KPT-9274 Inhibits Cellular NAD and Synergizes with NAD Depleting Enzymes to Induce Cancer Cell Death

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Abstract

Nicotinamide adenine dinucleotide (NAD) is an essential metabolite and an important cofactor for several biological processes that undergo significant alterations during malignant transformation, including metabolism and stability-processes. In cancer, NAD is rapidly turned over due to the high metabolic demand of rapidly proliferating cells and the increased activity of NAD consuming enzymes, such as sirtuins (SIRT1) and poly ADP ribose polymerase (PARP1). NAD can be generated de novo from tryptophan or regenerated by nicotinamide phosphoribosyl transferase (NAMPT) or nicotinate phosphoribosyl transferase 1 (NAPRT1) in NAD salvage pathways. However, cancer cells do not utilize the de novo or NAPRT1 pathways effectively. Instead, they rely on the NAMPT-dependent salvage pathway to generate NAD, making NAD depletion a promising anti-cancer therapy. We have previously described the PAK4 alkylator modulator, KPT-9274, a compound that also inhibits the enzyme activity of KPT-9274 (IP), and other NAMPT inhibitors rapidly deplete cellular NAD levels, ultimately leading to ATP depletion and cell death. Similarly, several studies have shown that hyperactivation of NAD consuming enzymes can lead to cell death. The purpose of this study is to determine whether co-administration of KPT-9274 with compounds that activate NAD depleting enzymes will enhance the efficacy of NAMPT inhibition.

Methods: CellTracker-Glo was used to measure ATP levels and viability of cells. NAD/NAMPT-Glo was used to measure total NAD levels in cells. Game and protein expression was measured using quantitative PCR and western blot analysis, respectively. Protein knockdown was accomplished using RNAi. 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. We have identified several mechanism-based combinations that increase the anti-tumor activity of PAK4/NAMPT dual inhibitor, KPT-9274, through the activation of NAD consuming enzymes (e.g. SIRT1 and PARP1). Specifically, we found that SIRT1/2, (activator of SIRT1) synergizes with KPT-9274 to increase cancer cell death. In contrast, RAD1/2 SIRT1 diminishes the efficacy of KPT-9274. Activation of PARP1 by DNA damaging agents (e.g. gemcitabine) significantly enhances the effectiveness of KPT-9274 mediated cell death. Finally, we show that PARP1 activating DNA damage agents enhance toxicity and anti-tumor properties of KPT-9274 in cancer models.

Conclusions: Here we report that KPT-9274 synergizes with NAD depleting enzymes to induce cancer cell death in vitro and in vivo. This noteworthy enhancement to the anti-cancer activity of KPT-9274, together with our previously observed PAK4 anti-tumor activity, support the continued development of this orally bioavailable small molecule in combination with current therapies.

KPT-9274: Dual Inhibitor of PAK4 and NAMPT

SIRT1 NAD Dependent Activity Contributes to KPT-9274 Cytotoxicity

SIRT1 Expression Affects KPT-9274 Cytotoxicity

KPT-9274 Activator SRT1720 Synergizes with KPT-9274 to Enhance Cancer Cell Death

KPT-9274 Disrupts the Localization of Hexokinase II (HKII) and Induces the Formation of SIRT1 Foci in the Cytoplasm

PARP1 Activation by Gemcitabine Enhances the Anti-Cancer Activity of KPT-9274 In vitro and In Vivo

NAD Levels

KPT-9274, a dual inhibitor of PAK4 and NAMPT, significantly reduces cellular NAD levels in a dose dependent manner by reversibly inhibiting the catalytic activity of NAMPT. The combination of NAD depletion and PAK4 inhibition ultimately leads ATP depletion and cell death.

- NAD is a critical metabolic fuel NPT production and has additional roles in various processes such as DNA damage repair, cell signaling and transcriptional regulation. NAMPT is an evolutionarily conserved enzyme that regulates the synthesis of NAD in cells.

- The NAD dependent deacetylase SIRT1 contributes to KPT-9274 mediated cell death. Silencing SIRT1 expression or reducing its activity reduces the efficacy of KPT-9274. In contrast, activating SIRT1 with the small molecule SIRT1D20 increases cell death when used in combination with KPT-9274.

- SIRT1, which also acts as an NAD sensor has been reported to regulate mitochondrial biogenesis and form cytosolic/perinuclear focal spots when cells are treated with KPT-9274. The observed SIRT1 focal spots coincide with the disruption of mitochondrial membrane polarity and the localization of mitochondrial proteins (e.g. HKII).

- PARP1 does not affect the cytotoxicity of KPT-9274 unless its activation through single strand breaks (e.g. gemcitabine treatment) both in vitro and in vivo.

Together these data suggest that enhancing NAD depletion by activating NAD consuming enzymes in cells may enhance the anti-tumor activity of KPT-9274 and further development may lead to novel anti-cancer combination therapies in the future.

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