CORRELATIVE SCIENCE PROCEDURE MANUAL

1. Purpose

This document describes the procedures required for the collection, shipping, and processing of biospecimens that are being submitted for patients enrolled or registered on A222101. This document also describes the procedures that will be followed subsequent to the receipt of biospecimens by the Alliance Biorepository at Mayo Clinic (ABMAYO) prior to their use for protocol-specified and future, unspecified correlative science research studies. This document should be used by staff involved with any aspect of the A222101 biospecimen collection, processing, and submission, including staff at satellite institutions.

2. Scope

This document applies to all biospecimens collected specifically for the A222101 clinical trial study. Please refer to the trial protocol-specific language for additional details regarding eligibility, participant enrollment, and data submission. **Please ensure that you are reading the most updated version of this document. This document may experience minor updates, revisions, and clarifications independent of a formal protocol amendment. The most recent version of this document may be found on the Alliance website and CTSU.**

3. Definitions

Term	Definition
ABMAYO	Alliance Biorepository at Mayo Clinic

4. Contact Information

4.1 For questions and problems related to protocol administration, eligibility, patient registration, and data submission, relevant contact information is listed on protocol pages 1 and 2.

4.2 For information on using the BioMS system, please refer to the 'Help' links on the BioMS webpage to access the online user manual, FAQs, and training videos. To report technical problems, such as login issues or application errors, please contact: 1-855-55-BIOMS or **bioms@alliancenctn.org**. For assistance in using the application or questions or problems related to specific specimen logging, please contact: 1-855-55-BIOMS or **bioms@alliancenctn.org**.

4.3 For all other questions regarding biospecimen procurement and shipping procedures, please contact:

ABMAYO

- Katie Halverson
  Phone: 1-507-293-7147
  Email: Halverson.katie@mayo.edu
  Please copy Dr. Dan Hertz on all correspondence with ABMAYO:
  dlhertz@med.umich.edu

5. Site Preparation

5.1 Please refer to A222101 protocol document for any specific requirements related to patient enrollment, data submission, registration, and regulatory compliance.

5.2 Please ensure that you have the appropriate log-in credentials and can successfully access the BioMS application. The BioMS application is used for logging the collection and shipment of biospecimens to the Alliance Biorepository at Mayo Clinic. For training and assistance in using the application or questions or problems related to specific specimen logging, please contact: 1-855-55-BIOMS or **bioms@alliancenctn.org**.

6. Collection Schema

The following biospecimens are to be collected at each of the time points listed in the table below. Please refer to individual biospecimen collection and processing methods and specific shipping procedures that are detailed in this manual.

Time Point	Biospecimen	Quantity	Collection / Processing Method	Shipping	Recipient	Notes
For patients consenting to biobanking, submit the following:						
After registration / prior to study treatment	Whole blood (lavender top EDTA tube)	1 x 10 ml	Whole blood for germline DNA (9.1)	Ambient	ABMAYO-BAP Freezer (10.2)	1
After registration / prior to study treatment	Whole blood for plasma (green top sodium heparin tube)	3 x 1 ml aliquots	Frozen plasma (9.2)	Dry Ice	ABMAYO-BAP Freezer (10.2)	1
After registration / prior to study treatment	Whole blood for serum (red top tube)	3 x 1 ml aliquots	Frozen serum (9.3)	Dry Ice	ABMAYO-BAP Freezer (10.2))	1
Day 1 of GM1 Dose 4	Whole blood for plasma (green top sodium heparin tube)	3 x 1 ml aliquots	Frozen plasma (9.2)	Dry Ice	ABMAYO-BAP Freezer (10.2)	1
Day 1 of GM1 Dose 4	Whole blood for serum (red top tube)	3 x 1 ml aliquots	Frozen serum (9.3)	Dry Ice	ABMAYO-BAP Freezer (10.2)	1
Day 1 of GM1 Dose 8	Whole blood for plasma (green top sodium heparin tube)	3 x 1 ml aliquots	Frozen plasma (9.2)	Dry Ice	ABMAYO-BAP Freezer (10.2)	1
Day 1 of GM1 Dose 8	Whole blood for serum (red top tube)	3 x 1 ml aliquots	Frozen serum (9.3)	Dry Ice	ABMAYO-BAP Freezer (10.2)	1
Day 1 of GM1 Dose 12	Whole blood for plasma (green top sodium heparin tube)	3 x 1 ml aliquots	Frozen plasma (9.2)	Dry Ice	ABMAYO-BAP Freezer (10.2)	1, 2
Day 1 of GM1 Dose 12	Whole blood for serum (red top tube)	3 x 1 ml aliquots	Frozen serum (9.3)	Dry Ice	ABMAYO-BAP Freezer (10.2)	1, 2

Notes:

1. Collection is optional for patients, but all sites are required to offer to patients to consent. Please see the U.S.-specific consent documents.
2. If study treatment is discontinued before finishing 12 doses of GM1, then end of treatment blood sample should be collected at time of discontinuation whenever deemed feasible.

7. Biospecimen Collection Kits

7.1 There are no "kits" provided for submission of specimens for this study. Sites are responsible for acquiring the materials for collection and shipping of these specimens to ABMAYO. Please see **Section 10 – Biospecimen Shipping** for specific instructions on shipping to ABMAYO.

8. Biospecimen Labeling and Tracking

8.1 All research biospecimens MUST be labeled with the Alliance study number (A222101), Alliance patient ID number, patient initials (Last, First, Middle), the date and time (if applicable) of collection and specimen type (e.g. serum, plasma). Specimen labels must match BioMS records.

8.2 Label all containers and vials with an indelible, solvent-resistant marker when they are at ambient temperature.

8.3 **Do not affix any labels to vials or tubes.** Label the collection containers directly with the marking pen.

8.4 All biospecimens that are collected and sent to ABMAYO must be **logged and tracked in BioMS** under A222101. Do not send samples to the Biorepository in which the patient has not been registered onto the study. The BioMS system is a web-based application that tracks the collection and shipping of biospecimens. Once individual biospecimens are logged and 'shipped' in the BioMS system, a packing manifest will be created by the system. **This manifest must be printed out and must accompany all biospecimen shipments**. To become familiar with the BioMS system and for further information about training, access, and use, please contact the BioMS Help desk at: 1-855-55-BIOMS or **bioms@alliancenctn.org**.

8.5 In the event that BioMS cannot be accessed, please complete a BioMS Specimen Log and Shipping Manifest form which can be found here- **http://tinyurl.com/alliance-bioms-contingency**.

9. Blood Collection Methods

9.1 Whole Blood- Lavender Top EDTA (No Processing)

9.1.1 Collect 10 ml of whole blood by standard venous phlebotomy technique into the EDTA tube. Immediately following collection, invert blood in lavender tube by hand 10 times to ensure the blood and EDTA are thoroughly mixed.

9.1.2 Store lavender top EDTA tube with whole blood at 4 degrees Celsius (i.e. refrigerated) until shipping. Do not freeze the tube. **The tube may be stored for up to 72 hours at refrigerated temperature before shipment. If blood must be collected on Friday, it can be shipped on Friday for Saturday delivery. Ensure that the EDTA tube is shipped with a refrigerant pack to ensure proper temperature and to avoid freezing.**

9.2 Plasma Processing

9.2.1 Collect 10 ml of whole blood by standard venous phlebotomy technique into the green top (sodium heparin) tube. Immediately after collection, invert the green top tube by hand 10 times to ensure the blood and sodium heparin are thoroughly mixed.

9.2.2 Immediately place tube in an ice-water bath. Label 3 cryovials as instructed in **section 8**. Make certain each vial is labeled completely and identically. Cryovials should be placed in an ice-water bath to pre-chill (for **step 9.2.5**).

9.2.3 Centrifuge the green top tube at 1,300 x g for 10 minutes at 4 degrees Celsius or at ambient temperature if a refrigerated centrifuge is not available. This separates plasma from red and white blood cells.

9.2.4 Immediately after centrifugation, return the green top tube to the ice-water bath.

9.2.5 Using a pipette, transfer ~1 ml of plasma into each of the pre-chilled, labeled cryovials. Discard any remaining plasma and blood cells.

9.2.6 Freeze plasma containing cryovials on dry ice or a -70 to -90 degree Celsius ultralow freezer. Store at -70 to -90 degrees Celsius until ready for shipment on dry ice. If a -70 to -90 degree Celsius ultralow freezer is not available, temporary storage (up to 30 days) in a -20 degree Celsius freezer is allowable.

9.3 Serum Processing

9.3.1 Collect 10 ml of whole blood by standard venous phlebotomy technique into the red top (plain glass with clot activator) tube. Do not collect whole blood into a "tiger top" / "SST" / "gel tube."

9.3.2 Following blood collection, the red top tube should be left upright and undisturbed at room temperature for at least 30 minutes but no more than 2 hours to allow a clot to form.

9.3.3 Label 3 cryovials as instructed in **section 8**. Make certain each vial is labeled completely and identically. Cryovials should be placed in an ice-water bath to pre-chill (for **step 9.3.5**).

9.3.4 After clot formation, centrifuge the red top tube at 1,300 x g for 10 minutes at 4 degrees Celsius or at ambient temperature if a refrigerated centrifuge is not available. This separates the serum from the blood cells. Immediately after centrifugation, place the red top tube in an ice-water bath.

9.3.5 Using a pipette, transfer ~1 ml of serum into each of the pre-chilled, labeled cryovials. Be careful not to disturb the red blood cell layer. Discard any remaining plasma and blood cells.

9.3.6 Freeze serum containing cryovials on dry ice or a -70 to -90 degree Celsius ultralow freezer. Store at -70 to -90 degrees Celsius until ready for shipment on dry ice. If a -70 to -90 degree Celsius ultralow freezer is not available, temporary storage (up to 30 days) in a -20 degree Celsius freezer is allowable.

10. Biospecimen Shipping

10.1 Overview

10.1.1 Place the original, completed copy of the BioMS packing manifest in the shipment. Do not send specimens without a completed BioMS Packing Manifest or substitute "BioMS Downtime Form." Biospecimens cannot be accepted without this completed form.

10.1.2 All biospecimens collected on the study should be shipped within the time frames specified in **section 9**. If patient or physician schedules do not allow biospecimens to be shipped within the time frame specified, please contact appropriate personnel listed in **section 4.3** for further instructions, at least 24 hours prior to anticipated collection.

10.2 Shipping to ABMAYO- BAP Freezer

10.2.1 Ship container for PRIORITY OVERNIGHT DELIVERY according to IATA guidelines and standard institutional policies and using the preferred vendor. **Ship specimens to ABMAYO on Monday through Friday only. Do not ship on Saturdays or on the day before a national holiday.**

Ship to:

Alliance Biorepository at Mayo Clinic- BAP Freezer
ST-SL-16
150 Third Street SW
Rochester, MN 55902
Phone: 507-538-0602

11. ABMAYO Biospecimen Receipt and Quality Assurance Measures

11.1 All biospecimens sent to ABMAYO will be accessioned into the informatics system BioMS, at ABMAYO Research Laboratory Information Management System (RLIMS) which interfaces with BioMS.

11.2 All biospecimens received at the ABMAYO will be logged, associated, and tracked by the patient study ID number.

11.3 Upon receipt, all physical biospecimens received will be reconciled with what is recorded on the BioMS packing manifest. Any discrepancies noted will be communicated to submitting site for reconciliation.

11.4 Upon receipt, any biospecimen received that is not in appropriate physical condition (broken vials, frozen samples that are thawed, ambient samples that are frozen) will be reported to the Program Manager, who will contact the submitting site for reconciliation.

11.5 All biospecimens submitted to ABMAYO will remain in storage until exhausted. In the event the patient withdraws consent, the specimens will be disposed/returned according to Alliance Policy and Procedure Chapter 11.

12. Document History

Version	Description and Justification of Change	Author	Effective Date
1.0	New	PAA, DL, NS	07/01/2022