Conclusions: We have identified a series of potent, selective S1P1R antagonists, which may have the potential to be novel antivascular drugs for the treatment of cancer.

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Targeting MKP1 with novel chemical inhibitors sensitizes melanoma and colon cancer cells to chemotherapeutics in vitro and in vivo

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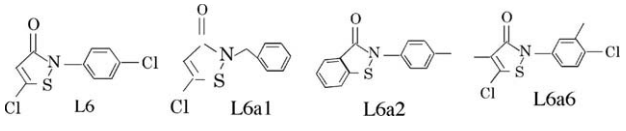
Phosphatase MKP1 is a putative cancer therapeutic target for inducing apoptosis and sensitizing cancer cells to chemotherapeutics, based on its over-expression in breast and lung cancer cells that is associated with an anti-apoptotic effect and chemo-resistance. However, chemical inhibitors of MKP1 with pre-clinical anti-tumor activity as a single agent or in combination with chemotherapeutics have not been reported. The significance of MKP1 in other malignancies (e.g., melanoma and colon cancer) remains undefined. From a library of drug-like small chemicals and chemical databases, we have identified lead compounds L6 and its more active analog L6a6 as novel MKP1 inhibitors with anti-cancer potential. Active against recombinant MKP1, the compounds selectively increased phosphorylation of MKP1 substrates in a dose-dependent manner (IC50 ~0.1 μg/ml) in melanoma and colon cancer cell lines in vitro and induced cell death (LD50 ~0.1 μg/ml) via apoptosis, in contrast to several structural analogs (e.g., L6a1) that had little effect on the substrates or cell growth. Consistent with its capacity to sensitize WM9 human melanoma cells to temozolomide (TMZ) or cisplatin in vitro, growth of WM9 xenograft tumors in mice was inhibited more efficiently (50%) by a tolerated combination of TMZ (80 mg/kg, ip) and L6a6 (10 mg/kg, oral) for two weeks (5 d/week) in comparison to TMZ alone (20%). L6a6 (3 mg/kg, oral, daily ×5 days/week for two weeks) also sensitized MC-26 colon cancer tumors in mice to 5-FU/LV, the standard regimen for colon cancer, inducing significantly better growth inhibition ($p < 0.01$) via combination (80%) than the chemotherapeutics (52%) or L6a6 alone (22%) in a tolerated manner. MKP1 expression in the cancer cell lines was verified by immunoblotting whereas high levels of MKP1 expression in advanced human melanoma tissues were detected by IHC. The compounds also sensitized WM9 melanoma cells to IFNa2b, a cytokine approved for melanoma treatment, and exhibited LD50 around 0.1 μg/ml toward human cell lines of breast cancer, lung cancer and prostate cancer in culture. In initial experiments with a promoter-insertion vector for up-regulating gene expression, L6a6-resistant WM9 clones have been isolated for mechanistic analysis. Taken together, these results provide direct evidence for the first time that support targeting MKP1 as a safe and efficacious cancer therapeutic strategy. Moreover, the pre-clinical anti-tumor activity of L6a6 as an orally active and well-tolerated lead compound suggests the potential of this class of chemicals for development into novel cancer therapeutics.



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GSK923925A, a novel and selective CENP-E inhibitor, induces pharmacodynamic effects and anti-tumor activity in human Colo205 xenografts

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Background: The mitotic kinesin centromere-associated protein E (CENP-E) integrates mitotic spindle mechanics with mitotic checkpoint signaling. CENP-E plays no known role outside of mitosis. CENP-E mRNA is over-expressed in a variety of human tumors relative to normal adjacent tissues suggesting it may play an important role in tumor cell proliferation. Inhibition of CENP-E in cultured human tumor cells leads to cell cycle arrest in mitosis with bipolar mitotic spindles and misaligned chromosomes and eventual cell death. GSK923295A is a novel and selective inhibitor of CENP-E ATPase activity that is currently in a Phase I clinical trial. The purpose of this study was to determine the pharmacokinetics (PK) and pharmacodynamics (PD) of GSK923295A in a human tumor xenograft model.

Methods: GSK923295A was administered intraperitoneally at 62.5, 125, or 250 mg/kg for two 3 day cycles separated by 4 days to nude mice with Colo205 tumor xenografts. Treated mice were divided into 2 cohorts: cohort 1 to monitor tumor growth and cohort 2 to determine PK in blood and tumor samples and PD (biomarkers) in tumor samples. PK was determined after the first dose of GSK923295A in each cycle.

Results: Tumor regression was observed at 125 and 250 mg/kg but no effect on tumor growth was observed at 62.5 mg/kg. Drug exposure was dose-dependent in both blood and tumors. An increase in drug exposure was observed in tumors, but not blood, between cycle 1 and 2. Examination of GSK923295A-treated tumors revealed a dose-dependent appearance of abnormal mitotic figures and a marked decrease in the presence of post-metaphase figures. Many of the abnormal mitotic figures had lagging chromosomes, a phenotype characteristic of CENP-E inhibition. GSK923925A also resulted in a dose-dependent increase in phosphohistone-H3 (pHH3) (a mitosis specific marker). The increase in pHH3 was transient and had largely disappeared by 48 h after dosing. PD effects were more pronounced after the second cycle of treatment with extensive tumor necrosis observed. The extent of pHH3 increase was related to the anti-tumor activity observed. At the lowest inactive dose, only a modest PD effect (increase in abnormal mitotic figures) was observed. At the active doses, more robust PD activity (both abnormal mitotic figures and increase in pHH3) was observed.

Conclusion: The observations made in vivo are consistent with previous cell based data and provide further insight into the potential of GSK923295A for the treatment of cancer.

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The ghrelin receptor agonist TZP-101 is a potent anti-tumor-cachexia agent in the human G361 melanoma mouse xenograft model

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TZP-101 is a small-molecule, ghrelin receptor agonist and prokinetic agent currently in Phase IIb clinical development for post-operative ileus and diabetic gastroparesis. Agonism of the ghrelin receptor has also been associated with increased food intake and the generation of a positive overall energy balance. The current study was intended to investigate the effect of TZP-101 as compared to ghrelin peptide on tumor cachexia in the G361 melanoma model grown as a subcutaneous xenograft in BALB/c nu/nu mice. Cachexia is considered the major reason for mortality, rapidly declining quality of life and limitation of therapy in advanced tumor patients. To this effect, 60 tumor-bearing mice were randomised 12 days after tumor cell inoculation into two sets of 5 groups containing 6 animals each. At initiation of treatment the average body weight loss of Set 1 (Groups 1−5) and Set 2 (Groups 6−10) animals was approximately 8.5% and 4.5%, respectively, of the initial average body weight. Treatment of Set 1 and 2 animals commenced on Days 12 and 16 after tumor inoculation, respectively. Groups 1 and 6 received vehicle s.c. bid alone, while Groups 5 and 10 were administered rat ghrelin peptide s.c. (1 mg/kg; bid, 6 h apart) as a positive control. TZP-101 was administered s.c. twice daily, 6 h apart, at doses of 3 (Groups 2 and 7), 10 (Groups 3 and 8) and 30 mg/kg (Groups 4 and 9) up to 33 (Groups 1−5) and 28 consecutive days (Groups 6−10). Mice were culled during the study according to predetermined criteria including >15% initial body weight loss and/or tumor volume in excess of 2000 mm3 and/or display of severe clinical signs.

As a result, TZP-101 treated animals of both Sets showed a dramatic increase in survival: while all vehicle treated control mice of Set 1 (Group 1) were dead on Day 5 after initiation of treatment, TZP-101 treated animals survived until Day 9, 28 and 30, at doses of 3, 10 and 30 mg/kg respectively. Similarly, the mean survival of Set 2 animals increased dose dependently from 17 days (vehicle treated controls) to 22, 26 and 27 days at TZP-101 doses of 3, 10 and 30 mg/kg, respectively. For comparison ghrelin treated mice survived for 33 (Set 1) and 22 days (Set 2). TZP-101 treatment was also associated with markedly increased food and water consumption, a clear tendency of increased body mass index, as well as dose dependently increased plasma concentrations of cholesterol, triglyceride and non-esterified fatty acids. In all cases treatment with TZP-101 caused a much greater response than ghrelin peptide. A concomitant 50% decrease in blood glucose levels may, in addition, support the notion of a change in metabolism.

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