e15096 **Publication Only**

Narazaciclib's kinase inhibitory activity is differentiated from approved CDK4/6 inhibitors in preclinical models.

Petros Kechagioglou, Camille Dupont, Hajime Yurugi, Ute Distler, Stefan Tenzer, Alexey Chernobrovkin, Kristina Riegel, Stephen Cosenza, Steven M. Fruchtman, Krishnaraj Rajalingam; Johannes Gutenberg University Medical Centre Mainz, Mainz, Germany; Institute of Immunology, University Medical Center Mainz, Mainz, Germany; Pelagobio Bioscience, Solna, Sweden; Onconova Therapeutics Inc, Newtown, PA; Onconova Therapeutics, Princeton, NJ

Background: Despite clinical benefit of CDK4/6 inhibitors in HR+Her2-mBC, patients progress. Neutropenia and diarrhea are safety concerns. Narazaciclib (ON 123300) is a multi-targeted kinase inhibitor of CDK4/6, ARK5 (NUAK1), CSF1R, and c-Kit at low nM concentrations designed to enhance efficacy and safety. Narazaciclib is in Ph I trials; NCT04739293 and CXHL1900340; in different regimens. Treatment of tumor cell lines with narazaciclib induces G1; G2 arrest and apoptosis. Mouse models suggest narazaciclib causes less neutropenia than palbociclib (palbo). Since signaling pathways are affected, we explored the activity of narazaciclib in direct comparisons with abemaciclib (abe), palbo and ribociclib (ribo) using *in vitro* and cell based assays. **Methods:** Comparison of narazaciclib's *in vitro* IC_{50} profile to abe, palbo and ribo was studied against a panel of 370 kinases (HotSpot). Kd values were determined by KINOMEscan. Intracellular IC_{50} kinase values were determined by NanoBret technology. Protein specific binding of narazaciclib and palbo was investigated by Cellular Thermal Shift Assay (CETSA). To investigate narazaciclib's effect on signaling pathways, integrative Inferred Kinase Activity (INKA) analysis was performed. **Results:** Narazaciclib and abe were found to be the most promiscuous *in vitro* kinase inhibitors and ribo the most specific. Abe and narazaciclib had similar profiles against the CDK family members. Kd values of CDK4/cyclinD1 binding show similar trends; abe (0.08 nM), narazaciclib (0.18 nM), palbo (0.75 nM) and ribo (1.3 nM). Narazaciclib and abe displayed nM activity against CDK2/cyclinA. The IC_{50} values against GSK3β, whose inhibition putatively causes diarrhea, was 374 nM for narazaciclib and 13 nM for abe. Although narazaciclib displayed nM IC_{50} values in the *in vitro* assays against many CDKs, narazaciclib was very specific in cellular kinase assays with highest activity against CDK4/6, CSF1R and NUAK1. CETSA-MS revealed more potential targets engaged by narazaciclib compared to palbo in both lysates and intact cells such as CHEK1, AAK1, BMP2K, GSK3α and GSK3β, but did not identify binding to NUAK1 or CSF1R. INKA analysis demonstrated that narazaciclib induced unique deregulated phosphorylation compared to palbo, including BUB1 (highly expressed in TNBC), NUAK1, CAMK2D, CDK16 and ULK1. Inhibition of autophagy, both at early and late stages, may sensitize cancer cells to narazaciclib and induce irreversible cell proliferation inhibition, providing a novel therapeutic approach. **Conclusions:** These studies identify important differences generated from assay models studying kinase inhibition, binding and pathway engagement and will guide future studies with narazaciclib targeting specific kinase driven tumors with the potential for improved safety. These preclinical data await confirmation from current and future clinical trials. Research Sponsor: Onconova Therapeutics, Inc, CRC 1292 TP05.