

## **NOTIFICATION TO SPONSOR/CRO OF BOARD ACTION**

<b>BOARD ACTION DATE:</b>	03/06/2017	<b>PANEL:</b>	1
<b>WIRB PROTOCOL NUMBER:</b>	20162603	<b>APPROVAL EXPIRES:</b>	11/12/2017
<b>WORK ORDER NUMBER:</b>	1-998773-1	<b>CONTINUING REVIEW:</b>	Annually

**SPONSOR:** Animated Dynamics, Inc.

**PROTOCOL NUM:** 002

**AMD. PRO. NUM:**

**TITLE:**

Feasibility Study of Motility Contrast Tomography for Predicting Therapeutic Response Among Bladder Cancer Patients Receiving Neoadjuvant Chemotherapy

**APPROVAL INCLUDES:**

Revised Protocol (03-01-2017)

Template Consent Form [S0]

**THE BOARD DIRECTED THE FOLLOWING INFORMATION BE PLACED ON THE WIRB CERTIFICATE OF APPROVAL DOCUMENT FOR ANY INVESTIGATOR APPROVED BY WIRB TO CONDUCT THIS RESEARCH:**

WIRB will begin reviewing investigator submissions upon written notice from you that the approved documents are acceptable. Please note that investigator submissions will not be reviewed until such notice is received by WIRB. If you have not provided us a list of the applicable investigators, please provide us with that information. To give approval to review investigator submissions, contact WIRB Client Services at [clientservices@wirb.com](mailto:clientservices@wirb.com).

**ALL WIRB APPROVED INVESTIGATORS MUST COMPLY WITH THE FOLLOWING:**

1. Conduct the research in accordance with the protocol, applicable laws and regulations, and the principles of research ethics as set forth in the Belmont Report.
2. Although a participant is not obliged to give his or her reasons for withdrawing prematurely from the clinical trial, the investigator should make a reasonable effort to ascertain the reason, while fully respecting the participant's rights.
3. Unless consent has been waived, conduct the informed consent process without coercion or undue influence, and provide the potential subject sufficient opportunity to consider whether or not to participate. (Due to the unique circumstances of research conducted at international sites outside the United States and Canada, when there is a local IRB and WIRB approved materials are reviewed by the local IRB and translated into the local language, the following requirements regarding consent forms bearing the WIRB approval stamp and regarding certification of translations are not applicable.)
  - a. Use only the most current consent form bearing the WIRB "APPROVED" stamp.
  - b. Provide non-English speaking subjects with a certified translation of the approved consent form in the subject's first language. The translation must be approved by WIRB unless other arrangements have been made and approved by WIRB.
  - c. Obtain pre-approval from WIRB for use of recruitment materials and other materials provided to subjects.
4. Enrollment of limited readers and non-readers: unless consent has been waived or the protocol excludes enrollment of limited readers or non-readers, involve an impartial witness in the consent process when enrolling limited or non-readers and document the participation of the impartial witness using the designated signature lines on the WIRB-approved consent form. In the absence of designated signature lines, download the WIRB standard impartial witness form from [www.wirb.com](http://www.wirb.com).

IF YOU HAVE ANY QUESTIONS, CONTACT WIRB AT 1-800-562-4789

This is to certify that the information contained herein is true and correct as reflected in the records of the Western Institutional Review Board (WIRB), OHRP/FDA parent organization number IORG 0000432, IRB registration number IRB00000533. WE CERTIFY THAT WIRB IS IN FULL COMPLIANCE WITH GOOD CLINICAL PRACTICES AS DEFINED UNDER THE U.S. FOOD AND DRUG ADMINISTRATION (FDA) REGULATIONS, U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES (HHS) REGULATIONS, AND THE INTERNATIONAL CONFERENCE ON HARMONISATION (ICH) GUIDELINES.



5. Enrollment of pregnant partners that do not have the capacity to consent for themselves and require consent be provided by a legally authorized representative: unless the protocol excludes the enrollment of pregnant partners that do not have capacity to consent for themselves, obtain consent from the pregnant partners legally authorized representative and document consent using the pregnant partner legally authorized representative signature lines on the WIRB-approved consent form. In the absence of designated signature lines, download the WIRB standard legally authorized pregnant partner form from [www.wirb.com](http://www.wirb.com).
6. Obtain pre-approval from WIRB for changes in research.
7. Obtain pre-approval from WIRB for planned deviations and changes in research activity as follows:
  - If the research is federally funded, conducted under an FWA, or is a clinical investigation of a drug or biologic, then all planned protocol deviations must be submitted to WIRB for review and approval prior to implementation except where necessary to eliminate apparent immediate hazards to the human subjects [(DHHS 45 CFR § 46.103(b)(4); (FDA 21 CFR § 56.108(a)(4); ICH 3.3.7].
  - However, if the research is a clinical investigation of a device and the research is not federally funded and not conducted under an FWA, then only planned protocol deviations that may adversely affect the rights, safety or welfare of subjects or the integrity of the research data should be submitted to WIRB for review and approval prior to implementation except where necessary to eliminate apparent immediate hazards to the human subjects [(DHHS 45 CFR § 46.103(b)(4); (FDA 21 CFR § 56.108(a)(4); ICH 3.3.7].

The reason for these different requirements regarding planned protocol deviations is that the Office for Human Research Protections (OHRP) and the Food and Drug Administration (FDA) drug and biologic divisions have adopted the regulatory interpretation that every planned protocol deviation is a change in research that needs prior IRB review and approval before implementation; however, the FDA device division operates under a distinct regulation (See 21 CFR 812.150(a)(4)).

Deviations necessary to eliminate apparent immediate hazards to the human subjects should be reported within 10 days.

8. Report the following information items to the IRB within 5 days:
  - a. New or increased risk
  - b. Protocol deviation that harmed a subject or placed subject at risk of harm
  - c. Protocol deviation made without prior IRB approval to eliminate an immediate hazard to a subject
  - d. Audit, inspection, or inquiry by a federal agency
  - e. Written reports of federal agencies (e.g., FDA Form 483)
  - f. Allegation of Noncompliance or Finding of Noncompliance
  - g. Breach of confidentiality
  - h. Unresolved subject complaint
  - i. Suspension or premature termination by the sponsor, investigator, or institution
  - j. Incarceration of a subject in a research study not approved to involve prisoners
  - k. Adverse events or IND safety reports that require a change to the protocol or consent
  - l. State medical board actions
  - m. Unanticipated adverse device effect
  - n. Information where the sponsor requires prompt reporting to the IRB

Information not listed above does not require prompt reporting to WIRB.

Please go to [www.wirb.com](http://www.wirb.com) for complete definitions and forms for reporting.

9. Provide reports to WIRB concerning the progress of the research, when requested.
10. Ensure that prior to performing study-related duties, each member of the research study team has had training in the protection of human subjects appropriate to the processes required in the approved protocol.

**Federal regulations require that WIRB conduct continuing review of approved research. You will receive Continuing Review Report forms from WIRB when the expiration date is approaching.**

**DISTRIBUTION OF COPIES:**

**Contact, Company**

Travis Morgan, MBA, Animated Dynamics, Inc.

**Protocol Title:**

Feasibility Study of Motility Contrast Tomography for Predicting  
Therapeutic Response Among Bladder Cancer Patients Receiving  
Neoadjuvant Chemotherapy

**Date:**

March 1, 2017

**Principal Investigator:**

Multiple

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**Resources:**

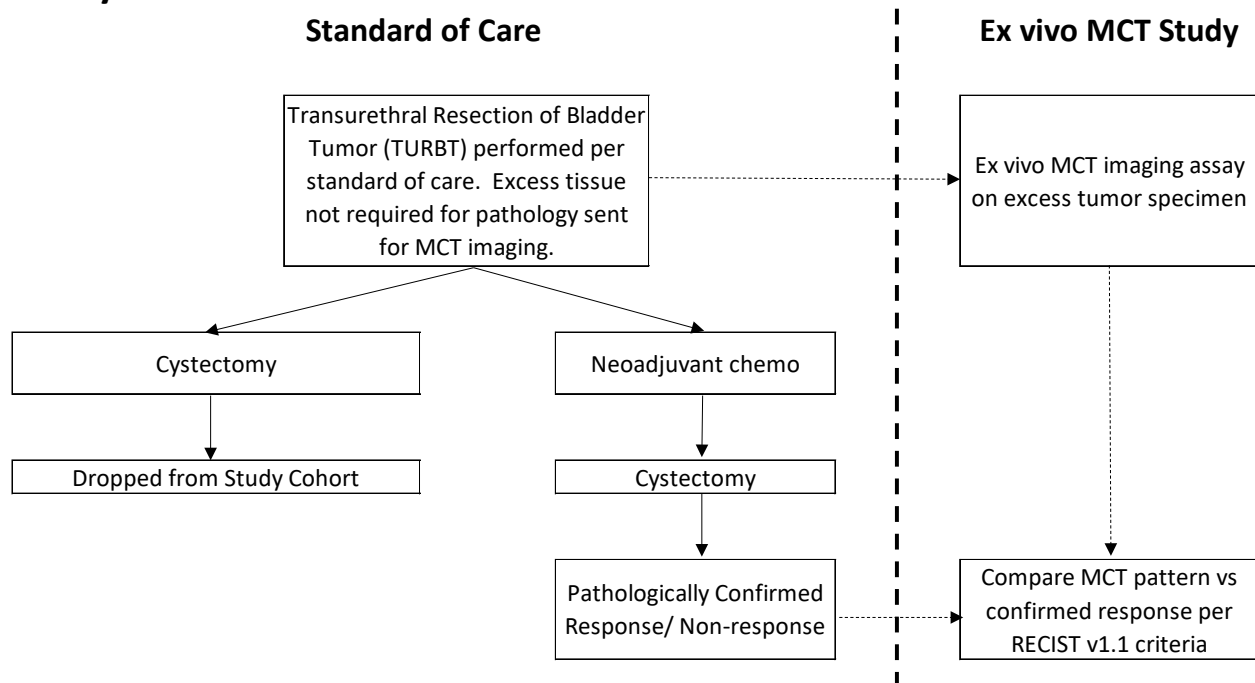
Funded by Animated Dynamics, Inc.

## PROJECT SUMMARY

In the US alone, millions of cancer patients every year receive chemotherapy with only a 20-60% probability of pathological response, while some experience adverse side effects that lower quality of life without necessarily prolonging it. Reliable identification of ineffective therapies can eliminate needless human suffering while increasing overall probability of positive response to treatment. Chemotherapy resistance profiling entails testing whether a patient exhibits strong resistance to a therapy prior to its final selection by the oncologist. However, there are no effective methods for quickly assessing patient chemotherapy resistance. Patient Derived Xenograft (PDX) models have replaced older Chemotherapy Sensitivity and Resistance Assays (CSRAs) to some degree, but both technologies suffer from long testing times, high cost, and/or low accuracy.

Motility Contrast Tomography (MCT) has recently emerged as a technology that measures the biodynamic response of intact tumor biopsies to applied therapeutics by using Doppler detection of infrared light scattered from intracellular motions inside living tissue. Several small scale animal, xenograft, and human studies have shown this phenotypic profiling technique to be highly accurate in prediction of response and resistance to chemotherapy. This project will be the first human trial of biodynamic phenotyping to predict chemotherapy response among bladder cancer patients. Specifically, the study cohort will include patients selected for neoadjuvant chemotherapy treatment, because this setting offers the opportunity for near-term outcome measurement at the time of post-chemo surgery. Pre-therapy fresh tumor specimens will be imaged using MCT, and the resulting bio-dynamic signatures will be compared to confirmed pathological response at the time of surgery. Observation of a high predictive value will provide the basis for expanded clinical trials and prompt commercialization of a biodynamic chemotherapy selection assay for bladder and other cancer patients.

### Study Schema:



## RATIONALE & BACKGROUND

**STUDY RATIONALE:** The demonstrated ability of MCT to accurately assess tumor xenografts may establish it as a reliable technique for patient tumor stratification based on predicted response to therapy, which could enable a treatment selection based on the personal needs of an individual patient. This study is designed to assess MCT as an assay for predicting chemosensitivity to treatment with chemotherapy agents routinely used in the neoadjuvant and adjuvant treatment of bladder cancer. If positive, the results of this study will provide the basis for expanded clinical trials and use of MCT in therapy selection.

**BACKGROUND:** Live cell imaging has become the standard for high-content analysis and drug discovery applications. The most common assays on live cells include viability, proliferation and cytotoxicity assays as cellular physiology and function are measured responding to applied perturbations of xenobiotics. Cellular and tissue viability assays are typically measured using exogenous vital dyes as biomarkers of membrane integrity or cellular metabolic activity. However, dyes are invasive, potentially toxic, and often require fixing of the tissue or permeabilization of the membranes [1, 2]. Furthermore, the common format of high content analysis and flow cytometry requires isolated cells, or cells distributed on flat hard surfaces. Isolated cells lack many of the biologically-relevant intercellular connections and communications that are hallmarks of healthy tissue [3, 4], and flattened cells on plates have pathological shapes and anisotropic cellular adhesions [5].

Discovery of technology that can predict response to cancer therapy is an urgent priority. While many technologies exist to evaluate early response to drugs *ex vivo*, the need to perform viability, cytotoxicity and proliferation assays in three-dimensional tissue or culture has become increasingly urgent [6, 7], as drug response in 2D is often not the same as drug response in biologically-relevant three dimensional culture. This is in part because genomic profiles are not preserved in monolayer cultures [8-10]. There have been several studies that have tracked the expression of genes associated with cell survival, proliferation, differentiation and resistance to therapy that are expressed differently in 2D cultures relative to three-dimensional culture. For example, cell lines of epithelial ovarian cancer [11, 12], hepatocellular carcinoma [13-15] or colon cancer [16] display expression profiles more like those from tumor tissues when measured in three-dimensional culture, but not when grown in 2D. In addition, the three-dimensional environment of 3D culture presents different pharmacokinetics than 2D monolayer culture and produce differences in cancer drug sensitivities [17-20]. Finally, most current technologies rely on destructive end-point assessment, preventing meaningful longitudinal observation of therapy response over time.

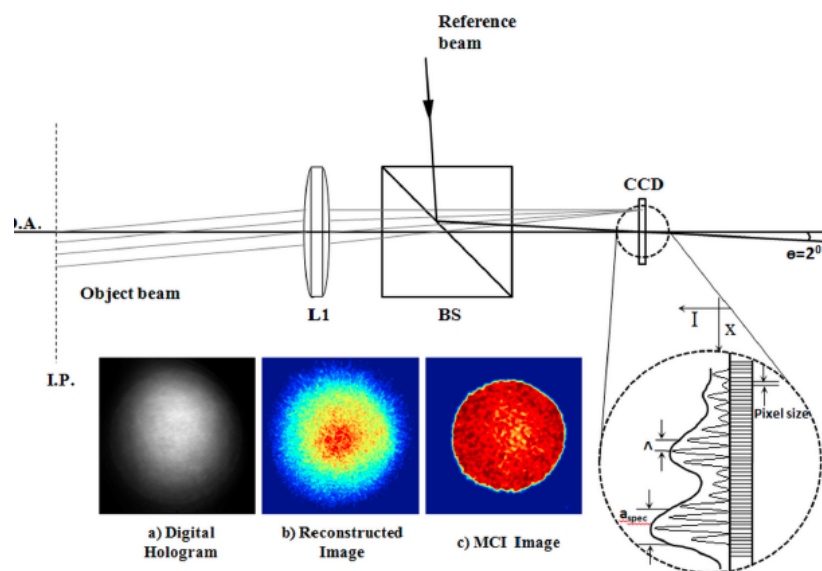
One of the main challenges to migrating drug-response assays to the third and fourth dimensions has been to find a means to extract vital information from deep (up to a millimeter) inside living tissue. Tissue is translucent and light can propagate diffusively many centimeters. Furthermore, the dynamic motions of living cells cause dynamic light scattering that produces phase fluctuations on the scattered light fields that can be measured as dynamic speckle in diffusely reflected light from tissue. This is the basis of diffusing wave spectroscopy (DWS) [21, 22] and diffusion correlation spectroscopy (DCS) [23-26], but these techniques lack depth resolution. A powerful tool in the characterization of light propagation in tissues is the use of interferometry [27]. Interferometric detection is the underlying process in optical coherence tomography (OCT) [28-31], which is a point-scanning technique that suppresses speckle [32-34], although speckle decorrelation in OCT data can provide similar information as provided by DCS. This has been used to measure intracellular rheology [35] and to find dynamic signatures of apoptosis [36]. Transport also can be detected at cellular resolution using phase contrast microscopy [38], but this approach cannot be used in thick tissues.

Dr. Nolte and colleagues have developed volumetrically-resolved tissue dynamic imaging that uses the advantages of depth selectivity from low-coherence interferometry, combined with high speckle contrast in broad-illumination digital holography. The technique is called Motility Contrast Tomography (MCT) and uses low-coherence digital holography to penetrate up to 1 millimeter into living tissue to measure speckle dynamics from light scattering from dynamic motion in living cells [37]. It was previously applied as a cytotoxicity assay to study the efficacy of anti-mitotic drugs [40]. In essence, the technology works by profiling the 'movement' of cellular organelles. Specific changes in organelle motion are detectable very early in cells undergoing response to chemotherapy treatment, and may be usable as an early predictor of chemotherapy response.

MCT is based on optical coherence imaging (OCI) [38]. OCI is a full-field short-coherence holography [39] that collects backscattered speckle. With the help of coherence gating, OCI can optically section tissue up to 1 mm deep. MCT specifically uses intracellular motion as the endogenous contrast to characterize submicron subcellular motion inside three-dimensional living tissue [42].

Figure 1 shows the holographic recording principle of MCT. After calibration, the short coherence light is first divided into an object beam and a reference beam. The object beam hits the living tissue sample, and backscattered speckles from the tissue are collected by the lens L1. The living tissue sample locates at the focal plane of the lens L1, so L1 also performs an optical Fourier transform of the backscattered light. The charge coupled device (CCD) locates at the other focal plane of L1, so it captures the Fourier transformed scattering light from the tissue. The reference beam is controlled by a delay stage (not shown in the figure) to adjust the path length of the reference beam to perform a zero-path match between the object and reference beams. The beam splitter combines both beams and because they are zero-path matched, they interfere at the CCD plane. The reference beam is tilted by  $20^\circ$  in an off-axis configuration, and the spacing of the interference fringes ( $\Lambda$ ) is 3 pixels ( $24\ \mu\text{m}$ ). The speckle size (aspec) is adjusted to be 3 fringes wide ( $70\ \mu\text{m}$ ). Additional details about the experimental setup can be found in reference [41].

Fig.1. The principle of MCT on multicellular tumor spheroids. The biological sample is located at the image plane of lens L1. The back scattered light from the sample is Fourier transformed by L1 and interfered with reference beam on the CCD chip. The speckle hologram is recorded on the Fourier plane with a  $20^\circ$  crossing angle with the reference beam. Examples of a) Raw digital hologram; b) reconstructed image; c) MCI image. O.A.: optical axis; I.P.: image plane; L1: lens; BS: beam splitter; CCD: charge coupled device.



**EX VIVO CANCER CHEMOSENSITIVITY ANALYSIS** MCT was previously applied to study the efficacy of anti-mitotic drugs using multicellular tumor spheroids [40]. When applying MCT to tumor xenografts, it is also capable of showing a significantly different response between two cell lines under cisplatin. After applying the drug, the normalized standard deviation (NSD) value of the platinum-sensitive cell line (A2780) drops from 0.7 to 0.1 in 8

hours. In contrast, the NSD value of the platinum-resistant cell line (A2780-CP70) remains nearly constant (0.81 to 0.80) 9 hours after applying drug. The NSD value of normal mouse tissue attached to the tumor xenograft decreases only a little (0.6 to 0.52) compared with A2780. Fig. 2 shows the cisplatin drug response curves. The NSD value of each point is averaged over the entire target. The time between measurements is 24 minutes for A2780-CP70 and normal mouse tissue and is 12 minutes for A2780. The 20  $\mu\text{M}$  cisplatin was applied at time  $t = 0$ , and the measurements lasted 9 hours for A2780-CP70 and normal mouse tissue, and lasted 8 hours for A2780. At time=0, the aggressive cell line A2780-CP70 has the highest NSD and the normal mouse mesenterium tissue has lowest NSD (0.6). The NSD of the platinum-sensitive cell line A2780 lies in the middle: 0.7. After applying cisplatin, the NSD curve of A2780 drops immediately. The NSD value of the A2780-CP70 almost doesn't change.

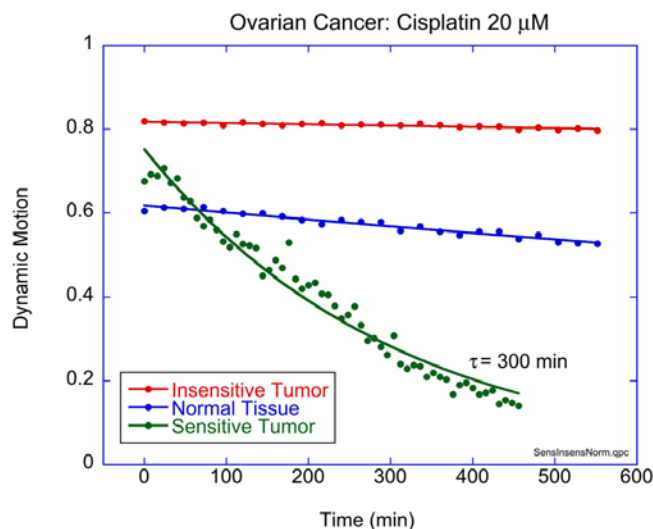


Fig. 2 Motility metric (NSD) of ovarian cancer tumor xenografts responding to 20  $\mu\text{M}$  cisplatin. The x-axis is time (minute), the y-axis is NSD value. The sensitive tumor is A2780, while the insensitive tumor A2780-CP70. Both tumor tissues begin with higher motility than normal mouse tissue. The cisplatin was added at time  $t=0$ . The NSD of A2780 dropped very fast and after 8 hours it dropped to 0.1. The NSD of the insensitive tumor A2780-CP70 didn't change during 9 hour period. The NSD of normal mouse tissue dropped a little compared with the A2780.

In a further study in a veterinary clinical setting, MCT has been used to predict patient outcome for canine non-Hodgkin's lymphoma. Canine non-Hodgkin's lymphomas are initially characterized by tumoral infiltration of peripheral lymph nodes. Canine non-Hodgkin's lymphomas are diverse in their clinical aggressiveness and response to chemotherapy. The only current biomarker for chemoresponsiveness is tumor cell immunophenotype (i.e. T-cell vs. B-cell origin), but chemoresponsiveness varies tremendously within immunophenotype, which reduces the clinical utility of this biomarker. In our study, we used MCT to measure the heterogeneous response of canine lymphoma biopsies to the standard-of-care doxorubicin. The biodynamic signatures of doxorubicin responsivity ex vivo were correlated with canine patient outcome. These studies have demonstrated, for the first time, the utility of label-free intracellular biodynamic markers to predict therapeutic efficacy for cancer treatment in dogs.

## SPECIFIC AIMS

The primary study objective is to examine the feasibility of using MCT as a chemosensitivity assay among bladder cancer patients receiving neoadjuvant chemotherapy by comparing MCT patterns consistent with chemotherapy response or resistance ex-vivo to confirmed response or resistance to chemotherapy as measured by Response Evaluation Criteria in Solid Tumors (RECIST) v1.1 criteria.

**PRIMARY ENDPOINT:** Objective pathological response measured at the time of surgery.

## RESEARCH DESIGN AND METHODS

**STUDY DESIGN:** Retrospective correlation study.



**PARTICIPANT IDENTIFICATION:** Patients of all races and ethnic groups are eligible for this study. Patients will be recruited from participating sites after interpretation of initial cystoscopy and/or radiological screening, but prior to performance of a second staging biopsy and/or Transurethral Resection of Bladder Tumor (TURBT) procedure per standard of care. Pre-screening of potential participants may be performed by the principal investigator or delegates under the principal investigator's direct supervision. Once all eligibility criteria are confirmed, potential participants will be offered the opportunity to participate. Potential participants will be presented with the purpose of the study and the potential risks and benefits of participation. All potential participants who are interested in participation will undergo the informed consent process privately at the time of their appointment or during a return appointment after they have an opportunity to review the consent. Participants will be considered registered to the study upon receipt of a signed informed consent statement and determination by the principal investigator post-cystoscopy procedure that sufficient tumor tissue will likely be available to allow for excess specimen to be used in the study.

Registration information will be maintained by the Principal Investigator. All participants will be assigned a unique study ID derived from the bar-coded specimen collection kit used to collect all specimens in the study. All subjects will adhere to inclusion and exclusion criteria as listed below.

**INCLUSION CRITERIA:** To be eligible for the study, patients must meet the following criteria:

1. Ability to understand and willingness to sign an informed consent and authorization for release of tissue not required for pathologic diagnosis to be used for research purposes
2.  $\geq 18$  years old at time of consent
3. Suspected muscle-invasive cancer and/or high probability of receiving neoadjuvant chemotherapy and a cystectomy

**EXCLUSION CRITERIA:** To be eligible for the study, patients must not have any of the following:

1. Women who are pregnant or breastfeeding
2. Known tumor genetics, comorbidities or other factors, which in the treating physician's professional judgement, make the patient an unlikely candidate to receive neoadjuvant chemotherapy or a cystectomy.

**METHODS:** See Appendix B for a provider workflow checklist. Patients will be identified as noted above in "Participant Identification." Patient confidentiality will be maintained during all chart reviews and data collection.

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#### PRE-TREATMENT BIOPSY PLAN

The standard of care for newly diagnosed or recurrent transitional cell carcinoma in the bladder is pathologic confirmation by TUR-BT (TransUrethral Resection-Bladder Tumor). This is done for both tissue confirmation and staging. Typically, a tumor that is likely to be muscle invasive is usually larger and more sessile than small and papillary. The most important part of the specimen procured is the base to confirm muscle invasive disease. Often, urologist procure enough tissue to make the diagnosis without resecting the entire tumor. Hence, often times there is plenty of tissue remaining or that can be procured for testing the study hypothesis. We suggest the surgeon procure at least 80mg of fresh tissue for this study (approximately 3-4 loops) then continue the TUR for pathologic confirmation as is standard of care.



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## BIOPSY SAMPLE COLLECTION PARAMETERS

Tumor biopsy samples will be collected using standardized specimen collection kits, each containing a unique bar code that will serve as the patient's primary study ID. Upon completion of a TURBT procedure on a consenting patient where sufficient excess tissue is available for MCT imaging (at least 80mg of excess tumor), the excess specimen will be placed in a bar coded 15mL vial containing transport medium, chilled using an ice pack provided with each collection kit, and conveyed to FedEx for next day delivery to Animated Dynamics' laboratory in Indianapolis. See Appendix B.

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## BIOPSY SAMPLE PROCESSING AND ANALYSIS

Upon receipt of tissue, researchers at Animated Dynamics will dissect prepared samples into small pieces up to 1 mm<sup>3</sup> in volume. Sample pieces will be immobilized in multi-well specimen plates using agarose. Prepared plates will be imaged using MCT. After a 2-4 hour baseline period, selected anti-cancer drugs will be applied. The dynamic spectra are then acquired for at least 9 hours to evaluate dynamic response to the applied therapeutics. Samples are then removed from the MCT system, fixed, and stored at 4° C.

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## CHEMOTHERAPY TREATMENT PLAN

**Administration and treatment schedule:** Registered participants will be treated per routine care guidelines and subject to treating physician discretion. Patients may be treated with an additional biologic agent if done within the confines of a separate clinical trial.

**General concomitant medication and support care guidelines:** Routine care guidelines should be followed at investigator discretion.

**Dose delays/modifications:** Dose adjustments for toxicity will be based on recommendations per the package insert and at the treating investigator's discretion.

**Duration of follow up:** Participants will be followed per routine care guidelines for progression.

**Criteria for removal:** Participants may withdraw from the study at any time at their own request, or they may be withdrawn at any time at the discretion of the investigator for safety, behavioral, or administrative reasons. Reasons for participant removal include: noncompliance with study procedures or follow-up procedures; participant withdrawal of consent and election to discontinue participation in the trial; any other reason which in the opinion of the investigator, would justify removing the participant from the study.

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## MEASUREMENT OF EFFECT - CHEMOTHERAPY

**Timing of response assessment:** In addition to a baseline scan (obtained within 35 days prior to registration), patients' response to chemotherapy will be evaluated immediately upon surgical tumor resection per routine care guidelines and according to institutional practice.

**Methods of response assessment:** Response and progression will be evaluated in this study using the new international criteria proposed by the revised RECIST guideline (version 1.1). Changes in the largest diameter (unidimensional measurement) of the tumor lesions are used in the RECIST criteria.

**Evaluable for objective response:** Only those patients who have measurable disease present at baseline, have received at least one cycle of neoadjuvant chemotherapy, and have had their disease re-evaluated upon surgical tumor resection will be considered evaluable for response. These patients will have their response classified

according to the definitions stated below. (Note: Patients who exhibit objective disease progression prior to the end of cycle 1 will also be considered evaluable.)

**Evaluable Non-Target Disease Response:** Patients who have lesions present at baseline that are evaluable but do not meet the definitions of measurable disease, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for non-target disease. The response assessment is based on the presence, absence, or unequivocal progression of the lesions.

**Measurable disease:** Measurable lesions are defined as bladder lesions that can be accurately measured in at least one dimension (longest diameter to be recorded) as  $\geq 2$  cm by cystoscopy or CT scan, MRI, or calipers by clinical exam. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

**Non-measurable disease:** All other lesions (or sites of disease), including small lesions (longest diameter  $< 2$  cm), are considered non-measurable disease for purposes of this study.

**Target lesions:** Any measurable bladder lesions up to a maximum of 2 lesions per bladder and 4 lesions in total, should be identified as target lesions and recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all lesions, but in addition should be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement in which circumstance the next largest lesion which can be measured reproducibly should be selected. A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum diameters. The baseline sum diameters will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease.

**Non-target lesions:** All other lesions (or sites of disease) including any measurable lesions over and above the target lesions should be identified as non-target lesions and should also be recorded at baseline. Measurements of these lesions are not required, but the presence, absence, or in rare cases unequivocal progression of each should be noted throughout follow-up.

**Methods for Evaluation of Measurable Disease:** All measurements should be taken and recorded in metric notation using a ruler or calipers. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 4 weeks before the beginning of the treatment.

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## RESPONSE CRITERIA

**Complete Response (CR):** Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to  $< 10$  mm.

**Partial Response (PR):** At least a 30% decrease in the sum of the diameters of target lesions, taking as reference the baseline sum diameters.

**Progressive Disease (PD):** At least a 20% increase in the sum of the diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progression.)

**Stable Disease (SD):** Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study.

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## MEASUREMENT OF EFFECT – MOTILITY CONTRAST TOMOGRAPHY

The foundational data structure that is obtained by MCT is a time-frequency spectrogram. This spectrogram captures how the Doppler frequencies of intracellular motion respond to the applied drug. This data structure is two-dimensional and can exhibit a variety of patterns that have been associated with mechanistic response of the tissue to the drug. To further improve the interpretation of the MCT results, numerous metrics have been developed that quantify the cellular response. These include: Backscatter brightness, NSD, change in NSD, dipole sine response, dipole cosine response, and quadrupole response. A further set of metrics, that are linearly dependent on the previous metrics, have more direct cellular mechanistic basis. These include: mitotic index, apoptotic index, necrotic index, metabolic activity, and membrane ruffling. These metrics are non-orthogonal, and a patient will often show strongly correlated responses among these metrics. Multiparameter logistic regression is performed on these metrics to provide a single-valued predictor of therapy response.

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## DRUG FORMULATION AND PROCUREMENT

**NOTE:** All therapy in this trial is standard of care. All drugs to be tested in this study are commercially available formulations procured, stored, and administered in accordance with routine care standards and institutional policy. The primary therapies that will be screened using MCT in this study will include the following list, but may be supplemented from time to time at the investigators discretion. Therapies are listed in order of descending priority, with lower priority therapies tested subject to availability of sufficient tissue quantity. When tissue quantity permits, both combination and monotherapies will be imaged using MCT.

- DDMVAC (dose-dense methotrexate, vinblastine, doxorubicin, and cisplatin)
- Gemcitabine and cisplatin
- CMV (cisplatin, methotrexate, and vinblastine)

**STUDY COSTS:** All non-routine care procedures including the preparation, handling, and transport of MCT specimens, MCT assay procedures, and reporting of outcome data will be paid for by the study.

**SAFETY CONSIDERATIONS:** **NOTE:** All procedures and therapy in this trial are standard of care. Since the study does not entail any intervention in patients' care, only adverse events related to the loss of patient confidentiality will be reported to the IRB at the time of continuing review.

## STATISTICAL CONSIDERATIONS

**STUDY DESIGN:** This is a non-randomized, multi-arm, multi-center, feasibility study, designed to examine the feasibility of using MCT as a chemosensitivity assay among bladder cancer patients by comparing ex-vivo MCT patterns consistent with chemotherapy response and resistance to confirmed pathological response or resistance as measured by Response Evaluation Criteria in Solid Tumors (RECIST) v1.1 criteria.

**DEFINITION OF PRIMARY OUTCOME ENDPOINT:** Feasibility is defined as a high statistical correlation between MCT-predicted response, and surgically confirmed response to neoadjuvant chemotherapy.

**ANALYSIS PLAN FOR PRIMARY OBJECTIVE:** The study will be considered successful, if a majority of specimens yield interpretable MCT results, and if MCT signatures are identified which demonstrate a high statistical correlation to confirmed patient response.

**EXPLORATORY ANALYSIS:** Due to the inadequate samples size, no formal analysis is planned. Descriptive analysis of the correlation between MCT parameters and clinical outcome measures will be performed.

**DETERMINATION OF SAMPLE SIZE:** A target sample size of 500 patients was selected for initial enrollment using the investigators' previous research experience to approximate the appropriate number needed to demonstrate the feasibility of this technique. It is anticipated that only 20-30% of enrolled patients will yield specimens evaluable for response. It is estimated that it will take approximately 6-12 months to complete accrual.

**EVALUATION FOR RESPONSE:** Participants who provide a bladder tumor specimen, have received at least one cycle of chemotherapy, and have had their disease re-evaluated upon surgical resection will be considered evaluable for response.

**REPLACEMENTS:** Participants who withdraw from the study prior to having the TURBT procedure or fail to complete at least one evaluation for response will be replaced. No reported data will be included for participants who are replaced.

## DATA AND RECORD KEEPING

**DATA COLLECTION:** Each subject will be assigned a unique study ID number once it has been determined they meet inclusion criteria. Subject outcome data will be collected using a secure web-based data collection form which will identify subjects only by their unique study ID and procedure date. Only the study investigators will have access to the list matching each subject ID to the patient's medical records, and only the PI and his immediate staff will enter follow-up data into the data collection form. The following data points will be collected for this study:

- pre-treatment clinical/ pathological description of disease
- TURBT date, location, and clinical description
- objective response, as confirmed upon surgical tumor resection,
- Neoadjuvant chemotherapy agent(s) and regimen used
- Pre and post-treatment CT scans (if available)

**RECORD KEEPING/CONFIDENTIALITY:** This information will be stored on password protected electronic systems managed by the study team. All hardcopies of study related documents will be kept in locked and secure files accessible only to the study team. The potential risk to subjects is the loss of confidentiality. To mitigate this risk, the data collection form contains no patient identifiers. As data are entered into the data collection form, the PI will create a master list that links the patient to her study identification number. This master list will be stored in a locked room in a locked cabinet with limited public access. The electronic files will be stored on a secure computer with password protection. To minimize the risk of lost confidentiality, only study investigators will have access to the list that links patient identifiers to the assigned study number. Once the patient is assigned a study identification number, that number will be used without additional patient identifiers whenever possible. All collected information will be stored electronically on an encrypted and password protected laptop.

**RISK ASSESSMENT:** This study involves only procedures and commercial therapeutic agents used in accordance with routine care practice and institutional guidelines. No increase in patient health risk is anticipated as a result of this study. There is a slight risk of loss of confidentiality of participant information. Every effort will be made to

keep participant information confidential. The PI is responsible for conducting continuous review of data and participant safety. At any time during the conduct of the trial, if it is the opinion of the investigators that the risk (or benefits) to the participants warrant early closure of the study, the study will be closed.

**CHANGES TO THE PROTOCOL:** Study procedures will not be changed without the mutual agreement of the Principal Investigator and co-investigators. If it is necessary for the study protocol to be amended, the amendment or a new version of the study protocol (amended protocol) will be generated by the Principal Investigator and must be approved by an IRB. Local requirements must be followed. If a protocol amendment requires a change to the Written Informed Consent Form, then the IRB must be notified. Approval of the revised Written Informed Consent Form by the IRB is required before the revised form is used. The Principal Investigator is responsible for the distribution of these documents to his IRB, and to the staff at his center. The distribution of these documents to the regulatory authority will be handled according to local practice.

## ETHICS:

The final study protocol, including the final version of the Written Informed Consent Form, must be approved by an IRB. The Principal Investigator is responsible for informing the IRB of any amendment to the protocol in accordance with local requirements. In addition, the IRB must approve any advertising used to recruit participants for the study. The protocol must be periodically re-approved by the IRB annually as local regulations may require. Progress reports and notifications of serious unexpected adverse events will be provided to the IRB according to local regulations and guidelines. The study will be performed in accordance with ethical principles originating from the Declaration of Helsinki, which are consistent with ICH/Good Clinical Practice, and applicable regulatory requirements.

**WRITTEN INFORMED CONSENT:** The investigator will ensure the participant is given full and adequate oral and written information about the nature, purpose, possible risks and benefits of the study. Participants must also be notified they are free to discontinue participation in the study at any time. The participant should be given the opportunity to ask questions and allowed time to consider the information provided. The participant's signed and dated informed consent must be obtained before utilizing any of the patient's health information or specimens for the study. The investigator must store the original, signed Written Informed Consent Form. A copy of the signed Written Informed Consent Form must be given to the participant. A copy of the Written Informed Consent Form is included in Appendix A.

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## **Appendix A – Written Informed Consent Form**

## Appendix B – Provider Workflow Checklist

### 1) Patient Screening

- a) Based on known tumor genetics, comorbidities, or other factors, is the patient highly unlikely to receive neoadjuvant chemotherapy? If “YES” then stop.
- b) Obtain patient informed consent document

### 2) Specimen Screening

- a) Is there at least 80mg of excess tumor specimen (approximately 3-4 loops) after preserving that which is necessary for routine pathology? If “YES” then proceed.

### 3) Specimen Preparation

- a) Open an Onco4D specimen collection kit (provided by Animated Dynamics) and apply the kit’s unique bar code identifier to patient paperwork using provided bar code labels. Place remaining bar code labels in patient file for use with additional paperwork in the future. This bar code will serve as the patient’s unique “Code” for purposes of identification throughout the study period, to reduce the risk of compromising protected health information.
- b) Place one copy of the patient informed consent signature in the collection kit (retaining one copy for the patient, and one for the practice).
- c) Collect two buccal swab samples using the matching bar coded swabs provided, replace in the zip top sleeve, and return to the collection kit.
- d) Dissect approximately 80mg of excess lesion:
  - i) Approximately 3-4 “loops” of tissue should yield sufficient specimen size
- e) Place fresh specimen(s) into bar coded vial in specimen collection kit ensuring that specimen is submerged in transport media, and seal vial.
- f) Place vial in zip top bag provided and seal bag.
- g) Place sealed bag in cooling kit along with buccal swabs.
- h) Place frozen ice pack in kit, close, and seal kit.
- i) Deliver Onco4D kit to Fedex courier as soon as possible (same day) for delivery to Animated Dynamics.).
- j) Record the association of the patient and the assigned Code for safekeeping.
- k) Forward pathology reports related to the TURBT procedure, as well as recommended chemotherapy regimen (if any) as soon as available.

### 4) Outcome Data

- a) As soon as practical after cystectomy, provide:

- i) neoadjuvant chemotherapy regimen given
- ii) pre and post-chemotherapy CT studies
- iii) pathology report from cystectomy specimen, including confirmed pathological response per RECIST v1.1 (if available)