

2023

MDACC U24 Web Resource SOP

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1.0 Animal Protocols – Implantation and Cryopreservation of Tissue for PDX Generation

1.1 Introduction/Safety

This SOP describes the procedures for patient-derived xenograft (PDX) tissue preparation, and cryopreservation of materials under BSL-2 safety criteria. This SOP is used/performed by the H. Charles Manning laboratory (HCM) at MD Anderson Cancer Center for Cancer Research (MDACC).

HCM treats all PDX tissue and mice carrying PDX tumors. Mice are housed in barrier facilities using the full micro-isolator technique with all work, including husbandry, provided by experienced technical staff. The mouse strains used are the NOD.Cg-Prkdcscid112rgtm1Wjl/SzJ (NSGTM), which are highly susceptible to infection due to their profound immunodeficiency and Nude with a genetic mutation that causes a deteriorated or absent thymus. All materials coming into the barrier facilities are decontaminated by autoclaving, or chemical means including the use of chlorine dioxide gas and Virkon[®] dip tanks for non-autoclavable items. All *in-vitro* work complies with the BSL-2 safety criteria.

1.2 Materials & Equipment

1.2.1 Implant Materials & Equipment

Sterile scalpel, forceps, scissors, sterile petri dishes, 11-gauge Trocar (tissue implant needle), Buprenorphine ER (0.5 mg/mL), NOD.Cg-Prkdcscid112rgtm1Wjl/SzJ (NSG) mice, Isoflurane and anesthesia machine.

1.2.2 Alternate Implant Methodology

Sterile PBS, sterile water, heating pad, wound closure sterile staples or sutures.

1.2.3 Cryopreservation Materials & Equipment

- Piptman 100 uL and sterile tips
- 2 mL Cryovials and tube holder
- Sterile scalpel, forceps and scissors
- Freeze medium: Medium 199 (gibco, 11150-059) containing 20% fetal bovine serum (FBS), 10% DMSO, and 1% penicillin-streptomycin (PS)
- Tumor material freshly harvested from PDX-bearing mice in PBS, maintained at 4°C by placing in sterile petri dish on ice.
- Bucket of ice
- Stepped Rate Cryopreservation Freezer*

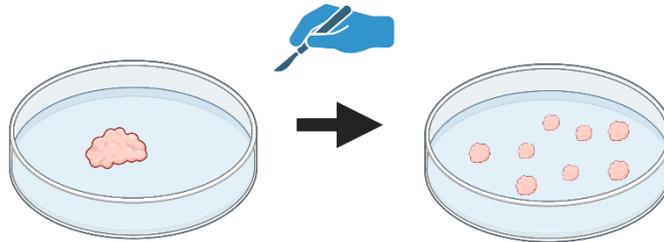
*Isopropanol-based freeze container can also be used for slow-rate freezing if a stepped rate freezer is not available.

1.3 Generation of Subcutaneous PDS in Immunocompromised Mice

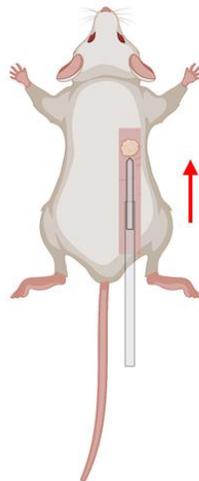
Animals should be ready for implantation immediately following tissue preparation. All tissue preparation should be performed in a Biological Safety Cabinet (BSC) using sterile instruments and techniques.

1.3.1 Steps for Implantation

- Shave the implantation site prior to anesthesia.
- Anesthetize a sex-matched NSG mouse by isoflurane inhalation until non-responsive to toe-pinch. Keep mouse warm using a heating pad and monitor breathing during procedure.
- Inject Buprenorphine ER (0.5mg/mL) into neck skin subcutaneously to relieve pain/discomfort.
- Tumor dissociation: Using sterile scalpels, cut patient tumor tissue or tumor tissue from PDX-bearing mice in proper size (~4mm)



- Disinfect implantation site with 70% Isopropyl alcohol swab (BD, 326895).
- Using scissors, make a small incision (3~4 mm) in the inguinal area (for standard SC implants placed in the posterior axillary area) and insert the Trocar through the skin opening.
- Slide the Trocar craniodorsally to about 0.5 cm caudal to the axillary area; the Trocar should slide smoothly under the skin.
- Inject the tissue subcutaneously. Forceps can be used over the skin to hold the fragment in place during trocar withdrawal.



- Close the incision using wound clips.
- Keep mice warm and monitor breathing until awakened from anesthesia.

1.4 Orthotopic Liver PDX Implantation

WILL BE AVAILABLE BY 5/1/23.

1.5 Cryopreservation of PDX Material

1.5.1 Tumor Preparation for Cryopreservation

- In a BSC, harvest the tumor into a sterile petri dish containing a small volume of the freeze media.
- Using a sterile scalpel, cut the tissue into ~3-4 mm³ fragments.
- With the cryovials in a tube rack held on ice, add 5-10 fragments to each tube then add 1 mL of the freeze medium to each tube without exceeding the tube fill volume.
- Apply the cap to the cryovials, seal well. Wipe the exterior with disinfectant then place into wet ice until ready to begin stepped-rate freezing.

1.5.2 Slow-rate Freezing (Isopropanol-based)

- PDX material should be held on wet ice in cryovial tubes until ready for placement into the slow-rate freeze container.
- The base of the cryo-container is filled with isopropanol per the manufacturer's recommendation and the tube holder is placed on top.
- Transfer the cryovials filled with fragments/freeze media from the ice-bucket into the tube holder of the cryo-container, screw the lid securely onto the cryo-container, and place at -80°C for a minimum of 4 hrs, but most commonly for overnight.

1.5.3 Observation Process

- Mice are weighed 1-3 times per week as a gauge of animal health and more frequently if clinical signs are noted.
- Mice are observed at least once daily, and tumor growth is monitored with caliper measurements at least once weekly. The tumor volume (mm³) is calculated using the following formula¹

$$Tumor\ volume = \frac{(tumor\ length) \times (tumor\ width)^2}{2}$$

- Potential growth of P0 (first passage in mouse) tumors is monitored for up to 300 days after implantation. Mice not showing progressive tumor growth at 300 days post- implant are euthanized.
- Antemortem determination for harvesting tumor tissues is based upon tumor size as well as clinical signs. Animals without palpable tumors are evaluated clinically and by monitoring body weights.

1.6 Generation of Humanized Mouse Model

WILL BE AVAILABLE 5/1/23

1.7 Generation of Subcutaneous PDS in Immune-Competent Humanized Mice

WILL BE AVAILABLE 5/1/23

2.0 RNA-Sequence Protocols

2.1 Tissue Processing for RNA

2.1.1 Tumor Harvest

- 1) Euthanize mice according to institutional guidelines, typically by sedation with 2.5% Isoflurane, followed by asphyxiation and physical confirmation of death by cervical dislocation.
- 2) Dislocation.
- 3) Harvest tumors using DEPC treated scissors and forceps
- 4) Wash tumors in cold PBS quickly
- 5) Place tumors in 6 cm dish on ice
- 6) Cut tumors into slices less than 0.5 cm thick as quickly as possible

2.1.2 Stabilization of RNA in Harvest Tumor Tissues

- 1) Before excising the tumor, estimate the volume (or weight) of the tumor to be stabilized in RNAprotect Tissue Reagent (Qiagen). At least 10 volumes of the reagent is required. Approximately 10 μ l reagent per 1 mg of tumor tissue. Pipet the correct amount of reagent into an appropriate collecting vessel.
- 2) Completely submerge the tumor pieces in the collection vessel containing RNAprotect Tissue Reagent.
- 3) For archival storage at -80°C , first incubate the tissue overnight in the reagent at 2-8 $^{\circ}\text{C}$, then remove the tissue from the reagent and transfer it to -80°C for storage.

2.1.3 Purification of Total RNA from Tumor Tissues (Based on Qiagen RNeasy Mini Kit)

- 1) Remove RNA protect stabilized tissue from the reagent using forceps. Determine the amount of tissue. Do not use more than 30 mg.
- 2) Place the tissue directly into a suitably sized vessel for disruption and homogenization.
- 3) Disrupt the tissue and homogenize the lysate in 600 μ l of Buffer RLT.
- 4) Immediately disrupt and homogenize the tissue using a conventional rotor-stator homogenizer until it is uniformly homogeneous (usually 20–40 s).
- 5) Centrifuge the lysate for 3 min at full speed. Carefully remove the supernatant by pipetting and transfer it to a new microcentrifuge tube. Use only this supernatant (lysate) in subsequent steps.
- 6) Add 1 volume of 70% ethanol (350 or 600 μ l) to the cleared lysate and mix immediately by pipetting. Do not centrifuge. Proceed immediately to next step.

- 7) Transfer up to 700 μ l of the sample, including any precipitate that may have formed, to and RNeasy spin column placed in a 2 ml collection tube. Close the lid gently, and centrifuge for 15 s at $\geq 8000 \times g$ ($\geq 10,000$ rpm). Discard the flow-through.
- 8) Add 700 μ l Buffer RW1 to the RNeasy spin column. Close the lid gently, and centrifuge for 15 s at $\geq 8000 \times g$ ($\geq 10,000$ rpm) to wash the spin column membrane. Discard the flow-through.
- 9) Place the RNeasy spin column in a new 2 ml collection tube (supplied), and discard the old collection tube with the flow-through. Close the lid gently, and centrifuge at full speed for 1 min.
- 10) Place the RNeasy spin column in a new 1.5 ml collection tube (supplied). Add 50 μ l RNase-free water directly to the spin column membrane. Close the lid gently, and centrifuge for 1 min at $\geq 8000 \times g$ ($\geq 10,000$ rpm) to elute the RNA.

2.1.4 Assessing Yield and Quality of Total RNA

- 1) The ratio of A_{260}/A_{280} value is a measure of RNA purity, and it should fall in the range of 1.8 to 2.1.
- 2) RNA Integrity Number will be measured using RNA 6000 Nano LabChip Kit (Agilent, CA, USA) and RIN number >7.0 will be for RNA-seq.

2.2 Sequencing of Samples of RNA

2.2.1 Poly(A) mRNA Isolation by using Invitrogen Magnetic Beads

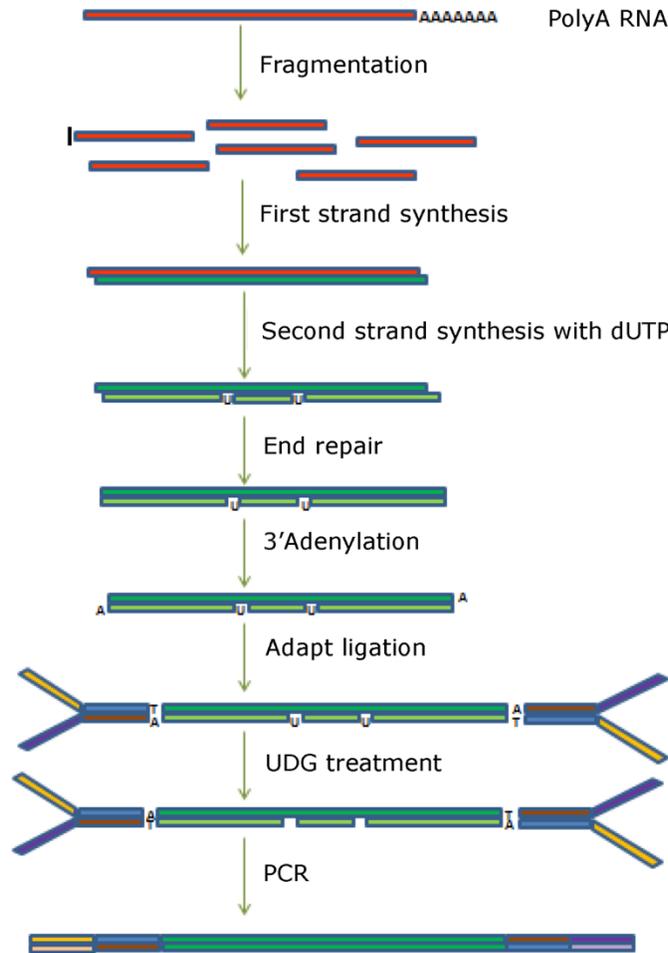
- 1) Prepare the Oligo(dtT) Magbeads.
- 2) Bind to Oligo(dtT) Magbeads.
- 3) Wash the Oligo(dtT) Magbeads.
- 4) Recover the poly(A) RNA and dissolve the RNA in 20-50 μ l THE RNA Storage Solution
- 5) Store the dissolved RNA at -70°C

2.2.2 Library Construction and Sequencing

- 1) RNA fragmentation: Fragment the RNA using RNA fragmentation reagents (NEBNext Magnesium RNA Fragmentation Module) to generate short fragments (about 200-300 bp).
- 2) cDNA synthesis: Synthesize first-strand cDNA using reverse transcriptase and random primers. Then, synthesize second-strand cDNA using DNA polymerase I, RNase H, and dUTP instead of dTTP to generate a double-stranded cDNA molecule with a blunt end and a defined strand orientation.
- 3) End repair and dA-tailing: End repair the double-stranded cDNA using a DNA end repair kit (NEBNext End Repair/dA-Tailing Module). Then, add a single 'A' base to the 3' end of the cDNA using Klenow fragment (3' to 5' exo-). Adapter ligation: Ligate sequencing adapters to the cDNA fragments using a ligation kit (NEBNext Ultra II DNA Library Prep Kit) to add unique indexes to each library for multiplexed sequencing. The adapters provide the primer binding sites needed for bridge amplification during cluster generation on the sequencer.
- 4) Size selection and PCR amplification: Select cDNA fragments of the desired size range (e.g., 200-300 bp) using gel electrophoresis or a bead-based size selection

method (AMPure XP beads). Amplify the size-selected cDNA fragments using PCR with primers that anneal to the adapter sequences.

- 5) Library quantification and quality control: Quantify the library using a fluorometer (Qubit) and assess its quality using a Bioanalyzer or similar tool.
- 6) Perform paired-end 2×150bp sequencing on an Illumina HiSeq 4000 and following the vendor's recommended protocol. (Sequence from both ends of the cDNA fragment to generate two overlapping reads).



Library construction and sequencing

2.3 Data Analysis for RNA

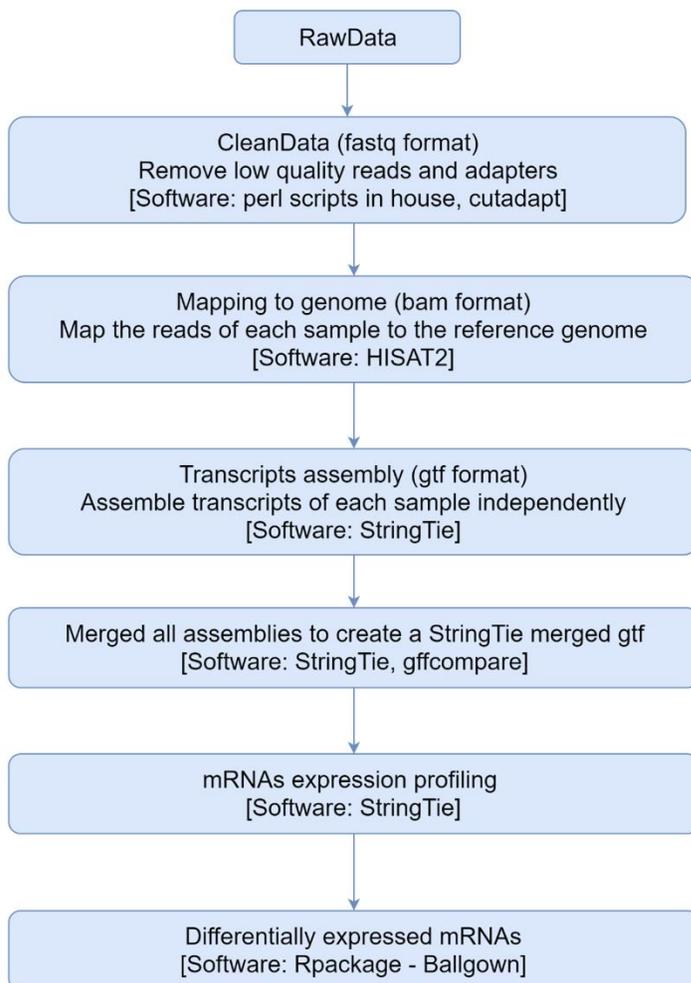
2.3.1 Transcripts Assembly

- 1) Cut, adapt and perl scripts in house were used to remove the reads that contained adaptor contamination, low quality bases and undetermined bases.
- 2) Sequence quality was verified using FastQC (<http://www.bioinformatics.babraham.ac.uk/projects/fastqc/>)
- 3) Used HISAT2[2] to map reads to the genome of *Oryza sativa* (Version: RGAP 7).

- 4) The mapped reads of each sample were assembled using StringTie.
- 5) All transcriptomes from 6 Samples were merged to reconstruct a comprehensive transcriptome using perl scripts and gffcompare (<https://github.com/gpertea/gffcompare/>).
- 6) After the final transcriptome was generated, StringTie[3] and Ballgown[4] was used to estimate the expression levels of all transcripts.

2.3.2 Different Expression Analysis of mRNAs

- 1) StringTie[3] was used to perform expression level for mRNAs by calculating FPKM ($FPKM = \frac{\text{total_exon_fragments}}{\text{mapped_reads(millions)} \times \text{exon_length(kB)}}$).
- 2) The differentially expressed mRNAs were selected with \log_2 (fold change) >1 or \log_2 (fold change) <-1 and with parametric F-test comparing nested linear models (p value < 0.05) by R package Ballgown.



Bioinformatics pipeline for RNA sequencing

3.0 Pathology Protocols

3.1 Tissue Processing for Histology

3.1.1 Tissue Harvest and Fixation'

- 1) Euthanize mice according to institutional guidelines, typically by sedation with 2.5% Isoflurane, followed by asphyxiation and physical confirmation of death by cervical dislocation.
- 2) Harvest tissue using cleaned scissors and forceps
- 3) Wash tissue in cold PBS
- 4) Cut tissue into slice about 2-4mm thick
- 5) Place tissue into 4% paraformaldehyde/PBS solution, fix tissue O/N at 4°C

3.1.2 Tissue Dehydration

- 1) Move tissue from 4% paraformaldehyde/PBS solution and place tissue into 70% ethanol for 1 h.
- 2) Place tissue into 80% ethanol for 1h
- 3) Place tissue into 95% ethanol for 1h
- 4) Repeat step 3 once, using fresh 95% ethanol
- 5) Place tissue into 100% ethanol for 1h
- 6) Repeat step 5 twice and using fresh 100% ethanol

3.1.3 Tissue Clearing

- 1) Remove tissue from 100% ethanol and place tissue into Xylene for 30 min
- 2) Repeat step 1 once

3.1.4 Tissue Infiltration and Embedding

- 1) Place tissue into 60°C paraffin for 1h
- 2) Repeat step 1 once and using fresh paraffin
- 3) Place tissue in properly oriented in a plastic mold filled with liquid paraffin
- 4) Place pathology cassette on top of the mold and completely fill the mold (The paraffin will solidify in 10-15 minutes at RT)
- 5) Remove embedded sample from the mold
- 6) Store at room temperature until ready to section

3.1.5 Sectioning

- 1) Paraffin embedded tissue blocks will be cut 5µM by using microtome
- 2) Sectioned slides will be warmed in a 60°C oven for 1-2h

3.2 Histological Staining of Samples

3.2.1 Hematoxylin and Eosin (H&E) Staining

- 1) Deparaffinize and hydrate paraffin embedded tissue sections
 - a). Xylene I 15 min
 - b). Xylene II 15 min
 - c). 100% ethanol I 5 min
 - d). 100% ethanol II 5 min

- f). 95% I ethanol 5 minX2
- e). 95% II ethanol 5 min
- g). 85% ethanol 5 min
- h). 80% ethanol 5 min
- i). 75% ethanol 5 min
- j). 70% ethanol 5 min
- k). water 5 min x 2
- 2) Hematoxylin staining
 - a). Harris Modified Hematoxylin Solution (Sigma, HHS32), 2min,
 - b). Rinse in running tap water 5 min
 - c). 1% HCl in 70% ethanol 4 dips
 - d). Rinse in tap water 2 min
- 3) Eosin staining
 - a). Eosin Y Solution (Sigma, HT110232), 30 sec
 - b). Resin in running tap water 30 sec
- 4) Dehydration and clearing
 - a). 90% ethanol 5 sec
 - b). 95% ethanol 5 sec
 - c). 100% ethanol I 2 min
 - d). 100% ethanol II 2 min
 - e). Xylene I 5 min
 - f). Xylene II 5 min
 - g). Mount sections with Permount Mounting Medium (Fisher Chemical, SP15-500)

3.2.2 Immunohistochemical Staining (IHC)

- 1) Deparaffinize and hydrate paraffin embedded tissue sections
 - a). Xylene I 15 min
 - b). Xylene II 15 min
 - c). 100% ethanol I 5 min
 - d). 100% ethanol II 5 min
 - f). 95% I ethanol 5 minX2
 - e). 95% II ethanol 5 min
 - g). 85% ethanol 5 min
 - h). 80% ethanol 5 min
 - i). 75% ethanol 5 min
 - j). 70% ethanol 5 min
 - k). water 5 min x2
- 2) Antigen retrieval
 - a). Add 750 ml of distill water to pressure cooker (2100 Retriever, Prestige Medical) and 45 ml of antigen retrieval buffer (Sodium citrate buffer, pH 6.0) to slide container
 - b). Heat 120°C 15 min
 - c). Cool the slides to room temperature

- d). Resin slides in distill water 5 min
- 3) Quenching of endogenous peroxidase activity
 - a). Submerge slides with 3% H₂O₂/methanol 30 min at room temperature
 - b). Resin slides in distill water 5 min
 - c). Resin slides in PBS buffer
- 4) Incubate sections with 2.5% normal goat serum 30 min at room temperature
- 5) Tip off excess serum from sections
- 6) Incubate sections with primary antibodies (rabbit or mouse anti-human) diluted in 2.5% normal goat serum/PBS 30 at 4°C O/N
- 7) Wash slides in PBS buffer 5 min X 2
- 8) Incubate sections with diluted biotinylated secondary antibody (Goat anti-rabbit or mouse, Vector Labs)
- 9) Wash slides in PBS buffer 5 min X 2
- 10) Incubate sections with prepared Avidin/Biotin-HRP complex (Vector Labs, PK-4000) 30 min at room temperature
- 11) Wash slides in PBS buffer 5 min X 2
- 12) Incubate in a peroxidase substrate solution (Vector Labs, SK-4105) until desired stain intensity develops
- 13) Rinse in tap water 2 min
- 14) Counterstain with Hematoxylin
 - a). Harris Modified Hematoxylin Solution (Sigma, HHS32), 2min,
 - b). Rinse in running tap water 5 min
 - c). 1% HCl in 70% ethanol 4 dips
 - d). Rinse in tap water 2 min
- 15) Dehydration, clearing and mounting
 - a). 70% ethanol 2 min
 - b). 80% ethanol 2 min
 - c). 90% ethanol 2 min
 - a). 95% ethanol I 2 min
 - b). 95% ethanol II 2 min
 - c). 100% ethanol I 2 min
 - d). 100% ethanol II 2 min
 - e). Xylene I 5 min
 - f). Xylene II 5 min
 - g). Mount sections with Permount Mounting Medium (Fisher Chemical, SP15-500)

4.0 Imaging QA/QC Protocols

4.1 Daily QC MicroPET

Daily detector checks are performed on the PET/CT by placing a Na-22 phantom into the scanner and running a quick scan. The system has an internal program that performs QA checks the scan to make sure each module is working appropriately.

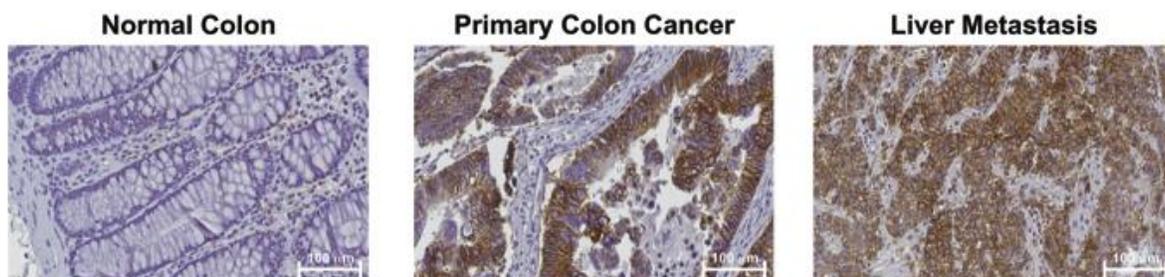
4.2 Annual QC MicroPET

Annual QC checks are performed by the manufacturer. Annual reports and PM are sent to Manager of DI Operations, Department of Imaging Physics. Contact Charles Kinsley @ charles.kinsley@manderson.org.

5.0 PET Imaging Protocols

5.1 Protocol for preclinical ^{18}F -4-fluoro-glutamine PET

ASCT2 Expression in Metastatic CRC. We have recently shown that the Gln transporter ASCT2 is highly expressed in primary colon tumors and liver metastases. Fig. 3 shows representative images of immunohistochemistry (IHC) staining for ASCT2 protein in a tissue microarray (TMA) constructed from archival colon tissue sections collected from 97 colon cancer patients representing primary and metastatic disease in the liver. The left panel consists of a representative micrograph of sections of normal colon tissue from patients with colon cancer which stained negative for ASCT2. The middle and right panels consist of representative micrographs of primary colon cancer sections and matched liver metastases from patients with colon cancer.



5.2 Protocol for preclinical ^{18}F -FSPG PET

Imaging metrics that are common to PET (e.g. SUVmax, peak or mean) as well as pharmacokinetic parameters of ^{11}C -Gln and ^{18}F -FSPG tracer uptake will be determined and associated with several measures. Archival tissue from these patients will be used for genomic analyses. RNAseq data from these tissues will be used to inform a risk-prediction model that can be used to construct a gene signature to then correlate Gln avidity with treatment response. Gln avidity will be defined based on SUVmax. This will allow genomic and Gln PET data to be correlated with treatment-induced changes in tumor volume at standard clinical intervals. This gene signature will be tested preclinically using highly-annotated PDX-derived mouse models.

This study will also allow the acquisition of much needed safety and biodistribution, as this tracer has only been used in a limited number of patients to date. To date, no adverse side effects have been observed. We do not anticipate any toxicity since this tracer is a

naturally-occurring essential amino acid in high abundance and is administered at sub-pharmacologic doses.

A long-term goal of these preliminary studies is to validate the Gln PET imaging metrics and gene signature as predictors of response to EGFR mAb therapy in retrospective and prospective cohorts of patients with Stage IV mCRC via multicenter collaborations

5.3 Protocol for clinical dual tracer ¹¹C-glutamine/¹⁸F-FSPG PET

Objective. The primary objective of this trial is to evaluate the ability of ¹¹C-Gln and ¹⁸F-FSPG PET imaging to predict response to EGFR-targeted therapy in patients with advanced CRC.

Hypothesis. ¹¹C-Gln and ¹⁸F-FSPG PET imaging metrics will be significantly associated with observed changes in tumor size among patients with advanced CRC.

Primary Endpoints. Change in tumor size from baseline to best response among patients with advanced CRC.

Study Design and Analysis Plan. The primary objective of this aim is to determine PET imaging metrics that predict clinical outcomes for patients with advanced CRC. To this end, dual tracer PET imaging with ¹¹C-Gln and ¹⁸F-FSPG will be conducted on 30 patients. We will estimate the imaging metrics' association with the change in tumor size, progression-free survival (PFS) and overall survival (OS) following EGFR-targeted therapy in a cohort of 30 patients with advanced CRC. We will also perform RNA-Seq on archived primary or metastatic CRC tumor tissue(s). From this data, we will create an imaging guided gene signature and estimate this signature's association with the change in tumor size.

6.0 PET Data Analysis Protocols Using Osirix Lite

6.1 Purpose/Scope

This Standing Operating Procedure (SOP) describes the procedures for preclinical PET data analysis. In this SOP, OsiriX¹ is used as a PET/CT image analysis tool. OsiriX is a medical imaging software that is widely used for viewing and analyzing various types of medical images, such as CT scans and MRIs. It was first developed in 2004 by Antoine Rosset and Osman Ratib at the University of Geneva in Switzerland, and has since become a popular tool for medical professionals around the world. OsiriX measures metabolic tumor volume in PET images of solid tumors². This SOP is used/performed by the H. Charles Manning laboratory (HCM) at MD Anderson Cancer Center for Cancer Research (MDACC).

6.2 Related Documents

Source	Title
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https://pixmeo.pixmeo.com/documents/OsiriXUserManualExtract.pdf	OsiriXUserManualExtract
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6.3 Materials & Equipment

6.3.1 MacOS installed PC or Laptop

OsiriX is fully optimized for macOS, and Windows PCs cannot run the software.

6.3.2 Software

OsiriX Lite (Free version) and OsiriX MD (Paid version) are both medical image viewers, but they have some key differences in terms of their features and intended use:

- **Features:** OsiriX Lite is a free, open-source medical image viewer with basic features, while OsiriX MD is a more advanced version with additional features, such as advanced 3D rendering, PET-CT fusion, and more powerful DICOM networking capabilities.
- **Intended use:** OsiriX Lite is intended for non-clinical use, such as research, education, or personal use, whereas OsiriX MD is intended for clinical use by medical professionals. In fact, OsiriX MD is a certified medical device in some countries, which means it meets certain standards and regulations for medical software.
- **Licensing:** OsiriX Lite is free to download and use, while OsiriX MD requires a license fee to use.

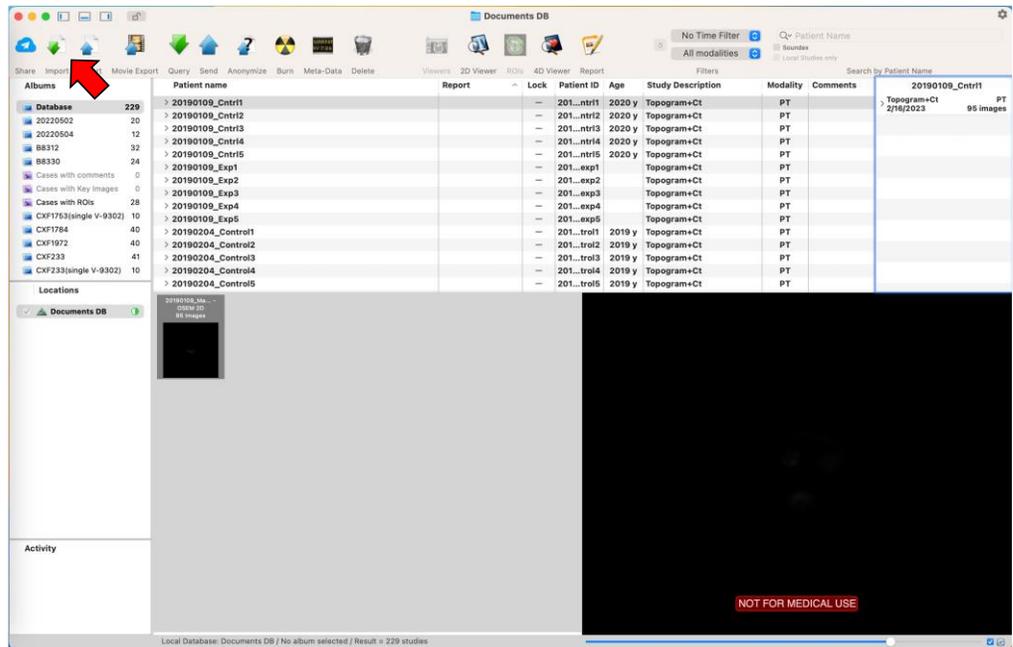
Overall, while both OsiriX Lite and OsiriX MD are medical image viewers, OsiriX MD is a more advanced and regulated version designed for clinical use, while OsiriX Lite is more suited for non-clinical use

6.3.3 Wacom Intuos

For drawing volume of interests (VOI) on OsiriX, using a Wacom Intuos pen tablet allows us to draw more effectively and more precisely than using a mouse.

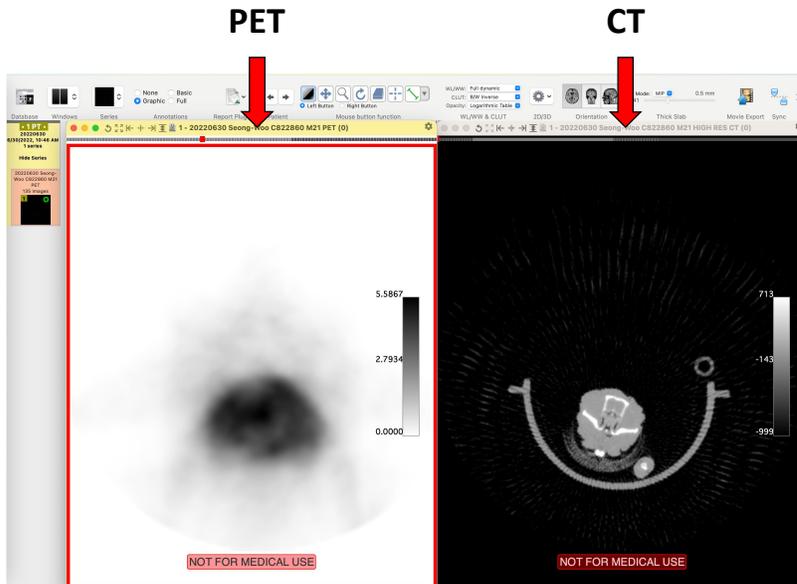
6.4 Analysis Procedures

- 1) Import digital imaging and communications in medicine (DICOM) PET/CT files*.

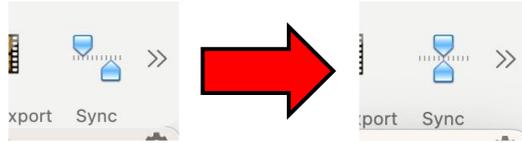


*Each DICOM file should be reconstructed after applying anatomical co-registration. As a preprocessing, SUV or %ID/cc unit should be applied to the PET DICOM based on the information such as injected dose, body weight, time, etc.

2) Open (hit "return" on Mac PCs) PET data and corresponding CT data.



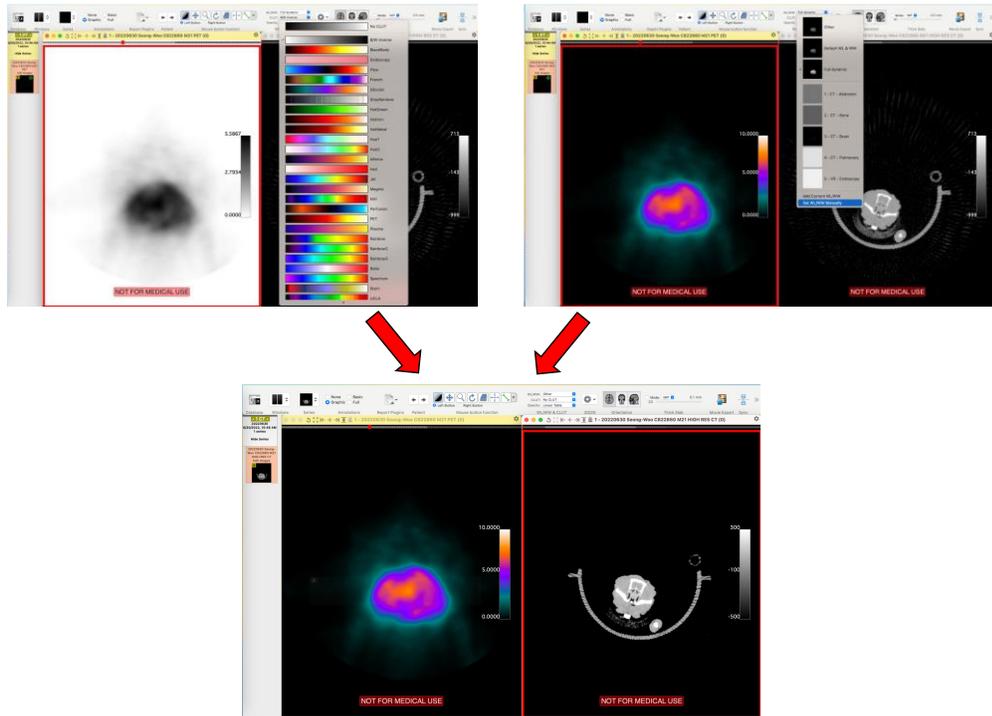
- 3) PET and CT images should be in the same position using the Sync function on the upper right.



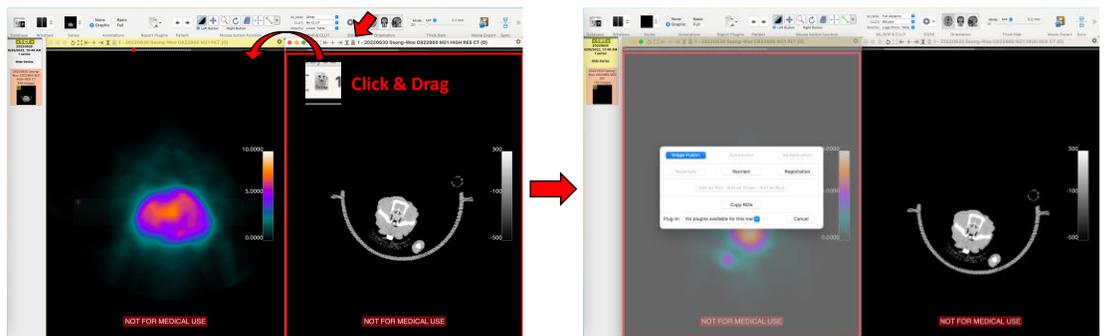
- 4) (Optional) Change image color and intensity.

The pseudo color can be changed in CLUT tab

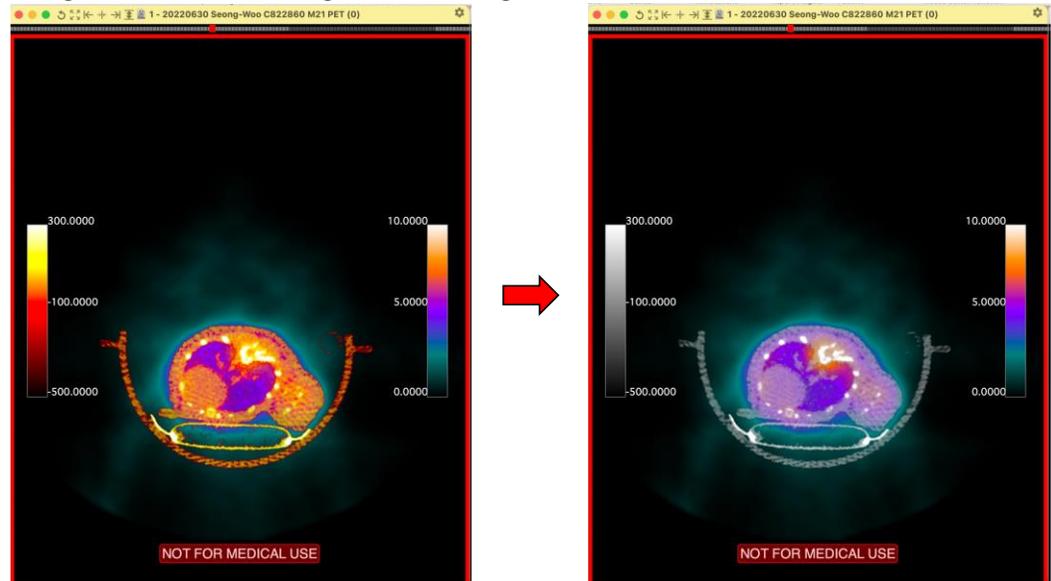
The intensity can be adjusted in "Set WL/WW Manually" of WL/WW tab.



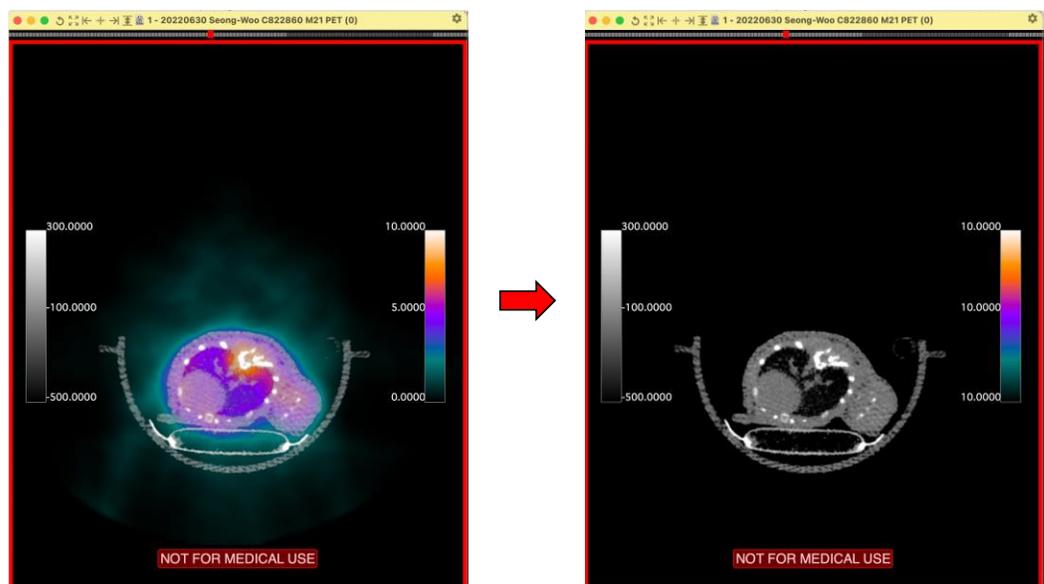
- 5) Merge PET and CT images.



6) Change the color of CT images on the merged window.

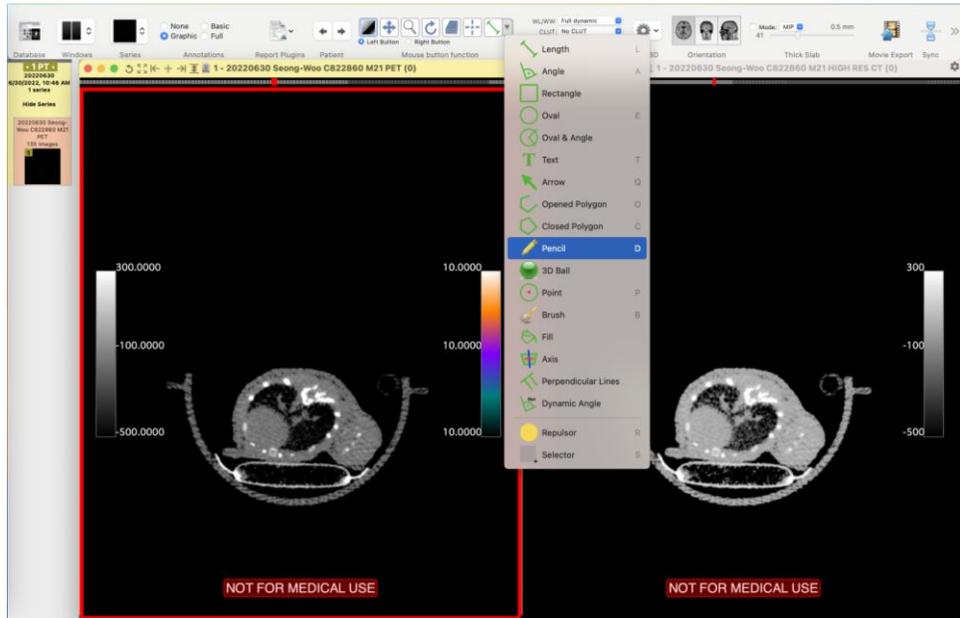


7) Using the “Set WL/WW Manually” function, decrease the PET color on the merged window.

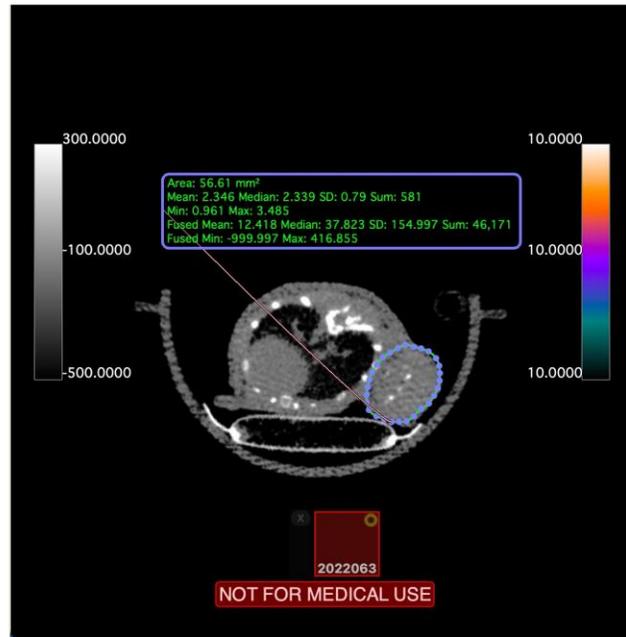


8) Scrolling down with the wheel of a mouse, figure out the top, middle, and bottom of the target (e.g. tumor). Draw region of interest (ROI) using a Wacom pencil or a mouse. The number of ROIs would be depending on the tumor size. The more we capture ROIs of a target, the final volume of interest (VOI) can provide a precise

value avoiding interfered signals from other organs such as the kidney, heart, and bladder. At the very least, 3 ROIs (top, middle, bottom) would be suggested.



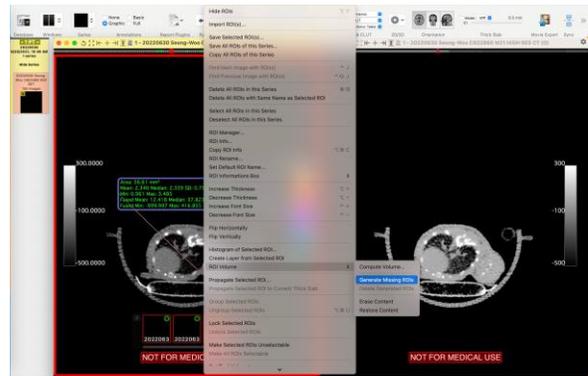
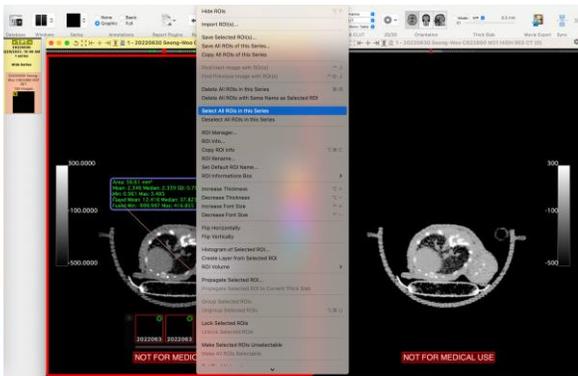
Select “Pencil” function among ROI-related tools* and start drawing a ROI.



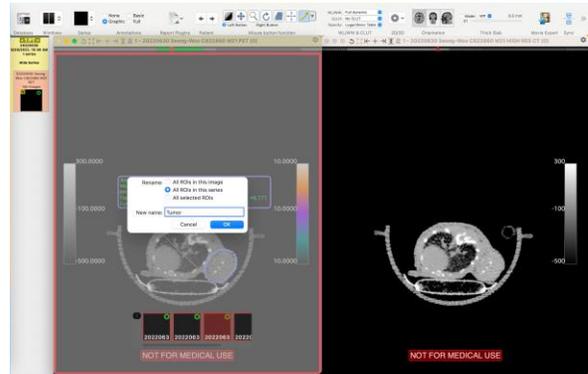
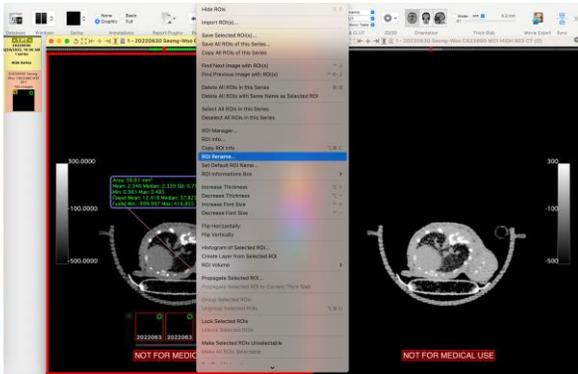
Example of a ROI on one slice of a target

*For precise polishing of ROI, “Repulsor” function would be useful.

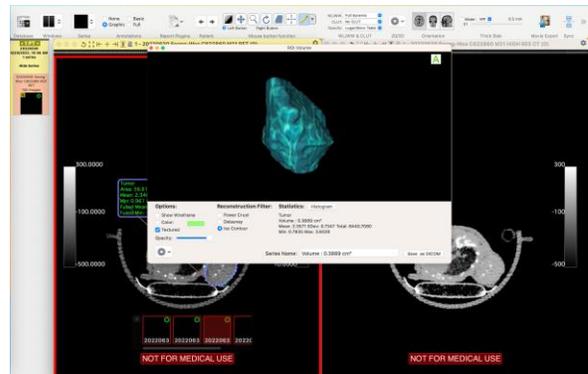
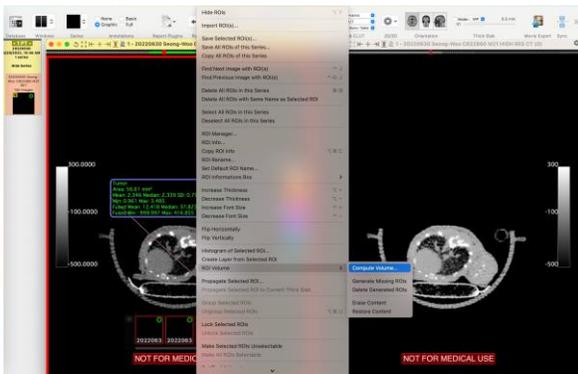
- 9) To cover all slices of the target, select “Select All ROIs in this Series” in the “ROI” tab, and run “Generate Missing ROIs” in the “ROI Volume”.



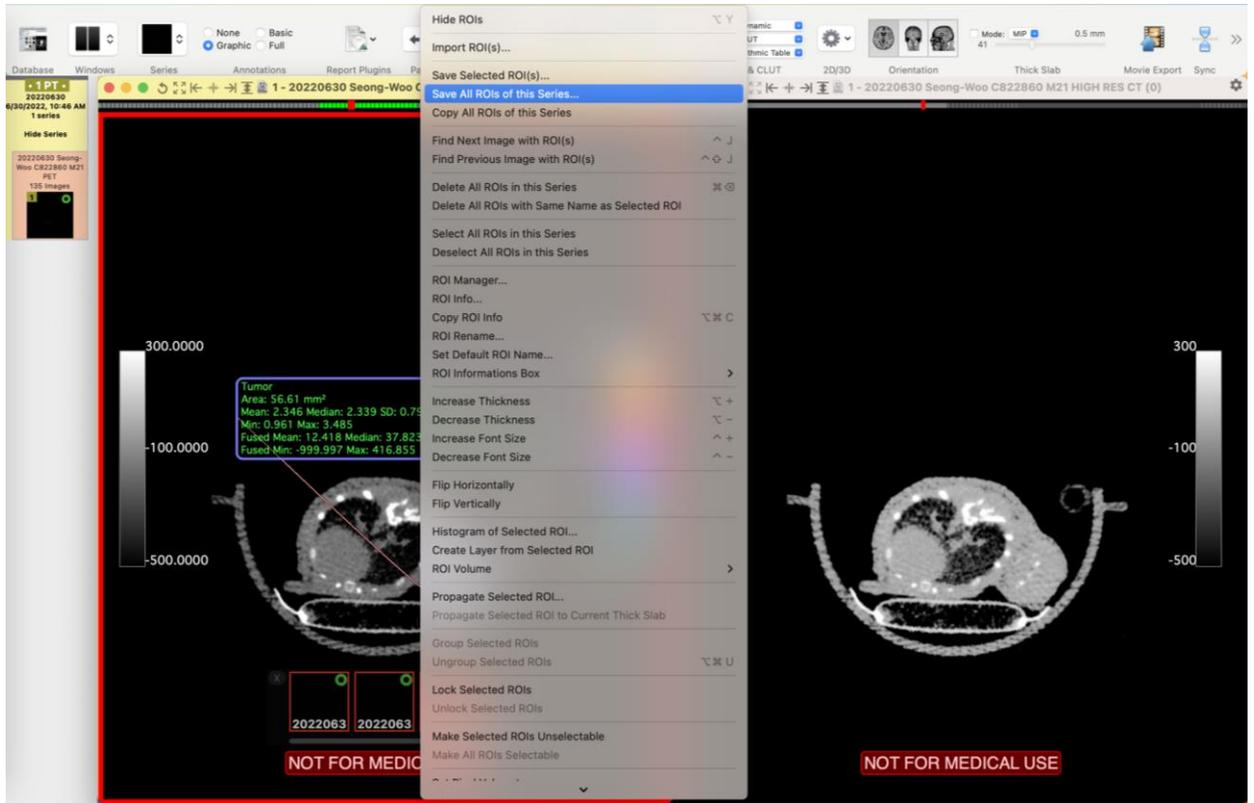
10) Select "ROI Rename" in the "ROI" tab and label new name for "All ROIs in this series".



11) Compute the PET uptake values (min, max, mean) and volume of the target.



12) Save the VOI in the "ROI" tab.



REFERENCES

- 1) Plowman J, D.D., Hollingshead M, et al., *Human tumor models in NCI drug development*. Anticancer Drug Development Guide: Preclinical Screening, Clinical Trials, and Approval. 1997.