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Female breast cancer classification using immunohistochemistry biomarkers staining in Botswana

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Abstract

Breast cancer remains the most diagnosed cancer among women world-wide and a leading cause of cancer-related deaths accounting for 15% of deaths in 2018. Worldwide, the incidence increased from 1.4 million in 2011 to over 2 million in 2018 with a concomitant increase in mortality from 458,400 to 626,679 in the same period. Low- and middle-income countries, such as Botswana, have a disproportionate burden of breast cancer incidence and mortality and there is an urgent need to characterise the unique tumour molecular profiles that may be influencing mortality in these populations. **Methods** A retrospective study of 125 archived mastectomy specimens (from 2006 to 2009) from women with breast cancer in Botswana was conducted. We determined molecular characteristics of breast cancers by carrying out four immunohistochemistry (IHC)classification (PR, ER, HER2 receptors and Ki 67), cytokeratin 5/6 and EGFR1.Statistical software STATA and SPSS were used to determine the relationship between histology, IHC of biomarkers of interest. **Results** Out of 125 breast cancer tissues, the distribution of molecular subtypes were as follows: Luminal A (44/125; 35.2%), Luminal B (and TNBC (23/125; 18,4%), HER2 Enriched (17/125; 13.6%), and Luminal B HER2 Enriched (9/125; 7.2%), Basal (9/125; 7.2%), and CK5/6 was expressed by 12.8% (16/125) of tumours. Furthermore 6% of the tumours were basal positive luminal tumours. Morphological 76% of tumours were IDC-NOS and 24% were special type, majority were grade 2 (40%) followed by grade 1(30.4%), grade 3 (23.2%) was and mucinous types were 6.4%. Clinical staging and tumour involvement data were incomplete. **Conclusion** The discovery of basal positive luminal breast tumours in women from Botswana original not accounted for in the four distinct molecular subtype calls for an expanded antibody panel 6-IHC panel) in order to stratify women of African descent patients into good/poor prognostic groups. Characterising tumour subtypes will better inform optimal therapeutic regimens for women with breast cancer in Botswana.

Keywords Female breast cancer, IHC

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Introduction

Breast cancer is a manifestation of a diverse group of malignancies which present themselves in various clinical and pathological forms, and their clinical outcomes emanate from their molecular complexity and biological diversity [1, 2] a heterogeneous morphologies observation noticed by pathologists histologically, which varies intra and intertumoral and within a single tumour [3]. Globally, female breast cancer is the most diagnosed malignancy (11.7%) among women with 2.3 million new cases recorded [3]. It is the leading cause of cancer related deaths among females, accounting for 685,000 (6.9%) of overall cancer deaths [3]. The number of new cases recorded during the 2018/2019 reporting was 2,088,849 (11.6% of all cancer) resulting in 626,679 death (6.6% of all cancer) [4] and in the latest data of 2022, new breast cancer cases stand at 2,308,897 (11.6 of all cancer cases) and mortality rate was 665,684 (6.9% of all cancers) [5]. Global, female breast cancer death rate increased from 12.4 to 15.0 per 100 000 population between the same reporting periods [3]. The incidence of breast cancer in high-income countries (HIC) and developing nation exceeds all other cancer incidences i.e. 55.9/100 00 and 29.7/100 000 population, respectively [3]. However, death from breast cancer is 17% higher in developing nations than developed nations (15/100 000 compared to 12.8/100 000 in developed nations [3]. This has been attributed to developed countries having more resources for early detection, treatment and care of breast cancer patients than developing countries where there are varying capacity to initiate and sustain care programs for breast cancer [6].

Breast cancer is characterized into four intrinsic molecular subtypes [7]. The 4 intrinsic subtypes are oestrogen receptor positive groups (Luminal A, Luminal B), oestrogen receptor negative group HER2-enriched (HER2 positive), basal-like (triple negative group) and normal breast-like group. St Gallen consensus of 2013 recommends that breast tumours be immunohistochemically assayed for Oestrogen (ER), Progesterone (PR), Human Epidermal growth factor 2 (HER2) and Ki67 for determination of these Molecular Subtypes [8]. Despite the successful use of this molecular classification inHICs [9], difference in tumour characteristics are still evident across different races and ethnicities which prompts a quest to identify other biomarkers which could explain the disparities in outcomes observed in genetically heterogenous populations [10–15]. A meta-analysis of 26 studies (n =4737) of IHC receptor-defined subtypes of breast cancer in Sub-Saharan Africa found that ER status varied from 20–70% across the region. There was also variability in PR and HER2 status [16]. Most studies confirmed a 20% or more prevalence of triple negative breast cancer, (TNBC) among women of African descent. A recent study revealed that ER prevalence in five eastern African countries stood at 55% [17].

In Botswana, breast cancer is the second most frequently diagnosed cancer among women, with an age adjusted ratio of 17.5/100,00 and a death rate of 8.55/100,000 according to the WHO 2022 cancer statistics sheet [18]. Despite this high incidence, the country still lacks quality data which could be used to develop context-relevant guidelines for early breast cancer diagnosis and treatment. Currently, publication on the subject matter has been drawn from secondary sources like the pathology registry where the data was obtained under routine conditions and do not account for duplication due to different times at which patients were biopsied [19]. The Breast Global Health Initiative (BGHI) calls for pilot research which can provide a baseline for priority allocation of resources and development of context-relevant guidelines of breast cancer care [20]. The lack of quality researched data impacts on the development of guidelines for treatment modalities and prognostication of breast cancer patients in developing nations [21] (and hence there is a clarion call for research and policies on cancers at large [22]. The objective of this study was to determine the4-IHC molecular subtypes, epidermal growth factor 1 and CK5/6 transcript among female breast cancer patients in Botswana.

Materials and methods

Study design, site and sample size

This was a retrospective cross-sectional study carried out between June 2015 and December 2016 on 182 archived formalin-fixed paraffin embedded (FFPE) tissue obtained from mastectomy specimens received between August 1st, 2006 and December 31 st, 2009. The study samples were collected from two public health pathology laboratory: the National Health Laboratory in Gaborone and from Nyangabwe Referrals Hospital Pathology Department, in Francistown Botswana. Immunohistochemistry analysis was conducted in the Division of Anatomical Pathology Research Laboratory, University of Cape Town, South Africa.

Ethical considerations

Ethical approval was obtained from the University of Botswana Office of Research and Development Institutional Review Board (RES/IRB/1380). A research permit was obtained from the Botswana Ministry of Health Research Division (PPME 13/18/1) and ethical clearance approvals were obtained from Princess Marina Hospital (PMH 5/75 &79) and Nyangabgwe Hospital Research and Ethics Committees (NRH/1/2).

Permission to transport residual FFPE tissue samples to South Africa to conduct research was granted by the Ministry of Health MH 3/87 III. Further ethical approval was also granted by the University of Cape Town, Faculty of Health Sciences, Human Research Ethics Committee (HREC 620/2013). The archived tumour blocks were de-identified before enrolment into the study in keeping with the provisions of ISO 15189.

Study samples

A total of 182 mastectomy specimen were obtained from the two facilities and all 182 FFPE breast cancer tissue blocks had adequate tissue for research. The tissue blocks were sectioned and stained with H&E along with control tissues that has been subjected to standard fixation protocol and only those which showed good morphological preservation were selected for the study. The 182 cases were reviewed and classified according to WHO histological classification by a second pathologist at UCT and 125 (69%) were confirmed as female breast cancer. Six(6) cases were either in-situ carcinomas or had insufficient tumour and fifty-one (51) had suboptimal fixation and were excluded from further analysis.

Laboratory investigations

Selected breast cancer and positive control FFPE tissue blocks were sectioned at 3 µm, using a Leica RM2125 RTS microtome. The sections were floated on a water bath and picked up on HistoBond adhesive glass slides (Paul Marienfeld GmbH & Co. KG, Germany). The sections were then dewaxed for 2 min in three changes of xylene, cleared in absolute alcohol and followed by washing under running tap water for 5 min. Antigen retrieval was performed under different conditions depending on the antibody (Table 1) in a Russell Hobbs RHPC 6L 4110 pressure cooker. Immunohistochemistry was performed by first blocking for endogenous peroxide using H_20_2, slides were rinsed in distilled water, incubated in PBS buffers followed by background blocking with 5% goat serum. The sections were then incubated with respective antibodies as shown in Table 1. The slides were washed with PBS to remove excess unbound antibody before being incubated with a secondary antibody for 30 min. The slides were washed with PBS to remove unbound secondary antibody, DAB was applied and incubated for 10 min followed by developing under running tap water. Slides were then counter stained with mayor haematoxylin, dehydrated in ascending grades of alcohol, cleared in xylene and mounted in synthetic resin. For detail please see appended protocol appendix 1 for ER, PR, HER2, Ki67, CK5/6, EGFR.

Scoring of ER and PR immunohistochemistry staining

Staining for nuclear associated hormone receptor markers ER and PR was evaluated using the Allred scoring system [23, 24]. HER2 immunohistochemical status (negative or positive) was evaluated according to the American Society for Oncologists (ASCO) and College of American Pathologists recommended guidelines [25]. The LightMix Kit HER2/neu TIB which employs the use of a LightCycler Instrument LC 480 software version 4.1 (TIB MOLBIOL Synthesealabor GmbH, Berlin, Germany) was used on all immunohistochemical HER2 2+ cases to determine *HER2* status. According to the fit point module of analysis of the Roche Quantification Software and an expression ratio of sample to reference gene greater than 2.0 was regarded as positive for HER2/neu amplification.Ki67 index was determined by counting the number of stained tumour nuclei per 100 tumour cells using high power field (× 400) of a light microscope as per Ki67 working group of 2010 [26]. EGFR and CK5/6 expression were analysed using the immune reactive scores (IRS),proportion of tumour cells stained were assigned values depending on the proportion of staining cell, are then multiplied by the tumour staining intensity value, giving a stain index ranging from 0–9 [27].

For heterogeneous tumours both proportion and intensity of stain were considered for IHC analysis for all biomarkers by following established standard protocols applicable to individual biomarkers.

Table 1 Antibodies and immunohistochemical staining conditions

Antibody	Clone	Control	Retrieval Buffer	Dilution	Incubation time at RT
Oestrogen Receptor	NCL-L-ER6 F11	Breast cancer	EDTA pH 8	1:75	60 min
Progesterone Receptor	NCL-L-PGR-312	Breast cancer	EDTA pH 8	1:50	60 min
c-erbB-2	Polyclonal	Confirmed breast cancer	EDTA pH 8	1:200	60 min
Ki67	MIB-1	tonsil	EDTA pH 8	1:50	60 min
CK 5/6	D5/16 B4	tonsil	Tris–EDTA pH 9	1:50	60 min
EGFR	NCL-L-EGFR-384	placenta	Tris–EDTA pH 9	1:50	60 min

Statistical analysis

Stata software, Version 15.1, StataCorp, Texas USA was used to conduct statistical analysis. Categorical data was summarised using frequencies. Bivariate analysis to test for association between clinicopathological variables (age at diagnosis, tumour size, lymph node involvement, histological type and grade) and each biomarker (ER, PR, HER2, Ki67, CK5/6, EGFR) was conducted using the Chi-square test. However, when the assumptions for the chi-square test were not met, a Fisher`s exact test was used for the analysis. Results were considered statistically significant when p-value was <0.05. The results were presented in tables.

Results

The mean age at mastectomy was 56.9 years and was higher than the reported mean age of other sub-Saharan African studies [23, 24]. Clinical staging was available for 60% of cases of which 75% presented with a clinical stage of III or IV at the time of diagnosis. Using the WHO histological classification of tumours, the 125 tumour specimens were classified into 12 classes, as presented in Table 2. Lymph node involvement and type of node was observed in 66.4% Most tumours were grade 2 and distant metastasis was observed in 56% of cases, 39.2% did not have metastasis, and 4.8% (6/125) metastasis status was unknown (Table 3).

Lymph node involvement was found in 83/125 tumours. In 31/83 (37%) tumours the cancer had spread to less than 3 internal mammary/auxiliary lymph node and in 28/83 (34%) of the tumours, the cancer had spread to more than 3 axillary lymph nodes. In 19/83 (23%) of the tumours, the cancer was observed in matted auxiliary nodes and those in other structures (near the collar bone, under arm and near breastbone), and in 5/83 (6%) tumours it was observed in more than 10 level III lymph nodes. The data for lymph node involvement was incomplete in 42/125 cases. Statistically analysis looked at overall lymph node involvement rather than type of lymph node involved (Table 3).

The tumour size was significantly associated with histological grade with a p-value of 0.027. Most of the high-grade tumours were large as presented in Table 4 below.

Table 2 Morphological types of breast cancers cases and their frequencies in the study

Morphologic type ($n=125$)	N	%
Invasive ductal carcinoma-NOS	95	76.0
Invasive mucinous carcinoma	8	6.4
Invasive papillary carcinoma	4	3.2
Micropapillary carcinoma	5	4.0
Oncocytic carcinoma	2	1.6
Signet ring	2	1.6
Cribriform	2	1.6
Invasive lobular carcinoma	2	1.6
Metaplastic carcinoma	1	0.8
Medullary	2	1.6
Neuroendocrine	1	0.8
Tubular	1	0.8
Total	125	100

Immunohistochemistry results

This table presents a classification of breast tumours into molecular subtypes using the 4-IHC panel of ER, PR, HER2 and Ki67 (shown in Figs. 1, 2, 3 and 4 respectively) as per the ST Gallen consensus agreement of 2013 [8] (which is concordant to the Genomic Health Recurrence score (GHI-RS) (Oncotype DX) [28, 29].

Discussion

This study presents findings for female breast cancers in Botswana in a cohort consisting of125 women who underwent a mastectomy after a breast cancer diagnosis. The mean age at mastectomy was 56.9 years and was higher than reported mean age from other studies done in sub-Saharan Africa [30–33]. Clinical staging was available for 60.0% of cases highlighting gaps in clinical documentation and 75% of the cases presented with a clinical stage of III or IV at time of diagnosis which is consistent with findings from other African cohorts [30–34]. In this study, 75% (83/111) of tumours were node positive and this frequency of lymph node metastases is consistent with findings from other African cohorts [31]. The data in Table 4, shows that the average tumour size in this study was 5.7 cm, and this finding is consistent with other studies from other SSA regions [30–34]. However, this is in sharp contrast to the cohorts from developed countries were most women present with tumours less than 2.0 cm [35, 36]. The late detection of tumours could be due to inadequate imaging facilities and dedicated breast clinics in Botswana and other SSA regions. Larger tumours size in women >50 years have been attributed to inadequate knowledge on breast self-examination technique and reduced mobility [37] since most of these tumours had low proliferation indexes. The most common histological type in this study was the IDC-NOS, (Table 2) and is consistent with the findings in other cohorts [30, 33, 38]. The proportion of mucinous carcinomas in this study was higher than the European and other African cohorts [30, 31]. However, lobular carcinoma accounted for 1.6% (2) of all cases which was lower than other African cohorts [30, 33, 39]. There was significant association between tumour grade and tumour size as shown in Table 5. Larger tumours tended to be grade 2 or 3 and

Table 3 Clinicopathological variables of Female breast cancer from Botswana

Variable	≤50 years (*n* = 45, 36.0%)	>50 years (*n* = 80, 64.0%)	Total (*n* = 125, 100%)
Tumour Grade (*n* =125) *			
I	15 (33.3%)	23 (28.8%)	38 (30.4%)
II	23 (51.1%)	27 (33.8%)	50 (40.0%)
III	7 (15.6%)	22 (27.5%)	29 (23.2%)
		*mucinous 8 (10%)	*mucinous 8 (6.4%)
Tumour Size, n (%) average 5.7 cm)			
<2 cm	1 (2.2%)	5 (6.2%)	6 (4.8%)
2–5 cm	22 (48.9%)	35 (43.8%)	57 (45.6%)
>5 cm	18 (40.0%)	34 (42.5%)	52 (41.6%)
Unknown	4 (8.9%)	6 (7.5%)	10 (8.0%)
pTStage n, (%)			
I	0 (0.0%)	3 (3.8%)	3 (2.4%)
II	6 (13.3%)	10 (12.5%)	16 (12.8%)
III	11 (24.4%)	17 (21.2%)	28 (22.4%)
IV	8 (17.8%)	20 (25.0%)	28 (22.4%)
Unknown	20 (44.4%)	30 (37.5%)	50 (40.0%)
Tumour Laterality			
Bilateral	1 (2.2%)	1 (1.3%)	2 (1.6%)
Left	15 (33.3%)	37 (46.2%)	52 (41.6%)
Right	22 (48.9%)	32 (40.0%)	54 (43.2%)
Not specified	7 (15.6%)	10 (12.5%)	17 (13.6%)
Lymph node involvement*			
Yes	25 (55.5%)	58 (72.5%)	83 (66.4%)
No	12 (26.7%)	14 (17.5%)	28 (22.4%)
Unknown	8 (17.8%)	6 (7.5%)	14 (11.2%)
Histology, n (%)			
Invasive ductal-NOS	36 (80.0%)	59 (73.8%)	95(76.0%)
Special type	8(17.8%)	20 (25.0%)	28 (22.4%)
Invasive lobular	1(2.2%)	1 (1.2%)	2 (1.6%)

*Mucinous carcinoma cases were not allocated grades (8 cases)

*refers to any lymph node positive for metastasis

*pT Stage refers to extent of the main tumour

Table 4 Tumour grade vs tumour size of samples enrolled in the study

Tumour Size	Grade (n)			*P*-value
	Grade 1 (35)	Grade 2 (46)	Grade 3 (26)	
≤5 cm (60)	20	31	9	0.027
>5 cm (47)	15	15	17	

these tumours tend to proliferate faster, increase in size and metastasize to adjacent organs asobserved in other studies [34, 39].

A triple negative breast cancer (TNBC)prevalence of 25.6% in this cohort was higher than the global estimates of 10–20% [7] but within the range encountered in women of African descent [31, 33]. Prevalence of TNBC in African cohorts has been confounded by suboptimal tissue fixation and lack of standardized protocols [16, 34]. This study identified TNBC basal-like (TNBC, CK5/6 + and EFGR ±) TNBC subtype and was the first study in Southern Africa to investigate CK5/6 status in breast carcinoma. CK5/6 has been investigated in several studies in SSA (East Africa and West Africa) and its prevalence ranged from 12.2% to 35.7% [40–44] and overall expression of CK5/6 in our cohort was 12.8% and is lower than in other studies. The prevalence of basal-like subtype (TNBC, CK5/6 + and EFGR ±) was 8% in the Botswana cohort and was lower

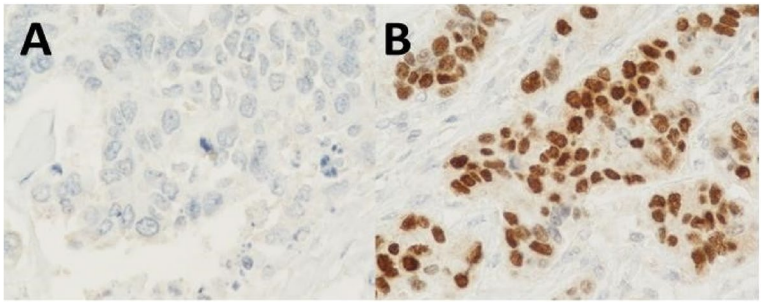

Fig. 1 showing Oestrogen Receptor (ER) expression (Allred score: A = 0 and B = 8)

than the Sudanese cohort [44]and the Nigerian cohorts of Agboola et al. (2012) [42], and comparable to the Nigerian cohort of Titloye et al (2016) [41]. It has been discovered that different phenotypes of TNBC could be susceptible or resistant to different therapies [45–47].

Classification of ER-PR-and HER2-, CK5/6+ and EFGR± as basal-like carcinomas is concordant with gene microarray expression [48, 49]. The absence of ER, PR, could be attributed elevated EGFR, (Table 6). EGFR has been associated with downregulation of ER and PR [50]. Nuclear EGFR expression has been associated with resistance to cancer therapeutics [51, 52] such as radiotherapy resistance [52]. In vivo models have demonstrated that nuclei EGFR aids resistance to chemotherapy e.g. platinum and offers resistance to cisplatin [53, 54]. Moreover, nuclear EGFR is associated with breast cancer resistance protein or ATP binding cassette subfamily G member 2 (ABCG2) which hinders drug transport into tumour cells [55, 56]. Nuclear EGFR has been found to modulate DNA repair following treatment by cisplatin and radiation [57].

The frequency of combined luminal B subtypes was 25.6% (18.4% luminal B and 7.2% luminal B Her2 positive). They were characterized by moderate to high expression of ER, PR, Ki67, EGFR and occasional CK5/6. The average age at diagnosis was lower than in most Western cohorts [35, 36], had a high average Ki67 index, decreased PR expression (average Allred score 2.6) and 41% of tumours expressed subcellar EGFR, Table 6. Their biology typifies the common observation of rapidly growing tumours in young women of African descent, [58–60]. HER2 induces proliferation, motility and invasion in breast cancer through the MAPK pathway in ER positive tumour [61].The frequency of *HER2* amplification was 20.8%,prevalence of HER2 enriched molecular subtype was 13.6% and prevalence of tumour heterogeneity especially in equivocal cases [62].HER2 enriched group was characterized by high proliferation, overexpression of HER2 and EGFR, Table 6. Eighty-eight percent of HER 2 enriched tumours had metastasis that can be attributed to an active EMT program and the Notch signalling pathways due to elevation of both EGFR (HER1) and HER2 [63]. Dimerization of HER2/HER2 or HER1/HER1 and HER1/HER2 [61]. EGFR translocation to the nucleus (Figs. 1, 2, 3, 4 and 6A), could be attributed to the notch signalling which affects transcription of other genes [64, 65].

HER2 drives breast cancer through the Notch, PI3 K, AKT, mTOR pathways [61, 66, 67] and MAPK promoting proliferation and resistance to apoptosis which is signalled by the high proliferation index and metastasis in this cohort. Co-expression of HER1/HER2 has been implicated in resistance to Herceptin and splice variants have been reported for both HER2 and HER1 [68, 69].

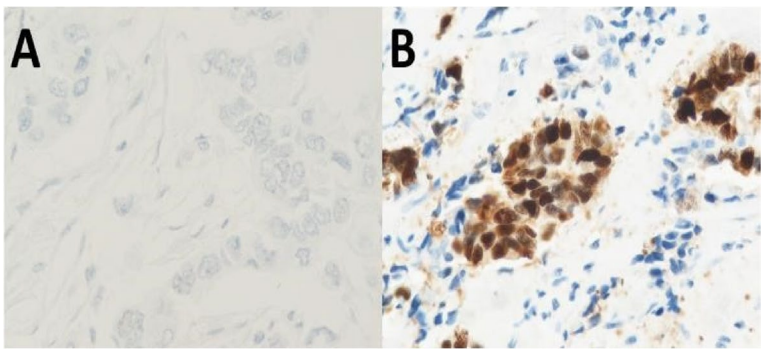

Fig. 2 showing Progesterone Receptor (PR) expression (Allred scores: A = 0, and B = 8)

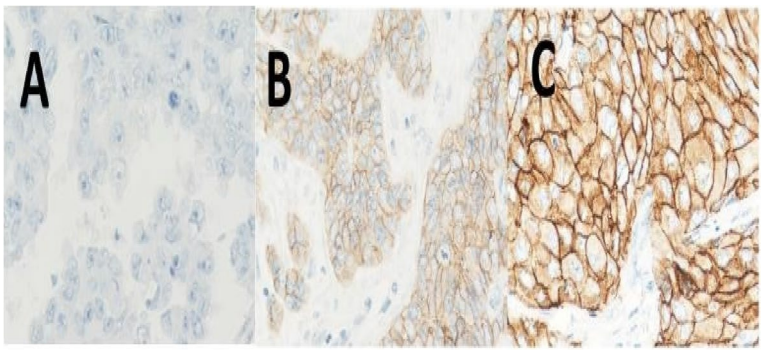

Fig. 3 showing human Epidermal growth factor 2 (HER2) expression (Allred scores: A = 0, B = 2 + and C = 3 +)

An active EGFR/HER2 notch signalling which was particularly observed in the luminal B HER2 positive group raises the question of medication induced EMT vs natural heterogeneity hence the need to employ EGFR antagonist in treatment. CK5/6 represents a more aggressive breast cancer phenotype and was expressed in 6% of luminal A and 4.3% of Luminal B tumours. The discovery of CK5/6 positive tumours was unexpected but have been identified in two studies (4% in a West African cohort and 4% in a Ugandan Cohort) [13, 27]. These tumours are termed basal positive luminaltumours [70] and in our cohort, they had low oestrogen expression. These mixed phenotypes depict breast cancer as a heterogeneous disease that perhaps obey the intrinsic classification to a limited point and beyond which they display their own unique identity as dictated by internal pressures: such as inherent mutations and clonal selectivity [71] and other external pressures such as treatment and epigenetic induced EMT [72, 73].

In our study we included Ki67 proliferation index which improves stratification. These mixed phenotypes could partially account for breast cancer aggression in women of African descent [27, 74]. Basal cytokeratin state represents an under-differentiated lineage while luminal represents a well differentiated lineage [75]. The finding of tumour cells with mixed phenotype could be due to retention of a subpopulation which express CK5/6 but has expanded (clonal evolution) after withstanding external pressures such as treatment regimen which wipes out luminal and leaving the basal-like to expand [72, 76]. Mixed cell population must be evaluated correctly as studies have shown that they are associated with resistance to therapy [77]. Intra tumour heterogeneity has been associated with familial history but recently it has been associated with race [78], EGFR expression was detected in the cell membrane, cytoplasm and nucleus singly or in various combinations. Nuclear EGFR staining had a strong relationship with tumour grade and molecular subtype. About a third (33.6%) of tumours showed membrane expression of EGFR. This result is consistent with that observed in the West African cohorts [13, 41]. EGFR positivity was associated with aggressive molecular subtypes. EGFR positive tumours were most frequent in TNBC, followed by luminal B, HER2 enriched, luminal A and luminal B HER2 positive. The Botswana cohort had a higher frequency of EGFR positive tumours than the Ugandan cohort [27].

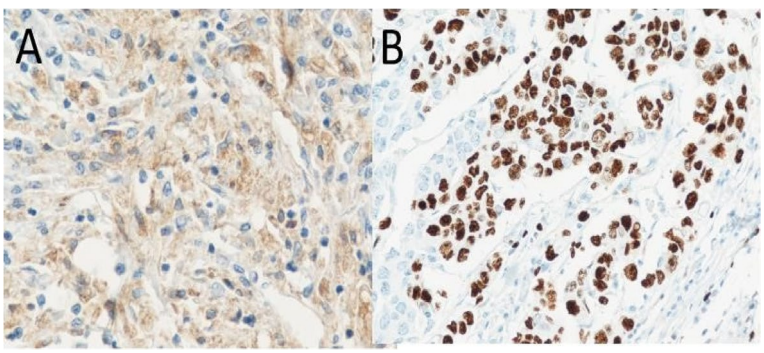

Fig. 4 showing Ki67 expression (Allred scores: A ≤ 14% and B > 14%)

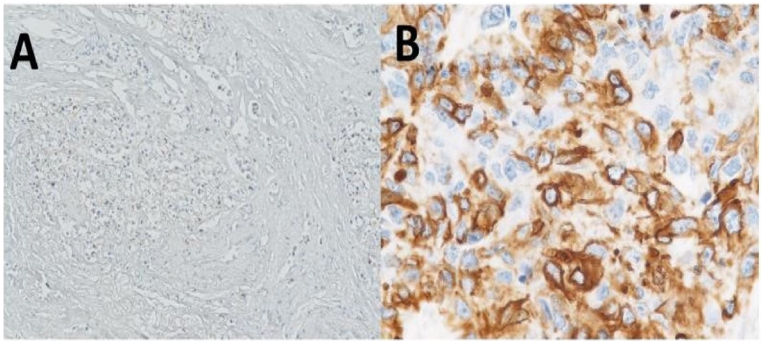

Fig. 5 showing CK5/6 IRS expression (A = 0 and B = 9)

In the current cohort, nucleic EGFR expression was 15.2% (19/125). Nuclear EGFR expression is associated with the development or acquisition breast cancer resistant protein (BCRP) [64, 79]and poor prognosis [80]. BCRP is associated with resistance to; radiotherapy [53], chemotherapy [51]and targeted therapy [79].

Nuclear EGFR is involved in repairing DNA damaged by chemotherapy, radiation, and activates survival pathways such RAS/MAPK, AKT/PI3 and other [57]. Suppression of DNA-PK expression has been found to render tumour cell susceptible to radiation therapy [81].

The Nielsen classification employed by most countries and the 4 IHC classification may not adequately stratify patients for proper treatment and could explain in part the high mortality observed in LMIC countries. Some countries assay for 3 antibodies [31, 39] due to limited or lack of resources. Refined molecular subtyping have been suggested to address disparities and to reduce numbers of unclassifiable patients [82]such as the Nottingham prognostic plus [36, 83], therefore, an expanded panel to include EGFR and CK5/6 would enhance patient stratification.

Despite African population being genetically heterogeneous [84], tumour heterogeneity has not been investigated for in African cohorts. A study by [83], found 27.4% (500/1852) had a mixed phenotype of luminal and basal cytokeratin [85]. Subsequent several studies have since confirmed existence of luminal tumours which express CK5/6 [70, 86]. Studies by Blows et al., (2010) [70, 85] which were based on reported studies from different centres did not stratify the Luminal B HER2- group according to proliferation status. The approach of this study differs from other Sub-Saharan African studies since it is one of the few studies which carried out a full 4-IHC classification panel which yields a more refined patient stratification able to guide therapy [8, 9, 36, 87–89]. Furthermore, it differentiates between luminal B subtype from luminal A (which are biologically distinct tumours) 86 based on Ki67 status as opposed to the Nielson classification which does include Ki67 in the panel [90]. The proportions of molecular subtypes in this cohort Table 5 are comparable to a study carried out in Southern Africa [30]. Luminal A were comparable to the average age of breast cancer at time of diagnosis in Western cohorts [9, 12, 13]. Tumours with large average diameter in this cohort could be attributed to delay in seeking medical

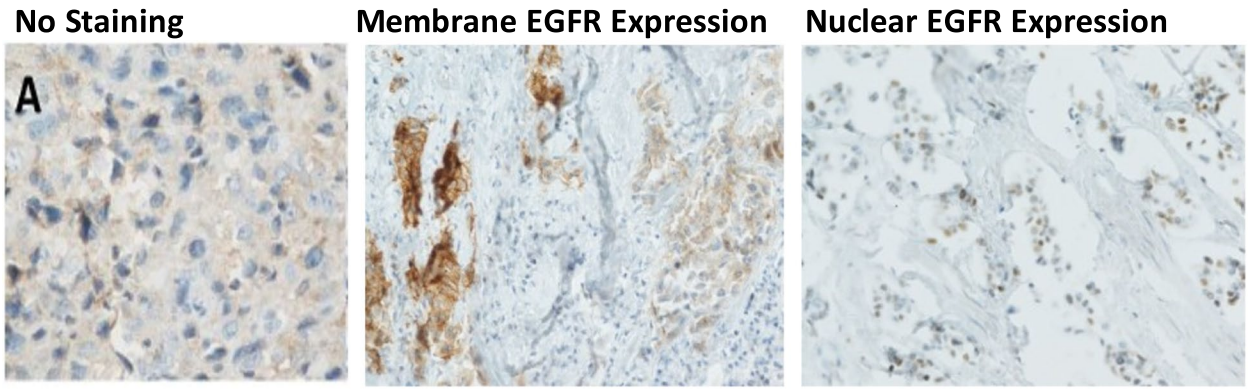

Fig. 6 showing EGFR Subcellular expression IRS (Allred score: A = 0 and B = 6)

Table 5 Presents the correlation between ER, PR, HER2, CK5/6 and EGFR staining and clinicopathological variables

IHC Marker $n = 100$ (100%)	Age		Tumour size		Histology type		Lymph Node involvement		Tumour grade		
	> 50 years ($n = 80$, 64%)	≤ 50 years ($n = 45$, 36%)	≤ 5 cm	> 5 cm	IDC NOS	Other	Yes	No	G1	G2	G3
ER, n (%)		P = 0.346	$p = 0.596$		$p = 0.678$		$P = 0.366$		**$P = 0.003$**		
Positive 72 (58%)									25 (66)	31 (62)	8 (28)
Negative 53 (42%)									13 (34)	19 (38)	21 (72)
PR, n (%)	$p = 0.166$		$p = 0.1.000$		$p = 0.503$		$p = 0.154$		**$p = 0.0012$**		
Positive 41 (33%)									16 (42)	17 (34)	3 (10)
Negative 84 (67%)									22 (58)	33 (66)	26 (90)
HER2, n (%)	p = 0.495		$p = 0.635$		**$p = 0.029$**		**$p = 0.789$**		**$p = 0.025$**		
Positive 26 (21%)	11 (24.4%)	15 (18.8%)			24 (25)	2 (6)			3 (8)	15 (30)	8 (28)
Negative 99 (79%)	34 (75.6%)	65 (81.2%)			71 (75)	28 (94)			35 (92)	35 (70)	21 (72)
Ki67, n 125 (100%) $p = 0.008$	**$p = 0.009$ $n = 80$ (%)**	**$p = 0.026$ $n = 45$ (%)**	$p = 0.852$		$p = 0.272$		$p = 0.275$		**$p < 0.001$**		
≤ 14% 64 (51)	48 (60)	16 (36)							32 (85)	20 (40)	5 (17)
> 14% 61 (49)	32 (40)	29 (64)							6 (15)	30 (60)	24 (83)
CK5/6, n (%)		$p = 1.000$	$p = 0.769$		$p = 0.252$		$p = 0.748$		$p = 0.189$		
Positive 16 (42%)											
Negative 109 (87%)											
EGFR, n (%)	$p = 0.844$		$p = 0,089$		$p = 0.825$		$p = 0118$		**$p = 0.001$**		
Positive 42 (34%)									4 (11)	20 (40)	16 (55)
Negative 83 (66%)									34 (89)	30 (60)	13 (45)
Molecular subtype classification ($n = 125$), **n (%)**	**$p = 0.017$**		$p = 0.922$		$p = 0.093$		$p = 0.696$		**$p < 0.0001$**		
Luminal A 44 (35%)	36 (45)	8 (18)							22 (58)	12 (24)	2 (7)
Luminal B 23 (18%)	11 (14)	12 (27)							5 (13)	14 (28)	4 (14)
Luminal B HER2 Positive 9 (7%)	5 (6)	4 (9)							2 (5)	6 (12)	1 (3)
Triple negative32 (26%)	18 (23)	14 (31)							8 (21)	9 (18)	15 (52)
HER2 enriched 17 (14%)	10 (12)	7 (16)							1 (3)	9 (18)	7 (24)

Table 6 Immunohistochemistry results for ER, PR, HER2, cCK5/6 and EGFR

IHC Marker	≤50 years (*n* = 45, 36.0%)	>50 years (*n* = 80, 64.0%)	Total (*n* = 125, 100%)
ER, n (%)			
Positive	23 (51.1%)	49 (61.3%)	72 (57.6%)
Negative	22 (48.9%)	31 (38.7%)	53 (42.4%)
PR, n (%)			
Positive	11 (24,4%)	30 (37.5%)	41 (32.8%)
Negative	34 (75.6%)	50 (62.5%)	84 (67.2%)
HER2, n (%)			
Positive	11 (24,4%)	15 (18.8%)	26 (20.8%)
Negative	34 (75.6%)	65 (81.2%)	99 (79.2%)
Ki67, n (%)			
≤ 14%	16 (35.6%)	48 (60.0%)	64 (51.2%)
> 14%	29 (64.4%)	32 (40.0%)	61 (48.8%)
CK5/6, n (%)			
Positive	6 (13.3%)	10 (12.5%)	16 (12.8)
Negative	39 (86.7%)	70 (87.5%)	109 (87.2%)
EGFR, n (%)			
Positive	16 (35.6%)	26 (32.5%)	42 (33.6%)
Negative	29 (64.4%)	54 (67.5%)	83 (66.4%)
Molecular classification (*n* =125), n (%)			
Luminal A	8 (17.8%)	36 (45.0%)	44 (35.2%)
Luminal B	12 (26.7%)	11 (13.7%)	23 (18.4%)
Luminal B HER2 Positive	4 (8.8%)	5 (6.3%)	9 (7.2%)
Triple negative	14 (31.1%)	18 (22.5%)	32 (25.6%)
HER2 enriched	7 (15.6%)	10 (12.5%)	17 (13.6%)

attention [37, 91], or inherent biological factors [10, 60, 78, 92].

Six percent (6.1%) of Luminal A tumours expressed CK5/6 (Figs. 1 and 5B) and EGFR was expressed membranous, cytoplasmic and nucleic (Figs. 1 and 6B&C) suggesting a possibility of enhanced Intratumoural heterogeneity. Tumour heterogeneity [15, 29] could result in aggressive traits especially in the ≤50 years old and may lead to relapse following treatment of therapy responsive phenotypes. IHC determination of this molecular subtype in African studies have often been underestimated due to suboptimal handling of tissues [12, 16]. In view of the high prevalence of EGFR and CK5/6, there is need to expand the 4 IHC panel currently in use to the 6 IHC panel by adding EGFR and CK5/6 stains to cater for tumour heterogeneity. This would help in stratifying patients into good/poor prognostic groups and allow clinicians to identify patients likely to relapse due to a switch from one phenotype to another following therapy (Fig. 6).

Limitations of study

This was retrospective study with a small sample size, comprised of samples dating back from 2006, 53/188 sample were poorly fixed were excluded. Poor records keeping and lack of information on important parameters such as metastasis status and clinical staging limited analysis for other factors.

Conclusions

There was high prevalence of TNBC in this study with most of the patients presenting with advanced disease at diagnosis. Over 60% of tumours of ER positive tumours had an ER of Allred score of 5/8 or less, accompanied by low PR expression and a high Ki67 expression. Tumour heterogeneity within the molecular subtypes was also high with Luminal subtypes positive for CK5/6 and EGFR which could relapse.

Abbreviations

NCD	Non-communicable diseases
LMIC	Low- and middle-income countries
GLOBOCAN	Global cancer statistics
IARC	International Agency for Research on Cancer
GNI	Gross national income
SSA	Sub-Saharan Africa
ASR	Age Standardized rate
UMIC	Upper Middle-Income country
BNCR	Botswana National Cancer Registry
DCIS	Ductal carcinoma in situ carcinoma
LCIS	Lobular carcinoma in situ
H&E	Haematoxylin and Eosin
IHC	Immunohistochemistry
ER	Oestrogen receptor
PR	Progesterone receptor
HER2/neu	Human Epidermal Growth Factor Receptor 2

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Authors' contributions
KN Primary experimental research, scoring of ihc, data analysis and manuscript drafting IK: Statistical analysis, data analysis, manuscript drafting and final editing PSR: Statical analysis and drafting, document formatting, data analysis and final editing DG Academic, project supervision and consulting pathologist for morphological and immunohistochemistry scoring, data analysis RN Academic supervision and scientific conceptualization, data analysis, manuscript drafting and final editing KS Clinical data scoping.

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Data availability
All data used/analysed during the study is available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Informed consent was not obtained as the study used residual samples that had been collected as part of clinical care. The study adhered to the 18th World Medical Assembly, Helsinki, Finland, June 1964, amended by the 29th World Medical Assembly, Tokyo, Japan, October 1975, and the 35th World Medical Assembly, Venice, Italy, October 1983 which guides physicians in biomedical research involving human subjects.
Ethical approval was obtained from the University of Botswana Office of Research and Development Institutional Review Board (RES/IRB/1380). A research permit was issued by the Botswana Ministry of Health Research Division (PPME 13/18/1) and further ethical clearance approvals and waiver of consent to enrol participants in the study was issued by from Princess Marina Hospital (PMH 5/75 &79) and Nyangabgwe Hospital Research and Ethics Committees (NRH/1/2).
Permission to transport residual FFPE tissue samples to South Africa to conduct research was granted by the Ministry of Health MH 3/87 III. Further ethical approval was also granted by the University of Cape Town, Faculty of Health Sciences, Human Research Ethics Committee (HREC 620/2013). The archived tumour blocks were de-identified before enrolment into the study in keeping with the provisions of ISO 15189.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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References

1. Polyak K. Heterogeneity in breast cancer. J Clin Invest. 2011;121(10):3786–8. https://doi.org/10.1172/JCI60534. Epub 2011 Oct 3. PMID: 21965334; PMCID: PMC3195489.
2. Guo L, Kong D, Liu J, Zhan L, Luo L, Zheng W, Zheng Q, Chen C, Sun S. Breast cancer heterogeneity and its implication in personalized precision therapy. Exp Hematol Oncol. 2023;12(1):3. https://doi.org/10.1186/s40164-022-00363-1.Erratum.In:ExpHematolOncol.2024Jan23;13(1):7.doi:10.1186/s40164-024-00472-z.PMID:36624542;PMCID:PMC9830930.
3. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin. 2021;71(3):209–49. https://doi.org/10.3322/caac.21660. Epub 2021 Feb 4 PMID: 33538338.
4. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68(6):394–424. https://doi.org/10.3322/caac.21492. Epub 2018 Sep 12. Erratum in: CA Cancer J Clin. 2020;70(4):313. https://doi.org/10.3322/caac.21609. PMID: 30207593.
5. Bray F, Laversanne M, Sung H, Ferlay J, Siegel RL, Soerjomataram I, Jemal A. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2024;74(3):229–263. https://doi.org/10.3322/caac.21834. Epub 2024 Apr 4. PMID: 38572751.
6. Anderson BO, Context-Relevant Guidelines in Cancer Care: Breast Cancer Early Detection, Diagnosis and Treatment in Low- And Middle-Income Countries. Glob Cancer Policy-Making. 2013:36–40. https://cancercontrol.info/wp-content/uploads/2014/08/cc2013_36-40-Benjamin-OAnderson_2013.pdf.
7. Perou CM, Sørlie T, Eisen MB, van de Rijn M, Jeffrey SS, Rees CA, Pollack JR, Ross DT, Johnsen HAkslen LA, Fluge Ø, Pergamenschikov A, Williams C, Zhu SX, Lønning PE, Børresen-Dale A-L, Brown PO, Botstein D. Molecular portraits of human breast tumours. Nature. 2000;406:747–752.
8. Goldhirsch A, Winer EP, Coates AS, Gelber RD, Piccart-Gebhart M, Thürlimann B, Senn HJ; Panel members. Personalizing the treatment of women with early breast cancer: highlights of the St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2013. Ann Oncol. 2013;24(9):2206–23. https://doi.org/10.1093/annonc/mdt303. Epub 2013 Aug 4. PMID: 23917950; PMCID: PMC3755334.
9. Inwald EC, Koller M, Klinkhammer-Schalke M, et al. 4-IHC classification of breast cancer subtypes in a large cohort of a clinical cancer registry: use in clinical routine for therapeutic decisions and its effect on survival. Breast Cancer Res Treat. 2015;153(3):647–58. https://doi.org/10.1007/s10549-015-3572-3.
10. Sivasubramaniam PG, Zhang BL, Zhang Q, Smith JS, Zhang B, Tang ZH, Chen GJ, Xie XM, Xu XZ, Yang HJ, He JJ, Li H, Li JY, Fan JH, Qiao YL. Breast Cancer Disparities: A Multicenter Comparison of Tumor Diagnosis, Characteristics, and Surgical Treatment in China and the U.S. Oncologist. 2015;20(9):1044–1050. https://doi.org/10.1634/theoncologist.2014-0290.
11. Adebamowo CA, Famooto A, Ogundiran TO, Aniagwu T, Nkwodimmah C, Akang E. E: Immunohistochemical and molecular subtypes of breast cancer in Nigeria. Breast Cancer Res Treat. 2008;110:183–8. https://doi.org/10.1007/s10549-0079694-5.
12. Adjei EK, Owusu- Afriyie O, Awuah B, Stalsberg H. Hormone Receptors and Her2 Expression in Breast Cancer in Sub- Saharan Africa. A Comparative Study of Biopsies from Ghana and Norway. Breast J. 2014;20:308–11. https://doi.org/10.1111/tbj.12261.
13. Agboola AJ, Musa AA, Wanangwa N, Abdel-Fatah T, Nolan CC, Ayoade BA, Oyebadejo TY, Banjo AA, Deji-Agboola AM, Rakha EA, Green AR, Ellis I. O: Molecular characteristics and prognostic features of breast cancer in Nigerian compared with UK women. Breast Cancer Res Treat. 2012;135:555–69. https://doi.org/10.1007/s10549-012-2173-7.
14. Allott EH, Geradts J, Cohen SM, Khoury T, Zirpoli GR, Bshara W, Davis W, Omilian A, Nair P, Ondracek RP, Cheng TD, Miller CR, Hwang H, Thorne LB, O'Connor S, Bethea TN, Bell ME, Hu Z, Li Y, Kirk EL, Sun X, Ruiz-Narvaez EA, Perou CM, Palmer JR, Olshan AF, Ambrosone CB, Troester MA. Frequency of breast cancer subtypes among African American women in the AMBER consortium. Breast Cancer Res. 2018;20(1):12. https://doi.org/10.1186/s13058-018-0939-5.PMID:29409530;PMCID:PMC5801839.
15. Guth AA, Kim JC, Schwartz S, Montes J, Snyder RA, Axelrod D, Schnabel F. The Relationship of Race, Oncotype DX, and Ki67 in a Population Highly Screened For Breast Cancer. 2017 Wiley Periodicals, Inc., 1075–122X/17. Breast J. 2017;23(2):177–181. https://doi.org/10.1111/tbj.12781.
16. Eng A, McCormack V, dos-Santos-Silva I. Receptor-Defined Subtypes of Breast Cancer in Indigenous Populations in Africa: A Systematic Reviewand Meta-Analysis. PLoS Med. 2014;11(9):e1001720. https://doi.org/10.1371/journal.pmed.1001720 Receptor-Defined Subtypes of Breast Cancer in Indigenous Populations in Africa: A Systematic Review and Meta-Analysis. Available from: https://www.researchgate.net/publication/265467850_Receptor-Defined_Subtypes_of_Breast_Cancer_in_Indigenous_Populations_in_Africa_A_Systematic_Review_and_Meta-Analysis#fullTextFileContent. Accessed 28 Oct 2024.

17. Popli P, Gutterman EM, Omene C, Ganesan S, Mills D, Marlink R. Receptor-Defined Breast Cancer in Five East African Countries and Its Implications for Treatment: Systematic Review and Meta-Analysis. JCO Glob Oncol. 2021;7:289–301. https://doi.org/10.1200/GO.20.00398.PMID:33591798; PMCID:PMC8081496.
18. https://gco.iarc.who.int/media/globocan/factsheets/populations/72-botswana-fact-sheet.pdf.
19. Sinha S, Bhatia R, Narasimamurthy M, Rayne S, Grover S. Epidemiology of Breast Cancer Presentation in Botswana, South Africa, and the United States. J Surg Res. 2022;279:533–539. https://doi.org/10.1016/j.jss.2022.04.071. Epub 2022 Jul 19. PMID: 35868037; PMCID: PMC10033457.
20. El Saghir NS, Adebamowo CA, Anderson BO, Carlson RW, Bird PA, Corbex M, Badwe RA, Bushnaq MA, Eniu A, Gralow JR, Harness JK, Masetti R, Perry F, Samiei M, Thomas DB, Wiafe-Addai B, Cazap E. Breast cancer management in low resource countries (LRCs): consensus statement from the Breast Health Global Initiative. Breast. 2011;20(Suppl 2):S3-11. https://doi.org/10.1016/j.breast.2011.02.006. PMID: 21392996.
21. Jemal A, Bray F, Center MM, O`Brien, Ferlay J, Ward E, Forman D. Global Cancer Statistics. CA Cancer J CLin. 2011 61:69–90. Vc 2011 American Cancer Society, Inc.
22. Wu Z, Xia F, Lin R. Global burden of cancer and associated risk factors in 204 countries and territories, 1980–2021: a systematic analysis for the GBD 2021. J Hematol Oncol. 2024;17:119. https://doi.org/10.1186/s13045-024-01640-8.
23. Hammond ME, Hayes DF, Dowsett M, Allred DC, Hagerty KL, Badve S, Fitzgibbons PL, Francis G, Goldstein NS, Hayes M, Hicks DG, Lester S, Love R, Mangu PB, McShane L, Miller K, Osborne CK, Paik S, Perlmutter J, Rhodes A, Sasano H, Schwartz JN, Sweep FC, Taube S, Torlakovic EE, Valenstein P, Viale G, Visscher D, Wheeler T, Williams RB, Wittliff JL, Wolff AC. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer. Arch Pathol Lab Med. 2010;134(6):907–22. https://doi.org/10.5858/134.6.907. Erratum. In: Arch Pathol Lab Med. 2010;134(8):1101. PMID: 20524868; PMCID: PMC3073033.
24. M, Perlmutter J, Perou CM, Regan MM, Rimm DL, Symmans WF, Torlakovic EE, Varella L, Viale G, Weisberg TF, McShane LM, Wolff AC. Estrogen and Progesterone Receptor Testing in Breast Cancer: ASCO/CAP Guideline Update. J Clin Oncol. 2020;38(12):1346–66. https://doi.org/10.1200/JCO.19.02309. Epub 2020 Jan 13. PMID: 31928404.
25. Wolff AC, Somerfield MR, Dowsett M, Hammond MEH, Hayes DF, McShane LM, Saphner TJ, Spears PA, Allison KH. Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer. Arch Pathol Lab Med. 2023;147(9):993–1000. https://doi.org/10.5858/arpa.2023-0950-SA. PMID: 37303228.
26. Dowsett M, Nielsen TO, A'Hern R, et al. Assessment of Ki67 in Breast Cancer: Recommendations from the International Ki67 in Breast Cancer Working Group. JNCI Journal of the National Cancer Institute. 2011;103(22):1656–64. https://doi.org/10.1093/jnci/djr393.
27. Nalwoga H, Arnes JB, Wabinga H, Akslen LA. Expression of EGFR and c-kit is associated with the basal-like phenotype in breast carcinomas of African women. APMIS. 2008;116(6):515–25. https://doi.org/10.1111/j.1600-0463.2008.01024.x. PMID: 18754326.
28. Eroles P, Bosch A, Pérez-Fidalgo JA, Lluch A. Molecular biology in breast cancer: intrinsic subtypes and signaling pathways. Cancer Treat Rev. 2012;38(6):698–707. https://doi.org/10.1016/j.ctrv.2011.11.005. Epub 2011 Dec 16 PMID: 22178455.
29. Cuzick J, Dowsett M, Pineda S, Wale C, Salter J, Quinn E, Zabaglo L, Mallon E, Green AR, Ellis IO, Howell A, Buzdar AU, Forbes JF. Prognostic value of a combined estrogen receptor, progesterone receptor, Ki-67, and human epidermal growth factor receptor 2 immunohistochemical score and comparison with the Genomic Health recurrence score in early breast cancer. J Clin Oncol. 2011;29(32):4273–8. https://doi.org/10.1200/JCO.2010.31.2835. Epub 2011 Oct 11 PMID: 21990413.
30. McCormack VA, Joffe M, van den Berg E, Broeze N, Silva Idos S, Romieu I, Jacobson JS, Neugut AI, Schüz J, Cubasch H. Breast cancer receptor status and stage at diagnosis in over 1,200 consecutive public hospital patients in Soweto, South Africa: a case series. Breast Cancer Res. 2013;15(5):R84. https://doi.org/10.1186/bcr3478.PMID:24041225;PMCID:PMC3978918.
31. Sayed S, Moloo Z, and Wasike R, et al. Is breast cancer from Sub Saharan Africa truly receptor poor? Prevalence of ER/ PR/HER2 in breast cancer from Kenya Breast. 2014;23:591–596. https://doi.org/10.1016/j.breast.2014.06.006 PMID: 25012.
32. Hadgu E, Seifu D, Tigneh W, Bokretsion Y, Bekele A, Abebe M, Sollie T, Merajver SD, Karlsson C, Karlsson MG. Breast cancer in Ethiopia: evidence for geographic difference in the distribution of molecular subtypes in Africa. BMC Womens Health. 2018;18(1):40. https://doi.org/10.1186/s12905-018-0531-2.PMID:29444670;PMCID:PMC5813361.
33. Miguel F, Lopes LV, Ferreira E, Ribas E, Pelaez AF, Leal C, Amaro T, Lopes P, Santos CM, Lopes C, Santos LL. Breast cancer in Angola, molecular subtypes: a first glance. Ecancermedicalscience. 2017;30(11):763. https://doi.org/10.3332/ecancer.2017.763.PMID:28900476;PMCID:PMC5574661.
34. Adjei EK, Owusu- Afriyie O, Awuah B, talsberg H. Hormone Receptors and Her2 Expression in Breast Cancer in Sub- Saharan Africa. A Comparative Study of Biopsies from Ghana and Norway. Breast J. 2014;20:308-311. https://doi.org/10.1111/tbj.12261.
35. Inwald EC, Koller M, Klinkhammer-Schalke M, et al. 4-IHC classification of breast cancer subtypes in a large cohort of a clinical cancer registry: use in clinical routine for therapeutic decisions and its effect on survival. Breast Cancer Res Treatment. 2015;153(3):647-658. https://doi.org/10.1007/s10549-015-3572-3.
36. Rakha EA, Soria D, Green AR, Lemetre C, Powe DG, Nolan CC, … Ellis IO. Nottingham Prognostic Index Plus (NPI+): a modern clinical decision making tool in breast cancer. British J Cancer. 2014;110(7):1688–1697. https://doi.org/10.1038/bjc.2014.120.
37. Dialla PO, Chu WO, Roignot P, Bone-Lepinoy MC, Poillot ML, Coutant C, Arveux P, Dabakuyo-Yonli TS. "Impact of age-related socio-economic and clinical determinants of quality of life among long-term breast cancer survivors." Maturitas. 2015;81(3): 362–70.
38. Galukande M, Wabinga H, Mirembe F, Karamagi C, Asea A. Molecular breast cancer subtypes prevalence in an indigenous Sub Saharan African population. Pan Afr Med J. 2014;5(17):249. https://doi.org/10.11604/pamj.2014.17.249.330.PMID:25309649;PMCID:PMC4189896.
39. Effi AB, Aman NA, Koui BS, Koffi KD, Traoré ZC, Kouyate M. Immunohistochemical determination of estrogen and progesterone receptors in breast cancer: relationship with clinicopathologic factors in 302 patients in Ivory Coast. BMC Cancer. 2017;17(1):115. https://doi.org/10.1186/s12885-017-3105-z.PMID:28173783;PMCID:PMC5297122.
40. Awadelkarim KD, Arizzi C, Elamin EOM, Hamad HMA, De Blasio P, Mekki SO, Osman I, Biunno I, Elwali NE, Barberis MC, Mariani-Costantini R. Basal-Like Phenotype in a Breast Carcinoma Case Series from Sudan: Prevalence and Clinical/Pathological Correlations Pathology Research International. 2011, Article ID 806831:10.https://doi.org/10.4061/2011/806831.
41. Titloye NA, Foster A, Omoniyi-Esan GO, Komolafe AO, Daramola AO, Adeoye OA, Adisa AO, Manoharan A, Pathak D, D'Cruz MN, Alizadeh Y, Lewis PD, Shaaban AM. Histological Features and Tissue Microarray Taxonomy of Nigerian Breast Cancer Reveal Predominance of the High-Grade Triple-Negative Phenotype. Pathobiology. 2016;83(1):24–32. https://doi.org/10.1159/000441949. Epub 2016 Jan 6 PMID: 26730581.
42. Agboola AJ, Musa AA, Wanangwa N, Abdel-Fatah T, Nolan CC, Ayoade BA, Oyebadejo TY, Banjo AA, Deji-Agboola AM, Rakha EA, Green AR, Ellis IO.Molecular characteristics and prognostic features of breast cancer in Nigerian compared with UK women; Breast Cancer Res Treat. 2012;135:555–569 DOI 10.1007/s10549-012-2173-7.
43. Nalwoga H, Arnes J, Wabinga H, et al. Expression of aldehyde dehydrogenase 1 (ALDH1) is associated with basal-like markers and features of aggressive tumours in African breast cancer. Br J Cancer. 2010;102:369–75. https://doi.org/10.1038/sj.bjc.6605488.
44. Huo D, Ikpatt F, Khramtsov A, Dangou JM, Nanda R, Dignam J, Zhang B, Grushko T, Zhang C, Oluwasola O, Malaka D, Malami S, Odetunde A, Adeoye AO, Iyare F, Falusi A, Perou CM, Olopade OI. Population differences in breast cancer: survey in indigenous African women reveals over-representation of triple-negative breast cancer. J Clin Oncol. 2009;27(27):4515–21. https://doi.org/10.1200/JCO.2008.19.6873. Epub 2009 Aug 24. PMID: 19704069; PMCID: PMC2754904.
45. Lehmann BD, Bauer JA, Chen X, Sanders ME, Chakravarthy AB, Shyr Y, Pietenpol JA. Identification of human triple-negative breast cancer subtypes and preclinical models for selection of targeted therapies. J Clin Invest. 2011;121(7):2750–67. https://doi.org/10.1172/JCI45014.PMID:21633166; PMCID:PMC3127435.
46. Davis SL, Eckhardt SG, Tentler JJ, Diamond JR. Triple-negative breast cancer: bridging the gap from cancer genomics to predictive biomarkers.

Ther Adv Med Oncol. 2014;6(3):88–100. https://doi.org/10.1177/1758834013519843.PMID:24790649;PMCID:PMC3987651.
47. Lindner R, Sullivan C, Offor O, Lezon-Geyda K, Halligan K, Fischbach N, Shah M, Bossuyt V, Schulz V, Tuck DP, Harris LN. Molecular phenotypes in triple negative breast cancer from African American patients suggest targets for therapy. PLoS ONE. 2013;8(11):e71915. https://doi.org/10.1371/journal.pone.0071915.PMID:24260093;PMCID:PMC3832509.
48. Sood N, Nigam JS. Correlation of CK5 and EGFR with Clinicopathological Profile of Triple-Negative Breast Cancer. Patholog Res Int. 2014;2014:141864. https://doi.org/10.1155/2014/141864. Epub 2014 Oct 23. PMID: 25400978; PMCID: PMC4225826.
49. Tang P, Tse GM. Immunohistochemical Surrogates for Molecular Classification of Breast Carcinoma: A 2015 Update. Arch Pathol Lab Med. 2016;140(8):806–14. https://doi.org/10.5858/arpa.2015-0133-RA. PMID: 27472239.
50. Li Yin, Zhao-Yi Wang; Roles of the ER-α36-EGFR/HER2 positive regulatory loops in tamoxifen resistance, Steroid. 2016;111:95–99. ISSN 0039–128X. https://doi.org/10.1016/j.steroids.2016.01.019. https://www.sciencedirect.com/science/article/pii/S0039128X16000209.
51. Hsu SC, Miller SA, Wang Y, Hung MC. Nuclear EGFR is required for cisplatin resistance and DNA repair. Am J Transl Res. 2009;1(3):249–58. PMID: 19956435; PMCID: PMC2776325.
52. Dittmann K, Mayer C, Rodemann HP. Nuclear EGFR as novel therapeutic target: insights into nuclear translocation and function. Strahlenther Onkol. 2010;186(1):1–6. https://doi.org/10.1007/s00066-009-2026-4. Epub 2009 Dec 28 PMID: 20082181.
53. Dittmann K, Mayer C, Paasch A, Huber S, Fehrenbacher B, Schaller M, Rodemann HP. Nuclear EGFR renders cells radio-resistant by binding mRNA species and triggering a metabolic switch to increase lactate production. Radiother Oncol. 2015;116(3):431–7. https://doi.org/10.1016/j.radonc.2015.08.016. Epub 2015 Aug 27 PMID: 26320552.
54. Aichler M, Motschmann M, Jütting U, Luber B, Becker K, Ott K, Lordick F, Langer R, Feith M, Siewert JR, Walch A. Epidermal growth factor receptor (EGFR) is an independent adverse prognostic factor in esophageal adenocarcinoma patients treated with cisplatin-based neoadjuvant chemotherapy. Oncotarget. 2014;5(16):6620–32. https://doi.org/10.18632/oncotarget.2268.PMID:25216514;PMCID:PMC4196151.
55. Mao and Unadakat 2015Mao Q, Unadkat JD. Role of the breast cancer resistance protein (BCRP/ABCG2) in drug transport--an update. AAPS J. 2015;17(1):65–82. https://doi.org/10.1208/s12248-014-9668-6. Epub 2014 Sep 19. PMID: 25236865; PMCID: PMC4287283.
56. Huang Y and Chang Y (2011) Epidermal Growth Factor Receptor (EGFR) Phosphorylation, Signaling and Trafficking in Prostate Cancer. Prostate Cancer - From Bench to Bedside. InTech. Available at: http://dx.doi.org/10.5772/27021.
57. Liccardi G, Hartley JA, Hochhauser D. EGFR nuclear translocation modulates DNA repair following cisplatin and ionizing radiation treatment. Cancer Res. 2011;71(3):1103–14. https://doi.org/10.1158/0008-5472.CAN-10-2384. Epub 2011 Jan 25. PMID: 21266349; PMCID: PMC3033323.
58. Bhikoo R, Srinivasa S, Yu TC, Moss D, Hill AG. Systematic review of breast cancer biology in developing countries (part 1): Africa, the middle East, eastern europe, Mexico, the Caribbean and South america. Cancers (Basel). 2011;3(2):2358–81. https://doi.org/10.3390/cancers3022358.PMID:24212814;PMCID:PMC3757422.
59. Newman LA, Kaljee LM. Health Disparities and Triple-Negative Breast Cancer in African American Women: A Review. JAMA Surg. 2017;152(5):485–93. https://doi.org/10.1001/jamasurg.2017.0005. PMID: 28355428.
60. Cubasch H, Dickens C, Joffe M, Duarte R, Murugan N, Chih MT, Moodley K, Sharma V, Ayeni O, Jacobson JS, Neugut N, McCormack V, Ruff P: Breast cancer survival in Soweto, Johannesburg, South Africa: A receptor defined cohort of women diagnosed from,. to 11. Cancer Epidemiol. 2009;52(2018):120–7.
61. Baker AT, Zlobin A, Osipo C. Notch-EGFR/HER2 Bidirectional Crosstalk in Breast Cancer. Front Oncol. 2014;12(4):360. https://doi.org/10.3389/fonc.2014.00360.PMID:25566499;PMCID:PMC4264417.
62. Nitta H, Kelly BD, Allred C, Jewell S, Banks P, Dennis E, Grogan TM. The assessment of HER2 status in breast cancer: the past, the present, and the future. Pathol Int. 2016;66(6):313–24. https://doi.org/10.1111/pin.12407. Epub 2016 Apr 7 PMID: 27061008.
63. Tai W, Mahato R, Cheng K. The role of HER2 in cancer therapy and targeted drug delivery. J Control Release. 2010;146(3):264–75. https://doi.org/10.1016/j.jconrel.2010.04.009. Epub 2010 Apr 10. PMID: 20385184; PMCID: PMC2918695.
64. Brand TM, Iida M, Luthar N, Starr MM, Huppert EJ, Wheeler DL. Nuclear EGFR as a molecular target in cancer. Radiother Oncol. 2013;108(3):370–7. https://doi.org/10.1016/j.radonc.2013.06.010. Epub 2013 Jul 3. Erratum in: Radiother Oncol. 2019 Jan;130:195. https://doi.org/10.1016/j.radonc.2018.10.011. PMID: 23830194; PMCID: PMC3818450.
65. Read J, Ingram A, Al Saleh HA, Platko K, Gabriel K, Kapoor A, Pinthus J, Majeed F, Qureshi T, Al-Nedawi K. Nuclear transportation of exogenous epidermal growth factor receptor and androgen receptor via extracellular vesicles. Eur J Cancer. 2017;70:62–74. https://doi.org/10.1016/j.ejca.2016.10.017. Epub 2016 Nov 22. Erratum in: Eur J Cancer. 2017;83:335. https://doi.org/10.1016/j.ejca.2017.07.009. PMID: 27886573.
66. Rakha EA, Reis-Filho JS, Baehner F, Dabbs DJ, Decker T, Eusebi V, … Ellis IO. Breast cancer prognostic classification in the molecular era: the role of histological grade. Breast Cancer Research : BCR. 2010;12(4):207. https://doi.org/10.1186/bcr2607.
67. Howe LR, Brown PH. Targeting the HER/EGFR/ErbB Family to Prevent Breast Cancer. Cancer Prev Res. 2011;4:1149–57.
68. Hart V, Gautrey H, Kirby J, Tyson-Capper A. HER2 splice variants in breast cancer: investigating their impact on diagnosis and treatment outcomes. Oncotarget. 2020;11(46):4338–57. https://doi.org/10.18632/oncotarget.27789.PMID:33245725;PMCID:PMC7679030.
69. Jackson C, Browell D, Gautrey H, Tyson-Capper A. Clinical Significance of HER-2 Splice Variants in Breast Cancer Progression and Drug Resistance. Int J Cell Biol. 2013;2013:973584. https://doi.org/10.1155/2013/973584. Epub 2013 Jul 1. PMID: 23935627; PMCID: PMC3713377.
70. Sung H, Garcia-Closas M, Chang-Claude J, Blows FM, Ali HR, Figueroa J, Nevanlinna H, Fagerholm R, Heikkilä P, Blomqvist C, Giles GG, Milne RL, Southey MC, McLean C, Mannermaa A, Kosma VM, Kataja V, Sironen R, Couch FJ, Olson JE, Hallberg E, Olswold C, Cox A, Cross SS, Kraft P, Tamimi RM, Eliassen AH, Schmidt MK, Bolla MK, Wang Q, Easton D, Howat WJ, Coulson P, Pharoah PD, Sherman ME, Yang XR. Heterogeneity of luminal breast cancer characterised by immunohistochemical expression of basal markers. Br J Cancer. 2016;114(3):298–304. https://doi.org/10.1038/bjc.2015.437. Epub 2015 Dec 17. PMID: 26679376; PMCID: PMC4742579.
71. McGranahan N, Swanton C. Cancer Evolution Constrained by the Immune Microenvironment. Cell. 2017;170(5):825–7. https://doi.org/10.1016/j.cell.2017.08.012. PMID: 28841415.
72. Gupta PB, Fillmore CM, Jiang G, Shapira SD, Tao K, Kuperwasser C, Lander ES. Stochastic state transitions give rise to phenotypic equilibrium in populations of cancer cells. Cell. 2011;146(4):633–44. Erratum. In: Cell. 2011;147(5):1197. Erratumin: Cell. 2011;146(6):1042. https://doi.org/10.1016/j.cell.2011.07.026. PMID: 21854987.
73. Turashvili G, Brogi E. Tumor Heterogeneity in Breast Cancer. Front Med (Lausanne). 2017;4:227. https://doi.org/10.3389/fmed.2017.00227. PMID: 29276709; PMCID: PMC5727049. Cuzick J, Dowsett M, Pineda S, Wale C, Salter J, Quinn E, Zabaglo L, Mallon E, Green AR, Ellis IO, Howell A, Buzdar AU, Forbes JF. Prognostic value of a combined estrogen receptor, progesterone receptor, Ki-67, and human epidermal growth factor receptor 2 immunohistochemical score and comparison with the Genomic Health recurrence score in early breast cancer. J Clin Oncol. 2011;29(32):4273–8. https://doi.org/10.1200/JCO.2010.31.2835. Epub 2011 Oct 11. PMID: 21990413.
74. Mansouri H, Leah FM, Erick PM, Elingarami S, Emmanuel AM. Ki-67, p53 and BCL-2 Expressions and their Association with Clinical Histopathology of Breast Cancer among Women in Tanzania. Sci Rep. 2019;9(1):9918.
75. Kim MS, Kwon S, Kim T, Lee ES, Park JK. Quantitative proteomic profiling of breast cancers using a multiplexed microfluidic platform for immunohistochemistry and immunocytochemistry. Biomaterials. 2011;32(5):1396–403. https://doi.org/10.1016/j.biomaterials.2010.10.040. Epub 2010 Nov 18 PMID: 21093044.
76. McGranahan N, Swanton C. Clonal Heterogeneity and Tumor Evolution: Past, Present, and the Future. Cell. 2017;168(4):613–28. https://doi.org/10.1016/j.cell.2017.01.018. PMID: 28187284.
77. Lavi O, Greene JM, Levy D, Gottesman MM. Simplifying the complexity of resistance heterogeneity in metastasis. Trends Mol Med. 2014;20(3):129–36. https://doi.org/10.1016/j.molmed.2013.12.005. Epub 2014 Jan 31. PMID: 24491979; PMCID: PMC3962236.
78. Keenan T, Moy B, Mroz EA, Ross K, Niemierko A, Rocco JW, Isakoff S, Ellisen LW, Bardia A. Comparison of the Genomic Landscape Between

Primary Breast Cancer in African American Versus White Women and the Association of Racial Differences With Tumor Recurrence. J Clin Oncol. 2015;33(31):3621–7. https://doi.org/10.1200/JCO.2015.62.2126. Epub 2015 Sep 14. PMID: 26371147; PMCID: PMC4979243.
79. Brand TM, Iida M, Li C, Wheeler DL. The Nuclear Epidermal Growth Factor Receptor Signaling Network and its Role in Cancer. Discov Med. 2011;12(66):419–32.
80. Brand TM, Iida M, Dunn EF, Luthar N, Kostopoulos KT, Corrigan K, LWleklinski MJ, Yang D, Wisinski KB, Salgia R, Wheeler DL. Nuclear Epidermal Growth Factor Receptor Is a Functional Molecular Target in TripleNegative Breast Cancer. Mol Cancer Ther. 2014(13) (5):1356–1368; https://doi.org/10.1158/1535-7163.MCT-13-1021.
81. Gustafsson AS, Abramenkovs A, Stenerlöw B. Suppression of DNA-dependent protein kinase sensitize cells to radiation without affecting DSB repair. Mutat Res. 2014;769:1–10. https://doi.org/10.1016/j.mrfmmm.2014.06.004. Epub 2014 Jun 22 PMID: 25771720.
82. Green A. R, Powe D. G, Rakha E. A, Soria D, Lemetre C, Nolan C. C, Barros F. F. T, Macmillan R. D, Garibaldi J. M, Ball R, and Ellis I. O: Identification of key clinical phenotypes of breast cancer using a reduced panel of protein biomarkers British Journal of Cancer. 2013;109:1886–1894 | https://doi.org/10.1038/bjc.2013.528.
83. Green AR, Soria D, Powe DG, Nolan CC, Aleskandarany M, Szász MA, … Ellis IO. Nottingham prognostic index plus (NPI+) predicts risk of distant metastases in primary breast cancer. Breast Cancer Res Treatment. 2016;157:65–75. https://doi.org/10.1007/s10549-016-3804-1.
84. Campbell MC, Tishkoff SA. African genetic diversity: implications for human demographic history, modern human origins, and complex disease mapping. Annu Rev Genomics Hum Genet. 2008;9:403–33. https://doi.org/10.1146/annurev.genom.9.081307.164258.PMID:18593304;PMCID:PMC2953791.
85. Blows FM, Driver KE, Schmidt MK, Broeks A, van Leeuwen FE, Wesseling J, … Huntsman D. Subtyping of Breast Cancer by Immunohistochemistry to Investigate a Relationship between Subtype and Short and Long Term Survival: A Collaborative Analysis of Data for 10,159 Cases from 12 Studies. PLoSMedicine. 2016;7(5):e1000279. https://doi.org/10.1371/journal.pmed.1000279.
86. Nakai T, Ichihara S, Kada A, Ito N, Moritani S, Kawasaki T, Uchiyama T, Itami H, Morita K, Takano M, Takeda M, Hatakeyama K, Ohbayashi C. The unique luminal staining pattern of cytokeratin 5/6 in adenoid cystic carcinoma of the breast may aid in differentiating it from its mimickers. Virchows Arch. 2016;469(2):213–22. https://doi.org/10.1007/s00428-016-1963-4. Epub 2016 May 30 PMID: 27240462.
87. Eroles P, Bosch A, Pérez-Fidalgo JA, Lluch A. Molecular biology in breast cancer: intrinsic subtypes and signaling pathways. Cancer Treat Rev. 2012;38(6):698-707. https://doi.org/10.1016/j.ctrv.2011.11.005. Epub 2011 Dec 16. PMID: 22178455.
88. Toss A, Cristofanilli M. Molecular characterization and targeted therapeutic approaches in breast cancer. Breast Cancer Res. 2015;17(1):60. https://doi.org/10.1186/s13058-015-0560-9.PMID:25902832;PMCID:PMC4407294.
89. Ades F, Zardavas D, Bozovic-Spasojevic I, Pugliano L, Fumagalli D, de Azambuja E, Viale G, Sotiriou C, Piccart M. Luminal B breast cancer: molecular characterization, clinical management, and future perspectives. J Clin Oncol. 2014;32(25):2794–803. https://doi.org/10.1200/JCO.2013.54.1870. Epub 2014 Jul 21 PMID: 25049332.
90. Nielsen TO, Hsu FD, Jensen K, Cheang M, Karaca G, Hu Z, Hernandez-Boussard T, Livasy C, Cowan D, Dressler L, Akslen LA, Ragaz J, Gown AM, Gilks CB, van de Rijn M, Perou CM. Immunohistochemical and clinical characterization of the basal-like subtype of invasive breast carcinoma. Clin Cancer Res. 2004;10(16):5367–74. https://doi.org/10.1158/1078-0432.CCR-04-0220. PMID: 15328174.
91. Abdulrahman Jnr. G. O. Rahman G.A. "Epidemiology of Breast Cancer in Europe and Africa," J Cancer Epidemiology. 2012;Article ID 915610:5. https://doi.org/10.1155/2012/915610.
92. Trinkaus ME, Sayed S, Gakinya SM, Moloo Z, Hanna W, Rahim Y. Triple negative and basal-like breast cancer in East Africa. Breast J. 2011;17(4):438–40. https://doi.org/10.1111/j.1524-4741.2011.01096.x. PMID: 21752138..

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