AWARD NUMBER: W81XWH-18-1-0474

TITLE: Molecular and Imaging Biomarkers for Precision Therapy of Breast Cancer

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CONTRACTING ORGANIZATION: Sunnybrook Research Institute

REPORT DATE: OCTOBER 2022

TYPE OF REPORT: Annual Report

PREPARED FOR: U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
OCTOBER 2022	Annual Report	15Sept2021- 14SEP2022

4. TITLE AND SUBTITLE

Molecular and Imaging Biomarkers for Precision Therapy of Breast Cancer

5a. CONTRACT NUMBER
W81XWH-18-1-0474

5b. GRANT NUMBER
BCRP-BREAKTHROUGH2-FL12

5c. PROGRAM ELEMENT NUMBER

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5d. PROJECT NUMBER
011197789

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

In this project, we are using 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 continued to collect tissue blocks from newly diagnosed cancer cases, and completed sequencing of 45 cases. Radiological images have also been annotated and radiomic analysis completed on 36 cases. 3 prospective cases have been whole-mounted to-date.

15. SUBJECT TERMS

NONE LISTED

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRDC
Unclassified	Unclassified	Unclassified	Unclassified	8	19b. TELEPHONE NUMBER *(include area code)*

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INTRODUCTION

Our project aims at integrating in vivo 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 are being analyzed retrospectively. Tissue blocks are retrieved for molecular analysis including targeted mutational sequencing and expression profiling. Mammographic images from the same cases are being studied to identify potentially informative 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 co-register 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

To date, we have identified 57 cancer cases from the TMIST Lead-In trial. Of these cases we excluded a total of 13 cases for molecular studies due to insufficient amount of DNA/RNA or other limitations (e.g. patients received neo-adjuvant treatment). **42** cases have been sequenced, analyzed and annotated, and **3** prospective whole-mount surgical cases completed to-date. There have been no new cases added during this reporting period.

DNA and RNA were harvested from 42 invasive screen-detected cancers and analysed with NGS targeted sequencing with the Oncomine Comprehensive Assay v3, RNA transcriptomic profiling, as well as the Nanostring 200-gene assay developed by our collaborators Drs. Bayani and Bartlett at OICR. Prognostication of the screen-detected tumors was conducted based on results from the Nanostring 200-gene assay, which provided estimated scores of Prosigna Risk of Recurrence, Mammaprint, OncotypeDx multi-parametric assays, as well as PAM50 intrinsic molecular subtype and Mammatyper subtype classifications. Our analysis indicated that over 70% (31/42) of the analyzed tumors were classified as Luminal A subtype based on PAM50. However, 11 out of 31 of these PAM50-defined Luminal A cancers were classified as LumBL (Luminal B-like, HER2-negative) or HER2+ with Mammatyper. Moreover, although PAM50-defined Luminal A cases usually have favorable prognosis and exhibit low recurrence risk, a large fraction (12/31) of these cases appeared to exhibit intermediate to high recurrence risk in the OncotypeDx assay. These data suggest that there could be underlying latent elements that could drive non-invasive cancer cells to become aggressive, or switch them from dormancy to active proliferation later on. PAM50-defined Luminal A cases which were either classified as LumBL or HER2+ subtypes by Mammatyper, or that demonstrated an intermediate/high risk OncotypeDx scores, are collectively referred to as high-risk Luminal A (Luminal A HR), while the

concordant PAM50 and Mammatyper Luminal A cases, or the ones that exhibited low risk in OncotypeDx, are referred as low-risk Luminal A (Luminal A LR).

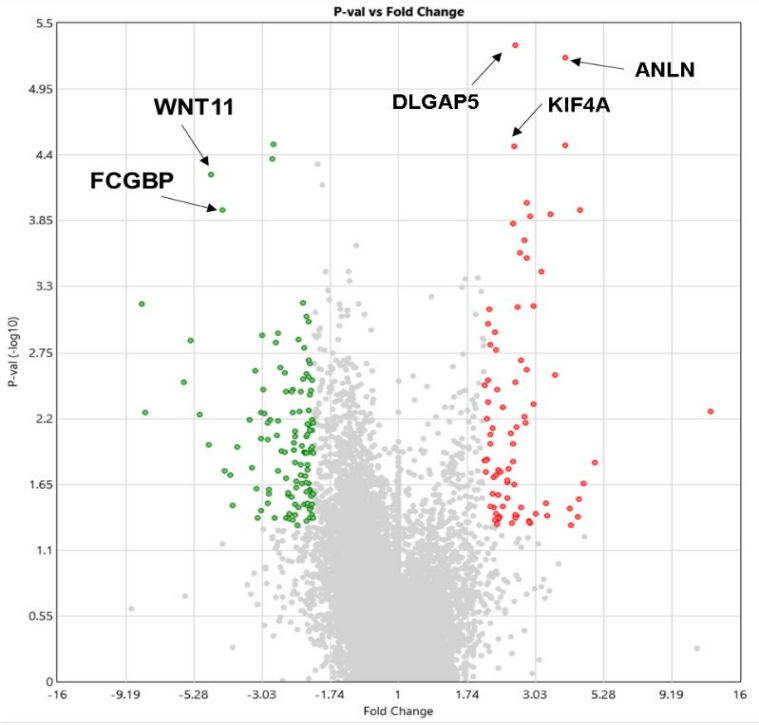

Figure 1: Volcano plot of differentially expressed genes between HR and LR Luminal A cancers. Red, genes that are up-regulated in HR Luminal A cancers compared to LR. Green, genes that are down-regulated. A few of the candidate genes were indicated on the plot.

We attempt, by means of differential gene expression analysis to identify what are the molecular elements that could differentiate between Luminal A HR cancers from those that were classified as Luminal A LR. Our comparison of differential gene expression between Luminal A LR and HR cancers have identified candidate target genes that contribute to the increased measured risk in HR Luminal A cases (Fig 1). Comparing the transcriptional profile of 13 HR Luminal A cases to 16 LR Luminal A cases, there were 82 upregulated- and 122 downregulated-genes. Some of the more prominent candidates (low P-value and high level of fold-change) that were up-regulated in HR cancers include Discs large homolog associated protein 5 (DLGAP5/HURP), Kinesin family member 4A (KIF4A) and Anillin actin binding protein (ANLN). Interestingly, these genes have been shown to be part of cell cycle machinery and involved in cell cycle progression or proliferation (Magnusson et al. 2016; Hou et al. 2018; Santarella et al. 2007). Genes that were down-regulated in HR cancers include Fc Gamma Binding Protein (FCGBP) and Wnt family member 11 (WNT11). Some of these potential targets have already been reported to be responsible for increased invasiveness and poor prognosis in cancer (Chen et al. 2014; Das et al. 2021). Contrary to our findings, both FCGBP and WNT11 were reported to promote aggressiveness in breast cancer and colorectal cancer respectively (Menck et al. 2021; Yan et al. 2022; Zhuang et al. 2021).

Our upcoming activities on protein multiplexing will focus on assessing the various cellular components in the tumor microenvironment (Tumor infiltrating lymphocytes TILs and macrophages, and cancer-associated fibroblasts) and how their composition and spatial arrangement with regards to the cancer epithelium could be correlated to radiomic signatures. We will also evaluate the protein markers of some of the differentially expressing genes between Luminal A LR and HR cases.

The radiomic team completed the development of a semi-automated pipeline for the registration of imaging-pathology-genomic 3D visualization. This pipeline will be applied in our some of our whole-mount processed breast specimens which would allow us to identify imaging/radiomic features which could be associated with particular molecular signatures from genomics and proteomics analysis.

IMPACT

Our analysis of a small sample size suggests that a majority of the screen-detected breast cancers are of the Luminal A intrinsic subtype, which usually have the most favorable prognosis. However, within Luminal A cancers there appears to be a subset of cancers that mimic the more aggressive Luminal B cancers, and exhibit moderate to high prognostic risk scores. This finding implicates that there is a need to further characterize Luminal A cancers, and identify the elements that drive aggressive changes in the long run. This will be one of the objectives of our application to the DoD BCRP FY22 Expansion Award.

CHANGES/PROBLEMS

We submitted a second no-cost extension (NCE) request for this project. Our project was delayed due to Covid-19 implications (reduced access to pathology samples and number of patient interaction/visits) and a disruption in the completion of the multiplexing studies due to failure of the older protein multiplexer. We have successfully replaced our old system with a new model (CellDIVE, Leica Microsystems). It was installed and tested in May and June of this year. We have replenished our supply of conjugated antibodies of interest. Due to improved image quality and throughput capacity of the new system we have some ability to catch up and we plan to complete biomarker multiplexing on select samples by the beginning of 2023.

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.
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:	Leading scientific activities; data analysis, reports, and team meetings.
Funding Support:	Ontario Institute for Cancer Research (OICR)

Name:	James Mainprize

Project Role:	Research Associate
Researcher Identifier	N/A
Nearest person-month worked:	1
Contribution to Project:	Radiomic data analysis of images and histology
Funding Support:	Ontario Institute for Cancer Research (OICR)

Name:	Heba Hussein
Project Role:	Radiology Intern
Researcher Identifier	N/A
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
Project Role:	Research Laboratory Technologist
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.

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