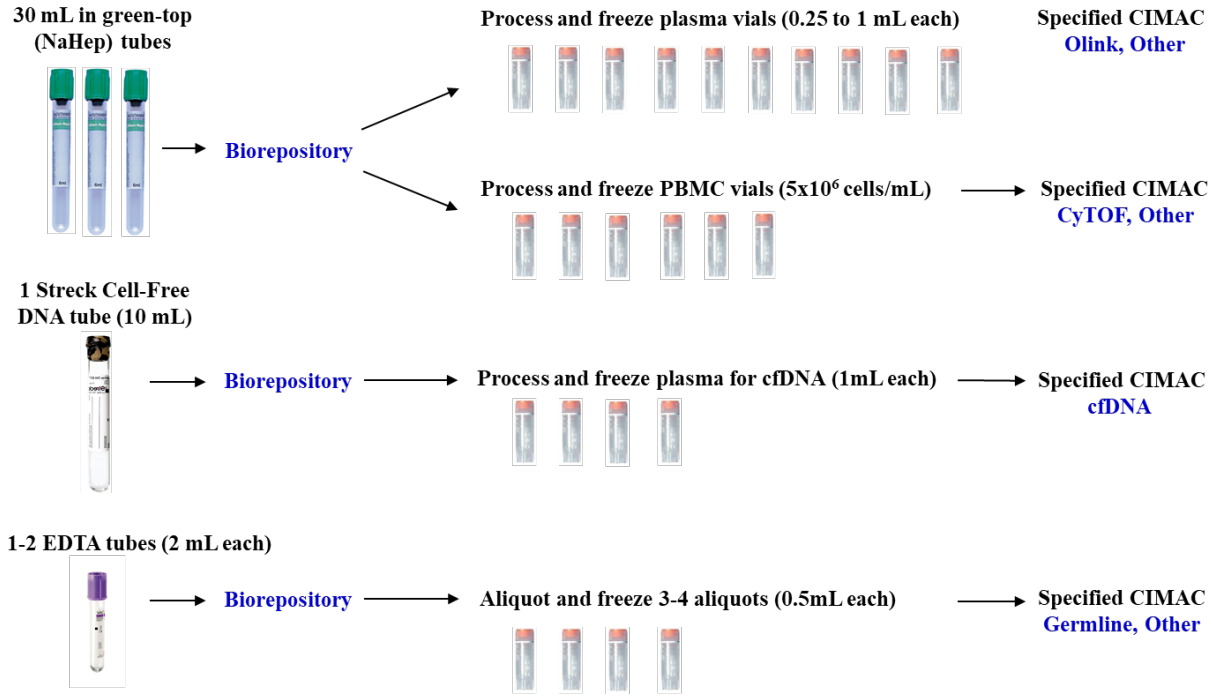
4.1.10 Stool Samples

Upon receiving a self-collection kit with a stool sample, the Biorepository should:

- [Homogenize frozen and DNA-stabilized stool samples according to study need].
- Make five 2 mL aliquots for DNA extraction and three 15 mL aliquots for RNA extraction and record the actual weight of the stool in each tube. [Determined by study need]
- Freeze aliquots at -80°C until request for shipment.

4.2 Blood Processing by Biorepository

Recommended Blood Processing Schema



4.2.1 Important Notes

- [Number and volume of aliquots may vary based on total blood volume, PBMCs collected, and assay needs, custom-aliquoting may be requested for each protocol].
- Any blood sample processed within 24 to 48 hours should be noted as non-compliant under "Comments" in STS. [ETCTN]
- Blood samples should be discarded by the Biorepository without further processing if more than 48 hours has passed since time of collection. A blanket permission-to-destroy method should be employed.
- Label sample vials ***to be banked*** with thermostable labels typically used by the Biorepository.
- [Each Biorepository may use their own labeling sample schema until a time when the CIMAC network provides custom-labeling instructions].
- Label sample aliquots ***to be shipped*** with a thermostable CIMAC network label.

4.2.2 Sodium Heparin Green-Top Tubes

Upon receiving the Sodium Heparin Green-Top Tubes from the collection site, the Biorepository will ***pool all samples*** from a 30 mL draw at one timepoint and prepare Plasma and PBMCs following a Ficoll-Paque protocol. [described in ***Appendix III***]

- Create ~12 plasma vials in 1 mL aliquots (or as many as can be obtained) and store at -80°C.
- Create ~6 PBMC vials in 10% DMSO/FBS (or as many as can be obtained) at 5 x 10^6 cells/mL depending on blood volume and study need. Typical recovery can expect 1 x 10^7 cells from each 10 mL tube.
- Slow-freeze PBMC aliquots at -80°C in a freezing container <24 hours (up to 14 days) followed by long-term cryopreservation in a liquid nitrogen vapor phase freezer.
- [If requested, the Biorepository will optionally extract DNA/RNA].

- [If both CyTOF and genomics are requested for PBMCs, nucleic acid extraction may need to be performed immediately on one vial of PBMCs before aliquots are frozen as PBMCs can only be thawed once for CyTOF].

4.2.3 Streck cfDNA Tubes

Upon receiving the Streck cfDNA Tube from the collection site, the Biorepository should prepare Plasma. [described in ***Appendix IV***]

- For each 10 mL Streck tube, create at least 4 plasma vials of 1 mL aliquots (or as many as can be obtained) and store at -80°C.

4.2.4 EDTA Purple-Top Tubes

Upon receiving EDTA Purple-Top Tubes from the collection site, the Biorepository should:

- For each 2 mL EDTA Purple-Top Tube, create 3-4 whole blood vials of 0.5 mL aliquots and store at -80°C, as follows:
  - Invert the tube gently about 5 times; excess inversion can cause changes in the integrity of the sample.
  - Aliquot 500 µL of whole blood cell pellet using a sterile pipet into each of three or four prelabeled 1.8 or 2 mL cryovials (discard as waste if less than 0.5 mL remains).
  - Store blood samples in a -80°C freezer.
- [If requested, the Biorepository will optionally extract DNA/RNA].

4.3 Shipment of Samples and Derivatives from Biorepository to CIMAC

BIOREPOSITORY WILL RECEIVE ONLY INFORMATION FOR IMMEDIATE PROCESSING AND LONG-TERM STORAGE OF CIMAC SPECIMENS. ADDITIONAL INFORMATION TO THAW, ALIQUOT AND DISTRIBUTE SAMPLE DERIVATIVES WILL BE ADDED AT A LATER TIME.

- As part of a ***formal sample request***, a Request Letter will be sent to Biorepository which will indicate shipping addresses for relevant CIMAC labs [listed in Table 6], these may change from the original protocol based on sample testing capacity.
- Ship samples as batches on dry ice (or Cryoport for ETCTN or equivalent container depending on practices) upon discretion based on shipping and receiving locations taking weather and other pending conditions into consideration.

Table 5. Shipping Conditions for Biorepository Samples

Sample	Shipping Schedule	Shipment conditions
All slides (imaging)	Upon discretion except before Federal Holidays, Monday through Wednesday (FedEx Priority Overnight)	Ambient, Storage box that prevents slide contact.
Frozen Tissue		Frozen, Cryoport/equivalent or dry ice
Stool Aliquots		
Plasma Aliquots		
PBMC Aliquots		
Whole Blood Aliquots		
DNA/RNA (from tissue, stool, blood, or bone marrow)		

Table 6. Contact Information for Shipping Samples from Biorepository to CIMAC Lab.

CIMAC Site Name	Study PI	Contact(s) (Attn to:)	Address
CIMAC 1--MD Anderson Cancer Center	Ignacio Wistuba iiwistuba@mdanderson.org Chantale Bernatchez cbernatchez@mdanderson.org Gheath Al-Atrash galatras@mdanderson.org	Elena Bogatenkova ebogatenkova@mdanderson.org Beatriz Sanchez-Espiridion bsanchez2@mdanderson.org 713-745-7047	Institutional Tissue Bank (ITB) 1515 Holcombe Blvd, Rm G1.3586 Houston, TX 77030
CIMAC 2--Icahn School of Medicine at Mount Sinai	Sacha Gnjatic Sacha.gnjatic@mssm.edu Adeeb Rahman Adeeb.rahman@mssm.edu Seunghee Kim-Schulze Seunghee.kim-schulze@mssm.edu	Diane Del Valle diane.delvalle@mssm.edu 212-824-9624 Jose Lacunza jose.lacunza@mssm.edu 212-824-9344	Hess Center for Science and Medicine 5th floor, rooms 310/313 Human Immune Monitoring Center (HIMC) Icahn School of Medicine at Mount Sinai 1470 Madison Avenue New York, NY 10029
CIMAC 3--Dana-Farber Cancer Institute	Catherine Wu cwu@partners.org Stephen Hodi Stephen_Hodi@dfci.harvard.edu	Mariano Severgnini Mariano_Severgnini@dfci.harvard.edu Srin Ranasinghe Srinika_Ranasinghe@dfci.harvard.edu	Dana-Farber Cancer Institute 450 Brookline Ave, Mayer Building Room 305 Boston, MA 02215 Tel: 617-632-2421
CIMAC 4--Stanford University	Holden Maecker maecker@stanford.edu Sean Bendall bendall@stanford.edu	Bita Sahaf bsahaf@stanford.edu Mina Pichavant minapich@stanford.edu	Human Immune Monitoring Core 1651 Page Mill Road, Palo Alto CA, 943041222
CIDC--Dana-Farber Cancer Institute	Xiaole Shirley Liu xsliu@jimmy.harvard.edu Ethan Cerami cerami@jimmy.harvard.edu	Joyce Hong jhong@jimmy.harvard.edu	Liu Lab Center for Life Science Building 3 Blackfan Circle, 11th Floor Boston, MA 02115

5 CIMAC ACTIVITIES (UNDER DEVELOPMENT)

5.1 Sample Processing by the CIMACs

- All H&E slides received will be scanned as whole slide images using an Aperio/Hamamatsu type system and the resulting image files will be stored centrally at CIDC.
- IHC/IF images will be generated and the resulting image files will be stored centrally at CIDC.
- Stool samples and their nucleic acid derivatives may be processed.

5.2 Quality Control Activities (QC) by the CIMACs

Any tissue specimens collected will be reviewed by reference pathologists, or qualified staff, at the individual CIMACs prior to biomarker analyses. The following QC activities ***may*** be performed on collected specimens:

- Cellular content of tissue may be evaluated from whole slide images as part of an initial sample assessment to inform how much material should be shipped for each assay. [refer to ***Appendix V*** for details]
- ***Histology/cytology examination*** may be performed on sample derivatives received for assay testing. [refer to ***Appendix V*** for details]
- Nucleic acid quality may be measured as part of the assay procedure.

APPENDICES

Appendix I. Processing and Paraffin Embedding of Tissue at Collection Sites and Biorepository

Core Needle Biopsy, Small Biopsy, and Surgical Resection Samples

- Tissue ***must be fixed*** in neutral-buffered formalin (no acid-based products).
- ***For collection sites shipping samples in Ethanol,*** formalin fixed tissue will be transferred to 70% ethanol at room temperature for **up to 72** hours before processing (Steps 3 to 13, Table 7) is completed at the Biorepository.
- The tissue will be processed on an ***automated tissue processor*** following Steps 3 to 12 ***as suggested*** in Table 7 so long ***as total time from ethanol to embedding (in gray) exceeds 4 hours.***
- Do ***not*** use a microwave processor.
- The tissue will be embedded in paraffin (Step 13, Table 7).

Table 7. **Main stages of tissue processing.** Steps 3-12 performed in an automated tissue-processor (no microwave processors).

Step/Process	Solution	Time
1. Fixation	10% buffered formalin	12-24 hours
2.Dehydration	70% Ethanol	30 minutes or up to 72 hours
3.Dehydration	95% Ethanol	30 minutes
4.Dehydration	95% Ethanol	30 minutes
5.Dehydration	100% Ethanol	30 minutes
6.Dehydration	100% Ethanol	30 minutes
7.Dehydration	100% Ethanol	30 minutes
8.Clearing	Xylene	30 minutes
9.Clearing	Xylene	30 minutes
10.Infiltration	Paraffin Wax	30 minutes
11.Infiltration	Paraffin Wax	30 minutes
12.Infiltration	Paraffin Wax	30 minutes
13.Blocking Out	Paraffin Wax	n/a

Appendix II. DNA/RNA Extraction from Tissue Samples

NOTE: For genomics assays, Biorepositories will perform DNA and RNA co-isolation using the following kits or equivalent:

- For Frozen Tissue: AllPrep DNA/RNA Kit (QIAGEN) plus MirVana Kit (Applied Biosystems).
- For FFPE: AllPrep DNA/RNA FFPE Kit (QIAGEN) plus High Pure (Roche).

Appendix III. Processing of Green-Top Tubes: Isolation of Plasma and PBMC

For ETCTN samples:

Please modify LAB-049 Protocol as follows:

Replace Section D step 4a with

Centrifuge the samples at 250 xg for 6 minutes at 18-20^{o}C with acceleration set at 9 and the brake turned off.

Add the following to Section D step 9

Centrifuge the collected plasma samples at 400 xg for 10 minutes at 18-20^{o}C with acceleration set at 9 and the brake turned off, to remove platelets.

Please modify LAB-002 Protocol as follows:

Replace Section VI B steps 27c AND 36a with

Centrifuge the PBMCs at 250 xg for 10 minutes.

i. Be sure the centrifuge is balanced.

PBMCs should be aliquoted into each cryovial at ***~5x10^{6} (5 million) cells per mL in 10% DMSO in heat-inactivated FBS (or equivalent).***

NOTE: The following protocols are given as examples; equivalent SOPs may be followed according to Biorepository practices.

Equipment

- Benchtop centrifuge (Allegra X-15R, Beckman Coulter) or equivalent
- Tali Image Based Cytometer (Invitrogen) or equivalent
- Pipette Gun (Drummond) or equivalent
- p200, p1000 micropipettes (Rainin) or equivalent

Materials

- Sodium Heparin Green-Top Tube (Fisher, # 367874)
- 1.8 mL Cryotube vials (Fisher, #375418)
- Micropipette tips
    - Sterile, filtered, p200 micropipette tips
    - Sterile, filtered, p1000 micropipette tips
- 50 mL conical tube (Fisher, #352070)
- Tali Cellular Analysis Slide (Invitrogen, #110794) or equivalent
- CoolCell (Fisher, #NC9883130 or Biocision Inc., BCS-405) and CoolBox or equivalent
- 2 mL, 5 mL, 10 mL, 25 mL, and 50 mL sterile serological pipettes (Fisher, #356507, #356543, #356551, #356525, #356550, respectively

Reagents

- Ficoll-©-Paque (GE Healthcare, 17144003)
- SepMate™ (StemCell Technologies, 85450)
- PBS (without Ca^{2+}, Mg^{2+}) (Invitrogen, 10010-049)
- Fetal Bovine Serum – Heat inactivated (Gibco, 14040)
- DMSO (Sigma-Aldrich, #154938)

PLASMA Isolation

1. Whole blood samples will be received by the laboratory collected in Sodium Heparin Vacutainer® Green-Top Tubes.
2. ***Note the condition of the samples upon receipt***. Observations may include labeling errors, obvious clotting, degree of hemolysis, low blood volume, leakage or breakage, etc.
3. Sodium Heparin tubes will not be processed if any of the following conditions exist:
    a. Samples which cannot be identified.
    b. Clotted or excessively hemolyzed (dark red/mahogany-colored plasma) samples.
    c. Compromised integrity of Sodium Heparin tubes (e.g. leaking or broken tubes).
4. In a 50 mL conical tube, ***pool all blood samples from each case*** (30 mL total volume) and measure the volume of heparinized whole blood and record it (in mL).
5. Pre-chill at 2-8°C or on ice cryo-vials that have been pre-labeled with pertinent patient and sample information.
6. Load the blood samples in the centrifuge such that the load is properly balanced. Tubes of the same type and size should be compared and balanced according to fill volumes. If an odd number of tubes will be centrifuged, “balance tubes” containing water must be used.
7. Centrifuge the samples at 250 xg for 6 minutes at 18-20°C with acceleration set at 9 and the brake turned off.

8. Following centrifugation, carefully transfer all upper (plasma) phase to a fresh conical tube making sure you do not disturb the lower phase. Mix the plasma-containing tube with a pipette. ***Save the lower part which will be used for the PBMC isolation.***
9. Centrifuge the plasma samples at 400 xg for 10 minutes at 18-20^{o}C with acceleration set at 9 and the brake turned off, to remove platelets.
10. Label sample cryovials with two thermostable labels: a CIMAC network label and a label typically used by the Biorepository.
11. Transfer all plasma to a fresh conical tube. Aliquot the plasma (***~12 x 1 mL***) into the pre-chilled pre-labeled cryovials. The exact aliquot volume may be adjusted based on requirements of the specific study.
12. Cryovials containing plasma will be stored at -80^{o}C until used, transferred, or shipped.
13. Record the storage location on the corresponding worksheet/database.

PBMC Isolation

Isolation of PBMCs will be performed using one of the following protocols selected for each correlative study based on technical requirements and resources available:

PBMC Isolation Using Ficoll-Paque

1. Dilute blood 1:1 with PBS (without Ca^{2+}, Mg^{2+}). (Blood amount should not exceed 25 mL per tube.)
2. Take 2 new 50 mL conical tubes and add 12 mL Ficoll-Paque (Cat# 17144003; GE Healthcare) per tube.
3. Slowly and gently layer the diluted blood on top of the Ficoll-Paque of the tube with a maximum volume of 35 mL. Minimize blood entering into the Ficoll layer and avoid air bubbles
4. Centrifuge the tube at 500 xg for 20 min at room temperature with slow acceleration (#7) and deceleration (#7) (Sorvall Legend XTR centrifuge).
5. A white ring of PBMC will be observed between the upper layer (diluted plasma) and middle layer (Ficoll-Paque). The lower layer is composed of pelleted red blood cells. Discard the upper layer (diluted plasma) carefully using a pipette. Remove the PBMC layer from the tube and transfer into a 50 mL conical tube. Do not transfer the red blood cell pellet.
6. Completely fill conical tube containing isolated PBMC with PBS, mixing well by inverting capped tube 2-3 times.
7. Centrifuge the PBMCs at 250 xg for 10 minutes.
8. Aspirate the supernatant and resuspend the cells in 48 mL of PBS.
9. Count the cells using the Tali Counter (or lab's preferred cell counting method) and record viable cell count and total count.
10. Centrifuge the conical vial at 250 xg for 10 minutes. Based off the viable cell count, calculate the number of vials and volume of freezing medium that will be needed
    a. PBMCs should be aliquoted into each cryovial at ***~5x10^{6} (5 million) cells per mL***. The total mL amount of freezing media needed is equal to the total number of aliquots needed.
    b. Label the appropriate number of empty cryovials with de-identified label and place in a CoolBox to chill for at least 10 minutes (alternatively 4^{o}C/wet ice can be used).
    c. Make enough "Freezing Media A" (100% FBS) and "Freezing Media B" (80% FBS and 20% DMSO) to create 1mL aliquots (heat inactivated serum).
    d. Pre-chill both media to 4^{o}C.
11. Aspirate the supernatant and discard.
12. Resuspend the cells in "Freezing Medium A" (FBS) equal to one half of the final aliquot volume needed.

13. Using a dropwise technique (1 drop/second) while swirling the sample, add “Freezing Medium B” equal to the remaining half of the total volume (e.g. to make six 1 mL aliquots, resuspend cells in 3 mL of FBS and dropwise add 2 mL of Freezing Medium B).
14. Label sample cryovials to be cryopreserved with two thermostable labels: a CIMAC network label and a label typically used by the Biorepository.
15. Quickly aliquot ***1 mL of cell suspension in each prelabeled cryovial*** with CIMAC label and biorepository label. on ice (delay in this step reduces viability).
16. Place the cryovials into a CoolCell (or equivalent container) and into a -80°C freezer for 2 hours or overnight (alternatively a Mr. Frosty or controlled rate freezer can be used).
17. Following this, immediately put the PBMCs cryovials into liquid nitrogen for long term storage.

Appendix IV. Processing of Streck Cell-Free DNA Tube: Isolation of Plasma

For ETCTN:

Please modify LAB-049 Protocol as follows:

Replace Section D step 4b with

Centrifuge the samples at 250 xg for 6 minutes at 18-20°C with acceleration set at 9 and the brake position off.

Replace Section D step 10c with

Centrifuge the collected plasma samples at 400 xg for 10 minutes at 18-20^{o}C with acceleration set at 9 and the brake turned off.

NOTE: The following protocols are given as examples; equivalent SOPs may be followed according to Biorepository practices.

Plasma Isolation

1. Blood samples will be received by the laboratory collected in Streck Cell-Free DNA Tubes.
2. ***Note the condition of the samples upon receipt***. Observations may include labeling errors, obvious clotting, degree of hemolysis, low blood volume, leakage or breakage, etc.
3. Streck tubes will not be processed if any of the following conditions exist:
    a. Samples which cannot be identified.
    b. Streck tube samples that have been refrigerated.
    c. Clotted or excessively hemolyzed (dark red/mahogany-colored plasma) samples.
    d. Compromised integrity of Streck tubes (e.g. leaking or broken tubes).
4. Load specimen tubes in the centrifuge. Ensure the centrifuge is balanced. Use an appropriate counterbalance if needed (i.e., Streck tube filled with water--Streck catalog # 218961, 218962, or 2189921).
5. Centrifuge the samples at 250 xg for 6 minutes at 18-20°C with acceleration set at 9 and the brake position off.
6. After centrifugation transfer the top layer (plasma) into a 15mL sterile conical tube.
7. Centrifuge the plasma samples collected in step 3 at 400 xg for 10 minutes at 18-20^{o}C with acceleration set at 9 and the brake turned off.
8. Label sample cryovials to be cryopreserved with two thermostable labels: a CIMAC network label and a label typically used by the Biorepository.
9. After second centrifugation aliquot ***1 ml of plasma*** cell-free DNA (cfDNA) into each prelabeled cryovial. Don't pipet the pellet built on the bottom of 15 ml tube, instead discard it all together after plasma collection. The exact aliquot volume may be adjusted based on requirements of the specific study.
10. Store all plasma cfDNA samples at -80°C.

Appendix V. Quality Control of Tissue Specimens

Histology/Cytology examination: H&E-stained sections from CNBs, surgical resections and archival tissues will be used to confirm the presence of tumor cells, as well as their relative abundance (tumor cellularity), and the composition of the tumor associated stroma and lymphocytic infiltrates.

The following pathological analysis will be performed by the Biorepository:

1. Use the pathology/clinical report provided with each sample timepoint to confirm the tumor diagnosis concordance and record the World Health Organization (WHO) classification on a Pathology Verification Form using established Biorepository practices.
2. Score the percentage of viable tumor cells comprising the tumor bed area ***to select the most suitable material for nucleic acid extraction using established practices at the Biorepository.***

The following pathological analysis will be performed by CIMACs:

1. Score Lymphocyte Infiltration (0-No infiltration; 1+ weak; 2+moderate; 3+high).
2. Score the percentage of viable tumor cells comprising the tumor bed area
3. Score the evaluation of stromal elements (this indicates the % area of tumor bed occupied by non-tumor cells, including inflammatory cells [lymphocytes, histiocytes, etc], endothelial cells, fibroblasts, etc); and
4. Score the percentage area of necrosis; and
5. Score the percentage area of fibrosis.
6. Percentages for items 2 through 5 should add up to 100%

Table 8. Quality Control Metadata for Tissue Samples

Diagnosis (WHO)	Lymphocyte Infiltration (0 to 3+)*	Viable Tumor (% area)	Viable Stroma (% area)	Necrosis (% area)	Fibrosis (% area)

* 0- No infiltration; 1+ weak; 2+ moderate; 3+ high

Highlighted columns each reflect percent area of viable and damaged tumor bed that should add up to 100%.

Appendix VI. Pre-Analytic Information

The following table lists the major pre-analytic variables that will be recorded by sample type. The example provided (blue) describes four cores, obtained pre-treatment from a primary tumor core needle biopsy, embedded at the clinical site and shipped to the biorepository in one batch of 5 FFPE-sectioned unstained slides. Green rows provide examples of clinical sites processing flash-frozen tissue samples and blood. Rows in peach indicate sample shipment and receipt conditions.

Pre-Analytic Variable	Example
Specimen timepoint	Baseline
Category of specimen	Formalin-fixed tissue
Type of specimen	FFPE Block
Type of tissue	Primary tumor
Ischemic start time	0800; 01/10/2018
Formalin start time	0900; 01/10/2018
Formalin end time	0900; 01/11/2018
Ethanol start time	0900; 01/12/2018
Ethanol end time	0900; 01/13/2018
# of samples collected	4
Block number	1
Core/Segment number	XXX-XXX-XXX-1
Flash freezing time; date	0900; 01/10/2018
Blood processing time; date	0900; 01/10/2018
Sample shipment time; date	1300; 01/12/2018 (pickup)
Sample receipt time; date	0900; 01/13/2018 (delivery)
Shipment condition	Ambient
Receipt condition	Ambient
# of samples received	5

Appendix VII. Checklist of Minimal CIMAC Biomarker and Specimen Collection Details Required for Trial Activation

Determine if dose escalation phase requires collection of specimens for CIMAC testing:

- Can trial be activated if samples from initial phase are not assayed by CIMAC.
- Specify which specimens are collected for each timepoint:
- Some cores may need to be earmarked for integral assays.
- Indicate desired number of tissue cores in formalin.
- Indicate desired number of tissue cores snap-frozen.
- Indicate blood, aliquot size volume and number for each tube type.
- Indicate if archival blocks, slides, punches, or scrolls are requested AND confirm quantity, minimal tumor % (genomics only), section thickness, and volume for each assay.
- Confirm that clear distinction is made between archival tissue, fresh-frozen tissue, and fresh tumor biopsy core (FFPE) for each timepoint.
- Confirm agreement between Biomarker Table and Specimen Summary Table.

Specify sample processing details at collection site:

- Indicate if special ischemic times are required for tissue excision and fixation.
- Specify what pre-analytical data is to be collected.
- Select how tissue is processed on-site (FFPE embedding on-site OR ship cores in ethanol). ***If on-site embedding is selected include a table of steps for tissue auto-processor.***
- Indicate processing time requirements for tissue (12-24 hours in formalin, <72 hours in Ethanol) and blood (no more than 48 hours for blood).
- Include "external sample label" language in shipping section to alert biorepository to prioritize processing *for tissue and blood samples*.
  "An external sample label should be fixed to the shipping container to alert the Biorepository of Formalin-fixed sample time and date it was placed into Ethanol (this helps to identify and prioritize received samples that have processing time requirements)"
- Confirm specimens are shipped to their intended biorepository.

Specify sample processing details at Biorepository:

- Specify in the Biorepository Section that "additional sample processing and sample request details will be provided at a later time".
- Specify that DNA/RNA is to be co-extracted at the biorepository for genomics assays.
- Number and size of blood aliquots, number and priority of tissue cores (FFPE and flash-frozen), and number of slides should be derived from the Intake Form.
- For trials where CyTOF and Genomics assays are performed on PBMCs: specify if nucleic extraction to be performed immediately on one vial PBMCs before aliquots are frozen.
- Processing of specialized material (stool, bone marrow aspirates etc.) should be requested on case-by-case basis.