

# AKT inhibitor has potent anti-tumor activity in human lung cancer xenograft models

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Compounds For Cures

## Summary

Lung cancer accounts for more than 215,000 new cases annually in the United States, and is the single largest cause of cancer death accounting for more than 130,000 deaths each year (NCL.gov). Non-small cell lung cancer (NSCLC) accounts for 80% of all bronchogenic neoplasms with 90% of diagnosed patients dying within five years; therefore, new therapies are strongly desired.

Effective therapies will come from a greater understanding of the molecular mechanisms underlying the disease and targeting specific signal transduction pathways (Le 2003, Petty 2004). In normal and cancer cells, the AKT signal transduction pathway, regulates both growth and survival mechanisms, e.g., cell cycle regulation, protein synthesis, glycogen metabolism, and apoptosis.

AKT is frequently up-regulated in a broad range of human tumors, thereby increasing the survival of cancer cells that would normally undergo apoptosis; therefore, down regulation of AKT signaling may be a viable strategy for cancer treatment and prevention (Altomare 2005, Toker 2006, Crowell 2007). Mitogen-activated protein (MAP) kinases also important regulators of cellular activity, such differentiation, proliferation, and cell survival and apoptosis and the action of oncogenes (Pearson 2001).

We have demonstrated that Kevetrin™, a novel small molecule, inhibited phosphorylation of AKT, inhibited p38 MAP kinase activity, and exerted potent anti-tumor activity against two human lung xenograft tumor models, NCI-H1975 and A549, at a dose and schedule that was well-tolerated. In addition, Kevetrin was more effective than paclitaxel in these drug resistant tumor models.

A Phase I trial with Kevetrin in solid tumors is planned to begin in 2010.

## Materials and methods

### Cytotoxicity assays

The effect of Kevetrin on the viability of A549 and NCI-H1975, both human lung carcinoma cell lines, and MDA-MB-231, human breast carcinoma, was determined by measuring the bioreduction of tetrazolium in the presence of an electron coupling reagent phenazine methosulfate by dehydrogenase enzymes found in metabolically active cells (Promega). Briefly, cells were seeded in 96-well tissue culture plates and incubated overnight. The exponentially growing cells were exposed to different Kevetrin concentrations in duplicate for 24 to 72 hours. The tetrazolium reagents were added and absorbance measured at 490nm after 2-3 hours at 37°C. The IC<sub>50</sub> value was derived from a 50% reduction in viability based on OD curves as a function of dose.

### AKT assay

Activated AKT, also known as protein kinase B (PKB), was measured by a sandwich ELISA specific for phosphorylated threonine<sub>308</sub> or serine<sub>473</sub> AKT proteins (PhosphoDetect AKT<sup>Thr308</sup>, Calbiochem). Tumor cells were incubated with Kevetrin overnight. To initiate IGF-1R mediated signal transduction activating the PI3K pathway, the cells were treated with 100 ng/ml IGF-1 for 30 minutes. Cells were lysed, centrifuged, and supernatants were then assayed by ELISA. The IC<sub>50</sub> value was derived from a 50% reduction phosphorylated protein based on OD curves as a function of dose.

### p38 MAP kinase assay

p38 MAP kinase activity, also known as RK (CDC-related protein kinase), was measured by a sandwich ELISA specific for phosphorylation of p38 at threonine<sub>180</sub> and tyrosine<sub>182</sub> in the TGY motif, resulting in p38 activation. Tumor cells were incubated with Kevetrin overnight. Cells were lysed, centrifuged, and supernatants were then assayed by ELISA. The IC<sub>50</sub> value was derived from a 50% reduction phosphorylated protein based on OD curves as a function of dose.

### Anti-tumor activity assays

Anti-tumor activity was assessed in NCI-H1975 and A549 xenograft tumor models. Nude mice were implanted with 5x10<sup>6</sup> cells subcutaneously on the flank. Once tumor became established, ~1 week, mice were treated with either 200 mg/kg Kevetrin intraperitoneally (IP) three times every other day or 22 mg/kg paclitaxel intravenously (IV) four times every other day. Tumors were measured in 2 dimension with calipers and volume was calculated using the formula: Volume (mm<sup>3</sup>) = (w<sup>2</sup> × l) / 2.

### Pharmacokinetics

A single IV dose of 50 mg/kg Kevetrin was administered to CD-1 mice. Blood samples were obtained via the retro orbital sinus using heparinized capillary tubes at 1, 3, 5, 10, 20, 40 min, 1, 2, 3, 4, 8, 24 hr post injection. Samples were immediately centrifuged after collection. The plasma was collected and stored at -80°C until analysis. Kevetrin plasma levels were determined using reverse phase chromatography. Pharmacokinetic parameters were determined based on drug concentration over time using PK Solutions 2.0 software (Summit Research Services, Montrose CO).

## Results

### Cytotoxicity

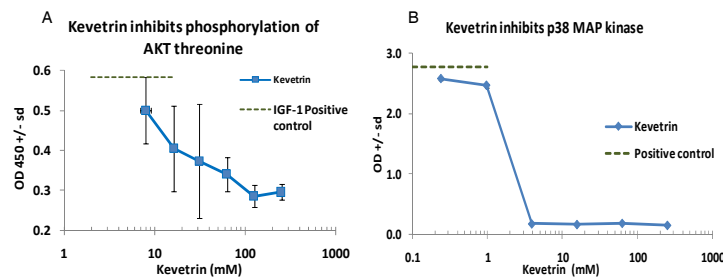
The cytotoxicity of Kevetrin and chemotherapy agents are shown in Table 1. Kevetrin was less cytotoxic than cisplatin, paclitaxel, or 5-FU.

**Table 1.** IC<sub>50</sub>s (uM) of Kevetrin, cisplatin, paclitaxel, and 5-FU in NCI-H1975, A549, MDA-MB-231 tumor cells after 24, 48, or 72 hour exposure.

	NCI-H1975 IC <sub>50</sub> (uM)			A549 IC <sub>50</sub> (uM)			MDA-MB-231 IC <sub>50</sub> (uM)		
	24 hr	48hr	72hr	24 hr	48hr	72hr	24 hr	48hr	72hr
Kevetrin	2738	226	211	3976	1107	565	2875	180	110
cisplatin	158	20	11	172	87	13	52	8	3
paclitaxel	65	5.7	0.1	311	138	2	10	0.3	0.001
5-FU	740	113	2	1313	1259	175	1527	59	3

### Kinase activity

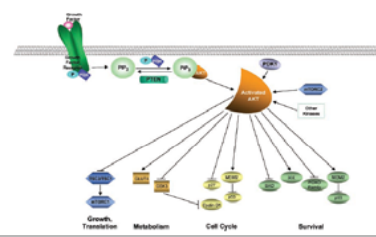
Kevetrin inhibited the phosphorylation of threonine<sub>308</sub> AKT at an IC<sub>50</sub> of 15mM; however, Kevetrin did not inhibit the phosphorylation of serine<sub>473</sub>. Kevetrin also inhibited p38 MAP kinase activity with an IC<sub>50</sub> of 2 mM.



**Figure 1.** A) Phosphorylation of threonine<sub>308</sub> AKT or B) p38 MAP kinase activity in MDA-MB-231 tumor cells as measured by ELISA

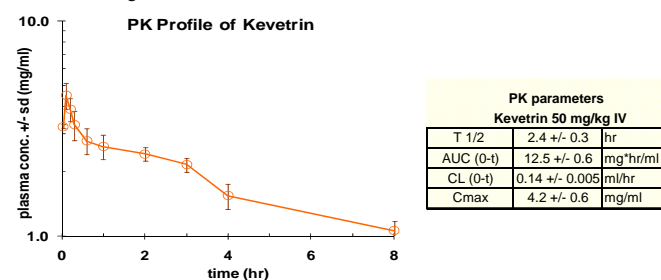
### AKT signaling pathway

Growth factor binds to receptor, PI3K is activated, PIP3 binds to AKT, AKT is phosphorylated on Thr<sub>308</sub> and Ser<sub>473</sub>. (Crowell 2007 Mol Cancer Ther 6: 2139-2148)



### Pharmacokinetics

Results of an initial study with Kevetrin given IV showed a biphasic time vs. drug concentration curve. The elimination half-life (t<sub>1/2</sub>) was 2.2 hrs and the AUC was 13.4 mg\*hr/ml.

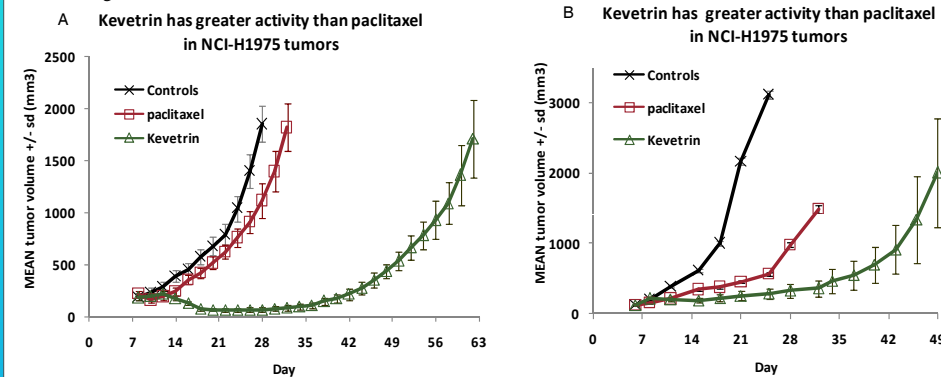


**Figure 2.** Pharmacokinetics profile of Kevetrin in mice following a single dose of 50 mg/kg Kevetrin IV in CD1 mice. Inset: Pharmacokinetics parameters based on data shown in Figure 2.

## Results

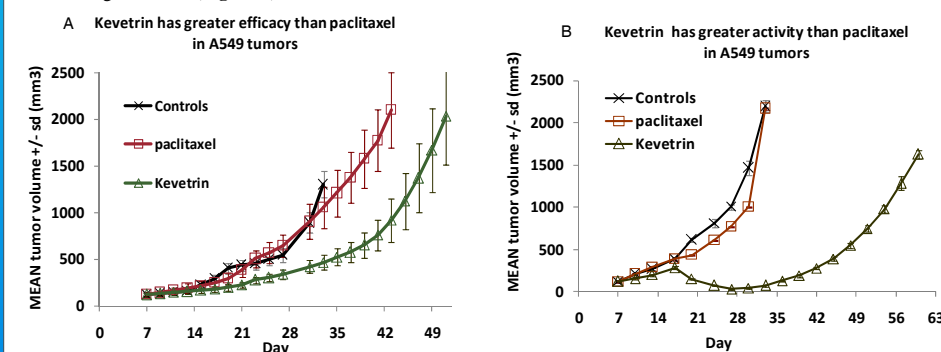
### Anti-tumor activity

In NCI-H1975 tumors, Kevetrin significantly delayed median tumor growth by 34 days (142%) in the first experiment (A) and 28 days (156%) in a repeat experiment (B), whereas the tumor growth delay (TGD) of paclitaxel was just 4 days (17%) and 14 days (78%), respectively (Figure 3). No weight loss occurred during treatment with Kevetrin.



**Figure 3.** Efficacy of Kevetrin in NCI-H1975 tumors human tumor xenografts. Tumor bearing nude mice were treated with either 200 mg/kg Kevetrin IP qod for 3 doses or 22 mg/kg paclitaxel IV qod for 4 doses after tumors reached ~160mm<sup>3</sup> in volume. [p < 0.01 for Kevetrin vs. control and vs. paclitaxel]

In A549 tumors, Kevetrin significantly delayed median tumor growth by 11 days (33%) in the first experiment (A) and 30 days (111%) in a repeat experiment (B), whereas the TGD of paclitaxel was just 0 days (0%) and 3 days (11%), respectively (Figure 4). In this study, Kevetrin resulted in <5% weight loss during treatment (Figure 5).

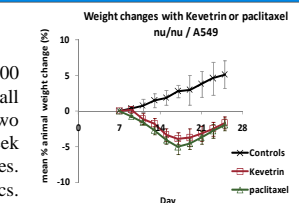


**Figure 4.** Efficacy of Kevetrin in A549 tumors human tumor xenografts. Tumor bearing nude mice were treated the same as in Figure 3 after tumors reached ~120 mm<sup>3</sup> in volume. [p < 0.01 for Kevetrin vs. control and vs. paclitaxel]

### Toxicity

CD1 mice were treated with a single dose of Kevetrin at 150, 200, or 300 mg/kg given IP. All mice survived all doses. Although there was a small dose dependent loss in body weight, the weight loss was <5% with the two higher doses. In addition, any weight lost was regained within one week after the lowest dose and within two weeks after the two highest doses. Myelosuppression was less compared to conventional chemotherapeutics. No dose dependent changes in any of blood chemistry parameters were measured. Histologically, organs showed few changes.

Rats were also treated with a single dose of Kevetrin at 160, 200, or 240 mg/kg given IP. 90% survived the lowest dose; 50% survived the medium dose; and 30% survived the highest dose. The highest dose produced a 12% weight loss and the lowest dose produced 4% weight loss. The lower doses recovered their weight losses within 8 days while the highest dose did not recover before the end of the study at day 15. Dose dependent decreases in total protein, globulin, and glucose and dose-dependent increases in alkaline phosphatase, AST(SGOT), bilirubin and creatinine were observed. These observations suggest some adverse effects on liver and kidney at the highest dose.



**Figure 5.** Animal weight changes in mice in Figure 4 A.

## Conclusions

Kevetrin exerted potent anti-tumor activity against two human lung xenograft tumor models, NCI-H1975 and A549, at a dose and schedule that was well-tolerated as indicated by a small transient weight loss during treatment and toxicity results at therapeutic doses. Kevetrin had greater anti-tumor activity compared to paclitaxel despite being less cytotoxic suggesting Kevetrin may be targeting specific components of the signal transduction pathway.

NCI-H1975 have the T790M and L858R mutations in epidermal growth factor receptor (EGFR) (Kwak 2005) and A549 have the K-ras mutation and overexpress MRP3, STAT3 and Nrf2 (Mahaffey 2009, Kim 2009, Homma 2009); these mutations are associated with resistance to standard chemotherapy.

The reduction of activated AKT and/ or p38 MAP kinase activity may be the mechanism(s) by which Kevetrin overcame resistance and showed efficacy in these tumor models. Although full activation of AKT requires phosphorylation of two conserved residues (Thr<sub>308</sub> and Ser<sub>473</sub>), Kevetrin had potent anti-tumor activity even though it inhibited the phosphorylation of only the threonine. Other targets may also be involved.

These studies demonstrate that Kevetrin has promising potential for the treatment of lung carcinoma, particularly in cases where tumors have become resistant to standard chemotherapy.

Based on the encouraging results, a Phase I clinical trial is planned to commence in 2010 in patients with solid tumors.

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### For further information

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