Abstract

We have previously described KPT-7523 and KPT-724 as PA44 allosteric modulators (PAMs) with potent anti-cancer activity. We recently found that PAMs also inhibit nicotinamide phosphoribosyltransferase (NAMPT) enzymatic activity, a protein that forms a complex with PAK4 to regulate cytoskeletal structures, cell adhesion, and migration. NAMPT and nicotinate phosphoribosyltransferase 1 (NAPRT1) catalyze the rate limiting steps in the nicotinamide adenine dinucleotides (NAD) salvage pathways, which are critical to cancer cells due to increased metabolic demands and the activity of NAD consuming enzymes. Curiously, while NAMPT is frequently overexpressed in cancer, NAPRT1 is often downregulated in certain cancers. Moreover, it is known that NAD depletion induces cell death and can result in tumor regression, indicating the potential benefit of the supplementation of nicotinamide (NA). The purpose of this study is to determine whether KPT-724 co-dosed with NA can reduce potential toxicities associated with NAD depletion, while enhancing NAMPT activity in cancer cells.

Methods: Cyclic NAMPT colormetric assay was used to examine NAMPT enzymatic activity in vitro. NAD/NADH-Glo and CellTiter-Glo were used to measure NAD and ATP levels, respectively. Enzyme activity in vitro and tumor cell culture. The role of NAMPT was determined using quantitative PCR and sequencing technologies. Western blot analysis was used to examine protein expression and protein-protein interactions.

Results: We have identified an orally bioavailable dual inhibitor of PAK4 and NAMPT, which demonstrated potent anti-cancer activity in a variety of cell lines, both in vitro and in vivo. In cell lines expressing NAPRT1 (Z-128, MV-4-11, COLO 205, and THP-1) cell death can be mediated with the supplementation of NA, while cell lines lacking NAPRT1 remain sensitive. However, a reduction of PAK4 protein levels and cell viability is still observed in these cells. In preliminary toxicology studies of KPT-724 co-administration with NA (in dogs), we observed a reduction in potential toxicity (e.g. gastrointestinal and hematological effects). Furthermore, KPT-724 showed potent anti-cancer activity in xenograft studies, although the suppression of anti-tumor activity by NA co-administration did not strictly correlate with NAPRT1.

Conclusions: Here we report that the therapeutic index of KPT-724, the first-in-class dual inhibitor of PAK4 and NAMPT, can be enhanced when co-dosed with NA in cancers lacking NAPRT1 protein expression in vitro. Furthermore, KPT-724 reduces steady-state levels of both NAPRT1 and NAMPT enzymatic activity, thus leading to the rapid depletion of NAD levels and cell death. Based on the in vitro and in vivo activity, co-administration of NA with KPT-724 may be beneficial for the treatment of a wide variety of cancers and will be tested in phase 1 clinical development.

KPT-724: Dual Inhibitor of PAK4 and NAMPT

KPT-9274 Enhances the Therapeutic Index of KPT-9274 in Cancer Cells

KPT-9274 Targets and Promotes Degradation of PAK4

KPT-9274 Inhibits NAD Synthesis by Inhibiting NAMPT Catalytic Activity

NAPRT1 is Epigenetically Silenced

Sensitivity of NAPRT1+/+ Tumor Xenografts to KPT-9274 Plus Nicotinic Acid

KPT-9274 Inhibits NAD Synthesis by Inhibiting NAMPT Catalytic Activity

Prolonged Exposure to KPT-9274 Leads to Cancer Cell Death

Nicotinic Acid Can Rescue KPT-9274 Cytotoxicity in NAPRT1 Expressing Cells In vitro