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ORIGINAL ARTICLE

High cyclin E1 protein, but not gene amplification, is prognostic for basal-like breast cancer

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Abstract

Basal-like breast cancer (BLBC) has a greater overlap in molecular features with high-grade serous ovarian cancer (HGSOC) than with other breast cancer subtypes. Similarities include *BRCA1* mutation, high frequency of *TP53* mutation, and amplification of *CCNE1* (encoding the cyclin E1 protein) in 6–34% of cases, and these features can be used to group patients for targeted therapies in clinical trials. In HGSOC, we previously reported two subsets with high levels of cyclin E1: those in which *CCNE1* is amplified, have intact homologous recombination (HR), and very poor prognosis; and a *CCNE1* non-amplified subset, with more prevalent HR defects. Here, we investigate whether similar subsets are identifiable in BLBC that may allow alignment of patient grouping in clinical trials of agents targeting cyclin E1 overexpression. We examined cyclin E1 protein and *CCNE1* amplification in a cohort of 76 BLBCs and validated the findings in additional breast cancer datasets. Compared to HGSOC, CCNE1 amplified BLBC had a lower level of amplification (3.5 versus 5.2 copies) and lower relative cyclin E1 protein, a lack of correlation of amplification with expression, and no association with polyploidy. BLBC with elevated cyclin E1 protein also had prevalent HR defects, and high-level expression of the cyclin E1 deubiquitinase ubiquitin-specific protease 28 (USP28). Using a meta-analysis across multiple studies, we determined that cyclin E1 protein overexpression but not amplification is prognostic in BLBC, while both cyclin E1 overexpression and amplification are prognostic in HGSOC. Overall *CCNE1* gene amplification is not equivalent between BLBC and HGSOC. However, high cyclin E1 protein expression can co-occur with HR defects in both BLBC and HGSOC, and is associated with poor prognosis in BLBC.

Keywords: cyclin E1; *CCNE1* amplification; 19q12; basal-like breast cancer; high-grade serous ovarian cancer; homologous recombination deficiency; USP28

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Introduction

Basal-like breast cancer (BLBC) and high-grade serous ovarian cancer (HGSOC) are both aggressive diseases with high frequency of *TP53* mutation, *BRCA1* mutation, and *CCNE1* gene amplification at 19q12 [1]. Overall, BLBC is more similar to HGSOC than to other breast cancer subtypes based on the comparison of copy number gains and losses, and the comparison of gene network activation by analysis of transcriptomes [1]. There is also a significant co-occurrence of these tumours in patients [2].

While the mainstay of treatment for BLBC and HGSOC is chemotherapy, the high frequency of *BRCA1* mutation and other homologous recombination (HR) defects has led to the introduction of PARP (poly adenosine diphosphate-ribose polymerase) inhibitors as a maintenance therapy in both cancers [3,4]. It is now common for clinical trials to group HGSOC and BLBC patients in order to access larger cohorts when testing new drug combinations with PARP inhibitors or PARP inhibitors in pre-treated patients (e.g. clinical trials NCT01623349, NCT00679783, NCT00892736, EMBRACE). *CCNE1* amplification is also being explored as a therapeutic biomarker for HGSOC and BLBC. This is because cells with very high cyclin E1 expression undergo replication stress and genomic instability, and these cancer cells can be forced into mitotic catastrophe and cell death through inhibition of the Chk1 and Wee1 cell cycle checkpoints [5]. Consequently, *CCNE1* amplification has been used as a biomarker to group patients with solid tumours for clinical trials of Chk1 inhibitor prexasertib (NCT02873975) and Wee1 inhibitor adavosertib (NCT03253679), with results of these trials pending. An alternative way to target the oncogenic activity of cyclin E1 is via the inhibition of its partner kinase, CDK2. Pre-clinical studies have demonstrated the potential of CDK2 inhibitors in combination with other therapies for treatment of *CCNE1*-amplified HGSOC and cyclin E1 overexpressing BLBC [6,7].

With the increasing use of biomarker-driven treatment for BLBC and HGSOC, it is imperative to ensure that each biomarker associates with similar biology and therapeutic vulnerabilities in these cancers. Recently, we described separate subsets of HGSOC, one where *CCNE1* amplification and *BRCA1/BRCA2* mutation were mutually exclusive (the 19q12 amplified subset), and another where *BRCA1/BRCA2* mutation co-occurred with high cyclin E1 protein overexpression (the high cyclin E1 protein subset) [8]. We subsequently investigated a patient cohort that is highly enriched for BLBC and identified the co-occurrence of *BRCA1* mutation and high cyclin E1 protein [7]. However, an unresolved question is whether *CCNE1* amplification and cyclin E1 protein expression in BLBC demarcate patients into subgroups that are similar to those that have been detected in HGSOC. This has important implications for the selection of BLBC patients to receive therapies such as PARP inhibitors, cell cycle checkpoint inhibitors, and CDK2 inhibitors.

Here, we aimed to determine the similarities and differences between BLBC and HGSOC in terms of cyclin E1 expression, *CCNE1* amplification, and patient survival. Accordingly, we assessed the overlap between BLBC and HGSOC subsets in terms of the relative expression of cyclin E1, *CCNE1* amplification, homologous recombination deficiency (HRD), and their association with outcome.

Materials and methods

Patient cohorts

Molecular and clinical data were obtained for breast cancer patients from the Kathleen Cunningham Foundation Consortium for research into Familial Breast cancer (kConFab), as described in Ref. [7], and for HGSOC from the Australian Ovarian Cancer Study (AOCS), as described in Ref. [8]. For both studies, ethics board approval was obtained at all institutions for patient recruitment, sample collection, and research studies. Written informed consent was obtained from all participants for participation in research studies. Both cohorts are described in Supplementary materials and methods, including the definition of BLBC for the kConFab cohort. Summary details of BLBC and breast cancers other than BLBC (non-BLBC) are shown in Table 1.

BLBC was identified from PAM50-defined subsets of The Cancer Genome Atlas (TCGA) [9] and Molecular Taxonomy of Breast Cancer International Consortium (METABRIC) [10], and HGSOC from the TCGA dataset [11]. Data for protein expression, gene amplification, and patient survival were downloaded via cBioPortal [12]. Cut-offs were ≥0 z-score on reverse phase protein array (RPPA) for high protein expression [13], and a Genomic Identification of Significant Targets in Cancer (GISTIC) score of 2 for gene amplification [14]. GISTIC scores of *CCNE1* amplification in cell lines were downloaded via cBioPortal [12] from the Cancer Cell Line Encyclopaedia [15]. Gene expression and ASCAT (Allele-Specific Copy number Analysis of Tumours) estimates of ploidy [16] from HGSOC and BLBC of the TCGA cohorts were accessed through COSMIC [17]. Homologous recombination deficiency-loss of heterozygosity (HRD-LOH) score is a measure of LOH through analysis of allele loss, and scores for TCGA samples were derived from Ref. [18].

Cyclin E1 analysis

Cyclin E1 immunohistochemistry (IHC) is described in Supplementary materials and methods and supplementary material, Figure S1. Detection of *CCNE1* amplification is described in Supplementary materials and methods.

Table 1. Patient and tumour characteristics of BLBC and non-BLBC

	BLBC		Non-BLBC	
Age, years	Median	Range	Median	Range
	40	19–74	43.5	29–73
Histological subtype	Number	%	Number	%
Infiltrating duct carcinoma, NOS	66	86.84	105	77.21
Medullary carcinoma	5	6.58	0	0.00
Carcinoma (unspecified)	4	5.26	11	8.09
Infiltrating lobular carcinoma	0	0.00	8	5.88
Infiltrating duct and lobular carcinoma	0	0.00	8	5.88
Other	1	1.32	4	2.94
Grade	Number	%	Number	%
1	1	1.32	18	13.24
2	9	11.84	50	36.76
3	54	71.05	59	43.38
No grade	12	15.79	9	6.62
Nodal status	Number	%	Number	%
N0	31	40.79	52	38.24
N1 (1–3)	11	14.47	26	19.12
N2 (4–8)	2	2.63	10	7.35
N3 (>8)	1	1.32	5	3.68
Not known	31	40.79	43	31.61
CK5/CK14/EGFR status	Number	%	Number	%
CK5 and/or CK14 positive, EGFR positive	6	7.89	0	0.00
CK5 and/or CK14 positive, EGFR negative	61	80.26	0	0.00
EGFR positive, CK5/CK14 negative	9	11.84	0	0.00
Germline *BRCA1/2* status	Number	%	Number	%
BRCA1 mutated	60	78.95	35	25.74
BRCA2 mutated	7	9.21	37	27.21
Wild-type	9	11.84	64	47.06
ER, PR and HER2 status	Number	%	Number	%
ER/PR positive, HER2 negative	6	7.89	79	58.09
ER/PR positive, HER2 positive	3	3.95	29	21.32
ER/PR negative, HER2 positive	14	18.42	4	2.94
Triple negative	51	67.11	22	16.18
Not tested	2	2.63	2	1.47
Overall survival	Events	Median (months)	Events	Median (months)
	21	163.18	33	161.38

EGFR, epidermal growth factor receptor; ER, oestrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2.

Meta-analyses

Publications reporting cyclin E1 protein expression, *CCNE1* amplification, and overall survival data were identified through searches with the terms 'cyclin E', 'cyclin E1', 'CCNE1' 'survival', 'breast cancer', and 'ovarian cancer' of the PubMed database, Google Scholar, and SCOPUS. To be included, studies needed to be able to report overall survival at 50 months for either HGSOC or BLBC, have 40 patients or more, and report the HGSOC and BLBC subgroups (defined by cytokeratin [CK] 5/6/14 staining or PAM50 status) as distinct entities within ovarian cancer and breast cancer, respectively. Study identification is shown with PRISMA diagrams in supplementary material, Figure S2. Bias within each meta-analysis was assessed by inspection of FUNNEL plots of treatment effects from individual studies plotted against a measure of study size [19]. The prognostic power of cyclin E1 expression and *CCNE1*

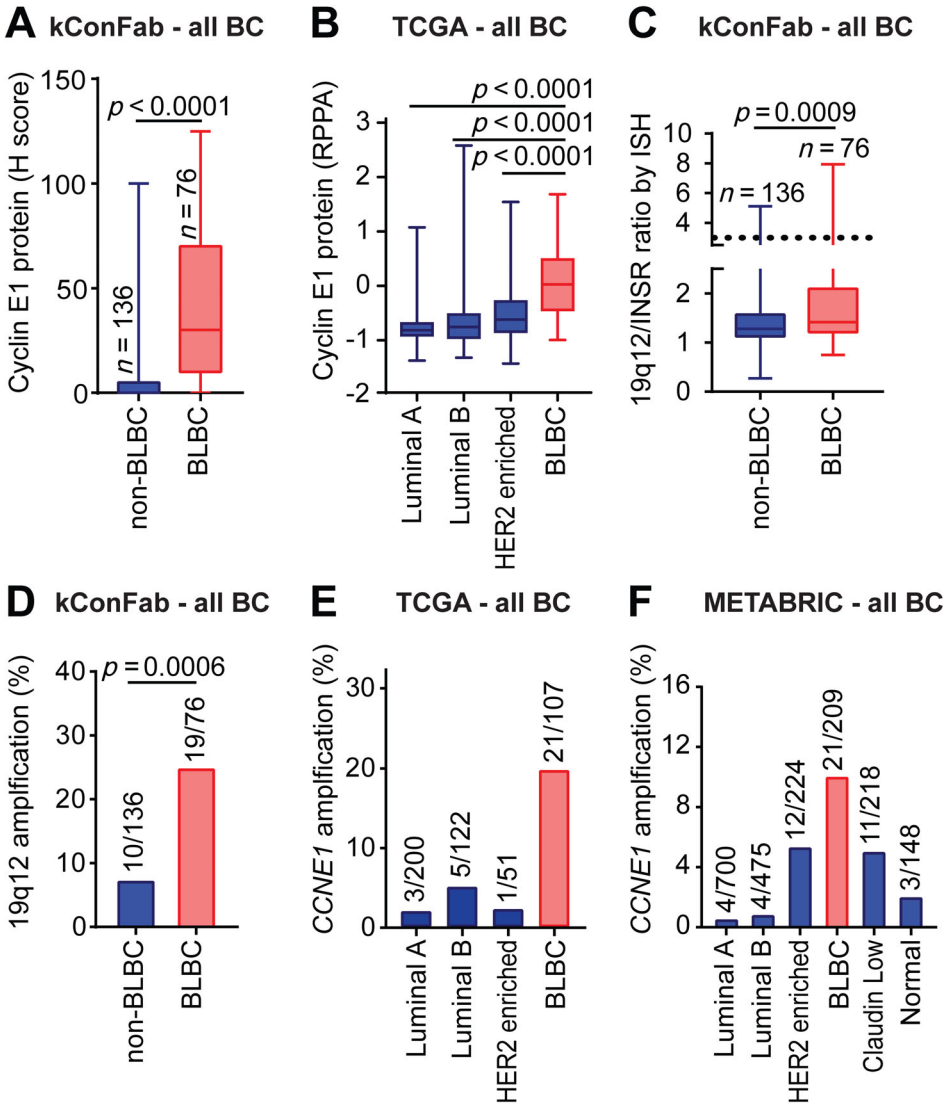


Figure 1. High protein expression of cyclin E1 and *CCNE1* amplification are frequent in BLBC. (A) Cyclin E1 protein expression in BLBC compared to non-BLBC in the kConFab cohort, analysed by Mann–Whitney test. (B) Cyclin E1 protein expression by RPPA in 273 TCGA breast cancer cases categorised into PAM50 defined subtypes: luminal A, basal-like, HER2 enriched, and luminal B. Data analysed by one-way ANOVA with Tukey test for multiple comparisons. (C) 19q12/INSR ratio (ISH) in BLBC compared to non-BLBC in the kConFab cohort, analysed by Mann–Whitney test. Data in (A)–(C) are box and whisker plots with error bars of minimum to maximum, where the box extends from the 25th to the 75th percentile, and the line in the middle of the box indicates the median. (D) Relative 19q12 amplification in the BLBC and non-BLBC subsets of the kConFab cohort, as defined by 19q12/INSR ratio of ≥3, and/or 19q12 score >6. Analysed by Fisher's exact test. (E) Relative *CCNE1* amplification across the molecular subtypes of breast cancer, including BLBC, based on amplification (GISTIC = 2) in the TCGA breast cancer dataset. (F) Relative *CCNE1* amplification across the molecular subtypes of breast cancer, including BLBC, based on amplification (GISTIC = 2) in the METABRIC dataset.

amplification were assessed by meta-analysis using Revman 5.0 software [20].

Cell line culture

Cell lines were obtained from ATCC and cultured in RPMI 1640, 5–10% foetal calf serum, and insulin (10 µg/ml). All cell lines were authenticated by STR profiling (CellBank Australia) and cultured for less than 6 months after authentication. Bona fide HGSOC and BLBC cell lines were classified as described [21,22]. The derivation of cyclin E1 overexpressing cell lines, ploidy analysis, and colony-forming assays is described in Supplementary materials and methods.

Statistical analysis

Statistical analyses were performed using Prism Software™ version 9.3.1 [23]. Statistical tests, *P* value calculation, and data presentation are described in detail in Supplementary materials and methods.

Results

High cyclin E1 protein expression and *CCNE1* amplification are frequent in BLBC

We explored the relationship between cyclin E1 protein expression and survival in BLBC by quantifying cyclin E1 expression across the kConFab familial breast cancer cohort [7]. Cyclin E1 expression was significantly higher in BLBC compared to non-BLBC cases ($p < 0.0001$) (Figure 1A). We validated these findings in the PAM50-defined BLBC cohort of TCGA using RPPA data [1]. This showed that cyclin E1 protein is significantly higher in BLBC compared to other PAM50-defined breast cancer subtypes including luminal A, luminal B, and HER2 enriched ($p < 0.0001$) (Figure 1B).

Amplification of the *CCNE1* gene located at 19q12 is a major contributor to high cyclin E1 expression in HGSOC [8,24] and therefore we assessed whether 19q12 amplification is associated with BLBC. Compared to non-BLBC breast cancers, BLBC had significantly higher copy number at the *CCNE1* gene locus, as measured by the ratio of 19q12 to chromosome 19 control probe, insulin receptor (INSR) ($p = 0.0009$) (Figure 1C). Twenty-five percent of BLBC patients in the kConFab cohort had 19q12 amplification, compared to 7.4% of non-BLBC ($p = 0.0006$, Fisher's exact test, Figure 1D). BLBC had the highest rate of *CCNE1* amplification in samples from the TCGA and METABRIC datasets (Figure 1E,F).

Meta-analysis identifies that *CCNE1* amplification and cyclin E1 protein expression are both prognostic for HGSOC, but only cyclin E1 protein expression is prognostic for BLBC

Cyclin E1 protein and *CCNE1* amplification have been reported as prognostic markers in subtypes of breast cancer [25–27] and in HGSOC, which has many similarities to BLBC. The kConFab, TCGA, and Metabric BLBC cohorts are small and hence underpowered for survival analyses, and the kConFab cohort also has inherent bias as it is highly enriched for mutation carriers for *BRCA1* and *BRCA2*. To address these limitations, we combined these cohorts in meta-analyses, along with other published cohorts, where additional studies were identified via the listed search terms (Figure 2A). In parallel, we examined studies of cyclin E1 protein and *CCNE1* amplification in HGSOC, identified by the same search terms.

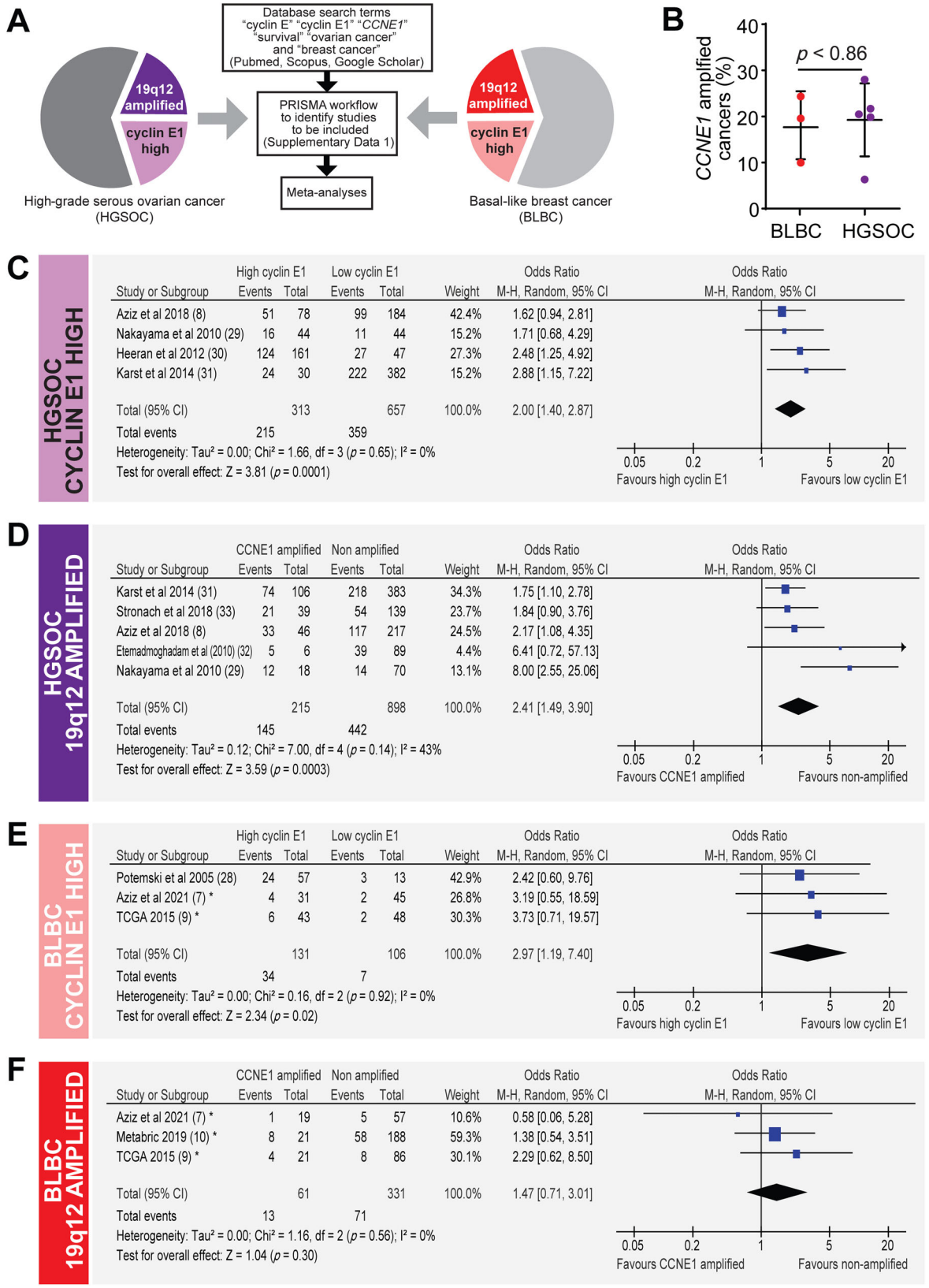
We identified three BLBC cyclin E1 protein studies [7,9,28], three BLBC 19q12/*CCNE1* amplification studies [7,9,10], four HGSOC cyclin E1 protein studies [8,29–31], and five HGSOC 19q12/*CCNE1* amplification studies [8,29,31–33], which were suitable for inclusion in the meta-analyses (PRISMA diagram, supplementary material, Figure S2). In these studies, there was a similar rate of *CCNE1* amplification across BLBC and HGSOC (Figure 2B), although this was highly variable. This variability could have arisen because *CCNE1* amplification can be measured by either estimates of copy number from exome/genome sequencing or by 19q12 ISH, and this may give rise to different sensitivity in the detection of amplification. Each meta-analysis was weighted to patient number and analysed using a random effects model.

High cyclin E1 protein expression and *CCNE1* amplification were both associated with greater risk of cancer death in HGSOC (Figure 2C,D). The risk of death was twice as high in patients with high cyclin E1 protein expression, with an odds ratio of 2.00 (95% CI 1.40, 2.87; $p = 0.0001$). Similarly, the risk was also increased for those patients with *CCNE1* amplification with an odds ratio of 2.41 (95% CI 1.49, 3.90; $p = 0.0003$). The studies across *CCNE1*-amplified ovarian cancer displayed some heterogeneity ($I^2 = 43\%$), although this was not significant, and FUNNEL plots did not show significant deviations overall in the meta-analyses (supplementary material, Figure S3).

In BLBC, only high cyclin E1 protein, and not *CCNE1* amplification, was associated with increased risk of death (Figure 2E,F). The risk of death was 2.97-fold higher in patients with high cyclin E1 protein expression (95% CI 1.19, 7.40; $p = 0.02$), whereas the risk of death for *CCNE1* amplification was not significantly increased (odds ratio 1.47; 95% CI 0.71, 3.01; p = 0.30). Individual Kaplan–Meier curves for the kConFab, TCGA, and Metabric cohorts are shown in supplementary material, Figure S4.

CCNE1-amplified HGSOC expresses higher cyclin E1 protein than *CCNE1*-amplified BLBC

The meta-analysis showed that *CCNE1* amplification is prognostic of poor overall survival for HGSOC but not for BLBC. This was despite a similar rate of



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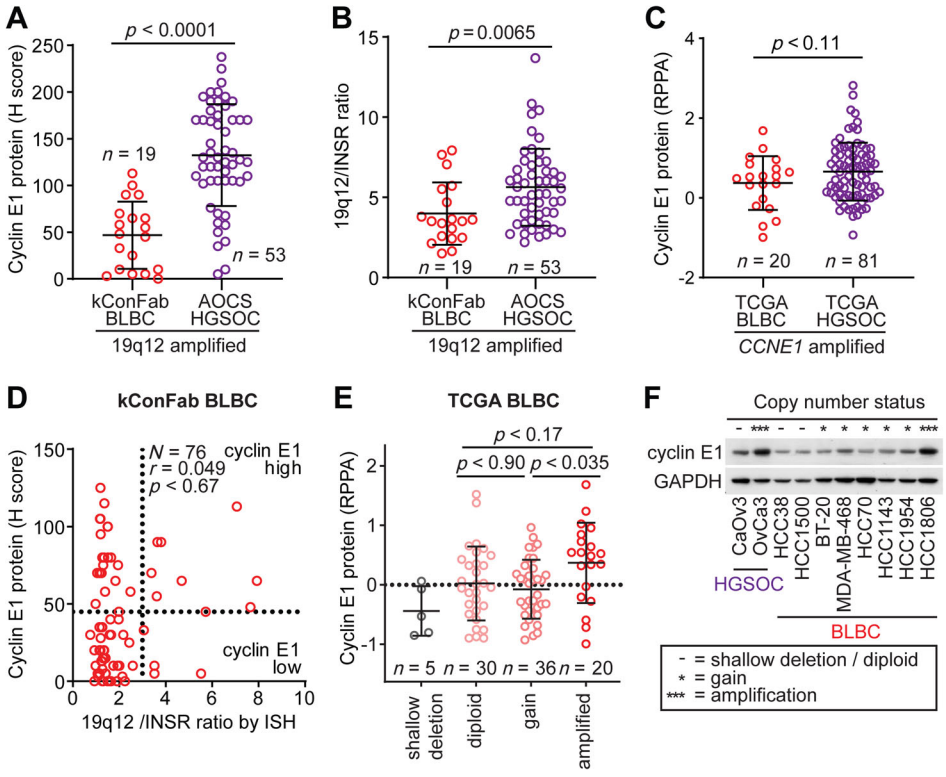

Figure 3. *CCNE1* undergoes low range amplification in BLBC, leading to moderate cyclin E1 protein expression. (A) Cyclin E1 protein expression in 19q12 amplified BLBC from kConFab and 19q12 amplified HGSOC from the AOCS. Analysed by *t*-test, error bars represent standard deviation (SD). (B) The relative 19q12/INSR ratio of the amplified subsets of HGSOC and BLBC. Analysed using a non-parametric Mann–Whitney test, error bars represent SD. (C) Cyclin E1 protein expression by RPPA in amplified BLBC and HGSOC from TCGA. Analysed by *t*-test, error bars represent SD. (D) Scatter plot of cyclin E1 protein expression versus 19q12/INSR ratio amplification status in BLBC subset of the kConFab cohort. r = Spearman coefficient for correlation analysis. Interrupted lines indicate cut-offs for high expression. (E) Relative cyclin E1 protein expression compared to copy number status in BLBC from TCGA. Analysed by one-way-ANOVA with Tukey test for multiple comparisons, error bars represent SD. (F) Western blots of HGSOC and BLBC cell lines with different degrees of *CCNE1* gene amplification. Amplification levels derived from [15]. *** = High amplification (GISTIC = 2), * = *CCNE1* gain (GISTIC = 1), – = unamplified or allelic loss (GISTIC = −1 or 0).

CCNE1 amplification across BLBC (10–25%) and HGSOC (6–34%) (Figure 2B).

To explore why *CCNE1* amplification has different prognostic value in these cancers, we examined how the level of protein expression was related to *CCNE1* amplification in BLBC and HGSOC using data from the kConFab and AOCS cohorts [7,8]. These cohorts were stained by the same protocol and used the same positive control, allowing their direct comparison. Protein expression was 2.8-fold higher in 19q12 amplified HGSOC than in 19q12 amplified BLBC ($p < 0.0001$, Figure 3A). As 19q12 amplified HGSOC had significantly more cyclin E1 protein than 19q12 amplified BLBC, we assessed the degree of 19q12 amplification

Figure 2. Meta-analysis identifies that only cyclin E1 protein expression is prognostic for BLBC, but both *CCNE1* amplification and cyclin E1 protein expression are prognostic for HGSOC. (A) Schematic of meta-analysis of cyclin E1 protein expression and *CCNE1* amplification in BLBC and HGSOC. (B) Amplification rates in studies of overall survival of BLBC ($n = 3$) and HGSOC ($n = 5$), analysed by *t*-test. Error bars represent standard deviation (SD). (C–F) Meta-analyses performed on studies using random-forest analysis of a dichotomous value of overall survival at 50 months. (C) Meta-analysis of association of high cyclin E1 protein with overall survival in HGSOC. (D) Meta-analysis of association of *CCNE1* amplification with overall survival in HGSOC. (E) Meta-analysis of association of high cyclin E1 protein with overall survival in BLBC. (F) Meta-analysis of association of *CCNE1* amplification with overall survival in BLBC. Data from studies indicated with (*) are shown in supplementary material, Figure S4.

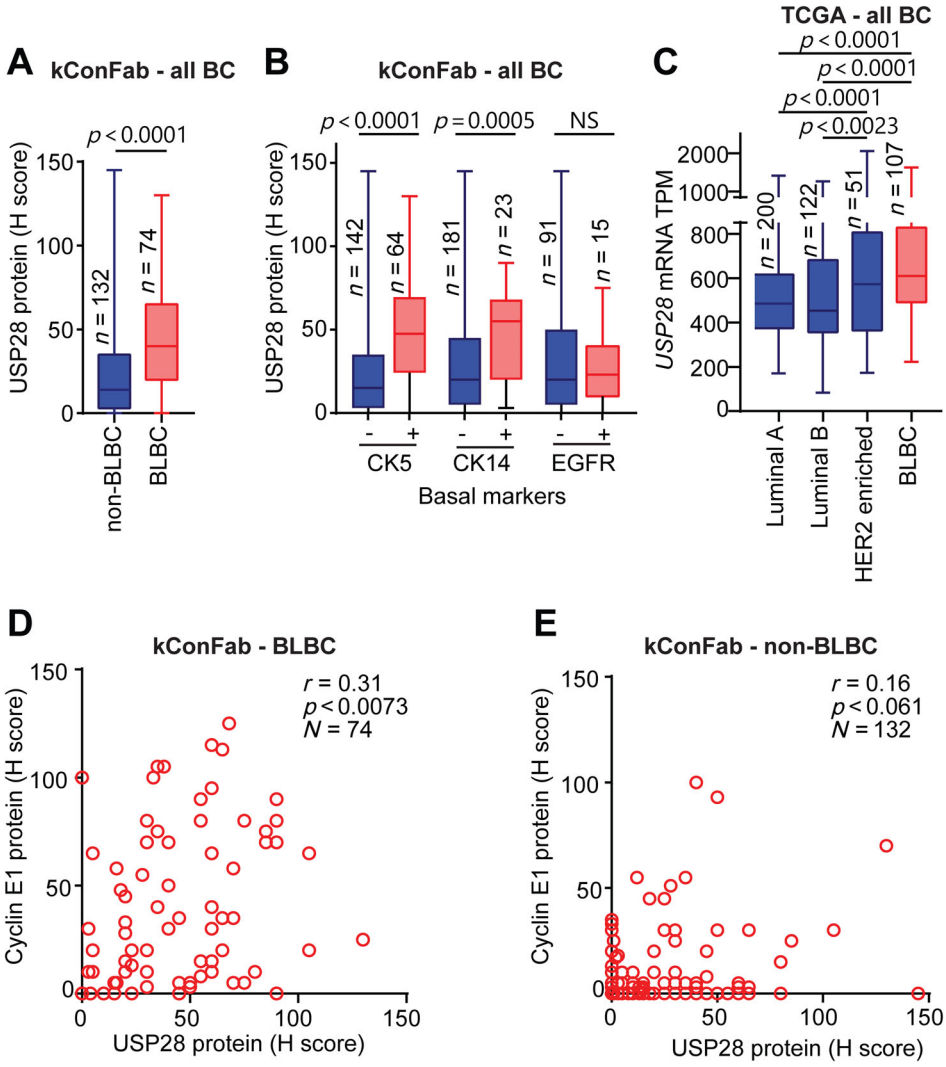

Figure 4. USP28 is high in BLBC, and correlates with high cyclin E1 protein. (A) USP28 protein expression in BLBC compared to non-BLBC in the kConFab cohort, analysed by Mann–Whitney test. (B) USP28 protein expression in breast cancers from the kConFab cohort that are positive or negative for the basal markers, CK5, CK14, and epidermal growth factor receptor (EGFR). Analysed by Mann–Whitney test. NS = non-significant. (C) *USP28* mRNA expression as normalised transcripts per million (TPM) in TCGA breast cancer cases categorised into PAM50 defined subtypes: luminal A, basal-like, HER2 enriched and luminal B. Data analysed by one-way ANOVA with Tukey test for multiple comparisons. Data in (A)–(C) are box and whisker plots with error bars of minimum to maximum, where the box extends from the 25th to the 75th percentile, and the line in the middle of the box indicates the median. (D) Scatter plot of cyclin E1 protein expression versus USP28 protein expression in BLBC subset of the kConFab cohort. (E) Scatter plot of cyclin E1 protein expression versus USP28 protein expression in non-BLBC subset of the kConFab cohort. r = Spearman coefficient for correlation analysis.

in each subset. We found that the median 19q12/INSR ratio in 19q12 amplified HGSOC was 5.2, which was significantly higher than the median ratio of 3.5 in BLBC ($p = 0.0065$, Figure 3B). In the TCGA dataset, we were unable to compare the degree of amplification, as all putative amplified cases are binned into a single amplification category of GISTIC = 2, and there was no significant difference between HGSOC and BLBC in cyclin E1 protein expression measured by RPPA ($p = 0.11$, Figure 3C).

We had previously observed that cyclin E1 protein expression is correlated positively with *CCNE1* amplification in HGSOC [8]. By contrast, there was no correlation between cyclin E1 protein expression and *CCNE1* amplification (19q12/INSR ratio) across the kConFab BLBC cohort ($r = 0.049$, $p < 0.67$, Figure 3D). Several

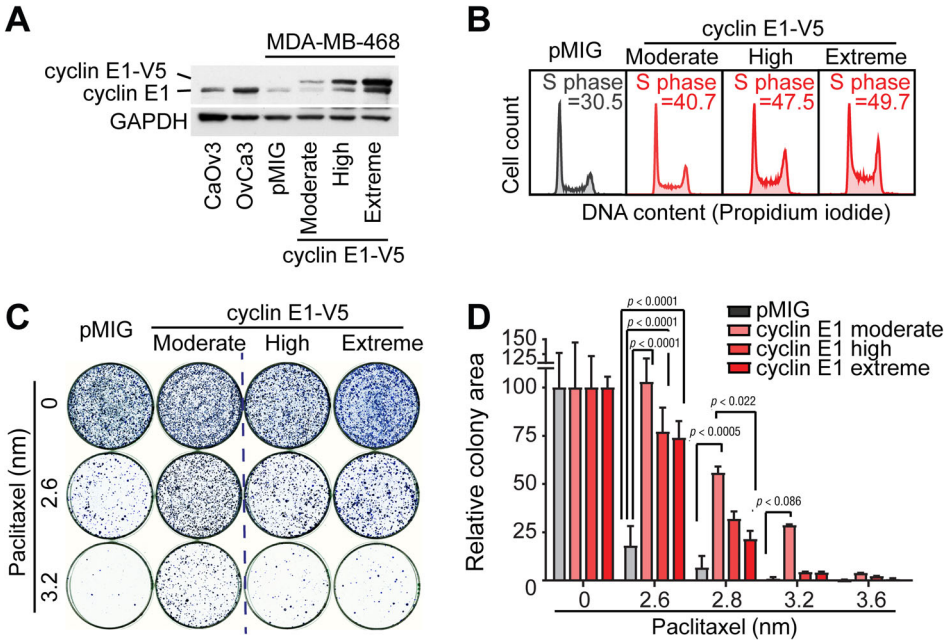

Figure 5. Moderate expression of cyclin E1 is sufficient for a survival benefit in BLBC cell line MDA-MB-468. (A) Western blot of cyclin E1 expression in HGSOC cell lines (CaOv3 and OvCa3) and in MDA-MB-468 BLBC cell line, with cyclin E1-V5 overexpression from the pMIG vector at moderate, high, and extreme levels. GAPDH is used as loading control. (B) Cell cycle phase of cyclin E1 overexpressing MDA-MB-468 cells determined by propidium iodide staining and flow cytometry. (C) MDA-MB-468 cells expressing different levels of cyclin E1 were treated with paclitaxel for 3 weeks, and analysed by colony-forming assay. (D) Quantitation of colony-forming assay performed in triplicate and analysed by two-way ANOVA with Tukey test for multiple comparisons. Error bars represent standard deviation.

BLBC showed low levels of amplification (19q12/INSR score between 3 and 6) associated with either low or high cyclin E1 protein expression. However, 3/76 (4%) BLBC showed a 19q12/INSR ratio of ≥6, indicative of high-level amplification. These three cases also had high cyclin E1 protein.

We then compared cyclin E1 protein expression in TCGA BLBC cases classed as having different copy number states. This showed that BLBC with *CCNE1* gene amplification does not have significantly higher cyclin E1 protein expression than BLBC with diploid *CCNE1* ($p < 0.17$, Figure 3E). We next examined the protein expression of BLBC and HGSOC in cell lines with different reported levels of amplification. Most BLBC cell lines had no amplification or low-level gene gain of copy number and we did not observe a substantial difference in protein expression dependent on copy number (Figure 3F). Cyclin E1 expression in these models was comparable to expression in the HGSOC model CaOv3 which lacks *CCNE1* amplification. One BLBC cell line with high-level *CCNE1* amplification (HCC1806) showed comparable cyclin E1 protein expression to the HGSOC *CCNE1*-amplified cell line, OvCa3 (Figure 3F). Overall, it appears that while cyclin E1 protein expression is frequently high in BLBC, it does not necessarily associate with *CCNE1* amplification.

High cyclin E1 protein is associated with high USP28 protein in BLBC

As *CCNE1* amplification was not associated with high cyclin E1 in BLBC, we investigated other mechanisms that could be leading to higher cyclin E1 protein. We previously showed that patients with *BRCA1* mutation, a BLBC-enriched population, have significantly higher expression of ubiquitin-specific protease 28 (USP28) [7]. USP28 is a deubiquitinase that stabilises cyclin E1 [34], and USP28 is also high in cyclin E1 high HGSOC that does not have *CCNE1* amplification [8].

We examined the expression of USP28 in BLBC and its relationship to cyclin E1 protein expression in the BLBC and non-BLBC patient subgroups. USP28 protein expression was assessed by immunostaining of formalin-fixed paraffin-embedded samples and H-score. USP28 was significantly higher in BLBC than non-BLBC in the kConFab cohort ($p < 0.0001$, Figure 4A). We also found that USP28 was significantly higher in cancers that were positive for basal

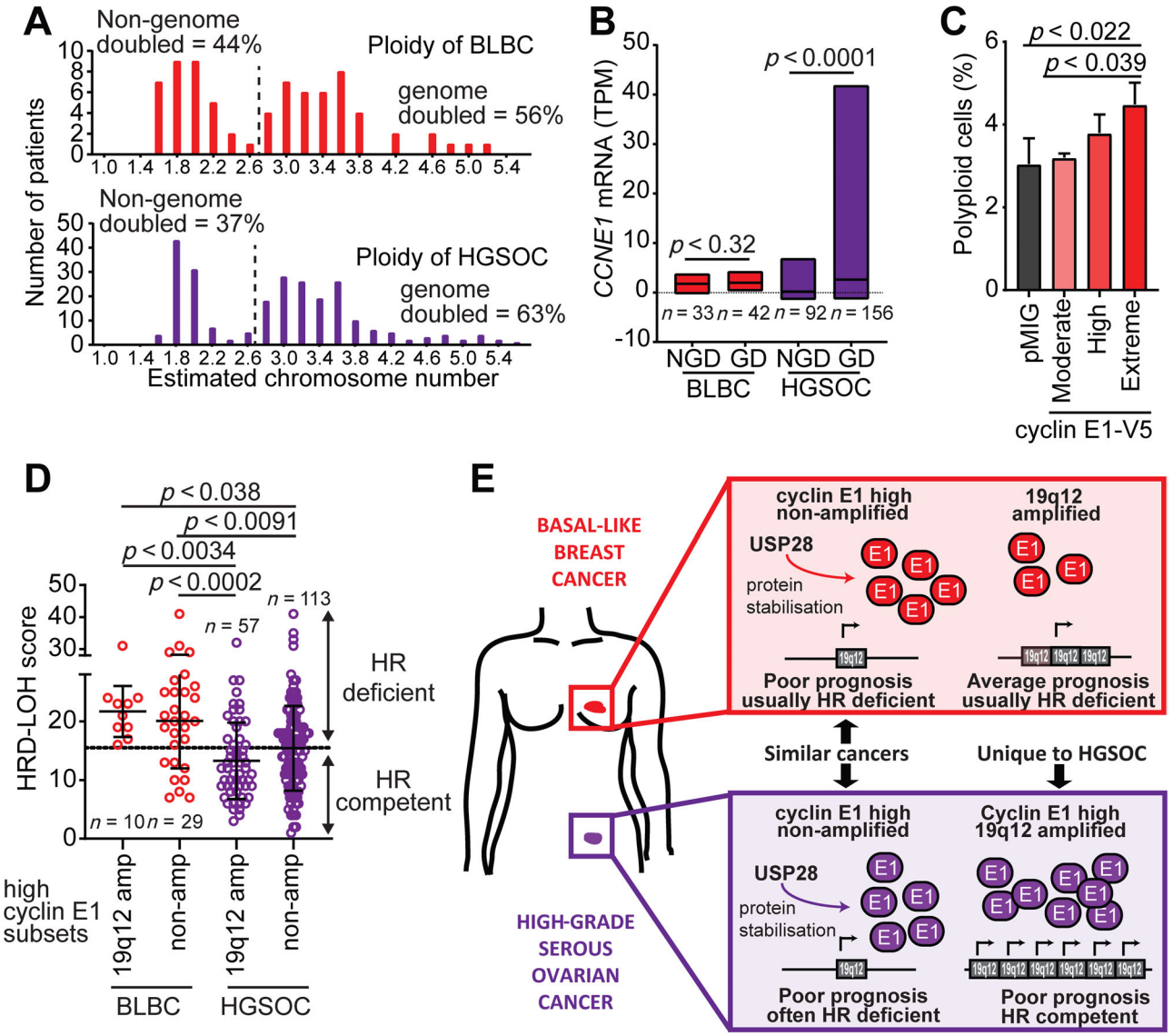

Figure 6. Increased cyclin E1 expression in the BLBC is not associated with polyploidy but is associated with homologous recombination deficiency (HRD). (A) Non-genome doubled (NGD) and genome doubled (GD) cancers of BLBC or HGSOC from the METABRIC dataset. (B) Expression of *CCNE1* mRNA in NGD and GD cancers of BLBC or HGSOC from the METABRIC dataset. Data shown with floating bars of minimum to maximum, and line is at the mean. Data analysed by Mann–Whitney test. TPM = transcripts per million. (C) Percentage of polyploid cells in cell lines overexpressing cyclin E1. Polyploidy measured by propidium iodide staining and identification of >4N populations on flow cytometry. Performed in triplicate and analysed by one-way ANOVA with Tukey post-tests, error bars represent standard deviation (SD). (D) Levels of HRD-LOH (signature score of cancers with homologous recombination deficiency) in cancer subsets of BLBC and HGSOC from TCGA. Data analysed by one-way ANOVA and error bars are SD. A dashed line is shown at HRD-LOH of 15.5 which is the cut-off for high HRD-LOH score. (E) Schematic of cyclin E1 expressing subsets of BLBC and HGSOC, and the relationship with USP28 expression, HR deficiency, and patient outcome.

markers CK5 and CK14, although not in cancers that were positive for the basal marker EGFR (epidermal growth factor receptor) (Figure 4B). We then examined the TCGA breast cancer cohort and found that *USP28* mRNA was significantly higher in BLBC than in luminal A and luminal B cancers, although HER2-enriched breast cancers had similar *USP28* mRNA expression levels to BLBC (Figure 4C).

We examined the correlation between expression of cyclin E1 and USP28 protein in BLBC and non-BLBC in the kConFab cohort. Cyclin E1 and USP28 protein expression showed a moderate and significant

correlation in BLBC ($r = 0.31$, $p < 0.0073$, Figure 4D) but had only a trend towards a poor correlation in non-BLBC ($r = 0.16$, $p < 0.061$, Figure 4E). Overall, these data suggest that USP28 is associated with the basal-like phenotype, and that cyclin E1 protein is possibly stabilised via USP28-mediated deubiquitination in BLBC.

Moderate expression of cyclin E1 is sufficient to increase survival advantage in a BLBC cell line

We had observed that *CCNE1*-amplified BLBC did not express cyclin E1 at the same level as in *CCNE1*-amplified HGSOC, and that that the overall expression of cyclin E1 protein in BLBC was lower than HGSOC regardless of amplification. This led us to speculate that very high expression of cyclin E1 that is commensurate with the expression seen in *CCNE1*-amplified HGSOC may not lead to a growth or survival advantage for some BLBC cells. We tested this hypothesis by overexpressing cyclin E1 at different levels in the BLBC cell line MDA-MB-468, which has moderate endogenous expression of cyclin E1 (Figure 3F). Using retroviral infection, we overexpressed cyclin E1.V5-fusion protein using the pMSCV vector, which co-expresses GFP using an IRES (internal ribosome entry site) sequence. Populations expressing different levels of cyclin E1.V5 were selected by flow cytometry and validated by western blot (Figure 5A). Moderate overexpression of cyclin E1 was commensurate with non-genomic overexpression seen in ovarian cancer CaOv3 cells, whereas high overexpression corresponded to the levels seen with *CCNE1* amplification in the HGSOC cell line, OvCa3 (Figure 5A). The third population, expressing 'extreme' levels of cyclin E1, was >2-fold higher than observed with amplification in OvCa3 cells.

We determined the effect that these expression levels had on the cell cycle by performing propidium iodide staining for DNA content. There was a dose-dependent effect, where the S phase fraction of the cells increased with increased expression of cyclin E1 (Figure 5B). We examined the colony-forming ability of these cell lines following paclitaxel treatment to determine the effect of cyclin E1 expression on survival. Paclitaxel reduces colony-forming ability, but cells overexpressing any level of cyclin E1 form more colonies at 2.6 nM paclitaxel (Figure 5C,D). Moderate-level cyclin E1 overexpression led to a marked increase in colony number at the higher doses of paclitaxel, but further increases in expression to 'high' and 'extreme' levels did not further increase survival (Figure 5C,D).

Overall, we found that elevated cyclin E1 expression gave a survival advantage in the MDA-MB-468 BLBC model, but that very high expression of cyclin E1 does not have additional survival benefit.

Amplification of *CCNE1* is associated with increased ploidy in HGSOC but not in BLBC

In HGSOC, the amplification of *CCNE1* is specifically associated with an increase in cancer cell ploidy [35] but this has not been determined for BLBC. We assessed the association between cyclin E1 expression and polyploidy in BLBC and HGSOC. In the METABRIC dataset, using ASCAT estimations of ploidy derived from COSMIC, 56% of BLBC and 63% of HGSOC are high ploidy cancers (Figure 6A). When the expression of *CCNE1* mRNA is compared between diploid and polyploid cancers for both cancer types, *CCNE1* mRNA is similar in both diploid and polyploid BLBC but is significantly higher in the HGSOC polyploid compared to diploid cancers (Figure 6B). We then examined our cyclin E1 overexpressing BLBC cell line for differences in ploidy by measuring >4N content by propidium iodide staining. We observed that moderate overexpression of cyclin E1 did not lead to a significant increase in polyploid cells, but polyploidy was significantly increased in the case of the 'extreme' overexpression (Figure 6C). The lack of polyploid cells in BLBC is therefore consistent with the generally lower level of *CCNE1* amplification and cyclin E1 expression observed compared to HGSOC.

High cyclin E1 protein co-occurs with homologous recombination deficiency in BLBC and HGSOC, except in HGSOC cases with *CCNE1* amplification

CCNE1 amplification is a driver of genomic instability in HGSOC and this is generally exclusive to HRD driven by *BRCA1/BRCA2* loss in HGSOC [24]. Our prior study across the kConFab cohort demonstrates that cyclin E1 high BLBC co-exists with *BRCA1* mutations and *BRCA1* methylation, suggesting that BLBC often exhibits both HRD and high cyclin E1 protein [7]. As the kConFab cohort is a familial cancer cohort that has natural enrichment for *BRCA1* and *BRCA2* mutations (Table 1) and may thus have an inherent bias, we investigated further in the TCGA datasets whether BLBC has co-occurrence of HRD and high cyclin E1 protein.

We used a genomic estimate of HRD called HRD-LOH to identify cancers that most likely have defective HR [36]. HRD-LOH quantitates the LOH regions across the genome that corresponds to damage caused

by HRD, and cancers with *BRCA1/BRCA2* mutation have high HRD-LOH (>15.5) [36]. HRD-LOH is a component of the MyChoiceHRD commercial HRD test (Myriad Genetics, Salt Lake City, UT, USA) which, in some trials, has identified patients who will have better PARP inhibitor responses [37]. Across TCGA datasets, we found high HRD-LOH in BLBC with *CCNE1* amplification/high cyclin E1 (mean HRD-LOH = 21.7) and non-amplified BLBC with high cyclin E1 (mean HRD-LOH = 20.1) (Figure 6D). *CCNE1* non-amplified HGSOC with high cyclin E1 had ∼50% high HRD-LOH (57/113, mean HRD-LOH = 15.42), and 70% (40/57) of *CCNE1*-amplified HGSOC had HRD-LOH scores less than 15.5 (mean HRD-LOH = 13.28). Thus, cyclin E1 high cancers have a consistent signature of HRD in BLBC and ∼50% of *CCNE1* non-amplified HGSOC also has high HRD-LOH score.

Discussion

Here, we describe that high expression of cyclin E1 protein by *CCNE1* amplification does not frequently occur in BLBC despite the 10–25% rate of *CCNE1* amplification in this cancer subtype. *CCNE1* amplification also does not provide any prognostic value for overall survival in BLBC, which can be explained by the lack of consistent associated protein expression.

CCNE1 amplification is associated with poor prognosis in a number of gynaecological malignancies, including HGSOC [8], endometrioid endometrial carcinomas [38], and ovarian clear cell carcinoma [39]. There are inconsistent reports on whether there is an association between *CCNE1* amplification and poor prognosis in breast cancers. *CCNE1* gene amplification was not associated with overall survival in ER+ breast cancer in the Metabric cohort [40], and was not prognostic in a study of 232 node-negative breast cancers [41]. *CCNE1* amplification was associated with poor progression-free survival in a small cohort of trastuzumab-treated HER2+ breast cancers (n = 34) [25], but not in a similar study of 185 patients [42]. In a cohort of 54 triple-negative breast cancers (TNBC) without *BRCA1/BRCA2* mutations, *CCNE1* amplification detected by targeted exome sequencing was associated with poor disease-free survival, although not poor overall survival [26]. In the study of Zhao *et al* on chemotherapy-treated TNBC disease (n = 59), which includes some BLBC cases, *CCNE1* amplification was associated with poor overall survival [27]. Confounding factors across all studies are their small size, the different cut-offs that may be used for *CCNE1* amplification, that there are other potential oncogenic driver genes in the 19q12 amplicon such as *URI* [43], and that *CCNE1* is not always included in the 19q12 amplicon as defined by arrayCGH [44]. Further larger scale studies would considerably improve our understanding of the relationship between *CCNE1* amplification and survival across different subtypes of breast cancer, including in BLBC.

A surprising observation was that *CCNE1* amplification was not significantly associated with high cyclin E1 protein expression in BLBC. *CCNE1* amplification and cyclin E1 protein expression are associated in HGSOC [8], ovarian clear cell carcinoma [39], and endometrial cancer [45]. However, in agreement with our study, an investigation of medullary-like BLBC found that two of four cancers with *CCNE1* amplification lacked cyclin E1 protein expression [46], and in colon adenocarcinoma *CCNE1* amplification and cyclin E1 protein expression are not correlated [47]. However, we note that three cases in the kConFab cohort had high-level *CCNE1/INSR* ratios of >6, and this co-occurred with high protein expression of cyclin E1 (Figure 3D). It is worth exploring if adjusting the threshold for *CCNE1* amplification could identify a poor prognosis subset of *CCNE1*-amplified BLBC, but larger cohorts are required for this to be assessed.

BLBC and HGSOC have similar rates of *CCNE1* amplification, but we found that it is only in BLBC that *CCNE1* amplification does not correlate with protein expression. BLBC also had lower relative cyclin E1 protein expression compared to HGSOC. We speculate that BLBC may have relatively lower cyclin E1 protein expression and a lack of correlation with gene amplification because of the prevalence of *BRCA1* mutation and the related 'Brcaness' phenotype in BLBC. 'Brcaness' describes tumours with *BRCA1/BRCA2*-like properties that most likely have similar HRD. Defects in HR and high genomic instability are synthetically lethal to cancer cells, with the consequence that it is highly unlikely that these aberrations are detected in the same cells. Indeed, *CCNE1* amplification occurs in ∼20% of HGSOC, and is mutually exclusive with *BRCA1/2* mutation [24] and with 'Brcaness' that occurs in up to 60% of other HGSOC cases [48]. By contrast, almost all of BLBC shows a HRD signature [49], which could preclude very high cyclin E1 protein expression through synthetic lethality [50]. We note that the MDA-MB-468 cell line used to model an example of high expression of cyclin E1 in BLBC has a 'Brcaness' phenotype [51] that is associated with a *BRCA2* mutation [52], and low

homologous repair function [53]. This cell line exhibited a survival advantage in paclitaxel with moderate cyclin E1 protein expression, but there was no further advantage in survival with very high levels of cyclin E1 commensurate with *CCNE1* amplification.

While *CCNE1* amplification is not associated with poor overall survival of BLBC patients, we show that elevated expression of cyclin E1 protein occurs in ~40% of patients and is associated with poor overall survival. It appears likely that increased cyclin E1 protein in BLBC is due in part to protein stabilisation via the deubiquitinase USP28, rather than via *CCNE1* amplification. Our study shows that USP28 is expressed at significantly higher levels in BLBC than non-BLBC (Figure 4). Prior work has also suggested that USP28 may contribute to the basal phenotype, as the downregulation of USP28 led to decrease of the basal marker CK14, in association with a shift from basal to luminal cellular morphology [54], and in lung cancer the deletion of USP28 reduces the number of lesions with expression of CK5 [55]. We find that USP28 is high in CK5/CK14-positive BLBC, and it correlates with high cyclin E1 protein. This suggests that USP28-mediated stabilisation may contribute to high cyclin E1 in BLBC, consistent with our prior observation that USP28 siRNA treatment led to increased cyclin E1 protein expression in a BLBC cell line, MDA-MB-468 [7]. Our previous work has also demonstrated that cyclin E1 protein is stabilised by loss of phosphorylation in association with *BRCA1* mutation [7]. As BLBC has high-frequency *BRCA1* mutation, it is likely that cyclin E1 protein is stabilised by multiple mechanisms in BLBC.

As part of this study, we have compared BLBC to HGSOC, which has two distinct subgroups with high cyclin E1 expression: *CCNE1* amplified and *CCNE1* non-amplified [8]. We conclude that *CCNE1* amplification has different associations with protein expression and patient outcome in BLBC and HGSOC, and that cyclin E1 high BLBC are most similar to *CCNE1* non-amplified HGSOC. Therapies and biomarkers developed for *CCNE1*-amplified HGSOC, an essentially *BRCA1/BRCA2* wild-type population, are unlikely to be transferable to BLBC. Importantly, the detection of *CCNE1* amplification in BLBC via commercial genomic testing platforms is less likely to offer any benefit for therapy matching, unless accompanied by IHC to confirm cyclin E1 protein overexpression.

While BLBC does not resemble *CCNE1*-amplified HGSOC, we can conclude that BLBC and *CCNE1* non-amplified HGSOC that overexpress cyclin E1 protein show similarity in their drivers. Common features are moderate/high cyclin E1 protein, *BRCA1* mutation, and possible 'Brcaness' phenotypes, as well as high expression of the deubiquitinase USP28 [8] (Figure 6E). Consequently, these cancers may have similar therapeutic targets such as combination Wee1 and PARP inhibitors [56,57], or combination CDK2 inhibitors and PARP inhibitors [7]. Overall, given the differences identified between HGSOC and BLBC subsets, a cautious approach should be taken rather than grouping these cancers for clinical trials on the basis of *CCNE1* amplification or cyclin E1 protein expression status alone.

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Author contributions statement

CEC and DA conceived and designed the study. kConFab and AOCS collated and provided materials, and updated patient data. DA and CEC collected, analysed and interpreted the data. CL, KF and ZP undertook experiments and analysed the data. CEC and DA extracted data for the meta-analysis, and performed the analysis. VC advised on design and implementation of the meta-analysis. PW provided intellectual input into the study design and interpretation. CEC and DA drafted the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Ethics board approval for the kConFab study is described in Mann *et al* (2008) [58], and was obtained for patients' recruitment, sample collection, and research studies. Written informed consent was obtained from all participants as described [58].

Data availability statement

Datasets generated from the AOCS and kConfab studies during the current study are not publicly available due to reasons of patient confidentiality. Processed and de-identified datasets are available from the corresponding author on reasonable request. Access to datasets and samples can be directly requested from AOCS (www.aocstudy.org) and kConFab (www.kConFab.org).

Publicly available datasets used to generate figures (Metabric, TCGA, COSMIC) are described in Materials and Methods section. Additional supporting datasets that are not restricted by patient confidentiality are also available from the corresponding author on reasonable request.

References

1. The Cancer Genome Atlas Network. Comprehensive molecular portraits of human breast tumours. *Nature* 2012; **490**: 61.
2. Begg CB, Rice MS, Zabor EC, *et al*. Examining the common aetiology of serous ovarian cancers and basal-like breast cancers using double primaries. *Br J Cancer* 2017; **116**: 1088.
3. Pothuri B, O'Cearbhaill R, Eskander R, *et al*. Frontline PARP inhibitor maintenance therapy in ovarian cancer: a Society of Gynecologic Oncology practice statement. *Gynecol Oncol* 2020; **159**: 8–12.
4. Grinda T, Delaloge S. Survival benefits of PARP inhibitors in advanced breast cancer: a mirage? *Ann Oncol* 2020; **31**: 1432–1434.
5. Aarts M, Sharpe R, Garcia-Murillas I, *et al*. Forced mitotic entry of S-phase cells as a therapeutic strategy induced by inhibition of WEE1. *Cancer Discov* 2012; **2**: 524–539.
6. Au-Yeung G, Lang F, Azar WJ, *et al*. Selective targeting of cyclin E1-amplified high-grade serous ovarian cancer by cyclin-dependent kinase 2 and AKT inhibition. *Clin Cancer Res* 2017; **23**: 1862–1874.
7. Aziz D, Portman N, Fernandez KJ, *et al*. Cyclin E1 protein is stabilized in *BRCA1* mutated breast cancers leading to synergy between CDK2 and PARP inhibitors. *npj Breast Cancer* 2021; **7**: 111.
8. Aziz D, Etemadmoghadam D, Caldon CE, *et al*. 19q12 amplified and non-amplified subsets of high grade serous ovarian cancer with overexpression of cyclin E1 differ in their molecular drivers and clinical outcomes. *Gynecol Oncol* 2018; **151**: 327–336.
9. Ciriello G, Gatza ML, Beck AH, *et al*. Comprehensive molecular portraits of invasive lobular breast cancer. *Cell* 2015; **163**: 506–519.
10. Curtis C, Shah SP, Chin SF, *et al*. The genomic and transcriptomic architecture of 2,000 breast tumours reveals novel subgroups. *Nature* 2012; **486**: 346–352.
11. Bell D, Berchuck A, Birrer M, *et al*. Integrated genomic analyses of ovarian carcinoma. *Nature* 2011; **474**: 609–615.
12. Chappuis PO, Donato E, Goffin JR, *et al*. Cyclin E expression in breast cancer: predicting germline *BRCA1* mutations, prognosis and response to treatment. *Ann Oncol* 2005; **16**: 735–742.
13. Sheehan KM, Calvert VS, Kay EW, *et al*. Use of reverse phase protein microarrays and reference standard development for molecular network analysis of metastatic ovarian carcinoma. *Mol Cell Proteomics* 2005; **4**: 346–355.
14. Mermel CH, Schumacher SE, Hill B, *et al*. GISTIC2.0 facilitates sensitive and confident localization of the targets of focal somatic copy-number alteration in human cancers. *Genome Biol* 2011; **12**: R41.
15. Barretina J, Caponigro G, Stransky N, *et al*. The Cancer Cell Line Encyclopedia enables predictive modelling of anticancer drug sensitivity. *Nature* 2012; **483**: 603–607.
16. Van Loo P, Nordgard SH, Lingjærde OC, *et al*. Allele-specific copy number analysis of tumors. *Proc Natl Acad Sci U S A* 2010; **107**: 16910–16915.
17. Tate JG, Bamford S, Jubb HC, *et al*. COSMIC: the Catalogue Of Somatic Mutations In Cancer. *Nucleic Acids Res* 2019; **47**: D941–D947.

18. Marquard AM, Eklund AC, Joshi T, *et al*. Pan-cancer analysis of genomic scar signatures associated with homologous recombination deficiency suggests novel indications for existing cancer drugs. *Biomark Res* 2015; **3**: 9.
19. Sterne JAC, Harbord RM. Funnel plots in meta-analysis. *Stata J* 2004; **4**: 127–141.
20. The Cochrane Collaboration. Review Manager (RevMan) [Computer Program] Version 5.4. 2020.
21. Domcke S, Sinha R, Levine DA, *et al*. Evaluating cell lines as tumour models by comparison of genomic profiles. *Nat Commun* 2013; **4**: 2126.
22. Neve RM, Chin K, Fridlyand J, *et al*. A collection of breast cancer cell lines for the study of functionally distinct cancer subtypes. *Cancer Cell* 2006; **10**: 515–527.
23. GraphPad Prism 9.3.1 for Windows. GraphPad Software. San Diego, CA, 2021.
24. Etemadmoghadam D, Weir BA, Au-Yeung G, *et al*. Synthetic lethality between *CCNE1* amplification and loss of *BRCA1*. *Proc Natl Acad Sci U S A* 2013; **110**: 19489–19494.
25. Scaltriti M, Eichhorn PJ, Cortés J, *et al*. Cyclin E amplification/overexpression is a mechanism of trastuzumab resistance in HER2+ breast cancer patients. *Proc Natl Acad Sci U S A* 2011; **108**: 3761–3766.
26. Huang X, Shao D, Wu H, *et al*. Genomic profiling comparison of germline BRCA and non-BRCA carriers reveals CCNE1 amplification as a risk factor for non-BRCA carriers in patients with triple-negative breast cancer. *Front Oncol* 2020; **10**: 583314.
27. Zhao Z-M, Yost SE, Hutchinson KE, *et al*. CCNE1 amplification is associated with poor prognosis in patients with triple negative breast cancer. *BMC Cancer* 2019; **19**: 96–96.
28. Potemski P, Kusinska R, Watala C, *et al*. Prognostic relevance of basal cytokeratin expression in operable breast cancer. *Oncology* 2005; **69**: 478–485.
29. Nakayama N, Nakayama K, Shamima Y, *et al*. Gene amplification CCNE1 is related to poor survival and potential therapeutic target in ovarian cancer. *Cancer* 2010; **116**: 2621–2634.
30. Heeran MC, Høgdall CK, Kjaer SK, *et al*. Limited prognostic value of tissue protein expression levels of cyclin E in Danish ovarian cancer patients: from the Danish 'MALOVA' ovarian cancer study. *APMIS* 2012; **120**: 846–854.
31. Karst AM, Jones PM, Vena N, *et al*. Cyclin E1 deregulation occurs early in secretory cell transformation to promote formation of fallopian tube-derived high-grade serous ovarian cancers. *Cancer Res* 2014; **74**: 1141–1152.
32. Etemadmoghadam D, George J, Cowin PA, *et al*. Amplicon-dependent CCNE1 expression is critical for clonogenic survival after cisplatin treatment and is correlated with 20q11 gain in ovarian cancer. *PLoS One* 2010; **5**: e15498.
33. Stronach EA, Paul J, Timms KM, *et al*. Biomarker assessment of HR deficiency, tumor BRCA1/2 mutations, and CCNE1 copy number in ovarian cancer: associations with clinical outcome following platinum monotherapy. *Mol Cancer Res* 2018; **16**: 1103–1111.
34. Wang X, Liu Z, Zhang L, *et al*. Targeting deubiquitinase USP28 for cancer therapy. *Cell Death Dis* 2018; **9**: 186–186.
35. Etemadmoghadam D, Au-Yeung G, Wall M, *et al*. Resistance to CDK2 inhibitors is associated with selection of polyploid cells in CCNE1-amplified ovarian cancer. *Clin Cancer Res* 2013; **19**: 5960–5971.
36. Abkevich V, Timms KM, Hennessy BT, *et al*. Patterns of genomic loss of heterozygosity predict homologous recombination repair defects in epithelial ovarian cancer. *Br J Cancer* 2012; **107**: 1776.
37. Pellegrino B, Mateo J, Serra V, *et al*. Controversies in oncology: are genomic tests quantifying homologous recombination repair deficiency (HRD) useful for treatment decision making? *ESMO Open* 2019; **4**: e000480.
38. Nakayama K, Rahman MT, Rahman M, *et al*. CCNE1 amplification is associated with aggressive potential in endometrioid endometrial carcinomas. *Int J Oncol* 2016; **48**: 506–516.
39. Ayhan A, Kuhn E, Wu R-C, *et al*. CCNE1 copy-number gain and overexpression identify ovarian clear cell carcinoma with a poor prognosis. *Mod Pathol* 2017; **30**: 297–303.
40. Milioli HH, Alexandrou S, Lim E, *et al*. Cyclin E1 and cyclin E2 in ER+ breast cancer: prospects as biomarkers and therapeutic targets. *Endocr Relat Cancer* 2020; **27**: R93–R112.
41. Callagy G, Pharoah P, Chin S-F, *et al*. Identification and validation of prognostic markers in breast cancer with the complementary use of array-CGH and tissue microarrays. *J Pathol* 2005; **205**: 388–396.
42. Luhtala S, Staff S, Tanner M, *et al*. Cyclin E amplification, overexpression, and relapse-free survival in HER-2-positive primary breast cancer. *Tumour Biol* 2016; **37**: 9813–9823.
43. Theurillat JP, Metzler SC, Henzi N, *et al*. URI is an oncogene amplified in ovarian cancer cells and is required for their survival. *Cancer Cell* 2011; **19**: 317–332.
44. Natrajan R, Mackay A, Wilkerson PM, *et al*. Functional characterization of the 19q12 amplicon in grade III breast cancers. *Breast Cancer Res* 2012; **14**: R53.
45. Noske A, Brandt S, Valtcheva N, *et al*. Detection of CCNE1/URI (19q12) amplification by in situ hybridisation is common in high grade and type II endometrial cancer. *Oncotarget* 2017; **8**: 14794–14805.
46. Rodríguez-Pinilla SM, Rodríguez-Gil Y, Moreno-Bueno G, *et al*. Sporadic invasive breast carcinomas with medullary features display a basal-like phenotype: an immunohistochemical and gene amplification study. *Am J Surg Pathol* 2007; **31**: 501–508.
47. Bondi J, Husdal A, Bukholm G, *et al*. Expression and gene amplification of primary (A, B1, D1, D3, and E) and secondary (C and H) cyclins in colon adenocarcinomas and correlation with patient outcome. *J Clin Pathol* 2005; **58**: 509–514.
48. Goundiam O, Gestraud P, Popova T, *et al*. Histo-genomic stratification reveals the frequent amplification/overexpression of CCNE1 and BRD4 genes in non-BRCAness high grade ovarian carcinoma. *Int J Cancer* 2015; **137**: 1890–1900.
49. Polak P, Kim J, Braunstein LZ, *et al*. A mutational signature reveals alterations underlying deficient homologous recombination repair in breast cancer. *Nat Genet* 2017; **49**: 1476–1486.
50. Bartek J, Hodny Z. PARK2 orchestrates cyclins to avoid cancer. *Nat Genet* 2014; **46**: 527–528.
51. Roig B, Rodríguez-Balada M, Samino S, *et al*. Metabolomics reveals novel blood plasma biomarkers associated to the BRCA1-mutated phenotype of human breast cancer. *Sci Rep* 2017; **7**: 17831.

52. Meulemans L, Mesman RLS, Caputo SM, *et al*. Skipping nonsense to maintain function: the paradigm of BRCA2 exon 12. *Cancer Res* 2020; **80**: 1374–1386.
53. Mao Z, Jiang Y, Liu X, *et al*. DNA repair by homologous recombination, but not by nonhomologous end joining, is elevated in breast cancer cells. *Neoplasia* 2009; **11**: 683–691.
54. Wu Y, Wang Y, Yang XH, *et al*. The deubiquitinase USP28 stabilizes LSD1 and confers stem-cell-like traits to breast cancer cells. *Cell Rep* 2013; **5**: 224–236.
55. Ruiz EJ, Pinto-Fernandez A, Turnbull AP, *et al*. USP28 deletion and small molecule inhibition destabilises c-Myc and elicits regression of squamous cell lung carcinoma. *Elife* 2021; **10**: e71596.
56. Chen X, Yang D, Carey JPW, *et al*. Targeting replicative stress and DNA repair by combining PARP and Wee1 kinase inhibitors is synergistic in triple negative breast cancers with cyclin E or BRCA1 alteration. *Cancers (Basel)* 2021; **13**: 1656.
57. Ha DH, Min A, Kim S, *et al*. Antitumor effect of a WEE1 inhibitor and potentiation of olaparib sensitivity by DNA damage response modulation in triple-negative breast cancer. *Sci Rep* 2020; **10**: 9930.
58. Mann GJ, Thorne H, Balleine RL, *et al*. Analysis of cancer risk and *BRCA1* and *BRCA2* mutation prevalence in the kConFab familial breast cancer resource. *Breast Cancer Res* 2006; **8**: R12.
59. Alexandrou S, George SM, Ormandy CJ, *et al*. The proliferative and apoptotic landscape of basal-like breast cancer. *Int J Mol Sci* 2019; **20**: 667.
60. Won JR, Gao D, Chow C, *et al*. A survey of immunohistochemical biomarkers for basal-like breast cancer against a gene expression profile gold standard. *Mod Pathol* 2013; **26**: 1438–1450.
61. Oliveras-Ferraros C, Vazquez-Martin A, Martin-Castilló B, *et al*. Pathway-focused proteomic signatures in HER2-overexpressing breast cancer with a basal-like phenotype: new insights into de novo resistance to trastuzumab (Herceptin). *Int J Oncol* 2010; **37**: 669–678.
62. Bagaria SP, Ray PS, Wang J, *et al*. Prognostic value of basal phenotype in HER2-overexpressing breast cancer. *Ann Surg Oncol* 2012; **19**: 935–940.
63. Vincent-Salomon A, Gruel N, Lucchesi C, *et al*. Identification of typical medullary breast carcinoma as a genomic sub-group of basal-like carcinomas, a heterogeneous new molecular entity. *Breast Cancer Res* 2007; **9**: R24.
64. Stenger M. Calculating H-Score. The ASCO Post. 2015. [Accessed 10 February 2022]. Available from: https://ascopost.com/issues/april-10-2015/calculating-h-score/
65. Young AIJ, Law AMK, Castillo L, *et al*. MCL-1 inhibition provides a new way to suppress breast cancer metastasis and increase sensitivity to dasatinib. *Breast Cancer Res* 2016; **18**: 125.
66. Caldon CE, Sergio CM, Burgess A, *et al*. Cyclin E2 induces genomic instability by mechanisms distinct from cyclin E1. *Cell Cycle* 2013; **12**: 606–617.
67. Caldon CE, Lee CS, Sutherland RL, *et al*. Wilms' tumor protein 1: an early target of progestin regulation in T-47D breast cancer cells that modulates proliferation and differentiation. *Oncogene* 2008; **27**: 126–138.
68. Chia K, Milioli H, Portman N, *et al*. Non-canonical AR activity facilitates endocrine resistance in breast cancer. *Endocr Relat Cancer* 2019; **26**: 251–264.
69. Guzmán C, Bagga M, Kaur A, *et al*. ColonyArea: an ImageJ plugin to automatically quantify colony formation in clonogenic assays. *PLoS One* 2014; **9**: e92444.
70. D'Agostino RB, Belanger A, D'Agostino RB Jr. A suggestion for using powerful and informative tests of normality. *Am Stat* 1990; **44**: 316–321.

References 59–70 are cited only in the supplementary material.

SUPPLEMENTARY MATERIAL ONLINE

Supplementary materials and methods

Figure S1. Distribution of cyclin E1 H-scores for BLBC and non-BLBC of the kConFab cohort

Figure S2. PRISMA schematics for identification of studies to include in meta-analyses

Figure S3. FUNNEL plots of meta-analyses

Figure S4. Kaplan–Meier analysis of BLBC cases from the kConFab, TCGA, and Metabric cohorts