SONOMA BIOTHERAPEUTICS

SBT777101 SYNOVIAL BIOPSY MANUAL

Study Number	SBT777101-01
Study Title	A Phase 1 Study to Evaluate the Safety, Tolerability, Pharmacokinetics, Pharmacodynamics, and Activity of Single Ascending Doses of SBT777101 in Subjects with Rheumatoid Arthritis
Investigational Product	SBT777101
Sponsor Name	Sonoma Biotherapeutics, Inc.
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LIST OF ABBREVIATIONS

Abbreviation	**Definition**
AGSB	arthroscopic guided synovial biopsy
CAR	chimeric antigen receptor
CRF	case report form
CTCAE	Common Terminology Criteria for Adverse Events
PBS	phosphate-buffered saline
PD	pharmacodynamic
PK	pharmacokinetic
PPE	personal protective equipment
RA	rheumatoid arthritis
SOA	Schedule of Assessments
T_{reg}	regulatory T lymphocyte
US	ultrasound

1. INTRODUCTION

SBT777101 is a genetically engineered chimeric antigen receptor (CAR) regulatory T lymphocyte (Treg) cell therapy, designed to treat patients with moderate-to-severe rheumatoid arthritis (RA). As part of this clinical trial, biopsies of inflamed synovial tissue affected by disease must be obtained. Whenever possible, synovial fluid from the same inflamed joint will be collected by arthrocentesis, both pre- and post-infusion of the cell product. The pre-treatment sample will be collected during screening and used to evaluate the presence of inflammatory cell infiltration as well as the SBT777101-reactive antigen to inform eligibility for the clinical trial. A post-treatment sample will be collected to inform pharmacokinetic (PK) and pharmacodynamic (PD) parameters.

2. SCOPE

The purpose of this biopsy manual is to provide standard procedures for the acquisition, handling, processing, storage, and shipment of synovial tissue and synovial fluid obtained by the clinical team at each clinical study site. It was developed and adapted from written materials provided by Dr. Andrew Filer (University of Birmingham).

3. BIOPSY PURPOSE

The aim of the biopsy is to obtain at least 6 fragments per synovial tissue core biopsy, and any synovial fluid that can be aspirated, from a single inflamed joint per subject, to establish and inform:

- Pre-treatment (Screening): Presence of inflammatory cells and citrullinated proteins (i.e. SBT777101-reactive antigen), as part of the study eligibility criteria
- Post-treatment: Presence of SBT777101 CAR Tregs at sites of inflammation as well as evaluation of inflammatory cells and citrullinated proteins (i.e. SBT777101-reactive antigen)
- Pre- and Post-treatment: Exploratory biomarkers aimed at better understanding intended therapeutic effect

4. GENERAL CONSIDERATIONS

The aim of this biopsy manual is to ensure standardized collection of specimens for this clinical study. The utility of the data obtained from the analysis of tissues will be directly related to the quality of the tissue specimen (and synovial fluid if obtained).

Any subject on any anti-coagulation medications (e.g. aspirin, clopidogrel, warfarin, etc) should be held at least 3 days prior to procedure to minimize risk of intra-articular bleeding.

The following factors must be considered to obtain and maintain tissue samples with suitable integrity for this clinical study and innovative research:

- Avoid contamination with surrounding histologically distinct tissue (i.e. adipose, muscle, tendon).
- Minimize the time between biopsy and preservation, in order to prevent the initiation of cell death mechanisms and subsequent degradation.

- Samples must be taken and processed into a 10% neutral-buffered formalin or freezing medium as soon as possible on the same day of collection.
- Where relevant, use materials provided by the sponsor (e.g. cryovials and controlled rate freezing devices) that are suitable for appropriate temperature storage, in order to prevent ice crystal formation and thus maintain cell viability.

Important: Never place tissue intended as a frozen specimen in formalin.

5. SUBJECT ELIGIBILITY

5.1. Re-biopsy Joint Evaluation

Per the Schedule of Assessments (SOA) table in Protocol SBT777101-01, subjects will have a joint evaluation at screening prior to being enrolled into the study. This assessment is required to confirm that the investigator has determined that the subject has a joint that is suitable for biopsy prior to them being enrolled into the study.

5.2. Pre-biopsy Subject Communication and Consent Procedures

The synovial tissue biopsy and arthrocentesis procedures will be described in the study Informed Consent Form, which will be signed by the study investigator and subject prior to screening.

6. SYNOVIAL TISSUE BIOPSY AND OPTIONAL ARTHROCENTESIS (SYNOVIAL FLUID ASPIRATION) SUMMARY

6.1. Overview

The synovial tissue and fluid acquisition goals are as follows:

- Arthrocentesis: At least 0.3 mL synovial fluid when possible.
- Synovial tissue biopsy: 6 to 12 fragments per core biopsy (Refer to Section 8 for prioritization)

6.2. Biopsy and Arthrocentesis Team

Biopsy/Arthrocentesis Team: The team should be comprised of members with the following functional roles, who will fulfil the following responsibilities (Note: one person may fulfill multiple of these roles):

Proceduralist (i.e., Rheumatologist, Interventional Radiologist, or designated qualified personnel):

1. Ensure patient is consented.
2. Confirm that the appropriate joint for biopsy and or/arthrocentesis has been identified either through personal examination or correspondence with the clinician who made the initial clinical assessment and determination at screening.
3. Perform procedure(s) per instructions provided here (Section 7).

Ultrasonographer (i.e. Radiologist, Interventional Radiologist, ultrasound technician, or designated qualified personnel):

1. Perform ultrasound to guide synovial tissue biopsy and/or arthrocentesis on scheduled procedure dates.

Designated Specimen Processor and Handler:

The designated Specimen Processor and the Specimen Handler should be notified with ample time prior to start of the procedure, in order to allow the Specimen Processor to bring necessary pre-labeled collection, processing, and transportation reagents and supplies to the procedure room, and in order to allow the Specimen Handler to prepare streamlined short-term temporary storage or shipment of samples.

Please use the Sponsor-provided kits (e.g. tubes and reagents) and associated labels per the Lab Manual or as referenced here.

1. Process synovial tissue and/or synovial fluid according to instructions provided in this manual (Section 8).
2. Once synovial tissue and/or synovial fluid is processed, ensure immediate shipment of specimen(s) according to instructions provided in this manual (Section 9).
3. If short-term temporary storage is necessary due to scheduling and shipment logistics (e.g. day of the week), ensure specimen(s) are stored appropriately according to instructions provided in this manual (Section 8).
4. If specimen(s) need to be stored temporarily, ensure shipment of specimen(s) as soon as possible according to instructions provided in this manual (Section 9).

6.3. Biopsy and Arthrocentesis Overview

Ultrasound (US)

The Biopsy samples will be obtained using ultrasound with Doppler imaging to guide the arthrocentesis and synovial tissue biopsy.

Arthrocentesis Method Overview

Local anesthesia and/or sedation may be used as indicated by institutional standard operating procedures based on method of tissue acquisition, if considered safe for the patient.

All medications used during the procedure should be recorded as concomitant medications on the study electronic case report form (eCRF).

Synovial fluid will be aspirated and collected in green-top (Sodium Heparin) tube as indicated in the relevant procedure.

Biopsy Method Overview

Local anesthesia and/or sedation may be used as indicated by institutional standard operating procedures based on method of tissue acquisition, if considered safe for the patient.

All medications should be recorded as concomitant medications on the study electronic case report form (eCRF).

Sites can utilize method for biopsy as they are comfortable with and per respective institutional standard operating procedures [e.g. ultrasound-guided portal and forceps, arthroscopic guided synovial biopsy (AGSB)]. Otherwise, synovial tissue biopsy will be obtained using a biopsy needle per instructions provided in this biopsy manual.

7. SYNOVIAL TISSUE BIOPSY AND OPTIONAL ARTHROCENTESIS (SYNOVIAL FLUID ASPIRATION) PROCEDURES

7.1. Equipment and Supplies for the Needle Biopsy

Materials necessary to perform ultrasound-guided synovial tissue biopsy and arthrocentesis should be per each clinical site's respective institutional standard operating procedures for methods other than needle biopsy. Required equipment and supplies needed for needle biopsy include:

- Ultrasound machine (For US guided Biopsy)
- 3 mL, 5 mL, 10 mL, and 20 mL syringes
- Long 27G needle (for local anesthesia), and 18G needle to make incision bigger for needle as necessary / aspirate fluid
- 1 % Lidocaine (max 4.5mg/kg)
- 0.9% normal saline (for joint inflation)
- Sterile alcohol swabs (for anesthesia draws)
- Sterile 0.9% normal saline (either 125 mL or 150 mL bag per biopsy, depending on size of joint)
- Antiseptic solution (viscous chlorhexidine, 4% 8oz, 1 bottle per biopsy)
- Sterile contact medium (e.g. Hibiscrub, or same as antiseptic solution)
- Sterile 4" x 4" 12-ply gauze pads (pack of 12)
- Sterile McKesson sticky XL drapes without hole (at least 8 per biopsy)
- Sterile ultrasound sheath (including gel)
- Sterile gown in sufficient quantity for all involved in procedure
- Sterile gloves in sufficient quantity to double glove for all involved in procedure
- Waterproof shoe covers in sufficient quantity for all involved in procedure
- Face masks and hair covers in sufficient quantity for all involved in procedure
- 3M Coban self-adherent wrap
- Bacitracin ointment packets in sufficient quantity for wound care x3 days post-procedure
- Sponsor-provided supplies
    - 12-well plate (for optional use)
    - Corning CoolCell LX Cell Freezing Container
    - Cryostor CS10 Freezing medium
    - TEMNO, Cook, or Bard biopsy needles with coaxial introducers (16G recommended for wrist/ankle and 14G recommended for knee, each with 10cm or 15cm length)
    - Visit-specific Biopsy Kits [containing green-top sodium heparin (NaHep) tubes; 2.0 mL Cryovials; Formalin-filled vials] and **Biopsy Visit Requisition Form** (see Lab Manual)

7.2. NEEDLE BIOPSY PROCEDURE

7.2.1. Knee

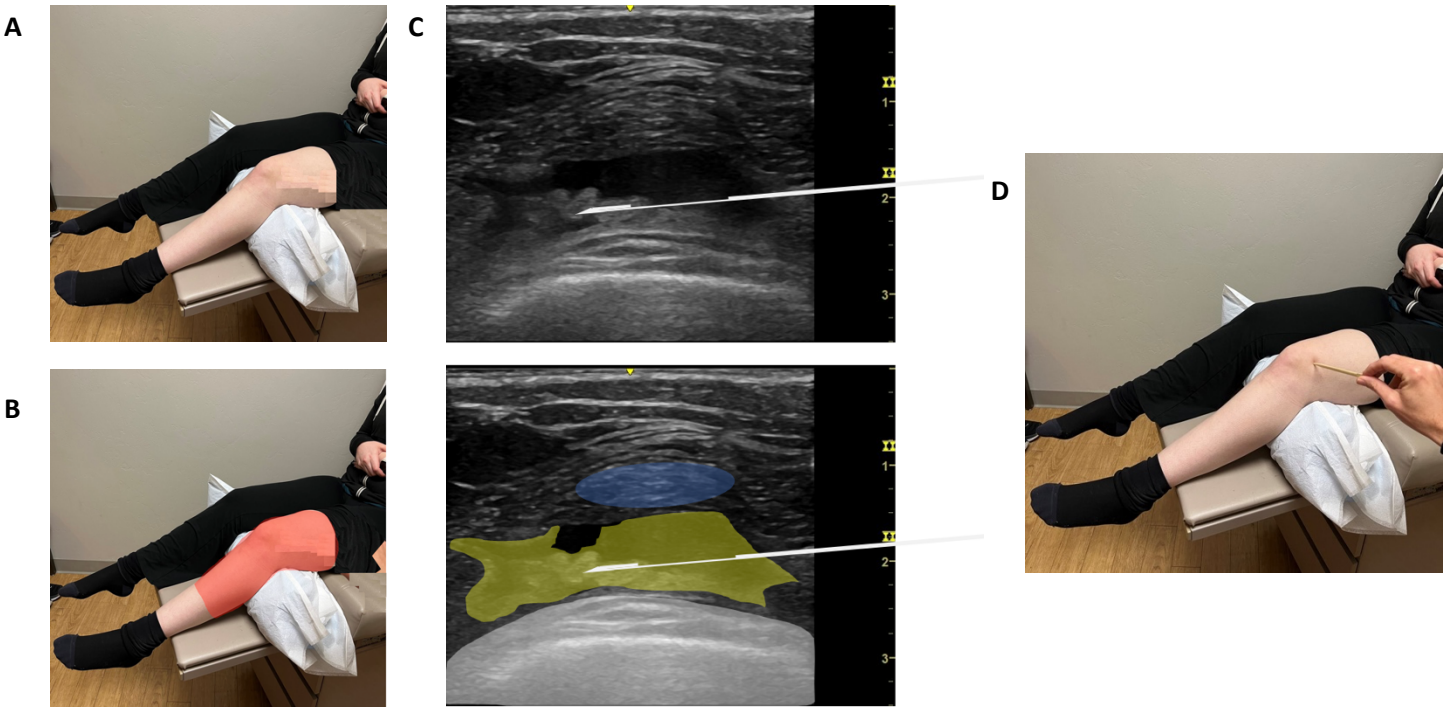

Figure 1: Ultrasound-guided knee biopsy

(A) Positioning. (B) Area to be sterilized (pink). (C) Ultrasound for guidance; needle (white line), tendon (blue), synovium (yellow). (D) Insertion of needle.

Orientation and Positioning of the Subject

1. The subject should be reclining at 45 degrees (with the option of lying supine if the patient prefers) on an appropriate surface during the procedure (Figure 1A).
2. To improve imaging of the supra-patella pouch, patient knee should be slightly flexed (25-30 degrees) (Figure 1A).

Preparation

1. All participating staff with close or direct patient contact (e.g. proceduralist, assistant, ultrasonographer) should commence personal preparations for the procedure which includes hand washing, and wearing double gloves, face mask, hair net/hat, sterile gown, and shoe covers. *Of note, there may significant fluid that comes of the joint upon entry of needle and/or introducer.*
2. Evaluate the equipment tray, including biopsy needles.
3. Place absorbent pads under the knee.
4. Sterilize the skin using appropriate sterilization fluid. A wide field should be sterilized more than the immediate area of interest (e.g. mid-thigh to mid-calf, both anteriorly and posteriorly) (Figure 1B).

5. Position the sterile drapes below and above the knee creating a sterile field sufficient for access to the supra-patella pouch and placement of the US probe for the purposes of imaging.
6. The ultrasonographer should place the US probe within the sterile sheath. US gel should be placed within the sheath, and the probe slowly lowered into the sheath. The upper end of the sheath should be secured with a sterile tie or elastic band usually provided with the sheath. *Of note, the sterile ultrasound gel should never come in direct contact with the subject's skin as this can damage the tissue.*
7. Prepare 3 syringes to be introduced into the skin and synovial space later (i.e. two syringes with 10 mL 1% lidocaine, and one syringe containing 10 mL normal saline). *Have the sterile source of saline present on the operative field in case more saline is needed.*
8. US examination of the lateral aspect of the knee should indicate a suitable area for needle insertion distal to the vastus lateralis muscle insertion into the patella (Figure 1C-D).

Biopsy Procedure

1. Using the initial US scan, determine point of insertion and inject 5 – 10 mL 1% lidocaine into the subcutaneous and deep tissue. Leave a minimum of 5 minutes for an effect.
2. Use US guidance and with 18G needle aspirate as much fluid as possible from the supra-patellar pouch. *If there is less than 0.3 mL synovial fluid present, proceed to Step 4*.
3. Disconnect the syringe leaving the needle in situ. Dispense the aspirated synovial fluid in 4ml green-top sodium heparin (NaHep) tube for further processing (Section 8.3).
4. Introduce 10mL 1% lidocaine into the joint, then introducing normal saline in order to give a clear view to subsequently insert the coaxial introducer and clearly identify the synovial tissue. *This step may be altered according to the size of the patient and volume of synovial fluid present (e.g. some knee joints may need more or less amounts of normal saline in order to expand the suprapatellar pouch)*.
5. Use US guidance and introduce the biopsy coaxial introducer into the supra-patellar pouch. Once the introducer is in place, withdraw the sharp obturator. The biopsy needle should be primed before its introduction into the synovial space.
6. The needle should be extended, and the throw identified on the US images. The throw of the needle should be placed against the surface of the synovium to maximize the opportunity for capturing the lining layer. Gentle pressure should be placed on the needle to oppose the throw and synovium. *Be mindful that if the tip of the extended needle is abutting a bony surface, backwards movement of the needle will occur at this stage with poor retrieval of tissue*.
7. When enough specimens are harvested, the remaining fluid should be aspirated, and up to 50 ml of normal saline (in units of 10 mL) should be used to briefly wash the joint clearing any debris or blood. Continue until any blood staining is obviously reducing.

7.2.2. Ankle

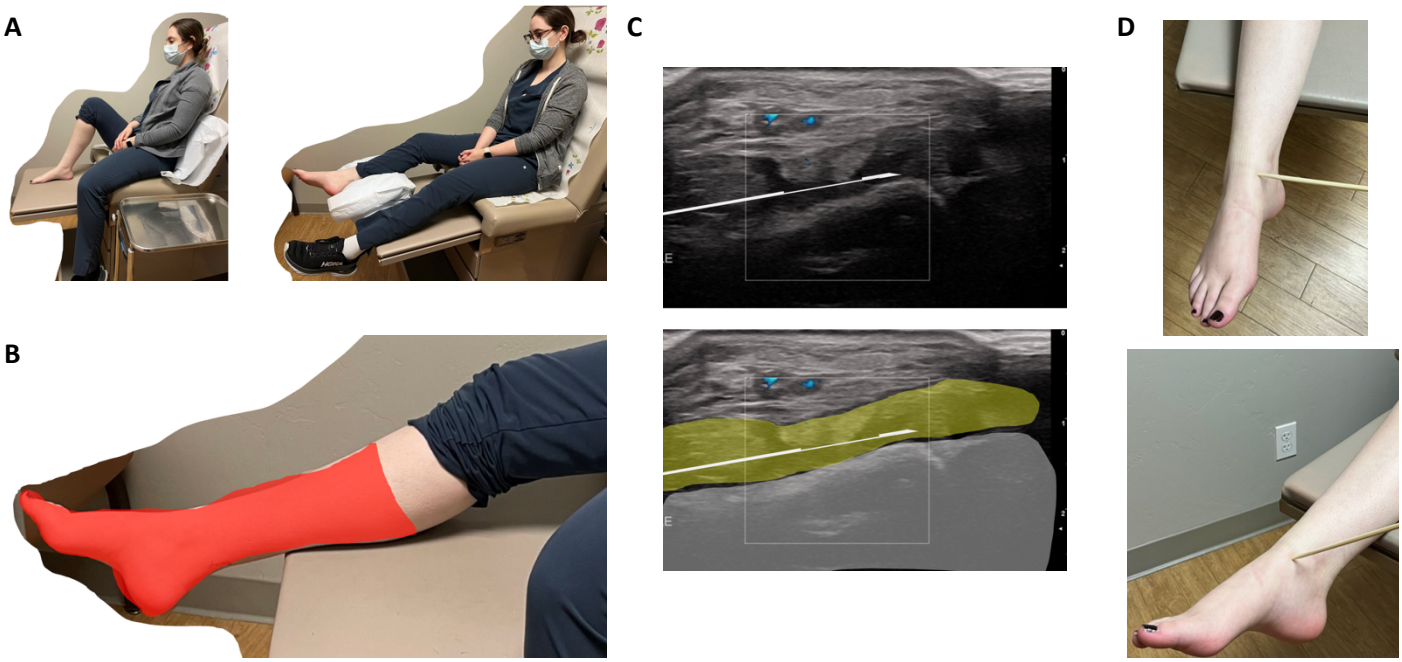

Figure 2: Ultrasound-guided ankle biopsy

(A) Positioning. (B) Area to be sterilized (pink). (C) Ultrasound for guidance; needle (white line), doppler (light blue), synovium (yellow). (D) Insertion of needle.

Orientation and Positioning of the Subject

1. Position the subject comfortably semi-recumbent or supine, with biopsy joint raised on pillow so that medial (inner) aspect is raised above contralateral limb (Figure 2A).

Preparation

1. All participating staff with close or direct patient contact (e.g. proceduralist, assistant, ultrasonographer) should commence personal preparations for the procedure which includes hand washing, and wearing gloves, face mask, hair net/hat, and sterile gown.
2. Evaluate the equipment tray, including biopsy needles.
3. Place absorbent pads under the ankle.
4. Sterilize the skin using appropriate sterilization fluid. A wide field should be sterilized in excess of the immediate area of interest (e.g. toes to mid-calf, both anteriorly and posteriorly) (Figure 2B).
5. Position the sterile drapes above, below, medial and lateral to the ankle leaving sufficient space for access to the tibio-talar joint and placement of the US probe for the purposes of imaging.
6. The ultrasonographer should place the US probe within the sterile sheath. US gel should be placed first upon the probe foot-print and the probe slowly lowered into the sheath. The upper end of the sheath should be secured with a sterile tie or elastic band usually provided with the sheath.

7. Prepare 3 syringes to be introduced into the skin and synovial space later (i.e. two syringes with 10 mL 1% lidocaine, and one syringe containing 10 mL normal saline).
8. US examination of the medial aspect of the ankle should indicate a suitable area for needle insertion deep to the tibialis anterior tendon, aiming laterally across the joint in line with the tibiotalar joint line (Figure 2C-D).

Biopsy Procedure

1. Using the initial US scan, determine point of insertion and inject 3 – 5 mL 1% lidocaine into the subcutaneous and deep tissue. Leave a minimum of 5 minutes for an effect.
2. Use US guidance and with a 21G needle aspirate as much fluid as possible from the joint. *If there is less than 0.3 mL synovial fluid present, proceed to Step 4.*
3. Disconnect the syringe leaving the needle in situ. Dispense the aspirated synovial fluid in 4mL green-top sodium heparin (NaHep) tube for further processing (Section 8.3).
4. Introduce 5—10 mL 1% lidocaine, allowing 5 minutes to take effect. Normal saline can be added to enable a better image to be acquired during the procedure and facilitate clear identification of synovial tissue.
5. Use US guidance and introduce the biopsy coaxial introducer into the joint space. Once the introducer is in place, withdraw the sharp obturator. The biopsy needle should be primed before its introduction to the synovial space.
6. The needle should be extended, and the throw identified on the US images. The throw of the needle should be placed against the surface of the synovium to maximize the opportunity for capturing the lining layer. Gentle pressure should be placed on the needle to oppose the throw and synovium. *Be mindful that if the tip of the extended needle is abutting a bony surface, backwards movement of the needle will occur at this stage with poor retrieval of tissue*.
7. When enough specimens are harvested, the remaining fluid should be aspirated, and 10 ml of normal saline should be used to briefly wash the joint clearing any debris or blood.

7.2.3. Wrist

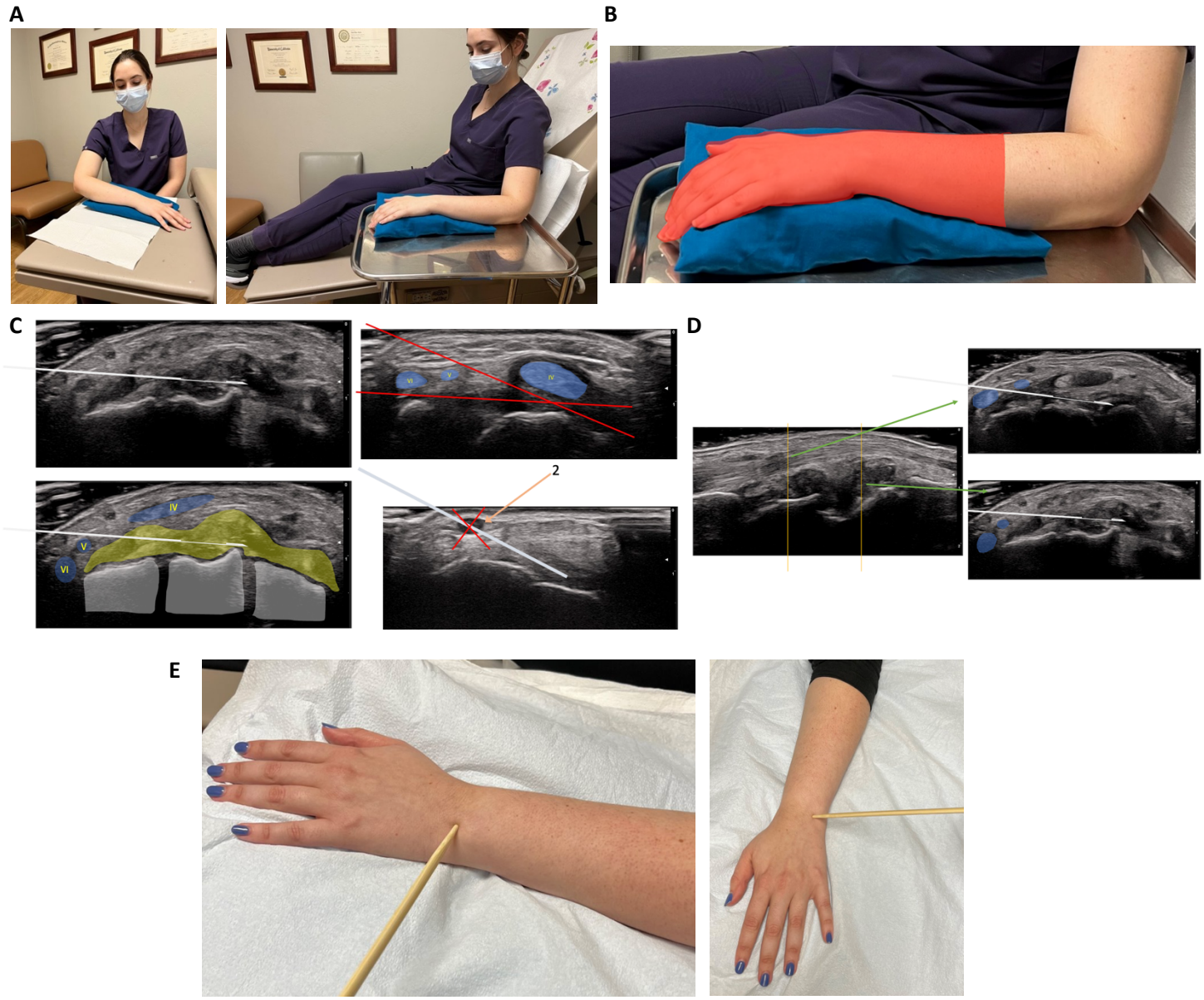

Figure 3: Ultrasound-guided wrist biopsy

(A) Positioning. (B) Area to be sterilized (pink). (C) Ultrasound for guidance; needle (white line), tendon (blue, with designated tendon groups IV-VI), synovium (yellow), possible angles of needle insertion to avoid tendons (red line), avoid superficial veins (red X). (D) Possibility of accessing the radio-carpal or mid-carpal joints. (E) Insertion of needle.

Orientation and Positioning of the Subject

1. The subject should either be in a seated position, reclining at 45 degrees, or lying supine per patient preference on an appropriate surface, with the hand placed on a table or dedicated adjacent surface. The hand should be pronated and resting in front of the patient, so that the ulnar wrist is facing away from the patient (Figure 3A).

2. Care should be taken not to elevate the hand or abduct the shoulder significantly as this will cause patient discomfort during the procedure. The hand should be place with the palm downwards.

Preparation

1. All participating staff with close or direct patient contact (e.g. proceduralist, assistant, ultrasonographer) should commence personal preparations for the procedure which includes hand washing, and wearing gloves, face mask, hair net/hat, and sterile gown.
2. Evaluate the equipment tray, including biopsy needles.
3. Place absorbent pads under the wrist.
4. Sterilize the skin using appropriate sterilization fluid. A wide field should be sterilized more than the immediate area of interest (e.g. hand to mid-forearm, dorsal and ventral aspects) (Figure 3B).
5. Position the sterile drapes below the wrist on the table and use a sterile drape as a cuff at the mid forearm. The wrist should be placed in slight palmar flexion to improve access and identification of the synovial recesses. This can be facilitated by placing a few sterile sections of gauze under the wrist.
6. The ultrasonographer should place the US probe within the sterile sheath. US gel should be placed first upon the probe foot-print and the probe slowly lowered into the sheath. The upper end of the sheath should be secured with a sterile tie or elastic band usually provided with the sheath.
7. Prepare a syringe with 10 mL 1% lidocaine.
8. US examination of the wrist should be performed prior to the biopsy to identify the extensor tendons of the 4th, 5th and 6th compartments, the scaphoid lunate junction and suitable synovial tissue. A suitable path should be planned as the normal anatomical relations may be disturbed in chronic Rheumatoid Arthritis. A suitable path can usually be identified inserting the needle superior to the Extensor Carpi Ulnaris (ECU) tendon and passing inferior to the Extensor Digiti Quinti Proprius (EDQ) and Long flexors (Extensor Digitum longus). The typical region for biopsy lies just superior to the scaphoid lunate ligament (Figure 3C-E).
9. A piece of sterile gauze may be placed under the wrist to elevate the joint to facilitate better access and maneuverability when performing the biopsy procedure.

Biopsy Procedures

1. Using the initial US scan, determine point of insertion and inject 2 – 5 mL 1% lidocaine into the skin and subcutaneous tissue. Leave a minimum of 3-5 mins for an effect.
2. Use US guidance and with 19G needle aspirate as much fluid as possible. *If there is less than 0.3 mL synovial fluid present, proceed to Step 4.*
3. Disconnect the syringe leaving the needle in situ. Dispense the aspirated synovial fluid in a 4 mL green-top sodium heparin (NaHep) tube for further processing (Section 8.3).

4. Inject 2 – 5 mL 1% lidocaine into the synovial recess where biopsy is planned, allowing 5 minutes to take effect.
5. Use US guidance to introduction of the biopsy coaxial introducer into the wrist. This is best achieved by imaging the needle along its long axis.
6. The needle should be extended, and the throw identified on the US images. The throw of the needle should be placed against the surface of the synovium to maximize the opportunity for capturing the lining layer. Gentle pressure should be placed on the needle to oppose the throw and synovium. *Be mindful that if the tip of the extended needle is abutting a bony surface, backwards movement of the needle will occur at this stage with poor retrieval of tissue.*
7. When enough specimens are harvested, the remaining fluid should be aspirated.

7.3. Aftercare

1. Apply bacitracin ointment at biopsy site, covered with 4”x4” gauze, and wrapped with Coban.
2. A neurovascular assessment should be made of the respective extremities following the procedure and documented in the patient’s notes.
3. The subject should remain in the department for a minimum of 30 minutes post procedure.
4. The subject should change dressing in the same manner as initially applied, daily and after getting wet (e.g. after shower) for 3 consecutive days after the biopsy.
5. Post-biopsy risks include but are not limited to: pain, soreness, redness, swelling, pseudogout flares, hamarthrosis. These risks and contact details in case of complications should be provided to the subject. Specifically, subjects should be asked to contact the team if there is significant pain, swelling or redness of the biopsied joint within the next 5-7 days.

7.4. Safety Reporting

Adverse events will be reported and tracked as required in the clinical study protocol and defined by Common Terminology Criteria for Adverse Events (CTCAE).

8. PROCESSING OF SPECIMENS

8.1. Overview

The following specimen types will be collected (see Figure 4):

- Synovial fluid: Processed by centrifugation to obtain supernatant and pelleted cell samples
- Synovial tissue: Fragments will be divided between formalin- or freezing medium-containing vials per specific guidelines below

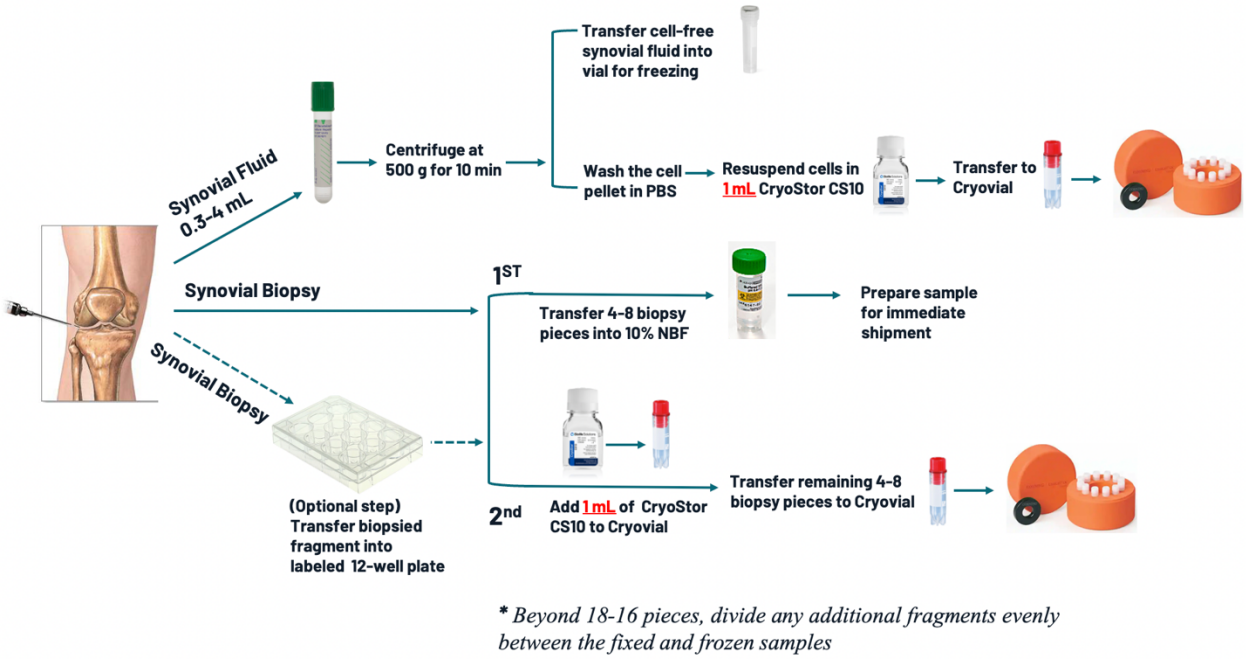

Figure 4: Tissue Processing Workflow

8.2. Equipment and Supplies for Tissue Processing

Required equipment and supplies needed for tissue processing include:

- Visit-specific Biopsy kit (**provided by sponsor**)
  - Green-top sodium heparin (NaHep) tubes
  - 2.0 mL Cryovials
  - Formalin-filled vials
- Bulk supplies (**provided by sponsor**)
  - CryoStor CS10 media (stored at 4°C)
  - CoolCell Controlled-rate freezing container (stored at 4°C)
  - Make sure the core (black ring) is at room temperature and seated in the bottom of the central cavity.
  - 12 well plate (for optional use)
  - Sterile forceps (for optional use)
  - Sterile pipettes for transfer of synovial fluid and cell resuspension medium
- Additional supplies needed (not provided by sponsor)
  - Tabletop centrifuge
  - PBS (4°C)

8.3. Processing of Synovial Fluid

1. The **Biopsy Visit Requisition Form** should be filled out completely by the proceduralist, who should note the date and time if synovial fluid is collected.
2. If arthrocentesis is done, synovial fluid should be collected as outlined in Section 7.2.

3. The fluid should be transferred from the collecting syringe into a 4.0 mL green-top sodium heparin (NaHep) tube. If more than 4.0 mL are collected, please discard extra fluid.
4. The green top tube should be inverted 10X, then centrifuged at room temperature at 500 g for 10 minutes within 2 hours of collection.
5. The supernatant should be transferred using a transfer pipette into new and labeled cryovials (provided in the kit) in 2 mL aliquots and placed at –80°C.
    a. If **0.3 mL to 4 mL** is acquired, split the total volume between two tubes.
    b. If **less than 0.3 mL** is acquired, freeze all the synovial fluid in one vial.
6. The cell pellet should be resuspended in 2 mL cold PBS using a transfer pipette. Centrifuge at room temperature at 500 g for 10 minutes. The new cell pellet should be resuspended using transfer pipettes in 1 mL cold CryoStor CS10 media (provided in the kit) and immediately transferred to 2.0 mL cryovial tube (Cryovial provided in the kit, **please use provided labels only**).
    a. Keep PBS and CryoStor CS10 media at 4°C until required for immediate use.
7. The controlled-rate freezing container (provided by the sponsor) should be used for viable freezing of resuspended cells. Make sure the core (black ring) is at room temperature and seated in the bottom. Place the labelled sample vial containing resuspended cells in an empty well, and then fill empty wells with dummy vials containing freezing medium so that each well contains a filled vial (Filler/dummy vials provided).
8. Place the CoolCell container at -80°C for at least 24 hours. Samples should then be removed from the CoolCell container and shipped to the central lab (MRL) on dry ice within 7 days post-freezing.

8.4. Processing of Synovial Tissue

1. The **Biopsy Visit Requisition Form** should be filled out completely by the Proceduralist, who should note the date and time of synovial tissue biopsy (see Lab Manual).
2. Prior to start of procedure, pipette 1 mL CryoStor CS10 media (provided in the kit) into a 2.0 mL cryovial tube (Cryovial provided in the kit, **please use provided labels only**).
3. Upon removal of each synovial tissue fragment, the proceduralist can **EITHER**:
    a. Place fragments directly into the vial with 10% neutral-buffered formalin (FFPE Synovial Biopsy) (with minimum and maximum number of core fragments depending on size of joint, see Section 8.5), and then place the remaining biopsy samples in CryoStor CS10 medium (Cryo Synovial Biopsy) (with minimum and maximum number of core fragments depending on size of joint, see Section 8.5); ***OR***
    b. Place individual fragments into individual wells of the 12-well plate (provided by the sponsor), with each well filled with sterile saline or PBS to maintain hydration. Then with sterile forceps, transfer fragments into the vial with 10% neutral-buffered formalin (FFPE Synovial Biopsy) (with minimum and maximum number of core fragments depending on size of joint, see Section 8.5), and then place the remaining biopsy

samples in CryoStor CS10 medium (Cryo Synovial Biopsy) (with minimum and maximum number of core fragments depending on size of joint, see Section 8.5).

4. Record numbers of biopsies taken on the Biopsy Visit Requisition Form (see Lab Manual).

8.5. Specimen Storage

Large Joints (Knee/Ankle):

	Intended analysis	Number of fragments	Format	Process
1	Fixed tissue (FFPE)	6 – 8[A]	Loose in 10% formalin in a pre-filled vial	Place tissue directly into pre-filled vial
2	Frozen tissue for subsequent disaggregation (Cryo)	6 – 8[A]	Loose in CryoStor CS10 medium in a cryovial	Place tissue into 2.0 mL cryovial with 1 mL cold Cryostor medium

[A] Allocate 6 fragments first for fixation and then allocate 6 fragments for freezing. Divide any additional fragments evenly between the fixed and frozen samples. Collect up to 16 fragments in total (even if using Portal and Forceps method, which would typically generate 20-30 fragments).

Small Joints (Wrist):

	Intended analysis	Number of fragments	Format	Process
1	Fixed tissue (FFPE)	4-6[B]	Loose in 10% formalin in a pre-filled vial	Place tissue directly into pre-filled vial
2	Frozen tissue for subsequent disaggregation (Cryo)	4-6[B]	Loose in CryoStor CS10 medium in a cryovial	Place tissue into 2.0 mL cryovial with 1 mL cold Cryostor medium

[B] Allocate 4 fragments first for fixation and then allocate 4 fragments for freezing. Divide any additional fragments evenly between the fixed and frozen samples. Collect up to 12 fragments in total.

8.6. FFPE Tissue

1. Remove tissue fragments from saline and promptly transfer to the pre-filled vial (FFPE biopsy) containing 10% neutral-buffered formalin. Ensure the vial is labelled per the kit instructions using provided labels only.
2. Ship tissue to the processing lab using the instructions provided in the lab manual on the day of collection to ensure that fixation is not prolonged past 48 hours from sample collection.

8.7. Frozen Tissue Disaggregation

1. Mince larger tissue fragments with a sterile scalpel until pieces are approximately 1mm in length.
2. For each set of fragments, use forceps and place into a single cryovial (this works best if you add them all to one side of the cryovial) and add 1 mL of cold CS10 freezing medium.
3. Gently shake or flick the vial to manually separate tissue pieces and move off the side of the tube into the freezing medium. Place the tube on ice for 10 min.
4. Make sure the CoolCell container core (black ring) is at room temperature and seated in the bottom. Place one filled sample vial per well, and then fill any empty wells with dummy vials containing freezing medium.
5. Place the CoolCell container at -80°C for at least 24 hours. Samples should then be removed from the CoolCell container and shipped to the central lab (MRL) on dry ice within 7 days post-freezing.

9. TRANSPORT OF SPECIMENS

Please refer to the Lab Manual for detailed information about:

- Completion of the **Biopsy Visit Requisition Form**
- Equipment and supplies for transport of respective specimens
- Shipping instructions