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.

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

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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.

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

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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.

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

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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.

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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

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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