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Hormonal Therapy Confers Clinically Relevant Benefit in Breast Cancers with Low Estrogen Receptor Expression

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Abstract

Background: Breast tumors with low Estrogen Receptor (ER) expression cluster more closely with ER-negative tumors on a molecular level and may not derive the same benefit from hormonal therapy as ER-positive tumors. In this study, we examined the effect of hormonal therapy on survival outcomes in low ER-positive tumors.

Methods: Retrospective review was done of 2872 women diagnosed with breast cancer at Tan Tock Seng Hospital from 2001 to 2012. Low ER-positive tumors were defined as tumors where 1% to 9% of cells stained positive for ER.

Results: Low ER-positive tumors were found in 171 women, 70% of whom received hormonal therapy. Compared to tumors demonstrating at least 10% ER expression, low ER-positive tumors were more common in younger (P<0.001), non-Chinese (P=0.018) women and were more likely to be high grade (P=0.003 and P<0.001 for DCIS and invasive cancers respectively), Progesterone Receptor (PR)-negative (P<0.001) and human epidermal growth factor receptor (HER)-2 over-expressing (P<0.001). Distant disease-free survival among low ER-positive tumors was significantly worse compared to tumors expressing at least 10% ER (P=0.005), but closely overlapped that of ER-negative tumors (P=0.574). Nevertheless, hormonal therapy was still found to reduce disease recurrence, both locoregional and distant, in women with low ER-positive tumors (P=0.042 and P<0.001 respectively) and improved 10-year distant disease-free and overall survival outcomes (P<0.001 and P=0.004 respectively). An advanced stage at presentation (P=0.002,), PR-negativity (P=0.042) and the omission of hormonal therapy (P<0.001) increased the risk of distant recurrence in women with low ER-positive tumors.

Conclusion: Hormonal therapy conferred clinical benefit in low ER-positive tumors and should be considered especially in those with advanced or PR-negative tumors who are at high risk of distant recurrence.

Keywords: Low ER expression; Hormonal therapy; Survival

Introduction

The recommendation for hormonal therapy in breast cancer is primarily based on tumor Estrogen Receptor (ER) status. Hormonal therapy is known to reduce the risk of disease recurrence and contralateral cancer and improves overall survival and is therefore the standard of care for ER-positive disease [1,2]. Tumor ER status is determined on Immunohistochemistry (IHC) and is interpreted according to the intensity and proportion of tumor cells staining positive on ER antibody evaluation, these are then combined to define the threshold for a positive result [3]. A high level of reliability and reproducibility is therefore essential and laboratory assays must demonstrate at least 90% concordance for ER/Progesterone Receptor (PR) positivity and 95% concordance for ER/PR negativity with known clinically validated assay models [4]. Traditionally, many institutes, including ours, considered tumors where 10% or more tumor cells stained positive for ER with at least moderate intensity as being ER-positive. This threshold was based on earlier ligand-binding assays where a cytosol protein level of 10 fmol/mg was considered positive [5]. Estrogen receptor testing and reporting thresholds of ER varied from institute to institute and even within Singapore, not all institutes adopted the same thresholds for a positive ER result [6,7]. The American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) recommendations published in 2010 sought to standardize these practices [4]. Based on the strength of existing data, ASCO/CAP recommended that tumors be deemed ER-positive as long as at least 1% of tumor cells

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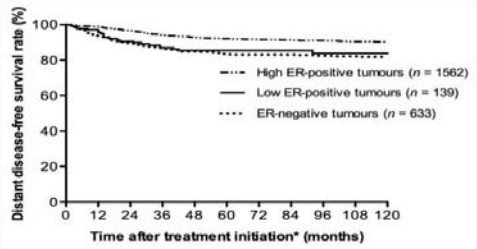
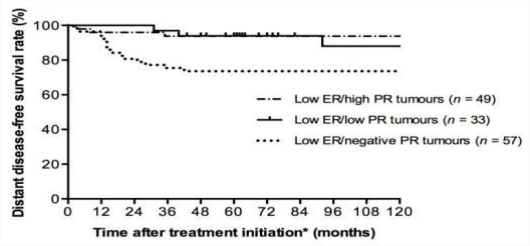


Comparison	Hazard Ratio	95% CI	P value
High ER-positive vs. Low ER-positive	0.485	0.273 - 0.862	0.005
High ER-positive vs. ER-negative	0.459	0.350 - 0.603	<0.001
Low ER-negative vs. ER-negative	1.137	0.562 - 1.376	0.574

Time (months)	0	12	24	36	48	60	72	84	96	108	120
Numbers at risk High ER-positive	1519	1503	1470	1430	1336	1201	1043	963	889	706	611
Low ER-positive	139	134	126	121	117	117	117	114	55	55	55
ER-negative	633	592	566	549	499	435	422	422	290	270	237

Figure 1: Kaplan Meier curves for 10-year distant disease-free survival stratified by the Estrogen Receptor (ER) expression levels among patients with non-metastatic invasive cancers. *Treatment initiation refers to definitive breast cancer surgery or the initiation of neoadjuvant chemotherapy.



Comparison	Hazard Ratio	95% CI	P value
Low ER/high PR vs. Low ER/low PR	1.399	0.141 - 3.624	0.685
Low ER/high PR vs. Low ER/negative PR	0.279	0.110 - 0.705	0.007
Low ER/low PR vs. Low ER/negative PR	0.373	0.145 - 0.961	0.041

Time (months)	0	12	24	36	48	60	72	84	96	108	120
Numbers at risk Low ER/high PR	49	48	47	46	43	37	27	22	22	19	17
Low ER/low PR	33	33	33	33	30	27	20	17	16	16	16
Low ER/negative PR	57	55	46	44	39	35	28	23	20	20	14

Figure 2: Kaplan Meier curves for 10-year distant disease-free survival stratified by tumor Estrogen Receptor (ER) and Progesterone Receptor (PR) status in patients with non-metastatic low ER-positive invasive cancers (*n* = 139). *Treatment initiation refers to definitive breast cancer surgery or the initiation of neoadjuvant chemotherapy.

stained positive for ER, regardless of the staining intensity [4,8]. This meant that tumors staining positive in only 1% to 9% of cells were now also considered ER-positive. Since our unit had previously adopted 10% as the positive threshold, alignment with these new recommendations resulted in more tumors being classified as ER-positive and consequently, more women being eligible for hormonal therapy.

However, some have questioned the benefit of hormonal therapy in low ER-positive tumors, tumors where less than 10% of cells stain ER-positive as there is data suggesting that such tumors are in fact more similar to ER-negative tumors [9,10]. Given that cells not expressing ER do not respond to hormonal therapy, low ER-positive tumors are not expected to be as hormone sensitive as tumors expressing higher levels of ER. The question then arises as to whether this smaller extent of benefit outweighs potential treatment related toxicity, this being especially significant since hormonal therapy is given for extended periods [11]. Large studies to define the benefit of hormonal therapy in low ER-positive tumors are difficult to conduct since low ER-positive tumors account for less than 10% of all breast cancers and the decision for hormonal therapy is often left to the discretion of the oncologist. In this present study, we examined the prevalence of low ER-positive tumors, the factors associated with low ER expression and also factors that likely influenced the recommendation for hormonal therapy. We also evaluated the effect of hormonal therapy on clinical outcomes, such as disease recurrence, overall survival and contralateral breast cancer, and determined whether clinicopathological indicators could identify low ER-positive tumors with a high recurrence risk that could be expected to derive a greater benefit from hormonal therapy.

Materials and Methods

Women diagnosed and treated for breast cancer, both Ductal Carcinoma *in Situ* (DCIS) and invasive carcinoma, at Tan Tock Seng Hospital between January 1, 2001 and December 31, 2012 were identified from our records. This study has Ethics Committee

Table 1: Univariate analyses of tumor Estrogen Receptor (ER) expression with standard clinicopathological parameters (n=2104). Women with unknown outcome at 10 years excluded.

Parameter	Low ER-positive tumors (n=171)	High ER-positive tumors (n=1933)	P value
Median Age (years, range)	50 (27-87)	56 (23- 98)	<0.001
Menstrual status Pre-menopausal Post-menopausal	 93(54.4) 75 (43.9)	 686(35.5) 1237(64.0)	<0.001
Ethnicity Chinese Malay Indian Others	 124(72.5) 23 (13.5) 15 (8.8) 9 (5.3)	 1583(81.9) 176 (9.1) 93 (4.8) 81 (4.2)	0.018
Disease stage at presentation DCIS I II III IV	 17 (9.9) 54 (31.6) 53 (31.0) 32 (18.7) 15 (8.8)	 225 (11.6) 565 (29.2) 603 (31.2) 289 (15.0) 151 (7.8)	0.372
DCIS tumor grade Low Intermediate High	 0 (0.0) 4 (2.3) 12 (7.0)	 42 (2.2) 94 (4.9) 85 (4.4)	0.003
DCIS tumor size (mm, range)	18.5 (3.0 - 40.0)	14.0 (1.0 - 105.0)	0.49
Invasive grade 1 2 3	 16 (9.4) 65 (38.0) 61 (35.7)	 339 (17.5) 766 (39.6) 402 (20.8)	<0.001
Invasive tumor size (mm, range)	20.0 (0.5 - 170.0)	21 (1.0 - 150.0)	0.937
Nodal involvement Positive Negative	 71 (41.5) 100 (58.5)	 763 (39.5) 1170 (60.5)	0.6
PR status Positive Negative	 83 (48.5) 88 (51.5)	 1437 (74.3) 485 (25.1)	<0.001
HER2 status Positive Negative	 44 (25.7) 77 (45.0)	 223 (11.5) 1057(54.7)	<0.001
Locoregional recurrence* Yes No	 11 (7.9) 128(92.1)	 79 (5.1) 1483(94.9)	0.15
Distant recurrence* Yes No	 21 (15.1) 118 (84.9)	 146 (9.3) 1416 (90.7)	0.029
Contralateral breast cancer* Yes No	 4 (2.9) 135 (97.1)	 47 (3.0) 1515 (97.0)	0.931
Death* Yes No	 17 (12.2) 122(87.8)	 182 (11.7) 1380(88.3)	0.839

Low ER-positive tumors: tumors with 1% to 9% cells staining positive for ER. High ER-positive tumors: tumors with 10% or more cells staining positive for ER. DCIS: Ductal Carcinoma *in situ*; PR: Progesterone Receptor; HER2: Human Epidermal Growth Factor Receptor 2. HER2 status was routinely determined only from 2006 onwards.

*Outcomes evaluated in 1701 non-metastatic invasive cancers (low ER-positive: 139 high ER-positive1562).

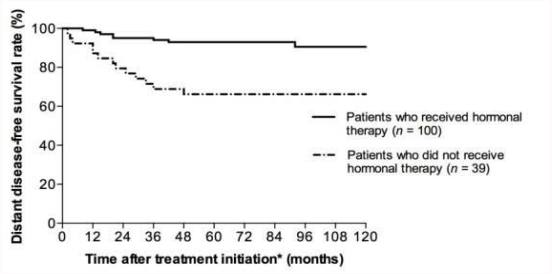
approval (DSRB2013/00597). Male patients, those with unknown tumor ER status or where the intensity and proportion of ER staining were not specifically reported and those who did not continue treatment at our unit after initial diagnosis were excluded. Data was collected from patient clinical records and included demographic data, standard clinicopathological parameters, treatment details and clinical outcomes. Tumors were classified as ER-positive or ER-negative according to the thresholds in use at the time of diagnosis.

In accordance with existing guidelines, women with ER-positive tumors were recommended hormonal therapy. Prior to 2006, all women were offered tamoxifen, administered as a daily dose of 20 mg. Aromatase inhibitors were increasingly recommended to post-menopausal women from 2006 onwards. Anastrozole (at a daily dose of 1 mg) was the most frequently used AI in the earlier years while letrozole (at a daily dose of 2.5 mg) became the preferred agent in later years. Exemestane was not used as first-line, with women being switched to it either because of intolerable side effects or disease recurrence or progression on the other agents. Hormonal therapy was recommended for duration of 5 years. Pre-menopausal women were generally offered tamoxifen; AI with concurrent Gonadotrophin-Releasing Hormone (GnRH) agonists was not used as first-line at our unit. In post-menopausal women, the decision to use tamoxifen or AI was made after discussion with the patient. Almost all women with tumors with at least 10% ER expression were offered hormonal therapy, unless there were specific contraindications, but the practice was more variable in those with low ER-positive tumors. Even after the ASCO/CAP guidelines were published, not all clinicians recommended hormonal therapy to women with low ER-positive tumors. Apart from hormonal therapy, the recommendations for surgery and other adjuvant treatments, including chemotherapy, radiation therapy and targeted therapy (after 2006 when HER2 testing became routine) were in accordance with existing guidelines. The decision between mastectomy and breast conservation was made following a discussion with the patient and was based on clinical eligibility and patient preference. Immediate breast reconstruction was offered to women treated with mastectomy who were deemed suitable. Chemotherapy was recommended to patients with node-

Table 2: Univariate analyses of standard clinicopathological parameters with the recommendation for hormonal therapy in 171 women with low Estrogen Receptor (ER)-positive tumors.

Parameter	Women recommended hormonal therapy (n=122)	Women not recommend hormonal therapy (n=49)	P value
Median age (years, range)	50 (30-79)	51 (27-87)	0.421
Tumor histology DCIS Invasive carcinoma	 7 (5.7) 115 (94.3)	 10 (20.4) 39 (79.6)	0.004
Invasive tumor grade 1 2 3	 11 (9.0) 57 (46.7) 38 (31.1)	 5 (10.2) 7 (14.3) 23 (46.9)	0.046
Invasive tumor size (mm, range)	21.0 (3.5-83)	21.0 (0.5-170)	0.585
Nodal involvement Positive Negative	 51 (41.8) 71 (58.2)	 20 (40.8) 29 (59.2)	0.906
PR status Positive Negative	 80 (65.6) 42 (34.4)	 3 (6.1) 46 (93.9)	<0.001
HER2 status Positive Negative	 35 (28.7) 58 (47.5)	 9 (18.4) 19 (38.8)	0.596
Surgery Yes No	 120 (98.4) 2 (1.6)	 44 (89.8) 5 (10.2)	0.021
Radiotherapy Yes No	 70 (57.4) 41 (33.6)	 28 (57.1) 20 (40.8)	0.573
Chemotherapy Yes No	 68 (55.7) 43 (35.2)	 25 (51.0) 24 (49.0)	0.226
Targeted therapy Yes No	 18 (14.8) 65 (53.3)	 7 (14.3) 30 (61.2)	0.73

DCIS: Ductal Carcinoma *in situ;* PR: Progesterone Receptor; HER2: Human Epidermal Growth Factor Receptor 2. HER2 status was routinely determined only from 2006 onwards.



Comparison	Hazard Ratio	95% CI	P value
Hormonal therapy versus no hormonal therapy	0.143	0.053 - 0.384	<0.001

Time (months)	0	12	24	36	48	60	72	84	96	108	120
Numbers at risk Hormonal therapy	100	100	970	95	78	59	46	40	39	36	32
No hormonal therapy	39	36	30	27	26	26	23	18	16	16	36

Figure 3: Kaplan Meier curves depicting 10-year distant disease-free survival stratified by the of hormonal therapy among patients with non-metastatic low Estrogen Receptor (ER)-positive invasive cancers (n=139). *Treatment initiation refers to definitive breast cancer surgery or the initiation of neoadjuvant chemotherapy.

Table 3: Cox proportional-hazards model stratified by distant recurrence-free survival in women with non-metastatic low Estrogen Receptor (ER)-positive tumors.

Parameter	Hazards Ratio	Standard Error	P value	95% CI
Age at diagnosis	1.034	0.021	0.1	0.994 – 1.077
Tumor PR status	0.353	0.181	0.042	0.129 – 0.963
Disease stage	13.587	11.2	0.002	2.703 – 68.313
Hormonal therapy	0.178	0.858	<0.001	0.069 – 0.458
Chemotherapy	0.538	0.377	0.376	0.137 – 2.121
Radiation therapy	0.492	0.292	0.232	0.154 – 1.576
Surgery*	0.697	0.397	0.526	0.228 – 2.127

PR: Progesterone Receptor. *mastectomy *versus* wide local excision

positive disease, as well as those with high-risk node-negative disease; co-existing morbidities and performance status were taken into consideration. Prior to 2006, Cyclophosphamide/Methotrexate/Fluorouracil (CMF) was the most commonly used regimen, though some patients received an anthracycline-based regimen (fluorouracil/doxorubicin/cyclophosphamide FAC or Fluorouracil/Epirubicin/Cyclophosphamide (FEC). From 2006 onwards, doxorubicin/cyclophosphamide followed by paclitaxel (AC/T) was the preferred regimen; the non-anthracycline based regimen of docetaxel/cyclophosphamide (TC) was used in some node-negative patients and those at increased risk of anthracycline-related cardiotoxicity. Trastuzumab was recommended to patients with HER2-over-expressing tumors from 2006 onwards. Whole breast radiation (total dose of 50 Gy in 25 fractions) with a 10 Gy boost to the tumor bed was routinely recommended after wide local excision, as part of breast conserving therapy. Some node-negative patients received a hyperfractionated regimen, 42.5 Gy in 16 fractions. Post-mastectomy radiation was recommended to patients with tumors larger than 5 cm and to pre-menopausal women with node-positive disease and to post-menopausal women with N2 disease (4 or more involved nodes). Post-mastectomy radiation was discussed on a case-by-case basis with post-menopausal women with N1 disease (1 to 3 involved nodes). The ipsilateral axillary nodal basin and the supraclavicular fossa were included in node-positive disease, while the internal mammary nodal basin was included only in medially located tumors and when there was evidence of internal mammary nodal involvement.

Tumor ER and PR status were assessed with Immunohistochemistry (IHC) using validated protocols at our laboratory. Tissue handling, fixation, validation with extrinsic and intrinsic controls, IHC interpretation and reporting were in accordance with existing standards and the laboratory was subjected to regulatory CAP accreditation audits. Formalin-fixed paraffin-embedded tumor sections were stained with anti-ER antibody (Neomarker MS750 Neomarkers Inc., Point Technologies, and Santa Clara, United States) and anti-PR antibody (Dako M3569, Dako, and Santa Clara, United States). Envision ChemMate (EnVision, Santa Clara, United States) was used as the detection system for ER and the Ventana detection kit (Ventana Medical Systems Inc., Tucson, United States) for PR. Up to 2010, prior to the adoption of the ASCO/CAP recommendations published in July 2010, tumors were considered ER and PR positive when 10% or more tumor cells stained positive with at least moderate intensity. After July 2010, antibodies and detection kits were switched to Novocastra NCL-ER-6F11 for ER (Novocastra Laboratories, Newcastle upon Tyne, United Kingdom), Ventana 1E2 for PR (Ventana Medical Systems Inc., Tucson, United States) and the Ventana Optiview DAB IHC detection kit. Tumors were considered ER and PR positive if at least 1% of tumor cells stained positive, regardless of intensity [12].

Routine HER2 testing began in 2006 at our unit. Immunohistochemistry was the first-line test. Sections were stained with anti-HER2 antibody (Neomarker MS-441Neomarkers Inc.) and tumors were considered positive if at least 10% of tumor cells exhibited intense membranous staining and equivocal if at least 10% of tumor cells exhibited moderate membranous staining. In 2007, the anti-HER2 antibody and thresholds were changed. Anti-HER2 antibody (Neomarker RM-9103 Neomarkers Inc.) was used instead and tumors were considered positive if more than 30% of tumor cells exhibited uniform intense membrane staining; equivocal when at least 10% of tumor cells exhibited complete circumferential membrane staining that was non-uniform or weak in intensity, or if less than 30% of tumor cells exhibited intense complete membrane staining. Tumors not fulfilling these criteria were considered HER2-negative. Tumors with an equivocal IHC result were then sent to another institute for fluorescent *in situ* Hybridization (FISH) where HER2/neu gene amplification was detected using Vysis PathVysion HER2 DNA Probe kit. Tumors were considered positive if the ratio

of HER2 gene signals to chromosome 17 signals was grater than or equal to 2.0.

In this study, we defined low ER-positive tumors as those where 1% to 9% of cells stained positive for ER; we had previously considered these tumors to be ER-negative prior to the ASCO/CAP recommendations. High ER-positive tumors referred to tumors where at least 10% of tumor cells stained positive for ER. Univariate analyses were performed to examine the association of standard clinicopathological parameters with ER expression, to determine the factors more frequently associated with low ER expression as well as factors that influenced the decision for hormonal therapy in such low ER-positive tumors. Univariate analyses were performed using the *Chi* square test, *Chi* square test for trend, one-way ANOVA where appropriate, using GraphPad Prism, version 6 (GraphPad Software, San Diego, United States). Cox regression was used to identify independent factors associated with distant recurrence in patients with low ER-positive tumors, and was carried out using the Stata package release 11.0 (Stata Corporation, College Station, Texas, USA). A full model was first created to include all potentially important explanatory variables. At each step, the variable with the smallest contribution to the model was removed, until a final backward stepwise model was obtained. The effects of ER and PR levels and of hormonal therapy on disease-free and overall survival were examined using Kaplan–Meier survival curves; these were calculated using GraphPad Prism as well. Distant disease recurrence was defined as the development of systemic disease in a previously non-metastatic patient; local recurrence was defined as recurrent disease in the ipsilateral breast or axilla (post-wide local excision) or chest wall (post-mastectomy); contralateral cancer was defined as the ocurrence of a metachronous cancer in the contralateral breast more than 6 months following the diagnosis of the first cancer. The starting point for follow-up was taken as the date of definitive cancer surgery or the date of chemotherapy initiation when given as neoadjuvant treatment. All patients were censored after 10 years of follow-up. Other censored observations included loss to follow-up and incomplete medical records with no available information on outcomes. A 2-tailed P value test was used for all analyses and a value of $P<0.05$ was considered statistically significant.

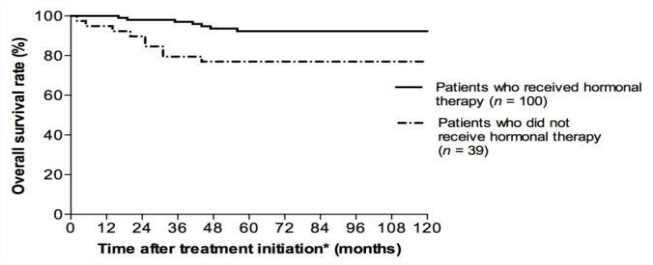
Results

A total of 2872 women were included in this study. Median patient age was 55 years (ranging from 23 to 98 years) and more than half the women (1810 of 2872, 63.0%) were post-menopausal. Majority of the women were of Chinese ethnicity (2296 of 2872, 79.9%) and more than 89.9% (2583 of 2872) were diagnosed with invasive carcinoma: 785 (30.4%) with Stage I disease, 968 (37.5%) with Stage II disease, 595 (23.1%) with Stage III disease and 235 (9.1%) with Stage IV disease. Median invasive tumor size was 21 mm (ranging from 1 mm to 210 mm), and 329 tumors involved the overlying skin or underlying chest wall (staged as T4). Median tumor grade was 2. Another 289 women (10.1%) were diagnosed with Ductal Carcinoma *in Situ* (DCIS); median DCIS tumor size was 21 mm (ranging from 1 mm to 120 mm), and 140 tumors (48.4%) were of high nuclear grade. Tumors in 2037 of the 2872 women (70.9%) were classified as being ER-positive at the time of diagnosis. More tumors were classified as ER-positive in the years following the 2010 ASCO/CAP update (P=0.032, OR 1.216, 95% CI 0.688 to 0.983) and when the updated ER threshold was applied to the preceding years, an additional 105 tumors would have been classified as ER-positive. In total, 171 tumors were considered low ER-positive (with 1% to 9% positively stained cells) according to the updated ASCO/CAP recommendations. More than half the tumors (1553 of 2872, 54.1%) were PR-positive and 539 of 1998 (26.9%) were HER2-postive; HER2 testing became routine only from 2006 onwards. Over a median follow-up period of 41.9 months (ranging from 3.1 to 1371.8 months), 166 women developed local recurrence (128 of them occurring within the first 5 years) and 282 women developed distant recurrence (183 occurring within the first 5 years). Of those who developed distant recurrence, 81 (28.7%) were also found with locoregional disease. Contralateral breast cancer was diagnosed in 83 women (60 occurring within the first 5 years). There were 459 deaths, of which 139 were women who had presented with metastatic disease. A non-breast related cause of death was recorded in 96 women.

Low ER-positive tumors occurred more frequently in younger women (P<0.001), and in women of Malay or Indian ethnicity (P=0.002, OR 1.803, 95% CI 1.226 to 2.652) (Table 1). Low ER expression tended to be associated with high tumor grade, in both *in-situ* (P=0.003) and invasive (P<0.001) cancers, absent PR expression (P<0.001, OR 3.142, 95% CI 0.232 to 0.437) and HER2-over-expression (P<0.001, OR 2.709, 95% CI 1.820 to 4.031) (Table 1). There was no association with disease stage at presentation (P=0.372) (Table 1).

Hormonal therapy was recommended to 1999 women (69.6%) in this study and the rate of uptake was high, with 1810 of 1999 (90.5%) women starting on treatment. Twenty women with ER-positive tumors were not recommended hormonal therapy: 5 women were of advanced age (older than 85 years), 13 were women who had undergone bilateral mastectomy for pure DCIS and there was no clear documentation in the remaining 2 women. In total, 122 of 171 (71.3%) of the women with low ER-positive tumors received hormonal therapy; 48 of these 122 patients had ER-negative/PR-positive tumors at the time of diagnosis (ER expression in these tumors were between 1% to 9% and considered ER-negative according to the threshold in use at the time of diagnosis). Women with invasive carcinomas (P=0.004, OR 4.212, 95% CI 0.085 to 0.666), rather than in-situ tumors, and women with tumors that were PR-positive (P<0.001, OR 29.210, 95% CI 8.567 to 99.570) or of high grade (P=0.046) were more often recommended hormonal therapy, as were those who had been treated with surgery (P=0.021, OR 6.818 95% CI 1.276 to 36.450). There was no significant association seen with any other treatment modalities.

We next examined the survival outcomes of the 1701 women who had received curative treatment for non-metastatic disease (Stage I to III cancers). Distant disease recurrence was almost 2-fold higher in women with low ER-positive tumors (P=0.029, OR 1.726, 95% CI 1.053 to 2.830) (Table 1), compared to those with tumors with more than 10% ER expression. Furthermore, distant disease-free survival appeared to vary according to tumor ER expression levels. Distant disease-free survival was best in women with tumors expressing at least 10% ER. Survival in women with low ER-positive tumors was significantly poorer (P=0.005) and in fact overlapped with ER-negative tumors, such that no survival difference was observed between low ER-positive and ER-negative tumors (P=0.574) (Figure 1). Low ER expression did not, however, show any significant association with locoregional recurrence (P=0.150) (Table 1), contralateral breast cancer (P=0.931) or mortality (P=0.839). Tumor PR status appeared to contribute to survival outcomes in low ER-positive disease, with distant disease-free survival being superior in tumors that also



Comparison	Hazard Ratio	95% CI	P value
Hormonal therapy versus no hormonal therapy	0.218	0.072 - 0.662	0.004

Time (months)	0	12	24	36	48	60	72	84	96	108	120
Numbers at risk Hormonal therapy	100	100	99	97	77	58	44	38	38	35	31
No hormonal therapy	39	37	35	33	31	31	27	20	18	17	12

Figure 4: Kaplan Meier curves depicting 10-year overall survival stratified by the receipt of hormonal therapy among patients with non-metastatic low Estrogen Receptor (ER)-positive invasive cancers (n=139). *Treatment initiation refers to definitive breast cancer surgery or the initiation of neoadjuvant chemotherapy.

Table 4: Clinical outcome in women with non-metastatic low ER-positive invasive cancers stratified by hormonal treatment (n=139).

	Patients who received hormonal therapy (n=100)	Patients who did not receive hormonal therapy (n=39)	P value
Local recurrence			
Yes	5	6	0.042
No	95	33	
Distant recurrence			
Yes	8	13	<0.001
No	92	26	
Contralateral cancer			
Yes	2	2	0.322
No	98	37	
Mortality			
Yes	7	10	0.003
No	93	29	

expressed PR, regardless of the level of PR expression (P=0.007, HR 0.279, 95% CI 0.110-0.705 for tumors with at least 10% PR expression and P=0.041, HR 0.373, 95% CI 0.145 to 0.961 for tumors with low PR expression) (Figure 2). In the multivariate analysis, tumor PR status emerged as an independent predictor of distant recurrence (P=0.042, HR 0.353, 95% CI 0.129 to 0.963) (Table 3), together with advanced disease (P=0.002, HR 13.587, 95% CI 2.703 to 68.313) and treatment with hormonal agents (P<0.001, HR 0.178, 95% CI 0.069 to 0.458) (Table 3). Hormonal therapy reduced both locoregional (P=0.042, OR 0.290, 95% CI 0.083-1.012) and distant recurrence (P<0.001, OR 0.140, 95% CI 0.065-0.465) (Table 4) in women with low ER-positive tumors and also improved distant-recurrence-free and overall survival (P=0.001, HR 0.143, 95% CI 0.053-0.384 and P=0.004, HR 0.218, 95% CI 0.072-0.662 respectively) (Figure 3 and 4).

Discussion

The benefit of hormonal therapy is well established in ER-positive tumors [2,13], with data now supporting even an extended treatment duration of 10 years [11,14]. The decision for hormonal therapy is based primarily on tumor ER status, as determined by the intensity and extent of ER antibody staining on immunohistochemistry. Scoring systems to assess ER staining varied across institutions and the threshold for a positive result was not consistent prior to the ASCO/CAP standardization [7,15-17]. The ASCO/CAP guidelines places greater importance on the proportion of cells staining positive, even though one study suggested that quantification of the average staining intensity was more clinically relevant at low levels of ER expression [18]. Overall, 6% of ER-positive tumors at our institute were classified as low ER-positive, consistent with other published reports [10,19]. Our adoption of the ASCO/CAP recommendations led to 4% more tumors being considered ER-positive.

There is a lack of consensus on whether women with low ER-positive tumors should receive hormonal therapy. One study reported that up to one third of such women did not receive hormonal therapy and reflected the prevailing opinion among some oncologists that the benefit of hormonal therapy was small in low ER-positive disease and did not justify the cost of treatment and potential adverse side effects [5,20,21]. Similarly, 29% of the women with low ER-positive tumors in our study were not recommended hormonal therapy; many of whom had DCIS or PR-negative tumors. The perception that hormonal therapy produces only a small benefit in low ER-positive tumors arises from evidence suggesting that these tumors are in fact more similar to ER-negative tumors. In our study, we found low ER expression more often associated with poor risk factors such as young age, high tumor grade and PR negativity, factors that were also common in ER-negative tumors [22]. Our observation that distant disease-free survival in low ER-positive tumors was no different from that of ER-negative tumors seems to further support this. On a molecular level, low ER-positive tumors tend to cluster with ER-negative tumors. Expression levels of the ER gene (ESR1) and co-expressed genes of the Sensitivity to Endocrine Therapy (SET) index were similar between low ER-positive and ER-negative tumors and the majority of low ER-positive tumors were classified as HER2 over-expressing or basal, rather than as Luminal A or B, as was the case with tumors expressing more than 10% ER [9,19,23].

More than two thirds of the women with low ER-positive tumors in our study received hormonal therapy. These women were more likely to have invasive cancers that were also PR-positive and were more likely to have undergone definitive surgery. Given the significant proportion of women who had received hormonal therapy, the absence of any survival difference between low ER-positive and ER-negative tumors would seem to support existing data that hormonal therapy conferred little benefit in low ER-positive disease [5,22]. However, when examining the women with low ER-positive tumors alone, we found that those who had received hormonal therapy were

less likely to develop disease recurrence and had better distant disease-free and overall survival. One possible explanation could be that low ER-positive tumors are molecularly diverse and vary in their response to hormonal therapy [24]. Our observation that tumor PR status was associated with survival would support this. We found distant disease-free survival to be superior in low ER-positive tumors that concurrently expressed PR, even at levels of less than 10%. Several other studies have also found tumor PR levels to be associated with recurrence-free and overall survival, [7,16,25] and taken together, these suggest that PR may not merely be a downstream target of ER. One study in fact found evidence of PR modulating ER activity [26]. But while the loss of PR expression indicated poor prognosis [27,28]. Levels of PR expression did not predict hormonal therapy response [5,29,30].

Our study suggests that hormonal therapy continues to confer benefit to women with low ER-positive tumors, though the benefit is likely smaller than that seen with tumors expressing more than 10% ER. The clinical benefit could also be more relevant to tumors with an increased risk of distant recurrence, such as those that are advanced or PR-negative. Despite this, the benefit-risk ratio is likely to still favor hormonal therapy since it is generally well tolerated. Thromboembolic events and endometrial cancer are potential adverse evens in women taking tamoxifen, but overall, the incidence of both remains low. Thromboembolic events occurred in 1.7% of women tamoxifen, compared to 0.4% of women on placebo, and the incidence of endometrial cancer was 1.26 per 1000 patient years in women on tamoxifen, compared to 0.58 per 1000 patient years in those on placebo [31,32]. A review of our own data found an overall incidence of 0.4% in our women treated for breast cancer and endometrial cancer not more frequent in those taking tamoxifen (unpublished data). In more recent years, aromatase inhibitors have replaced tamoxifen as the first-line agent in post-menopausal women. Osteoporosis from accelerated bone loss is a primary concern, but osteoporotic bone fractures are in fact relatively uncommon [33].

One of the main limitations of our present study is the small numbers of low ER-positive tumors, given that such tumors generally account for less than 10% of all ER-positive tumors. While our study follow up of 10 years would be adequate to capture late recurrences, which are more common in ER-positive tumors, many of these women had received 5 years of tamoxifen, the standard of care at that time. The effect of extended therapy and of aromatase inhibitors, which were increasingly used only after 2006, cannot therefore be adequately examined. The small study numbers also preclude further conclusions as to whether ER expression follows an ethnic variation. We found low ER-positive tumors to be more common in Malay and Indian (non-Chinese) women, which was somewhat in keeping with an earlier study that also found triple negative tumors (ER-negative tumors also negative for PR and HER2 expression) more common in Malay and Indian women [34]. Interestingly, one study had also reported low ER-positive tumors to be more prevalent among non-white women [22].

Conclusion

Standardization in accordance with ASCO/CAP guidelines has provided confidence that low ER levels detected with IHC is reliable and not merely a false-positive result. While low ER-positive tumors may share several features and survival outcomes in common with ER-negative tumors, hormonal therapy still confers clinical benefit. Hormonal therapy reduces distant recurrence and confers overall survival advantage in low ER-positive tumors. Given that the potential for adverse events from hormonal therapy is low, hormonal therapy should be recommended to women with low ER-positive tumors, particularly those with advanced or PR-negative tumors in view of the higher risk of distant recurrence.

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