(PB2540) SYNERGISTIC THERAPEUTIC EFFICACY OF CDK9 INHIBITOR WITH CX-4945 BY REPRESSING ASNS TRANSCRIPTION IN HIGH-RISK B-ALL

Topic: 01. Acute lymphoblastic leukemia - Biology & translational research

Huimin Xiang[1], Qi Han[1], Chunhua Song[2], Zheng Ge*[1]

[1]Zhongda Hospital Southeast University, Institute of Hematology Southeast University, Department of Hematology, Nanjing, China; [2]Division of Hematology, The Ohio State University Wexner Medical Center, The James Cancer Hospital, Columbus, United States of America;

Background

Acute lymphoblastic leukemia (ALL) is caused by the clonal proliferation of lymphoid stem cells or progenitor cells, with more than 80% originating from B-cell progenitors (B-ALL). As a member of the cyclin-dependent kinase family, CDK9 plays an important role in the occurrence and development of leukemia. CDKI-73, as a new highly selective CDK9 inhibitor, has been shown to exhibit antitumor effects in hematologic malignancies. CX-4945, a selective CK2 inhibitor, has shown strong CK2 activity inhibition as well as anti-leukemia efficacy in both in vivo and in vitro experiments. However, it remains unclear whether the combination of the two drugs exerts a synergistic anti-tumor effect.

Aims

To investigate the synergistic effect and the underlying mechanism of CX-4945 combined with CDKI-73 in B-ALL.

Methods

CCK-8 cell proliferation assay, Annexin V apoptosis assay, and colony formation assay were performed in B-ALL cells in the presence of CX-4945 only, CDKI-73 only, CX-4945 + CDKI-73, and vehicle control for 48h. Gene knockdown was performed by lentiviral shRNA. Student *T-test* was employed for statistical analysis to determine the significant difference between groups. A synergistic effect was determined by the combination indices (CIs) with CalcuSyn software. The B-ALL patient-derived xenograft (PDX) mouse models were applied for in vivo assay.

Results

Treatment of CDKI-73 significantly results in cell proliferation arrest of Nalm6 cells in a dose-dependent manner. The combination of various doses of CX-4945 with 1/2IC50 of CDKI-73 significantly decreases the cell viability of the cells *versus* single-drug control, and CalcuSyn analysis showed the significant synergistic effects of the two drugs (**Fig.1A-B**). CDKI-73 increased the % apoptotic cells in a dose-dependent manner, and the combination also significantly increased the % apoptotic cells compared to the single-drug controls in the Nalm6 cells (P<.001 or .01, **Fig.1C-D**). Moreover, the combination produced significantly less colony-forming *vs* either single drug in Nalm6 cells (**Fig.1E-F**). The combination significantly decreases the cell viability and apoptosis in the patient samples compared with single-drug controls; CalcuSyn analysis showed the synergistic effects of the two drugs on the proliferation arrest (**Fig.1G-H**). Moreover, the combination significantly reduced the spleen size, decreased % $hCD45^{+}hCD19^{+}$ cells in the spleen and bone marrow, and prolonged the survival of the B-ALL PDX mouse models (**Fig.1I-L**). To further understand the underlying mechanism of the synergy, RNA-seq analysis was performed in Nalm6 cells treated with either CX-4945 or CDKI-73 *vs* vehicle control, respectively. The overlapped altered DEGs were identified, and asparagine synthetase (ASNS) is one of the top DEGs; its mRNA level is dramatically down-regulated. The combination of the two drugs *vs* either single drug controls significantly suppressed the *ASNS* expression in Nalm6 cells (P<0.001, **Fig.1M-N**). Moreover, *ASNS* knockdown significantly suppresses the proliferation of Nalm6 cells (P<0.001, **Fig.1O**).

Summary/Conclusion

Combination of CX-4945 with CDKI-73 has a synergistic efficacy in B-ALL cells by down-regulation of *ASNS* in B-ALL. Our data provide experimental evidence for the combination as a potential option for high-risk B-ALL therapy.

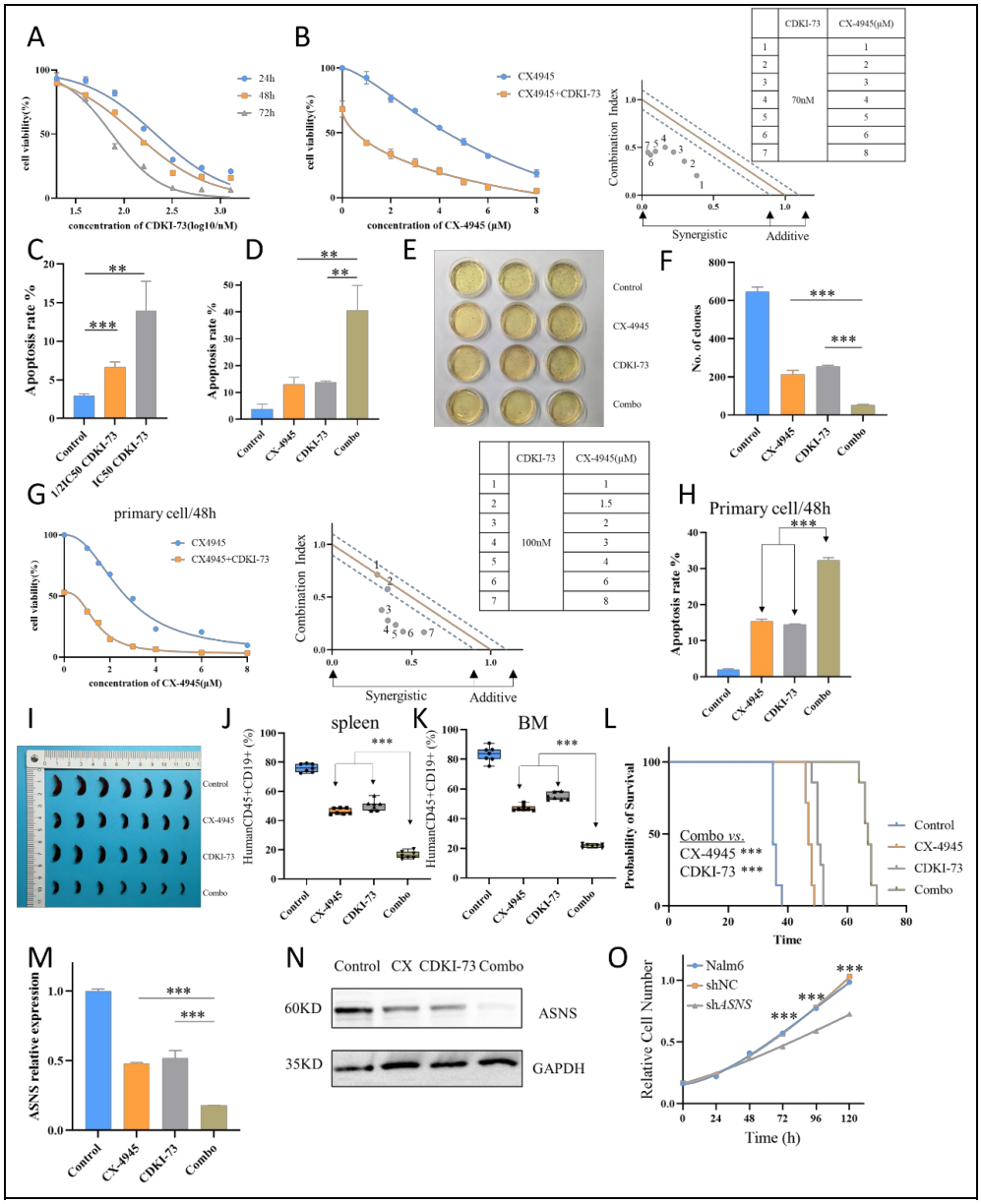

Fig. 1 Synergy of CX-4945 with CDKI-73 on therapeutic efficacy by repressing ASNS on transcription in high-risk B-ALL (A-B) The cell proliferation arrest effect of CDKI-73 alone with gradient concentration for 24, 48, and 72h in Nalm6 cells (A). Effect of the combination of CX-4945 and CDKI-73 vs single drug on cell proliferation and the synergistic analysis of the combination in Nalm6 cells(B). (C-D) The statistic histogram of apoptosis in Nalm6 cells treated by CDKI-73 alone with ½ IC50 and IC50 concentration for 48h. The statistic histogram of apoptosis in Nalm6 cells that were treated with CX-4945, CDKI-73 alone, and two drugs combination for 48h (D). (E-F) Effect of the combination of CX-4945 and CDKI-73 vs single drug on colony-forming and the statistic histogram of colony-forming in Nalm6 cells. (G) Effect and synergistic analysis of the combination of CX-4945 and CDKI-73 vs single drug on cell proliferation in B-ALL patient primary cells. (H) The statistic histogram of apoptosis in B-ALL patient primary cells that were treated with CX-4945, CDKI-73 alone, and two drugs combination for 48h. (I) Spleen image of 4 groups of mice post-treatment period. (J-K) The percentage of hCD45+ hCD19+ cells in the spleen (J) and bone marrow (K) from 4 groups of mice post-treatment period. (L) Comparison of Kaplan–Meier survival curves in the combination of CX-4945 with CDKI-73 compared with either single drug controls. (M-N) The expression of ASNS in Nalm6 cells treated with CX-4945 alone, CDKI-73 alone, and two drugs combination for 48 h detected by RT-qPCR (M) and western blot (N). (O) Effect of ASNS knockdown versus shNC on cell proliferation in Nalm6 cells.