In vivo efficacy of the PAK4 Allosteric Modulator, KPT-9274, against a triple negative breast cancer model.

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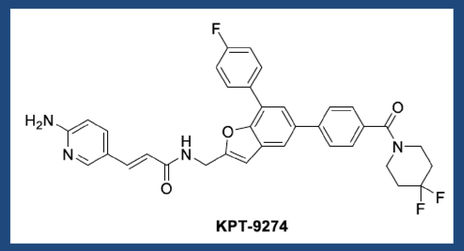
INTRODUCTION

The p21-activated kinases (PAK) belong to a family of serine threonine kinases that promote cell survival and play an important role in cell proliferation, cell cycle regulation and cell shape determination. There are six mammalian PAK proteins which can be subdivided into two groups by sequence homology and mode of activation- Group A PAKs consisting of PAK 1, 2 and 3 and Group B PAKs consisting of PAK 4, 5 and 6. We have found that PAK4 protein levels are elevated in breast cancer, including Her2 positive and triple negative breast cancers, while it is expressed at low levels in normal mammary tissue, making it an attractive drug target. PAK inhibitors are being tested for effectiveness against solid tumors, but generation of highly specific PAK4 inhibitors has been a challenge. Furthermore, PAK4 has been reported to have kinase-independent functions. Therefore inhibiting its kinase activity alone might not be sufficient in blocking its tumorigenic potential. Our lab has previously reported the effectiveness of PAK4 allosteric modulators (PAM; KPT-8752 and KPT-9274) against multiple breast cancer cell lines. These novel PAK4 inhibitors reduce steady state protein levels and were able to block cell growth, cell migration and induce apoptosis in breast cancer cell lines, without affecting the control cells. Here, we tested the efficacy of the orally bioavailable PAM, KPT-9274 against tumors formed by the triple negative breast cancer cell line, MDA-MB-231. Following six weeks of treatment with orally administered KPT-9274 (150 mg/kg bidx4), there was almost a five-fold reduction in tumor volume and tumor weight in the treatment group as compared to the control group (Average Tumor Volume in Control group, 539.5 mm^3 ± 20 S.E.; Average Tumor Volume in Treatment group, 120.6 mm^3 ± 8 S.E; Average Tumor Weight in Control group, 300.15 mg ± 15 S.E.; Average Tumor Weight in Treatment group, 61.4 mg ± 4 S.E.). The treatment did not significantly affect mice body weight. After six weeks of treatment, the tumors were excised and analyzed for PAK4 levels. We observed a significant decrease in PAK4 levels in excised tumors from the treatment group as compared to those from the control group. PAK1 levels were monitored to see any off-target effects, but their levels were unchanged. Our results indicate that an orally administered KPT-9274 was capable of specifically binding and inhibiting PAK4, and consequently reducing tumor growth. These results suggest that PAK4 plays a key functional role in triple negative breast cancer and treatment with Future studies analyzing the effects of KPT-9274 in blocking PAK4 mediated functions that promote tumorigenesis are ongoing. Additional studies of the effectiveness of KPT-9274 on tumors formed by other triple negative breast cancer cell lines are under investigation.

METHODS

- Female nude mice were injected subcutaneously with MDA-MB-231 cells on both the flanks at 10^6 cells per site; animals were sacrificed 6 weeks after injection, at which tumors were excised.
- Western Blot analysis was performed to measure protein levels.
- MTT assay was performed to monitor the effects of PAK4 inhibition on cell growth by incubating cells with PAMs at specified concentrations.
- Flow cytometry analysis was performed to monitor the effects of PAK4 inhibition on apoptosis induction using Annexin V- Propidium Iodide staining.
- Wound healing assay was performed to monitor the effects of PAK4 inhibition on cell migration of breast cancer cell



PAK4 protein levels upregulated in triple negative breast cancer cell lines

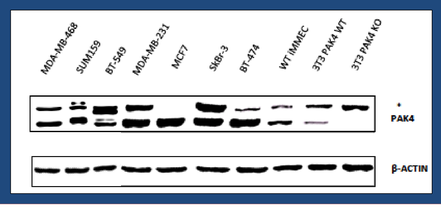


Fig 1. **PAK4 levels up regulated in breast cancer cells**
PAK4 protein levels were up regulated in, MDA-MB-468, SUM159, BT-549 and MDA-MB-231 (Triple Negative); SkBr-3 (HER2+); BT-474 (PR+/HER2+); MCF7 (ER+/PR+));

PAK4 Allosteric Modulators block cell growth in triple negative breast cancer cell lines

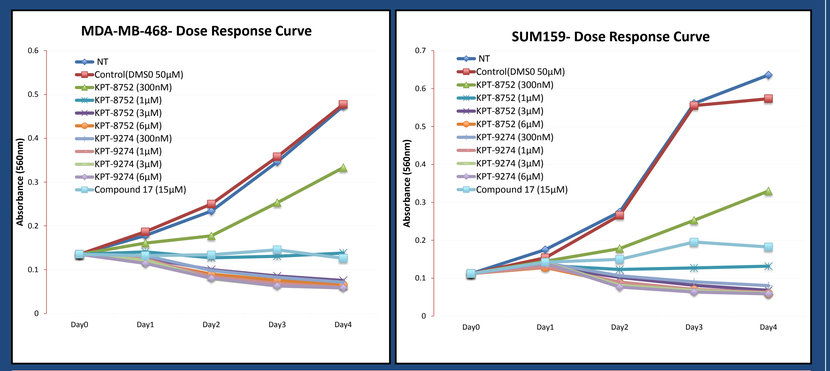


Fig 2. **Treatment with PAMs block cell growth of triple negative breast cancer cells**
Effect of PAK4 inhibition on cell growth was analyzed by MTT assay. MDA-MB-468 and SUM159 cells were plated in tissue culture plates, treated with PAMs, KPT-8752 and KPT-9274; and Genentech Inhibitor, Compound 17 at the specified concentrations, and absorbance was measured at 560nm which corresponds to the number of viable cells. Treatment with PAMs blocked cell growth of MDA-MB-468 and SUM159 cells

PAK4 Allosteric Modulators induce apoptosis in triple negative breast cancer cells

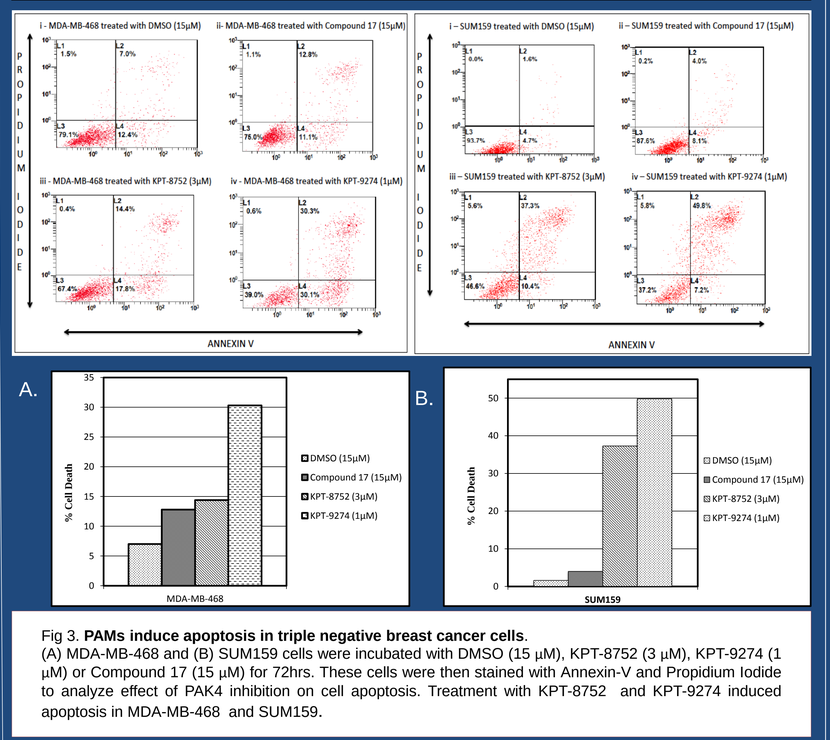


Fig 3. **PAMs induce apoptosis in triple negative breast cancer cells**.
(A) MDA-MB-468 and (B) SUM159 cells were incubated with DMSO (15 μM), KPT-8752 (3 μM), KPT-9274 (1 μM) or Compound 17 (15 μM) for 72hrs. These cells were then stained with Annexin-V and Propidium Iodide to analyze effect of PAK4 inhibition on cell apoptosis. Treatment with KPT-8752 and KPT-9274 induced apoptosis in MDA-MB-468 and SUM159.

PAK4 Allosteric Modulators block cell migration in triple negative breast cancer cell lines

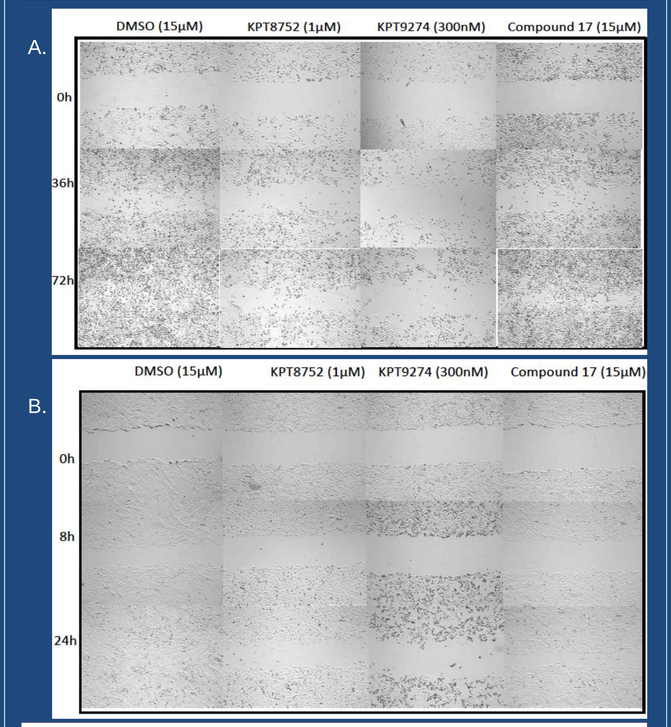
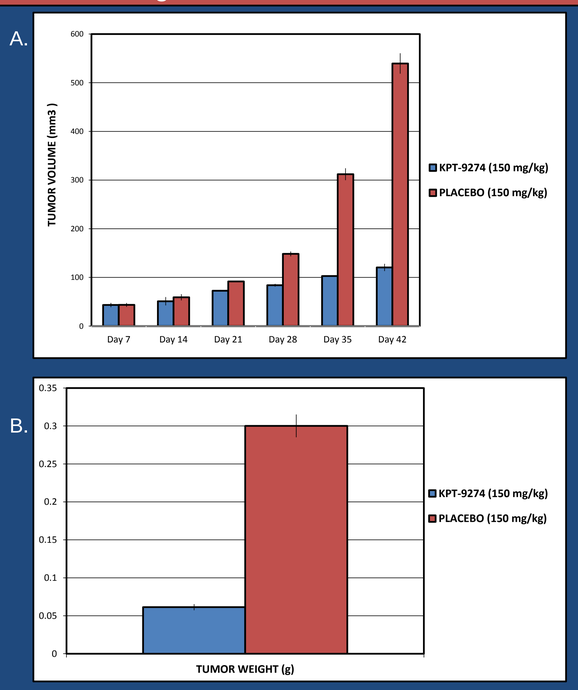


Fig 4. **Treatment with PAMs impair motility of triple negative breast cancer cells**
Effect of PAK4 inhibition on cell migration of (A) MDA-MB-468 and (B) SUM159 was analyzed by Wound Healing Assay. Confluent monolayers of cells treated with DMSO (15 μM), KPT-8752 (1 μM), KPT-9274 (300 nM) or Compound 17 (15 μM) for 72 h were scratched using sterile pipet tips. Phase contrast micrograph images were recorded at the indicated time after wounding, to monitor migration of cells into the wounded area.

Treatment with orally available KPT-9274 significantly reduces tumor growth in female nude mice



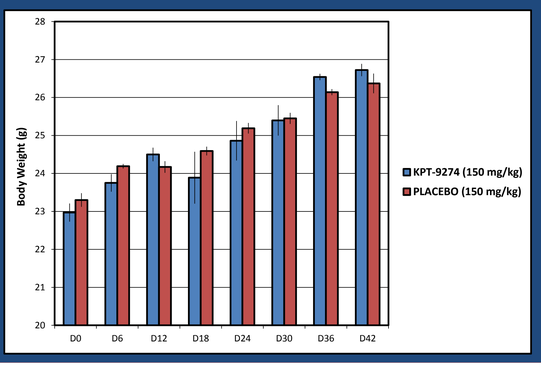


Fig 5. **Orally administered KPT-9274 significantly reduces MDA-MB-231 tumor growth.** 10^6 MDA-MB-231 cells injected on both the flanks of female nude mice (n = 8, Treatment group; n = 10, Control group). 7 days following tumor injection, mice were treated with orally bioavailable KPT-9274 or placebo (150 mg/kg) b.i.d. four days a week. Following 6 weeks of treatment with orally administered KPT-9274, there was almost a five-fold reduction in the (A) Tumor Volume and (B) Tumor Weight of treatment group as compared to the control group. (Average Tumor Volume in Control group, 539.5 mm^3 ± 20 S.E.; Average Tumor Volume in Treatment group, 120.6 mm^3 ± 8 S.E; Average Tumor Weight in Control group, 300.15 mg ± 15 S.E.; Average Tumor Weight in Treatment group, 61.4 mg ± 4 S.E.). (C.) Treatment with KPT-9274 did not significantly affect the body weight of the mice.

Treatment with KPT-9274 significantly reduces PAK4 levels, but does not affect PAK1 levels

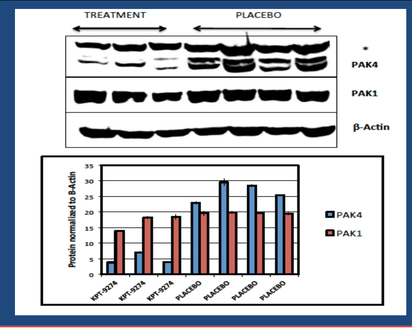


Fig 2. **KPT-9274 treatment blocks PAK4 protein levels, without affecting PAK1 levels**. MDA-MB-231 tumors were analyzed by Western Blot to monitor PAK4 and PAK1 protein levels. Treatment with KPT-9274 significantly reduced PAK4 protein levels without affecting PAK1 in the treatment group versus the control group..

CONCLUSIONS

- PAK4 plays an important role in driving MDA-MB-231 tumor growth.
- Treatment with orally administered KPT-9274 blocks PAK4 protein levels and significantly reduces tumor load in athymic mice.
- Triple negative breast cancer cells, MDA-MB-468 and SUM159, expressing high levels of PAK4,respond effectively to KPT-9274 in vitro with significant reduction in cell proliferation, cell migration and induction of apoptosis.
- Orally available PAK4 inhibitor,KPT-9274 can be utilized as a potential clinical agent for triple negative breast cancer treatment, which form aggressive tumors and have a poor prognosis.

FUTURE DIRECTIONS

- Understand the mechanism of action of PAK4 driving tumor growth.
- Analyze in vivo efficacy of KPT-9274 on additional triple negative breast cancer models .
- Validating the use of PAK4 targeting compounds as a clinical method for triple negative breast cancer treatment .

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