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POSTER ABSTRACTS

622.LYMPHOMAS: TRANSLATIONAL-NON-GENETIC

CDK9 Inhibition Overcomes Ibrutinib Resistance in Mantle Cell Lymphoma (MCL)

Carly Roleder[1], *Edward C. Dominguez, PhD*[1], *Andrew Chen*[1], *Brian J. Ball, MD*[2], *Olga Danilova, MD PhD*[1], *Alexey Danilov, MD*[3]

[1] City of Hope, Duarte, CA
[2] Hematology/HCT, City of Hope National Medical Center, Duarte, CA
[3] Department of Hematology and HCT, City of Hope National Medical Center, La Canada Flintridge, CA

Introduction: Bruton tyrosine kinase (BTK) inhibitors (e.g., ibrutinib [ibr]) have shown clinical efficacy in MCL. However, resistance to BTK inhibitors is inevitable. Cyclin-dependent kinases (CDKs)are Ser/Thr kinases that associate with specific cyclins during the cell cycle to regulate its progression. We and others have shown that selective targeting of CDK9, a pTEFb component which facilitates RNA transcription, downmodulates MYC and MCL1 in malignant B-cells. Here, we investigated the effects of a selective CDK9 inhibitor, AZD4573, in ibr-naive and ibr-resistant MCL *in vitro* and *in vivo*.
Methods: MCL cell lines (Mino, JeKo-1) were cultured in increasing doses of ibr for ~6 months to generate resistance. MCL cell lines were treated with AZD4573 and assayed for apoptosis/proliferation, metabolic phenotype (Seahorse), and protein and RNA expression. Primary MCL cells were incubated for 24 h in CD40L/BAFF-conditioned media prior to drug treatment. Two MCL PDX models were used (chemo-resistant and ibr-resistant). MCL cells were injected IV in NSG mice and tracked by flow cytometry ($CD5^+$ $CD19^+$ $CD45^+$). Upon MCL detection in the peripheral blood (~1-2%), mice began treatment with AZD4573 (25 mg/kg IP once or twice weekly) or vehicle control. Splenocytes were harvested and RNA was isolated and analyzed by RNA-Seq/RT-PCR. Additionally, primary circulating MCL cells were collected from a patient treated with one dose of AZD4573 (at baseline; 4 and 7 h post-infusion and day 7) and analyzed by scRNA-Seq.
Results: Treatment with AZD4573 potently inhibited proliferation and induced apoptosis of both ibr-sensitive and resistant MCL cell lines *in vitro* (IC_{50} ~ 2-15 nM). AZD4573 also induced apoptosis of primary MCL cells in stromal conditions. Loss of RNA polymerase phosphorylation at Ser2 residue, a CDK9-dependent site, preceded apoptosis as measured by cleaved PARP. AZD4573 did not inhibit CDKs 2, 4/6, and 7 (as measured by pRb and pRNApol [Ser5]), confirming its selective effect. CDK9 inhibition resulted in rapid and dose-dependent downregulation of MYC and MCL1 mRNA and protein levels in MCL cell lines and primary cells.
Treatment with AZD4573 but not with ibr extended survival of ibr-resistant PDX mice without significant toxic events. RNA-Seq analysis of the MCL splenocytes harvested from these mice after 3 weeks of AZD4573 treatment demonstrated that gene sets related to transcription-factor activity were most enriched in the treated samples, consistent with known drug mechanism. TNFa/NFκB, TGF-β and PI3K/AKT/mTOR signaling pathways were most downregulated. We have shown that CDK9 inhibition leads to epigenetic reprogramming in neoplastic B-cells, resulting in upregulation of a subset of superenhancer-associated oncogenes, i.e. MYC, PIM kinaseand others (Thieme et al, 2023). Consistent with this notion, splenocytes derived from AZD4573-treated mice exhibited upregulation of MYC-dependent targets and OxPhos at the time of sacrifice. A number of genes relating to components of the electron transport chain were upregulated (*ATP5F1D*, *NDUFS7*, *ATP5MC1* as the top three). This suggested that these pathways may govern resistance to CDK9 inhibition in MCL over time. To further explore this, parental and ibr-resistant MCL cell lines were treated with low doses of AZD4573 (~2 nM). After 48 h, CDK9 inhibition was associated with increased oxygen consumption rates (OCR) in Seahorse analysis. This was not accompanied by increased cell proliferation by cell cycle analysis or Ki-67 staining. IACS-010759, an inhibitor of complex I of the ETC which targets OxPhos, demonstrated synergy with AZD4573 in parental and ibr-resistant MCL cell lines.
Single-cell RNA-Seq analysis of primary MCL cells obtained from a patient treated with AZD4573 on a clinical trial revealed rapid downmodulation of NFκB signaling (at 4 h) and MYC transcriptional targets and PI3K/AKT/mTOR signaling pathways (at 7 h). Interestingly, we observed partial recovery of MYC, mTOR pathways and induction of OxPhos by day 7 (prior to next planned dose), validating our pre-clinical findings.

Conclusion: CDK9 inhibition with AZD4573 induced apoptosis, downregulated MYC and MCL1 and NFκB signaling, and overcame ibr resistance in preclinical MCL models. This was also noted in a sample obtained from a clinical trial. Prolonged CDK9 inhibition led to metabolic reprogramming towards OxPhos, which thus can serve as a therapeutic target in MCL.

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