CDK4/6 and IGF1 Receptor Inhibitors Synergize to Suppress the Growth of p16<sup>INK4A</sup>-Deficient Pancreatic Cancers

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Abstract

Loss-of-function mutations in p16<sup>INK4A</sup> (CDKN2A) occur in approximately 80% of sporadic pancreatic ductal adenocarcinoma (PDAC), contributing to its early progression. Although this loss activates the cell-cycle-dependent kinases CDK4/6, which have been considered as drug targets for many years, p16<sup>INK4A</sup>-deficient PDAC cells are inherently resistant to CDK4/6 inhibitors. This study searched for targeted therapies that might synergize with CDK4/6 inhibition in this setting. We report that the IGF1R/IR inhibitor BMS-754807 cooperated with the CDK4/6 inhibitor PD-0332991 to strongly block proliferation of p16<sup>INK4A</sup>-deficient PDAC cells in vitro and in vivo. Sensitivity to this drug combination correlated with reduced activity of the master cell growth regulator mTORC1. Accordingly, replacing the IGF1R/IR inhibitor with the rapalog inhibitor temsirolimus broadened the sensitivity of PDAC cells to CDK4/6 inhibition. Our results establish targeted therapy combinations with robust cytostatic activity in p16<sup>INK4A</sup>-deficient PDAC cells and possible implications for improving treatment of a broad spectrum of human cancers characterized by p16<sup>INK4A</sup> loss.

Introduction

The retinoblastoma tumor suppressor (pRB) pathway is a key negative regulator of cell proliferation and functionally inactivated in many cancers (1). During the G<sub>1</sub> phase of the cell cycle, cyclin-dependent kinases phosphorylate pRB, allowing E2F transcription factors to drive S-phase entry and to promote cell-cycle progression (2). The regulation of pRB is frequently compromised in tumor cells by loss of the CDKN2A locus (3). CDKN2A encodes p16<sup>INK4A</sup>, an inhibitor of CDK4 and CDK6, and p14<sup>ARF</sup>, an inhibitor of MDM2-mediated p53 degradation (4). Loss of p16<sup>INK4A</sup> results in hyperactive cyclin D–CDK4/6 kinase complexes and pRB is the best-known member of a plethora of CDK4/6 substrates (5). In normal cells, cyclin D–CDK4/6 kinases integrate mitogenic signals. Elevated CDK4/6 activity promotes tumor growth by countering tumor suppressor mechanisms, such as senescence and apoptosis (6–8). Because the great majority of cancers with CDKN2A loss retain intact RB1, there is considerable interest in targeting CDK4/6 as a treatment strategy for these tumors, and in identifying drug combinations that potentiate the therapeutic effect.

PD-0332991 (palbociclib) is an orally active CDK4/6-specific inhibitor, which selectively inhibits CDK4 and CDK6 with in vitro IC<sub>50</sub> values of 11 and 16 nmol/L, respectively (9). In cells that express intact pRB, PD-0332991 blocks pRB phosphorylation at sites normally targeted by CDK4/6 (including serines S807/811 and S780) and causes G<sub>1</sub> cell-cycle arrest (9, 10). PD-0332991 has been well tolerated in phase I clinical trials and is effective in mantle cell lymphoma as well as in estrogen receptor (ER)–positive breast cancer (10–13). By contrast, single-agent treatment with PD-0332991 has produced only modest responses in most other malignancies regardless of CDKN2A mutational status.

The mechanisms of resistance to PD-0332991 in tumors predicted to have hyperactive CDK4/6 are poorly understood. Mutant forms of pRB that lack CDK phosphorylation sites give a dominant arrest in tumor cell lines (14). A combination of PD-0332991 and drugs that converge on the pRB pathway might lead to more effective CDK4/6 suppression and more stable pRB reactivation. Indeed, recent preclinical studies have shown CDK4/6 inhibition to cooperate with therapeutics targeting oncogenic drivers of p16<sup>INK4A</sup>-mutant cancers such as pediatric astrocytoma and malignant melanoma (15, 16).

Pancreatic ductal adenocarcinoma (PDAC) is the fourth leading cause of cancer-related death in the United States and highly resistant to existing treatments. Because CDKN2A inactivation occurs in 80% to 95% of cases and contributes to the early progression of PDAC precursor lesions whereas RB1 remains intact, CDK4/6 is an attractive target in these tumors (17–21). Here we sought to identify compounds showing synergy with CDK4/6 inhibitors in PDAC. Analysis of a comprehensive screen identifying genomic markers for drug sensitivities in cancer cell lines (22) suggested that mutational status of CDKN2A may correlate with sensitivity to inhibitors...
Figure 1. CDK4/6 and IGF1R/IR inhibitors synergize to reduce viability of PDAC cells with CDKN2A deletion. A, MIA-PaCa-2 cells were treated with BMS-754807 (BMS), PD-0332991 (PD), or their fixed-ratio (1:10) combination (BMS/PD) over a concentration range (μmol/L) for 72 hours and cell viability was measured relative to DMSO-treated controls. (Continued on the following page.)
that selectively target the insulin-like growth factor I receptor (IGF1R) and the related insulin receptor (IR). We found that concurrent targeting of CDK4/6 and IGF1R/IR resulted in synergistic effects on proliferation of CDKN2A-mutant PDAC cell lines in vitro and potent suppression of tumor growth in vivo. pRB depletion or activation of the key IGF1R/IR down-stream effector mTORC1 was sufficient to render cells resistant to the synergistic drug combination, suggesting that alterations in these pathways converge to promote the growth of CDKN2A-deleted PDAC cells.

Materials and Methods

Cell lines and drugs
All cell lines were provided and authenticated by The Center of Molecular Therapeutics (Massachusetts General Hospital Cancer Center) and cultured in RPMI medium containing 5% fetal bovine serum (FBS), except for YAPC, HPAC, HPAF-II, and Panc1, which were grown in DMEM with 10% FBS, and Mia-PaCa-2, which was grown in DMEM/F12 with 5% FBS. Cell media were supplemented with penicillin/streptavidin and glutamine, and cells were maintained at 37°C and 5% CO2. BMS-754807, OSI-906, and PD-0332991 were purchased from ChemieTek, temsirolimus, and AZD8055 were purchased from Selleck Chemicals. The compounds used to treat cells were dissolved in DMSO at 10 mmol/L and stored at −80°C.

Cell viability and synergy assays
Cells were seeded in 96-well plates at 2,000 to 4,000 cells/well depending on the growth characteristic of the cell line so that each was in growth phase at the end of the assay (60%–80% confluency). The following day, cells were treated in duplicate with single agents and their fixed-ratio combination for 72 hours over an 8-point, 128-fold concentration range, which was centered on the single-agent concentrations that inhibited viability by 50% (IC50). Cell viability was measured by staining the cells for 4 hours with 100 μg/mL resazurin (Sigma) whose conversion was detected at wavelengths of 510/595 nm using a SpectraMax M5 plate reader (Molecular Devices). The R software package mixlow (23) was applied to derive Loewe synergy indexes from the sensitivities to the single agents and their combination.

Mouse treatment study
One million YAPC cells in 100 μL PBS were injected subcutaneously into each flank of 10-week-old female CB17/ker-Prkdc–/–/kerCrl mice (Charles River Laboratories). After 1 week, tumor volumes were determined using electronic calipers to measure the length (L) and width (W) and calculated according to the formula $(L \times W^2)/2$. The mice were separated into four groups matched for tumor volume (50–60 mm3), which were randomly assigned to treatment arms. For oral administration, BMS-754807 was dissolved in sterile polyethylene glycol 400 (PEG400)/water (4:1, v/v) and PD-0332991 was dissolved in sterile 50 mmol/L sodium lactate (pH 4). The drugs or their vehicles were administered by gastric gavage every other day starting from day 8 after injection, with PD-0332991 at 75 mg/kg being fed in the morning and BMS-754807 at 15 mg/kg in the evening (minimum of 6 hours between PD-0332991 and BMS-754807). Tumor volumes were assessed twice weekly as described above. For pharmacodynamic evaluation at the study endpoint, tumor tissue was harvested 3 hours after the final BMS-754807 dose and frozen in liquid nitrogen or fixed in 10% formalin. All mouse studies were conducted through Institutional Animal Care and Use Committee (IACUC #2005N000148)-approved animal protocols in accordance with institutional guidelines.

Additional Materials and Methods are described in the Supplementary Methods.

Results

CDK4/6 and IGF1R/IR inhibitors synergize in CDKN2A-deleted PDAC lines
To identify targeted therapies that sensitize cancer cells to the CDK4/6 inhibitory PD-0332991, we examined drug sensitivity data from a comprehensive cancer cell line screen (22). As expected, sensitivity to PD-0332991 was associated with CDKN2A loss and lack of RB1 mutations. We noted that CDKN2A was the cancer gene whose mutational status most frequently correlated with differential drug response (Supplementary Fig. S4 of ref. 22). The correlations with CDKN2A status were particularly evident in PDAC cell lines where 32 drugs showed a trend toward decreased efficacy in the context of mutant CDKN2A versus wild-type CDKN2A. However, the number of CDKN2A wild-type PDAC lines included in the screen was small (N = 3), thus any hypothesis coming from this data needed to be verified experimentally. We reasoned that if the difference in drug sensitivity was indeed associated with inactivation of CDKN2A then it should be possible to recapitulate this effect by combining these drugs with PD-0332991 in CDKN2A-mutant PDAC cells, because this would mimic functional p16Ink4a (Supplementary Fig. S1).

The drugs with decreased sensitivities in CDKN2A-mutant PDAC cells included three different small molecule IGF1R/IR kinase inhibitors (Supplementary Table S1 and Fig. S2). BMS-
Figure 2. The combination of PD-0332991 and BMS-754807 enhances G1 arrest and senescence. A, bivariate flow cytometry analyses of MIA-PaCa-2 cells treated 24 hours with 50 nmol/L BMS-754807 (BMS), 500 nmol/L PD-0332991 (PD), their combination (BMS/PD), 50 nmol/L/500 nmol/L, or DMSO. The x-axis denotes DNA content as detected with PI, the y-axis shows cells undergoing active S-phase as indicated by BrdUrd labeling. Inset values show percent cells in G1, G0, S, and G0-M phase. B, MIA-PaCa-2 cells were treated for 6, 16, and 24 hours as described in A. Bar graphs show percent cells in G1, G0, S, and G0-M phase as determined by BrdUrd/PI staining (N = 2, P < 0.05, 2-tailed t test). C, PSN1, Hup-T3, and YAPC cells were treated for 24 hours and analyzed as in A and B. D, representative of the MIA-PaCa-2 staining. Bar graphs show mean percentage of SAβ-gal positive cells (P < 0.05, 2-tailed t test). E, population doubling of Hup-T3 and YAPC cells that were continuously cultured for 12 days in BMS-754807, PD-0332991, their combination, or DMSO and counted every 4 days. Data are from two independent replicate assays and error bars represent SD for all experiments.

Figure 3. Concurrent treatment with PD-0332991 and BMS-754807 is synergistic in vivo. A, YAPC xenografts were treated with 15 mg/kg BMS-754807 (BMS; N = 5), 75 mg/kg PD-0332991 (PD; N = 6), their combination (BMS/PD; N = 5), or vehicle (N = 5) on days 8, 10, 12, 14, 17, 19, and 21 after subcutaneous tumor implant and tumor volumes (mean ± SEM) were measured every 3 days from day 7. P values were calculated by 2-tailed t test for BMS/PD versus the other groups (P < 0.05) and the single-agent treatments versus the vehicle group (not significant). B, tumors were lysed following completion of the treatment study and analyzed by Western blotting with the indicated antibodies; a representative of at least two independent experiments is shown.
sufficient to generate sensitivity to the drug combination. Because p16INK4A is frequently lost by means other than genetic deletion, we used siRNA against CDK4/6 to verify that the proliferation of 2 sensitive lines depends on CDK4/6, whereas the proliferation of 2 CDKN2A wild-type lines does not (Fig. 1G). In conclusion, we have identified a drug combination that synergistically inhibits viability in more than half (4/7) of the CDKN2A-deleted PDAC cell lines tested.

**Concurrent inhibition of CDK4/6 and IGF1R/IR enhances G1 arrest and senescence**

Cyclin D–CDK4/6 kinase complexes drive G1–S cell-cycle progression. We performed cell-cycle analysis to examine how the CDK4/6 inhibitor affects viability when combined with IGF1R/IR inhibitor. Treatment of MiaPaCa-2 cells with PD-0332991 for 24 hours resulted in an increased G1–G0 population (from 41% to 61%) and reductions in the proportion of cells in S-phase (45% to 30%) and G2–M (14% to 8%; Fig. 2A). In combination with BMS-754807, G1–G0 arrest was markedly augmented (15% S, 77% G1–G0, and 7% G2–M), whereas BMS-754807 alone did not significantly affect cell-cycle distribution (Fig. 2A). To determine the timing of this enhanced G1–G0 arrest, we investigated shorter treatment durations. PD-0332991 reduced the S-phase population more potently to 13% and 10% after 8 and 16 hours, respectively (Fig. 2B). BMS-754807 addition decreased this fraction further to 4% at 16 hours, but had no added effect at 8 hours. These data suggest that IGF1R/IR inhibition synergizes with CDK4/6 inhibition by enhancing the PD-0332991 induced G1–G0 arrest starting from 16 hours after addition of the drugs and by maintaining the G1–S block more efficiently. The enhanced G1–G0 arrest at 24 hours was confirmed for the sensitive PSN1, HuP-T3, and YAPC lines (Fig. 2C).

Prolonged G1–G0 arrest can lead to senescence and PD-0332991 has previously been shown to cause senescence in solid tumors (6). Correspondingly, staining for senescence-associated β-galactosidase (SA-β-gal; ref. 27) revealed that combination treatment induced high levels of senescence compared with the single agents (combination treatment 61% SA-β-gal positive cells vs. BMS-754807 13% and PD-0332991 42%; Fig. 2D). Comparable results were obtained for HuP-T3 and YAPC cells, in which the drug combination induced a significant increase of SA-β-gal. These findings were further corroborated by counting population doublings of HuP-T3 and YAPC cells cultured in the presence of the inhibitors and passed every 4 days. Combination treatment prevented population doubling in HuP-T3 cells and strongly slowed it in YAPC cells treated with half the drug doses compared with the HuP-T3 experiments, consistent with induction of a cytostatic senescence response, whereas BMS-754807 had no significant effect on cell proliferation and PD-0332991 showed an intermediate effect (Fig. 2E).

**Combined CDK4/6 and IGF1R/IR inhibition is synergistic in vivo**

We evaluated whether combined CDK4/6 and IGF1R/IR inhibition is synergistic in vivo and could be a potential therapeutic approach against PDAC. Xenograft tumors were generated with YAPC cells, which are sensitive to the drug combination in vitro and form tumors within a week after subcutaneous injection into SCID mice. Once tumors reached 50 mm3 in volume, the mice were grouped and treatment was initiated with submaximal tolerated doses of each inhibitor. Administration of the single agents had no significant effect on the tumor volume, paralleling the in vitro results (Fig. 3A). In contrast, the combination therapy slowed tumor growth approximately 2.5-fold and significantly reduced tumor burden as compared with both the vehicle and the monotherapy cohorts (Fig. 3A), suggesting that concurrent targeting of CDK4/6 and IGF1R/IR is synergistic in vivo. This efficacy was achieved in the absence of any apparent adverse effects. Consistent with the in vitro findings, we observed increased staining for the senescence marker trimethylated lysine 9 histone H3 in PD-0332991 treated tumors, which was further enhanced by the drug combination (Supplementary Fig. S6).

To confirm that the drugs inhibited their respective targets in vivo, we analyzed the tumors at the study endpoint for CDK4/6 and IGF1R/IR signaling. BMS-754807 alone or in combination with PD-0332991 reduced tyrosine autophosphorylation of IGF1R/IR as well as phosphorylation of the IGF1R/IR downstream effector AKT (Fig. 3B). Single-agent PD-0332991 did not affect these phosphorylation events. To read out the activity of mechanistic target of rapamycin complex 1 (mTORC1) downstream of IGF1R/IR-AKT, we detected S6K1 phosphorylation at the mTORC1 site T389. Although BMS-754807 alone had only a slight effect, the drug combination abrogated S6K1 phosphorylation, suggesting that IGF1R/IR-AKT inhibition only translated to effective mTORC1 suppression in the presence of the CDK4/6 inhibitor, which did not decrease S6K1 activation by itself. To evaluate CDK4/6 activity in the tumors, we detected pRB phosphorylation at the CDK4/6 sites S807/S811. The drug combination notably reduced phosphorylation levels, whereas either single-agent treatment led to a minimal decrease in pRB phosphorylation. In accordance with a previous study noting that PD-0332991 stabilizes cyclin D1 (28), we observed elevated cyclin D1 levels in tumors that had been treated with the CDK4/6 inhibitor alone or in combination, indicating that PD-0332991 targeted CDK4/6 in both regimes. In vitro experiments confirmed that BMS-754807 enhances PD-0332991-mediated inhibition of pRB phosphorylation in YAPC cells even under conditions optimal for drug delivery (Supplementary Fig. S7). Together, these data indicate that the single agents affected their respective targets, but did not suppress tumor growth. In contrast, the drug combination elicited a tumorstatic response corresponding with enhanced S6K1 inhibition and pRB reactivation.

**The drug combination specifically reduces S6K1 phosphorylation**

To elucidate the mechanistic basis for the drug synergy, we investigated the phosphorylation of the CDK4/6 substrate pRB and of IGF1R/IR effectors after addition of BMS-754807, PD-0332991, and their combination. PSN1 cells were treated with PD-0332991, which abrogated pRB phosphorylation 16 hours after drug addition (Fig. 4A). This effect was not present at time points as early as 2 hours, most likely because of the fact that asynchronously cycling cell populations were used. CDK4/
6 inhibition was combined with low doses of BMS-754807 that dampened AKT activation in a dose-dependent manner (Fig. 4A). As observed in the xenograft tumors, BMS-754807–mediated inhibition of S6K1 T389 phosphorylation was enhanced by PD-0332991 (Fig. 4A). Thus, these compounds cooperate to inactivate mTORC1 in PDAC cells in vitro and in vivo.

To address whether this effect may be responsible for the drug synergy, we compared the effects on S6K1 phosphorylation in two lines that displayed the synergy with two lines that did not show the synergistic effects. Although concurrent treatment with BMS-754807 and PD-0332991 significantly reduced S6K1 phosphorylation in the sensitive MIA-PaCa-2 and HuP-T3 cell lines (Fig. 4B), it had no effect in PANC-03-27 or CFPAC-1 cells, in which the inhibitors do not synergize. The inhibitors did not affect ERK1/2 activity, suggesting that the signaling alterations were specific to the PI3K-mTOR pathway (Fig. 4B).

**Cyclin D1 or CDK4/6 depletion sensitizes PDAC cells to S6K1 inhibition**

The cooperation of CDK4/6 inhibitor with IGF1R/IR targeting on the level of mTORC1 raised the question whether this effect of PD-0332991 was through inhibition of CDK4/6 or off-target. To distinguish between these possibilities, cyclin D1 or CDK4/6 were depleted with siRNA followed by BMS-754807 treatment for 2 hours. Cyclin D1 knockdown sensitized MIA-PaCa-2 and YAPC cells to BMS-754807–mediated inhibition of S6K1 phosphorylation (Fig. 5A). Identical results were obtained in YAPC cells after CDK4/6 depletion. CDK4/6 knockdown in MIA-PaCa-2 cells lead to increased phospho-S6K1 that was strongly reduced by BMS-754807, which had no effect on control siRNA–transfected cells (Fig. 5B). These findings indicate that the PD-0332991–mediated sensitization to mTORC1 inhibition is specific and could thus be shared by other CDK4/6 inhibitors.

**CDK4/6 and mTOR inhibitors synergize in CDKN2A-deleted PDAC lines**

The observed effect on phosphorylation of S6K1, a primary substrate of mTORC1, raised the question whether mTORC1 inhibition would suffice to synergize with CDK4/6 inhibition. Indeed, PD-0332991 synergized with temsirolimus, an allosteric mTOR inhibitor that specifically targets mTORC1, to reduce
viability of not only the PDAC lines sensitive to BMS-754807/PD-0332991, but also the additional lines PANC-03-27, BxPC-3, and PANC-10-05 (Fig. 6A and Supplementary Fig. S8). IGF1R/IR signaling may not be required for mTORC1 activity in these lines. Remarkably, combined CDK4/6 and mTORC1 inhibition synergized, at least partially, in all of the CDKN2A-deleted PDAC lines tested (Fig. 6B).

Catalytic mTOR kinase inhibitors, such as AZD8055, have recently been developed in order to target both mTOR complexes and abolish mTORC1 activities more effectively than rapalogs. AZD8055, which is two orders of magnitude more potent than temsirolimus in HuP-T3 cells, synergized with PD-0332991 for 72 hours, followed by 2-hour treatment with BMS-754807 and temsirolimus, which blocks mTORC1 downstream of TSC2 (Fig. 7A).

The best-established cyclin D–CDK4/6 substrates are the pRB family members. Because pRB fulfills a unique role among the pocket proteins in mediating oncogene-induced senescence, we tested whether loss of pRB would render cells insensitive to the synergistic drug combination. We made stable MIA-PaCa-2 and HuP-T3 lines, in which pRB was depleted with two different shRNAs, and determined their sensitivities to BMS-754807, PD-0332991, and their combination. The pRB-depleted cell pools were less sensitive to either drug alone as well as to the drug combination (Fig. 7B), with the shRNA construct that gave the stronger reduction in pRB levels (shRB1-2) conferring a higher degree of resistance. These data suggest that both IGF1R/IR and CDK4/6 inhibitors engage the pRB tumor suppressor pathway and that pRB loss can alleviate the synergistic effects of the drug combination.

**Discussion**

Here we identify inherent resistance of CDKN2A-mutant PDAC cells to CDK4/6 inhibition alone, because PD-0332991 failed to induce sustained growth arrest and senescence. CDKN2A loss is frequent in PDAC and contributes to PDAC progression by bypassing oncogenic RAS-induced senescence (29–31). Although several CDKN2A-mutant PDAC cell lines indeed depend on CDK4/6, only 1 of 13 cell lines with CDKN2A mutations is sensitive to PD-0332991 (AsPC-1 IC_{50} 0.25 μmol/L; Supplementary Table S1). Our data suggest that CDKN2A-deleted PDAC cells are intrinsically resistant to CDK4/6 inhibition alone, because PD-0332991 failed to induce sustained growth arrest and senescence at concentrations that initially block CDK4/6 activity as evidenced by decreased pRB phosphorylation. This inherent resistance to PD-0332991 is likely because of elevated PI3K-mTOR activity. KRAS-driven PDAC formation and maintenance has been demonstrated to depend on active PI3K signaling (26) that suppresses oncogenic RAS-induced senescence in pancreatic cancer (30). The results described here show that targeting of IGF1R/IR, which
potently inhibited the PI3K effector AKT, in combination with CDK4/6 inhibition resulted in prolonged cell-cycle arrest and increased SA-β-gal activity in vitro as well as tumor stasis in vivo, in accordance with the drug combination reinstating oncogene-induced senescence.

Despite the evidence for IGF1R being an important target in PDAC, clinical trials with IGF1R-blocking antibodies have been disappointing (32). Our study provides evidence that CDKN2A-mutant PDAC cells are inherently insensitive to IGF1R/IR inhibition alone. CDK4/6 has long been known for its role to promote growth in model organisms and its constitutive activation following p16INK4A loss may provide growth signals in the absence of IGF1R/IR signaling. Although the IGF1R/IR inhibitor BMS-754807 repressed AKT activation at low
nanomolar doses, it failed to efficiently inhibit the mTORC1 substrate S6K1 at these concentrations and impacted viability only at doses that were 2 orders of magnitude higher. Persistent activation of mTOR has recently been described to correlate with resistance of breast cancers to PI3K inhibitor (33) as well as with resistance of melanoma to RAF or MEK inhibitor (34). Similarly, here we found that the response to concurrent CDK4/6 and IGF1R inhibition correlated well with decreased mTORC1 activity. PD-0332991–mediated sensitization to mTORC1 inhibition could be recapitulated with siRNA against either cyclin D1 or CDK4/6, suggesting that other CDK4/6 inhibitors could share this activity. Overexpressed cyclin D has been previously shown to bind the mTORC1 negative regulator TSC2 (35), providing evidence of a direct physical connection between cyclin D1–CDK4/6 and regulation of mTORC1. To date, we have been unable to confirm this interaction between the endogenous proteins. Our results do, however, suggest that TSC2 likely plays a critical role in this synergistic drug response, as its depletion rendered cells resistant to the combination of CDK4/6 and IGF1R inhibitors. Furthermore, PD-0332991 synergized with the rapalog temsirolimus. This drug combination may be more easily translatable, because it expanded the number of sensitive CDKN2A-mutant lines and rapalogs have been well tolerated in clinical settings.

The synergistic effects seen by combining PD-0332991 with other treatments apply to other cancer types. A recent drug synergy screen and network modeling study suggested that hyperactive CDK4 and IGF1R signaling also cooperate in liposarcoma (36). Targeting additional cancer types may require the identification of context-specific oncogenic drivers that cooperate with CDK4/6 deregulation (37). Apart from the clinical benefit of a combination of PD-0332991 and letrozole seen in ER-positive breast cancer, several preclinical studies have shown PD-0332991 to be effective in combination with drugs targeting mitogenic drivers in pediatric astrocytoma, melanoma, and prostate cancer (15, 16, 28). In conclusion, our work suggests that CDKN2A loss in PDAC can be exploited by concurrent targeting of CDK4/6 and the IGF1R/IR-mTOR axis in order to efficiently reactivate pRB tumor suppressor functions and suppress growth.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.
Drug Combinations against p16<sup>INK4A</sup>-Deficient Pancreatic Cancers

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References
31. Carrière C, Gore a J, Norris AM, Gunn JR, Young AL, Longnecker DS, et al. Deletion of Rb accelerates pancreatic carcinogenesis by onco-
genic Kras and impairs senescence in premalignant lesions. Gastro-
32. Guha M. Anticancer IGF1R classes take more knocks. Nat Rev drug
33. Elkabets M, Vora S, Juric D, Morse N, Mino-Kenudson M, Muranen T,
et al. mTORC1 inhibition is required for sensitivity to PI3K p110α
5:196ra99.
34. Corcoran RB, Rothenberg SM, Hata AN, Faber AC, Piris A, Nazarian
RM, et al. TORC1 suppression predicts responsiveness to RAF and
MEK inhibition in BRAF-mutant melanoma. Sci Transl Med 2013;5:
196ra98.
35. Zacharek SJ, Xiong Y, Shumway SD. Negative regulation of TSC1-
TSC2 by mammalian D-type cyclins. Cancer Res 2005;65:
11354–60.
synergy screen and network modeling in dedifferentiated liposarcoma
identifies CDK4 and IGF1R as synergistic drug targets. Sci Signal
2013;6:ra85.
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