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Award Number: W81XWH-11-1-0214

TITLE: Tumor Microenvironment and Progression to Invasion after a Diagnosis of
Ductal Carcinoma In Situ

PRINCIPAL INVESTIGATOR: Amy Trentham-Dietz, Ph.D.
Patricia J. Keely, Ph.D.

CONTRACTING ORGANIZATION: University of Wisconsin
Madison, WI 53715-1218

REPORT DATE: March 2013

TYPE OF REPORT: Annual

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

DISTRIBUTION STATEMENT: Approved for Public Release;
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REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
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1. REPORT DATE (DD-MM-YYYY) March 2013		2. REPORT TYPE Annual		3. DATES COVERED (From - To) 1 March 2012 - 28 February 2013	
4. TITLE AND SUBTITLE Tumor Microenvironment and Progression to Invasion after a Diagnosis of Ductal Carcinoma In situ				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER W81XWH-11-1-0214	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Amy Trentham-Dietz and Patricia Keely E-Mail: trentham@wisc.edu; pjkeely@wisc.edu				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) University of Wisconsin Madison, WI 53715-1218				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel 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 Ductal carcinoma in situ (DCIS) makes up 18% of all new breast cancer diagnoses, and is considered a precursor to invasive breast cancer even though the majority of cases—almost 70%—may never progress to invasive disease. Markers that identify which patients are most likely to experience progression are critically needed so that fewer patients are over-treated. This study is evaluating two novel tumor markers that may indicate greater risk of tumor progression based on recent work that suggests that stromal syndecan-1 expression induces an extracellular matrix with aligned collagen fiber architecture, and that this collagen alignment in turn facilitates malignant cell invasion. We are using archived tumor tissue from 267 cases of DCIS of the breast to evaluate syndecan-1 expression and collagen alignment. These DCIS cases, diagnosed between 1995 and 1999, have been followed for breast cancer outcomes; to-date, 13% of cases have experienced a second breast cancer diagnosis. Analysis of syndecan-1 expression and collagen alignment patterns will be completed soon, and preliminary data analysis has begun. Findings from this research are expected to be disseminated through peer-reviewed journals and for presentation at national conferences by the end of the year.					
15. SUBJECT TERMS Ductal carcinoma in situ, breast cancer, tumor microenvironment, collagen alignment, syndecan-1, disease-free survival, second harmonic generation					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT UU	18. NUMBER OF PAGES 11	19a. NAME OF RESPONSIBLE PERSON USAMRMC
a. REPORT U	b. ABSTRACT U	c. THIS PAGE U			19b. TELEPHONE NUMBER (include area code)

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INTRODUCTION

Ductal carcinoma in situ (DCIS) makes up 18% of all new breast cancer diagnoses, and is considered a precursor to invasive breast cancer. It is estimated that almost 70% of DCIS cases may never progress to invasive disease. However, since the transition from DCIS to invasive breast cancer is a critical progression step associated with a substantial drop in survival, patients are uniformly treated with aggressive therapy, and thus many are being over-treated. Unfortunately, relatively little is known about the factors that govern this progression, and so markers that isolate patients likely to progress have not been identified. An emerging approach in tumor biology focuses on important changes in the stromal tissue surrounding malignant cells during tumor progression. The recent work of Drs. Patricia Keely and Andreas Friedl with invasive breast carcinoma suggests that stromal syndecan-1 expression induces an extracellular matrix with an aligned collagen fiber architecture, and that this collagen alignment in turn facilitates malignant cell invasion. These changes have not been investigated in DCIS. We hypothesize that re-alignment of the extracellular matrix, triggered by syndecan-1 induction in stromal fibroblasts, plays a major role in the progression from DCIS to invasive breast cancer, and thus can be used as a marker to predict outcome. Our objective is to evaluate this hypothesis using archived tumor samples and follow-up data from Dr. Amy Trentham-Dietz's cohort study of 267 DCIS cases with available tumor samples who were recruited upon their diagnosis between 1995 and 1999. Collagen patterns and stromal expression of syndecan-1 will be evaluated from archived unstained tumor slides using state-of-the-art methods by Drs. Keely and Friedl, respectively.

BODY

The approved Statement of Work for this grant includes:

Task 1. Obtain and maintain regulatory approval, Months 1-24:

a. Obtain initial IRB/Human Subjects approvals, Months 1-6.

Progress report: Initial IRB/human subjects approval was obtained in August 2011 (month 6) from both the University of Wisconsin (UW) Health Sciences IRB and the DOD Human Research Protection Office (HRPO). .

b. Obtain continuing review annual approval from IRB, Month 12.

Progress report: An annual progress report was submitted to the UW Health Sciences IRB. Approval was obtained with the expiration date of 3 May 2013. Another annual progress report form will be submitted to the Health Sciences IRB before 1 April 2013. A copy of the continuing review approval notification by the University of Wisconsin Madison Minimal Risk IRB (Health Sciences) will be submitted to the HRPO as soon as possible after receipt of approval.

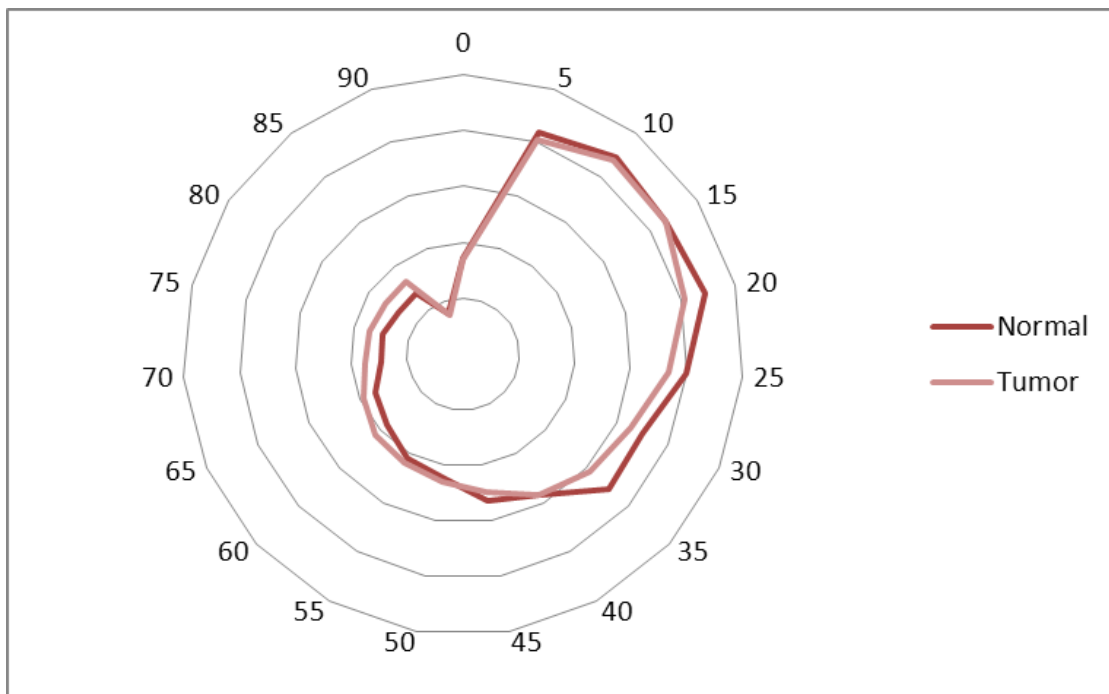
Task 2. Evaluate tumor microenvironment in 267 DCIS samples, Months 7-10;

a. Evaluate collagen alignment patterns, Months 5-10.

Progress report: Evaluation of the collagen alignment patterns for the tumor tissue slides has been completed. Collagen fibers were imaged using second harmonic generation (SHG) microscopy, which is a non-linear optical imaging form of microscopy. The technique takes advantage of the unique non-centrosymmetric structure of collagen in combination

with the multiphoton absorbance of laser light by the peptide bonds of collagen to act as a frequency doubler. The net effect is that the emitted light is of exactly one-half the wavelength of the incident light upon interaction with collagen. In this way, an image of the collagen extracellular matrix (specifically) is acquired. These images were then transformed in the frequency space into curvelets, which are essentially vector representations of individual collagen fibers. A boundary between the tumor and stroma was drawn in the image by the user and software program (CurveAlign2) then measured the angle at which each curvelet crossed the border. These individual measurements were compiled to create a histogram of the angles at which collagen fibers are oriented with respect to the tumor boundary. Since there are many fibers at any given lesion, this automated analysis is highly useful. The multiphoton microscope and curvelet analysis program used were both custom created through established collaborations here at the University of Wisconsin.

Collagen alignment was evaluated in 3-7 tumor lesions for 229 cases. (Collagen alignment could not be evaluated for 38 cases.) We compared the angles at which the collagen fibers were configured relative to the tumor boundary. A similar assessment was completed for normal tissue from 95 cases. Statistical analysis designed for compositional data demonstrates that the distribution of collagen fiber angles for the tumor lesions was significantly different than the pattern of collagen fiber angles for the adjacent normal cells ($P=0.01$). As shown in the figure below, in both normal and tumor, many of the fibers were aligned at 5 to 15-degree angles; relatively few of the lesions were aligned at 60 to 85-degree angles, and even fewer fibers were aligned at 90-degree angles. Compared to normal, tumor lesions had relative increases at 55-85-degree angles. The other angles show relative drops (fiber alignments were lower for tumor relative to normal for 15 to 40-degree angles).



The CurveAlign2 program calculates the mean curvelet angle (collagen alignment measurement #1) in addition to creating the distribution histogram (collagen alignment measurement #2) for each image. The program has recently been updated where it now creates a new image for each round of analysis where individual curvelets are superimposed upon the SHG image and colored based on the angle at which they cross the tumor/stroma boundary. Additionally, spatial information is taken into account when creating this image such that only groups of curvelets in close proximity to each other who share a similar angle are colored; in this way we have created a “heat map” image that easily identifies aligned collagen. We can use information from this new image to further quantify the amount of aligned collagen present in each image (collagen alignment measurement #3).

We have also evaluated by eye whether lesions were characterized by the Tumor Associated Collagen Signature-3 (TACS-3) phenotype (collagen alignment measurement #4), with the presence of radially aligned collagen fibers that are hypothesized to facilitate invasion.(Ref: Provenzano et al. BMC Med 2006: 4:38). Other tumor signatures include the presence of dense collagen around the tumor (TACS-1), and the presence of straightened collagen fibers stretched around the tumor, indicating an increased mechanical load (TACS-2).

It is expected that the remaining normal tissue samples will be evaluated for collagen alignment (according to angles) as well as the 3-category TACS assessment by the end of March 2013. Progress on evaluation of tissue slides required more time than expected due to microscope maintenance problems. However, problems were overcome and we expect to complete all collagen assessments by the end of March 2013.

b. Evaluate syndecan-1 expression, Months 5-10.

Progress report: Following initial optimization, immunohistochemical labeling for syndecan-1 has been completed for all 267 cases. We have begun quantitatively evaluating stained tissue slides for syndecan-1 expression by fibroblasts located in the periductal stroma using the Nuance image analysis system. We expect completion of the analysis by the end of May 2013.

Task 3. Determine outcome status among 267 DCIS cohort subjects, Months 7-8.

a. Clean study cohort dataset to determine 2nd breast cancer events, Months 7-8.

Progress report: We have ascertained 32 second breast cancer diagnoses (13%) among 255 DCIS cases.

b. Categorize 2nd breast cancer events by invasive/in situ stage, ipsilateral/contralateral location, and estrogen receptor status, Months 7-8.

Progress report: Among the 32 second diagnoses among the 255 DCIS cases, 34% were invasive breast cancer, 53% were *in situ* breast cancer, and 13% (N=4) have unknown extent of disease (See table). A slight majority of second diagnoses were ipsilateral (53%). Estrogen receptor (ER) status is unknown for most of the second diagnoses (69%); 90% of the second diagnoses with known ER status were ER-positive.

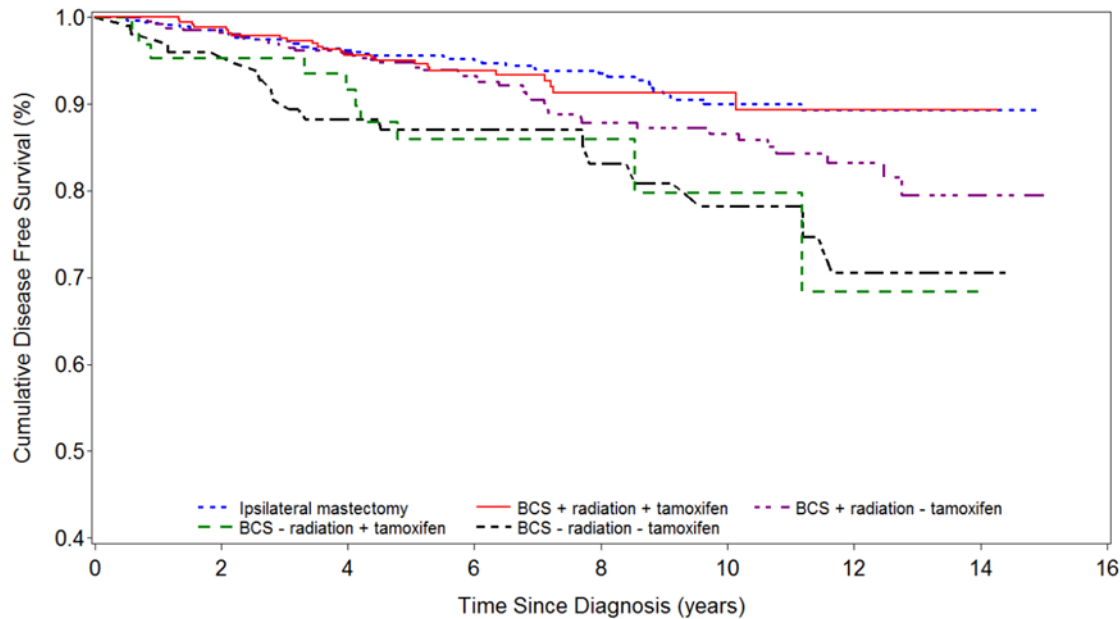
<i>Summary of DCIS cases (median length of follow-up = 11.8 years)</i>		
	N	%
Total	255	
Second diagnoses	32	12.5%
Extent of disease		
Invasive	11	34%
In situ	17	53%
Unknown	4	13%
Laterality		
Ipsilateral	17	53%
Contralateral	13	40%
Both	2	6%
Unknown	1	3%
Estrogen receptor status		
Positive	9	28%
Negative	1	3%
Unknown	22	69%

Task 4. Statistical analyses, Months 11-17:

- Link the tumor microenvironment data from Task 2 to the cohort data of Task 3, Month 11.
- Characterize the collagen alignment patterns and syndecan-1 expression levels in the 267 DCIS samples, Months 11-12.
- Evaluate the association between the tumor microenvironment data and tumor/patient characteristics, Months 13-15.
- Determine the relation between the tumor microenvironment data and disease-free survival, Months 16-17.

Progress report: Statistical analyses utilizing data for collagen alignment (fiber angles and TACS) of tumor samples has begun; analysis of syndecan-1 expression levels will commence once data collection is complete. Data collection is expected to be complete for collagen alignment measures for the remaining normal tissue samples by the end of March 2013; assessment of syndecan-1 expression may require until May 2013. A six month no-cost extension has been approved for this project through 31 August 2013 for these data analysis activities.

We have continued to prepare for these analyses by characterizing disease-free survival in the WISC Cohort from which the DCIS cases with tumor tissue samples were drawn. There are 1,959 DCIS cases in the WISC Cohort. After an average of 7.1 years of follow-up, 143 second breast cancer events occurred. Overall five-year disease-free survival was similar among women treated with ipsilateral mastectomy (95.6%; 95% CI: 93.5, 97.0) compared to women treated with BCS and radiation (94.8%; 95% CI: 92.8, 96.1), though women receiving BCS without radiation experienced poorer overall disease-free survival (87.0%; 95% CI: 80.6, 91.5; see figure below). Among women treated with BCS and radiation, the addition of tamoxifen was associated with a 30% (HR = 0.70; 95% CI: 0.41, 1.19) reduction in risk of second events. Women treated with BCS, radiation, and tamoxifen had comparable risk of a second event as those treated with ipsilateral mastectomy (HR=1.20; 95% CI: 0.71, 2.02).



We have also examined tumor and patient characteristics in relation to DCIS disease-free survival in the WISC Cohort. DCIS cases detected symptomatically were more likely to have a recurrence than cases detected by screening mammography (HR=1.6; 95% CI 0.9-3.0). Tumor size, grade, and histologic subtype were not strongly associated with disease-free survival. Similarly, little variation in disease-free survival was observed by age, family history of breast cancer, body mass index, parity, postmenopausal hormone use, and education. These results provide information as to the inclusion of potentially confounding factors in the analyses of Task 4.d. Our findings suggest that treatment received and mode of detection are important variables for inclusion in multivariable models of tumor microenvironment in relation to disease-free survival. The overall disease-free survival rates in the parent cohort will also be informative in comparing the disease-free survival rates observed among the DCIS cases with tumor tissue samples (i.e., to guide the generalizability and interpretation of the results).

Preliminary analysis has evaluated recurrence in the DCIS according to the presence of TACS-3 in the tumor tissue samples. Of cases with zero lesions with TACS-3, 87.2% did not have a recurrence and 12.8% did have a recurrence. Of cases with any lesions with TACS-3, 83.9% did not have a recurrence and 16.1% did have a recurrence. This preliminary analysis suggests that certain collagen alignment patterns may be associated with increased likelihood for disease progression.

Task 5. Communication of results, Months 12-24:

- a. Submit annual progress report to the DOD, Month 12
- b. Prepare manuscripts describing the results found in Task 4, Months 18-24.
- c. Present the study results at the DOD Era of Hope meeting and other national conferences, Months 12-24.
- d. Deliver final report to the DOD, Month 24.

Progress report: The first annual progress report was submitted and approved 13 June 2012. Communication of results will commence once data are collected and analyzed. No

manuscripts have been prepared although we are currently drafting a description of our laboratory methods and statistical analysis plan. Tasks 5b and 5c will be conducted upon completion of Tasks 1-4. We aim to submit an abstract to the CTRC-AACR San Antonio Breast Cancer Symposium, planned for December 10-14, 2013. Abstracts are due by June 11, 2013.

KEY RESEARCH ACCOMPLISHMENTS

- IRB approval obtained
- Procedures finalized for evaluating collagen fiber alignment in DCIS samples including imaging and quantification of angles as well as qualitative assessment (TACS)
- Collagen alignment for all tumor tissue samples has been evaluated, and assessment of collagen alignment for all normal tissue samples is expected to be completed by the end of March 2013
- Tumor slides have been stained for syndecan-1 expression
- Assessment of syndecan-1 expression levels has begun
- Second breast cancer diagnoses (invasive and *in situ*) have been identified among cohort participants, and the relations between disease-free survival and treatment patterns have been described
- Methods for statistical analysis have been established and preliminary data analysis has begun

REPORTABLE OUTCOMES

Preliminary findings regarding DCIS disease-free survival in the parent WISC Cohort were presented as a poster at the AACR Frontiers in Cancer Prevention Research annual meeting¹ and as an oral presentation at the annual breast cancer conference at the Vermont Cancer Center.²

Based on procedures established in this project, we successfully obtained a new grant from the NIH ("Vermont PROSPR Research Center", U54 CA163303); collaborators are now members of the Population-based Research Optimizing Screening through Personalized Regimens (PROSPR) consortium organized by the Applied Research Program within the Division of Cancer Control and Population Sciences at the NIH.

CONCLUSION

All study procedures have been finalized. Human subjects' protection approval for this study was obtained August 2011 from the University of Wisconsin Institutional Review Board, and on-going approval has been maintained. Using H&E stained slides, tumors were imaged using second harmonic generation microscopy and fiber alignment patterns were evaluated for 229 cases. Preliminary analysis suggests that fiber alignment patterns are significantly different for DCIS lesions than for adjacent normal duct cells. Tumor slides have been stained for syndecan-1, and assessment of staining intensity has begun. Statistical data analysis of the study aims will ensue upon completion of the assessment of collagen fiber alignment patterns in both tumor and normal tissue using several different measures of collagen alignment and syndecan-1 expression. Since data collection is not complete, no scientific knowledge or reportable outcomes have been produced yet, although preliminary data analysis has begun and methods sections of manuscripts are being drafted. While we have experienced some delays with establishing procedures for evaluating fiber alignment and evaluating syndecan-1

expression, and one microscope did stop functioning, methods are firmly in place and we expect to achieve the proposed Tasks within the project period since a 6-month no-cost extension has been approved.

REFERENCES

¹McLaughlin V, Sprague BL, Hampton J, Newcomb P, Trentham-Dietz A. DCIS disease-free survival in the population-based Wisconsin In Situ Cohort. Presented at the Tenth AACR International Conference on Frontiers in Cancer Prevention Research, October 22-25, 2011, Boston, MA.

²Sprague BL. Predictors of recurrence after a DCIS diagnosis. Presented at the Vermont Cancer Center 15th Annual Breast Cancer Conference, October 5, 2012, Burlington, VT.

APPENDICES

1. Poster¹



DCIS Disease-Free Survival in the Population-Based Wisconsin In Situ Cohort



Vicki McLaughlin^{1,2}, Brian Sprague¹, John Hampton³, Polly Newcomb⁴, Amy Trentham-Dietz³

¹University of Vermont, ²University of Massachusetts Amherst, ³University of Wisconsin, ⁴Fred Hutchinson Cancer Research Center

Introduction

- Ductal carcinoma in situ (DCIS) is the earliest form of breast cancer. DCIS constitutes 20% of all new breast cancer diagnoses and every year over 50,000 women are diagnosed with DCIS in the United States.
- A number of randomized trials have demonstrated the effectiveness of radiation and tamoxifen in reducing the risk of second events after a DCIS diagnosis. However, few population-based studies have examined disease-free survival according to treatment in community practice.

Study Aim

We describe the risk of second breast cancer events among women diagnosed with DCIS in the state of Wisconsin over the past 15 years. We determine overall disease-free survival among treatment groups and examine the frequency of ipsilateral and contralateral second events.

Methods

The Wisconsin In Situ Cohort (WISC) study was designed to evaluate breast cancer outcomes among a large population-based cohort of women with in situ breast cancer.

Eligible
Women age 20-74 years with a first primary diagnosis of breast carcinoma in situ reported to the Wisconsin Reporting System 1995-2006

Enrolled
78% of eligible women (N=2,281)

Study Population
Women diagnosed with DCIS (N=1,959)

Final Analytic Sample
Women with DCIS and complete treatment information (N=1,689)

Treatment Information

Surgical, radiation, and hormone treatment was self-reported at a baseline interview approximately 1 year after diagnosis and during follow-up interviews conducted every 2 years.

Breast Cancer Recurrence

Recurrences were self-reported at follow-up interviews conducted at two year intervals and were confirmed via pathology reports.

Statistical Methods

Descriptive statistics were used to compare baseline characteristics among women with each treatment type. Unadjusted disease-free survival was determined using Kaplan-Meier survival estimates. Cox proportional hazards regression was used to estimate hazard ratios by treatment, adjusting for confounders.

Results

Baseline Characteristics

The median age at the time of diagnosis was 56.0 years and most women in the study were postmenopausal (59.2%). A majority of breast cancers were detected via mammography (85.4%). Use of tamoxifen was reported by 38.0% of women, most frequently among women undergoing breast conserving surgery (BCS) (Figure 1).

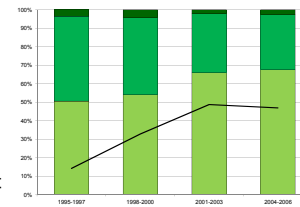


Figure 1: Surgical treatment rates and tamoxifen use by year of diagnosis, Wisconsin DCIS Cohort, 1995-2010

Risk of Recurrence

Over a median follow-up of 6.3 years, 133 second breast cancer events were recorded. The distribution of these recurrences by laterality is shown in Figure 2. Estimated hazard ratios for the risk of a second event by treatment group are shown in Table 1. The risk of a second ipsilateral event was significantly higher for women treated with BCS without radiation or biopsy only.

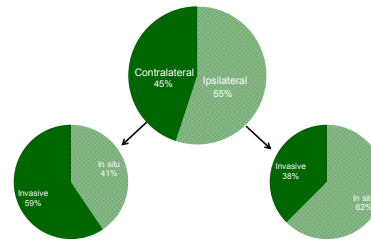


Figure 2: Distribution of second breast cancer events, Wisconsin DCIS Cohort, 1995-2010

Table 1: Estimated hazard ratios for the risk of 2nd breast cancer events according to treatment group, Wisconsin DCIS Cohort, 1995-2010.

Treatment type	Any second event				Any ipsilateral event				Invasive ipsilateral events				In situ ipsilateral events			
	No. Total	No. events	HR*	95% CI	No. events	HR*	95% CI		No. events	HR*	95% CI		No. events	HR*	95% CI	
BCS with radiation ^a	861	63	1	-	27	1	-		7	1	-		10	1	-	
BCS without radiation ^a	181	20	1.44	0.86, 2.40	14	2.24	1.15, 4.35		3	1.84	0.45, 7.54		5	2.25	0.74, 6.86	
Ipsilateral mastectomy	526	43	0.83	0.54, 1.26	13	0.52	0.26, 1.05		0	-	-		2	0.25	0.05, 1.19	
Bilateral mastectomy	75	0	-	-	0	-	-		0	-	-		0	-	-	
Biopsy only	46	7	1.82	0.82, 4.06	6	3.13	1.24, 7.86		2	4.42	0.81, 24.2		3	4.01	0.99, 16.2	

* Multivariable model adjusts for age group, menopausal status, screening history, mode of detection, tumor size, grade, tamoxifen use, and year of diagnosis. Unknown values of covariates were estimated using multiple imputation (m=10).

^a BCS = Breast conserving surgery.

Acknowledgements

This project is supported by the National Cancer Institute (CA67264, CA47147), the Department of Defense (BC102357), and a fellowship grant from the American Society of Preventive Oncology sponsored by the Susan G. Komen Foundation and the Prevent Cancer Foundation.

Results

Disease-free Survival

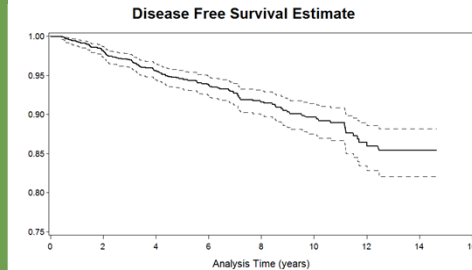


Figure 3: Overall disease-free survival with 95% confidence bands, Wisconsin DCIS Cohort, 1995-2010.

Disease Free Survival Estimate by Treatment Type

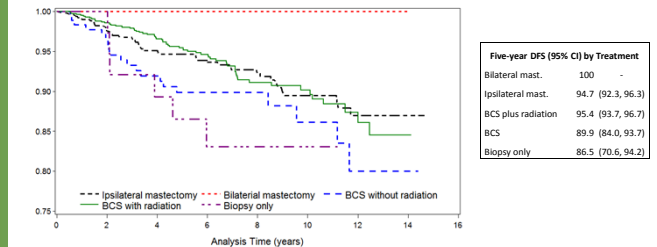


Figure 4: Disease-free survival by treatment type, Wisconsin DCIS Cohort, 1995-2010.

Discussion

- In this large population-based cohort study, we observed high disease-free survival rates among women diagnosed with DCIS. At five years after diagnosis, 94.6% of subjects had not experienced a second breast cancer event.
- The highest rates of disease-free survival were observed in women treated with bilateral mastectomy (100%) and the lowest rates in women treated with biopsy only (86.5%). Tamoxifen use reduced the risk of a second event by about 20% (data not shown).
- Limitations of this study included lack of ER/PR status and reliance on self-reported treatment.
- In general we found that the effectiveness of radiation therapy and tamoxifen in reducing risk of second events was comparable to that observed in randomized trials. The results of this study provide population-based data that can be used to guide treatment for DCIS.