Title: Assessment of the androgen receptor in older women with primary breast cancer - association with a panel of biomarkers and breast cancer specific survival

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Abstract

Introduction

Breast cancer in older women tends to have more favourable biology, compared to younger women. Androgen receptor (AR) is significant for breast tumour carcinogenesis, however its role in older women has not been fully explored.

Methods

Surgical specimens were obtained from an existing series of 1,758 older women (≥70 years) with primary breast cancer, treated in a single institution with long-term (37+ years) follow-up. As part of previous work, it was possible to construct good quality tissue microarrays (TMAs) in 575 surgical specimens and a panel of 24 biomarkers has been measured by immunohistochemistry (IHC) in these TMAs. AR positivity was assessed by IHC and defined as H-score ≥40. The relationship between AR in this cohort was compared to an equivalent group of younger women (<70 years, n=1,708); the panel of 24 biomarkers and breast cancer specific survival (BCSS) in the older cohort.

Results

AR was assessed in 509 samples. Overall, 59% of the older women cohort had positive expression of AR, compared to 63% in the younger cohort. AR positivity (regardless of age) was associated with smaller size of tumour, lower grade of tumour, lower tubule formation, lower nuclear polymorphism and lower mitotic frequency. AR positivity was associated with positive expression of oestrogen receptor (ER), progesterone receptor (PR), breast cancer gene 1 (BRCA1), cytokeratin (CK) 7/8, CK18, CK19, B-cell lymphoma (Bcl)2 and Mucin 1 (Muc1) expression. Conversely, AR-positive expression was associated with negative expression of human epidermal growth factor receptor-2 (HER2), Ki-67, CK5, CK17, epidermal growth factor receptor (EGFR), and CD44 expression. Patients with AR-positive tumours in older women had better BCSS compared to AR-negative tumours (p=0.009).

Conclusions

There was no difference in AR expression between older and younger women with breast cancer. AR has prognostic potential in terms of BCSS. Further work is needed to investigate AR as a therapeutic target.

Keywords: androgen receptor, biomarkers, breast cancer, older women

Summary Points

Why carry out this study?

- Older women with breast cancer tend to have more favourable biology compared to their younger counterparts.

  Androgen receptor (AR) is a biomarker which has been shown to be significant for breast tumour carcinogenesis however its role specifically in older women with breast cancer has not been fully explored.

What was learned from this study?

- This research assessed expression of AR in older compared to younger women with breast cancer, and its role as a prognostic factor related to breast cancer specific survival (BCSS).
- No difference was found in the expression of AR between older and younger women, however, AR had prognostic potential in terms of BCSS. AR was also associated with different subtypes of breast cancer as it was significantly related to ER, PR, and HER-2 status.
- Further work needs to be done to investigate the therapeutic role of AR.

Introduction

Breast carcinoma is a heterogeneous disease, with varying biological subtypes resulting in differing responses to treatment. Risk of developing breast cancer increases with age (1), with over a third of all breast cancer patients presenting aged 70 or over (2).

Current management guidance for breast cancer emphasises the importance of tailoring treatments based on biology, which is especially important in the context of older women given the important differences between breast cancer in older compared to younger women when it comes to biology; the biology of breast cancer in older women seems to be more favourable for prognosis than their younger counterparts (3, 4). This is seen in the increased expression of biomarkers with good prognostic significance such as oestrogen receptor (ER) and progesterone receptor (PR), and decreased expression of those with poor prognostic significance such as epidermal growth factor receptor (EGFR) and tumour protein p53 (p53) (5).

Additionally, there are a number of factors that play a role in choosing treatment, especially for older women. Increased frailty, number of comorbidities and medication for older women are some of the reasons why older women may prefer non-surgical treatment such as primary endocrine therapy (PET) to surgery, which could pose additional risks for frail groups of patients. Studies have shown that comorbidities have a major role to play in influencing the choice of treatment for breast cancer in older women (6-9).

The remarkable clinical efficacy of treatments that target the biomarkers ER and human epithelial growth factor receptor (HER)2 has shaped current management of breast cancer and has led to increased emphasis on discovering novel targeted therapies (10, 11). This has led to much research into other biomarkers with potential predictive and prognostic significance, such

as Ki-67 (12), B-cell lymphoma (BCL)2 (13) and EGFR (14), to name a few. One such biomarker of interest is the androgen receptor (AR).

The AR is a transcription factor activated by steroid hormones which belong to the nuclear receptor superfamily along with ER and PR (15) and is expressed in around 70-90% of all breast cancers (16, 17). Steroid hormone receptors are critical components of signalling pathways and play an important role in controlling gene expression as transcription factors. Several androgens, including androstenedione (A) and dehydroepiandrosterone (DHEA), which are produced from the female ovaries and adrenal glands, bind to AR (18, 19) and are, in turn, involved in both cell proliferation and apoptosis, which is dependent on which signalling pathways are activated (20-23).

Given the high expression of AR in many breast cancer phenotypes, its utility as a biomarker has been of recent interest. Many studies have evaluated the significance of AR and have shown that it is linked to factors such as smaller tumour size, lower histological grade, lower clinical stage and lower proliferation index (24-27), however, the majority of these studies have been conducted in younger women. Due to the uniqueness of breast cancer biology in older women, it is important to understand the role of AR specific to this cohort of patients.

The exponential progress in the emergence of molecular statistical tools has made it easier for several prognostic classifications of breast cancer to arise. Of date, the main ones that are used clinically to classify human breast cancers are based on the presence of ER, PR, and HER2, or by their absence in triple-negative breast cancer (TNBC). Given the heterogeneity of breast cancer, abundant research has investigated breaking up these groups to get more specific classifications, which has now given rise to five molecular subtypes, including normal-like, luminal A, luminal B, basal-like, and HER 2-enriched. The significance of AR,

especially in this context, comes into play when regarding TNBC, as most studies looking into this topic do so with an interest in finding novel therapies for this group of breast cancers since it is unreceptive to the treatments used for other classifications of breast cancer. Lehmann et al (28) identified seven clusters of TNBC, namely basal-like 1, basal-like 2, immunomodulatory, mesenchymal, mesenchymal stem-like, luminal androgen receptor (LAR), and unstable (28). The subtype of interest, namely LAR subtype, comprises of 10-15% of all TNBC, with low proliferation rates and is inherently resistant to chemotherapy (28-30), hence making it important to study. Given the potential of AR as a target for drug therapy, especially in these tumours where established treatment regimens do not yield beneficial results, many studies have looked into this but with controversial results (31-34). A recent phase II clinical study testing bicalutamide, a first-generation AR antagonist, in metastatic TNBC has reported a modest clinical benefit rate of 20% (NCT00468715). Another trial looking at the effect of bicalutamide in AR+ advanced breast cancers showed a clinical benefit rate of 19% at 6 months as well (35). A phase II clinical study looking at a second-generation AR antagonist, enzalutamide, in combination with trastuzumab in patients with HER2+ and AR+ metastatic or locally advanced breast cancer showed a clinical benefit rate of 23.6% (NCT02091960). These trials show that more needs to be done to look at the potential AR has as a therapeutic target for breast cancer. Although targeting this receptor has demonstrated a potential value in AR-positive TNBC, the predictive role of AR expression alone needs a better characterization (36). In other types of breast cancer however, AR has been shown to be an independent predictor of good prognosis in BC, particularly in grade 3 and Luminal A tumours (37). Given the use of AR in this context, it makes it even more important to study it concerning survival markers and as a prognostic factor.

The aims of this present study are to i) describe expression of AR in a cohort of older women with breast cancer and compare it to a cohort of younger women ii) compare the expression of AR to a panel of other biomarkers in older women with breast cancer and iii) correlate expression of AR to breast cancer specific survival (BCSS) in older women.

Methodology

Patient cohort

A total of 1,758 women aged ≥70 years with early operable breast cancer (T-0, N0-1, M0) were managed in a dedicated clinic over 37 years (1973-2010) with clinical information available from diagnosis till death/last follow-up (38). Overall, 813 patients underwent primary surgery with optimal adjuvant therapy as per unit policy at the time (38). All patients underwent the same management with the same management guidelines, which evolved due to the long study period. From the cohort, 267 (46.6%) patients received adjuvant endocrine therapy and 105 (18.3%) received postoperative radiotherapy. None of these patients received chemotherapy. For comparison, a previously characterised series of younger (<70 years) patients (N=1708) was available (39).

From the cohort who underwent surgery (N=813), it was possible to obtain enough tissue to construct tissue microarrays (TMAs) in 575 cases.

Construction of TMA

TMAs of formalin-fixed paraffin-embedded tumour (FFPE) sections were constructed as described (40). Briefly, 0.6mm diameter cores of the representative part of the tumour blocks

were implanted in the TMA blocks using Beecher's manual tissue microarrayer (MP06 Beecher Instruments Inc., Sun Prairie, WI, USA) (41, 42)

Measurement of a panel of biomarkers

Immunohistochemical (IHC) staining of 25 biomarkers was performed using StreptAvidin Biotin Complex and EnVision methods (43). The biomarkers measured were: AR, ER, PR, HER2, Ki-67, p53, Breast Cancer Gene (BRCA)1, BRCA 2, Cytokeratin (CK)5, CK 5/6, CK 7/8, CK14, CK17, CK18, CK19, EGFR, HER3, HER4, B-cell lymphoma (Bcl)2, E-cadherin, Mucin 1 (Muc1), Vascular Endothelial Growth Factor (VEG-F), CD44, Liver Kinase B1 (LKB1), and MDM4.

The expression of biomarkers (with the exception of HER2) was assessed using the H-score scoring system (range 0 – 300) (44, 45). The Herceptest scoring system (46) was used to score HER2, which involved scoring the staining of the membrane (0-3). Within a TMA specimen, not all of the samples were equally robust to allow IHC staining of each individual sample. Therefore, only the results of successful staining will be presented.

Measurement of the panel of biomarkers was performed in a research laboratory setting and was conducted independently of clinicopathological analysis conducted by the Histopathologist in the clinical setting. Therefore, definitions of positive/negative cut-offs for each biomarker are unique to this individual piece of work

Assessment of immunoreactivity

In order to view the TMAs, a NanoZoomer slide scanner by Hamamatsu Photonics was used to capture high-resolution digital images. These images were then viewed via computer for easy viewing, using the software mentioned above.

The expression of biomarkers was assessed using the H-score scoring system. The cut-off value used for AR was 40, which was calculated using X-Tile software.

Statistical methods

Breast cancer specific survival (BCSS) was calculated from data of definitive surgery to the date of death due to breast cancer. Patients dying from other causes were censored at the time of death. The Kaplan-Meier method was used to compare prognostic significance of AR status and AR/ER statuses. The log rank test was used to test the survival differences, *P*-values <0.05 were considered to be statistically significant. For comparison of AR expression and other biomarkers, chi square test was used when comparing frequencies.

BCSS was defined as all patients that died from breast cancer specifically. This was chosen as the outcome measure to determine the significance of AR in relation to the prognosis of breast cancer, with death as a result of breast cancer being the endpoint.

Statistical analyses were carried out using the SPSS version 24.

Ethical Approval

The research Nottingham Research Ethics Committee 2 approved the study under ethical approval number C2020313. Individual patient consent was not required as determined by the review board.

Results

Patients and tumour characteristics

Clinicopathological information was available for 531 women aged ≥70 years and compared to 1653 women aged <70 years from previous work (39). The age of the total number of patients (N=2184) ranged from 18 to 91 years with a median of 59 years. A summary of the clinicopathological characteristics of included patients is given in Table 1.

Expression of AR in the older women cohort compared to younger women

Of the total number of patients (N=2184), 1357 were AR-positive (59.4%) and 827 were AR-negative (36.2%). When broken down by age, 58.7% of older women had AR-positive breast cancers, while 63.2% of younger women had AR-positive cancers. There was no significant difference in AR expression between the older and younger series of patients (p=0.065). Table 2 summarises the clinicopathological characteristics of patients based on AR status.

Comparison of AR status to a panel of biomarkers in the older women cohort

The results of comparison of AR status in older women to a panel of 24 other biomarkers are shown in Table 3. Patients with AR-positive expression also had positive expression of ER, PR, BRCA1, CK 7/8, CK18, CK19, Bcl2, and Muc1 expression. Conversely, AR-positive expression was associated with negative expression of HER2, Ki-67, CK5, CK17, EGFR, and CD44 expression.

Relationship of AR status with BCSS in the older women cohort

Positive expression of AR, as shown in Figure 1, was associated with significantly better BCSS in the older women cohort (*p*-value < 0.000).

The subtype AR-/ER- (Figure 2.) was associated with significantly poorer BCSS (*p*-value: <0.000).

Relationship of AR status with Breast Cancer Subtypes

AR status was compared to subtypes of breast cancer, as shown in Figure 3. It was seen that a majority of AR+ cases were of luminal A subtype (154, 27%), followed by luminal B (30, 0.5%), HER2+ (19, 0.3%), and lastly TNBC (15, (0.26%)). AR- cases were majority of TNBC subtype (55, 0.9%), followed by luminal A (41, 0.7%), HER2+ (29, 0.5%), and then luminal B (25, 0.4%). Complete data sets were only available in 383 cases, out of which 218 cases were

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AR+, 150 cases were AR- and 15 cases were only PR+. The percentage of cases that fit in these subtypes according to AR status is given in Figure 3.

Discussion

Current literature surrounding AR shows it to be key in our understanding of the biology of breast cancer, as well as having the potential to serve as a therapeutic target. It was found in this present study that AR-positive tumours were associated with better BCSS, tubule formation, mitotic frequency as well as lower grades than that of AR-negative tumours. In addition, there was no association found between age and AR expression.

Patient and tumour characteristics

A majority of older and younger women had AR-positive carcinomas, which is in line with previously reported findings (47, 48). Recent studies show that the average age of patients with AR-positive tumours was higher than that of those with AR-negative tumours (49) and that positive AR expression is associated with older age (50, 51). In this study, while we did see that a higher percentage of younger women had AR-positive carcinomas, this result was statistically insignificant and the proportion of older women with AR-positive tumours only slightly differs from younger women with the same (58.7% vs 63.2%).

The percentage of tumours that express AR has been reported in various studies to either be higher than what is given in this study (70 – 80%) (17, 52) or around the same (50 – 60%)(53, 54), or even lower (55). This range may be due to the lack of standardisation for the cut-off of AR positivity, which also vastly differed in these studies (56). Leading from this, studies with lower cut-off scores could also have had higher proportion of patients with tumours that expressed AR compared to our study simply because their standard for AR-positivity was lower (17, 57, 58). The method that was used to calculate the cut-off for AR via the H-score (45)was through the X-tile bioinformatics software, which has been shown to be effective in

determining cut-offs for biomarkers in this context (59-61). Another method commonly seen was nuclear immunostaining (17, 48, 58).

The present study also found that AR-negative tumours were associated with higher grade, tubule formation and mitotic frequency than that of AR-positive patients. This correlates with the findings that AR-positive patients have better BCSS than AR-negative patients. While this study did not find any associations between AR expression and tumour size, other studies have found that AR expression is generally associated with smaller tumour size (62, 63). Other studies have also found an association between AR expression, coupled with lower histological stage and grade (60, 64).

Expression of AR in the older women cohort compared to younger women

There was no association found between AR and age. This suggests that AR does not play a role in the biological difference between these two age groups. The majority of studies, however, have also shown that AR expression is associated with older age (49-51), which was not seen in this study. Some studies have also shown that AR expression has an association with older age and postmenopausal status (16), while others have demonstrated that AR expression has no prognostic significance for postmenopausal women (65, 66). However, this study adds to the literature in that it compares AR expression for both younger and older women, which can prove to be extremely helpful when deciding upon guidelines for the management and treatment of breast cancer for these two groups of patients.

Comparison of AR status to a panel of biomarkers in the older women cohort

Another interesting finding from this study was that AR was associated with good prognostic markers (Bcl2, ER, Muc1 and PgR) as well as poor prognostic markers (EGFR, HER2 and Ki-67). This provides an overall complex picture of whether AR can be used as a prognostic marker on its own. This may suggest that AR is more relevant in some subtypes of breast

cancer, but not others. In a large population study conducted by Collins et al (17), androgen receptor expression was seen in a majority of luminal A and B types of invasive breast cancer. Similarly, it has been noted in other studies that luminal subtypes commonly express AR and that AR is frequently co-expressed in ER-positive and PR-positive tumours (26, 53), like is seen in this study.

Relationship of AR status with BCSS in the older women cohort

It was found that positive expression of AR was associated with significantly better BCSS in the older women cohort. This seems to agree with previous studies, one study having found that AR expression was associated with improved BCSS in the first 5-10 years following diagnosis (67). Furthermore, AR status has also been shown to have an association with survival, with a meta-analysis of over 10,000 patients demonstrating that AR positive patients had longer disease free-survival (DFS) and overall survival (OS) (68). These studies, however, do not differentiate between older and younger women. While Kensler et al (67) shows that trends regarding BCSS were similar between post- and pre-menopausal women, this cohort does not necessarily differentiate between older and younger women the way this study has. In the meta-analysis conducted by Bozovic-Spasojevic et al (68), it is seen that AR-positivity was associated with women above the age of 50 and longer DFS and OS, but no correlation was made between older women and longer survival as this study has shown.

While the majority of studies looking at TNBC have found that AR-expression was associated with better survival (27, 68, 69), others have shown either the opposite or no association (50, 66). In the few studies looking at older women specifically, AR positivity was related to lower likelihood of recurrence (70), however, this has not been studied intensively in this group of

patients. This study adds to the literature in that AR positivity is not only associated with better BCSS but also other factors that have been shown to improve patient outcome.

Patients with AR-negative/ER-negative breast cancer had a significantly poorer BCSS than all other AR/ER status combination patients in this study. However, this is a small number of patients in this series, given that this is a cohort of older women with primary breast cancer who generally have ER-positive tumours. This supports previous findings, which demonstrate that AR-positive/ER-positive patients have a better prognosis than AR-negative/ER-negative patients (71-73). Co-expression of ER and AR was also associated with lower pathological T stage and lower tumour grade due to the inhibitory effect of AR signaling (72, 73). Androgens are involved in activating cell proliferation in ER-negative breast cancer cells and inhibit cell proliferation in ER-positive breast cancer cells (74, 75), which could rationalise why better BCSS is seen in AR/ER positive patients.

Relationship of AR status with breast cancer subtypes

Given the number of studies looking at AR as a potential molecular target for therapy in TNBC (31-34), it is incredibly beneficial to look at what number of cases actually fall within the category of being AR-positive and of TNBC subtype. While these studies have found that a majority of TNBC cases are also AR-positive, this study did not find that, rather showing that a majority of TNBC samples were AR-negative rather than positive (55 vs 15). However, given the heterogeneity within the TNBC subtype itself, this could be attributed to AR being differentially expressed in these molecular subtypes of TNBC (35). More needs to be done to look at AR expression in specific TNBC subtypes in order to develop targeted therapies for this population.

With regards to the other subtypes of breast cancer, multiple studies have shown AR's relation with ER and Luminal subtypes, with luminal A being the most common subtype (17, 76, 77),

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which is what is seen in this study. However, an analysis with a data set as large as in this study is one of the firsts to our knowledge. The relevance of this study comes into play when looking at AR as a potential prognostic tool for this large group of tumours, or as a potential targeted therapy if these tumours become resistant to current therapies.

Limitations

The tumour samples from the older patients in this study were taken from surgically treated patients which could add some sampling bias; there may be bias towards patients with ER-negative disease as they would not have the alternative option of PET, to surgery. Additionally, we were unable to make comparisons between different subtypes of breast cancer; it would be interesting to see this comparison to see if the association between AR expression and BCSS is specific to some types of breast cancer or not.

Conclusions

This study aimed to describe AR expression in a cohort of older women with breast cancer and compare it to younger women, while comparing the expression of AR to a panel of biomarkers and BCSS. This study found that while AR expression is similar in older and younger women, it is associated with a number of biomarkers and AR positivity is related to better BCSS. While there are limitations, there is novelty in this study as it has shown that there is no difference in older and younger women with breast cancer, despite evidence showing differences in cancer biology between these two populations. This study also looked at the prognostic significance of AR expression, showing that AR expression was positively correlated to improved BCSS and associated with other biomarkers, which can tell us significantly more about the characteristics of the breast cancer itself. It is paramount that more research is done around AR and breast cancer, especially around older women given the increased expression seen in this

population. More needs to be done to look at specific prognostic factors related to AR that play in role in the survival of older women with breast cancer.

Furthermore, studying diagnostic CNBs to prevent sampling bias in order to cover all patients, including those who do not undergo surgery and investigation into different subtypes of breast cancer, would be helpful.

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Disclosures

Jahnavi Kalvala, Ruth Parks, Jamal Abdi, Andrew Green, and Kwok-Leung Cheung have nothing to disclose.

Author Contributions

Conceptulisation – Kwok-Leung Cheung, Andrew Green, Jamal Abdi

Methodology – Kwok-Leung Cheung, Andrew Green, Jamal Abdi

Formal analysis and investigation – Jamal Abdi

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Writing – review and editing – Jahnavi Kalvala, Ruth Parks

Supervision – Ruth Parks, Andrew Green, Kwok-Leung Cheung

Compliance with Ethics Guidelines

The research Nottingham Research Ethics Committee 2 approved the study under ethical approval number C2020313.

Data Availability

All data generated or analyzed during this study are included in this published article/as supplementary information files.

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Figure Legend

Figure 1. Breast cancer specific survival of early operable primary breast cancer in older women by AR status.

Figure 2. Breast cancer specific survival of early operable primary breast cancer in older women by AR/ER status.

Figure 3. Breast cancer subtypes in relation to AR status.

627 **Table 1.** Summary of clinicopathological characteristics of patients included in study.

Character	Number of patients	Percentage
Age (years)	**N=2184**	
<70	1653	75.7
≥70	531	24.3
Clinical size	**N=2200**	
≤2cm	1223	55.6
>2cm	977	44.4
Grade	**N=2125**	
1	356	16.8
2	726	34.2
3	1043	49.1
Tubule formation	**N=1948**	
1	117	6.0
2	628	32.2
3	1203	61.8
Nuclear pleomorphism	**N=1944**	
1	43	2.2
2	738	38.0
3	1163	59.8
Mitotic frequency	**N=1948**	
1	744	38.2
2	360	18.5

3	844	43.3
Nodal Stage	**N=2018**	
1	1256	62.2
2	587	29.0
3	175	8.7

† Grade observed from diagnostic core needle biopsy.

647 **Table 2.** Summary of clinicopathological criteria of patients included in study, based on AR
648 status.

Character	AR-positive		AR-negative		p-value
Age (years)	**N=1357**	**%**	**N=827**	**%**	
<70	1045	77	608	73.5	0.065
≥70	312	23	219	26.5	
Clinical size	**N=1372**	**%**	**N=828**	**%**	
≤2cm	815	59.4	408	49.3	$4x10^{-6}$
>2cm	557	40.6	420	50.7	
Grade	**N=1330**	**%**	**N=795**	**%**	
1	284	21.4	72	9.1	$8.13x10^{-58}$
2	574	43.2	152	19.1	
3	472	35.5	571	71.8	
Tubule formation	**N=1233**	**%**	**N=715**	**%**	
1	91	7.4	26	3.6	$7.87x10^{-13}$
2	457	37.1	171	23.9	
3	685	55.6	518	72.4	
Nuclear pleomorphism	**N=1231**	**%**	**N=713**	**%**	
1	33	2.7	10	1.4	$3.02x10^{-32}$
2	587	47.7	151	21.2	
3	611	49.6	552	77.4	
Mitotic frequency	**N=1233**	**%**	**N=715**	**%**	

1	612	49.6	132	18.5	$1.49x10^{-60}$
2	258	20.9	102	14.3	
3	363	29.4	481	67.3	
Nodal Stage	**N=1268**	**%**	**N=750**	**%**	
1	792	62.5	464	61.9	0.243
2	376	29.7	211	28.1	
3	100	7.9	75	10	

Table 3. Summary of 24 biomarkers compared with AR status.

Biomarker Expression	**AR-positive N(%)**	**AR-negative N(%)**	***P*-value**
ER	**N=246**	**N=172**	
Positive	211 (85.8)	79 (45.9)	$3.41x10^{-18}$
Negative	35 (14.2)	93 (54.1)	
PgR	**N=272**	**N=195**	

Positive	197 (72.4)	70 (35.9)	$3.62x10^{-15}$
Negative	75 (27.6)	125 (64.1)	
HER2	**N=283**	**N=201**	
Positive	23 (8.1)	30 (14.9)	0.018
Negative	260 (91.9)	171 (85.1)	
Ki-67	**N=296**	**N=213**	
Positive	101 (34.1)	92 (43.2)	0.037
Negative	195 (65.9)	121 (56.8)	
p53	**N=259**	**N=182**	
Positive	108 (41.7)	76 (41.8)	0.99
Negative	151 (58.3)	106 (58.2)	
BRCA1	**N=257**	**N=180**	
Positive	229 (89.1)	130 (72.2)	$6.0x10^{-6}$
Negative	28 (10.9)	50 (27.8)	
BRCA2	**N=224**	**N=164**	
Positive	181 (80.8)	125 (76.2)	0.275
Negative	43 (19.2)	39 (23.8)	
CK5	**N=277**	**N=196**	
Positive	65 (23.5)	78 (39.8)	$1.39x10^{-4}$
Negative	212 (76.5)	118 (60.2)	
CK5/6	**N=253**	**N=188**	
Positive	120 (47.4)	89 (47.3)	0.985
Negative	133 (52.6)	99 (52.7)	
CK7/8	**N=275**	**N=196**	

Positive	274 (99.6)	182 (92.9)	$3.6x10^{-5}$
Negative	1 (0.4)	14 (7.1)	
CK14	**N=248**	**N=185**	
Positive	50 (20.2)	51 (27.6)	0.071
Negative	198 (79.8)	134 (72.4)	
CK17	**N=269**	**N=192**	
Positive	42 (15.6)	50 (26)	0.006
Negative	227 (84.4)	142 (74)	
CK18	**N=266**	**N=186**	
Positive	265 (99.6)	171 (91.9)	$1.3x10^{-5}$
Negative	1 (0.4)	15 (8.1)	
CK19	**N=271**	**N=194**	
Positive	265 (97.8)	178 (91.8)	0.003
Negative	6 (2.2)	16 (8.2)	
EGFR	**N=269**	**N=182**	
Positive	28 (10.4)	48 (26.4)	$9.0x10^{-6}$
Negative	241 (89.6)	134 (73.6)	
HER3	**N=265**	**N=190**	
Positive	261 (98.5)	186 (97.9)	0.633
Negative	4 (1.5)	4 (2.1)	
HER4	**N=267**	**N=186**	
Positive	252 (94.4)	165 (88.7)	0.028
Negative	15 (5.6)	21 (11.3)	
Bcl2	**N=269**	**N=191**	

Positive	251 (93.3)	138 (72.3)	$7.28x10^{-10}$
Negative	18 (6.7)	53 (27.7)	
E-cadherin	**N=269**	**N=192**	
Positive	171 (63.6)	121 (63)	0.904
Negative	98 (36.4)	71 (37)	
Muc1	**N=273**	**N=197**	
Positive	260 (95.2)	169 (85.8)	$3.4x10^{-4}$
Negative	13 (4.8)	28 (14.2)	
VEGF	**N=243**	**N=164**	
Positive	202 (83.1)	143 (87.2)	0.263
Negative	41 (16.9)	21 (12.8)	
CD44	**N=274**	**N=197**	
Positive	46 (16.8)	54 (27.4)	0.005
Negative	228 (83.2)	143 (72.6)	
LKB1	**N=227**	**N=163**	
Positive	179 (78.9)	128 (78.5)	0.938
Negative	48 (21.1)	35 (21.5)	
MDM4	**N=228**	**N=157**	
Positive	227 (99.6)	156 (99.4)	0.79
Negative	1 (0.4)	1 (0.6)	

Estrogen Receptor (ER), Progesterone Receptor (PgR), Human Epithelial Growth Factor Receptor (HER)2, Ki-67, Tumour Protein p53 (p53), Breast Cancer Gene 1 (BRCA1), Breast Cancer Gene 2 (BRCA2), Cytokeratin 5 (CK5), Cytokeratin 5/6 (CK5/6), Cytokeratin 7/8 (CK7/8), Cytokeratin 14 (CK14), Cytokeratin 17 (CK17), Cytokeratin 18 (CK18),

Cytokeratin 19 (CK19), Endothelial Growth Factor Receptor (EGFR), HER3, HER4, B-cell Lymphoma 2 (Bcl2), E-cadherin, Mucin 1 (Muc1), Vascular Endothelial Growth Factor (VEG-F), CD44, Liver Kinase B1 (LKB1), and MDM4.