In conclusion, TZP-101 demonstrated impressive anti-cachexia activity in the mouse G361 melanoma xenograft model. The results of this study clearly warrant further investigation of TZP-101 in tumor patients.

**339** POSTER

**Identification of XL413, a selective Cdc7 kinase inhibitor which induces cell cycle arrest and exhibits potent antitumor activity**

S. Robertson[1]. [1]*Exelixis, Clinical Development, San Francisco, CA, USA*

**Background:** Cdc7 is a serine-threonine kinase that plays a critical role in the initiation of DNA synthesis. Inhibition of Cdc7 by small molecules or siRNAs leads to a block in replication and a halt in cell cycle progression. Additionally, in many tumor cell lines, apoptotic induction follows Cdc7 inhibition. Cdc7 also functions in the checkpoint response, and may be required for the activation of Chk1 in response to DNA damage. Thus, inhibition of Cdc7 may have utility in the treatment of cancer, as either a single agent or in combination with DNA damaging agents.
**Methods and Results:** XL413 was identified via high throughput screening and medicinal chemistry optimization as a potent and selective inhibitor of Cdc7 kinase activity (IC50 = 3.4 nM), and acts in an ATP-competitive and reversible manner. In multiple tumor cell lines, XL413 exhibits potent inhibition of Cdc7-dependent phosphorylation of MCM2, a component of the replicative helicase. Cell profiling by flow cytometry demonstrates an accumulation of cells in the S/G2 phases of the cell cycle following XL413 treatment, consistent with a block in DNA replication. An accumulation of cells with sub-2N DNA content is observed in many tumor cell lines but not in normal fibroblasts, indicating a selective induction of apoptosis by XL413 in tumor cells. Consistent with a role in cell cycle checkpoint response, treatment of U-2 OS cells with XL413 inhibits hydroxyurea-induced Chk1 phosphorylation. Dosing of rodent and non-rodent species demonstrates that XL413 exhibits significant oral bioavailability and dose-linear plasma exposures. In vivo pharmacodynamic studies show that oral administration of XL413 causes potent and dose-dependent inhibition of Cdc7-dependent MCM2 phosphorylation in multiple xenograft tumor models. The pharmacodynamic effects of XL413 translate into potent tumor growth inhibition in the same xenograft models at well-tolerated doses. Exploration of different dosing schedules demonstrates a good relationship between pharmacodynamic inhibition of Cdc7 and anti-tumor activity.
**Conclusions:** XL413 is a potent, selective Cdc7 inhibitor that shows excellent inhibition of Cdc7 substrate phosphorylation in preclinical tumor models following oral dosing. XL413 also demonstrates significant anti-tumor activity combined with excellent tolerability in vivo, suggesting that clinical exploration of this compound is warranted.

**340** POSTER

**Anti-tumor activity of YM753, a histone deacetylase inhibitor, against hormone refractory prostate cancer**

M. Mori[1], N. Shindoh[1], N. Amino[1], A. Kita[1], Y. Terada[1], M. Taniguchi[1], K. Sohda[1], Y. Sowa[2], T. Sakai[2], M. Sasamata[1]. [1]*Astellas Pharma Inc., Drug Discovery Research, Tsukuba, Japan;* [2]*Kyoto Prefectural University of Medicine, Department of Molecular-Targeting Cancer Prevention, Kyoto, Japan*

**Background:** YM753 is a novel cyclic-peptide-based histone deacetylase (HDAC) inhibitor. To investigate the anti-tumor activity of YM753 against hormone refractory prostate cancer (HRPC), we evaluated its effects on cultured cells and in animal models.
**Material and Methods:** An HDAC inhibition assay was performed using recombinant human HDAC1−7 and acetylated histone H4 peptide. Anti-proliferative activity was assessed against DU 145, PC-3, PPC-1, and 22Rv1 human HRPC cell lines using the sulforhodamine B assay. Anti-tumor activity was evaluated in male nude mice subcutaneously or orthotopically implanted with PC-3. Acetylated histone was detected in culture cells or tumors by immunoblotting.
**Results:** YM753 inhibited all HDAC subtypes examined with $IC_{50}$ values ranging from 0.2 to 6 nM, except for HDAC6. YM753 induced the accumulation of acetylated histones and showed potent anti-proliferative activity against various HRPC cell lines with $GI_{50}$ (50% growth inhibitory concentration) values ranging from 3.4 to 29 nM. *In vitro* analysis using PC-3 cells also indicated that YM753 induced $G_1$ and $G_2$/M arrest as well as caspase-dependent apoptosis and caspase-independent cell death. In nude mice with a subcutaneously xenografted PC-3 tumor, YM753 (0.3, 1, 3, and 10 mg/kg/day i.v.) induced the accumulation of acetylated histones and significantly inhibited tumor growth dose-dependently; treatment with 10 mg/kg/day resulted in tumor regression without a significant decrease in body weight. YM753 also induced tumor regression in PPC-1 xenografted mice. These anti-tumor effects were superior to those of other well-known HDAC inhibitors. In a PC-3 orthotopic xenograft model, YM753 at doses of 1, 3, and 10 mg/kg/day i.v. significantly inhibited tumor growth by 66%, 76%, and 83%, respectively, compared with the control. Moreover, YM753 induced the acetylation of histone H3 in orthotopically xenografted PC-3 tumors in a dose-dependent manner, which indicated that YM753 was distributed to the prostate tumor, and its anti-tumor activity was based on the induction of histone acetylation. In a combination study using a PC-3 xenograft model, tumor volume decreased significantly in mice treated with YM753 in combination with docetaxel, compared to treatment with each compound alone.
**Conclusions:** YM753 showed potent anti-tumor activity against culture cells and animal models of HRPC. These findings indicate that this novel HDAC inhibitor may be another way to treat HRPC.

**341** POSTER

**CX-5461, a novel, orally bioavailable selective small molecule inhibitor of RNA polymerase I transcription, induces autophagy and shows potent antitumor activity**

D. Drygin[1], A. Lin[1], M. Haddach[2], C. Ho[1], J. Bliesath[1], C. Proffitt[1], M.T.K. Schwaebe[2], W.A.K. Rice[1], K.L. Anderes[1]. [1]*Cylene Pharmaceuticals Inc., Cancer Biology, San Diego, CA, USA;* [2]*Cylene Pharmaceuticals Inc., Chemistry, San Diego, CA, USA*

Cancer is a disease of uncontrolled proliferation. The rate of cellular growth and proliferation is directly proportional to the rate of ribosomal biogenesis. The rate-limiting regulatory process in ribosome formation is transcription of the ribosomal RNA (rRNA) genes in ribosomal DNA (rDNA) by RNA Polymerase I (Pol I). Pol I transcription is initiated by SL1, a five-subunit protein complex that together with UBF anchors Pol I to rDNA promoter and is required for specific initiation of rRNA synthesis. Knock down of SL1 subunit expression inhibits rRNA synthesis. Mitotic silencing of rRNA synthesis occurs through inactivation of SL1. In addition, tumor suppressors p53, Rb and PTEN are often lost during tumorigenesis and have been shown to control rRNA synthesis by interfering with SL1 function. Collectively these findings underscore the importance of SL1 and Pol I function in regulating cell proliferation via initiation of rRNA synthesis. It follows that inhibitors of the SL1/Pol I complex may be effective anticancer agents. We employed a nuclear lysate-based cell-free system to identify selective Pol I inhibitors. CX-5461 was found to be a potent inhibitor of Pol I that exhibited more than ten-fold selectivity against Pol I versus RNA Polymerase II (Pol II). Further characterization of CX-5461 in cell culture confirmed potent inhibition of Pol I and showed antiproliferative activity with IC50 < 100 nM for multiple cell lines. qRT-PCR analysis of pancreatic carcinoma MIA PaCa-2 and melanoma A375 cells treated with CX-5461 demonstrated that CX-5461 inhibited rRNA synthesis with IC50 = 50–100 nM and exhibited ~200-fold selectivity over inhibition of Pol II transcription. Order of addition studies demonstrated that CX-5461 acts at the initiation step of Pol I transcription. ChIP and EMSA studies showed that CX-5461 interferes with SL1 function by disrupting SL1-rDNA promoter interaction. In vitro mechanism of action studies indicate CX-5461 induces autophagy. CX-5461 shows oral bioavailability in multiple species and demonstrated significant antitumor efficacy in xenografts. CX-5461 is a first in class agent designed to selectively inhibit Pol I transcription and represents a molecularly targeted approach to selectively kill cancer cells by halting the production of excess ribosomes and inducing autophagic cell death. The preclinical data support the development of CX-5461 as an anticancer drug with potential for activity in many types of cancer.

**342** POSTER

**2-[18F] fluoro-2-deoxy-d-glucose positron emission tomography is an early biomarker for tumor growth inhibition of human Colo205 xenografts by the novel and selective CENP-E inhibitor, GSK923295A**

D. Sutton[1], R.A. Fleming[2], K.W. Wood[3], J.R. Jackson[1]. [1]*GlaxoSmithKline, Oncology CEDD, Collegeville, PA, USA;* [2]*GlaxoSmithKline, Oncology Discovery Medicine, Research Triangle Park, NC, USA;* [3]*Cytokinetics, Cancer Biology and Therapeutics, South San Francisco, CA, USA*

**Background:** Positron emission tomography (PET) using 2-[18F] fluoro-2-deoxy-d-glucose (FDG) as a marker of tumor metabolism is well established in the diagnosis and clinical management of various malignancies. Clinical studies have demonstrated that changes in tumor glucose metabolism precede changes in tumor size and may therefore reflect drug effects at the cellular level. Thus, FDG-PET provides a relatively non-invasive means for evaluating pharmacological activity in tumors which may help in the drug development process. The purpose of this study was to evaluate FDG-PET imaging as a biomarker for GSK923295A, a novel and selective inhibitor of centromere-associated protein E (CENP-E) ATPase activity that is currently in a Phase I clinical trial.
**Methods:** Nude mice with advanced human colon Colo205 xenografts were treated with GSK923295A administered intraperitoneally at either 125 or

62.5 mg/kg for 3 consecutive days (days 0, 1, 2). PET imaging was carried out on days −1 (pretreatment baseline), 1 (24 h after dose 1) and 3 (24 h after dose 3).
**Results:** During the first treatment cycle, mean tumor volume in the placebo group increased by approximately 49% vs a 6% decrease and 7% increase in the groups treated with GSK923295A at 125 mg/kg and 62.5 mg/kg treated groups, respectively. Subsequently (day 7 onwards), tumor regression (>50% decrease in tumor volume relative to pre-treatment volume) was observed in 11/13 mice dosed at 125 mg/kg, but only 4/14 dosed at 62.5 mg/kg. FDG-PET imaging showed significant decreases in mean FDG standardized uptake values (SUV) relative to placebo at both dose levels. On day 3, the mean SUV's in GSK923295A-treated tumors decreased by approximately 25% and 30% at 62.5 and 125 mg/kg respectively. The day 3 SUV's for both treatment groups were significantly lower than either day −1 (pre-treatment) ($p < 0.05$) or placebo ($p < 0.001$).
**Conclusion:** The results demonstrate that in a preclinical setting, GSK923295A treatment significantly affected FDG uptake early after onset of therapy and prior to tumor regression. FDG-PET may provide a means of evaluating pharmacodynamic activity in patients treated with GSK923295A.

**343** POSTER

**Preclinical validation of the TrpM8 ion channel as a cancer target**

D.F. Duncan[1], N. Provost[2], O. Moreno[1], M.W. Frohlich[3], D. Urdal[4].
*[1]Dendreon Corporation, Chemistry, Seattle, USA; [2]Dendreon Corporation, Product Development, Seattle, USA; [3]Dendreon Corporation, Clinical Affairs, Seattle, USA; [4]Dendreon Corporation, Research, Seattle, USA*

**Background:** TrpM8, a transmembrane cation channel protein, is expressed in cancers including prostate, breast, lung, and colon. In normal tissues, its expression is primarily confined to prostate and a subset of sensory neurons. We have previously reported that small molecule agonists of TrpM8 can selectively kill cells that express TrpM8.
**Methods:** Activity and selectivity of agonists were measured in vitro in cell killing assays using CHO cells and CHO cells stably transfected to express TrpM8 (CHO/TrpM8). Cell viability was used to determine $EC_{50}$ values. Plasma pharmacokinetics (PK) was determined in mice. Compounds were assessed in vivo in murine xenograft models using CHO and CHO/TrpM8 cells. Tumor growth inhibition (%TGI) was defined as the difference between the control and treated tumor volumes as a % of control. The plasma PK of the lead compound was evaluated in rats and dogs. In vivo activity was evaluated in human prostate cancer (LuCap) xenograft models and in a rat model of androgen-induced, benign prostate hyperplasia (BPH).
**Results:** Several compounds were identified that were potent and selective in vitro and efficacious in vivo via oral administration. Compound D-3263 proved to be the most potent and preliminary PK studies conducted in mice, rats and dogs suggest the plasma half-life ranges from 10 hrs (mice and rats) to 27 hrs (dogs). The compound appears to inhibit LuCap growth in Nude mice ($p = 0.004$, 123.7 $mm^3$ for treated vs 207.6 $mm^3$ for vehicle on Day 4) and inhibited androgen-induced hyperplasia of normal rat prostates (mean prostate weights for treated 620.1 g vs 1009.9 g for untreated; $p = 0.004$).

Table 1

| Compound | MW | *In vitro* EC50 (uM) | | *In vivo* %TGI |
|---|---|---|---|---|
| | | CHO/TrpM8 | CHO | |
| D-3263 | 373 | 0.003 | >10 | 70% |
| D-3457 | 368 | 0.01 | >10 | 62% |
| D-3517 | 346 | 0.01 | >10 | 48% |

**Conclusions:** These results establish TrpM8 as a tractable therapeutic target and endorse the likelihood of an agonist demonstrating clinical activity against cancers that express TrpM8. The orally bioavailable small molecule agonist D-3263 has been selected for clinical development.

**344** POSTER

**A phase Ib dose escalation study to evaluate safety and tolerability of the combination of the aminopeptidase inhibitor CHR-2797 and paclitaxel in patients with advanced or treatment refractory tumors**

C. van Herpen[1], F. Eskens[2], M. de Jonge[2], I. Desar[1], L. Hooftman[3], E. Bone[3], A. Timmer-Bonte[1], J. Verweij[2]. *[1]Radboud University Medical Centre Nijmegen, Department of Medical Oncology, Nijmegen, The Netherlands; [2]Erasmus University Medical Center Rotterdam, Department of Medical Oncology, Rotterdam, The Netherlands; [3]Chroma Therapeutics, Oxford, United Kingdom*

**Background:** CHR-2797 is an orally bioavailable aminopeptidase inhibitor. In a single agent phase I study in solid tumors the recommended daily dose was 240 mg. The active metabolite, CHR-79888 accumulates intracellularly. In vitro and in vivo experiments confirmed good synergy between CHR-2797 and paclitaxel.
**Methods:** Patients with histologically confirmed advanced solid tumors refractory to standard therapy and performance score $\leqslant 2$ were treated every 21 days with paclitaxel given i.v. over 3 hrs (escalating from 135 mg/$m^2$ in the first cohort to 175 mg/$m^2$ in subsequent cohorts) and escalating doses of CHR-2797 (90–240 mg). The first 21 day cycle (Cy) constituted the dose finding phase. Patients received up to 6 Cy of paclitaxel and could remain on CHR-2797 therapy until evidence of PD or unacceptable toxicity.
**Results:** 22 patients (median age 59 years [range 34−72], 18M/4F) were treated. At the 1st dose level (DL) 3 patients were given paclitaxel 135 mg/$m^2$ and 90 mg CHR-2797. DLs 2−5 received paclitaxel 175 mg/$m^2$ and CHR-2797 at 90, 130, 180, and 240 mg (4, 3, 9, and 3 patients respectively). One DLT (dyspnea G3) was seen at DL 4. Common Grade 1−3 toxicities during treatment included alopecia and fatigue (each in 95% of patients), sensory neuropathy (59%), myalgia (50%), anorexia and dizziness (each in 45% of patients), rash (32%). Infusion reactions developed in 13 (59%) patients. At DL 4 and 5 CHR-2797 was withheld for 5 days, from d18 of each Cy, in an attempt to decrease the risk of infusion reactions. However, this had no clear effect on the number of infusion related reactions. Six patients continued CHR-2797 after discontinuation of paclitaxel. Neither agent influenced the pharmacokinetics of the other. PR was achieved in 3 patients (melanoma, non small cell lung cancer, esophageal squamous cell).
**Conclusions:** Except for an unexpected high number of infusion reactions to paclitaxel the combination of paclitaxel-CHR-2797 was otherwise well tolerated. Further investigation into the potential immunological mechanisms is warranted. Formal MTD was not reached. Anti-tumor activity was observed in several patients.

**345** POSTER

**Myofibrillogenesis regulator 1 as a potential target for cancer therapy**

R. Shao[1], K. Ren[1], Y. Wang[1]. *[1]Institute of Medicinal Biotechnology CAMS, Department of Oncology, Beijing, China*

**Background:** The phosphorylation of myosin light chain-2 (MLC2) is pivotal in the regulated assembly and disassembly of focal adhesions and adherens junctions contributed to cell motility and tumor invasion. MLC kinase inhibitor abrogates MLC2 phosphorylation, cell polarization and migration, and MLC2 dephosphorylation induces apoptosis. Our previous data show that MLC2 interacts with human myofibrillogenesis regulator 1 (MR-1). Thus, we investigate whether MR-1 is associated with the proliferation and migration of cancer cells.
**Material and Methods:** The transcription and expression of MR-1 were detected by RT-PCR and Western blot analysis. Functional analysis of MR-1 small hairpin RNA (shRNA) was conducted in HepG2 cells using Lipofectamine-mediated gene transfer. The changes of cell spreading, adherence and migration in response to the treatments were evaluated by immunofluorescent staining, immunohistochemistry and Boyden chamber invasion assay. The tumorigenicity of HepG2 cells stably transfected with MR-1-shRNA was assessed by transplantation into nude mice.
**Results:** RT-PCR and Western blot analysis showed that MR-1 was overexpressed in human cancer cells and especially in hepatoma HepG2 cells. Transient treatment of cells with shRNA against MR-1 or stable transfection of cells with plasmid expressing MR-1-shRNA led to impairment of cell proliferation, adhesion and migration. Following inhibition of MR-1 by MR-1-shRNA, the phosphorylations of MLC2, focal adhesion kinase (FAK) and Akt were decreased dramatically and formation of stress fiber was destroyed. In the same condition, MLC kinase inhibitor could block exogenous MR-1-induced phosphorylations of MLC2, FAK and Akt and F-actin polymerization inhibitor also decreased phosphorylations of FAK and Akt, indicating that activation of MLC2 and intact actin cytoskeleton was upstream of FAK and Akt in MR-1 modulating pathway. *In vivo* data showed that knockdown of MR-1 markedly inhibited the tumorigenicity of human