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Expression of MAGE-A and NY-ESO-1 cancer/testis antigens is enriched in triple-negative invasive breast cancers

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Aims: A better understanding of the expression of cancer/testis antigens (CTAs) in breast cancer might enable the identification of new immunotherapy options, especially for triple-negative (TN) tumours, which lack expression of the conventional therapeutic targets oestrogen receptor, progesterone receptor, and human epidermal growth factor receptor 2. The aim of this study was to quantify the expression of MAGE-A and NY-ESO-1 CTAs in breast cancer, and relate this to known clinicopathological parameters.

Methods and results: We surveyed MAGE-A and NY-ESO-1 expression in an unselected cohort of 367 breast tumours (of which 65 were TN), with accompanying clinical follow-up data, by using immunohistochemical analysis of tissue microarrays. Relevant to their potential as vaccine targets in breast cancer, MAGE-A was expressed in 13% of cases, and NY-ESO-1 in 3.8%, with the majority of tumours showing fairly homogeneous staining within individual tissue cores (~85% of cases with staining in >75% of tumour cells). Most NY-ESO-1-positive cases also expressed MAGE-A ($P = 2.06 \times 10^{-9}$), and both were strongly associated with the TN phenotype ($P < 0.0001$), with the most proliferative and poorly differentiated cases, in paticular, showing genomic instability. This was characterised by coexpression of c-Kit and TTK, and overexpression of p53.

Conclusions: MAGE-A and NY-ESO-1 are frequently expressed in TN breast cancer (~47% and 17% of TN cases, respectively), suggesting that targeting them could be feasible in this patient group. Expression is reasonably homogeneous in positive cases, suggesting that immunohistochemical analysis of tissue biopsies would be a reliable companion biomarker.

Keywords: cancer/testis antigens, MAGE-A, NY-ESO-1, TNBC

Introduction

Breast cancer is the most frequently diagnosed cancer in women. It is also a predominant cause of mortality, and the global burden of breast cancer rises every year.[1] Approximately 10–20% of breast tumours belong to the triple-negative (TN) subtype, defined by lack of expression of oestrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2), which are companion biomarkers and/or therapeutic targets of hormone and anti-HER2 therapy. Cytotoxic chemotherapy is the standard-of-care therapy, and is very effective in a subset of patients, but responses

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are not durable in all cases,[2] which ultimately relapse with the shortest progression-free and overall survival of all breast cancers.[3] Accordingly, efforts are ongoing to identify predictive markers of chemotherapy efficacy in TN breast cancer (TNBC), as well as alternative treatments for patients who are most likely to develop resistance.

High-resolution genetic and cellular profiling of TNBCs has revealed subgroups characterised by chromosomal instability (CIN), DNA repair defects, and androgen receptor (AR) signalling.[4–6] The immune microenvironment is also a strong determinant of both the molecular profile and the clinical outcome in TNBC patients, with the poorest prognostic group being characterised by stromal restriction of tumour-infiltrating lymphocytes (TILs), or a general deficiency of TILs in tumour and stromal compartments.[7–9] Therapeutic strategies retargeting the immune system to cancer cells have proven successful in liquid cancers[10–13] as well as in a few solid tumours.[14–18] This is achieved by manipulating the endogenous immune response[19–22] and/or bypassing it altogether by genetically reprogramming T cells with adoptive transfer of chimeric antigen receptor (CAR) T cells, which are reprogrammed to target tumour antigens.[10–18] Therapeutic cancer vaccines may also be useful as part of regimens aimed at boosting antitumour immunity. Cancer vaccines and CAR T cells can be directed against proteins expressed by many cancers (shared antigens) or neoantigens encoded by mutant transcripts.

Cancer/testis antigens (CTAs) belong to a group of proteins that are expressed in the developing embryo, are restricted to the testis in the adult, and are aberrantly re-expressed in malignancy, particularly high-grade and advanced-stage tumours, including TNBC.[23–28] Members of the melanoma-associated antigen (MAGE) family and New York Esophageal Squamous Cell Carcinoma-1 (NY-ESO-1) are among the CTAs being actively investigated as cancer immunotherapy targets. They have been shown to evoke spontaneous cytotoxic T-cell responses in melanoma, oesophageal carcinoma, bladder cancer, and non-small-cell lung carcinoma.[29,30] Several studies have evaluated MAGE-A and NY-ESO-1 expression in breast cancer, with variable reports on expression frequency.[23,31–34] Their therapeutic potential for immunotherapy and the staining homogeneity observed in diverse cohorts led us to investigate the expression of MAGE-A and NY-ESO-1 in our historical cohort of breast cancer patients.

Materials and methods

TISSUE MICROARRAYS (TMAS) AND HISTOPATHOLOGY REVIEW

This study made use of the Queensland Follow-Up (QFU) cohort, a resource comprising formalin-fixed paraffin-embedded breast tumours from patients undergoing surgical resection at the Royal Brisbane Women's Hospital (RBWH) between 1987 and 1994, with accompanying long-term (up to 30 years) clinical follow-up information.[35–39] Ethical approval from the Human Research Ethics Committees of the RBWH and the University of Queensland was obtained prior to the commencement of the study. Tumours were sampled in duplicate on TMAs for biomarker studies, and haematoxylin and eosin-stained whole tissue sections were available for histopathological review.

The review was conducted by an experienced breast pathologist (S.R.L.; parameters included histological subtype, grade, and the presence of lymphovascular invasion and lymphocytic infiltrate; Table 1). We also considered patient age, tumour size, and lymph node (LN) status (whether disease had spread to the LNs at the time of surgery), extracted from diagnostic pathology reports. We selected a range of breast tumour biomarkers implicated in the prognosis and/or pathobiology of breast cancer: (i) hormone receptors, i.e. ER and PR; (ii) Ki67, a marker of proliferation; and (iii) a range of biomarkers implicated in TNBC, including markers of basal/myoepithelial-like phenotype [epidermal growth factor receptor (EGFR) and the high molecular weight cytokeratins (CKs) CK5/6 and CK14], vimentin (mesenchymal marker), androgen receptor (AR) (can confer luminal-like intracellular signalling and a luminal-like phenotype in a proportion of TNBCs with a favourable outcome),[40,41] c-Kit (associated with primitive, progenitor states[42]), p53 (overexpression is associated with genomic instability),[43] and mitosis-independent expression of the dual-specificity protein kinase TTK (implicated in chromosomal instability and poor clinical outcome.[35]

IMMUNOHISTOCHEMICAL (IHC) ANALYSIS

Analysis of the CTAs were performed by staining the QFU TMA slides with antibodies against MAGE-A (Santa Cruz Biotechnology, Santa Cruz, CA, USA; sc-20034, 1:500 dilution), NY-ESO-1 (Santa Cruz Biotechnology; sc-53869, 1:30 dilution), p53 (Dako, Santa Clara, CA, USA; M7001, 1:150 dilution), c-Kit

Table 1. Association of MAGE-A and NY-ESO-1 expression with histopathological parameters in breast cancer.

	n			% of cases				*n*			% of cases		
MAGE-A staining:	Total	MAGE-A-positive	MAGE-A-negative	Negative	Positive	*P*-value	NY-ESO-1 staining:	Total	NY-ESO-1-positive	NY-ESO-negative	Negative	Positive	*P*-value*
Age (years)							Age (years)						
>40	273	237	36	87	13	NS	>40	300	287	13	96	4	NS
≤40	35	27	8	77	23		≤40	38	38	0	100	0	
n	308						*n*	338					
Grade							Grade						
G1	46	46	0	100	0	<0.0001	G1	50	50	0	100	0	0.023
G2	176	157	19	89	11		G2	170	166	4	98	2	
G3	140	106	34	76	24		G3	134	124	10	93	7	
n	362						*n*	354					
Mitotic score							Mitotic score						
1	43	43	0	100	0	<0.0001	1	199	196	3	98	2	0.0255
2	154	137	17	89	11		2	47	44	3	94	6	
3	126	95	31	75	25		3	107	99	8	93	7	
n	323						*n*	353					
Histological type							Histological type						
Ductal NOS	188	161	27	86	14	NS	Ductal NOS	207	198	9	96	4	NS
Lobular/variants	37	35	2	95	5		Lobular/variants	42	42	0	100	0	
Mixed ductolobular	30	26	4	87	13		Mixed ductolobular	32	31	1	97	3	
Mixed	30	25	5	83	17		Mixed ductolobular	31	28	3	90	10	
Metaplastic	14	9	5	64	36		Metaplastic	15	15	0	100	0	
Special types	24	20	4	83	17		Special types	27	26	1	96	4	
n	323						*n*	354					

Table 1. *(Continued)*

MAGE-A staining:	*n*			% of cases		*P*-value	NY-ESO-1 staining:	*n*			% of cases		*P*-value*
	Total	MAGE-A-positive	MAGE-A-negative	Negative	Positive			Total	NY-ESO-1-positive	NY-ESO-negative	Negative	Positive	
Lymph node status							Lymph node status						
Negative	97	84	13	87	13	NS	Negative	102	100	2	98	2	NS
Positive	82	69	13	84	16		Positive	88	83	5	94	6	
n	179						*n*	190					
Tumour size (mm)							Tumour size (mm)						
< 20	140	125	15	89	11	NS	<20	152	147	5	97	3	NS
20–50	121	98	23	81	19		20–50	132	127	5	96	4	
>50	21	18	3	86	14		>50	22	20	2	91	9	
n	282						*n*	306					
Lymphovascular invasion							Lymphovascular invasion						
Absent	236	202	34	86	14	NS	Absent	262	252	10	96	4	NS
Present	87	73	14	84	16		Present	92	88	4	96	4	
n	323						*n*	354					
Lymphocytic infiltrate							Lymphocytic infiltrate						
Absent	108	105	3	97	3	<0.0001	Absent	123	123	0	100	0	NS
Mild	145	117	28	81	19		Mild	157	150	7	96	4	
Moderate-severe	57	42	15	74	26		Moderate-severe	61	55	6	90	10	
n	310						*n*	341					
Central scarring/fibrosis							Central scarring/fibrosis						
Absent	289	248	41	86	14	NS	Absent	319	306	13	96	4	NS
Present	34	27	7	79	21		Present	35	34	1	97	3	
n	323						*n*	354					

Table 1. *(Continued)*

MAGE-A staining:	*n*			% of cases		*P*-value	NY-ESO-1 staining:	*n*			% of cases		*P*-value*
	Total	MAGE-A-positive	MAGE-A-negative	Negative	Positive			Total	NY-ESO-1-positive	NY-ESO-negative	Negative	Positive	
Tumour border							Tumour border						
Infiltrative	281	247	34	88	12	0.0023	infiltrative	310	299	11	96	4	NS
Pushing	45	31	14	69	31		Pushing	44	41	3	93	7	
n	326						*n*	354					
HR status							Hormone receptor (HR) status						
Negative	77	46	31	59.7	40.3	<0.0001	Negative	68	66	2	97.1	2.9	<0.0001
Positive	244	227	17	93.0	7.0		Positive	282	270	12	95.7	4.3	
n	321						*n*	350					
ER status							ER status						
Positive	241	225	16	93	7	<0.0001	Positive	270	268	2	99	1	<0.0001
Negative	83	51	32	61	39		Negative	85	73	12	86	14	
n	324						*n*	355					
HER2 status (CISH)							HER2 status (CISH)						
Negative	286	244	42	85	15	NS	Negative	314	301	13	96	4	ns
Positive	30	27	3	90	10		Positive	31	31	0	100	0	
n	316						*n*	345					
Ki67							Ki67						
Negative	192	177	15	92	8	<0.0001	Negative	192	188	4	98	2	0.0386
Positive	124	93	31	75	25		Positive	124	115	9	93	7	
n	316						*n*	316					

Table 1. *(Continued)*

MAGE-A staining:	*n* Total	MAGE-A-positive	MAGE-A-negative	% of cases Negative	Positive	*P*-value	NY-ESO-1 staining:	*n* Total	NY-ESO-1-positive	NY-ESO-negative	% of cases Negative	Positive	*P*-value*
Basal marker: CK14, CK5, EGFR							Basal marker: CK14, CK5. EGFR						
Negative	216	199	17	92	8	<0.0001	Negative	236	235	1	100	0	<0.0001
Positive	87	58	29	67	33		Positive	91	78	13	86	14	
n	303						*n*	327					
Vimentin							Vimentin						
Negative	284	253	31	89	11	<0.0001	Negative	316	308	8	97	3	0.0016
Positive	38	21	17	55	45		Positive	38	32	6	84	16	
n	322						*n*	354					
Androgen receptor							Androgen receptor						
Negative	35	19	16	54	46	<0.0001	Negative	38	30	8	79	21	<0.0001
Positive	278	248	30	89	11		Positive	305	300	5	98	2	
n	313						*n*	343					

CISH, chromogenic *in-situ* hybridisation; CK, cytokeratin; EGFR, epidermal growth factor receptor; ER, oestrogen receptor; HER2, human epidermal growth factor receptor 2; HR, hormone receptor; MAGE, melanoma-associated antigen; NOS, not otherwise specified; NS, not significant; NY-ESO-1, New York Esophageal Squamous Cell Carcinoma-1. st.
*Chi-square test or Fisher's exact test.

(Dako; A4502, 1:1000 dilution), TTK (Abcam, Cambridge, UK; ab11108, 1:100 dilution), HER2 (Dako; A0485, 1:200 dilution), ER (Novocastra, Newcastle upon Tyne, UK; NCL-L-ER-6F11, 1:100 dilution), PR (Novocastra; NCL-L-PGR-312, 1:200 dilution), vimentin (Dako; M0725, 1:400 dilution), AR (Dako; M3562, 1:50 dilution) and Ki67 (Dako; M724001-2, 1:200 dilution) with the MACH 1 Universal HRP-Polymer (Biocare Medical; (Pacheco, CA, USA; Cat. no. M1U539 G, L10) or Vectastain Universal ABC (Vector Laboratories, Burlingame, CA, USA) kit. Slides were scanned with the Aperio ScanScope T2 digital system (Buffalo Grove, IL, USA), and core images were then segmented into individual images for scoring (Aperio Spectrum TMA module).

Samples were assessed in a blinded manner by two observers (A.P. and P.K.dC). For MAGE-A and NY-ESO-1, scoring was based purely on intensity, owing to its homogeneity of staining in the tissue cores. A score in a range of 0–3 (0 = negative; 1 = weak; 2 = moderate; 3 = strong) was assigned for each cellular compartment—cytoplasm and nucleus. However, the majority of the cases (65%) expressed CTAs homogeneously throughout the tumour compartment of duplicate cores, and there were no obvious subcellular expression patterns. Hence, cytoplasmic and nuclear component scoring were not considered separately for further analysis. Tumour cell percentage staining was subsequently stratified as either >75% or <75%, as this adequately described the positive cases. IHC analysis was performed for the markers, the results for some of which have been published previously.[35,36,38,39] The cut-off value for HER2 positivity was based on a silver *in-situ* hybridisation (SISH) score of >6 and an IHC score of 3+ if SISH was unsuccessful. Once HER2 positivity had been determined, ER status and PR status were examined; staining of ≥1% of the tumour cell nuclei was considered to be positive. After scoring for ER positivity if the sample was negative for both ER/PR and HER2, it was assigned to the TN subtype. For distinction between TN, basal-like, and non-basal, if ≥1% of tumour cells were positive for either EGFR or CK14 or CK5/6, the tumour was considered to be TN basal-like. However, with relevance to the clinical context, TN/basal-like is not a clinically defined subtype, and we therefore assigned any tumours that fell into the TN category to the TN group.

Once HER2 status and ER status had been determined, Ki67 expression was scored high if staining was observed in 10% of the tumour cell nuclei, and low if it was observed in <10% of the tumour cell nuclei. Biomarkers such as TTK and c-Kit were scored purely on intensity, owing to the homogeneity of staining in the tissue cores. A score in a range of 0–3 (0 = negative; 1 = weak; 2 = moderate; 3 = strong) was assigned. Scoring for p53, vimentin and AR was based on an IHC score, which was derived by multiplying the intensity and percentage of the tumour cell staining. It was further stratified into a binary scoring system, i.e. 0–1 (<60 = 0/negative and >60 = 1/positive for p53; >0 = 1/positive for AR and vimentin).

Statistical analysis was performed with PRISM (v7). Associations between MAGE-A, NY-ESO-1 and clinicopathological parameters were evaluated with the chi-square test and Fisher's exact test. Relationships with breast cancer-specific survival were investigated with Kaplan–Meier analysis, with log-rank tests being used to assess significance. *P*-values of <0.05 were considered to be significant.

Results

COHORT DEMOGRAPHICS AND CLINICOPATHOLOGY

Among the 367 cases, the median patient age at diagnosis was 59 years, the median follow-up was 5.2 years, and the median follow-up of patients who were alive was 21.5 years. These cases were collected between 1987 and 1994. Most of the cases were grade 2 (49%), and LN status was available for 56% of the cohort (27% LN-positive; 29% LN-negative) (Figure 1A). Invasive ductal carcinoma (IDC) was the major histological subtype (58%), followed by lobular variants (12%), mixed histologies (ductolobular, 9%; others collectively, 9%), metaplastic (5%), and other special types (collectively, 7%; Figure 1A). IHC staining showed that 75% of the cohort were ER-positive, and SISH analysis identified *ERBB2* amplification in 10% of cases.

Cases were also categorised according to their expression of a panel of prognostic biomarkers: ER, PR, HER2, Ki67, and expression of EGFR and/or high molecular weight cytokeratins (CK5/6 and CK14), which are associated with a basal-like phenotype. According to this scheme,[35] the majority of the cohort was categorised as ER-positive/Ki67-low (65%), followed by TN/basal-like (15%), HER2-positive (10%), ER-positive/Ki67-high (7%) and TN/non-basal (3%) (Figure 1A).

EXPRESSION OF MAGE-A AND NY-ESO-1 IN INVASIVE BREAST CANCER

MAGE-A and NY-ESO-1 showed homogeneous staining, with positivity in both cytoplasmic and nuclear

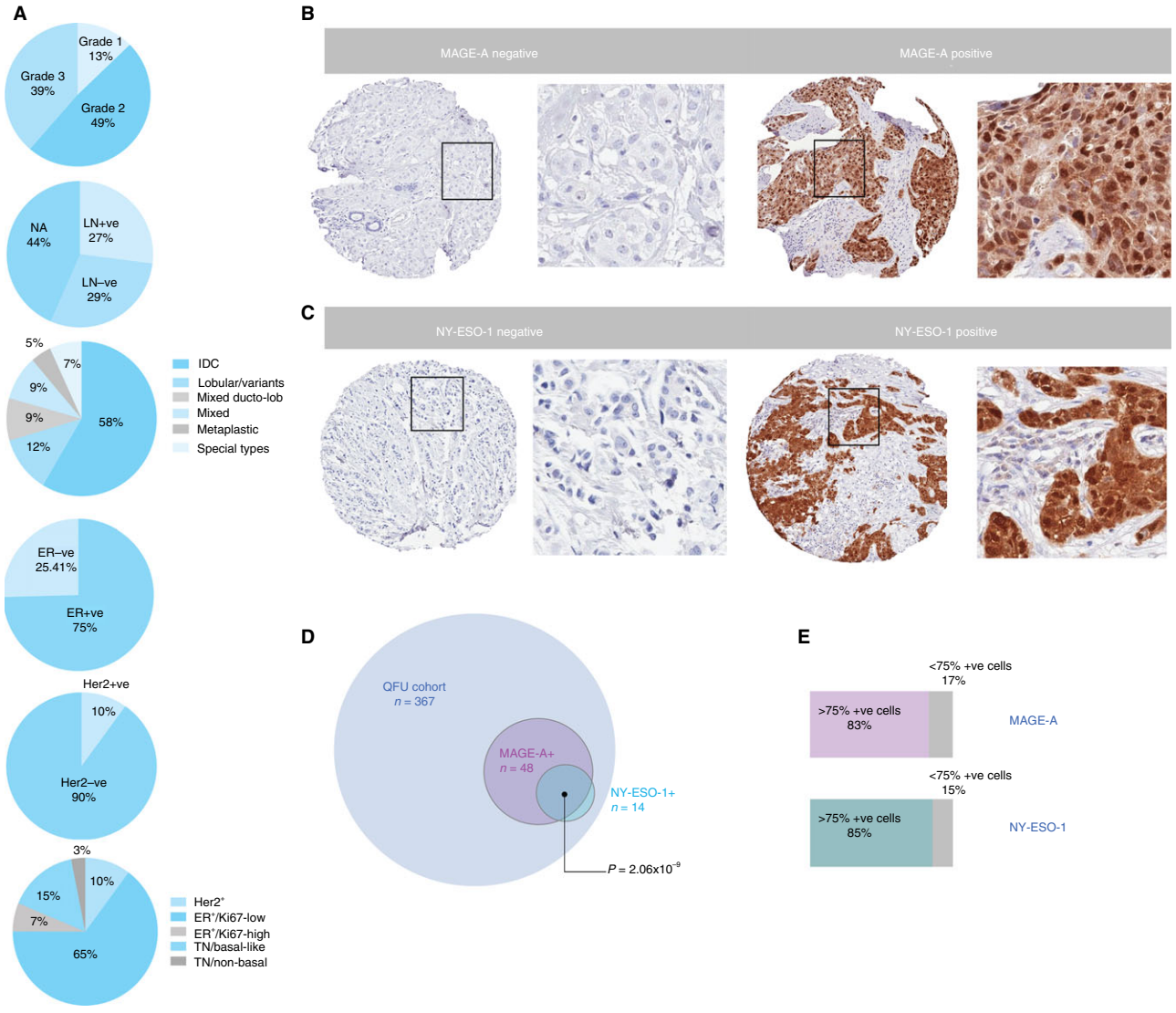

Figure 1. **A**, Distribution of the Queensland Follow-Up patient population according to: grade, lymph node involvement, histological subtype, oestrogen receptor positivity, HER2 status, and various prognostic subtypes. **B**,**C**, Representative images of MAGE-A and NY-ESO-1 staining on breast tumour tissue microarray cores, shown at low and high magnification. **D**, Concomitant expression of MAGE-A and NY-ESO-1. **E**, Proportion of MAGE-A-positive and NY-ESO-1-positive tumours expressing the respective antigens in >75% of cells within duplicate cores.

tumour cell compartments in 13% ($n = 48$) and 3.8% ($n = 14$) of cases, respectively (Figure 1B–D). CTA staining was relatively homogeneous, with >75% of tumour cells being stained in the majority of the MAGE-A-positive (83%) and NY-ESO-1-positive (85%) cases (Figure 1E). This criterion was employed as a threshold to note homogeneity, and not as a cut-off for positivity. Interestingly, 12 of the 14 NY-ESO-1-positive cases coexpressed MAGE-A (Figure 1D; $P = 2.06 \times 10^{-9}$). Analysis of clinicopathological parameters showed that expression of both CTAs was associated with Ki67 expression and grade (driven mostly by the mitotic score component; Table 1). This was underpinned by strong enrichment of expression in TN tumours (86% of which were TN/basal-like for MAGE-A, and all of which were TN/basal-like for NY-ESO-1 (Figure 2Ai,ii). Taking advantage of data generated as part of previous QFU cohort studies,[35–39] we further examined the phenotypic features of MAGE-A-expressing and NY-ESO-1-expressing TNBCs, and found positive associations with c-Kit, p53, and mitosis-independent TTK expression (Figure 2B). The CTAs (particularly NY-ESO-1) were also associated with expression of vimentin (a marker of mesenchymal differentiation), and were inversely associated with

AR expression (Table 1), although these associations did not reach statistical significance within the TN group (not shown).

EXPRESSION OF MAGE-A AND NY-ESO-1 IN BREAST CANCER IS NOT ASSOCIATED WITH SURVIVAL

The median overall survival time of all cases in this present study was 13.6 years. According to Kaplan–Meier survival analysis, MAGE-A-positive cases within the whole cohort showed a 40% decrease in breast cancer-specific survival (BCSS) at 25 years (Figure 3A), and MAGE-A-positive cases within the TN group showed a 35% decrease in BCSS at 5 years (Figure 3C), which was not significantly different from the MAGE-A-negative cases. NY-ESO-1-positive cases showed a trend towards a poorer outcome (Figure 3B), but there was no difference after accounting for TN status (Figure 3D).

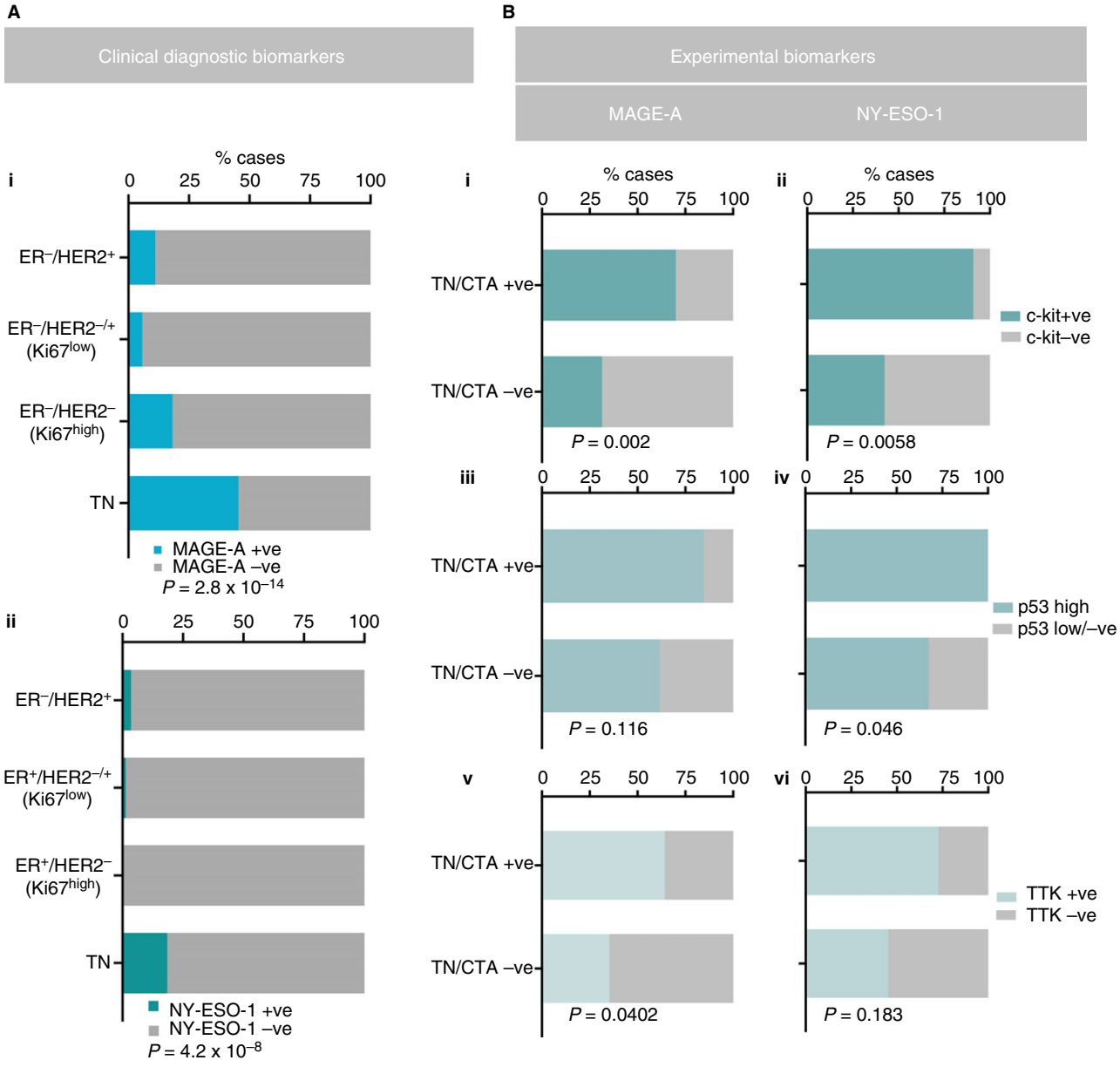

Figure 2. **A**,**B**, Chi-square or Fisher's exact test analysis of associations between expression of MAGE-A/NY-ESO-1 and clinical diagnostic or experimental biomarkers (c-Kit, p53, and TTK1).

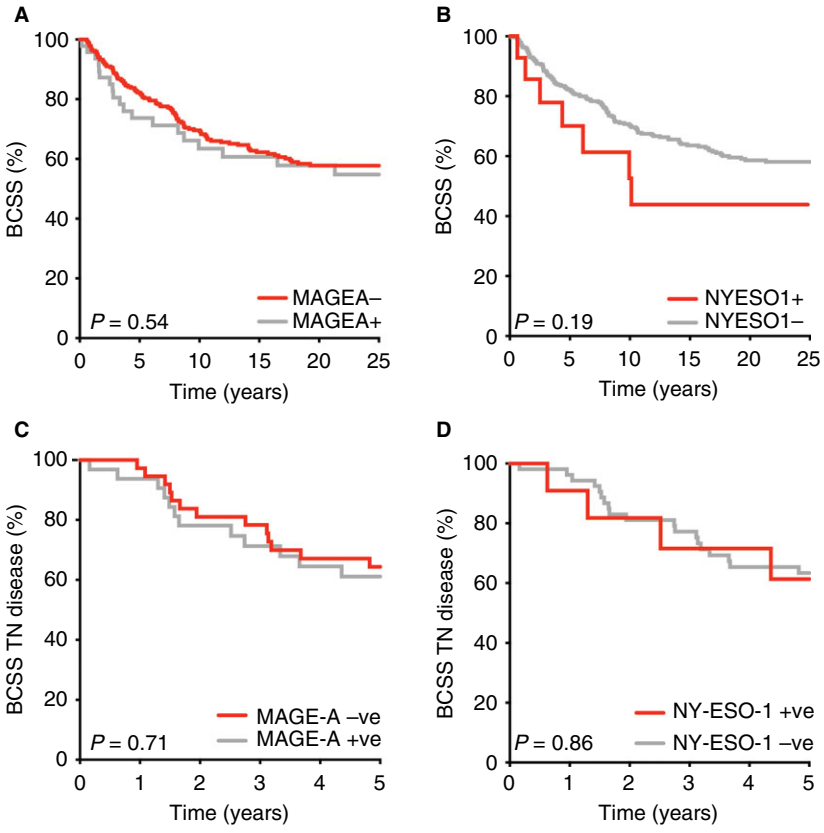

Figure 3. Kaplan–Meier survival analysis of the whole Queensland Follow-Up cohort (**A**,**B**) or triple-negative breast cancer (TNBC) cases (**C**,**D**) according to MAGE-A and NY-ESO-1 expression. For TNBC, the analysis focuses on the first 5 years after diagnosis, which is the period that is most determinant of long-term outcome. [Colour figure can be viewed at wileyonlinelibrary.com]

Discussion

We investigated the expression of MAGE-A and NY-ESO-1 in invasive breast cancer, and found prevalent expression within the TN group. Previous reports have shown variable expression of MAGE-A and NY-ESO-1, ranging between 17% and 74%, and between 2% and 40%, respectively,[31,44] and the expression levels of both were higher in TNBC,[31,44–48] which corroborates our findings. Taking advantage of the large pre-existing dataset on experimental biomarkers in the QFU cohort, we found that the CTAs are expressed most prevalently in the TN tumours showing salient features of poor differentiation/primitive phenotype, proliferation, and genomic instability. This was indicated by coexpression of c-Kit and TTK, and overexpression of p53. In addition, we found that these CTAs were expressed concomitantly and in the majority of the cells within a tumour, suggesting that they have favourable features as cancer vaccine targets. Our study strengthens the rationale for targeting CTAs to broaden the therapeutic options for TNBC.

Mutations in p53 can result in a stable non-functional protein that accumulates in the nucleus, and that gives rise to an IHC phenotype mimicking overexpression;[43,49] thus, p53 overexpression in cancer is a surrogate for functional abrogation. CIN and aneuploidy are interrelated and are crucial hallmarks of cancer, which is partly contributed to by inadequacy of p53.[50–55] This promotes aberrant DNA damage repair and augments mutagenesis.[56] Through association with p53 coexpression, our findings imply that NY-ESO-1-positive tumours reflect these chromosomal abnormalities, and have a proliferative advantage. However, we observed no association between MAGE-A and p53, which, perhaps, could be attributed to MAGE-A inhibiting its function;[57] one possible suggested mechanism is blocking of the interaction between p53 and chromatin, thus making p53 unable to regulate tumour cell proliferation and apoptosis.[57,58] Furthermore, we found a strong association between MAGE-A expression and mitosis-independent expression of the spindle assembly checkpoint protein TTK, which is crucial for chromosomal alignment and centrosome duplication, and is a marker for CIN in TNBC.[35,59,60]

Re-expression of CTAs in cancer is thought to give cancer cells stem cell-like properties.[61] Interestingly, we found striking coexpression of both CTAs with the mammary luminal progenitor marker c-Kit

(Figure 2Bi,ii), and enrichment with vimentin (Table 1), suggesting that these tumours are in relatively primitive states of differentiation.[62] Basal-like breast cancers often show luminal progenitor-like phenotypes, and may even originate from this cell type in the premalignant breast.[42] Therefore, this striking coexpression could be part of the association with the basal-like phenotype (Table 1). This strong correlation might also indicate a programmed state of dedifferentiation involving multiple stem cell markers, whereby a demethylation programme drives the expression of these CTAs, as is evident in ovarian and colon cancer.[63,64]

If cancer vaccine or CAR T-cell therapies are to be implemented for treatment in certain cases, efficacy would be determined, in part, by antigen abundance and heterogeneity. An extensive IHC analysis of the expression of eight CTAs in 454 IDCs revealed frequent coexpression in ER-positive as compared with ER-negative tumours,[47] consistent with our findings on MAGE-A and NY-ESO-1 coexpression. This raises the possibility that therapies simultaneously targeting multiple CTAs may elicit more efficient antitumour responses than single-antigen approaches. In terms of expression heterogeneity, we found that the majority of cases expressed MAGE-A and NY-ESO-1 in >75% of tumour cells within duplicate cores, which is also a potentially favourable feature in terms of therapeutic use. To maximise the efficacy of vaccination-based strategies, it would be informative to analyse the distribution of multiple 'actionable' CTAs within individual tumours in a larger cohort, in order to identify combinations that could achieve the greatest breadth of coverage.

Immunotherapy is an attractive strategy to target TNBC, especially in patients with minimal residual disease after neoadjuvant chemotherapy, because of their statistically poor prognosis.[46,48] Analysing MAGE-A and NY-ESO-1 expression in post-neoadjuvant chemotherapy surgical samples may therefore aid in recognising patients who are suitable for vaccination strategies. Interestingly, adoptive T-cell therapy targeting MAGE-A3 has shown promise in the metastatic setting,[65] and another trial is ongoing (Identifier no. NCT02111850).[66] We hypothesise that CTAs may be expressed more frequently in the chemoresistant cells selected after neoadjuvant therapy, owing to their association with features that promote clonal selection (primitive phenotype, high proliferation, and CIN). Features of high proliferation, evasion of apoptosis, and a primitive/stem-like state, which is considered to be an epithelial–mesenchymal transition state, perhaps confer chemoresistance to these cells, as is evident in multiple myeloma.[67]

Finally, our study was performed on a small number of TN tumours, so our findings are worth following up in a larger cohort. Nonetheless, given the promising benefits of immunotherapy for this group of patients with currently limited interventional strategies, it provides the rationale for targeting CTAs in TNBC.

Conflicts of interest

The authors declare no conflicts of interest.

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Author contributions

A. Raghavendra, J. M. Saunus and S. R. Lakhani designed the study. P. Kalita-de Croft, A. Raghavendra, A. C. Vargas and C. E. Smart performed the research. P. Kalita-de Croft analysed the data and wrote the manuscript. J. M. Saunus, P.T. Simpson and S. R. Lakhani contributed to writing and editing of the manuscript.

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