

Table 3 Image and Target Descriptions

Image Type	Name	Description
CT	Exhale or EX1	Initial Exhale CT planning dataset
CT	Inhale or IN1	Initial Inhale CT planning dataset
PET	PET1	Initial Free Breathing PET dataset
CT	CTPET1	Initial Free Breathing or Exhale PET CT dataset
CT	Exhale or EX2	During Exhale CT planning dataset
CT	Inhale or IN2	During Inhale CT planning dataset
PET	PET2	During Free Breathing PET dataset
CT	CTPET2	During Free Breathing or Exhale PET CT dataset

Dataset	Structure Name	Description	Dose in 2Gy equiv
EX1	EX1GTV	as entered by the physician	
IN1	IN1GTV	as entered by the physician	
EX1	PREGTV	composite of Exhale and Inhale CT GTV's + PET1	
EX1	PRECTV	0.5 cm expansion of GTV	60 Gy
EX1	PREPTV	Minimum of 0.5 cm expansion of CTV	50 Gy
PET1	PET1GTV	as defined by the treating physician	
PET2	PET2GTV	autotracked with same parameters as PET1GTV	
PET2	PET2PTV	Minimum of 0.5 cm expansion of PET2 GTV	50 Gy + Boost to 17.2% NTCP
CT2	CT2GTV	as entered by the physician	
CT2	CT2PTV	~1 cm expansion of CT2GTV	70 Gy
EX1	EXTERNAL	Autotracked external surface	
EX1	R_LUNG	Autotracked Rt lung	17.2%NTCP to R_Lung + L_Lung - GTV
EX1	L_LUNG	Autotracked Lt lung	
EX1	HEART	Drawn in by DOSIM	≤1.00 Veff to 45 Gy ≤0.34 Veff to 65 Gy
EX1	ESOPHAGUS	Drawn in by DOSIM	≤0.34 Veff to 72 Gy
EX1	CORD	Drawn in by DOSIM	≤50 Gy

Example

<p>EX1GTV + IN1GTV + PET1GTV = PREGTV</p> <p>PREGTV + 0.5cm = PRECTV Tx to 60 Gy</p> <p>PRECTV + (min)0.5cm = PREPTV Tx to 50Gy</p> <p>PET2GTV auto tracked using the same ratio as previous PET</p> <p>PET2GTV + (min)0.5cm = PET2PTV Boost Tx up to 17.2% NTCP for lung</p> <p>CT2GTV + ~1cm = CT2PTV Tx to 70Gy</p>
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APPENDIX E

Blood Sample Collection and Correlative Research Procedures

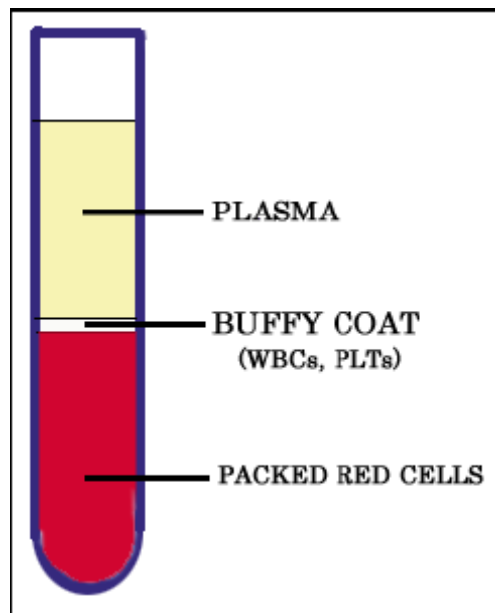
1. Blood Sample Collection for Translational Research

1.1 Blood Sample Drawing and Handling

Samples for TGF β 1 and other cytokine measurement and proteomic analysis need to be handled gently and carefully to avoid platelet degradation or contamination [65]. Needles of large gauge (19-21G) should be used to minimize platelet-contamination from hemolysis. Blood will be collected in two standard blood collection tubes (one purple top and one red top). Blood samples can be drawn in the clinic or blood lab, and temporarily placed vertically at a 4°C until plasma/serum are prepared within 2 hours of collection.

1.2 Collection of Plasma and Buffy Coat Samples

- a. Collect one 5-10 ml tube of blood using one EDTA (purple top) tube and Invert gently one to two times to mix with anticoagulant
- b. Store the blood at 4° or ice as soon as possible (the time of samples setting in room temperature should be less than 5 minutes).
- c. Centrifuge under ~ 3000xG at 4° for 30 minutes
- d. Carefully pipette and transfer ~1ml aliquots of plasma into 4-5 CRYOVIAL® Polypropylene Tubes, keeping the pipet tip at least 5 mm above the buffy coat (at the level of the thickest arrow) to avoid platelets contamination.
- e. Remove the buffy coat cells carefully and place into one 1ml CRYOVIAL® Polypropylene Tubes labeled “buffy coat” (*it is okay if a few packed red cells are inadvertently collected in the process*).
- f. Place tops on CRYOVIAL® Polypropylene Tubes and make sure tops of CRYOVIAL® Polypropylene Tubes are on securely.
- g. Tubes should be clearly labeled as indicated.
- h. Place tubes in a Styrofoam holder and then place into a zip lock bag.
- i. Store samples at -80°C until assay



1.3 Serum Sample Preparation

- a. Collect one 5-10 ml tube of blood without coagulants (Red-topped tube).
- b. Sit at room temperature for 30 min to allow clot formation.
- c. Centrifuge in a standard clinical centrifuge ~ 3000xG RPM at 4° for 30 minutes
- d. Transfer ~1ml aliquots of separated serum into 4-5 CRYOVIAL® Polypropylene Tubes
- e. Place tops on CRYOVIAL® Polypropylene Tubes and make sure tops are secured.
- f. Tube should be clearly labeled as serum as indicated.
- g. Place tubes in a Styrofoam holder and then place into a zip lock bag.
- h. Store samples at -80°C until assay.

1.4 Sample Labeling

Each label will contain the following:

Protocol Number:

Patient ID number (Case number):

Date of Sampling: (mm/dd/yy)

Time of blood collection:

Time of sample collection:

Sample Type (plasma, serum, or buffy coat):

Site ID Number:

1.5 Plasma, serum, and buffy coat will be saved in:

Radiation Oncology/Biology Laboratory of Dr. Feng-Ming Kong
Room 4410, Med Sci I / SPC 5637
1301 Catherine Street, Ann Arbor, MI 48109-5637
Telephone: 734-764-3324/ 734-330-3284/ 734-709-6888
Pager: 734-936-6266 extension 13022 or 14613

1.6 Confidentiality/Storage

All samples will be de-identified and stored at -80°C until assay.

1.6.1 Upon receipt, the specimen will be labeled with the protocol number and the patient's case number only. The blood/database will only include the following information: the number of specimens received and the date the specimens were received. No clinical information will be kept in the database.

1.6.2 Specimens will be stored for an indefinite period of time. Specimens for the translational research component of this protocol will be retained until the study is terminated, unless the patient has consented to storage for future studies. If at any time the patient withdraws consent to store and use specimens, the material will be trashed.

2.0 Assays for cytokine, proteomic and genomic markers will be performed in the core services of the institution, or the laboratories of the principal investigator, co-investigator or their collaborators. Updated technique will be used to secure the best results of the research