Abstract: P1272

Title: THE MULTIKINASE CDK4/6 INHIBITOR NARAZACICLIB OVERCOMES BTK-I RESISTANCE IN MANTLE CELL LYMPHOMA BY TARGETING USP24-P53 SIGNALING AXIS

Abstract Type: Poster Presentation

Topic: Lymphoma biology & translational research

Background:

Bruton tyrosine kinase inhibitors (BTK-i) have transformed the therapeutic landscape of mantle cell lymphoma (MCL); yet the emergence of primary and acquired resistance to these agents remains a challenge. Previous studies have suggested that narazaciclib (ON123300), an orally administered second-generation CDK4/6 inhibitor currently in clinical development, could help overcome BTK-i refractoriness.

Aims:

Our aim was to compare the efficacy and safety profiles of narazaciclib with three health authority-approved CDK-i (palbociclib, abemaciclib and ribociclib), as monotherapy or in combination with covalent (ibrutinib, acalabrutinib) and non-covalent (pirtobrutinib, ARQ-531) BTK-i, across a panel of preclinical models of MCL with distinct sensitivity to these latter, and to determine the molecular bases of the interaction between these two classes of drugs.

Methods:

A panel of n=12 MCL cell lines and primary samples were cultured either in 2D or 3D configuration in the presence of the different drugs, and subjected to CellTiter-Glo proliferation assay, FACS-mediated quantification of cell cycle and apoptosis, RT-PCR and western blot analyses. We integrated RNA sequencing and gene set enrichment analysis (GSEA) coupled to phospho-proteomics and kinase-substrate enrichment analysis (KSEA) to explore the molecular bases of BTKi-CDKi drug interaction in these models, followed by CRISPR-Cas9-mediated validation assays. Safety and efficacy of narazaciclib/ibrutinib combination were evaluated in vivo in a set of n=5 cell line-derived- or patient-derived and immune-competent xenograft models of MCL, using the chicken embryo chorioallantoic membrane (CAM) assay.

Results:

We found that narazaciclib exhibited high antitumor activity among MCL cell lines (mean IC50: 3.61 ± 2.1 μM), regardless of their sensitivity to ibrutinib. As expected, transcriptomic and phenotypic analyses revealed a predominant downregulation of E2F target genes and G2/M checkpoint response following narazaciclib treatment. This effect was associated with intracellular accumulation of p21, p16, and phospho-p27, decreased mitotic index, G1 cell cycle blockade, and apoptosis onset.

Combining narazaciclib with ibrutinib, but not with other BTK-i, resulted in enhanced antitumor activity, demonstrating synergistic effects in both BTK-sensitive and BTK-resistant MCL models. The integration of transcriptomics and phospho-proteomics further revealed that narazaciclib-ibrutinib combo modulated signatures related to DNA repair, P53 signalling, and glycolytic activity. The phosphorylation of Ubiquitin Specific Peptidase 24 (USP24), a deubiquitinase regulating DNA damage response by directly targeting P53, was identified as a key mechanism involved in narazaciclib-ibrutinib synergistic activity in MCL, enabling to bypass BTK-independent drug refractoriness.

In vivo studies then validated the effectiveness of this combination, allowing a 65% tumor growth inhibition in ibrutinib-unresponsive xenografts, and achieving a 50% reduction in malignant B cell infiltration into the bone marrow. This outcome was consistent across both cell line-derived and patient-derived xenograft models, without any observable toxicity.

Summary/Conclusion:

Our findings demonstrate that narazaciclib is safe and effective as a single agent in preclinical models of MCL, including BTKi-resistant cases. Its combination with ibrutinib achieved a synergistic tumoricidal effect in vitro and in vivo, accelerating cell cycle blockade and, specifically in the BTK-resistant cases, promoting the phosphorylation of USP24, followed by the activation of P53 and the modulation of DNA repair pathway.

Keywords: Drug interaction, Mantle cell lymphoma, Bruton's tyrosine kinase inhibitor (BTKi), Proteomics