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LEA-135 Expression: Its Association with a Lower Risk of Recurrence and Increased Overall Survival of Patients with Lymph Node-positive Primary Invasive Breast Cancer

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Abstract. *A retrospective study was undertaken to determine and compare the prognostic significance of LEA-135 protein expression by immunohistochemistry with other prognostic pathological parameters, with respect to recurrence and overall survival. This study was conducted in freshly-frozen tissue sections from a cohort of 367 patients having primary invasive breast cancer, with axillary lymph node metastasis. The association of LEA-135 expression was compared with estrogen and progesterone receptor status, segmentectomy or radical mastectomy and hormonal therapy or chemotherapy in terms of recurrence or disease-free survival. Pathologic parameters including tumor size, histological tumor type and histological grade, as well as age of patients at the time of initial diagnosis, and the treatments, together with a median follow-up of 8.8 years were contemplated for the study. Among these parameters, tumor size and histological grade were individually and significantly associated with an increased probability of recurrence (log rank $p<0.001$ in both cases) and short survival (log ranks $p<0.001$ and $p=0.002$, respectively), whereas age was only significantly associated with an increased probability of recurrence (log rank $p=0.002$) by univariate analysis. By multivariate analysis, both tumor size and histological grade remained statistically significant for recurrence (log rank $p<0.001$ and $p=0.013$, respectively) and overall survival (log ranks $p<0.001$ and $p=0.016$, respectively). Among the prognostic biomarkers, both ER and PR expression were associated with a decreased rate of recurrence (log ranks $p<0.001$ and $p=0.008$, respectively) and overall survival (log ranks $p<0.001$ and $p=0.002$, respectively) by univariate analysis. By multivariate analysis, only the ER expression remained significantly associated with a decreased recurrence and increased overall survival (log ranks $p=0.023$ and $p=0.002$, respectively). Patients with high (>50% positive cells) or moderate (5-50% positive cells) number of LEA-135-positive cells had a lower probability (46%) of recurrence at 10 years after surgery compared to 76% in LEA-135-negative patients (log rank $p<0.001$) by univariate analysis. Moreover, the probability of overall survival was higher in patients with high or moderate expression of LEA-135 (46% and 47%, respectively) compared to LEA-135-negative patients (24%) by univariate analysis (log rank $p=0.009$). By multivariate analysis, the association remained statistically significant for recurrence (log rank $p<0.001$) and survival (log rank $p=0.002$). However, there was no significant association between LEA-135 and any of the pathological parameters, age, hormone receptor status, the mode of surgery or the form of therapy (chemo- and/or hormonal) received by this cohort of patients. The results show that an improved prognosis was directly associated with the density of LEA-135-positive cancer cells, while loss of LEA-135 expression was associated with an aggressive phenotype of cancer cells during breast cancer progression. Thus, LEA-135 expression can be implicated as a significant and independent biomarker to identify and distinguish high- from low-risk patients with lymph node-positive invasive breast cancer for an aggressive treatment. Moreover, according to the present results, LEA-135 expression appears to be associated with the tumor cells that have retained certain normal biological characteristics, leading to their lack of aggressiveness and hence a better prognosis.*

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Table I. *Summary of clinicopathological characteristics of patients with invasive breast cancer.*

Factor	No. of Patients	Percent
Total Patients	367	
Age at Diagnosis (Year)		
≤50	89	24%
51-60	90	25%
>61	188	51%
Median (Range)	61 (27-94)	
Tumor Size (mm)		
≤20	138	38%
21- 50	207	56%
>50	22	6%
Median (Range)	25 (6-100)	
Histological Tumor Type		
Infiltrating Ductal Ca	330	90%
Infiltrating Lobular Ca	29	8%
Other	8	2%
Histological Grade		
High/ Moderate	128[1]	35%
Low	239	65%
Treatment		
Chemo + Tamoxifen	61	17%
Chemotherapy Only	114	31%
Tamoxifen Only	168	46%
No Treatment	24	7%
Segmentectomy		
Yes	29	8%
Radical Mastectomy		
Yes	338	92%
Outcomes		
Alive	164	
Recurrence	29	
Dead	203	
Recurrence	155	

[1]Including 4 patients with highly-differentiated tumor

Lymph node status has been shown to be a significant prognostic factor for patients with lymph node-positive breast cancer. However, the biology of tumor cells and their response to therapeutic agents is not revealed by the patient's lymph node status. In this regard several biomarkers such as Her2-neu (1-3), mutated p53 (4,5) cathepsin-D (6) and Ki-67 (7,8) have been evaluated for their ability to identify high-risk patients for recurrence and survival. Among these molecules, only Her2-neu could be successfully used as a prognostic indicator, which, however, has restricted use for being over-expressed in only 25% of the patients (9). With regard to treatment, prescreening of patients based on their steroid hormone receptor (10-13) or Her2/neu status for effective treatment with anti-steroid hormone receptor (tamoxifen) or Herceptin has proved to be beneficial. However, owing to the lack of any other reliable biomarker, patients negative for steroid hormone or Her2/neu cannot be prescreened to determine a specific therapy. In this context, we conducted a retrospective study to evaluate the usefulness of LEA-135, a cell-surface sialoglycoprotein, as a prognostic biomarker to identify high-risk *vs.* low-risk patients with primary breast cancer (14). In the previous studies LEA-135 was shown to be present in the apical plasma membrane of the epithelial cells lining the ducts and lobules of normal breast tissue, while its expression varied greatly in the cancerous counterpart, irrespective of the morphology of the tumor cells (14, 15). Moreover, LEA-135 expression was associated with significant reduction in the rate of recurrence and consequent ascent in the rate of overall survival in lymph node-negative patients with primary breast cancer, independent of size and histological grade of tumor as well as age of the patient (16). Encouraged by the above findings, we extended our study to evaluate the prognostic significance of LEA-135 expression in freshly-frozen tissue biopsy specimens from a larger cohort (n=367) of well-characterized patients with lymph node-positive primary invasive breast cancer. Moreover, the association of LEA-135 expression was compared with other established pathological prognostic parameters, hormone receptor status, mode of surgery and treatments.

Materials and Methods

Patients. Freshly-frozen tissue biopsy sections were obtained from 367 patients undergoing surgery for primary invasive breast cancer. The tissue specimens from these patients were obtained by Dr. G. Carlsson, Sahlgrenska Universitetssjukhuset/Östra, Göteborg, Sweden, according to the policies and procedure of the Faculty Ethical Board. Numbers were assigned to each tissue specimen to ensure that the principal investigator and his staff were blinded to the identity of the patients associated with the tissue specimens. For each patient, pathological prognostic parameters, which included tumor size, histological tumor type, histological grade, as well as age at the initial diagnosis, hormone receptor status, mode of surgery, and treatments (chemotherapy or anti-hormone therapy) were known, together with a median follow-up for 8.8 years (Table I). All patients had axillary lymph node metastasis.

Immunostaining of tissue sections. Prior to immunostaining, representative sections were stained with haematoxylin and eosin to confirm the diagnosis. LEA-135 was localized in these frozen sections with a mouse monoclonal anti-LEA-135 antibody using an indirect immunohistological method described before (14). Briefly, the 5-μm-thick sections were air-dried for 30 min and then fixed in chilled acetone for another 10 min. Firstly the slides were rehydrated with progressively lower dilutions of ethanol, followed by incubation with normal horse serum for 20 min, to avoid non-specific binding by the subsequent antibodies. LEA-135 antibody was applied at a

Table II. *Univariate analysis of recurrence with clinicopathological characteristics of patients with invasive breast cancer.*

Factor	No. of Patients	Percent	Relative Risk[1] (95% CI)	Prob. of not recurring at 10 Years	*P*-value[2]
Overall	367	100%		0.46 ± 0.03	
Age at Diagnosis (Year)					0.002
≥61	188	51%	1.00	0.52 ± 0.05	
51-60	90	25%	1.62 (1.14, 2.30)	0.36 ± 0.06	
≤50	89	24%	1.76 (1.25, 2.49)	0.39 ± 0.05	
Tumor Size (mm)					<0.001
≤20	138	38%	1.00	0.55 ± 0.06	
21- 50	207	56%	1.87 (1.34, 2.60)	0.42 ± 0.04	
>50	22	6%	4.02 (2.31, 7.01)	0.104 ± 0.09	
Histological Tumor Type					0.40
Infiltrating Ductal Ca	330	90%	1.00	0.45 ± 0.03	
Infiltrating Lobular Ca	29	8%	1.09 (0.65, 1.86)	0.46 ± 0.10	
Other	8	2%	0.41 (0.10, 1.65)	0.75 ± 0.15	
Histological Grade					<0.001
High/ Moderate[3]	128	35%	1.00	0.55 ± 0.05	
Low	239	65%	1.71 (1.24, 2.36)	0.41 ± 0.04	
Treatment					0.003
Chemo ± Tamoxifen	61	17%	1.00	0.47 ± 0.07	
Chemotherapy Only	114	31%	1.56 (1.01, 2.39)	0.34 ± 0.05	
Tamoxifen Only	168	46%	0.85 (0.55, 1.31)	0.54 ± 0.05	
No Treatment	24	7%	1.26 (0.64, 2.47)	0.44 ± 0.11	
Segmentectomy					0.48
Yes	29	8%	1.00	0.56 ± 0.10	
No	338	92%	1.24 (0.69, 2.22)	0.45 ± 0.03	
Radical Mastectomy					0.48
No	29	8%	1.00	0.56 ± 0.10	
Yes	338	92%	1.24 (0.69, 2.22)	0.45 ± 0.03	
LEA-135					<0.001
High	222	60%	1.00	0.54 ± 0.04	
Moderate	46	13%	1.21 (0.75, 1.95)	0.54 ± 0.08	
Negative	99	27%	2.19 (1.61, 2.99)	0.24 ± 0.05	
ER					<0.001
Positive	275	78%	1.00	0.48 ± 0.04	
Negative	78	22%	1.80 (1.29, 2.5)	0.37 ± 0.06	
Missing	14				
PR					0.008
Positive	205	58%	1.00	0.52 ± 0.04	
Negative	148	42%	1.49 (1.11, 2.00)	0.39 ± 0.05	
Missing	14				

[1]Relative risk can be thought of as the average increased risk of recurring at any point in time if the relative risk is greater than 1. The group with the ratio equal to 1.00 is the reference group.
[2]Based on log rank test.
[3]Including 4 patients with highly-differentiated tumor.
[4]Probability of survival at 6 years after surgery.

concentration of (0.1mg/ml) for 1 h. Any endogenous peroxidase activity was quenched by 0.3% H_2O_2 for 20 min. A biotinylated horse anti-mouse secondary antibody (1:200) (Vector Laboratories, Burlingame, CA, USA), followed by avidin-biotin-peroxidase conjugate (Vector Laboratories) was applied to the sections. Diaminobenzidine was used as the chromogen and haematoxylin as the counterstain. To ensure specificity of the reaction, negative controls where sections were incubated with the primary antibody, which were pre-absorbed with LEA-135 protein (1mg/ml), were included in each experiment.

Evaluation of the immunostaining. The LEA-135-immunostained sections were examined independently by investigators (C.R.T., W.Y.N., B.C., S.A.I.) blinded to the patients' follow-up data. Based on the percentage of tumor cells positive for LEA-135, patients were divided into three subgroups, *High* (>50% positive cells), *Moderate* (5%-50% positive cells) and *Negative* (less than 5% cells positive). This criterion of selecting the cut-off point of 5% LEA-135-positive cells was found to be optimal for statistical analysis, as described in previous studies (16).

Table III. *Univariate analysis of survival with clinicopathological characteristics of patients with invasive breast cancer.*

Factor	No. of Patients	Percent	Relative Risk[1] (95% CI)	Prob. of not recurring at 10 Years	*P*-value[2]
Overall	367	100%		0.39 ± 0.03	
Age at Diagnosis (Year)					0.23
≤50	89	24%	1.00	0.36 ± 0.04	
51-60	90	25%	1.26 (0.84, 1.90)	0.38 ± 0.06	
≥61	188	51%	1.36 (0.96, 1.94)	0.36 ± 0.04	
Tumor Size (mm)					<0.001
≤20	138	38%	1.00	0.50 ± 0.05	
21- 50	207	56%	1.53 (1.13, 2.07)	0.36 ± 0.04	
≥50	22	6%	3.79 (2.22, 6.48)	0.274 ± 0.10	
Histological Tumor Type					0.88
Infiltrating Ductal Ca	330	90%	1.00	0.39 ± 0.03	
Infiltrating Lobular Ca	29	8%	0.95 (0.56, 1.60)	0.38 ± 0.12	
Other	8	2%	0.79 (0.29, 2.14)	0.63 ± 0.17	
Histological Grade					0.002
High/ Moderate[3]	128	35%	1.00	0.49 ± 0.05	
Low	239	65%	1.59 (1.18, 2.15)	0.34 ± 0.04	
Treatment					0.37
Chemo ± Tamoxifen	61	17%	1.00	0.43 ± 0.08	
Chemotherapy Only	114	31%	1.04 (0.67, 1.61)	0.46 ± 0.05	
Tamoxifen Only	168	46%	1.21 (0.81, 1.83)	0.33 ± 0.05	
No Treatment	24	7%	1.56 (0.86, 2.83)	0.36 ± 0.10	
Segmentectomy					0.39
Yes	29	8%	1.00	0.45 ± 0.13	
No	338	92%	1.28 (0.73, 2.24)	0.39 ± 0.03	
Radical Mastectomy					0.39
No	29	8%	1.00	0.45 ± 0.13	
Yes	338	92%	1.28 (0.73, 2.24)	0.39 ± 0.03	
LEA-135					0.009
High	222	60%	1.00	0.46 ± 0.04	
Moderate	46	13%	1.10 (0.70, 1.73)	0.47 ± 0.08	
Negative	99	27%	1.58 (1.17, 2.14)	0.24 ± 0.05	
ER					<0.001
Positive	275	78%	1.00	0.44 ± 0.04	
Negative	78	22%	1.93 (1.41, 2.65)	0.26 ± 0.06	
Missing	14				
PR					0.002
Positive	205	58%	1.00	0.44 ± 0.04	
Negative	148	42%	1.56 (1.18, 2.07)	0.34 ± 0.05	
Missing	14				

[1]Relative risk can be thought of as the average increased risk of dying at any point in time if the relative risk is greater than 1. The group with the ratio equal to 1.00 is the reference group.
[2]Based on log rank test.
[3]Including 4 patients with highly-differentiated tumor.
[4]Probability of survival at 6 years after surgery.

Statistical analysis. The final outcome of the disease was described in terms of overall survival (OS) and the time of recurrence of breast cancer, if any. The overall survival indicated the time interval between surgery (segmentectomy or radical lumpectomy) and death or the last follow-up, while time to recurrence was calculated as the time interval between surgery and first recurrence of breast cancer. All deaths, regardless of cause, were counted. Patients who died prior to their first recurrence were censored at the time of their death, while patients who never recurred were censored at the time of last follow-up, when survival analysis for recurrence was used alone.

For all patients, the data about the adjuvant therapy received (tamoxifen alone, chemotherapy alone, combination of the two or none at all) was available for statistical analysis.

The relationship between LEA-135 expression and other clinicopathological features (tumor size, histological diagnosis, histological differentiation, age at diagnosis, hormone receptor status, mode of surgery and treatment) was assessed by Fisher's exact test. The probability of recurrence or overall survival was calculated using the Kaplan-Meier's plot and the difference between the Kaplan-Meier's curves was tested by log rank test.

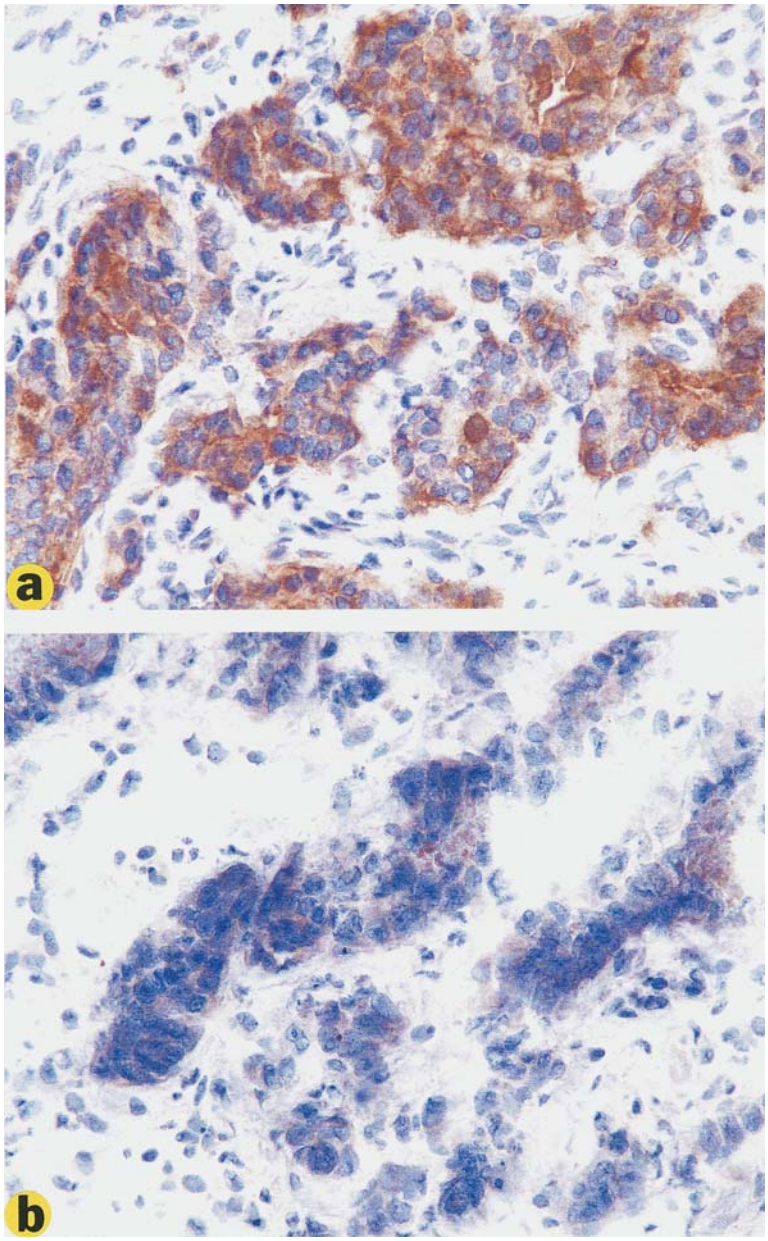

Figure 1. *Immunohistochemical localization of LEA-135 expression in patients with lymph node-positive primary invasive breast cancer. Frozen tissue sections were stained with anti-LEA-135 MAb. Representative case from patients who did not experience recurrence exhibited a high level of LEA-135 expression (a), whereas that from patients with recurrence showed undetectable LEA-135 expression (b). The connective tissue cells were consistently negative. The tissue sections were counterstained with Meyer's hematoxylin. Original magnification X 400.*

Standard errors were based on Greenwood's formula (17). The relative risk indicated the risk associated with recurrence or death in a greater risk category compared to that in a lower risk category. In univariate analyses, LEA-135, along with the clinicopathological parameters mentioned above, was examined for possible association with recurrence or overall survival.

The stratified log rank test and the Cox-proportional hazards model (17) were used for multivariate analysis to determine whether LEA-135 could act as an independent prognostic marker.

Results

Association of survival and recurrence with age, clinicopathological parameters, hormone receptor status, surgery and treatment. Among the clinicopathological parameters, tumor size and histological grade were found to be significantly associated with an increased probability of recurrence (log rank $p<0.001$ in both cases) and short survival (log ranks

Table IV. *Multivariable analysis of recurrence of patients with breast cancer.*

Characteristics	Relative Risk[1] (95% CI)	*P*-value[2]
Age at Diagnosis (Year)		0.11
≥61	1.00	
51-60	1.48 (0.98, 2.23)	
≤50	1.55 (0.95, 2.51)	
Tumor Size (mm)		<0.001
≤20	1.00	
21-50	1.75 (1.24, 2.49)	
>50	4.37 (2.40, 7.96)	
Histological Grade		0.013
High/Moderate	1.00	
Low	1.54 (1.09, 2.17)	
Treatment		0.51
Chemo + Tamoxifen	1.00	
Chemotherapy Only	1.13 (0.67, 1.91)	
Tamoxifen Only	0.98 (0.60, 1.59)	
No Treatment	1.62 (0.78, 3.36)	
LEA-135		<0.001
High	1.00	
Moderate	1.17 (0.71, 1.93)	
Negative	2.31 (1.65, 3.24)	
ER		0.023
Positive	1.00	
Negative	1.62 (1.08, 2.44)	
PR		0.49
Positive	1.00	
Negative	1.13 (0.80, 1.62)	

[1]Relative risk equal to 1 is as the reference group
[2]The final model includes the variables of tumor size, histological differentiation, LEA-135 and ER status, which were significant at the 0.05 level in the univariate analysis. *P*-value was based on likelihood ratio Chi-square test.

Table V. *Multivariable analysis of survival of patients with breast cancer.*

Characteristics	Relative Risk[1] (95% CI)	*P*-value[2]
Tumor Size (mm)		<0.001
< 20	1.00	
21-50	1.41 (1.03, 1.94)	
>50	3.62 (2.06, 6.34)	
Histological Grade		0.016
High/Moderate	1.00	
Low	1.46 (1.07, 2.00)	
LEA-135		0.002
High	1.00	
Moderate	1.07 (0.67, 1.72)	
Negative	1.76 (1.29, 2.42)	
ER		0.002
Positive	1.00	
Negative	1.80 (1.24, 2.61)	
PR		0.26
Positive	1.00	
Negative	1.21 (0.87, 1.68)	

[1]Relative risk equal to 1 is as the reference group
[2]The final model includes the variables of tumor size, histological differentiation, LEA-135 and ER status, which were significant at the 0.05 level in the univariate analysis. *P*-value was based on likelihood Ratio Chi-square test.

Median follow-up 8 years with a range of (0.2-12) years.

$p<0.001$ and $p=0.002$, respectively), whereas age was only significantly associated with an increased probability of recurrence (log rank $p=0.002$) by univariate analysis (Tables II and III). A positive status for estrogen and progesterone receptors indicated a significantly reduced probability of recurrence (log ranks $p<0.001$ and $p=0.008$, respectively) and an increased survival (log ranks $p<0.001$ and $p=0.002$, respectively) by univariate analysis (Tables II and III). By multivariate analysis, both tumor size and histological grade but not age remained significant for recurrence (log rank $p<0.001$ for tumor size and $p=0.013$ for histological grade) and overall survival (log ranks $p<0.001$ for tumor size and $p=0.016$ for histological grade) (Tables IV and V). In addition, only estrogen receptor status reached statistically significant values for both recurrence and survival ($p=0.023$ and $p=0.002$, respectively) by multivariate analysis, whereas the progesterone receptor status failed to do so (Tables IV and V).

Consideration of the outcome of surgical treatment in the patients showed that there was no significant association between either segmentectomy or radical mastectomy and recurrence or overall survival by univariate analysis (Tables II and III). With regard to the treatments, patients with tamoxifen therapy exhibited a significantly decreased probability of recurrence (log rank $p=0.003$), but showed no association with overall survival by univariate analysis (Tables II and III). However, the association of treatment with disease recurrence did not reach a statistical significance (log rank $p=0.51$) by multivariate analysis (Table IV).

Immunohistological localisation of LEA-135. Immunohistochemical localisation of LEA-135 protein expression was found mainly in the apical plasma membrane of the luminal epithelial cells lining the ducts and lobules of the normal breast tissue, in accordance with previous studies (18,19). A strong expression of LEA-135 was also found in

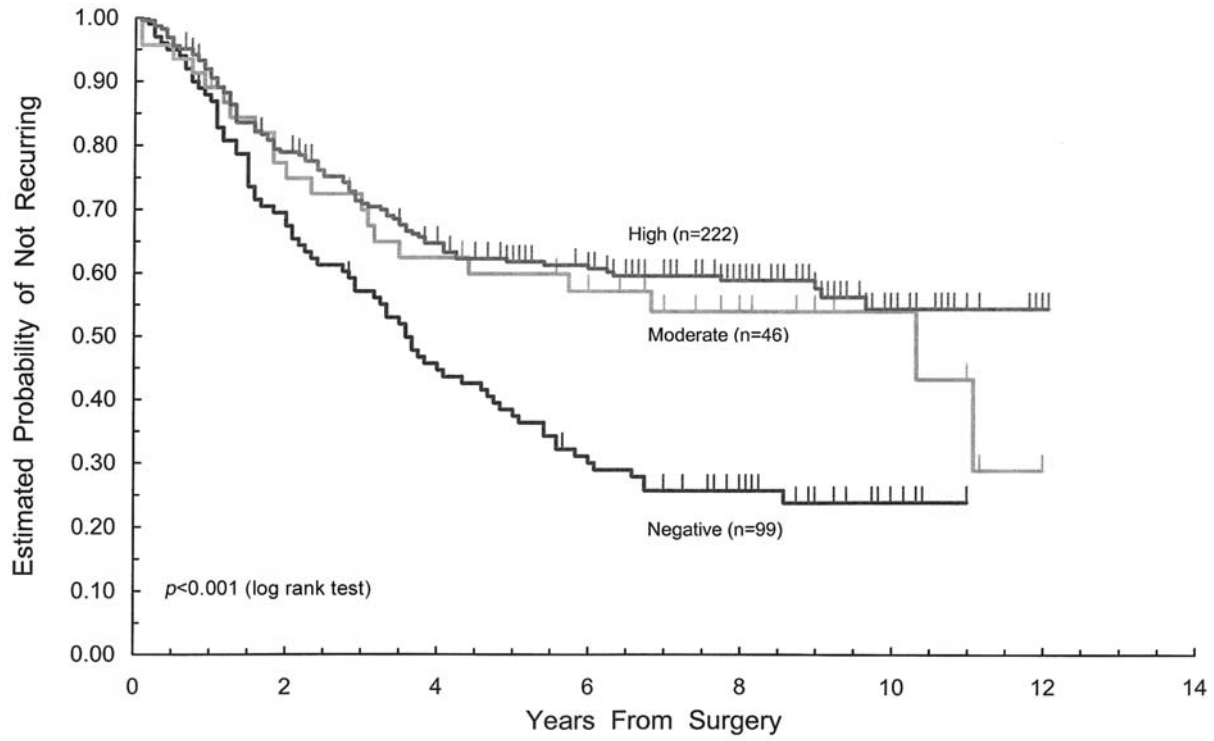

Figure 2. *Kaplan-Meier plot of the probability of recurrence of patients (n=367) with high, moderate or negative LEA-135 expression. Patients with LEA-135 (high or moderate)-positive cancer cells experienced a lower risk of recurrence as compared to those with LEA-135-negative cancer cells (log rank p<0.001). Time was measured from the surgery to the last follow-up in years.*

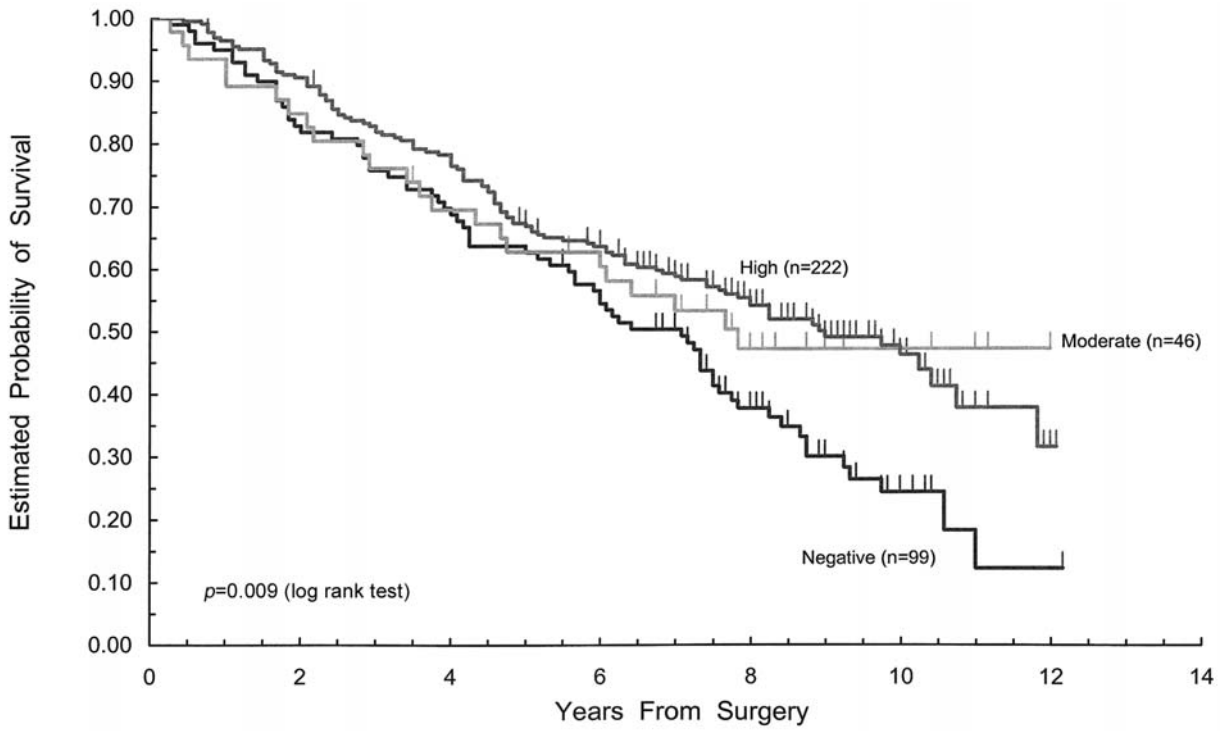

Figure 3. *Kaplan-Meier plot of the probability of overall survival of patients (n=367) with high, moderate or negative LEA-135 expression. Patients with LEA-135 (high or moderate)-positive cancer cells experienced a longer overall survival as compared to those with LEA-135-negative cancer cells (log rank p=0.009). Time was measured from the surgery to the last follow-up in years.*

Table VI. *Relationship of LEA-135 activity with breast cancer patients.*

Factor	LEA-135 Activity Negative (N=99) n	(%)	Moderate(N=46) n	(%)	High (N=222) n	(%)	*p*-value[1]
Age at Diagnosis (Year)							0.30
≤50	25	(25)	13	(28)	51	(23)	
51-60	31	(31)	10	(22)	49	(22)	
≥61	43	(43)	23	(50)	122	(55)	
Tumor Size (mm)							0.74
≤20	34	(34)	17	(37)	87	(39)	
21-50	61	(62)	26	(57)	120	(54)	
>50	4	(4)	3	(7)	15	(7)	
Histological Tumor Type							0.31
Infiltrating Ductal Ca	88	(89)	39	(85)	203	(91)	
Infiltrating Lobular Ca	7	(7)	6	(13)	16	(7)	
Other	4	(4)	1	(2)	3	(1)	
Histological Grade							0.99
High/Moderate	35	(35)	162	(35)	772	(35)	
Low	64	(65)	30	(65)	145	(65)	
Treatment							0.55
Chemo + Tamoxifen	18	(18)	7	(15)	36	(16)	
Chemotherapy Only	36	(36)	17	(37)	61	(27)	
Tamoxifen Only	41	(41)	19	(41)	108	(49)	
No Treatment	4	(4)	3	(7)	17	(8)	
Segmentectomy							0.27
No	90	(91)	40	(87)	208	(94)	
Yes	9	(9)	6	(13)	14	(6)	
Radical Mastectomy							0.27
No	9	(9)	6	(13)	14	(6)	
Yes	90	(91)	40	(87)	208	(94)	
ER							0.10
Positive	81	(85)	35	(76)	159	(74)	
Negative	14	(15)	9	(20)	55	(26)	
Missing	4		2		8		
PR							0.35
Positive	54	(57)	30	(68)	121	(57)	
Negative	41	(43)	14	(32)	93	(43)	
Missing	4		2		8		

[1]based on Fisher's exact test
[2]total 4 patients with highly-differentiated tumor

the normal and/or hyperplastic epithelial cells of the investigated cases, thus providing the internal control. Among the 367 cases studied, 222(60 %) had a high expression of LEA-135 in their cancer cells, while 46 (13%) had moderate and 99(27%) were negative for the same. Representative cases of invasive breast ductal carcinoma cells expressing a high level of LEA-135 and negative for LEA-135 are shown in Figure 1a and b.

Association of survival and recurrence with LEA-135 expression. By univariate analysis, patients with high (>50% positive cells) or moderate (5-50% positive cells) number of LEA-135-positive cells showed a statistically significant probability of not recurring (0.54±0.04 for high and 0.54±0.08 for moderate) at 10 years after surgery and survival (0.46±0.04 for high and 0.47±0.08 for moderate) compared to LEA-135-negative patients, who showed

probabilities of 0.24±0.05 and 0.24±0.05, at 10 years after surgery for recurrence and survival, respectively (log rank $p<0.001$ for recurrence and $p=0.009$ for overall survival) (Figures 2 and 3, Tables II and III). Furthermore, the association of LEA-135 expression remained significant by multivariate analysis for recurrence (log rank $p<0.001$) and survival (log rank $p=0.002$) (Tables IV and V).

Association of LEA-135 with age, clinicopathological characteristics and treatment. There was no statistically significant association between LEA-135 expression and age, hormone receptor status, mode of surgery as well as the clinicopathological parameters included in this study (Table VI). Moreover, in this cohort of patients, LEA-135 expression was not associated with any of the three types of therapies to which they were subjected, in terms of recurrence or overall survival (Table VI).

Discussion

Prognostic biomarkers, which can consistently identify patients with low- *vs.* high-risk of recurrence or lowered overall survival and a favorable response to a chemotherapeutic agent, can be of immense use for effective management of patients with primary invasive breast cancer. Moreover, the responsiveness of patients to a particular type of adjuvant therapy cannot be assured beforehand and thus patients are often subjected unnecessarily to toxicity, discomfort, expense and other potential long-term consequences of therapy.

The lymph node status, histological grade and tumor size are considered to be the major prognostic markers for patients with primary breast cancer. Although histological grade is known to work fairly well, determination of it remains highly subjective and varies among different pathologists (20-22). Tumor size at diagnosis can predict the recurrence rate in the first ten years but, after that, the frequency of recurrence seems to be unrelated to tumor size (23). The lymph node status of patients, though shown to be a significant predictor of outcome of the disease (24, 25), neither reveals the biological characteristics of tumor cells nor predicts their response to a therapeutic agent. Therefore, confinements of the present approach leave scope for improvising new risk- indicative biomarkers that are consistently expressed in breast tissue, can be reproducibly evaluated by pathologists and relate to the pathological behavior and therapeutic responsiveness of the tumor cells.

This retrospective study was undertaken to assess the efficacy of LEA-135, a newly identified cell surface glycoprotein, as a favorable prognostic marker for patients with lymph node-positive primary invasive breast cancer. The study was also conducted to confirm and extend the findings of our previous studies, which indicated LEA-135 to be a favorable prognostic marker for patients with primary invasive breast cancer with or without metastasis to axillary lymph node and/or bone marrow (14, 16, 18, 19). Moreover, instead of formalin-fixed paraffin-embedded tissues, the use of freshly-frozen sections obtained from a larger cohort of well- characterized patients was anticipated to contribute to the added accuracy of the results of this study.

In this study, LEA-135 emerged as a favorable prognostic marker for patients with lymph node-positive primary breast cancer. A high to moderate level of LEA-135 protein expression in the cancer cells predicted a decreased probability of recurrence and increased overall survival. The results suggest that LEA-135-protein may identify a subgroup of patients whose tumor cells have retained some of the normal biological characteristics and a less aggressive behavior, which result in better prognosis. However, there was no significant association between LEA-135 expression and any of the pathological parameters, age, hormone receptor status or the mode of surgery, thus suggesting LEA-135 as an independent favorable prognostic marker in this cohort of patients.

Next, statistical analyses were performed to determine whether LEA-135 expression was associated with any of the three forms of therapy (hormonal, chemotherapy or a combination of the two) received by this cohort of patients. There was no statistically significant association between LEA-135 expression and the various forms of therapies. One of the attributing factors for the lack of association may have been the lack of an appropriate control population. To be precise, patients who underwent hormonal/chemotherapy had more aggressive features (larger tumor size and poorer differentiation) than those who were not administered any such therapy. Therefore, an adequately randomized clinical trial in future may substantiate any association of LEA-135 expression with responsiveness towards a particular type of treatment.

The advantage of LEA-135 lies in the fact that it is consistently expressed in cancer cells of patients through all stages of breast cancer progression, irrespective of their other clinicopathological features. Moreover, determination of LEA-135 expression in both paraffin and frozen tissue sections by immunohistochemistry, which is an easy and inexpensive technique with a rapid turn-around time, may add to its potential for use as a diagnostic tool in adjunct to the currently used parameters. In this context, with further advancement of research ideas to compartmentalize patients according to their extent of risk, the status of LEA-135 expression may be worthy of consideration.

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