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Mesothelin expression and survival outcomes in triple receptor negative breast cancer

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Abstract

Background—Mesothelin is an ideal tumor-associated marker for the development of targeted therapy due to its limited expression in normal tissues. The aim of this study was to evaluate mesothelin expression in triple-negative breast cancer (TNBC) and its correlation with survival outcomes.

Methods—Mesothelin expression was completed using immunohistochemistry, and quantified by H-score. An H-score >10 was considered positive. Patient's characteristics were compared by mesothelin expression. Kaplan-Meier product limit method was used to estimate survival outcomes. Cox proportional hazards models was used to adjust for patient and tumor characteristics.

Results—Median age was 52 years. Of the 109 TNBC, 37 (34%) were positive for mesothelin expression. There were no differences on patient/tumor characteristics by mesothelin expression with the exception of high frequency of lymphovascular space invasion in mesothelin-negative tumors ($P=0.03$). At a median follow up of 75.8 months 20 (18.3%) patients had experienced a recurrence and 22 (20.2%) had died. Five-year progression-free survival was 87% and 92% in patients with mesothelin-positive and mesothelin-negative tumors ($P=0.43$). Five-year overall survival was 85% and 91% in patients with mesothelin-positive and mesothelin-negative tumors ($P=0.57$), respectively. Mesothelin expression was not an independent predictor of survival outcomes.

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Conclusion—Mesothelin expression was identified in 34% of TNBC. Mesothelin expression did not correlate with survival outcomes in TNBC.

Keywords

Mesothelin expression; triple-negative breast cancer; prognosis

Introduction

Mesothelin is a cell surface glycoprotein present on normal mesothelial cells lining the pleural, peritoneum and pericardium. The mesothelin gene encodes a precursor protein of 71 kDa that is processed to a 31 kDa shed protein called megakaryocyte potentiating factor (MPF) and a 40 kDa membrane bound protein, mesothelin.¹ The biologic function of mesothelin is not well known. Previous studies, reported no detectable abnormalities in growth and reproduction in mesothelin mutant mice.² However, recent studies in ovarian cancer demonstrated that mesothelin may play a role in cell adhesion by interaction with CA-125 suggesting that mesothelin and CA-125 may have an important role in the metastatic spread of ovarian cancer.³ Overexpression of mesothelin has been identified in various cancers including mesothelioma, ovarian, biliary and pancreatic adenocarcinoma and lung cancer by using an immunohistochemistry staining.⁴⁻⁸ As a result of its limited expression in normal tissues, mesothelin has been reported as an ideal tumor-associated marker for the development of targeted therapy⁹.

Recent studies have reported the association of mesothelin expression and clinical outcomes in different tumor types.¹⁰⁻¹⁵ Cheng and colleagues evaluated mesothelin mRNA levels and their correlation to clinic-pathologic features and outcomes in 139 patients with epithelial ovarian cancer. The authors found higher mesothelin mRNA levels in advanced stage tumors. Further, mesothelin levels were independent predictors of PFS (HR: 2.03, p=0.006) and OS (HR: 3.72, p=0.002).¹⁰ Einama and colleagues measured mesothelin and CA125 expression in pancreatic ductal adenocarcinoma and found that the co-expression of both markers was associated with an unfavorable patient outcome.¹¹ A single study has evaluated mesothelin expression in all breast cancer subtypes and showed that mesothelin was expressed in 30% of the cases, and that high mesothelin levels in the tumor cells were significantly associated with decreased overall survival¹⁶.

Triple-negative breast cancer (TNBC), an aggressive subtype of breast cancer, is defined by the lack of expression of estrogen, progesterone and HER2 receptors and accounts for 10-20% of invasive breast cancers. TNBC are associated with a higher recurrence rate, particularly in the first 3 years after diagnosis and shorter survival outcomes. This breast cancer subtype has not only distinct epidemiology, histologic features and clinical behavior, but lacks effective targeted therapies that can improve on chemotherapy benefit.¹⁷⁻²⁰ Therefore, a better understanding the molecular biology of this challenging breast cancer subgroup may assist leading the path to improve and customize drug development with the goal of improve outcomes. Data on mesothelin expression in breast cancer, particularly in TNBC is limited. Only one published report has examined mesothelin expression in TNBC, and show that mesothelin was overexpressed in the majority of TNBC (67%) but rarely present in ER-positive or HER2-positive breast cancers.²¹ In the same study, investigators suggested that using adoptive transfer of T-cells expressing a chimeric antigen receptor specific for mesothelin (mesoCAR T-cell) in mesothelin-expressing primary breast cancer cell lines resulted in significantly higher cell killing than achieved with non-transduced T-cells. The authors did not reported data on mesothelin expression and clinical outcomes.²¹ Based on the above data, and due to the potential use of anti-mesothelin biological therapies including antibody-drug conjugates (ADC) already under evaluation in clinical trials clinic,

we sought to evaluate the frequency of mesothelin expression in TNBC and its correlation with clinical characteristics and disease outcome.

Patients and Methods

We identified 109 with primary TNBC diagnosed between 1993 and 2009 and whose tissues were available in the breast cancer tissue bank were identified. Patient's data were collected by chart review. Tumors were considered triple receptor-negative if nuclear staining $\geq 5\%$ of both estrogen receptor and progesterone receptor, and HER2 negative receptor expression on IHC staining (membranous staining in less than 10% of cells), and/or negative gene amplification found on FISH. All patients were treated with a multidisciplinary approach. After definitive surgery, all patients received adjuvant chemotherapy with an anthracycline-based, a taxane-based, or an anthracycline/taxane based and non-anthracycline/taxane base regimens. The Institutional Review Board of The University of Texas, MD Anderson Cancer Center approved the laboratory retrospective study.

Pathology methods

Paraffin tissue blocks from the patients' archived TNBC available from the MDACC Breast Cancer Tumor Bank were used to construct tissue microarrays (TMAs). These arrays consisted of two 1-mm cores from each TNBC tumor block and additional 10 1-mm cores from normal breast tissue as control.

The polymeric biotin-free horseradish peroxidase (HRP) method on the Leica Microsystems Bond Max stainer (Leica Microsystems, Buffalo Grove, IL) was used for mesothelin immunohistochemical staining. An unstained tissue section slide 4- μ m thick prepared from TMA paraffin block of triple negative invasive carcinoma was incubated at 60 °C for twenty minutes. Following heat-induced epitope retrieval with TRIS-EDTA for 20 minutes at 100 °C, slides were incubated with antibody against mesothelin at 1:30 dilution (clone 5B2, Novocastra, Buffalo Grove, IL). The Refine Polymer Detection kit was used for detection of bound antibody, with 3,3-diaminobenzidine serving as the chromagen (Leica Microsystems, Buffalo Grove, IL). Slides were counterstained with Mayer's hematoxylin. Results were evaluated with known positive and negative tissue controls. Immunostained tissue sections were evaluated by one breast cancer specialized pathologist and positive staining was scored by proportion of tumor cells with positive stain percentage and intensity of the stain (1+, 2+, 3+), H-score,²²⁻²⁴ with a maximal score of 300. H-score more than 10 was considered positive, cut off established based on data distribution on a histogram.

Statistical Analyses

Patients with triple negative breast cancer (TNBC) were categorized according to their H scores into one of two groups: Mesothelin negative (H score ≤ 10), and mesothelin positive (H score > 10). Patients characteristics including age, race, tumor size, lymph nodes, pathologic stage, histology, grade, lymphovascular invasion, and adjuvant chemotherapy were tabulated and compared between groups using Fisher's exact test. Overall survival (OS) was measured from the date of surgery to the date of death or lost to follow-up. Relapse-free survival (RFS) was measured from the date of surgery to the date of first documented local or distant recurrence or lost follow-up. Patients who died before experiencing the relevant events were considered censored at their dates of last follow-up date. The Kaplan-Meier product limit method was used to estimate the survival outcomes of all patients by time to chemotherapy groups; groups were compared using the log-rank statistic.

Cox proportional hazards models were fit to determine the association of mesothelin with survival outcomes after adjustment for other patient and disease characteristics including

grade (II, III), pathologic stage (I, II, III), lymphovascular invasion (negative, positive), and adjuvant chemotherapy (anthracycline-based, Taxane-based, anthracycline/Taxane-based, other). Results are expressed in hazard ratios (HRs) and 95% CIs. P-values less than 0.05 were considered statistically significant; all tests were two-sided. Statistical analyses were carried out using SAS 9.2 (SAS Institute, Cary, NC) and S-Plus 7.0 (Insightful Corporation, Seattle, WA).

Results

A total of 109 women with TNBC were identified. There were 63 (58%) patients whose H-scores were 0, and another 9 (8%) patients who had H-scores between 1 and 10. The rest 37 (34%) patients had H-scores sparsely distributed between 10 and 300. Figure 1 showed examples of tumors with positive or negative mesothelin staining. Patient characteristics stratified by mesothelin groups are summarized in Table 1. There were no differences on patient/tumor characteristics by mesothelin expression with the exception of high frequency of lymphovascular space invasion in mesothelin-negative tumors ($P=0.03$). At a median follow up of 75.8 months (range, 12-341 months), 20 women (18.3%) had experienced a recurrence and 22 (20.2%) had died. Five-year relapse-free survival was 87% and 92% in patients with mesothelin-negative and mesothelin-positive tumors ($P=0.43$), and 5-year overall survival was 85% and 91% in patients with mesothelin-negative and mesothelin-positive tumors ($P=0.57$), respectively. Table 2 summarizes the 5-year RFS and 5-year OS estimates by patients and tumor characteristics and Figures 2A-2B illustrate the Kaplan-Meier curves for RFS and OS by mesothelin expression. In multivariable Cox proportional hazards models, mesothelin expression was not independently associated with RFS or OS in TNBC (HR: 1.0; 95%CI 0.33-3.06; $P=1$ for RFS and HR: 1.02; 95%CI 0.35-2.98, $P=0.97$ for OS) after adjustment for other patient and disease characteristics including grade, pathologic stage, lymphovascular invasion, and adjuvant chemotherapy. Table 3 summarizes the multivariable Cox proportional hazards models of RFS and OS adjusted for mesothelin group, grade, stage, lymphovascular invasion, adjuvant chemotherapy.

Discussion

In this analysis of 109 patients with TNBC, we found that the frequency of mesothelin expression was 34%, and that mesothelin expression did not correlate with survival outcomes in TNBC. The first report that studied mesothelin expression in breast cancer reported negative results.²⁵ Investigators used immunohistochemistry to stain a variety of tumor types including 12 breast cancers, but no information on breast cancer subtype was reported. The largest report of mesothelin expression in breast cancer to date, includes 99 primary breast cancers, of which 43 were TNBC. Investigators found that 67% of TNBC exhibit at least 5% mesothelin staining in tumor cells in contrast to 1/29 and 1/27 hormone receptor-positive and HER2-positive tumors respectively.²¹ Compared with our study, the frequency of mesothelin expression in this report higher than in ours. These differences can in part be explained by the fact that we used TMA which can limit the evaluation of expression due to tumor heterogeneity, and different mesothelin immunoreactivity scoring. Compared with standard immunohistochemistry percentage scoring, our study used the more accurate scoring system, H-score,²² which evaluates not only the proportion of tumor cells with positive stain but also the intensity of the stain (1+, 2+, 3+) creating a more accurate linear score. Additionally, in our study, we used cut off values of ER, PR expression different that the current ASCO/CAP guidelines considered as positive (1%)²⁶ since we have shown the lack of efficacy of adjuvant endocrine therapy in patients with < 5% ER or PR expression.²⁷ We don't believe this influence our results on mesothelin expression. Recent studies reported an association between mesothelin expression and clinical outcomes in patients with ovarian cancer, pancreatic cancer and gastric cancer.¹⁰⁻¹⁵ However, to the

best of our knowledge, no study has investigated the correlation between mesothelin expression with patient characteristic and clinical outcomes in TNBC. Although our study showed that mesothelin levels were not independently associated with outcomes. (HR: 1.0 for RFS and HR: 1.02 for OS), the results could be limited by the small sample size. Although small, our cohort of TNBC shows not even a trend to such correlation. Further, a sensitivity analysis excluding patients with H-score 1-10 was carried out, and showed no difference in survival outcomes. This decreases the interest of studying the mechanistic role of this protein in the disease; however, if one third of TNBC overexpress mesothelin, it can be exploited as an indirect target for drug delivery.

Currently, agents targeting mesothelin expressing tumors are being investigated in the clinic. Different approaches include SS1P (anti-mesothelin immunotoxin), MORAb-009 (a high-affinity chimeric monoclonal IgG1/k Ab), anti-mesothelin antibody drug conjugate (ADC) BAY94-9343, a mesothelin tumor vaccine (CRS-207) as well as a mesothelin adoptive T-cell immunotherapy using mesothelin-specific CARs, are being evaluated in phase I clinical trials.^{9,19,28-32} Some of these compounds have shown promising results without unfavorable toxicities in preclinical or early clinical trials and should be tested in patients with TNBC. These ongoing clinical trials will help customizing the potential treatment of mesothelin-expressing malignancies. However, a standardized and validated test for mesothelin expression needs to be available before using it as a predictive biomarker.

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References

1. Chang K, Pastan I. Molecular cloning of mesothelin, a differentiation antigen present on mesothelium, mesotheliomas, and ovarian cancers. *Proc Natl Acad Sci USA*. 1996; 93:136–40. [PubMed: 8552591]
2. Bera TK, Pastan I. Mesothelin is not required for normal mouse development or reproduction. *Mol Cell Biol*. 2000; 20:2902–6. [PubMed: 10733593]
3. Rump A, Morikawa Y, Tanaka M, et al. Binding of ovarian cancer antigen CA125/MUC16 to mesothelin mediates cell adhesion. *J Biol Chem*. 2004; 279:9190–8. [PubMed: 14676194]
4. Chang K, Pastan I, Willingham MC. Isolation and characterization of a monoclonal antibody, K1, reactive with ovarian cancers and normal mesothelium. *Int J Cancer*. 1992; 50:373–81. [PubMed: 1735605]
5. Chang K, Pai LH, Pass H, et al. Monoclonal antibody K1 reacts with epithelial mesothelioma but not with lung adenocarcinoma. *Am J Surg Pathol*. 1992; 16:259–68. [PubMed: 1599018]
6. Argani P, Iacobuzio-Donahue C, Ryu B, et al. Mesothelin is overexpressed in the vast majority of ductal adenocarcinomas of the pancreas: identification of a new pancreatic cancer marker by serial analysis of gene expression (SAGE). *Clin Cancer Res*. 2001; 7:3862–8. [PubMed: 11751476]
7. Swierczynski SL, Maitra A, Abraham SC, et al. Analysis of novel tumor markers in pancreatic and biliary carcinomas using tissue microarrays. *Hum Pathol*. 2004; 35:357–66. [PubMed: 15017593]
8. Hassan R, Laszik ZG, Lerner M, et al. Mesothelin is overexpressed in pancreaticobiliary adenocarcinomas but not in normal pancreas and chronic pancreatitis. *Am J Clin Pathol*. 2005; 124:838–45. [PubMed: 16416732]
9. Hassan R, Ho M. Mesothelin targeted cancer immunotherapy. *Eur J Cancer*. 2008; 44:46–53. [PubMed: 17945478]
10. Cheng WF, Huang CY, Chang MC, et al. High mesothelin correlates with chemoresistance and poor survival in epithelial ovarian carcinoma. *Br J Cancer*. 2009; 100:1144–1153. [PubMed: 19293794]

11. Einama T, Kamachi H, Nishihara H, et al. co-expression of mesothelin and CA125 correlates with unfavourable patient outcome in pancreatic ductal adenocarcinoma. *Pancreas*. 2011; 40:1276–82. [PubMed: 21775916]
12. Baba K, Ishigami S, Arigami T, et al. Mesothelin expression correlates with prolonged patient survival in gastric cancer. *J Surg Oncol*. 2012; 105:195–9. [PubMed: 21780126]
13. Yen MJ, Hsu CY, Mao TL, et al. Diffuse mesothelin expression correlates with prolonged patient survival in ovarian serous carcinoma. *Clin Cancer Res*. 2006; 12:827–31. [PubMed: 16467095]
14. Einama T, Homma S, Kamachi H, et al. Luminal membrane expression of mesothelin is a prominent poor prognostic factor for gastric cancer. *Br J Cancer*. 2012; 107:137–42. [PubMed: 22644300]
15. Roe OD, Creaney J, Lundgren S, et al. Mesothelin-related predictive and prognostic factors in malignant mesothelioma: a nested case-control study. *Lung Cancer*. 2008; 61:235–43. [PubMed: 18281122]
16. Wang L, Niu Z, Zhang L, et al. Clinicopathological significance of mesothelin expression in invasive breast cancer. *J Int Med Res*. 2012; 40:909–16. [PubMed: 22906263]
17. Rakha EA, Putti TC, Abd El-Rehim DM, et al. Morphological and immunophenotypic analysis of breast carcinomas with basal and myoepithelial differentiation. *J Pathol*. 2006; 208:495–506. [PubMed: 16429394]
18. Nguyen PL, Taghian AG, Katz MS, et al. Breast cancer subtype approximated by estrogen receptor, progesterone receptor, and HER-2 is associated with local and distant recurrence after breast-conserving therapy. *J Clin Oncol*. 2008; 26:2373–8. [PubMed: 18413639]
19. Rakha EA, Reis-Filho JS, Ellis IO. Basal-like breast cancer: a critical review. *J Clin Oncol*. 2008; 26:2568–81. [PubMed: 18487574]
20. Kennecke H, Yerushalmi R, Woods R, et al. Metastatic behavior of breast cancer subtypes. *J Clin Oncol*. 2010; 28:3271–7. [PubMed: 20498394]
21. Tchou J, Wang LC, Selven B, et al. Mesothelin, a novel immunotherapy target for triple negative breast cancer. *Breast Cancer Res Treat*. 2012; 133:799–804. [PubMed: 22418702]
22. Tabernero J, Rojo F, Calvo E, et al. Dose- and schedule-dependent inhibition of the mammalian target of rapamycin pathway with everolimus: a phase I tumor pharmacodynamic study in patients with advanced solid tumors. *J Clin Oncol*. 2008; 26:1603–10. [PubMed: 18332469]
23. McCarty KS Jr, Miller LS, Cox EB, et al. Estrogen receptor analyses: correlation of biochemical and immunohistochemical methods using monoclonal antireceptor antibodies. *Arch Pathol Lab Med*. 1985; 109:716–721. [PubMed: 3893381]
24. Shousha S, Peston D. Immunohistochemical demonstration of oestrogen and progesterone receptors in paraffin sections of breast carcinoma. *Curr Diag Pathol*. 1997; 4:176–180.
25. Ordóñez NG. Value of mesothelin immunostaining in the diagnosis of mesothelioma. *Mod Pathol*. 2003; 16:192–7. [PubMed: 12640097]
26. Hammond ME, Hayes DF, Dowsett M, et al. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer. *J Clin Oncol*. 2010; 28:2784–95. [PubMed: 20404251]
27. Raghav KP, Hernandez-Aya LF, Lei X, et al. Impact of low estrogen/progesterone receptor expression on survival outcomes in breast cancers previously classified as triple negative breast cancers. *Cancer*. 2012; 118:1498–506. [PubMed: 21837669]
28. Hassan R, Bera T, Pastan I. Mesothelin: a new target for immunotherapy. *Clin Cancer Res*. 2004; 10:3937–42. [PubMed: 15217923]
29. Hassan R, Cohen SJ, Phillips M, et al. Phase I clinical trial of the chimeric anti-mesothelin monoclonal antibody MORAb-009 in patients with mesothelin-expressing cancers. *Clin Cancer Res*. 2010; 16:6132–8. [PubMed: 21037025]
30. Kelly RJ, Sharon E, Pastan I, et al. Mesothelin-targeted agents in clinical trials and in preclinical development. *Mol Cancer Ther*. 2012; 11:517–25. [PubMed: 22351743]
31. Ma J, Tang WK, Esser L, et al. Recognition of mesothelin by the therapeutic antibody MORAb-009: structural and mechanistic insights. *J Biol Chem*. 2012; 287:33123–31. [PubMed: 22787150]

32. Wang K, Bodempudi V, Liu Z, et al. Inhibition of mesothelin as a novel strategy for targeting cancer cells. PLoS One. 2012; 7:e33214. [PubMed: 22485139]

Figure 1. Mesothelin staining: A: Positive mesothelin expression (H-score >10); B: Negative mesothelin expression (H-score 10)

Figure 2. Relapse-free survival (A), and overall survival (B) by mesothelin expression in triple receptor-negative breast cancer

Table 1

Patient and Clinical Characteristics by Mesothelin Expression

All Patients (N = 109)		Mesothelin Negative (H-score = 10) (N = 72)		Mesothelin Positive (H-score > 10) (N = 37)	
	N (%)	N	%	N	P-value
Age, years					
Age ≤ 50	48(44%)	31	43.1	17	45.9
Age > 50	61(56%)	41	56.9	20	54.1
					0.84
Race					
White	71(65.1%)	49	68.1	22	59.5
African-American	21(19.3%)	11	15.3	10	27.0
Hispanic	9(8.3%)	6	8.3	3	8.1
Other	8(7.3%)	6	8.3	2	5.4
					0.54
Tumor Size					
T1	55(53.4%)	37	52.9	18	54.5
T2	44(42.7%)	30	42.9	14	42.4
T3-4	4(3.9%)	3	4.3	1	3.0
					1.0
Lymph Nodes					
N0	69(66.3%)	45	64.3	24	70.6
N1	27(26%)	19	27.1	8	23.5
N2-3	8(7.7%)	6	8.6	2	5.9
					0.84
Pathologic Stage					
1	40(39.2%)	25	36.2	15	45.5
2	59(57.8%)	42	60.9	17	51.5
3	3(2.9%)	2	2.9	1	3.0
					0.67
Histology					
Ductal	91(83.5%)	59	81.9	32	86.5
Other	18(16.5%)	13	18.1	5	13.5
					0.60
Nuclear Grade					
II	9(8.7%)	7	10.1	2	5.7
III	95(91.3%)	62	89.9	33	94.3
					0.71
Lymphovascular Invasion					

	All Patients (N = 109)		Mesothelin Negative (H-score ≤ 10) (N = 72)		Mesothelin Positive (H-score > 10) (N = 37)		P-value
	N (%)	N	%	N	%		
Negative	88(83%)	55	77.5	33	94.3		
Positive	18(17%)	16	22.5	2	5.7	0.03	
Adjuvant chemotherapy							
Anthracyclines	43(41.7%)	25	36.8	18	51.4		
Anthracyclines/Taxanes	49(47.6%)	35	51.5	14	40.0		
Taxanes	5(4.9%)	4	5.9	1	2.9		
Other	6(5.8%)	4	5.9	2	5.7	0.56	

Table 2

Survival Estimates by Patients Characteristics

	N Patients	N Events	5-Year Overall Survival Estimate (95% CI)	P-value	N Events	5-Year Relapse-Free Survival Estimate (95% CI)	P-value
All Patients	109	22	0.87(0.79, 0.92)		20	0.89(0.81, 0.93)	
Mesothelin							
Negative	72	15	0.85(0.74, 0.92)		14	0.87(0.76, 0.93)	
Positive	37	7	0.91(0.75, 0.97)	0.57	6	0.92(0.77, 0.97)	0.43
Age, years							
Age ≤ 50	48	10	0.94(0.81, 0.98)		10	0.91(0.79, 0.97)	
Age > 50	61	12	0.82(0.68, 0.9)	0.10	10	0.87(0.75, 0.93)	0.52
Race							
White	71	12	0.88(0.77, 0.94)		12	0.88(0.78, 0.94)	
African-American	21	6	0.85(0.6, 0.95)		5	0.9(0.67, 0.98)	
Hispanic	9	3	0.89(0.43, 0.98)		2	0.89(0.43, 0.98)	
Other	8	1	0.88(0.39, 0.98)	0.82	1	0.88(0.39, 0.98)	0.98
Tumor Size							
T1	55	6	0.92(0.81, 0.97)		6	0.92(0.81, 0.97)	
T2	44	12	0.84(0.68, 0.93)		9	0.89(0.75, 0.95)	
T3-4	4	3	0.25(0.01, 0.67)	<0.001	3	0.25(0.01, 0.67)	<0.001
Lymph Nodes							
N0	69	9	0.89(0.78, 0.95)		9	0.89(0.79, 0.95)	
N1	27	6	0.92(0.73, 0.98)		6	0.89(0.69, 0.96)	
N2-3	8	6	0.5(0.15, 0.77)	0.002	3	0.75(0.31, 0.93)	0.33
Pathologic Stage							
1	40	3	0.92(0.76, 0.97)		2	0.94(0.78, 0.99)	
2	59	16	0.85(0.73, 0.92)		14	0.86(0.75, 0.93)	
3	3	2	0.33(0.01, 0.77)	0.001	2	0.33(0.01, 0.77)	<0.001
Histology							
Ductal	91	18	0.88(0.79, 0.93)		15	0.9(0.81, 0.95)	
Other	18	4	0.83(0.55, 0.94)	0.99	5	0.82(0.55, 0.94)	0.33
Nuclear Grade							
II	9	3	0.88(0.39, 0.98)		2	1	

	N Patients	N Events	5-Year Overall Survival Estimate (95% CI)	P-value	N Events	5-Year Relapse-Free Survival Estimate (95% CI)	P-value
III	95	18	0.86(0.77, 0.92)	0.58	16	0.87(0.78, 0.92)	0.86
Lymphovascular Invasion							
Negative	88	14	0.9(0.82, 0.95)		14	0.91(0.82, 0.95)	
Positive	18	7	0.67(0.37, 0.85)	0.14	5	0.78(0.51, 0.91)	0.17
Adjuvant chemotherapy							
Anthracycline	43	7	0.93(0.79, 0.98)		8	0.86(0.71, 0.93)	
Anthracycline/Taxane	49	10	0.86(0.72, 0.94)		8	0.92(0.79, 0.97)	
Taxane	5	3	0.57(0.1, 0.87)		2	0.8(0.2, 0.97)	
Other	6	2	0.67(0.19, 0.9)	0.02	1	0.83(0.27, 0.97)	0.54

Table 3

Multivariable Cox Proportional Hazards Model

	Overall Survival			Relapse-Free Survival		
	Hazard Ratio	95% CI	P-value	Hazard Ratio	95% CI	P-value
Mesothelin: positive v. negative	1.02	0.35 - 2.98	0.97	1.00	0.33 - 3.06	1
Grade: 3 v. 2	0.45	0.11 - 1.81	0.26	0.70	0.14 - 3.58	0.66
Pathologic stage: II v. I	4.23	0.53 - 33.92	0.17	6.32	0.8 - 50.11	0.08
Pathologic stage: III v. I	33.72	2.64 - 430.1	0.007	59.08	4.35 - 803.04	0.002
Lymphovascular invasion: yes v. no	1.48	0.46 - 4.73	0.51	1.33	0.41 - 4.32	0.63
Adjuvant Chemo: Anthracyclines/Taxanes v. Anthracyclines	2.61	0.76 - 8.9	0.13	0.89	0.29 - 2.75	0.83