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ORIGINAL ARTICLE

Molecular profiling of breast cancer in Nigerian women identifies an altered p53 pathway as a major mechanism underlying its poor prognosis compared with British counterpart

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Abstract

Background: Advances in breast cancer (BC) research have demonstrated differences between black and white women with regarding tumour behaviour, patient outcome and response to treatment which can be explained by underlying genetic changes. The tumour suppressor gene p53 has been speculated to be involved in tumour biology of triple negative and/or basal –like BC and more commonly observed in black than caucasian women. **Materials and methods:** In this study, the protein expression of p53 was investigated in tissue samples from a series of 308 Nigerian women, prepared as a tissue microarray (TMA), using immunohistochemistry. Clinicopathological parameters, biomarkers of functional significance in BC and patient outcome of tumours expressing p53 in Nigerian women were correlated with UK grade matched series. **Results:** A significantly large proportion of BC from Nigerian women showed high p53 expression compared with UK women ($p<0.001$). In those tumours showing positive p53 in the Nigerian series, a significant proportion were premenopausal, diagnosed before 50 years, larger in size, with evidence of metastasis into lymphatic vessels (all $p<0.001$). In addition, p53 positive expression was also significantly correlated with negative expression of ER and PgR ($p<0.001$, $p<0.03$ respectively), BRCA1, MDM2 (all $p<0.001$), p21 ($p=0.006$) and E-cadherin ($p=0.001$) and positively associated with P-cadherin ($p=0.001$), triple negative phenotype, basal cytokeratin (CK) 5/6 expression ($p<0.04$) and basal phenotype compared with the UK series ($p<0.001$). Survival analyses showed Nigerian women with BC were significantly associated with poor BC specific survival ($p<0.001$, but no significant association with disease free interval was observed**. Conclusion:** In this study, protein expressions of p53 pathways are different between Nigerian and UK BC women and this may also contribute to differences in tumour biology. Therefore, targeting these p53 pathways for therapeutic usage might improve the poor outcome observed in Black Nigerian women.

Key words: p53, independent prognostic factor, breast cancer, Nigerian, ethnicity

INTRODUCTION

Advances in breast cancer (BC) research have shown that differences exist between black and white women with respect to patient outcome and response to treatment.[1-5] Studies show that BC arising in black women is more likely to be diagnosed at advanced tumour stage, with higher histological grade and larger tumour size, which may contribute to shorter survival compared to white women.[5-8] In addition, the majority of the BC from black women belong to triple negative (oestrogen negative, progesterone negative and HER-2 negative) and/or basal-like BC (ER, PR, HER-2 negative and CK5/6 and or EGFR positive) where the optimum mode of treatment is still disputed. Unfortunately, in the early stage of triple negative and basal like BC about 30% improvement on disease free interval (DFI) and 15% on overall survival (OS) can be achieved using chemotherapy, whereas a large proportion

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will be resistant to this therapy. Therefore, there is a need to carefully select patients for specific therapeutic regimens.[1-3] Contrary to the well-established predictive factors for hormonal therapy, less information is available for the chemo-sensitivity been used for the treatment of triple negative and basal l-like BC, particularly among ethnicity.[4,5] Based on these observations, a concept of personalised medicine is evolving, with the sole aim of more effective treatment for the women with BC.[6]

The tumour suppressor gene p53 plays a major role in the development of cancer, particularly breast cancer, most especially in medullary carcinoma as well as *BRCA*1 mutation mediated breast carcinoma.[7,8] The function of p53 ranges from controlling transcription,[9] the cell cycle through the stimulation of WAF1/Cip1 that encodes for p21,[10-12] apoptosis,[13] DNA repair,[14] inhibition of angiogenesis[15] to elimination and inhibition of the proliferation of abnormal cells.[9,12-16]

p53 is activated in response to cellular stress; (extreme temperature, exposure to toxins and mechanical damage), central to the mechanism of action is the p53-MDM2 pathway interactions with three distinct mechanisms.[17,18] *In vitro*, the Murine Double Minute 2 (MDM2) oncogene function was demonstrated through transcriptional up-regulation of p53.[19] In DNA damage caused by ionising radiation and other endogenous means, MDM2 is activated through the MDM2 phosphorylation of Ataxia Telangiectasia Mutation (ATM) and c-ABL, thereby inhibiting the degradation of p53.[20] Conversely, it can also down regulate p53 function by promoting the rapid elimination of p53 through ubiquitin–proteasome pathways and/or by silencing Alternative Reading Frame (ARF) expression.[19,21,22]

Although, just like other p53 binding proteins, Murine Double Minute 4 (MDM4) shares significant homology sequence with MDM2, it does not however possess intrinsic ubiquitin ligase 3 (E3) linkage activity and therefore, does not have direct ability to degrade p53. Moreover, it interacts with p53 indirectly by binding to MDM2 through the C-terminal ring domain and stimulates MDM2 to ubiquitinate and degrades p53. MDM4 is therefore speculated to contribute to the suppression of p53.[23-27]

Although, many studies have adduced different reasons for the increased likelihood of black women presenting with advanced stage at diagnosis, the tumour biology that may contribute to the differences observed in the ethnic nationalities is not completely understood.[28,29] We therefore, hypotheses that alteration of the p53 pathway may contribute to the tumour biology observed among the Black Nigerian women with BC.

Thus, the aim of this study is to investigate the p53 pathway expression using immunohistochemistry in breast tumours from Black Nigerian women and to compare them to a well-characterised series of BC from Caucasian women living in the UK, in order to establish whether the differences between the two Nationalities are due to p53 pathway tumour biology.

MATERIAL AND METHODS

Patients

The Nigerian patient cohort comprised formalin-fixed paraffin embedded (FFPE) breast cases from 308 women presenting at the Olabisi Onabanjo University Teaching Hospital, Sagamu, and Histopathology Specialist laboratory, Idi-Araba Lagos, Nigeria from January 2002 to December 2008. Clinical history and tumour characteristics including age, menopausal status, tumour type, histological grade, tumour size, lymph node status and vascular invasion were assessed in a standardised manner for all the patients.

The tissue sections were re-evaluated for histological features such as tumour grade and type. Patient outcome and treatment data were retrieved from the patient's records. All patients were treated with combination of classical chemotherapy cyclophosphamide, methotrexate and 5FU and hormonal therapy (tamoxifen). Eighty-five of the patients (42.5%) received radiotherapy. Patients were followed up for at least 60 months. Two hundred and two (202) patients outcome records were available for this study while one hundred and six patients were lost to follow up. Out of 202 patients, one hundred and seventy patients (84.2%) died, 32 patients remained alive (15.8%) during the follow-up period.

A UK patient cohort of 1,902 primary operable invasive breast carcinoma cases, were from the well-characterised Nottingham-Tenovus Primary Breast Carcinoma Series consisting of women presenting between 1986-1998. All patients were assessed in a standardised manner for clinical history and tumour characteristics.[30-33] Patient management was based on tumour characteristics by the Nottingham Prognostic Index (NPI)[34] and

hormone receptor status (ER and PgR status). Patients with an NPI score ≤ 3.4 received no adjuvant therapy, those with a NPI score >3.4 received Tamoxifen if oestrogen receptor (ER) positive (± Zoladex if pre-menopausal) or classical cyclophosphamide, methotrexate and 5-fluorouracil if ER negative and fit enough to tolerate chemotherapy.[35] Survival data including breast cancer specific survival (BCSS) and disease-free interval (DFI) were maintained on a prospective basis. DFI was defined as the interval (in months) from the date of the primary surgical treatment to the first locoregional (including invasive malignancy and ductal carcinoma in situ) or distant recurrence. BCSS was taken as the time (in months) from the date of the primary surgical treatment to the time of death from breast cancer.

Of this series, 841 cases had complete data on the following immunohistochemical markers: oestrogene receptor (ER), progesterone receptor (PgR), cytokeratins (CK5/6, CK14,) EGFR (HER1), HER2, BRCA1, placental- cadherin (p-cadherin), Epithelial- cadherin (E-cadherin), p53, p21, Bcl-2, MDM2 and MDM4.[30-33,36] In order to compare the Nigerian and UK series with respect to biomarkers and patient outcome, a grade-matched UK control groups to the Nigerian cohort, comprising of 308 patients, was generated from the above patients. Within the UK tumour grade-matched cohort, 202 were randomly selected for patient outcome due to 106 that were lost to follow up in Nigeria series. 57/202 (28.9%), 170/202 (84.2%) had died at 60 months and 145/202 (71.1%), 32/202(15.8%) remained alive respectively.

The Reporting Recommendations for Tumour Marker Prognostic Studies (REMARK) criteria, recommended by McShane *et al*,[37] were followed. This study was approved by the Medical Advisory Committee, Olabisi Onabanjo University Teaching Hospital and by the Nottingham Research Ethics Committee 2 under the title of "Development of a molecular genetics classification of breast cancer".

Tissue Microarray Array Construction

Three hundred and eight samples from Nigerian cohort were constructed as tissue microarrays (TMA) as previously described.[38] Breast tumour cores were taken from each FFPE donor tissue block that has been marked for the most representative points of tumour areas (both peripherally and centrally). A precision instrument (ALPHELYS MiniCore®) was used to take representative cores of tissue (0.6mm diameter, 3mm height) from each sample, which was then arrayed into a recipient paraffin block in 11x15 core format.

Immunohistochemistry

Nigerian series immunohistochemistry staining was performed in the same Nottingham laboratory using the same antibodies and detection kits used on UK series for easy standardisation. Briefly, all the biomarkers required antigen retrieval except HER-2 and EGFR. Antigen retrieval was performed by microwaving the slides at 800W for 10 minutes followed by 560W for 10 minutes in citrate buffer (1M Sodium Citrate at pH of 6.0) followed by cooling in running water immediately. The primary antibody for each biomarker (Table 1) was incubated for 60 minutes at room temperature. ABC detection system was used. Diaminobenzidine tetrahydrochloride (DAB) solution was incubated for 10 minutes after which copper-sulphate solution (0.5% Copper Sulphate in 0.8% Sodium Chloride) were applied to the slides and incubated for 10 minutes each and sections counter stained with haematoxylin for 3 minutes, followed by rinsing in tap water. Slides were de-hydrated by immersing in three alcohol baths for 10 seconds and cleared in two xylene baths followed by application of cover slip. Negative and positive controls were performed by omitting the primary antibody and including control tissues as specified by the antibody supplier respectively.

Immunohistochemical scoring

The scoring was performed using the modified Histochemical score (H-score), a semi-quantitative assessment. Staining intensity was scored from 0, 1, 2 to 3 and the percentage of positive cells was determined for each score to produce a final score in the range 0–300. The cases were scored without knowledge of the patient outcome. The TMA samples were scored twice by one observer (JA). The mean of the scores were calculated to reach a final score. A proportion of these were counter scored by an observer (AG) to ensure reproducibility. Table 1 shows the cut off points used for the biomarkers analysis.[36,39] For c-HER2, the American Society of Clinical Oncology/ College of American Pathologists Guideline Recommendations for Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer was used for assessment.[40] Equivocal (2+) cases were

TABLE 1: Sources, dilution, distribution, cut-offs point and pre-treatment used for revalidation

Antibody	Clone	Source	Dilution	Distribution	Scoring System	Cut-offs	Pre-treatment	Positive control	Negative control
Bcl-2	124	Dako-Cytomation	1:100	Cytoplasm	% of positive cells	>10% (positive)	Antigen retrieval Microwave	Normal breast acini	Omitting the antibody
BRCA1	Ab-1 (MS110)	Calbiochem	1:150	Nuclear	% of positive cells	<25% (negative)	Antigen retrieval Microwave	MCF 7 cells	Omitting the antibody
Ck5/6	M7237	Dako-cytomation	1:60	Cytoplasm	% of positive cells	≥10% (positive)	Antigen retrieval Microwave	Known case of CK56 BC	Omitting the antibody
E–cadherin	NCH-38	Dako-Cytomation	1:100	Cytoplasm and membrane	% of positive cells	≥100 H score (positive)	Antigen retrieval Microwave	Normal gastric mucosa	Omitting the antibody
EGFR	31G7	Novocastra	1:30	Membrane	% of positive cells	≥10% (positive)	Not required	Myoepithelial cells of normal duct in normal mammary gland	Omitting the antibody
erbB2	Polyclonal	Dako-Cytomation	1:100	Membrane				Known case of erbB2 strong BC expression	Omitting the antibody
ER	1D5	Dako-Cytomation	1:200	Nuclear	% of positive cells	≥0(positive)	Antigen retrieval Microwave	Normal breast acini	Omitting the antibody
MDM2	IB10	Novocastra	1:200	Nuclear	% of positive cells	≥ 10% (positive)	Antigen retrieval Microwave	Liver	
MDM4	IHC-00108	Bethyl Labs	1:100	Nuclear	% of positive cells	0% (negative) <1-20% (low) > 20% (strong)	Antigen retrieval Microwave	Known case of MDM4 strong BC expression	Omitting the antibody
P-cadherin	NCL-P-cad	Novocastra	1:200	Cytoplasm	% of positive cells	≥5% (positive)	Antigen retrieval Microwave	Known case of P-cadherin strong BC expression	Omitting the antibody
PgR	PgR	Dako-Cytomation	1:150	Nuclear	% of positive cells	≥0 (positive)	Antigen retrieval Microwave	Normal breast acini	Omitting the antibody
p21	EA10	Abcam	1:25	Nuclear	% of positive cells	≥10% (positive)	Antigen retrieval Microwave	Normal breast acini	Omitting the antibody
p53	DO7	Novocastra	1:50	Nuclear	% of positive cells	>10% (negative)	Antigen retrieval Microwave	Normal breast acini	Omitting the antibody

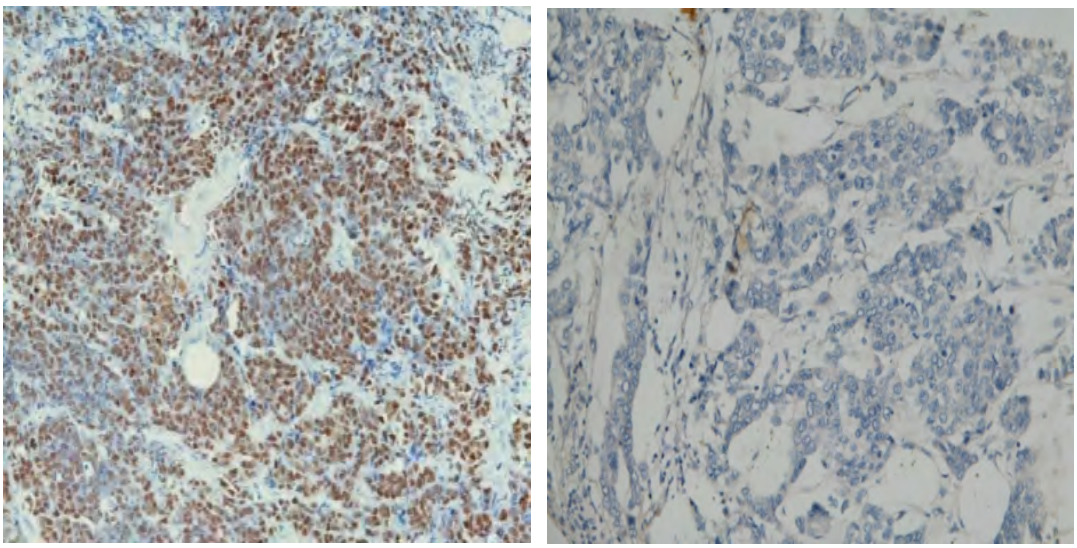

FIG. 1: Immunochemistry of p53 expression. Mag X20

confirmed by CISH as previously described.[41] For molecular classification, Nielsen's method[42] was used. This comprises of Luminal A (ER, PR positive and HER 2negative), Luminal B (ER, PR HER 2 positive), Basal (ER, PR, HER-2 negative and CK5/6 and or EGFR positive), HER2 (ER negative and HER2 positive) and an unclassified group (ER, PR, HER2 CK5/6 and EGFR negative).

Statistical analysis

Statistical analysis was performed using SPSS 16.0 statistical software. Chi-squared analyses were used for inter-relationships between the Nigerian and UK series and for comparison with clinicopathological parameters. The Kaplan–Meier survival method and the log-rank test were used for survival curves. Multivariate analyses using Cox proportional hazard regression models were performed and from the model both the risk factor and 95% confidence intervals were generated. A two-sided *p*-value of <0.05 was considered significant.

RESULTS

p53 expression in Nigerian compared with UK series

p53 immunoreactivity showed nuclear staining with different degrees of staining intensities (Figure 1). After the exclusion of uninformative cores (not having up to 15% of the core areas as tumour) in Nigerian series, p53 was highly expressed and accounted for 63.3%. While in UK breast tumours, p53 expression was observed in 25.8%. The relationship between p53 expression in a randomly grade matched breast cancer between Nigerian and UK women showed a significantly large proportion of breast tumours from Nigerian women showed high p53 expression compared with UK women ($p<0.001$) (Table 2).

Correlation of p53 expression between Nigerian and UK breast cancer in relation to clinicopathological features

In those tumours showing positive p53, a significant proportion of breast cancers from the Nigerian series were from patients that were premenopausal ($p<0.001$) and diagnosed

TABLE 2: Relationship between p53 expression in Nigeria and UK series

Biomarkers	Nigeria (%)	UK (%)	χ^2 value	p-value
p53				
Negative	80 (36.7)	222 (74.2)	**73.18**	**<0.001**
Positive	138 (63.3)	77 (25.8)		

TABLE 3: Relationship between clinico-pathological parameters in Nigerian and UK tumours expressing p53

Variables	p53 positive expression		χ^2 value	P-value
	Nigeria (%)	UK (%)		
Age (years)				
≤50	93 (67.4)	30 (39.0)	**16.32**	**<0.001**
>50	45 (32.6)	47 (61.0)		
Menopausal				
Pre	99 (71.7)	29 (37.7)	**23.82**	**<0.001**
Post	39 (29.3)	48 (62.3)		
Sizes (cm)				
≤ 2	13 (9.4)	33 (42.9)	**32.86**	**<0.001**
> 2	125 (90.6)	44 (57.1)		
Lymph node involvement				
Negative	8 (5.8)	50 (64.9)	**87.74**	**<0.001**
Positive	130 (94.2)	27 (35.1)		
Vascular invasion				
Negative	36 (26.1)	49 (63.6)	**29.15**	**<0.001**
Positive	102 (72.9)	28 (36.4)		
Tumour type				
Typical medullary	0 (0.0)	4 (5.2)		
Atypical medullary	2 (1.4)	3 (3.9)		
Tubular	2 (1.4)	0 (0.0)		
Lobular	0 (0.0)	3 (3.9)		
Ductal NST	118 (85.5)	57 (75.0)	20.19	0.020
Mucinous	3 (2.2)	0 (0.0)		
Tubulolobular	1 (0.7)	0 (0.0)		
Lobular mixed	2 (1.4)	2 (2.6)		
Tubular mixed	10 (7.7)	6 (7.9)		
Mixed NST	1 (1.3)	1 (1.3)		
Others	0 (0.0)	0 (0.0)		

NST: no special type
Others: metaplastic, spindle and alveolar lobular histological type

before 50 years (p<0.001). Also, the tumours were significantly larger in size (p<0.01) with evidence of metastasis into lymph node (p<0.001) and vascular invasion (p<0.001) compared with the UK series (Table 3).

Correlation of p53 expression between Nigerian and UK breast cancer in relation to other biomarkers expression

In the Nigerian series, p53 positive expression was significantly correlated with negative expression of the steroid hormone receptors ER and PgR (p<0.001, p<0.03 respectively), BRCA1, MDM2 (all p<0.001), p21 (p=0.006) and E-cadherin (p=0.001). Conversely, p53 positive expression tumours were significantly associated with P-cadherin (p=0.001) and basal cytokeratin CK 5/6 (p<0.04) expression.

In addition, a greater proportion of the tumour expressing p53 was associated with basal phenotype using Nielsen's classification compared to UK. On the contrary, majority of the UK series were positively correlated with luminal phenotype compared with tumour expressing p53 arising from Nigerian women (p<0.001).

However, a significantly larger proportion of Nigerian tumours that showed a positive EGFR expression had a trend with p53 in the majority of Nigerian tumours compared with UK series (p=0.05). There was no significant difference between Nigerian and UK p53 positive tumours in the expression of Bcl-2 MDM4 and HER-2 (Table 4).

TABLE 4: Relationship between biomarker expression in UK and Nigerian tumours expressing p53

Variables	p53 positive expression			
	Nigeria (%)	UK (%)	χ^2 value	p-value
Bcl-2				
Negative	63 (58.3)	30 (49.2)	2.86	0.25
Positive	45 (41.7)	31 (50.8)		
BRCA1				
Negative	89 (80.2)	13 (20.3)	59.84	**<0.001**
Positive	22 (19.8)	51 (79.7)		
CK5/6				
Negative	71 (58.7)	56 (72.7)	4.04	**0.04**
Positive	50 (41.3)	21 (27.3)		
E-cadherin				
Negative	72 (64.3)	31 (40.8)	10.09	**0.001**
Positive	40 (35.7)	45 (59.2)		
EGFR				
Negative	60 (55.0)	50 (69.4)	3.77	0.05
Positive	49 (45.0)	22 (30.6)		
ER				
Negative	108 (83.7)	36 (48.6)	28.06	**<0.001**
Positive	21 (16.3)	38 (51.4)		
HER-2				
Negative	98 (82.4)	64 (85.3)	0.30	0.58
Positive	21 (17.6)	11 (14.7)		
MDM2				
Negative	92 (77.3)	3 (6.1)	71.58	**<0.001**
Positive	27 (22.7)	46 (93.9)		
MDM4				
Negative	89 (80.2)	43 (84.3)	0.39	0.52
Positive	22 (19.8)	8 (15.7)		
PgR				
Negative	81 (76.4)	46 (61.3)	4.77	**0.03**
Positive	25 (23.6)	29 (38.7)		
P21				
Negative	106 (86.9)	30 (68.2)	7.64	**0.006**
Positive	16 (13.1)	14 (31.8)		
P-cadherin				
Negative	49 (40.8)	12 (17.9)	10.2	**0.001**
Positive	71 (59.2)	55 (82.1)		
Triple negative				
No	45 (45.0)	46 (61.3)	4.58	**0.03**
Yes	55 (55.5)	29 (38.7)		
Classification				
Basal	45 (32.6)	9 (11.7)		
HER-2	17 (12.3)	6 (7.8)	67.76	**< 0.001**
Luminal A	20 (14.5)	52 (67.5)		
Luminal B	3 (2.2)	3 (3.9)		

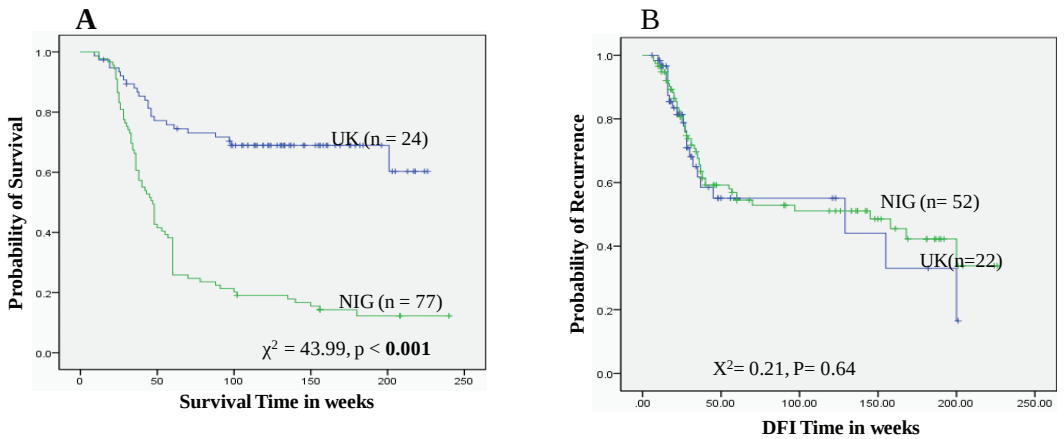

FIG. 2: (A) p53 expression in relation to breast cancer survival (A) and recurrence (B) between UK and Nigerian series

Patient outcome in tumour expressing p53 in Nigerian compared with UK series

Survival analyses were performed comparing the Nigerian and UK series in relation to both DFI and BCSS. Nigerian women were significantly associated with BCSS ($p<0.001$), but no significant association with DFI was observed (Figure 2). Cox multivariate regression showed Nigerian women have worsen survival than British women was an independent predictor of BCSS in the tumour expressing p53 (Table 5).

p53 transcription pathways in Nigerian compared with UK breast cancer

The frequency of expression and co-expression of p53 and other related biomarkers: Bcl-2, MDM2 and p21 in Nigerian breast cancer was determined and compared with the UK series. In the p53/Bcl-2 phenotypes in both series; 67 Nigerian cases (42.1%) were $p53^{pos}$ /Bcl-2^{neg} whereas in the UK series this was significantly lower at 13% frequency. The most common phenotype observed in the UK series was $p53^{neg}$ /Bcl-2^{pos} (53.9%) which was not frequent in the Nigerian series (14.5%). Similarly, with co-expression of p53/MDM2, there were significantly more Nigerian tumours with $p53^{pos}$ /$MDM2^{neg}$ (50.5%) than in the UK series where this was a rare occurrence (1.5%, $p<0.001$). In the UK series $p53^{neg}$ /$MDM2^{pos}$ (69.5%) was the most common phenotype which was in low frequency in the Nigerian series (7.1%) . Likewise, when

TABLE 5: Cox multivariate analysis of probability of survival in tumours expressing p53 in Nigerian and UK breast cancer

Variables	p53 positive expression				p53 negative expression,			
	p-value	Hazard ratio	95 % CI Lower	Upper	p-value	Hazard ratio	95 % CI Lower	Upper
Racial differences								
Nigeria vs UK	**<0.001**	4.16	2.04	7.34	**< 0.001**	10.41	5.43	19.92
Lymph node	0.33	0.77	0.38	1.38	0.81	0.94	0.58	1.52
Tumour Size	0.18	1.17	0.93	1.38	0.49	1.07	0.87	1.32
ER	0.06	0.68	0.30	3.26	0.42	1.25	0.45	1.39

comparing the p53/p21 transcriptional pathways, a significant number of Nigerian cases expressed $p53^{pos}/p21^{neg}$ (56.0%) compared with the UK series where $p53^{neg}/p21^{neg}$ (27.9%) and $p53^{neg}/p21^{pos}$ (17.2%) were more commonly expressed (Table 6).

Patient outcome of p53 transcription pathways in Nigerian compared with UK breast cancer

The Kaplan-Meier estimates of BCSS for the groups of combined co-expression of p53 with any of the three biomarkers expression: Bcl-2 MDM2 and p21 showed a significant positive correlation between reduced BCSS in Nigerian women in all the p53/Bcl-2/MDM2/p211/ phenotypes, except $p53^{pos}/p21^{pos}$, $p53^{pos}/MDM2^{pos}$ and $p53^{pos}/MDM2^{neg}$ ($p<0.001$), Figures 3-5). However, no significant correlation was observed between UK and Nigerian breast tumours between the cell-cycle phenotypes and DFI. Cox multivariate estimates showed race was an independent predictor of BCSS in $p53^{neg}/Bcl\text{-}2^{pos}$, $p53^{neg}/p21^{neg}$ and $p53^{neg}/MDM2^{pos}$ (Tables 7-9).

DISCUSSION

Cells are tightly controlled by different regulator genes within the check point of the cell cycle for the maintenance of chromosomal level and therefore, any abnormalities in their expression might result in passing mutated genes or chromosomes into daughter cells which may serve as impetus for the development of oncogenesis with severe implications for tumour development and behaviour.[43-45] The prognostic significance of the p53 pathway, in view of its importance in the development, proliferation, cell migration and clinical outcome in BC is well documented.[46-48] However, there is no consensus regarding the significance of p53 pathway in BC among different ethnicities.

In this study, expression of the p53 regulatory proteins in 308 breast cancer cases from Nigerian women were evaluated and compared with histological tumour grade matched British women BC. Although, there is a paucity of information on the p53 expression in Nigerian breast cancer, the results presented in this study showed that the tumour specimens obtained from Nigerian compared with British women were more likely to express p53. This is in support of other data on p53 protein expression of diagnosis in black women. Porter *et* (2004r) *al* reported high expression of p53 in African-American compared with Caucasian women.[49] Similar results were also reported by Jones et al observing differences in p53 IHC expression between African American and Caucasian women.[50] Consistent with the tumours of aggressive behaviour (i.e. diagnosed earlier in life, premenopausal, tumours were larger than 2cm, metastasis into lymph node and lymphatic vessels). p53 expression was associated with unfavourable tumour characteristics in Nigerian compared with British counterpart and this is similar to findings regarding p53 over expression in BC.[36] In addition, Nigerian tumours expressing p53 had strong inclination towards basal phenotype but lack hormone receptors and BRCA1 compared with Caucasian and this is in agreement with previous studies on p53

TABLE 6: Relationship between p53 and BCL2, MDM2, p21 co-expression in Nigerian compared with UK breast cancer

Variables	Nigeria (%)	UK (%)	χ^2 value	p-value
$p53^{neg}$ / $BCL2^{neg}$	28 (17.6)	56 (22.0)	**148.30**	**<0.001**
$p53^{neg}$ / $BCL2^{pos}$	23 (14.5)	137 (53.9)		
$p53^{pos}$ / $BCL2^{neg}$	67 (42.1)	33 (13.0)		
$p53^{pos}$ / $BCL2^{pos}$	41 (25.8)	28 (11.0)		
$p53^{neg}$ / $MDM2^{neg}$	50 (27.5)	10 (5.2)	**215.39**	**<0.001**
$p53^{neg}$ / $MDM2^{pos}$	13 (7.1)	135 (69.6)		
$p53^{pos}$ / $MDM2^{neg}$	92 (50.5)	3 (1.5)		
$p53^{pos}$ / $MDM2^{pos}$	27 (14.8)	46 (23.7)		
$p53^{neg}$ / $p21^{neg}$	51 (26.7)	86 (47.0)	**68.54**	**<0.001**
$p53^{neg}$ / $p21^{pos}$	19 (9.9)	53 (29.0)		
$p53^{pos}$ / $p21^{neg}$	107 (56.0)	30 (16.4)		
$p53^{pos}$ / $p21^{pos}$	14 (7.3)	14 (7.7)		

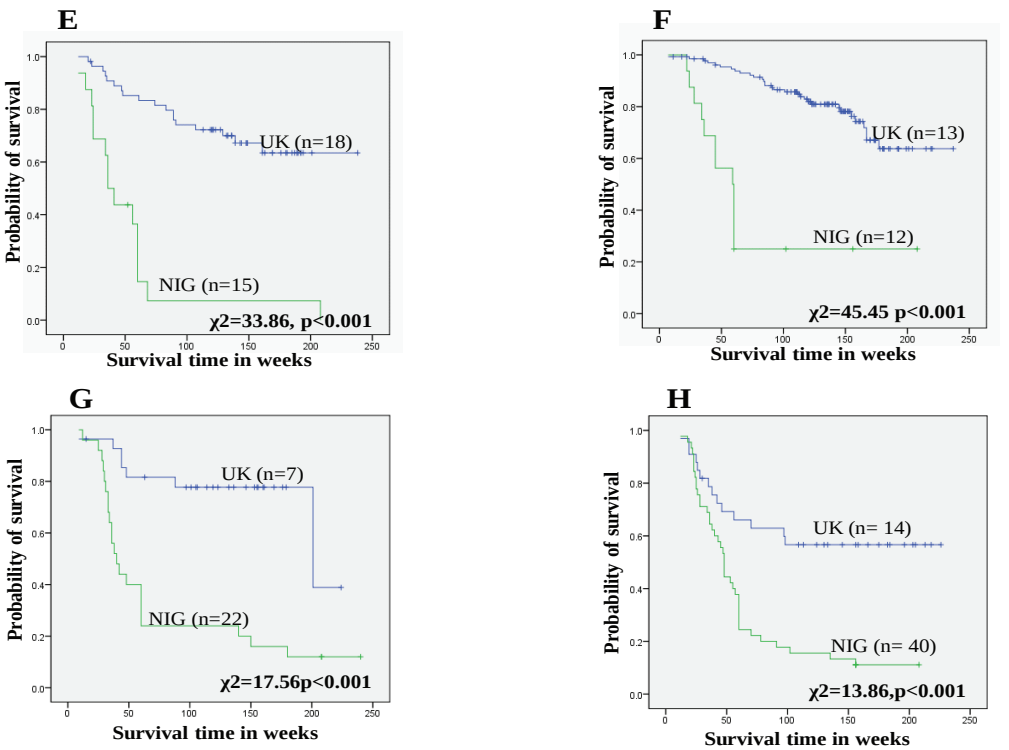

FIG. 3: BCSS of patients with breast tumours in the Nigerian and UK series showing the following phenotypes: E) $p53^{neg}$/Bcl-2^{neg} (F) $p53^{neg}$/ Bcl-2^{pos} (G) $p53^{pos}$ / Bcl-2^{pos} (H) $p53^{pos}$ / Bcl-2^{neg}

dysfunction roles in aggressive tumours.[51,52] Also, a greater percentage of Nigerian tumours with p53 expression had altered E-and P-cadherin expression. Altered expression of E- and p-cadherin (loss or low expression of E- and over expression p-cadherin) are needed for the epithelial to mesenchymal transition, which is a hallmark of tumour metastasis to the distance sites in breast cancer.[53] Therefore, over expression of p53 might have contributed to the high degree of metastasis in Nigerian tumours.

Studies also suggest that p53 dysfunction contributes to the modulation of the efficacy of the chemotherapy and therefore considered as

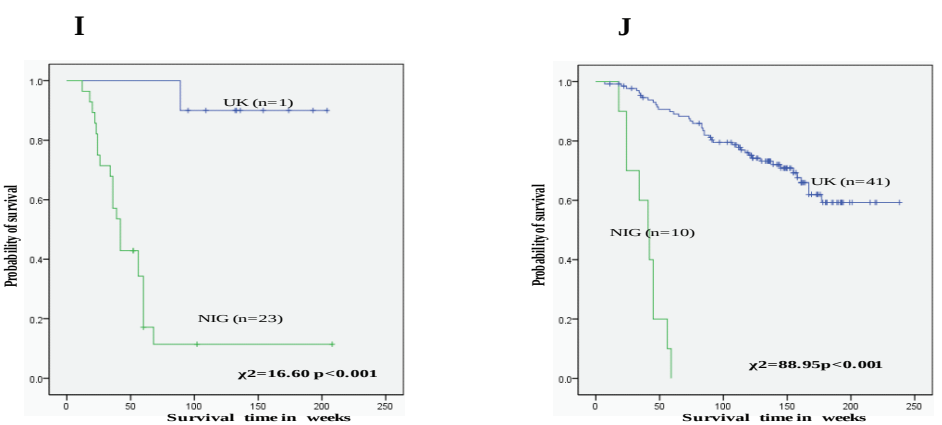

FIG. 4: BCSS of patients with breast tumours in the Nigerian and UK series showing the following phenotypes: (I) $p53^{neg}$/ $MDM2^{neg}$ (J) $p53^{neg}$/ $MDM2^{pos}$ expression

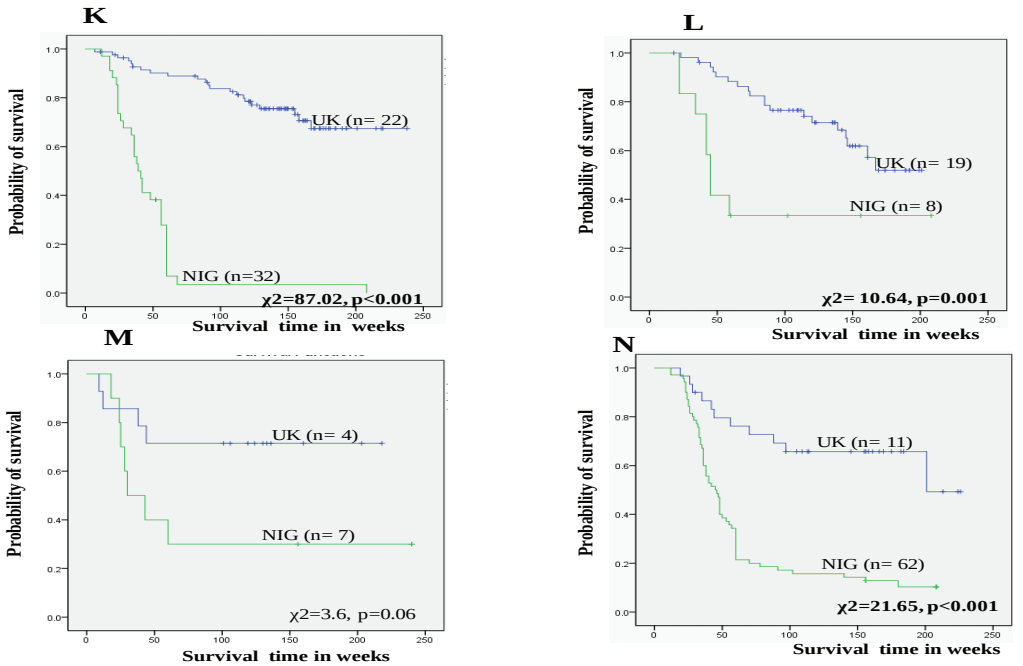

FIG. 5: BCSS of patients with breast tumours in the Nigerian and UK series showing the following phenotypes: (K) $p53^{neg}/p21^{neg}$ (L) $p53^{neg}/p21^{pos}$ (M) $p53^{pos}/p21^{pos}$ (N) $p53^{pos}/p21^{neg}$

prime factor for the chemotherapy failure.[54-56] Consistent with findings that tumour expressing p53 contributes immensely towards poor clinical outcome, significant number of Nigerian with p53 tumours had short BCS compared with British women. Based on the above observations, p53 dysfunction might have contributed to the aggressive nature and poorer clinical outcome of breast tumours arising from Nigerian women compared with those from British women as a result of lack of cell cycle control, modulation of the efficacy of the standard chemotherapy, and non response to hormonal therapy. Therefore, p53 dysfunctions have lots of implications for therapeutic decision on BCs arising from different ethnicity and the p53 roles observed in this study has enhanced our understanding on the racial disparity in BCSS.

It has been hypothesised that combining protein expression of p53 with three downstream targets (p21, MDM2 and Bcl-2) may be a more sensitive and specific method for p53 pathway dysfunctional roles in breast cancer rather than p53 alone.[36,57] MDM2 protein functions as a regulator of the p53 in an auto regulatory negative feedback cross talk; a loss of p53 function is indicated by either p53 mutation or by nuclear accumulation of functionally impaired p53 protein that usually leads to a decrease in MDM2 protein expression.[58] Furthermore, abnormal p53 proteins can invariably affect the biological function of p53 as well as other controlling proteins such as MDM2, MDM4, ATM and ER.[57,59-61] Tumours that express MDM4, MDM2, p21 and Bcl-2 without p53 expression are associated with an excellent prognosis and favourable clinicopathological characteristics, while p53 positive tumours with negativity of any of these markers MDM2, MDM4, BLC2 and p21 are linked with features of poor clinicopathological variables and outcome.[36] In line with features associated with poor clinical outcome with p53 downstream pathways, p53 was expressed while majority of the associated cell cycle markers were lost in Nigerian breast cancer while the opposite were observed in British women. In addition, majority of Nigerian BC that express p53/Bcl-2/MDM2/p21 phenotypes, except $p53^{pos}/p21^{pos}$, $p53^{pos}/MDM2^{pos}$ and $p53^{pos}/MDM2^{neg}$ were associated with poor outcome compared with British counterpart. The small numbers in the different groups made the analysis impossible in this study after stratification of the tumours

TABLE 7: Cox multivariate regression analyses of p53/Bcl-2 co-expressions in relation to BCSS in Nigerian and UK series

Variables	$p53^{neg}$ / $Bcl\text{-}2^{neg}$				$p53^{neg}$ / $Bcl\text{-}2^{pos}$				$p53^{pos}$/ $Bcl\text{-}2^{pos}$				$p53^{pos}$ / $Bcl\text{-}2^{neg}$			
	p-value	Hazard ratio	95 % CL Lower	Upper	p-value	Hazard ratio	95 % CL Lower	Upper	p-value	Hazard ratio	95 % CL Lower	Upper	p-value	Hazard ratio	95 % CL Lower	Upper
Racial differences																
Nigeria vs UK	**0.004**	4.79	1.64	13.98	**<0.001**	9.92	3.39	28.99	0.14	2.41	0.74	7.88	**0.004**	3.09	1.43	6.71
Lymph node	0.97	1.01	0.39	2.58	0.92	0.96	0.48	1.91	0.66	0.78	0.25	2.39	0.53	0.77	0.35	1.72
Tumour Size	0.02	1.46	1.04	2.05	0.95	1.01	0.71	1.43	0.14	1.32	0.90	1.93	0.84	1.02	0.78	1.34
ER	0.57	1.26	0.56	2.80	0.21	2.42	0.60	9.65	0.03	0.33	0.12	0.93	0.32	0.72	0.38	1.37

TABLE 8: Cox multivariate regression analyses of p53/MDM2 phenotypes in relation to BCSS in Nigerian and UK series

Variables	$p53^{neg}$ /$MDM2^{neg}$				$p53^{neg}$ / $MDM2^{pos}$			
	p-value	Hazard ratio	95% CL Lower	Upper	p-value	Hazard ratio	95% CL Lower	Upper
Racial differences								
Nigeria vs UK	**0.006**	24.78	2.55	239.9	**< 0.001**	19.25	4.79	77.38
Lymph node	0.62	0.72	0.19	2.69	0.40	1.30	0.69	2.43
Tumour Size	0.37	0.85	0.60	1.20	0.02	1.41	1.03	1.93
ER	0.12	0.51	0.22	1.2	0.13	2.38	0.77	7.37

TABLE 9: Cox multivariate regression analyses of p53/p21 phenotypes in relation to BCSS in Nigerian and UK breast cancer

Variables	$p53^{neg}$ / $p21^{neg}$				$p53^{neg}$ / $p21^{pos}$				$p53^{pos}$ / $p21^{neg,}$			
	p-value	Hazard ratio	95 % CL Lower	Upper	p-value	Hazard ratio	95 % CL Lower	Upper	p-value	Hazard ratio	95 % CL Lower	Upper
Racial differences												
Nigeria vs UK	**< 0.001**	7.74	3.38	17.68	**0.004**	7.61	1.93	29.89	**0.002**	3.49	1.57	7.78
Lymph node	0.36	1.42	0.66	3.07	0.06	0.43	0.18	1.04	0.58	0.80	0.37	1.73
Tumour Size	0.20	1.20	0.90	1.60	0.83	1.04	0.70	1.54	0.36	1.10	0.88	1.38
ER	0.74	0.89	0.47	1.70	0.14	2.66	0.70	10.01	0.01	0.46	0.25	0.86

using Abdel-fatah *et al*[36] hypothesed biological pathway. However, several studies have reported similar findings using the combinations that were used in the analysis of p53 transcription pathways in this study. For instance, Ihemaladu *et al* reported $p53^{pos}$/Bcl-2^{neg} was significantly associated with the basal phenotype and may have also contributed to aggressiveness observed in African American.[62] Race was observed as an independent predictor of BCSS in $p53^{neg}$/Bcl-2^{pos}, $p53^{neg}$/$p21^{neg}$ and $p53^{neg}$/$MDM2^{pos}$ phenotypes. Based on these observations, BC arising from the Black and Caucasian women might have been influenced by p53 transcription pathways.

In conclusion, in this study, protein expressions of p53 pathways are different between Nigerian and British breast cancer and these may also contributes to the differences in the tumour biology that exists between Nigerian and British breast cancer women. Therefore, targeting these p53 pathways for therapeutic usage might reduce the poor BCSS in Nigerian women.

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Competing interests
The authors declared no competing interest

Authors' contributions
Ayodeji Johnson Agboola participated in the design of the study, performed the immunohistochemistry and manuscript write up, Adewale A. Musa and Babatunde A Ayoade contributed to patients' management and outcome follow up, Adekumbiola A. Banjo and Charles C.Anunobi performed the histological diagnosis of the samples in Nigeria, Emad Rakha and Ian.O.Ellis contributed re-evaluation of the histological diagnosis of the samples from Nigeria at UK histopathology laboratory and Ian. O.Ellis also contributed immersely towards the design of the study and manuscript development. Chris C. Nolan and Andrew Green performed the TMA and Additionally, Andrew Green participated in the design of the study, immunohistochemical scoring and also edited the manuscript. Anotu Mopelola Deji Agboola performed the statistical analysis. T. Abdel-Fatah performed immunohistochemical scoring on UK cohort.

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