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Inhibition of prostate xenograft growth by two novel orally bioavailable microtubule disruptors

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Background: Treatment options for hormone independent prostate cancer remain limited, with only the taxanes offering some benefit to patients with advanced disease. 2-Methoxyestradiol-3,17-O,O-bis-sulfamate (2-MeOE2bisMATE) and 2-ethylestradiol-3,17,O,O-bis-sulfamate (2-EtE2bisMATE) are novel compounds which possess anti-tumor and anti-angiogenic activities.
Materials and Methods: Proliferation was quantified in vitro by MTS 96 well plate assays. FACS analysis was used to study both cell cycle arrest and to quantify apoptosis. Hif-1 alpha and Cyclin B1 expression was determined by immunoblotting of nuclear and whole cell extracts respectively. To assess in vivo efficacy, mice bearing PC-3 and DU-145 xenografts (80–100 mm^3) were treated for up to 60 days. The microtubule disruptors, Taxotere and Vinorelbine, and the anti-angiogenic agents 2-MeOE2 and Avastin were included in the study for comparison.
Results: In vitro studies demonstrated that both 2-MeOE2bisMATE and 2-EtE2bisMATE inhibited proliferation of PC-3 and DU-145 cells. These compounds induced cell cycle arrest and subsequent apoptosis in the prostate cell lines. Furthermore, there was decreased expression of Hif-1 alpha protein. In vivo both compounds are efficacious by daily oral administration, with 22 mg/kg 2-MeOE2bisMATE and 50 mg/kg 2-EtE2bisMATE causing significant regression in the PC-3 model. In contrast, the DU-145 tumours were more resistant to all the therapies studied, especially taxotere (30 mg/kg i.v.). 2-EtE2bisMATE (50 mg/kg p.o.) was more potent than 2-MeOE2bisMATE (22 mg/kg p.o.) in these tumors. 2-EtE2bisMATE (50 mg/kg p.o.) was equipotent to navelbine (80 mg/kg i.v.) but without the associated toxicity. Avastin (5 mg/kg i.v.) was only efficacious in the DU-145 model, indicating that VEGF may play an important role in this cell line.
Discussion: Here we describe and characterise two novel compounds, which combine anti-tumor and anti-angiogenic activities in one orally bioavailable molecule. Both compounds directly target the tumor by causing cell cycle arrest and apoptosis. The previously described anti-angiogenic activity of these compounds may, in part, be explained by the down-regulation of Hif-1 alpha protein expression. These properties may translate into a significant clinical improvement over existing agents used to treat advanced prostate cancer, such as the taxanes.

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Comparative antiproliferative activities and cellular distribution of the third-generation epothilone ZK-EPO and taxanes

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Background: ZK-EPO, a synthetic third-generation epothilone in Phase II clinical trials, is a microtubule stabilizer that induces cell-cycle arrest in G2/M. Preclinical data indicate more potent antitumor activity than taxanes (eg paclitaxel) and second-generation epothilones such as ixabepilone (BMS-247550). ZK-EPO eludes the cellular efflux pumps normally responsible for multidrug resistance (MDR) and has activity against both taxane-resistant and taxane-sensitive tumor models. We compared the subcellular distribution of ZK-EPO and paclitaxel to assess the efficiency of cellular uptake and binding to subcellular compartments.
Materials and Methods: A panel of cultured human breast and lung tumor cells (including taxane-sensitive and -resistant models) was treated with ZK-EPO or other tubulin-binding agents, at a range of concentrations, to determine antitumor activity and uptake/distribution characteristics. Cells were incubated with tritiated ZK-EPO or paclitaxel, and radioactivity measured in the whole cell and in the subcellular fractions. For immunofluorescence (IF) studies, cultures were exposed to agents prior to fixation and stained with antibodies for tubulin isotypes.
Results: ZK-EPO was significantly more active than paclitaxel against breast and lung tumor cell proliferation in all cell lines examined. Activity against MCF7 cells was high for ZK-EPO (IC_{50} < 1 nM) compared with paclitaxel or ixabepilone (IC_{50} > 3 nM). Against MDR NCI/ADR cells, ZK-EPO also had subnanomolar potency, unlike epothilone B (IC_{50} > 1 nM), paclitaxel or ixabepilone (IC_{50} > 1 μM). In radiolabeling experiments, ZK-EPO almost completely localized to the nuclear/cytoskeletal fraction compared with the cytosolic fraction. In contrast, a majority of the tritiated paclitaxel was detected in the cytosolic fraction in all cell types examined. In MaTu/ADR and NCI/ADR cells, total cellular uptake of ZK-EPO was 7.5 and 290 times greater than uptake of paclitaxel, respectively, suggesting that ZK-EPO is not recognized by MDR mechanisms in taxane-resistant cells. IF studies showed that ZK-EPO induces microtubulin polymerization.
Conclusions: ZK-EPO displays a rapid cellular uptake compared with paclitaxel, and preferentially accumulates in the nucleus in a range of tumour cell types. ZK-EPO uptake may be unaffected by MDR-pump activity, suggesting efficient maintenance within tumor cells. ZK-EPO may be clinically effective as an alternative to taxanes.

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Randomized phase II study of BMS-247550 (NSC 710428) given daily X 5 days or weekly in patients with metastatic or recurrent squamous cell cancer of the head and neck: E2301

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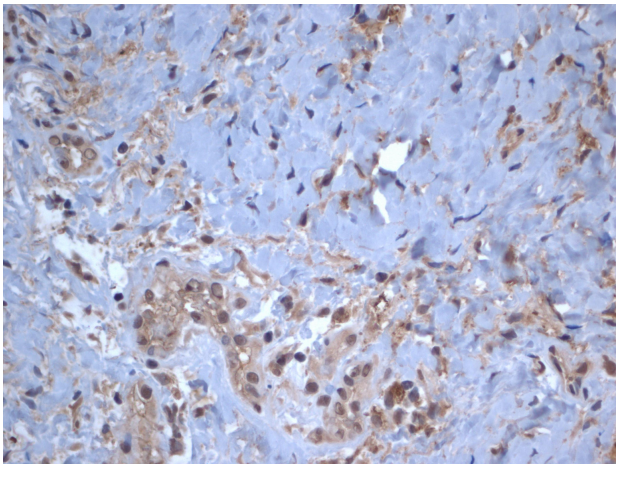
Background: Ixabepilone is a novel tubulin-polymerizing agent, with potential activity in SCCHN.
Methods: Patients (pts) were eligible who had incurable, measurable SCCHN. ⩽2 prior regimens for metastatic/recurrent disease were permitted, including taxanes. ECOG PS 0 or 1, ANC > 1500/ml and adequate renal/hepatic function were required. Pts were randomized to ixabepilone 6 mg/m^2/d 60 minute (min) infusion × 5 days (d) q 21 d [Arm A], or 20 mg/m^2 60 min infusion d 1, 8, and 15 q 28 d [Arm B], with diphenhydramine premedication. Tumor assessment was q 6 weeks. Each arm accrued taxane-naive (Tax-N) and -exposed (Tax-E) patients as separate strata, in a 2-stage design. The primary endpoint was response. 16 pts entered each group in the first stage. If 1 response was observed in any group, it continued to 32 pts.



Results: 84 eligible pts entered. Male 68, ECOG PS (1) 57, metastatic disease 65. 15 Tax-E and 17 Tax-N eligible pts entered stage 1 of Arm A. There was 1 response (6.7%) among the Tax-E pts and none among the Tax-N with this dose and schedule. 34 eligible Tax-N pts were accrued to Arm B and 5 had partial responses (14.7%, 90% CI: 6.1%, 28.8%), 11 stable and 9 progressive disease. 9 in this group were inevaluable (5 died before restaging, 2 never treated). 18 eligible Tax-E pts entered Arm B and none responded. On Arm A, 2/16 Tax-N pts developed grade (gd) 3/4 anemia (13%), and 4/16 (25%) developed gd 3 fatigue. Other gd 3/4 toxicities occurred in <8% of pts on Arm A. On Arm B, 9/33 (27%) Tax-N pts experienced gd 3/4 leukopenia, 3/33 (9%) gd 3/4 anemia, 7 gd 3/4 fatigue (21%), 4 gd 3 nausea (12%), 9 (27%) gd 3 sensory and 2 (6%) gd 3 motor neuropathy. 2/21 Tax-E pts on Arm B experienced gd 4 neutropenia (10%), 6 gd 3 fatigue (29%), 3 gd 3 motor (14%) and 2 gd 3 sensory neuropathy (10%). Median follow up for eligible pts is 31.7 months (mo). 77 have died. Median progression-free survival for Arm A Tax-N is 1.5 mo, Arm A Tax-E is 1.8 mo, Arm B Tax-N 1.9 mo, Arm B Tax-E 1.6 mo. Median survival for Arm A Tax-N is 5.62 mo (CI 4.0, 10.0); for Arm A Tax-E is 6.5 mo (CI 2.7, 8.8); for Arm B Tax-N is 7.8 mo (CI 3.8, 9.9) and Arm B Tax-E is 6.8 mo (CI 2.0, 11.3). Baseline tissue is stained for survivin and

data correlating survivin expression with response and survival will be presented.

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Effect of estrogen on cathepsin B activity and antitumor efficacy of Paclitaxel Poliglumex in human tumor xenografts

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Background: Paclitaxel poliglumex (PPX, XYOTAX™) is a novel macromolecular chemotherapeutic that covalently links paclitaxel to poly-L-glutamic acid. Retrospective analysis of 2 phase III studies in chemo-naïve pts with advanced non-small cell lung cancer (NSCLC) suggests that PPX is more active in female than in male pts, particularly in those presumed to be pre-menopausal. Our working hypothesis is that estrogens may play an important role in the metabolic release of paclitaxel from the polymeric backbone and, therefore, in the improvement of the antitumor efficacy of PPX. The estrogen-modulated proteolytic enzyme cathepsin B appears to be the major contributor to proteolysis of PPX, resulting in PTX release.
Methods: This study examined the influence of 17β-estradiol (E2) on cathepsin B activity and PPX antitumor efficacy in the estrogen receptor β (ERβ) positive human xenograft tumors HT-29 (colon) and H460 (NSCLC). E2 or placebo pellets were subcutaneously implanted in female nude mice on day 0, and at day 5 animals were transplanted with tumor fragments. A single PPX iv dose of 90 mg/Kg PTX equivalents was then administered on day 21. At different time points, tumor, lung, liver and blood were collected to evaluate E2 plasma levels, ER activation status and cathepsin B activity.
Results: The growth of both tumors was substantially enhanced by E2, with the greatest effect on H460. ER analysis showed that the form is predominant in both tumors and that the higher tumor growth rate is sustained by ERβ activation. Cathepsin B activity was enhanced by E2 (by 35−40%) in both H460 and HT-29 tumors. The antitumor efficacy of PPX in HT-29 tumor was greater in E2-supplemented mice and greater in female than in male mice. The effect of E2 and gender on PPX efficacy in the H460 tumor model, and the effect of E2 on PPX metabolism, are currently under study and will be included.
Conclusions: These *in vivo* findings indicate a correlation with enhanced estrogen-dependent tumor growth (mediated by ERβ activation) increased cathepsin B activity and greater PPX antitumor efficacy in xenograft tumors. These preclinical data provide a mechanistic rationale that partly explains the clinical evidence of enhanced PPX efficacy in women, especially premenopausal women, with advanced NSCLC.

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Safety and Pharmacokinetic (PK) Trial of KOS-1584, a Novel Analog of Epothilone D

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Background: Several epothilones are progressing through phase 1–3 clinical trials treating various solid malignancies. KOS-1584 is a third generation analog of EpoD, with 3–12 fold increased potency (as measured by *in vitro* cytotoxicity, *in vivo* xenografts or induction of G2/M arrest by flow cytometry) and improved pharmacologic/PK profile (enhanced tumor tissue penetration and reduced exposure to the CNS). We report the results of the initial dose-escalation trial in which KOS-1584 was administered to patients (pts) with advanced solid malignancies.
Methods: KOS-1584 was administered as a 3-hour intravenous infusion every 3 weeks. PK of KOS-1584: after the 1st and 2nd infusion. Pharmacodynamics: assessed by serial sampling of peripheral blood mononuclear cells (PBMC) for microtubule bundle formation (MTB).
Results: 37 pts (23 F; median age 60; ECOG PS 0–1; prior regimens 4 (range 0–7) enrolled in 10 dose levels (between 0.8−20 mg/m^2). No Cycle 1 DLT has been observed so far. Toxicities (n = 35) did not show obvious dose dependency. Common toxicities (grade 1–2) included fatigue, and GI (diarrhea, constipation, nausea, anorexia). Drug-related Grade 3 toxicity: increased PTT, fatigue and increased AST (1 each). Drug-related neurotoxicity was not notable. PK/parent (n = 33): t* 18.9±6.3 h, Vz 677±347 L and CL 26.6±15.8 L/h. 20 mg/m^2 Cmax 274±37 ng/mL; AUCtot 2676±1110 ng/mL*h. Cmax and AUCtot increased linearly with dose. Dose dependent increases in %MTB were observed (20 mg/m^2: 55% at end of infusion, compared to 50−60% for Epothilone D using the same assay). Sigmoidal Emax model described the relationship between plasma concentration and %MTB. Antitumor activity included an unconfirmed partial response in a patient with ovarian cancer and declines in CA125 (n = 2).
Conclusions: Accrual is continuing to define the optimal dose on the 3-week regimen.

Dose level	KOS1584 (mg/m^2)	# pts	DLT	Comments	Time on study/Response
1	0.8	1	N		Adrenocortical: 4 cycles
2	1.5	4	N		Leiomyosarcoma: 6 cycles Colon 5 cycles
3	2.5	4	N		NSCLC: 4 cycles
4	3.7	3	N		Ovarian: 6 cycles (28% ↓CA125)
5	5.0	4	N		Liposarcoma: 4 cycles
6	6.5	6	N	Septic pneumonia/ NTP (DLT later ruled out)	Ovarian: 6 cycles Ovarian: 4 cycles (unconfirmed PR, 62% ↓CA125)
7	8.5	3	N		
8	11.3	4	N	Grade 3 fatigue/ weakness (DLT later ruled out)	Ovarian: 7 cycles Gastric: 4 cycles
9	15.0	4	N		Leiomyosarcoma: 4+ cycles
10	20.0	4	Pending		

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Synthesis and cytotoxic activity of novel water-soluble peptide-based paclitaxel conjugates

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Background: Paclitaxel (PTX) is a microtubule stabilizing drug with proven therapeutic activity in breast, lung and ovarian cancers. However, the commercially available Cremophore-based PTX formulation suffers from significant drawbacks such as aqueous insolubility, hypersensitivity reactions and dose-limiting toxicity. These effects are attributed to the presence Cremophore. The aim of the study was to develop a new water-soluble Cremophore-free PTX formulation by conjugation of original tumour-derived peptide with PTX molecule and to investigate it's cytotoxic properties.
Materials and Methods: Identification and isolation of two peptides – TCTP-1 and Thx – were made using phage display method using both linear and cyclic peptide libraries. The peptides were synthesized by means of standard Fmoc solid phase peptide synthesis techniques. PTX was conjugated to the peptides via a covalent bond. The conjugation step was completely regioselective and high yielding. The cytotoxicity of TCTP-1-PTX and Thx-PTX was tested on C8161 human melanoma, A549 and NCI H460 human non-small cell lung cancer (NSCLC) cells, and pulmonary artery smooth muscle (PASMC) cells using MTT test. IC_{50} values were evaluated after 72-hour incubation.
Results: TCTP-1 peptide sequence was found in *in vitro* screening against murine fibrosarcoma cell line, *in vivo* screening against melanoma lung metastases in mouse and *in vitro* screening against matrix metalloproteinase -9. Thx peptide sequence was identified by screening against tissue samples from patients with NSCLC. TCTP-1 is a cyclic octapeptide and Thx is a linear heptapeptide. Both peptide-PTX conjugates were highly water-soluble (>50 mg/mL). Pure TCTP-1 and Thx were not cytotoxic. The cytotoxic IC_{50} values of conjugates and PTX are presented in the table.

Cell type	TCTP-1-PTX	Thx-PTX	PTX
C8161	4.8 nM	Not evaluated	8.7 nM
A549	Not evaluated	15 nM	7.5 nM
NCI H460	Not evaluated	15 nM	10 nM
PASMC	6.3 nM	35 nM	7.9 nM

Conclusion: TCTP-1-PTX and Thx-PTX are highly water-soluble peptide-based PTX conjugates with cytotoxic activity against human melanoma and NSCLC cells. TCTP-1-PTX is more active than PTX against melanoma cells.