determined by an anti-proliferative assay using hematological and solid tumor cell lines shows IC50 values as low as 54 nM. MV-4−11 leukemia cells treated with SGI-1776 shows a dramatic decrease in phospho-BAD levels (a direct substrate of the Pim kinases) as determined by western blot with an EC50 value of <10 nM. In conjunction with the anti-proliferative and phospho-BAD data, cell death via apoptosis was observed in cells treated with SGI-1776. Pharmacokinetic analysis of SGI-1776 in rats has demonstrated good oral bioavailability of the drug. Treatment of xenograft tumor models showed decrease tumor growth rates and in some models complete regression of the tumors at doses as low as 67 mg/kg. To determine if SGI-1776 was modulating Pim-1 activity in xenograft tumor in vivo pharmacodynamic studies were performed. Bad phosphorylation levels were determined in xenograft tumors by western blot and IHC. These studies showed that oral delivery of SGI-1776 modulated PD markers and could do so in a dose dependent manner. SuperGen's SGI-1776 exhibits potent inhibition of Pim kinase activity, translating into potent inhibition of cellular signaling pathways, cancer cell proliferation, and in vivo tumor progression in non-clinical models.

333 POSTER

Effectiveness of 6-(7-nitro-2,1,3-benzoxadiazol-4-ylthio)hexanol (NBDHEX) on human osteosarcoma and melanoma tumours

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Background: NBDHEX is a promising anticancer drug which activates the c-Jun N-terminal kinase (JNK) pathway through the binding to glutathione S-transferase P1−1 (GSTP1−1). The disruption of the GSTP1−1-JNK complex is followed by a remarkable pro-apoptotic effect in tumor cells. In the present work we assessed the in vitro and in vivo effectiveness of NBDHEX on poorly responsive/resistant human osteosarcoma (OS) and melanoma models.
Material and Methods: NBDHEX was tested on 10 human OS cell lines and on 20 U-2OS or Saos-2 variants resistant to cisplatin (CDDP), doxorubicin or methotrexate and on two human melanoma cell lines (A375 and Me501 cells). Apoptosis was evaluated by chromatin fragmentation and caspase activation tests. GSTP1−1 levels and activation of the JNK/cJun pathway were tested by Western blot. The activity of the NBDHEX-CDDP combination was evaluated in OS cell lines sequentially exposed to equitoxic concentrations of the two drugs. In vivo experiments were performed on SCID mice implanted with A375 or Me501 tumors. Mice were treated orally with different doses of NBDHEX. Tumor growth inhibition (TI) was monitored three times a week. Toxicity was evaluated on the basis of weight loss and the autopsy findings. The mitotic index (MI) was determined by microscopy.
Results: NBDHEX was very active on both OS and melanoma cells with IC50 values in the micromolar range, independently of GSTP1−1 levels. In these cell lines NBDHEX induced the activation of the JNK/cJun pathway and a strong pro-apoptotic effect. Drug combination studies on OS cells, showed that NBDHEX can be used in association with CDDP. Anti-tumor efficacy was observed in vivo against Me501 human melanoma. NBDHEX showed 70% TI after oral (daily $\times$25) administration. Tolerability was good at all tested doses with no significant changes in body weight. The tumor MI value was decreased by 50% after NBDHEX treatment. Similar results were obtained on advanced human melanoma, A375 model, with 63% TI after oral (daily $\times$10) treatments. Tests on in vivo efficacy of NBDHEX on OS models are still ongoing.
Conclusions: The low responsive/resistant OS and melanoma tumor cells are efficiently committed to death by NBDHEX. This drug is effective and well tolerated in in vivo tumor models. These findings indicate that activation of JNK/cJun pathway, through a selective GSTP1−1 targeting, could prove a promising new strategy for treating tumors that respond poorly to conventional therapies.

334 POSTER

Molecular modelling and synthesis of novel CYP26A1 inhibitors

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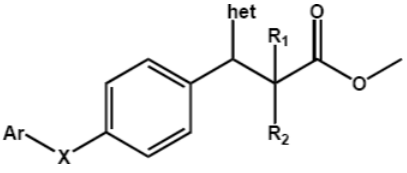
Cancer is one of the major causes of death in the world nowadays. The treatment of cancer could be accomplished through surgery or radiation followed by application of chemotherapy, however the main drawback of most chemotherapeutic agents is the side effects which accompany their use, many of which arise owing to the inability of chemotherapeutic agents to differentiate between the malignant cells and the fast dividing normal cells. One of the major targets of the scientist is to find drugs which can affect cancer cells without impairing the normal cells. One approach to fulfill this criterion is through drugs which act upon Cytochrome P450, such as CYP19A1, CYP24A1 and CYP26A1.
Our main target is CYP26A1, which is the enzyme responsible for the metabolism of retinoic acid. Retinoic acid (RA), the active metabolite of vitamin A, binds with nuclear receptors RAR/RXR to regulate cell growth and differentiation in a variety of cell types, and can reverse malignant growth in vitro and in vivo. These properties have led to RA being used effectively in a number of clinical situations including APL and neuroblastoma. However, in both cases resistance to RA can limit the therapeutic benefits observed. Evidence is growing that this resistance is related to up-regulation of CYP26 resulting in accelerated metabolism of RA. Inhibition of CYP26A1 results in the increase of the tissue level of retinoic acid and/or maintenance of a high therapeutic level of retinoic acid. Liarozole is the most studied and first CYP26A1 inhibitor to undergo clinical investigation for the treatment of ichthyosis. Liarozole also showed promising results in the treatment of prostate cancer compared with cyproterone acetate, however it lacked CYP selectivity. A model for CYP26A1 enzyme, developed within our group, has been used for docking of our compounds. Also, we have generated a pharmacophore model for CYP26A1 inhibitors. The docked compounds have been synthesized using different chemical methods, and analyzed for their CYP26A1 inhibitory activity within our laboratory using MCF-7 breast cancer cells. The tested compounds have shown good to moderate activity against CYP26A1, with nanomolar to low micromolar activities and good selectivity for CYP26A1.



General structure formula of the tested compounds. Ar = naphthyl, phenyl; X = O, NH; R_1 = H, CH_3; R_2 = H, CH_3.

335 POSTER

Identification of potent, selective sphingosine-1-phosphate 1 receptor (S1P1R) antagonists with antitumor activity

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Background: Bioactive lipids, including sphingosine-1-phosphate (S1P), have been shown to play important roles as signaling and regulatory molecules. Many, if not all, of the effects of S1P are mediated by five distinct but related G-protein coupled receptors, S1P1−5. Despite the large body of evidence for the role of S1P1R in angiogenesis, proliferation, and cell migration, the development and application of potent, selective, small molecule S1P1R antagonists is lacking. The goal of this study was to identify and characterize selective S1P1R antagonists using in vitro and in vivo angiogenesis and tumor models.
Methods: High-throughput screening (HTS) of approximately 4.5 million compounds was conducted using HEK-293 cells overexpressing S1P1R and a modified cyclic nucleotide gated channel that served as a biosensor for intracellular cAMP. Compounds identified from screening were further optimized by medicinal chemistry and characterized using a variety of bioassays. Migration assays were conducted with SK-Hep-1 cells using a Boyden chamber assay. S1P1R selectivity assays were performed using β-arrestin recruitment assays in cell lines that expressed S1P1R, S1P2R or S1P3R. Tube formation assays were conducted using conditioned media from DU145 cells in a co-culture system consisting of primary human fibroblasts and endothelial cells. The effects of S1P1R antagonists were assessed in vivo using human tumor xenograft models in nude mice.
Results: Several potent, selective compounds were identified from HTS. Optimization by medicinal chemistry yielded S1P1R antagonists with in vitro IC50 values <10 nM in the cAMP biosensor assay that were highly selective for S1P1R compared to the related S1P2R and S1P3R receptors. Moreover, compounds were equally potent in inhibiting migration of SK-HEP-1 cells and blocking in vitro tube formation. Selected compounds had high oral bioavailability in rodents, and administration to nude mice bearing MDA-MB-231 tumor xenografts produced significant changes in tumor microvessel structure and evidence of antitumor activity.

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.

336 POSTER

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

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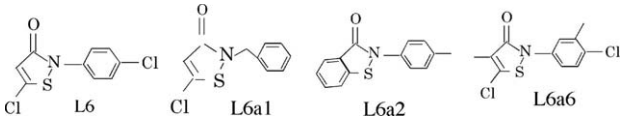
Phosphatase MKP1 is a putative cancer therapeutic target for inducing apoptosis and sensitizing cancer cells to chemotherapeutics, based on its over-expression in breast and lung cancer cells that is associated with an anti-apoptotic effect and chemo-resistance. However, chemical inhibitors of MKP1 with 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.



337 POSTER

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.

338 POSTER

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.