636 POSTER

The antiproliferative activity of ENMD-1420, a diaryl inhibitor of tubulin polymerization, is selective for the Z isomer

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Background: ENMD-1420 (previously CC-5079) is a synthetic diaryl compound that inhibits tubulin polymerization and TNF-α production with in vitro and in vivo antitumor activity. One strategy to optimize the effects of these compounds has included defining molecular stereoselectivity for activity.
Material and Methods: ENMD-1420 was synthesized at EntreMed by a Wittig reaction to give an E/Z mixture which was purified by chromatography to give individual isomers. Alternatively, pure E and Z isomers were prepared directly though a Heck coupling reaction. Cell proliferation was assessed in cancer cell lines and endothelial cells (HUVEC) using the WST-1 reagent. The in vivo antitumor activity of ENMD-1420 was evaluated in the Lewis lung carcinoma (LLC) experimental pulmonary metastatic model.
Results: For the 1:1 E/Z isomer mixture, the IC50 values of ENMD-1420 were determined to be 21, 16 and 12 nM against MDA-MB-231, Lewis lung carcinoma and HUVEC, respectively. The Z isomer (ENMD-1427) was statistically more potent than the mixture against these cell lines (IC50 = 6, 6 and 4 nM against MDA-MB-231, LLC, and HUVEC, respectively, $p < 0.01$). The IC50 values of the E isomer (ENMD-1916) were statistically less potent against MDA-MB-231, LLC and HUVEC (IC50 = 64, 73 and 63 nM, $p < 0.01$). The increased potency observed with the Z isomer of ENMD-1420 was also observed with 5 additional analogs tested. Treatment of B6 mice bearing metastatic pulmonary Lewis lung carcinoma with the E/Z isomer mixture of ENMD-1420 (25 mg/kg qd × 5) resulted in an 84% inhibition of tumor growth with minimal body weight loss. As a single administered dose of 25 mg/kg, the pharmacokinetics of the E/Z isomer of ENMD-1420 gave a Cmax of 151 ng/mL and an AUC of 445 ng/mL*h. The in vivo antitumor activity of 5 analogs have also been assessed in this model with tumor growth inhibition ranging from 78−96% The impact of the Z isomer of ENMD-1420 and analogs on in vivo tolerability and antitumor activity is now being evaluated and will be reported.
Conclusions: The stereoselectivity of ENMD-1420 and analogs for in vitro antiproliferative activity against tumor and endothelial cells resides in the Z isomer which indicates that a synthetic strategy targeting this particular configuration should be used to optimize antitumor activity and lead compound identification.

637 POSTER

Paclitaxel poliglumex cellular uptake by normal tissues and human tumor xenograft: an IHC study in nude mice

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Background: Paclitaxel poliglumex (PPX, XYOTAX™) is a novel chemotherapeutic that links paclitaxel to a biodegradable polymer, poly-L-glutamic acid. Biodistribution studies demonstrate that PPX accumulates in reticuloendothelial organs and in syngeneic or xenogeneic tumor tissues. The aim of this study was to investigate PPX localization at the cellular level using immunohistochemistry (IHC) techniques in xenograft tumor bearing mice.
Methods: Female nude mice were subcutaneously implanted with NCI-H460 (NSCLC) human tumor fragments. Animals were administered PPX (90 mg/kg i.v. paclitaxel equivalents) and sacrificed 24 hours post-treatment. Lung, liver, spleen and tumor were collected, formalin fixed and paraffin embedded. IHC staining of tissue sections was performed with an anti-PPX monoclonal antibody (CT-2D5) which recognizes full length PPX but not PPX fragments, poly-L-glutamic acid or free paclitaxel.
Results: Positive staining for CT-2D5 was found in the cytoplasm of cells in all collected organs, with the ratio of positive cells higher in liver and spleen compared to tumor and lung. Morphology of positive cells was consistent with that of liver macrophages (Kupffer cells), spleen resident macrophages, type II pneumocytes and tumor-associated macrophages (TAMs). Positive staining for CD45 (common leukocyte antigen) and F4/80 (specific macrophage antigen) in the same organs confirmed that CT-2D5 positive cells were of macrophage lineage. CT-2D5 positive cells were mainly localized in the tumor capsule and F4/80 staining confirmed that TAMs infiltration was limited to that area. Strong CT-2D5 positive staining, mainly extracellular but in some cases intracellular, was observed in perinecrotic areas. The few positive cells were positive for CD45, but negative for F4/80. Morphologic analysis and specific staining (Myeloperoxidase) suggests that they are infiltrating polymorphonuclear leukocytes (PMN) and intracellular PPX is due to their phagocitic activity in the necrotic area.
Conclusions: Our results confirm that PPX is largely taken up by macrophages in the reticulo-endothelial organs and TAMs. In this tumor model, the role of TAMs in the delivery of PPX in areas different from the capsule was not elucidated. However, the presence of extracellular PPX in the perinecrotic areas suggests that other mechanisms of PPX penetration, such as the vascular leak and the consequent enhanced permeability and retention (EPR) of macromolecules might be involved.

638 POSTER

Phase 1 trial of a novel epothilone, KOS-1584, using a weekly dosing schedule

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Background: KOS-1584 (9,10-didehydroepothilone D) was discovered as part of a screening program to develop a new generation of epothilones with higher potency and an improved pharmacologic/pharmacokinetic (PK) profile. Epothilones stabilize microtubule polymerization, inducing rapid G2/M arrest and apoptosis. Antitumor activity of KOS-1584 (Chou *et al* 2003) is approximately 3–12 fold more potent compared to the structurally-related Epothilone D. KOS-1584 demonstrates enhanced tumor tissue penetration and reduced exposure to selected tissues (including CNS). This dose-escalation trial explores a weekly administration schedule of KOS-1584.
Methods: Define MTD, toxicity profile and PK of KOS-1584 administered to patients with advanced solid malignancies via 1-hour infusion on Days 1, 8 and 15 every 4 weeks. Pharmacodynamics are assessed by serial sampling of PBMCs for soluble and polymerized microtubules by immunoblot.
Results: 27 pts (17 F; median age 57; median ECOG PS 1; median prior regimens 4, range 1–13) enrolled in 9 dose levels (0.8, 1.5, 2.5, 5.0, 7.5, 10, 13, 16 and 20 mg/m^2). To date, no Cycle 1 DLT has been seen; one Grade 3 episode of arthritis occurred in Cycle 2. Drug-related toxicities, all Grade 1 or 2 severity (n = 24): nausea (n = 9), fatigue (n = 8), diarrhea/vomiting/constipation/anorexia/neuropathy (all n = 6). Neutropenia is more commonly observed, starting at 16 mg/m^2. Neurotoxicity is not a notable toxicity (no observations greater than grade 1 severity). PK/parent (n = 24): t1/2 23.3±7.2 h, Vz 557±217 L and CL 17.4±6.3 L/h with no evidence of dose dependency. 16.0 mg/m^2 Cmax 567±206 ng/mL; AUCtot 3108±2248 ng/mL*h. Dose proportional increase in exposure and Cmax was observed over the range tested to date. Metabolite (Seco-D KOS-1584) AUCtot was 8.9±3.2% of parent. Comparison between 1-h and 3-h infusions shows slightly slower clearance for the shorter infusion (17.4 versus 26.6 L/h) and similar Vz. Dose-dependent increases in polymerized microtubules were observed, with maximal effect at the end of infusion. Antitumor activity: patient with ovarian cancer (40% decrease in CA125), NSCLC (minor response), and stable disease (patients with head & neck and breast cancer of 5 and 4 months, respectively).
Conclusions: Accrual is continuing in order to define the optimal dose on this regimen. Exposure and Cmax remain linear within this dose range; slower systemic clearance is observed for the same dose administered over 1 vs 3 h.

639 POSTER

RTA 301 (peloruside): a novel microtubule stabilizer with potent in vivo activity against lung cancer and resistant breast cancer

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RTA 301 (peloruside) is a novel microtubule stabilizer isolated from a marine sponge. It potently inhibits the growth of many human tumor cell lines, including those that express high levels of P-glycoprotein (P-gp) or have mutations in β-tubulin. It is much more water soluble than standard taxanes and binds to a different site on β-tubulin than the taxanes and epothilones. Although the activity of RTA 301 has been characterized *in vitro*, it has never before been tested *in vivo*. We performed three xenograft studies in athymic *nu/nu* mice to assess the efficacy of RTA 301 compared to standard anticancer agents. RTA 301 was administered by intraperitoneal (i.p.) injection. The first study examined the effect of two doses of RTA 301 compared to docetaxel and paclitaxel on the growth of NCI-H460 non small cell lung carcinoma (NSCLC) tumors. RTA 301 was administered at 5 and 10 mg/kg every day for five days (QDx5), docetaxel was administered at 6.25 mg/kg i.v. every other day for a total

of three doses (Q2Dx3), and paclitaxel was administered at 8 mg/kg i.p. every day for 5 days (QDx5). RTA 301 treatment caused sustained tumor regression and resulted in tumor/control (T/C) values of 5% and 16% for the high and low dose groups, respectively, while treatment with docetaxel or paclitaxel was much less active (T/C of 82% and 50%, respectively). In the second study, different doses and schedules of RTA 301 administration were compared to docetaxel and paclitaxel using the A549 NSCLC cell line. RTA 301 was administered at 5 or 10 mg/kg i.p. QDx5, or at 10 or 15 mg/kg Q2Dx3. Docetaxel was administered at 13.5 mg/kg i.v. Q2Dx3 and paclitaxel was administered at 16 mg/kg i.p. QDx5. RTA 301 treatment again out-performed docetaxel and paclitaxel, with T/C in the best performing group of 23%, compared with 51% and 59% for these other agents. The third study was performed in P-gp overexpressing NCI/ADR-RES breast tumors. RTA 301 was administered at 5 mg/kg i.p. QDx5 or 15 mg/kg i.p. Q2Dx4 and doxorubicin was administered at 2.5 mg/kg i.p. QDx5. Treatment with RTA 301 was much better tolerated than doxorubicin, allowing administration of multiple cycles. RTA 301 inhibited tumor growth to a greater extent than doxorubicin and was better tolerated in this model, suggesting that this compound may be efficacious in drug-resistant tumors. In summary, RTA 301 significantly inhibited the growth of NSCLC tumors and P-gp overexpressing breast tumors. RTA 301 exhibited greater activity and tolerability than paclitaxel, docetaxel and doxorubicin when dosed at their MTDs in these models. Based on its significant *in vivo* activity, advanced preclinical development of RTA 301 is underway.

640 POSTER

Phase I study of ispinesib (SB-715992), a kinesin spindle protein inhibitor, in combination with capecitabine in patients with advanced solid tumors

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Background: Kinesin spindle protein (KSP) is required early in mitosis for establishment of mitotic spindle bipolarity and for cell cycle progression through mitosis. Ispinesib (ISP), a KSP inhibitor, blocks assembly of a functional mitotic spindle by inhibiting spindle pole separation and leads to mitotic arrest. In a MX-1 tumor xenograft model, sub-MTD doses of both capecitabine (CAP) and ISP led to a 2.5 to 3-fold delay in tumor growth compared to CAP alone (ISP alone was inactive). This provided the impetus for the current study.
Material and Methods: Patients (pts) with advanced solid tumors, PS < 1, and < 4 prior therapies were included in this study. Escalating oral doses of CAP (750–1250 mg/m^2) were administered bid for 14 days along with escalating doses of ISP (12−18 mg/m^2) administered as a 1 hour infusion on day 1 of a 21-day cycle. Three pts were treated at each dose level, with expansion to 6 pts in the event of dose-limiting toxicity (DLT). The (OTR) was defined as the highest dose level for which $\leqslant$1/6 pts experience a DLT. Limited pharmacokinetic (PK) samples were obtained. Clinical response assessments per RECIST criteria were performed every 2 cycles.
Results: 22 pts [(12 M/10 F); median age 60.5, ECOG PS 1, median prior regimens 4], were evaluated at 5 dose levels. A median of 2 cycles were administered (range 1–11) for a total of 68 cycles. The most common toxicities (n = 15), regardless of attribution, included fatigue (5 pts), hand foot syndrome (4), diarrhea (4), pain (3), leukopenia (3), and neutropenia (3); all were Grade (Gr) 1/2 except neutropenia (Gr 4–2 pts; Gr 3–1 pt) and leukopenia (Gr 3–3 pts). DLT of prolonged (>5 days) Gr 4 neutropenia was observed at ISP doses of 15 mg/m^2 (1 pt) and 18 mg/m^2 (1 pt) with a CAP dose of 1000 mg/m^2 bid. The OTR has yet to be defined and evaluation of 18 mg/m^2 of ISP and 1250 mg/m^2 bid of CAP is ongoing. Based on preliminary PK assessment of ISP (n = 12), the concentration of ISP is not affected by the presence of CAP when compared to data from previous single agent studies of ISP. A total of 8 pts (3 breast, 1 each head & neck, bladder, tongue, colon, thyroid) had a best response of SD (duration 2.25−8.25 mo).
Conclusions: ISP has an acceptable tolerability profile at doses up to its monotherapy MTD when combined with a therapeutic dose of CAP. Determination of the OTR is ongoing. Based on the preliminary data, there is no apparent PK interaction between ISP and CAP.

641 POSTER

Efficacy and prediction of response to the new oral taxane DJ-927 in anthacycline pre-treated advanced breast cancer (ABC)

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Background: DJ-927 is a novel taxane, which was selected for low toxicity, oral bioavailability, and potent antitumour activity. This phase 2 study aimed to assess the efficacy of DJ-927 in anthracycline pre-treated ABC and also assess cross-resistance to other taxanes.
Methods: 34 patients with measurable disease were recruited by August 2005. One patient was not evaluable. DJ-927 was given orally at a dose of 27 or 35 mg/m^2 every 3 weeks. 8 patients were subsequently treated with single agent docetaxel. The primary end-point was response rate (assessed by RECIST criteria). Secondary end-points included: duration of response, time to tumour progression, time to treatment failure, subsequent best response to docetaxel and pharmacokinetics of DJ-927 in plasma.
Results: The median age of this cohort of 33 patients evaluable for response was 50. All had prior anthracycline: 18 received it for ABC and 15 as adjuvant or neoadjuvant therapy. Overall, the patients received 163 treatment cycles of DJ-927 (median = 4 per patient). There were 7 confirmed partial responses (21.2%); and 17 stable disease (51.5%). 2 patients withdrew consent and 7 discontinued due to toxicity. The dose of 35 mg/m^2 administered initially to 7 patients was discontinued due to 1 grade 4 haematological (neutropenia >5 days) and 1 grade 4 non-haematological toxicity (pulmonary embolism). DJ-927-related toxicity (grade 3) included: neutropenia (75%), anaemia (6%), thrombocytopenia (6%), constipation (9%), anorexia (9%), sensory neuropathy (3%), motor neuropathy (3%) and neutropenic sepsis (3%). One patient died from a non drug-related event (acute pulmonary embolism). Of those subsequently treated with single agent docetaxel (on average, 4cycles per patient), 5 (62.5%) achieved partial response and 1 (12.5%) had stable disease, suggesting significant (75%) non-cross-resistance. Currently, tumour samples of the patients are being examined by immunohistochemistry for established resistance markers to taxanes, viz. p-glycoprotein, and redox proteins (thioredoxin, thioredoxin reductase, peroxiredoxins and glutaredoxin) to derive a comparative protein profile suggesting sensitivity or resistance to DJ-927.
Conclusion: Significant antitumour activity, tolerability and non-cross resistance of DJ-927 to docetaxel was observed in this study. A molecular protein profile for identifying sensitivity to DJ-927 in comparison to other taxanes may help to personalise the choice of taxane in ABC.

642 POSTER

Pharmacological profile of SVT004703, a new oral proapoptotic compound for the treatment of cancer

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Background: We have discovered a novel structural family of substituted imidazopyrimidines (Patent no. WO 2005/014598), which exhibits high antitumorogenic efficacy both *in vitro* and *in vivo*. Members of this family have shown antiproliferative and proapoptotic activity *in vitro* through the inhibition of tubulin polymerisation. The *in vitro* and *in vivo* pharmacological profile of SVT004703, the most advanced candidate, is reported.
Material and Methods: Antiproliferative activity was assessed by the sulforhodamine B (SRB) assay in two adenocarcinoma cell lines, HCT 116 and HT-29, and the squamous cell line A-431. Apoptosis was determined by the quantification of nucleosomes of treated *versus* control cells (treated with the vehicle) through ELISA. *In vitro* tubulin polymerisation assays were performed in the presence and absence of paclitaxel, known to inhibit tubulin depolymerisation. *In vivo* efficacy was assessed in a human tumor xenograft model with A-431 cells in athymic nude mice, in which the percent change in tumor size for the treated tumors compared to control (T/C) was calculated, and also in a model of UV light-induced skin cancer in hairless Skh:HR-1 mice.
Results: Cell growth inhibition assays revealed that SVT004703, and its analogs SVT004353 and SVT004352, showed high antiproliferative activity against all the tumor cell lines tested in the submicromolar range. SVT-compounds induced apoptosis of HCT 116 cells at concentrations equal or higher than their respective GI_{50} and inhibited microtubule formation by 100% and 75% of both spontaneous or paclitaxel-induced tubulin polymerisation, respectively. Oral SVT004703 treatment of UV-irradiated hairless mice significantly inhibited tumor multiplicity. After 7 weeks of