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CORRESPONDENCE

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Cyclin dependent kinase 4/6 inhibitor palbociclib synergizes with BCL2 inhibitor venetoclax in experimental models of mantle cell lymphoma without *RB1* deletion

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Abstract

Background Mantle cell lymphoma (MCL) is a chronically relapsing malignancy with deregulated cell cycle progression. We analyzed efficacy, mode of action, and predictive markers of susceptibility to palbociclib, an approved CDK 4/6 inhibitor, and its combination with venetoclax, a BCL2 inhibitor.

Methods A panel of nine MCL cell lines were used for in vitro experiments. Four patient derived xenografts (PDX) obtained from patients with chemotherapy and ibrutinib-refractory MCL were used for in vivo proof-of-concept studies. Changes of the mitochondrial membrane potential, energy-metabolic pathways, AKT activity, and pro-apoptotic priming of MCL cells were evaluated by JC-1 staining, Seahorse XF analyser, genetically encoded fluorescent AKT reporter, and BH3 profiling, respectively. MCL clones with gene knockout or transgenic (over) expression of *CDKN2A, MYC, CDK4*, and *RB1* were used to estimate impact of these aberrations on sensitivity to palbociclib, and venetoclax.

Results Co-targeting MCL cells with palbociclib and venetoclax induced cytotoxic synergy in vitro and in vivo. Molecular mechanisms responsible for the observed synthetic lethality comprised palbociclib-mediated downregulation of anti-apoptotic MCL1, increased levels of proapoptotic BIM bound on both BCL2, and BCL-XL and increased pro-apoptotic priming of MCL cells mediated by BCL2-independent mechanisms, predominantly palbociclib-triggered metabolic and mitochondrial stress. Loss of *RB1* resulted in palbociclib resistance, while deletion of *CDKN2A* or overexpression of *CDK4*, and *MYC* genes did not change sensitivity to palbociclib.

Conclusions Our data strongly support investigation of the chemotherapy-free palbociclib and venetoclax combination as an innovative treatment strategy for post-ibrutinib MCL patients without *RB1* deletion.

Keywords Cyclin-dependent kinase (CDK) inhibitors, Palbociclib, BCL2, Venetoclax, RB1, Mantle cell lymphoma (MCL)

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To the editor

Mantle cell lymphoma (MCL) is a subtype of B-cell lymphomas characterized by deregulation of cell cycle progression at the G_1-S phase transition as a consequence of cyclin D1 overexpression [1, 2]. Palbociclib, a highly selective cyclin-dependent kinase (CDK) 4/6 inhibitor demonstrated single-agent activity in patients with relapsed and/or refractory (R/R) MCL. Venetoclax, a BCL2 inhibitor also demonstrated clinical activity in MCL, but the remissions were short calling for rational drug combinations [3–6]. We analyzed efficacy, mode of action, and predictive markers of susceptibility to palbociclib, and provide sound preclinical rationale for its combination with venetoclax.

Anti-proliferative and cytotoxic effect of palbociclib was analyzed using a panel of nine MCL cell lines (Supplemental Table 1) with promising anti-proliferative effect (median IC50 13.6 nM), however the cytotoxic effect was detected only after extremely high concentrations of palbociclib with LD50 above 10μM in all cell lines.

In vitro, palbociclib increased number of cells arrested in the G1 phase, and downregulated critical cell cycle regulators including RB1 protein, while expression of CDKs and BCL2 family proteins remained without significant changes (Supplemental Figs. 1, 2 A). Despite this, in vitro screen demonstrated synergistic efficacy between palbociclib and venetoclax (Supplemental Table 2). The synergy was subsequently confirmed in vivo using a panel of 4 patient-derived xenograft (PDX) models derived from patients with R/R MCL including ibrutinib resistant cases (Fig. 1, Supplemental Table 3). Similar observations were recently reported by Yuxuan Che et al. [7]. Except for moderate downregulation of MCL1, ex vivo protein expression analysis did not significantly differ from in vitro results. Co-immunoprecipitation analyses after palbociclib revealed increased amounts of BCL2 and BCL-XL bound to BIM ex vivo and in vitro (but only after prolonged cultivation). (Supplemental Fig. 2B, 3).

Because the mode of action behind the synergy could not be explained by significant changes in the levels of free or bound BCL2 proteins, we speculated that cell cycle arrest induced by palbociclib might lead to complex changes that prime MCL cells to venetoclax-triggered apoptosis. Indeed, reactive oxygen species (ROS) and JC-1 analyses revealed increased mitochondrial ROS production and increased mitochondrial depolarization of MCL cells after exposure to palbociclib (Supplemental Fig. 4A, B). Enhanced mitochondrial proapoptotic priming by complex mechanisms independent of BCL2 proteins was confirmed by intracellular BH3 profiling (Supplemental Fig. 4C). Although the SeaHorse analysis did not reveal any specific impact of palbociclib on oxidative phosphorylation, both basal and maximal glycolytic activity was decreased in 6 out of 7 analyzed MCL cell lines (Supplemental Figs. 5 and 6). Using a genetically encoded AKT activity biosensor we demonstrated that AKT activity corresponded with the change of glycolytic activity- it was decreased in MINO and UPF1H cells but increased in JEKO-1 (Supplemental Fig. 7).

These data point to the fact that palbociclib leads to complex deregulation of key energy metabolic pathways

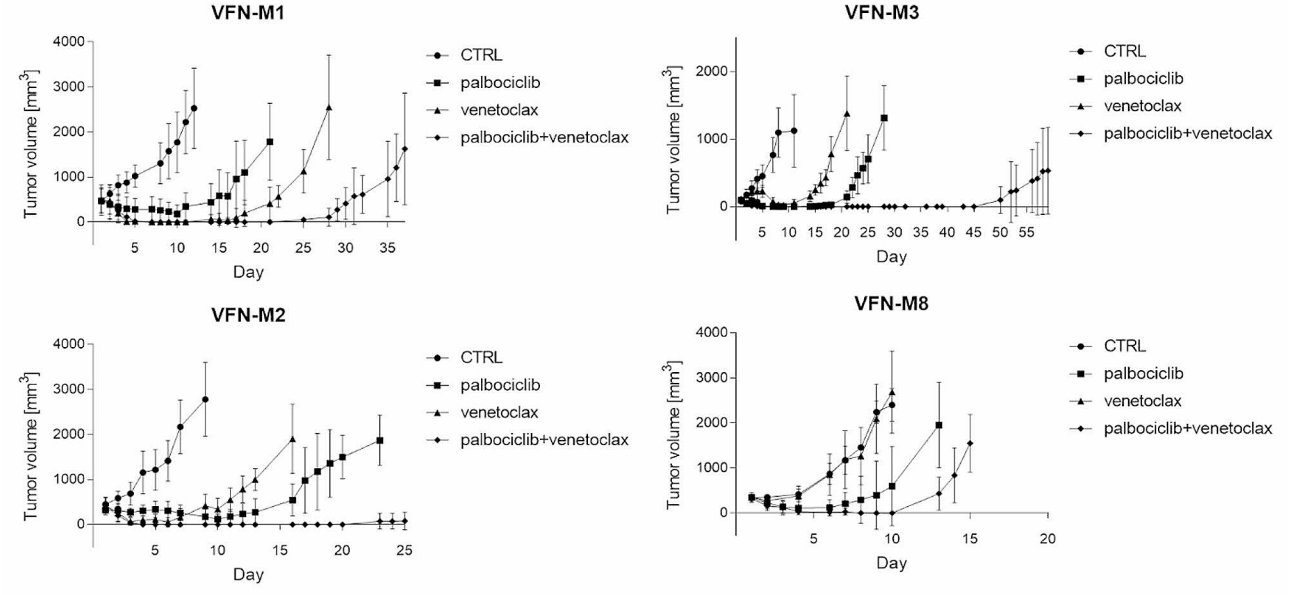

Fig. 1 Effect of palbociclib and venetoclax on PDX models of R/R MCL. Legend: Synergistic effect of CDK4/6 inhibition with palbociclib and BCL2 blockage with venetoclax on a panel of 4 PDX models of R/R MCL. X-axis shows days from initiation of therapy. Y-axis shows calculated tumor volume of PDX tumors (means ± standard deviations)

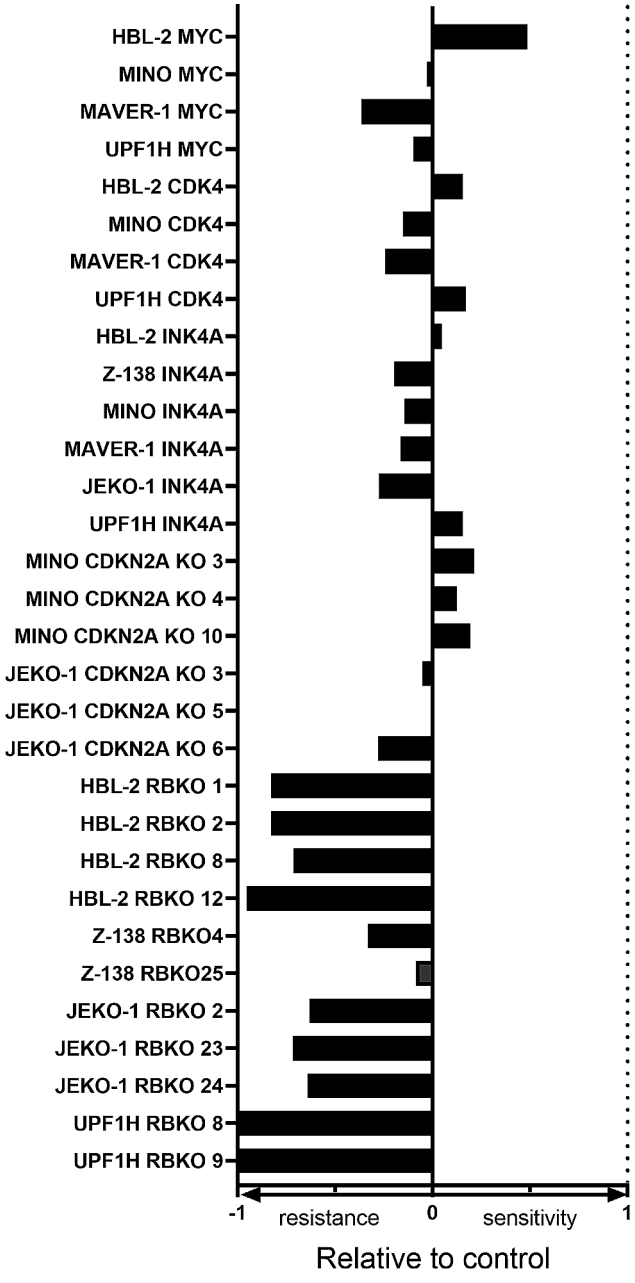

Fig. 2 Effect of palbociclib on clones with overexpression of *MYC, CDK4, CDKN2A and K/O of CDKN2A and RB*. Legend: the figure shows relative comparison of half-maximal inhibitory concentration (IC50, in nM) in response to palbociclib between MCL clones ($IC50_{clone}$) and corresponding controls ($IC50_{CTRL}$). The bars were constructed according to the following formulas: $1 + IC50_{CTRL} / IC50_{clone}$ in the cases when $IC50_{clone} > IC50_{CTRL}$; $1 - IC50_{clone} / IC50_{CTRL}$ in the cases when $IC50_{CTRL} > IC50_{clone}$. Positive and negative bars represent more sensitive and resistant clones, respectively (compared to controls)

and priming to mitochondrial apoptosis. However, precise molecular mechanisms that mediate the observed palbociclib-mediated priming of MCL cells to venetoclax remain to be elucidated.

MCL is characterized by several other recurrent molecular / cytogenetic lesions that deregulate G1-S cell cycle transition, including deletions of *CDKN2A* or *RB1*, and amplification of *CDK4* or *MYC* genes. To evaluate the impact of these aberrations on palbociclib (and venetoclax) sensitivity, we derived MCL clones with transgenic (over)expression of p16INK4A, MYC, and CDK4, as well as MCL clones with knockout of *CDKN2A* and *RB1* genes. We demonstrated that *RB1* gene knockout leads to acquired resistance to palbociclib, which is in line with previous reports in breast cancer [8, 9]. Other tested aberrations did not significantly change sensitivity to palbociclib. Of note, overexpression of MYC was associated with increased sensitivity to venetoclax (Fig. 2, Supplemental Fig. 8).

To sum up, the innovative, chemotherapy-free combination of an FDA-approved CDK4/6 inhibitor palbociclib, and a BCL2 inhibitor venetoclax is synthetically lethal in vitro and in vivo on a panel of chemotherapy and ibrutinib-refractory MCL. Molecular mechanisms behind the observed synergy include palbociclib-triggered downregulation of anti-apoptotic MCL1, increased levels of proapoptotic BIM bound on both BCL2, and BCL-XL and increased pro-apoptotic priming mediated by BCL2-independent mechanisms, predominantly palbociclib-induced metabolic and mitochondrial stress. Deletion of *RB1* represents a predictive marker of palbociclib resistance. In translation, our data strongly support investigation of palbociclib in combination with venetoclax as an innovative treatment strategy for R/R MCL patients without *RB1* deletion.

Abbreviations

CDK	Cyclin-dependent kinase
IC50	Half maximal inhibitory concentration
LD50	Median lethal dose
MCL	Mantle cell lymphoma
PDX	Patient derived xenograft
ROS	Reactive oxygen species
R/R	Relapsed and/or refractory

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40164-024-00499-2.

Supplementary Material 1

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Author contributions
PK and DM designed the study. DM, RJ, KK, JS, AD, EP, KN, DS, CD performed experiments. DK and OH prepared clones with knockout or overexpression of selected genes. KH performed statistical analyses. DCH, LA, OH, MK and PK contributed to interpretation of results and discussion. DM and PK wrote the manuscript. VK, MS, MT provided feedback of the manuscript. All authors discussed the results and contributed to the final version of manuscript.

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Data availability
All data generated or analyzed during this study are included in this published article, figures and tables (and its supplementary information files). Raw data are available from the corresponding author on reasonable request.

Declarations

Competing interests
The authors declare that they have no competing interests.

Ethics approval and consent to participate
All animal procedures were performed in accordance with protocols approved by the Institutional Animal Welfare Committee and Czech Ministry of Agriculture under number MSMT-37330/2020-2.

Consent for publication
Not applicable.

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