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Cdk 4/6 inhibitors revolutionized breast cancer therapy

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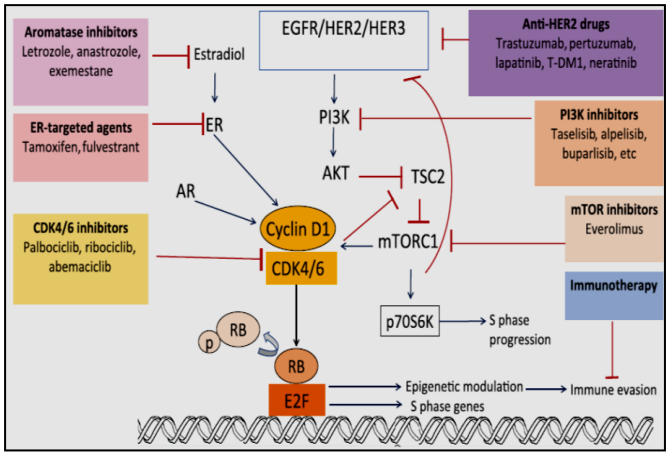
Abstract
The cyclin-CDK inhibitors of the Cip/Kip family p21Cip1, p27Kip1, and p57Kip2 have emerged as multifaceted proteins with functions beyond cell cycle regulation.
They are used to treat cancers by preventing over proliferation of cancer cells. In metazoans, two CKI gene families have been defined based on their evolutionary origins, structure, and CDK specificities. A vast body of literature has described the importance of p21, p27, and p57 in restraining proliferation during development, differentiation, and response to cellular stresses, although each has specific biological functions that distinguish it from the other family members. Thus, different anti-proliferative signals tend to cause elevated expression of only a subset of the Cip/Kip proteins. Following a recent and detailed review on the subject. We concentrate our attention on an updated list of compounds under clinical evaluation (phase I/II/III) and discuss their mode of action as ATP-competitive inhibitors Also, tentative progress for forthcoming potential ATP non-competitive inhibitors and allosteric inhibitors will be discussed.

Keywords: cyclin-CDK, ATP-competitive inhibitors, ATP non-competitive, metazone

Introduction
Breast Cancer (BC) is the most common cancer type in women. It is estimated that it will be responsible for 40,450 deaths in the US in 2016, representing the second cause of cancer-related death after lung cancer [1]. Amongst novel possible therapeutical targets, proteins involved in the control of the cell cycle attracted a lot of interest in the last 10 years: cyclins and cyclin-dependent kinases (CDKs) were firstly discovered in yeast and then in humans [2]. In normal tissues, cell proliferation is tightly regulated by the cell cycle machinery, a group of proteins that controls a cell's orderly procession from one phase of the cell cycle to the next. In breast cancer, much attention has been given to particular members of the cell cycle machinery. The D-type cyclins and their partner kinases, cyclin-dependent kinase 4 (CDK4) and CDK6. Indeed, a wealth of preclinical research has shown that tumor cell proliferation in many breast cancers is underpinned by hyperactivity of the cyclin D–CDK4/6 axis, making pharmacological blockade of this axis an attractive therapeutic strategy [3-5]. First-generation CDK inhibitors tended to be less specific, targeting other CDKs in a broad fashion and were associated with chemotherapy-like toxicities and unacceptable safety profiles. More recently, a new generation of very specific CDK 4/6 inhibitors have been developed [6-7].

Mechanism of cyclind1–CDK4/6–RB pathway
Potent, selective, orally bioavailable inhibitors of CDK4/6 have only become available as cancer therapeutics in the last decade. By directly blocking the activity of the cyclin D–CDK4/6 holoenzyme, these agents act to restrain proliferation of sensitive tumor cells, in particular preventing cell cycle progression from the G1 to the S phase of the cell cycle (see below and Figure 1). In sensitive cells, CDK4/6 inhibition typically induces a phenotype resembling cellular senescence [8], consistent with the critical role of the retinoblastoma (RB) tumor suppressor in mediating senescence [9].



The role of the cyclin D1–CDK4/6–RB pathway in breast cancer

The role of the cyclin D1–CDK4/6-RB pathway in breast cancer cells, including cross talk with other oncogenic signaling pathways. Mitogenic forces including ER transcriptional activity and signaling through ERBB2/PI3K/AKT/mTOR increase cyclin D1 levels, activating CDK4/6 and promoting cellular progression to the S phase. There is extensive crosstalk between the PI3K and CDK4/6 pathways: not only does PI3K pathway activity increase cyclin D1 levels, but the cyclin D–CDK4/6 complex can modulate TSC2 phosphorylation and hence mTORC1 activity. Combined inhibition of CDK4/6 and nodes in the PI3K pathway can thus maximally suppress mTORC1 activity as well as RB phosphorylation, inhibiting two promoters of S phase progression. Furthermore, suppression of E2F activity can modulate the tumor cell epigenome, rendering tumor cells more immunogenic and providing a rationale for CDK4/6-immunotherapy combinations. AR, androgen receptor; EGFR, epidermal growth factor receptor; ER, estrogen receptor; HER, human epidermal growth factor receptor; mTOR, mammalian target of rapamycin; PI3K, phosphoinositide-3-kinase; RB, retinoblastoma protein, TSC2, tuberous sclerosis complex 2 (tuberin).

The cell cycle constitutes a series of tightly controlled events that drive DNA replication and cell division. The cell cycle is divided into phases: G0 (quiescence) followed by G1 (pre-DNA synthesis), S (DNA synthesis), G2 (pre-division), and M (cell division) [10]. The progression from G1 to S is a critical checkpoint in protecting the cell from abnormal replication, and a key regulator of this process is the cyclin D–CDK 4/6–INK4–Rb pathway. A variety of mitogenic signaling pathways, including steroid hormones (such as the ER pathway), PI3K/AKT/mTOR, MAPKs, wnt/β-catenin, STATs, and NF-κB/IKK, upregulate the expression of cyclin D, which associates with CDK 4/6 [11]. Activation of the cyclin D-CDK 4/6 complex contributes to the hyperphosphorylation of the Rb protein, which causes inactivation of its growth-inhibitory function by decoupling it from E2F transcription factors. E2F transcription factors allow the transcription of genes promoting entry into the S phase hence, cell-cycle progress. Association of cyclin D with CDK 4/6 is tightly regulated by various inhibitors, such as INK4, Cip, and Kip proteins [12].

Drug discovery

CDK4/6 inhibitors have shown both preclinical and clinical activity in ER-positive breast cancer, and data suggest synergy when combining antiestrogen therapy with CDK4/6 inhibitors.13, 14 the fact that cyclin D1 is a downstream effectors of estrogen stimulation highlighted the potential role of targeting the cell cycle in ER positive BCs. For their serine/threonine kinase activity, the cyclin-dependent kinases represented the perfect therapeutic target. The first generation of CDK4/6 inhibitors (i.e. flavopiridol) gave disappointing results in clinical trials due to lack of selectivity for the target (pan-CDK inhibitors), thus a range of drug-mediated dose-limiting side-effects [15]. Also, the route and time of administration (IV) represented other relative limitations for these drugs, adding complexity to the treatment regimes. Flavopiridol, the most studied compound of this group, showed scarce activity as a single agent and a moderate activity in combination with chemotherapy [16]. It is worth noting that the three approved CDK4/6 inhibitors present distinct relative potencies for CDK4 and CDK6 inhibition, pharmacokinetics, dosing schedules and toxicity profiles they are: Palbociclib, Ribociclib, Abemaciclib.

Table 1

points	Palbociclib	Ribociclib	Abemaciclib
Action on resistance	The first CDK4/6 inhibitor described to show activity against breast cancer cells, when Finn and colleagues demonstrated synergy between palbociclib and endocrine therapy in ER-positive cell lines [17].	Reversible Cdk4/6 Inhibitor [21].	Most potent Cdk4/6 inhibitors [23].
Action	palbociclib inhibits the growth of tamoxifen-resistant ER-positive xenografts *in vivo* when added to selective ER degraders (SERDs) [18].	Same as that of palbociclib.	Abemaciclib has also been shown to have inhibitory activity against other kinases *in vitro* including, but not limited to, CDK9 and PIM1. It able to cross blood brain barrier hence showing some activity in central nervous system.
Drug profile	A first-in-human phase I study of palbociclib in patients with solid tumors showed a favorable safety profile with myelosuppression, particularly neutropenia, being the main dose-limiting toxicity – presumably a consequence of CDK6 inhibition in granulocyte precursors [19].	The toxicity profile of ribociclib is very Similar to palbociclib, with neutropenia and Thrombocytopenia being the most frequent grade 3/4 adverse events in large trials Ribociclib therapy can Prolong the QT interval as measured by electrocardiography, Requiring monitoring of this parameter In clinical practice.	Hematopoietic toxicity is less common with abemaciclib than with palbociclib or ribociclib Diarrhea has emerged as the most common abemaciclib toxicity [24].
Administration	Dose of 125 mg daily 3 weeks on, 1 week off, in conjunction with endocrine therapy [20].	Starting dose of 600 mg daily, 3 weeks on, 1 week off [22].	Starting at 200 mg bid as a monotherapy Or 150 mg bid when given with endocrine Therapy [25].

Three trials including patients with hormone receptor (HR)-positive, HER2-negative BC are currently ongoing in the adjuvant setting (table 2) [2]. Ongoing trials with preoperative or adjuvant CDK4/6 inhibitors in primary BC. Following are the results obtained:

Clinical trial.gov identifier	Therapy	Phase	Patient characteristics	Number of patients	Primary end points	Estimated study completion
Adjuvant Palbociclib						
NCT02040857	Palbociclib + AI or tamoxifen	II	HR+, HER2- stage 2 or 3 (+ men)	160	Treatment discontinuation rate	June 2019, recruiting
NCT18644746	Palbociclib (13 cycles) + Standard ET Placebo + Standard ET	III, PENELOPE-B	HR+, HER2- Residual invasive disease after neoadjuvant chemotherapy; adequate surgery High CPS-EG score	1100	Invasive DFS	November 2023, recruiting
NCT02513394	Palbociclib 2 years + standard ET Standard ET	III, PALLAS	HR+, HER2- Stage 2 or 3 (+men)	4600	Invasive DFS	September 2025, recruiting
Presurgical Palbociclib						
NCT01709370	Palbociclib + letrozole (16 weeks)	II	OR+, HER2- Postmenopausal tumour≥2 cm Not T3N1, T4, N2 or N3	45	RR	NR, study status last verified October 2012
NCT01723774	Anastrozole + goserelin (if premenopausal) + palbociclib	II	OR+, HER2- stage 2 or 3	29	Complete cell cycle arrest in women without PIK3CA hot spot mutation	February 2016, recruiting
NCT02296801	Letrozole Letrozole → palbociclib + letrozole Palbociclib → palbociclib +letrozole Palcociclib + letrozole 14 weeks	II, PALLET neoadjuvant	OR+, HER2- postmenopausal operable, tumour≥2 cm	306	Proliferation (Ki67)	January 2015, recruiting
NCT02400567	FEC→ docetaxel palbociclib + letrozole	II, NeoPAL Randomised,	Luminal A + nodal involvement or luminal B postmenopausal stage −2–3A	132	Number with residual tumour in breast or lymph node	April 2019, recruiting
Eudract number 2014-000809-12	Palbociclib + standard ET standard ET	II, PREDIXLumA (part of a translational study based of molecular subtypes)	Luminal A >2 cm, no lymph node metastases	200 (whole trial)	pCR	NR, recruiting
Eudract number 2014-000810-12	Palbociclib + standard ET standard ET	II, PREDIXLumB (part of a translational study based of molecular subtypes)	Luminal B>2 cm and/or lymph node metastases	200 (whole trial)	pCR	NR, recruiting
NCT02008734	Control palbociclib (125 m/day for 14 days) Palbociclib (100 mg/d for 21 days)	II, POP Randomised (3:1)	Untreated, operable early BC (≥15 mm) Not candidate for neoadjuvant chemotherapy	105	Antiproliferative response	January 2016, recruiting
Abemaciclib						
NCT02441946	Abemaciclib + loperamide 2 weeks Abemaciclib + loperamide + anastrozole 2 weeks Anastrozole 2 weeks Followed by 14 weeks abemaciclib + anastrozole + loperamide	II, NeoMONARCH	ER+, HER2- Postmenopausal tumour≥1 cm, ET deemed suitable	220	Ki67 expression at 2 weeks	February 2017, recruiting
Ribociclib						
NCT01919229	Ribociclib (400 mg) + letrozole Ribociclib (600 mg) + letrozole Letrozole	II, MONALEESA-1	HR+, HER2- Postmenopausal, tumour≥1.0 cm	14	Cell cycle response rate	Completed, no results published

Future aspects

Trials of approved medicines continue to see who may benefit from which combinations. Results from the MONALEESA-7 trial showed ribociclib could help women who haven with or without menopause though no symptoms of metastatic cancer growth. In the study ribociclib was given with hormonal

therapy as well as ovarian suppression, medicine to stop periods and prevent the ovaries from working. Meanwhile, the PALLAS trial are testing if the addition of CDK 4/6 inhibitors to hormonal therapy works in early-stage breast cancer. Researchers are also studying whether CDK 4/6 inhibitors help immunotherapy medicines work better and there is an urgent need for prospective biomarker-driven trials to identify patients for whom these treatments are cost-effective.

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