Annals of Oncology Advance Access published August 1, 2013

original article

Annals of Oncology 00: 1–7, 2013
doi:10.1093/annonc/mdt277

Bcl2 is an independent prognostic marker of triple negative breast cancer (TNBC) and predicts response to anthracycline combination (ATC) chemotherapy (CT) in adjuvant and neoadjuvant settings

T. M. A. Abdel-Fatah[1], C. Perry[1,2], P. Dickinson[1], G. Ball[3], P. Moseley[1], S. Madhusudan[1,2], I. O. Ellis[4] & S. Y. T. Chan[1*]

[1]Clinical Oncology Department, Nottingham University Hospitals, Nottingham; [2]Laboratory of Molecular Oncology, Academic Unit of Oncology, School of Molecular Medical Sciences, University of Nottingham, Nottingham; [3]School of Science and Technology, Nottingham Trent University, Clifton campus, Nottingham; [4]Division of Pathology, School of Molecular Medical Sciences, University of Nottingham, Nottingham University Hospitals, Nottingham, UK

Received 1 March 2013; revised 28 May 2013; accepted 10 June 2013

Background: TNBC represents a heterogeneous subgroup of BC with poor prognosis and frequently resistant to CT.
Material and methods: The relationship between Bcl2 immunohistochemical protein expression and clinico-pathological outcomes was assessed in 736 TNBC-patients: 635 patients had early primary-TNBC (EP-TNBC) and 101 had primary locally advanced (PLA)-TNBC treated with neo-adjuvant- ATC-CT.
Results: Negative Bcl2 (Bcl2-) was observed in 70% of EP-TNBC and was significantly associated with high proliferation, high levels of P-Cadherin, E-Cadherin and HER3 (P's < 0.01), while Bcl2+ was significantly associated with high levels of p27, MDM4 and SPAG5 ($P < 0.01$). After controlling for chemotherapy and other prognostic factors, Bcl2- was associated with 2-fold increased risk of death ($P = 0.006$) and recurrence ($P = 0.0004$). Furthermore, the prognosis of EP-TNBC/Bcl2- patients had improved both BC-specific survival ($P = 0.002$) and disease-free survival ($P = 0.003$), if they received adjuvant-ATC-CT. Moreover, Bcl2- expression was an independent predictor of pathological complete response of primary locally advanced triple negative breast cancer (PLA-TNBC) treated with neoadjuvant-ATC-CT ($P = 0.008$).
Conclusion: Adding Bcl2 to the panel of markers used in current clinical practice could provide both prognostic and predictive information in TNBC. TNBC/Bcl2- patients appear to benefit from ATC-CT, whereas Bcl2+ TNBC seems to be resistant to ATC-CT and may benefit from a trial of different type of chemotherapy with/without novel-targeted agents.
Key words: anthracyclin chemotherapy, Bcl2, predictive marker, prognostic marker, theraputic targets, triple negative breast cancer

introduction

BC is the most commonly occurring cancer in women. One hundred and seventy thousand (12.2%) of the newly 1.38 million diagnosed BC cases each year are defined TNBC [1, 2]. TNBC represents a subgroup of BCs that lack significant expression of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor-2 (HER2) TNBCs are generally considered to be aggressive tumours that often occur at a young age [3]. Recent studies, however, suggest that TNBC may represent a heterogeneous group of tumours with regards to gene expression, clinical presentation, epidemiology, histology, radiology, prognosis and response to treatment [2–5].

The management of TNBC remains a significant clinical challenge, hindered by the inability of these tumours to respond to targeted therapeutic agents [2–4]. As TNBC appears to represent a more diverse population than originally thought, further sub-stratification may help identify patients with a subtype of TNBC more or less likely to respond to chemotherapy.

Recent studies have shown Bcl2 protein and gene expression to be a promising prognostic and predictive marker in human cancers [6–17] especially in hormone receptor-positive, node-negative BC [16, 17]. High Bcl2 level is associated with improved BC outcome.

The role of Bcl2 in TNBC, however, is not well defined. In addition, few studies have investigated the association between Bcl2 status and response to chemotherapeutic agents, including anthracycline in BC patients [10]. For instance, in diffuse large B-cell lymphoma (DLBCL) with MYC/Bcl2 co-expression a worse prognosis was seen in patients treated with an ATC-CT

*Correspondence to: Dr StephenY.T. Chan, Clinical Oncology Department, University of Nottingham City Hospital NHS Trust, Hucknall Road, Nottingham NG51PB, UK. Tel: +44-115-9691169; Fax: +44-115-9628047; E-mail: steve.chan@nuh.nhs.uk

(R-CHOP; Rituximab-cyclophosphamide, doxorubicin, vincristine and prednisolone).

In this study, we have investigated the role of Bcl2 as a prognostic marker in a large cohort of TNBC. We have also studied the role of Bcl2 as a surrogate predictive marker of response to chemotherapy prescribed in the adjuvant or neo-adjuvant setting in patients with either early primary-TNBC (EP-TNBC) or primary locally advanced triple negative breast cancer (PLA-TNBC).

materials and methods

For materials and methods section, see supplementary material, available at *Annals of Oncology* online.

results

early primary-TNBC cohort

EP-TNBC patients were all female and their median age was 51 years (range 28–71 years). Their median follow-up was 107 months (range 2–243 months).

clinico-pathological features of bcl2 expression in EP-TNBC. Negative Bcl2 expression (Bcl2-; Figure 1C–E) was observed in 421 of 600 (70.2%) of EP-TNBC tumours and was significantly associated with high mitotic index ($P = 0.005$), high levels of P-Cadherin ($P = 0.007$), E-Cadherin ($P = 0.045$), CK 19 ($P = 0.001$) and HER3 ($P < 0.0001$). While Bcl2 positive expression (Bcl2+) was significantly associated with high expression of p27 ($P = 0.01$), MDM4 ($P < 0.0001$), and sperm associated antigen 5 (SPAG5; $P = 0.004$) (Table 1).

bcl2 is an independent prognostic biomarker in EP-TNBC. Bcl2- was strongly associated with an adverse outcome at 10 years and nearly doubles the risk of death from EP-BC (HR 1.71; 95% confidence interval (CI) 1.21–2.41; $P = 0.002$) and recurrence (HR 1.79; 95% CI 1.34–2.38; $P = 0.0005$); (Figure 1F–H). After controlling for chemotherapy, traditional pathological prognostic factors and other potential confounders, Bcl2 was confirmed as an independent prognostic factor for both DFS (HR 1.69; 95% CI 1.26–2.27; $P = 0.0004$) and BCSS (HR 1.64; 95% CI 1.15–2.32; $P = 0.006$) at 10 years (Table 2). The EP-TNBC cohort was further subdivided into three patient

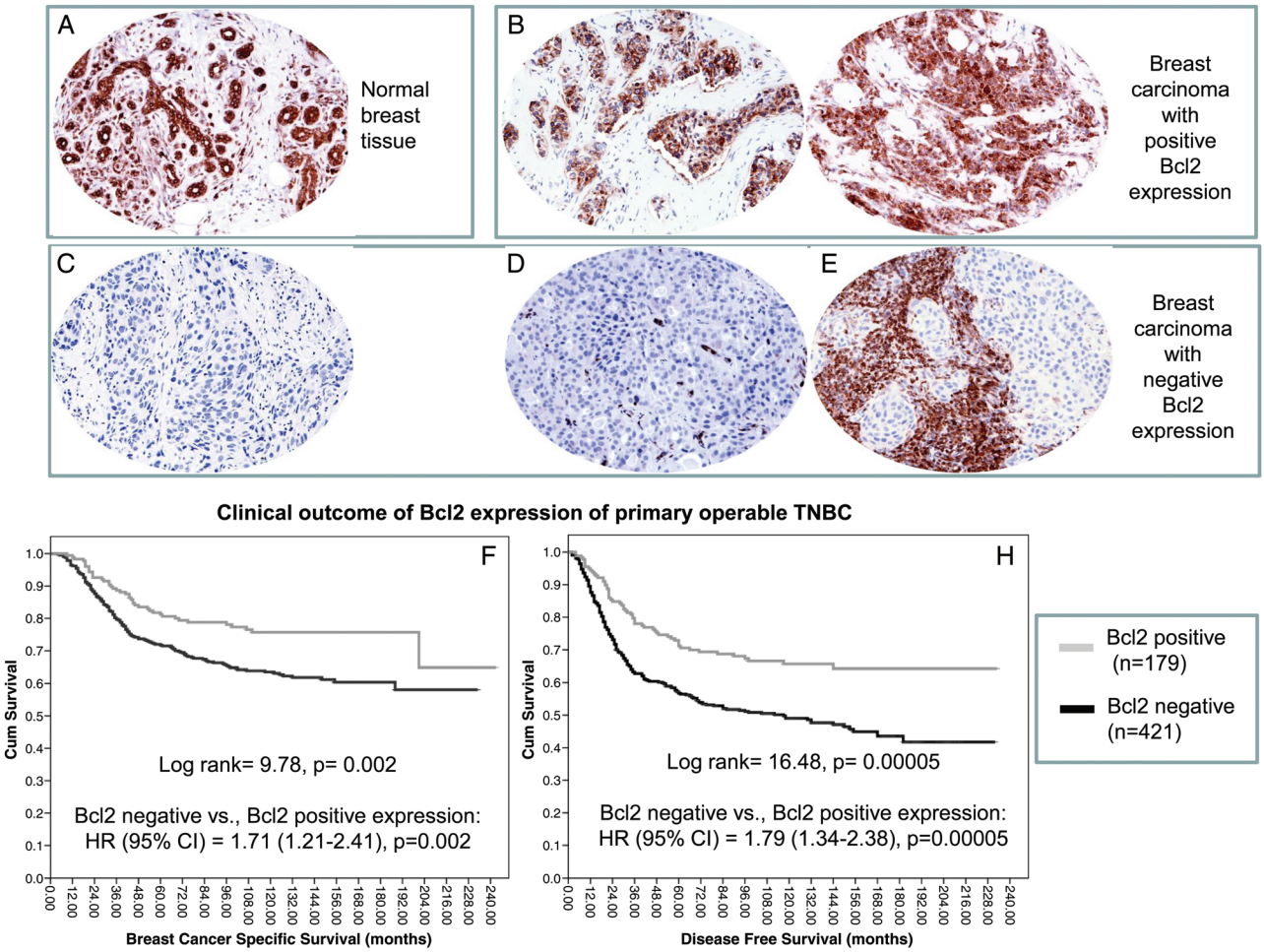

Figure 1. (A–E) Microphotographs of Bcl2 expression in normal breast tissue showing strong cytoplasmic expression in the neoplastic cells and in the stromal cells (A), breast cancer tissue (B–E) showing complete absence of any staining in both neoplastic and stromal cells (C), negative expression of Bcl2 in the neoplastic cells, with few positive stromal lymphocyte cells reaction (D), negative expression of Bcl2 in the neoplastic cells with positive stromal extensive lymphocyte reaction (E) (magnification × 200). (F–H): Kaplan–Meier curves showing breast cancer-specific survival (F) and disease-free survival (H) in the primary operable triple negative breast cancer (PO-TNBC). See text for details.

Table 1. Clinico-pathological characteristics of Bcl2 protein expression in primary operable triple negative breast cancer ($n = 600$)

Variables	Bcl2 protein expression		*P*–value
	Negative ($n = 421$)	Positive ($n = 179$)	
Mitotic index			
M1 (low; mitoses <10)	19 (4.5)	16 (8.9)	0.005**
M2 (medium; mitoses 10–18)	54 (12.9)	10 (5.6)	
M3 (high; mitoses >18)	346 (82.6)	153 (85.5)	
P27			
Negative	272 (85.3)	89 (74.8)	0.01**
Positive	47 (14.7)	30 (25.2)	
SPAG5			
Negative	151 (44.9)	42 (30.7)	0.004**
Positive	185 (55.1)	95 (69.3)	
HER3			
Negative	194 (56.1)	105 (72.9)	<0.0001**
Overexpression	152 (43.9)	39 (27.1)	
MDM4			
Negative	337 (96.3)	127 (85.4)	<0.0001**
Overexpression	14 (3.7)	22 (14.6)	
CK19			
Negative	25 (12.1)	23 (28.8)	0.001**
Positive	182 (87.9)	57 (71.3)	
E-cadherin			
Negative	107 (29.3)	59 (38.3)	0.045**
Positive	258 (70.7)	95 (61.7)	
P-cadherin			
Negative	45 (13)	33 (22.9)	0.007**
Positive	300 (87)	111 (77.1)	
Tumour grade			
G1	2 (0.5)	5 (2.8)	0.055
G2	34 (8.1)	14 (7.8)	
G3	383 (91.4)	160 (89.4)	
Tumour size			
T1 *a* + *b* (≤1.0)	25 (6)	13 (7.5)	
T1 *c* (>1.0–2.0)	183 (43.9)	77 (44.3)	0.577
T2 (>2.0–5.0)	187 (44.8)	79 (45.4)	
T3 (>5)	22 (5.3)	5 (2.9)	
Lymph node stage			
Negative	273 (65.0)	121 (67.6)	0.237
Positive (1–3 nodes)	100 (23.8)	46 (25.7)	
Positive (>3 nodes)	47 (11.2)	12 (6.7)	
Tumour type			
IDC-NST	346 (86.5)	133 (79.6)	0.225
Medullary/atypical	23 (5.8)	5 (3.0)	
Tubular carcinoma	5 (1.3)	11 (6.6)	
Invasive lobular carcinoma	12 (3.0)	9 (5.4)	
Others	14 (3.5)	9 (5.4)	
Lymphovascular invasion			
Yes	212 (50.5)	85 (48.0)	0.584
No	208 (49.5)	92 (52.0)	
EGFR			
Negative	197 (53.5)	90 (59.2)	0.236
Overexpression	171 (46.5)	62 (40.8)	
HER4			
Negative	131 (37.4)	60 (40.5)	0.514
Overexpression	219 (62.6)	88 (59.5)	
P53			
Negative	167 (42.3)	67 (42.4)	0.978
Positive	228 (57.7)	91 (57.6)	
Ki67			
Negative	51 (13.4)	27 (16.7)	0.324
Positive	329 (86.6)	135 (83.3)	
BRCA1			
Negative	218 (64.9)	81 (58.7)	0.205
Positive	118 (35.1)	57 (41.3)	
Basal like phenotype			
No	126 (34.9)	56 (38.4)	0.463
Yes	235 (65.1)	90 (61.6)	

**Statistically significant.

groups based on the chemotherapy given: (i) no chemotherapy (supplementary Figure S1A–B, available at *Annals of Oncology* online), (ii) CMF (Supplementary Figure S1C–D, available at *Annals of Oncology* online) or (iii) anthracycline-combination (FEC – 5FU, epirubicin, cyclophosphamide) (supplementary Figure S1E–F, available at *Annals of Oncology* online). Again Bcl2- was associated with approximately twice the risk of death from EP-BC (HR 2.25; 95% CI 1.40–3.59; $P = 0.001$) and also of recurrence (HR 2.45; 95% CI 1.61–3.71; $P = 0.00002$) in patients who did not receive chemotherapy. In patients treated with CMF a significant association was demonstrated between Bcl2 status and clinical outcome; Bcl2- had a shorter DFS (log rank 2.66, $P = 0.031$) and BCSS (log rank 3.43, $P = 0.05$).

bcl2 is a predictor of outcome after adjuvant ATC-CT. In EP-TNBC patients who received *ATC-CT*, Bcl2- tumours had a clinical outcome similar to those with Bcl2+ phenotype (supplementary Figure S1E–F, available at *Annals of Oncology* online). Both BCCS and DFS were longer in Bcl2- patients treated with an *ATC-CT* when compared with those Bcl2- patients who didn't receive chemotherapy or who were treated with CMF (Figure 2A–D).

Bcl2-TNBC patients had prolonged BCSS and DFS when they were exposed to *ATC-CT* compared with those who either received CMF or did not received any chemotherapy (Figure 2A–D). For example 42% of patients who were Bcl2- and either did not receive any chemotherapy or received CMF had died of BC at 10 years compared with 25% of Bcl2- patients who were treated with *ATC-CT*. For Bcl2-/TNBC patients, exposure to *ATC-CT* reduced the risk of death (HR 0.53; 95% CI 0.35–0.80, $P = 0.002$) and recurrence (HR 0.61; 95% CI 0.44–0.84, $P = 0.003$) by 40%–50%.

No benefit was demonstrated from prescribing *ATC-CT* in Bcl2+ EP-TNBC (supplementary Figue S2A–C, available at *Annals of Oncology* online). Even those of high-risk (NPI >3.4) Bcl2+ EP-TNBC patients who were exposed to *ATC-CT* had double the risk of recurrence (HR 1.85; 95% CI 1.03–3.34,

Table 2. Multivariate analysis using Cox regression analysis confirms that Bcl2 protein expression and lymph node stage are independent prognostic factors in triple negative breast cancer

Clinico-pathological variables	Breast cancer-specific survival at 10 years		Progression-free survival at 10 years	
	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Bcl2 expression				
High	1	0.006**	1	0.0004**
Low	1.64 (1.15–2.32)		1.69 (1.26–2.27)	
Lymph node stage				
Negative	1	0.001**	1	2.2×10^{-8}**
Positive (1–3 nodes)	1.13 (0.81–1.60)		1.17 (0.88–1.56)	
Positive (>3 nodes)	2.23 (1.48–3.37)		2.79 (1.98–3.91)	
Tumour grade				
Grade 1(low)	1	0.739	1	0.999
Grade 2 (intermediate)	0.69 (1.14–3.39)		1.03 (0.29–3.63)	
Grade 3 (high)	0.89 (0.15–5.28)		1.04 (0.25–4.29)	
Tumour size (continuous)	1.01 (0.97–1.04)	0.620	1.01 (0.98–1.040)	0.593
Lymphovascular invasion				
No	1	0.325	1	0.582
Yes	0. 87 (0.65–1.16)		0.93 (0.73–1.19)	
Mitotic index				
M1 (low; mitoses <10)	1	0.319	1	0.503
M2 (medium; mitoses 10–18)	1.55 (0.57–4.22)		1.24 (0.58–2.65)	
M3 (high; mitoses >18)	1.14 (0.38–3.41)		0.99 (0.42–2.32)	
Chemotherapy				
No	1	0.006**	1	0.109
Yes	0.78 (0.65–0.93)		0.89 (0.77–1.03)	

**Statistically significant.

$P = 0.041$) compared with those who did not (Supplementary Figue S2D, available at *Annals of Oncology* online).

p LA-TNBC patients treated with neoadjuvant ATC-CT

All LA-TNBC patients were female and their mean age was 51 years (range 25–76 years). All patients received neoadjuvant ATC-CT either without (67 of 101; 66.3%) or with Taxane (34 of 101; 33.7%) [supplementary Table S2, available at *Annals of Oncology* online]. Sixty-seven of 101(66.3%) of patients received six cycles of an anthracycline-based therapy (FEC: 5-fluorouracil (5-FU) 500 mg m^{-2}, epirubicin 75–100 mg m^{-2}, cyclophosphamide 500 mg m^{-2}, on day 1 of a 21-day cycle). Patients were scheduled to undergo surgery 4 weeks after the sixth cycle. Twenty-eight of 101 (27.7%) of LA-TNBC patients achieved pCR.

bcl2 is a predictor of outcome in patients who receive neoadjuvant ATC-CT. Among 101 patients with PLA-TNBC treated with neoadjuvant ATC-CT, 52 of 101 (51.5%) were Bcl2-. Twenty-one of 52 (40%) of TNBC patients with Bcl2- achieved a pCR versus 7 of 49 (14%) of those with Bcl2+ ($P = 0.003$). Similar to the situation in adjuvant setting, LA-TNBC patients who received neoadjuvant ATC-CT, Bcl2- tumours who achieved a pCR had a clinical outcome similar to those with Bcl2+ phenotype (Supplementary Figue S3C, available at *Annals of Oncology* online). In contrast, among 73 patients who had residual disease after neoadjuvant ATC-CT, 31 patients with Bcl2- had a worse BCSS (HR 2.39; 95% CI 1.15–4.98, $P = 0.02$) and DFS (HR 2.4; 95% CI 1.16–5.04, $P = 0.018$) compared with those with Bcl2+ expression (supplementary Figue S3D, available at *Annals of Oncology* online).

Multivariate regression analysis, after controlling for patient age at diagnosis, tumour stage, grade, size and p53 status, showed that Bcl2-expression is an independent predictor of pCR of LA-TNBC (HR 4.53; 95% CI 1.5–13.8 $P = 0.008$); (supplementary Figue S3B, available at *Annals of Oncology* online).

discussion

TNBC encompasses a subpopulation of BC patients traditionally considered to have a poor prognosis [3]. Neither the classical pathological variables nor the modern molecular assays, have shown prognostic value in this patient group [2–5, 17]. Current research is focused on the identification of biological markers that could be used to tailor treatment to individual patients with TNBC. The results of our study show Bcl2 to be a promising prognostic and predictive marker in TNBC. This adds to an increasing body of evidence to support that routine assessment of Bcl2 status in TNBC patients may refine the outcome, both as a prognostic and predictive factor.

Although the potential importance of Bcl2 as a prognostic factor in BC has been investigated in previous studies [8, 9, 15, 16], our study has reported the prognostic importance of this marker in a large number of TNBC cases (over 700 patients).

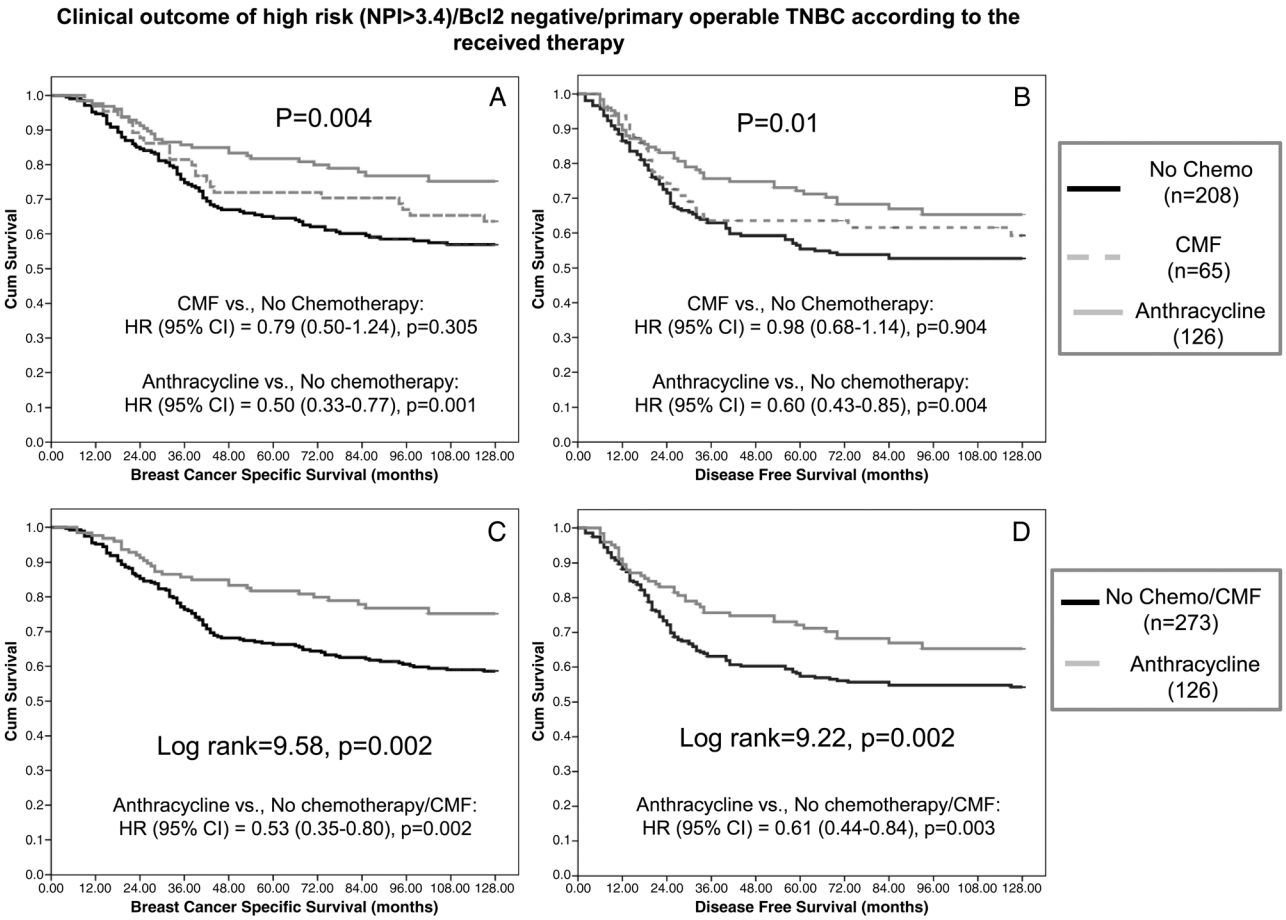

Figure 2. Kaplan–Meier curves showing breast cancer-specific survival (BCSS) and disease-free survival (DFS) in high-risk (NPI > 3.4) Bcl2 negative primary operable triple negative breast cancer (Bcl2-/PO-TNBC) stratified according to the received adjuvant chemotherapy protocols; No chemotherapy, CMF and anthracycline (A–B). As no significant difference was seen between either BCSS or DFS in high-risk Bcl2 negative patients treated with CMF when compared with no chemotherapy, we chose to combine the no chemotherapy/CMF groups and to compare this new combination group with patients who received an anthracycline-based regimen (C–D). See text for details.

We have shown that loss of Bcl2 nearly doubles the risk of both death and recurrence from TNBC in agreement with earlier studies in mixed BC cohorts [8, 9, 15, 16]. Contrary to our findings, Tawfik et al. found Bcl2+ TNBC had a worse prognosis than Bcl2-TNBC. The reason for these differences is not clear but the smaller patient sample, differences in treatment protocols or other factors in the study by Tawfik et al. may play a role.

Our study has also shown that Bcl2 status could help predict the outcome of treatment. In Bcl2-/TNBC patients, both BCSS and DFS are improved if patients are treated with an anthracycline. This association is not seen in Bcl2+/TNBC patients. In fact in the latter population there is some evidence to suggest that these patients have worse DFS when treated with an anthracycline. Similar results have been reported by Bouchalova et al. who analysed the response to AC-ACT in a small cohort of TNBC patients. The findings of this study are in agreement with our results demonstrating that high Bcl2 expression is associated with worse relapse free and overall survival (OS) in patients treated with adjuvant anthracycline-based treatment. Again the results from the multivariate analysis of this study show Bcl2 status, size and LN stage to be predictive of both OS and DFS [26] in common with our findings. Furthermore, similar associations have been seen between Bcl2 status and anthracycline response in other tumours. For example DLBCL patients with both MYC and Bcl2 co-expression had a worse DFS and OS when treated with standard R-CHOP chemotherapy [6].

Interestingly in our study the benefit from chemotherapy in the Bcl2- patient group was only seen when they were treated with an anthracycline and not from CMF. All patients who received an anthracycline-based regimen will have been given FEC- combination. As FEC contains 5-FU and cyclophosphamide in common with CMF the only different agent between the two regimens is the anthracycline, in this case epirubicin. The significant difference observed between the no chemotherapy/CMF group and the anthracycline treated group is therefore most likely due to the addition of epirubicin, although could be a combination effect of the drugs used. Recently, Buchholz et al. found that 27% of Bcl-2 negative BC had achieved pCR to Doxorubicin-based neoadjuvant chemotherapy versus 4% of Bcl-2 positive ($P = 0.004$).

We have shown Bcl2-expression to be an independent predictor of pCR in PLA-TNBC. Bcl2-/TNBC patients were

significantly more likely to have a pCR than patients with Bcl2+ expression. These findings are similar to early reports in mixed BC cohorts (i.e. both TNBC and non-TNBC [7]. Von Minckwitz et al also found Bcl2- to be associated with pCR in patients with primary-BC treated with dose-dense doxorubicin and docetaxel chemotherapy with or without endocrine treatment. As a pCR following neoadjuvant ATC-CT has been shown to be associated with an improved outcome, our findings suggest that staining for Bcl2 status in TNBC patients could help identify a cohort of patients who would be ideal for neoadjuvant ATC-CT.

As evidenced by our results, and those from earlier studies, patients with Bcl2-/TNBC tumours gain significant benefit from anthracycline treatment. The explanation behind this finding is likely to be complex and involve the multiple functions of Bcl2. The Bcl2 protein acts as both an anti-apoptotic factor and also prolongs cell cycle at G0 [11, 13, 14], and therefore, Bcl2+ cells are more likely to recover from damage caused by chemotherapy. Bcl2- tumours however respond to treatment, probably through accumulation of DNA damage, abnormal mitoses and subsequent mitotic catastrophe. The mechanisms of mitotic catastrophe are unknown, but it likely results from a combination of deficient cell-cycle checkpoints (in particular the DNA structure checkpoints and the spindle assembly checkpoint) and cellular damage catastrophe. Conversely, non-responding Bcl2- tumours could escape the response through accumulation of genetic abnormalities that would not lead to a mitotic catastrophe but rather to aneuploidy and subsequent growth advantage. Interestingly, we found that loss of Bcl2 in TNBC was associated with higher mitotic index, low levels of p27 (G0/G1 check protein), MDM4 (p53-inhibitory factor) and SPAG5 which is essential for cell cycle progression and fidelity of chromosomal segregation.

Bcl2-/LANBC patients treated with neoadjuvant ATC-CT are more likely to have a pCR than Bcl2+ patients. However, in those patients who do not achieve a pCR prognosis is worse in the Bcl2- group. Clearly, these Bcl2- patients represent a cohort who has demonstrated limited response to anthracycline-based treatment and targeting alternative pathways in these patients warrants further investigation.

We found that loss of Bcl2 expression in TNBC was significantly related to increased levels of CK19, P-cadherin, E-cadherin and HER3. The differential expression of these biomarkers could be a reflection of the molecular subclasses of TNBC and could also have biological and therapeutic implications. Previous studies have suggested P-cadherin as a promising antibody therapeutic target or as a possible target for immunotherapy of pancreatic, gastric and colorectal cancers relapse. In agreement with an early study in basal BC, 75% of our TNBC showed overexpression of P-cadherin and that is highly associated with loss of Bcl2 compared with 35% of mixed BC. Given the high incidence of P-cadherin overexpression in TNBC Bcl2- tumours and that was associated with poor clinical outcome (data not shown), P-cadherin targeting may warrant further investigation in this group of TNBC.

Given that ~40% of our TNBC cases show overexpression of HER3, and that this is highly associated with the absence of Bcl2, suggests a potential role of HER3 targeting agents in specific subtypes of TNBC. Recent studies suggest that combination treatment with trastuzumab and pertuzumab may be particularly efficacious in HER2-amplified BC; however, no data are available for TNBC particularly in the HER2-enriched subgroup.

Although prognosis is better in Bcl2+/TNBC mortality rates can still be in excess of 20% at 15 years. Our data demonstrate that the benefit seen in Bcl2-/TNBC in response to anthracycline-based treatment is not observed in Bcl2+ patients. This would suggest that a different treatment strategy is required in these patients. In conclusion, adding Bcl2 to the panel of markers used in current clinical practice (ER, PR and HER2) could provide both prognostic and predictive information in patients with TNBC. Patients with Bcl2-/TNBC phenotype have a worse prognosis than Bcl2+/TNBC patients but appear to benefit from ATC-CT. The optimal management of Bcl2+/TNBC tumours is yet to be defined. A prospective study is required to validate these results and to investigate potential therapeutic targets in Bcl2+/TNBC patients. In addition, Bcl2 status could be investigated as a possible predictive marker for chemotherapy in non-TNBC high-risk BC subsets.

funding

This work was supported by the Nottingham University Hospitals (NUH) Trust, Research and Innovation (R&I) Breast Cancer Research Charitable Fund.

disclosure

The authors have declared no conflicts of interest.

references

1. WHO. Globocan 2008: Estimated cancer incidence, mortality, prevalence and disability-adjusted life years (DALYs) worldwide in 2008. 2nd January 2013]; Available from: http://globocan.iarc.fr/ (22 February 2013, date last accessed).
2. Ossovskaya V, Wang Y, Budoff A et al. Exploring molecular pathways of triple-negative breast cancer. Genes Cancer 2011; 2: 870–879.
3. Brouckaert O, Wildiers H, Floris G et al. Update on triple-negative breast cancer: prognosis and management strategies. Int J Womens Health 2012; 4: 511–520.
4. Amos KD, Adamo B, Anders CK. Triple-negative breast cancer: an update on neoadjuvant clinical trials. Int J Breast Cancer 2012; 2012: 385978.
5. Perou CM. Molecular stratification of triple-negative breast cancers. Oncologist 2010; 15(Suppl 5): 39–48.
6. Barrans S, Crouch S, Smith A et al. Rearrangement of MYC is associated with poor prognosis in patients with diffuse large B-cell lymphoma treated in the era of rituximab. J Clin Oncol 2010; 28: 3360–3365.
7. von Minckwitz G, Sinn HP, Raab G et al. Clinical response after two cycles compared to HER2, Ki-67, p53, and bcl-2 in independently predicting a pathological complete response after preoperative chemotherapy in patients with operable carcinoma of the breast. Breast Cancer Res 2008; 10: R30.
8. Callagy GM, Pharoah PD, Pinder SE et al. Bcl-2 is a prognostic marker in breast cancer independently of the Nottingham Prognostic Index. Clin Cancer Res 2006; 12: 2468–2475.
9. Dawson SJ, Makretsov N, Blows FM et al. BCL2 in breast cancer: a favourable prognostic marker across molecular subtypes and independent of adjuvant therapy received. Br J Cancer 2010; 103: 668–675.
10. Gasparini G, Barbareschi M, Doglioni C et al. Expression of bcl-2 protein predicts efficacy of adjuvant treatments in operable node-positive breast cancer. Clin Cancer Res 1995; 1: 189–198.

11. Sierra A, Castellsague X, Escobedo A et al. Bcl-2 with loss of apoptosis allows accumulation of genetic alterations: a pathway to metastatic progression in human breast cancer. Int J Cancer 2000; 89: 142–147.
12. Reed JC. Bcl-2 and the regulation of programmed cell death. J Cell Biol 1994; 124: 1–6.
13. O'Reilly LA, Huang DC, Strasser A. The cell death inhibitor Bcl-2 and its homologues influence control of cell cycle entry. EMBO J 1996; 15: 6979–6990.
14. van Slooten HJ, van de Vijver MJ, van de Velde CJ et al. Loss of Bcl-2 in invasive breast cancer is associated with high rates of cell death, but also with increased proliferative activity. Br J Cancer 1998; 77: 789–796.
15. Abdel-Fatah TM, Powe DG, Ball G et al. Proposal for a modified grading system based on mitotic index and Bcl2 provides objective determination of clinical outcome for patients with breast cancer. J Pathol 2010; 222: 388–399.
16. Ali HR, Dawson SJ, Blows FM et al. A Ki67/BCL2 index based on immunohistochemistry is highly prognostic in ER-positive breast cancer. J Pathol 2012; 226: 97–107.
17. 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 2004; 351: 2817–2826.

References 18 to 34 are available in the supplementary material, available at *Annals of Oncology* online.