Chapter

CLINICAL RELEVANCE OF BIOMARKERS IN OESTROGEN RECEPTOR POSITIVE BREAST CANCER

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ABSTRACT

Some biomarkers have been examined extensively in the context of breast cancer, including the oestrogen receptor (ER) and human epidermal growth factor receptor 2 (HER2). They are now routinely measured and used clinically for selection of primary, (neo)adjuvant endocrine therapy and/or chemotherapy. In this chapter, we will review the literature, including the work of our group, on the analysis of biomarkers (including those that are not routinely measured clinically but may be relevant in ER positive breast cancer e.g., B-cell lymphoma (BCL) 2-protein, Ki-67, and p53). We will specifically look at the relationship of these biomarkers with

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age and their potential clinical relevance, notably their prognostic value and ability to predict therapeutic response. Furthermore, we will discuss the potential technology of high-throughput measurement of these biomarkers at diagnosis for use as tools for clinical assessment and research.

Keywords: oestrogen receptor, epidermal growth factor receptor, biomarkers, immunohistochemistry

ABBREVIATIONS

Amplified in breast cancer (AIB);
B cell lymphoma (BCL);
Breast cancer gene (BRCA);
Cytokeratin (CK);
Epidermal growth factor receptor (EGFR);
Human epidermal growth factor receptor (HER);
liver kinase B1 (LKB1);
(mTOR);
Mammalian target of rapamycin;
mucin (MUC);
neoadjuvant chemotherapy (NACT);
Oestrogen receptor (ER);
pathological complete response (pCR);
Poly (ADP-ribose) polymerases (PARP);
Progesterone receptor (PgR);
Phosphatase and tensin homolog (PTEN);
Tumour protein 53 (Tp53);
Vascular endothelial growth factor (VEGF)

INTRODUCTION

The majority of patients will have core needle biopsy (CNB) performed at diagnosis of breast cancer. This sample is usually analysed on site by a resident histopathologist. It is routine for two types of histological analysis to be completed. Firstly haematoxylin and eosin (H&E) staining is performed to provide information on histological type and grade and secondly, immunohistochemistry to measure the expression of oestrogen receptor (ER), progesterone receptor (PgR) and human epidermal growth factor receptor (HER)-2. These features are important for treatment planning at all stages of therapy (neoadjuvant, primary, adjuvant and advanced) and to help predict recurrence risk and survival outcomes.

Although, ER, PgR and HER2 are now routinely measured, numerous other 'biomarkers' may have a potential role in breast cancer. A 'biomarker' is a characteristic that can be objectively measured and evaluated as an indicator of a normal biological or pathogenic process or pharmacological response to a therapeutic intervention [1]. A number of biomarkers, for example, B-cell lymphoma (BCL)-2, Ki-67 and p53 are well recognised in the literature to be relevant in breast cancer, however they are usually only measured in a research setting.

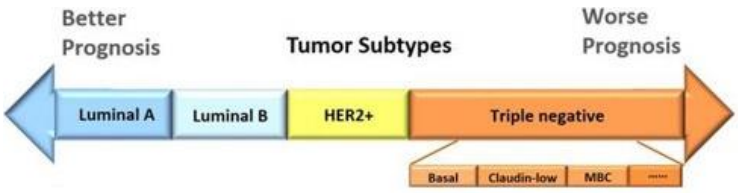

Figure 1. Our current understanding of subtypes of breast cancer [7] (permission to use not required).

We now understand breast cancer to be a heterogeneous complex of diseases, with a spectrum of many subtypes with distinct biological features [2-4] which leads to differences in response patterns to various treatment modalities. Therefore, traditional treatment plans based on routinely

measured biomarkers and our current understanding of disease subtypes (Figure 1) may no longer be adequate [5, 6].

In recent years, clinical risk stratification tools such as Adjuvant Online [8] and PREDICT [9] and genomics tool such as Oncotype DX [10] have taken advantage of this concept to assist in decision making regarding adjuvant systemic therapy including chemotherapy. The use of molecular profiling more widely in these settings or in other treatment dilemmas is currently only used in the research setting. A key component of molecular profiling is understanding which biomarkers and in what combination are likely to be most relevant to answer specific treatment questions. In the next section, we will explore individual biomarkers in more detail.

Over two-thirds of all breast cancers are ER positive [11, 12]. Endocrine therapies targeting the ER are utilised at every stage of the treatment pathway including chemoprevention in high-risk patients, primary treatment for patients who are unfit for or decline surgery, in the (neo)adjuvant and advanced settings. However, resistance to endocrine therapy is well recognised but not completely understood. Therefore, it is hypothesised that other biomarkers must pay a role in translating measured ER-positivity to clinically relevant hormone responsiveness. It is for these reasons that we have focused on ER-positive cancer in this chapter. The studies presented in the remainder of this chapter have used immunohistochemistry (IHC) methods to measure biomarkers on patient tumour tissue, unless otherwise stated. These studies include a wide range from the literature, also including those from our group (Nottingham, UK) [6, 13-19]. We possess a large cohort of 1,758 consecutive patients aged ≥70 years with primary operable breast cancer, treated at a single centre with long-term follow-up data available [20]. We have full clinico-pathological data, histological information based on CNB and surgical excision (SE) (if surgery was primary treatment) as well as tissue microarrays (TMAs) constructed from SE. In the same unit, we have a comparative cohort of younger patients (<70 years) again with long-term follow up data, which has been extensively described [21-23].

BIOMARKERS – ASSOCIATION WITH AGE, PREDICTIVE AND PROGNOSTIC VALUES, FUTURE DIRECTIONS

Table 1. Summary of biomarkers associations with age, efficacy of endocrine therapy and survival

Rece-ptor	Association with age	Association with efficacy of endocrine therapy	Association with survival
ER	Increases with age	Increases	Positivity confers better outcome
PgR	No association confirmed	Increases	Negativity linked to poor outcome
HER2	Decreases with age	Established use of trastuzumab	Positivity confers poor outcome
HER3	Possible increase with age	Linked to resistance	Conflicting evidence, possible poor outcomes
HER4	Possible increase with age	Expression may be linked to resistance	Expression associated with good prognosis
EGFR	Positivity more likely in younger age	Associated with therapy resistance	Poor clinical outcome in terms of survival
VEGF	Conflicting evidence	Anti-VEGF therapy established	~~Positivity~~Positivity associated with lower survival
Tp53	Chance of mutation increases with age	Linked to therapy resistance	High expression associated with lower survival
BRCA	Possible loss of expression with age	BRCA 1 associated with resistance	Conflicting results
PTEN	Unknown	Downregulation associated with poor response to tamoxifen	PTEN loss predicts worse survival
LKB1	Possible increase with age	Not enough evidence	Conflicting evidence
Ki-67	Lower expression in older women	High expression is an independent predictor of pCR	Positivity associated with worse survival
BCL-2	Conflicting evidence	Better response to tamoxifen	High expression associated with better survival
Cytoker atins	Higher expression of CK5/6, CK5, CK18 in the older population and lower expression of CK 7/8	Unknown – little evidence at present	Basal cytokeratins linked to poor prognosis
MUC1	Unknown	Associated with resistance	High levels associated with poor outcome
AIB1	Unknown	Associated with good response	High levels associated with poor outcome

Following on from the well-documented correlation between ER-positivity and age, we will discuss the relationship with age of other biomarkers. Measurement of a biomarker can give both predictive information (how the cancer will respond to a certain therapy), and prognostic information (what the biomarker can tell us about clinical outcome). In order to advance the development of this field, we will discuss future work in the research and clinical settings.

A summary of all biomarkers discussed in given in Table 1.

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Hormonal Receptors

If present and activated in breast cancer cells, hormonal receptors will stimulate growth of that cell.

Oestrogen Receptor (ER)

The ERs are a group of proteins found inside cells, which are activated by the hormone oestrogen. Around 70% of breast cancers overall are ER-positive [24].

Association with Age

ER positivity increases with age with the highest proportion of ER-positive cases in patients >65 years of age [25, 26] (Table 2).

Table 2. Percentage of ER-positive by age group in a cohort of 18,586 breast cancer patients by Kerklikowske et al. [25]

Age group (years)	ER-positive cancer (%)
35-39	73.4
40-44	78.0
45-49	76.3
50-54	80.9
55-59	81.6
60-64	83.6
65-69	84.4

70-74	84.3
Overall	*80.5*

Predictive Value

Endocrine agents targeting the ER are effective treatment for breast cancer.

Tamoxifen and raloxifene are approved by the Food and Drug Administration (FDA) for chemoprevention in high risk women [27]. In the International Breast Cancer Prevention Trial [28], 13,388 women deemed to be at an increased risk of breast cancer received tamoxif en or placebo. Tamoxifen reduced the risk of invasive breast cancer by 49% overall, however there was an increase in side effects.

Neoadjuvant endocrine therapy (NAET) has been used in postmenopausal women with ER-positive breast cancer when chemotherapy is not suitable, either due to tolerability or patient choice, with the aim to improve operability whilst simultaneously treating presumed micrometastases. However, it is noted that the pathological complete response (pCR) of NAET is less than with chemotherapy [29], longer durations of treatment may be required [30] and there is greater disease progression when compared to chemotherapy [31].

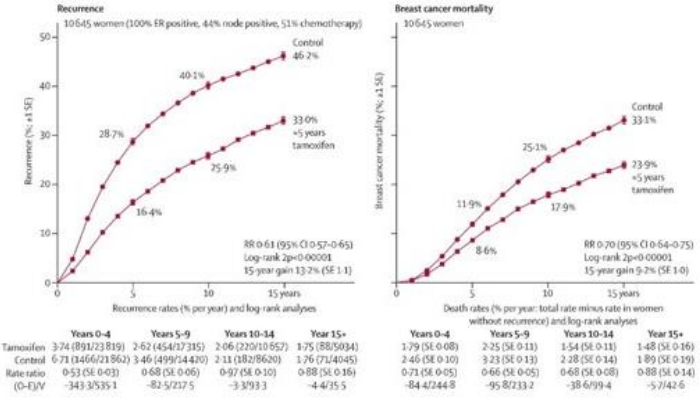

Figure 2**.** Effects of about 5 years of tamoxifen on the 15-year probabilities of recurrence and of breast cancer mortality, for ER-positive disease [34] (permission not required to use).

Primary endocrine therapy (PET) has been shown to be equivalent in terms of survival outcome compared to surgery [32] and is frequently used in the older population with multiple comorbidity or who decline surgery [33].

Adjuvant endocrine therapy is given for at least 5 years after surgery. The Early Breast Cancer Trialists' Collaborative Group (EBCTCG) meta-analysis published in 2011 [34] showed a 30% reduction in breast cancer mortality (absolute reduction in mortality of 9% maintained for 15 years, following 5 years of adjuvant tamoxifen (Figure 2).

Prognostic Value

Above the age of 40 years, positive ER status is associated with improved breast cancer specific survival (BCSS) and overall survival (OS) [35]. Patients with ER-positive tumours generally have lower risk of mortality compared to those with ER-negative tumours [36], although this is not uniform across all groups. In BRCA1/2 carriers, there is work to suggest that ER-positivity confers a negative outcome [37].

Future Directions

As endocrine therapies are well established as outlined above, current research is involved in combination work with newer targeted agents, for example, cyclin-dependent kinase (CDK) 4/6 inhibitors. This combination is currently utilised in the setting of advanced disease and in clinical trials as adjuvant therapy [38, 39].

Progesterone Receptor (PgR)

PgR is a nuclear receptor, activated by the hormone progesterone. Between 65-75% of breast cancers are PgR positive [40]. Most PgR positive tumours are also ER-positive, so its significance is often downplayed. Progesterone receptor serves as an indicator of a functionally intact nuclear ER pathway because adequate levels of oestrogen and nuclear ER are

required to transcribe PgR; therefore it may help predict which patients will respond to hormonal therapy [41].

Association with Age

There is no confirmed relationship between expression of PgR and age to date [6, 26, 42]. Biological characterisation of 14,007 primary breast cancer cases in a single series [43] has suggested that PgR expression may increase with age, but this was not statistically significant.

Predictive Value

In the adjuvant setting, PgR expression is usually associated with greater benefit from endocrine therapy [43, 44]. One study which found conflicting results was the Arimidex, Tamoxifen Alone, or in Combination (ATAC) trial [45]. A total of 9,366 postmenopausal patients with primary breast cancer were found to have significantly improved disease-free survival (DFS) and time to recurrence (TTR) when treated with adjuvant anastrozole compared to tamoxifen. The TTR was longer for patients who were ER-positive and PgR-positive or negative, however the overall survival benefit was greater in the PgR-negative subgroup). The significance of this retrospective subgroup analysis remains controversial.

Prognostic Value

A lack of PgR positivity has been linked to poor survival regardless of age and independent of ER status [46] and increases the risk of relapse and death from breast cancer [47].

In a recent study by Van Asten et al. [48] in 9,647 patients with primary operable ER-positive, HER2-negative breast cancer, absent PgR expression predicted worse outcome in terms of disease free recurrence interval (DFRI) and the group suggested that this effect was more important in high compared with low proliferative tumours.

In a study performed by Albanghali et al. [15] of 536 older women with ER-positive primary breast cancer, PgR expression was associated with better BCSS.

Future Directions

Both the ATAC trial [45] and the study by Van Asten et al. [48] have shown results which require validating in larger prospective cohorts.

Growth Factor Receptors

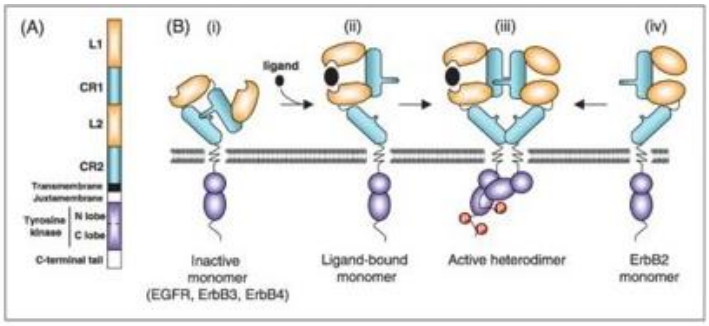

Figure 3. Structure of ErbB receptors [49] (permission to reuse not required).

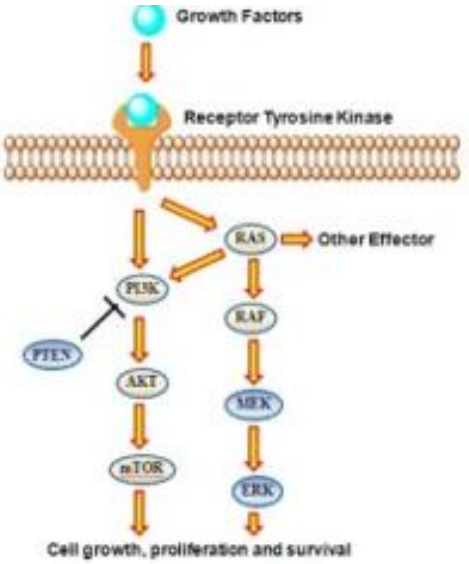

Figure 4. PI3K/Akt/mTOR and Ras/Raf/MEK/ERK signalling pathways [51] (permission to use requested 29/10/2019).

Breast cancer cells require activated growth factor receptors to proliferate, invade and metastasise. Growth factor receptors transduce extracellular signals through activation of intracellular messengers. The human epidermal growth factor (HER) family is one of the most extensively studied and consists of four members: EGFR, HER2, HER3 and HER4. The receptors are structurally related. The receptors require dimerisation (Figure 3) activation of RAS-MAPK and PI3K-AKT-mTOR downstream signalling pathways [49, 50] (Figure 4).

Epidermal Growth Factor Receptor (EGFR)

In an American database of 2,567 breast cancer patients on whom EGFR expression was measured, 18% were EGFR positive [52].

Association with Age

In the same study, EGFR expression was more common in breast tumours in younger (<50 years) compared to older women [52].

Predictive Value

EGFR expression is associated with lower hormone receptor (HR) levels, higher tumour proliferation and HER2 overexpression [52]; which correlated with higher risk of relapse in patients receiving adjuvant treatment. This has been seen in other studies where high levels of EGFR contributed to acquired endocrine resistance with tamoxifen [53].

Prognostic Value

High expression of EGFR correlates with poor clinical outcome in breast cancer [54-56]. In a study by Tsutsui et al. [55] expression of EGFR was analysed in 241 patients with recurrent breast cancer. Patients with positive EGFR expression had worse post-relapse survival than those with negative expression.

Future Directions

There are two types of EGFR inhibitors on the market now; these include tyrosine kinase inhibitors for lung and pancreatitis cancer and monoclonal antibodies for colorectal and head and neck squamous cell carcinoma. For breast cancer however, clinical trials of EGFR inhibitors as monotherapy and in combination with chemotherapy, have not been as successful in universally producing good response rates. Only a handful of trials have noted a good response to EGFR inhibitors in a small number of patients [57, 58]. A phase II trial performed by Johnston et al[58] tested lapatinib, an oral inhibitor of EGFR and HER2 in 45 patients. A clinical response was seen in 50% of HER2-positive patients. Going forward, it may be necessary to stratify patients to enhance the efficacy of EGFR inhibitors and consider combination therapies [59].

Human Epidermal Growth Factor Receptor 2 (HER2)

Approximately 15 of breast cancers have positive expression of HER2 [60, 61]. There is an inverse relationship between HER2 and ER positivity, leading to approximately 10% of ER-positive tumours being HER2-positive and around 50% of HER2-positive tumours being ER-positive [62].

Association with Age

There is lower expression of HER2 with increasing age [6, 60, 63]; HER2 positive tumours are less common over the age of 70 years [63].

Predictive Value

Clinical trials have shown that trastuzumab (Herceptin), a monoclonal antibody targeting the HER2 receptor, significantly improves OS in women with HER2-positive breast cancer and this has been confirmed with long-term follow-up data from the HERA (HERceptin Adjuvant) trial [64]. National Institute for Health and Care Excellence (NICE) guidelines recommend 1 year of adjuvant trastuzumab for stage T1c and above HER2-positive invasive breast cancer in combination with chemotherapy [65].

Retrospective studies have suggested that HER2 overexpression may have a predictive role for response to chemotherapy and endocrine therapy, but results are conflicting [66].

Other HER2 directed therapies approved for clinical use include the monoclonal antibody, pertuzumab, the small molecule kinase inhibitor lapatinib [67] and the toxin-carrying antibody, trastuzumab emtansine [68].

Prognostic Value

HER2 overexpression in general confers an aggressive phenotype and poor patient outcome [69]. Overexpression is associated with increased tumour aggressiveness, increased rates of recurrence and increased mortality in patients with node-positive disease [66].

Future Directions

Pharmacological resistance to trastuzumab and other HER2 directed therapies is recognised. It is hypothesised that HER2-positive breast cancer can be targeted by immunotherapeutic interventions and compounds are currently in the clinical trial setting [50]. One example is immune checkpoint inhibitors, which include CTLA-4 inhibitors, anti-PD-1 and anti PD-L1 antibodies and are currently in Phase I and II trials for early and advanced HER2-positive breast cancer. HER-2 specific peptide-based vaccines have been developed to treat cancers by enhancing the antitumour immune response. One example is Nelipepimut-S (E75) which is currently under investigation in Phase II and III clinical trials [50].

Human Epidermal Growth Factor Receptor 3 (HER3)

Co-expression of HER2 and HER3 is common in breast cancer [70]. The rate of overexpression of HER3 in breast cancer is widely varied according to the literature and ranges from 30-3-75.1% [71].

Association with Age

High expression has been noted in older women (≥70 years) with primary breast cancer, however, no direct comparison to younger patients has been made [6].

Predictive Value

HER3 has been linked to resistance to tamoxifen, which is presumed to be due to the ability of a breast cancer cell to bypass a response to normal endocrine therapies; this is more likely with co-expression of HER2 and HER3 [72]. Although there are currently no specific therapeutic targets to HER3, several studies indicate that activation of HER3 is a major cause of failure of anti-HER2 or anti-ER based therapies. Overexpression of HER3 has been shown to predict resistance to trastuzumab and lapatinib [73].

Prognostic Value

The prognostic value of HER3 is unknown. Studies have shown conflicting evidence and suggest that overexpression of HER3 may be associated with worse survival or of no prognostic use [74]. In a study of 177 primary breast cancers, low HER3 expression predicted recurrence in HER2 amplified breast cancer [74] and more aggressive clinicopatho-logical features.

Future Directions

There are a number of HER3-targeted biologic therapies in preclinical phases of study in breast, colorectal, lung and ovarian cancer. One example is Seribantumab (MM-121) [75], a monoclonal antibody that inhibits HER3 activation, which is in Phase II testing for preoperative triple negative breast cancer (TNBC) and HR-positive, HER2-negative breast cancer in combination with chemotherapy. However, concerns have been raised regarding intolerable side effects of HER3-targeted therapies, resulting in discontinuation of a previous study [72, 75, 76].

Human Epidermal Growth Factor Receptor 4 (HER4)

HER4 mediates both anti-proliferative and pro-apoptotic activity; its function is not yet fully understood [77, 78] and therefore, its overall expression in breast cancer remains unknown.

Association with Age

As with HER3, high expression has been noted in older women (≥70 years) with primary breast cancer, but no direct comparison to younger patients has been made [6].

Predictive Value

Recent studies have shown that expression of HER4 improves OS of breast cancer patients with ER-positive tumours, raising the possibility that HER4 influences response to endocrine therapy [79]. This has been shown in mouse models where HER4 suppression has been associated with tamoxifen resistance [80].

Prognostic Value

Expression of HER4 is associated with good prognosis, with better BCSS, in early breast cancer and correlates with ER-positivity and lower tumour grade [81-83].

Future Directions

There are few clinical trials to investigate anti-HER4 therapies, compared to anti-HER3 therapies. Anti-HER4 antibodies, such as clone P6-1, have been shown to cause growth inhibition of breast cancer cells in vitro [73].

Figure 5 summarises therapeutic targets against the HER family, currently under investigation.

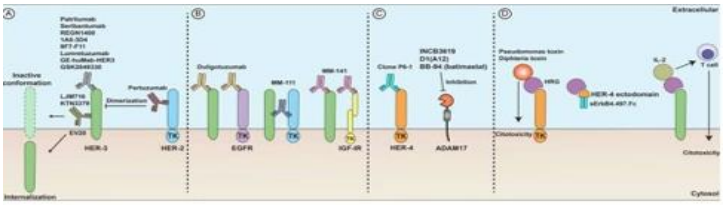

Figure 5. A summary of therapeutic targets including HER3, HER2, HER4 and EGFR (permission to reuse not required).

Vascular Endothelial Growth Factor (VEGF)

VEGF is a signalling protein expressed on vascular endothelial cells as well as carcinoma cells. Isoform VEGF-A has been shown to have an essential role in angiogenesis and tumour blood supply which is a major element in tumour growth and metastasis [84, 85] (Figure 6) and is overexpressed in approximately 72-98% of breast cancers [86].

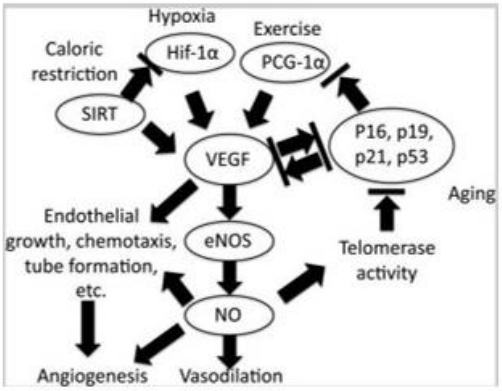

Figure 6. Overview of angiogenic pathways (permission to reuse not required).

Association with Age

Advancing age is associated with a decline in VEGF in normal tissue [87] Conversely, in a study of 575 surgical specimens from older women with primary breast cancer by Syed et al. [6], high levels were found in these patients.

Predictive Value

High VEGF expression has been linked to failure of tamoxifen therapy [88]. Foekens et al. [88] measured levels of VEGF in primary breast tumours from 845 patients who developed recurrence during follow-up. Patients with higher or intermediate levels of VEGF showed a poor rate of response to first-line tamoxifen therapy compared to those with low VEGF levels.

The benefits of anti-VEGF therapy, for example, bevacizumab (a monoclonal antibody) when combined with standard chemotherapy for first-line treatment of metastatic HER2-negative breast cancer have been confirmed in the Phase III RIBBON-1 trial (Regimens in Bevacizumab for Breast Oncology) [89]. The combination of bevacizumab with chemotherapy improved clinical benefit in terms of increased progression-free survival (PFS).Bevacizumab is approved for use in the US.

Prognostic Value

In the same study by Foekens et al. [88], higher levels of VEGF were associated with shorter PFS and OS. This has been confirmed in other breast cancer studies [86, 90].

Future Directions

Following RIBBON-1 [89], clinical trials of bevacizumab are ongoing. The current focus is on identifying subgroups of patients, based on expression of other biomarkers, who may benefit from this therapy [91].

Tumour Suppressor Genes

These are a group of genes prone to genetic change, which results in tumorigenesis. Tp53 mutations are estimated to occur in 50% of all cancers [92], however, a comprehensive meta-analysis revealed that only approximately 20% of all breast cancer cases express mutant p53 [93].

Tumour Suppressor Protein 53 (p53)

A mutation of the p53 gene results in the ability of damaged cells to induce apoptosis [94], resulting in cell death.

Association with Age

The likelihood of a p53 mutation increases with age. A study based on the GLOBOCAN and International Agency for Research on Cancer (IARC) databases showed that p53 mutations accounted for approximately 25% of the aging-related rise in breast cancer worldwide [95].

Predictive Value

The impact of Tp53 status on antihormone treatments has been investigated in two retrospective series, which provided evidence that Tp53 mutation may affect tamoxifen response [96].

Yamashita et al. [97] found that p53 protein accumulation was associated with greater likelihood of resistance to endocrine therapy [97] in metastatic breast cancer, as well as reduced post-relapse survival, compared to cases where p53 expression was low.

Prognostic Value

Low expression of mutant p53 has been linked to better DFS and OS in breast cancer [98]. These finding have been found to be similar across all age groups [6, 19].

Future Directions

Several retrospective studies have investigated a potential prognostic and therapeutic predictive role for mutant p53 in breast cancer, however with conflicting results [99]. Several compounds are being investigated which can reactivate the mutant p53 protein and convert it to a conforming type. Some of these compounds (e.g., PRIMA-1) have been found to exhibit anticancer activity in preclinical models of breast cancer [99].

Breast Cancer Gene (BRCA) 1 and 2

BRCA1 and 2 are genes that produce tumour suppressor proteins. Specific inherited mutations in BRCA1 and BRCA2 increase the risk of breast, ovarian and pancreatic cancers. BRCA mutations are the most common genetically inherited mutations which increase the risk of breast cancer; around 5% of breast cancers are caused by a BRCA mutation [24].

Associations with Age

Estimates from pooled data of 8,139 patients, of whom 500 had a germline mutation in BRCA1 and BRCA2, give an average cumulative risk of BRCA1 mutation carriers by age 70 years of 65% and 45% for BRCA 2. The risk of developing breast cancer with a BRCA mutation increased with

age up to 70 years and then plateaued [100]. Although the relative risk of developing breast cancer is the same, there is a loss of expression of BRCA1 and BRCA2 in older women [6] compared to their younger counterparts.

Predictive Value

There is some laboratory and clinical data to suggest that BRCA1 associated breast cancer may be resistant to tamoxifen [101]. A retrospective study, comparing outcomes in early sage BRCA mutation and sporadic breast cancer treated with endocrine therapy, notes a lower OS in the BRCA carrier group [102].

Prognostic Value

In an analysis of 1940 breast cancer cases [22], altered BRCA1 was associated with shorter disease-free interval, as well as development of recurrence. A systematic review and meta-analysis by van den Broek et al. [103] examined the evidence for breast cancer prognosis of BRCA1/BRCA2 mutation carriers. They concluded that current evidence did not support worse breast cancer survival of mutation carriers. This is contradicted in a recent retrospective cohort study by Vocka et al. [37] that analysed the effect of clinicopathological features on prognosis in BRCA1/2 carriers. They found that mutation carriers with ER-positive disease were high-risk patients in terms of risk of breast cancer death and recurrence.

Future Directions

Poly (ADP-ribose) polymerases (PARPs) are important enzymes in DNA damage repair mechanisms, which BRCA1/2 mutations rely on (Figure 7). The PARP inhibitor, olaparib, has been approved for use in HER2-negative metastatic breast cancer with an inherited BRCA1/2 mutation. The phase III OlympiAD trial has shown a PFS benefit of olaparib compared to single-agent chemotherapy in this setting, but no OS benefit [104]. There are currently over 30 actively recruiting trials involving olaparib to determine the exact benefit as well as comparison with other immunotherapy agents.

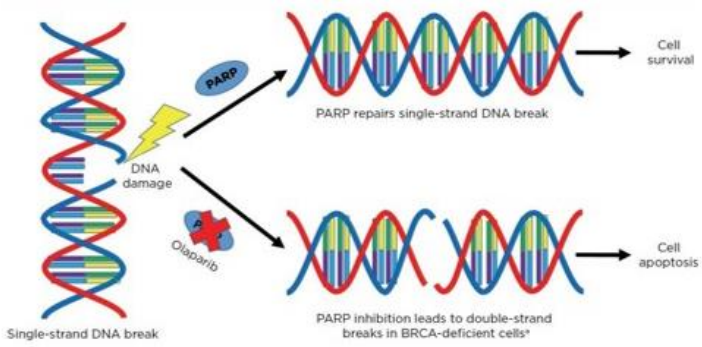

Figure 7. Mechanism of action of olaparib [104] (permission to reuse not required).

Phosphatase and Tensin Homolog (PTEN)

PTEN is a negative regulator of the PI3K/ALT signalling pathway (Figure 4) which is associated with tumorigenesis in multiple cancers including breast. Less than 5% of breast cancers occur because of a PTEN mutation, however, around 25% have significantly low levels of PTEN [105].

Association with Age

Association of PTEN with age is not clearly understood. Dean et al. [106] studied 101 breast cancers and found that PTEN loss was associated with younger age at the onset of breast cancer. Shoman et al. [107] found no association between PTEN expression and age (range 28-93 years) in 100 patients.

Predictive Value

PTEN expression is postulated to have an effect on acquired resistance to tamoxifen. Shoman et al. [107] found an association between downregulation of PTEN expression in ER-positive tumours and failure to respond to adjuvant tamoxifen.

Prognostic Value

Reduced PTEN expression has been associated with more advanced disease [107] and PTEN gene deletion has shown direct association with HER2 amplification and poorer prognosis in HER2 positive breast cancer overall [108].

A study of 49 primary breast cancer patients treated with adjuvant tamoxifen by Tanic et al. [109] found that the loss of PTEN expression was associated with shorter DFS, BCSS and OS, compared to high expression.

A meta-analysis by Li et al. [110] based on 27 studies involving 10,231 breast cancer patients found that PTEN loss was associated with worse DFS and OS, larger tumour size, lymph node metastasis and triple-negative phenotype.

Future Directions

Each stage of the downstream signalling pathway following on from PTEN (Figure 4) consists of potential therapeutic targets (Figure 4). Mammalian target of rapamycin (mTOR) is a serine/threonine protein kinase, downstream of AKT. Once activated it is responsible for limiting the proliferative signals transmitted by upstream effectors [111].

Everolimis is a rapamycin analogue that targets mTOR and was the first drug to be approved for the treatment of HR-positive/HER2-negative metastatic breast cancer which has progressed on first-line therapy with an aromatase inhibitor (AI) [112]. The Breast Cancer Trials of OraL EveROlimus-2 (BOLERO-2) trial [113] demonstrated that the addition of everolimus to exemestane (a steroidal AI) markedly prolonged PFS in HR positive, advanced breast cancer with disease recurrence following prior non-steroidal AIs (Figure 8).

An invitro study by Owusu-Brackett et al. [114] tested TAK228 (mTOR inhibitor). TAK228 enhanced the efficacy of eribulin (a chemotherapy agent) in TNBC, but further investigation is require to determine patient selection for this combination therapy.

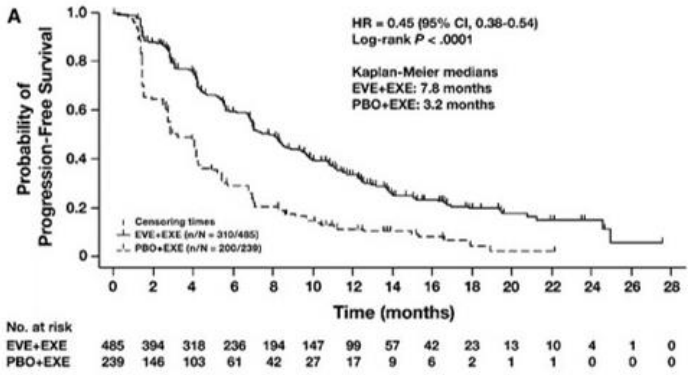

Figure 8. Kaplan-Meier estimates of progression-free survival of patients treated with everolimus plus exemestane versus exemestane alone. *CI* confidence interval, *HR* hazard ratio, *EVE* everolimus, *EXE* exemestane, *PBO* placebo [113] (permission to reuse not required).

Liver Kinase B1 (LKB1)

LKB1 is a serine-threonine kinase described in the development of Peutz Jagher's syndrome where around 45% of patients develop breast cancer in their lifetime [14], among other cancers.

Association with Age

High expression of LKB1 has been noted in older women (>70 years) compared to younger patients [6, 14].

Predictive Value

There is little in the present literature about the predictive value of LKB1 in breast cancer. In a study of older breast cancer patients undergoing surgery [14], LKB1 expression was associated with better survival outcome among patients receiving adjuvant endocrine therapy.

Prognostic Value

In the same study [14], within the older age group, LKB1 expression was associated with poor prognostic factors including high tumour grade and high expression of Ki-67 and HER2.

Contrary to this, Chen et al. [115] found that high LKB1 expression was predictive of better survival in patients with HER2-positive tumours.

Future Directions

Future studies are required to investigate and compare the expression of LKB1 in the younger compared to older patient population and delineate the precise role of LKB1 as a therapeutic target. Metformin has been shown to selectively inhibit proliferation of LKB1 positive cancers including breast cancer [116], which needs further evaluation.

Cell Proliferative Markers

Ki-67

Ki-67 is a protein that is associated with cell proliferation. It is difficult to determine the overall high expression of Ki-67 in breast cancer due to the lack of consensus over the method of detection and definition of 'high' expression [117, 118].

Association with Age

In a cohort of 462 patients with stage I-III breast cancer [119], younger age (≤40 years) was associated with high expression of Ki-67. In a cohort of 575 older women (≥70 years), low expression of Ki-67 was seen [6]. These findings suggest a less proliferative disease in older women.

Predictive Value

Measurement of Ki-67 has been utilised to monitor the tumour proliferation index in neoadjuvant settings, particularly for chemotherapy treatment. A high Ki-67 proliferation index is an independent predictor factor for pCR after neoadjuvant chemotherapy (NACT) [120].

The IMPACT trial was a neoadjuvant randomised trial evaluating adjuvant treatment with anastrozole, tamoxifen or in combination, in 330 postmenopausal patients with ER-positive breast cancer [121]. A secondary objective of the study was to evaluate changes in Ki-67 expression after two weeks of treatment. Higher expression of Ki-67 after two of endocrine therapy was associated with shorter recurrence free survival (RFS), larger tumour size at baseline and lower ER level.

Prognostic Value

A meta-analysis by Petrelli et al. [122] exploring the prognostic value of Ki-67 in early breast cancer analysed data of 64,196 patients from 41 studies. Ki-67 was an independent prognostic indicator in terms of OS and expression of >25% was associated with greater risk of death compared with lower expression rates.

A meta-analysis by de Azambuja et al. [123] identified 12,155 patients from 46 studies where Ki-67 was measured. Ki-67 positivity was associated with higher rate of relapse and worse survival in patients with early breast cancer.

Future Directions

Despite endorsements by several international guidelines, measurement of Ki-67 is yet to gain widespread application as a prognostic or predictive marker, which is mainly to do with wide variation in methodology of detection and lack of standardisation [118].

Recent studies in renal cancer have focused on Ki-67 targeted therapy but this is not yet done in breast cancer [124]; therapies targeting Ki-67 in the setting of renal carcinoma are in the preclinical stages.

Anti-Apoptosis Markers

B Cell Lymphoma 2 (BCL-2)

BCL-2 gene is the founding member of the BCL-2 family of regulator proteins that regulate cell death by either inhibiting or inducing apoptosis.

In a retrospective study of 605 cases of breast cancer, 53.8% showed BCL-2 expression [125].

Association with Age

In the same study [125], BCL-2 positive expression was associated with young age (<50 years). High expression of BCL-2 has been found in a cohort of women ≥70 years with primary breast cancer treated by surgery [6], compared to younger patients (<70 years) and also in a series of older women with TNBC [13] (≥70 years). Biological characterisation of 14,007 primary breast cancers by Daidone et al. [43] showed an increase in BCL-2 expression with age (≥65 years compared to <65 years).

Predictive Value

High BCL-2 has been linked to better clinical response to tamoxifen in ER-positive metastatic breast cancer [126].

Tamoxifen induces apoptosis in breast cancer cells. BCL-2 genes interfere with apoptosis in various ways. A study by Zhang et al. [127] investigated the competing effects of tamoxifen and BCL-2 gene products and found that tamoxifen induced down-regulation of BCL-2, thus inducing apoptosis.

Prognostic Value

A meta-analysis by Callagy et al. [128] reviewed 5,892 cases of female breast cancer where BCL-2 had been measured. They concluded that patients with high expression of BCL-2 had better OS and DFS. This has been reproduced in other studies [129, 130].

Specifically in older women, BCL-2 has been shown to correlate with better DFS and BCSS [6, 131].

Future Directions

Therapeutic compounds have been developed to attempt to disrupt the action of BCL-2, known as BH3-mimentics (Figure 9). These BCL-2

inhibitors have shown ability to restrict tumour growth in xenograft models [132] and there are a number currently under further investigation in the clinical trial setting for example, Navitoclax [133].

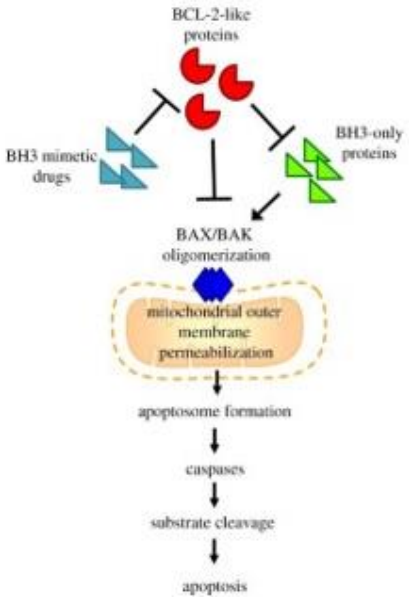

Figure 9. Action of BH3 mimetic drugs [132] (permission to reuse not required).

Cell Differentiation Markers

Cytokeratins (CKs)

Breast cancer originates from basal (myoepithelial) or luminal (glandular) tissue that express specific cytokeratins. In normal breast tissue, CK 5, CK 5/6and CK14 are expressed in myoepithelial cells and CK7, CK8, CK18, CK19, CK20 in ductal epithelium [134].

Association with Age

High expression of CK5/6, CK5, CK18 and low expression of CK7/8 has been seen in the older population of primary breast cancer patients [6], compared to younger counterparts (<70 years). In a cohort of patients with TNBC, CK7/8 positivity was lower and CK18 higher, compared to a comparative younger series [135]. A meta-analysis to determine the clinicopathological significance of CK18 in breast cancer, confirmed higher expression in the older population ≥50 years, compared to <50 years [136].

Predictive Value

There is minimal evidence in the literature to support the predictive values of cytokeratins, which encompass a large heterogeneous group of markers. In a study of CK20 in a human cell line, CK20 expression enhanced the metastatic potential of breast cancer cells and was thought to be a potential therapeutic target in tamoxifen-resistant breast cancer [137].

An experimental study by Bozionellou et al. [138] has shown that chemotherapy resistant CK19 mRNA-positive cells in the peripheral blood and bone marrow can be effectively targeted by trastuzumab administration.

Prognostic Value

A study of 611 breast cancers by van de Rijn et al. [139] found that expression of CK 17 and CK 5/6 in tumour cells was associated with poor clinical outcome. Multivariate analysis showed that in node-negative cancer, expression of CK 17 and C5/6 was a prognostic factor independent of size and grade.

A literature review by Haupt et al. [140] looked at the literature relating to basal-like breast carcinomas and confirmed that expression of basal CKs was an independent prognostic factor in lymph node negative cases, but in lymph node positive cases this was unclear. Overall, the evidence was suggestive that basal-like breast carcinomas are associated with worse clinical outcome.

Future Directions

Cytokine expression has been found to be associated with response to PET in a small cohort of older women with primary operable breast cancer [15] and these findings need validating in a larger cohort.

OTHERS

Mucin 1 (MUC1)

MUC 1 is an epithelial cell surface protein that is aberrantly overexpressed in over 90% of breast cancer as well as other cancers [141].

Association with Age

There is little in the current literature to describe the association of the expression of MUC1 with age in breast cancer. A study in older compared to younger women having surgery did not show any statistically significant difference in expression [6].

Predictive Value

In ER-positive patients, increased expression of MUC1 is associated with tamoxifen resistance [142].

Prognostic Value

Breast cancers which exhibit increased and mislocalised expression of MUC1 are more likely to metastasise [142].

High levels of MUC1 have been associated with poor clinical outcome [143-145]. Jing et al. [143] accessed expression levels of MUC1 in breast cancer and normal tissues from the Oncomine database. Abnormally high levels of MUC1 was associated with poor prognosis, with reduced FRS and disease-specific survival (DSS).

An analysis of 171 cases of invasive breast cancer by McGuckin et al. [144] showed patients whose tumours had high expression of MUC1 has

significantly poorer DFS and OS, when compared with those with lower expression.

Future Directions

MUC1 targeted therapies have been in development for the last 30 years, however effective benefit in clinical trials has not yet been achieved [146].

A monoclonal antibody GGSK-1/30 targeting human tumour-associated MUC 1 has shown promising initial results in human breast tissue analysis at determining the severity of disease [141].

Amplified in Breast Cancer 1 (AIB1)

AIB1 is a member of the steroid receptor coactivator family and mediates the activities of nuclear receptors including ER and PgR. AIB1 is overexpressed in 30-60% of breast cancers [147].

Association with Age

AIB1 expression was measured in 185 breast cancer surgical resection specimens; there was no difference noted between expression and age (≤50 years compared to >50 years) [148].

Predictive Value

In postmenopausal women with primary breast cancer, high AIB1 has been implicated as a marker of good response to tamoxifen treatment as adjuvant therapy. Furthermore, it was a prognostic marker of decreased RFS in patients who did not receive adjuvant systemic therapy [149].

Osborne et al. [150] measured AIB1 and HER2 levels in 316 breast cancer patients. In patients who received tamoxifen, high AIB1 expression was associated with worse DFS, indicative of tamoxifen resistance. Patients whose tumours expressed AIB1 and HER2 had worse outcomes with tamoxifen therapy than other combinations.

These two studies are conflicting in their findings.

Prognostic Value

The International Breast Cancer Study Group's trial BIG 1-98 randomised 1,396 Danish patients to adjuvant tamoxifen and/or AIs [151]. AIB1 expression correlated to expression of HER2 and high grade as well as poor DFS and OS.

In a study of 185 breast cancers by Lee at al [148], high AIB1 expression was associated with lower DFS and OS. These findings were most significant in the ER-negative cohort.

Future Directions

Steroid receptor coactivators such as AIB1 lack a high affinity ligand binding domain and therefore are considered difficult drug targets [152].

An AIB1 small molecule inhibitor, bufalin, has been shown to effectively inhibit tumour growth in TNBC in a mouse model [153], suggesting a potential target for TNBC treatment. Bufalin and other small molecule inhibitors are still in pre-clinical phases of investigation [152].

Other Potential Biomarkers

It is not possible to discuss every relevant biomarker in breast cancer in this chapter, of which are there many hundreds. We have described so far a number of biomarkers which are known to the authors to be relevant in ER-positive breast cancer and which are under further investigation in this setting.

Some more recent biomarkers which have been discovered to have a prognostic role in breast cancer and involvement in acquired endocrine resistance include glutaminases [18], zinc transporters [154], Cathepsin [155], cell division regulators (such as CDC20) [17] and other members of the nuclear receptor superfamily (such as RXRG) [156].

With such a plethora of literature of copious numbers of biomarkers, it can be daunting to understand how we should use this information in clinical practice. In the next section of the chapter, we will discuss high throughput measurement of biomarkers in the research and clinical settings.

HIGH THROUGHPUT MEASUREMENT OF BIOMARKERS

The development of high-throughput technologies to investigate genetic, epigenetic and proteomic changes has helped to unravel the complexity of breast cancer biology [157]. The realisation of breast tumour heterogeneity prompted the development of a molecular classification system, which constituted the first step towards the establishment of personalised medicine in breast cancer.

Concept of Tissue Microarray (TMA)

The majority of studies to date that have profiled the biology of breast cancer have used (SE specimens to do so and analyse individual patient specimens one at a time.

This creates potential bias in having not included the large group of patients who do not have surgery and furthermore, cannot provide any insight when considering neoadjuvant therapy. Performing IHC analysis on patient specimens one at a time is costly and time-consuming.

As an alternative to SE profiling, CNB, which is usually obtained at diagnosis in breast cancer patients, should be considered for the study of tumour biology. CNB samples can be obtained from all older patients diagnosed with breast cancer regardless of primary treatment. Another advantage of CNB compared to SE is that preservation of CNB tissue leads to faster penetration of the tissue by a fixative agent, resulting in less chance of enzyme degradation and thus, better preservation of biological features [158, 159].

The technique of TMA for use in breast cancer was first reported by Kononen et al. [160] in 1998. The construction of TMAs involves embedding multiple fragments of tumour tissue in a single paraffin block for the purpose of high-throughput analysis. In addition to maximising tissue resources, TMA has the advantage of facilitating analysis and evaluation of

tissue-based assays in an efficient, cost-effective and uniform condition [161]. Tissue micro-array has become standard technique to examine tissue biology in detail but primarily in SE specimens. There are many challenges faced when manipulating TMA technique to utilise CNB for profiling tumour biology, including complex construction given the small diameter of the biopsy and erosion of biopsy after sectioning for initial diagnosis. After a comprehensive systematic literature review on the subject [16], our team has successfully managed to develop an optimal technique to construct TMAs from CNB samples [15]; this gives us the unique opportunity to utilise CNB for profiling tumour biology.

We therefore suggest that CNB TMA is the optimum method for studies assessing biology in breast cancer.

Prediction Tools Available

There are a number of prediction tools currently available on the market, which utilise either measurement of tissue protein or gene assay.

Tools Based on Tissue Protein Analysis

PREDICT [9] is a validated tool to predict overall and BCSS survival for women treated with early breast cancer in the UK. It considers the following factors: age, menopausal status, ER status, HER2 status, Ki-67 status, tumour size, tumour grade, number of positive nodes. Using the Eastern Cancer Registration and Information Centre dataset, information was collated for 5,694 women who had surgery for invasive breast cancer. Breast cancer mortality models were derived from this data using the PREDICT tool and validated against an external dataset. Model calibration was good for both data sets.

Adjuvant! Online [8] was developed in patients aged ≤69 years and aids decision making regarding adjuvant therapies. The tool considers the following information: age, menopausal status, comorbidity estimate, tumour size, number of positive axillary nodes, ER status. Outcomes for OS

and DFS seen in clinical trials are reasonably modelled by Adjuvant Online! in the younger age group [8], but not for patients ages ≥65 years [162].

These tools have been developed and validated based on a collection of clinical and pathological information from large patient populations, not measurement of protein-based biomarkers from individual patients.

Tools Based on Gene Assay

There are a number Of genomic tests which analyses the activity of certain genes in breast cancer, these include Oncotype DX [10], Mammaprint [163] and EndoPredict [164]. These tools are based on personalised measurement of biomarkers in the individual patient's tumour sample.

Only Oncotype DX has been recommended for use by NICE in the UK [165]. Oncotype DX is a 21-gene assay to assist in decision making regarding adjuvant chemotherapy in patients with ER positive and HER2 negative tumours. In has been validated in a prospective trial setting [10].

FUTURE DIRECTIONS SUMMARY

There is a potential for extensive biomarker research, as we have described in this chapter, to be used in detection, treatment and measurement of recurrence risk of breast cancer. Current tools are not validated for used in older women (>70 years) and are primarily concerning adjuvant treatment. Once we have a better understanding of the complex roles and interactions of biomarkers in breast cancer there is potential for prediction tools to be employed at all stages of the treatment pathway.

In order to produce a tool which could be generalised to all patients with breast cancer, we need consensus regarding how to measure currently undefined biomarkers, such as Ki-67 and uniform agreement on definitions of high expression. Despite the ageing population and increased likelihood of ER-positive breast cancer in older women, there is a lack of research focusing on the biology of breast cancer in this cohort of patients. Increasing ER-positivity in this group is of importance at all stages of the treatment

patients ((neo)adjuvant, primary and advanced) as they are more likely to consider less aggressive alternative therapies compared to surgery and chemotherapy.

In the future, optimal personalised treatment for the individual patient with breast cancer based on the unique biological characteristics of that cancer is achievable.

CONCLUSION

ER, PgR and HER2 are biomarkers routinely measured by IHC on biopsy samples taken at diagnosis for breast cancer. Many other biomarkers are associated with predicting prognosis and response to therapy in breast cancer, but are not currently measured outside of a research setting. Differences in expression of biomarkers between younger and older women has been confirmed for ER, HER2, Tp53 and cytokeratins. The mechanism of endocrine resistance is complex and biomarkers found to be associated with this are HER3, HER4, EGFR, Tp53, BRCA1, PTEN, BCL-2, MUC1 and AIB1. The biomarkers ER, PgR, HER2, HER4, EGFR, VEGF, Tp53, PTEN, Ki-67, BCL-2, cytokeratins, MUC1 and AIB1 have been shown to have an association with survival. Once we have a better understanding of the complex roles and interactions of biomarkers in breast cancer, there is potential for prediction tools to be developed and employed at all stages of the treatment pathway [166].

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