REVIEW

Cyclin E1 and cyclin E2 in ER+ breast cancer: prospects as biomarkers and therapeutic targets

H H Milioli1,2, S Alexandrou1, E Lim1,2 and C E Caldon1,2

1Garvan Institute of Medical Research, Darlinghurst, Sydney, New South Wales, Australia
2St Vincent's Clinical School, UNSW Sydney, Sydney, New South Wales, Australia

Correspondence should be addressed to C E Caldon: l.caldon@garvan.org.au

This article was commissioned following sponsorship of the 7th PacRim Breast and Prostate Meeting by Endocrine-Related Cancer

Abstract

Cyclin E1 is one of the most promising biomarkers in estrogen receptor positive (ER+) breast cancer for response to the new standard of care drug class, CDK4/6 inhibitors. Because of its strong predictive value, cyclin E1 expression may be used in the future to triage patients into potential responders and non-responders. Importantly, cyclin E1 is highly related to cyclin E2, and both cyclin E1 and cyclin E2 are estrogen target genes that can facilitate anti-estrogen resistance and can be highly expressed in breast cancer. However, cyclin E1 and E2 are often expressed in different subsets of patients. This raises questions about whether the expression of cyclin E1 and cyclin E2 have different biological drivers, if high expressing subsets represent different clinical subtypes, and how to effectively develop a biomarker for E-cyclin expression. Finally, several pan-CDK inhibitors that target cyclin E-CDK2 activity have reached Phase II clinical trials. In this review, we outline the data identifying that different cohorts of patients have high expression of cyclins E1 and E2 in ER+ cancer and address the implications for biomarker and therapeutic development.

Cyclin E1 and E2 are core cell cycle proteins

The cell cycle is the core machinery that a cell engages in order to undergo a round of DNA replication, replicate its cellular contents, and undergo a cell division. Each cell cycle consists of functional phases: G1 is the phase where cells ready themselves for DNA replication and commit to a cell cycle, during S phase cells undergo DNA replication, and G2/M coordinates mitosis. The G1 to S phase of the cell cycle has particular importance in cancer, as this is the point where cells commit to another round of cell division, and this threshold is weakened in many cancer cells. The gatekeeper for the G1/S phase transition is the Retinoblastoma (Rb) protein which in its unphosphorylated form inhibits progression into S phase. During G1/S phase, cyclin dependent kinases (CDKs) phosphorylate Rb; first CDK4/6 is activated by cyclin D1, D2, or D3 to phosphorylate Rb, followed by phosphorylation by CDK2 in complex with cyclin E1 or cyclin E2 (Table 1). Phosphorylated Rb releases E2F transcription factors to upregulate genes necessary for DNA replication and mitosis. The cyclin E1/E2-CDK2 complexes, as well as phosphorylating Rb, phosphorylate a large number of other target proteins involved in DNA replication and histone transcription. In this way the CDK2 kinase synchronises multiple cell cycle events to
allow for successful DNA replication and cell division. A further level of control is added through the CDK inhibitor proteins p27Kip1 and p21Waf1, which can inhibit the CDK4, CDK6, and CDK2 complexes to fine tune their activity (Fig. 1).

In addition to their canonical S phase roles, cyclins E1 and E2 coordinate other cell cycle functions, often without a requirement for CDK2. Cyclin E1 and E2 physically localize to centrosomes (Rogers et al., 2015), where cyclin E1 binds to Minichromosome Maintenance Complex Component 5 (MCM5) (Ferguson & Maller, 2008) and cyclins E1 and E2 facilitate loading of MCM replicative helicases in cells exiting quiescence (Geng et al., 2007). Cyclin E1 promotes proliferation in a CDK2 independent manner in hepatocytes and hepatocellular carcinoma, and this may be due to the kinase independent roles at the centrosome and during re-entry from quiescence (Geng et al., 2015).

The major isoform of cyclin E1 is a 395 amino acid protein transcribed from the 19q12 locus, and cyclin E2 encodes a 69.3% similar protein of 404 amino acids, transcribed from an independent gene located at chromosome 8q22. As we have described previously (Caldon & Musgrove, 2010), the E-cyclin genes are conserved in vertebrates, but a single E-type cyclin is encoded in invertebrates such as Drosophila melanogaster. There are some critical differences in function between the two E-cyclins, the most striking of which is that cyclin E2/-/- male mice are infertile, while cyclin E1/-/- mice have normal fertility. There are also some examples of CDK targets that appear unique to either cyclin: SAMHD1, which controls dNTP cellular pools, is a specific target of cyclin E2-CDK2 (Hu et al., 2018), and NPAT, a master regulator of histone transcription, which preferentially co-localises with cyclin E2 (Rogers et al., 2015). Another key difference between cyclin E1 and E2 is that cyclin E1 can be cleaved into a low molecular weight (LMW) protein, which hyperactivates CDK2, has increased cytoplasmic localisation and altered substrate interactions (Caruso et al., 2018).

Despite these differences, cyclins E1 and E2 can substitute for many of each other’s function (Geng et al., 2003) and show similar cell cycle expression (Gudas et al., 1999). What has become increasingly apparent is that cyclin E1 and E2 diverge in their expression in cancer (Caldon et al., 2009, 2013a,b, Caldon & Musgrove, 2010). This could represent activation by distinct transcriptional cascades or regulators, independent amplification events, or it may represent differences in function.

**Table 1** Cyclin-CDK complexes in the cell cycle.

<table>
<thead>
<tr>
<th>Phase of cell cycle</th>
<th>Cyclin</th>
<th>CDK binding partner</th>
<th>CDK inhibitors</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>S phase transition</td>
<td>Cyclin E1/E2</td>
<td>CDK2</td>
<td>p21Cip1/Waf1, p27kip1, p57kip2</td>
<td>(Prall et al., 1997)</td>
</tr>
<tr>
<td>G2/M phase transition</td>
<td>Cyclin A2, Cyclin B1</td>
<td>CDK1 (CDC2)</td>
<td>WEE1, MYT1</td>
<td>(Yam et al., 2002, Fung &amp; Poon, 2005)</td>
</tr>
</tbody>
</table>

**Figure 1**

Activation and de-activation of cyclin E1/E2-CDK2. CDK2 binds with either cyclin E1 or cyclin E2 to form cyclin E-CDK2 complexes. Cyclin E1 monomers can be ubiquitinated by Cul3 and SPOP and cyclin E1/E2 monomers by SCF complexes, leading to proteosomal degradation. Cyclin E-CDK2 complexes can be inhibited by CDK inhibitors p21Waf1/Cip1, p27kip1, and p57kip2, but cyclin D-CDK4/6 complexes compete for these inhibitors. Active cyclin E-CDK2 complexes autophosphorylate cyclin E, priming for further GSK-3β mediated phosphorylation. Phosphorylated cyclin E is recognised for SCF mediated ubiquitination, followed by proteosomal degradation.

**Altersations to cyclin E1 and cyclin E2 in cancer**

**Cyclin E1 and cyclin E2 drive proliferation and genome instability in cancer**

As core cell cycle regulators, cyclins E1 and E2 have the capacity to drive excessive proliferation when deregulated...
in cancer. Cyclin E1 can act as a bona fide oncogene to drive the formation of tumours when constitutively overexpressed in the mouse mammary gland, albeit with low penetrance (10%) and long latency (Bortner & Rosenberg 1997). In many cancer cell line models cyclin E1 overexpression will increase the proportion of cells in S phase, leading to increased Rb phosphorylation and cell proliferation (Hwang & Clurman 2005). Cyclin E2 overexpression has not been assessed in mouse models, but in cell line models its overexpression also leads to increased S phase fraction and proliferation (Zariwala et al. 1998, Gudas et al. 1999). Inhibition of CDK2 has a profound anti-proliferative effect (Horiuchi et al. 2012), and downregulation of either cyclin E1 or E2 can inhibit proliferation (Caldon et al. 2009).

The cyclic expression of cyclins E1 and E2 is important to its cell cycle functions, and perturbing the degradation of the E-cyclins in S phase can lead to genomic instability. Excess cyclin E1-CDK2 in S phase causes replication stress resulting in the under-replication of DNA in late S phase and genomic deletions (Teixeira et al. 2015). Persistent expression of cyclin E1-CDK2 at mitosis can then inhibit the APC/C^{cdh1} complex and proper mitotic exit, leading to the misalignment of chromosomes at the metaphase plate. This results in chromosome mis-segregation and polyploidy (Keck et al. 2007). Excess mitotic cyclin E1-CDK2 also results in persistent CENPA Ser18 phosphorylation, which disrupts proper centromere formation and leads to mitotic defects (Takada et al. 2017).

Cyclin E2-CDK2 also causes genomic instability, but its mechanism is less well characterised (Caldon et al. 2013b). Unlike cyclin E1, genomic instability via cyclin E2 does not occur downstream of APC/C^{cdh1} (Caldon et al. 2013b), but its overexpression causes the mis-segregation of chromosomes (Duffy et al. 2016).

Increased expression through increased transcription

The cyclin E1 and E2 genes, CCNE1 and CCNE2, are frequently observed at high levels in solid cancers, as a result of gene amplification and gene deregulation. CCNE1 occurs in the large 19q12 amplicon, and 19q12 amplification associated with cyclin E1 overexpression is believed to be a driver event in about 20% of high grade serous ovarian cancers (Karst et al. 2014). The role of cyclin E1 amplification as a driver in breast cancer is less certain (Natrajan et al. 2012). Chromosome region 8q22, which includes the CCNE2 gene, is also frequently amplified, although several other genes from this region have been highlighted as driver oncogenes for this amplification event (Li et al. 2010).

Cyclins E1 and E2 undergo strong cyclical regulation due to their pivotal roles in cell cycle progression. In G1 and G2, the CCNE1 and CCNE2 genes are repressed via inhibitory deacetylation and methylation of their promoter regions (Caldon & Musgrove 2010). Both cyclin E1 and E2 are then upregulated during late G1, following E2F transcription factor activation, along with the recruitment of co-factors such as CARM1, SRC, and CHD8 (reviewed in Caldon & Musgrove 2010). Consequently, cyclin E1 and E2 gene expression is responsive to growth factor signalling downstream of SRC, and other factors. Further differential expression of CCNE1 and CCNE2 expression is driven through miRNA networks that individually target each gene (reviewed in Caldon & Musgrove 2010).

Increased expression through altered degradation

As cyclical proteins, both cyclin E1 and cyclin E2 are downregulated during late S phase through ubiquitin-mediated proteasomal degradation (Fig. 1). This is first primed by phosphorylation of the cyclin E1 and cyclin E2 proteins on regions of the protein called ‘phospho- degrons’. The phosphorylation sites of cyclin E1 have been described in detail (Welcker et al. 2003) and these sites facilitate recognition by the F-box protein FBXW7. Several sites are autophosphorylated as part of the cyclin E-CDK2 complex, and these modifications then prime for additional phosphorylation by GSK3β (Welcker et al. 2003). Downregulation of GSK3β via PI3K/Akt signaling can, in this way, disrupt cyclin E1 stability (Siu et al. 2012).

Despite these common phospho-degrons in the cyclin E1 and E2 proteins, the degradation of cyclins E1 and E2 is asynchronous, implying different mediators of degradation (Caldon et al. 2013a). Cyclins E1 and E2 are both targeted by Skp1-Cullin-F-box (SCF) E3 ubiquitin ligases, where the F-box adaptor protein recognises and binds the cyclin protein. The F-box protein FBXW7 (Klotz et al. 2009) binds to cyclin E-CDK2 complexes, though FBXW7 targeting of cyclin E2 is ineffective in breast cancer cells (Caldon et al. 2013a). Cyclin E1 monomers are additionally targeted by Skp2 (Nakayama et al. 2000), SPOP (Ju et al. 2019), and CUL3 (Davidge et al. 2019) for ubiquitination and subsequent proteosomal degradation. In the case of CUL3 this is specific to cyclin E1, as CUL3 does not ubiquitinate cyclin E2 or LMW cyclin E1 (Davidge et al. 2019). For cyclin E2 turnover, other ubiquitin ligases are likely to be important, as indicated
by the cyclin E2-specific turnover mediated by calcium dependent signaling (Perez-Neut et al. 2015).

Failure to degrade cyclin E1 and cyclin E2 can lead to increased proliferation and genomic instability. Cyclin stabilisation can occur through changes to the ubiquitin ligases that target cyclins E1 and E2. Fbxw7 can be disabled by mutation or gene methylation and occurs at low levels in ER+ breast cancer, although its loss is not particularly correlated with survival for these patients (Wei et al. 2012). SPOP is frequently reduced in cancer and associated with either mutation or loss of heterozygosiy events (Li et al. 2011), whereas CUL3 is generally elevated in breast cancer and associated with advanced stage disease (Haagenson et al. 2012). SKP2 is also elevated in breast cancer (Ravaioli et al. 2008) and while this may increase cyclin E1/E2 turnover, this is counteracted by SKP2’s other effects. SKP2 facilitates degradation of the cyclin E-CDK2 inhibitor protein p27Kip1 (Nakayama et al. 2000), and it can transactivate the activity of E2F1 on the cyclin E1 promoter (Salon et al. 2007).

Relative expression and amplification of cyclin E1/cyclin E2 in ER+ breast cancers

ER+ breast cancers are highly dependent on estrogen to stimulate passage of cells through the G$_{1}$/S phase transition to commit to ongoing proliferation. Since CDK2 has a pivotal role in the G$_{1}$/S phase transition, cyclins E1 and E2 are central players in ER+ breast cancer. In this review we have performed primary analyses of the Molecular Taxonomy of Breast Cancer International Consortium (METABRIC) dataset, and a metastatic breast cancer dataset, to illustrate the differences and similarities between cyclin E1 and E2 across ER+ breast cancer and discuss this in the context of previous findings (Figs 2, 3, 4 and 5). METABRIC is a genomic and transcriptomic study of >2000 primary breast cancers (available at http://www.ebi.ac.uk/ega/, under accession number EGAS00000000083) that includes 1175 samples of a luminal type and allows for a comprehensive analysis across the luminal A and luminal B types of ER+ breast cancer (Curtis et al. 2012).

Cyclin E1 mRNA has moderate expression in luminal B type ER+ breast cancers, but it is expressed at the highest level in the ER-negative (ER-) basal-like breast cancers (Fig. 2A). Likewise, 19q12 amplification is predominantly observed in ER- breast cancers (Natraj et al. 2012), and it occurs in only 0.7% of ER+ cancers (Fig. 2B).

In contrast to cyclin E1, cyclin E2 mRNA occurs at its highest levels in luminal B ER+ breast cancers and also at high levels in basal-like and HER2 overexpressing breast cancers (Fig. 2A). The 8q22 chromosomal locus, which includes the $CCNE2$ gene, is amplified in 15–20% of breast cancers (Li et al. 2010) and this amplification event occurs in 28.6% of luminal B breast cancers (Fig. 2B). 8q22 amplification is associated with increased risk of metastasis and anthracycline chemotherapy resistance via expression of the genes LAPTM4B and YWHAZ (Li et al. 2010). However, it should be noted that chemotherapy is not used as therapy in the majority of early stage ER+ breast cancer so other driver events, such as $CCNE2$ amplification, are possible in ER+ breast cancer.

One of the earliest publications on cyclin E2 reported a high correlation between cyclin E1 and E2 mRNA in ten breast cancer samples (Payton et al. 2002). With the availability of much larger datasets such as METABRIC, we can now see that there is only a moderate correlation in mRNA expression between cyclin E1 and cyclin E2 in ER+ cancers ($r=0.470$, Fig. 3A). $CCNE2$ expression correlates

![Figure 2](https://www.ebi.ac.uk/ega/studies/EGAS00000000083 and relative expression of $CCNE1$ and $CCNE2$ was plotted across breast cancer subtypes (luminal A, luminal B, Her2-enriched, basal-like, and claudin-low) as annotated in METABRIC (Curtis et al. 2012). (B) Gene amplification data were obtained from ‘Breast Cancer METABRIC via CBioPortal (http://cbioportal.org) for $CCNE1$ and $CCNE2$ and determined for luminal A ($n=700$) and luminal B ($n=475$) cancers. The percentage of $CCNE1$ and $CCNE2$ amplification is plotted for both ER+ breast cancer subtypes.)
more with the proliferation markers CCNA2 and MKI67 than it does with CCNE1, and CCNE1 correlates better with CCNA2 than it does with CCNE2 expression (Fig. 3B, C, D and E). This indicates that cyclin E1 and cyclin E2 are not tightly co-regulated in ER+ breast cancer, and we speculate that they could be driving the proliferation of different subsets of ER+ cancers.

**Cyclin E1 and cyclin E2 in endocrine resistance**

Due to the strong mitogenic effects of estrogen, endocrine therapies in the form of anti-estrogens (tamoxifen, fulvestrant) and aromatase inhibitors have been a standard of care for ER+ breast cancer for over 30 years. Resistance to these therapies (endocrine therapy resistance) occurs in up to 30% of patients. This is an important clinical problem: it is a very large cohort of patients, and once patients fail endocrine therapy they are usually refractory to standard chemotherapies. As a result, biomarkers of therapeutic resistance and therapeutic targets are required to identify and treat these patients.

Cyclins E/CDK complexes are acutely responsive to estrogen and anti-estrogens (Caldon et al. 2009) and have thus been investigated as drivers and biomarkers of endocrine therapy resistance. Both the CCNE1 and CCNE2 genes are stimulated by E2F-mediated transcription (El Messaoudi et al. 2006, Caldon et al. 2009), which is a major conduit of estrogen-induced proliferative signaling (Miller et al. 2011). Cyclin E1-CDK2 activity is, however, more downstream of c-Myc activation,
Figure 5
Patient overall survival associated with CCNE1 and CCNE2 in the METABRIC ER+ breast cancer cohort. Kaplan–Meier curves show estimated survival over time associated with CCNE1/CCNE2 gene expression or gene amplification in patients with ER+ breast cancers \( n = 1140 \). (A) High CCNE1 (top quartile, \( n = 275 \)) vs low CCNE1 (bottom quartile, \( n = 275 \)). (B) CCNE1 amplified (\( n = 8 \)) vs CCNE1 non-amplified (\( n = 1132 \)). (C) High CCNE2 (top quartile, \( n = 275 \)) vs low CCNE2 (bottom quartile, \( n = 275 \)). (D) CCNE2 amplified (\( n = 217 \)) vs CCNE2 non-amplified (\( n = 923 \)). Gene expression data and survival outcomes were downloaded from https://www.ebi.ac.uk/ega/studies/EGAS00000000083. To determine the significant differences between the two independent groups, we computed the \( P \)-value using the Kaplan–Meier (log rank) test. The analysis was performed in the \( R \) environment using the packages survival and survminer.

whereas CCNE2 lies downstream of estrogen-mediated activation of Cyclin D-CDK4 (Caldon et al. 2009). This is because of the co-activation of CCNE2 by the chromatin remodeler CHD8, which has an important role in steroid hormone control of G1/S phase genes (Caldon et al. 2009, Menon et al. 2010). By contrast, the CCNE1 gene is co-activated by CARM1 (El Messaoudi et al. 2006), which is an ER co-activator that drives proliferation (Al-Dhaferi et al. 2011).

Data from experimental models have provided strong evidence that cyclins E1 and E2 have roles in endocrine therapy resistance. The proliferation of tamoxifen-resistant cells is inhibited by RNAi-mediated knockdown of cyclin E1, cyclin E2, or CDK2 (Caldon et al. 2012), and cyclin E1 and cyclin E2 overexpression can reduce antiestrogen sensitivity in vitro (Dhillon & Mudryj 2002, Hui et al. 2002, Akli et al. 2004, Caldon et al. 2012). The LMW form of cyclin E1 appears more effective at preventing fulvestrant-mediated arrest than the full length version (Akli et al. 2004) and sustaining S phase entry when aromatase is inhibited (Akli et al. 2010). The downregulation of CDK inhibitors p21\(^{Cip1/Waf1}\) or p27\(^{Kip1}\) also abrogates anti-estrogen mediated arrest (Cariou et al. 2000). Notably, cyclin E2 is commonly detected at elevated levels in models of anti-estrogen resistance compared to parental models (Huang et al. 2011, Caldon et al. 2012, Tu et al. 2013). Cyclin E1 is occasionally elevated (Louie et al. 2010), or it is reported as unchanged (Post et al. 2019).

Using the data available from the PALOMA-3 trial (NCT01942135), we were able to compare expression of CCNE1 and CCNE2 in patients with metastatic breast cancer who have progressed on endocrine therapy, derived from primary samples and metastatic samples \( n = 159 \) primary, \( n = 142 \) metastatic, \( n = 302 \) total: data to identify individual patient subsets is not available) (Turner et al. 2019). In this cohort, there was no correlation between CCNE1 and CCNE2 expression \( (r = 0.026, \text{Fig. 4A}) \). Both CCNE1 and CCNE2 correlated to proliferative markers: CCNE1 correlates weakly with CCNA2 \( (r = 0.326) \) and MKI67 \( (r = 0.226) \) (Fig. 4B and C), whereas CCNE2 has a moderate correlation with both these markers \( \text{CCNA2: } r = 0.493, \text{MKI67: } r = 0.534; \text{Fig. 4D and E}) \). For this cohort, we conclude that the regulation of CCNE1 and CCNE2 is dominated by individual regulatory networks rather than co-regulation, but each gene is involved in proliferation independently. In addition, there are a significant proportion of cancers which are either CCNE1 high/CCNE2 low or CCNE1 low/CCNE2 high. This aligns with the data from in vitro models, where either cyclins E1 and E2 can act as putative drivers of endocrine therapy resistance.

Cyclin E1 as a biomarker for ER+ breast cancer and endocrine resistance
CCNE1 mRNA is generally predictive of poor outcome for ER+ patients (Fig. 5A). High CCNE1 was also predictive across three cohorts of tamoxifen treated patients, including shorter disease-free interval for patients on adjuvant therapy (Span et al. 2003, Desmedt et al. 2006) and time to progression (Jansen et al. 2012). However, in a composite cohort of patients treated with either tamoxifen or goserelin, CCNE1 was not predictive of a shorter disease-free interval (Caldon et al. 2012). It is unlikely that CCNE1 amplification has a significant role in ER+ breast cancer: only a very small proportion of patients show CCNE1 amplification (Fumagalli et al. 2016), and this is not associated with improved overall survival (Fig. 5B).

Surprisingly, high cyclin E1 protein has not been indicative of poor survival across ER+ cohorts of patients. In a study of 2020 patients, high cyclin E1 did not predict
poorer overall survival or disease-free survival in ER+ or ER- patients, using a nuclear staining cyclin E antibody (Porter et al. 2006). Using a nuclear/cytoplasmic staining antibody (raised against the HE12 epitope), Cyclin E1 did not predict for poor outcome in a cohort of 51 ER+ patients (Agarwal et al. 2009), nor did it predict tamoxifen response (Berglund et al. 2008).

There are several caveats to the interpretation of studies on cyclin E1 protein which may explain the conflicting results from studies on CCNE1 mRNA and cyclin E1 protein in ER+ breast cancer. First, not all antibodies have been demonstrated to be specific to cyclin E1, and these antibodies may also detect cyclin E2. Second, the LMW isoform of cyclin E1 is not detected by all cyclin E1 antibodies (Karakas et al. 2016). The LMW isoform of cyclin E1 is detected in ~22% of ER+ breast cancers and associated with increased disease recurrence (Akli et al. 2010). Fortunately most studies, including standard RPPA analysis in the TCGA datasets, use the antibody raised against the HE12 epitope, which does not detect cyclin E2 (Caldon et al. 2009) and is able to detect LMW cyclin E1 (Akli et al. 2010, Karakas et al. 2016). In addition, LMW cyclin E1 preferentially localises to the cytoplasm. Cytoplasmic cyclin E, when used as a marker in combination with phosphorylated CDK2, associates strongly with poor recurrence-free survival (Karakas et al. 2016). Consequently, if cohorts are scored solely on nuclear staining, the outcome of patients may be biased toward effects that are more associated with the full length cyclin E1 protein.

**Cyclin E2 as a biomarker for ER+ breast cancer and endocrine resistance**

Cyclin E2 mRNA (CCNE2) expression is associated with poor outcome in ER+ breast cancer (Desmedt et al. 2006, Sieuwerts et al. 2006) (Fig. 5C), although in subgroup analyses of the tamoxifen-only treated patients of one cohort (Desmedt et al. 2006), high CCNE2 levels did not predict treatment resistance. In other large cohorts, CCNE2 is included in gene signatures that predict disease progression in either tamoxifen-resistant breast cancer or metastatic breast cancer, whereas cyclin E1 does not feature in these same signatures (van’t Veer et al. 2002, Sotiriou et al. 2006, Bosco et al. 2007, Musgrove et al. 2008, Huang et al. 2011). Our previous analysis of a composite cohort of six studies showed that high CCNE2 predicted poor metastasis-free survival in endocrine therapy treated patients (Caldon et al. 2012).

Despite its high prevalence (18.5% of ER+ cancers, Fig. 2B), Cyclin E2 gene amplification has not been independently examined as a prognostic marker in ER+ breast cancer to date. However, our analysis of METABRIC shows that CCNE2 amplification is significantly associated with poor outcome (Fig. 5D). Given the large percentage of luminal B ER+ cancers that are CCNE2 amplified (28.6%, Fig. 2B) and the propensity for luminal B cancers to be intrinsically endocrine resistant (Ades et al. 2014), it is feasible that CCNE2 amplification has a role in endocrine therapy resistance and disease recurrence.

Currently there are no reports of cyclin E2 immunohistochemistry across ER+ breast cancer, most likely due to the lack of suitable antibodies.

**Cyclin E driven CDK2 activity in CDK4/6 inhibitor sensitivity and resistance**

The current standard of care for metastatic ER+ breast cancer is CDK4/6 inhibitors in combination with endocrine therapy (Portman et al. 2019). Inhibitors such as palbociclib, ribociclib, and abemaciclib are now used routinely in metastatic breast cancer that has progressed on endocrine therapy, after the demonstration in multiple phase III trials that these inhibitors extend progression-free survival (Finn et al. 2015, Hortobagyi et al. 2016, Goetz et al. 2017). While CDK4/6 inhibitors have a significant effect on disease progression, resistance is inevitable in most patients (Portman et al. 2019).

The current predictive biomarker for use of CDK4/6 inhibitors is ER, but there has been a concerted effort to identify further biomarkers that could refine the patient cohort into responders and non-responders. CDK4/6 inhibitor resistance is also now emerging as a significant clinical problem, and there are not yet effective biomarkers for the emergence of resistance (Portman et al. 2019). Both CCNE1 and CCNE2 have been identified as potential biomarkers that alter sensitivity to combination endocrine therapy and CDK4/6 inhibitors and could indicate an increased likelihood of resistance (Arnedos et al. 2018, Turner et al. 2019), which we discuss in detail.

**CDK4/6 inhibitor resistance is associated with increase in CDK2 activation**

Current efforts to understand the emergence of CDK4/6 inhibitor resistance have shown that numerous mechanisms converge to drive proliferation through CDK2 activation, including upregulation of cyclin E1 and cyclin E2. Studies across laboratory cell line models,
mainly performed in the ER+ MCF-7 and T-47D breast cancer cell lines, have identified cyclin E1 and E2 mRNA and protein upregulation in multiple models (Table 2). The overexpression of either cyclin E1 or cyclin E2 is able to reduce sensitivity to palbociclib (Caldon et al. 2013). Although full length cyclin E1 overexpression does not affect all models, LMW cyclin E1 overexpression can be more effective at reducing sensitivity to palbociclib (Doostan et al. 2017).

Other pathways that converge on CDK2 activation are changes to the CDK inhibitor proteins p21Cip1/Waf1 and p27Kip1. This includes reduction of p21Cip1/Waf1 (lida et al. 2019) and p27Kip1 protein (Dean et al. 2010). Increased expression of components of the cyclin D-CDK4/6 complexes such as CDK6, observed in (Yang et al. 2017) and (Cornell et al. 2019), could also increase CDK2 activity by engaging the p21Cip1/Waf1 and p27Kip1 inhibitory proteins and hence remove these inhibitors from CDK2 complexes (Tsubari et al. 1999). Since CDK4/6 inhibitors bind in the ATP pocket of CDK4/6, p21Cip1/Waf1/p27Kip1 binding is not prevented in the presence of drug (Paternot et al. 2014), allowing CDK4/6 to sequester p21Cip1/Waf1 and p27Kip1.

The role of CDK2 in the transition to senescence must also be considered, as CDK4/6 inhibitors drives breast cancer cells into a senescent growth arrested state (Klein et al. 2018). Cellular senescence is a state of permanent proliferative arrest and exit from the cell cycle which is characterised by enlarged cellular morphology and production of markers such as senescence-associated β-galactosidase (Campisi 2013). It is mediated by the CDK inhibitors p16INK4a and p21Cip1/Waf1 and tumour suppressors p53 and Rb in response to oncogenic stresses or DNA damage (Coppe et al. 2010). In this response, cyclin-CDK complexes are inactivated by CDK inhibitor binding: p16INK4a binds to cyclin D-CDK4/6 and p21Cip1/Waf1 to cyclin E-CDK2 and cyclin D-CDK4/6 (Table 1) (Prall et al. 1997, Sherr & Roberts 1999, Sheppard & McArthur 2013). CDK2 transcription is also downregulated downstream of p53 and Rb as part of the senescence response (Zalzati et al. 2015). Downregulation of CDK2 is necessary for senescence entry (Zalzati et al. 2015), but conversely high levels of CDK2 can inhibit senescence entry (Campaner et al. 2010). Residual CDK2 activity may, however, be useful to drive cells into senescence following a DNA damage-induced G2 arrest (Müllers et al. 2017). In light of these findings, the persistence of high CDK2 in CDK4/6 inhibitor resistance setting would most likely prevent cells from entering senescence and promote continual proliferation.

### Cyclin E1 and cyclin E2 as biomarkers of response to CDK4/6 inhibitors

The PALOMA-2 and PALOMA-3 trials show that irrespective of the expression levels of CCNE1 and CCNE2 in the pre-treated primary tumour samples, the addition of palbociclib to endocrine therapy demonstrates a benefit on recurrence-free survival compared to endocrine therapy alone. However, those patients with the longest and shortest recurrence-free survival can be differentiated by the expression of CCNE1 (Table 3). In both the MONALEESA-2 (letrozole/ribociclib) and the PALOMA-3 (fulvestrant/palbociclib) trials, high expression of CCNE1 mRNA was associated with poor progression-free survival (Formisano et al. 2019, Turner et al. 2019). There was a trend for CCNE1-high patients to have poor progression-free survival in the MONALEESA-7 and CLEE011X2106 trials. Finally, patients expressing cytoplasmic cyclin E1 protein, thought to be representative of LMW cyclin E1, were found to have worse survival than patients expressing only nuclear cyclin E1 protein (Vijayaraghavan et al. 2017). In PALOMA-3, high pre-existing CCNE2 mRNA expression was not associated with any difference in progression-free survival (Turner et al. 2019). CCNE2 was not included in the gene panels for MONALEESA-2, MONALEESA-7, or CLEE011X2106.

The degree of change of proliferative markers in response to CDK4/6 inhibitors can be correlated to either CCNE1 or CCNE2 across several studies. In the POP trial of pre-operative palbociclib, CCNE2 expression was significantly decreased in anti-proliferative responders vs non-responders measured over 15 days (P<0.006) (Arnedos et al. 2018). The POP study also showed a non-significant trend for CCNE1 to be high in non-responders (P=0.09) (Arnedos et al. 2018). Similar observations were made in the NeoPalAna study (CCNE1 was high in patients who maintained high Ki67 after 15 days of treatment) (Ma et al. 2017) and the PALLE study (low Ki67 at 14 weeks was significantly less frequent in CCNE1 high patients) (Martins et al. 2019). CCNE2 was not measured in the NeoPalAna or PALLE studies.

It is unlikely that cyclin E1 amplification predisposes to CDK4/6 inhibitor resistance, as the frequency is not high in ER+ breast cancer (0.7%, Fig. 2B). There is no evidence that CCNE1 amplification is acquired during progression to resistance: in the PALOMA-2 trial it was noted that there were insufficient CCNE1 amplified patients to examine the relationship with resistance (Finn et al. 2019), and in the PALOMA-3 trial CCNE1 amplification was not acquired in 28 patients who progressed on palbociclib plus fulvestrant.
Table 2  Pre-clinical HR+ HER2- breast cancer models of CDK4/6 inhibitor resistance.

<table>
<thead>
<tr>
<th>Study</th>
<th>ER+ Model</th>
<th>Generation of CDK4/6 resistance</th>
<th>CCNE1/cyclin E1</th>
<th>CCNE2/cyclin E2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Abemaciclib</strong> (Yang et al. 2017)</td>
<td>MCF-7</td>
<td>Acquired resistance to abemaciclib (100 nM) after 21 weeks (resistant cells maintained with 200 nM)</td>
<td>No change to CCNE1 mRNA; Cyclin E1 protein not reported</td>
<td>Increased expression of cyclin E2 protein downstream of CDK6 amplification</td>
</tr>
<tr>
<td></td>
<td>T47D</td>
<td>Acquired resistance to abemaciclib (500 nM) after 21 weeks (resistant cells maintained with 1 μM)</td>
<td>No change to CCNE1 mRNA; Cyclin E1 protein not reported</td>
<td>Increased expression of cyclin E2 protein downstream of CDK6 amplification</td>
</tr>
<tr>
<td></td>
<td>CAMA-1</td>
<td>Acquired resistance to abemaciclib (100 nM) after 21 weeks (resistant cells maintained with 100 nM)</td>
<td>Significant overexpression of CCNE1 mRNA; Cyclin E1 protein not reported</td>
<td>No change to CCNE2 mRNA</td>
</tr>
<tr>
<td><strong>Palcocibib</strong> (Herrera-Abreu et al. 2016)</td>
<td>MCF-7</td>
<td>Acquired resistance to palbociclib (3–4 months; 1 μmol/L)</td>
<td>Amplification of CCNE1 (on chromosome 19q12); increase in cyclin E1 protein expression; sensitive to CCNE1 siRNA</td>
<td>Sustained cyclin E2 protein in the presence of palbociclib Not sensitive to CCNE2 siRNA</td>
</tr>
<tr>
<td></td>
<td>T47D</td>
<td></td>
<td>No change</td>
<td>Sustained cyclin E2 protein in the presence of palbociclib Not sensitive to CCNE2 siRNA</td>
</tr>
<tr>
<td>(Vijayaraghavan et al. 2017)</td>
<td>Panel of cell lines classified as being sensitive or resistant to palbociclib</td>
<td>MCF-7 6 days of palbociclib (1 μM) and letrozole (1 μM) treatment</td>
<td>High CCNE1 expression in cell lines intrinsically resistant to palbociclib</td>
<td>Overexpression of LMW cyclin E1 mediated resistance to palbociclib + letrozole</td>
</tr>
<tr>
<td></td>
<td>T47D</td>
<td>6 days of palbociclib (1 μM) treatment</td>
<td>Overexpression of LMW cyclin E1 mediated resistance to palbociclib</td>
<td>Overexpression of LMW cyclin E1 mediated resistance to palbociclib</td>
</tr>
<tr>
<td>(Michaloglou et al. 2018)</td>
<td>MCF-7</td>
<td>Acquired resistance to palbociclib (increasing concentration up to 1 μM) after 4–9 months</td>
<td>Not reported</td>
<td>Not reported</td>
</tr>
<tr>
<td>(Lypova et al. 2019)</td>
<td>MCF-7</td>
<td>Acquired resistance to palbociclib (0.1–4 μM) after 6 months (resistant cells maintained with 1 μM)</td>
<td>Increase in full-length cyclin E1 and LMW cyclin E1</td>
<td>Not reported</td>
</tr>
<tr>
<td>(Ji et al. 2019)</td>
<td>MCF-7</td>
<td>Acquired resistance to palbociclib (increasing concentration up to 30 μM) after 1 year</td>
<td>Elevated expression of cyclin E1 downstream of AR activation by DHT treatment</td>
<td>Sustained expression of cyclin E2 protein in the presence of palbociclib shAR decreased cyclin E2 protein expression</td>
</tr>
<tr>
<td>(Kong et al. 2019)</td>
<td>MCF-7</td>
<td>Acquired resistance to palbociclib (increasing concentration up to 900 nmoL/L) after 8 weeks</td>
<td>High cyclin E1 protein in 6/11 MCF-7 resistant clones</td>
<td>Not reported</td>
</tr>
<tr>
<td>(Kettner et al. 2019)</td>
<td>MCF-7</td>
<td>Acquired resistance to palbociclib (increasing concentration up to 5 μmoL/L) after 6 months</td>
<td>Increased cyclin E1 kinase activity; increase in LMW cyclin E1</td>
<td>Not reported</td>
</tr>
<tr>
<td></td>
<td>T47D</td>
<td></td>
<td>CNV amplification of CCNE1; increase in LMW cyclin E1</td>
<td></td>
</tr>
</tbody>
</table>

(Continued)
R102  

**Table 2 Continued**

<table>
<thead>
<tr>
<th>Study</th>
<th>ER+ Model</th>
<th>Generation of CDK4/6 resistance</th>
<th>CCNE1/cyclin E1</th>
<th>CCNE2/cyclin E2</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Cornell et al. 2019)</td>
<td>T47D</td>
<td>Acquired resistance to palbociclib (100-500 nM) after 12 weeks</td>
<td>No significant decrease in CCNE1 mRNA (100 nM palbociclib); no significant variation in CCNE1 copy number; small increase in cyclin E1 protein expression with increasing concentration of palbociclib (200–300 nM); significant decrease in CCNE1 mRNA when resistant cells are cultured with no palbociclib (7 weeks) compared to continuous treatment</td>
<td>Small (but not significant) decrease in CCNE2 mRNA (100 nM palbociclib)</td>
</tr>
<tr>
<td>Ribociclib (Jansen et al. 2017)</td>
<td>MCF-7 T47D</td>
<td>Acquired resistance to ribociclib (increasing concentration up to 1 µM)</td>
<td>Higher levels of cyclin E protein in resistant cell lines mediated by upstream PI3K/PDK1 signaling pathway</td>
<td>Unchanged cyclin E1 protein expression</td>
</tr>
<tr>
<td>(Iida et al. 2019)</td>
<td>Estrogen-deprived MCF-7-E10</td>
<td>Acquired resistance to ribociclib (100 nM) after 7 months</td>
<td>Not reported</td>
<td>Sustained expression of cyclin E2 protein in resistant tumours</td>
</tr>
<tr>
<td><strong>In vivo</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Palbociclib (Formisano et al. 2019)</td>
<td>TM00386 (ER+ HER2- FGFR1- amplified breast cancer PDX)</td>
<td>Treatment with fulvestrant (5 mg/week; s.c.) + palbociclib (30 mg/kg/day; oral gavage) (3 weeks) supplemented with 21-day release of 0.25 mg 17β-estradiol</td>
<td>Downregulation of CCNE1 expression using NanoString 770-gene nCounterPanCancer Pathways panel</td>
<td>Downregulation of CCNE2 expression using NanoString 770-gene nCounterPanCancer Pathways panel</td>
</tr>
<tr>
<td>Ribociclib (Herrera-Abreu et al. 2016)</td>
<td>PDX244 (CDK4/6-sensitive PDX)</td>
<td>Acquired resistance to ribociclib (100 days; 75 mg/kg, 6 days/week) supplemented with continuous treatment with 17β-estradiol (1 µM)</td>
<td>Not reported</td>
<td>Sustained expression of cyclin E2 protein in resistant tumours</td>
</tr>
</tbody>
</table>

Treatment: Enzalutamide (second generation AR antagonist).

AR: androgen receptor; CNV: copy-number amplification; DHT: 5-α-dihydrotestosterone; IHC: immunohistochemistry; LMW cyclin E1: low-molecular weight cyclin E1.
### Table 3  Clinical trials with CDK4/6 inhibitors in combination with endocrine therapy for HR+HER2- breast cancer.

<table>
<thead>
<tr>
<th>Trial</th>
<th>Study</th>
<th>Phase</th>
<th>Enrolment</th>
<th>Treatment History</th>
<th>Description</th>
<th>Outcome measured</th>
<th>CCNE1</th>
<th>CCNE2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palbociclib (PALOMA-2: NCT01740427)</td>
<td>(Finn et al. 2016)</td>
<td>III</td>
<td>n = 666</td>
<td>ER+ HER2: ABC No prior treatment for advanced disease Postmenopausal</td>
<td>palbociclib + letrozole vs letrozole</td>
<td>PFS: 24.8 months vs 14.5 months (HR: 0.58; 95% CI: 0.46-0.72; P &lt; 0.001) 38 month follow up PFS: 27.6 months vs 14.5 months (HR: 0.563; 95% CI: 0.461-0.687; P &lt; 0.001)</td>
<td>Not reported</td>
<td>Not reported</td>
</tr>
<tr>
<td>Palbociclib (PALOMA-3: NCT01942135)</td>
<td>(Turner et al. 2015)</td>
<td>III</td>
<td>n = 521</td>
<td>HR+ HER2: ABC Progression on prior endocrine therapy ≤ 1 line of chemotherapy for advanced/metastatic disease Any menopausal status</td>
<td>palbociclib + fulvestrant vs fulvestrant</td>
<td>PFS: 9.2 months vs 3.8 months (HR: 0.42; 95% CI: 0.32-0.56; P &lt; 0.001) -8.9 month follow up PFS: 9.5 months vs 4.6 months (HR: 0.46; 95% CI: 0.36-0.59; two-sided P = 0.0001) ctDNA analysis of PIK3CA mutation at baseline OS: 34.9 months vs 28 months (HR: 0.81; 95% CI: 0.64-1.03; P = 0.09)</td>
<td>Not reported</td>
<td>Not reported</td>
</tr>
<tr>
<td></td>
<td>(Cristofanilli et al. 2016)</td>
<td>Retrospective study</td>
<td>Samples analysed: n = 194 (combination arm) n = 108 (placebo)</td>
<td>ctDNA analysis in paired baseline, 15 days of treatment and EOT plasma samples</td>
<td>clonal evolution occurs frequently during combination therapy (RB1 mutations in 6/127 patients) and PIK3CA (P = 0.00097) and ESR1 (P = 0.0037) driver mutations after treatment in both arms</td>
<td>3/24 patients had existing CCNE1 amplifications, but no patients acquired CCNE1 amplification at EOT (palbociclib + fulvestrant; n = 24)</td>
<td>Not reported</td>
<td>Not reported</td>
</tr>
<tr>
<td></td>
<td>(O'Leary et al. 2018)</td>
<td>Retrospective study</td>
<td>EOT plasma samples</td>
<td>ctDNA analysis of paired baseline and EOT plasma samples</td>
<td>mRNA expression correlated with PFS to determine markers of resistance to combination therapy</td>
<td>4/18 patients had existing CCNE2 amplifications, but no patients acquired CCNE2 amplification at EOT (palbociclib + fulvestrant; n = 18)</td>
<td>High expression of CCNE1 was associated with reduced response to palbociclib</td>
<td>Not reported</td>
</tr>
<tr>
<td></td>
<td>(Turner et al. 2019)</td>
<td>Retrospective study</td>
<td>Biomarker analysis using EdgelQ Oncology 254-gene panel on primary and metastatic tumour samples</td>
<td>Clonal evolution occurs frequently during combination therapy (RB1 mutations in 6/127 patients) and PIK3CA (P = 0.00097) and ESR1 (P = 0.0037) driver mutations after treatment in both arms</td>
<td>High expression of CCNE1 was associated with reduced response to palbociclib</td>
<td>Palbociclib arm vs 4 months (placebo) (HR: 0.85; 95% CI: 0.58-1.26)  Low expression of CCNE1 associated with greater efficacy of palbociclib</td>
<td>Palbociclib arm vs 4.8 months (placebo) (HR: 0.32; 95% CI: 0.2-0.5)</td>
<td>Not reported</td>
</tr>
</tbody>
</table>

(Continued)
### Table 3 Continued

<table>
<thead>
<tr>
<th>Trial</th>
<th>Study</th>
<th>Phase</th>
<th>Enrolment</th>
<th>Treatment history</th>
<th>Description</th>
<th>Outcome measured</th>
<th>CCNE1</th>
<th>CCNE2</th>
</tr>
</thead>
<tbody>
<tr>
<td>PALLET</td>
<td>(Johnston et al. 2019)</td>
<td>II</td>
<td>n = 307</td>
<td>ER+ HER2- EBC</td>
<td>(A) palbociclib (2 weeks) → palbociclib + letrozole (14 weeks)</td>
<td>Anti-proliferative response (Ki67, IHC) between baseline and EOT: • (A–C): −4.1 vs (D) −2.2 (one-sided P &lt; 0.001) • CRR: • (A–C): 54.4% patients had complete or partial response vs (D) 49.5% patients had complete or partial response (P = 0.20)</td>
<td>Not reported</td>
<td>Not reported</td>
</tr>
<tr>
<td>NCT01037790</td>
<td>(DeMichele et al. 2015)</td>
<td>II</td>
<td>n = 33</td>
<td>HR+ HER2+ Rb+ ABC Unil</td>
<td>palbociclib</td>
<td>CBR: 7/33 patients (21%) had stable disease for ≥6 months. CB was seen exclusively in patients (7/24; 29%) who progressed on ≥2 prior lines of hormonal therapy</td>
<td>Not reported</td>
<td>Not reported</td>
</tr>
<tr>
<td>NCT02008734</td>
<td>(Arnedos et al. 2018)</td>
<td>II</td>
<td>n = 74</td>
<td>HR+ HER2+ EBC</td>
<td>palbociclib (2 weeks) → surgery vs surgery</td>
<td>Anti-proliferative response (Ki67) between baseline and EOT: Higher Ki67 decrease following palbociclib treatment (58% vs 12%; P &lt; 0.001)</td>
<td>CNE1 expression was not significantly more decreased in anti-proliferative responders vs non-responders (P = 0.5) Persistent elevated expression of CCNE1 at EOT indicates resistance</td>
<td>CCNE2 expression was significantly more decreased in anti-proliferative responders vs non-responders (P = 0.006) but not in the HR+ HER2- subgroup (P = 0.43)</td>
</tr>
<tr>
<td>POP</td>
<td>(Turner et al. 2019)</td>
<td>Retrospective study</td>
<td>n = 61</td>
<td>Gene expression analysis using GeneChip Human Gene Array 372.1</td>
<td>mRNA expression correlated with anti-proliferative response</td>
<td>CNE1 expression in luminal A tumours was significantly lower than in luminal B tumours (P &lt; 0.001)</td>
<td>High CCNE1 expression was associated with lower Ki67 in response to palbociclib</td>
<td>Not reported</td>
</tr>
</tbody>
</table>

Biomarker analysis of tumour samples at baseline and EOT (14 weeks) by IHC and/or FISH: n = 124 patients (A–C) and n = 64 (D) 90% vs 59% achieved CCA (Ki67 < 2.7%)
| NeoPalAin NCT01723774 | (Ma et al. 2017) | II | n = 50 | ER+ HER2- invasive breast cancer | Neooadjuvant therapy; no prior treatment | Pre- and postmenopausal | Anastrozole (4 weeks) + palbociclib + anastrozole (16 weeks) + palbociclib + anastrozole (1.5 weeks if no side effects) → surgery | Anti-proliferative response (Ki67) between baseline, day 1 and 15 of palbociclib treatment and surgery | CCNA was higher on day 1 (87%) vs day 15 of palbociclib treatment (26%) (P < 0.001) | CNE1 expression was significantly elevated during palbociclib resistance (96/72.7%, 5/41 patients) on day 15 of palbociclib treatment (P = 2.25e⁻⁶) | Not reported |
| (Formisano et al. 2019) | Retrospective study | n = 34 | ER+ HER2- BC | palbociclib + endocrine therapy | Post-progression ctDNA analysed from patients using Guardant360 assay | • 2/14 patients had CNE1 amplification | Not reported |
| (Keptner et al. 2019) | Prospective patient database from MDACC | n = 25 patients who progressed on therapy | ER+ HER2- MBC | palbociclib + endocrine therapy | IHC analysis of patient samples pre- and post-progression | LMWE increased from 8/25 patients (pre-treatment) to 15/25 patients (post-progression) (P = 0.023) | Not reported |
| (Vijayaghain et al. 2017) | Prospective patient database from MDACC | n = 78 | ER+ HER2- ABC | palbociclib + letrozole | IHC analysis of patient samples pre- and post-progression | • PFS: RB+/LMWE- and RB+/LMWE+ patients (>36.5 months vs 17 months) | Not reported |
| | | n = 31 | palbociclib + fulvestrant | | | • 1.3/35 patients with progressive disease had RB+/LMWE+ tumours | Not reported |

### Ribociclib

| MONALEESA-1 NCT01919229 | (Curigliano et al. 2016) | II | Window of opportunity | n = 14 | HR+ HER2- EBC | No prior therapy | Postmenopausal | Ribociclib (600 mg A) or 600 mg (B) + letrozole vs letrozole (C) (12 weeks) → surgery | Anti-proliferative response (Ki67) between baseline and EOT | KI67 levels decreased from baseline: 96% (range 78-100%; n = 6) (A), 92% (range 75-100%; n = 3) (B), 69% (range 38-100%; n = 2) (C) | Decreased expression of CNE1 was observed following ribociclib treatment | Not reported |
| MONALEESA-2 NCT01958021 | (Hortobagyi et al. 2016) | III | n = 668 | HR+ HER2- MBC | No prior therapy for advanced disease | Postmenopausal | Ribociclib (400 mg (A) or 600 mg (B)) + letrozole vs letrozole (C) (12 weeks) → surgery | Anti-proliferative response (Ki67) between baseline and EOT | KI67 levels decreased from baseline: 96% (range 78-100%; n = 6) (A), 92% (range 75-100%; n = 3) (B), 69% (range 38-100%; n = 2) (C) | Increased expression of CNE1 was observed following ribociclib treatment | Not reported |
| (Hortobagyi et al. 2018) | | | | | | | | | | | Not reported |
| (Andre et al. 2017) | | | | | | | | | | | | |
| (Formisano et al. 2019) | Retrospective study | n = 391 | Archival patient samples pre- and post progression | CDK4/6 | Tumour samples assessed for mRNA expression using NanoString 230 gene targeted panel | Benefit from combination therapy was observed irrespective of baseline RB, p16, KI67, CDK2, CDKN2A, p16, ESR1 | Not reported |

**Continued**
### Table 3 Continued

<table>
<thead>
<tr>
<th>Trial</th>
<th>Study</th>
<th>Phase</th>
<th>Enrolment</th>
<th>Treatment History</th>
<th>Description</th>
<th>Outcome measured</th>
<th>CCNE1</th>
<th>CCNE2</th>
</tr>
</thead>
<tbody>
<tr>
<td>MONALEESA-7</td>
<td>(Tripathy et al. 2018)</td>
<td>III</td>
<td>n = 672</td>
<td>HR+/HER2; ABC No prior endocrine therapy for advanced disease ≤ 1 line of chemotherapy for advanced disease</td>
<td>Pre/perimenopausal Ribociclib + tamoxifen / NSAI + goserelin vs tamoxifen / NSAI + goserelin</td>
<td>PFS: 23.8 months vs 13 months (HR: 0.55; 95% CI: 0.44–0.69; one-sided P &lt; 0.001)</td>
<td>Not reported</td>
<td>Not reported</td>
</tr>
<tr>
<td></td>
<td>(Im et al. 2019)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>OS at 48 months: 70.2% vs 46% (HR: 0.71; 95% CI: 0.54–0.95; P = 0.00973)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(Lu et al. 2019)</td>
<td></td>
<td>n = 360</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CLEE011X2106</td>
<td>(Bardia et al. 2018)</td>
<td>I/II</td>
<td>n = 132</td>
<td>ER+/HER2; ABC Resistant to NSAI No prior CDK4/6i (cohort 1) or progressed within 1 month of CDK4/6i therapy (cohort 2)</td>
<td>Pre/perimenopausal Ribociclib (300 mg) + everolimus + exemestane vs ribociclib + exemestane</td>
<td>Baseline tumour samples were assessed using NanoString 230 gene nCounter GA Human Reference panel</td>
<td>PFS benefit seen with ribociclib also trended to be greater in patients with low vs high expression of CCNE1 (HR: 0.38 vs 0.65)</td>
<td>Not reported</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Customised NanoString nCounter GX 800-gene panel</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Notes:**
- Treatment administration: Abemaciclib: every 12 h for 28 days (oral); Anastrazole: 1 mg daily (oral); Everolimus: 2.5 mg daily (oral); Exemestane: 25 mg daily (oral); Fulvestrant: 500 mg every 14 days for three cycles and then every 28 days (i.m. injection); Goserelin: 3.6 mg every 28 days (s.c. injection); Letrozole: 2.5 mg daily (oral); Palbociclib: 125 mg daily for 21 consecutive days, followed by 7 days off (oral); Ribociclib: 600 mg daily for 21 consecutive days, followed by 7 days off (oral); Tamoxifen: 20 mg daily (oral).
- ABC: advanced breast cancer; CB: clinical benefit; CBR: clinical benefit rate; CCCA: complete cell cycle arrest; CDK4/6i: cyclin-dependent kinase 4/6 inhibitor; CI: confidence interval; CRR: clinical response rate; ctDNA: circulating tumour DNA; EBC: early breast cancer; ER+: estrogen receptor; EOT: end of treatment; FISH: fluorescent in situ hybridisation; HER2: human EGF receptor 2; HR: hazard ratio; HR+: hormone receptor; IHC: immunohistochemistry; LMW: low-molecular weight cyclin E1; MBC: metastatic breast cancer; MDACC: MD Anderson Cancer Centre (University of Texas); NCT: ClinicalTrials.gov Identifier; NSAI: nonsteroidal aromatase inhibitors, for example, letrozole, anastrozole; ORR: objective response rate; OS: overall survival; PFS: progression-free survival.
In the study of Formisano et al. (2019), 2/34 patients had CCNE1 amplifications following progression on palbociclib (CCNE2 status was not measured). However, this cohort was not analysed for pre-existing CCNE1 amplifications, so it is not known if this was acquired during the development of resistance. CCNE2 amplification is much more prevalent in ER+ breast cancer, so it could predispose to resistance or be acquired to facilitate resistance. CCNE2 amplification was observed in 4/18 patients at commencement of the PALOMA-3 trial, but no further amplification events occurred during disease progression (O’Leary et al. 2018).

Overall, there is a growing body of evidence that CCNE1 could be a significant biomarker for poorer response to CDK4/6 inhibitors combined with endocrine therapy. Given the poor performance of cyclin E1 protein as a biomarker for endocrine therapy resistant breast cancer, it seems unlikely that full length cyclin E1 protein could be used as an immunohistochemistry marker in the context of CDK4/6 inhibitor resistance. Insufficient data has been collected regarding the potential of CCNE2 as a biomarker in CDK4/6 inhibitor resistance, but given the prevalence of gene amplification and strong correlations with proliferation and resistance (DeMichele et al. 2016, Arnedos et al. 2018), further investigation is warranted.

**Conclusions**

Metastatic ER+ breast cancer remains a clinical challenge because of the high frequency of resistance to endocrine therapies and the emerging resistance to CDK4/6 inhibitors. These drug classes directly or indirectly target CDK4/6 activity in the G1 to S phase transition of the cell cycle, meaning that the re-routing of proliferation to rely more heavily on CDK2 activity is a prominent mechanism of resistance. Cyclin E1 and E2 are both dysregulated in ER+ breast cancer, providing a potential route to increased CDK2 activity. As shown in the preceding sections, there is now substantial evidence that dysregulation of these two proteins does occur in both endocrine therapy and CDK4/6 inhibitor resistance, but often not concurrently.

An unresolved issue in understanding the biology of cyclins E1 and E2 is the detection of their respective proteins in tissue samples. The detection of cyclin E1 is hampered by difficulties in completely distinguishing the full length and truncated (LMW) versions of the protein which can have different activities. Cyclin E2 has not yet been effectively examined by immunohistochemistry
across ER+ patient cohorts. The development of better tools for the detection of each protein would aid in understanding the effect of each protein on ER+ breast cancer biology pre-, during-, and post-treatment with endocrine therapies and CDK4/6 inhibitors.

Irrespective of cyclin E1/E2 expression in metastatic ER+ breast cancers, patients will benefit from CDK4/6 inhibitor therapy (Finn et al. 2019). There is also no evidence that high expression of cyclins E1 and E2 would prevent any benefit from adjuvant endocrine therapy. However, the identification of pre-existing high expression of cyclin E1/E2 may identify those patients who will recur earlier and require another line of therapy. When patients stop responding to CDK4/6 inhibitors, high cyclin E1 and E2 could be alternative pathways to high CDK2 activity in resistant disease. Consequently, it would be ideal for future studies to include both biomarkers to detect potential perturbations of cyclin E-CDK2 activity.

Declaration of Interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

Funding
This work was supported by the National Breast Cancer Foundation Australia (ECF17-002 CEC) and the Cancer Institute of NSW (ID: ECF171156 HHM).

References


Caldon CE, Sergio CM, Sutherland RL & Musgrove EA 2011a Differences in degradation lead to asynchronous expression of cyclin E1 and cyclin E2 in cancer cells. *Cell Cycle* 10 596–605. (https://doi.org/10.4161/cc.10.3.13499)


Keck JM, Summers MK, Tedesco D, Ekholm-Reed S, Chuang LC, Jackson PK & Reid SI 2007 Cyclin E overexpression impairs progression through mitosis by inhibiting APC*(catalytic domain)*. *Journal of Cell Biology* 178 371–385. (https://doi.org/10.1083/jcb.200703202)


Received in final form 11 February 2020
Accepted 13 February 2020
Accepted Manuscript published online 14 February 2020