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

633 POSTER

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

S. Di Giovine[1], P. De Feudis[1], D. Torriani[1], G. Colella[1], M. Cassin[1], L. Piazzoni[1], U. Bastrup[1], M. Natangelo[1], G. Pezzoni[1], J. Singer[2]. *[1]Cell Therapeutics (Europe) S.r.l., Preclinical R&D, Bresso (MI), Italy; [2]Cell Therapeutics Inc., Discovery & Research, Seattle (WA), USA*

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.

634 POSTER

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

M. Villalona-Calero[1], S. Goel[2], L. Schaaf[1], B. McCracken[1], K. Desai[2], G. Cropp[3], K. Kersey[3], R. Johnson[3], A. Hannah[3], S. Mani[2]. *[1]Ohio State University, Columbus, USA; [2]Albert Einstein, Bronx, USA; [3]Kosan Biosciences, Hayward, USA*

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		

635 POSTER

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

K. Gurbanov, M. Huttunen, A. Koivistoinen, K. Karisalmi, H. Peräkylä, M. Bergman. *Karyon Oy, Helsinki, Finland*

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.