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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.

15. SUBJECT TERMS

NONE LISTED

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INTRODUCTION

Our project aims at integrating imaging information on breast cancer to correlate to the underlying molecular alterations in the tissue in order to improve characterization and prognostication of detected lesions and subsequent management of the disease. Our data come from breast cancers diagnosed from the Tomosynthesis Mammographic Imaging Screening Trial (TMIST) Lead-In component. Existing breast cancers will be analyzed retrospectively. Tissue blocks will be retrieved for molecular analysis including targeted mutational sequencing and expression profiling. Mammographic images from the same cases are being studied to identify radiomic imaging patterns. In addition to analyzing 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 radiomic features that would potentially be useful for predicting the aggressiveness of cancer.

KEYWORDS

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

ACCOMPLISHMENTS

Working closely with the pathologists and clinical trial coordinators, we have identified to-date **57** cancer cases that originated from the TMIST Lead-In trial. Of these cases, **6** had their surgeries done outside of Sunnybrook, and therefore have been excluded from this study. Out of the **51** eligible cases, 6 cases had insufficient amount of DNA/RNA or other limitations (NAT) for molecular studies and had to be further excluded from sequencing and comparative radiomic analysis.

An updated list of all breast cancer cases assessed to-date is included in Table 1.

Table I: Breast cancer detected in TMIST Lead-In study as of October 1, 2021.

Breast Cancer pathology type	**Count**
Invasive ductal carcinoma (IDC), including Mammary	27
Invasive lobular carcinoma (ILC)	4
Ductal carcinoma in situ (DCIS)	5
Lobular carcinoma in situ (LCIS)	5
Invasive mucinous	1
IDC/DCIS	5
Tumour bed post NAT	4
Total	*51*

The team completed RNA and DNA profiling of 12 new cases. In all, 45 cases have been sequenced and analyzed to-date.

To-date we completed 3 whole-mount prospective surgical cases surgeries. One of the cases processed in June 2021 presented a particular challenge as it was a large specimen that required processing in batches. As a result of this case, a new standard operating procedure was developed to deal with large and difficult cases. The team took a course on radioactive seed handling and received a permit to handle and store radioactive seeds in the laboratory.

The Radiomic team developed a semi-automated radiomic pipeline for imaging-pathology-genomic correlation that will be applied to the images in this project.

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). RNA has been sent to Diagnostic Development at OICR for conducting their NanoString nCounter 200 gene assay (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). The analysis has provided us with multiple risk stratification indices, including the Prosigna ROR stratification, RS-Risk from Oncotype Dx, Mammoprint Risk score, IHC4 score and the 95 Gene score (Table I). Molecular subtype information was also provided with Prosigna PAM50 and with Mammatyper (Table II). While a majority of cancers were classified as Luminal A cancers with PAM50 assay, a fraction of them were identified as Luminal B-like (LumBL) by Mammatyper, and more HER2+ were detected as well. We will use this information to study 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).

Multi-parametric assay	Risk Stratification	Number of specimens
Prosigna		
RISK ROR-PT	Low	28
	Intermediate	8
	High	1
RISK ROR-P	Low	27
	Intermediate	8
	High	2
MammaPrint		
Risk.Mammaprint	Low	24
	High	13
Oncotype Dx		
RS-RISK	Low	24
	Intermediate	9
	High	4
IHC4		
Risk.IHC4	Low	7
	Intermediate	25
	High	5
95 Gene	Low	30
	High	7
Genomic Grade	Low	26
GGI.risk	High	11

Table I: Summary of the multi-parametric prognostic assays and the number of specimens that were assigned to different risk stratification scores.

Multiparametric Assay	Subtype classification	No of Specimen
Prosigna		
PAM50	Luminal A	30
	Luminal B	5
	HER2+	1
	TNBC	1
Mammatyper	LumA	20
	LumBL	11
	LumBH	1
	HER2+	5
	TN	0

Table II: Summary of subtype classification of cohort following PAM50 and Mammatyper.

Genomic targeted sequencing with Oncomine v3 and RNAseq have also been completed by the Genomics Core Facility at Sunnybrook Research Institute (Aim 2 Tasks 3 and 5). A number of common driver mutations in breast cancer were identified. Transcriptomic analysis has also identified cases with differential expression patterns. These data will soon be integrated with the prognostic scores quantified from the Nanostring assays.

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 fluorescent 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)), 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. Phenotype of tumor-associated fibroblasts (FAP) will be determined using the staining of validated antibodies for smooth muscle actin (αSMA) and Fibroblast Activation Protein (FAP). Quantitative methods to identify different clusters of various biomarker co-expression patterns and spatial analysis have been developed and will be applied on these data

Radiological images from 36 cancer cases diagnosed in TMIST Lead-In have been annotated by a radiologist (Aim1 Task 3). Once annotations are analyzed, 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 ongoing, 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. During this reporting period, we have identified 3 cases of breast cancer into our prospective WM processing study. We have obtained informed consent from one patient where the surgical specimen was subsequently processed

with WM techniques. For one other case, the patient declined to provide consent, and one case was not processed due to Covid constraints (see below “Changes/Problems”).

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 at the present time.

CHANGES/PROBLEMS

We submitted a no-cost extension (NCE) request for this project due to Covid-19 implications including reduced access to pathology samples and number of patient interaction/visits. The NCE was approved until September 2022.

There is a delay with multiplexing experiments as the multiplexer was under repair. We project that the multiplexing experiments will initiate in November 2021.

PRODUCTS

Nothing to Report.

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

Name:	Martin Yaffe
Project Role:	Principal Investigator
Researcher Identifier	N/A
Nearest person-month worked:	1
Contribution to Project:	Oversight of all related scientific activities for TMIST Lead-In and DOD projects, no-cost extension request and chairing team meetings
Funding Support:	Ontario Institute for Cancer Research (OICR)

Name:	Alison Cheung
Project Role:	Research Associate
Researcher Identifier	N/A
Nearest person-month worked:	3
Contribution to Project:	Co-ordination of all day-to-day scientific activities; renewal of IRB and ICF, progress/update meetings (February 16 ,2021), sequencing data analysis
Funding Support:	Ontario Institute for Cancer Research (OICR)

Name:	James Mainprize
Project Role:	Research Associate

Researcher Identifier	N/A
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Contribution to Project:	Radiomic data analysis of images and histology
Funding Support:	Ontario Institute for Cancer Research (OICR)

Name:	Heba Hussein
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Nearest person-month worked:	2
Contribution to Project:	Annotations on patient images, review of medical histories and reports
Funding Support:	Ontario Institute for Cancer Research (OICR)

Name:	Rachel Peters
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Researcher Identifier	N/A
Nearest person-month worked:	1
Contribution to Project:	Clinical data and reports, microtomy, whole-mount processing, 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, pathology annotations for HE slides/images for molecular studies
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.