Co-Administration of Nicotinic Acid (NA) Enhances the Therapeutic Index of KPT-9274 in Cancer Cells

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Abstract

We have previously described KPT-7523 and KPT-9274 as PAK4 allosteric modulators (PAMs) with potent anti-cancer activity. We recently found that PAMs also inhibit nicotinamide phosphoribosyl transferase (NAMPT) enzymatic activity, a protein that forms a complex with PAK4 to regulate cytoskeletal structures, cell adhesion and migration. NAMPT and nicotinate phosphoribosyl transferase 1 (NAPRT1) catalyze the rate limiting steps in the nicotinamide adenine dinucleotide (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 induced cell death can be mitigated in a NAPRT1 dependent manner with the supplementation of nicotinic acid (NA). The purpose of this study is to determine whether KPT-9274 co-dosed with NA can reduce potential toxicities associated with NAD depletion, while enhancing anti-neoplastic activity in cancers lacking NAPRT1.

Methods: Cyclex NAMPT colorimetric 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. Gene expression and gene promoter methylation 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-138, MV-4-11, COLO 205, and THP-1) cell death can be mitigated 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-9274 co-administration with NA (in dogs), we observed a reduction in potential toxicity (e.g. gastrointestinal and hematological effects). Furthermore, KPT-9274 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-9274, 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-9274 reduces steady state PAK4 levels and inhibits 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-9274 may be beneficial for the treatment of a wide variety of cancers and will be tested in phase 1 clinical development.

KPT-9274: Dual Inhibitor of PAK4 and NAMPT

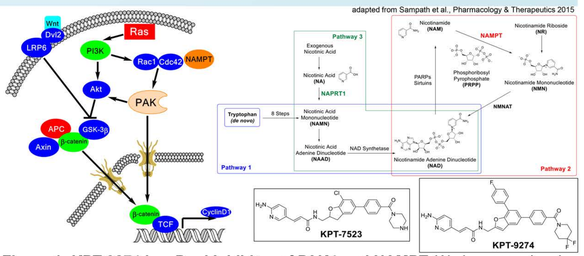


Figure 1: KPT-9274 is a Dual Inhibitor of PAK4 and NAMPT. We have previously highlighted the anti-cancer properties of KPT-9274. In addition to specifically binding and targeting PAK4, KPT-9274 and analogues inhibit the catalytic activity of NAMPT.
(Cross reference with abstract #3016, April 19, 8 to 12 PM, Poster Session 17)

KPT-9274 Targets and Promotes Degradation of PAK4

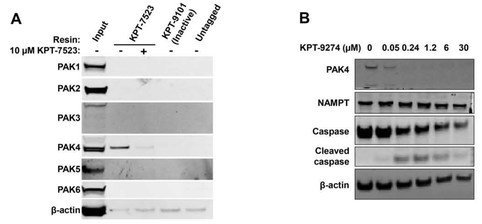
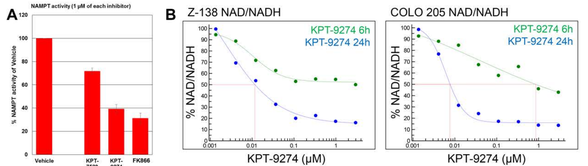


Figure 2: (A) MDA-MB-231 cells were treated with either DMSO or 10 μM KPT-7523 for 48 hours then collected by lysing. The lysates were incubated with either KPT-7523-resin, KPT-9101-resin (inactive compound) or untagged resin overnight. Resin was washed, run on SDS-PAGE and probed with PAK1-6 antibodies. KPT-7523-resin bound specifically to PAK4. KPT-9101 and untagged resin did not bind. (B) COLO 205 cells treated with increasing concentrations of KPT-9274 for 72 hours exhibit a dose dependent loss of PAK4 but not NAMPT.

KPT-9274 Inhibits NAD Synthesis by Inhibiting NAMPT Catalytic Activity



C

Z-138 Time point (hr)	NAD/NADH Glo IC50 (nM)	Celltiter-Glo IC50 (nM)	COLO 205 Time point (hr)	NAD/NADH Glo IC50 (nM)	Celltiter-Glo IC50 (nM)
6	NA	NA	6	859	NA
24	11.6	NA	24	7.6	NA
48	49.1	323.6	48	8.7	86.6
72	88.9	40.2	72	26.8	93.4

Figure 3: (A) The Cyclex NAMPT colorimetric assay was used to measure NAD synthesis. DMSO and FK866 were used as a negative and positive controls, respectively. KPT-9274 and KPT-7523 inhibit NAD synthesis. Z-138 and COLO 205 cells were plated in 96-well plates and then treated with varying concentrations of KPT-9274 for 6, 24, 48, and 72 hours. Cell viability (ATP) and NAD levels were measured using the Celltiter-Glo and NAD/NADH-Glo assay, respectively. (B and C) KPT-9274 inhibits NAD synthesis as early as 6 hours which leads to ATP depletion within 24 and 48 hours.

Prolonged Exposure to KPT-9274 Leads to Cancer Cell Death

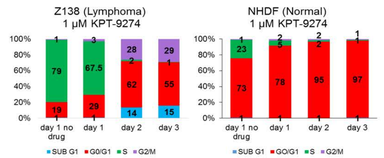


Figure 4. Z-138 cells and normal human dermal fibroblasts (NHDF) were treated with KPT-9274 for 24, 48, or 72 hours and BrdU cell cycle analysis was performed using flow cytometry. DNA synthesis is halted after 48 hours and leads to cancer cell death while normal cells remain in cell cycle arrest.

NAPRT1 is Epigenetically Silenced

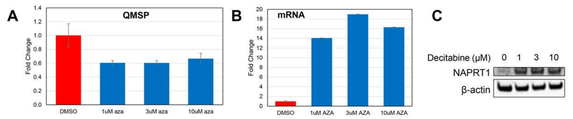
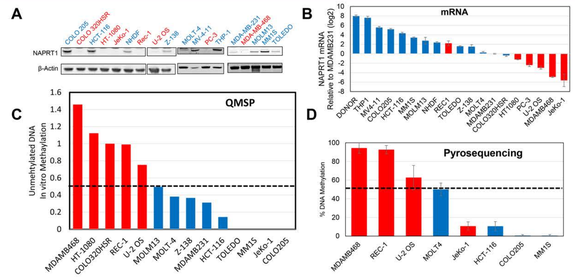


Figure 5: (A) U-2 OS cells were treated with decitabine for 72 hours. NAPRT1 promotor methylation was measured using a QMSP (Quantitative Methylation Specific PCR) assay. (B) Gene expression was measured using a Taqman assay for NAPRT1. (C) Protein expression was determined by Western blot analysis. Decitabine reduces DNA promoter methylation and restores NAPRT1 expression.

NAPRT1 Promoter Methylation Can be Used to Predict Nicotinic Acid Rescue



E

Human NAPRT1 Methylation Analysis - Results in % Methylation													
From ATG	-62	-52	-47	-45	-40	-34	-29	-24	-22	-22	-17	-13	-10
Location	5-UTR	5-UTR	5-UTR	5-UTR	5-UTR	5-UTR	5-UTR	5-UTR	5-UTR	5-UTR	5-UTR	5-UTR	5-UTR
Sample ID	CpG#-11	CpG#-10	CpG#-9	CpG#-8	CpG#-7	CpG#-6	CpG#-5	CpG#-4	rs201214521 - /CCGGC insertion	rs201214521 Creates CpG#-4a	CpG#-3	CpG#-2	CpG#-1
MDAMB468	97.2	89.5	97.3	91.6	89.4	100.0	82.8	100.0	Homozygous	96.0	100.0		
COLO205	1.9	2.7	1.9	0.0	0.0	0.0	0.0	0.0			0.0	0.0	0.0
HCT-116	8.1	11.7	10.7	12.1	12.0	9.5	11.3	8.9			21.2	0.0	11.4
JeKo-1	7.3	10.8	8.1	5.8	8.5	10.4	5.8	16.0	Homozygous	15.2	19.0		
MM1S	0.0	3.0	0.0	0.0	0.0	2.7	0.0	0.0			0.0	0.0	0.0
MOLT4	48.6	49.6	47.5	44.8	51.5	45.8	49.3	63.5	Heterozygous	40.1	59.5		
REC-1	96.2	91.3	96.6	90.3	91.1	99.2	89.1	96.3			95.9	84.1	89.2
U-2 OS	78.1	66.8	76.0	64.3	64.5	67.8	72.0	52.6			66.2	34.1	48.7
25%	35.9	30.3	36.2	28.8	27.2	34.7	31.3	33.8			26.5	30.5	32.2
50%	57.9	57.6	56.3	42.4	58.3	56.2	57.5	47.9			64.3	46.9	56.7
75%	80.1	84.1	78.2	78.2	79.5	76.5	76.5	88.4	Homozygous	71.8	68.2		
100%	96.9	95.4	94.0	93.8	94.3	100.0	90.6	100.0	Heterozygous	78.2	100.0		

Figure 6: NAPRT1 (A) protein and (B) mRNA expression in various cell lines. (C) QMSP was used to determine NAPRT1 promotor methylation in cell lines with gene expression cutoff ratio of 0.5. (D) Pyrosequencing analysis confirms QMSP data. (E) Individual CpG island methylation can be quantified using pyrosequencing. Outliers (JeKo-1) contain NAPRT1 mutations.

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

Cell line	IC_{50} (nM)	IC_{50} (nM) + 100 μM Niacin	NAPRT1 mRNA	NAPRT1 protein
COLO 205 (Colorectal)	40	> 3000	+	+
COLO 320HSR (Colorectal)	211	220	-	-
JeKo-1 (MCL)	75	79	-	-
REC-1 (MCL)	116	110	+	-
Z-138 (MCL)	18	> 3000	+	+
THP-1 (AML)	12	> 3000	+	+
MV-4-11 (AML)	81	> 3000	+	+
U-2 OS (Osteosarcoma)	100	79	-	-
PC-3 (Prostate cancer)	80	81	-	-

Sensitivity of NAPRT1+/- Tumor Xenografts to KPT-9724 Plus Nicotinic Acid

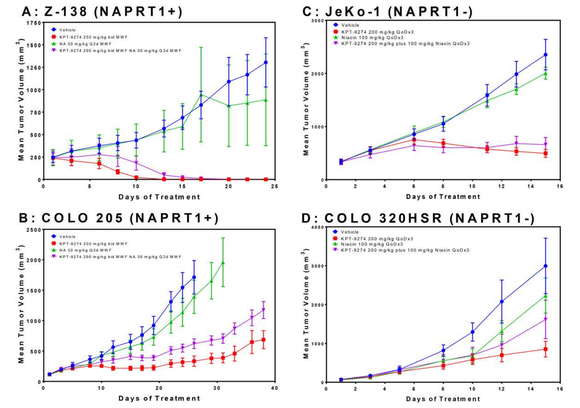


Figure 7: NAPRT1 expression correlates with NA rescue in cells lines. Mice were injected with (A) Z-138, (B) COLO 205, (C) JeKo-1, or (D) COLO 320HSR cells. When tumors reached ~100 - 200 mm^3, mice were randomized then treated with oral administration of vehicle or KPT-9274 with or without co-administration of NA at the concentrations indicated above. Mean tumor volume in mice treated with KPT-9274 significantly reduced when compared to vehicle. Co-administration of NA partially suppressed KPT-9274 anti-tumor activity in NAPRT1- models, in most cases behaving as predicted by *in vitro* results.

Conclusions

- 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 to ATP depletion and cell death.
- KPT-9274 targets both PAK4 and NAMPT in different manners. KPT-9274 binds to PAK4 resulting in the degradation of PAK4 protein. In contrast, KPT-9274 binds to NAMPT and inhibits its catalytic activity. Moreover, nicotinic acid can rescue NAD depletion and but not PAK4 stability.
- NAD is a critical metabolite for ATP 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 biosynthesis of NAD in cells from nicotinamide (NAM).
- In an alternative pathway, NAPRT1 is the first step in the conversion of NA (niacin) to NAD. NAPRT1 is epigenetically silenced in certain cancer types (especially sarcomas and lymphomas), increasing tumor dependence on NAMPT. Gene expression can be restored by the global hypo-methylating agent, decitabine, confirming epigenetic silencing.
- NAPRT1 gene expression can be predicted by quantifying gene specific promotor region methylation and can be measured using Quantitative Methylation Specific PCR. Methylation predicts gene silencing and can be used to predict nicotinic acid rescue. Additional promoter sequencing can be done to identify specific mutations affecting NAPRT1 expression.
- NA mitigates KPT-9274 activity in cell lines expressing NAPRT1. Similar results were observed in xenograft studies. Further optimization of this effect is ongoing.
- KPT-9274 +/- NA (niacin ER) will be evaluated in a FIH Phase 1 clinical trial in patients with solid malignancies and NHL (NCT02702492).

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