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

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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. SIRT 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 p31-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 PKA through DNA damaging agents (e.g. doxorubicin) 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 = 6; sarcoma, n = 6; mast cell tumor, n = 2) were enrolled in a prospective phase 1 dose escalation study of KPT-9274 given q3d x 3 per week. Plasma samples were used for pharmacokinetics (PK) and tumor biopsies were used to assess pharmacodynamic (PD; i.e. KPT, 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 3/4 toxicities. At 4.5 mg/kg one dog exhibited severe diarrhea, anorexia, and thrombocytopenia, establishing a 4 mg/kg as MTD. The PK of KPT-9274 was dose proportional and in agreement with healthy dogs. PD 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 naïve 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 well tolerated without evidence of 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

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.

Figure 2: KPT-9274 depletes PAK4 and NAMPT activity in Tumor Cell Lines

Figure 3: Best responses to KPT-9274 in Dogs with Cancer

Figure 4: Plasma levels of KPT-9274 at days 0 and 7 in DOX-treated dogs. Doses enrolled in the phase 1 study received KPT-9274 on a MAF (q3d)x2 dose schedule. PK sampling was performed on (A) Day 0 (solid line) after the first dose and (B) Day 7 (dashed line) after the fourth dose. Doses of 4.5 mg/kg resulted in significantly higher plasma drug levels.

KPT-9274 Decreases PAK4 and NAMPT Activity in Tumor Cells

Figure 5: PnB markers from patient dog tumor tissue. Fine needle aspirates were extracted from tumors pre-dose, 24 hrs post first dose, and 7 days post final dose of KPT-9274. (A) Western blot analysis of target proteins (PAK4 and NAMPT) and pathway proteins (β-catamin and GAPDH). (B) IHC staining of proliferation (Ki67), apoptosis (Apoptag) and target pathway markers (PAK4, GEF-H1, GEF-H2, and PKA). Staining revealed a decrease in Ki67 and increase in Apoptag 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.

Pharmacokinetics of KPT-9274 in Dog Plasma

Pharmacodynamics of KPT-9274 in Dog Tumor Biopsies

KPT-9274 and Doxorubicin in Dogs with NHL

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KPT-9274 and Doxorubicin in Dogs with NHL

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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 PnB markers in dogs showed increased apoptosis (Apoptag) 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 (NCT03793492)

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