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Prognostic molecular markers in women aged 35 years or younger with breast cancer: is there a difference from the older patients?

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ABSTRACT

Background Women aged ≤35 years with breast cancer have a poor prognosis, but their prognostic factors have not been clearly defined.

Aims To evaluate whether the molecular markers used in age-unspecified breast cancer could also be applied to women ≤35 years.

Methods Archival tumours from patients aged ≤35 years with stage I–III breast cancer were collected. Oestrogen receptor (ER), progesterone receptor (PR), HER2, Ki67 and P53 protein expression profiles in paraffin-embedded tissue sections were determined by immunohistochemistry. Tumours with an HER2 score of 2+ were further evaluated by fluorescence in situ hybridisation. Mutational analysis of exons 4–9 of the TP53 gene and exons 9 and 20 of the PIK3CA gene was carried out using direct sequencing analysis.

Results 116 patients with a median follow-up duration of 62.7 months were included. In addition to tumour size and axillary lymph node status, univariate analysis showed that high Ki67 expression, ER-negative, HER2 overexpression, and TP53 mutations were associated with shorter overall survival. Multivariate analysis showed that high Ki67 expression (HR=3.93, p=0.005), HER2 overexpression (HR=3.21, p=0.013) and TP53 mutations (HR=4.44, p=0.005) were associated with shorter overall survival. PR expression and PIK3CA mutations were not associated with survival.

Conclusions For women ≤35 years, TP53 mutations, Ki67 and HER2 expressions are strong prognostic factors. The limited prognostic value of hormone receptors suggests that the prognostic markers used in age-unspecified breast cancer may not be completely fit for this population.

INTRODUCTION

Women under 35 years of age comprise a small proportion of patients with breast cancer[1] and have significantly worse survival than older patients.[2 3] Breast cancers in these young women are more frequently poorly differentiated, oestrogen receptor (ER) negative, and display a high proliferation index. However, these adverse pathological factors have been shown to only partially explain the survival difference between older and younger patients. Age younger than 35 years remained an independent predictor of poor outcome[4 5] and these cancers may be considered as a distinct disease entity.

Due to its relative rarity, the prognostic factors in women aged ≤35 years with breast cancer have yet to be firmly established. For example, the prognostic impact of ER and human epidermal growth factor receptor 2 (HER2) status, the two well established markers in age-unspecified breast cancer, remain controversial in this young population.[6–11]

TP53 and *PIK3CA* mutations are two of the most common genetic alterations in human breast cancer. In age-unspecified patients, numerous studies have shown that *TP53* mutations are predictive of poor prognosis.[12 13] Activating mutations in *PIK3CA*, the gene encoding the p110 catalytic subunit of PI3K, have been identified as novel mechanisms of inducing oncogenic PI3K signalling,[14] and therefore have become an attractive target for cancer treatment.[15] However, the prognostic role of these two common genetic alterations has also not been studied in women aged ≤35 years with breast cancer.

The present study aimed to comprehensively evaluate whether these common prognostic markers identified in age-unspecified breast cancer could be applied to women aged ≤35 years with breast cancer.

METHODS

Patients and sample collection

During the period January 1997 to December 2005, incident breast cancer stage I–III was diagnosed in 181 consecutive female patients aged ≤35 at the National Taiwan University Hospital. Among them, archival breast tumour tissues from 116 patients were available for immunohistochemical and mutation analyses. Fifty-six patients had been included in our previous study describing association of age with molecular subtypes defined by immunohistochemistry.[16]

The staging of breast cancer followed the American Joint Committee on Cancer (AJCC, 7th edition) criteria. Histological grade was categorised as grade I, II or III according to the Nottingham modification of the Scarff–Bloom–Richardson criteria by a single pathologist. Patients' characteristics and clinical data were extracted from medical charts. In patients who were lost to follow-up, disease status and survival outcomes were obtained from medical charts, hospital cancer registry records and the National Death Certificate Registry system. The survival data used in this study were current as of 30 June 2009.

Immunohistochemistry and fluorescence in situ hybridisation

The ER, progesterone receptor (PR) and HER2 status were evaluated as previously described.[16] For ER and PR, tumours with ≥10% positively stained nuclei were considered positive.[17] The HER2 status was considered positive if scored 3+ by immunohistochemical analysis or 2+ with gene amplification on fluorescence in situ hybridisation (FISH).[18]

The immunohistochemical methods used for staining Ki67 and P53 protein expression have been previously reported[19 20]; the primary antibodies were anti-Ki67 (1:200 dilution, clone

Table 1 Clinical and pathological characteristics of patients by *TP53* and *PIK3CA* mutations

	No. (%)				No. (%)		
Characteristics	**All (n=116)**	**TP53 wild (n=84)**	**TP53 mutant (n=32)**	**p Value**	**PIK3CA wild (n=94)**	**PIK3CA mutant (n=22)**	**p Value**
Family history of breast cancer				0.187			0.826
None	92 (89)	70 (92)	22 (81)		76 (89)	16 (89)	
First-degree relative	3 (3)	2 (3)	1 (4)		3 (4)	0 (0)	
Second-degree relative	7 (7)	4 (5)	3 (11)		5 (6)	2 (11)	
First- and second-degree relatives	1 (1)	0 (0)	1 (4)		1 (1)	0 (0)	
Unknown	13	8	5		9	4	
Histology type				1.00			1.00
Ductal carcinoma	108 (93)	78 (93)	30 (94)		87 (93)	21 (95)	
Other	8 (7)	6 (7)	2 (6)		7 (7)	1 (5)	
Histological grade				0.325			0.172
1	21 (18)	18 (22)	3 (9)		20 (22)	1 (5)	
2	59 (51)	41 (49)	18 (56)		46 (49)	13 (59)	
3	35 (30)	24 (29)	11 (34)		27 (29)	8 (36)	
Unknown	1	1	0		1	0	
Ki67 expression				0.126			0.476
≤20%	71 (61)	55 (65)	16 (50)		59 (63)	12 (55)	
>20%	45 (39)	29 (35)	16 (50)		35 (37)	10 (45)	
Tumour size				0.654			0.292
<2 cm	44 (38)	33 (39)	11 (34)		33 (35)	11 (50)	
2–5 cm	57 (49)	39 (46)	18 (56)		48 (51)	9 (41)	
>5 cm	15 (13)	12 (14)	3 (9)		13 (14)	2 (9)	
Axillary lymph node				0.309			0.203
None or cN0	63 (54)	49 (58)	14 (44)		53 (56)	10 (45)	
1–3 or cN1	28 (24)	20 (24)	8 (25)		23 (24)	5 (23)	
4–9 or cN2	13 (11)	5 (6)	8 (25)		10 (11)	3 (14)	
≥10 or cN3	12 (10)	10 (12)	2 (6))		8 (9)	4 (18)	
AJCC stage				0.081			0.564
I	32 (28)	27 (32)	5 (16)		26 (28)	6 (27)	
II	56 (48)	39 (46)	17 (53)		47 (50)	9 (41)	
III	28 (24)	18 (21)	10 (31)		21 (22)	7 (32)	
ER status				0.004			0.742
Negative	35 (30)	19 (23)	16 (50)		29 (31)	6 (27)	
Positive	81 (70)	65 (77)	16 (50)		65 (69)	16 (73)	
PR status				0.839			0.888
Negative	49 (42)	35 (42)	14 (44)		40 (43)	9 (41)	
Positive	67 (58)	49 (58)	18 (56)		54 (57)	13 (59)	
HER2 status				0.055			0.586
Negative	87 (75)	67 (80)	20 (63)		69 (73)	18 (82)	
Positive	29 (25)	17 (20)	12 (38)		25 (27)	4 (18)	
Triple negative				0.324			0.756
No	97 (84)	72 (86)	25 (78)		79 (84)	18 (82)	
Yes	19 (16)	12 (14)	7 (22)		15 (16)	4 (18)	
Hormone therapy*				0.077			0.709
None	30 (26)	18 (21)	12 (38)		25 (27)	5 (23)	
Yes	86 (74)	66 (79)	20 (63)		69 (73)	17 (77)	
Chemotherapy*				0.545			0.211
None	30 (26)	23 (27)	7 (22)		22 (23)	8 (36)	
Yes	86 (74)	61 (73)	25 (78)		72 (77)	14 (54)	
P53 expression				<0.001			0.197
0	29 (25)	27 (32)	2 (6)		24 (26)	5 (23)	
1	24 (21)	21 (25)	3 (9)		18 (19)	6 (27)	
2	34 (29)	23 (27)	11 (34)		31 (33)	3 (14)	
3	29 (25)	13 (15)	16 (50)		21 (22)	8 (36)	

*Neoadjuvant and/or adjuvant therapy.
AJCC, American Joint Committee on Cancer; ER, oestrogen receptor; HER2, human epidermal growth factor receptor 2; PR, progesterone receptor.

MIB-1, DakoCytomation, Denmark) and anti-P53 (1:200 dilution, clone DO-7, DakoCytomation), respectively. The expression of Ki67 was regarded as positive if at least 20% of invasive cancer cells stained positive.[19] The intensity of P53 staining was rated semi-quantitatively on a four-point scale (0=no staining, 1=light staining, 2=staining of moderate intensity and 3=intense or maximum staining). The maximum intensity of staining in ≥10% positive staining tumour cells was scored for each tissue specimen; score 3 was considered positive.[20]

PCR and direct sequencing of *TP53* and *PIK3CA*

H&E-stained slides of the tumours were reviewed and areas of tumour were marked for macrodissection to enrich tumour DNA. H&E-stained tissue sections (15 μm sections) were scraped off from glass slides. The genomic DNA of the macrodissected tumour specimens was isolated using the QIAamp DNA Mini Kit (Qiagen, Valencia, California, USA), amplified by PCR, and sequenced for the exons known to contain mutational hotspots. These exons included exons 4–9 of the *TP53* gene and exons 9 and 20 of the *PIK3CA* gene.[21 22] The primers are listed in the supplementary table online. The amplified DNA was subjected to forward and reverse sequencing using an autosequencer (Applied Biosystems, CA, USA) with sequencing or corresponding PCR primers.

Statistical analysis

Disease-free survival was defined as the duration from diagnosis to either confirmation of disease recurrence, including local, regional and distant recurrences, or death due to breast cancer. Overall survival was defined as the duration from diagnosis to death due to any cause.

Survival curves were constructed using the Kaplan–Meier method. The association between each of the categorical variables and survival was analysed by the log-rank test. Cox's proportional hazards analysis was used to determine the relative contribution of various factors to disease-free and overall survival. A p value ≤0.05 was used to indicate statistical significance; all tests were two-tailed. All statistical analyses were performed with the statistical package SPSS for Windows V.17.0.

RESULTS

Clinical and pathological characteristics of patients

Table 1 summarises the demographic data of the 116 patients with and without *TP53* and *PIK3CA* mutations. Eleven (11%) of 103 patients with available records had a family history of breast

Table 2 *TP53* mutations detected by genomic sequencing

Type of mutation	Codon	Normal sequence	Mutant sequence	Amino acid change	P53 expression	Histology type	AJCC stage
Missense*	106	AGG	AGC	Arg→Ser	2	Ductal	II
Missense	109	TTC	GTC	Phe→Val	3	Ductal	II
Missense†	138	GCC	ACC	Ala→Thr	2	Ductal	II
Missense	138	GCC	ACC	Ala→Thr	2	Ductal	II
Missense	147	GTT	GGT	Val→Gly	3	Ductal	I
Missense	148	GAT	AAT	Asp→Asn	2	Ductal	III
Missense	158	CGC	AGC	Arg→Ser	2	Ductal	II
Missense	158	CGC	AGC	Arg→Ser	2	Ductal	II
Missense	175	CGC	CAC	Arg→His	3	Ductal	II
Missense	175	CGC	AGC	Arg→Ser	1	Ductal	III
Missense	175	CGC	CAC	Arg→His	3	Ductal	III
Missense	185	AGC	AAC	Ser→Asn	2	Ductal	II
Missense	205	TAT	TGT	Tyr→Cys	3	Ductal	II
Missense	215	AGT	TGT	Ser→Cys	1	Ductal	II
Missense	220	TAT	TGT	Tyr→Cys	3	Ductal	II
Missense	230	ACC	ACA	Thr→Thr	3	Ductal	III
Missense	231	ACC	GCC	Thr→Ala	2	Ductal	I
Nonsense	231	CGA	TGA	Arg→stop	2	Ductal	III
Missense	232	ATC	AAC	Ile→Asn	0	Ductal	II
Missense	233	CAC	CAA	His→Gln	3	Medullary	III
Missense	236	TAC	TGC	Tyr→Cys	3	Ductal	II
Missense	238	TGT	TAT	Cys→Tyr	3	Ductal	III
Missense	241	TCC	TTC	Ser→Phe	3	Ductal	II
Missense	248	CGG	CAG	Arg→Gln	3	Ductal	II
Missense	248	CGG	CAG	Arg→Gln	3	Ductal	III
Missense	250	CCC	TCC	Pro→Ser	1	Ductal	I
Missense	258	GAA	AAA	Glu→Lys	0	Ductal	II
Missense	280	AGA	AGC	Arg→Ser	3	Ductal	I
Missense	283	CGC	CGT	Arg→Arg	3	Mucinous	II
Missense	283	CGC	CGT	Arg→Arg	3	Ductal	III
Missense	296	CAC	TAC	His→Tyr	2	Ductal	III
Missense	304	ACT	ATT	Thr→Ile	2	Ductal	I
Nonsense†	306	CGA	TGA	Arg→stop	2	Ductal	II
Missense*	325	GGA	GAA	Gly→Glu	3	Ductal	II

*This tumour contained two missense mutations.
†This tumour contained one missense and one nonsense mutation.
AJCC, American Joint Committee on Cancer.

cancer; none had a family history of ovarian cancer. One of four patients who received *BRCA1* and *BRCA2* mutation analyses was identified to have *BRCA1* mutation (T1691K). After surgery, the majority of patients (86 of 116, 75%) received adjuvant hormone treatment, including tamoxifen (n=75), ovarian suppression plus tamoxifen (n=6) and ovarian suppression plus aromatase inhibitors (n=5). Eighty-six (74%) patients received neoadjuvant and/or adjuvant chemotherapy. The regimens included cyclophosphamide/methotrexate/5-fluorouracil in 4 patients, anthracycline-based regimens in 60 patients, anthracycline plus taxane-based regimens in 20 patients, and taxane plus cisplatin in 2 patients. Because the use of trastuzumab in the adjuvant setting had not been reimbursed by the national health insurance in Taiwan until 1 January 2010, only 2 patients with HER2-positive tumours received adjuvant trastuzumab in the present study.

Table 2 presents details of mutations in the coding region of *TP53*. A total of 34 mutations, including 30 missense mutations and 2 nonsense mutations, were identified in 32 tumours. All of the *TP53* mutations detected in this study had been previously reported in the IARC *TP53* database (http://www-p53.iarc.fr/MutationValidation Criteria.asp). As shown in table 1, *TP53* mutations were negatively associated with ER expression (p=0.004), marginally associated with HER2 overexpression

Table 3 Correlation of clinical and pathological variables with disease-free and overall survival by univariate analysis

		DFS		OS	
	No.	HR (95% CI)	p Value	HR (95% CI)	p Value
Histology grade			0.646		0.452
1	21	1.00		1.00	
2	59	1.63 (0.55 to 4.83)		1.34 (0.37 to 4.88)	
3	35	1.68 (0.53 to 5.36)		2.09 (0.56 to 7.78)	
Ki67 expression			0.009		0.005
≤20%	71	1.00		1.00	
>20%	45	2.56 (1.26 to 5.19)		3.48 (1.45 to 8.34)	
Tumour size			0.097		0.033
≤2 cm	62	1.00		1.00	
2–5 cm	98	1.54 (0.66 to 3.58)		2.56 (0.72 to 9.09)	
>5 cm	21	3.05 (1.10 to 8.43)		6.08 (1.52 to 24.38)	
Axillary lymph node			0.003		0.025
None or cN0	68	1.00		1.00	
1–3 or cN1	23	1.64 (0.65 to 4.17)		1.35 (0.43 to 4.27)	
4–9 or cN2	13	3.00 (1.09 to 8.25)		2.99 (0.94 to 9.51)	
≥10 or cN3	12	5.28 (2.18 to 14.25)		5.28 (1.64 to 17.02)	
ER status			0.176		0.027
Negative	35	1.00		1.00	
Positive	81	0.61 (0.30 to 0.25)		0.39 (0.17 to 0.90)	
PR status			0.941		0.548
Negative	49	1.00		1.00	
Positive	67	0.97 (0.47 to 2.01)		0.77 (0.33 to 1.82)	
HER2 status			0.072		0.007
No	87	1.00		1.00	
Yes	29	1.93 (0.94 to 3.95)		3.16 (1.37 to 7.31)	
Triple negative			0.690		0.542
No	97	1.00		1.00	
Yes	19	1.21 (0.47 to 3.16)		1.40 (0.47 to 4.17)	
Hormonal therapy*			0.951		0.564
No	30	1.00		1.00	
Yes	86	0.98 (0.44 to 2.17)		0.77 (0.31 to 1.90)	
Chemotherapy*			0.055		0.069
No	30	1.00		1.00	
Yes	86	2.79 (0.98 to 7.98)		3.87 (0.90 to 16.57)	
P53 expression			0.085		0.089
Negative	85	1.00		1.00	
Positive	31	1.88 (0.92 to 3.86)		2.11 (0.89 to 4.98)	
TP53 mutation			0.010		0.035
Wild	84	1.00		1.00	
Missense mutant	30	2.93 (1.44 to 5.96)		3.18 (1.24 to 8.14)	
Nonsense mutant†	2	3.15 (0.42 to 23.90)		4.99 (0.62 to 40.49)	
PIK3CA mutation			0.732		0.952
Wild	94	1.00		1.00	
Exon 9 mutant	4	1.82 (0.43 to 7.70)		0.86 (0.11 to 6.54)	
Exon 20 mutant	18	1.17 (0.48 to 2.86)		1.16 (0.39 to 3.44)	

*Neoadjuvant and/or adjuvant therapy.
†Including the tumour harbouring one missense and one nonsense mutation.
DFS, disease-free survival; ER, oestrogen receptor; HER2, human epidermal growth factor receptor 2; OS, overall survival; PR, progesterone receptor.

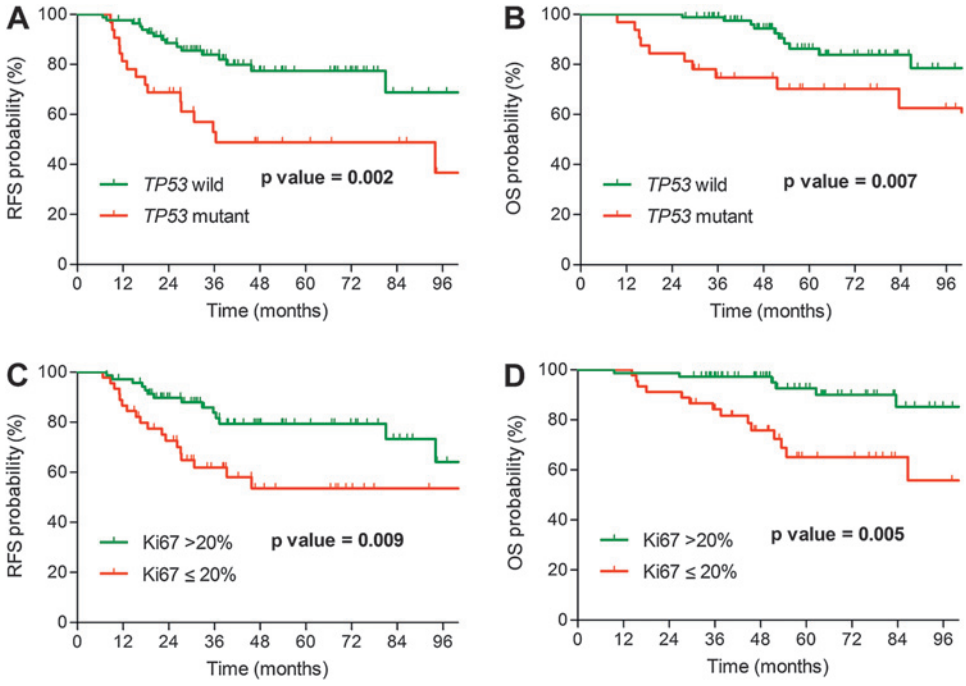

Figure 1 Kaplan–Meier estimates of: (A) relapse-free survival (RFS), and (B) overall survival (OS) survival curves by *TP53* mutation; and (C) RFS, and (D) OS curves by Ki67 expression (unadjusted analysis).

(p=0.055) and significantly associated with P53 expression (p<0.001). *PIK3CA* mutations found in this study included the H1047R missense mutation in exon 20 (n=18), the E542K mutation in exon 9 (n=2) and the E545K mutation in exon 9 (n=2). As shown in table 1, tumours with *PIK3CA* mutations had a higher frequency of P53 protein expression (p=0.027), but were not associated with the presence of *TP53* mutations (data not shown, p=0.622).

Univariate survival analyses of prognostic factors

The median follow-up duration was 62.7 months (95% CI 66.4 to 68.9). The 5-year disease-free survival rate was 89% for stage I disease, 71% for stage II disease and 48% for stage III disease. The 5-year overall survival rate was 96% for stage I disease, 81% for stage II disease and 68% for stage III disease. Univariate analysis showed that larger tumour size, greater axillary lymph node involvement, high Ki67 proliferative index and *TP53* mutations were significantly associated with poor disease-free and overall survival (table 3 and figure 1). HER2 overexpression and ER negativity were significantly associated with poor overall survival. PR and *PIK3CA* exon 20 and exon 9 mutations were not significantly associated with disease-free or overall survival.

Multivariate survival analyses of prognostic factors

Table 4 presents results of logistic regression and Cox's proportional hazards analyses for disease-free and overall survival. In addition to axillary lymph node status and tumour size, *TP53* mutation (HR=3.76, p=0.001) was an independent prognostic factor for poor disease-free survival, and high Ki67 expression (HR=2.09, p=0.052) was marginally associated with shorter disease-free survival. HER2 overexpression (HR=3.21, p=0.013), high Ki67 expression (HR=3.93, p=0.005) and *TP53* mutations (HR=4.44, p=0.005) were independent predictors of poor overall survival.

DISCUSSION

This study showed that high Ki67 expression, HER2 overexpression and *TP53* mutations were independent predictors of poor prognosis in women aged ≤35 years with breast cancer. Both Ki67 expression and *TP53* mutation were better indicators of poor prognosis than histological grade and P53 expression. ER

Table 4 Multivariate Cox hazard regression models for risk factors

	DFS		OS	
Characteristic	**HR (95% CI)**	**p Value**	**HR (95% CI)**	**p Value**
Tumour size		0.065		0.010
>5 cm vs ≤5 cm	2.31 (0.95 to 5.65)		4.42 (1.44 to 13.60)	
Lymph node		0.018		0.180
Lymph nodes 1–9 vs 0	1.47 (0.64 to 3.38)		1.10 (0.39 to 3.05)	
Lymph nodes ≥10 vs 0	4.37 (1.56 to 12.26)		3.23 (0.88 to 11.95)	
HER2 overexpression		0.385		0.013
Yes vs no	1.40 (0.66 to 2.96)		3.21 (1.28 to 8.03)	
Ki67 status		0.052		0.005
≥20% vs <20%	2.09 (0.99 to 4.39)		3.93 (1.51 to 10.23)	
TP53 status		0.001		0.005
Mutant vs wild*	3.76 (1.67 to 8.46)		4.44 (1.58 to 10.23)	

*Missense and nonsense mutations.
DFS, disease-free survival; HER2, human epidermal growth factor receptor 2; OS, overall survival.

expression was associated with longer overall survival in the univariate analysis but not in the multivariate analysis. PR and *PIK3CA* mutations were not associated with survival in this population.

A meta-analysis involving 15 790 patients showed that high Ki67 expression was predictive of shorter overall survival (HR=1.73, 95% CI 1.37 to 2.17).[23] In 2009, the St Gallen Consensus recommended using markers of proliferation, such as Ki67, to determine the optimum treatment for early breast cancer.[24] The present study shows that the prognostic value of Ki67 expression is superior to that of histological grade and supports the use of Ki67 assessment in women aged ≤35 years.

In this study, the rate of mutations in exons 4–9 of the *TP53* gene was 28%, a mutation rate similar to that reported in age-unspecified populations,[12] and the prognostic impact is consistent with a meta-analysis of 3500 age-unspecified patients which showed that patients with *TP53* mutations were more likely to develop disease recurrence (HR=2.0, 95% CI 1.7 to 2.5).[12] Although P53 protein expression was significantly associated with the presence of *TP53* mutation (p<0.001), P53 protein expression was only marginally associated with survival in our univariate analysis. A similar finding was reported in a meta-analysis involving more than 9800 patients, which showed that the prognostic value of P53 immunohistochemical expression in breast cancer was weak.[25]

In this study, ER expression was associated with better overall survival in the univariate analysis but not in the multivariate analysis (p=0.201, data not shown). When we controlled for *TP53* mutations in the multivariate analysis, ER expression was still only marginally associated with longer overall survival (p=0.065, data not shown). Our finding is consistent with results from the Danish Breast Cancer Cooperative Group who showed that very young patients with ER-positive tumours have significantly longer overall survival in the first 5 years but not 5–10 years after diagnosis.[8] The marginal prognostic value of ER and the lack of association between PR and survival in this study suggest that hormone receptor status is not a reliable predictor of prognosis in women ≤35 years of age.

The frequency of HER2 overexpression (25%) in this study is close to that in an age-unspecified breast cancer population.[26] The strong prognostic significance of HER2 shown in this study is consistent with the findings reported in two previous studies,[9 10] but differs from the frequency reported in a study conducted at the M.D. Anderson Cancer Center.[11]

The *PIK3CA* mutation rate in this study was 19%, which is lower than the mutation rate in age-unspecified populations from previous reports (20–40%).[22 27 28] Therefore, we additionally examined the frequency of *PIK3CA* mutations in breast carcinoma specimens from 74 patients aged >35 years and found that the *PIK3CA* mutation frequency was 31% (data not shown). The association of *PIK3CA* mutations with older age is consistent with that reported by Kalinsky *et al*.[22] Although the presence of *PIK3CA* mutations has been linked to both favourable[22 29 30] and unfavourable[27] prognosis, the present study and two previous reports show no association between the presence of *PIK3CA* mutations and patients' prognosis.[28 31]

In summary, we found a strong prognostic impact of *TP53* mutations, and Ki67 and HER2 expression, and limited prognostic value of hormone receptor expressions in women ≤35 years. The limited prognostic value of hormone receptors suggests that the prognostic molecular markers used in age-unspecified breast cancer may not be completely suitable for this young population.

Take-home messages

- High Ki67 expression, human epidermal growth factor receptor 2 (HER2) overexpression and *TP53* mutations are strong predictors of poor prognosis in women ≤35 years with breast cancer.
- The prognostic value of oestrogen receptors is only marginal, and progesterone receptor expression and *PIK3CA* mutations are not associated with survival in the young population.
- The common prognostic molecular markers used in age-unspecified breast cancer may not be completely suitable for women ≤35 years.

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Competing interests None declared.

Patient consent Obtained.

Ethics approval The study was approved by the institutional review board of the National Taiwan University Hospital (NTUH).

Contributors CHL has made substantial contributions to conception, experimental design, data analysis and manuscript writing of this study. YSL and CSH conceived and designed the study. SLY was responsible for the statistical analysis. DYC participated in the experimental design. KTK performed analysis of TP53 and PIK3CA mutations. CCW performed immunohistochemical analysis of Ki67 and P53 protein expressions. PHL collected the family history and the results of BRCA1 and BRCA2 mutations. WHK and KJC supplied tissue samples and collected clinical data. ALC participated in the conception and design of the study, guided the data analysis and manuscript preparation, and reviewed the manuscript. All authors read and approved the final manuscript.

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REFERENCES

1. **Shannon C,** Smith IE. Breast cancer in adolescents and young women. *Eur J Cancer* 2003;**39**:2632–42.
2. **Albain KS,** Allred DC, Clark GM. Breast cancer outcome and predictors of outcome: are there age differentials? *J Natl Cancer Inst Monogr* 1994:35–42.
3. **Winchester DP,** Osteen RT, Menck HR. The National Cancer Data Base report on breast carcinoma characteristics and outcome in relation to age. *Cancer* 1996;**78**:1838–43.
4. **Nixon AJ,** Neuberg D, Hayes DF, *et al*. Relationship of patient age to pathologic features of the tumor and prognosis for patients with stage I or II breast cancer. *J Clin Oncol* 1994;**12**:888–94.
5. **Kim JK,** Kwak BS, Lee JS, *et al*. Do very young Korean breast cancer patients have worse outcomes? *Ann Surg Oncol* 2007;**14**:3385–91.
6. **Aebi S,** Gelber S, Castiglione-Gertsch M, *et al*. Is chemotherapy alone adequate for young women with oestrogen-receptor-positive breast cancer? *Lancet* 2000;**355**:1869–74.
7. **Goldhirsch A,** Gelber RD, Yothers G, *et al*. Adjuvant therapy for very young women with breast cancer: need for tailored treatments. *J Natl Cancer Inst Monogr* 2001:44–51.
8. **Bentzon N,** During M, Rasmussen BB, *et al*. Prognostic effect of estrogen receptor status across age in primary breast cancer. *Int J Cancer* 2008;**122**:1089–94.
9. **Agrup M,** Stal O, Olsen K, *et al*. C-erbB-2 overexpression and survival in early onset breast cancer. *Breast Cancer Res Treat* 2000;**63**:23–9.
10. **Guerra I,** Algorta J, Diaz de Otazu R, *et al*. Immunohistochemical prognostic index for breast cancer in young women. *Mol Pathol* 2003;**56**:323–7.
11. **Gonzalez-Angulo AM,** Broglio K, Kau SW, *et al*. Women age < or = 35 years with primary breast carcinoma: disease features at presentation. *Cancer* 2005;**103**:2466–72.
12. **Pharoah PD,** Day NE, Caldas C. Somatic mutations in the p53 gene and prognosis in breast cancer: a meta-analysis. *Br J Cancer* 1999;**80**:1968–73.
13. **Borresen-Dale AL.** TP53 and breast cancer. *Hum Mutat* 2003;**21**:292–300.
14. **Samuels Y,** Wang Z, Bardelli A, *et al*. High frequency of mutations of the PIK3CA gene in human cancers. *Science* 2004;**304**:554.
15. **Gustin JP,** Cosgrove DP, Park BH. The PIK3CA gene as a mutated target for cancer therapy. *Curr Cancer Drug Targets* 2008;**8**:733–40.

16. **Lin CH,** Liau JY, Lu YS, *et al*. Molecular subtypes of breast cancer emerging in young women in Taiwan: evidence for more than just westernization as a reason for the disease in Asia. *Cancer Epidemiol Biomarkers Prev* 2009;**18**:1807—14.
17. **Thurlimann B,** Keshaviah A, Coates AS, *et al*. A comparison of letrozole and tamoxifen in postmenopausal women with early breast cancer. *N Engl J Med* 2005;**353**:2747—57.
18. **Geyer CE,** Forster J, Lindquist D, *et al*. Lapatinib plus capecitabine for HER2-positive advanced breast cancer. *N Engl J Med* 2006;**355**:2733—43.
19. **Penault-Llorca F,** Andre F, Sagan C, *et al*. Ki67 expression and docetaxel efficacy in patients with estrogen receptor-positive breast cancer. *J Clin Oncol* 2009;**27**:2809—15.
20. **Done SJ,** Eskandarian S, Bull S, *et al*. p53 missense mutations in microdissected high-grade ductal carcinoma in situ of the breast. *J Natl Cancer Inst* 2001;**93**:700—4.
21. **Deng G,** Chen LC, Schott DR, *et al*. Loss of heterozygosity and p53 gene mutations in breast cancer. *Cancer Res* 1994;**54**:499—505.
22. **Kalinsky K,** Jacks LM, Heguy A, *et al*. PIK3CA mutation associates with improved outcome in breast cancer. *Clin Cancer Res* 2009;**15**:5049—59.
23. **Stuart-Harris R,** Caldas C, Pinder SE, *et al*. Proliferation markers and survival in early breast cancer: a systematic review and meta-analysis of 85 studies in 32,825 patients. *Breast* 2008;**17**:323—34.
24. **Goldhirsch A,** Ingle JN, Gelber RD, *et al*. Thresholds for therapies: highlights of the St Gallen International Expert Consensus on the primary therapy of early breast cancer 2009. *Ann Oncol* 2009;**20**:1319—29.
25. **Barbareschi M.** Prognostic value of immunohistochemical expression of p53 in breast carcinomas. A review of the literature involving over 9,000 patients. *Appl Immunohistochem* 1996;**4**:106—16.
26. **Owens MA,** Horten BC, Da Silva MM. HER2 amplification ratios by fluorescence in situ hybridization and correlation with immunohistochemistry in a cohort of 6556 breast cancer tissues. *Clin Breast Cancer* 2004;**5**:63—9.
27. **Li SY,** Rong M, Grieu F, *et al*. PIK3CA mutations in breast cancer are associated with poor outcome. *Breast Cancer Res Treat* 2006;**96**:91—5.
28. **Stemke-Hale K,** Gonzalez-Angulo AM, Lluch A, *et al*. An integrative genomic and proteomic analysis of PIK3CA, PTEN, and AKT mutations in breast cancer. *Cancer Res* 2008;**68**:6084—91.
29. **Perez-Tenorio G,** Alkhori L, Olsson B, *et al*. PIK3CA mutations and PTEN loss correlate with similar prognostic factors and are not mutually exclusive in breast cancer. *Clin Cancer Res* 2007;**13**:3577—84.
30. **Maruyama N,** Miyoshi Y, Taguchi T, *et al*. Clinicopathologic analysis of breast cancers with PIK3CA mutations in Japanese women. *Clin Cancer Res* 2007;**13**:408—14.
31. **Saal LH,** Holm K, Maurer M, *et al*. PIK3CA mutations correlate with hormone receptors, node metastasis, and ERBB2, and are mutually exclusive with PTEN loss in human breast carcinoma. *Cancer Res* 2005;**65**:2554—9.

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Notes