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CONTRACTING ORGANIZATION: Sunnybrook Research Institute, Toronto, Canada

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

In this project, we will use surgical specimens from the TMIST Lead-In in an attempt to more completely define the underlying molecular, phenotypic and radiological features associated with cancers detected with digital breast tomosynthesis (DBT) vs. digital mammography (DM) or interval cancers, and their surrounding tissue (micro/macroenvironment). We will extract and characterize radiological features including mammographic density, parenchymal texture, lesion morphology, etc. from cancers in DBT and DM images. We will compare the molecular classifications of cancers detected by DBT vs. DM and test correlation with imaging patterns. We will process a subset of the breast cancer specimens using 3D whole-mount processing, in combination with protein marker multiplexing to study the cancer, the tumor micro-environment and the proximal "macro-environment". A protein biomarker panel reporting on: hormonal receptors, proliferative capacity, functional status of tumor infiltrating lymphocytes (TILs), macrophages and cancer-associated fibroblasts (CAFs) in the microenvironment, and mammographic density-related markers will be studied. We believe that this integrated cross-platform study will identify novel imaging signatures that could inform clinicians the biological characteristics of the cancer detected, and facilitate more appropriate, precise and efficient intervention utilizing targeted and conventional therapeutics. In the past year, we have collected tissue blocks from diagnosed cancer cases, and extracted molecular materials for analysis. Radiological images have also been retrieved and radiomics analysis will begin shortly.

15. SUBJECT TERMS

None listed.

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INTRODUCTION

Our project aims at integrating imaging information of breast cancer to correlate to the underlying molecular alterations in order to improve characterization and prognostication of detected lesions and subsequent management of the disease. We will be studying breast cancers diagnosed from the Tomosynthesis Mammographic Imaging Screening Trial (TMIST) Lead-In component. Existing breast cancers will be retrospectively analyzed. Tissue blocks will be retrieved for molecular analysis including targeted mutational sequencing and expression profiling. Mammographic images from the same cases will be studied to identify radiomic imaging patterns. In additional to studying the existing specimens, we will also prospectively recruit newly diagnosed breast cancer patients from the TMIST Lead-In for whole-mount (WM) histopathological processing of their surgical specimens. Since the spatial context of tumor and surrounding stromal tissue are preserved with WM processing, we can coarsely locate the findings from our molecular examinations to imaging data to identify imaging features that would potentially be useful for predicting the aggressiveness of cancer.

KEYWORDS

Breast cancer characterization, imaging, tomosynthesis, molecular analysis, biomarker, radio-histo-genomics

ACCOMPLISHMENTS

Working closely with our study pathologist Dr. Elzbieta Slodkowska (Department of Anatomic Pathology, Sunnybrook Health Sciences Centre) and Dr. Kela Liu (Sunnybrook Research Institute), H&E stained sections of the existing diagnosed cancer cases from the TMIST Lead-In study were carefully reviewed for eligibility to be included into our molecular analysis study. These criteria include grade of tumors, size and cellularity. Tumors that were deemed too small in size for extraction of molecular material were excluded. A total of 42 cases were reviewed and 36 cases were selected to be included into our study (Aim 2, Task 1). From these cases, the formalin-fixed, paraffin-embedded (FFPE) tissue blocks with the best representation of the tumor were retrieved and microtomed for another H&E section for annotation, followed by 6 ten micron-thick sections for use in molecular extraction, Two more sections were collected for whole slide protein multiplexing studies and an additional H&E slide for annotations for constructing a tissue microarray (see below).

The first batch of 36 samples included invasive ductal carcinoma (IDC), invasive lobular carcinoma, ductal carcinoma *in situ* (DCIS), lobular carcinoma *in situ* (LCIS) and mixed IDC/DCIS (Table 1). Since these specimens are also part of the main TMIST study, a detailed study protocol was devised by the lab to ensure that microtomy and slide stainings were performed to satisfy the histological analysis requirements for both studies. Clinical information from these cases were also collected from pathology reports.

Table I: Breast cancer detected in TMIST Lead-In study

Breast Cancer pathology type	Count
Invasive ductal carcinoma (IDC)	16
Invasive lobular carcinoma (ILC)	3
Ductal carcinoma in situ (DCIS)	10
Lobular carcinoma in situ (LCIS)	1

IDC/DCIS	4
Tumour bed post NAT	2
Total	*36*

DNA and RNA have been extracted from these cases by Dr. Yutaka Amemiya at the Genomics Core Facility at Sunnybrook Research Institute (Aim 2, Task 2). At a study meeting held on July 6th, 2020, discussion with our collaborators recommended to first conduct the NanoString nCounter 200 gene assay developed by Dr. John Bartlett's laboratory at the Ontario Institute for Cancer Research (OICR) (Aim 2 Task 4). This is a "research-based" expression profiling assay of 200 genes which includes the genes studied in multiple widely accepted multi-parameter prognostic assays including PAM50 (Parker JS et al), results which would allow one to assign intrinsic subtype and compute a risk score for each case. This will provide us with the information to determine the number of cancers with favorable vs poor prognosis, and whether 3D tomosynthesis mammography is more sensitive in detecting more aggressive breast cancer which is one of the objectives our proposed study (Aim 2). We have sent the 37 RNA samples from 32 cases to OICR and this work is expected to be completed by November 2020.

Tissue sections from FFPE blocks of TMIST Lead-In cancers were also prepared and will be studied using protein multiplexing imaging to determine the cellular phenotype of cancer and tumor micro- and macroenvironment (Aim 3 Task 2). A number of protein markers for cellular phenotype have also been validated in the lab including breast biomarkers (Estrogen Receptor, Progesterone Receptor, Epidermal Growth Factor Receptor 2 (HER2/neu)). We also have validated Ki67, P53, P21, P16, Cytokeratins CK8/18 and Pan-cytokeratin PCK26. An immune panel of CD3, CD4, CD8, CD20, macrophage markers CD68 and CD163, Treg marker FoxP3 has been validated, allowing us to study the composition and distribution of tumor-infiltrating lymphocytes. Our lab is currently validating the markers SMA and Fibroblast Activation Protein (FAP) to phenotype cancer-associated fibroblasts in the cancer. Multiplexing experiments are projected to initiate around December 2020 – January 2021.

Radiological images from 36 cancer cases diagnosed in TMIST Lead-In have been retrieved from the Sunnybrook hospital clinical PACS (Aim1 Task 2). A breast radiologist is being recruited into our study to annotate the lesion on the images. Once annotations are completed, radiomics quantification of imaging features such as breast density, parenchymal texture and lesion morphology will be conducted (Aim 1 Tasks 2-4). The pipeline for this analysis is nearing completion.

As the TMIST Lead In study is still on-going, newly detected breast cancer patients will be asked (and informed consent requested as per our IRB) to enroll into our whole-mount histopathology study, where their lumpectomy or mastectomy surgical specimens will be processed with a whole-mount (WM) protocol developed in our lab (Aim 3, Task 1). Preservation of spatial context of tumor and the surrounding stromal tissue with WM processing of surgical specimens will allow us to coarsely map our imaging features onto the pathological section, and link molecular findings to the radiomic phenotypes. Thus far, we have identified 3 cases of breast cancer into our prospective WM processing study. We have collected informed consent from one patient where the surgical specimen was subsequently processed with WM techniques. One other case has declined consent, and one case was not proceeded due to Covid resulted in limited lab activities (see below).

Reference:

Parker, J. S. *et al.* Supervised Risk Predictor of Breast Cancer Based on Intrinsic Subtypes. *J. Clin. Oncol.* **27,** 1160–1167 (2009).

IMPACT

Nothing to Report

CHANGES/PROBLEMS

Research activities at our laboratory at Sunnybrook Research Institute were temporarily halted when there was a Covid lockdown in Toronto, significantly impacting our progress. Recruitment in TMIST main and Lead-In studies were also suspended during that time. Research activities have now resumed with Covid precautions in place (limited/shift work allowed with physical distancing protocols). Trial recruitment is resumed at 40% level. We anticipate that these precautions will be in place until the pandemic is over.

PRODUCTS

Nothing to Report.

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

Name:	Martin Yaffe
Project Role:	Principal Investigator
Researcher Identifier	N/A
Nearest person month worked:	3
Contribution to Project:	Oversight of all related scientific activities for TMIST Lead-In and DOD projects; reviewed and approval of IRB and Data Use and Sharing Agreements (DUAs) with OICR and Dr. Troester, chairing of the annual team meeting (July 6, 2020).
Funding Support:	Ontario Institute for Cancer Research (OICR)

Name:	Alison Cheung
Project Role:	Research Associate
Researcher Identifier	N/A
Nearest person month worked:	2
Contribution to Project:	Project planning, co-ordination of scientific activities; review of IRB and DUAs documents, planning of the annual team meeting (July 6, 2020), sample review and selection.
Funding Support:	Ontario Institute for Cancer Research (OICR)

Name:	Rachel Peters
Project Role:	Research Laboratory Technologist
Researcher Identifier	N/A
Nearest person month worked:	1
Contribution to Project:	Clinical data/reports/images collection and organization; slide scanning, microtomy, clinical data entry, QC/QA.
Funding Support:	Ontario Institute for Cancer Research (OICR)

Name:	Kela Liu
Project Role:	Lab Manager/Pathology (Foreign Medical Grad.)
Researcher Identifier	N/A
Nearest person month worked:	1
Contribution to Project:	Whole mount tissue processing and preparation (1 case processed out of 3 planned: 2 cases cancelled due to Covid and other restrictions), slide annotations for DNA/RNA extraction (n=36)
Funding Support:	Ontario Institute for Cancer Research (OICR)

There are no changes in the support of PI or senior key personnel. There are no changes in partnering organizations.