Clinical Research Protocol

Study Title: Radiogenomics of muscle invasive bladder cancer

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PROTOCOL SUMMARY

Title:	**Radiogenomics of muscle invasive bladder cancer**
Protocol number:	IIT2020-22-Rosser-Radiogenomics
Phase:	Pilot
Study Design:	Single center clinical trial evaluating identifying a distinct radiogenomic signature of muscle invasive bladder cancer (MIBC)
Study Duration:	84 weeks
Objective:	**Primary Objective:** To show feasibility, which we will define as know-how to create a radiogenomic workflow and to learn about the correlation structure between the radiomic and genomic parameters of interest, which will allow us to design future studies with adequate power.
Number of subjects:	20 subjects
Main Inclusion criteria:	Adults with sessile bladder masses, suspected of harboring MIBC
Study product, dose, route of administration:	N/A
Statistical Analysis:	We will apply a deconvolution algorithm to the genomic data from patients with MIBC in conjunction with magnetic resonance imaging (MRI) data to search for genomic and radiomic parameters that predict tumor stage and outcomes.

1.0 BACKGROUND, RATIONALE

In the US, radical cystectomy is the treatment of choice of muscle invasive bladder cancer (MIBC). MIBC comprises approximately 1/3 of bladder cancer (BCa) and is associated with significant morbidity and mortality. Accurate staging of BCa is essential to identifying the optimal treatment. Current NCCN guidelines recommend contrast-enhanced CT of the abdomen and pelvis to assess the local extent of the cancer, including regional lymph nodal. However, 1/3 of patients are understaged, while 1/3 of patients are overstaged with CT imaging (1). Thus, more accurate non-invasive staging modality is necessary, which could improve medical decision making between various treatments (*e.g.*, radical cystectomy vs. bladder preservation).

Radiomics

Multiparametric magnetic resonance imaging (mpMRI) is emerging as the imaging modality of choice in tumor staging, with a reported sensitivity of 90% in detecting BCa and 76% sensitivity with 89% specificity of detecting metastatic lymph nodes (2). The superior soft tissue contrast and versatile imaging sequences of MRI can facilitate margin definitions critical in cystectomy and bladder preservation strategies. In addition, 4D-MRI techniques have provided high isotropic resolution that was previously unachievable with standard MRI (3-5). With these images, extensive quantitative imaging metrics can be collected. Recently, radiomics, defined as the high-throughput extraction of quantitative imaging metrics, has emerged as a potential diagnostic, capable of assessing the tumor's aggressiveness and extent. Thus, 4D-mpMRI offers an opportunity to reduce staging errors through better anatomical visualization (6, 7) compared to traditional CT imaging (8, 9).

Genomics

Genomics has long been the mechanism of assessing a tumor's clinical characteristics. With the advent of early versions of gene expression profiling and RNA sequencing, there has been a large amount of data available for assessing tumor morphology, molecular structure, and behavior. Furthermore, TCGA has reported clustering of mRNA expression converged into distinct subsets with differential epithelial-mesenchymal transition status (*e.g.*, basal, luminal and neuroendocrine), which have been linked to outcomes and survival (10). While we may have more knowledge and understanding of BCa, a different approach to query its molecular underpinning is warranted in hopes of identifying clinically useful biomarkers to guide medical decision making.

A previous study using single cell RNA sequencing (scRNA-seq) demonstrated transcriptional heterogeneity of BCa (11), suggesting that the traditional bulk RNA sequencing (RNA-seq), similar to what is in TCGA, may have limitations in BCa. In particular, scRNA-seq of BCa revealed an interesting transcriptome, a platinum resistance gene, COX7B, and its surrogate marker, CD63, which was previously not identified (12). Consequently, employing scRNA-seq of MIBC, we have identified unique gene clusters including but not limited to EGFR, NRG1, NRXN1, PTPRC, VWF, COL6A2, CD247, and ITGAX (unpublished data). Thus, we believe scRNA-seq hold a key to not only better analyze tumors for genetic defects, but to identify key cell lines within the tumor microenvironment.

Radiogenomics

Radiogenomics is a relatively new area of research, emerging because of the ability to associate radiologic imaging data to genomics data from tumors to garner a better tumor phenotype and predict clinical outcomes (13). Results from these radiogenomic studies have reported a strong

association between radiologic parameters and genomic features, *e.g.*, DNA mutations, mRNA expression, and copy number variations. Imaging parameters with strong associations to genomic data may serve as reliable markers for tumor diagnosis (*i.e.*, staging), prognosis and treatment response. In addition, integrating radiogenomic data may a) allow the exploration of molecular interconnections that explain various tumor features captured by the radiologic parameters and b) validate the mechanistic pathways supporting such interconnections, and thereby, allowing us to gain a deeper understanding of the cancer and its pathophysiology.

The Cancer Genome Atlas (TCGA) is a large publicly available genomic database of various human tumors. Akin to TCGA is The Cancer Imaging Archive (TCIA), a publicly available radiomic database of various human tumors. There are many tumors within TCGA and TCIA with both genomic and radiomic data. For example, Zhu *et al.* integrated multi-omics molecular data with magnetic resonance imaging (MRI) data from 91 invasive breast cancers and reported miRNA expression was associated with tumor size and tumor enhancement texture on imaging (14). Furthermore, Vargas *et al*. evaluated radiogenomic data from 92 high-grade serous ovarian adenocarcinomas and noted a significant association between mRNA expression, miRNA expression, promoter methylation and DNA copy number data with CT-based characteristics of primary ovarian masses, including size, presence of definable mesenteric implants and infiltration, etc. (15). Lastly, Karlo *et al.* reported the radiogenomic results of 233 clear cell renal cell carcinoma cases, which revealed VHL was associated with ill-defined tumor margins, nodular tumor enhancement and gross appearance of intratumoral vascularity (16).

Interestingly, when conducting a PubMed literature search for radiogenomic and BCa, only one article is noted. In this study, Lin *et al.* report the results of bulk RNA-seq, radiomics features and clinical parameters of 62 BCa patients and found that the radiomics and transcriptomics signatures significantly stratified patients into high- and low-risk groups in terms of the progression-free survival (PFS) (17).

Current trends in bladder cancer

The landscape for BCa treatment options have changed dramatically over the past 5 years with the approval of five checkpoint inhibitors as well as Erdafitinib and Enfortumab. For MIBC, more attention is turning towards bladder preservation (BP). Despite improvements in systemic therapy options and radiation therapy technology (*e.g.*, image guide radiation therapy (IGRT), intensity modulated radiation therapy (IMRT), the optimal outcomes (*i.e.*, maintaining one's bladder, good quality of life and overall survival; a.k.a. 'trifecta') is still elusive. There are several factors which may contribute to the inability to obtain this 'trifecta'; including inadequate staging and inability to predict who would respond favorably to radiation therapy. Thus, a more thoughtful, phased approach to BP strategies need to develop and evolve in order to improve long-term outcomes for the growing need of patients who are ineligible for cisplatin based neoadjuvant chemotherapy and/or are either refusing or ineligible for radical cystectomy.

In particular, 5-year OS for MIBC remains poor at 50% (18). However, a patient's quality of life could be negatively affected by radical cystectomy (19), thus the continued intrigue with BP. Multiple BP options exist, although the approach of maximal transurethral resection of bladder tumor (TURBT) performed along with chemoradiation therapy (CRT) is the most favored (20, 21). BP strategies need to develop and evolve if they are to address issues related to OS. Specifically, improved modalities to stage patients with MIBC are needed to ensure we are identifying the optimal patient for BP. With this said, Cedars-Sinai Cancer (CS Cancer) has identified BP strategies as a major initiative from which to begin to develop a BCa center of excellence.

We aim to build upon the current knowledge by correlating radiomics findings with that of genetic signature in order to identify a radiogenomic profile of MIBC which could be used to

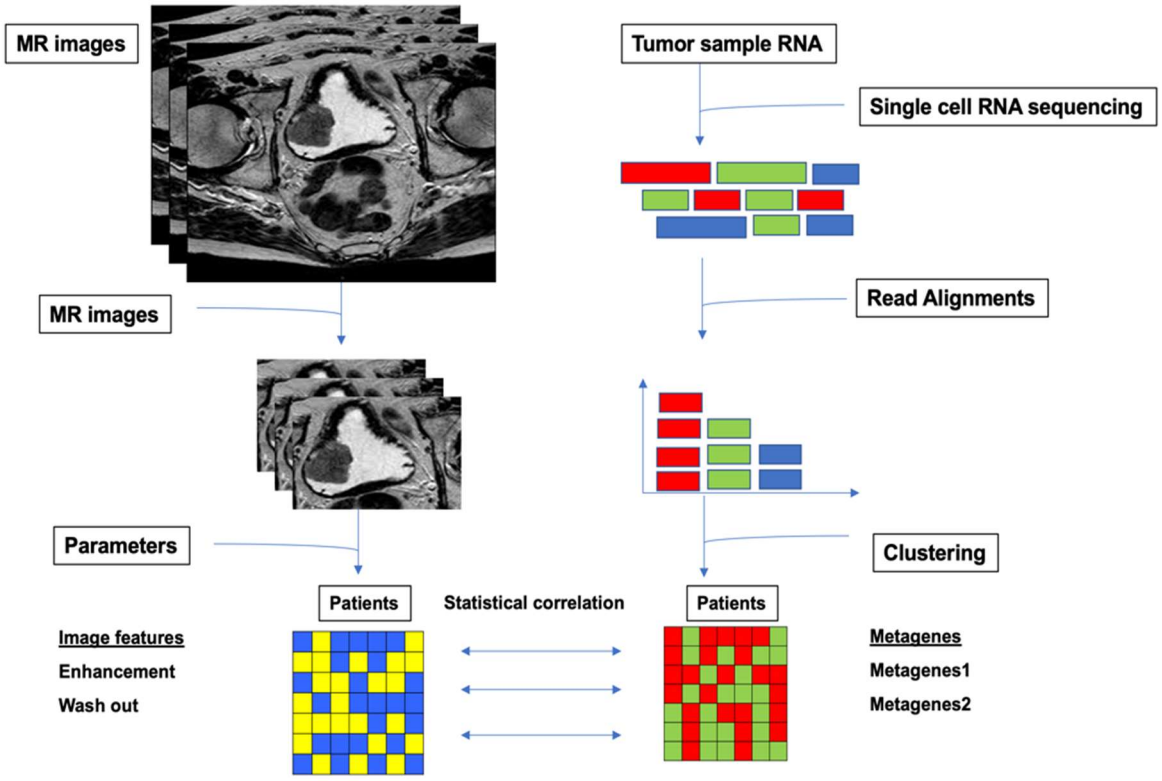

Fig. 1 Schematic diagram summarizing the interactions of the project

accurately stage patients (**Fig. 1**) leading to better medical decision making as it relates to BP techniques. Our central hypothesis is that a radiogenomic profile exists that a) is specifically associated with BCa and b) can be utilized to more effectively and accurately stage BCa. We seek to develop a computational algorithm that predicts, from radiographic imaging data and genetic alterations from RNA-seq data of a tumors, the relative proportions of predefined cancer cell populations, which lends to aggressiveness and BCa extent. To begin to test this hypothesis, we propose to conduct a pilot study of integrating radiomics data with genomics data in patients with localized MIBC and correlating to final pathology at the time of radical cystectomy. By improving our ability to better stage patients with MIBC, this work could facilitate future studies to explore a radiogenomic signature associated with MIBC and improve patient outcomes.

2.0 STUDY OBJECTIVES

To show feasibility, which we will define as know-how to create a radiogenomic workflow and to learn about the correlation structure between the radiomic and genomic parameters of interest, which will allow us to design future studies with adequate power.

3.0 STUDY POPULATION

3.1 SELECTION OF THE STUDY POPULATION

The study will accrue patients with sessile appearing bladder masses who are felt by the treating physician to harbor MIBC.

Prior to TURBT at Cedars-Sinai, **ALL** subjects will undergo axial imaging for clinical staging in the form of contrast enhanced MRI of the abdomen and pelvis (standard of care). The pelvic MRI will be multiparametric (mp)-4D MRI incorporating high resolution diffusion weighted imaging (HR-DWI). Both the abdominal and pelvic MRI will have an official intrepretation by a radiologist, thus both can be used in the care of the subject. Next, **ALL** subjects will undergo TURBT at which time, voided urine, blood and fresh frozen bladder tumor will be collected.

Many subjects present to Cedars-Sinai after their transurethral resection of bladder tumor (TURBT) at an outside facility and a repeat TURBT is not planned at Cedars-Sinai. Such subjects will undergo axial imaging for clinical staging in the form of contrast enhanced MRI of the abdomen and pelvis (standard of care). The pelvic MRI will be multiparametric (mp)-4D MRI incorporating high resolution diffusion weighted imaging (HR-DWI). Both the abdominal and pelvic MRI will have an official interpretation by a radiologist, thus both can be used in the care of the subject. Next, **ALL** subjects will have their outside TURBT formalin fixed paraffin embedded (FFPE) requested, specifically 1 H&E slide and 10 unstained slides.

Subsequently, those patients deemed to have muscle invasive, yet localized disease and are considered candidates for radical cystectomy by their treating physicians will proceed to radical cystectomy at which time another voided urine, blood and fresh frozen bladder tumor will be collected. Pathologic stage will be determined and reported on both the TURBT specimen and radical cystectomy specimen. Finally, subjects will be followed for 2 years to elicit outcome data.

Inevitably, a portion of subjects with bladder masses who are suspected of harboring BCa will not have bladder cancer (10%), BCa diagnosed may not be MIBC (20%) and some subjects may not desire radical cystectomy for their MIBC (20%), thus we anticipate the need to enroll and image 20 subjects to get to our final study population of 10 MIBC who have undergone mp-4D MRI, TURBT and subsequent radical cystectomy.

3.2 INCLUSION CRITERIA

1. Patients must be > 18 years of age.

2. Patient must have a sessile mass noted within the bladder on cystoscopy or imaging worrisome for bladder cancer, specifically MIBC.

3. Patient must agree to undergo staging which will include mp-4D MRI HR-DWI of the pelvis in addition to standard axial imaging of the abdomen.

4. Patient must have undergone TURBT or agree to undergo planned TURBT as part of the normal treatment course.

5. Patients must not have known or suspected primary urothelial carcinoma of the ureter, urethra, or renal pelvis.

6. Patients must not have known distant metastatic disease (*e.g.* pulmonary or hepatic

metastases). Subjects with malignant lymphadenopathy in the abdomen or pelvis considered appropriate for radical cystectomy and lymphadenectomy with the goal of complete resection of all malignant disease are allowed.

7. Patients must not have had prior definitive treatment for bladder cancer.

8. Patients must not have clinically significant active infection or uncontrolled medical condition that would preclude participation in study.

9. Patients must not have any active malignancy other than urothelial carcinoma of the bladder that, in the opinion of the treating investigator, which could interfere with protocol treatment.

10. Patient must have adequate renal function: Serum creatinine < 2 mg/dL OR calculated CrCl > 30ml/min.

11. Patients must not have allergy or contraindication for MRI contrast/contrast dye.

12. Patients must not be under treatment with systemic immunosuppressive medications (including but not limited to prednisone, cyclophosphamide, azathioprine, methotrexate, thalidomide, and anti-tumor necrosis factor [anti-TNF] agents) within 2 weeks prior to TURBT.

13. Patient must not be adverse to undergo radical cystectomy as part of the normal treatment course if found to have MIBC.

14. Patients must have the ability to understand and willingness to sign a written informed consent.

3.3 EXCLUSION CRITERIA

1. Persons with allergy to animal dander or animal-instigated asthma.

2. Patient must not have undergone a bladder biopsy or limited (incomplete) TURBT within 3 weeks (21 days) of the MRI.

3.4 SUBJECT SCREENING AND ENROLLMENT

Subjects will be recruited by investigators within the outpatient Urology Clinics at Cedars-Sinai Medical Center (CSMC). Subjects will be evaluated by a clinical investigator for eligibility. The study rationale, and potential risks and benefits will be clearly explained; study investigators will obtain informed consents and enroll subjects. Only subjects who meet the inclusion/exclusion criteria will be enrolled. Enrolled subjects will be assigned a unique, non-identifying study subject ID number.

4.0 STUDY DESIGN AND METHODS

This is a single center non-randomized pilot clinical trial assessing radiogenomics in a cohort of subjects with MIBC recruited at CSMC.

After signing informed consent, the subject will be scheduled for standard of care axial imaging of the abdomen and pelvis to include pelvic MRI imaging in the radiologic suite in Davis Building at CSMC.

mp-4D pelvic MRI incorporating high resolution diffusion weighted imaging (HR-DWI)

1. All imaging will be performed on a 3T clinical MR scanner (Biograph mMR, Siemens Medical Solutions, Erlangen, Germany) with a 32-channel phase array surface coil.
2. During the imaging session, routine protocols including 3D T1W volumetric interpolated breath-hold examination (T1-VIBE) with Dixon fat saturation in axial orientation, multi-slice T2W half-Fourier acquisition single-shot turbo spin-echo sequence (T2-HASTE) in axial and coronal orientations, and multi-slice single-shot echo-planar imaging (SS-EPI) DWI sequence will be first acquired for pancreas delineation and tumor definition.
3. 2D Modified Look-Locker Inversion Recovery (MOLLI) sequence in oblique planes to cover as much PDAC tumor mass will be obtained as the reference for in-vivo T1 mapping.
4. Following these sequences, the Multitasking DCE 4D-MRI will be performed. The technique has been designed to cover the entire pelvis. Each SR period lasted 500 ms with 84 TIs at TR 5.6 ms. One readout line will be collected per TI. The SR period will be repeated 1200 times in a scan of 10 min. The DCE temporal footprint was selected to be 1 second by combining two SR periods to balance between the temporal resolution and noise (22).
5. Gd contrast agent (Gadavist, 0.1 mmol/kg, Bayer Schering Pharma) will be administrated intravenously 2 minutes into the scan at a rate of 2 mL/s, followed by a 20 mL saline flush at the same rate. Immediately at the end of this sequence, post-contrast MOLLI at the same slice location as pre-contrast one will be collected. Detailed imaging parameters for the protocols are summarized in “Variables collected”.
6. At the same time the pelvic MRI is being completed, the abdominal MRI can be performed.

Next, subjects who have not had an outside TURBT will undergo TURBT in the operating room (mandatory). In pre-operative holding, a voided urine sample (50 mL) will be collected, while blood (15 mL in ACD-containing tube) and fresh frozen tumor tissue will be collected in the operating room by the BioBank staff. The results of the evaluation of the TURBT will determine if the subject has bladder cancer and if so the histology, clinical stage and grade will be noted. If the subject is found not to have MIBC, then he/she would be off study. For the subjects who had an outside TURBT, tumor material from the TURBT will be requested from the local hospital/pathology, specifically, 1 H&E slide and 10 unstained slides will be requested.

However if the subject is found to have MIBC and is deemed to be a candidate for definitive therapy by radical cystectomy without neoadjuvant chemotherapy or immunotherapy, then he/she will be scheduled for radical cystectomy within 4 weeks. At the time of radical cystectomy, additional voided urine (approximately 50 mL) will be collected in pre-operative holding and blood (15 mL in ACD-containing tube) and fresh frozen tumor tissue will be

ciollected in the operating room by the Biobank staff. The results of the histological evaluation of the cystectomy specimen will be reported noting histological type of bladder cancer, pathologic stage and grade.

Correlative studies
Embedded within will be crucial correlative studies that will begin to shed light on MIBC in hopes of identifying a radio-molecular staging system, which is more accurate than the standard clinical staging.

These studies will involve analyses of:

(1) **Peripheral blood specimen:**
An additional specimen of blood will be obtained beyond what is needed for clinical care. The amount of blood will be approximately 15 ml of extra blood collected from a vein into ACD-containing tubes. In the BioBank, the blood will separated by centrifugation into the plasma fraction and the blood cell fractions. The fractions will be divided into aliquots for measurements which will be done either at the time of the blood sample acquisition or at a designated time later on stored specimens. Storage will be at -80^0 C in a secure biorepository freezer in CSMC BioBank.

(2) **Biofluid Specimens (Urine):**
Urine sample(s) will be obtained by asking the patient to provide urine into a provided container or via urinary catheter placed at the time of surgery. Approximately 50 mL will be obtained, centrifuged, separated into cellular pellet and supernatant and stored at -80^0 C, in a secure biorepository freezer in CSMC BioBank.

(3) **Tissue Specimens:**
A fresh frozen tissue sample from TURBT and subsequent cystectomy speciumens will be obtained by CSMC BioBank personnel. The tissue samples will be snap-frozen in liquid nitrogen and stored in -80C freezers in CSMC BioBank. When needed for analysis, BioBank will send a portion of the de-identified TURBT tumor ($5mm^3$) to CS Genomic Core for RNA extraction, and RNA-seq.
Or FFPE tissue sample from a TURBT will be requested. Specifically we will request 1 H&E from the FFPE block with tumor as well as 10 unstained slides.

Using standard techniques, CS Genomic Core will perform RNA-seq. Biostatisticians and Bio-informaticians will assess correlations between radiomics, genomics and pathology data.

Table 8 Correlative Studies timing and requirements

Specimen	Quantity	MRI	TURBT	Cystectomy
Whole Blood ACD tube	1x15 mL+		X+	X+
Urine	50 mL		X+	X+

Bladder tissue (Fresh)	~5mm^3		X~	X+
Bladder tissue (FFPE)	1 H&E 10 unstained		X~	

~, used for sequencing
+, banked in Cedars' Biobank for future use

5.0 CLINICAL ASSESSMENTS

5.1.1 CONCOMITANT MEDICATIONS

All concomitant medication and concurrent therapies will be documented at Baseline/Screening, at each study visit, and at early termination when applicable, as part of routine medical care/treatment. Dose, route, unit frequency of administration, and indication for administration and dates of medication will be captured.

5.1.2 DEMOGRAPHICS

Demographic information (date of birth, gender, race) will be recorded at Screening.

5.1.3 MEDICAL HISTORY

Relevant medical history, including history of current disease and information regarding underlying diseases will be recorded at Screening.

5.1.4 PHYSICAL EXAMINATION

A complete physical examination will be performed by physician at screening.

5.1.5 VITAL SIGNS

Body temperature, blood pressure and pulse will be performed at screening.

5.1.6 OTHER STANDARD OF CARE CLINICAL PROCEDURES

- TURBT
- Axial imaging of the abdomen
- Axial imaging of the chest or 2 view chest x-rays, determined by treating physician
- Bone scan, if medically indicated
- Radical cystectomy. This will occur according to established clinical guidelines (e.g. NCCN) within 6 weeks of consenting

6.0 ADVERSE EVENT REPORTING AND DOCUMENTATION

It is unlikely any subject will experience a study related adverse event as all elements of the study are part of standard of care. However if such events occur the information regarding the occurrence will be captured throughout the study using the CTCAE v5.0 criteria. Duration (start and stop dates and times), severity/grade, outcome, treatment and relation to study device and radiation treatment will be recorded on the case report form (CRF).

6.1 Adverse Events (AE)

An **adverse event** is any symptom, sign, illness or experience that develops or worsens in severity during the course of the study. Intercurrent illnesses or injuries should be regarded as adverse events. Abnormal results of diagnostic procedures are considered to be adverse events if the abnormality:

- results in study withdrawal
- is associated with a serious adverse device effect
- is associated with clinical signs or symptoms

- leads to additional treatment or to further diagnostic tests
- is considered by the investigator to be of clinical significance

SERIOUS ADVERSE EVENT (SAE)
An SAE is defined as any AE occurring that results in any of the following outcomes:

- death
- a life-threatening adverse experience
- inpatient hospitalization or prolongation of existing hospitalization
- a persistent or significant disability/incapacity
- a congenital anomaly/birth defect

Other important medical events may also be considered an SAE when, based on appropriate medical judgment, they jeopardize the subject or require intervention to prevent one of the outcomes listed.

6.2 Unanticipated Problems Involving Risk to Subjects or Others (UPIRSO)

Any of the above-listed events could classify as a UPIRSO if they meet the following criteria:

- Unexpected in nature, severity, or frequency (*i.e.* not described in study-related documents such as the IRB-approved protocol or consent form, the investigators brochure, etc.)
- Related or possibly related to participation in the research (*i.e.* possibly related means there is a reasonable possibility that the incident experience, or outcome may have been caused by the procedures involved in the research)
- Suggests that the research places subjects or others at greater risk of harm (including physical, psychological, economic, or social harm).

6.3 RECORDING OF UNANTICIPATED ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

The Investigator will probe, via discussion with the subject, for the occurrence of UADEs, AEs, and SAEs during each subject visit and record the information in the site's source documents. Adverse events will be recorded on the study CRF/eCRF. These events will be described by duration (start and stop dates and times), severity, outcome, treatment and relation to study drug, or if unrelated, the cause. All clearly related signs, symptoms, and abnormal diagnostic procedures results should be recorded in the source document, though should be grouped under one diagnosis. Data will be collected on logs and related CRFs or eCRFs. The clinical course of each event should be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause. Serious adverse device effects that are still ongoing at the end of the study period must be followed up to determine the final outcome.
All SAEs that occur (whether or not related to study device) will be documented - the collection period for all SAEs will begin after informed consent is obtained and continue until after the completion of cystectomy.

6.4 AE SEVERITY & GRADING

The National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0 should be used to assess and grade AE severity, including laboratory abnormalities judged to be clinically significant. The guidelines shown below should be used to grade severity when the event does not fit within a term/diagnosis available in the CTCAE criteria. It should be

pointed out that the term "severe" is a measure of intensity and that a severe AE is not necessarily serious.

TABLE AE SEVERITY GRADING

Severity (Toxicity Grade)	Description
Grade (1)	Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
Grade (2)	Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL*. *Instrumental ADL refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.
Grade (3)	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL**. **Self-care ADL refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.
Grade (4)	Life-threatening consequences; urgent intervention indicated.
Grade (5)	Death related to AE.

6.5 AE Relationship to MRI

The relationship of an AE to the study MRI should be assessed using the following guidelines in the table below

TABLE AE RELATIONSHIP TO DEVICE OR RADIATION

Definite	The AE is clearly related to the study MRI
Probable	The AE is likely related to the study MRI
Possible	The AE may be related to the study MRI
Unlikely	The AE is doubtfully related to the study MRI
Unrelated	The AE is clearly NOT related to the study MRI

Note: This includes all events that occur within 30 days of the MRI. Any event that occurs more than 30 days after the MRI and is attributed (possibly, probably, or definitely) to the agent(s) must also be reported accordingly.

Determine the prior experience (expectedness) of the adverse event.

Expected events are those that have been previously identified as resulting from TURBT and cystectomy. An adverse event is considered unexpected, for expedited reporting purposes only, when either the type of event or the severity of the event is not listed in:

- The current known adverse events listed below

TURBT Expected Adverse Events

- Genitourinary: Frequency, nocturia, acute or chronic bleeding from the bladder mucosal surface
- Gastrointestinal: Rectal irritation, bowel obstruction or bleeding, rectal ulcers, hematochezia, fistula formation, colitis, mucous-like stools
- Dermatologic: Erythema, loss of pubic hair which could be permanent
- Gynecologic: Dyspareunia, ovarian failure and sterility

Cystectomy Expected Adverse Events

- Genitourinary: urinary tract infection (UTI), septicemia, stones in conduit/neobladder, incontinence, hydronephrosis, azotemia
- Gastrointestinal: bowel obstruction, diarrhea
- Wound: infection, dehiscence, hernia

6.6 Reporting of SAEs

Any significant adverse event will be reported to the IRB in accordance with the standard operating procedures and policies of the local Institutional Review Board (IRB) / Independent Ethics Committee (IEC).

The IRB must be notified within 10 business days of "any unanticipated problems involving risk to subjects or others."

The Principal Investigator must report all SAEs promptly to **within 48 hours of first becoming aware of the event.** This report will be accomplished by completing a Medwatch 3500A form which is available on the FDA website at http://www.fda.gov.

At the time of first notification of an SAE, the following information should be provided by the site, if available:

- Patient's study number and initials
- Date of first dose of test article
- Date of last dose of test article, if applicable
- Protocol description (and number, if assigned)
- Treatment regimen (dosing frequency, combination therapy)
- Adverse event term
- Time and date of occurrence of the event
- A brief description of the event, severity, outcome to date and any actions taken
- The seriousness criteria(on) that were met
- Concomitant medication at onset of the event
- Relevant past history information
- Relevant laboratory test findings
- Investigator's assessment of the relationship to test article

Any missing or additional relevant information concerning the SAE should be provided in a written follow-up report.

It will be left to the investigator's clinical judgment whether or not an AE is of sufficient severity to require the subject's removal from treatment. If the subject was permanently withdrawn from the study or investigational product due to an SAE, this information must be included in either the initial or follow-up SAE Report Form.

The Investigator is required to comply with applicable regulations regarding the notification of his/her IRB or Ethics Committee.

6.7 Protocol Defined Important Medical Findings Requiring Real Time Reporting

Events that are to be reported in an expedited manner that may or may not meet the definition of serious are listed below. For any of these events, the Principal Investigator and Data Safety and Monitoring Committee (DSMC) must be notified within 24 hours:

1. SAE deemed to be related to the protocol.
2. On-study deaths, including death of a research subject unless the death is expected (e.g. due to disease progression).

6.8 Deviations from the study protocol

A protocol deviation occurs when the subject or investigator fails to adhere to significant protocol requirements affecting the inclusion, exclusion, subject safety, and primary endpoint criteria. Please refer to the CSMC IRB intranet webpage to obtain IRB protocol deviation reportable to the IRB:
https://web.csmc.edu/research-and-education/research/institutional-review-board/

Protocol deviations for this study may include, but are not limited to, the following:

- Failure to meet inclusion/exclusion criteria
- Non-compliance by the study subject with required tests and procedures (TURBT, mp-MRI HR-DWI study, radical cystectomy, etc.)
- Failure to comply with Good Clinical Practice (GCP) guidelines
- When a protocol deviation occurs, Protocol Deviation Form detailing the events will be generated. This form will be signed by the clinical site investigator and submitted to the local IRB per institutional policy. A copy of the form will be filed in the site's regulatory binder and sent to the DSMC.

7.0 DISCONTINUATION AND REPLACEMENT OF SUBJECTS

7.1 Early Discontinuation of Study Participation

A subject may be discontinued from study treatment at any time if the subject or the investigators feel that it is not in the subject's best interest to continue. The following is a list of possible reasons for study treatment discontinuation:

- Subject withdrawal of consent (or assent) for any reason
- Because the subject is too ill to proceed with radical cystectomy
- Because the subject changes their mind about treatment and elects to not undergo radical cystectomy.
- Subject is not compliant with study procedures

- Adverse event that in the opinion of the investigator would be in the best interest of the subject to discontinue study
- Protocol violation requiring discontinuation of study
- Lost to follow-up
- DSMC request for early termination of study

If a subject is withdrawn from treatment due to an adverse event, the subject will be followed and treated by the clinical site investigator until the abnormal parameter or symptom has resolved or stabilized.

All subjects are free to withdraw from participation at any time, for any reason, specified or unspecified, and without prejudice.

Reasonable attempts will be made by the investigator to provide a reason for subject withdrawals. The reason for the subject's withdrawal from the study will be specified in the subject's source documents.

7.2 Withdrawal of Subjects from the Study

A subject may be withdrawn from the study at any time if the subject or the investigator feels that it is not in the subject's best interest to continue.
All subjects are free to withdraw from participation at any time, for any reason, specified or unspecified, and without prejudice.

7.3 Replacement of Subjects

Subjects who withdraw from the study before radical cystectomy will be replaced.

8.0 SCHEDULE OF PROCEDURES

Procedures	Screening Visit	Staging	TURBT	Cystectomy	Follow-up
Informed consent	X				
Demographic data	X				
Medical history, baseline conditions	X				
Vital signs ^					
Physical examination	X				
Survival follow-up					Every 6 months for 3 years
Chest CT†		X			
Abdomen CT/MRI		X			
Chest x-ray†		X			
Bone scan (if indicated)		X			
Pelvis MRI		X^			
Pregancy test	X				
Hematology	X				
Chemistry	X				
TURBT#			X		
Research urine			X	X	
Research blood			X	X	
Fresh frozen tissue or FFPE#			X	X	
Cystectomy				X	

^Heart rate, systolic and diastolic blood pressure while the patient is in a seated position, and temperature.
† Patients who have received a chest CT (or MRI) need not have a chest x-ray. Patients who have findings suspicious for metastatic disease on a chest x-ray at baseline should have a confirmatory CT of the chest at baseline. Patients who have findings suspicious for metastatic disease at baseline on CT should have CT scans at follow-up tumor assessments
^ mp-4D MRI high resolution with DWI
# could be outside TURBT with FFPE tissue

9.0 DATA COLLECTION AND MANAGEMENT

9.1 DATA PROCUREMENT

- Patient medical history will be extracted from the Cedars-Sinai Medical Center EMR system.
- Pathologic (histology, grade, stage) and MRI data will be extracted from the Cedars-Sinai Medical Center EMR system.
- Biological specimens (blood, urine, tissue) will be collected during patients' visits.
- Additional information in regard to diseases status will be obtained via follow-up visits and chart review.
- Information will be kept for 5 years after completion of the study.

9.2 VARIABLES COLLECTED

The following data points/variables will be collected:

1. RNA-seq of bladder tumor
2. Imaging Measurement Tools:

- MRI Pelvis protocol with modified sequences
- T1 and T2 imaging (relaxation differences between tumor/tissue)
    - 3D imaging of abdomen (w/o contrast)
- DWI multi-b value (cellularity, density)
    - 3D imaging of abdomen (w/o contrast)
    - ADC maps for slow and fast diffusion components
- DCE-MRI (microvascular density, vascularity, vascular permeability)
    - 3D imaging of abdomen (w/ contrast)
    - Kinetic parameter maps, K^{trans}, k_{ep}, v_e, AUC
    - Fractional blood volume, v_p

9.3 SOURCE DOCUMENTS

Cedars-Sinai Medical Center EMR system will constitute source documents. In particular, EMR will be reviewed for patient demography and indication for procedure, MRI data, pathology data, clinical diagnosis information and outcomes.

9.4 DATA COLLECTION AND STORAGE

All electronic data will be stored electronically in password protected Cedars-Sinai Medical Center encrypted computers within a dedicate RedCap database. Hard copy research records will be stored in secure location (e.g. locked cabinet, office). Biological specimens will be stored in freezers at secure location (locked room or space accessible only to study team) within Cedars-Sinai BioBank.

The data will be accessible only by the study team.

On the day of consent, a de-identifier ID will be generated and assigned to each patient. The study database will not include identifiers. Only designated study team members will have the key that links the de-identifier ID with patient name, MRN or other direct identifiers.

9.5 CONFIDENTIALITY AND SECURITY OF DATA

The following measures will be taken in order to maintain confidentiality of any information obtained from or about the subjects:

<u>Identifiable Information and Linking Lists/Files</u>

- A de-identifier ID will be generated and assigned to each patient. The linking lists with de-identifier ID, name and Medical Record Number of the patient will be maintained separately from research data in RedCap. Access to the list/file will be limited to approved research personnel. The study data coded upon abstraction, and any direct identifiers, will be maintained separately from data
- Collection of sensitive information about subjects (PHI) is exclusively limited to the amount necessary to achieve the aims of the research study and no unneeded sensitive information will be collected
- Only the minimum health information pertaining to the study will be collected. This will include clinical, procedure, and disease-related factors affecting the study outcomes.

- Identifiable information will be destroyed as soon as possible after it is no longer needed for research purposes
- Identifiable information will not be used or disclosed for any purposes not described in this application

Research Records and Data Storage

- Electronic research records will be stored in shared network folder on Cedars-Sinai network (no storage on non-CSMC computer)
- All laptops used for collecting and storing research data or patient information will be encrypted in accordance with EIS standards. Laptops purchased with grant or other Cedars-Sinai funds will be processed through EIS to register and encrypt the laptop
- The data will only be made available to certified research personnel with IRB approval for access, through the use of passwords and encryption (No downloading to unencrypted portable devices, such as flash drive, personal laptop, etc.)
- Access will be only at Cedars-Sinai or through approved VPN access
- Hard copy research records will be stored in secure location (e.g. locked cabinet, office); Paper records with PHI will not be removed from Cedars-Sinai premises.

10.0 DATA AND SAFETY MONITORING

10.1 DATA MONITORING AND QUALITY ASSURANCE

Minimal Risk Monitoring

Adherence to the protocol, Good Clinical Practices (GCP), and institutional policy will be monitored by the PI during the course of the study through routine Disease Research Group (DRG) meetings (or equivalent). In addition, the SOCCI Cancer Clinical Trials Office (CCTO) Quality Management Core (QMC) will conduct focused internal monitoring visits and audits for data quality and protocol adherence. QMC reports will be forwarded to the SOCCI Data and Safety Monitoring Committee (DSMC). Refer to the DSMC Charter for more details. For any protocol, QMC has the authority to request more frequent reviews or focused safety monitoring if it is deemed appropriate for any reason.

10.2 SAFETY MONITORING

Minimal Risk Monitoring

Oversight of the progress and safety of the study will be provided by the PI. The PI will maintain continuous safety monitoring for the duration of the study by reviewing subject/study data. Adverse events and unanticipated problems are not expected, but if they occur, they will be documented and reported according to CS-IRB policies and procedures. If the PI becomes aware of any new safety information that may place subjects at increased risk than what was previously known, the IRB will be promptly notified and if warranted, enrollment may be held until the PI determines whether a modification to the study is necessary and/or the informed consent documents are updated accordingly. It is the responsibility of the principal investigator to adhere to the Data Safety Monitoring Plan throughout the life of the study.

In addition, this protocol will utilize the SOCCI Data and Safety Monitoring Committee will provide another layer of data and safety oversight. DSMC membership and responsibilities are governed by the committee charter. The annual DSMC findings and recommendations will be

reported in writing to the Principal Investigator as a summary letter which will be forwarded by the Principal Investigator or designee to the CS-IRB. The DSMC may increase or decrease the frequency of study review, at their discretion. Refer to the DSMC Charter for details of the DSMC review.

11.0 STATISTICAL CONSIDERATIONS

11.1 STUDY OUTCOME MEASURES

We have previously performed RNA-seq on 25 MIBCs, to identify 3 key bladder tumor cell populations that are differentially represented across tumors. These cells show major molecular differences and their proportions within a tumor are strong predictors of the aggressiveness of the disease, as measured by time to recurrence. Further, we have applied this data to develop a deconvolution algorithm that is able to predict their proportions in other tumors within TCGA based on bulk RNA-seq data. Now, we will apply this deconvolution algorithm to the RNA-seq data from 10 tumors in conjunction with imaging data to search for image and genomic parameters that predict tumor stage, outcomes and the presence of each of the cancer cell types, as well as fibroblasts and different immune infiltrates.

Querying a small population for numerous parameters can lead to high false positive rates, thus from our retrospective work, we will apply 30 radiomics parameters and no more than 40 genes in our network analyses, prioritization of genes and their interactions will be done using Lynx, PINTA and String algorithms to perform annotation, clustering, enrichment analysis, and prediction of high-confidence triggers and mediators in various cell populations of interest. Predictions of gene interactions and reconstruction of molecular pathways for the phenotypes of interest will be done using PINTA and STRING algorithms. These pipelines will be executed at scale using cloud resources. In addition to cloud resources, we will leverage the existing infrastructure available to the Knott lab. The results will be annotated with information from Lynx knowledge base integrating > 35 biological databases and publicly presented for revision and analysis via Web-based user interface developed in the course of the project. Biostatistical and bioinformatic comparisons of the molecular profiles with MRI imaging characteristics will be performed using a variety of publicly available and locally developed software applications.

11.2 SAMPLE SIZE CONSIDERATIONS

The main hypothesis to be tested is whether there is association between radiogenomic features and pathologic stage. The prospective study is a pilot study to: a) generate high quality data and b) show feasibility, which we will define as know-how to create a radiogenomic workflow and to learn about the correlation structure of the radiogenomics parameters of interest, which will allow us to design future studies with adequate power. Pearson's Correlation hypothesis tests with 5% significance level (two-sided) and 80% power allows sample sizes of 10 patients to detect a difference between the null hypothesis correlation of zero and the alternative hypothesis correlation of 0.76 (23). In addition, sensitivity and specificity of several potential predictors will be estimated. The maximum width of the 95% confidence interval for proportions (sensitivities or specificities) associated with sample sizes of 10 patients are 0.63 (24). We anticipate 50% of subjects presumed to have MIBC at the time of TURBT and/or presumed not to have metastatic disease to not complete the study, i.e., undergo radical cystectomy, which will denote true pathologic stage. Thus, our final sample size to enroll is 20.

12.0 REFERENCES

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13.0 STUDY SCHEMA