VOLUME 27 · NUMBER 28 · OCTOBER 1 2009

JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

Prediction of Local Recurrence, Distant Metastases, and Death After Breast-Conserving Therapy in Early-Stage Invasive Breast Cancer Using a Five-Biomarker Panel

Ewan K.A. Millar, Peter H. Graham, Sandra A. O'Toole, Catriona M. McNeil, Lois Browne, Adrienne L. Morey, Sarah Eggleton, Julia Beretov, Constantine Theocharous, Anne Capp, Elias Nasser, John H. Kearsley, Geoff Delaney, George Papadatos, Christopher Fox, and Robert L. Sutherland

From the Cancer Research Program, Garvan Institute of Medical Research, and Department of Pathology (SydPath), St Vincent's Hospital, Darlinghurst; Department of Anatomical Pathology, South Eastern Area Laboratory Service, and Department of Radiation Oncology, Cancer Care Centre, St George Hospital, Kogarah; Department of Medical Oncology, University of Sydney, Westmead Hospital, Westmead; Department of Radiation Oncology, Liverpool Hospital, Liverpool; Department of Radiation Oncology, Wollongong Hospital, Wollongong; St Vincent's Clinical School, University of New South Wales, and University of Western Sydney, Sydney; and Department of Anatomical Pathology, Royal Prince Alfred Hospital, Camperdown, Australia.

Submitted December 23, 2008; accepted April 22, 2009; published online ahead of print at www.jco.org on August 31, 2009.

Supported by grants from the National Health and Medical Research Council of Australia, the Cancer Institute New South Wales, the Petre Foundation, and the R.T. Hall Trust.

Authors' disclosures of potential conflicts of interest and author contributions are found at the end of this article.

Corresponding author: Ewan K.A. Millar, MD, Cancer Research Program, Garvan Institute of Medical Research, 384 Victoria St, Darlinghurst, New South Wales 2010, Australia; e-mail: e.millar@garvan.org.au.

The Acknowledgment is included in the full-text version of this article, available online at www.jco.org. It is not included in the PDF version (via Adobe® Reader®).

0732-183X/09/2728-4701/$20.00

DOI: 10.1200/JCO.2008.21.7075

ABSTRACT

Purpose
To determine the clinical utility of intrinsic molecular phenotype after breast-conserving therapy (BCT) with lumpectomy and whole-breast irradiation with or without a cavity boost.

Patients and Methods
Four hundred ninety-eight patients with invasive breast cancer were enrolled into a randomized trial of BCT with or without a tumor bed radiation boost. Tumors were classified by intrinsic molecular phenotype as luminal A or B, HER-2, basal-like, or unclassified using a five-biomarker panel: estrogen receptor, progesterone receptor, HER-2, CK5/6, and epidermal growth factor receptor. Kaplan-Meier and Cox proportional hazards methodology were used to ascertain relationships to ipsilateral breast tumor recurrence (IBTR), locoregional recurrence (LRR), distant disease-free survival (DDFS), and death from breast cancer.

Results
Median follow-up was 84 months. Three hundred ninety-four patients were classified as luminal A, 23 were luminal B, 52 were basal, 13 were HER-2, and 16 were unclassified. There were 24 IBTR (4.8%), 35 LRR (7%), 47 distant metastases (9.4%), and 37 breast cancer deaths (7.4%). The overall 5-year disease-free rates for the whole cohort were: IBTR 97.4%, LRR 95.6%, DDFS 92.9%, and breast cancer–specific death 96.3%. A significant difference was observed for survival between subtypes for LRR ($P = .012$), DDFS ($P = .0035$), and breast cancer–specific death ($P = .0482$), but not for IBTR ($P = .346$).

Conclusion
The 5-year and 10-year survival rates varied according to molecular subtype. Although this approach provides additional information to predict time to IBTR, LRR, DDFS, and death from breast cancer, its predictive power is less than that of traditional pathologic indices. This information may be useful in discussing outcomes and planning management with patients after BCT.

J Clin Oncol 27:4701-4708. © 2009 by American Society of Clinical Oncology

INTRODUCTION

Breast conserving therapy (BCT) is the preferred option in the management of early breast cancer (EBC). Surgical excision to clear margins combined with local radiotherapy can successfully lower the rate of local recurrence to approximately 5% at 5 years.[1] However, prediction of outcome for individual patients is uncertain and the development of new biomarkers to guide clinical decision making is needed. Specifically, no established biomarkers that predict ipsilateral breast tumor recurrence (IBTR) after BCT have been validated. Current radiotherapy regimens typically include treatment of the whole breast with 40 to 50 Gy with or without a cavity boost of 16 Gy. The importance of optimal local control is highlighted by meta-analysis which shows that overall mortality is reduced with improved local control, which equates to one fewer death for every four local recurrences prevented after 5 years.[2] IBTR has a significant impact on overall survival with 5-year survival rate of approximately 60%.[1]

Gene expression profiling has identified breast cancer subtypes with five main gene expression profiles,[3,4] which divides patients into groups with distinct tumor phenotypes and outcomes.[5] Recent immunohistochemical validation of these intrinsic

molecular phenotypes have suggested that a five-marker panel of estrogen receptor (ER), progesterone receptor (PR), HER-2, CK5/6, and epidermal growth factor receptor (EGFR) can predict distant failure and death.[6,7] A recent study of conservatively treated breast cancer confirmed the value of this approach in predicting IBTR, using a simplified panel of ER, PR, and HER-2.[8] Further refining this classification and translating these features into a useful panel for routine pathology is a priority. However, it is now believed that the triple-negative phenotype (TNP; ie, ER−, PR−, and HER2−) is a heterogeneous group comprising basal-like, normal-like breast cancer, and other unclassified breast cancers and does not equate with a basal-like phenotype[9] as originally suggested. Positive expression of basal markers are needed to better define this group of tumors, as evidenced by a recent study showing that the poor prognosis of TNP tumors was conferred by those tumors expressing basal markers CK5/6 and/or EGFR with a specificity of 100% and a sensitivity of 76%.[7]

Among women with EBC, management decisions regarding local therapy are generally made without regard to breast cancer subtype and more refined data regarding risk of local and distant failure would allow for better patient-specific tailoring of therapy. However, identifying risk factors for failure in BCT is problematic as current local recurrence rates in early-stage breast cancer are low and therefore large numbers of patients are required for sufficient statistical power to detect a significant difference.

In this study, we compared the clinical utility of intrinsic molecular phenotype as assessed by the five-biomarker panel, ER, PR, HER-2, CK 5/6, and EGFR with traditional pathologic indices, in predicting local or distant failure and death in conservatively treated EBC.

Table 1. Patient Baseline Characteristics, Treatments, and Outcomes

Characteristic	Patients No.	Patients %	Median	Range
Length of follow-up, months	498		84	1-134
Age, years			61	24-84
Tumor size, mm			16	1-60
T1a (1-5)	4	0.8		
T1b (6-10)	77	16.3		
T1c (11-20)	270	54.2		
T2 (21-50)	136	27.3		
T3 (> 50)	1	0.2		
Tumor grade				
1	167	33.5		
2	185	37.1		
3	145	29.1		
Lymph node metastases	146	29.3		
N0	339	69.9		
N1 (1-3)	128	25.7		
N2 (4-10)	17	3.5		
N3 (> 10)	2	0.4		
LN unsampled	12	2.4		
ER+	393	78.9		
PR+	334	68.3		
HER-2 amplified (FISH)	36	7.2		
Subtype				
Luminal A	394	79.1		
Luminal B	23	4.6		
Basal-like	52	10.4		
HER-2	13	2.6		
Unclassified	16	3.2		
Triple negative	68	13.6		
Treatment and outcome				
Margin+	17	3.4		
Cavity boost positive	247	49.5		
Cavity boost negative	251	50.5		
Endocrine therapy	223	44.7		
Chemotherapy	117	23.4		
Endocrine and chemotherapy	48	9.6		
Patients with IBTR	24	4.8		
Patients with LRR	35	7		
Patients with distant metastases	47	9.4		
Breast cancer–specific deaths	37	7.4		
5-year survival				
IBTR free		97.4		
LRR free		95.6		
DDFS		92.9		
Breast cancer–specific		96.3		

Abbreviations: ER, estrogen receptor; PR, progesterone receptor; FISH, fluorescent in situ hybridization; IBTR, ipsilateral breast tumor recurrence; LRR, locoregional recurrence; DDFS, distant disease free survival.

PATIENTS AND METHODS

Patient Selection

All patients were enrolled into a randomized clinical trial that compared the benefit of the addition of a local cavity boost of radiotherapy to BCT. The complete study cohort included 688 women with breast cancer, 629 of whom had invasive carcinoma, with an additional 59 patients with ductal carcinoma in situ (Clinical Trials Registry NCT00138814). Formalin-fixed paraffin-embedded tissue blocks were available for 498 patients with invasive carcinoma. The clinicopathologic characteristics of this cohort are summarized in Table 1. HER-2 status was unknown at the time of treatment. Seventy-three percent of patients were postmenopausal, 20% were premenopausal, and 7% were perimenopausal. This study was approved by the Human Research Ethics Committee of St George Hospital, Sydney, Australia (ref No: 96/84).

Treatment

All patients with invasive carcinoma received local excision and axillary sentinel node biopsy or axillary clearance. Adjuvant chemotherapy (adriamycin and cyclophosphamide, or cyclophosphamide, methotrexate, and 5-fluorouracil) was given to 23.7% of patients and 44.9% received adjuvant tamoxifen. No patients received adjuvant trastuzumab. Patients were randomly assigned to whole-breast radiotherapy of 50 Gy in 25 fractions or whole-breast radiotherapy of 45 Gy in 25 fractions plus a tumor bed boost of 16 Gy in 8 fractions. Supraclavicular fields were not added unless there were four or more nodes positive. Seventeen patients had positive margins (clearance = 0 mm), 65 had clearance of smaller than 1 mm, and an additional 86 had smaller than 2 mm clearance, the remainder being well clear.

Follow-Up

Patients were assessed 6 weeks after radiation therapy, every 6 months for 2 years, then annually thereafter with annual breast imaging. Follow-up time was calculated from the date of the first surgical procedure to the date of the first event (see End Points) or to the last known confirmed date of breast cancer disease-free status. Median follow-up was 84 months (range, 1 to 134 months).

End Points

The primary end point was time to ipsilateral breast tumor recurrence (IBTR). This included any ipsilateral in-breast recurrence (invasive or noninvasive). The secondary end points were locoregional recurrence (LRR, which included patients with IBTR and regional recurrences in the axilla, chest wall, internal mammary, or supraclavicular fossa lymph nodes) and time to distant metastases and death.

Table 2. Patient Tumor Characteristics and Event Rates Classified by Intrinsic Molecular Phenotype

Patient Tumor Characteristics	Whole Cohort		Luminal A		Luminal B		Basal		HER-2		Unclassified	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
No. of patients	498		394	79.1	23	4.6	52	10.4	13	2.6	16	3.2
Size < 20 mm	357	70.3	289	73.4	17	73.9	31	59.6	7	53.8	7	43.7
LVI+	79	15.8	62	15.7	4	17.4	9	17.3	2	15.3	2	12.5
LN+	146	29.0	117	29.6	5	21.7	13	25	8	61.5	4	25
Grade 3	145	29.1	65	16.5	16	69.5	47	90.3	9	69.2	8	50
EIC+	45	9.0	29	7.4	5	21.7	6	11.5	3	23.1	1	6.3
Median age, years	61		62		57		54		53		50	
Events												
Median follow-up	84		83.5		71		85		83		72.5	
IBTR	24	4.8	15	3.8	2	8.7	5	9.6	1	7.6	1	6.3
LRR	35	7	20	5.1	2	8.7	9	17.3	2	15.4	2	12.5
DDFS	47	9.4	30	7.6	2	8.7	8	15.3	2	15.4	5	31
Breast cancer death	37	7.4	23	5.8	2	8.7	7	13.5	2	15.4	3	18.8

Abbreviations: LVI, lymphatic/vascular invasion; LN+, lymph node positive; EIC, extensive intraduct carcinoma; IBTR, ipsilateral breast tumor recurrence; LRR, locoregional recurrence; DDFS, distant disease–free survival.

Tissue Microarray Construction Immunohistochemistry and Fluorescent In Situ Hybridization

Immunohistochemistry for ER, PR, CK 5/6, and EGFR was performed on tissue microarrays (TMAs), assessed by one breast pathologist (E.K.A.M.) blinded to clinical outcome. Tumors were considered HER-2 positive if amplified on fluorescent in situ hybridization (FISH) using a HER-2: chromosome 17 ratio higher than 2.2.

Classification of Intrinsic Molecular Phenotype

Patients were categorized based on the status of their primary tumor as previously described[10]: luminal A (ER+ and/or PR+ and HER2–), luminal B (ER+ and/or PR+ and HER-2+), HER-2 (ER− and PR− and HER-2+), and basal (ER− and PR−, HER-2−, CK 5/6+, and/or EGFR+), unclassified (negative for all five markers). The TNP was assigned on the basis of ER−, PR−, HER-2−.

Statistical Analysis

Kaplan-Meier analyses for IBTR, LRR, distant disease-free survival (DDFS), and breast cancer–specific death were estimated for each subtype and compared using the log-rank test. Crude rates of survival by subtype for each end point were also calculated at 5 and 10 years. We used Cox proportional hazards univariate analysis to analyze the association between prognostic variables and molecular subtype with IBTR, LRR, metastases, and breast cancer–specific death. Those variables significant in univariate analysis were used in multivariate analysis to construct models identifying variables which were independently prognostic and not the result of confounding factors. Subsequently step-wise removal of redundant variables was employed until resolution. Further analyses characterized how IBTR influenced DDFS and mortality using Kaplan-Meier analysis where survival times were reported using the times from the IBTR event until distant disease or death. Patients who developed distant metastases within 3 months of IBTR were excluded from the analysis. Five-year results were reported for these end points. All analyses were performed using Statview 5.0 (Abacus Systems, Berkeley, CA). Competing risks proportional hazards models (Fine and Gray) were constructed using ACCoRD software (http://boffinsoftware.com).

RESULTS

IBTR

The clinicopathologic characteristics, number of events, crude rates, and median follow-up within each subtype of invasive cancer are summarized in Table 2, median times to event in Table 3, and crude rates at 5 and 10 years in Table 4. At a median follow-up of 84 months IBTR was observed in 24 patients. The 5-year recurrence-free rate was 97.4% for the whole cohort, 98.8% for luminal A, 95.5% for luminal B, 90% for basal, 92.3% for HER-2, and 92.9% for unclassified. Consistent with the overall randomized 6-year analysis,[11] in this biomarker study cohort, no reduction in IBTR was observed in patients treated with a radiotherapy boost either in the whole cohort ($P = .214$ or between subtypes). The luminal A phenotype was associated with a lower rate of IBTR, compared to all other groups at 5 and 10 years (Table 4; hazard ratio [HR], 0.433; 95% CI, 0.186 to 1.005; $P = .051$; Table 5). Kaplan-Meier analysis comparing survival of all five molecular subtypes was not statistically significant ($P = .346$; Fig 1A). The median times to event (Table 3) was significantly shorter for basal, unclassified, and HER-2 compared to luminal A and B. The only variables that predicted IBTR in univariate and multivariate analysis were grade 3 (HR, 3.372; 95% CI, 1.488 to 7.642; $P = .004$) and positive margins (HR, 5.838; 95% CI, 1.690 to 20.172; $P = .005$, Tables 5 and 6). Of the 24 patients with IBTR, eight recurrences were located in the same quandrant as the primary tumor (four luminal A, three basal, one HER-2) and 16 were elsewhere (11 luminal A, two luminal B, two basal, one unclassified). All 17 patients with positive margins were luminal A.

Table 3. Median Time to Event, in Months, According to Molecular Subtype

Event	Whole Cohort	Luminal A	Luminal B	Basal	HER2	Unclassified
IBTR	60	80.5	78	20	23	30
LRR	49	72	78	26	25	28.5
DDFS	33	44	59	23	25	27
Breast cancer death	61	66	94.5	23	33	18

Abbreviations: IBTR, ipsilateral breast tumor recurrence; LRR, locoregional recurrence; DDFS, distant disease–free survival.

Table 4. 5- and 10-Year Event Rates According to Molecular Phenotype

	IBTR				LRR				DDFS				Breast Cancer–Specific Death			
	5 Year		10 Year		5 Year		10 Year		5 Year		10 Year		5 Year		10 Year	
Parameter	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
No. of patients	24				35				47				37			
Whole cohort (n = 498)																
	12/498	2.4	23/498	4.6	21/498	4.2	35/498	6.8	34/498	6.8	47/498	9.4	18/498	3.6	37/498	7.4
	12/24	50	23/24	95.8	21/35	60	35/35	100	34/47	72.3	47/47	100	18/37	48.6	37/37	100
Luminal A (n = 394)																
	4/394	1	14/394	3.6	8/394	2	19/394	4.8	19/394	4.8	30/394	7.6	7/394	1.8	23/394	5.8
	4/15	26.6	14/15	93.3	8/20	40	19/20	95	19/30	63.3	30/30	100	7/23	30.4	23/23	100
Luminal B (n = 23)																
	1/23	4.3	2/23	8.7	1/23	4.3	2/23	8.6	2/23	8.6	2/23	8.6	1/23	4.3	2/23	8.6
	1/2	50	2/2	100	1/2	50	2/2	100	2/2	100	2/2	100	1/2	50	2/2	100
Basal (n = 52)																
	5/52	9.6	5/52	9.6	8/52	14.8	9/52	17.3	7/52	13.5	8/52	14.8	6/52	11.5	7/52	13.5
	5/5	100	5/5	100	8/9	88.8	9/9	100	7/8	87.5	8/8	100	6/7	85.7	7/7	100
HER-2 (n = 13)																
	1/13	7.7	1/13	7.7	2/13	15.3	2/13	15.3	2/13	15.3	2/13	15.3	2/13	15.3	2/13	15.3
	1/1	100	1/1	100	2/2	100	2/2	100	2/2	100	2/2	100	2/2	100	2/2	100
Unclassified (n = 16)																
	1/16	6.3	1/16	6.3	2/16	12.6	2/16	12.6	4/16	25	5/16	31.3	2/16	12.6	3/16	18.8
	1/1	100	1/1	100	2/2	100	2/2	100	4/5	80	5/5	100	2/3	66.7	3/3	100

NOTE. Rates are presented relating to the whole cohort and molecular subtype and as the relative rate of all events for each subtype.
Abbreviations: IBTR, ipsilateral breast tumor recurrence; LRR, locoregional recurrence; DDFS, distant disease–free survival.

LRR

Thirty-five patients developed LRR with a 5-year disease-free rate of 95.6% for the whole cohort, luminal A of 99%, basal of 93.7%, HER-2 of 84.6%, and unclassified of 92.9% with a significant difference in survival between subtypes (Fig 1B). Crude recurrence rates of luminal A were less than one third of those of basal (5% *v* 17.3%; Table 5). In a resolved multivariate model, grade 3 (HR, 3.365; 95% CI, 1.848 to 7.151; $P = .001$), lymph node positivity (HR, 1.986; 95% CI, 1.01 to 3.906; $P = .047$) and extensive intraduct carcinoma (HR, 3.212; 95% CI, 1.382 to 7.463; $P = .007$) were independently predictive of LRR.

DDFS

Forty-seven patients developed distant metastases with a 5-year DDFS rate for the whole cohort of 92.9%, luminal A of 95%, luminal B of 90%, basal of 86.3%, HER-2 of 84.6%, and unclassified of 75%. Luminal A had a crude event rate of less than half that of basal, HER-2, and unclassified tumors (Table 4), with a statistically significant difference in survival between subtypes ($P = .0035$, Fig 1C). Lymph node positivity (HR, 3.558; 95% CI, 1.937 to 6.536; $P < .001$), lymphatic invasion (HR, 1.977; 95% CI, 1.054 to 3.710; $P = .034$), grade 3 (HR, 1.912; 95% CI, 1.046 to 3.495; $P = .035$), and PR (HR, 0.523; 95% CI, 0.287 to 0.952; $P = .034$) were independently significant in a resolved multivariate analysis (Table 7).

Breast Cancer–Specific Death

There were 37 deaths attributable to breast cancer with a 5-year breast cancer–specific survival for the whole cohort of 96.3%, luminal A of 98.2%, luminal B of 95.7%, basal of 88.3%, HER-2 of 84.6%, and unclassified of 87.5%. Luminal A (5.8%) had a crude death rate less than half that of basal (13.5%) and less than one third of HER-2 (15.4%) and unclassified (18.8%), with a statistically significant difference in survival between subtypes ($P = .048$; Fig 1D). PR (HR, 0.369; 95% CI, 0.189 to 0.722; $P = .004$), size larger than 20 mm (HR, 2.178; 95% CI, 1.050 to 4.521; $P = .037$), lymph node involvement (HR, 3.984; 95% CI, 1.850 to 8.851; $P = .001$), lymphatic invasion (HR, 2.858; 95% CI, 1.336 to 6.113; $P = .007$), and IBTR (HR, 3.608; 95% CI, 1.341 to 9.706; $P = .011$) were significant in a resolved multivariate analysis (Table 8).

Competitive Risks Modeling

Further analyses showed no significant alteration in the final resolved models presented above (Tables 6-8) for IBTR, DDFS and breast cancer–specific death. For LRR the final resolved model contained grade 3 (HR 3.423, 95% CI 1.746-6.709, $P < .001$) and extensive intraduct carcinoma (HR 2.950, 1.250-6.963, $P = .014$) with lymph nodal status no longer significant (HR 1.748, 0.890-3.433, $P = .105$).

DDFS and Overall Survival After IBTR and Effect of Subtype, Endocrine Therapy, and Chemotherapy on Outcome

Two of 24 patients developed distant metastases within 3 months of IBTR and were not included in this analysis. After IBTR, the 5-year DDFS rate was 81% and breast cancer–specific survival rate was 77.3%. IBTR did not predict distant metastases but was significant for breast cancer–specific death in univariate and multivariate analysis (HR, 3.608; 95% CI, 1.341 to 9.706; $P = .011$; Table 8).

No association between subtype, treatment, and outcome was observed.

Table 5. Univariate Crude Rates and Cox Analysis for IBTR, LRR, DDFS, and Breast Cancer–Specific Death

	IBTR (n = 24)				LRR (n = 35)				DDFS (n = 47)				Breast Cancer–Specific Death (n = 37)			
	Crude Rate				Crude Rate				Crude Rate				Crude Rate			
Parameter	No.	%	HR	*P*	No.	%	HR	*P*	No.	%	HR	*P*	No.	%	HR	*P*
ER+	16/393	4.1	0.564	.195	21/393	5.3	0.401	**.009**	29/393	7.4	0.427	**.005**	22/393	5.6	0.398	**.006**
PR+	15/334	4.5	0.810	.622	19/334	5.7	0.559	.089	23/334	6.9	0.450	**.006**	17/334	5.1	0.395	**.005**
G3	13/145	8.9	3.025	**.007**	20/145	13.8	3.441	**.001**	24/145	16.6	2.632	**.001**	21/145	14.5	3.270	**.001**
LN+	11/146	7.5	2.041	.082	15/146	10.3	1.791	.089	29/146	19.9	4.046	**< .001**	24/146	16.4	5.068	**< .001**
LVI	4/79	5	1.208	.731	8/79	10.1	1.752	.166	16/79	20.2	3.026	**.001**	15/79	18.9	3.966	**< .001**
Size	10/147	6.8	1.906	.125	13/147	8.8	1.531	.228	24/147	16.3	2.614	**.001**	23/147	15.6	4.011	**< .001**
HER2+	3/36	8.3	1.661	.421	4/36	11.1	1.544	.421	4/36	11.1	1.218	.706	4/36	11.1	1.611	.368
Age < 50 years	8/102	7.8	1.998	.114	12/102	11.8	2.085	**.041**	14/102	13.7	1.635	.123	13/102	12.7	1.959	.051
EIC+	4/45	8.8	1.887	.249	7/45	15.6	2.474	**.033**	—	—	—	—	—	—	—	—
Marg+	3/17	17.6	4.437	**.016**	4/38	10.5	1.744	.282	—	—	—	—	—	—	—	—
Boost+	15/247	6.1	1.674	.226	19/247	7.7	1.195	.599	—	—	—	—	—	—	—	—
Endo	7/223	3.1	0.545	.181	11/223	4.9	0.587	.146	18/223	8.1	0.774	.393	15/223	6.7	0.876	.692
Chemo	9/117	7.7	1.921	.128	13/117	11.1	1.919	.066	19/117	16.2	2.379	**.004**	18/117	15.4	3.194	**.001**
IBTR	—	—	—	—	—	—	—	—	4/21	19	2.281	.116	5/22	22.7	3.602	**.008**
LA	15/394	3.8	0.433	**.051**	20/394	5	0.333	**.002**	30/394	7.6	0.446	**.008**	23/394	5.8	0.414	**.009**
LB	2/23	8.7	2.132	.307	2/23	8.7	1.365	.669	2/23	8.7	0.963	.958	2/23	8.7	1.258	.753
Basal	5/52	9.6	2.182	.126	9/52	17.3	3.025	**.005**	8/52	15.4	1.745	.151	7/52	13.5	1.662	.108
HER2	1/13	7.7	1.056	.959	2/13	15.4	1.725	.475	2/13	15.4	1.612	.509	2/13	15.4	2.103	.307
Uncls	1/16	6.2	1.905	.530	2/16	12.5	2.621	.188	5/16	31.2	4.533	**.001**	3/16	18.8	3.419	**.042**
TNP	6/68	8.8	2.227	.093	11/68	16.2	3.194	**.002**	13/68	19.1	2.538	**.004**	10/68	14.7	2.446	**.016**

Abbreviations: IBTR, ipsilateral breast tumor recurrence; LRR, locoregional recurrence; DDFS, distant disease-free survival; HR, hazard ratio; ER, estrogen receptor; PR, progesterone receptor; G3, grade 3; LN+, lymph node positive; LVI, lymphatic vascular invasion; Size, tumor size > 20 mm; EIC, extensive intraduct component; Marg+, resection margin positive; Boost+, cavity boost of 16 Gy given; endo, endocrine therapy; chemo, chemotherapy; LA, luminal A; LB, luminal B; uncls, unclassified; TNP, triple negative phenotype. Bold indicates significance.

DISCUSSION

The importance of achieving optimal local control in BCT is highlighted by its association with improved overall survival; conversely, IBTR is a poor prognostic indicator for subsequent distant failure and death.[12-14] The recent trend of progressive decline in local recurrence rates to approximately 5% is likely the result of several factors, including improved preoperative breast imaging, greater emphasis on pathological margin assessment, achieving clear surgical margins, and more frequent use of adjuvant systemic therapies. Local failure has been associated with young age (< 50), tumor size (> T2), negative hormone receptor status, and lymph node involvement, although these vary between studies,[15-18] and an algorithm to define risk of IBTR has been described.[19] However, there is a need to improve predictive and prognostic information to better tailor discussion regarding recurrence risk and hence treatments to individual patients.[20]

The subclassification of breast cancer into five main intrinsic subtypes correlates with outcome but there are limited data available regarding its predictive value. Specific subtypes, such as basal-like cancers, have no specific targeted therapy, unlike ER+ and HER-2+ disease, and their identification is important for therapeutic decision making. The recent validation of the intrinsic molecular signature using a panel of five markers, ER, PR, HER-2, CK 5/6, and EGFR, demonstrated its superiority over ER, PR, and HER-2 (TNP) alone, as it identifies basal-like tumors with a specificity of 100% and sensitivity of 75%, compared with classification by gene expression profiling.[7] Specifically, the inclusion of the latter two antigens as basal markers is now important as triple-negative status alone is not synonymous with this group of tumors.[9]

We applied the five-biomarker panel to assess its predictive and prognostic value in locally treated EBC in a clinical trial setting. We found very low rates of IBTR with 97.4% recurrence-free survival at 5 years for the whole cohort, which is predominantly comprised of luminal A cancers (79.1%) with small components of luminal B (4.6%), basal-like (10.4%), HER-2 (2.6%), and unclassified (3.2%). Our Australian cohort is similar to a population-based study from North America[7] (64.8% luminal A, 5.5% luminal B, 6.4% HER-2, 9% basal, 8% unclassified), although we have lower rates of HER-2, luminal B and unclassified cancers, which may reflect the selection of cases in this trial setting. We found that molecular subtype was associated with differences in IBTR, LRR, DDFS, and breast cancer–specific death, although this was not significant for IBTR, which is likely the result of insufficient numbers of events. Our 5-year subtype IBTR-free rates (98.8% luminal A, 95.5% luminal B, 92.3% HER-2, 90% basal, 92.9% unclassified) are similar to those described in a recent study which utilized this approach with a simplified triple-marker assessment of ER, PR, and HER-2.[8] This latter cohort of 793 patients was of similar composition to ours, but their use of the triple assessment method for classification did not require positive basal marker expression and did not include an unclassified group. Two other studies that also examined the predictive utility of the TNP and IBTR in BCT did not find an association, compared with non–triple-negative cancers[21,22] although the mean time to local recurrence was shortened (2.8 *v* 4.2 years).[22] Using the TNP as a classifier, our cohort contained

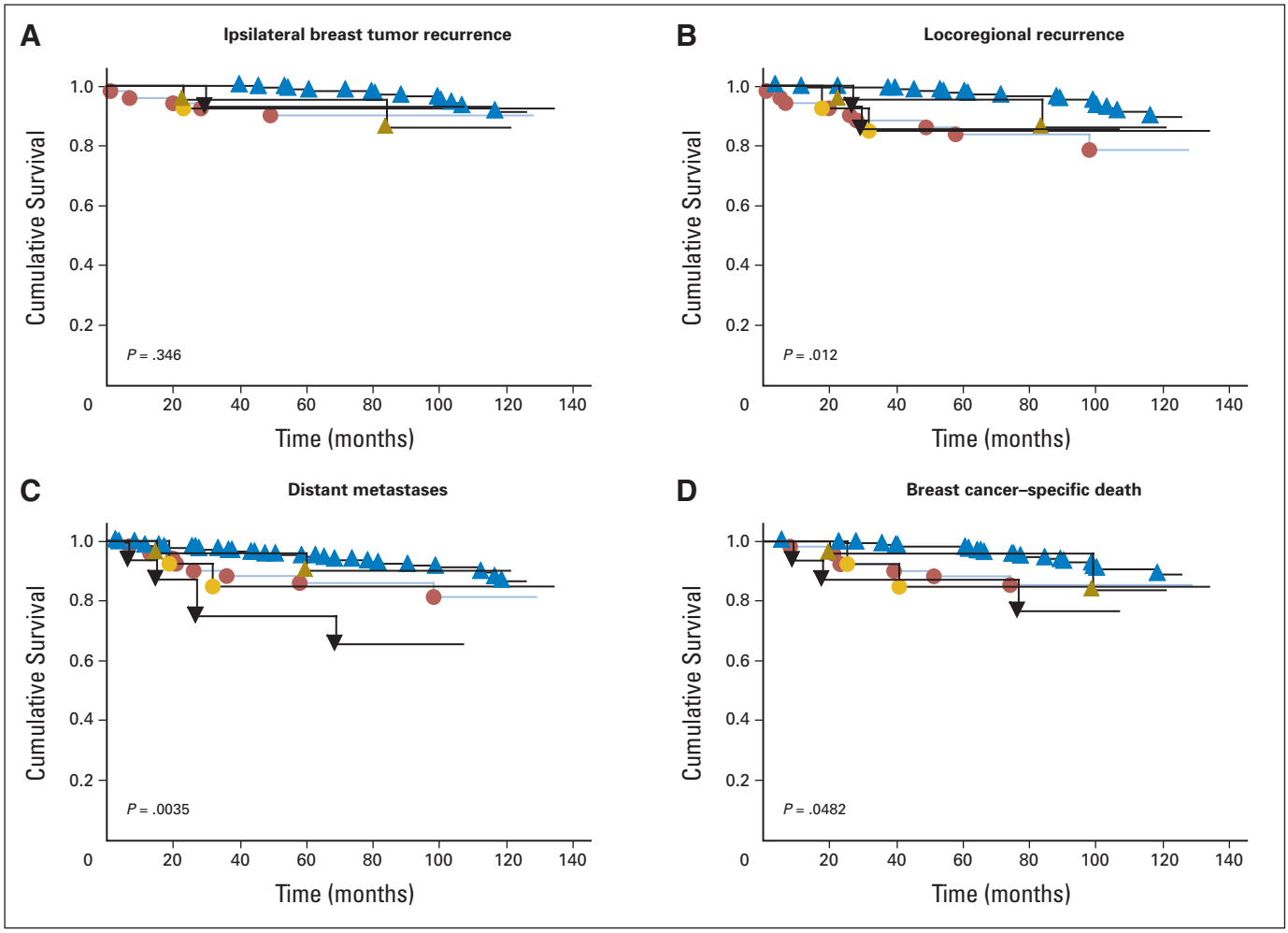

Fig 1. Kaplan-Meier estimates (log-rank test) for (A) ipsilateral breast tumor recurrence (24 events), (B) locoregional recurrence (35 events), (C) distant metastases (47 events), and (D) breast cancer–specific death (37 events), according to intrinsic molecular subtype. Luminal A (blue triangle; n = 394), luminal B (yellow triangle; n = 23), basal (red circle; n = 52), HER2 (yellow circle; n = 13), unclassified (black triangle; n = 16).

68 (13.6%) of 498, but there was no association with IBTR, although it predicted LRR, distant metastases, and death, and it out-performed the basal group. In a large Danish cohort of lymph node–positive patients treated with mastectomy, TNP was significantly associated with LRR whether treated with radiotherapy or not.[23]

Tumor subtype identifies groups with divergent behavior associated with differing recurrence rates, times to event, and overall survival. Significant differences between groups was observed in terms of median time to event for all measures of outcome with greatly

Table 6. Cox Proportional Hazards Multivariate Model for IBTR

Variable	HR	95% CI	*P*
ER+	0.831	0.260 to 2.649	.754
PR+	1.152	0.390 to 3.404	.798
Grade 3	3.206	1.281 to 8.024	**.013**
Size > 20 mm	1.214	0.510 to 2.888	.661
LN+	1.952	0.826 to 4.614	.128
LVI	0.804	0.262 to 2.470	.704
Margin+	4.508	1.248 to 16.285	**.022**
Resolved model			
Grade 3	3.372	1.488 to 7.642	**.004**
Margin+	5.838	1.690 to 20.172	**.005**

Abbreviations: IBTR, ipsilateral breast tumor recurrence; HR, hazard ratio; ER, estrogen receptor; PR, progesterone receptor; LN+, lymph node positive; LVI, lymphatic vascular invasion. Bold indicates significance.

Table 7. Cox Proportional Hazards Multivariate Model for DDFS

Variable	HR	95% CI	*P*
ER+	0.559	0.245 to 1.272	.165
PR+	0.631	0.316 to 1.260	.191
Grade 3	1.758	0.876 to 3.530	.112
Size > 20 mm	1.331	0.709 to 2.498	.373
LN +	3.815	2.008 to 7.250	**< .001**
LVI	2.115	1.092 to 4.097	**.026**
Chemotherapy	0.648	0.313 to 1.33	.241
Resolved model			
PR	0.523	0.287 to 0.952	**.034**
Grade 3	1.912	1.046 to 3.495	**.035**
LN+	3.558	1.937 to 6.536	**< .001**
LVI	1.977	1.054 to 3.710	**.034**

Abbreviations: DDFS, distant disease–free survival; HR, hazard ratio; ER, estrogen receptor; PR, progesterone receptor; LN+, lymph node positive; LVI, lymphatic vascular invasion. Bold indicates significance.

Table 8. Cox Proportional Hazards Multivariate Model for Breast Cancer–Specific Death

Variable	HR	95% CI	P
ER+	0.742	0.278 to 1.979	.550
PR+	0.477	0.209 to 1.088	.786
Grade 3	1.978	0.868 to 4.505	.104
Size > 20 mm	1.927	0.905 to 4.104	.089
LN+	3.984	1.850 to 8.581	**.001**
LVI	2.858	1.336 to 6.113	**.007**
Chemotherapy	0.624	0.230 to 1.697	.356
IBTR	2.800	0.994 to 7.889	**.051**
Age < 50 years	1.237	0.502 to 3.049	.643
Resolved model			
PR	0.369	0.189 to 0.722	**.004**
Size > 20 mm	2.178	1.050 to 4.521	**.037**
LN+	3.342	1.597 to 6.993	**.001**
LVI	2.773	1.330 to 5.780	**.007**
IBTR	3.608	1.341 to 9.706	**.011**

Abbreviations: HR, hazard ratio; ER, estrogen receptor; PR, progesterone receptor; LN, lymph node; LVI, lymphatic vascular invasion; IBTR, ipsilateral breast tumor recurrence. Bold inidcates significance.

shortened recurrence times for the more aggressive subtypes: basal, HER-2, and unclassified. However, although they do identify behavioral differences in terms of biology, their role as predictive markers appears to be inferior to traditional pathologic variables such as high grade, tumor size, lymph node status, lymphatic invasion, and hormone receptor status. Luminal A and unclassified groups were associated with improved or poor outcome for LRR, distant metastases, and breast cancer–specific death. For IBTR, only grade 3 and positive margins were of predictive value, although there was a strong trend for reduced risk with luminal A subtype ($P = .051$).

One of the main shortcomings of this study in assessing the predictive value of molecular subtype is the relatively low number of events. This may reflect the very low rate of margin involvement (3.4%), eliminating an important contributing factor for IBTR. Thus, we would expect lower recurrence rates but increased sensitivity to intrinsic biologic predictors of IBTR risk. The relative success of BCT requires large numbers of patients to be accrued to have enough events to provide sufficient statistical power. We have provided results with 5- and 10-year event rates, but in a predominantly luminal A, T1 cohort. Longer follow-up will be needed to assess outcomes at 10 and 15 years. As a result of the relatively small size of the luminal B, HER-2 and unclassified groups (23, 13, and 16 patients, respectively), the confidence interval for outcome estimates for these groups widens. In addition, the relative importance of the luminal B and HER-2 subtypes is lessened by the fact that they would now receive anti-HER-2 therapies which would alter the outcome results.

Molecular classifications of invasive carcinoma have been assessed in predicting IBTR. The Oncotype Dx (Genomic Health Inc, Redwood City, CA) assay which was developed to predict distant failure in ER+ cancers treated with tamoxifen[24] has also been shown to predict risk of local recurrence.[25] This assay and other signatures of grade[26-27] that predict relapse support our finding of an association of grade with IBTR. Several other gene signatures were also recently assessed, with only the Wound signature being significant.[28,29] Thus, the clinical utility of this approach requires further validation in defined patient cohorts.

In summary, this study identifies that the molecular subtype of breast cancer, as approximated by the five-biomarker panel, identifies differences in behavior for IBTR, LRR, DDFS, and death after BCT. This additional information may assist in planning ongoing management and suggests that those more aggressive subtypes that have shorter recurrence times and most events occurring within 5 years (HER-2, basal, and unclassified) should have more frequent breast imaging and follow-up (eg, every 6 months for the first 2 years). In addition for these high risk subtypes, it may also be prudent to consider the addition of a local cavity boost, which was of benefit in three previous randomized studies.[30-32] Although tumor subtype is of less predictive value than existing histopathologic parameters, such as grade and lymph node status, it does provide further information to complement these indices and may be useful in routine practice to help better inform both clinician and patient about their anticipated outcome after BCT.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

AUTHOR CONTRIBUTIONS

Conception and design: Ewan K.A. Millar, Peter H. Graham, Sandra A. O'Toole, Catriona M. McNeil, Lois Browne, Robert L. Sutherland
Provision of study materials or patients: Ewan K.A. Millar, Peter H. Graham, Lois Browne, Sarah Eggleton, Julia Beretov, Constantine Theocharous, Anne Capp, Elias Nasser, John H. Kearsley, Geoff Delaney, George Papadatos, Christopher Fox, Robert L. Sutherland
Collection and assembly of data: Ewan K.A. Millar, Peter H. Graham, Sandra A. O'Toole, Lois Browne, Adrienne L. Morey, Sarah Eggleton, Julia Beretov, Constantine Theocharous, Anne Capp, John H. Kearsley, Robert L. Sutherland
Data analysis and interpretation: Ewan K.A. Millar, Peter H. Graham, Sandra A. O'Toole, Catriona M. McNeil, Lois Browne, Adrienne L. Morey, Robert L. Sutherland
Manuscript writing: Ewan K.A. Millar, Peter H. Graham, Sandra A. O'Toole, Catriona M. McNeil, Lois Browne, Julia Beretov, Constantine Theocharous, Anne Capp, John H. Kearsley, Geoff Delaney, Robert L. Sutherland
Final approval of manuscript: Ewan K.A. Millar, Peter H. Graham, Sandra A. O'Toole, Catriona M. McNeil, Lois Browne, Adrienne L. Morey, Sarah Eggleton, Julia Beretov, Constantine Theocharous, Anne Capp, Elias Nasser, John H. Kearsley, Geoff Delaney, George Papadatos, Christopher Fox, Robert L. Sutherland

REFERENCES

1. Wapnir IL, Anderson SJ, Mamounas EP, et al: Prognosis after ipsilateral breast tumor recurrence and locoregional recurrences in five National Surgical Adjuvant Breast and Bowel Project node-positive adjuvant breast cancer trials. J Clin Oncol 24:2028-2037, 2006

2. Clarke M, Collins R, Darby S, et al: Early Breast Cancer Trialists' Collaborative Group: Effects of radiotherapy and of differences in the extent of surgery for early breast cancer on local recurrence and 15-year survival: An overview of the randomised trials. Lancet 366:2087-2106, 2005

3. Perou CM, Sorlie T, Eisen MB, et al: Molecular portraits of human breast tumors. Nature 406: 747-752, 2000

4. Sorlie T, Perou CM, Tibshirani R, et al: Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. Proc Natl Acad Sci U S A 98:10869-10874, 2001

5. Carey LA, Perou CM, Livasy CA, et al: Race, breast cancer subtypes and survival in the Carolina breast cancer study. JAMA 295:2492-2502, 2006

6. Neilsen TO, Hsu FD, Jensen K, et al: Immunohistochemical and clinical characterization of the basal-like subtype of invasive breast cancer. Clin Cancer Res 10:5367-5374, 2004

7. Cheang MC, Voduc D, Bajdik C, et al: Basal-like breast cancer defined by five biomarkers has superior prognostic value than triple-negative phenotype. Clin Cancer Res 14:1368-1376, 2008

8. Nguyen PL, Taghian AG, Katz MS, et al: Breast cancer subtype approximated by estrogen receptor, progesterone receptor, and HER-2 is associated with local and distant recurrence after breast-conserving therapy. J Clin Oncol 26:2373-2378, 2008

9. Rakha EA, Reis-Filho JS, Ellis IO: Basal-like breast cancer: A critical review. J Clin Oncol 26: 2568-2581, 2008

10. Millar EKA, Anderson LR, McNeil CM, et al: BAG-1 predicts patient outcome and tamoxifen responsiveness in ER-positive invasive ductal carcinoma of the breast. Br J Cancer 100:123-133, 2009

11. Graham P, Browne L, Capp A, et al: The St George, Wollongong and Liverpool Breast boost trial: 1st planned analysis at 6 year median follow-up. Australasian Radiology 51:A85, 2007 (suppl 3)

12. Haffty BG, Fischer D, Beinfield M, et al: Prognosis following local recurrence in the conservatively treated breast cancer patient. Int J Radiat Oncol Biol Phys 21:293-298, 1991

13. Whelan T, Clark R, Roberts R, et al: Ipsilateral breast tumor recurrence post lumpectomy is predictive of subsequent mortality: Results from a randomized trial. Int J Radiat Oncol Biol Phys 30:11-16, 1994

14. Haffty BG, Reiss M, Beinfield M, et al: Ipsilateral breast tumor recurrence as a predictor of distant disease: Implications for systemic therapy at the time of local relapse. J Clin Oncol 14:52-57, 1996

15. Calle R, Vilcoq JR, Zafrani B, et al: Local control and survival of breast cancer treated by limited surgery followed by irradiation. Int J Radiat Oncol Biol Phys 12:873-878, 1986

16. Arriagada R, Le MG, Contesso G, et al: Predictive factors for local recurrence in 2006 patients with surgically resected small breast cancer. Ann Oncol 13:1404-1413, 2002

17. Early Breast Cancer Trialists' Collaborative Group: Favourable and unfavourable effects on long-term survival of radiotherapy for early breast cancer: An overview of the randomised trials. Lancet 355: 1757-1770, 2000

18. Fowble BL, Schultz DJ, Overmoyer B, et al: The influence of young age on outcome in early stage breast cancer. Int J Radiat Oncol Biol Phys 30:23-33, 1994

19. Sanghani M, Balk E, Cady B, et al: Predicting the risk of local recurrence in patients with breast cancer: An approach to a new computer-based predictive tool. Am J Clin Oncol 30:473-480, 2007

20. Graham P, Fourquet A: Placing the boost in breast-conservation radiotherapy: A review of the role, indications and techniques for breast boost radiotherapy. Clin Oncol 18:210-219, 2006

21. Haffty BG, Yang Q, Reiss M, et al: Locoregional relapse and distant metastasis in conservatively managed triple negative early-stage breast cancer. J Clin Oncol 24:5652-5657, 2006

22. Dent R, Trudeau M, Pritchard KI, et al: Triple negative breast cancer: Clinical features and patterns of recurrence. Clin Cancer Res 13:4429-4434, 2007

23. Kyndi M, Sorensen FB, Knudsen H, et al: Estrogen receptor, progesterone receptor, HER-2, and response to post mastectomy radiotherapy in high-risk breast cancer: The Danish Breast Cancer Cooperative Group. J Clin Oncol 26:1419-1426, 2008

24. Paik S, Shak S, Tang G, et al: A multigene assay to predict recurrence of tamoxifen treated, node-negative breast cancer. N Engl J Med 351: 2817-2826, 2004

25. Mamounas EP, Tang G, Bryant J, et al: Association between the 21-gene recurrence score assay (RS) and risk of locoregional failure in node-negative, ER-positive breast cancer: Results from NSABP B-14 and NSABP B-20. Presented at the 28th Annual San Antonio Breast Cancer Symposium Meeting, December 8-11, 2005, San Antonio, TX

26. Musgrove EA, Sergio MS, Loi S, et al: Identification of functional networks of estrogen and c-Myc responsive genes and their relationship to response to tamoxifen therapy in breast cancer. PLoS ONE 3:e2987, 2008

27. Sotiriou C, Wirapati P, Loi S, et al: Gene expression profiling in breast cancer: Understanding the molecular basis of histologic grade to improve prognosis. J Natl Cancer Inst 98:262-272, 2006

28. Nuyten DSA, Kreike B, Hart AAM, et al: Predicting local recurrence after breast-conserving therapy by gene expression profiling. Breast Cancer Res 8:R62, 2006

29. van de Vijver MJ, He YD, van't Veer LJ, et al: A gene-expression signature as a predictor of survival in breast cancer. N Engl J Med 347:1999-2009, 2002

30. Romestaing H, Lehingue Y, Carrie C, et al: Role of a 10 Gy boost in the conservative treatment of early breast cancer: Results of a randomised clinical trial in Lyon, France. J Clin Oncol 15:963-968, 1997

31. Bartelink H, Horiot JC, Poortmans PM, et al: Impact of a higher radiation dose on local control and survival in breast-conserving therapy of early breast cancer: 10-year results of the randomized boost versus no boost EORTC 22881-10882 trial. J Clin Oncol 25:3259-3265, 2007

32. Polgar C, Fodor J, Orosz Z, et al: Electron and high dose rate brachytherapy boost in the conservative treatment of stage I-II breast cancer: First results of the randomised Budapest boost trial. Strahlenther Onkol 178:615-623, 2002

