CDK4 inhibitors an emerging strategy for the treatment of melanoma

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Practice points

- Dysregulation of the RB pathway (p16^{INK4A}: cyclin D-CDK4/6:RB) may be an early activating oncogenic event.
- CDK4 and CCND1 may represent independent oncogenes. Tumors with these activating events may be 'addicted' to this pathway.
- Loss of p16^{INK4A} via genetic alterations in *CDKN2A* is associated with increased sensitivity to CDK4 inhibition in melanoma cell lines.
- Overexpression or amplification of CDK4 is associated with increased CDK4 inhibitor therapeutic activity.
- Loss of RB is associated with CDK4 inhibitor resistance.
- Clinical trials with combined CDK inhibition and MEK inhibition are underway to investigate if this dual approach will be effective for managing *NRAS*-mutant melanomas.
- The use of CDK inhibitors in combination with other small molecule targeted agents is a potential treatment strategy under investigation for overcoming acquired therapeutic resistance.

Research into the cyclin-dependent kinases and their inhibitors is finally coming into the forefront of clinical research in cancer. Targeted therapies such as BRAF inhibitors have led the way in improving treatment outcomes in advanced melanoma. Based on detailed genomic knowledge of melanoma it is now time to extend targeted therapies beyond BRAF to fulfill the vision of precision medicine. The p16^{INK4A}-cyclin D-CDK4/6-retinoblastoma protein pathway (RB pathway) is dysregulated in more than 90% of melanomas and interacts biochemically and genetically with the RAS/RAF/MEK/ERK pathway. Recognizing and understanding these processes that drive melanomagenesis is essential to rationally develop new therapies. This paper reviews the mechanisms, background and progress of small molecule CDK4 inhibitors in the management of melanoma.

The incidence of melanoma over the last four decades has increased at a greater rate (3–7%) than in any other solid tumor [1]. It is now one of the most common malignancies in young adults. When detected and managed at an early stage, there is a 90% survival rate at 5 years. However, in the advanced or metastatic setting, the prognosis has been far more limited with a median

KEYWORDS

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survival of 6–8 months and <10% survival rates at 5 years [2].

Recent developments in the therapeutic landscape for advanced melanoma have brought new hope to the horizon. Within the last 3 years, six new therapies have been approved by the US FDA: ipilimumab (anti-CTLA4), vemurafenib (BRAF inhibitor), dabrafenib (BRAF inhibitor) and trametinib (MEK inhibitor) and most recently pembrolizumab (anti-PD1) and nivolumab (anti-PD1). Despite significantly improving survival, issues of drug resistance and limited periods of treatment benefit continue to challenge patients and clinicians. In order to overcome the obstacles, recognizing and understanding the processes that drive melanomagenesis is essential in order to identify rational combination treatments. The increasing availability of next-generation sequencing is allowing identification of the critical genomic drivers of a patient's melanoma that makes precision medicine a real option in patient management. Research and development into the cyclin-dependent pathway and its inhibitors has been ongoing for the last 20 years, but it has only been in the last few years that cyclin-dependent kinase inhibitors (CDKI) are finally coming into the forefront of clinical research. In part this is being driven by the use of next-generation sequencing that has highlighted the frequencies of aberrations of the p16^{INK4A}-cyclin D-CDK4/6-retinoblastoma protein pathway (RB pathway) in melanoma in cohorts such as The Cancer Genome Atlas and is also being incorporated into targeted sequencing panels that are being applied to patients with advanced disease undergoing therapy. For example, one Phase II multicenter open label study in patients with advanced BRAF V600 melanoma examines sequential BRAF and MEK inhibition with the encorafenib (LGX818) plus binimetinib (MEK162) performs targeted next-generation sequencing at baseline and progression that is to identify a rational combination with a targeted agent including the CDK4 inhibitor LEE011 after progression to overcome resistance if the genomic profile suggests activation of CDK4 (NCT01820364) [3].

Aberrations in the p16-cyclin D-CDK4/6-retinoblastoma protein pathway (RB pathway) have been reported in more than 90% of melanomas [4,5]. Moreover the RB pathway is activated downstream of the RAS/RAF/MEK/ERK pathway, another frequently dysregulated

pathway in melanoma [6]. Interactions between these two pathways are currently being investigated and may have therapeutic implications in both NRAS-mutated melanoma that occurs in up to 20% of melanomas [4] and inactivating *NFI* mutations that lead to RAS activation [6]. Furthermore, the challenge of overcoming acquired treatment resistance to BRAF and MEK inhibition remains an issue. Combination therapy with different types of small molecular inhibitors via complementary oncogenic pathways such as the RB pathway is a potential strategy to surpass this resistance. Understanding the role of CDK4 in the RB pathway and its relation to progression of melanoma maybe the next critical step in the management of advanced melanoma with targeted therapies. This paper reviews the mechanisms, background and progress of CDK4 inhibitors in the management of melanoma.

CDK4 in cell cycle control & regulation

Regulation of the cell cycle involves a complex choreographed interplay between cyclin-dependent kinase (CDK), CDKIs, that control levels of phosphorylated retinoblastoma suppressor gene protein (RB1) and transcriptional activity of the E2F family of transcription factors in addition to activity of tumor suppressor protein, TP53, in some situations.

CDKs are protein kinases involved in driving cell cycle progression, controlling transcription processes and regulating cell proliferation. Each of the CDKs controls a specific point of the cell cycle and function as checkpoints to halt cell cycle progression in response to defects in the mitotic spindle or DNA damage. Mitogenic signalling from stimuli such as the RAS/RAF pathway, triggers quiescent cells to enter the G1 phase of the cell cycle from G0. This occurs via an increase in D-type cyclin expression, which prompts the release of inhibitory INK4 proteins, p15 and p16, from the CDK4/6 complexes and inhibitory CIP/KIP proteins, p21 and p27, from the CDK2 complexes. The activated D-type cyclin-CDK4/ CDK6 complexes and the cyclin E (CCNE1/E2)- CDK2 complexes phosphorylate RB1, which release the RB-dependent inhibition of the E2F transcription factors and prompt cells to enter the cell cycle and transition through the G1-S restriction point. Cyclin A-CDK1 and CDK2 complexes and CCNB1-CDK1 complexes are responsible for maintaining the hyperphosphorylated RB1 state throughout

S phase. At the end of mitosis, RB1 is dephosphorylated and E2F recombines with RB, thus preventing unregulated cell proliferation. The specific checkpoints controlled by individual CDKs during each stage of the cell cycle ensure fidelity of the replicated genome.

Mechanisms of cyclin-dependent kinase dysregulation in melanoma

Of particular interest among the CDKs is the role of CDK4/6, which mediates progression of cells through G1 phase of the cell cycle. CDK4/6 appears to play a significant role in melanomagenesis with up to 90% of melanoma cases being associated with activating genomic changes affecting various components of the p16-cyclin D-CDK4/6-RB pathway (RB pathway) [4,7]. Germline mutations in *CDKN2A* [8] and *CDK4* [9–12] both appear to influence the risk of developing melanoma. Other mechanisms of RB pathway dysregulation that have been identified in melanoma include germline mutations in p16^{INK4} binding domain of CDK4 [9,13], inactivation of INK4 and CIP/KIP inhibitors [14,15], amplification of *CDK4* and *CCND1* [4] and amplification or overexpression of CDK4/6 [16].

The p16 INK4a protein encoded by the *CDKN2A* locus [8,17] acts as a tumor suppressor that induces G1 cell cycle arrest as described above (Figure 1). Mutations or deletion of *CDKN2A* (p16^{INK4a}) is reported to occur in 22–78% of melanomas and 80–90% of metastatic tumors. This occurs frequently in BRAF- and NRAS-mutated melanomas, but is also reported in KIT amplified melanomas [18]. Loss of p16^{INK4a} occurs via gene deletion (50–80%) [14,19–20] inactivating mutations (16%) [15] and epigenetic silencing (20%) [21] including promoter methylation (20–30%) [21,22]. The sequential loss of p16^{INK4a} protein expression has been demonstrated to correlate with progression to melanoma with positive p16^{INK4a} protein expression detected in 70–100% of benign naevi, 30–80% of primary melanomas but only 10–20% of metastatic tumors [23]. This strongly suggests that the aberrations in the RB pathway maybe an early activating event in the development of melanoma.

It has been hypothesized that the loss of p16^{INK4a} results in loss of the negative feedback control on the RB pathway, resulting in a rise in CDK4/6 activity which enhances cell progression and proliferation [7]. Increase in CDK4 activity or *CCND1* due to amplification or overexpression may also dysregulate the negative

feedback control and increase proliferation [7]. CDK4 mutations have been reported in 5–37% of melanomas including p16^{INK4A}-CDK4 binding domain mutations [11,13,15], *CDK4* amplification in 3% [4], and amplifications of *CCND1* in 11–18% [4,7]. p16^{INK4A} and CDK4 proteins normally bind together with a 1:1 ratio. A reduction in this 1:1 ratio appears to promote entry into the cell cycle.

Amplification of CDK4 may obviate the need for loss of p16^{INK4A} (deletion of *CDKN2A*), such that either genetic variation will disrupt the balance and over-ride the check point control for entry into the S phase, leading to unregulated cell proliferation that is independent of normal extracellular cues [10,13,21,24]. However, gain of *CCND1* in combination with gain in *CDK4* and or loss of p16^{INK4A} (*CDKN2A*) has been reported to lead to significantly poorer survival than in cases with single gene variations [7]. Deletions or epigenetic alterations in RB have also been identified in 6–14% of melanomas [18].

It appears likely that the alterations that dysregulate the RB pathway are critical oncogenic events that drive melanomagenesis [7]. It has also been suggested that melanomas with these activating events are ‘addicted’ to this pathway [20]. With these findings in mind, further investigation and research into the therapeutic targeting of CDKs in melanoma has been undertaken (Table 1).

Development of cyclin-dependent kinase inhibitors

Research and development of CDKs began in the mid 1990s and resulted in the investigation of hundreds of small molecules and patent applications [25]. The inhibition of CDKs has been thought to be particularly attractive therapeutic targets given their roles in cell progression, transcription and apoptosis coupled with the almost universal dysregulation and activation of CDKs in cancer. On the other hand it has also been argued that, as normal cells also require CDKs for proliferation that may limit therapeutic index of CDK inhibition. Ultimately *in vivo* studies and clinical trials are required to address these alternate hypotheses. CDKI investigated to date cover a wide range of compounds with various selectivity, targets and mechanisms of actions. However, it is becoming increasingly apparent that combination of CDKI with other pathways or compounds is most likely to yield the greatest clinical antitumor efficacy. Furthermore, work

Figure 1. Regulation of the cell cycle and cyclin-dependent kinase inhibitors in development.

into identifying cancer biomarkers for response to CDKI is also underway. Current preclinical data suggest that markers of particular interest include truncated cyclin E (particularly in breast cancer), MYC transcription factor and MCL-1 survival factor (particularly in hematological cancers) [26], although none have yet been validated in clinical trials of CDKI.

All CDKI developed to date can be divided into two main categories – broad range inhibitors and selective inhibitors. Drugs within the broad range inhibitors category include flavopiridol, olomucine, roscovitine, kenpaullone, SNS-032,

AT7519, AG-024322, (S)-Roscovitine and R547. Selective CDKI being developed for melanoma treatment include SU 9516, PD0332991, LEE011 and P276-00. Other selective CDKI being developed for other malignancies include faspaplysin, ryuvindine, purvalanol A, NU2058 and BML-259. Therapeutic success with early CDKI was only modest and the development of many of these compounds was not taken forward due to issues with potency, selectivity, poor pharmacological and physiochemical properties as well issues with the toxicities profile of the broad range CDKI. In comparison, the more

selective CDKI have improved toxicity profiles and are showing evidence of clinical benefit. In particular, palbociclib (PD-0332991), an oral CDK4/6 inhibitor was recently FDA approved in the setting of advanced estrogen receptor-positive breast cancer. There is also significant interest in developing CDKI in the melanoma.

First-generation CDK inhibitors

Olomucine is a plant secondary metabolite that was originally isolated from the radish and synthesized in 1986 [27]. Olomucine was found to inhibit CDK with IC₅₀ values in the low micromolar range. Subsequent development of the olomucine structure produced roscovitine (also known as CYC202, seliciclib). Roscovitine was found to be a potent inhibitor of CDK2, phosphorylation of retinoblastoma protein and RNA polymerase II. This first-generation CDKI non-selectively inhibited multiple kinases (CDK1, CDK2, CDK5, CDK7 and CDK9) inducing apoptosis in multiple cell cycle phases. These data are consistent with the observation of significant side effects of R-Roscovitine including fatigue, skin rash, emesis, abnormal liver function tests, hyponatraemia, hypokalemia and renal impairment reported in a Phase I clinical trial [28].

Flavopiridol (also referred to as alvocidib, L-868275, HMR-1275 and NSC-649890) is another first-generation broad range CDKI that inhibits CDK1, CDK2, CDK4 and CDK7 [29]. It was the first CDKI used in human clinical trials with evidence of antitumor activity. Flavopiridol arrests the cell cycle at two points, either during the G1 phase or the boundary between G2 phase and M phase [30]. There were two studies investigating its use in melanoma that have been reported – NCT00005971 [31] and NCT00003690 [32]. NCT00005971 was a

Phase II study looking specifically at its use in melanoma, while NCT00003690 was a Phase I study assessing its use in combination with carboplatin/cisplatin in advanced solid tumors including melanoma [33]. The main adverse effects reported with the use of flavopiridol include secretory diarrhea, proinflammatory syndrome, neutropenia, nausea and vomiting. Interim results from the Phase II study in untreated metastatic melanoma concluded that flavopiridol was well tolerated, with acceptable, primarily GI, toxicity profile. Stable disease was seen in 7 out of 16 patients ranging from 1.8 to 9.2 months, but there was no evidence of significant clinical activity in melanoma by objective response criteria [34]. As a result further development of flavopiridol in melanoma has not been pursued.

In the search for more active CDKI compounds, analysis of the National Cancer Institute anticancer drug screen data was used to identify other compounds with similar properties to flavopiridol. Kenpaullone, a potent inhibitor of CDK1/cyclin B, CDK2/cyclin A, CDK2/ cyclinE and CDK5 was identified [35]. Kenpaullone also inhibits the α - and β -isoforms of GSK-3 in submicromolar concentrations. Paullones do not inhibit CDK4. Subsequent development of this compound has focused more on its reprogramming properties in neurodegenerative diseases rather than its anticancer properties [36].

P276-00 is an analog of flavopiridol. It is a highly specific inhibitor of CDK2 but is less selective toward CDK1 and CDK4 [37]. Major adverse effects reported include fatigue, hypotension, nausea, sweating and dry mouth [38]. The Phase II trials ended in 2012/13 but no results have yet been reported.

Table 1. Frequency of genetic alterations in the p16^{INK4A}: cyclin D-CDK4/6-retinoblastoma protein pathway.

Mechanism	Frequency	Ref.
CDK4 mutation–insensitivity to p16 ^{INK4} inhibition	5–37%	[7,11]
p16 ^{INK4A} -binding domain mutation in CDK4	Rare	[9,13]
Amplification of CDK4 (more frequent in <i>BRAF</i> , <i>NRAS</i> wild-type)	3%	[4]
Amplification CCND1	11–18%	[4,7,18]
Overexpression CCND1 (more frequent in <i>BRAF</i> , <i>NRAS</i> wild-type)	30–65%	
CDKN2A mutation (frequent in <i>BRAF</i> - and <i>NRAS</i> -mutated, <i>KIT</i> amplified and familial melanoma)	22–78%	[7,14–15,19,21–22]
Loss of p16 ^{INK4A} : deletions	50–80%	
Loss of p16 ^{INK4A} : inactivation mutations	16%	
Loss of p16 ^{INK4A} : epigenetic silencing	20%	
Deletion or epigenetic alterations to RB	6–14%	[18,24]

Roniciclib, previously known as BAY1000394, is an oral pan-CDK inhibitor, which targets CDK1, CDK2, CDK3, CDK4, CDK7 and CDK 9. Preclinical data in xenograft model resistant to standard cytotoxic therapies such as doxorubicin, cisplatin and paclitaxel demonstrated antitumor activity [39]. This compound has entered Phase I clinical trials in refractory solid tumors including melanoma (NCT02047890) [40].

AT7519 is a multiple CDKI of CDK1, CDK2, CDK4, CDK5 and CDK9 and a potent inhibitor of RNA polymerase II-dependent transcription. CDK9 phosphorylates the C-terminal domain of RNA polymerase 2 to promote its activity [41]. AT7519 has potent preclinical antitumor activity and a favorable toxicity profile demonstrated in the Phase I study in refractory solid tumors. Clinical and pharmacodynamics activity was seen in the Phase I study that assessed 28 patients with refractory solid tumors including melanoma. Four patients exhibited stable disease for greater than 6 months and one had a prolonged partial response. Antiproliferative activity was reported at a dose of 28 mg/m² [42]. Further Phase I and II clinical trials, NCT00390117, [43] are currently investigating the activity of AT7519 in hematological cancer.

A number of CDKI which were assessed across a range of tumors including melanoma, have subsequently been found to be more active in hematological cancers. These include dinaciclib, SNS032, and AT7519 and terameprocol. Dinaciclib (SCH727965) is a potent inhibitor of CDK1, CDK2, CDK5 and CDK9, which has demonstrated superiority compared with ofatumumab in refractory chronic lymphocytic leukemia. SNS032 (BMS-387032) selectively inhibits CDK2, CDK7 and CDK9. It has confirmed inhibitory activity against cell cycle progression and transcription [44]. Terameprocol is being evaluated in Phase I and II clinical trials in refractory solid tumors and leukemia [45].

Second-generation: specific cyclin-dependent kinases

Second-generation CDK have achieved greater therapeutic success and are generating renewed interest in the role of CDK in the RB pathway. Of particular interest is palbociclib (PD-0332991, Pfizer), an oral bioavailable highly selective inhibitor of CDK4 and CDK6, which targets the ATP binding site of the CDK4 [46]. It is an effective antiproliferative agent against

retinoblastoma-positive tumor cells with intact RB1 (retinoblastoma protein). In preclinical studies palbociclib was shown to be an inhibitor of cell proliferation, and a suppressor of DNA replication, through preventing cells from entering S phase [47]. Interestingly, palbociclib can also induce a senescence response that is dependent on inhibition of the transcription factor FOX-M1 [48] indicating that the therapeutic outcome of CDK4 inhibition can be more than simple G1 arrest that may offer more effective therapeutic outcomes *in vivo*. Palbociclib has emerged to be more tolerable than previous first-generation CDKI and at therapeutic doses resulted in marked reduction of phosphorylated RB and the proliferative marker ki-67 in tumor tissues by instigating G1 arrest. Two Phase I clinical trials have established tolerated dosing levels of 200 mg daily, when administered for 2 weeks followed by a 1 week break or an alternative daily dosing of 125 mg for 3 weeks with a 1 week break [47,49]. The most common toxicity encountered in both studies was neutropenia, which was manageable with treatment breaks. Serious sequelae of infection were rare that clearly distinguishes neutropenia induced by inhibition of CDK from that induced by cytotoxic chemotherapy. When combined with letrozole versus letrozole alone in a Phase II study involving ERBB2-negative/ER-positive postmenopausal metastatic breast cancer patients, the combination achieved a doubling of the progression free survival to 20.2 months in the combination arm versus 10.2 months in the letrozole monotherapy arm [47,49–51]. In April 2013, palbociclib received Breakthrough Therapy designation by the FDA for the potential treatment of resistance in breast cancer. Then in February 2015, the FDA-approved palbociclib as the first-ever CDKI therapy for the treatment of ER-positive metastatic breast cancer.

In the melanoma setting, palbociclib is being evaluated as part of a novel combination therapy with GSK's trametinib in patients with advanced/metastatic melanoma (NCT02065063) [52]. This study will also evaluate the effect of combination therapy on tumor biomarkers, safety and antitumor activity in patients with BRAFV600 wild-type melanoma including those with NRAS mutations.

Another second-generation specific CDKI in advanced stages of clinical trial development is LEE011 (Novartis/Astex). This is the most selective CDK4/6 inhibitor to date. It

exhibits inhibitory activity against CDK4/cyclin D1 and CDK6 complexes at submicromolar concentrations [53,54]. LEE011 is currently being investigated as a single agent in three studies (NCT01237236 [55], NCT01898845 [56], NCT02187783 [57], and in four combination studies (NCT01777776 [58], NCT01820364 [59], NCT02159066 [60], NCT01781572) [61]. LEE011 inhibits growth of tumor cells at G1. As of July 2013, 78 patients on a Phase I study (NCT01777776) [58] had been treated with increasing doses of once daily oral LEE011. This was administered daily for 21 days followed by a 1 week rest (28 day cycle). Doses ranging from 50 to 1200 mg were assessed on this scheduled. Continuous dosing of 600 mg once daily for 28 days of a 28 day cycle was also assessed. Preliminary data from Phase I trials have shown a tolerable toxicity profile when administered at 600 mg/day. The most common side effect was grade 1 or 2 neutropenia occurring in 23% at doses of 280 mg or higher. Grade 3 or 4 neutropenia was seen in 21% at doses of 400 mg or higher. There were a total of 10 dose-limiting treatment events including grade 3 mucositis (n = 1) at 50 mg, grade 3 pulmonary embolus (n = 1) at 280 mg, grade 3 hyponatremia (n = 1) and prolonged grade 3/4 neutropenia (n = 1) at 400 mg, prolonged grade 2 elevated creatinine (n = 1) at 600 mg, grade 4 thrombocytopenia (n = 1) at 750 mg, grade 3 asymptomatic QTcF prolongation with grade 3 neutropenia (n = 1) at 900 mg and grade 4 febrile neutropenia (n = 1) at 600 mg on the continuous dosing schedule. Other common toxicities include nausea (42%), anemia (41%), leukopenia (37%), fatigue (35%), diarrhea (31%), thrombocytopenia (28%) and hyperglycemia (17%) [53,62–63]. When paired with other targeted therapies in preclinical studies, LEE011 has been shown to reduce resistance to the other partnered compound such as the BRAF inhibitor encorafenib and the PI3K inhibitor BYL719 [64].

LY2835219 (Eli Lilly) is another highly selective oral CDK4 and CDK6 inhibitor. LY2835219 is reported to have antitumor activity and permeability across the blood–brain barrier [65]. The maximum tolerated dose established in the Phase I study was a continuous dosing of 200 mg twice daily. The main toxicities encountered were mild-to-moderate diarrhea, fatigue and neutropenia [66]. There are currently three ongoing Phase I clinical trials investigating LY2835219 in advanced cancers including melanoma (NCT02117648 [67], NCT01394016 [68]

and NCT02014129) [69]. Further development of LY2835219 in Phase II and III studies is currently focused on the management of breast and lung tumors (Table 2).

Patient selection in CDK4/6 inhibition

Observations from preclinical studies have noted that CDK4/6 inhibitors are generally ineffective in cells lacking RB [7,20,46]. Sensitivity to CDK4/6 inhibition correlates with high expression of RB and low expression of INK4A in both ovarian and glioblastomas [81]. If this is the case, then more careful patient selection and a shift toward molecular profiling patients prior to treatment may become an increasing practical reality. Preclinical data suggest that melanomas with p16 loss may be more sensitive to CDK4 inhibition [7,20], while data from Phase II and III clinical trials with selective CDK4/6 inhibitors in melanoma have confirmed that loss of RB is linked to drug resistance [64,66].

Cross talk & combination therapies

Based on the prevalence of the dysregulation of the RB pathway, and its relationship with other dysregulated pathways in metastatic melanoma, CDK inhibition has emerged as an attractive therapeutic target not only in its own right but also as a combination strategy for treating melanoma.

The RB pathway potently interacts with mutant BRAF or NRAS to promote malignant transformation of melanocytes [82]. Preclinical studies demonstrated decreased median tumor latency from 12 to 7 months and increased penetrance from 54 to 80% at 1 year when the loss of function *CDKN2A* mutation was added to murine melanocytes possessing activating mutations in BRAF [82]. The cross talk between the RAS/RAF/MEK/ERK pathway and the RB pathway is also evident in the increased *CCND1* expression following dysregulation of the RAS/RAF/MEK/ERK pathway in 65–90% of metastatic melanoma. Shortened duration of progression-free survival with dabrafenib therapy correlates with elevated *CCND1* and reduced *CDKN2A* copy number [83]. Thus demonstrating that increased *CCND1* expression mediates one mechanism of reduced sensitivity of a BRAF inhibitor in BRAFV600-mutated melanomas. Furthermore, *in vivo* studies have reported a statistically significant augmentation of response in BRAFV600E-mutated melanomas when MEK inhibition is combined with

Table 2. Cyclin-dependent kinase inhibitors in clinical trials in melanoma.

Compound, company	Target	Formulation	Phase and status	Clinical trial details	Clinical trial identifier
Broad range CDK inhibitors					
Flavopiridol: Alvocidib L-868275, HMR-1275 NSC649890, Sanofi-Aventis	CDK1, CDK2, CDK4, CDK7, CDK9	Intravenous	Phase II, completed Phase I, completed	Advanced melanoma Alvocidib plus cisplatin/carboplatin, solid tumors including melanoma	NCT00005971 [31] NCT00003690 [32]
Roscovitine: Seliciclib CYC202, Cyclacel	CDK1, CDK7	Oral	Phase II, unknown	Solid tumors including melanoma	NCT00999401 [70]
AT7519: Astex, Novartis	CDK1, CDK2, CDK4, CDK5, CDK9	Intravenous	Phase I/II, completed	Solid tumors including melanoma. Focusing on hematological cancer	NCT00390117 [43]
SNS032: BMS-387032, Sunesis	CDK2, CDK9	Intravenous	Phase I, completed	Solid tumors including melanoma	NCT00292864 [71]
P1446A: Piramal Enterprises	CDK1, CDK4, CDK9	Oral	Phase I/II, suspended Phase I, completed	Advanced melanoma with BRAF mutation, P1446A + vemurafenib Solid tumors including melanoma	NCT01841463 [72] NCT00840190 [73]
Dinaciclib: SCH727965, Merck	CDK1, CDK2, CDK5, CDK9	Intravenous	Phase I/II, active, not recruiting Phase II, active, not recruiting	Advanced stage 3–4 melanoma Advanced stage 4 melanoma	NCT01026324 [74] NCT00937937 [75]
Specific CDK inhibitors					
Rivaciclib: P276-00, Piramal Enterprises	CDK1, CDK4, CDK9	Intravenous	Phase II, completed	Malignant melanoma positive for cyclin D1 expression (ENVER)	NCT00835419 [76]
EM-1421: Erimos	CDK1	Intravenous	Phase I/II, completed	Solid tumors including melanoma	NCT00259818 [77]
Roniciclib: BAY-1000394, Bayer	CDK1, CDK2, CDK3, CDK4, CDK7, CDK9	Oral	Phase I, recruiting	Solid tumors including melanoma	NCT02047890 [40]
Palbociclib: PD0332991, Pfizer	CDK4, CDK6	Oral	Phase II, recruiting Phase I, active, not recruiting Phase I and II Recruiting	Solid tumors including melanoma Advanced solid tumors, evaluation of two schedules Solid tumors including melanoma, trametinib (MEK inhibitor) + palbociclib	NCT01037790 [78] NCT00141297 [79] NCT02065063 [80]
LEE011: Novartis, Astex	CDK4, CDK6	Oral	Phase I and II, active, not recruiting Phase II, recruiting Phase I, active, not recruiting Phase II, recruiting Phase I and II, recruiting	Locally advanced or metastatic BRAF- mutant melanoma Advanced melanoma (LOGIC trial) LGX818 (BRAF inhibitor) + LEE011 Solid tumors including melanoma Solid tumors including melanoma in Asians Locally advanced or metastatic NRAS- mutant melanoma, LEE011 + MEK162 (MEK inhibitor)	NCT01777776 [58] NCT01820364 [59] NCT01237236 [56] NCT01898845 [55] NCT01781572 [61]
Abernaciclib: LY2835219, Eli Lilly	CDK4, CDK6	Oral	Phase I, active, not recruiting Phase I, active, not recruiting Phase I, active, not recruiting	Solid tumors including melanoma Solid tumors including melanoma Solid tumors including melanoma in Japanese patients	NCT02117648 [67] NCT01394016 [68] NCT02014129 [69]

CDK4 inhibition against different melanoma cell lines [84].

Enhanced activity of CDK4/6 has been seen in melanomas with other oncogenic lesions in genes encoding mutations in EGFR amplification, PIK3CA mutations, KRAS mutations and PTEN inactivation [85,86]. CDKI may have therapeutic utility in melanomas harboring other oncogenic drivers including those with acquired resistance to other targeted therapies.

CDK4/6 inhibition with BRAF inhibitor

Co-inhibition of CDK4/6 and BRAF kinases has been shown to have greater efficacy in pre-clinical studies on BRAF V600-mutated melanoma models than with the single agents alone. Furthermore, combination therapy delayed the development of treatment resistance compared with monotherapy with vemurafenib or encorafenib alone [87]. LEE011 is being evaluated in combination with the novel BRAF inhibitor, encorafenib (LGX818) in three Phase II studies (NCT01777776 [58], NCT018020364 [59] and NCT02159066) [60].

CDK4/6 inhibition & MEK inhibition

Preclinical network modeling by Kwong *et al.* [88] suggests that disruptions in NRAS and MEK lead to differential activation of the RB cell cycle checkpoint. CDK4 is believed to be the most differentiating target in this pathway able to achieve extinction of NRAS signaling when combined with MEK inhibition. The combination of MEK inhibition with CDK inhibition appears to be synergistic, with inhibition of the MAPK and CDK4 pathways complementing each other by affecting different oncogenic circuits and approximating NRAS inhibition.

Phase Ib/II (NCT01781572) [61] clinical trial in advanced NRAS-mutant melanoma is currently underway investigating CDKI, LEE011 in combination with binimetinib (MEK162). Preliminary data presented at the American Society of Clinical Oncology annual conference, 2014, showed promising antitumor activity in 18 out of 22 (86%) cases enrolled so far demonstrating some tumor regression. A maximum tolerated dose of 200 mg once daily of LEE011 for 3 weeks on and 1 week off with 45 mg twice daily of binimetinib administered continuously was established although other doses and schedules are also being explored [89]. Reported toxicities include fatigue, skin reactions, edema, elevated creatine phosphokinases and creatinine, hematological and gastrointestinal events.

As previously mentioned, a Phase I and II (NCT02065063) [80] clinical trial investigation palbociclib in combination with the trametinib is also underway.

CDK4/6 inhibition & other combinations

Preclinical data demonstrate that LEE011 when combined with RAD001 (mTORC1 inhibitor, Afinitor) induces synergistic growth inhibition in multiple tumor models. There are presently no clinical trials combining a CDKI with an mTOR inhibitor in the melanoma setting.

Several preclinical studies have shown synergistic benefit when pan-CDK inhibitors are administered in conjunction with cytotoxic therapy (cisplatin, 5-fluorouracil, doxorubicin and paclitaxel), indicating that the CDKI may be more effective when cells are arrested in specific cell phases. However, the incidence and grade of toxicities also increased with combination of cytotoxic and CDKI therapy as inhibition of CDK2 inhibits DNA repair [90]. CDK4/6 inhibitors may also be more efficacious when combined with other therapies.

Conclusion

The dysregulation of the RB pathway is central to the development of many human cancers, and melanoma may have innate dependency or 'addiction' to the RB pathway that is being therapeutically exploited with various CDK4/6 inhibitors currently in clinical trials. The ability to genomically profile a patient's cancer may lead to a personalized approach to the use of CDK4/6 inhibitors in melanoma. Whether these therapies will fair better alone or in combination remains to be seen, but it is becoming increasingly apparent that combination of CDKI with other pathways or compounds is most likely to yield the greatest clinical antitumor efficacy. However, issues with toxicities when combining these treatments may factor into management decisions.

Future perspective

The recent developments in immunotherapy and targeting of the MAPK pathway with BRAF and MEK inhibitors have significantly altered treatment outcomes and expectations in advanced melanoma. However, despite these advances, additional targets are needed for patient subpopulations whose melanomas are not addicted to MAPK signaling or have developed resistance to the current therapies. The use of CDK4/6

inhibitors in combination with other small molecule targeted agents is a potential treatment strategy under investigation for overcoming acquired therapeutic resistance. Furthermore, a dual approach with combined CDK4/6 inhibition and MEK inhibition maybe an effective managing strategy for *NRAS*-mutant melanomas. The results from these studies are awaited.

Future research needs to focus on identifying mechanisms of resistance to current treatments and predictive biomarkers for treatment response. With ever increasing novel targeted therapies, immunotherapies and advancing genomic technology, a combination of therapies is likely to be used to devise treatment

strategies tailored to patients' genomic profiles. It is hoped that better long-term control or even cure may one day become a possibility in a significant proportion of patients with advanced melanoma.

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