Original paper

Correlation of Ki-67 proliferative index with oncotype DX recurrence score in hormone receptor-positive, human epidermal growth factor receptor 2-negative early breast cancer with low-burden axillary nodal disease – a review of 137 cases

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The use of chemotherapy in breast cancer management has significantly contributed to the decrease in its mortality. Currently, the prognosis is determined by molecular biomarkers, such as oestrogen receptors, and human epidermal growth factor receptor 2. However, the increasing use of advanced molecular technologies, including oncotype DX recurrence score (ODX-RS), has provided the ability to estimate the risk of recurrence. Research has demonstrated that the ODX-RS helps to predict recurrence risk and the potential benefit of chemotherapy in breast cancer. As a result, it can assist clinicians in making decisions regarding using the chemotherapy. The goal of work is to explore the correlation between the ODX-RS and Ki-67 proliferative index (Ki-67-PI).

This study included 137 patients with oestrogen positive, human epidermal growth factor receptor 2-negative early breast cancer, and had non- or early axillary disease. Patients with low Ki-67-PI were as follows: low ODX-RS in 17%, intermediate ODX-RS in 80%, and high ODX-RS in 2%. In the high Ki-67-PI group: low ODX-RS in 12%, intermediate ODX-RS in 48%, and high ODX-RS in 40%.

In conclusion, the results show no significant correlation between the ODX-RS and Ki-67-PI ($r = 0.511$, p-value < 0.9).

Key words: chemotherapy, breast cancer, radiotherapy, oncotype DX recurrence score, oestrogen receptors, Ki-67 proliferative index.

Introduction

About 75% of breast cancers fall into the oestrogen receptor-positive (ER^+), human epidermal growth factor receptor 2-negative ($HER2^-$) phenotype category [1]. Radical surgical treatment is currently considered the gold standard for early breast cancer management. However, the prognosis and recurrence patterns can vary significantly among survivors of the disease. The use of adjuvant hormonal manipulation therapy (HMT) in addition to adjuvant chemotherapy has led to a reduction in both local and distant disease recurrence rates as well as associated mortality. However, chemotherapy itself is not without adverse effects and costs, and not all patients will derive equal benefit from it. Traditional

tumour biomarkers, such as tumour size, histological grade, lymph node metastasis, ER expression, Ki-67 proliferative index (Ki-67-PI), and HER2 status, vary in their reliability as predictors of recurrence risk in early breast cancer. To better assess the benefit of adjuvant chemotherapy, several prognostic multigene expression assays have been developed, including RecurIndex, EndoPredict, and oncotype DX recurrence score (ODX-RS) [2]. These assays aim to estimate the risk of breast cancer recurrence after radical surgery, taking into account HMT, and help determine the necessity of adjuvant chemotherapy [3]. The association of Ki-67-PI with breast cancer prognosis and its response to treatment has been investigated. However, the currently available evidence is insufficient to recommend the routine use of Ki-67-PI as a prognostic factor and predictor of responsiveness to adjuvant chemotherapy in patients with early-stage breast cancer [4]. Additionally, the relationship between Ki-67-PI and ODX-RS remains unclear. Some authors have reported a moderate agreement between high Ki-67-PI and high ODX-RS values [5]. The primary aim of the study was to assess the correlation between ODX-RS and Ki-67-PI in the study population. Furthermore, the study aimed to assess the impact of incorporating ODX-RS into the decision-making process for adjuvant chemotherapy planning following primary breast cancer surgery.

Material and methods

The study cohort included 137 female patients with early breast cancer who had ER^+, $HER2^-$ tumours. Both Ki-67-PI and ODX-RS tests were performed on these patients between 2016 and 2022. All patients in the study group underwent breast-conserving surgery or mastectomy, along with axillary surgery, as appropriate. The enrolled patients had luminal-type breast cancer (ER+/$HER2^-$) and were classified as either node-negative (N0) or having low-burden axillary nodal disease with 1–3 positive nodes (N1) (Fig. 1). Certain exclusion criteria were applied, including tumours classified as T4, HER2+ or triple-negative breast cancer, patients with nodal disease classified as ≥ N2, and those with upfront endocrine or chemotherapy treatment or distant metastasis at initial presentation. The oestrogen receptor/progesterone receptor (PR) positivity was defined using the Quick Score, which measures immunohistochemistry (IHC) staining with a threshold set at > 4. The nodal status assessment was based on postoperative histopathology reports. The immunohistochemistry staining is done utilising Ki-67 (MM1 clone) monoclonal antibody (a mouse anti-human monoclonal antibody) in sections of formalin-fixed paraffin-embedded tumour tissue. We used the International Ki-67 in Breast Cancer Working Group

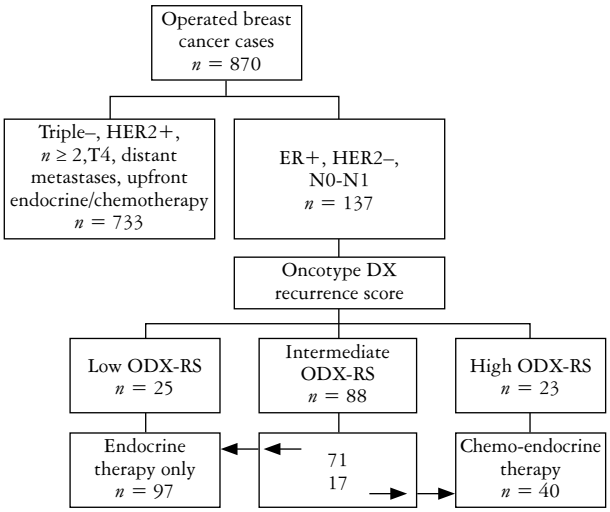

Fig. 1. Oncotype DX recurrence score testing pathway

ER – oestrogen receptor, HER – human epidermal growth factor receptor, N0 – node-negative, N1 – node-positive, ODX-RS – Oncotype DX recurrence score

(IKWG) recommended standardised visual counting using light microscopy or from a digital image method. An initial assessment performed by eyeballing the slide, selecting the most active area to cells with nuclear positivity, is carried out. Then the total number of positive-staining tumour cells in each image/field is visually counted followed by counting the total number of tumour cells in each image/field, counting a minimum of 500 such cells. A simple calculation of the Ki-67 index is done as follows: number of positive tumour cells/total number of tumour cells × 100. The Ki-67 proliferative index is reported in percentage format. The Ki-67-PI results were then categorised into 3 groups based on the guidelines provided by IKWG. The categories were defined as follows: low (≤ 5%), intermediate (6–29%), and high Ki-67-PI (≥ 30%). To investigate the relationship between Ki-67-PI and ODX-RS, the correlation between these 2 variables was assessed. The oncotype DX assay is conducted centrally in the clinical reference laboratory of Genomic Health, Inc., located in Redwood City, California. It is specifically optimised for formalin-fixed, paraffin-embedded resected tumour samples. The assay evaluates the activity of 16 cancer-related genes and 5 control genes using polymerase chain reaction in the excised breast cancer tissue (Table 1). The oncotype DX recurrence score is generated by an automated algorithm. It assigns a score to the expression of the 16 cancer-related genes, multiplied by a factor specific to each gene's assigned group. This score is then compared to the mean expression of 5 reference genes to calculate the overall ODX-RS. The oncotype DX recurrence score is presented as a value ranging 0–100, indicating the disease recurrence risk. In cases of multifocal/multi-centric disease, each lesion is individually tested, and the highest ODX-RS score among the lesions is considered. The cohort in this study was di-

Table I. Genes included in oncotype DX recurrence score assay [2]

Gene	Gene function	Scoring group	Multiplication factor
Grb7	Signalling protein recruited to various tyrosine kinases, including HER2/neu	HER2	0.47
HER2	Growth factor receptor. Overexpression leads to conversion to an oncogene		0.47
ER	Membrane receptors which can modify intracellular signalling	Oestrogen	–0.34
PR	Membrane receptors which can modify intracellular signalling		–0.34
BCL-2	Anti-apoptotic oncogene		–0.34
SCUBE2	Glycoprotein with a role in sonic hedgehog pathway signalling		–0.34
Ki-67	Proliferation marker	Proliferation	1.04
STK15	Stabilization of chromosomes during mitotic segregation		1.04
Survivin	Inhibition of apoptosis		1.04
Cyclin B1	Component of maturation-promoting factor, stimulation of the M phase of the cell cycle		1.04
MYBL2	Cell cycle progression		1.04
MMP11	Encodes stomelysin 3, important in tissue remodelling	Invasion	0.1
CTSL2	Encodes cathepsin L2, stimulates hydrogen peroxide production		0.1
CD68	Upregulated in breast cancer lines that have a high capacity to metastasize to bone		0.05
GSTM1	Glutathione S-transferase, detoxification		–0.08
BAG1	BCL-2-associated athanogene; enhances the anti-apoptotic effects of BCL-2		–0.07
B-actin	Cytoskeletal actin, important for cell motility and structure	Reference gene	1
GAPDH	Carbohydrate metabolism		1
RPLPO	Encodes components of ribosomal 60S subunit		1
GUS	β-glucuronidase		1
TFRC	Transferrin receptor		

vided into 3 risk groups: low-risk (ODX-RS < 11), intermediate-risk (ODX-RS 11–25), and high-risk (ODX-RS > 25), following the categorisation used in the TAILORx study and the National Comprehensive Cancer Network guidelines. Demographic and clinicopathological data were obtained from the patients' clinical records. The recommendations of the multidisciplinary team regarding adjuvant endocrine treatment and adjuvant chemotherapy were also analysed. Statistical analysis was performed using Microsoft Excel 2010, with continuous variables presented as mean and standard deviation. Student's *t*-test and the χ^2 test were used to compare categorical data. A *p-v*alue of ≤ 0.05 was considered statistically significant for all analyses.

Results

The cohort included 137 female patients with an age range 34–78 years. The mean age at diagnosis was 56 years (SD ±11). The patients' demographic data, tumour characteristics, and management details are demonstrated in Table II. In terms of age distribution, 40% of the patients were 50 years of age or younger, 45% were between the ages of 51 and 70 years, and 15% were over 70 years old. Regarding ODX-RS classification, 19% of the patients had a low-risk ODX-RS, 64% had an intermediate-risk ODX-RS, and 17% had a high-risk ODX-RS. Most of the lesions (74%) were T2 tumours, with sizes ranging from > 20 to ≤ 50 mm. T1 tumours, with sizes ≤ 20 mm, accounted for 18% of cases, while T3 tumours, with sizes > 50 mm, were observed in only 7% of cases. Tumour grade analysis showed that 73% of the tumours were grade 2, followed by grade 3 in 23% of cases, and grade 1 in only 4% of cases. Regarding pathomorphology, most cases (78%) were classified as invasive breast carcinoma of no special type (formerly known as invasive ductal carcinoma). Invasive lobular carcinoma was encountered in 14%

Table II. Patient and tumour characteristics based on categorised oncotype DX recurrence score

Parameters	All patients, $n = 137$	RS < 11, $n = 26$	RS 11–25, $n = 88$	RS > 25, $n = 23$	*p*-value (low/intermediate RS vs. high RS)
Mean age (SD) (years)	56 (11)	56 (13)	55 (10)	55 (11)	0.57
Age category, *n* (%)					
≤ 50 years	55 (40)	10 (40)	38 (43)	07 (29)	
> 50 to ≤ 70 years	62 (45)	08 (32)	41 (47)	13 (54)	
> 70 years	20 (15)	07 (28)	09 (10)	04 (17)	
The largest tumour size mean (SD) [mm]	30 (12)	34 (16)	29 (12)	32 (9)	
Histological tumour size category, *n* (%)					
T1: ≤ 20 mm	25 (18)	4 (16)	19 (21)	2 (9)	
T2: > 20 to ≤ 50 mm	101 (74)	16 (64)	65 (73)	20 (87)	
T3: > 50 mm	11 (7.3	5 (20)	5 (6)	1 (4)	
Surgical procedure, *n* (%)					
WLE + SLNB	59 (43)	7 (28)	42 (47)	10 (43)	
WLE + ALNC	12 (9)	3 (12)	9 (10)	1 (4)	
Mx + SLNB	53 (38)	13 (52)	34 (38)	6 (26)	
Mx + ALNC	13 (9)	2 (8)	5 (6)	6 (26)	
Tumour histology, *n* (%)					
IBC, NST	107 (78)	19 (73)	67 (85)	21 (95)	
ILC	19 (14)	1 (4)	18 (23)	0	
Mixed	5 (3.6)	2 (8)	3 (4)	0	
Mucinous	4 (3)	3(12)	1 (1)	0	
Papillary	2 (1.5)	1 (4)	0	1 (5)	
Tumour grade category, *n* (%)					
Grade I	6 (4)	1 (4)	5 (5)	0	
Grade II	100 (73)	22 (88)	70 (79)	8 (35)	
Grade III	31 (23)	2 (8)	14 (16)	15 (65)	
Nodal status, *n* (%)					
N0	102 (74)	19 (79)	68 (76)	15 (65)	
Nmic	9 (7)	1 (4)	6 (7)	2 (9)	
N1 (1–3)	25 (18)	4 (17)	15 (17)	6 (26)	
N2 (4–9)	1 (0.7)	0	1 (1)	0	
N3 (≥ 10)	0	0	0	0	

Table II. Cont.

Parameters	All patients, $N = 137$	RS < 11, $N = 26$	RS 11–25, $N = 88$	RS > 25, $N = 23$	*p*-value (low/intermediate RS vs. high RS)
Ki-67-PI, *n* (%)					
Low (≤ 5%)	46 (34)	8 (32)	37 (42)	1 (4)	0.9
Intermediate (6–29%)	49 (36)	12 (48)	32 (36)	5 (22)	
High (≥ 30%)	42 (30)	5 (20)	20 (22)	17 (74)	
Lymphovascular invasion, *n* (%)					
Present	44 (32)	14 (58)	22 (24)	8 (35)	0.19
Not present	93 (68)	10 (42)	68 (76)	15 (65)	
Adjuvant endocrine therapy	97 (71)	25 (100)	72 (81)	0	0.46
Adjuvant Chemo-endocrine therapy	40 (29)	0	17 (19)	23 (100)	
Adjuvant radiotherapy, *n* (%)					
Adjuvant radiotherapy given	84 (61)	14 (58)	56 (62)	14 (64)	0.01
Adjuvant radiotherapy not given	53 (39)	10 (42)	35 (38)	8 (36)	

ALNC – axillary lymph node clearance, IBC – invasive breast cancer, ILC – invasive lobular carcinoma, Mx – mastectomy, NST – no special type, SLNB – sentinel lymph node biopsy, WLE – wide local excision

of cases, and the mixed subtype was present in 3.6% of cases. In regard to the Ki-67-PI, 34% of the cases had a level of ≤ 5%, more than 80% of this group had intermediate-risk ODX-RS (11–25), 17% low-risk ODX-RS (0–10), and only 2% had high-risk ODX-RS (26-100). Around 36% of the cohort with Ki-67-PI were intermediate level (6–29%), 65% of them had intermediate-risk ODX-RS, and only 10% had high-risk ODX-RS. The group with high Ki-67-PI (≥ 30%) comprised 30% of the cohort, 12% of the group had low-risk ODX-RS, 48% had intermediate-risk ODX-RS, and 40% had high-risk ODX-RS. The statistical analysis revealed that there is no significant correlation between ODX-RS and Ki-67-PI ($r = 0.511$, p-value < 0.9) (Fig. 2, 3). Among T2 tumours, the most frequent lesions, 87% had high-risk ODX-RS, and 73% had intermediate-risk ODX-RS (Fig. 4). The low-risk ODX-RS group had the largest mean tumour size and the highest number of mucinous carcinoma cases (75%). The high-risk ODX-RS group constituted approximately 20% of invasive breast carcinoma of no special type (IBC, NST) lesions, 50% of grade 3 tumours, and about 40% of cases with Ki-67-PI ≥ 30%. In terms of surgical procedures, 43% of the cohort underwent wide local excision with sentinel lymph node biopsy (WLE + SLNB), while mastectomy with sentinel lymph node biopsy (Mx + SLNB) was performed in 38% of cases. Most of the cases (74%) were node negative (N0), 7% had lymph node micrometastases, and 18% were classified as N1 disease (Fig. 5). Lymphovascular invasion was present in 32% of cases, with 50% of this group having an intermediate-risk ODX-RS and only 18% having a high-risk ODX-RS. In terms of adjuvant treatment, most patients with intermediate-risk ODX-RS received HMT only (81%). In contrast, all patients in the high-risk ODX-RS group received both endocrine therapy and chemotherapy. Patients in the low-risk ODX-RS group received HMT only (Fig. 6). Radiotherapy was administered to 58% of the patients in the low-risk ODX-RS group, 62% of the patients in the intermediate-risk ODX-RS group, and 64% of the patients in the high-risk ODX-RS group.

Discussion

The GLOBOCAN (World Health Organisation Global Cancer Institute) database reports reveal that breast cancer has become the most commonly diagnosed cancer globally, surpassing lung cancer. Unfortunately, one in every 8 women of reproductive age and beyond is diagnosed with breast cancer. In 2020, there were approximately 2.3 million new cases of breast cancer in both genders (11.7%). This was followed by lung cancer (11.4%), colorectal cancer (10.0%), prostate cancer (7.3%), and stomach cancer

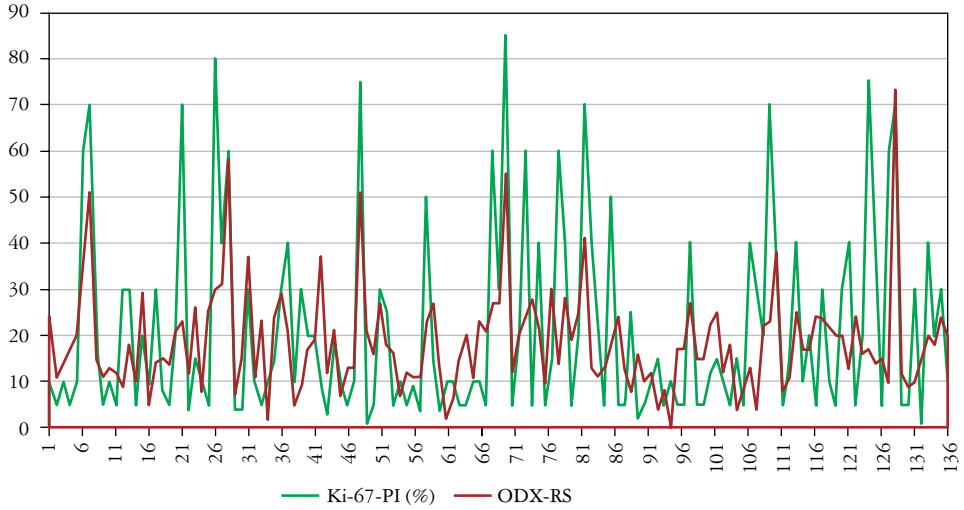

Fig. 2. Ki-67 proliferative index and oncotype DX recurrence score in each patient
Ki-67-PI – Ki-67 proliferative index, ODX-RS – Oncotype DX recurrence score

(5.6%) [6]. Breast cancer represents a significant portion of female cancer cases, accounting for one-quarter of all cases. It continues to have a substantial impact on cancer-related mortality worldwide, with an estimated 685,000 deaths attributed to breast cancer, representing 30% of all cancer-related deaths. This translates to approximately one in every 6 cancer-related female deaths [7, 8]. In the UK, there are around 56,000 new cases of breast cancer each year, averaging more than 150 new cases *per* day. Breast cancer is the most common cancer in both genders, accounting for 15% of all new cancer cases. It is followed by prostate cancer (14%) and lung cancer (13%). Bowel cancer ranks as the fourth most frequent cancer in the UK, accounting for 11% of all newly diagnosed cases [8, 9]. Globally, lung cancer remains the leading cause of cancer-related deaths,

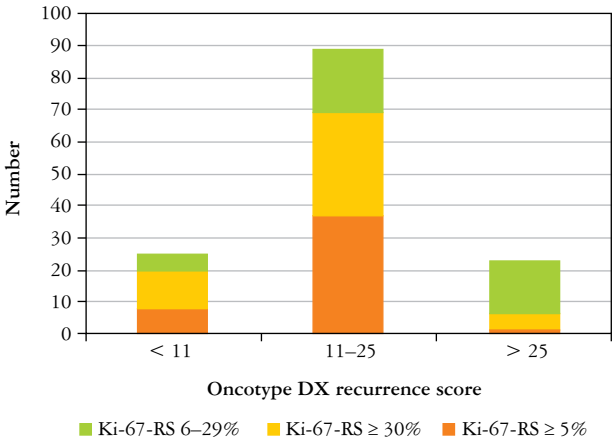

Fig. 3. Ki-67 proliferative index distribution in the categorised groups of oncotype DX recurrence score
RS – recurrence score

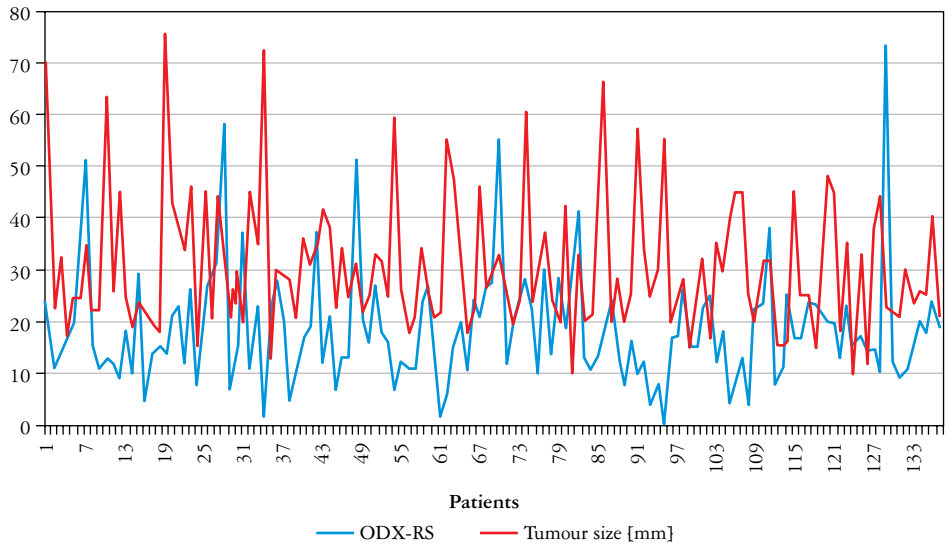

Fig. 4. Tumour size and oncotype DX recurrence score in each patient
ODX-RS – Oncotype DX recurrence score

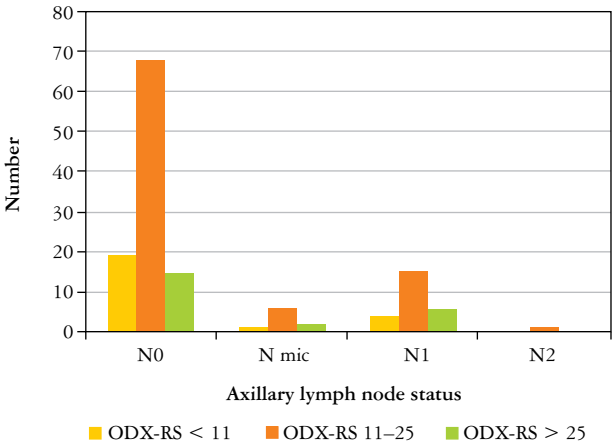

Fig. 5. Axillary lymph node status in the categorised groups of oncotype DX recurrence score
N – node, ODX-RS – Oncotype DX recurrence score

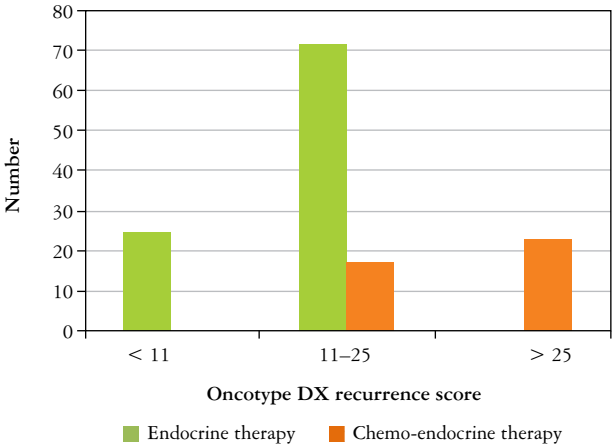

Fig. 6. Impact of oncotype DX recurrence score on adjuvant therapy decision making in 137 early breast cancer cases

with 1.8 million deaths (18%). This is followed by colorectal cancer (9.4%), liver cancer (8.3%), stomach cancer (7.7%), and female breast cancer (6.9%) [6]. Breast cancer poses a significant burden on the global population, particularly among individuals aged 50 years and older. More than 70% of all newly diagnosed cases and 81% of all deaths related to breast cancer occur in this age group. Unfortunately, transitioning countries bear a disproportionate share of breast cancer deaths [7]. In the UK, lung cancer is the most common cause of cancer-related deaths, accounting for 21% of all cancer deaths. Bowel cancer ranks second, responsible for 10% of all cancer deaths. Prostate cancer is the third leading cause of cancer mortality in the UK, with a rate of 7.3%. Breast cancer is recognised as the fourth most common cause of cancer death in the UK, with around 11,500 deaths occurring each year, equivalent to 32 deaths every day. Breast cancer accounts for 6.8% of all cancer deaths in the UK [8, 9]. The incidence and mortality rates of newly diagnosed breast cancer vary significantly across different regions and age categories. In Middle Africa, postmenopausal women account for 43% of new cases and 49% of breast cancer-related deaths. In Northern America, Western Europe, and Northern Europe, these figures increase to 80% of new cases and 90% of breast cancer-related deaths occurring in the postmenopausal age group [7, 10]. Western countries continue to exhibit the highest age-standardised incidence rates of breast cancer, with rates exceeding 30 *per* 100,000 for premenopausal women and exceeding 300 *per* 100,000 for postmenopausal women. The highest breast cancer-related mortality rates in the premenopausal age group were reported in Melanesia, Middle Africa, and Western Africa (8.2–9.8 *per* 100,000), while the lowest rates were observed in New Zealand and Australia (2.9 *per* 100,000) [7, 10].

The distribution of Ki-67 protein within cells varies during different stages of the cell cycle. It is strongly expressed in proliferating cells during the late G1, S, G2, and M phases, which are active phases of the cell cycle. However, its expression is not detected at the same level during the G0 and early G1 phases, which are resting or quiescent phases of the cell cycle. Ki-67 plays roles in both the interphase and mitotic stages of the cell cycle. During interphase, it is crucial for maintaining the normal cellular distribution of heterochromatin antigens and their association with the nucleolus. During mitosis, Ki-67 is involved in the formation of the perichromosomal layer, which is a ribonucleoprotein sheath that coats the condensed chromosomes. In this context, Ki-67 prevents the aggregation of mitotic chromosomes, contributing to proper chromosome segregation [11]. Ki-67, as a marker of cell proliferation, plays a role in the management of malignant diseases due to its significantly higher expression in tumour cells compared to normal cells [12]. Several studies have shown the association between Ki-67-PI expression and the response to systemic cancer treatments, as well as prognosis, in both the neoadjuvant and adjuvant settings [13]. Some authors have suggested that Ki-67-PI may be used as a prognostic indicator in ER+ early breast cancer [14]. However, its use as a predictive measure of adjuvant chemotherapy benefit is not currently recommended in clinical practice. This is due to limitations such as variability in laboratory methods for measuring Ki-67-PI and the use of different cut-off points, which hinder its consistent applicability [5]. The 2013 St. Gallen Consensus Conference in 2013 recommended utilisation of Ki-67-PI in categorising $ER^+/HER2^-$ early breast cancer into Luminal A and Luminal B subtypes [15].

The Consensus Conference proposed classifying breast cancer into 5 subgroups:

1. Luminal A: ER$^+$, PR$^+$, HER2$^-$, and low Ki-67-PI;
2. Luminal B (HER2$^-$): ER$^+$, HER2$^-$, and either high Ki-67-PI or low PR expression;
3. Luminal B-like (HER2$^+$): ER$^+$, HER2 overexpressed or amplified, any Ki-67-PI, and any PR;
4. HER2-enriched: HER2 overexpressed or amplified; ER and PR$^-$;
5. Triple-negative: ER and PR absent and HER2$^-$ [12, 15].

One of the recent significant advancements in the medical treatment of ER+, HER2$^-$ early breast cancer is the approval of cyclin-dependent kinase 4 (CDK4) and cyclin-dependent kinase 6 (CDK6) inhibitors, such as abemaciclib, in combination with adjuvant HMT [16, 17]. Cyclin-dependent kinase 4 is an enzyme encoded by the *CDK4* gene and is a critical component of the protein kinase complex involved in the G1 phase of the cell cycle. Abemaciclib is a selective small-molecule inhibitor of CDK4 and CDK6. It is 14 times more potent against CDK4 than CDK6 in enzymatic assays. In the United States, the current recommendations advise the use of abemaciclib for patients with ER$^+$, HER2$^-$, node-positive disease who are at high risk of recurrence and have a Ki-67-PI (measured by a US Food and Drug Administration [FDA]-approved test) of ≥ 20% [16, 17]. The USA FDA has approved the above recommendations for breast cancer cases where the Ki-67 has been tested using the Ki-67 IHC MIB-1 pharmDx kit, which is an IHC test. This was based on the findings of the monarchE study, which investigated the use of endocrine therapy with or without abemaciclib (LY2835219) following surgery in participants with breast cancer. In a study involving 5637 randomly assigned patients, a cohort of 323 invasive disease-free survival (IDFS) events were observed in the intent-to-treat group. After a median observation period of

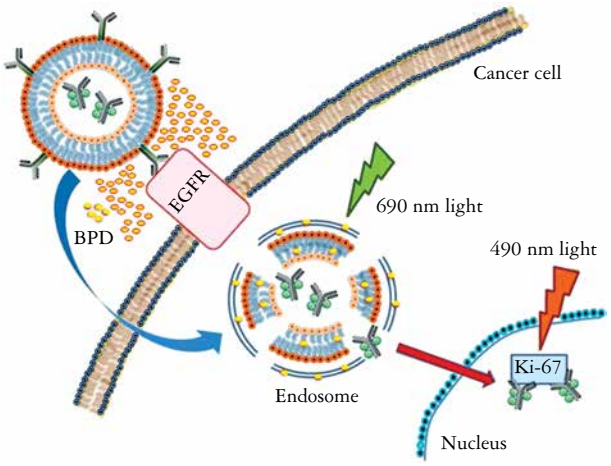

Fig. 7. Dually targeted strategy against the membrane protein epidermal growth factor receptor and the nuclear protein Ki- 67 [19]
BPD – benzoporphyrin derivatives, eGFR – epidermal growth factor receptor

15.5 months, the addition of abemaciclib to HMT demonstrated superior IDFS compared to HMT alone ($p = 0.01$; HR: 0.75; 95% CI: 0.60–0.93). The 2-year IDFS rates were 92.2% in the abemaciclib group vs. 88.7% in the HMT alone group, resulting in an absolute improvement of 3.5% at 2 years [16, 17]. This study has demonstrated that the CDK4 and CDK6 inhibitor abemaciclib, when added to standard adjuvant HMT, significantly improves IDFS in patients with ER$^+$, HER2$^-$, node-positive early breast cancer at high risk of early recurrence [17]. It has been reported that the nuclear protein Ki-67 can trigger cell death after light inactivation using the tubulin β-9 chain antibody (TUBB-9). In 2016, Wang *et al*. published a paper demonstrating a dual targeting approach for the selective and efficient light-controlled killing of cells expressing both epidermal growth factor receptor (eGFR) and Ki-67. Erbitux is an eGFR

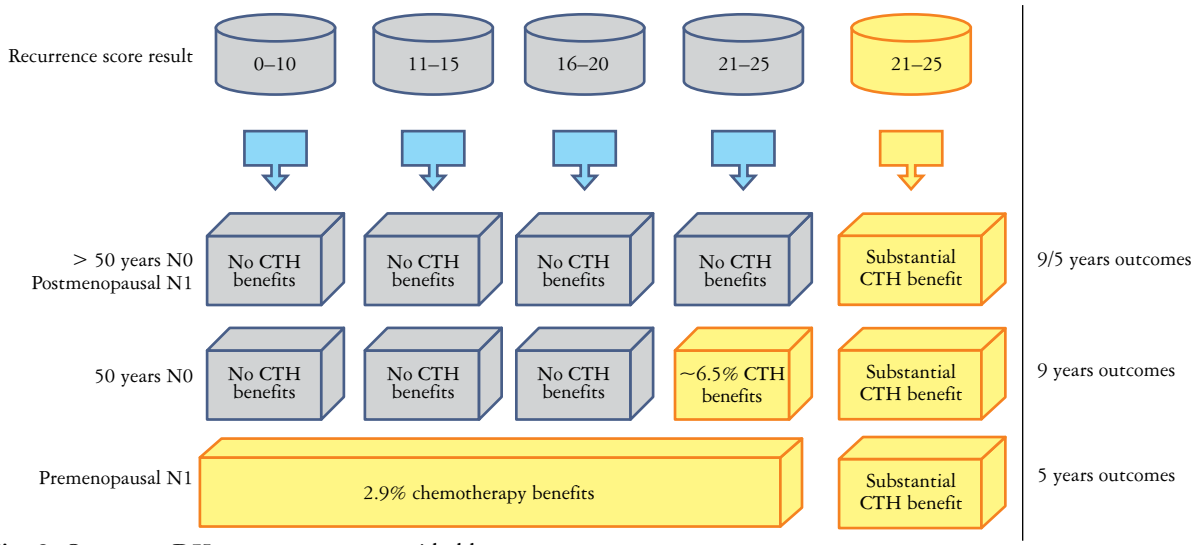

Fig. 8. Oncotype DX recurrence score guided breast cancer management
CTH – chemotherapy

monoclonal antibody clinically indicated for the treatment of metastatic head and neck cancer as well as colorectal cancer [18, 19]. In the context of targeted cancer therapy, the covalently linked eGFR inhibitor Erbitux complexed with liposomes allows eGFR-positive tumour cells pre-loaded with the photoactive dye benzoporphyrin derivatives to selectively uptake the fluorescein isothiocyanate-labelled Ki-67 antibody TUBB-9 [20–22]. This indicates the promising use of Ki-67 as a site for targeted cancer therapy (Fig. 7). The standard treatment for early breast cancer typically involves surgical tumour resection, which may be accompanied by axillary lymph node surgery if necessary. In addition to surgery, systemic adjuvant treatments are commonly employed to reduce the risk of distant recurrence and improve breast cancer-specific survival. These treatments include HMT and chemotherapy. The probability of overexpression of certain genes related to breast cancer can potentially characterise the risk of disease recurrence as well as distant metastasis. Multi-gene panel testing is utilised to personalise early breast cancer adjuvant systemic treatment. These tests include various gene panels, such as the 21-gene assay (ODX-RS), the 70-gene panel (MammaPrint), the 50-gene panel (Prosigna), the 12-gene panel (EndoPredict), and the 7-gene panel (Breast Cancer Index). In the 21-gene assay, the quantitative analysis of gene expression involves the measurement of 5 reference genes (*ACTB*, *TFRC*, *GAPDH*, *GUSB*, and *RPLP0*) and 16 cancer-related genes (Table 1). These cancer genes include those coding for HER2 (HER2 and GRB7), ER-related genes (*ER*, *PGR*, *BCL2*, and *SCUBE2*), proliferation-related genes (*MKI-67*, *STK15*, BIRC5, *CCNB1*, and *MYBL2*), and invasion-related genes (*MMP11* and *CTSL2*), as well as *GSTM1*, *CD68*, and *BAG1* [23, 24]. A 21-gene assay is a valuable tool in predicting the risk of distant breast cancer recurrence and determining the potential benefit of adjuvant chemotherapy in addition to endocrine therapy after surgery. It serves as an established prognostic indicator for malignant tumours and has been extensively studied in clinical research [23–29]. This can aid clinicians in planning personalised management plans for patients with early-stage breast cancer (Fig. 8). The utilisation of genomic assay tests in early breast cancer patients is crucial to avoiding the administration of adjuvant chemotherapy with negligible benefit. Without these tests, some patients may receive chemotherapy unnecessarily, exposing them to potentially harmful side effects. On the other hand, patients who require chemotherapy but do not receive it may be at an increased risk of recurrence; additionally, utilising genomic assay tests improves cost-effectiveness and ensures better utilisation of healthcare resources [1, 24]. The West German Study Group Phase III Plan B Trial report, published in 2016, demonstrated the use of ODX-RS to identify a subgroup of patients who could safely omit adjuvant chemotherapy and receive HMT alone. The study involved 3198 patients with a median age of 56 years, of whom 32.5 had grade 3 tumours and 41% had node-positive disease. Adjuvant chemotherapy was omitted in 15.3% of patients with an ODX-RS ≤ 11. After a median follow-up period of 35 months, the 3-year disease-free survival (DFS) was 98% for patients with an ODX-RS ≤ 11 who received HMT only. The disease-free survival was also 98% for patients with an ODX-RS between 12 and 25 who received adjuvant chemotherapy and 92% for patients with an ODX-RS > 25 who received adjuvant chemotherapy. The study found that ODX-RS, along with traditional biomarkers such as ER status, tumour size, lymph node involvement, tumour grade, and the Ki-67 protein, were significant univariate prognostic factors for DFS. The same report revealed that ODX-RS was positively but moderately correlated with the Ki-67 protein encoded by the *MKI67* gene and tumour grade. However, it was negatively correlated with PR and ER expression. The study concluded that patients with 1–3 node involvement and high traditional biomarkers who omitted adjuvant chemotherapy based on an ODX-RS ≤ 11 had excellent 3-year DFS. In addition, the observed correlation between ODX-RS and survival rate supports the findings of other retrospective studies. Another important finding from the authors is that the clinical outcome results, as well as the prospective results of the TAILORx trial low-risk arm published in 2015, suggest that patients with a low ODX-RS ≤ 11 derive no clinical benefit from the adjuvant chemotherapy [24, 25]. In the REHAB study conducted in our institution, ODX-RS altered treatment recommendations in 29% of patients. In the REHAB study, which is limited by a relatively small sample size, it was found that 16% of patients were initially recommended for chemo-endocrine therapy without considering their ODX-RS results. However, when ODX-RS was utilised, it resulted in de-escalating treatment for 65% of patients from the initial chemotherapy group to hormonal therapy only. Additionally, 14% of patients who would have initially received adjuvant endocrine therapy had chemotherapy added based on their ODX-RS results. On the other hand, before ODX-RS utilisation, HMT alone was recommended for 84% of the cohort. However, after ODX-RS results, 28% of these patients had their treatment changed to chemo-endocrine therapy [24]. In the current study, most patients had a low or intermediate ODX-RS score (83%), and 71% of the cohort did not receive chemotherapy (Fig. 1). Patients with high-risk ODX-RS scores received chemo-endocrine therapy, and only 20% of those with intermediate ODX-RS scores received this treatment. All patients with low-risk ODX-RS scores did not receive chemotherapy.

The study also found that the concordance rate between Ki-67-PI and ODX-RS was low. Only 4% of cases showed intermediate Ki-67-PI with high ODX-RS, and 12% of cases showed high Ki-67-PI with high ODX-RS. Conversely, a minimal number of cases with high-risk ODX-RS scores showed low Ki-67-PI expression. In 2018, Tan *et al*. from Wahroonga in Australia analysed results of 58 luminal-type node-negative early breast cancer (T1-2, N0-1mi, M0, ER^+, $HER2^-$) patients and found that Ki-67-PI and conventional prognostic markers do not correlate with ODX-RS [30]. Durrani *et al*., in their research conducted in Saudi Arabia in 2021, analysed the data of 156 early breast cancer patients; the study revealed that age, size of the tumour, Ki-67-PI, and axillary nodal status did not have a statistically significant impact on ODX-RS in the targeted patient population [31]. In another larger study in Mount Sinai/Tisch Cancer Institute-New York, Patel *et al*. in 2022 published a paper looking at the relationship between Ki-67-PI and ODX-RS in 525 patients with hormone-positive breast cancer. In this study there was no significant correlation between Ki-67-PI and ODX-RS in the overall cohort population, and there was fair agreement between high Ki-67 and high ODX-RS values [5]. Conversely, Copur *et al*. from Morrison Cancer Centre in the USA in 2022 presented results of 43 postmenopausal early stage 0–3 node/hormone-positive breast cancer patients, and their data support a linear, statistically significant, positive correlation between Ki-67-PI and ODX-RS (Pearson correlation coefficient $= 0.49, p\text{-value} < 0.001$) [32]. Looking to the results from this study, we can accept that no significant correlation exists between ODX-RS and Ki-67-PI ($r = 0.511, p\text{-value} < 0.9$); this observation is supported by the majority of the published studies.

Conclusions

The oncotype DX recurrence score is a reliable tool for de-escalating breast cancer management from chemo-endocrine therapy to hormonal therapy alone. In addition, it is reliable in determining prognosis and assisting in making chemotherapy decisions for patients who would otherwise only receive adjuvant endocrine therapy. Although our study is limited by a relatively small cohort, it demonstrates that there is no significant correlation between ODX-RS and Ki-67-PI.

The authors declare no conflict of interest.

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