PAK4 Allosteric Modulators (PAMs) Repress the Wnt/β-catenin Signaling Pathway and Tumor Growth

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Abstract

Background: Wnt/β-catenin signaling is a ubiquitous pathway conserved throughout evolution and plays a role in embryonic and cancer development. However, this pathway has proven to be difficult to target therapeutically. Another intractable target is oncogenic Ras which is highly mutated in many cancer types, including pancreatic, colon and lung. An available target at the intersection of both of these genes is G12/13-activated Ras (PAK4). PAK4 (1 of 8 PAK proteins) that is downstream of the Ras oncogene and a direct kinase that regulates transcriptional activity of β-catenin. Therefore, therapeutically targeting PAK4 could be beneficial in a broad range of cancer types. PAK4 Allosteric Modulators (PAMs) represent a novel and selective class of compounds that inhibit PAK4 allosterically.

Methods: Flow cytometry and CellTiter Blue assays (MTS) were used to determine compound effects on cell cycle distribution and proliferation. CCLE, COSMIC and other databases were used for bioinformatics analysis of mutations in cancer genes. Studies on Ras signaling and its inhibitors were used to study phosphorylation signatures of whole cell proteins and total protein steady state levels.

Results: We identified a selective, orally bioavailable small molecules, (PAK4, KPT-654, KPT-732, KPT-6723, and KPT-7824) that were validated in a panel of cancer cell lines (IC50 values from 0.005 - 1 μM). Bioinformatics revealed that sensitivity to PAMs was directly correlated with mutations in APC or Nras and inversely correlated with mutations in β-catenin. K-ras, or P53. KPT-732 and other PAMs reduced phosphorylation and steady state levels of PAK4 protein while reducing Phospho-S657 and total β-catenin. PAMs also reduced β-catenin transcriptional activity (i.e. CCND1, WNT3A, and WNT16). PAMs arrested cancer cell cycle at the G1 and G2 phases and induced apoptosis through Caspase and PARP cleavage. KPT-7824 (100 mg/kg/day only) has demonstrated potent antitumor activity against hemaglobulins (Z-138, Mol-4, MN1) and solid (MDA-MB-231, MDA-MB-468, H520, Hep 3B and Colo-205) xenograft models in mice. PAM-treated xenografts showed reduction of PAK4, β-catenin and cyclin-D1 proteins.

Conclusions: PAK4 represents a novel anti-cancer target at the crosstalk of Ras and β-catenin signaling axes. This identified selective small molecule PAMs with anti-cancer activity both in vitro and in vivo. These allosteric modulators induce tumor cell growth arrest and apoptosis. Bioinformatics helped identify target specific predictive markers in the Wnt signaling pathway while deep sequencing and proteomics revealed possible PDK markers. Based on the in vitro and in vivo activity, KPT-7824 may be beneficial for the treatment of a wide variety of cancers and preclinical studies are ongoing.

Bioinformatics Analysis Suggest PAM In Vitro Sensitivity is Predicted to be though Ras and Wnt Pathways

Clinical Candidate, KPT-9274, Potently Cytotoxic to Cancer Cells and Inhibits PAK4 Wnt/β-catenin Signaling in Vitro

PAK4 Display In Vivo Efficacy in Colo-205 with a Reduction in Biomarkers

PAK4 Displays In Vivo Efficacy in PC3 Xenograft in Rats

PAK4 Display In Vivo Efficacy in HCC (Hep G2) in Mice

Figure 2: Bioinformatics analysis predicts mutations in the Ras and Wnt signaling pathway influence sensitivity to PAMs. (A) Label the HCC cell line as a 72 pairs test cell (HCC-MBA, TPA or Caco2). PAMs were derived from ONCOBEAT analysis, and were shown to be affected in the HCC-MBA cell line. (B) A recent KPT-9274 case study showed that PAMs were more effective in xenografts with mutations in β-catenin (K-ras, or P53). KPT-732 and other PAMs reduced phosphorylation and steady state levels of PAK4 protein while reducing Phospho-S657 and total β-catenin. PAMs also reduced β-catenin transcriptional activity (i.e. CCND1, WNT3A, and WNT16). PAMs arrested cancer cell cycle at the G1 and G2 phases and induced apoptosis through Caspase and PARP cleavage. KPT-7824 (100 mg/kg/day only) has demonstrated potent antitumor activity against hemaglobulins (Z-138, Mol-4, MN1) and solid (MDA-MB-231, MDA-MB-468, H520, Hep 3B and Colo-205) xenograft models in mice. PAM-treated xenografts showed reduction of PAK4, β-catenin and cyclin-D1 proteins.

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PAK4 Display In Vivo Efficacy in Colo-205 with a Reduction in Biomarkers

Figure 3: a. PAMs show antitumor activity in colon cancer model. Mice were treated with vehicle or 100 mg/kg/day of KPT-9274 for 6 weeks (on-bus). a, b. PAM-treated tumor volume (t-test) is smaller than vehicle-treated tumors (P<0.0001). c. PAMs reduced tumor weight (Student's t-test P<0.0001). d. PAMs reduced tumor weight and volume (Student's t-test P<0.0001). e. PAMs reduced tumor weight and volume (Student's t-test P<0.0001)

Conclusions: KPT-9274 is our clinical candidate with IND expected in 2H2015

PAK4 Display In Vivo Efficacy in HCC (Hep G2) in Mice

Figure 4: a. PAMs show antitumor activity in colon cancer model. Mice were treated with vehicle or 100 mg/kg/day of KPT-9274 for 6 weeks (on-bus). a, b. PAM-treated tumor volume (t-test) is smaller than vehicle-treated tumors (P<0.0001). c. PAMs reduced tumor weight (Student's t-test P<0.0001). d. PAMs reduced tumor weight and volume (Student's t-test P<0.0001). e. PAMs reduced tumor weight and volume (Student's t-test P<0.0001)

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PAK4 Display In Vivo Efficacy in PC3 Xenograft in Rats

Figure 5: a. PAMs show antitumor activity in colon cancer model. Mice were treated with vehicle or 100 mg/kg/day of KPT-9274 for 6 weeks (on-bus). a, b. PAM-treated tumor volume (t-test) is smaller than vehicle-treated tumors (P<0.0001). c. PAMs reduced tumor weight (Student's t-test P<0.0001). d. PAMs reduced tumor weight and volume (Student's t-test P<0.0001). e. PAMs reduced tumor weight and volume (Student's t-test P<0.0001)

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PAK4 Display In Vivo Efficacy in Colo-205 with a Reduction in Biomarkers

Figure 6: a. PAMs show antitumor activity in colon cancer model. Mice were treated with vehicle or 100 mg/kg/day of KPT-9274 for 6 weeks (on-bus). a, b. PAM-treated tumor volume (t-test) is smaller than vehicle-treated tumors (P<0.0001). c. PAMs reduced tumor weight (Student's t-test P<0.0001). d. PAMs reduced tumor weight and volume (Student's t-test P<0.0001). e. PAMs reduced tumor weight and volume (Student's t-test P<0.0001)

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PAK4 Display In Vivo Efficacy in HCC (Hep G2) in Mice

Figure 7: a. PAMs show antitumor activity in colon cancer model. Mice were treated with vehicle or 100 mg/kg/day of KPT-9274 for 6 weeks (on-bus). a, b. PAM-treated tumor volume (t-test) is smaller than vehicle-treated tumors (P<0.0001). c. PAMs reduced tumor weight (Student's t-test P<0.0001). d. PAMs reduced tumor weight and volume (Student's t-test P<0.0001). e. PAMs reduced tumor weight and volume (Student's t-test P<0.0001)

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PAK4 Display In Vivo Efficacy in PC3 Xenograft in Rats

Figure 8: a. PAMs show antitumor activity in colon cancer model. Mice were treated with vehicle or 100 mg/kg/day of KPT-9274 for 6 weeks (on-bus). a, b. PAM-treated tumor volume (t-test) is smaller than vehicle-treated tumors (P<0.0001). c. PAMs reduced tumor weight (Student's t-test P<0.0001). d. PAMs reduced tumor weight and volume (Student's t-test P<0.0001). e. PAMs reduced tumor weight and volume (Student's t-test P<0.0001)

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- PAMs demonstrate anti-tumor activity across a broad range of hematological and solid malignancies in vitro while sparing normal cells.
- PAMs decrease PAK4 protein levels and signaling pathways of treated cells.
- PAMs decrease activity of Wnt/β-catenin both in vitro and in vivo.
- PAMs are orally bioavailable and display anti-tumor activity in hematological and solid xenograft mouse and rat models with excellent tolerability.

KPT-9274 is our clinical candidate with IND expected in 2H2015