

REVIEW



Potential role of cyclin-dependent kinase 4/6 inhibitors in the treatment of squamous cell carcinoma of the head and neck

Gabrielle van Caloen^a and Jean-Pascal Machiels^{a,b}

Purpose of review

Human papillomavirus (HPV)-negative squamous cell carcinoma of the head and neck (SCCHN) is mainly driven by genetic aberrations involved in the cell cycle pathway resulting in cyclin-dependent kinase (CDK) 4 and 6 activation. This supports the investigation of the activity of CDK4/6 inhibitors in this disease. We review the therapeutic potential of CDK4/6 inhibitors in SCCHN.

Recent findings

CDK4/6 inhibitors in monotherapy have demonstrated cytostatic activity in HPV-negative SCCHN. Combination with epidermal growth factor inhibitors, with phosphatidylinositol-3-kinase/protein kinase B/mammalian target of rapamycin pathways inhibitors or with immunotherapy, have shown promising preclinical efficacy. No strong predictive biomarkers of response or resistance have been firmly identified. Phase I clinical trials have demonstrated that palbociclib or ribociclib in combination with cetuximab is well tolerated. A phase II single-arm trial combining palbociclib/cetuximab has shown promising results.

Summary

Inhibition of CDK4/6 represents a new potential treatment for HPV-negative SCCHN patients. Randomized clinical trials that investigate these compounds in an unbiased manner are needed to fully evaluate their efficacy. However, it is unlikely that all the patients will benefit from this new approach. To determine a molecular profile/phenotype that will predict CDK4/6 inhibitor activity, researchers will have to take into account simultaneously occurring events in the cyclin-D/CDK4/CDK6/retinoblastoma and associated pathways.

Keywords

cyclin-dependent kinase 4/6 inhibitors, head and neck cancer, predictive biomarkers

INTRODUCTION

Curative treatment of squamous cell carcinoma of the head and neck (SCCHN) is based on surgery and/or (chemo)radiation, depending on the location of the primary tumour and disease stage. Approximately one-third of these patients will relapse [1–5]. Palliative treatments include platinum-based chemotherapy, cetuximab, and pembrolizumab or nivolumab [6–10]. However, the median overall survival for patients with locally recurrent and/or metastatic (R/M) SCCHN remains low (around 12–15 months), and the treatment is mainly palliation.

New treatment modalities are therefore needed. SCCHN is characterized by several molecular dysregulations in the cell cycle. Here, we review the therapeutic potential of cyclin-dependent kinase (CDK) 4 and 6 inhibitors.

THERAPEUTIC POTENTIAL OF CYCLIN-DEPENDENT KINASE 4/6 INHIBITION IN SQUAMOUS CELL CARCINOMA OF THE HEAD AND NECK TUMOURS

CDK4/6–cyclin-D complexes regulate the transition of early G₁ to S phase predominantly through the retinoblastoma-family proteins (Rb, p107 and

^aInstitut de Recherche Clinique et Expérimentale (Laboratory of Medical Oncology, Pole MIRO), UCLouvain and ^bService d'Oncologie Médicale, Institut Roi Albert II, Cliniques Universitaires Saint-Luc, Brussels, Belgium

Correspondence to Jean-Pascal Machiels, Service d'Oncologie Médicale, Institut Roi Albert II, Cliniques Universitaires Saint-Luc; Institut de Recherche Clinique et Expérimentale, UCLouvain, Avenue Hippocrate 10, 1200 Brussels, Belgium. Tel: +3227645457; fax: +3227645428; e-mail: jean-pascal.machiels@uclouvain.be

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KEY POINTS

- Genetic aberrations involved in the cell cycle pathway frequently occur in SCCHN.
- CDK4/6 inhibitors have shown promising preclinical results in HPV-negative, but not in HPV-positive, SCCHN.
- Ongoing umbrella trials will provide necessary information on the activity of CDK4/6 inhibitors in SCCHN.
- In the clinic, most investigations have failed to identify predictive biomarkers to CDK4/6 inhibitors as monotherapy.
- A phase II single-arm trial combining palbociclib/cetuximab has shown promising results that need to be confirmed in randomized trials.

p130). CDK4/6–cyclin-D complexes are phosphorylated by CDK-activating kinases that in turn phosphorylate their substrates including retinoblastoma. Hyperphosphorylated retinoblastoma proteins release the transcriptional factors E2F1-3 family proteins that drive the transcription of genes involved in DNA replication and cell cycle progression. Negative regulation of CDK4/6 is controlled by inhibitors of CDK4 (INK4) family members (p16-INK4a, p15-INK4a, p18-INK4a, and p19-INK4a) and by p21-kinase inhibitory protein (KIP1) and p27-KIP1. Similarly, p53 can also induce cell cycle arrest in the G₁ phase by driving the gene transcription of p21-KIP1, which can inactivate the CDK2/cyclin-E complex. This complex can also phosphorylate retinoblastoma proteins and stimulate cell cycle transition to S phase [11].

Dysregulation of cell cycle progression by upregulation of cyclin activities is a hallmark of cancer [12]. Human papillomavirus (HPV)-positive oropharyngeal cancer is mediated by the expression of the viral E6 and E7 oncoproteins which cause deregulation of the cell cycle by inactivation of p53 and retinoblastoma, respectively. In contrast, HPV-negative SCCHN is mostly driven by the inactivation of p16-INK4a and p53 [2,13]. The Tumor Cancer Genome Atlas consortium sequenced 243 HPV-negative SCCHN and identified alterations in genes implicated in the cell cycle in nearly every tumour. These genetic alterations included the tumour suppressor genes *TP53* and *CDKN2A* in 84% and 58%, and the amplification of the proto-oncogenes *CCND1* and *MYC* in 31% and 14%, respectively [13]. c-Myc is a proto-oncoprotein that directly regulates cell cycle transition by inducing the transcription of genes involved in the cyclin-D/CDK4/CDK6/retinoblastoma pathway as *CDK4*, *CCND2*, and *CCNE1-2* [14]. Moreover, micro-

ribonucleic acid let-7c is downregulated in 40% of HPV-negative SCCHN. let-7c inhibits cell proliferation and induces apoptosis by repressing directly or indirectly the expression of various proteins, including *CDC25A*, *CDK6*, *cyclin-D1*, *retinoblastoma*, and *E2F1-2* [15–18] (Fig. 1).

These genetic alterations support the investigation of the activity of CDK4/6 inhibitors in HPV-negative SCCHN. In addition, *CDKN2A* alterations and *CCND1* amplification are associated with poor prognosis [19–21]. Moreover, loss of function mutation or deletion in *RB1* is rare in SCCHN and this provides another reason why CDK4/6 inhibition could be beneficial as CDK4/6–cyclin-D complexes regulate cell cycle progression predominantly through retinoblastoma phosphorylation.

PRECLINICAL AND CLINICAL ACTIVITIES OF CYCLIN-DEPENDENT KINASE 4/6 INHIBITORS AS MONOTHERAPY IN SQUAMOUS CELL CARCINOMA OF THE HEAD AND NECK

Palbociclib, ribociclib, and abemaciclib are CDK4/6 kinase inhibitors approved for the treatment of metastatic breast cancer (mBC). These agents have been reviewed elsewhere [22]. However, only a few studies have been conducted in SCCHN.

Ku *et al.* [23] evaluated the activity of abemaciclib in three HPV-negative SCCHN cell lines *in vitro* and *in vivo*. Abemaciclib reduced phospho-retinoblastoma, leading to cell cycle arrest with tumour growth stabilization [23]. Ribociclib had a cytostatic effect in HPV-negative cell lines but not in those that were HPV positive [24]. Gong *et al.* [25[¶]] studied the efficacy of abemaciclib and palbociclib in 560 and 492 cell lines, respectively, from 54 cancer types in an attempt to identify new types of cancers in which CDK4/6 inhibitors may have activity. Twenty-eight of these cell lines involved HPV-negative SCCHN cells which showed response to abemaciclib and palbociclib with various degrees of sensitivity. Although these results support the investigation of CDK4/6 inhibitors in HPV-negative SCCHN, further research is needed to more precisely evaluate the potential of CDK4/6 inhibitors.

Only a few trials are evaluating single agent CDK4/6 inhibitors in patients with R/M SCCHN (Table 1). One of these trials is investigating palbociclib in patients with p16-negative and *CCND1*-amplified SCCHN (European Organization for Research and Treatment of Cancer-1559-HNCG, NCT03088059) [26[¶]]. Another Korean trial is investigating abemaciclib in patients with SCCHN characterized by p16-negative disease and genetic alterations in the CDK4/6 pathway (NCT03292250).

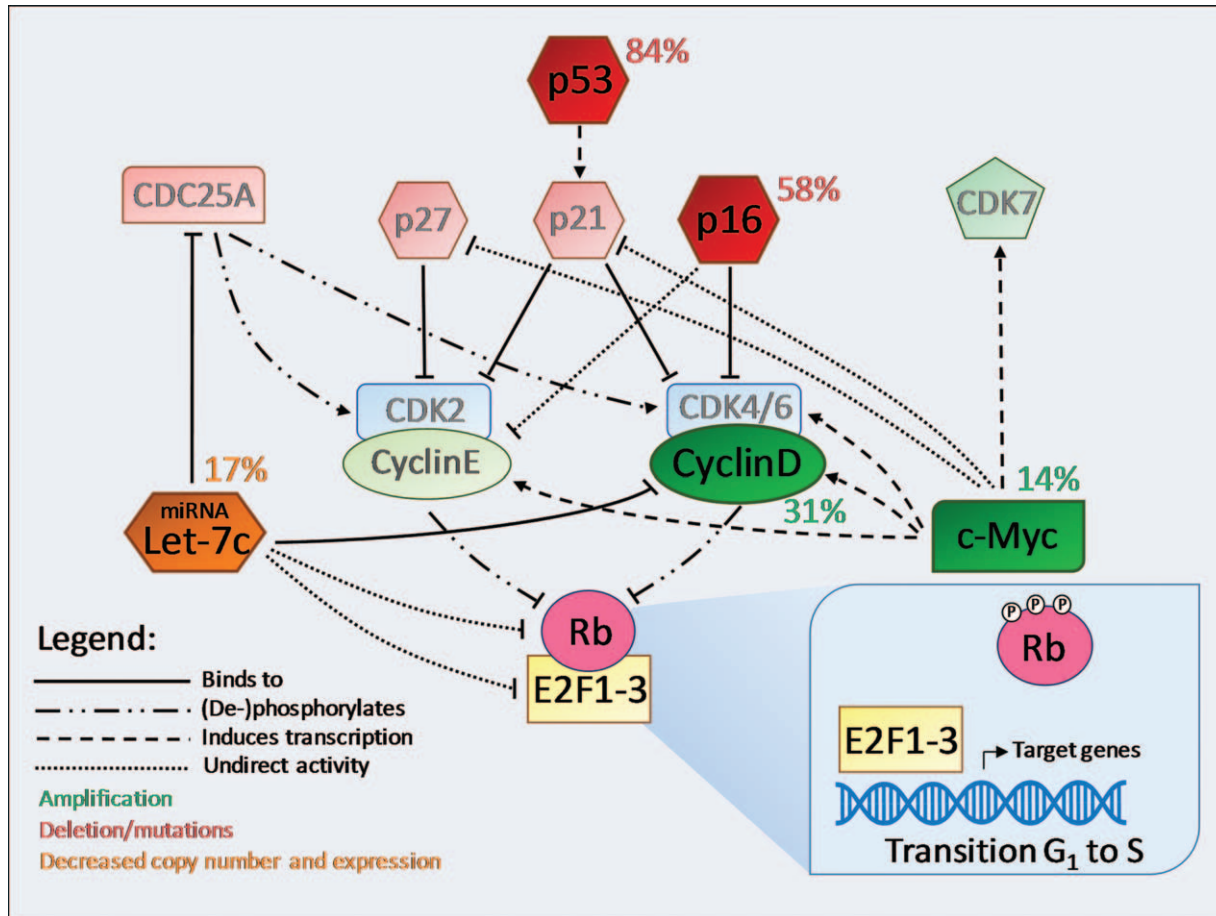


FIGURE 1. The most frequent molecular alterations involved in the cell cycle occurring in HPV-negative SCCHN. CDK, cyclin-dependent kinase; CDC, cell division cycle; miRNA, micro-ribonucleic acid; Rb, retinoblastoma; c-Myc, v-myc myelocytomatosis viral related oncogene; HPV, human papillomavirus; SCCHN, squamous cell carcinoma of head and neck.

Table 1. Ongoing trials investigating a cyclin-dependent kinase 4/6 inhibitor in squamous cell carcinoma of the head and neck (not exhaustive)

Study	Drugs investigated	Study design/regimen	Population
UPSTREAM ^a EORTC1559	Palbociclib	Randomized phase II trial: palbociclib versus investigator choice	Platinum failure, p16-negative and <i>CCND1</i> amplified
NCT03356223	Abemaciclib	Single-arm phase II study	Platinum and cetuximab failure, <i>CDKN2A</i> homozygous deletion and/or <i>CCND1</i> amplification and/or <i>CDK6</i> amplification
TRIUMPH ^{a,b} NCT03292250	Abemaciclib	Single-arm phase II study	Platinum failure, p16-negative and genetic alteration in the CDK4/6 pathway
NCT03655444	Abemaciclib + nivolumab (mAb anti-PD1)	Phase I and single-arm phase II	Platinum failure
NCT02499120	Palbociclib + cetuximab	Randomized phase II trial: palbociclib + cetuximab versus cetuximab	Platinum failure, p16-negative
NCT03065062	Palbociclib + gedatolisib (PI3K/mTOR inhibitor)	Phase I (multiple tumours) and one expansion cohort scheduled for SCCHN	p16-negative SCCHN

CCND, cyclin; CDK, cyclin-dependent kinase; EORTC, European Organization for Research and Treatment of Cancer; mTOR, mammalian target of rapamycin; PD1, programmed cell death protein 1; PI3K, phosphatidylinositol-3-kinase; SCCHN, squamous cell carcinoma of head and neck; TRIUMPH, translational biomarker driven umbrella project for head and neck; UPSTREAM, personalized strategy for recurrent and/or metastatic SCCHN

^aUmbrella trials.

^bThis study is also including oesophageal cancer.

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PREDICTIVE BIOMARKERS FOR CYCLIN-DEPENDENT KINASE 4/6 INHIBITORS

The identification of biomarkers to predict treatment activity or resistance is important to spare patients from worthless treatment and unnecessary side-effects [27,28]. Although alterations associated with the cyclin-D/CDK4/CDK6/retinoblastoma pathway have been widely investigated in mBC, no clinical studies to date have been able to associate these alterations with CDK4/6 inhibitor efficacy. Predictive biomarkers that have been investigated are briefly discussed below.

Retinoblastoma protein loss to predict resistance to cyclin-dependent kinase 4/6 inhibition

The level of retinoblastoma protein expression can vary across SCCHN cell lines and patients (van Caloen *et al.*, unpublished). It has been speculated that the absence of retinoblastoma proteins is associated with resistance to CDK4/6 inhibitors. However, the predictive role of retinoblastoma remains unclear. Although retinoblastoma protein expression was required for cell cycle inhibitory response to palbociclib treatment in some preclinical models [29–36], retinoblastoma deficiency was not sufficient to bypass CDK4/6 inhibition in other models and cell lines [37–39,40^{***},41]. In ovarian cell lines, hemizygous or homozygous deletions of *RB1* occurred in 50% of resistant cell lines to palbociclib but, interestingly, 10% of the sensitive cell lines were retinoblastoma deficient. Moreover, Michaud *et al.* [34] evaluated the activity of palbociclib in two clones of U87MG cells transfected with siRNA targeting *RB1* and found that palbociclib was still able to mediate cell cycle arrest, although this response was attenuated. Unfortunately, these preclinical studies did not investigate the exact mechanisms of CDK4/6 inhibitors in these retinoblastoma-deficient cell lines. Importantly, some cell lines resistant to palbociclib and abemaciclib were retinoblastoma positive, demonstrating that other molecular mechanisms are involved [25[†]].

Apart from retinoblastoma, CDK4/cyclin-D1 and CDK6/cyclin-D3 complexes are able to phosphorylate at least 70 proteins [42] and this may explain the difficulty in determining the role of retinoblastoma status to predict response to CDK4/6 inhibition. The relative stabilization of p107 (a retinoblastoma-related protein) in retinoblastoma-deficient cells exposed to palbociclib could be responsible for the cell cycle arrest observed in hepatoma cells [43]. This functional compensation for the lack of retinoblastoma proteins by p107 has been previously described [44,45]. Forkhead Box M1 (FOXM1), a transcriptional factor directly phosphorylated by CDK4/6 proteins and involved in the

regulation of the expression of G₁–S phase genes, is also a candidate to bypass the retinoblastoma protein in cell cycle regulation [42,46–48]. Loss of retinoblastoma has been shown to induce FOXM1 activation in bladder cells. In addition, a low level of FOXM1 proteins conferred resistance to CDK4/6 inhibition only in *RB1* mutant cells [49].

These findings may explain why phase II and III clinical trials, in the search for molecular profiles as response predictors to CDK4/6 inhibitors, failed to demonstrate the predictive role of retinoblastoma proteins [50–52,53^{***},54,55,56^{***}]. In addition, from a panel of over 400 kinases, 1 μM of abemaciclib, palbociclib or ribociclib was able to inhibit at least 50% of the activity of 81, 23 or 9 wild-type protein kinases respectively [33,40^{***}]. These findings suggest a possible role of off-target proteins in response to these CDK4/6 inhibitors in cancer cells.

Alterations in the cyclin-D/cyclin-dependent kinase 4/cyclin-dependent kinase 6/retinoblastoma pathway to predict cyclin-dependent kinase 4/6 inhibitor activity

A strong correlation between *CDKN2A* inactivation and cell sensitivity to CDK4/6 inhibition has been reported in multiple cancer types [33,35,37–39,41]. These findings are of great interest in HPV-negative SCCHN treatment given the high frequency of *CDKN2A* alterations [13]. Nonetheless, *CDKN2A* inactivation associated with relative resistant cell lines has also been observed in preclinical and clinical studies [25[†],48,50]. This observation could be because of the ability of p16 to inhibit CDK2 activity as CDK2–cyclin-E1 complexes are also able to phosphorylate retinoblastoma. Interestingly, in SCCHN, *CDKN2A* deletion has been associated with high CDK2 activity, and this activity was attenuated when p16 was restored [57]. Furthermore, high expression of *CCNE1* (encoding cyclin-E1) was positively correlated with CDK2 activity and was associated with cancer cell resistance to CDK4/6 inhibition in *in-vitro* studies and in a phase III clinical trial in patients with mBC [25[†],33,36,38,53^{***},58,59]. Therefore, the potential role of cyclin-E1 status to predict response to CDK4/6 inhibition should be investigated in SCCHN even though *CCNE1* is not frequently altered [60].

TP53 is the most frequently altered gene in SCCHN (84%) [13]. p53 induces cell cycle arrest through p21 transcription (a CDK2–cyclin-E inhibitor). Therefore, *TP53* and/or *CDKN1A* (encoding p21) alterations might promote cell cycle progression independently of CDK4/6 inhibition. However, p21 status did not predict CDK4/6 inhibitor efficacy [39,52,56^{***},61]. Association between *TP53* mutation and resistance to CDK4/6 inhibition has been

suggested in few studies [25[•],33,39], but has not been confirmed in clinical trials [56^{••},61].

PIK3CA amplification and/or mutations occur in 34% of HPV-negative SCCHN [13], and have been shown to induce cyclin-D1 upregulation in preclinical models, which might confer resistance to CDK4/6 inhibition [13,62,63]. In addition, mutations in *PTEN* or *PI3KCA* that activate the phosphatidylinositol-3-kinase/protein kinase B (PI3K/Akt) pathway seemed to confer resistance to CDK4/6 inhibitors in preclinical studies [33,58,59]. Although this correlation was not confirmed in patients with mBC treated with a CDK4/6 inhibitor, it is yet to be investigated in SCCHN [55,64].

CCND1 (encoding cyclin-D1) is frequently amplified in SCCHN. There is some controversy about cyclin-D1 status and predictive response to CDK4/6 inhibition. Correlation between high *CCND1* expression and sensitivity to palbociclib has been demonstrated in BC cell lines [37] but has not been confirmed in recent preclinical and clinical studies [25[•],41,50–52,56^{••}]. In addition, association between *CCND1* copy number gain and relative resistance to CDK4/6 inhibitors has been reported *in vitro* [25[•],39].

The difficulty in identifying molecular events able to predict the response of cancer cells to CDK4/6 inhibition is probably because of the simultaneous occurrence of several events in the cyclin-D/CDK4/CDK6/retinoblastoma and associated pathways. For instance, Young *et al.* [38] observed cell cycle arrest following treatment with CDK4/6 inhibitors in two p16-positive cell lines harbouring an activating *CDK4* mutation. A set of genetic alterations, called D-cyclin activating features (DCAFs), have also been shown to increase D-type cyclins expression. DCAFs have been associated with response to CDK4/6 inhibition in preclinical models [25[•]]. This finding led to a clinical trial investigating abemaciclib in solid tumours harbouring DCAFs or the amplification of *CDK4* or *CDK6* (NCT03310879). Several other potential predictive biomarkers are reported in Table 2 [65].

In the clinic, most investigations have failed to identify predictive biomarkers. In an attempt to find other potential predictive biomarkers to CDK4/6 inhibition, a phase II clinical study is currently investigating palbociclib as a single agent in adults with recurrent or refractory advanced cancers with aberration(s) in CDK/cyclin signalling (NCT03123744).

COMBINATION THERAPY WITH CYCLIN-DEPENDENT KINASE 4/6 INHIBITORS IN SQUAMOUS CELL CARCINOMA OF THE HEAD AND NECK

CDK4/6 inhibitors are cytostatic and induce tumour growth stabilization in preclinical SCCHN models,

but these inhibitors are not cytotoxic [23,24,25[•]]. A clinical need therefore exists to find a partner which can be used in combination with CDK4/6 inhibitors to provoke tumour regression.

Despite that, epidermal growth factor receptor (EGFR) is overexpressed in 90% of SCCHN tumours [66], the response rate of cetuximab is low and treatment resistance occurs. EGFR activation induces cell cycle progression through the activation of multiple signalling pathways including the mitogen-activated protein kinases (MAPK)/extracellular signal-regulated kinases (ERK) pathway, the PI3K/Akt/mammalian target of rapamycin, the Src tyrosine kinase, and the signal transducers and activators of transcription pathways [11,67,68]. Activation of these pathways can mediate cyclin-D1 transcription or stabilization. EGFR inhibitors may then be interesting compounds to combine with CDK4/6 inhibitors. Furthermore, preclinical studies have reported that *CCND1* overexpression is associated with resistance to EGFR inhibition in SCCHN cell lines [69]. Accordingly, afatinib or lapatinib combined with a CDK4/6 inhibitor seems synergistic in terms of cell viability reduction in HPV-negative cell lines [40^{••},70].

A phase I trial evaluated the combination of ribociclib and cetuximab in HPV-negative SCCHN patients [71]. The ribociclib recommended dose was 600 mg daily on a 3 weeks on with 1-week off schedule in combination with cetuximab at its standard dose. In the phase I part of the study, this combination was shown to be well tolerated, although the activity of the combination seemed to be low as no objective response was observed. The expansion cohort still needs to be reported [71]. Another phase I trial investigated the palbociclib/cetuximab combination [72]. This trial showed that palbociclib 125 mg daily (3 weeks on and 1-week off) with cetuximab was well tolerated. Nine R/M patients were included. Six out of nine patients had cetuximab-resistant SCCHN. A partial response was observed in two p16-negative SCCHN patients including one cetuximab-resistant patient. Six other patients had stable disease [72].

Recently, a phase II trial evaluated the efficacy of palbociclib combined with cetuximab in 30 patients with platinum-resistant R/M SCCHN and reported a complete response rate of 11% and partial response of 29% for an objective response rate of 39%; the median progression-free survival was 5.4 months and the median overall survival was 9.5 months [73^{••}]. In contrary to the two phase I trials reported above, this last study excluded patients previously treated with cetuximab, which could explain the greater sensitivity of these cetuximab-naïve patients to this combination. A randomized phase II trial that compared cetuximab to cetuximab/palbociclib

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Table 2. Potential predictive biomarkers to CDK4/6 inhibitors investigated in preclinical and clinical studies (not exhaustive)

Predictive biomarkers of sensitivity to CDK4/6 inhibitors		Predictive biomarkers of resistance to CDK4/6 inhibitors		Failed to predict response to CDK4/6 inhibition	
Preclinical					
Molecular alterations	References	Molecular alterations	References	Molecular alterations	References
High <i>RB1</i> expression	[33,36,37,39]	<i>RB1</i> deletions/mutations ^a	[33–35,38,39]	<i>RB1</i> expression	[25 [■] ,41]
Rb high level	[39]	Rb protein deficient ^a	[29,30–32,38,40 [■]]	Rb protein level	[43,49]
<i>CDKN2A</i> deletion/mutation	[33,38,39,41]	<i>CDKN2A</i> mutation ^a	[25 [■]]	<i>CDKN2A</i> deletion/mutation	[48]
Low <i>CDKN2A</i> expression	[33,36,37,39]	<i>CCND1</i> copy number gain	[39]	p16 protein level	[25 [■]]
Low p16 protein level	[31,38,39]	<i>CCND1</i> amplification ^a	[25 [■]]	<i>CDKN1A</i> expression	[39]
Low <i>CDKN2B</i> expression	[33]	High <i>CCNE1</i> expression	[33,36,39]	<i>CDKN1B</i> expression	[39]
<i>CDKN2B</i> deletion	[41]	<i>CCNE1</i> copy number gain	[39]	<i>CDKN2B</i>	[39]
<i>CDKN2A/C</i> codeletion	[35]	<i>TP53</i> mutation ^a	[25 [■] ,33,39]	<i>CDKN2C</i> expression	[39]
High <i>CCND1</i> expression	[37]	High <i>E2F1</i> expression	[41]	<i>CDKN2D</i> expression	[39]
Phospho-CDK4 on T172	[65]	<i>PIK3CA</i> mutation ^a	[25 [■] ,33]	<i>CDK4</i> expression	[35,36,39,41]
D-cyclin activating features	[25 [■]]	<i>PTEN</i> mutation	[33]	<i>CDK6</i> expression	[35,36,39,41]
<i>MYCN</i> amplification	[46]			<i>CCND1</i> expression	[25 [■] ,36,41]
<i>CTNNB1</i> mutation	[33]			Cyclin D1 protein level	[25 [■]]
<i>EZH2</i> mutation	[33]			<i>CCND2</i> expression	[25 [■] ,39]
High <i>RAD51D</i> expression	[33]			<i>CCND3</i> expression	[25 [■] ,39]
High <i>HGF</i> expression	[33]			<i>CCNE1</i> copy number gain	[25 [■]]
				<i>CCNE1</i> expression	[41]
				<i>E2F1</i> expression	[39]
				<i>RBL1</i> expression	[25 [■] ,39]
				<i>RBL2</i> expression	[25 [■] ,39]
				<i>CDK1</i> expression	[39]
				<i>BRAF</i> mutation	[38]
				<i>NRAS</i> mutation	[38]
				<i>TK1</i> expression	[39]
				<i>PARK2</i> expression	[25 [■]]
				<i>FBXO4</i> expression	[25 [■]]
				<i>FBXW8</i> expression	[25 [■]]
Clinical					
		<i>TP53</i> mutation in BC	[61]	<i>RB1</i> expression in BC	[52,53 [■]]
		High <i>CCNE1</i> expression in BC	[53 [■]]	Rb protein level in BC	[51,56 [■]]
				<i>CDKN2A</i> deletion/mutation in BC	[50]
				<i>CDKN2A</i> expression in BC	[52,56 [■]]
				p16 protein level in BC	[51,56 [■]]
				<i>CCND1</i> expression in BC	[52,53 [■] ,56 [■]]
				<i>CCND1</i> amplification in BC	[50]
				Cyclin D1 protein level in BC	[51]
				<i>CCNE1/2</i> expression in BC	[52]
				<i>CDK2</i> expression in BC	[52]
				<i>CDK4/6</i> expression in BC	[52,53 [■]]
				<i>TP53</i> mutations in BC	[56 [■]]
				<i>TP53</i> mutations in NSCLC, GBM, colorectal, melanoma	[61]
				<i>PIK3CA</i> mutation in BC	[56 [■]]
				Ki67 protein level in BC	[51,56 [■]]

BC, breast cancer; BRAF, b-raf proto-oncogene serine/threonine kinase; CCN, cyclin; MYCN, v-myc myelocytomatosis viral related oncogene; CDK, cyclin-dependent kinase; CDKN, cyclin-dependent kinase inhibitor; CTNNB1, gene coding β-catenin; EZH2, enhancer of zeste homolog 2; FBXO4, f-box protein 4; FBXW8, f-box and WD repeat domain containing 8; GMB, glioblastoma.; HGF, hepatocyte growth factor; NRAS, NRAS proto-oncogene GTPase; NSCLC, non-small cell lung cancer; PARK2, parkin RBR E3 ubiquitine protein ligase; PIK3CA, phosphatidylinositol 3-kinase catalytic alpha; PTEN, phosphatase and tensin homolog; RAD51D, DNA repair protein homolog D; Rb, retinoblastoma; RBL, retinoblastoma-like; TK, thymidine kinase.

^aWeak or controversial evidence in the experiment performed.

Table 3. Clinical trials that have investigated a cyclin-dependent kinase 4/6 inhibitor in squamous cell carcinoma of the head and neck

References	Study design	Study regimen	N	ORR	PFS (months)	OS (months)	Population
[71]	Phase I	Ribociclib/cetuximab	10	0%	NA	NA	Cetuximab resistant (60%); platinum resistant (100%)
[72]	Phase I	Palbociclib/cetuximab	9	22%	NA	12	Cetuximab resistant (67%); platinum resistant (44%)
[73 ^{***}]	Phase II (single arm)	Palbociclib/cetuximab	30	39%	5.4	9.5	Platinum resistant (100%); cetuximab naïve (100%)

CDK, cyclin-dependent kinase; N, number of patients; NA, not available; ORR, objective response rate; OS, overall survival; PFS, progression-free survival.

in HPV-negative SCCHN patients, who progress after platinum therapy, has completed its enrolment (NCT02499120). Results of randomized trials are eagerly awaited to fully assess the potential of cetuximab/CDK4/6 combinations as single-arm trials have bias in patient selection, which may explain the discrepancy in objective response rate across the studies reported above (Table 3).

As previously mentioned, *PIK3CA* is frequently mutated or amplified in SCCHN. Preclinical studies have described, *in vitro* and *in vivo*, synergy between CDK4/6 and PI3K pathway inhibitors in *PIK3CA* mutated cancer cells [48,58,59,74,75^{*},76]. Furthermore, the adaptation of cancer cells to CDK4/6 inhibition could depend on the PI3K/Akt/mammalian target of rapamycin pathway that can activate CDK2. PI3K/Akt pathway inhibitors might therefore prevent acquired resistance to CDK4/6 inhibitors [59,77]. The combination of CDK4/6 and PI3K/Akt signalling inhibitors has also been shown to lead to cell cycle arrest and enhanced immunogenic cell death with induced tumour immunogenicity [78]. Although the mechanism is still unclear, this provides a strong rationale for combining CDK4/6 inhibitors with immunotherapy such as checkpoint inhibitors. The combination of CDK4/6 and programmed cell death protein 1 inhibitors has shown promising preclinical results [79^{*},80–83]. The efficacy of these combination therapies is evaluated in patients with SCCHN (Table 3).

CONCLUSION

Owing to frequent alterations occurring in the cyclin-D/CDK4/CDK6/retinoblastoma and associated pathways in HPV-negative SCCHN, further studies investigating the activity of CDK4/6 inhibitors are warranted. Randomized clinical trials that investigate these compounds in an unbiased manner are ongoing. In our search for a molecular profile/phenotype that will predict CDK4/6

inhibitor activity, we need to take into account several simultaneously occurring events in the cyclin-D/CDK4/CDK6/retinoblastoma and associated pathways. Some genomic alterations or gene expression/proteomic profiles can also vary from one cancer histology to another [84]. It is therefore important to also consider the origin of the cancer. This may lead to the discovery of different predictive biomarkers across cancers.

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Conflicts of interest

There are no conflicts of interest.

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- of special interest
- of outstanding interest

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