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Natishkah Johnson Date

Stanniocalcin 1 (STC1) Expression as a Predictor of Late Breast Cancer Recurrence: Evaluating STC1 in Recurrent Tumors

By

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Global Epidemiology

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BSc, University of Richmond, 2017

Faculty Thesis Advisor: Dr. Timothy L. Lash, DSc, MPH

An abstract of
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2019

Abstract

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By Natishkah Johnson

Background: Expression of the Stanniocalcin 1 (STC1) hormone is associated with increased cancer risk and progression, and may increase the likelihood of recurrence. We investigate the hypothesis that STC1 expression is higher in recurrent tumors of patients experiencing late recurrence compared to early recurrence.

Methods: A total of 194 estrogen receptor-positive, tamoxifen-treated (ER^+/TAM^+) and 116 estrogen receptor-negative, tamoxifen-untreated (ER^-/TAM^-) breast cancer recurrence patients who experienced recurrence within 10 years post diagnosis were selected from a cohort of 11,251 Danish breast cancer patients diagnosed from 1985 to 2001. The association between IHC expression of STC1 in recurrent tumor tissues was evaluated within intervals of time to recurrence (2 to <3 years, 3 to <4 years, 4 to <6 years, 6 to <10 years, ref: 1 to <2 years).

Results: Dichotomized STC1 expression (positive /negative) was not associated with recurrence at any time interval including late recurrence, 6 to 10 years (aOR = 0.43; 95% CI, 0.1-1.74).

Conclusion: Our results do not suggest an association between recurrent tumor STC1 expression and breast cancer recurrence. While the evidence is insufficient to draw conclusions, there was a trend of decreasing odds of recurrence over increasing time to event intervals that may suggest that STC1 expression in the primary tumor may be important to potentiate late recurrence, but not necessary to maintain that potential.

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Table of Contents

Chapter I: Background/Literature Review

As a leading cause of cancer among women, breast cancer has been a major focus of cancer research in recent years. Improvements in screening guidelines, detection and treatment technology has lead to a decrease in mortality and a growing number of cancer survivors [1]. In fact, this number is estimated to be roughly 3.1 million women in the US alone [2]. Dependent on a number of factors, many of these women are at risk for recurrence [3]. While the majority of relapses occur three or more years after diagnosis, as many as 5% to 7% of these recurrences occur six to fifteen years after diagnosis [4, 5]. While research on cancer prevention and treatment continue to be of significant value, growing attention to recurrence research may be useful for improving outcomes among cancer patients [6]. In evaluating recurrence risk, prognostic methods that can distinguish those at risk of late recurrence uniquely are of special interest given the success of current prognostic methods in predicting early recurrence [7, 8].

Recurrence research has entailed a growing interest in biomarkers of outcome prediction, such as Stanniocalcin 1 (STC-1). STC-1 is a glycoprotein hormone involved in stabilizing cells in stressed conditions [9, 10]. Expression of Stanniocalcin 1 is associated with increased cancer risk and progression among various cancer types. A 2003 case-control study conducted in the United States by Wascher et al., identified a correlation between STC-1 mRNA expression in blood and bone marrow of breast cancer patients and prognostic factors such as tumor size and stage [18]. In a mouse model, a positive association of STC-1 expression with the development and progression of breast cancer was found in which tumor cell lines with different degrees of metastatic capacity were injected into the mammary glands of the mice [11].

Expression of STC-1 was associated with risk and poor cancer prognosis among different cancer types. In a 2015 cohort study of 60 Chinese glioma patients, high STC-1 expression was associated with worse overall survival [17]. Further, a case-control study involving 62 laryngeal squamous cell carcinoma patients and 30 healthy controls found that STC-1 mRNA expression in the peripheral blood of the participants was significantly higher among the cases than among the healthy controls [12]. Among Chinese clear cell renal cell carcinoma patients in a 2015 case-control study, STC-1 mRNA and protein expression was significantly upregulated among the 146 tumors cases relative to the 48 healthy controls [13]. Similarly, a 2016 case-control study involving 88 lung adenocarcinoma patients and 27 healthy controls, found that elevated expression of STC-1 protein for stage III and IV adenocarcinoma patients was positively associated with cancer progression [14].

Expression of STC-1 was also associated with poor cancer prognosis among breast cancer patients. A 2008 cohort study involving 72 breast cancer patients in Finland found higher expression of STC-1 in recurrent tumors and relapse, at 5 years ($p= 0.0012$) and 10 years ($p=0.0017$) post surgery relative to STC-1 expression in primary tumors [15]. Moreover, a 2018 nested case control study involving 841 Danish breast cancer patients and matched recurrence-free controls, found a positive association of STC-1 expression with late recurrence (6 – 10 years) aOR: 2.70 (1.22, 5.98) [16].

While these studies provide significant for the use of STC-1 as a predictor of risk and cancer prognosis in varying cancer types, more research is needed on its potential as an indicator for breast cancer recurrence. This research would need to more closely investigate early versus late recurrence, and to investigate expression levels in both

primary and recurrent tumors. Additionally, many previous studies had small sample sizes, so investigations in larger cohorts are needed.

Chapter II: Manuscript

Stanniocalcin 1 (STC1) Expression as a Predictor of Late Breast Cancer Recurrence: Evaluating STC1 in Recurrent Tumors

By Natishkah Johnson

Abstract

Background: Expression of the Stanniocalcin 1 (STC1) hormone is associated with increased cancer risk and progression, and may increase the likelihood of recurrence. We investigate the hypothesis that STC1 expression is higher in recurrent tumors of patients experiencing late recurrence compared to early recurrence.

Methods: A total of 194 estrogen receptor-positive, tamoxifen-treated (ER^+/TAM^+) and 116 estrogen receptor-negative, tamoxifen-untreated (ER^-/TAM^-) breast cancer recurrence patients who experienced recurrence within 10 years post diagnosis were selected from a cohort of 11,251 Danish breast cancer patients diagnosed from 1985 to 2001. The association between IHC expression of STC1 in recurrent tumor tissues was evaluated within intervals of time to recurrence (2 to <3 years, 3 to <4 years, 4 to <6 years, 6 to <10 years, ref: 1 to <2 years).

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Introduction

As the leading cause of cancer death among women world wide, breast cancer has been a major focus of cancer research in recent years. Improvements in screening, detection and treatment technology have led to a decrease in breast cancer-related mortality and an increase in the population of breast cancer survivors [1]. In the US alone, this number is estimated to be roughly 3.1 million women [2]. Influenced by factors such as cancer subtype and treatment, many of these women are at risk for recurrence [3]. While the majority of relapses occur three or more years after diagnosis, as many as 5% to 7% of these recurrences occur six to fifteen years after diagnosis [4, 5]. Research on cancer prevention and treatment are of significant value, however increasing interest in recurrence risk may be useful for improving outcomes among cancer patients [6]. In evaluating recurrence risk, prognostic methods that can distinguish those at risk of late recurrence uniquely are of special interest given the success of current prognostic methods in predicting early recurrence [7, 8].

This interest in recurrence research has meant a growing interest in biomarkers of outcome prediction, such as Stanniocalcin 1. Stanniocalcins are glycoprotein hormones involved in stabilizing cells in stressed conditions [9, 10]. Expression of the Stanniocalcin 1 (STC1) hormone is associated with increased cancer risk and progression among various cancer types, including: glioma, laryngeal squamous cell carcinoma, renal cell carcinoma, lung adenocarcinoma, and breast cancer [11-16]. Within a cohort of 72 Finish breast cancer patients, Joensuu et al. found that compared to primary tumors with early relapse and their metastases, the expression of STC1 was associated with higher relapse 5 years and 10 years post-surgery [15]. Moreover, a nested case control study from our

group by Brantley et al. identified a positive association between STC-1 expression and late recurrence (6 – 10 years) in the primary tumors of a cohort of 841 Danish breast cancer patients [16].

Although these studies do provide support for the use of STC-1 as a predictor of risk and prognosis in different cancer types, more support for its usefulness as a prognostic tool for breast cancer recurrence specifically may be useful for distinguishing early and late breast cancer recurrence risk. Further investigating STC1 expression as a predictor of late recurrence and evaluating expression patterns in recurrent tumors also, are important next steps. Consistent with the study by Brantley et al., using the same cohort of Danish breast cancer patients, we investigate the hypothesis that STC1 expression is higher in recurrent tumors of patients experiencing late recurrence compared to early recurrence.

Methods

Study population

Recurrent cases were identified from a source population of 11,251 female breast cancer patients registered with the Danish Breast Cancer Cooperative Group (DBCG). Women diagnosed with stage I, II or III breast cancer between 1985 and 2001, between 35 and 69 years old at diagnosis, whose estrogen receptor (ER) and tamoxifen (TAM) status were known, and who survived one or more years without recurrence were included in the study. Patients for whom STC1 expression could not be determined were excluded from the study (n=310). After exclusions, there were 194 ER^+/TAM^+ and 116 ER^-/TAM^- study subjects.

All patients in the DBGG were followed-up for 10 years from time of diagnosis. Follow-up occurred every three to six months during the first five years, and yearly for

years five to ten post diagnosis. Subjects identified as recurrences included patients that received a diagnosis of breast cancer or metastases by December 31, 2006, after initial course of treatment. STC1 expression, the exposure of interest, was ascertained by IHC staining conducted on tissue samples from recurrent tumors.

TMA Construction and IHC staining

Formalin-fixed, paraffin-embedded recurrent breast tumor specimens were collected from the pathology labs of participating hospitals. From these specimens, blocks with invasive carcinoma were identified by a pathologist blinded to patients' clinical information. Tissue microarrays (TMA) were constructed using a TMA Master (3DHISTECH), with 1-mm tissue cores from each specimen. Tissue samples of 2.5μm were stained for STC1 by the pathology laboratory at the Rollins School of Public Health at Emory University (Atlanta, GA). To stain, slides were deparaffinized in xylene, hydrated in graded alcohols, and blocked for endogenous peroxidase for 5 minutes in UltraVision hydrogen peroxidase block (Thermo Fisher Scientific, ref. TA-125H202Q). Heat-induced epitope retrieval was performed in a decloaking chamber (PT Link, Agilent). Before staining, and in between each step, slides were washed with Tween 20 buffer (Cell Marque, ref. 935B-09). Automated staining was carried out at room temperature using the Dako AutostainerPlus. Following UltraV block (Thermo Fisher Scientific, ref. TA-125-UB), sections were incubated for 30 minutes with the primary antibody [rabbit polyclonal anti-STC1 (Sigma Aldrich, cat. HPA023918) at 1:500 dilution, followed by UltraVision Goat Polyvalent Secondary (Thermo Fisher Scientific, ref. TL-125- BN) for 15 minutes, UltraVision Streptavidin Horseradish Peroxidase (Thermo Fisher Scientific, ref. TL-125-HR) for 15 minutes, and by diaminobenzidine

(DAB; Thermo Fisher Scientific, ref. TA-125-HDX) for 5 minutes]. Slides were counterstained with hematoxylin (Thermo Fisher Scientific, ref. 7211), dried for at least 24 hours, and then digitalized using the Panoramic Scan 150 whole slide image scanner (3DHISTECH) [16].

IHC expression was evaluated by one of us (NJ) at Rollins School of Public Health, Emory University (Atlanta, GA) using digital slides. STC1 expression intensity was assigned on a scale of 0 to 3. Values of 0 indicated no staining, while 1 indicated low intensity staining and 3 indicated intense staining. Staining was also scored by proportion on a scale of 0 to 100%, where 0% was assigned to tissue samples without staining and 100% to those with complete stain coverage. The STC1 expression intensity and proportion scores were then multiplied to obtain sample H-scores (0 to 300). To determine H-sores among patients with more than one sample (up to 3), an average of the H-scores were taken. Patients whose H-scores could not be determined as a result of incomplete tissue sample, were excluded from the study.

Definition of Analytic Variables

STC1 expression was dichotomized as positive or negative using patient H-scores. H-scores less than or equal to 50 were considered STC1 negative, while scores 51 to 300 were categorized as STC1 positive. Time to recurrence was categorized as: 1 to <2 years; 2 to <3years, 3 to <4 years; 4 to <6 years; and 6 to 10 years. Data on potential covariate factors determined by *a priori evidence* were obtained from DBCG records. Covariates included: ER expression at diagnosis, whether prescribed tamoxifen during treatment, menopausal status, UICC stage at diagnosis (I, II, III), year of diagnosis (1985-1993,

1994-1996, and 1997-2001), county of residence, age, primary treatment (radiation and chemotherapy), systemic chemotherapy receipt (yes/no) and Charlson comorbidity score.

Statistical Analysis

Proportions of STC1 positive and negative cases were calculated for both ER^+ /TAM^+ and

ER^-/TAM^- groups within each time to recurrence category, as well as all covariates. To evaluate the hypothesis that STC1 expression is higher in recurrent tumors of patients experiencing later recurrences compared with early recurrences, crude, age-adjusted and adjusted ORs for the association of STC1 expression with recurrence were calculated at each category of time to recurrence within ER^+ /TAM^+ and ER^-/TAM^- groups. Polytomous logistic regression was used to calculate ORs given the ordinal outcome of interest, time to recurrence. The adjusted ORs include adjustment for age group, year group of diagnosis, menopausal status, stage, county of treatment, radiation treatment, chemotherapy, duration of tamoxifen therapy, and CCI score. Age-adjusted OR included adjustment for the age group covariate only. All statistical analyses were conducted using SAS 9.4.

Results

The majority of women in this study were postmenopausal (81%), a distribution also observed within positive and negative ER/TAM groups individually (92% and 63% respectively). Distribution of age and stage at diagnosis did differ between ER/TAM groups however. Women with ER^+ /TAM^+ statuses were mostly older, 55-64 years old (57%) and diagnosed with stage III breast cancer at time of diagnosis (Table 1). In contrast, the majority of ER^- /TAM^- women were 35-54 years (62%) with stage I or II

breast cancer at diagnosis. Mastectomy was most common among both ER/TAM status groups, as well as radiotherapy. Most participants were also without comorbidities (Table 1).

To determine whether STC1 expression was differentially associated with time to recurrence, adjusted ORs were calculated for each time to recurrence period. Among ER^+ /TAM^+ women, STC1 expression was not notably associated with higher odds of breast cancer recurrence 6 to 10 years (aOR = 0.43; 95% CI, 0.1-1.74; Table 2) post diagnosis. It was not notably associated with time to recurrence for any period post diagnosis (ref. 1 to <2 years). The same was observed among ER^- /TAM^- women: STC1 expression was not associated with time to recurrence at any period post diagnosis (Table 3). Odds of recurrence for years 6 to 10 was aOR = 2.79; 95%CI, 0.24-31.92. Adjusting for age solely did not change the OR estimates substantially (Table 2 & 3).

Discussion

In this cohort of Danish breast cancer patients, no association was found between STC1 expression in recurrent tumors and categorized time to recurrence. Further, there is a trend of decreasing odds of recurrence over increasing time to event intervals. These findings are not consistent with previous studies that identified STC1 expression as a predictor of late recurrence [15, 16]. Most important to note in comparing these studies however, is that STC1 expression levels in recurrent tumors were measured for this study, while previous studies measured expression in primary tumors. Moreover, follow-up duration is greater (up to 23 years) for the Joensuu et al. study compared to 10 years of follow-up in this study [15]. While the evidence provided is insufficient to draw

conclusions on observed trends, the decreasing odds of recurrence observed during later recurrence periods may suggest that STC1 expression in the primary tumor may be important to potentiate late recurrence, but not necessary to maintain that potential.

A comparison of the descriptive characteristics of breast cancer recurrence patients in the study population to those in the source population [16] revealed a similar distribution within both ER^+/TAM^+ and ER^-/TAM^- groups with just two exceptions (Table 4). The proportion of ER^-/TAM^- women receiving systemic adjuvant chemotherapy within populations varied by about 10%. Most notable however, were the differences in proportions of STC1expression among ER^-/TAM^- women. The distribution of tumors with positive STC1 expression was 25% higher in this study population than in the source population (Table 4), which resulted in a shifted majority. The majority (65%) of tumors in the source population were STC1 negative, while the majority (60%) of tumors in this study population were STC1 positive (Table 4). This may suggest selection bias, but can also be a result of differential tumor scoring. Using the STC1 expression scores designated in the source population study [16] would produce results more appropriate for comparison.

This study is also limited by a small sample size (n=310) that is further reduced by conducting separate analyses for the ER^+/TAM^+ (n=194) and ER^-/TAM^- (n=116) groups. Moreover, the definitions of negative and positive STC1 expression was based on an H-score cutoff that may not best represent STC1 expression. Because very few samples were without any sign of IHC staining, an H-score cutoff of 50 was established to dichotomize STC1 expression exposure. Considering a different way to better represent

STC1 expression -such as lowering the H-score cut-off may provide a more accurate measure of association.

Strengths of this study include consistent and standardized follow-up data available for all study patients over the 10-year period of interest. Data on potential covariates are also available for almost all patients, which allow for reduction of potential confounding during statistical analyses. Additionally, the standardized care provided by Denmark's universal healthcare system is also important as quality care among patients at baseline can be assumed.

In addition to addressing limitations such as sample size and STC1 expression categorization, future work should compare STC1 expression among both primary and recurrent tumors of breast cancer patients to better understand differences in expression over the course of recurrence follow-up, and potentially provide more information on mechanisms involved in their observed similarities or differences.

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Tables

Table 1. Frequency and proportion of breast cancer recurrent patients with positive and negative STC1 expression within ER/TAM group strata (n=310)

	ER+/TAM+, no. (%) or mean (SD)		ER-/TAM-, no. (%) or mean (SD)	
Patient characteristics	STC1 Expression, Negative[a]	STC1 Expression, Positive[a]	STC1 Expression, Negative[a]	STC1 Expression, Positive[a]
Recurrence Time				
1 to <2 years	19 (21)	23 (22)	13 (28)	24 (34)
2 to <3 years	15 (17)	20 (19)	17 (37)	19 (27)
3 to <4 years	12 (13)	18 (17)	6 (13)	13 (19)
4 to <6 years	24 (27)	27 (26)	6 (13)	6 (9)
6 to 10 years	19 (21)	17 (16)	4 (9)	8 (11)
Diagnosis year				
1985–1993	28 (31)	43 (41)	14 (30)	21 (30)
1994–1996	23 (26)	19 (18)	13 (29)	20 (29)
1997–2001	38 (43)	43 (41)	19 (41)	29 (41)
Age category at diagnosis, years				
35–54	25 (28)	23 (22)	28 (61)	44 (63)
55–64	46 (52)	62 (59)	8 (17)	19 (27)
65–69	18 (20)	20 (19)	10 (22)	7 (10)
Menopausal status at diagnosis				
Premenopausal	9 (10)	6 (6)	14 (30)	29 (41)
Postmenopausal	80 (90)	99 (94)	32 (70)	41 (59)
UICC tumor stage at diagnosis				
I - II	48 (54)	42 (40)	23 (50)	40 (66)
III	41 (46)	63 (60)	23 (50)	24 (34)
Histologic grade				
1	20 (22)	22 (21)	6 (13)	7 (10)
2	36 (40)	35 (33)	20 (43)	23 (33)
3	14 (16)	27 (26)	13 (28)	26 (37)
Missing	19 (21)	21 (20)	7 (15)	14 (20)
Surgery type				
Mastectomy	80 (90)	98 (93)	41 (89)	63 (90)
Breast Conserving Surgery	9 (10)	7 (7)	5 (11)	6 (9)
Radiotherapy				
Yes	28 (31)	30 (29)	17 (37)	28 (40)
No	61 (69)	75 (71)	29 (63)	39 (56)
Tamoxifen protocol, years (only ER+)				
1	39 (44)	44 (42)	-	-
2	14 (16)	21 (20)	-	-
5	36 (40)	40 (38)	-	-
Systemic adjuvant chemotherapy				
Yes	11 (12)	9 (9)	37 (80)	51 (73)
No	78 (88)	96 (91)	9 (20)	19 (27)
CCI score				
0	50 (56)	74 (70)	38 (83)	48 (69)
1	5 (6)	5 (5)	0 (0)	5 (7)
2+	14 (16)	9 (9)	6 (13)	9 (13)

[a]Dichotomous STC1 expression defined as negative if $\leq$50 h-score, positive if >50 h-score.

Table 2. Association of time to breast cancer recurrence with STC1 expression, ER+/TAM+

	ER+/TAM+			
Time to Recurrence	**STC1 Expression positive/negative**	**Crude OR (95% CI)**	**Age-Adjusted OR (95% CI)**	**Adjusted OR (95% CI)***
1 to <2 years	23/19	ref.	ref.	ref.
2 to <3 years	20/15	1.10 (0.45 - 2.72)	1.13 (0.45 - 2.82)	0.55 (0.17 - 1.83)
3 to <4 years	18/12	1.24 (0.48 - 3.20)	1.29 (0.49 - 3.39)	0.86 (0.22 - 3.36)
4 to <6 years	27/24	0.93 (0.41 - 2.11)	0.91 (0.39 - 2.13)	0.84 (0.26 - 2.75)
6 to 10 years	17/19	0.74 (0.30 - 1.81)	0.72 (0.29 - 1.81)	0.43 (0.11 - 1.74)

*Adjusted OR includes adjustment for covariates: age group, year group of diagnosis, menopausal status, stage (I–III), county of treatment, chemotherapy, radiation, CCI group, and tamoxifen duration (ER+)

Table 3. Association of time to breast cancer recurrence with STC1 expression, ER-/TAM-

	ER-/TAM-			
Time to Recurrence	**STC1 Expression positive/negative**	**Crude OR (95% CI)**	**Age-Adjusted OR (95% CI)**	**Adjusted OR (95% CI)***
1 to <2 years	24/13	ref.	ref.	ref.
2 to <3 years	19/17	0.61 (0.24 - 1.55)	0.57 (0.21 - 1.53)	0.65 (0.19 - 2.25)
3 to <4 years	13/6	1.17 (0.36 - 3.82)	0.85 (0.24 - 2.95)	0.39 (0.05 - 2.87)
4 to <6 years	6/6	0.54 (0.15 - 2.02)	0.48 (0.11 - 2.05)	0.06 (0.00 - 1.06)
6 to 10 years	8/4	1.08 (0.27 - 4.29)	1.26 (0.27 - 5.87)	2.79 (0.24 - 31.92)

*Adjusted OR includes adjustment for covariates: age group, year group of diagnosis, menopausal status, stage (I–III), county of treatment, chemotherapy, radiation, CCI group, and tamoxifen duration (ER+)

Table 4. Comparison of the descriptive characteristics of breast cancer recurrent patients from the source population[a] and the study population of patients with available tissue samples.

	ER+/TAM+, no. (%) or mean (SD)		ER-/TAM-, no. (%) or mean (SD)	
Patient characteristics	Source Population	Study Population	Source Population	Study Population
STC1 expression, dichotomous				
Negative	222 (50)	89 (46)	165 (65)	46 (40)
Positive	218 (50)	105 (54)	87 (35)	70 (60)
Diagnosis year				
1985–1993	187 (42)	71 (37)	89 (35)	35 (30)
1994–1996	90 (20)	42 (22)	67 (26)	33 (28)
1997–2001	169 (38)	81 (42)	97 (38)	48 (41)
Age category at diagnosis, years				
35–54	113 (25)	48 (25)	156 (61)	72 (62)
55–64	229 (51)	108 (56)	70 (28)	27 (23)
65–69	104 (23)	38 (20)	27 (11)	17 (15)
Menopausal status at diagnosis				
Premenopausal	30 (7)	15 (8)	100 (40)	43 (37)
Postmenopausal	416 (93)	179 (92)	153 (60)	73 (63)
UICC tumor stage at diagnosis				
I - II	210 (47)	90 (46)	153 (61)	69 (59)
III	236 (53)	104 (54)	100 (39)	47 (41)
Histologic grade				
1	84 (19)	42 (22)	17 (7)	13 (11)
2	199 (45)	71 (37)	111 (44)	43 (37)
3	77 (17)	41 (21)	90 (36)	39 (34)
Missing	86 (19)	40 (21)	35 (14)	21 (18)
Surgery type				
Mastectomy	403 (90)	178 (92)	217 (86)	104 (90)
Breast Conserving Surgery	43 (10)	16 (8)	35 (14)	11 (9)
Radiotherapy				
Yes	151 (34)	58 (30)	104 (42)	45 (39)
No	295 (66)	136 (70)	145 (58)	68 (59)
Tamoxifen protocol, years (only ER+)				
1	208 (47)	83 (43)	-	-
2	75 (17)	35 (18)	-	-
5	163 (36)	76 (39)	-	-
Systemic adjuvant chemotherapy				
Yes	61 (14)	20 (10)	215 (85)	88 (76)
No	385 (86)	174 (90)	38 (15)	28 (24)
CCI score				
0	338 (76)	124 (64)	199 (79)	86 (74)
1	41 (9.2)	10 (5)	15 (6)	5 (4)
2+	67 (15)	23 (12)	39 (15)	15 (13)

[a] The source population is descried in the Brantley et al. study [16]

Chapter III: Summary, Public Health Implications, Possible Future Directions

Although this study did not find an association between STC1 expression in recurrent tumors and time to recurrence, we cannot dismiss the potential usefulness of STC1 for predicting breast cancer recurrence as it is associated with recurrence in primary tumors. While the evidence provided is insufficient to draw conclusions on observed trends, the decreasing odds of recurrence observed during later recurrence periods may suggest that STC1 expression in the primary tumor may be important to potentiate late recurrence, but not necessary to maintain that potential. Future work should compare STC1 expression among both primary and recurrent tumors of breast cancer patients to better understand differences in expression over the course of recurrence follow-up, and potentially provide more information on mechanisms involved in their observed similarities or differences.