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CDK4/6 inhibitors in breast cancer – from in vitro models to clinical trials

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Abstract

Background: Breast cancer (BC) is one of the leading causes of cancer-related deaths worldwide. **Standard therapies aim to disrupt pathways that regulate the growth and survival of BC cells. Therapeutic agents such as endocrine therapy target hormone dependent cancer cells and have shown to be suitable approaches in BC treatment. However, i**n the case of metastatic BC, curative options are limited, thus strategies have been explored to improve survival and clinical benefit. In this review we provide an up to date overview of the development of anti-cancer agents, particularly the newly developed CDK4/6 inhibitors.

Material and methods: A search of PubMed was conducted to identify preclinical data surrounding the development of endocrine therapy and CDK4/6 inhibitors in early and metastatic BC. Clinical data were also sought using PubMed and clinicaltrials.gov.

Results: Agents targeting estrogen and its receptor have demonstrated positive outcomes in clinical trial with improvements in objective responses and overall survival. However, patients do exhibit adverse effects and some will eventually fail to respond to endocrine therapy. Subsequently, the development and success of 3rd generation CDK4/6 inhibitors in preclinical studies have allowed for their use in clinical studies. In patients with ER+ BC, CDK4/6 have demonstrated dramatic improvements in progression free survival when in combination with endocrine therapies. Similar findings were also observed in metastatic disease. Adverse effects were minimal in CDK4/6 treated patients demonstrating their safety.

Conclusion: CDK4/6 inhibitors are highly specific making them a safe and viable therapeutic for BC. Although a number of trials have demonstrated this, as a lone therapy or in combination, optimisation of treatment scheduling are still required in further clinical investigations.

Keywords: CDK4/6; pre-clinical; breast cancer; metastasis

Introduction

As the most common malignancy diagnosed in women, Breast Cancer (BC) is the second leading cause of cancer-related deaths worldwide [1]. BC is diagnosed in one in eight women with a 3.4% risk of mortality. It also accounts for 29% of new cancer cases

in the USA with 5-10% presenting with metastatic disease [2]. The 5 year overall survival rates are relatively high (90%) in early stage disease. However in metastasis, the 5 year overall survival is dramatically decreased to 25%. In the metastatic setting, therapies for patients are mainly palliative and often accompanied with adverse effects. Depending on the subtype of BC, patients have a broad spectrum of therapies in an attempt to provide better outcomes. For example, endocrine receptor positive BCs are heavily dependent on the expression of the estrogen (ER) and/or progesterone (PR) receptors. Upon activation, these receptors initiate signalling pathways that regulate cellular processes such as proliferation, apoptosis and angiogenesis that contribute to cancer progression [3] (figure 1). This dependence on ER/PR signalling has resulted in the development of endocrine therapies that target these receptors and their downstream pathways, including ER/PR blockers, aromatase inhibitors and ER down-regulators.

As little as 5% of patients are diagnosed with stage IV BC at initial diagnosis, most cases arise after endocrine therapy or chemotherapy [4]. Although endocrine therapies are associated with high rates of clinical benefit, disease progression is often observed after one year of first line treatment [5]. ER/PR positive disease cease to respond to endocrine treatment due to acquired resistance caused by either the loss of endocrine receptors and/or activation of ER/PR-independent signalling pathways [6,7]. This resistance may in part result from the tumour losing the dependency on hormone-ER signalling and instead utilising alternative survival pathways that act through the cyclin-dependent kinase (CDK)-cyclin and retinoblastoma (Rb) interaction [3]. The CDKs are a family of serine/threonine kinases that are key regulators of the cell cycle. Subgroups of CDKs, such as CDK4/6, are shown to have a critical role in BC pathogenesis and tumourigenesis, leading to the development of CDK targeting agents. Known as CDK inhibitors (CDKi), these drugs have been shown to improve clinical outcome and delay the onset of breast tumour progression, particularly when used in combination with endocrine therapy [8,9].

Mechanisms underlying BC- the role of cyclins

Cyclin-CDK pathways regulate the cell cycle

The cell cycle is split into four phases, growth phase (G1), DNA synthesis (S), a second growth phase (G2) and mitosis (M) (figure 2). Passage from one phase to the other is tightly regulated by molecular checkpoints, essential for the detection of DNA damage as well as

providing the opportunity to repair defects prior to mitosis [10]. The checkpoints are regulated by a number of cyclins, regulatory subunits of the CDK-cyclin holoenzyme, and their respective CDKs. In particular, the irreversible transition from G1-S phase is heavily dependent on the cyclin D-CDK4/6-Rb pathway [11]. The cyclin family is divided into A-, B-, D- and E- types, of which, cyclin D and cyclin D1 are the best characterised of the cyclin subtypes [12]. During cell division, cyclin D1 is found to have strong interactions with CDKs, specifically CDK4 and CDK6. Another major checkpoint regulator is the retinoblastoma protein (Rb), which has a role in inhibiting the cell from entering the cell cycle by repressing the E2F family of transcriptional factors. When initiating the cell cycle process, the cell produces cyclin D1 that goes on to form an activating complex with CDK4/6. The cyclin D1-CDK4/6 complex then promotes the phosphorylation of Rb, which, in its phosphorylated state lifts its transcriptional repression of E2F. E2F then proceeds with the transcription of S-phase genes, such as cyclin E, which forms a complex with CDK2. The cyclin E-CDK2 complex further phosphorylates Rb and other regulators of the G1/S phase checkpoint, establishing a positive feedback loop resulting in the irreversible transition and progression through the cell cycle [13].

Cyclin-CDK pathways in BC pathogenesis

Uncontrolled cell division is a defining characteristic, and a hallmark of cancer [14]. Mutations in cell cycle regulators, particularly within the cyclin-CDK-Rb pathway, have been found to be an underlying cause in the disruption of controlled proliferation. In fact, the overexpression of cyclin D1 is evident in 50% of all BC cases, resulting in aberrant phosphorylation of Rb, demonstrating its crucial role in the development of disease [15-17]. Analysis of BC subtypes by the Cancer Genome Atlas has shown that cell cycle genes vary in different subtypes [18]. For example, cyclin D1 amplification is most frequently found in LBC-A (29%), -B (58%) and HER2 positive (38%) subtypes. Moreover, both cyclin D1 and CDK4 were observed to be highly amplified in LBC-B and HER2 cancers (58% and 25%, respectively). It is also known that the loss of heterozygosity of the Rb gene locus occurs in 20-30% of BC [19]. In the case of ER positive BCs, estrogen signalling is essential for the growth, proliferation and survival of the tumour. Estrogen signalling also leads to the upregulation of cyclin D1, which in turn activates CDK4/6 and downstream signalling molecules [20]. As mentioned previously, endocrine therapy is the first-line approach in the treatment of metastatic ER positive BC, however not all ER positive cancers respond and

resistance is common. Thus, it has been proposed that inhibition of cell cycle regulators may be an effective method of targeting ER positive BC.

Therapeutic targeting of BC

Endocrine therapy

The foundations of endocrine-directed cancer therapy were first established in the late 1800's by George Thomas Beatson. He showed that oophorectomy (removal of the ovaries) conducted on BC patients resulted in improved outcomes hypothesising that ovaries maintained the existence of the cancer within the breast [21]. In fact, he was explaining the stimulatory effect of the female ovarian hormone, estrogen, before its discovery. Thus, endocrine therapies commonly used in ER positive BCs target ERs by either blocking them (selective estrogen-receptor modulators (SERMs)), inhibiting estrogen production (aromatase inhibitors AIs), or actively downregulating them (selective estrogen-receptor degraders (SERDs)).

SERMS are a group of ER antagonists that block the receptor, thereby preventing downstream signalling pathways in the presence of estrogen (figure 1). Tamoxifen was identified in the 1970s as a potential anti-ER in induced mammary carcinogenesis [22]. Due to the success and safety of tamoxifen in preclinical studies [23], a large scale clinical trials in early BC in the late 1990's was initiated. The EBCTCG studied tamoxifen as an adjuvant therapy in around 30,000 women and found dramatic falls in recurrence rates after a 10 year follow-up, proportional to the duration of treatment (fall in recurrence at 1 year 21%, 2 years 29% and 5 years 47%). In addition, mortality rates also dropped in proportion to treatment duration, suggesting that prolonged exposure is more effective in targeting BC with tamoxifen [24]. Of the 30,000 women enrolled in the trials, 8,000 exhibited ER low/negative tumours. In these cases, it was found that tamoxifen had little effect, instead showing benefits for ER positive pre- and postmenopausal women [24]. Parallel investigations were undertaken to determine the safety and treatment scheduling [25-27]. Postmenopausal symptoms were the most commonly observed side effects, including hot flushes, night sweats, irregular menses and vaginal dryness, discharge or irritation [27]. Life-threatening side effects were minimal, however an increase in the risk of a thromboembolic event occurring was greater in tamoxifen treated patients as described in the P1 study by The National Surgical Adjuvant Breast and Bowel Project (NSABP) [28,29]. From these studies, it was concluded that the benefits of

tamoxifen treatment outweighed the adverse effects. Furthermore, adjuvant tamoxifen therapy was determined to be administered for a duration between 2 and 5 years providing a significant survival advantage [30]. More recently however, the ATLAS study showed continued tamoxifen treatment for 10 years resulted in a reduction in the risk of BC recurrence and significant reductions in BC mortality [31]. These findings were conformed by the aTTOm trial conducted between 1991-2005 [32]. Since these studies show benefit with continued 10-year treatment with tamoxifen compared to 5 years, most oncologists now recommend 10 years as a standard of care.

Aromatase inhibitors (AIs) inhibit the production of estrogen by targeting and suppressing the aromatase enzyme (figure 1). Prior to the menopause, ovarian-derived aromatase is responsible for the production of circulating estrogen. Post menopause, circulating estrogen production predominantly occurs in fat and muscle as well as other estrogen-sensitive organs such as breast, uterus and vagina. Thus, AI therapy is commonly used in postmenopausal women since the inhibition of ovarian-aromatase decreases ovary-derived estrogen setting up a negative feedback loop to the pituitary gland, eliciting further production of LH and subsequently ovarian estrogen in the pre-menopause setting, rendering the AI ineffective.

Studies using AIs have been reviewed extensively in the early BC setting [33,34]. When compared to tamoxifen, 10 year mortality rates are reduced by 15% in AI treated patients over 5 years of treatment [35]. Further studies such as the Breast International Group (BIG) 1-98 and Arimidex Tamoxifen Alone or in Combination (ATAC) trials demonstrated the advantage of adjuvant AI (letrozole and anastrozole respectively) therapy compared to tamoxifen [36,37]. The Intergroup Exemestane Study (IES) trial introduced exemestane (an AI) therapy following 2-3 years of tamoxifen treatment for the remainder of the 5 year regimen [38]. The overall survival rate was improved on AI treatment compared to continued tamoxifen treatment [39]. A phase3 randomised double-blinded study, MA. 17, was conducted to investigate whether treatment with the AI letrozole after 5 years on tamoxifen reduces the risk of late recurrences [40]. Improved disease free survival and improved overall survival in node-positive patients with extended letrozole treatment was observed in comparison to placebo [41]. Importantly, the above studies illustrate the safety of AI therapy as the lack of documented adverse events such as cardiac-related complications and endometrial cancers, otherwise observed in tamoxifen treated patients [42]

AIs have also been extensively used as a therapeutic strategy in targeting metastatic BC in postmenopausal women. Around 35 trials have investigated the use of AIs as first- and second line treatments [43]. Anastrozole and letrozole have shown to have good efficacy and tolerability in phase 2 and phase 3 trials. When compared to tamoxifen, anastrozole demonstrated improved clinical benefit (defined as complete or partial response ≥ 24 weeks) in 59% of postmenopausal women with advanced BC, compared to 46% of the tamoxifen treated patients, as a first-line therapy [44]. Similar results were observed in a randomised phase 3 trial in ER positive advanced BC patients, reporting that median time to disease progression was significantly higher in anastrozole treated patients compared to tamoxifen treated patients [45]. It was also demonstrated that letrozole significantly improved median time to progression, objective response and overall survival in a number of phase 3 studies when compared to tamoxifen in advanced BC patients [46-48].

The SERD, fulvestrant, was first compared to anastrozole as an adjuvant therapy in a phase 3 trial [49,50]. No significant differences were observed in clinical outcomes such as median time to progression and objective response rate and this is not routinely used in this setting. In the metastatic setting, when compared to tamoxifen as first line treatment, little difference was observed in the median time to progression between treatment groups [51]. The combination of anastrazole and fulvestrant was also investigated in two trials [52,53]. In these studies, a large proportion of the patients had received prior tamoxifen treatment and both showed that combination therapy did not result in improved overall survival compared to anastrazole alone. The results observed in these earlier trials were limited by the low dose of fulvestrant administered. Due to its poor solubility properties, fulvestrant is administered by intramuscular injection at 250mg every 28 days. To identify more effective approaches, the dose effect of fulvestrant on a range of biomarkers was explored. ER positive BC patients were given 500mg every 28 days and an additional 500mg on day 14 of the first week as compared to 250mg every 28 days. On day 28, a significant increase in down-regulation of Ki-67 was observed in patients receiving the higher dose compared to the initial treatment plan (78.4% vs 47.4%) [54]. It was also discovered that a 25% reduction in ER expression was associated with the 500mg dose compared to 13.5% in the 250mg dose. A comparison between the two doses was undertaken by the CONFIRM phase III trial, showing that although ORR were similar between patients receiving 500mg and 250mg and both doses were well tolerated, progression free survival and overall survival were significantly improved in the 500mg arm [55]. With this, a phase II study consisting of 205 patients compared 500mg

fulvestrant to 1mg anastrozole **as first line treatment** [56]. The median time to progression in fulvestrant patients was significantly higher (23.4 months) compared to anastrozole (13.1 months). Overall survival was also higher in the fulvestrant group (54.1 months) compared to anastrozole treated patients (48.4 months). These findings led to the development of a 462 patient double-blind phase 3 trial, the FALCON study. Here, to confirm the efficacy of fulvestrant (500mg), it was compared with anastrazole in endocrine therapy-naïve women [57]. Patients receiving fulvestrant showed significantly longer disease free progression (median 16.6 months) compared to the anastrazole group (median 13.8 months) with similar adverse event rates. This study demonstrates the superior efficiency of fulvestrant in patients, predominantly with metastatic BC to the bone, that have not previously undergone hormone therapy.

From the trials described above, it is clear that endocrine therapy is an effective approach in ER positive BC. However, response to endocrine therapy heavily relies on ER expression, thus the loss of ERs results in de novo resistance which is not uncommon (15-20%) [3]. However, a high proportion of ER positive BC patients (30-50%) will relapse following endocrine therapy [58]. One of the underlying causes of resistance-mediated relapse are mutations of the ER gene, ESR1. Gain of function ESR1 mutations are found in 20% of patients treated with endocrine therapies. Moreover, mutations in the ligand-binding domain result in ER-independent tumour growth and partial resistance to endocrine therapy [59]. Point missense mutations in the ESR1 gene are also associated with constitutively active ER activity, however these are rarely found in the primary tumour. Instead their frequency is 30-times higher in metastatic lesions [60]. Meta-analysis conducted by Zhang et al confirmed that patients with plasma ESR1 mutations exhibited worse progression free survival and overall survival compared to wild-type ESR1. In addition, they showed that ESR1 mutations were associated with shortened progression free survival after AI therapy, though this correlation was not observed in fulvestrant-treated patients [61]. Since endocrine signalling dependence is abolished in these cases, agents that target alternative pathways, such as the cell cycle process and its downstream pathways, have been sought.

CDK inhibitors

Preclinical studies of CDK inhibitors

The success of CDK inhibitors is a result of decades of research aiming to identify the most effective and safest molecule that could be used to target CDKs and inhibit their actions. CDK

inhibitors are separated into 3 categories, first-, second- and third generation, defined by their discovery and targets (Table 1).

First generation inhibitors exert their effects through non-specific targeting of CDKs, they are therefore termed 'pan-CDK' inhibitors. Flavopiridol (alvociclib), one of the most studied compounds of this class, has been shown to target CDK1, CDK2, CDK4, CDK6, CDK7 and CDK9, demonstrating its pan-CDK inhibitory potential [62,63]. Initially discovered to inhibit proliferation in haematological cancer cell lines in vitro [64], subsequent studies demonstrated that flavopiridol inhibited cell cycle progression in G1 or G2 phase of the human breast carcinoma MCF-7 cell line in a CDK1-dependent manner [65]. A later study showed that in addition to its anti-proliferative effects, flavopiridol also exhibited pro-apoptotic potential in vitro and in vivo [66]. Disappointingly, flavopiridol did not prove to be as successful as expected in solid tumours due to toxicity issues and lack of efficacy [67], although some benefits were observed in haematological malignancies, including improved response rates and progression free survival [68]. As a result, no phase 3 studies have been undertaken and the development of flavopiridol has been discontinued.

Second generation CDK inhibitors were developed in an attempt to target specific CDKs, aiming to improve efficacy and clinical outcomes. One example is dinaciclib, identified as a potent inhibitor of CDK1 and CDK2 and demonstrating superior potential in supressing Rb phosphorylation when compared to flavopiridol [69]. Dinaciclib showed increased pro-apoptotic activity as well as inhibition of bromodeoxyuridine incorporation in over 100 tumour cell lines. In the same study, xenograft models of solid tumours demonstrated significant tumour regression following treatment with dinaciclib [69]. These promising results prompted its introduction into a phase 1 study in which dinaciclib was determined to be safe and tolerated in a range of solid tumours, including colorectal, ovarian and breast [70]. However, a subsequent phase 2 trial demonstrated that dinaciclib showed poor efficacy compared to the chemotherapeutic agent capecitabine [71]. The trial was ceased after an interim analysis revealed that the time to disease progression in dinaciclib treated patients was inferior to that of patients receiving capecitabine [71]. The lack of success of these inhibitors may be attributed to the limited understanding we have of their precise mechanism of action. Due to the low specificity of the agents, it is difficult to identify which CDKs are inhibited and thus its underlying anti-tumour mechanism. It was therefore imperative to identify molecules that were highly specific in targeting certain CDKs involved in particular pathways. For

example, purely targeting CDK4 and CDK6 would theoretically result in cytostatic G0/G1 arrest by inhibiting Rb phosphorylation. Subsequently, chemical screening and optimization discovered potent CDK4/6 inhibitors composed of pyrido-[2,3-d]pyrimidin-7-one with a 2-amino pyridine side chain at the C2 position [72]. These compounds are known as third generation CDK inhibitors (figure 2).

Preclinical studies of third generation CDK4/6 inhibitors

Upon further development and optimization of third generation inhibitors, palbociclib (PD-0332991, Pfizer), ribociclib (LEE011, Novartis) and abemaciclib (LY-2835219, Eli Lilly) were identified as selective CDK4/6 inhibitors [73,74]. These compounds act by targeting the ATP-binding pocket of CDK4 and CDK6, thereby inhibiting their ability to phosphorylate Rb, resulting in cell cycle arrest at the G1-S checkpoint [75].

Palbociclib is a highly specific inhibitor of CDK4 with an IC50 value of 0.011 μmol/L (to CDK4/cyclin D1 and D3), without activity against other CDKs or to 32 other protein kinases tested [73]. Moreover, palbociclib inhibited CDK6 with the same affinity as CDK4 (0.015 μmol/L), demonstrating its specificity for CDK4/6. Rb phosphorylation was found to decrease in response to palbociclib treatment in MDA-MB-435 breast carcinoma cells. In addition, palbociclib inhibited proliferation of breast, colon and lung carcinoma cells, with 95% of the cells arrested in the G1 phase [73]. Further analysis conducted on several BC cell lines showed that palbociclib inhibited growth in a cytostatic manner, with luminal ER positive and HER2 positive cancer cell lines showing the highest sensitivity [76]. It was also demonstrated that following 48 hours of incubation with palbociclib, Ki67 staining is suppressed in ex vivo human BC samples [77]. These results were reproduced in human xenograft mouse models [73]. Mice were transplanted subcutaneously with a range of cancer cells (including breast, lung and colon) and once the tumours were palpable, palbociclib treatment commenced using a dose of 150mg/kg daily for 14 to 28 days. Analysis of tumours after treatment showed evidence of inhibition of Rb phosphorylation and reduced Ki67 staining. Furthermore, tumour regression and a delay in tumour growth was also observed in response to palbociclib. An interesting finding was described when palbociclib was studied in combination with tamoxifen in resistant cells. Tamoxifen-resistant MCF-7 breast adenocarcinoma cells showed sensitivity to palbociclib alone and increased sensitivity to tamoxifen when treated in combination [76]. These results provide foundational evidence for the development of palbociclib as a BC therapeutic.

Ribociclib has also shown to exhibit antitumour effects , when screened against 50 BC cell lines, ribociclib demonstrated inhibitory activity, mostly against ER positive cell lines [78]. Inhibition of tumour growth was also observed in response to ribociclib treatment in in vivo xenograft models of ER positive BC [78]. Interestingly, using patient derived xenograft (PDX) models, ribociclib has shown to be a potent antitumour agent when combined with alpelisib (a PI3K inhibitor) as well as letrozole and fulvestrant, significantly improving response rates and progression free survival [79].

Abemaciclib was identified by researchers at Eli Lilly and Company Research Laboratories [74]. Like palbociclib, abemaciclib showed high specificity for CDK4 and CDK6 with low affinity for CDK9. An initial study investigated the anti-proliferative effects of abemaciclib in **BC** cells. As expected, inhibition of Rb phosphorylation was observed and in turn decreased proliferation of cells that were arrested in G1 [74,80]. In a panel of 44 BC cell lines, ER positive/HER2 negative and Her2-amplified cancer cell lines demonstrated highest sensitivity to abemaciclib [80]. When investigated in vivo, daily abemaciclib treatment at a dose of 75mg/kg resulted in regression of BC xenografts [80]. However, within the first week of withdrawal of abemaciclib, tumour growth resumed. BC xenograft tumours from animals treated with abemaciclib showed evidence of inhibited Rb phosphorylation [74]. It has also been demonstrated that abemaciclib treatment increases its effects on tumour regression (breast and colon tumours) when used in combination with cytotoxic agents [74,80]. These data support the development of abemaciclib in clinical trials, either alone or in combination in ER positive BC, as discussed below.

Clinical trials of CDK4/6 inhibitors in metastatic BC as monotherapy or in combination with endocrine therapy

Palbociclib

As combination therapy with endocrine treatment was supported by positive findings in preclinical studies, 12 menopausal women with ER positive metastatic BC were enrolled in a phase 1 study investigating the effects of palbociclib combined with the AI inhibitor letrozole. Nine patients demonstrated tumour stabilisation and 3 patients exhibited partial response, with few dose-limiting toxicities and no drug-drug interactions [81].

The success of the activity and safety of palbociclib phase 1 trials allowed the initiation of phase 2 trials. A non-randomised, single-arm phase 2 trial using palbociclib as a monotherapy was conducted in 37 patients with Rb positive, pre-treated, advanced BC (33 with ER positive disease) [82]. Patients with ER positive tumours showed a median progression-free survival of 4.5 months compared to 1.5 months in patients with ER negative tumours. In addition to this, seven patients, all within the ER positive group, showed clinical benefit in response to palbociclib. Interestingly, patients that had undergone fewer previous treatment lines showed better clinical benefit rates compared to those who were heavily pre-treated. Following the phase 1 trial by Slamon et al, a larger, open-label randomized phase 2 study was undertaken further assessing the safety and efficacy of palbociclib plus letrozole compared to letrozole alone [83]. The PALOMA-1 study (palbociclib: on going trials in the management of breast cancer-1) enrolled 165 ER positive metastatic BC patients with no prior systemic treatment and evidence of local recurrent disease not amenable to surgery. Patients were divided into two cohorts; patients in cohort 1 were assigned based on their ER positive, HER2 negative status whereas cohort 2 included patients that exhibited cyclin D1 amplification and/or loss of p16. Having two cohorts would allow for the assessment of both the efficacy of palbociclib in combination with letrozole, and whether additional biomarkers were required for patient selection. Within each cohort, patients were assigned two stratification factors, disease site (bone only, visceral and non-visceral) and disease-free interval (> 12 months from the end of adjuvant treatment to recurrence vs < 12 months or less from the end of adjuvant treatment to recurrence or de novo metastatic disease). Initial exploratory analysis was intended for cohort 1 and primary endpoint analysis for cohort 2. However, results from an interim analysis resulted in primary endpoint analysis being conducted in the combined populations of cohort 1 and 2. This was due to the suspension of enrolment into cohort 2 because of 'clinical meaningful activity' demonstrated by patients in cohort 1. The interim analysis also demonstrated that patient selection would not be improved based on cyclin D1 amplification and/or loss of p16. When analysed separately at the cut-off date, it was shown that patients receiving letrozole alone had a median progression-free survival of 10.2 months, this was significantly higher (20.2 months) in the cohort receiving combination therapy [83]. Within cohort 1, the median progression-free survival was 5.7 months in the letrozole group and 26.1 months in the palbociclib plus letrozole group. In cohort 2, the median progression-free survival for letrozole and combined therapy was 11.1 and 18.1 months, respectively. The data show that patients with ER positive advanced BC had improved clinical outcome in response to palbociclib treatment combined with letrozole. Again, as observed in previous clinical

trials, neutropenias and other adverse events such as leukopenia, fatigue and diarrhoea were common, more so in the combined therapy group, suggesting that palbociclib exerts CDK inhibitory effects on haematological progenitor cells.

Further analysis on safety and quality of life was undertaken in the phase 3, PALOMA-2, trial. This double-blind study enrolled post-menopausal women with ER positive, HER2 negative, advanced BC (n=666) who had received no previous systemic endocrine therapy [84]. Patients were treated with palbociclib plus letrozole, or letrozole plus placebo. In the palbociclib plus letrozole group, median progression-free survival was 24.8 months, considerably higher than the 14.5 months in the letrozole alone group. Neutropenia was the most common adverse effect, reported in 66.4% of palbociclib plus letrozole patients, compared to 1.4% of the placebo-letrozole group. However, less than 2% of palbociclib-letrozole patients suffered from febrile neutropenia, confirming the safety of the combination as a first line therapy. In a proceeding phase 3 study, the PALOMA-3 trial, palbociclib was investigated in combination with fulvestrant as second line therapy in patients with metastatic BC [85]. Unlike the PALOMA-2 trial, patients aged 18 years or older with previous endocrine therapy and disease relapse or progression were eligible to take part. Patients were treated with palbociclib and fulvestrant (n=347) or with fulvestrant and placebo (n=174). Palbociclib plus fulvestrant treated patients had a median progression free survival of 9.5 months, significantly higher than the fulvestrant and placebo group (4.6 months). Moreover, grade 3 or 4 adverse effects were observed in 73% of the palbociclib and fulvestrant patients compared to the 22% in the fulvestrant and placebo group, however severe adverse effects were 13% and 17% respectively [85]. In a more recent follow up of the PALOMA-3 study, it was shown that the median overall survival was 34.9 months in the palbociclib plus fulvestrant group compared to 28 months in the placebo group [86]. In addition to this, subgroup analyses showed that patients that were sensitive to the previous line of therapy exhibited a higher degree of benefit of palbociclib treatment. Contrarily, patients that demonstrated endocrine resistance did not obtain such benefit. It was also found that the median overall survival of patients with visceral metastatic disease was 27.6 months in palbociclib plus fulvestrant group, 2.9 months higher than for patients receiving placebo plus fulvestrant. In the parallel ‘no visceral metastatic disease’ group, palbociclib plus fulvestrant group demonstrated a median overall survival of 46.9 months, significantly higher that the placebo plus fulvestrant group (35.4 months). These results demonstrate the improved progression free survival in response to palbociclib treatment, regardless of previous endocrine therapy, menopausal or metastatic status.

Abemaciclib

Similar observations to those obtained with palbociclib were documented in phase 1 trials with abemaciclib, showing delayed disease progression and stabilised disease in the BC setting [87,88]. Patnaik et al sought to determine dose-limiting toxicity and safety of abemaciclib applying two dosing strategies, a once-daily dose of 225 mg or a 12-hourly dose of 200 mg. Patients receiving 225 mg/daily did not reach the maximum tolerated dose, however one out of 7 patients treated 12-hourly experienced grade 3 fatigue [88]. This established the 12-hourly dosing regimen for the first phase 2, MONARCH-1, trial with abemaciclib monotherapy in patients who had progressed on or after prior endocrine therapy and had 1 or 2 chemotherapy regimens in the metastatic setting [89]. Median progression-free survival of treated patients was 6 months and clinical benefit rate was 42.4%. Diarrhoea, fatigue and nausea were described as the most common adverse effect, but abemaciclib was found overall to be safe and effective. Consequently, a randomised phase 3 trial, MONARCH-2, was carried out to investigate the safety of abemaciclib plus the selective ER degrader (SERD) fulvestrant [90] in patients who had progressed while receiving neoadjuvant or adjuvant endocrine therapy (ET), ≤ 12 months from the end of adjuvant ET, or while receiving first-line ET for metastatic disease. Of the 669 patients enrolled, 446 were assigned to abemaciclib and fulvestrant treatment, whereas the remaining 223 were treated with placebo plus fulvestrant. Progression-free survival was significantly increased in the former group (16.4 versus 9.3 months, respectively). Objective response rate (ORR) was also observed to be considerably higher in patients treated with abemaciclib plus fulvestrant, 48.1%, compared to the control group, 21.3% [90]. A subsequent phase 3 trial of abemaciclib, MONARCH-3, was conducted in 2017. Unlike in MONARCH-2, postmenopausal women were enrolled without having received previous systemic treatment for their metastatic disease and were treated with abemaciclib or placebo in combination with a non-steroidal AI (letrozole or anastrazole) [91]). Again, progression-free survival was reported to be significantly longer in the abemaciclib than the placebo cohort, this was also the case for the ORR (59% versus 44% respectively). Adverse events included neutropenia (21.1% versus 1.2%), diarrhoea (9.5% versus 1.2%) and leukopenia (7.6% versus 0.6%) [91]. Abemaciclib (plus fulvestrant or AI) treatment resulted in a lower proportion of patients suffering with neutropenia compared to those reported in the PALOMA trials of palbociclib, but a higher incidence of diarrhoea, reflecting the high specificity of abemaciclib.

Ribociclib

The first in-human phase I clinical trial of ribociclib was conducted in 2014, investigating its safety and tolerability as a lone treatment [92]. 132 patients were enrolled to determine the maximum tolerated dose (MTD), the recommended dose for expansion (RDE) and safety of ribociclib in advanced Rb positive solid tumours (including ER positive BC). It was established that the MTD and RDE were 900 mg/day and 600 mg/day respectively. Although treatment-related adverse events were observed, such as neutropenia, leukopenia, fatigue and nausea, these were more frequently observed at the MTD compared to the RDE (neutropenia (46%; 27%), leukopenia (43%; 17%), fatigue (45%; 2%), and nausea (42%; 2%) respectively). The anti-proliferative effects of ribociclib were confirmed by the reduced Ki67 levels in biopsies, demonstrating its acceptable safety profile and clinical activity. An early phase 1b trial conducted in 2016 showed similar safety and tolerability results in postmenopausal patients with advanced ER positive BC treated with ribociclib in combination with letrozole [93]. Although the same adverse events were observed, the most interesting finding was that the ORR and clinical benefit rates were considerably higher in patients that had no previous treatment (39% and 73%, respectively) compared to treated patients (5% and 32%, respectively). In light of these results, a larger phase 3 trial was conducted, named MONALEESA-2, consisting of 668 postmenopausal women with ER positive metastatic BC [94]. The aim of the MONALEESA-2 trial was to determine the safety of ribociclib in combination with letrozole as first-line therapy. Patients treated with ribociclib and letrozole demonstrated a progression free survival rate of 63%, significantly higher than that observed in the placebo plus letrozole treated group (42%). Overall response rates were also improved in the ribociclib and letrozole group compared to the placebo group (52.7% and 37.1% respectively). Adverse events were observed at higher rates in the ribociclib group compared to the placebo group (neutropenia (59.3% vs 0.9%) and leukopenia (21.0% vs. 0.6%)). Follow up results were published at a later interim and it was shown that patients treated with ribociclib and letrozole exhibited a median progression free survival of 25.3 months, whereas the placebo group showed a median of 16 months [95]. Similar results were also observed in premenopausal women treated with ribociclib and tamoxifen (and other combinations of aromatase inhibitors) in the MONALEESA-7 trial [96]. More recent analysis of the MONALEESA-7 trial was able to assess the overall survival in ribociclib (in combination with endocrine therapy) treated patients [97]. Here, it was sown that patients treated with ribociclib exhibited longer overall survival at 42 months (70.2%) compared to that of the placebo group (46.0%). This was also reflected by the lower number of deaths in the ribociclib

group (83/335 (24.8%) compared to 109/337 (32%) in the placebo group). Results from this analysis is the first of the CDK 4/6 clinical trials to report an overall survival benefit. Overall survival outcomes from other studies are awaited. However, considering the progression free survival benefit is very similar between all agents it is anticipated that OS benefit may also be similar.

Fulvestrant was also investigated in combination with ribociclib as second line treatment in 484 postmenopausal women [98]. This trial, MONALEESA-3, saw patients' progression free survival improve with ribociclib plus fulvestrant (20.5 months) as opposed to fulvestrant and placebo (12.8 months). Overall response rates were also higher in the ribociclib and fulvestrant group (40.9% vs 28.7%). Interestingly, the treatment effects of ribociclib were consistent in both patients that were treatment-naïve and those who had received prior therapy. These results demonstrate the safety of ribociclib and its promising option as first- and second-line therapy in advanced metastatic BC.

Clinical trials of CDK4/6 inhibitors in early BC in combination with endocrine therapy

The promising results of the CDK4/6 inhibitors in the advanced setting has forced for their investigation in early BC (summarised in Table 2). A large phase 3 trial currently recruiting approximately 5600 patients aims to study the effects of palbociclib in addition to adjuvant endocrine therapy in patients with stage 2 or stage 3 BC (ClinicalTrials.gov identifier: NCT02513394 (PALLAS)). In this study, a comparison will be made between a 2-year palbociclib and adjuvant endocrine therapy group and an adjuvant 5-year endocrine therapy only group. Initially, the primary outcome measure will be invasive disease free survival followed by further outcomes such as the safety of 2 years palbociclib with endocrine therapy and distant recurrence-free survival. Ribociclib and abemaciclib are also under investigation in combination with endocrine therapies in stage 3 high risk BC (ClinicalTrials.gov identifier: NCT03078751 (EarLEE-1) and NCT03155997 (monarchE) respectively). The EarLEE-1 and monarchE studies, will investigate the safety and efficacy of ribociclib (2 years) and abemaciclib, respectively, in combination with adjuvant endocrine therapy compared to endocrine therapy alone. Similarly to the above study, invasive disease free survival will primarily be measured followed by distant recurrence free survival and overall survival.

Palbociclib will also be investigated in another phase 3 trial, recruiting patients with residual disease and high risk of relapse (ClinicalTrials.gov identifier: NCT01864746 (PENELOPE-B)). In this study however, the assessment of palbociclib (standard 21 days on, 7 days off for 13 treatment cycles) will be conducted as a post-neoadjuvant (chemotherapy) treatment in pre- and postmenopausal women. Here, invasive disease free survival for palbociclib will be compared to a placebo after neoadjuvant chemotherapy. A single-arm trial has also been designed to study palbociclib as a neoadjuvant therapy in stage 2-3 ER positive BC [99]. In this study, 50 patients were treated with anastrazole for 4 weeks followed by the addition of palbociclib for four 28 day cycles. Complete cell cycle arrest (CCCA) was used as a primary end point. Patient biopsies showed that palbociclib treated patients had significantly higher CCCA rates compared to anastrazole monotherapy (87% vs 26% respectively). It was also noted that the anti-proliferative effects of palbociclib were quickly diminished when treatment was withheld prior to surgery, suggesting that the dormant effects are dependent on continuous treatment. The neoMONARCH phase 2 trial, abemaciclib was used in combination with anastrazole in 220 postmenopausal women (ClinicalTrials.gov identifier: NCT02441946). Similar results were observed as the previous study where cell cycle arrest was more profound (i.e. reduced Ki67 and higher CCCA rates) in abemaciclib alone, and in combination, compared to anastrazole alone. These studies demonstrate the rising interest in CDK inhibitors for early and advanced ER positive breast cancer. They also provide strong arguments to consider their use in neo- and adjuvant settings.

Combining CDK4/6 inhibitors with other systemic anti-cancer treatments.

Two interesting pathways exist that are involved in the regulation of cell survival, growth and proliferation. The phosphatidylinositol-3-kinase (PI3K)/Akt and the target of rapamycin (mTOR) signalling systems are highly interconnected and often considered as a single pathway. The PI3K/Akt pathway has a vital role in survival during cellular stress [100]. Similarly, the mTOR pathway is dependent on external signals, such as growth factors, oxygen and amino acids. It functions by translating these signals through downstream signalling molecules, by phosphorylating S6K1 and 4EBP, which in turn promote the production of proteins, lipids and nucleotides that are essential for growth, survival and proliferation (figure 3) [101]. It is also known that mTOR activation leads to the production of cyclin D1 [102]. These pathways play important roles in cellular processes and dysregulation or mutations can result in oncogenic outcomes. For example, the overexpression of the receptor tyrosine kinase, ErbB2, is found in approximately 25% of BCs. In these tumours, the

PI3K/Akt pathway is constitutively activated resulting in uncontrolled proliferation [103]. Thus, the mTOR pathway has become an interesting target for anti-cancer therapeutics, with the use of mTOR inhibitors previously reviewed [104]. Numerous mTOR inhibitors have been investigated in a range of cell lines, including BC, and have progressed into clinical trial [105,106].

mTOR inhibitors have been recently proposed to be used in combination with CDK inhibitors. In 2016, an in vitro study investigated the effects of the mTOR inhibitor rapamycin combined with the CDK inhibitor purvalanol on androgen dependent, and independent, prostate cancer cells. Co-treatment with rapamycin and purvalanol resulted in apoptosis through the modulation of upstream targets of PI3K/Akt/mTOR signalling pathways [107]. More recently in ER positive BC setting, combined inhibition of the mTOR and CDK pathways using vistusertib and palbociclib resulted in tumour regression in cell lines and xenografts [108]. Interestingly, inhibition of mTOR caused a decrease in cyclin D1 expression and consequently Rb phosphorylation, effects that were more profound in combination with palbociclib. Treatment with the mTOR inhibitor in CDK4/6 inhibitor-resistant cell lines inhibited cell growth via the inhibition of Rb and decreased E2F-dependent genes, effects not seen with palbociclib treatment alone. From these studies, it is clear that the targeting of multiple pathways may prove to be important in identifying new biomarkers or providing therapeutic strategies for BC development and progression. However many challenges must still be overcome, as these pathways are essential in the regulation and maintenance of normal functions in non-cancerous cells. Thus, clinical trials to investigate the efficacy and safety of multiple pathway-targeting drugs are imperative for the advancement of combination therapy.

The incorporation of triple combinations has also been explored. The use of a PIK3 inhibitor (BYL719) in combination with CDK4/6 inhibitor synergistically reduced BC cell viability [109]. Thus, the combination of ribociclib, BYL719 and letrozole is currently being investigated in a phase 1b trial (ClinicalTrials.gov : NCT 01872260). In addition, everolimus (mTOR inhibitor) combined with ribociclib and exemestane (AI) have also been trialled in phase 1b for the treatment of ER positive BC (ClinicalTrials.gov : NCT 01857193). The outcomes of these trials may give an insight on the safety and efficacy of combined treatment strategies and could prove to be an effective approach as a combination agent.

Since chemotherapy require cycling cells to be effective, CDK4/6 inhibition antagonises their effects and they are therefore not advised to be used in conjunction [110,111]. Thus, efforts have been made to investigate suitable scheduling for cytotoxic administrations in between CDK4/6 inhibition so that cycling cells are targeted without the antagonising effects of the inhibitor [112]. However, translating this into the clinic may still pose a challenge as proliferation rates within tumours are highly variable.

Conclusions

Although the progress of CDK4/6 inhibitors from preclinical studies to clinical trials has proved successful, a number of concerns remain over the use of CDK4/6 inhibitors. For example, treatment schedules are still in need of optimisation, particularly when used in combination with cytotoxic chemotherapy and/or radiotherapy. It is also important to recognise that cancers will have the ability to acquire resistance to highly specific CDK4/6 inhibitors, already observed in a number of solid tumours as well as BC [88,113,114]. That high specificity towards CDK4/6 may constitute a drawback is supported by data from preclinical in vitro and in vivo studies showing that using non-specific CDK inhibitors (dinaciclib and purvalanol) result in an elevated lethal effect in triple negative BC [115,116]. Resistance may be due to other CDKs are compensating in response to CDK4/6 inhibition (amplification of CDK6 or suppression of CDK2 inhibitors (p27kip1) for example), however this mechanism is poorly understood.

Preclinical data has provided vital information on CDK inhibitors and has proved pivotal for their subsequent clinical development. Although the safety and efficacy has improved with the development of more specific CDK inhibitors, moderate adverse events remain an issue. Combination therapy with the CDK inhibitors show comparable improvements in progression free survival in BC patients to those obtained using endocrine monotherapy. Despite the very promising improvement in progression free survival in response to CDK4/6 inhibitors, most studies have yet to demonstrate a corresponding positive effect on overall survival (due to the lack of reported deaths), generating some concern that aggressive treatment-resistant tumours would emerge on therapy. However, many trials were not powered to assess effects on overall survival, and for others longer follow up is required before positive effects can be established. In support of this, a recent pre-planned interim analysis of the MONALEESA-7 trial reported significantly longer overall survival when the CDK4/6 inhibitor ribociclib was added to endocrine therapy in patients with advanced, hormone-receptor–positive, HER2-negative

breast cancer [97]). It is highly promising that the improved overall survival in patients receiving ribociclib compared to placebo was consistent with the improved progression free survival previously reported in this trial [96]. As further trials mature, it will become clear whether prolonged progression free survival translates into improved overall survival in different patient populations and with different combinations of agents.

Table 1. Summary of the development of CDK inhibitors

Table 2. Clinical development of third generation CDK4/6 inhibitors

Figure1. The role of estrogen in endocrine-mediated cell proliferation and survival

Upon estrogen (E) binding, the estrogen receptor (ER) recruits co-activator complexes (CoA) and localises on specific DNA sites to initiate the transcription of growth factor genes as well as receptor tyrosine kinases that go on to mediate proliferation, survival and growth. Cytoplasmic ER can also bind to receptor tyrosine kinases in response to estrogen stimulation resulting in activation of downstream kinase pathways. Aromatase inhibitors act by inhibiting the aromatase enzyme. This enzyme is responsible for the conversion of androgens into estrogens. This results in the decreased levels of estrogen. Selective estrogen receptor modulators/ degraders (SERM and SERD respectively) target the ER. SERMs bind and antagonise the ER thereby preventing estrogen-mediated signalling. SERDs also bind to ERs and inhibits dimerization, thus it is unable to form complexes or function correctly and consequently degraded.

Figure 2. Cell cycle phases

The cell cycle progresses through a number of phases in order to commence with a successful division process. Cells prepare for cell division by using the gap phases to duplicate proteins, organelles and DNA. During the first gap phase, G1, the cell assesses the internal and external environments to determine its ability to progress through to the next phase. If conditions are unfavourable, the cell will leave the cell cycle and remain in a resting phase, G0, where it will remain dormant until stimulation from external factors. If, however, the conditions are favourable, the cell will progress into the S phase where DNA synthesis occurs. The second gap phase, G2, commences to allow for DNA replication. Provided that there have been no delays, damage or inhibition at previous stages, the cell will continue into the final stage of division, the mitotic (M) phase, where mitosis and cytokinesis are initiated. Passage from one phase to another is tightly regulated by checkpoints. These ensure that essential checks have been undertaken in order for the cell to correctly reproduce its genetic material with no errors that may result in the death of the cell, or in the case of cancer, aberrant cell proliferation. Under normal, resting, conditions, Rb exists in an inhibitory complex with E2F and other corepressors. If cell division is initiated, cyclin/CDK complexes are activated to phosphorylate RB, lifting its inhibitory function and allowing transcription of cell cycle genes. CDK

inhibitors target CDKs and inhibit their function, this results in Rb remaining in an unphosphorylated state and the cell remains in the resting phase.

Figure 3. Schematic overview of the PI3K/AKT/mTOR pathway.
Upon ligand binding, the receptor tyrosine kinase (RTK) activates PI3K which in turn phosphorylates PI(4,5)P2 and converts it to PI(3,4,5)P3. PI(3,4,5)P3 activates AKT by phosphorylating its serine-308 and threonine-473 residues. Once activated, AKT stimulates a cascade of downstream signals, including the nuclear accumulation of cyclin D, that promote cellular growth, survival and proliferation. Phosphorylated AKT also activates mTOR which forms the TORC1 complex. mTOR then targets downstream signalling molecules that result in the synthesis of proteins that play a role in maintaining survival and promoting proliferation. Genetic mutations lead to constitutive activation of the PI3K/AKT/mTOR pathway and are common in BC. Thus, the development of inhibitors (yellow and red hexagons) have allowed for the disruption of these pathways in an attempt to impede aberrant cell behaviour as shown by the dashed lines.

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7. References

1. Toriola AT, Colditz GA. Trends in breast cancer incidence and mortality in the United States: implications for prevention. Breast cancer research and treatment. 2013 Apr;138(3):665-73.
2. Siegel R, Naishadham D, Jemal A. Cancer statistics, 2012. CA: a cancer journal for clinicians. 2012 Jan-Feb;62(1):10-29.
3. Osborne CK, Schiff R. Mechanisms of endocrine resistance in breast cancer. Annual review of medicine. 2011;62:233-47.
4. Marino N, Woditschka S, Reed LT, et al. Breast Cancer Metastasis: Issues for the Personalization of Its Prevention and Treatment. Am J Pathol. Vol. 1832013. p. 1084-95.
5. Matutino A, Joy A, Brezden-Masley C, et al. Hormone receptor–positive, HER2-negative metastatic breast cancer: redrawing the lines. Curr Oncol. Vol. 252018. p. S131-41.
6. Austreid E, Lonning PE, Eikesdal HP. The emergence of targeted drugs in breast cancer to prevent resistance to endocrine treatment and chemotherapy. Expert opinion on pharmacotherapy. 2014 Apr;15(5):681-700.
7. DiPippo AJ, Patel NK, Barnett CM. Cyclin-Dependent Kinase Inhibitors for the Treatment of Breast Cancer: Past, Present, and Future. Pharmacotherapy. 2016 Jun;36(6):652-67.
8. Coit DG, Thompson JA, Algazi A, et al. Melanoma, Version 2.2016, NCCN Clinical Practice Guidelines in Oncology. Journal of the National Comprehensive Cancer Network : JNCCN. 2016 Apr;14(4):450-73.
9. Rugo HS, Rumble RB, Macrae E, et al. Endocrine Therapy for Hormone Receptor-Positive Metastatic Breast Cancer: American Society of Clinical Oncology Guideline. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2016 Sep 1;34(25):3069-103.
10. Malumbres M, Barbacid M. Cell cycle, CDKs and cancer: a changing paradigm. Nature reviews Cancer. 2009 Mar;9(3):153-66.
11. Johnson A, Skotheim JM. Start and the restriction point. Current opinion in cell biology. 2013 Dec;25(6):717-23.
12. Musgrove EA. Cyclins: roles in mitogenic signaling and oncogenic transformation. Growth factors (Chur, Switzerland). 2006 Mar;24(1):13-9.
13. Skotheim JM, Di Talia S, Siggia ED, et al. Positive feedback of G1 cyclins ensures coherent cell cycle entry. Nature. 2008 Jul 17;454(7202):291-6.
14. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011 Mar 4;144(5):646-74.
15. Ishii Y, Pirkmaier A, Alvarez JV, et al. Cyclin D1 overexpression and response to bortezomib treatment in a breast cancer model. Journal of the National Cancer Institute. 2006 Sep 6;98(17):1238-47.
16. Eeckhoute J, Carroll JS, Geistlinger TR, et al. A cell-type-specific transcriptional network required for estrogen regulation of cyclin D1 and cell cycle progression in breast cancer. Genes & development. 2006 Sep 15;20(18):2513-26.
17. Dickson C, Fantl V, Gillett C, et al. Amplification of chromosome band 11q13 and a role for cyclin D1 in human breast cancer. Cancer letters. 1995 Mar 23;90(1):43-50.

18. Koboldt DC FR, McLellan MD, Schmidt H, Kalicki-Veizer J, McMichael JF, Fulton LL, Dooling DJ, Ding L, Mardis ER, Wilson RK, Ally A, Balasundaram M, Butterfield YS, Carlsen R, Carter C, Chu A, Chuah E, Chun HJ. Comprehensive molecular portraits of human breast tumours. Nature. 2012 Oct 4;490(7418):61-70.
19. Knudsen ES, Knudsen KE. Tailoring to RB: tumour suppressor status and therapeutic response. Nat Rev Cancer. Vol. 8. England2008. p. 714-24.
20. Watts CK, Sweeney KJ, Warlters A, et al. Antiestrogen regulation of cell cycle progression and cyclin D1 gene expression in MCF-7 human breast cancer cells. Breast cancer research and treatment. 1994;31(1):95-105.
21. Beatson GT. On the Treatment of Inoperable Cases of Carcinoma of the Mamma: Suggestions for a New Method of Treatment, with Illustrative Cases. Transactions Medico-Chirurgical Society of Edinburgh. 1896;15:153-179.
22. Jordan VC, Allen KE. Evaluation of the antitumour activity of the non-steroidal antioestrogen monohydroxytamoxifen in the DMBA-induced rat mammary carcinoma model. European journal of cancer (Oxford, England : 1990). 1980 Feb;16(2):239-51.
23. Jordan VC, Phelps E, Lindgren JU. Effects of anti-estrogens on bone in castrated and intact female rats. Breast cancer research and treatment. 1987 Oct;10(1):31-5.
24. EBCTCG. Tamoxifen for early breast cancer: an overview of the randomised trials. Early Breast Cancer Trialists' Collaborative Group. Lancet. 1998 May 16;351(9114):1451-67.
25. Fisher B, Dignam J, Bryant J, et al. Five versus more than five years of tamoxifen therapy for breast cancer patients with negative lymph nodes and estrogen receptor-positive tumors. J Natl Cancer Inst. 1996 Nov 6;88(21):1529-42.
26. Fisher B, Costantino J, Redmond C, et al. A randomized clinical trial evaluating tamoxifen in the treatment of patients with node-negative breast cancer who have estrogen-receptor-positive tumors. N Engl J Med. 1989 Feb 23;320(8):479-84.
27. Day R, Ganz PA, Costantino JP, et al. Health-related quality of life and tamoxifen in breast cancer prevention: a report from the National Surgical Adjuvant Breast and Bowel Project P-1 Study. J Clin Oncol. 1999 Sep;17(9):2659-69.
28. Fisher B, Costantino JP, Wickerham DL, et al. Tamoxifen for the prevention of breast cancer: current status of the National Surgical Adjuvant Breast and Bowel Project P-1 study. J Natl Cancer Inst. 2005 Nov 16;97(22):1652-62.
29. Fisher B, Costantino JP, Wickerham DL, et al. Tamoxifen for Prevention of Breast Cancer: Report of the National Surgical Adjuvant Breast and Bowel Project P-1 Study. JNCI: Journal of the National Cancer Institute. 1998;90(18):1371-1388.
30. Jordan VC. The role of tamoxifen in the treatment and prevention of breast cancer. Curr Probl Cancer. 1992 May-Jun;16(3):129-76.
31. Davies C, Pan H, Godwin J, et al. Long-term effects of continuing adjuvant tamoxifen to 10 years versus stopping at 5 years after diagnosis of oestrogen receptor-positive breast cancer: ATLAS, a randomised trial. Lancet. 2013 Mar 9;381(9869):805-16.
32. Gray GR, Rea D, Handley K, et al. aTTom: Long-term effects of continuing adjuvant tamoxifen to 10 years versus stopping at 5 years in 6,953 women with early breast cancer. Journal of Clinical Oncology. 2013;31(15):5-5.
33. Chung CT, Carlson RW. The role of aromatase inhibitors in early breast cancer. Curr Treat Options Oncol. 2003 Apr;4(2):133-40.

34. Smith IE, Dowsett M, Yap YS, et al. Adjuvant aromatase inhibitors for early breast cancer after chemotherapy-induced amenorrhoea: caution and suggested guidelines. J Clin Oncol. 2006 Jun 1;24(16):2444-7.
35. EBCTCG. Aromatase inhibitors versus tamoxifen in early breast cancer: patient-level meta-analysis of the randomised trials. Lancet. 2015 Oct 3;386(10001):1341-1352.
36. Thurlimann B, Keshaviah A, Coates AS, et al. A comparison of letrozole and tamoxifen in postmenopausal women with early breast cancer. N Engl J Med. 2005 Dec 29;353(26):2747-57.
37. Howell A, Cuzick J, Baum M, et al. Results of the ATAC (Arimidex, Tamoxifen, Alone or in Combination) trial after completion of 5 years' adjuvant treatment for breast cancer. Lancet. 2005 Jan 1-7;365(9453):60-2.
38. Coombes RC, Hall E, Gibson LJ, et al. A randomized trial of exemestane after two to three years of tamoxifen therapy in postmenopausal women with primary breast cancer. N Engl J Med. 2004 Mar 11;350(11):1081-92.
39. Coombes RC, Kilburn LS, Snowdon CF, et al. Survival and safety of exemestane versus tamoxifen after 2-3 years' tamoxifen treatment (Intergroup Exemestane Study): a randomised controlled trial. Lancet. 2007 Feb 17;369(9561):559-70.
40. Goss PE, Ingle JN, Martino S, et al. Randomized trial of letrozole following tamoxifen as extended adjuvant therapy in receptor-positive breast cancer: updated findings from NCIC CTG MA.17. J Natl Cancer Inst. 2005 Sep 7;97(17):1262-71.
41. Goss PE, Ingle JN, Martino S, et al. Efficacy of letrozole extended adjuvant therapy according to estrogen receptor and progesterone receptor status of the primary tumor: National Cancer Institute of Canada Clinical Trials Group MA.17. J Clin Oncol. 2007 May 20;25(15):2006-11.
42. Braithwaite RS, Chlebowski RT, Lau J, et al. Meta-analysis of vascular and neoplastic events associated with tamoxifen. J Gen Intern Med. 2003 Nov;18(11):937-47.
43. Kümler I, Knoop AS, Jessing CAR, et al. Review of hormone-based treatments in postmenopausal patients with advanced breast cancer focusing on aromatase inhibitors and fulvestrant. ESMO Open. Vol. 12016.
44. Nabholtz JM, Buzdar A, Pollak M, et al. Anastrozole is superior to tamoxifen as first-line therapy for advanced breast cancer in postmenopausal women: results of a North American multicenter randomized trial. Arimidex Study Group. J Clin Oncol. 2000 Nov 15;18(22):3758-67.
45. Milla-Santos A, Milla L, Portella J, et al. Anastrozole versus tamoxifen as first-line therapy in postmenopausal patients with hormone-dependent advanced breast cancer: a prospective, randomized, phase III study. Am J Clin Oncol. 2003 Jun;26(3):317-22.
46. Smith IE. Letrozole versus tamoxifen in the treatment of advanced breast cancer and as neoadjuvant therapy. J Steroid Biochem Mol Biol. 2003 Sep;86(3-5):289-93.
47. Mouridsen H, Sun Y, Gershanovich M, et al. Superiority of letrozole to tamoxifen in the first-line treatment of advanced breast cancer: evidence from metastatic subgroups and a test of functional ability. Oncologist. 2004;9(5):489-96.
48. Goss PE, Ingle JN, Pritchard KI, et al. Extending Aromatase-Inhibitor Adjuvant Therapy to 10 Years. N Engl J Med. 2016 Jul 21;375(3):209-19.

49. Howell A, Robertson JF, Quaresma Albano J, et al. Fulvestrant, formerly ICI 182,780, is as effective as anastrozole in postmenopausal women with advanced breast cancer progressing after prior endocrine treatment. J Clin Oncol. 2002 Aug 15;20(16):3396-403.
50. Osborne CK, Pippen J, Jones SE, et al. Double-blind, randomized trial comparing the efficacy and tolerability of fulvestrant versus anastrozole in postmenopausal women with advanced breast cancer progressing on prior endocrine therapy: results of a North American trial. J Clin Oncol. 2002 Aug 15;20(16):3386-95.
51. Howell A, Robertson JF, Abram P, et al. Comparison of fulvestrant versus tamoxifen for the treatment of advanced breast cancer in postmenopausal women previously untreated with endocrine therapy: a multinational, double-blind, randomized trial. J Clin Oncol. 2004 May 1;22(9):1605-13.
52. Bergh J, Jonsson PE, Lidbrink EK, et al. FACT: an open-label randomized phase III study of fulvestrant and anastrozole in combination compared with anastrozole alone as first-line therapy for patients with receptor-positive postmenopausal breast cancer. J Clin Oncol. 2012 Jun 1;30(16):1919-25.
53. Mehta RS, Barlow WE, Albain KS, et al. Combination anastrozole and fulvestrant in metastatic breast cancer. N Engl J Med. 2012 Aug 2;367(5):435-44.
54. Kuter I, Gee JM, Hegg R, et al. Dose-dependent change in biomarkers during neoadjuvant endocrine therapy with fulvestrant: results from NEWEST, a randomized Phase II study. Breast Cancer Res Treat. 2012 May;133(1):237-46.
55. Di Leo A, Jerusalem G, Petruzelka L, et al. Final overall survival: fulvestrant 500 mg vs 250 mg in the randomized CONFIRM trial. J Natl Cancer Inst. 2014 Jan;106(1):djt337.
56. Robertson JF, Lindemann JP, Llombart-Cussac A, et al. Fulvestrant 500 mg versus anastrozole 1 mg for the first-line treatment of advanced breast cancer: follow-up analysis from the randomized 'FIRST' study. Breast Cancer Res Treat. 2012 Nov;136(2):503-11.
57. Robertson JFR, Bondarenko IM, Trishkina E, et al. Fulvestrant 500 mg versus anastrozole 1 mg for hormone receptor-positive advanced breast cancer (FALCON): an international, randomised, double-blind, phase 3 trial. Lancet. 2016 Dec 17;388(10063):2997-3005.
58. Szostakowska M, Trębińska-Stryjewska A, Grzybowska EA, et al. Resistance to endocrine therapy in breast cancer: molecular mechanisms and future goals. Breast Cancer Res Treat. Vol. 1732019. p. 489-97.
59. Jeselsohn R, Buchwalter G, De Angelis C, et al. ESR1 mutations as a mechanism for acquired endocrine resistance in breast cancer. Nat Rev Clin Oncol. 2015 Oct;12(10):573-83.
60. De Mattos-Arruda L, Weigelt B, Cortes J, et al. Capturing intra-tumor genetic heterogeneity by de novo mutation profiling of circulating cell-free tumor DNA: a proof-of-principle. Ann Oncol. 2014 Sep;25(9):1729-35.
61. Zhang K, Hong R, Xu F, et al. Clinical value of circulating ESR1 mutations for patients with metastatic breast cancer: a meta-analysis. Cancer Manag Res. 2018;10:2573-2580.
62. Sedlacek H, Czech J, Naik R, et al. Flavopiridol (L86 8275; NSC 649890), a new kinase inhibitor for tumor therapy. Int J Oncol. 1996 Dec;9(6):1143-68.
63. Shapiro GI. Cyclin-dependent kinase pathways as targets for cancer treatment. J Clin Oncol. 2006 Apr 10;24(11):1770-83.

64. Czech J, Hoffmann D, Naik R, et al. Antitumoral activity of flavone L-86-8275. Int J Oncol. 1995 Jan;6(1):31-6.
65. Carlson BA, Dubay MM, Sausville EA, et al. Flavopiridol induces G1 arrest with inhibition of cyclin-dependent kinase (CDK) 2 and CDK4 in human breast carcinoma cells. Cancer Res. 1996 Jul 1;56(13):2973-8.
66. Parker BW, Kaur G, Nieves-Neira W, et al. Early induction of apoptosis in hematopoietic cell lines after exposure to flavopiridol. Blood. 1998 Jan 15;91(2):458-65.
67. Schwartz GK, O'Reilly E, Ilson D, et al. Phase I study of the cyclin-dependent kinase inhibitor flavopiridol in combination with paclitaxel in patients with advanced solid tumors. J Clin Oncol. 2002 Apr 15;20(8):2157-70.
68. Bose P, Simmons GL, Grant S. Cyclin-dependent kinase inhibitor therapy for hematologic malignancies. Expert Opin Investig Drugs. 2013 Jun;22(6):723-38.
69. Parry D, Guzi T, Shanahan F, et al. Dinaciclib (SCH 727965), a novel and potent cyclin-dependent kinase inhibitor. Mol Cancer Ther. 2010 Aug;9(8):2344-53.
70. Nemunaitis JJ, Small KA, Kirschmeier P, et al. A first-in-human, phase 1, dose-escalation study of dinaciclib, a novel cyclin-dependent kinase inhibitor, administered weekly in subjects with advanced malignancies. J Transl Med. 2013 Oct 16;11:259.
71. Mita MM, Joy AA, Mita A, et al. Randomized phase II trial of the cyclin-dependent kinase inhibitor dinaciclib (MK-7965) versus capecitabine in patients with advanced breast cancer. Clin Breast Cancer. 2014 Jun;14(3):169-76.
72. VanderWel SN, Harvey PJ, McNamara DJ, et al. Pyrido[2,3-d]pyrimidin-7-ones as specific inhibitors of cyclin-dependent kinase 4. J Med Chem. 2005 Apr 7;48(7):2371-87.
73. Fry DW, Harvey PJ, Keller PR, et al. Specific inhibition of cyclin-dependent kinase 4/6 by PD 0332991 and associated antitumor activity in human tumor xenografts. Mol Cancer Ther. 2004 Nov;3(11):1427-38.
74. Gelbert LM, Cai S, Lin X, et al. Preclinical characterization of the CDK4/6 inhibitor LY2835219: in-vivo cell cycle-dependent/independent anti-tumor activities alone/in combination with gemcitabine. Invest New Drugs. Vol. 322014. p. 825-37.
75. Asghar U, Witkiewicz AK, Turner NC, et al. The history and future of targeting cyclin-dependent kinases in cancer therapy. Nat Rev Drug Discov. 2015 Feb;14(2):130-46.
76. Finn RS, Dering J, Conklin D, et al. PD 0332991, a selective cyclin D kinase 4/6 inhibitor, preferentially inhibits proliferation of luminal estrogen receptor-positive human breast cancer cell lines in vitro. Breast Cancer Res. Vol. 112009. p. R77.
77. Dean JL, McClendon AK, Hickey TE, et al. Therapeutic response to CDK4/6 inhibition in breast cancer defined by ex vivo analyses of human tumors. Cell Cycle. Vol. 112012. p. 2756-61.
78. O'Brien N, Tomaso ED, J. SD, editors. Abstract 4756: In vivo efficacy of combined targeting of CDK4/6, ER and PI3K signaling in ER+ breast cancer. 105th Annual Meeting of the American Association for Cancer Research; 2014; San Diego, CA.: AACR; Cancer Res.
79. Gao H, Korn JM, Ferretti S, et al. High-throughput screening using patient-derived tumor xenografts to predict clinical trial drug response. Nat Med. 2015 Nov;21(11):1318-25.

80. O'Brien N, Conklin D, Beckmann R, et al. Preclinical Activity of Abemaciclib Alone or in Combination with Antimitotic and Targeted Therapies in Breast Cancer. Mol Cancer Ther. 2018 May;17(5):897-907.
81. Slamon D, Crown J, Lang I, et al. Long-term safety profile of palbociclib (P) in combination with letrozole (L) as first-line treatment for postmenopausal patients with ER+ and HER2- advanced breast cancer (ABC) (PALOMA-1/TRIO-18). [meeting-report]. http://dxdoiorg/101200/jco20153315_suppl570. 2015 2017-01-31.
82. DeMichele A, Clark AS, Tan KS, et al. CDK 4/6 inhibitor palbociclib (PD0332991) in Rb+ advanced breast cancer: phase II activity, safety, and predictive biomarker assessment. Clin Cancer Res. 2015 Mar 1;21(5):995-1001.
83. Finn RS, Crown JP, Lang I, et al. The cyclin-dependent kinase 4/6 inhibitor palbociclib in combination with letrozole versus letrozole alone as first-line treatment of oestrogen receptor-positive, HER2-negative, advanced breast cancer (PALOMA-1/TRIO-18): a randomised phase 2 study. Lancet Oncol. 2015 Jan;16(1):25-35.
84. Finn RS, Martin M, Rugo HS, et al. Palbociclib and Letrozole in Advanced Breast Cancer. N Engl J Med. 2016 Nov 17;375(20):1925-1936.
85. Cristofanilli M, Turner NC, Bondarenko I, et al. Fulvestrant plus palbociclib versus fulvestrant plus placebo for treatment of hormone-receptor-positive, HER2-negative metastatic breast cancer that progressed on previous endocrine therapy (PALOMA-3): final analysis of the multicentre, double-blind, phase 3 randomised controlled trial. Lancet Oncol. 2016 Apr;17(4):425-439.
86. Turner NC, Slamon DJ, Ro J, et al. Overall Survival with Palbociclib and Fulvestrant in Advanced Breast Cancer. N Engl J Med. 2018 Nov 15;379(20):1926-1936.
87. Shapiro G, Rosen LS, Tolcher AW, et al. A first-in-human phase I study of the CDK4/6 inhibitor, LY2835219, for patients with advanced cancer. [meeting-report]. http://dxdoiorg/101200/jco20133115_suppl2500. 2013 2017-01-30.
88. Patnaik A, Rosen LS, Tolaney SM, et al. Efficacy and Safety of Abemaciclib, an Inhibitor of CDK4 and CDK6, for Patients with Breast Cancer, Non-Small Cell Lung Cancer, and Other Solid Tumors. Cancer Discov. 2016 Jul;6(7):740-53.
89. Dickler MN, Tolaney SM, Rugo HS, et al. MONARCH 1, A Phase II Study of Abemaciclib, a CDK4 and CDK6 Inhibitor, as a Single Agent, in Patients with Refractory HR(+)/HER2(-) Metastatic Breast Cancer. Clin Cancer Res. 2017 Sep 1;23(17):5218-5224.
90. Sledge GW, Jr., Toi M, Neven P, et al. MONARCH 2: Abemaciclib in Combination With Fulvestrant in Women With HR+/HER2- Advanced Breast Cancer Who Had Progressed While Receiving Endocrine Therapy. J Clin Oncol. 2017 Sep 1;35(25):2875-2884.
91. Goetz MP, Toi M, Campone M, et al. MONARCH 3: Abemaciclib As Initial Therapy for Advanced Breast Cancer. J Clin Oncol. 2017 Nov 10;35(32):3638-3646.
92. Infante JR, Cassier PA, Gerecitano JF, et al. A Phase I Study of the Cyclin-Dependent Kinase 4/6 Inhibitor Ribociclib (LEE011) in Patients with Advanced Solid Tumors and Lymphomas. Clin Cancer Res. 2016 Dec 1;22(23):5696-5705.
93. Juric D, Munster, P.N., Campone, M., Ismail-Khan, R., García-Estevez, L., Hamilton, E.P., Becerra, C., De Boer, R.H., Hui, R., Goncalves, A. and Wang, Y. Ribociclib (LEE011) and letrozole in estrogen receptor-positive (ER+), HER2-negative (HER2−) advanced breast cancer (aBC): Phase Ib safety, preliminary efficacy and molecular analysis. Journal of Clinical Oncology. 2016;34:568-568.

94. Hortobagyi GN, Stemmer SM, Burris HA, et al. Ribociclib as First-Line Therapy for HR-Positive, Advanced Breast Cancer. N Engl J Med. 2016 Nov 3;375(18):1738-1748.
95. Hortobagyi GN, Stemmer SM, Burris HA, et al. Updated results from MONALEESA-2, a phase III trial of first-line ribociclib plus letrozole versus placebo plus letrozole in hormone receptor-positive, HER2-negative advanced breast cancer. Ann Oncol. 2018 Jul 1;29(7):1541-1547.
96. Tripathy D, Im SA, Colleoni M, et al. Ribociclib plus endocrine therapy for premenopausal women with hormone-receptor-positive, advanced breast cancer (MONALEESA-7): a randomised phase 3 trial. Lancet Oncol. 2018 Jul;19(7):904-915.
97. Im SA, Lu YS, Bardia A, et al. Overall Survival with Ribociclib plus Endocrine Therapy in Breast Cancer. N Engl J Med. 2019 Jun 4.
98. Slamon DJ, Neven P, Chia S, et al. Phase III Randomized Study of Ribociclib and Fulvestrant in Hormone Receptor-Positive, Human Epidermal Growth Factor Receptor 2-Negative Advanced Breast Cancer: MONALEESA-3. J Clin Oncol. 2018 Aug 20;36(24):2465-2472.
99. Ma CX, Gao F, Luo J, et al. NeoPalAna: Neoadjuvant Palbociclib, a Cyclin-Dependent Kinase 4/6 Inhibitor, and Anastrozole for Clinical Stage 2 or 3 Estrogen Receptor-Positive Breast Cancer. Clin Cancer Res. 2017 Aug 1;23(15):4055-4065.
100. Datta SR, Brunet A, Greenberg ME. Cellular survival: a play in three Akts. Genes Dev. 1999 Nov 15;13(22):2905-27.
101. Saxton RA, Sabatini DM. mTOR Signaling in Growth, Metabolism, and Disease. Cell. 2017 Mar 9;168(6):960-976.
102. Grewe M, Gansauge F, Schmid RM, et al. Regulation of cell growth and cyclin D1 expression by the constitutively active FRAP-p70s6K pathway in human pancreatic cancer cells. Cancer Res. 1999 Aug 1;59(15):3581-7.
103. Holbro T, Beerli RR, Maurer F, et al. The ErbB2/ErbB3 heterodimer functions as an oncogenic unit: ErbB2 requires ErbB3 to drive breast tumor cell proliferation. Proc Natl Acad Sci U S A. 2003 Jul 22;100(15):8933-8.
104. Faivre S, Kroemer G, Raymond E. Current development of mTOR inhibitors as anticancer agents. Nat Rev Drug Discov. Vol. 5. England2006. p. 671-88.
105. Zarogoulidis P, Lampaki S, Turner JF, et al. mTOR pathway: A current, up-to-date mini-review (Review). Oncol Lett. Vol. 82014. p. 2367-70.
106. Matar P, Rojo F, Cassia R, et al. Combined epidermal growth factor receptor targeting with the tyrosine kinase inhibitor gefitinib (ZD1839) and the monoclonal antibody cetuximab (IMC-C225): superiority over single-agent receptor targeting. Clin Cancer Res. 2004 Oct 1;10(19):6487-501.
107. Berrak O, Arisan ED, Obakan-Yerlikaya P, et al. mTOR is a fine tuning molecule in CDK inhibitors-induced distinct cell death mechanisms via PI3K/AKT/mTOR signaling axis in prostate cancer cells. Apoptosis. 2016 Oct;21(10):1158-78.
108. Michaloglou C, Crafter C, Siersbaek R, et al. Combined Inhibition of mTOR and CDK4/6 Is Required for Optimal Blockade of E2F Function and Long-term Growth Inhibition in Estrogen Receptor-positive Breast Cancer. Mol Cancer Ther. 2018 May;17(5):908-920.
109. Vora SR, Juric D, Kim N, et al. CDK 4/6 inhibitors sensitize PIK3CA mutant breast cancer to PI3K inhibitors. Cancer Cell. 2014 Jul 14;26(1):136-49.
110. Dean JL, McClendon AK, Knudsen ES. Modification of the DNA damage response by therapeutic CDK4/6 inhibition. J Biol Chem. 2012 Aug 17;287(34):29075-87.

111. Roberts PJ, Bisi JE, Strum JC, et al. Multiple roles of cyclin-dependent kinase 4/6 inhibitors in cancer therapy. J Natl Cancer Inst. 2012 Mar 21;104(6):476-87.
112. Witkiewicz AK, Cox D, Knudsen ES. CDK4/6 inhibition provides a potent adjunct to Her2-targeted therapies in preclinical breast cancer models. Genes Cancer. 2014 Jul;5(7-8):261-72.
113. Dean JL, Thangavel C, McClendon AK, et al. Therapeutic CDK4/6 inhibition in breast cancer: key mechanisms of response and failure. Oncogene. 2010 Jul 15;29(28):4018-32.
114. Herrera-Abreu MT, Palafox M, Asghar U, et al. Early Adaptation and Acquired Resistance to CDK4/6 Inhibition in Estrogen Receptor-Positive Breast Cancer. Cancer Res. 2016 Apr 15;76(8):2301-13.
115. Kang J, Sergio CM, Sutherland RL, et al. Targeting cyclin-dependent kinase 1 (CDK1) but not CDK4/6 or CDK2 is selectively lethal to MYC-dependent human breast cancer cells. BMC Cancer. 2014 Jan 20;14:32.
116. Horiuchi D, Kusdra L, Huskey NE, et al. MYC pathway activation in triple-negative breast cancer is synthetic lethal with CDK inhibition. J Exp Med. 2012 Apr 9;209(4):679-96.