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13. SUPPLEMENTARY NOTES

14. ABSTRACT The project is designed to test whether genetic and/or tumor environmental heterogeneity is a driving force in progression of breast DCIS. Our project, a collaboration between Duke and ASU, has made substantial progress on all 4 aims and we met our 48 month milestones. Primary achievements for 48 months are:
1) Continued Case and control identification (43 Pure DCIS & 43 adjacent DCIS with invasion) through extensive database and searching at Duke 2) Deep and comprehensive full exome sequencing for 86 cases from 30-160ng of DNA isolated from archival FFPE specimens, 3) Comparison of analytic methods to characterize somatic mutations from this full exome sequencing, 4) Application of sequencing data for copy number assessment 5) Development of dual immune-staining on DCIS lesions using 7 pairs of antibodies, 6) Imaging analysis of these stains, including quantitative analysis, 7) Identification of upstaged DCIS cases for the radiology aim, 8) Development of image analysis methods for digital mammograms, 9) Validation Aim (4) approval of the Duke IRB/ TBCRC038 protocol at 13 sites, including DOD approval to initiate collection of DCIS that either did or did not progress to invasive cancer, 10) Full integration of team members over the past year via frequent conferencing, face to face meetings, and constant communication. This multi-disciplinary progress puts our group into an ideal position to fully implement the aims of the project and reach our year 4 goals.

15. SUBJECT TERMS
DCIS, intra-tumor heterogeneity, genetic diversity, phenotypic diversity, somatic evolution, microenvironment, mammographic biomarkers

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1. INTRODUCTION

Ductal carcinoma in situ (DCIS) of the breast is an increasingly common diagnosis that is related to aggressive screening patterns (mammography). This "pre-invasive" lesion may progress to invasive cancer, but does so at a relatively low frequency. Nonetheless, it is commonly treated with extensive surgery, radiation, and hormonal therapy even though most of these lesions would never progress to invasive cancer. Thus, there is a pressing clinical need to stratify the risk of DCIS tumors into those in need of intervention and those that can be safely monitored without intervention. Our project is designed to address this need by characterizing the evolvability of DCIS, detecting those that have a high likelihood of evolving to malignancy versus those that are likely to remain indolent.

2. KEYWORDS

DCIS, cancer progression, intra-tumor heterogeneity, genetic diversity, phenotypic diversity, somatic evolution, microenvironment, mammographic biomarkers

3. ACCOMPLISHMENTS

What were the major goals of the project?

Aim 1. *Determine whether genetic diversity of DCIS is greater in DCIS with adjacent invasive disease compared to DCIS without progression.* Diversity measures must be derived from geographically distinct areas of tumor. Genetic divergence of the DCIS component of tumors will be measured based on exome sequencing and SNP arrays run on two separate regions of the tumor, as well as normal tissue, in patients with DCIS either with or without adjacent invasion to determine the association between genetic diversity and progression to malignancy. Genetic diversity will be measured by the genetic divergence between the tumor samples, that is, the proportion of the genome that differs between the two samples from the same tumor.

48 Month Milestones:

- Protocol preparation, IRB submission and approval: **Completed** (Duke eIRB Pro00054515, initial Duke approval, 5/27/2014 and renewed for the current year), DOD IRB approval in place.

- Case identification and tissue block selection: Through a variety of available databases, we identified a large number of cases and controls with tissue available in the Duke Pathology archives. Each potential case and control requires extensive chart and pathology review in order to determine final eligibility and usability. For example, there is sufficient amount of the DCIS lesion (>2mm size) for isolation and DCIS is not too close to invasive cancer (it extends outside the invasive component). There must be two blocks with DCIS present that are >0.8cm apart. To date we have identified **86** cases, with pathology reviewed.

- Sectioning of tissue blocks: New sections from candidate paraffin blocks are cut, stained to include one H&E at the beginning and end of each set and then reviewed by the study pathologist. Remaining sections from candidate blocks (containing a sufficient amount of the DCIS lesion of interest) are used for macro-dissection and subsequent DNA extraction. Additional sections (every other one) are also stored for immunohistochemical (IHC) analysis of key measures of tumor and micro-environmental heterogeneity. These slides are scanned for analytic and archival purposes. This process has been fully implemented and we are moving through both cases and controls in this manner.

- DNA extraction of test cases: **Completed**.

- Exome sequencing of test cases: **Completed.** We chose the Genome Center at Washington University where cutting-edge methods for producing high quality data from these FFPE specimens have been developed and refined. Over the past two years, **Wash U. sequenced 30-160ng from 255 individual DNA samples derived from 85 subjects** (germ line sample plus 2 DCIS containing samples). They were able to derive interpretable sequence data (minimum of 40X depth at 50% coverage) from 30-160ng of FFPE DNA with qualities summarized in Figure 1, 2, 3 and 4.

Figure 1: Exomic variants in Outer Track is genome; Middle Track is pure DCIS and Inner Track is adjacent DCIS.

- Development of a pipeline for identification of somatic genetic alterations: **Completed.** In order to assess and minimize artefacts induced by the FFPE procedure and the small amounts of DNA obtained from FFPE samples we developed a strategy based on 28 sequencing technical replicates. We used a 5-fold cross-validation procedure. We

partitioned the patients into 5 complementary subsets. The patients were randomly assigned to the groups but each group is composed of 5 samples and each sample has a different amount of DNA: 20, 40, 60, 80, 100 ng. One subset (training set) was taken as hold out and evaluated against the rest of the patients (training data set). 5 rounds of cross-validation were performed using different partitions (Figure 2). Although our pipeline has been completed and is fully functional, we continue to work to improve it. These improvements have been statistically significant as seen in Figure 3, Improved SNV Bioinformatics Pipeline (Wilcoxon signed-rank test, p=0.008).

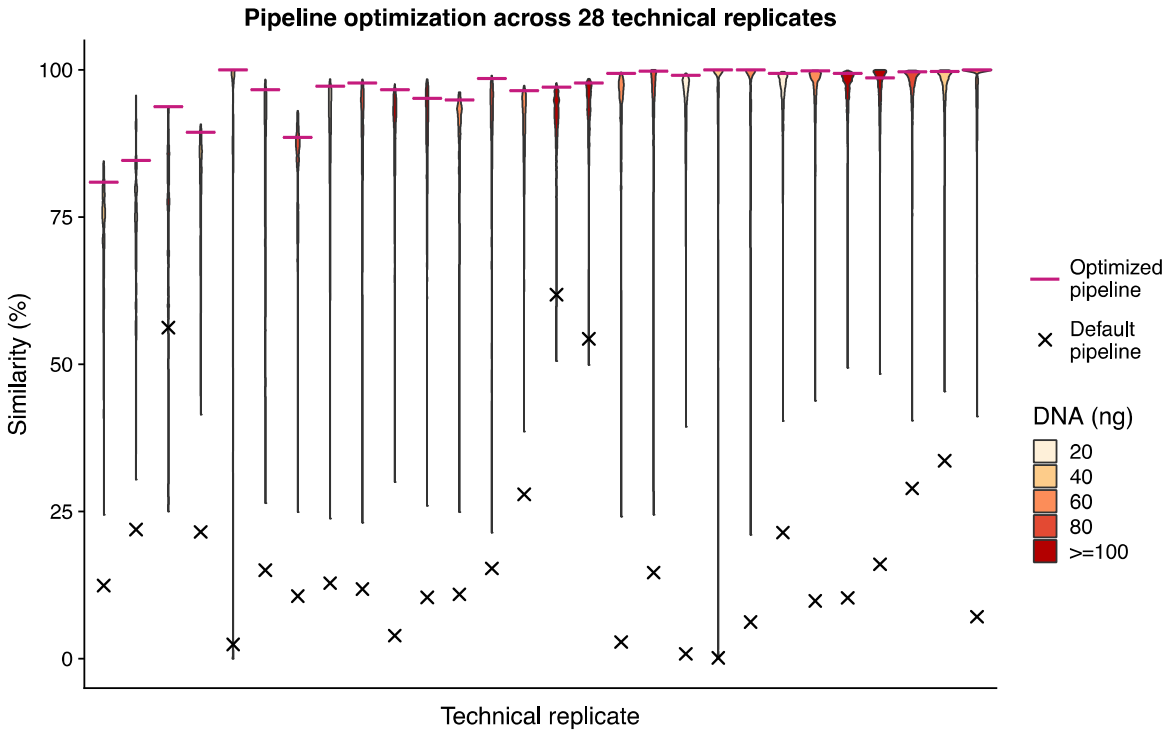


Figure 2: We developed an empirical method for optimizing the analysis algorithm through the comparison of sequences of the same DNA sample. We used a combination of filtering parameters on 28 different DNA samples extracted from 25 different patients. The same DNA analyzed twice with the same methodology should give the same results and detect the same somatic mutations in both analyses, ideally scoring 100% similarity. Of course, technical noise in the sequencing process interferes with achieving that ideal. After parameter optimization the similarity between the technical replicates was 96.8% ± 0.04 SD in average (x= similarity before optimization; — : similarity after optimization; colors indicate the amount (ng) of DNA used as template).

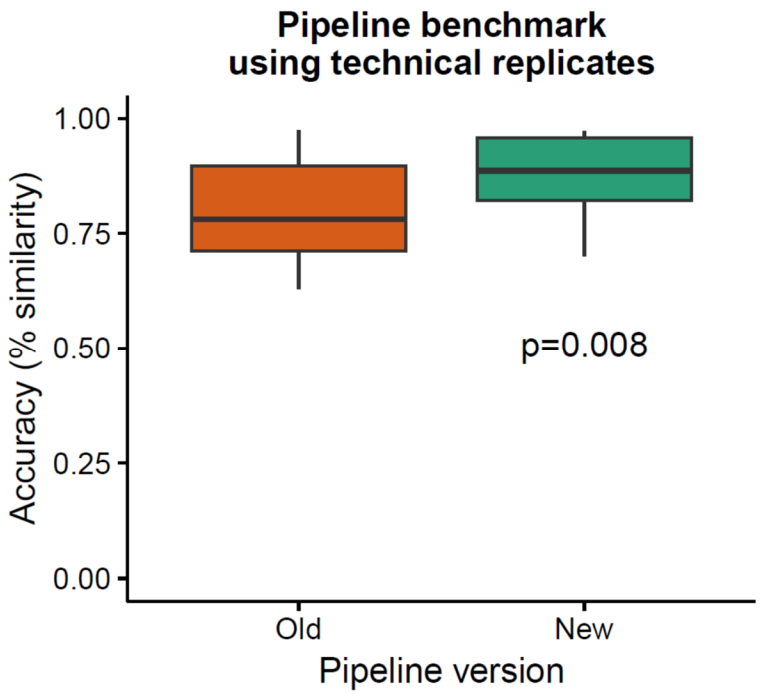


Figure 3: Improved SNV Bioinformatics Pipeline. We achieved a significantly better concordance between technical replicates of exon sequenced samples using our adjusted mutation calling pipeline.

- Calculation of genetic diversity scores for the pilot cohort: **Completed**. The percent of mutations different between two regions of the same tumor (called genetic divergence) is slightly (but not statistically significant) higher in DCIS adjacent to invasive disease than Pure DCIS (Figure 4, Exonic variants, Protein-coding variants, and Protein-altering variants). We found mutated genes in all patients. Cell adhesion, development, and differentiation processes are statistically significantly enriched in both groups. Pure DCIS patients have an enrichment of cell-matrix adhesion, sensor perception of chemical stimuli, and signal transduction. Current analysis of genetic diversity suggests that the genetic variability in DCIS adjacent samples was accumulated in the early phase of cancer development and then maintained during the subsequent tumor expansion. These are preliminary results and will be updated when we complete the sequencing of the cohorts for Aim 1.

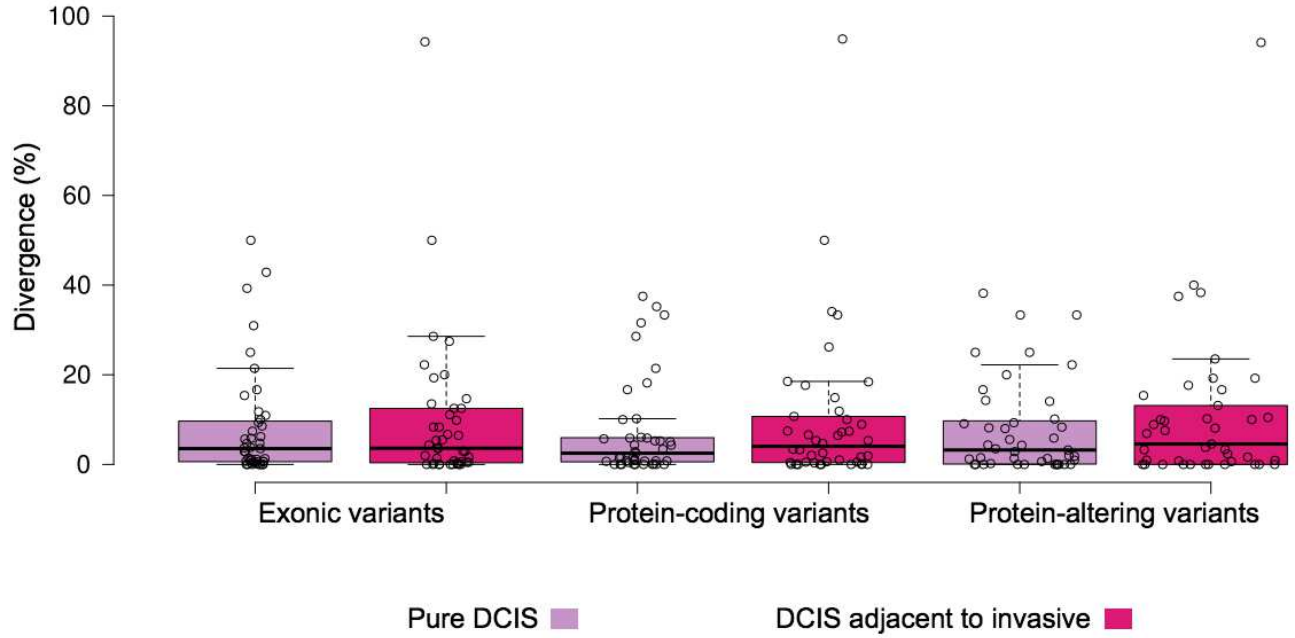


Figure 4: Exonic variants in Pure DCIS vs. DCIS adjacent to invasive disease comparing (% Divergence) the two regions sequenced from each case. Variants are categorized based on their protein coding consequences.

Table 1: Cohort Demographics

	Pure DCIS	Adjacent DCIS	
Age (mean)	57.9	57.4	
Race			
White	24	31	
Black	16	9	
Other	3	2	
Tumor Size (mean, cm)	3.5	3.6	
Nodal Status			
Positive	0	19	
Negative	43	23	
Grade		*DCIS*	*Invasive*
1	0	0	6
2	16	14	13
3	27	28	23
Surgery			
Lumpectomy	24	23	
Mastectomy	19	19	
Estrogen Receptor			
Positive	29	28	
Negative	9	14	
Equivocal	0	0	
Progesterone Receptor			
Positive	26	25	
Negative	10	16	
Equivocal	1	1	
HER2 Status			
Positive	0	9	
Negative	0	33	

Aim 2. Determine whether phenotypic diversity of DCIS and the tumor microenvironment (TME) is greater in DCIS with adjacent IDC compared to DCIS without IDC. Since genomics is not the sole driver of tumor behavior, we will phenotypically characterize DCIS and its microenvironment including markers of hypoxia, migration, proliferation, matrix organization, and immune signaling in the same samples used in Aim 1. We will compute microenvironmental divergence to determine if specific components of the TME, or the divergence between TMEs from the same tumor, differs between DCIS with and DCIS without adjacent IDC.

In the past 12 months, we have analyzed our phenotypic diversity markers on a total of 85 cases (43 pure DCIS, 42 mixed invasive/DCIS, Table 1). To evaluate these elements, we have used a detailed expert scoring that captures the distribution of intensity of staining. This allows us to fully evaluate heterogeneity between regions of the cancer following the original study design and the genetic analyses.

This work is still in progress, both staining the last few cases and expert quantitation of the staining. However, we have performed an interim analysis of the data to date specifically with respect to heterogeneity in the DCIS component. We calculated earth mover distance, Manhattan distance, and Euclidean distance between the two areas that are genetically defined by exome sequencing (Aim 1). Similar results were obtained using the three computational methods for defining distance. The results of these analyses are shown below (Table 2).

Significant differences are observed with the ALDH1 staining associated with stromal elements and CA9 staining indicative of tissue hypoxia. Again, these results are preliminary and will be finalized in the next 6 months.

Among the challenges of quantifying and interpreting either genetic or phenotypic parameters in DCIS is the approach to segmentation of these diverse and heterogeneous tissues. Automated identification of areas of DCIS and surrounding stroma will remove much of the subjectivity associated with any analysis. To this end, we have made substantial progress in using convoluted neural networks for semantic spatial segmentation that is geared specifically for evaluating the micro-ecology of the disease.

Table 2. Heterogeneity metrics for phenotypic diversity in DCIS

	EMD					Manhattan					Euclidean				
	mean_inv	mean_pure	p-value	rank	sig	mean_inv	mean_pure	p-value	rank	sig	mean_inv	mean_pure	p-value	rank	sig
ALDH_stroma	0.220	0.569	0.026	1	0.012	0.209	0.575	0.009*	1	0.012	0.130	0.351	0.011*	1	0.012
CA9	0.037	0.225	0.051	3	0.035	0.043	0.345	0.025	2	0.024	0.025	0.225	0.021*	2	0.024
GLUT1	0.423	0.255	0.045	2	0.024	0.530	0.336	0.065	3	0.035	0.322	0.209	0.079	3	0.035
COL15	0.113	0.250	0.079	4	0.047	0.225	0.494	0.087	4	0.047	0.159	0.348	0.089	4	0.047
RANK	0.060	0.172	0.187	5	0.059	0.120	0.330	0.201	6	0.071	0.085	0.227	0.212	5	0.059
PR	0.263	0.393	0.366	8	0.094	0.210	0.361	0.173	5	0.059	0.136	0.215	0.259	6	0.071
p63_percent	0.111	0.084	0.280	6	0.071	0.222	0.168	0.280	7	0.082	0.157	0.119	0.280	7	0.082
FOXP3_lymphs_percent	0.023	0.016	0.320	7	0.082	0.046	0.032	0.320	8	0.094	0.032	0.023	0.320	8	0.094
ER_DCIS	0.196	0.238	0.632	13	0.153	0.225	0.309	0.355	9	0.106	0.136	0.189	0.329	9	0.106
FASN	0.392	0.318	0.640	14	0.165	0.601	0.402	0.402	10	0.118	0.369	0.235	0.357	10	0.118
ALDH_DCIS	0.063	0.039	0.469	11	0.129	0.086	0.054	0.457	12	0.141	0.057	0.032	0.360	11	0.129
HER2_DCIS	0.261	0.272	0.891	17	0.200	0.383	0.457	0.511	13	0.153	0.235	0.292	0.385	12	0.141
Ki67	0.063	0.051	0.456	9	0.106	0.126	0.102	0.456	11	0.129	0.089	0.072	0.456	13	0.153
CD68_mpIndex	0.047	0.038	0.597	12	0.141	0.095	0.076	0.597	14	0.165	0.067	0.054	0.597	14	0.165
PFAK	0.522	0.591	0.462	10	0.118	0.784	0.806	0.847	16	0.188	0.493	0.500	0.916	17	0.200
HER2	0.151	0.141	0.869	16	0.188	0.218	0.236	0.815	15	0.176	0.146	0.158	0.812	15	0.176
CD31	0.443	0.475	0.854	15	0.176	0.545	0.538	0.968	17	0.200	0.325	0.306	0.863	16	0.188

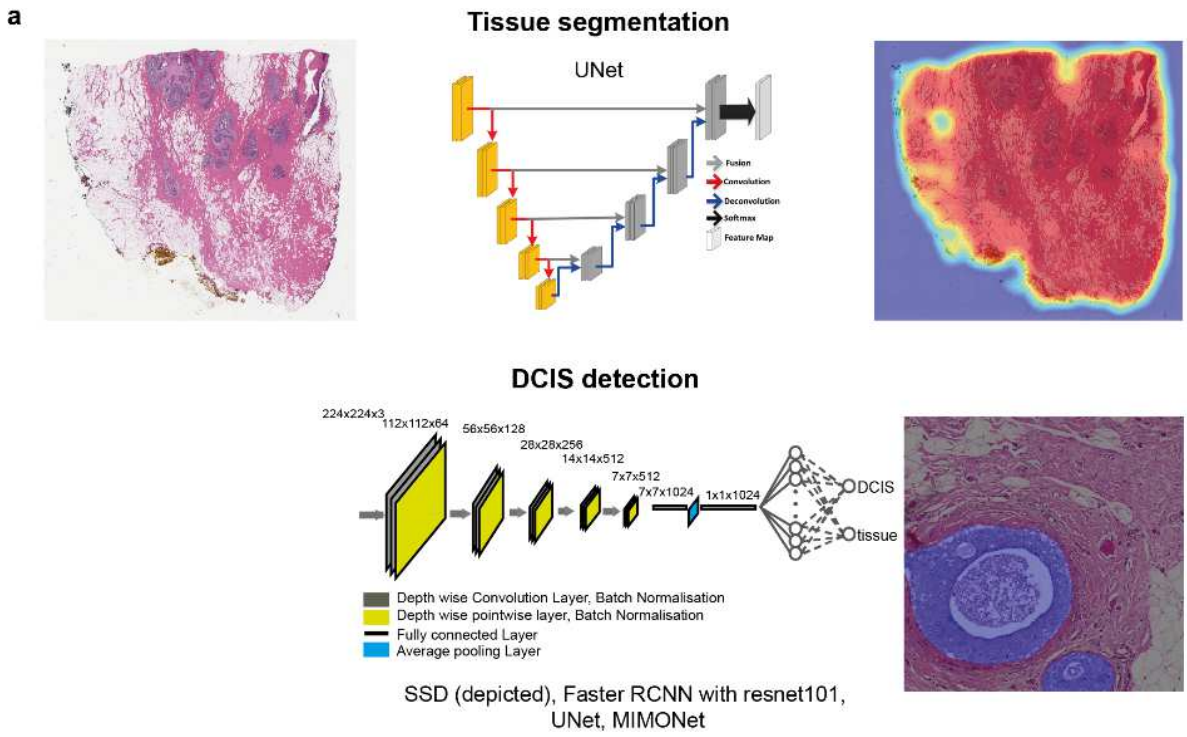
* Significant under Benjamini-Hochberg procedure; Benjamini-Hochberg procedure: $(i/m)Q$, where i is the rank, m is the total number of test and $Q = 0.2$ is the false discovery rate (FDR, or q-value: false positive in significant samples)

We hypothesize that local tumor ecology for individual DCIS creates differential selective forces and ultimately influences its potential for progression to invasive cancers. To characterize the local ecological features for each DCIS component within the tissue, we first designed a deep learning pipeline for automated detection and simultaneous segmentation of DCIS. Comparison of multiple cutting-edge convolutional neural networks including SSD, faster RCNN, showed that MIMOnet was the most accurate in identifying and delineating individual DCIS.

To further explore the ecological features immediately adjacent to each duct, we used the topological context to investigate whether deep learning extracted useful image features from carcinoma in situ to learn the difference in biology between cases with DCIS adjacent to invasive cancers versus cases with pure DCIS (Figure 5).

Spatial tessellation centered at each DCIS created the boundary in which local ecology can be studied. Subsequently, a deep learning method was used to classify single cells into lymphocytes, epithelial, stroma cells and others.

These developments in methodologies enable us to quantify the spatial relationship between lymphocytes and DCIS. Our preliminary results indicate that, while pure DCIS cases have overall more lymphocytes, the lymphocytes in adjacent cases tend to co-localize with DCIS, suggesting a more inflamed ecology locally to DCIS in tissue adjacent to invasive breast cancer.



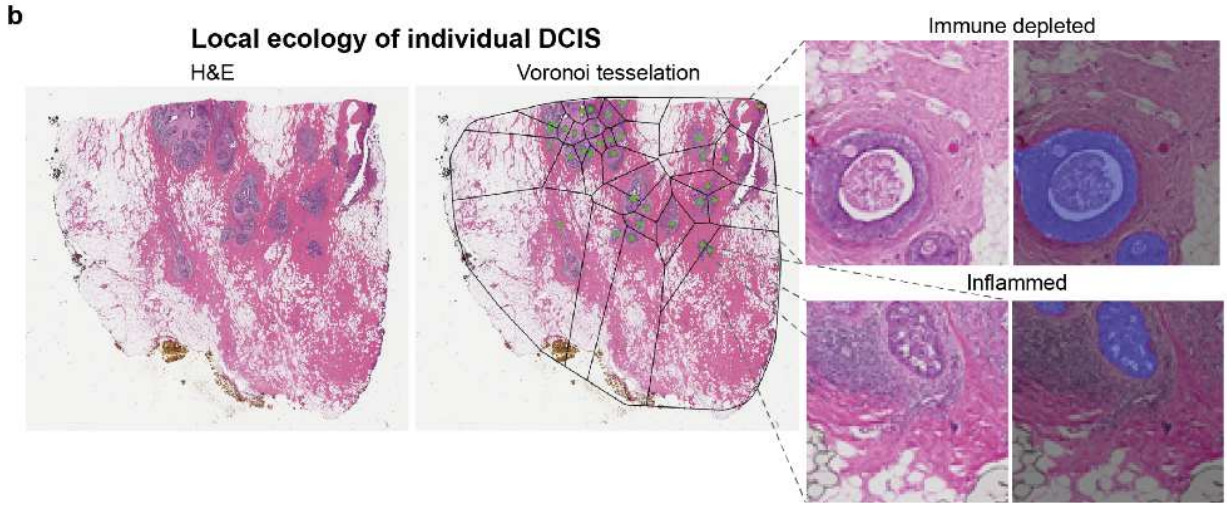


Figure 5. Overview of schematic pipeline depicting the individual steps in estimation of lymphocyte co-localisation between pure and adjacent DCIS cases. a. U-Net architecture for tissue segmentation and Single shot detector architecture for DCIS detection. b. Voronoi tessellation to quantify the spatial heterogeneity and context of DCIS.

48 Month Milestones:

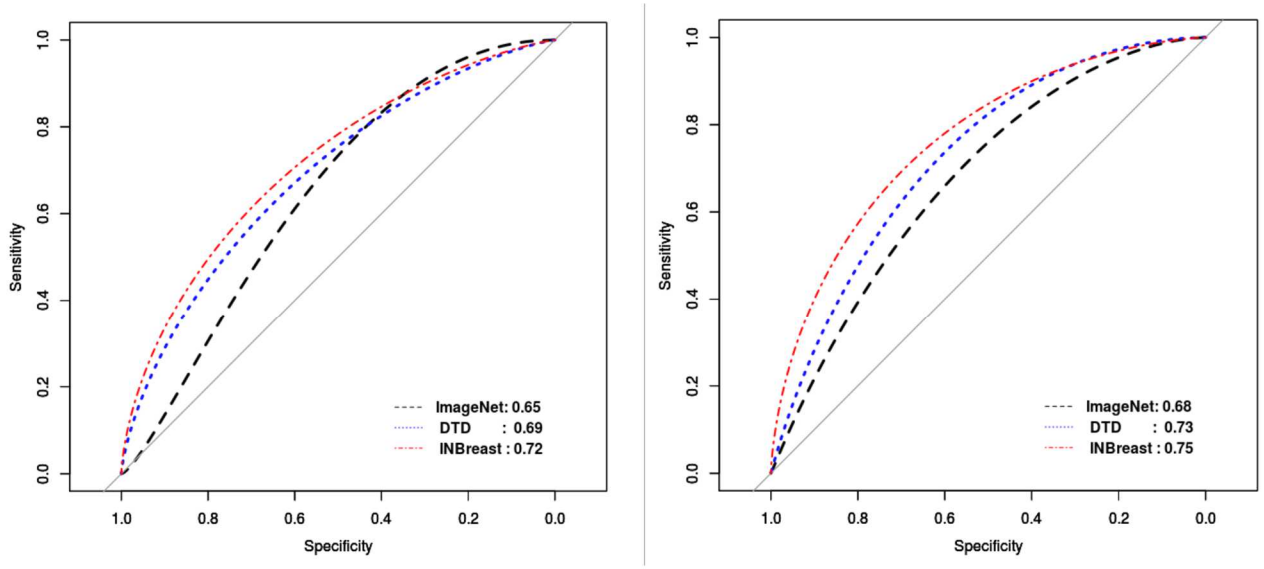
- IHC staining of candidate markers on all cases
- Expert scoring of all markers on all cases
- Data analysis using distance metrics to determine which markers demonstrate significant heterogeneity that distinguishes pure DCIS from mixed DCIS/invasive cases
- Stain Aim 4 cases with the most promising markers of tumor and microenvironmental heterogeneity
- Scan IHC and H&E stained slides for Automated image analysis (AIA)
- Training and validation of AIA for the identification and enumeration of cell types (epithelial, stromal, lymphocytes, blood vessels). Computer algorithms are trained by expert identification of cell types (study pathologist, Allison Hall). Accuracy of the computer identification is evaluated by comparison back to the expert scoring. Apply methods for quantitative image analysis
- Test computer vision methods for measuring nuclear size as a surrogate for tumor grade

Aim 3. *Create and test a computational learning algorithm to compare mammographic characteristics and diversity measures in pure DCIS compared to DCIS with IDC.* A weighted computational algorithm using mammographic features of lesional and stromal characteristics as well as heterogeneity measures derived from Aims 1 and 2 will be constructed. The tool will be designed to allow for radiologic discrimination between good and poor prognosis DCIS, and will be evaluated in a validation set.

48 Month Milestones:

- We concluded the second study on using deep learning features to predict upstaging of DCIS, and published in a special journal issue focusing on deep learning in medical imaging (Shi *et al.*, Journal of American College of Radiology 2018, PMID: 29398498). In a process known as "transfer learning," a deep learning model was trained on several unrelated data sets, then the model was used as a feature extractor. Specifically, DCIS images were fed into the model, and the "deep weights" from its internal layers were statistically pooled and then synthesized by logistic regression to predict upstaging. The three pre-trained deep learning models were trained using three different databases: natural images (ImageNet), image textures (DTD), and mammograms with lesions (INbreast).
- All of our studies to date were limited by the relatively small data set of only 99 DCIS cases (including 25 upstaged). For subsequent studies, we therefore expanded the data set to 140 cases, including 35 upstaged or the same 25% rate as the smaller data set. Using the same deep feature modeling approach, we repeated the analysis for this expanded data

set of 140 cases. This study was presented at SPIE Medical Imaging 2018 conference and published as a full-length proceedings paper (Shi *et al.*, Proc. SPIE Medical Imaging 2018). As with the previous smaller study, the more similar the unrelated data was to our task, the higher the performance. With feature selection, the model using mammography features resulted in receiver operating characteristic (ROC) area under the curve (AUC) of 0.75. The ROC curves are shown in Figure 6.



(a) (b)

Figure 6. Comparison of ROC curves of logistic regression models to merge deep features from ImageNet, DTD, and INbreast data sets: (a) without feature selection; (b) with feature selection.

- We initiated a third study to improve the prediction of pure DCIS (negative) versus upstaged (positive) cases. We investigated the adjunctive roles of two related classes, i.e. Atypical Ductal Hyperplasia (ADH) and Invasive Ductal Carcinoma (IDC). The hypothesis is that pure DCIS is more ADH-like, while upstaged DCIS is more IDC-like, and the use of those two related classes can improve DCIS classification. Similar to the previous study, we first presented an earlier version with 99 cases at SPIE Medical Imaging 2018 and published it as a full-length proceedings paper (Hou *et al.,* SPIE Medical Imaging 2018).

- We then repeated the study using ADH and IDC to improve prediction of DCIS upstaging, but now using the expanded 140 cases. A manuscript is currently in preparation for *IEEE Transactions in Biomedical Engineering*. The following data are selected from that manuscript. Adjunctive cohorts of 130 ADH and 65 IDC cases were used for training, resulting in up to 335 total subjects. For all studies, we focused on the computer vision features merged with logistic regression, as the deep features were still under development. The baseline Model A was created using only the 140 DCIS cases. Model B was transfer feature selection, where the regression classifier was built using only the adjunctive ADH and IDC cases, and then selected features were transferred to classify DCIS cases. Model C was forced labeling, where training was augmented by

adding ADH cases re-labeled as pure DCIS (negative) cases and IDC re-labeled as upstaged (positive) cases. Model D was domain adaptation, where the classifier was first trained only on ADH and IDC, and then directly applied to classify DCIS.

Based on ROC analysis, all three models outperformed the baseline Model A with feature selection's AUC of 0.614 (95% CI, 0.496-0.733): transfer feature selection AUC=0.638 (95% CI, 0.528-0.748), p=0.856; forced labeling AUC =0.668, (95% CI, 0.568-0.767), p=0.111; and domain adaptation AUC= 0.697 (95% CI, 0.595-0.797), p=0.017. These performances are summarized in Figure 7. In summary, although the adjunctive classes of ADH and IDC were clinically different from DCIS, they shared some similarities in feature spaces. The best performance was Model D, which was trained only on ADH and IDC and then transferred to the independent task of classifying pure vs. upstaged DCIS. This was also the only approach that significantly outperformed the baseline model.

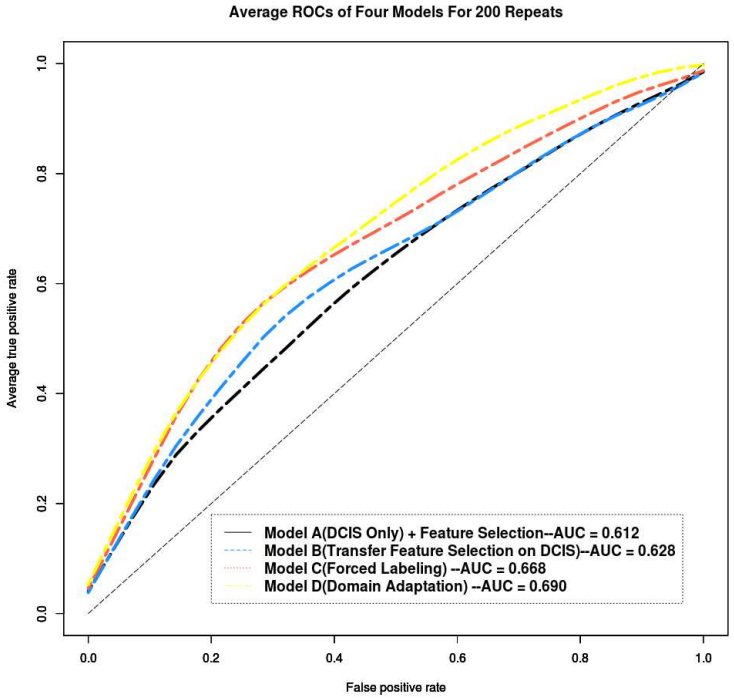


Figure 7. ROC Curves showing classification performances of the baseline Model A, Model B transfer feature selection, Model C forced labeling, and Model D domain adaptation. The models differ in how the adjunctive classes of ADH and IDC were utilized to enhance the classification of DCIS vs. upstaged DCIS.

- We are now initiating the next study to investigate the generalizability of all the aforementioned models. We are expanding the data set to include approximately 600 total cases. This will allow us to split the data into approximately 300 for training and validation, while still reserving 300 for final, independent testing. The increased number of cases will reduce variability due to case sampling as well as tendency of models to overtrain. We will also investigate the additional contributions from human-derived features, including radiological, pathological, and clinical data.

Aim 4. *Test the predictive performance of the best diversity measures in an independent validation set of pure DCIS with and without subsequent invasive recurrence.* Genotypic and phenotypic measures of diversity derived from Aims 1-2 will be applied to an independent case-control, longitudinal, tissue bank of DCIS with and without invasive recurrence to validate their utility. The Duke IRB approved protocol has been approved at 13 sites. For the next budget year, we will continue to accrue cases of pure DCIS that are long term disease free or recurred with invasive cancer. Slides are being shipped to Duke for macrodissection for DNA analysis and for immunodetection of phenotypic heterogeneity.

48 Month Milestones: This aim will be carried out after aims 1-3 are complete. We obtained approval to obtain these specimens through the Translational Breast Cancer Research Consortium (TBCRC) and Duke IRB approval. We have identified 13 high volume academic medical center members of the consortium who obtained regulatory approval, DOD approval, MTA's and completed an SIV training.

The REDCap database for data entry online and slide inventory control, has been populated for cases from 6 of the 13 external sites. To date we have obtained cases from 6 of the 13 sites. We have received 40 cohort 0, 41 cohort 1 and 28 cohort 2 cases to date. Overall, this aspect of the project is adhering to our proposed timeline and should achieve its accrual and analysis goals.

Below is the list of approved centers participating in this study and accrual to date.

Table 3: Multicenter Site Update for Aim 4

Site Name	IRB	Contract	MTA	DOD Approval	Cohort 0	Cohort 1	Cohort 2
Baylor	Approved	Executed	Executed	Approved			
Chicago	Approved	Executed	Executed	Approved			
DFCI	Approved	Executed	Executed	Approved	**0**	**6**	**2**
Duke	Approved	Executed	Executed	Approved	**35**	**18**	**13**
Georgetown	Approved	Executed	Executed	Approved			
Indiana	Approved	Executed	Executed	Approved			
Mayo	Approved	Executed	Executed	Approved			
MDACC	Approved	Executed	Executed	Approved	**0**	**1**	**3**
Montefiore	Approved	Executed	Executed	Approved	**0**	**3**	**0**
Pittsburgh	Approved	Executed	Executed	Approved			
UNC	Approved	Executed	Executed	Approved	**5**	**3**	**2**
UWashington	Approved	Executed	Executed	Approved	**0**	**6**	**4**
UPENN	Approved	Executed	Executed	Approved	**0**	**4**	**4**
UAB	Approved	Executed	Executed	Approved			
Total					**40**	**41**	**28**

What was accomplished under these goals?

Our primary goals have been met including, most importantly, identifying the most efficient method of sequence generation from small amounts of fixed DNA. We have acquired more radiology imaging data sets and established the computer vision algorithms for their analysis. Further, based on our databases, we are confident of accruing sufficient cases and controls at Duke to fulfill the Aim 1 and 2 goals of the project. Overall, we are in excellent position to complete the proposed work in the project period along the time line that was provided.

What opportunities for training and professional development has the project provided?

We hired several new post-doctoral fellows in the previous year to continue expanding our analysis. Priya Narayananhas acquired new skills in deep learning methods and attended a conference on breast cancer diagnosis. Yuantong Ding has acquired new skills in deep learning and continues to learn about the complexities of breast cancer diagnosis.

How were the results disseminated to communities of interest?

We had two DCIS abstracts based on aims 1 and 2 presented at the San Antonio Breast Cancer Symposium in December 2017.

Bibo Shi presented a poster at SPIE Medical Imaging 2018. Rui Hou was accepted for a talk at SPIE Medical Imaging 2018.

What do you plan to do during the next reporting period to accomplish the goals?

Aim 1: We will complete the identification, extraction and DNA sequencing for cases and controls (n=100). Diagnostic slides from candidate subjects will be evaluated by our study pathologist to determine if there is sufficient material for all planned analyses and ones that pass this metric will be included in the study. New unstained slides will be ordered from these cases for macrodissection and immunohistochemical staining. DNA extracted from these slides will be exome sequenced and applied to SNP arrays. Returned data from these assays will be analyzed using our current pipeline in order to scale up from the pilot study to a study with a bigger sample size, which will allow us to get more insights from the data. Moreover, we will investigate the biological meaning of the most common variants of the two different tumor types. We will apply our optimized sequencing analysis pipeline to determine the degree of genetic heterogeneity. These data will be prepared for publication during this period. In addition, we are preparing a manuscript to detail our methodologic approaches to sequence analysis focused on the technical replicates and the pipeline developed from these samples.

Aim 2:
We will complete the dual IHC staining on the remaining cases, as they come off line after pathology review. We will refine methods for agnostic computer scoring of IHC stains. These

methods will be implemented on all images. Further, we will develop computer vision methods to measure nuclear size of the epithelial component. These methods have been developed by Dr. Yuan's team and are in testing phase. All cases will be analyzed for this parameter by Dr. Narayanan.

Aim 3:
We will complete another paper describing the final results of the transfer learning of deep features. We will complete the analysis of the forced labeling study to improve classification by addition of neighboring classes, and submit that as an additional paper. We will then perform the majority of the final modeling studies using all cases from our institution, as well as begin to analyze cases from other institutions.

Aim 4:
This multicenter validation arm of the project is set up through the Translational Breast Cancer Research Consortium (TBCRC), a collaborative group set up to conduct innovative and high-impact breast cancer clinical trials.

The validation protocol has been approved by both the TBCRC and the Duke IRB (3/18/2016). Thirteen (14 including Duke) external sites have obtained local IRB approval. Sites have both IRB as well as DOD approval and completed an SIV call training session with key personnel from each site,

We will finalize the collection of cases from sites. We have 142 cases in the RedCap database from all sites. We currently participate in monthly calls with TBCRC participating sites (13) where clinical coordinators, from all active TBCRC studies, provide updates and questions are addressed. These cases will be analyzed using the genetic, informatic, and phenotypic approaches developed in Aims 1 and 2. These data will constitute the validation of the results from the first two aims and will be prepared for publication.

4. IMPACT

Successful completion of this project will lead to a variety of biomarkers (genetic, IHC and radiographic) to distinguish high risk from low risk DCIS. This would reduce patient suffering and conserve clinical resources for the women with low risk DCIS, and focus management efforts and clinical resources on women with high risk disease, potentially justifying the risks of interventions. As the project is in its initial stages, these important impacts await in the future.

What was the impact on the development of the principal discipline(s) of the project?

We continue to advance the field's understanding of DCIS progression and the impact of tumor heterogeneity on the fate of DCIS. The final deliverables of this proposal will impact how DCIS is regarded both by the scientific and clinical communities.

What was the impact on other disciplines?

We have contributed to emerging knowledge regarding the digital radiographic characteristics of DCIS and continue to extend the applications for machine learning in breast cancer. We are one of the most active teams in the field, as evidenced by numerous publications and invited talks.

What was the impact on technology transfer?

Nothing to report.

What was the impact on society beyond science and technology?

Nothing to report.

5. CHANGES/PROBLEMS

Changes in approach and reasons for change

There have been no changes in approach.

Actual or anticipated problems or delays and actions or plans to resolve them

So far the problems that have emerged have been primarily technical. Full exome sequencing from small amounts of FFPE tissue has been the primary challenge, and is now proceeding smoothly at Wash U.

Changes that had a significant impact on expenditures

None

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

None

Significant changes in use or care of human subjects

None

Significant changes in use or care of vertebrate animals.

Not applicable.

Significant changes in use of biohazards and/or select agents

Not applicable

6. PRODUCTS

Publications

1. Walther, V., Hiley, C.T., Shibata, D., Swanton, C., Turner, P.E., and **Maley, C.C.**: Can oncology recapitulate paleontology? Lessons from species extinctions. Nature Reviews Clinical Oncology, 12:273-285, 2015. doi:10.1038/nrclinonc.2015.12. Published. Acknowledged federal support.
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9. Shi B, Grimm LJ, Mazurowski MA, Marks JR, King LM, Maley CC, Hwang ES, **Lo JY**, "Can upstaging of ductal carcinoma in situ be predicted at biopsy by histologic and mammographic features?" Proc. SPIE 10134, Medical Imaging 2017: Computer-Aided

Diagnosis, Armato SG, Petrick NA, Eds., 101342X (2017). Published. Acknowledged federal support.

10. Abegglen, L.M., Caulin, A.F., Chan, A., Lee, K., Robinson, R., Campbell, M.S., Kiso, W.K., Schmitt, D.L., Waddell, P.J., Bhaskara, S., Jensen, S.T., **Maley, C.C**.†, Schiffman , J. D.†: Potential Mechanisms for Cancer Resistance in Elephants and Comparative Cellular Response to DNA Damage in Humans. JAMA, 314:1850-1860, 2015. Published. Acknowledged federal support.

11. Li, X., Paulson, T.P., Galipeau, P.C., Sanchez, C.A., Liu, K., Kuhner, M.K., **Maley, C.C**., Self, S.G., Vaughan, T.L., Reid, B.J., Blount, P.L.,: Assessment of esophageal adenocarcinoma risk using somatic chromosome alterations in longitudinal samples in Barrett's esophagus. Cancer Prevention Research, 8:845-56, 2015. Published. Acknowledged federal support.

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Technologies or techniques

Nothing to report

Inventions, patent applications, and/or licenses

Nothing to report

Other Products

Case report forms for Duke and outside cases and databases to efficiently capture this information

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Co-PI: Dr. Shelley Hwang (M.D., M.P.H.): Duke University (no change)
Co-PI: Dr. Carlo C. Maley (Ph.D.): Arizona State University (no change)

Co-Investigators:
Dr. Jeffrey Marks (Ph.D.): Duke University (no change)
Dr. Joseph Geradts (M.D.): Duke University (departed during year one)
Dr. Allison Hall (M.D.): Duke University, replacing Dr. Geradts.
Dr. Joseph Lo (Ph.D.): Duke University (no change)
Dr. Jay Baker (M.D.): Duke University (no change)
Dr. Yin Yin Yuan (PhD): Institute for Cancer Research, UK (no change)
Dr. Lars Grimm (M.D.): Duke University (no change)
Dr. Trevor Graham (Ph.D.): Barts Cancer Institute, Queen Mary University of London (no change)
Dr. C. Athena Aktipis (Ph.D.): Arizona State University (no change)
Dr. Shane Jensen (Ph.D.): University of Pennsylvania (departed during year one)

Post-Docs:
Dr. Mengyu Wang (PhD): Duke University (departed during year one)
Dr. Violet Kovacheva (PhD): Institute for Cancer Research, UK (departed during year two)
Dr. Narayanan (PhD): Institute for Cancer Research, UK, replacing Dr. Kovacheva.
Dr. Lorraine King (PhD): Duke University (no change)
Dr. Bibo Shi (PhD): Duke University (departed during year two)
Rui Hou, ECE Ph.D. student, Duke University (no change)
Dr. Angelo Fortunato (PhD): Arizona State University (no change)
Dr. Diego Mallo (PhD): Arizona State University (no change)