

4181 Narazaciclib, a Differentiated CDK4/6 Antagonist, Prolongs Cell Cycle Arrest and Metabolomic Reprogramming, Enabling Restoration of Ibrutinib Sensitivity in Btki-Resistant Mantle Cell Lymphoma

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Bruton tyrosine kinase inhibitors (BTKi) have transformed the therapeutic landscape of mantle cell lymphoma (MCL); however, primary and acquired resistance to these agents remains a challenge. Previous studies have suggested that narazaciclib (ON123300), a second-generation, orally bioavailable and clinical-stage CDK4/6 inhibitor (CDKi), may trigger cell cycle arrest and significant tumor growth inhibition (TGI) in BTKi-resistant MCL.

We compared the efficacy and safety profiles of narazaciclib with three health authority-approved CDKi (palbociclib, abemaciclib or ribociclib) in association with covalent (ibrutinib, acalabrutinib) and non-covalent (pirtobrutinib, ARQ-531) BTKi, across a panel of 10 MCL cell lines with distinct sensitivity to the first-in-class BTKi, ibrutinib. We integrated RNA sequencing and gene set enrichment analysis (GSEA) coupled to phospho-proteomics and kinase-substrate enrichment analysis (KSEA) to unravel the molecular bases of BTKi-CDKi drug

interaction in MCL. CellTiter-Glo proliferation assay, FACS-mediated quantification of cell cycle and apoptosis, RT-PCR and western blot approaches followed by Seahorse-mediated metabolic assays were carried out for validation purposes. Finally, we evaluated the safety and efficacy of narazaciclib/ibrutinib combo *in vivo* in three immune-competent chicken embryo chorioallantoic membrane (CAM) MCL xenograft models.

We found that narazaciclib exhibited the highest antitumor activity among MCL cell lines (mean IC_{50} : $3.61 \pm 2.1 \mu M$), regardless of the sensitivity of these to ibrutinib. Although there was no correlation between CDKi sensitivity and direct repression of the CDK4/CDK6-pRb pathway in MCL, transcriptomic and phenotypic analyses revealed a predominant downregulation of E2F target genes and G2/M checkpoint response upon narazaciclib treatment. This feature was linked to intracellular accumulation of p21, p16, and phospho-p27, decreased mitotic index, G1 cell cycle blockade, and apoptosis onset. When combined with ibrutinib, but not with the second-generation acalabrutinib, narazaciclib achieved significant synergistic antitumor activity in both BTKi-sensitive and BTKi-resistant cells. The combination was not associated with improved apoptosis, but rather with a slight but constant augmentation in G1 phase blockade and the down-modulation of cell cycle-associated transcriptome, although similar features were found when substituting narazaciclib with abemaciclib. The integration of transcriptomic and phospho-proteomic synergistic signatures further revealed that narazaciclib-ibrutinib combo modulated signatures related to DNA repair, P53 signaling, and glycolytic activity. Accordingly, in this model, narazaciclib/ibrutinib-exposed cells underwent a metabolic switch from glycolytic to oxidative phosphorylation (OxPhos) phenotype accompanied by intracellular accumulation of mitochondria and increased mtDNA copy number. This switch appeared to be specific to MCL cells rendered refractory to ibrutinib upon activation of alternative NFkB signaling, as it was not found in MCL-BTKmut cells. *In vivo*, while narazaciclib single agent achieved a moderate TGI in the CAM model, the narazaciclib/ibrutinib combo reduced tumor spreading by 65% and allowed a 50% reduction in malignant B cell infiltration into host bone marrow, with no detectable toxicity.

In 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 reverting the metabolic reprogramming characterizing MCL refractoriness to BTKi therapy.

Disclosures: **Makovski Silverstein:** *Onconova Therapeutics:* Current Employment. **Cosenza:** *Onconova Therapeutics:* Current Employment. **Fruchtman:** *Onconova Therapeutics:* Current Employment. **Esteller:** *Quimatrix:* Consultancy; *Ferrer International:* Consultancy. **Roue:** *Onconova Therapeutics:* Research Funding.