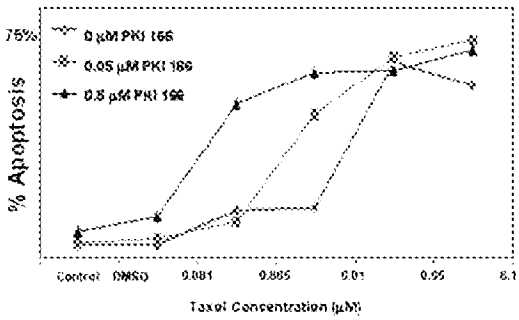
PKI166 [0, 0.05, 0.5 mM]. The addition of 0.5mM PKI166 increased tumor cell death in a synergistic fashion, shifting the amount of paclitaxel needed to induce apoptosis in 50% of cells from 0.1 to 0.001 mM.



Graph 1. Synergistic induction of apoptosis.

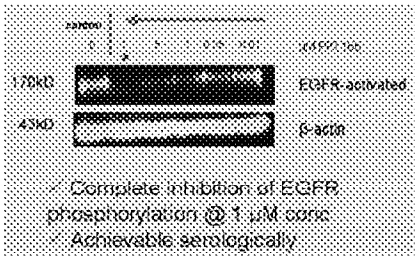


Image 1. PKI166 inhibits EGFR signalling.

Next, JMAR were pretreated for 1 hour and then stimulated with EGF. As measured by Western immunoblotting, EGF-R-specific tyrosine kinase autophosphorylation was inhibited by PKI166 in a dose-dependent fashion and at all doses tested (0.01 to 1 mM). PKI166 also inhibited phosphorylation of AKT(ser473)for in a dose-dependent manner.

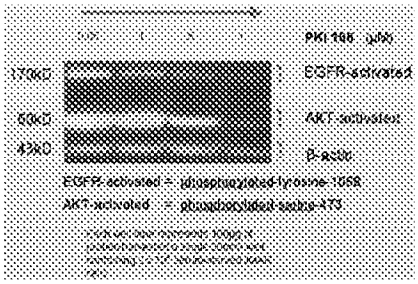
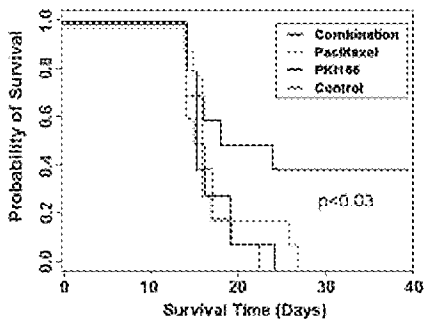
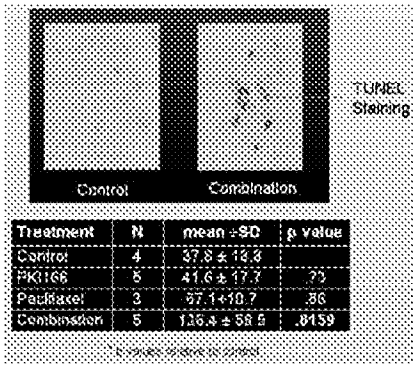


Image 2

Finally, these findings were confirmed using an orthotopic model of oral cancer. JMAR oral cancer cells were implanted in the tongues of nude mice. After lingual tumors developed, 40 mice were randomized into four groups. The animals were treated with 1) oral PKI166 [100mg/kg]; 2) intraperitoneal paclitaxel [2 mg/kg]; 3) PKI166 and paclitaxel; or 4) placebo and followed for survival. Mice treated with PKI166/paclitaxel demonstrated a significant increase in survival (p=0.028).



After necropsy, all tongue tumors were evaluated for apoptosis by TUNEL assay and examined under fluorescence microscopy. Tumor specimens from mice treated with paclitaxel and PKI166 revealed a greater apoptotic fraction of tumor cells than mice receiving placebo or single-drug therapy (136.4 vs. 37.8; p=0.016).



Treatment	N	mean ±SD	p value
Control	4	37.8 ± 18.8	
PKI166	5	41.6 ± 17.7	.73
Paclitaxel	3	97.1±10.7	.86
Combination	5	136.4 ± 58.5	.0139

Conclusions: Combination therapy with paclitaxel and PKI166 leads to prolonged survival in an orthotopic preclinical model of tongue cancer. This targeted molecular therapy increases programmed cell death of oral cancer *in vitro* and *in vivo* and may be mediated through abrogation of PI3kinase/AKT signaling.

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β2-Microglobulin induces apoptosis in CCRF-HSB-2 leukemia cells by molecular mechanisms different than doxorubicin- and Taxol-induced apoptosis mechanisms

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We have previously been shown that β2-microglobulin (β2m) triggers apoptosis in cancer cells, but the molecular mechanism of β2m-induced apoptosis is not clear. In this study, we investigated the mechanism of β2m-induced apoptosis in the CCRF-HSB-2 human lymphoblastic leukemia cell line and compared it to the apoptotic effects of doxorubicin (DOX) and Taxol in these cells. We found that exogenous β2m induces significant apoptosis in this cell line. β2m treatment induced the release of cytochrome c from the mitochondria, but no change in mitochondrial membrane potential was observed during apoptosis, suggesting that cytochrome c may be released through a mechanism independent of mitochondrial permeability transition (MPT) pore formation. Moreover, the β2m-induced release of cytochrome c from the mitochondria in these cells was caspase-independent, since Z-VAD-fmk, a general inhibitor of caspases, did not block the release of these factors. However, Z-VAD-fmk treatment significantly blocked β2m-induced apoptosis while Western blot analysis revealed that caspases-1, -2, -3, -6, -8, -9, and -10 are not activated during β2m-induced apoptosis in these cells. These results indicate that a caspase-dependent mechanism independent of these caspases is involved in β2m-induced apoptosis. Interestingly, both DOX and Taxol induced apoptosis and activated caspases-6 and -8, indicating that these drugs cause apoptosis by a different mechanism than β2m. Moreover, β2m significantly enhanced the production of reactive oxygen species (ROS) during 12-48 hr treatment, and β2m-induced apoptosis was almost totally inhibited in cells pre-treated with the antioxidant N-acetylcysteine (NAC), providing evidence that β2m-induced apoptosis in these cells is ROS-dependent. Therefore, these results reveal that β2m-induced apoptosis in CCRF-HSB-2 cells occurs through an unknown caspase-dependent, ROS-dependent mechanism(s) which is associated with cytochrome c release from mitochondria. Based on their different apoptosis mechanisms, combination use of β2m and these anticancer agents may provide a more effective therapeutic strategy to eliminate cancer cells. (Supported by grant CA080734 from NCI).