Characteristics and prognostic significance of polo-like kinase-1 (PLK1) expression in breast cancer

Aim: Polo-like kinase-1 (PLK1) plays a crucial role in cell cycle progression, and it is considered a potential therapeutic target in many cancers. Although the role of PLK1 is well established in triple-negative breast cancer (TNBC) as an oncogene, its role in luminal BC is still controversial. In this study, we aimed to evaluate the prognostic and predictive role of PLK1 in BC and its molecular subtypes.

Methods: A large BC cohort (n = 1208) were immunohistochemically stained for PLK1. The association with clinicopathological, molecular subtypes, and survival data was analysed. PLK1 mRNA was evaluated in the publicly available datasets (n = 6774), including The Cancer Genome Atlas and the Kaplan-Meier Plotter tool.

Results: 20% of the study cohort showed high cytoplasmic PLK1 expression. High PLK1 expression was significantly associated with a better outcome in the whole cohort, luminal BC. In contrast, high PLK1 expression was associated with a poor outcome in TNBC. Multivariate analyses indicated that high PLK1 expression is independently associated with longer survival in luminal BC, and in poorer prognosis in TNBC. At the mRNA levels, PLK1 expression was associated with short survival in TNBC consistent with the protein expression. However, in luminal BC, its prognostic value significantly varies between cohorts.

Conclusion: The prognostic role of PLK1 in BC is molecular subtype-dependent. As PLK1 inhibitors are introduced to clinical trials for several cancer types, our study supports evaluation of the pharmacological inhibition of PLK1 as an attractive therapeutic target in TNBC. However, in luminal BC, PLK1 prognostic role remains controversial.

Keywords: breast cancer, ER positive, luminal, PLK1, polo-like kinase-1, TNBC, triple-negative

Introduction

Carcinogenesis is a complex multistep process that requires accumulation of genetic and epigenetic alterations, where tumour cells often acquire different mutations affecting oncogenes, tumour-suppressor
genes, and other alterations that are required for cancer maintenance and progression.\textsuperscript{1,2}

Polo-like kinases (PLKs) are important members of the serine/threonine protein kinase family, which control many crucial biological processes, including regulation of the cell cycle.\textsuperscript{3} PLK1, which is a key component of the PLK family, promotes neoplastic transformation, and it has a critical role in cell division; it has a key role in all stages of mitosis, including centrosome maturation, bipolar spindle formation, chromosome segregation, and cytokinesis. PLK1 also plays a role in DNA replication during the S phase, and it acts as a modulator of the DNA damage response and maintenance of genome stability during replication.\textsuperscript{4,5} Overexpression of PLK1 enables tumour cells to override mitotic checkpoints leading to genetic instability.\textsuperscript{6,7} Therefore, PLK1 was considered a potential oncogene; it is overexpressed in a variety of tumours and tumour cell lines, hence, PLK1 inhibitors have been developed and are being tested as potential anticancer agents.\textsuperscript{1,8} To overcome the resistance to chemotherapy, PLK1 inhibition combined with chemotherapy agents were used in clinical trials and this combination induced apoptosis, inhibited the viability of cancer cells, and did not affect the normal cells.\textsuperscript{9} In triple-negative (TN) BC cell lines, inhibition of PLK1 activity with a small molecule (BI-2536) induced an increase in phosphorylated H2AX, G2-M arrest, and apoptosis.\textsuperscript{10}

However, recent data showed that downregulation of PLK1 can induce aneuploidy and tumourigenesis, whereas lower levels of PLK1 causes cell proliferation defect, improper segregation, a delay in centrosome maturation, and mitosis. Therefore, it may act as a tumour suppressor gene.\textsuperscript{11} Notably, PLK1 has been demonstrated to be tightly regulated by TP53, an established tumour suppressor that controls cell proliferation through induction of DNA repair, cell cycle arrest, and apoptosis.\textsuperscript{12,13}

High PLK1 expression is observed in many types of cancer, including gastric,\textsuperscript{14} colorectal,\textsuperscript{15} hepatocellular,\textsuperscript{16} prostate,\textsuperscript{17} ovarian,\textsuperscript{18} and non-small cell lung carcinomas.\textsuperscript{19} In breast cancer (BC), elevated PLK1 expression is associated with aggressive tumour characteristics, including vascular invasion, high proliferation, and enhanced growth in TNBC.\textsuperscript{20,21} However, the role of PLK1 in luminal BC is still controversial, while some studies showed that oestrogen receptor (ER)-positive tumours with higher PLK1 expression generally have improved prognosis.\textsuperscript{22,23} However, others found that knockdown of PLK1 inhibited ER expression and the PLK1 mRNA overexpression correlates with poor prognosis in luminal BC.\textsuperscript{24,25} In a recent study, we found that PLK1 was significantly associated with atypical mitosis in BC.\textsuperscript{26}

In this study, we hypothesised that the function of PLK1 is diverse among the different molecular subtypes of BC. Therefore, we aimed to explore the role of PLK1 at the protein level in BC utilising a large cohort at the protein and transcriptomic levels with consideration of the molecular subtypes.

**Materials and Methods**

**Study cohort**

In this study, two cohorts were used: the local Nottingham cohort ($n=1208$), which was used for the immunohistochemistry (IHC) part of the study. The other cohort is a publicly available cohort with mRNA results available ($n=6774$) that was used to study the expression and prognostic value of PLK1 mRNA levels in BC (Breast Cancer Gene-Expression Miner v4.9).

PLK1 protein expression was evaluated on a well-characterised primary BC series ($n=1208$) from patients presented at the Nottingham City Hospital, NHS Trust, Nottingham, United Kingdom. Clinical and tumour characteristics including patient’s age at diagnosis, histologic tumour type, grade, tumour size, lymph node status, Nottingham Prognostic Index (NPI), and lymphovascular invasion (LVI) were available (Supplementary Table S1). In addition, the outcome data in the form of BC-specific survival (BCSS), defined as time (in months) from the date of primary surgical treatment to the time of death by BC, and distant metastasis-free survival (DMFS) defined as the time (in months) from the surgery until the first event of distant metastasis were also collected from patients’ records. The mean follow-up time was 135 months, median was 158 months, and ranged from 7–256 months. During the follow-up 23% (276/1193) developed distant metastasis. Adjuvant treatment was given according to the institutional protocols. Information regarding ER, progesterone receptor (PR), and human epidermal growth factor 2 (HER2)\textsuperscript{27–30} were available. Tumours were also classified based on ER, PR, and HER2 into three molecular subtypes (luminal, TN, HER2-enriched) as the following: ER+ HER2- (luminal BC), ER-, PR- and HER2- (TNBC), while tumours with HER2+ (HER2 enriched).\textsuperscript{31} In view of the link between p53 and PLK1 regulation,\textsuperscript{26} data regarding IHC expression of p53 were available and was used for a comparative analysis with PLK1.\textsuperscript{32,33}
**PLK1 protein expression evaluation**

Prior to the IHC staining of the tissue sections, the specificity of the Anti-PLK1 antibody (ab109777, Abcam, UK) was validated by western blotting using cell lysates of MCF10, MCF7, HeLa, and SKBR3 human BC cell lines obtained from the American Type Culture Collection (Rockville, MD, USA). The PLK1 primary antibody was used at 1:1000 dilution. Proteins were detected using IRDye 800CW fluorescent secondary antibodies (1:5 000 dilution, LI-COR Biosciences, Lincoln, NE, USA) and the Odyssey Fc with Image Studio 4.0 (LI-COR Biosciences) was used to visualize the bands. Anti-β-actin primary antibody (Sigma-Aldrich, St. Louis, MO, USA) was used as a loading control (1: 5000). A single specific band for PLK1 protein was observed at the predicted molecular weight (68 kDa) (Supplementary Figure S1), confirming the specificity of the antibody.

Full-face sections of BC cases (n = 14), representative of several molecular subtypes and tumour grades, were used to evaluate the distribution of PLK1 expression. Tumour samples were arrayed using the Grand Master (3D HISTECH, Budapest, Hungary) as described previously.22,33 Tissue sections using the Novoceastra Novolink™ Polymer Detection Systems kit (Code: RE7280-K, Leica, Biosystems, Newcastle, UK) of 4 μm thick were dewaxed and endogenous peroxidase activity was blocked with 0.3% hydrogen peroxide in methanol for 10 min. Antigen retrieval was performed in citrate buffer pH 6.0 using a microwave (Whirlpool JT359 Jet Chef 1000 W, Benton Harbor, MI, USA) for 20 min. Rabbit polyclonal was diluted at 1:250 in Leica antibody diluent (RE AR9352, Leica, Biosystems, UK) and incubated for 30 min at room temperature. Normal testicular tissue was used as a positive control, while the negative control was obtained by omitting the primary antibody. The sections were counterstained with haematoxylin. The percentage of positive tumour cells was calculated. For assessing the interobserver concordance, a second observer (L.W.) scored 20% of the cohort and the intraclass correlation coefficient was calculated. In this study we used the Histochemical score (H-score) to assess the staining intensity and percentage of expression and the final H-score was obtained by giving a range of 0 to 300.34–36 Cases were then categorised into low and high expression based on X tile software, which defines the optimum cutoff associated with patient outcome.

**PLK1 mRNA expression**

PLK1 mRNA was analysed at the transcriptomic level in different publicly available cohorts, including the Molecular Taxonomy of BC International Consortium (METABRIC) cohort (n = 1980), the TCGA BC dataset (n = 843), and the Kaplan–Meier (KM) Plotter (n = 3951) online dataset (https://kmplot.com/analysis/).37

**P53 staining and scoring**

The data regarding the IHC expression of p53 were available from a previous study.33 Briefly, monoclonal mouse antihuman p53 (clone DO7, Novocastra, Deer Park, IL, USA) was used and was diluted at 1:50 in Leica antibody diluent (RE AR9352, Leica, UK) and incubated for 30 min at room temperature. Immunostaining for p53 showed nuclear expression and its score was evaluated using a semiquantitative system. H-score from 0–300 was calculated for each case. A 10% cutoff was used as the optimal cutoff of categorisation of p53 expression into negative (wild-type) and positive (mutant) tumours. For the interaction between p53, PLK1 markers, the whole cohort was categorised according to p53 status into wildtype tumours (p53−) and p53 mutant tumours (p53+) and clinicopathological variables were investigated.

**Statistical analysis**

Statistical package for the Social Sciences software v. 27.0 (SPSS, Chicago, IL, USA) was used for statistical analysis. An H-score of 170 was the optimal cutoff based on X-tile bioinformatics software v. 3.6.1 (School of Medicine, Yale University, New Haven, CT, USA) was used.38 For statistical analysis, the expression of P53 and PLK1 was divided into four groups: low PLK1 with negative P53, high PLK1 with positive P53, high PLK1 with negative P53, and low PLK1 with negative P53. The chi-square test was used for analysis of categorical data. Outcome analysis was assessed using Kaplan–Meier curves and the log-rank test. The association of PLK1 expression with the response of adjuvant therapy was also evaluated. Cox regression models were used for the multivariate analysis. Estimated hazard ratio (HR) and their 95% confidence interval (95% CI) were calculated. For all tests, P < 0.05 (two-tailed) were statistically significant.

This study was approved by the Yorkshire & the Humber-Leeds East Research Ethics Committee (REC Reference: 19/YH/0293) under the IRAS Project ID: 266925. Data collected were fully anonymised.

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Results

PLK1 protein expression

Full-face sections of BC showed an even distribution for PLK1 protein expression, which indicated the suitability of TMA to assess PLK1 protein expression. PLK1 showed cytoplasmic subcellular localization only. The mean ± SD of PLK1 H-score was 151 ± 80, the median was 100%, (range 0%–300%). There was excellent interobserver concordance in scoring of PLK1 H-scores ($k = 0.8$). Low PLK1 expression was observed in 80% (971/1208) of patients, while 20% showed high PLK1 expression (Figure 1). There was a significant association between high PLK1 expression and TNBC, while low expression was associated with the HER2-enriched and luminal subtype ($P = 0.005$; Table 1). The percentage of PLK1 expression and correlation with the different molecular subtypes are shown in Supplementary Table S2. In addition, when the HER2-enriched group was excluded and PLK1 expression levels were compared in luminal versus TNBC, high PLK1 remained significant in TNBC, and low expression was significant in luminal BC (Supplementary Table S3).

There was no association between PLK1 and tumour grade, tumour size, lymph node invasion, or LVI. However, a significant association was observed between high PLK1 expression and high mitosis scores, ($P = 0.008$).

Outcome analysis

High PLK1 expression showed a significant association with longer BCSS in the whole cohort ($P = 0.032$; Figure 2). With classification into different molecular subtypes, high PLK1 expression was associated with prolonged survival in both luminal and HER2-enriched subtypes ($P = 0.030$ and 0.045, respectively). In TNBC, high PLK1 expression showed a significant association with poor outcome ($P = 0.026$; Figure 3).

Multivariate Cox regression analysis indicated that high PLK1 expression is an independent risk factor of good prognosis in luminal BC (Table 2). Conversely, high PLK1 expression showed a significant association with poor survival in TNBC, independent of tumour grade and stage while the tumour grade lost its significance in that group of patients (Table 3).

Interaction between TP53 and PLK1

Although there was no association between PLK1 expression and P53, when the cohort was stratified based on the P53 status, low PLK1 expression showed a significant association with poor outcome in P53 mutated tumours ($p53^+$) ($P = 0.01$) (Supplementary Figure S2), while in P53 wildtype tumours ($p53^-$), there was no significant difference in patient outcome between low and high PLK1 expression tumours. Furthermore, with a combination of PLK1 and P53 expressions, tumours with low PLK1 and mutated P53 showed a strong association with parameters characteristic of aggressive tumour behaviour, including high tumour grade, the presence of vascular invasion, poor NPI, and high Ki67 expression ($P < 0.0001$). Additionally, low PLK1 with P53 mutated tumours showed the worst outcome compared to the other groups in the whole cohort ($P = 0.028$) and in luminal BC patients ($P = 0.019$; Figure 4).
Table 1. Relationship between PLK1 H. score and clinicopathological parameters

<table>
<thead>
<tr>
<th>Variables</th>
<th>PLK1 expression</th>
<th>( \chi^2 )</th>
<th>( P )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low H. score ≤ 170</td>
<td>High H. score &gt; 170</td>
<td></td>
</tr>
<tr>
<td>Age at diagnosis (years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;50</td>
<td>300 (78%)</td>
<td>82 (22%)</td>
<td>1.2</td>
</tr>
<tr>
<td>≥50</td>
<td>671 (81%)</td>
<td>155 (19%)</td>
<td></td>
</tr>
<tr>
<td>Menopausal state</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Premenopausal</td>
<td>327 (78%)</td>
<td>94 (22%)</td>
<td>3.1</td>
</tr>
<tr>
<td>Postmenopausal</td>
<td>644 (82%)</td>
<td>143 (18%)</td>
<td></td>
</tr>
<tr>
<td>Tumour size (cm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤2 cm</td>
<td>576 (79%)</td>
<td>154 (21%)</td>
<td>2.5</td>
</tr>
<tr>
<td>&gt;2 cm</td>
<td>395 (83%)</td>
<td>83 (17%)</td>
<td></td>
</tr>
<tr>
<td>Histologic tumour grade</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade 1</td>
<td>144 (80%)</td>
<td>35 (20%)</td>
<td>0.01</td>
</tr>
<tr>
<td>Grade 2</td>
<td>384 (81%)</td>
<td>93 (19%)</td>
<td></td>
</tr>
<tr>
<td>Grade 3</td>
<td>443 (80%)</td>
<td>109 (20%)</td>
<td></td>
</tr>
<tr>
<td>Histologic tumour types</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No special type (NST)</td>
<td>635 (81%)</td>
<td>147 (19%)</td>
<td>2.1</td>
</tr>
<tr>
<td>Lobular</td>
<td>77 (80%)</td>
<td>19 (20%)</td>
<td></td>
</tr>
<tr>
<td>Other special types</td>
<td>32 (73%)</td>
<td>12 (27%)</td>
<td></td>
</tr>
<tr>
<td>NST mixed</td>
<td>277 (80%)</td>
<td>59 (20%)</td>
<td></td>
</tr>
<tr>
<td>Molecular subtypes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Luminal</td>
<td>791 (81%)</td>
<td>188 (19%)</td>
<td>10.5</td>
</tr>
<tr>
<td>HER2 enriched.</td>
<td>52 (93%)</td>
<td>4 (7%)</td>
<td></td>
</tr>
<tr>
<td>Triple negative</td>
<td>119 (73%)</td>
<td>43 (27%)</td>
<td></td>
</tr>
<tr>
<td>Lymph node status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>580 (80%)</td>
<td>143 (20%)</td>
<td>0.03</td>
</tr>
<tr>
<td>Present</td>
<td>391 (81%)</td>
<td>94 (19%)</td>
<td></td>
</tr>
<tr>
<td>Lymphovascular invasion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>687 (80%)</td>
<td>173 (20%)</td>
<td>0.47</td>
</tr>
<tr>
<td>Present</td>
<td>284 (82%)</td>
<td>64 (18%)</td>
<td></td>
</tr>
<tr>
<td>Nottingham prognostic index</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Good prognostic group</td>
<td>314 (79%)</td>
<td>83 (21%)</td>
<td>4.7</td>
</tr>
<tr>
<td>Moderate prognostic group</td>
<td>490 (81%)</td>
<td>117 (19%)</td>
<td></td>
</tr>
<tr>
<td>Poor prognostic group</td>
<td>167 (82%)</td>
<td>37 (18%)</td>
<td></td>
</tr>
</tbody>
</table>

Significant \( P \) values are in bold.
PLK1 mRNA expression and patients' outcome

In the Kaplan–Meier (KM) Plotter dataset, high PLK1 mRNA levels showed a significant association with good outcome in the whole cohort \((P = 0.0004)\), while in TNBC, high PLK1 expression had a significant association with poor outcome \((P = 0.01; \text{Figure 5})\). Consistently, in both the TCGA cohort and METABRIC cohort, high PLK1 expression was significantly higher in TNBC compared with luminal BC (Supplementary Figure S3).
Discussion

BC is the leading cause of cancer-related deaths in women worldwide and the therapeutic decision of BC patients currently depends on histopathological data and molecular biomarkers. However, many patients show resistance to treatment due to the clinical heterogeneity of BC. For these reasons, research into the molecular biology of treatment response is often aimed at identifying genes with prognostic and
predictive impact, especially for TNBC patients, who do not benefit from targeted therapy and show a high rate of recurrence.43

PLK1 is a promising prognostic surrogate in BC, which is a key regulator of mitotic cell division.44 Also, PLK1 was one of the associated genes with atypical mitosis in a previous study.26 It has been proposed that overexpression of PLK1 is associated with poor prognosis in BC, hence PLK1 was considered an oncogene.45 However, in vivo studies using a mouse mammary gland tumour model, PLK1 overexpression prevented tumour development in most of the mouse colony and significantly delayed tumour latency in the rest of it.22 Similarly, in BC induced by the HER2 oncogene, the number of tumours per animal was significantly reduced after PLK1 overexpression due to defective mitosis and enhanced apoptosis.46

Looking at the role of PLK1 in BC molecular classes, it is more expressed in TNBC tissues as compared with normal breast tissue, HER2+ and/or luminal BC cells, proposing that PLK1 acts as an oncogene in TNBC and this was supported in many studies.47,48 On the other hand, the role of PLK1 in luminal BC remains controversial, whereas some studies revealed that high PLK1 was associated with a good outcome in luminal BC.22,23 Others showed a poor prognostic role for PLK1 in those tumours.24,25 In view of this controversial role of PLK1 in luminal BC, we aimed to assess the prognostic and predictive value of PLK1 in each of BC molecular subtype and to explore whether PLK1 acts as an oncogene or as a tumour suppressor gene in luminal BC.

In this study, a significant association was found between high PLK1 expression and high mitotic score, supporting a previous study findings.26 This confirms the role of PLK1 in regulation of mitosis process and its function to ensure the correct execution by phosphorylating centromere protein A (CENPA) to promote its assembly during the metaphase-anaphase transition in mitosis.49,50

In addition, a strong association was observed between high PLK1 expression and TNBC subtype at both the proteomic and transcriptomic levels, confirming previous studies.10,48,51 With dichotomisation of the whole cohort into molecular classes, high PLK1 expression had a significant association with longer BCSS and DMFS in luminal BC. These findings are in line with recent studies that showed that PLK1 has tumour suppressor functions in ER-positive tumours.22,23 Other studies revealed that PLK1 shows poor survival in luminal BC; however, this resulted from a limited number of samples that was used or due to the use of mRNA levels from the publicly available data, which seems diverse between these cohorts and different cutoffs for PLK1 were used in each cohort.20,45,52 This controversial role of PLK1 was studied previously in other tumours, including colorectal cancer.53,54

On the contrary, in this study high PLK1 expression was associated with a poor prognosis in TNBC, and it has been shown that PLK1 promotes proliferation and angiogenesis in TNBC cell lines by inducing a specific signalling pathway.47,55 Therefore, PLK1 can work as a therapeutic target for TNBC, especially in those patients who are chemoresistant.10,48

The discrepancy between the prognostic value of PLK1 in luminal and TNBC can be explained by the different actions that PLK1 may play in those molecular subtypes. For example, in luminal BC, PLK1...
mediates ER-regulated gene transcription participating in the expression of genes involved in developmental and tumour-suppressive functions; when PLK1 activity was inhibited, the ER-dependent gene sets were downregulated.\textsuperscript{56} Also, high expression of these genes correlates positively with a clinical benefit in BC patients.\textsuperscript{23,56,57} In TNBC, PLK1 acts as an oncogene, with its role of regulation of cell cycle and mitosis and inhibition of PLK1 decreases the viability of various TNBC cell lines, and this suggests that PLK1 is essential for the survival of TNBC cells.\textsuperscript{48} Therefore, it is implied that inhibition of PLK1 may be especially effective for the aggressive type of TNBC.\textsuperscript{47,58,59} It was reported that the resistance to some chemotherapeutic drugs has been linked to PLK1 overexpression, and PLK1-mediated mitotic events, which was found to reduce the efficacy of chemotherapeutic agents.\textsuperscript{8} Consistently, it was found that PLK1 inhibition synergised with chemotherapy in TNBC significantly decreased tumour volume compared to chemotherapy alone.\textsuperscript{58}

The potential of PLK1 inhibitors as cancer therapeutics has been widely investigated and previous studies have revealed that targeting PLK1 may be a novel approach for overcoming drug resistance in cancer chemotherapy in many cancer types, especially for TNBC patients.\textsuperscript{60–62} Currently, there are more than 10 commercially available PLK1-specific inhibitors, of which at least four have been evaluated in clinical trials in combination with chemotherapy in TNBC.\textsuperscript{63,64}

Evidence shows that the proteasome inhibitor bortezomib (Velcade), which is an experimental small molecule, can reduce PLK1 protein levels and has been used to treat multiple myeloma and mantle cell lymphoma.\textsuperscript{64} In addition, a PLK1 Inhibitor (Olvanibertib), is currently used for the treatment of relapsed small cell lung cancer patients, who have either not responded to or are unable to tolerate chemotherapy. Recently, a novel PLK1 inhibitor (RK-10) was developed in TNBC cells and has been shown to have antiproliferative and antimigratory properties causing $S$ phase and $G2/M$ cell cycle arrest.\textsuperscript{51}

It is known that PLK1 is downregulated by P53 as part of the $G2/M$ cell cycle checkpoint.\textsuperscript{65–67} and in the meantime, PLK1 has a pivotal role both in the p53-mediated regulation of DNA damage repair and mitosis.\textsuperscript{20} In this study, we found a significant association between low PLK1 expression and poor outcome in P53 mutated tumours. With a combination of PLK1 and P53 expression, we found that tumours with low PLK1 with mutated P53 showed the worst outcome in luminal BC patients. This suggests that the effect of PLK1 depends on the mutation of the TP53 gene.

**Limitations**

This was a retrospective study, where patients were not treated within the rigorous setting of a clinical trial. The number of HER2-enriched BC patients is small, which may affect the association with PLK1 in those patients.

**Conclusion**

Our findings suggest a reconsideration of the role of PLK1 in the different molecular subtypes in BC. We confirmed the role of PLK1 in TNBC as an oncogene, whereas treatment of TNBC patients with PLK1 inhibitors is recommended; caution should be considered when PLK1 inhibitors that could be used in a clinical trial in luminal BC patients, as its role in this group remains controversial.

**Author contributions**

AL scored the cases and wrote the article draft, data analysis, and interpretation, LW helped in double scoring. AL, MT, NM, AG, and MS agree with the article results and conclusions and critically reviewed the article. E.A. Rakha: conceived and planned the study, contributed to data interpretation, made critical revisions, and approved the final version.

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**Conflict of interest**

The authors declare that they have no conflicts of interest.
Data availability statement

All data used in this study are archived and are available upon a reasonable request.

References


Supporting Information
Additional Supporting Information may be found in the online version of this article:
Table S1. Clinicopathological characteristics of the study cohort.

Table S2. Low versus high PLK1 expression within the different molecular classes.

Table S3. Low versus high PLK1 expression within luminal and triple-negative molecular classes.

Figure S1. Western blot of PLk1 antibody shows a specific band at a molecular weight of 68 kDa while the control antibody, B-actin at 42 kDa.

Figure S2. a-Kaplan–Meier plots showing significant association between low PLK1 protein expression and short survival in P53 mutated (p53<sup>+</sup>) tumours.

Figure S3. a-Kaplan–Meier plots showing significantly higher PLK1 mRNA expression and in Basellike BC (TNBC) compared to other molecular classes in the METABRIC cohort.