KPT-9274 Inhibits Cellular NAD and Synergizes with Doxorubicin to Treat Dogs with Lymphoma

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Abstract #LB-308

Abstract

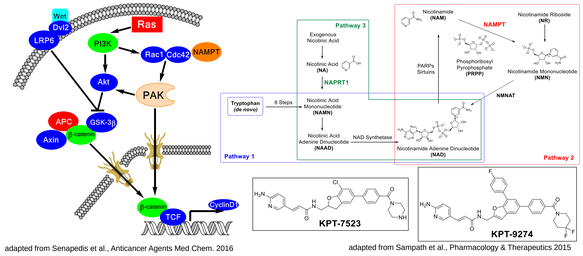
Nicotinamide adenine dinucleotide (NAD), an essential metabolite and cofactor of several biological processes (e.g. genomic stability), undergoes significant alterations during malignant transformation. In cancer cells, the high metabolic demands of proliferating cells and increased activity of NAD consuming enzymes (i.e. SIRT1 and PARP1) lead to rapid NAD turnover. 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 rely mainly on the NAMPT-dependent pathway, making NAD depletion a promising anti-cancer therapy. KPT-9274 is a novel first-in-class orally bioavailable dual inhibitor of p21-activated kinase 4 (PAK4) and NAMPT that demonstrates potent anti-tumor activity in a variety of cancer cell lines. KPT-9274 treatment rapidly depletes cellular NAD levels, leading to ATP loss and cell death. Similarly, hyper activation of PARP1 through DNA damaging agents (e.g. doxorubicin; DOX) can lead to apoptosis. Here we present results from a phase 1 study of KPT-9274 as a single agent and in combination with DOX for the treatment of dogs with solid tumors or lymphomas.

Methods: Dogs with cancer (lymphoma, n=4; sarcoma n=6; mast cell tumor n=2) were enrolled into a prospective phase 1 dose escalation study of KPT-9274 given qod x 3 per week. Plasma samples were used for pharmacokinetics (PK) and tumor biopsies were used to assess pharmacodynamics (PDn; i.e. PAK4, NAMPT and downstream effectors). In an expansion cohort, dogs with lymphoma (n=6) received 4 doses of KPT-9274 at 2 mg/kg prior to administration of a single dose of DOX.

Results: Doses up to 4 mg/kg KPT-9274 were well tolerated with no grade ≥3 toxicities. At 4.5 mg/kg one dog exhibited severe vomiting, diarrhea, collapse, anemia and thrombocytopenia, establishing 4 mg/kg as MTD. The PK of KPT-9274 was dose proportional and in agreement with healthy dogs. PDn markers (NAD levels) in tumors showed target engagement through NAD depletion as well as changes in PAK4 pathway biomarkers using IHC. Four dogs exhibited stable disease during treatment (3 soft tissue sarcomas, 1 mast cell tumor) at doses ranging from 3 – 4.5 mg/kg. Of the 6 dogs with naive lymphoma that received KPT-9274 and a single dose of DOX, 5 achieved a complete response, one of which lasted for over 3 months. No unexpected toxicities were noted with the combination when compared to those expected from DOX alone.

Conclusions: KPT-9274 exhibits single agent activity in canine spontaneous cancers. Moreover, the combination of KPT-9274 and DOX has substantial biologic activity against canine Non-Hodgkin lymphoma, likely through the activation of NAD consuming enzymes such as PARP1 by DOX. Importantly, the drug combination was safe with no enhanced toxicity over DOX alone. This data could be applied directly to the current trial of KPT-9274 in a Phase 1 clinical trial in patients with advanced solid malignancies or NHL (NCT02702492).

KPT-9274: Dual Inhibitor of PAK4 and NAMPT



adapted from Senapedis et al., Anticancer Agents Med Chem. 2016

adapted from Sampath et al., Pharmacology & Therapeutics 2015

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.

KPT-9274 Decreases PAK4 and NAMPT Activity in Tumor Cell Lines

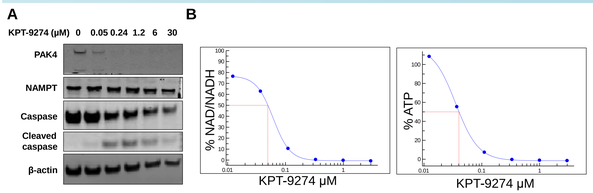
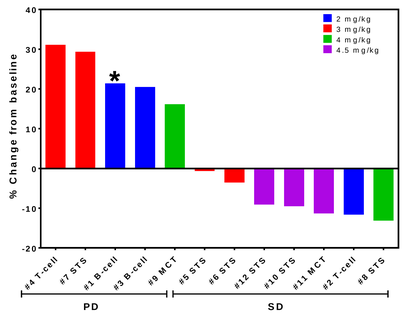


Figure 2: **KPT-9274 downregulates PAK4 and NAMPT activity.** U2OS and COLO 205 cells were treated with different concentrations of the PAK4/NAMPT inhibitor KPT-9274 for 72 hours. **(A)** Western blot of COLO 205 cells. **(B)** U2OS cells were incubated with NAD/NADH-Glo or Celltiter-Glo reagent in order to measure NAD or ATP levels, respectively. KPT-9274 reduces PAK4, NAD and ATP resulting in cancer cell death.

Best Response to KPT-9274 in Dogs with Cancer



* Dog #1 with B-cell lymphoma progressed on KPT-9274 alone

Received low dose doxorubicin after only 4 doses of KPT-9274.

Achieved a CR that was maintained for >3 months when unfortunately succumbed to an unrelated heart infection.

At necropsy there was no trace of lymphoma.

Figure 3. Best responses to KPT-9274 in the phase 1 study. Shown is a waterfall plot detailing the best responses across all tumors treated in the Phase 1 study. Stable disease was noted across all doses used, and was more likely to occur in the treatment of solid tumors rather than hematopoietic neoplasia (lymphoma) with a possible measure of dose response. Tumor types: **T- and B-cell**: lymphoma; **STS**: Soft tissue sarcoma; **MCT**: Mast cell tumor.

Pharmacokinetics of KPT-9274 in Dog Plasma

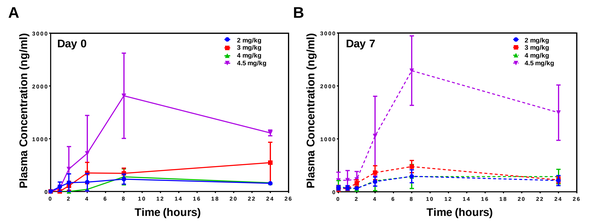


Figure 4. Plasma levels of KPT-9274 at days 0 and 7 of dosing. Dogs enrolled in the phase 1 study received KPT-9274 on a MWF (qodx3/week) dose schedule. PK sampling was performed on (**A**) Day 0 (solid lines) after the first dose and (**B**) Day 7 (dashed lines) after the fourth dose. Doses of 4.5 mg/kg resulted in significantly higher plasma drug levels.

Pharmacodynamics of KPT-9274 in Dog Tumor Biopsies

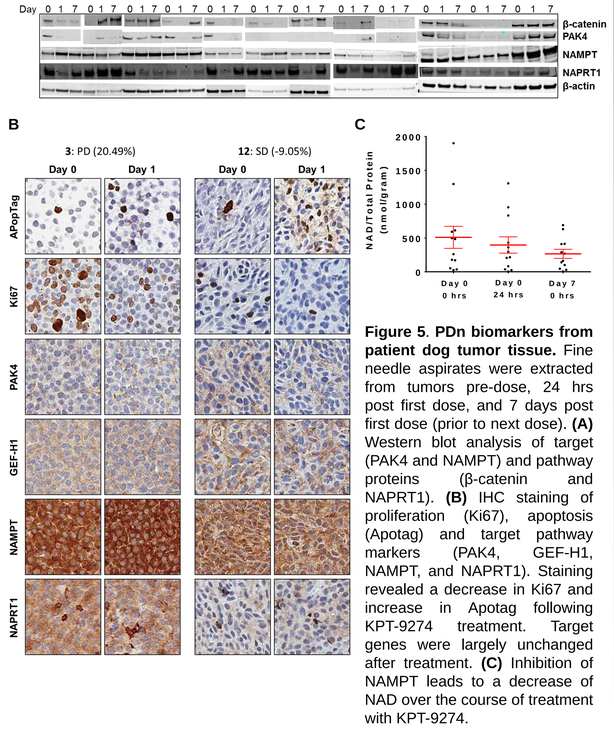


Figure 5. PDn biomarkers from patient dog tumor tissue. Fine needle aspirates were extracted from tumors pre-dose, 24 hrs post first dose, and 7 days post first dose (prior to next dose). **(A)** Western blot analysis of target (PAK4 and NAMPT) and pathway proteins (β-catenin and NAPRT1). **(B)** IHC staining of proliferation (Ki67), apoptosis (Apotag) and target pathway markers (PAK4, GEF-H1, NAMPT, and NAPRT1). Staining revealed a decrease in Ki67 and increase in Apotag following KPT-9274 treatment. Target genes were largely unchanged after treatment. **(C)** Inhibition of NAMPT leads to a decrease of NAD over the course of treatment with KPT-9274.

KPT-9274 and Doxorubicin in Dogs with NHL

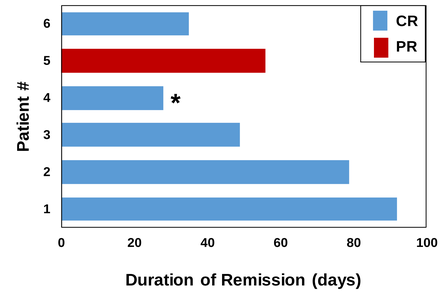


Figure 6. Response to combined KPT-9274 and doxorubicin. Dogs with naive NHL (B cell, n=5) were treated with 4 doses of KPT-9274 (MWFM) at 2 mg/kg. They were then treated with 30 mg/m^2 doxorubicin 24 hrs after the last KPT-9274 dose and followed weekly to assess remission status. All dogs achieved a CR except for Dog #5 who achieved a durable PR. *Dog #4 was withdrawn from study while in CR as the owner elected to pursue standard CHOP therapy.

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 ATP depletion and cell death.
- The PK of KPT-9274 was relatively dose proportional until 4.5 mg/kg where the plasma levels were disproportionately higher. Based on the day 7 results there is not a significant amount of accumulation.
- The PDn markers in dog tumors showed increased apoptosis (Apotag) and reduced proliferation (Ki67) with little change in target genes. In addition, there was marked reduction in NAD (product of NAMPT activity) in frozen tumor tissue.
- Stable disease was noted across all single agent doses used, and was more likely to occur in the treatment of slower growing solid tumors rather than the aggressive lymphoma with a possible measure of dose response.
- However, the combination of 4 doses of KPT-9274 plus one of DOX in NHL was highly efficacious (5/6 CRs) with no cumulative toxicity making this a promising regimen for companion dogs as well as their human counterparts.
- Data generated in dogs with spontaneous cancer will allow rapid implementation of this combination in the current human phase 1 trial of KPT-9274 in solid malignancies and NHL (NCT02702492)

Acknowledgements

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