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Senescence as a therapeutically relevant response to CDK4/6 inhibitors

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Abstract

Cyclin-dependent kinases 4 and 6 (CDK4/6) phosphorylate and inhibit retinoblastoma (RB) family proteins. Hyperphosphorylated RB releases E2F transcription factors, activating a transcriptional program that initiates S phase. Due to the critical role that this pathway has in regulating cell cycle progression, inhibiting CDK4/6 is an attractive therapeutic strategy. Indeed, CDK4/6 inhibitors in combination with anti-estrogens produce a significant benefit in patients with $ER^+/HER2^-$ breast cancer. Clinical trials are currently investigating if the use of CDK4/6 inhibitors alone or in combination can be extended to other cancer types. Inhibition of CDK4/6 can result in different cell fates such as quiescence, senescence or apoptosis. Senescence is a stress response that can be induced by stimuli that include oncogenic activation, chemotherapy, irradiation and targeted therapies such as CDK4/6 inhibitors. Senescent cells undergo a stable cell cycle arrest and produce a bioactive secretome that remodels their microenvironment and engages the immune system. In this review, we analyze the therapeutic relevance of senescence induction by CDK4/6 inhibitors. We also discuss how different therapies, including checkpoint inhibitors and drugs targeting MEK or PI3K, can be used in combination with CDK4/6 inhibitors to reinforce or exploit senescence. Recently, a lot of effort has been put into identifying compounds that selectively kill senescent cells (termed senolytics). Thus, sequential treatment with senolytics might be an additional strategy to potentiate the antitumor effects of CDK4/6 inhibitors.

Keywords

Senescence; CDK4; CDK6; CDK4/6 inhibitors; Palbociclib; Abemaciclib; Ribociclib

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Competing Interests

J. Gil has acted as a consultant for Unity Biotechnology and Geras Bio and Merck KGaA; owns equity in Unity Biotechnology and Geras Bio and is a named inventor in an MRC patent related to senolytic therapies.

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CDK4/6 inhibitors: overview

Cyclin-dependent kinases 4 and 6 (CDK4/6) play a crucial role in mammalian cell proliferation. When enzymatically activated by D-type cyclins (D1, D2 and D3), CDK4/6 phosphorylate members of the retinoblastoma (RB) family proteins (Figure 1). These include the p110 retinoblastoma protein (encoded by *RB1*) and the related pocket proteins p107 (encoded by *RBL1*) and p130 (encoded by *RB2*). Sequential phosphorylation of RB1 by CDK4/6 and cyclin E-dependent CDK complexes leads to release of E2F transcription factors, initiation of S phase and commitment to the cell cycle. CDK4/6 can be inhibited by proteins belonging to the CIP/KIP(such as $p21^{CIP}$, encoded by *CDKN1A*) and INK4 (such as $p16^{INK4a}$, encoded by *CDKN2A*) families of cyclin dependent kinase inhibitors. Both,RB1 and $p16^{INK4a}$, are tumor suppressors and their loss of function is frequent in cancer. Conversely, Cyclin D1acts as an oncogene and it is frequently amplified in tumors [1–3]. Overall, alterations of the “RB pathway” are a hallmark of cancer, whichhighlights the rationale for therapeutic intervention.

First attempts to develop a CDK4 inhibitor started even before the RB pathway was completely understood in the early 1990s [4]. As there are 20 different human CDKs, identification of a specific CDK4/6 inhibitor turned out to be a critical challenge, and initial trials with pan-CDK inhibitors were problematic due to serious side effects [5, 6]. It took more than a decade to overcome these issues, but eventually a specific CDK4/6 inhibitor, Palbociclib (PD0332991), was shown to induce G1 cell cycle arrest in cell lines and mouse models with functional RB [7, 8]. In the years since, other oral CDK4/6 inhibitors such as Abemaciclib (LY2835219) and Ribociclib (LEE011) have been described [9, 10]. In 2015, the phase II PALOMA-1 trial led to provisional FDA-approval of Palbociclib and letrozole for patients with estrogen receptor (ER)-positive, human epidermal growth factor receptor 2 (HER2)-negativemetastatic breast cancer [11]. As of today, Palbociclib, Abemaciclib and Ribociclibhave all been granted FDA-approval and other CDK4/6 inhibitors are being testedfor the treatment of ER+/HER2- breast cancer [5]. In addition, there are currently numerous clinical trials investigating the efficacy of CDK4/6 inhibitors alone or in combination to treat other cancers types.

Palbociclib, Abemaciclib and Ribociclib are all orally bioavailable and show great specificity for CDK4 and CDK6. Ribociclib is structurally very similar to Palbociclib, whereas the structure of Abemaciclib is different from the two other compounds [12]. Abemaciclib might also differ functionally, as multiomics profiling revealed a wider spectrum of secondary targets and a pan-CDK transcriptional signature [13]. Apart from that, there are differences in pharmacokinetics, dosing schedules and toxicity profiles between the three drugs [12]. Current preclinical and clinical research on CDK4/6 inhibitors focusses on a better understanding of treatment responses, identification of biomarkers to predict sensitivity or resistance, and designing combinations that could enhance their antiproliferative effects or engage the immune system for tumor clearance. The ongoing research might help to enhance patient stratification, allow for a wider but more targeted use of CDK4/6 inhibitors, and significantly improve patient outcome on the long term.

Senescence, quiescence, or resistance: biomarkers and cell fates

In cells with functional RB, CDK4/6 inhibitors arrest cells in the G1 phase of the cell cycle. Depending ondifferent factors, this arrest can lead to quiescence or senescence. Whereas quiescent cells retain the ability to exit the arrest in response to mitogenic signaling, senescent cells are characterized by a highly stable growth arrest accompanied by specific phenotypic alterations (e.g. a senescence-associated secretory phenotype/SASP andmetabolic, epigenetic and morphologic changes) [14] (Figure 2). In fact, CDK4/6 inhibitors have proven to induce quiescence or senescence in a variety of different cell types *in vitro* and *in vivo*: breast cancer [15–17], melanoma [18, 19], hepatocellular carcinoma [20], gastric and oesophageal cancer [21, 22], liposarcoma [23, 24], leukaemia [25] and neuroblastoma [26]. The extent to which senescent cancer cells can be found in patients after treatment with CDK4/6 inhibitors remains unknown, since broadly applicable diagnostic tests to detect senescent cells are still lacking.

Alongside quiescence and senescence, CDK4/6 inhibitors can also induce apoptosisin certain settings. In T-cell acute lymphoblastic leukemia (T-ALL), Palbociclib inhibits CDK6-mediated phosphorylation of glycolytic enzymes, ultimately leading to depletion of antioxidants, increased levels of reactive oxygen species (ROS), and apoptosis [27–29]. In tumors like T-ALL, where CDK6 is a major oncogenic driver, unique functions of CDK6 can influence treatment outcome of CDK4/6 inhibitors independent of RB. Apart from its enzymatic activity, CDK6 is also a transcriptional regulator and as such can promote expression of VEGF or p53 antagonists [30, 31]. It has been suggested that CDK4/6 inhibitor therapy might therefore favor the outgrowth of p53 mutant clones [30].

Whereas quiescence, senescence and apoptosis are favorable outcomes in cancer treatment, some cells/tumors are less sensitive or even resistant to CDK4/6 inhibitors. RB is the main biomarker to predict benefit of CDK4/6 inhibitor treatment and loss of RB function is a cause of primary and secondary resistance [7, 32, 33]. Other reported resistance mechanisms are activating alterations in AKT1, RAS, AURKA, CCNE2, ERBB2, and FGFR2, as well as high Cyclin E1 expression and CDK2 activity [15, 33–36]. Loss of p53 function might also be associated with reduced sensitivity to CDK4/6 inhibitors [34, 37]. Cells with specific genomic alterations, on the other hand, are particularly sensitive (CCND1 translocation, CCND1-3 3′UTR loss, and CCND2 or CCND3 amplification) [34]. Notably, high expression of Cyclin D1 alone, CCND1 amplification or loss of $p16^{INK4a}$ do not predict increased sensitivity [34]. A comprehensive review article about the mechanisms that underlie the sensitivity or resistance to treatment with CDK4/6 inhibitors has been published recently [5].

Senescence induced by CDK4/6 inhibitors

Cellular senescence was first described as a response limiting the proliferative capacityof human fibroblasts in culture [38]. Senescence is now considered as a stress response triggered by stimuli like telomere shortening (replicative senescence), oncogene activation, oxidative stress, irradiation or chemotherapy (therapy-induced senescence) [39]. Overall,

senescence is a powerful mechanism of tumor suppression preventing the uncontrolled proliferation of preneoplasticcells [40].

In addition, induction of senescence in tumor cells is relevantduring cancer therapy. Irradiation, chemotherapeutic agents and targeted anti-cancer therapies, including CDK4/6 inhibitorscan induce senescence in different cancer types *in vitro* and *in vivo* [41, 42]. In a lymphoma mouse model, senescence was associated with a better prognosis after chemotherapy [43]. Senescent breast and lung cancer cells have also been found in biopsies of patients who had received neoadjuvant chemotherapy [44, 45]. Importantly, clinical studies have confirmed that senescence is a predictor of treatment outcome [46] and a low SASP correlates with resistance to cancer therapies [47].

Thesenescence-associated growth arrest is orchestratedby activation of the p53 and p16/RBtumor suppressor pathways[48]. Two CDK inhibitors, $p21^{CIP1}$ and $p16^{INK4a}$, are induced during senescence. $p21^{CIP1}$ is upregulated by p53 as a result of the DNA damage associated with senescence induction [49]. The CDK4/6 inhibitors $p16^{INK4a}$ and $p15^{INK4b}$are amongst the best characterized markers of senescence [50, 51]. $p16^{INK4a}$and $p15^{INK4b}$ are expressed from the *INK4/ARF* locus together with ARF, which inhibits MDM2 and thereby activates p53. The *INK4/ARF* locus, epigenetically repressed by Polycomb proteins in normal cells, is activated during senescence [52].

Since the p16/RB pathway is a major component of the senescence machinery, it is not surprising that CDK4/6 inhibitionor overexpression of $p16^{INK4a}$result in senescence [18, 53–55]. Accordingly, CDK4 activity islinked to suppression of senescence and is necessary for Ras- or HER2-driven tumorigenesis [56, 57]. Senescence induction by CDK4/6 inhibition seems to depend on a functional RB. The transcription factor Forkhead Box M1 (FOXM1) is anothertarget of CDK4/6 [18]. Destabilization of FOXM1 upon CDK4/6 inhibition leads to elevated levels of ROS, priming cells for senescence induction.

Nevertheless, treatment with Palbociclib, Abemaciclib or Ribociclib does not uniformly lead to senescence in cancer cells. Complex genomic alterations and phenotypes in different tumorscould be responsible forthe particular treatment responses. CDK4/6 inhibitorsmight create a senescence permissive status, with additional intrinsic or external factors influencing the cell fate. The RAS and PI3K/AKT signaling pathways, for example, regulate the transcription of D cyclins, their assembly with CDK4/6 and their intracellular location [4]. Downregulation of MDM2 and repression of HRAS have been shown to be necessary for senescence induction in liposarcoma cells treated with CDK4/6 inhibitors [24]. In melanoma cells, mTOR inhibition has been demonstrated to be crucial for the implementation of senescence, and combining mTOR inhibition with Palbociclib intensified the cell cycle arrest [19, 58]. In another study, Palbociclib-induced senescence was associated with increased proteasome activity [59], further highlighting that multiple factors can influence cell fate. Uncovering the mechanisms that guide cells toundergo quiescence or senescence in response to CDK4/6 inhibition will help to further improve therapy planning and outcome.

Engaging the immune system

The stable growth arrest associated with senescence induction is appealing for cancer therapy. Although senescent cells have exited the cell cycle, they are highly bioactive cells. One of the most prominent characteristics of senescent cells, apart from the growth arrest, is the so-called senescence-associated secretory phenotype (SASP). The extent and composition of the cytokines and factors secreted by senescent cells can vary and is celltype- and stimulus-dependent [60]. The SASP of senescent stroma cells is associated with detrimental phenotypes such as the promotion of tumor growth, angiogenesis, metastasis and immunosuppression [61–63]. On the other hand, the SASP can induce senescence in autocrine and paracrine manners (thereby inhibiting proliferation) [64, 65] and stimulate the immune clearance of senescent cells [66]. Both the adaptive and the innate immune system are involved in the elimination of senescent (pre)malignant cells [66–69].

The positive outcomes achieved by checkpoint inhibitor treatment in clinical studieshave evoked great interest in therapies promoting immune clearance of cancer cells. So far, 7 antibodies have been FDA-approved (e.g. Ipilimumab, Nivolumab, Pembrolizumab) for the treatment of several different cancers. As for CDK4/6 inhibitors, a few studies have shown activation of the immune system and/or beneficial effects in combination with checkpoint inhibitors. Interestingly, these effects are at least partially related to their ability to induce senescence.

In a study investigating CDK4/6 inhibitor treatment in breast cancermodels, Abemaciclib and Palbociclib induced growth arrest and tumor regression *in vivo*, which was not attributable to apoptosis. Instead, the authors found that cytotoxic T cells were recruited into the tumors, which could be explained by increased antigen presentation on the tumor cells themselves and suppression of regulatory T cells (Figure 2). While the authors describe an increase in senescence-associated β-Galactosidase positive cells, expression of inflammatory SASP components like IL6, IL1A and IL1B was not detected [17]. Enhanced T-cell infiltration was also observed in other studies. In one of them, CDK4/6 inhibitors were identified as activators of PD-1 suppressed T cells in a small molecule screen [70]. Another study found synergistic and immunogenic effects when combining Ribociclib with the PI3Kα inhibitor Alpelisib [71]. Of note, CDK4/6 inhibition has been described to promote expression of PD-L1on cancer cells by inhibiting its proteasomal degradation [72]. Nonetheless, the combination of CDK4/6 inhibitors with checkpoint inhibitorsresulted in enhanced anti-tumor effects in all these studies. In addition, CDK4/6 inhibitors have been shown to repress an intracellular program associated with resistance to anti-PD-1 therapy [47]. Clinical trials are still ongoing (Table 1), but preclinical data give reason to hope that this treatment regimen could be transferred to the clinic.

While the combination with checkpoint inhibitors aims to boost the adaptive immune response, the innate immune system has been described to play a major role in lung cancer mouse models treated with Palbociclib and the MEK inhibitor Trametinib. Regardless of the p53 status of the cells, this combination led to tumor cell senescence and induction of a robust SASP [73]. The authors showed that SASP induction served to recruit and activate NK cells, enabling NK cell-mediated cytotoxicity and tumor regression (Figure 2). Another

study of the same group showed different effects of the SASP on the tumor microenvironment in pancreatic cancer [74]. Like previously seen in lung cancer cells, treatment with Palbociclib and Trametinib induced senescence and a robust SASP *in vitro* and *in vivo*. However, this did not result in recruitment of NK cells, but led to increased vascularization, which improved delivery of conventional chemotherapeutics (gemcitabine) (Figure 2). In addition, the recruitment of cytotoxic T cells was enhanced, and combination with checkpoint inhibitors led to tumor regression and prolonged survival.

Taken together, there is clear evidence that CDK4/6 inhibitors alone or in combination with other drugs have immunogenic effects. Whereas induction of senescence might not be a general requirement therefore, it seems that senescent tumor cells can mediate profound changes in the tumor microenvironment through their SASP. This underlines that induction of senescence can yield expanded anti-tumor effects beyond cell cycle arrest, making it a desirable treatment outcome.

Other combination therapies

Despite the clear advantages of CDK4/6 inhibitors (interference with key regulators of proliferation, high specificity and bioavailability, manageable side effects), there is increasing understanding that combination therapies might be necessary to achieve significant anti-tumor effects. Potential benefits of combination therapieslie in improved inhibition of tumor growth, shiftingfrom quiescence toapoptosis or senescence, overcoming resistance, engaging the immune system or reducing side effects by lowering the single agent doses.

As the RAS/MEK/ERK and the PI3K/AKT signaling pathwaysare involved in Cyclin D regulation and CDK4/6 inhibitor resistance (see above), several studies have been focusing on combining CDK4/6 inhibitors with inhibition of these pathways. Activating mutations in RAS or RAF are frequent in cancer, especially in pancreatic cancer (KRAS, 91%), colorectal cancer (KRAS, 42%), NSCLC (KRAS, 33%) and malignant melanoma (BRAF, 66%; NRAS 27%) [75, 76]. The development of potent RAF inhibitors (e.g. Vemurafenib, Dabrafenib) and MEK inhibitors (e.g. Trametinib, Binimetinib) has opened new opportunities. Despite their beneficial effects in BRAF V600E mutant melanoma, resistance mediated by feedback loops is a major problem, urging the need to find novel combination therapies. In an NRAS-driven melanoma mouse model, genetic ablation of NRAS, but not treatment with a MEK inhibitor, led to tumor regression. Further analysis revealed that CDK4 was the main driver of continued cell proliferation after MEK inhibition. Combining Palbociclib and a MEK inhibitor was synergistic, resulting in apoptosis, cell cycle arrest and tumor regression [77]. Other studies also found that this combination caused apoptosis in melanoma but induced senescence in pancreatic and lung cancer models (Table 2) [73, 74, 78, 79]. First results of a phase Ib/II trial combining Ribociclib and Binimetinib in patients with NRAS mutant melanoma were promising, as it resulted in significant tumor regression, however, serious side effects were also reported [80]. More clinical trials are ongoing (Table 3).

Like the RAS/MEK/ERK signaling pathway, the PI3K/AKT pathway is one of the signaling pathways most frequently activated in cancer [81]. 40% of patients with $ER^+/HER2^-$ breast cancer have tumors with *PI3KCA* mutations. In 2019, the PI3Kα inhibitor Alpelisib was FDA-approved for the treatment of these tumors [82]. Testing the combination of CDK4/6 inhibitors and PI3K inhibitors therefore seems natural. Whereas many clinical trials are still ongoing (Table 3), preclinical studies have shown that CDK4/6 inhibitors sensitize PI3KCA mutant breast cancer cells to PI3K inhibitors and that the combination of both drugs is synergistic *in vitro* and *in vivo* [83, 84]. Another groupdemonstratedthat a PI3K inhibitor could preventthe resistance to CDK4/6 inhibitors caused by increased CDK2 activity [85]. Interestingly, the combination of PI3K inhibitors and CDK4/6 inhibitors has been reported to lead to cell cycle arrest and apoptosis rather than senescence (Table 2) [79, 85].

Whether CDK4/6 inhibitors can be combined with conventional chemotherapy or irradiation remains controversial, as many cytotoxic therapies rely on cycling cells and are effective in S or M phase. In fact, Palbociclib has been shown to reduce the effect of carboplatin in RB-competent breast cancer mouse models, but at the same time mitigated chemotherapy-induced myelotoxicity [86, 87]. On the other hand, there are reports that CDK4/6 could be combined with gemcitabine, taxanesor platinum-based compounds in pancreatic or ovarian cancer, respectively [74, 88, 89]. A recent study investigatedthe use of CDK4/6 inhibitors after cytotoxic chemotherapy in pancreatic cancer models [90]. Chemotherapy followed by treatment with CDK4/6 inhibitors led to prolonged inhibition of proliferation and improved therapeutic effects, as CDK4/6 inhibitors repressed the DNA-repair machinery, preventing cells from recovering from DNA damaging agents.

Two-Hit strategy: Inducing apoptosis in senescent cells

Despite all the benefits of CDK4/6-induced senescence described above, the aberrant persistence of senescent cells has detrimentaleffects that might affect outcome on the long term. Senescent cells within thyroid tumors, for example, have been reported to lead invasion [91]. Besides, cancer recurrence is a major concern, as therapy-induced senescence has been shown to provoke stem cell-like features leading to more aggressive tumorbehavior [92]. In addition, the induction of senescence in stromal cells and normal tissues contributes to the side effects of chemotherapy andpromotes chronic inflammation [93, 94].

Elimination of senescent cells -through stimulation of immune cell-mediated clearance orthrough senolytic approaches- can alleviate these detrimental effects. Targeted induction of apoptosis in senescent cells is an appealing treatment strategy. In 2011, it was first shown that the selective elimination of senescent cells can increase lifespan in a progeroid mouse model [95]. Since then, senolytic approaches have been demonstrated to be beneficial in a widerange of diseases [48]. Therapy-induced senescence can also prepare the ground for subsequent elimination of cancer cells. This was first demonstrated in a preclinical study investigating the phenotype of lymphoma cells treated with chemotherapy [96]. Senescent lymphomas were more sensitive to inhibition of glucose utilization or autophagy, and addition of an autophagy inhibitor after chemotherapy improved treatment outcome in mice. This “double-hit” strategy (Figure 3) has also proven effective in a recently published study

in liver cancer [97]. Combination of a pro-senescence therapy with a senolytic drug resulted in marked reduction of tumor growth *in vivo*.

ABT-263 (also known as navitoclax), a specific inhibitor of the anti-apoptotic proteins BCL-2 and BCL-xL, was the first described prototypical senolytic drug [98]. As its clinical use is limited by side effects, especially thrombocytopenia, there is a need to identify novel senolytic compounds. Cardiac glycosides (such as digoxin or digitoxin)are widely used in clinic and have been recently described as broad-spectrum senolytics [99]. Interestingly, they also induced apoptosis in different senescent cancer cells treated with Palbociclib and could therefore be used to improve the outcome of CDK4/6 inhibitor therapies.

Conclusion

In 2015, the FDA approved Palbociclib in combination with letrozole for the treatment of$ER^{+}/HER2^{-}$metastatic breast cancer. Since then, the possibility to expand the use of CDK4/6 inhibitors to other tumor types has contributed to build excitement in this family of drugs. While the potential is huge, the challenge resides in understanding what patients will respond to CDK4/6 inhibitors and what drug combinations might be better suited to achieve maximum benefit.

A frequent (but not universal) outcome of treatment with CDK4/6 inhibitors is the induction of senescence. Senescence is a powerful tumor suppressor mechanism and influences the outcome of chemo- and radiotherapy. Cells undergoing senescence not only implement a stable cell cycle arrest, but produce a secretome (the SASP) that directs their immune-mediated clearance. The SASP is a key mediator of the effects exerted by senescent cells. The composition of the SASP differs depending on cell type and how senescence is induced [60]. Characterizing the secretome of cancer cells treated with CDK4/6 inhibitors is therefore important.

Senescence has recently been seen as a favorable outcome during cancer treatment. The ability to target senescent cells with senolytic drugs makes the scenario even more attractive, as the detrimental effects associated with the aberrant accumulation of senescent cells can be eliminated. To be able to fully exploit this strategy, we will need techniquesto detect senescent cells in patients. While markers and guidelines to identify senescent cells *in vitro* and *in vivo* are clear [14], diagnostic tests or imaging methods for patients are lacking. Hopefully, the identification of novel markers and the development of non-invasive diagnostics to monitor senescent cells [100] will help to facilitate the translation of this approach to the clinic. Given the myriad of active clinical trials with CDK4/6 inhibitors, the next years will better define the utility of these drugs and clarify the relevance of senescence induction for their efficacy.

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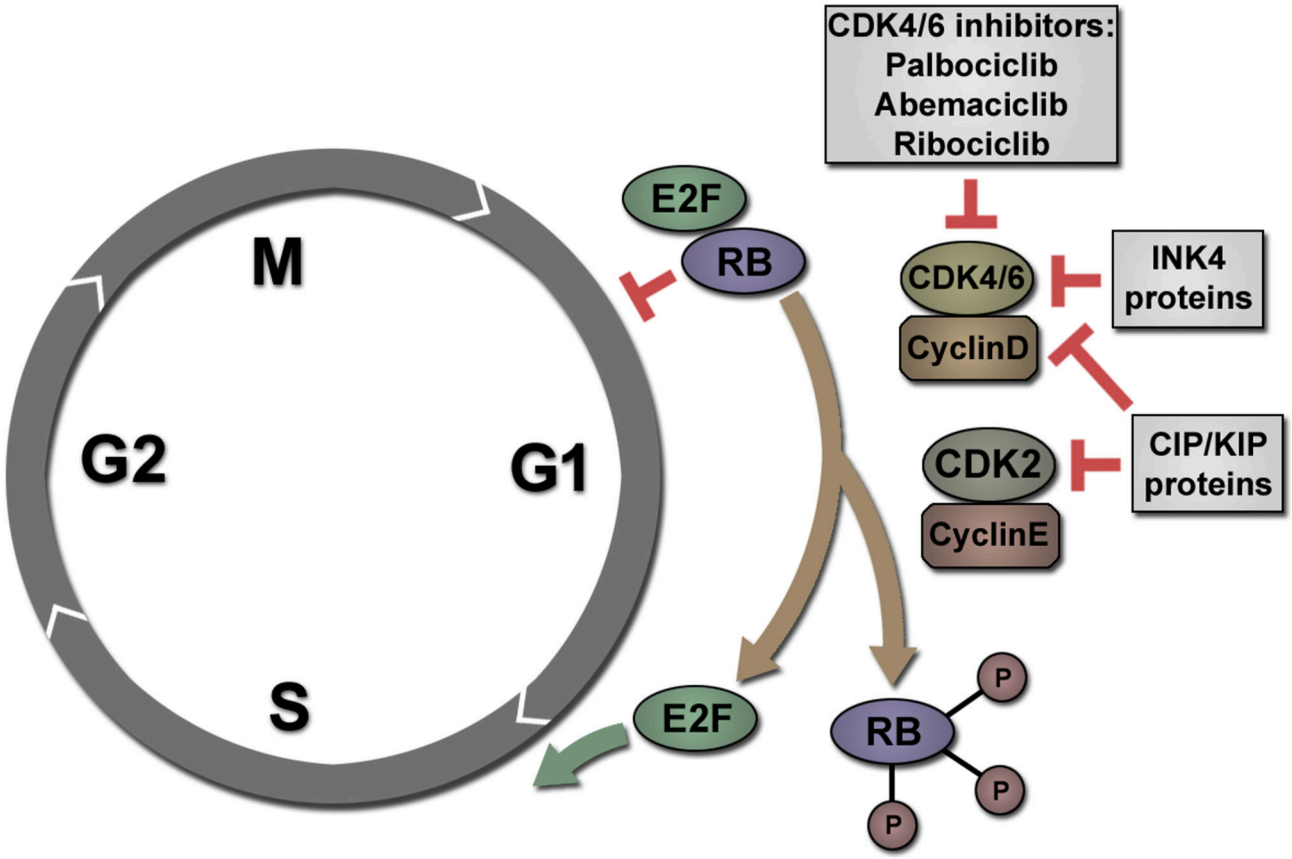

Figure 1. Inhibition of CDK4/6 induces cell cycle arrest in G1 phase.

After M phase, RB is hypophosphorylated and can bind and inhibit transcription factors of the E2F family. When activated by Cyclin D, CDK4/6 phosphorylate RB. RB can subsequently be phosphorylated by CDK2/Cyclin E. Hyperphosphorylated RB releases E2F transcription factors that activate a transcriptional programme promoting transition to S phase. Cyclin dependent kinase inhibitors of the CIP/KIP family (such as $p21^{CIP}$) can inhibit both CDK2 and CDK4/6 activity. INK4 proteins, like $p16^{INK4a}$, specifically inhibit CDK4/6 activity leading to cell cycle arrest in G1 phase. CDK4/6 inhibitors (such as Palbociclib, Abemaciclib or Ribociclib) exert similar effects, also inducing an arrest in G1.

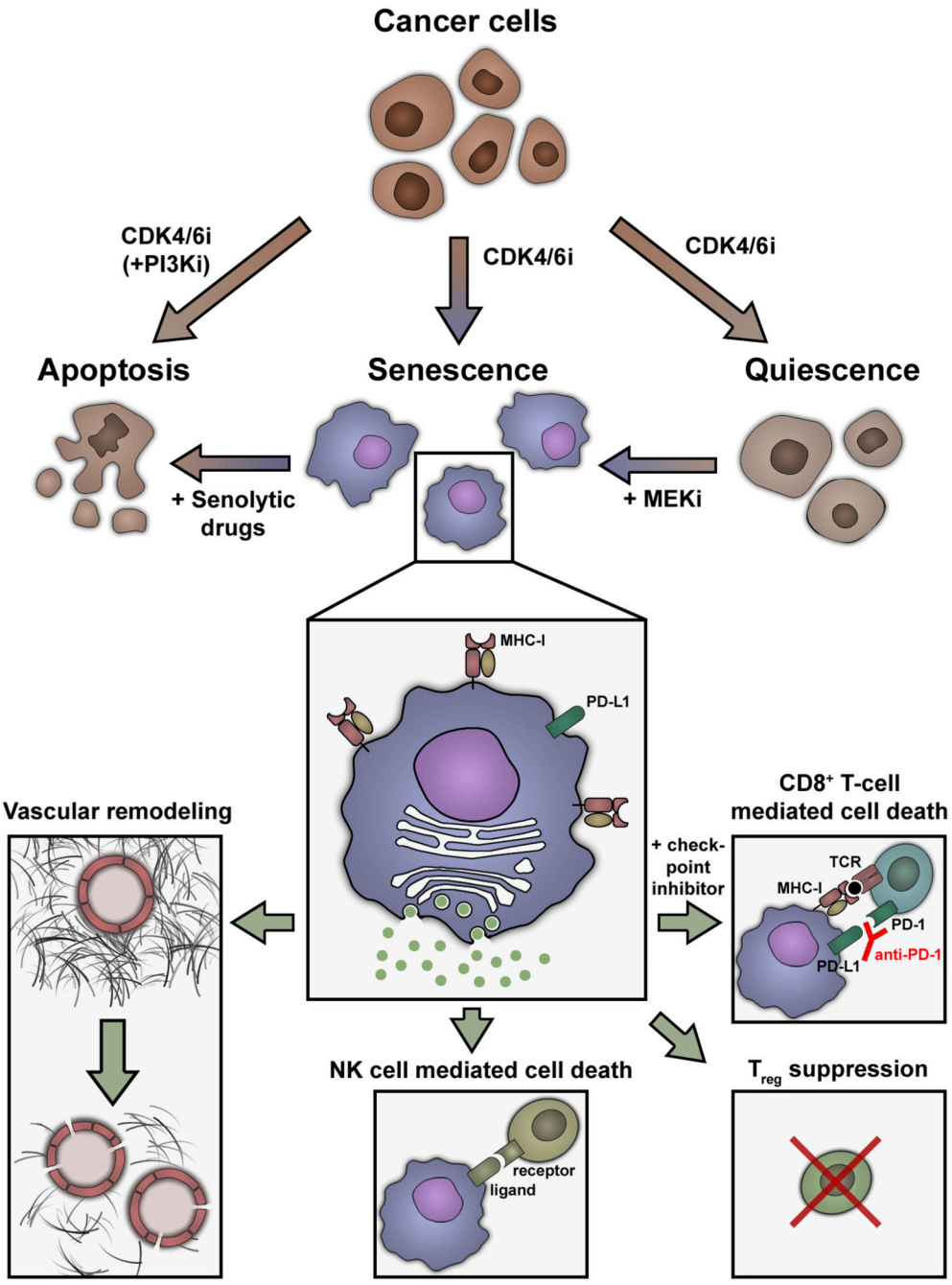

Figure 2. Different cell fates caused by CDK4/6 inhibitors influence treatment outcome.

In cancer cells with a functional RB pathway, treatment with CDK4/6 inhibitors usually induces quiescence or senescence. In some tumor types, senescence can be enforced by combined treatment with MEK inhibitors. The combination of a CDK4/6 inhibitor with a PI3K inhibitor can change the cell fate towards apoptosis, which is rarely seen in a monotherapy setting. Senescent cells have a characteristic phenotype that includes an enlarged and flattened morphology, increased senescence-associated β-Galactosidase activity (“blue cells”) and the senescence-associated secretory phenotype (SASP). The

SASP promotes changes in the tumor microenvironment, like vascular remodeling (allowing for increased uptake of chemotherapy in pancreatic cancer) or recruitment of cytotoxic T cells. CDK4/6 inhibitors also induce enhanced expression of MHC-I complexes on cancer cells, facilitating activation of cytotoxic T cells. Combination treatment with checkpoint inhibitors (e.g. an anti-PD-1 antibody) can further improve the immune-mediated clearance of senescent cancer cells. In some tumors, treatment with CDK4/6 inhibitors can also reduce levels of immunosuppressive regulatory T cells or attract NK cells and promote NK-cell mediated cancer cell death.

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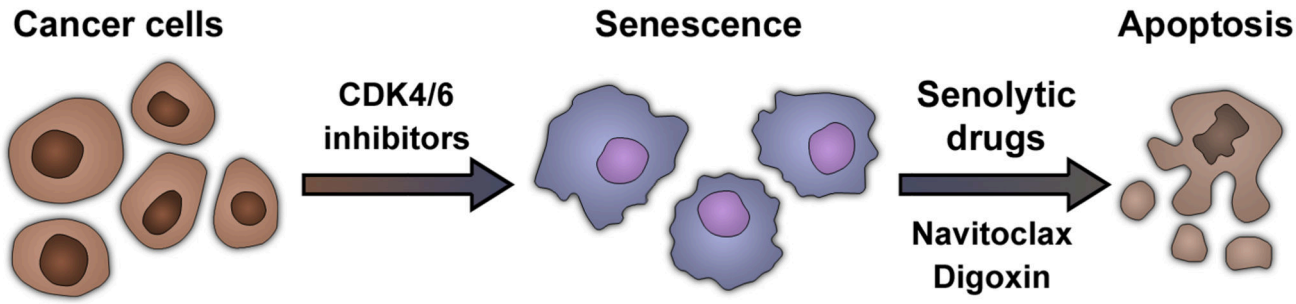

Figure 3. Sequential treatment with CDK4/6 inhibitors and senolytics.

Treatment with CDK4/6 inhibitors induces senescence in many cancer cells. Senescent cells share specific vulnerabilities that can be targeted by senolytic drugs. Examples of senolytic drugs include BCL2-family inhibitors such as navitoclax (ABT-263) or cardiac glycosides (e.g. digoxin). Senolytic drugs induce apoptosis in senescent cells. The stepwise combination of drugs able to induce senescence (pro-senescence therapy) such as CDK4/6 inhibitors and a senolytic drug constitutes an emerging therapeutic strategy.

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Table 1

Clinical trials combining CDK4/6 inhibitors and checkpoint inhibitors.

Drugs	Title	Condition(s)	Phase	NCT Number
Palbociclib Cetuximab (anti-EGFR) **Avelumab** (anti-PD-L1)	Avelumab, Cetuximab, and Palbociclib in Recurrent or Metastatic Head and Neck Squamous Cell Carcinoma	Head and Neck Squamous Cell Carcinoma	Phase 1	NCT03498378
Palbociclib Tamoxifen **Avelumab** (anti-PD-L1)	Neoadjuvant Tamoxifen, Palbociclib, Avelumab in Estrogen Receptor Positive Breast Cancer	Breast Cancer	Phase 2	NCT03573648
Palbociclib Letrozole **Pembrolizumab** (anti-PD-1)	Pembrolizumab, Letrozole, and Palbociclib in Treating Postmenopausal Patients with Newly Diagnosed Metastatic Stage IV Estrogen Receptor Positive Breast Cancer	Breast Cancer	Phase 2	NCT02778685
Palbociclib Anastrozole **Nivolumab** (anti-PD-1)	A Study of Neoadjuvant Nivolumab + Palbociclib + Anastrozole in Post-Menopausal Women and Men with Primary Breast Cancer	Breast Cancer	Phase 2	NCT04075604
Abemaciclib **Nivolumab** (anti-PD-1)	Modulation of the Tumor Microenvironment by Abemaciclib in Operable HPV-Negative Head and Neck Cancer (HNC)	Head and Neck Squamous Cell Carcinoma	Phase 2	NCT04169074
Abemaciclib **Nivolumab** (anti-PD-1)	Abemaciclib + Nivolumab in Patients with Recurrent/Metastatic Head and Neck Squamous Cell Carcinoma That Progressed or Recurred Within Six Months After Platinum-based Chemotherapy	Head and Neck Squamous Cell Carcinoma	Phase 1/2	NCT03655444
Abemaciclib **Atezolizumab** (anti-PD-L1)	Abemaciclib With or Without Atezolizumab in Metastatic Castration Resistant Prostate Cancer	Prostate Cancer	Phase 2	NCT04272645
Abemaciclib Nivolumab (anti-PD-1)	Abemaciclib and Nivolumab for Subjects with Hepatocellular Carcinoma	Hepatocellular Carcinoma	Phase 2	NCT03781960
Abemaciclib **Pembrolizumab** (anti-PD-1)	Abemaciclib + Pembrolizumab In Glioblastoma	Glioblastoma	Phase 2	NCT04118036
Abemaciclib **Pembrolizumab** (anti-PD-1)	Pilot Study of Pembrolizumab Combined with Pemetrexed or Abemaciclib for High Grade Glioma	High Grade Glioma	Early Phase 1	NCT04220892
Abemaciclib **Pembrolizumab** (anti-PD-1)	Abemaciclib and Pembrolizumab in Locally Advanced Unresectable or Metastatic Gastroesophageal Adenocarcinoma	Gastro-oesophageal Adenocarcinoma	Phase 2	NCT03997448
Abemaciclib **Durvalumab** (anti-PD-L1)	Neoadjuvant Study of Abemaciclib, Durvalumab, and an Aromatase Inhibitor Early Stage Breast Cancer	Breast Cancer	Early Phase 1	NCT04088032
Ribociclib **Spartalizumab** (anti-PD-1)	Ribociclib and Spartalizumab in R/M HNSCC	Head and Neck Squamous Cell Carcinoma	Phase 1	NCT04213404

Drugs	Title	Condition(s)	Phase	NCT Number
Ribociclib **Spartalizumab** (anti-PD-1)	Ribociclib + PDR001 (Spartalizumab) in Breast Cancer and Ovarian Cancer	Breast Cancer Ovarian Cancer	Phase 1	NCT03294694

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Table 2

Preclinical studies combining CDK4/6 inhibitors and MEK or PI3K inhibitors.

Cancer type	Drugs	Cell fate	Study design and overall outcome	Reference
Melanoma	**Palbociclib** **MEK inhibitor** (Selumetinib)	quiescence andapoptosis	NRAS driven melanoma mouse model; combination leads to tumor regression	[77]
Melanoma	**CDK4i** (219476) **MEK inhibitor** (PD98059)	apoptosis	combination leads to apoptosis in melanoma cell lines	[78]
Pancreatic cancer	**Palbociclib** **MEK inhibitor** (Trametinib)	senescence	pancreatic cancer mouse models; combination leads to senescence and remodeling of the tumor microenvironment(see also Fig. 2 and “Engaging the immune system”)	[74]
Pancreatic cancer	**Palbociclib** **MEK inhibitor** (Selumetinib)	senescence	combination leads to senescence in pancreatic cancer cell lines	[79]
Lung cancer	**Palbociclib** **MEK inhibitor** (Trametinib)	senescence	lung cancer mouse model; combination leads to senescence, NK cell recruitment and tumor regression	[73]
Pancreatic cancer	**Palbociclib** **PI3K/MTOR inhibitor** (BEZ235)	apoptosis	combination leads to apoptosis *in vitro* and suppression of tumor growth in patient-derived xenograft models of pancreatic cancer *in vivo*	[79]
Breast cancer	**Ribociclib** **PI3K(α) inhibitor** (Alpelisib and Pictilisib)	primarily quiescence	breast cancer cell lines and xenograft mouse models; combination is synergistic, tumor regression in *PI3KCA* mutant breast cancer	[83]
Breast cancer	**Ribociclib** **PI3Kα inhibitor** (Alpelisib)	quiescence and apoptosis	combination is synergistic *in vitro* and in patient-derived xenograft models *in vivo* and stimulates the immune system (see also paragraph “Engaging the immune system”)	[71]
Breast cancer	**Palbociclib** **PI3Kα inhibitor** (Taselisib)	apoptosis	breast cancer cell lines and xenograft mouse models; combination increases apoptosis in *PI3KCA* mutant cells *in vitro*, tumor regression *in vivo*	[84]
Breast Cancer (ER positive)	**Palbociclib** Fulvestrant **PI3K inhibitor** (Pictilisib)	apoptosis	cell lines and patient-derived xenograft models; triple combination induces apoptosis and leads to tumor regression *in vivo*	[85]

Table 3

Selection of ongoing clinical trials combining CDK4/6 inhibitors and MEK inhibitors or PI3K inhibitors.

Drugs	Title	Condition(s)	Phase	NCT Number
Palbociclib **Binimetinib** (MEK inhibitor)	Study of the CDK4/6 Inhibitor Palbociclib (PD-0332991) in Combination with the MEK Inhibitor Binimetinib (MEK162) for Patients with Advanced KRAS Mutant Non-Small Cell Lung Cancer	KRAS mutant NSCLC	Phase 1/2	NCT03170206
Palbociclib **Trametinib** (MEK inhibitor)	A Study to Investigate the Safety, Pharmacokinetics, Pharmacodynamics, and Anti-Cancer Activity of Trametinib in Combination with Palbociclib in Subjects with Solid Tumors	Solid Tumours	Phase 1	NCT02065063
Palbociclib **Binimetinib** (MEK inhibitor)	Binimetinib and Palbociclib or TAS-102 in Treating Patients with KRAS and NRAS Mutant Metastatic or Unresectable Colorectal Cancer	KRAS mutant or NRAS mutant Colorectal Cancer	Phase 2	NCT03981614
Ribociclib **Trametinib** (MEK inhibitor)	Study of Safety and Efficacy of Ribociclib and Trametinib in Patients With Metastatic or Advanced Solid Tumors	Solid Tumours	Phase 1	NCT02703571
Ribociclib **Binimetinib** (MEK inhibitor)	A Phase Ib/II Study of LEE011 in Combination with MEK162 in Patients with NRAS Mutant Melanoma	NRAS mutant melanoma	Phase 1/2	NCT01781572
Ribociclib Letrozole **Alpelisib** (PI3Kαinhibitor)	Study of LEE011, BYL719 and Letrozole in Advanced ER+ Breast Cancer	Breast Cancer	Phase 1	NCT01872260
Ribociclib Fulvestrant **Alpelisib** (PI3Kα inhibitor)	Study of LEE011 With Fulvestrant and BYL719 or BKM120 in Advanced Breast Cancer	Breast Cancer	Phase 1	NCT02088684
Palbociclib Fulvestrant **GDC-0077** (PI3Kα inhibitor)	A Study Evaluating the Efficacy and Safety of GDC-0077 + Palbociclib + Fulvestrant vs Placebo + Palbociclib + Fulvestrant in Patients with *PIK3CA*-Mutant, Hormone Receptor-Positive, Her2-Negative, Locally Advanced or Metastatic Breast Cancer	*PIK3CA* mutant breast cancer	Phase 2/3	NCT04191499
Ribociclib Letrozole **Buparlisib** (PI3K inhibitor)	Dose Escalation Study of LEE011 in Combination with Buparlisib and Letrozole in HR+, HER2-negative Post-menopausal Women with Advanced Breast Cancer.	Breast Cancer	Phase 1	NCT02154776
Abemaciclib Fulvestrant **Copanlisib** (PI3Kα inhibitor)	Testing the Addition of Copanlisib to Usual Treatment (Fulvestrant and Abemaciclib) in Metastatic Breast Cancer - Dose-Finding Study	Breast Cancer	Phase 1/2	NCT04088032
Palbociclib **Gedatolisib** (PI3K/mTOR inhibitor)	Phase I Study of Combination of Gedatolisib With Palbociclib and Faslodex in Patients With ER+/HER2- Breast Cancer	Breast Cancer	Phase 1	NCT02626507
Palbociclib **Gedatolisib** (PI3K/mTOR inhibitor)	A Study to Assess The Tolerability And Clinical Activity Of Gedatolisib In Combination With Palbociclib/Letrozole Or Palbociclib/Fulvestrant In Women With Metastatic Breast Cancer	Breast Cancer	Phase 1	NCT02684032
Palbociclib	Study of the CDK4/6 Inhibitor Palbociclib (PD-0332991) in Combination with the PI3K/mTOR Inhibitor Gedatolisib	Solid Tumours	Phase 1	NCT03065062

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Drugs	Title	Condition(s)	Phase	NCT Number
Gedatolisib (PI3K/mTOR inhibitor)	(PF-05212384) for Patients with Advanced Squamous Cell Lung, Pancreatic, Head & Neck and Other Solid Tumors			
Palbociclib **Taselisib** (PI3Kα inhibitor) OR **Pictilisib** (PI3K inhibitor)	Combination of PI3 Kinase Inhibitors and Palbociclib	Solid Tumours	Phase 1	NCT02389842