

# Targeting cyclin-dependent kinase 4/6 as a therapeutic approach for mucosal melanoma

Chao-ji Shi<sup>a,b,c,d,\*</sup>, Sheng-ming Xu<sup>a,b,c,\*</sup>, Yong Han<sup>a,b,c</sup>, Rong Zhou<sup>a,b,c</sup> and Zhi-yuan Zhang<sup>a,b,c,d</sup>

Mucosal melanoma is a rare but devastating subtype of melanoma which typically has a worse prognosis than other melanoma subtypes. Large-scale next-generation sequencing studies, including our recent research, have also proved that the molecular landscape and potential oncogenic drivers of mucosal melanoma remain distinct from that of cutaneous melanoma. Recently, a number of selective cyclin-dependent kinase 4 (CDK4)/6 inhibitors have been approved for clinical application in breast cancer or entered phase III clinical trial in other solid tumors. Additionally, we have revealed that the dysregulation of cell cycle progression, caused by CDK4 amplification, is a key genetic feature in half of mucosal melanoma and targeting of CDK4 in selected mucosal melanoma patients is a potentially promising direction for precision cancer treatment by using molecular-characterized mucosal melanoma patient-derived-xenograft models. This review summarizes the current literature regarding CDK4/6 dysregulation in mucosal melanoma, preclinical and clinical studies of CDK4/6

inhibitors and potential combinational strategies in treating mucosal melanoma. *Melanoma Res* 31: 495–503  
Copyright © 2021 The Author(s). Published by Wolters Kluwer Health, Inc.

*Melanoma Research* 2021, 31:495–503

**Keywords:** cyclin-dependent kinase 4/6 inhibitors, clinical trial, cyclin-dependent kinases, mucosal melanoma

<sup>a</sup>Department of Oral and Maxillofacial-Head Neck Oncology, Shanghai Ninth People's Hospital, College of Stomatology, Shanghai Jiao Tong University School of Medicine, <sup>b</sup>National Center for Stomatology, National Clinical Research Center for Oral Diseases, <sup>c</sup>Shanghai Key Laboratory of Stomatology & Shanghai Research Institute of Stomatology and <sup>d</sup>Research Unit of Oral and Maxillofacial Regenerative Medicine, Chinese Academy of Medical Sciences, Shanghai, China

Correspondence to Zhi-yuan Zhang, PhD, Department of Oral Maxillofacial-Head and Neck Oncology, Shanghai Ninth People's Hospital, College of Stomatology, Shanghai Jiao Tong University School of Medicine, No. 639, Zhizaoju Rd, Shanghai 200011, China  
E-mail: Zhiyuan.Zhang@sh9hospital.org.cn

\*Chao-ji Shi and Sheng-ming Xu contributed equally to the writing of this article.

Received 17 April 2021 Accepted 2 August 2021

## Introduction

Mucosal melanoma originates from melanocytes located in the mucosal membranes [1,2]. The nasal cavity and paranasal sinuses, oral cavity and oropharynx, genital tract, anorectal region and any other part of the mucosal surface lining are the primary sites for mucosal melanoma [3]. Mucosal melanoma accounts for about 1% of all melanomas among Caucasians [4,5], whereas its incidence among East Asian patients is approximately 25%, potentially due to the lower prevalence of cutaneous melanoma in Asian populations [6,7]. Mucosal melanoma is an extremely aggressive malignancy, which typically has a significantly worse prognosis than other melanoma subtypes, with 5-year survival rates ranging from only 20–25% [8,9]. Adding to the problem, in patients with metastatic mucosal melanoma, the 5-year survival rate is lower than 16% [4]. The clinicopathology and bioinformation of mucosal melanoma are distinct from that of cutaneous melanoma. Although exposure to ultraviolet radiation is a major risk factor contributing to cutaneous melanoma, it has not been directly associated with the development of mucosal

melanoma [8]; thus far, the definitive risk factors for the development of mucosal melanoma remain unknown [10].

Recent development of immunotherapies has provided promising therapeutic approaches for advanced melanoma cancer patients, such as immune checkpoint inhibitors (ICIs), especially anti-PD-1 monoclonal antibodies alone and in combination with anti-cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) mAb [11–14]. There have been several studies showing that mucosal melanoma patients treated with ICIs appeared to have longer overall survival (OS) when compared with patients treated with chemotherapy [15]. In addition, the combination of ICI and anti-CTLA-4 (e.g. nivolumab and ipilimumab) appears to have greater efficacy than ICI (e.g. nivolumab) monotherapy in mucosal melanoma [12,13,16]. Those reports suggested that patients with mucosal melanoma could benefit from immunotherapy while several clinical studies demonstrated that mucosal melanoma patients seemed to be less responsive to ICI than patients with cutaneous melanoma [11]. Thus, to date, no significant breakthrough has been made in the treatment or clinical outcomes for patients with mucosal melanoma to some extent. Due to the limited numbers of mucosal melanoma patients available for inclusion in clinical trials, mucosal melanoma still lacks

This is an open-access article distributed under the terms of the Creative Commons Attribution-Non Commercial-No Derivatives License 4.0 (CCBY-NC-ND), where it is permissible to download and share the work provided it is properly cited. The work cannot be changed in any way or used commercially without permission from the journal.

recommendations and specific clinical guidelines for systemic therapy. According to the 2020 report by the American Society of Clinical Oncology: patients with mucosal melanoma may be offered the same therapies recommended for cutaneous melanoma, and should be offered or referred for enrollment in clinical trials if possible [17]. Considering the serious therapeutic dilemma, it is, therefore, urgent to develop more effective treatment strategies and reliable, predictive biomarkers for mucosal melanoma.

To this end, next-generation sequencing techniques and other bioinformatics analyses have identified genetic alterations involved in mucosal melanoma pathogenesis [1,2,7]. Variations in the p16-cyclin D-cyclin-dependent kinase 4 (CDK4)/6-retinoblastoma protein (Rb) pathway have been reported in the majority of melanoma cases [18–20]. Previous studies conducted by our team [2], as well as other published data, have shown that aberrations of CDK4 and aberrant activation of the CDK4 signaling pathway are frequently found in mucosal melanoma [1,7,21]. The clinical application of therapies targeting CDK4/6 in different types of malignant tumors, especially in breast cancer, has led to great hope among patients, doctors, and scientists in recent years [22–25]. In the light of the clinical potential for targeting CDK4 in malignancy, the next critical directions for the development of treatments will necessarily explore the role of CDK4 and its signaling pathway in contributing to mucosal melanoma.

In this review, we discuss what is known about the CDK4 pathway and its dysregulation in mucosal melanoma. We also detail the preclinical and clinical studies of CDK4/6 inhibitors, as well as potential novel combinational strategies for the treatment of mucosal melanoma.

### Cyclin-dependent kinase 4 pathway and the cell cycle

One of the hallmarks of cancer cells is the ability to maintain continuous proliferation while evading the signals of growth suppressors [26]. For a cell to proliferate, it must pass through a predetermined number of phases, which are regulated by complex networks requiring a variety of regulatory factors [27]. This cyclic process is highly conserved among eukaryotes [28,29]. CDKs are involved in regulating cell cycle progression, controlling transcription processes and participating in cell proliferation [30]. Each of the CDKs functions as a checkpoint to control a specific point at which the cell cycle progression is halted in response to abnormal events during mitosis (Fig. 1). The cyclin family proteins, D-cyclins, integrate mitogenic signals to direct G1/S cell cycle transition [31]. Among these, CDK4 was first discovered and described in 1992, and CDK6, which has similar properties, was identified 2 years later [32,33]. CDK4 and its close homolog CDK6 together play a pivotal role in the cyclin D-CDK4/6-inhibitor of cyclin-dependent kinase (INK4)-Rb pathway, which participates in driving cells into the DNA synthetic (S) phase of the cell-division cycle [23].

Specifically, D-type cyclins (cyclin D) can bind and activate CDK4/6, forming a complex and phosphorylating retinoblastoma-associated protein 1 (Rb1). Hypophosphorylated, that is, active Rb1 functions as a negative regulator for the cell cycle via binding of E2F transcription factors to inhibit G1 transition [34]. Hyperphosphorylation of Rb inactivates this protein, resulting in the release of E2F, which in turn drives the gene expression and protein synthesis necessary for S phase entry and subsequent progression through the cell cycle [35,36]. These proteins and genes include the E-type cyclins, which activate CDK2 and other proteins. The cyclin E-CDK2 complex further phosphorylates Rb1, thereby reducing inhibition of E2F. These factors thus form a positive feedback loop to promote S phase entry (Fig. 2a) [37].

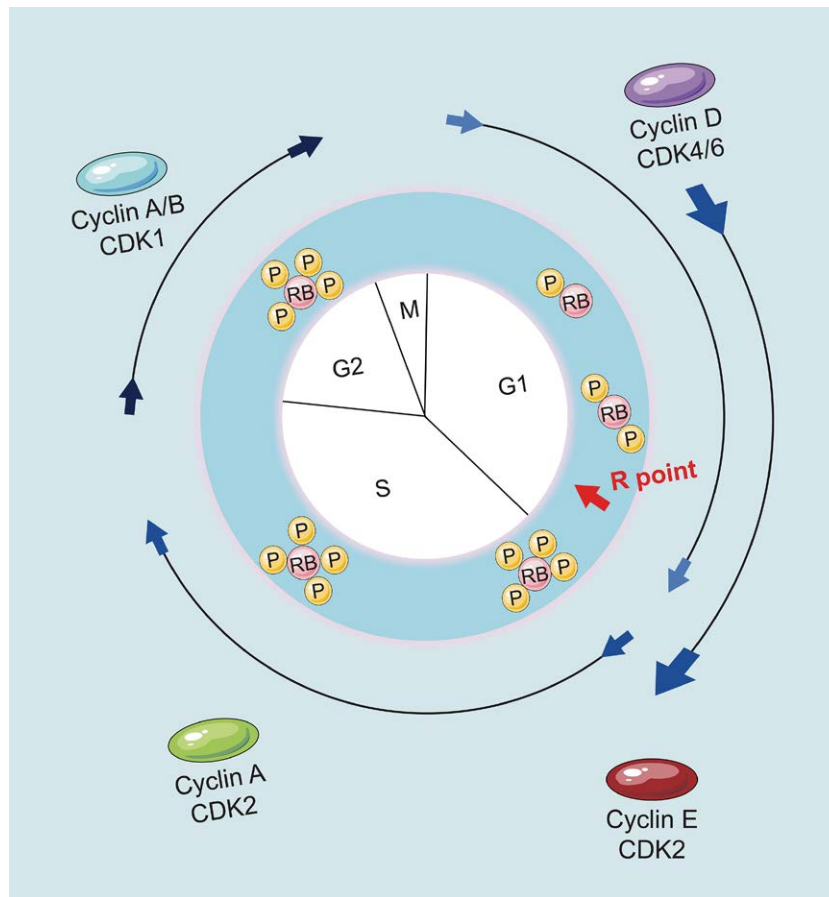
Typically, the activity of cyclin D-CDK4/6 is regulated by two distinct families: the INK4 family and the cyclin-dependent kinase inhibitor 1/kinase inhibitory protein (CIP/KIP) families [38]. The INK4 family consists of four members, including p15INK4b, p16INK4a, p18INK4c and p19INK4d, which specifically inhibit cyclin D-CDK4/6 activity by binding directly to the CDKs [39,40]. Among these, p16INK4a, encoded by the *CDKN2A* gene, is apparently required for tumor suppression [41,42] and can directly bind with CDK4 to inhibit its catalytic activity [3]. The CIP/KIP family, including p21CIP1, p27KIP1 and p57KIP2, regulates an array of cyclin-CDK complexes, including CDK2, CDK3, CDK4 and CDK6 [38,43]. In particular, p21CIP1 and p27KIP1 are ubiquitously expressed across several different tissues, and play both positive or negative regulatory roles on CDK4/6 depending on different conditions [44].

Apart from the above proteins, the cyclin D-CDK4/6-INK4-Rb pathway is regulated by several other mechanisms at various levels. For instance, cyclin D transcription and its assembly with CDK4/6 is highly dependent on mitogenic signaling by mitogen sensors that govern G1 phase progression [23]. Additionally, cyclin D1 levels can be upregulated by estrogen receptor signaling, which thereby further enhances the upregulation of CDK4/6 activity [45], while other signal transduction pathways, such as the PI3K/Protein kinase B, also known as PKB, Wingless/ $\beta$ -catenin, mitogen-activated protein kinase (MAPK) and nuclear factor kappa-B pathways, also lead to activation of the cyclin D-CDK4/6-INK4-Rb pathway [46].

### Cyclin-dependent kinase 4/6 inhibitors for cancer treatment

Given their key role in the process of cell proliferation, CDKs represent attractive targets for tumor therapy in a range of tumor types. Over the past two decades, great progress has been made in the discovery and development of CDK inhibitors [47]. Following the discovery of the role of CDK4/6 in tumorigenesis, a number of inhibitors targeting CDK have been developed for clinical use. In late 1993, David Beach *et al.*, first endeavored to identify a drug specifically intended to inhibit CDK4 [23]. Since

Fig. 1

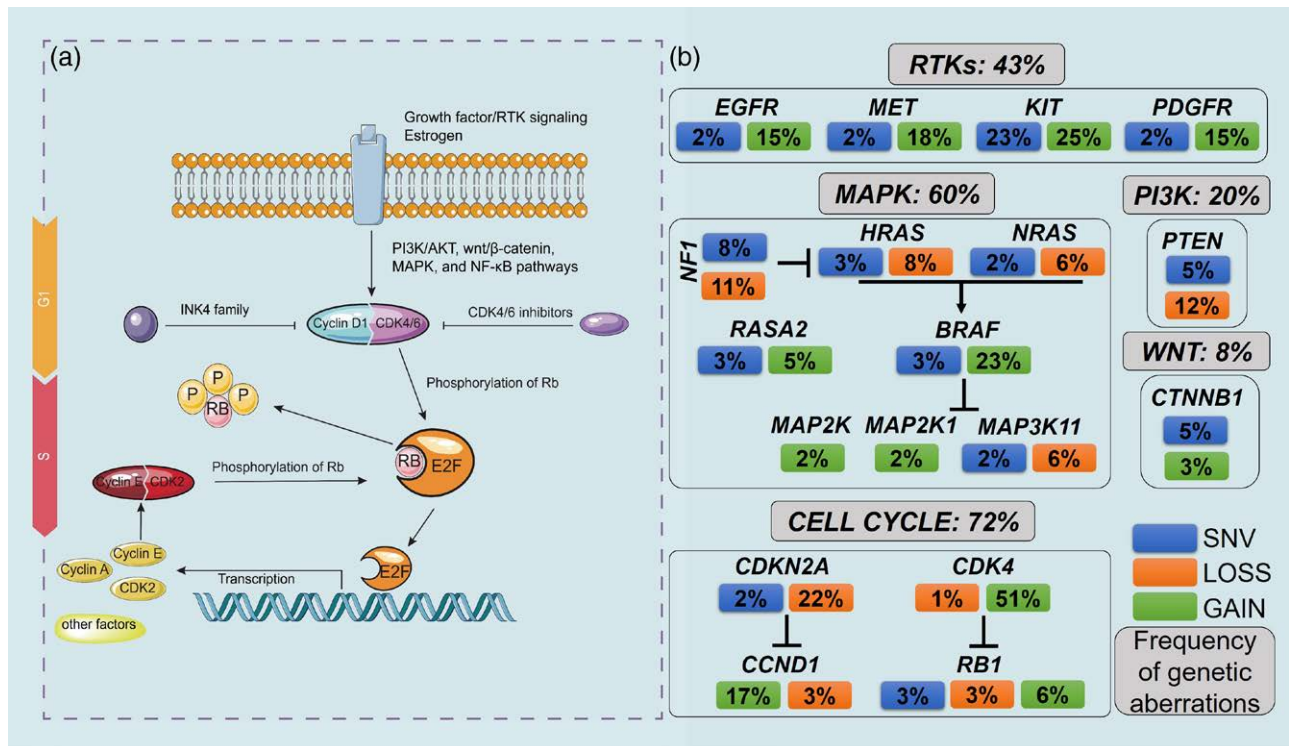


The cell cycle and cyclin-dependent kinase (CDK)/cyclin complexes. Different CDK/cyclin complexes participate in distinct phases of cell cycle (G0/G1, S, G2 and M) through regulating states of Rb phosphorylation. First, RB is phosphorylated by cyclin D-CDK4/6 in G1 and then further by cyclin E-CDK2. In late G1, RB becomes fully phosphorylated ('the restriction point', R point, red arrow) and drive cell cycle transition from the G1 phase to the S phase. After S-phase entry, RB phosphorylation is maintained by the progressive activation of other CDK/cyclin complexes, and promotes the transition from S phase to G2 phase. RB is dephosphorylated in M phase in mitosis with the degradation of cyclin A/B-CDK1 complex [23,24,38].

then, numerous inhibitors have been reported that target the CDK4/6 signaling pathway by blocking the activity of CDK4/6, Cyclin D and CDK2 [38]. Selective inhibitors of both CDK4 and CDK6 have markedly changed the perception of CDKs as therapeutic targets in cancer, and the approval of CDK4/6 inhibitors for breast cancer represents a major milestone in cancer therapeutics [24]. To date, three orally bioavailable, highly selective, small molecule inhibitors of CDK4/6, including palbociclib (Ibrance, Pfizer), abemaciclib (Verzenio, Lilly) and ribociclib (Kisqali, Novartis), have been approved for the treatment of advanced hormone receptor-positive breast cancer [24,48,49]. Among these, Palbociclib was approved by the State Food and Drug Administration of China in 2018. The selectivity of all three agents reflects the structural preference for the specialized ATP-binding pocket of CDK4/6 via specific interactions with binding cleft residues [50].

The great success of CDK4/6 inhibitors in breast cancer offers promise for improved anticancer therapies, including mucosal melanoma. Theoretically, CDK4/6 inhibitors may prove useful in the treatment of different tumor types which express fully functional Rb and require cyclin D-CDK4/6 complex to overcome the tumor suppressor. Currently, there exists an enormous body of published reports and clinical trials regarding the use of CDK4/6 inhibitors for the treatment of cancer patients exhibiting dysregulation of the CDK4 pathway [51,52], such as patients with metastatic pancreatic neuroendocrine tumors (NCT02806648), Ewing sarcoma (NCT04129151), non-small cell lung cancer (NCT03170206), head and neck squamous cell carcinoma (NCT03194373) and other solid tumors including melanomas (NCT02065063). To some extent, this wide range of clinical applications for CDK4/6 inhibitors in cancer treatment provides substantial validation for the concept of targeting CDK4 in mucosal melanoma.

Fig. 2



Cyclin-dependent kinase 4 (CDK4) pathway and the frequency of genetic aberrations within CDK4 related pathways altered in mucosal melanoma. (a) Mitogenic signals (e.g. the growth factor/RTK, estrogen, and PI3K signaling pathways) stimulate the accumulation of D-type cyclins in early G1 phase. In response to mitogenic signaling, cyclin D-CDK4/6 phosphorylate the RB. The INK4 protein family, including p16, p15, p18 and p19, act as a brake on the activation of CDK4/CDK6. These D-type cyclins form a complex with CDK4/6 to phosphorylate RB, which regulates E2F transcriptional activity. Hypophosphorylated RB can inhibit transcription by binding to E2F transcriptional regulators, suppressing target gene transcription. Cyclin D-CDK4/6 complex phosphorylates RB, and then partially activating the E2F-family proteins, which results in the E2F target gene transcription, including cyclins A and E and CDK2. With the accumulation of cyclin E, cyclin E-CDK2 complex further phosphorylate RB, forming a positive feedback loop via E2F, releasing and fully activating E2F, to push the cell from G1 to S phase. CDK4/6 inhibitors can prevent cell cycle progression by inhibiting CDK4/6 kinase activity. (b) Deregulation of genes and the frequency of genetic aberrations within the CDK4 related signal pathways in mucosal melanomas: single-nucleotide variants (SNV, blue), copy number gain (GAIN, green), homozygous deletion (LOSS, yellow), frequency of genetic aberrations in pathways (gray). Genomic data of mucosal melanomas derived from our previous study [2]. INK4, inhibitor of cyclin-dependent kinase; RTK, receptor tyrosine kinases.

## Dysregulation of the cyclin-dependent kinase 4 pathway in mucosal melanoma

### Genetic aberrations of the cyclin-dependent kinase 4 pathway in mucosal melanoma

Recent genomic analyses have revealed that the major genetic variations underlying mucosal melanoma are large-scale structural amplification or deletions of DNA, distinct from the point mutation hotspot found in cutaneous melanoma [7]. The mutation rate of the common factors driving cutaneous melanoma, such as V-raf murine sarcoma viral oncogene homolog B1 and neuroblastoma RAS viral (v-ras) oncogene homolog (NRAS), were lower in mucosal melanoma. In contrast, the mutation rate of SF3B1 and KIT in mucosal melanoma was higher than in cutaneous melanoma [7,53]. In addition, compared to cutaneous melanoma, the mutational burden has been shown to be much lower in mucosal melanoma patients [1,2,21]. Newell *et al.*, [1] conducted a large cohort ( $n=112$ ) genomic analysis of mucosal

melanomas from across geographically diverse regions, including China, Australia, the USA and Europe. Their results showed that all mucosal melanomas have a low number of single-nucleotide variants but high numbers of structural variants. Other studies showed that genetic alterations in cell cycle pathways were frequently found in cases of mucosal melanoma (20%) [51]; several studies specifically report that mutations in the CDK4 pathway, an integral component of cell cycle regulation, are common in mucosal melanoma. The main differences in the frequency of genomic aberrations in the CDK4 pathway between cutaneous melanoma and mucosal melanoma was shown in Tables 1 and 2.

The amplification of the *CDK4* and *CCND1* genes, as well as the deletion of *CDKN2A*, have been uniformly reported across many independent studies of mucosal melanoma, thus intimating that the aberrations in CDK4 signaling may be potential factors driving mucosal



**Table 1** The main differences of the single-nucleotide variants in cyclin-dependent kinase 4 pathway between cutaneous melanoma<sup>a</sup> and mucosal melanoma<sup>b</sup>

| Gene   | Cutaneous melanoma (TCGA database) (n=440) |           | Mucosal melanoma (n=65) |           |
|--------|--|-----------|-------------------------|-----------|
|        | Mutations                                  | Frequency | Mutations               | Frequency |
| CDKN2A | 165  | 37.5%     | 1                       | 1.5%      |
| RB1    | 26   | 5.9%      | 2                       | 3.1%      |
| BRAF   | 235  | 53.4%     | 2                       | 3.1%      |
| TP53   | 74   | 16.8%     | 2                       | 3.1%      |
| PTEN   | 70   | 15.9%     | 3                       | 4.6%      |
| NF1    | 78   | 17.7%     | 5                       | 7.7%      |
| KIT    | 36   | 8.2%      | 15                      | 23.1%     |
| NRAS   | 126  | 28.6%     | 1                       | 1.5%      |
| KRAS   | 14   | 3.2%      | 1                       | 1.5%      |
| HRAS   | 9  | 2.0%      | 2                       | 3.1%      |

BRAF, V-raf murine sarcoma viral oncogene homolog B1; CDK4, cyclin-dependent kinase 4; HRAS, v-Ha-ras Harvey rat sarcoma viral oncogene homolog; KRAS, v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog; NRAS, neuroblastoma RAS viral (v-ras) oncogene homolog; PTEN, phosphatase and tensin homolog deleted on chromosome ten.

<sup>a</sup>Genomic data of cutaneous melanomas was downloaded from the TCGA database using the cBioPortal (<http://www.cbioportal.org>) with available mutation and CNV data.

<sup>b</sup>Genomic data of mucosal melanomas derived from our previous study [2].

melanoma. Our own previous whole-genome sequencing (WGS) study of 65 mucosal melanomas originated from the head and neck region also identified significant amplification of oncogenes: over 50% of the mucosal melanoma patients harbored recurrent focal amplification

**Table 2** The main differences of the CNVs in cyclin-dependent kinase 4 pathway between cutaneous melanoma<sup>a</sup> and mucosal melanoma<sup>b</sup>

| Gene   | Cutaneous melanoma (TCGA database) (n=367) |      |              |                 | Mucosal melanoma (n=65) |      |              |                 |
|--------|--|------|--------------|-----------------|-------------------------|------|--------------|-----------------|
|        | Gain                                       | Loss | Total number | Total frequency | Gain                    | Loss | Total number | Total frequency |
| CDKN2A | 1  | 112  | 113          | 30.8%           | 0                       | 14   | 14           | 21.5%           |
| CCND1  | 23   | 1    | 24           | 6.5%            | 11                      | 2    | 13           | 20.0%           |
| CDK4   | 14   | 0    | 14           | 3.8%            | 33                      | 1    | 34           | 52.3%           |
| RB1    | 1  | 3    | 4            | 1.1%            | 4                       | 2    | 6            | 9.2%            |
| KIT    | 10   | 0    | 10           | 2.7%            | 16                      | 0    | 16           | 24.6%           |
| EGFR   | 2  | 1    | 3            | 0.8%            | 10                      | 3    | 13           | 20.0%           |
| MET    | 6  | 0    | 6            | 1.6%            | 12                      | 1    | 13           | 20.0%           |
| PTEN   | 0  | 28   | 28           | 7.6%            | 2                       | 8    | 10           | 15.4%           |
| BRAF   | 15   | 1    | 16           | 4.4%            | 15                      | 1    | 16           | 24.6%           |
| TP53   | 0  | 3    | 3            | 0.8%            | 0                       | 4    | 4            | 6.2%            |
| NF1    | 2  | 0    | 2            | 0.5%            | 1                       | 7    | 8            | 12.3%           |
| NRAS   | 11   | 0    | 11           | 3.0%            | 2                       | 4    | 6            | 9.2%            |
| KRAS   | 5  | 0    | 5            | 1.4%            | 10                      | 3    | 13           | 20.0%           |
| HRAS   | 1  | 0    | 1            | 0.3%            | 0                       | 5    | 5            | 7.7%            |

BRAF, V-raf murine sarcoma viral oncogene homolog B1; CCND1, cyclin D; EGFR, epidermal growth factor receptor; HRAS, v-Ha-ras Harvey rat sarcoma viral oncogene homolog; KIT, v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog; KRAS, v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog; MET, cellular-mesenchymal to epithelial transition factor; NRAS, neuroblastoma RAS viral (v-ras) oncogene homolog; PTEN, phosphatase and tensin homolog deleted on chromosome ten.

<sup>a</sup>Genomic data of cutaneous melanomas was downloaded from the TCGA database using the cBioPortal (<http://www.cbioportal.org>) with available mutation and CNV data.

<sup>b</sup>Genomic data of mucosal melanomas derived from our previous study [2].

of several oncogenes (CDK4, MDM2 and AGAP2) at 12q13-15, which significantly co-occurred with amplification of telomerase reverse transcriptase (TERT) at 5p15. Our study also revealed that enrichment of structural variations between chromosomes 5 and 12 defined a patient subgroup with significantly worse clinical outcomes [2]. Additionally, we also identified recurrent copy number variation (CNVs) in other well-recognized genes in cell cycle pathway, including gains of cyclin D (CCND1), along with significant losses in CDKN2A/B and TP53. These genomic features were again demonstrated in an independent validation cohort of 80 mucosal melanoma samples by using droplet digital PCR. Another small WGS study of oral MMs also identified that 11 out of 19 MM samples (57.9%) harbored amplifications of CDK4 [54]. Similarly, a large-cohort study of 213 Chinese patients with mucosal melanoma showed a 47.0% amplification rate for *CDK4* and *CCND1* was 27.7% [55]. However, the ratio of CDK4 amplification in cutaneous melanomas was only 4.3% (7/140) according to Hayward *et al.* reports [21].

More recently, some reports on genetic aberrations found in the CDK4 pathway, although using small sample size, could provide measures of clinical guidance for the management of mucosal melanoma. For example, one study reported that the most frequently deleted region was D9S171 in the location of the p16/CDKN2A gene on 9p21, and that codons 225 and 226 were mutational hot spots of the p16/CDKN2A gene in mucosal melanoma [56]. Circulating tumor DNA (ctDNA) is the fraction of cell-free DNA derived from tumor cells, which can provide valuable information about treatment response, recurrence and drug resistance in patients with cancer [57,58]. Another study found that gains in the plasma copy numbers of CDK4, cKit and CCND1 may also prove useful for evaluating treatment responses in patients with melanoma, including mucosal melanoma [59]. Thus, these data collectively suggest that amplification of genes in the CDK4 pathway is a common genetic event in melanoma development, and variation in the copy number of CDK4 pathway genes represents a potentially reliable therapeutic target for mucosal melanoma. The frequency of genetic aberrations within the CDK4 related signal pathways in mucosal melanoma is shown in Fig. 2b.

### Expression of cyclin-dependent kinase 4 pathway in mucosal melanoma

Although only a few reports have systematically examined the clinical relevance of the expression of genes in the CDK4 pathway in mucosal melanoma, several studies have demonstrated that transcription of CDK4 itself is increased. For example, Wang *et al.*, [60] showed that increased CDK4 protein expression predicts a poor prognosis and is significantly associated with reduced 3-year survival in mucosal melanoma and CDK4 expression was increased in elderly patients (>73 years) compared to patients in other age groups. However, another study in

a series of 35 primary oral mucosal melanoma by tissue microarray reported that CDK4 protein expression was negative in 67.64% [56]. Recent findings of Lyu *et al.*, [61] showed that CDK4 amplification tended to occur in combination with TERT amplification in head and neck mucosal melanoma and amplifications of CDK4 and TERT were associated with greater CDK4 and TERT protein expression levels.

Loss of p16 is also a frequent event in different tumor types. One study described the loss of p16 protein expression in 75% of mucosal melanoma, and was associated with recurrent/metastatic cases [62]. In agreement with that finding, Franchi *et al.*, [63] also observed the loss of p16 expression in 73% of sinonasal melanomas, which was not apparently associated with prognosis. Loss or negative expression of p16 has also been reported in oral mucosal melanoma [56,64]. However, one study showed, in contrast, that more than half of oral mucosal melanoma patients (52.5%) exhibit increased p16 expression, which was significantly associated with reduced 3-year survival [65]. Interesting work conducted by Mihic-Probst *et al.*, [66] on primary cutaneous melanoma suggested that the level of p16 expression gradually decreased from benign nevi to melanoma. The above-mentioned phenomenon may be related to the progression of melanoma development although this relationship has not yet been thoroughly studied.

## Treatment of mucosal melanoma with cyclin-dependent kinase 4/6 inhibitors

### Preclinical studies of cyclin-dependent kinase 4/6 inhibitors for mucosal melanoma

Previous studies have shown that CDK inhibitors, as a single agent or in combination with other strategies, can inhibit the growth of a variety of tumors, including melanoma [67,68]. Through WGS and exome sequencing analysis of different types of melanomas, in conjunction with the Cancer Genome Interpreter website, Newell *et al.*, [1] found that the majority of mucosal melanomas are potentially susceptible to CDK4/6 and MEK inhibitors, as well as their combination. Previous work has also shown that combined *in vivo* application of PD0332991 (Palbociclib, CDK4 inhibitor) and GSK1120212 (Trametinib, MEK inhibitor) led to a remarkable synergistic therapeutic effect compared with respective single treatments against melanomas carrying an NRAS mutation [69]. Moreover, the MEK inhibitor binimetinib plus the CDK4/6 inhibitor ribociclib suppressed MAPK pathway activation and cell cycle checkpoint dysregulation in NRAS-mutant melanomas in a phase IB trial (NCT01781572). Due to the lack of mucosal melanoma patients available for inclusion in clinical trials, it remains unknown whether these treatments will be effective for mucosal melanoma patients.

Several studies now point to potential therapeutic opportunities for mucosal melanoma via inhibition of

CDK4 pathway activity. For example, increased therapeutic activity by CDK4 inhibitor has been effectively demonstrated in cell lines with increased CDK4 expression or copy number. In HMVII cells, a human vaginal melanoma cell line with CDK4 amplification, treated with different concentrations of palbociclib (PD0332991) and AT7519 (a pan-CDK inhibitor), showing that both inhibitors significantly decreased the viability of HMVII cells. In addition, tumor growth was significantly inhibited by AT7519 or PD0332991 in patient-derived xenograft (PDX) models with CDK4 amplification plus P16INK4a loss [70]. In contrast, AT7519 and PD0332991 showed no inhibitory effect on tumor growth in an acral melanoma PDX model with a normal CDK4 pathway. In our previous work, we investigated the antitumor activity of palbociclib in molecularly-defined mucosal melanoma PDX models harboring CDK4 amplification compared with that in CDK4 wild-type PDX models [2]. This ‘PDX trial’ included 24 PDX models (14 cases with CDK4 amplification and 10 cases without CDK4 amplification) established from 24 mucosal melanoma patients using a format of one mouse per patient per treatment group (1 × 1 × 1), a preclinical antitumor drug evaluation strategy based on a drug screening study using large-scale PDX cohort. Among the 14 cases with CDK4 amplification, 8 cases (57%) showed effective tumor suppression or regression, while among the 10 cases without CDK4 amplification, only 1 (10%) had effective tumor suppression. In summary, these data indicate that CDK4 aberration can dictate the sensitivity to CDK inhibitors in mucosal melanoma-derived PDX models or cell lines. Given that a subset of mucosal melanomas harbor CDK4 amplification, these patients may be promising candidates for CDK4 inhibitor treatments combined with or without other potentially active agents.

Multiple studies have demonstrated that selective CDK4/6 inhibitors not only induced tumor cell cycle arrest, but also enhanced immune-mediated antitumor response in preclinical models [71–73]. The immunomodulatory effects of CDK4/6 inhibitors are multifaceted and complex, which may involve in tumor-intrinsic effects and direct effects on cells of the immune system [74]. For example, CDK4/6 inhibitor could enhance the expression of antigen-presenting genes, as well as the expression of PD-L1 on tumor cells [72,75]. Recent studies have also indicated that the inhibition of CDK4/6 could stimulate the T-cell activation, induce the phenotypic and functional acquisition of immunological T-cell memory and promote memory formation [73,76,77]. Indeed, results from these basic researches suggest the promising therapeutic efficacy of CDK4/6 inhibitors when combined with immune checkpoint blockade, which provides a rationale for the clinical investigation of this drug combination in patients with mucosal melanoma [72,73,76–78]. A previous study on Chinese patients with noncutaneous

melanoma found that genetic aberrations in the CDK4 pathway were related to innate resistance to anti-PD-1 therapy [79]. Based on data from 85 patients, including 13 mucosal melanomas, their results showed that patients with CDK4 amplification were more prone to develop resistance to anti-PD-1 immunotherapy. In addition, by using C57BL/6-hPD-1 and humanized immune system PDX models, palbociclib and anti-PD-1 antibody combined therapy showed enhanced efficacy on tumor growth inhibition compared to either monotherapy alone in PDX models with CDK4 amplification [79]. Given the characteristics of the CDK4 pathway in mucosal melanoma, these results provide a rationale for combining CDK4/6 inhibitors with ICIs to improve antitumor efficacy in patients with mucosal melanoma.

Collectively, the preclinical studies reviewed above validate that these CDK4/6 inhibitor-based therapeutic strategies for mucosal melanoma offer promising results and a solid rationale for testing in clinical trials.

### Clinical trial and application of cyclin-dependent kinase 4/6 inhibitor for mucosal melanoma

Guo *et al.* (2019) [80] first reported two metastatic melanoma patients (one case is a mucosal melanoma patient) with CNVs of CDK4 pathway-related genes, who were treated with CDK4/6 inhibitor palbociclib and achieved tumor control for over 6 months. Recently, the initiation of several, small clinical studies to assess the treatment efficacy of CDK inhibitors in mucosal melanoma and other melanomas have been encouraging for doctors and patients, alike. Guo *et al.*, (2018) [81] sponsored a phase II trial (NCT03454919) to evaluate the efficacy of palbociclib in acral melanoma bearing alterations in cell cycle pathways, including *CDK4* amplification, and/or *CCND1* amplification, and/or P16 (*CDKN2A*) loss, with a primary endpoint of overall response rate. This study is a single-arm phase II trial and the latest report of the trial showed that palbociclib monotherapy demonstrated preliminary efficacy and an acceptable safety profile in advanced acral melanoma patients with CDK4 pathway aberrations [81]. Similarly, a phase II trial (NCT00937937) to assess the efficacy and safety of dinaciclib, an inhibitor of CDK1, CDK2 and CDK9, in the treatment of patients with stage IV melanoma (including mucosal melanoma) is also currently active but not yet recruiting patients.

The highly selective CDK4/6 inhibitor, SHR6390, produced by Jiangsu Hengrui Medicine Co., Ltd (Jiangsu, China) [82,83] has entered phase III clinical trials for the treatment of breast cancer (NCT03966898). Recently, we initiated a new clinical trial (ChiCTR2000031608) to assess the efficacy of SHR6390 in treating patients with recurrent and/or metastatic mucosal melanoma of the head and neck harboring CDK4 amplification. To

our knowledge, this is the first clinical trial of a CDK4/6 inhibitor for mucosal melanoma with amplification of CDK4. However, it should be noted that this trial is currently underway and the results remain to be seen.

In short, the results from these preclinical studies and case reports, in combination with our increased understanding of the genomic profiles of mucosal melanoma, will further accelerate the progress of CDK4/6 inhibitor application against this lethal and intractable disease.

### Conclusion and future perspectives

Due to the rarity of mucosal melanoma, its study has been relatively neglected compared with other cancers, and there remains a dearth of prospective randomized trials to guide effective therapy; to date, well-established therapeutic guidelines for the treatment of mucosal melanoma do not exist [76]. Major advances in systemic therapy for melanomas have been achieved over recent years. However, as of yet, there has been no substantial progress in the treatment for mucosal melanoma. In this review, we have focused on the CDK4 pathways as the primary, potential therapeutic target for mucosal melanoma. Dysregulation of the CDK4 pathways is a key feature of many malignancies, and especially in melanomas. Owing to frequent alterations occurring in the CDK4 pathway and its associated pathways in mucosal melanoma, further preclinical and clinical research needs to be conducted to identify the most effective targeted therapy for mucosal melanoma carrying mutations in the CDK4 pathway. Moreover, further research is needed to learn about the optimal use of CDK4/6 inhibitors for patients with such a disease. In conclusion, inhibition of CDK4/6 offers a novel and promising direction for future mucosal melanoma treatment, thus providing great hope to doctors and patients with mucosal melanoma seeking a viable solution to this devastating disease.

On a final note, although this review focuses exclusively on the CDK4 pathway as a target for therapeutic intervention, it is likely that combining CDK4/6 inhibitors with existing and/or future treatment strategies will result in a substantial improvement for individuals suffering from mucosal melanoma. During recent years, many studies have demonstrated that CDK4/6-targeted therapies actually promote anti-tumor immunity through a complex immune regulatory network. CDK4/6 inhibitors act on both cancer cells and immune cells and augment the cancer-killing potential of checkpoint blockers, thus providing a strong rationale for new combination regimens of CDK4/6 inhibitors with immunotherapy in mucosal melanoma treatments. Beyond combined treatments, despite promising clinical outcomes in other tumors, the possible intrinsic or acquired resistance to CDK4/6 inhibitors should also be carefully investigated to determine the full effects of these drugs in the treatment of mucosal melanoma.



## Acknowledgements

This study was supported by the Shanghai Municipal Key Clinical Specialty (shslczdzk01601), Shanghai Clinical Research Center for Oral Diseases (19MC1910600), CAMS Innovation Fund for Medical Sciences (CIFMS) (2019-I2M-5-037), National Natural Science Foundation of China (grant No. 82002862) and the China Association for Science and Technology (grant No. 2019QNRC001).

S.C., Z.-Y.Z. and R.Z. contributed to the study concepts and design, and also critically revised the manuscript. S.C. drafted the manuscript.

S.-M.X and Y.H participated in collecting data and scientific discussion. All the authors agree to be accountable for all aspects of the work and approved the final version of the manuscript.

## Conflicts of interest

There are no conflicts of interest.

## References

- Newell F, Kong Y, Wilmott JS, Johansson PA, Ferguson PM, Cui C, *et al.* Whole-genome landscape of mucosal melanoma reveals diverse drivers and therapeutic targets. *Nat Commun* 2019; **10**:3163.
- Zhou R, Shi C, Tao W, Li J, Wu J, Han Y, *et al.* Analysis of mucosal melanoma whole-genome landscapes reveals clinically relevant genomic aberrations. *Clin Cancer Res* 2019; **25**:3548–3560.
- Goldemberg DC, Thuler LCS, de Melo AC. An update on mucosal melanoma: future directions. *Acta Dermatovenerol Croat* 2019; **27**:11–15.
- Chang AE, Karnell LH, Menck HR. The National Cancer Data Base report on cutaneous and noncutaneous melanoma: a summary of 84,836 cases from the past decade. The American College of Surgeons Commission on Cancer and the American Cancer Society. *Cancer* 1998; **83**:1664–1678.
- McLaughlin CC, Wu XC, Jemal A, Martin HJ, Roche LM, Chen VW. Incidence of noncutaneous melanomas in the U.S. *Cancer* 2005; **103**:1000–1007.
- Chi Z, Li S, Sheng X, Si L, Cui C, Han M, Guo J. Clinical presentation, histology, and prognoses of malignant melanoma in ethnic Chinese: a study of 522 consecutive cases. *BMC Cancer* 2011; **11**:85.
- Nassar KW, Tan AC. The mutational landscape of mucosal melanoma. *Semin Cancer Biol* 2020; **61**:139–148.
- Tacastacas JD, Bray J, Cohen YK, Arbesman J, Kim J, Koon HB, *et al.* Update on primary mucosal melanoma. *J Am Acad Dermatol* 2014; **71**:366–375.
- Bishop KD, Olszewski AJ. Epidemiology and survival outcomes of ocular and mucosal melanomas: a population-based analysis. *Int J Cancer* 2014; **134**:2961–2971.
- Postow MA, Hamid O, Carvajal RD. Mucosal melanoma: pathogenesis, clinical behavior, and management. *Curr Oncol Rep* 2012; **14**:441–448.
- Rossi E, Schinzari G, Maiorano BA, Indelicati G, Di Stefani A, Pagliara MM, *et al.* Efficacy of immune checkpoint inhibitors in different types of melanoma. *Hum Vaccin Immunother* 2021; **17**:4–13.
- Rose AAN, Armstrong SM, Hogg D, Butler MO, Saibil SD, Arteaga DP, *et al.* Biologic subtypes of melanoma predict survival benefit of combination anti-PD1+anti-CTLA4 immune checkpoint inhibitors versus anti-PD1 monotherapy. *J Immunother Cancer* 2021; **9**:e001642.
- Li J, Kan H, Zhao L, Sun Z, Bai C. Immune checkpoint inhibitors in advanced or metastatic mucosal melanoma: a systematic review. *Ther Adv Med Oncol* 2020; **12**:1758835920922028.
- Hodi FS, Chiarion-Sileni V, Gonzalez R, Grob JJ, Rutkowski P, Cowey CL, *et al.* Nivolumab plus ipilimumab or nivolumab alone versus ipilimumab alone in advanced melanoma (CheckMate 067): 4-year outcomes of a multicentre, randomised, phase 3 trial. *Lancet Oncol* 2018; **19**:1480–1492.
- Mignard C, Deschamps Huvier A, Gillibert A, Duval Modeste AB, Dutriaux C, Khammari A, *et al.* Efficacy of immunotherapy in patients with metastatic mucosal or uveal melanoma. *J Oncol* 2018; **2018**:1908065.
- D'Angelo SP, Larkin J, Sosman JA, Lebbé C, Brady B, Neyns B, *et al.* Efficacy and safety of nivolumab alone or in combination with ipilimumab in patients with mucosal melanoma: a pooled analysis. *J Clin Oncol* 2017; **35**:226–235.
- Seth R, Messersmith H, Kaur V, Kirkwood JM, Kudchadkar R, McQuade JL, *et al.* Systemic therapy for melanoma: ASCO guideline. *J Clin Oncol* 2020; **38**:3947–3970.
- Curtin JA, Fridlyand J, Kageshita T, Patel HN, Busam KJ, Kutzner H, *et al.* Distinct sets of genetic alterations in melanoma. *N Engl J Med* 2005; **353**:2135–2147.
- Walker GJ, Flores JF, Glendening JM, Lin AH, Markl ID, Fountain JW. Virtually 100% of melanoma cell lines harbor alterations at the DNA level within CDKN2A, CDKN2B, or one of their downstream targets. *Genes Chromosomes Cancer* 1998; **22**:157–163.
- Lee B, McArthur GA. CDK4 inhibitors: an emerging strategy for the treatment of melanoma. *Melanoma Manag* 2015; **2**:255–266.
- Hayward NK, Wilmott JS, Waddell N, Johansson PA, Field MA, Nones K, *et al.* Whole-genome landscapes of major melanoma subtypes. *Nature* 2017; **545**:175–180.
- Sheppard KE, McArthur GA. The cell-cycle regulator CDK4: an emerging therapeutic target in melanoma. *Clin Cancer Res* 2013; **19**:5320–5328.
- Sherr CJ, Beach D, Shapiro GI. Targeting CDK4 and CDK6: from discovery to therapy. *Cancer Discov* 2016; **6**:353–367.
- Spring LM, Wander SA, Andre F, Moy B, Turner NC, Bardia A. Cyclin-dependent kinase 4 and 6 inhibitors for hormone receptor-positive breast cancer: past, present, and future. *Lancet* 2020; **395**:817–827.
- Spring L, Bardia A, Modi S. Targeting the cyclin D-cyclin-dependent kinase (CDK) 4/6-retinoblastoma pathway with selective CDK 4/6 inhibitors in hormone receptor-positive breast cancer: rationale, current status, and future directions. *Discov Med* 2016; **21**:65–74.
- Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell* 2011; **144**:646–674.
- Barabási AL, Oltvai ZN. Network biology: understanding the cell's functional organization. *Nat Rev Genet* 2004; **5**:101–113.
- Kastan MB, Bartek J. Cell-cycle checkpoints and cancer. *Nature* 2004; **432**:316–323.
- Walworth NC. Cell-cycle checkpoint kinases: checking in on the cell cycle. *Curr Opin Cell Biol* 2000; **12**:697–704.
- Sherr CJ. The Pezcoller lecture: cancer cell cycles revisited. *Cancer Res* 2000; **60**:3689–3695.
- Rojas A, Kong SW, Agarwal P, Gilliss B, Pu WT, Black BL. GATA4 is a direct transcriptional activator of cyclin D2 and Cdk4 and is required for cardiomyocyte proliferation in anterior heart field-derived myocardium. *Mol Cell Biol* 2008; **28**:5420–5431.
- Matsushime H, Ewen ME, Strom DK, Kato JY, Hanks SK, Roussel MF, Sherr CJ. Identification and properties of an atypical catalytic subunit (p34<sup>PSK</sup>/J3/cdk4) for mammalian D type G1 cyclins. *Cell* 1992; **71**:323–334.
- Meyerson M, Harlow E. Identification of G1 kinase activity for cdk6, a novel cyclin D partner. *Mol Cell Biol* 1994; **14**:2077–2086.
- Sheldon LA. Inhibition of E2F1 activity and cell cycle progression by arsenite via retinoblastoma protein. *Cell Cycle* 2017; **16**:2058–2072.
- Ovejero-Benito MC, Frade JM. p27(Kip1) participates in the regulation of endoreplication in differentiating chick retinal ganglion cells. *Cell Cycle* 2015; **14**:2311–2322.
- Weinberg RA. The retinoblastoma protein and cell cycle control. *Cell* 1995; **81**:323–330.
- Harbour JW, Luo RX, Dei Santi A, Postigo AA, Dean DC. Cdk phosphorylation triggers sequential intramolecular interactions that progressively block Rb functions as cells move through G1. *Cell* 1999; **98**:859–869.
- O'Leary B, Finn RS, Turner NC. Treating cancer with selective CDK4/6 inhibitors. *Nat Rev Clin Oncol* 2016; **13**:417–430.
- Cánepa ET, Scassa ME, Ceruti JM, Marazita MC, Carcagno AL, Sirkin PF, Ogara MF. INK4 proteins, a family of mammalian CDK inhibitors with novel biological functions. *IUBMB Life* 2007; **59**:419–426.
- Goel S, DeCristo MJ, McAllister SS, Zhao JJ. CDK4/6 inhibition in cancer: beyond cell cycle arrest. *Trends Cell Biol* 2018; **28**:911–925.
- Kratzke RA, Greatens TM, Rubins JB, Maddaus MA, Niewoehner DE, Niehans GA, Geradts J. Rb and p16INK4a expression in resected non-small cell lung tumors. *Cancer Res* 1996; **56**:3415–3420.
- Tsuda H, Yamamoto K, Inoue T, Uchiyama I, Umesaki N. The role of p16-cyclin D/CDK-pRb pathway in the tumorigenesis of endometrioid-type endometrial carcinoma. *Br J Cancer* 2000; **82**:675–682.
- LaBaer J, Garrett MD, Stevenson LF, Slingerland JM, Sandhu C, Chou HS, *et al.* New functional activities for the p21 family of CDK inhibitors. *Genes Dev* 1997; **11**:847–862.
- Paternot S, Bockstaele L, Bisteau X, Kooken H, Coulonval K, Roger PP. Rb inactivation in cell cycle and cancer: the puzzle of highly regulated activating phosphorylation of CDK4 versus constitutively active CDK-activating kinase. *Cell Cycle* 2010; **9**:689–699.



- 45 Zwijsen RM, Wientjens E, Klompaker R, van der Sman J, Bernards R, Michalides RJ. CDK-independent activation of estrogen receptor by cyclin D1. *Cell* 1997; **88**:405–415.
- 46 Ameratunga M, Kipps E, Okines AFC, Lopez JS. To cycle or fight-CDK4/6 inhibitors at the crossroads of anticancer immunity. *Clin Cancer Res* 2019; **25**:21–28.
- 47 Sánchez-Martínez C, Lallena MJ, Sanfeliciano SG, de Dios A. Cyclin dependent kinase (CDK) inhibitors as anticancer drugs: recent advances (2015–2019). *Bioorg Med Chem Lett* 2019; **29**:126637.
- 48 Tamura K. Differences of cyclin-dependent kinase 4/6 inhibitor, palbociclib and abemaciclib, in breast cancer. *Jpn J Clin Oncol* 2019; **49**:993–998.
- 49 Álvarez-Fernández M, Malumbres M. Mechanisms of sensitivity and resistance to CDK4/6 inhibition. *Cancer Cell* 2020; **37**:514–529.
- 50 Asghar U, Witkiewicz AK, Turner NC, Knudsen ES. The history and future of targeting cyclin-dependent kinases in cancer therapy. *Nat Rev Drug Discov* 2015; **14**:130–146.
- 51 Miller TW, Traphagen NA, Li J, Lewis LD, Lopes B, Asthagiri A, *et al.* Tumor pharmacokinetics and pharmacodynamics of the CDK4/6 inhibitor ribociclib in patients with recurrent glioblastoma. *J Neurooncol* 2019; **144**:563–572.
- 52 Billard-Sandu C, Tao YG, Sablin MP, Dumitrescu G, Billard D, Deutsch E. CDK4/6 inhibitors in P16/HPV16-negative squamous cell carcinoma of the head and neck. *Eur Arch Otorhinolaryngol* 2020; **277**:1273–1280.
- 53 Hintzsche JD, Gorden NT, Amato CM, Kim J, Wuensch KE, Robinson SE, *et al.* Whole-exome sequencing identifies recurrent SF3B1 R625 mutation and comutation of NF1 and KIT in mucosal melanoma. *Melanoma Res* 2017; **27**:189–199.
- 54 Lyu J, Song Z, Chen J, Shepard MJ, Song H, Ren G, *et al.* Whole-exome sequencing of oral mucosal melanoma reveals mutational profile and therapeutic targets. *J Pathol* 2018; **244**:358–366.
- 55 Xu L, Cheng Z, Cui C, Wu X, Yu H, Guo J, Kong Y. Frequent genetic aberrations in the cell cycle related genes in mucosal melanoma indicate the potential for targeted therapy. *J Transl Med* 2019; **17**:245.
- 56 Hsieh R, Nico MM, Coutinho-Camillo CM, Buim ME, Sangueza M, Lourenço SV. The CDKN2A and MAP kinase pathways: molecular roads to primary oral mucosal melanoma. *Am J Dermatopathol* 2013; **35**:167–175.
- 57 Lu L, Bi J, Bao L. Genetic profiling of cancer with circulating tumor DNA analysis. *J Genet Genomics* 2018; **45**:79–85.
- 58 Zhang L, Liang Y, Li S, Zeng F, Meng Y, Chen Z, *et al.* The interplay of circulating tumor DNA and chromatin modification, therapeutic resistance, and metastasis. *Mol Cancer* 2019; **18**:36.
- 59 Mikoshiba A, Ashida A, Sakaizawa K, Kiniwa Y, Okuyama R. Detecting copy number alterations of oncogenes in cell-free DNA to monitor treatment response in acral and mucosal melanoma. *J Dermatol Sci* 2020; **97**:172–178.
- 60 Wang F, Chen G, Quinn MJ, Chen S, Ji X, Shentu Y, Li Y. Increased CDK4 protein expression predicts a poor prognosis in mucosal melanoma associated with the p16INK4a-CDK4-pRb pathway. *Int J Clin Exp Pathol* 2019; **12**:2819–2825.
- 61 Lyu J, Miao Y, Yu F, Chang C, Guo W, Zhu H. CDK4 and TERT amplification in head and neck mucosal melanoma. *J Oral Pathol Med* 2021; doi: 10.1111/jop.13180 [Epub ahead of print]
- 62 Prasad ML, Patel SG, Shah JP, Hoshaw-Woodard S, Busam KJ. Prognostic significance of regulators of cell cycle and apoptosis, p16(INK4a), p53, and bcl-2 in primary mucosal melanomas of the head and neck. *Head Neck Pathol* 2012; **6**:184–190.
- 63 Franchi A, Alos L, Gale N, Massi D, Paglierani M, Santucci M, *et al.* Expression of p16 in sinonasal malignant melanoma. *Virchows Arch* 2006; **449**:667–672.
- 64 Tanaka N, Odajima T, Mimura M, Ogi K, Dehari H, Kimijima Y, Kohama G. Expression of Rb, pRb2/p130, p53, and p16 proteins in malignant melanoma of oral mucosa. *Oral Oncol* 2001; **37**:308–314.
- 65 Chen H, Li Y, Long Y, Tang E, Wang R, Huang K, *et al.* Increased p16 and p53 protein expression predicts poor prognosis in mucosal melanoma. *Oncotarget* 2017; **8**:53226–53233.
- 66 Mihic-Probst D, Mnich CD, Oberholzer PA, Seifert B, Sasse B, Moch H, Dummer R. p16 expression in primary malignant melanoma is associated with prognosis and lymph node status. *Int J Cancer* 2006; **118**:2262–2268.
- 67 Schettini F, De Santo I, Rea CG, De Placido P, Formisano L, Giuliano M, *et al.* CDK 4/6 inhibitors as single agent in advanced solid tumors. *Front Oncol* 2018; **8**:608.
- 68 Ascierto PA, Flaherty K, Goff S. Emerging strategies in systemic therapy for the treatment of melanoma. *Am Soc Clin Oncol Educ Book* 2018; **38**:751–758.
- 69 Kwong LN, Costello JC, Liu H, Jiang S, Helms TL, Langsdorf AE, *et al.* Oncogenic NRAS signaling differentially regulates survival and proliferation in melanoma. *Nat Med* 2012; **18**:1503–1510.
- 70 Kong Y, Sheng X, Wu X, Yan J, Ma M, Yu J, *et al.* Frequent genetic aberrations in the CDK4 pathway in acral melanoma indicate the potential for CDK4/6 inhibitors in targeted therapy. *Clin Cancer Res* 2017; **23**:6946–6957.
- 71 Lai AY, Sorrentino JA, Dragnev KH, Weiss JM, Owonikoko TK, Rytlewski JA, *et al.* CDK4/6 inhibition enhances antitumor efficacy of chemotherapy and immune checkpoint inhibitor combinations in preclinical models and enhances T-cell activation in patients with SCLC receiving chemotherapy. *J Immunother Cancer* 2020; **8**:e000847.
- 72 Goel S, DeCristo MJ, Watt AC, BrinJones H, Sceneay J, Li BB, *et al.* CDK4/6 inhibition triggers anti-tumour immunity. *Nature* 2017; **548**:471–475.
- 73 Deng J, Wang ES, Jenkins RW, Li S, Dries R, Yates K, *et al.* CDK4/6 inhibition augments antitumor immunity by enhancing T-cell activation. *Cancer Discov* 2018; **8**:216–233.
- 74 Lelliott EJ, McArthur GA, Oliaro J, Sheppard KE. Immunomodulatory effects of BRAF, MEK, and CDK4/6 inhibitors: implications for combining targeted therapy and immune checkpoint blockade for the treatment of melanoma. *Front Immunol* 2021; **12**:661737.
- 75 Jin X, Ding D, Yan Y, Li H, Wang B, Ma L, *et al.* Phosphorylated RB promotes cancer immunity by inhibiting NF- $\kappa$ B activation and PD-L1 expression. *Mol Cell* 2019; **73**:22–35.e6.
- 76 Heckler M, Ali LR, Clancy-Thompson E, Qiang L, Ventre KS, Lenehan P, *et al.* Inhibition of CDK4/6 promotes CD8 T-cell memory formation. *Cancer Discov* 2021. doi: 10.1158/2159-8290.CD-20-1540 [Epub ahead of print]
- 77 Lelliott EJ, Kong IY, Zethoven M, Ramsbottom KM, Martelotto LG, Meyran D, *et al.* CDK4/6 inhibition promotes anti-tumor immunity through the induction of T cell memory. *Cancer Discov* 2021. doi: 10.1158/2159-8290.CD-20-1554 [Epub ahead of print]
- 78 Schaefer DA, Beckmann RP, Dempsey JA, Huber L, Forest A, Amaladas N, *et al.* The CDK4/6 inhibitor abemaciclib induces a T cell inflamed tumor microenvironment and enhances the efficacy of PD-L1 checkpoint blockade. *Cell Rep* 2018; **22**:2978–2994.
- 79 Yu J, Yan J, Guo Q, Chi Z, Tang B, Zheng B, *et al.* Genetic aberrations in the CDK4 pathway are associated with innate resistance to PD-1 blockade in Chinese patients with non-cutaneous melanoma. *Clin Cancer Res* 2019; **25**:6511–6523.
- 80 Tang B, Sheng X, Kong Y, Chi Z, Si L, Cui C, *et al.* Palbociclib for treatment of metastatic melanoma with copy number variations of CDK4 pathway: case report. *Chin Clin Oncol* 2018; **7**:62.
- 81 Mao L, Dai J, Cao Y, Bai X, Sheng X, Chi Z, *et al.* Palbociclib in advanced acral melanoma with genetic aberrations in the cyclin-dependent kinase 4 pathway. *Eur J Cancer* 2021; **148**:297–306.
- 82 Wang J, Li Q, Yuan J, Wang J, Chen Z, Liu Z, *et al.* CDK4/6 inhibitor-SHR6390 exerts potent antitumor activity in esophageal squamous cell carcinoma by inhibiting phosphorylated Rb and inducing G1 cell cycle arrest. *J Transl Med* 2017; **15**:127.
- 83 Long F, He Y, Fu H, Li Y, Bao X, Wang Q, *et al.* Preclinical characterization of SHR6390, a novel CDK 4/6 inhibitor, *in vitro* and in human tumor xenograft models. *Cancer Sci* 2019; **110**:1420–1430.