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POSTER ABSTRACTS

605.MOLECULAR PHARMACOLOGY AND DRUG RESISTANCE: LYMPHOID NEOPLASMS

Combination of CDK4/6 Inhibitor Palbociclib and PI3K Inhibitor Idelalisib Synergistically Induces an Anti-Tumor Effect in B-Cell Lymphoma and Overcomes Ibrutinib Resistance

Dingyao Hu[1], *Jiaowu Cao*[1], *Dedao Wang*[1], *Jiajin Wu*[1], *Lan Mi*[1], *Lingyan Ping*[1], *Ning Ding*[1], *Yuqin Song*[1], *Jun Zhu, PhD*[2]

[1] Key Laboratory of Carcinogenesis and Translational Research (Ministry of Education), Department of Lymphoma, Peking University Cancer Hospital & Institute (Beijing Cancer Hospital), Beijing, China
[2] Department of Lymphoma, Key laboratory of Carcinogenesis and Translational Research (Ministry of Education), Peking University Cancer Hospital & Institute, Beijing, China

Introduction: Aberrant activity of cyclin-dependent kinases 4/6 (CDK4/6) is frequently detected in B-cell non-Hodgkin's lymphoma (B-NHL), indicating the therapeutic implications of CDK4/6 inhibitors for B-cell malignancies. What's more, the introduction of agents targeting B cell receptor signaling, especially phosphatidylinositol 3-kinase (PI3K) isoform-specific inhibitors, is rapidly changing how B cell malignancies are treated. In this study, we aim to investigate whether the combination of CDK4/6 inhibitor palbociclib (PAL) and PI3Kδ inhibitor idelalisib (IDE) has synergistic anti-tumor effects in B cell lymphoma. Besides, we also evaluated the effect of co-administration PAL and IDE in Bruton's tyrosine kinase inhibitor (BTKi) resistant model.
Methods: *In vitro*, cell viability was analyzed using Cell Titer-Glo ® Luminescent Cell Viability Assay. Flow cytometry was used to detect apoptosis and cell cycle arrest induction. Western Blotting analysis was used to detect the changes in essential proteins in related signaling pathways. We also conducted RNA-seq to evaluate the whole transcriptome changes brought by co-treatment with PAL and IDE. The synergistic anti-tumor effects of PAL and IDE were also evaluated *in vivo*. BTKi secondary resistant cell lines were generated by long-time exposure to ibrutinib and BTK C481S mutation cell line was conducted by lentivirus infection and puromycin selection.
Results: Firstly, we analyzed diffuse large cell lymphoma (DLBCL) gene expression profile results from GEO database, and there were more than 50% patients carried cell cycle-related genes alterations, especially for CDKN2A-CDK4/6/CCND1-Rb machinery. Then, it was noteworthy that the high level of CDK4/6 mRNA and protein expression correlated with worse survival of DLBCL patients. Next, we illustrated that pharmacological inhibition of CDK4/6 kinase activity induced inhibition of cell proliferation, tumor cell apoptosis, and cell senescence. Meanwhile, PAL treatment upregulated the expression of PI3Kδ, p-AKT (S473), and p-mTOR (S2448), suggesting compensatory activation of the PI3K-AKT-mTOR signaling pathway. Thus, combination treatment of PAL and PI3K inhibitor IDE was assessed, and this combination synergistically induced a more potent anti-proliferative effect in several B-NHL cell lines, including mantle cell lymphoma cell lines (Z-138 and Jeko-1), DLBCL cell lines (U2932 and OCI-Ly8) and Burkitt lymphoma cell line (Raji). Meanwhile, co-administration treatment could also trigger synergistic anti-tumor activity in BTK inhibitor ibrutinib-resistant HBL-1 BTK C481S and HBL-1 IR cells (long time exposure to ibrutinib). Next, the flow cytometry results indicated that the B-NHL cells became apoptotic after the PAL and IDE treatment. The activation of apoptosis-related proteins (cleaved-caspase3 and cleaved-PARP) and decrease of anti-apoptosis proteins expression (BCL-2, BCL-xL, XIAP, and MCL-1) were induced by combination of CDK4/6 and PI3K inhibitor treatment. The combination of PAL and IDE also substantially increased the cell population of G0/G1 phase and elevated expression of CDK4, CDK6, and CyclinD1. Furthermore, the gene expression profile analysis demonstrated that the mRNA expression level of PLK1 was significantly decreased by co-administration treatment, rather than in PAL or IDE treatment alone. Finally, the combination treatment also showed synergistic anti-tumor activity in multiple tumor-bearing mice models.
Conclusion: The combination of CDK4/6 inhibitor palbociclib and PI3K inhibitor idelalisib synergistically induced anti-tumor activity in B-cell lymphoma through downregulation of PLK1 expression, suggested a new combination direction for the treatment of B-NHL and even BTK inhibitor-resistant patients.

Disclosures No relevant conflicts of interest to declare.

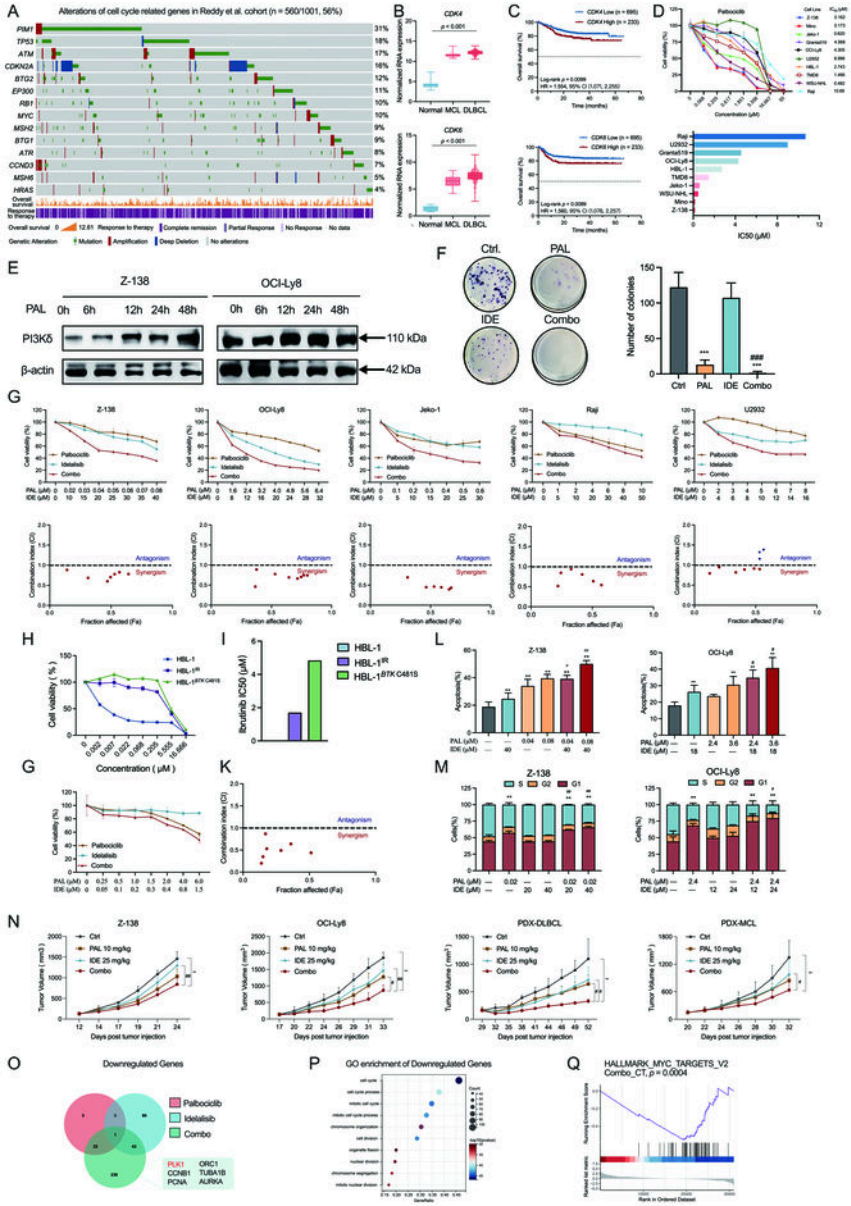

Figure 1. Combination of CDK4/6 inhibitor palbociclib and PI3Kδ inhibitor idelalisib synergistically induces an anti-tumor effect in B-cell lymphoma and overcomes ibrutinib resistance

(A) Percentage aberrations in cell cycle-related genes shown in the Reddy et al. cohort; (B) mRNA expression levels of *CDK4* and *CDK6* in DLBCL and MCL compared with normal B cells; (C) Overall survival of DLBCL patients based on CDK4 or CDK6 expression in GSE117556; (D) Dose-response curves for PAL in B-NHL cell lines, IC50 was shown in the bottom; (E) Western blotting images showing the protein level of PI3Kδ after PAL treatment; (F) Z-138 was treated with PAL (25 mM) and/or IDE (12.5 μM) by soft agarose colony forming assay. Colonies were stained with MTT after 14 days, and colony numbers were calculated by ImageJ; (G-K) The synergistic effects on multiple B-NHL cell lines, Combination Index (CI) was calculated by CompuSyn software and CI values < 1 was considered to be synergistic; (H) and (I) IC50 of ibrutinib in the indicated cell lines was assessed by CellTiter-Glo assays at 72 h; (L) and (M) OCI-Ly8 and Z-138 were treated with indicated PAL and/or IDE for 48h and subjected to cell-apoptosis analysis (L) and cell-cycle analysis (M) using Annexin V-PI staining or PI staining, respectively; (N) NOD/SCID mice bearing OCI-Ly8 or Z-138 xenografts and DLBCL or MCL patient-derived xenografts (PDX) were treated with vehicle, PAL (10 mg/kg), IDE (25 mg/kg), or the combination. Tumor volumes were measured every 2 days by caliper; (O-Q) Gene expression profile analysis demonstrated that the mRNA expression level of PLK1 was significantly decreased by co-administration treatment.

* p< 0.05, compared with vehicle group; # p<0.05, compared with PAL

Figure 1

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