Methods: Kinase inhibition and cell viability assays, immunoblotting, flow cytometry, immunofluorescence microscopy and transcription assays were performed in the colorectal human cancer cell line, HCT-116.
Results: Compound LGR1492 was found to potently inhibit CDK2/cyclin E with nanomolar potency in an enzyme assay. Consistent with its inhibition of CDK2, the antiproliferative activity of the compound is connected with cell cycle arrest in the late S phase and with a decreased population of cells actively replicating DNA. Inhibition of transcription was observed by measuring the levels of mRNA and RNA. The compound also induces apoptosis in treated cells, as assessed by activation of caspases and fragmentation of PARP. In addition, the compound increases cellular levels of the tumor suppressor protein p53, stabilizes its nuclear localization and activates transcription of some p53-regulated genes.
Conclusion: The studied pyrazolo[4,3-*d*]pyrimidines significantly surpasses other purine bioisosteres in terms of its antiproliferative and anticancer properties and could become a lead structure for development of potential new anticancer therapeutics.
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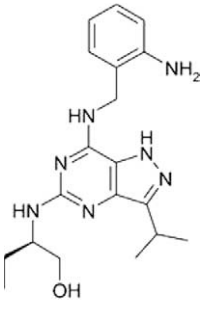

LGR1492

501 POSTER

4SC-207, a novel and highly potent anti-mitotic agent, active also on P-gp expressing tumor cells resistant to other chemotherapeutic drugs, induces complete tumor stasis in vivo

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Background: 4SC-207 is a novel small molecule of the tetrahydro-pyrido-thiophene chemotype with strong anti-mitotic activity derived from a cellular screening campaign. The purpose of this study was to investigate the potency of 4SC-207 to inhibit the proliferation of different tumor cell lines *in vitro* including chemotherapeutically resistant P-gp-expressing cells and to confirm these observations in *in vivo* xenograft tumor models.
Material and Methods: *In vitro proliferation assay:* 50 ATCC cell lines were grown in 96-well microtiter plates. After a 24 h pre-growth period cells were incubated with 4SC-207 at different concetrations for 72 hours. After treatment cells were precipitated and stained with 0.4% wt/v sulforhodamine B solution in 1% acetic acid. Measurement of optical density was performed at 520 nm. Proliferation inhibition was determined as growth inhibition of 50% (GI_{50}).
In vivo xenograft model: 4SC-207 (30% captisol solution) was tested both i.v. and p.o. in a xenograft NMRI mouse model using colon adenocarcinoma cell line RKOp27. In the i.v. study 4SC-207 was administered at a dose of 40 mg/kg BID on days 1−7 and SID on days 8 and 11−14. In the p.o. study 4SC-207 was administered at a dose of 80 mg/kg SID on days 1, 2, 6, 7, 11, and 12. Tumor growth in relation to control animals, body weight, hematologic parameters and lethality were determined.
Results: *In vitro activity on cell lines:* 4SC-207 effectively inhibited the proliferation of most tested tumor cell lines with average GI_{50} values between 4 nM and 12 nM. 4SC-207 was also active on many cell lines such as HCT-15 and DLD-1 which are known to express P-gp and to be resistant to a large set of conventional anti-cancer agents (e.g. taxanes).
In vivo xenograft model: 4SC-207 displayed a strong anti-tumor activity *in vivo*, both after intravenous or oral administration. Treatment with 4SC-207 induced complete tumor stasis (i.v.: T/C = 0.09; p.o.: T/C = 0.1). As expected, treatment with 4SC-207 had an effect on the hematopoetic system in terms of reduced white blood cells and platelets. Effects on body weight were mild and other signs of overt toxicity were not observed.
Conclusions: 4SC-207 is a very potent, novel anti-mitotic compound with strong *in vitro* and *in vivo* anti-tumor activity. Since 4SC-207 is also active on P-gp expressing tumor cells the compound could offer the opportunity to be used for hematological and solid tumor types which are resistant to many other anti-cancer agents.

502 POSTER

Polyploidy, senescence and apoptosis: distinctive phenotypic features of cancer cells treated with BI 811283, a novel Aurora B kinase inhibitor with anti-tumor activity

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Background: Aurora B kinase coordinates critical steps in mitosis, including chromosome condensation, segregation and cytokinesis. The key functions of this serine/threonine kinase and its over-expression in multiple tumor types render Aurora B an attractive target for cancer therapy.
Methods: Cell proliferation was quantified by Alamar Blue™ metabolic labeling and thymidine incorporation assays. The cellular phenotype was determined by DNA content analysis (FACS or Cellomics). PARP cleavage (Western Blots) and nuclear fragmentation (microscopy) were monitored to detect apoptosis. Senescent cells were identified by SA-b-Gal staining. Nude mice were grafted s.c. with NSCLC or CRC tumor cells (cell lines Calu-6 and HCT116, respectively). BI 811283 was administered once weekly to mice bearing established tumors by 24 h s.c. infusion using osmotic mini-pumps. In this schedule, the MTD was 20 mg/kg.
Results: BI 811283 potently inhibited Aurora B kinase (IC_{50} = 9 nM) and blocked the proliferation of cells of diverse origin in a large cancer cell line panel (all EC_{50} < 14 nM). In four cell lines tested by FACS, polyploid cells accumulated within 48 h of treatment (up to 80% of the population). In NCI-H460 cultures, ~ 25% of the cells expressed the senescence marker after 96 h of treatment, while apoptosis was only observed in 7%. In nude mouse xenograft models of human NSCLC and CRC, BI 811283 dose-dependently inhibited tumor growth and at the MTD, tumor regression was observed in a subset of animals. Histological examination of treated tumors showed an accumulation of enlarged, multi-nucleated cells in accordance with the expected mechanism of action.
Conclusions: Treatment of tumor cells with BI 811283, a potent Aurora B kinase inhibitor, induces a mitotic checkpoint override resulting in non-proliferating, polyploid cells that show hallmarks of senescence and apoptosis. Aurora B inhibition thus defines a new mechanistic paradigm for M-phase targeting agents. Final data from phase I clinical evaluation of BI 811283 in patients with advanced solid tumors will be presented at the EORTC–NCI–AACR Symposium 2010.

503 POSTER

In vitro characterization of TAK-960, a novel, small molecule inhibitor of Polo-like kinase 1

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Background: Polo-like kinase 1 (PLK1) is a serine/threonine protein kinase involved in key processes during mitosis. Human PLK1 has been shown to be overexpressed in various human cancers, and has been associated with poor prognosis. Several reports demonstrated that PLK1 depletion caused obvious cell cycle arrest at mitosis and induced apoptosis in a broad range of cancer cell lines, but not in normal diploid cells or non-dividing cells. To further explore the therapeutic potential of PLK1 inhibition in oncology, we have developed TAK-960, a novel, small molecule PLK1 inhibitor.
Materials and Methods: Inhibition activity for PLK1 was assessed using time-resolved fluorescence resonance energy transfer (TR-FRET). The dissociation rate of TAK-960 from PLK1 was measured using time-resolved fluorescence. Other protein kinases were assayed by transfer of ^{33}P phosphate to a peptide or protein substrate. The cell cycle distribution and phospho-Histone H3 (pH3) in the cells were measured by flow cytometry and ELISA, respectively. The anti-proliferative activity of TAK-960 was determined using CellTiter Glo assays.
Results: The mean IC_{50} values for TAK-960 inhibition of PLK1 activity at low (3 uM) and high (1000 uM) ATP concentrations were <3 and 6.5 nM, respectively. The dissociation rate constant (k_{off}) indicate that TAK-960 demonstrates slow-dissociation kinetics upon binding to PLK1. The results of kinase panel assay indicate that TAK-960 is a highly potent and selective inhibitor of PLK1 among 288 kinases tested. Consistent with selective PLK1 inhibition, TAK-960 treatment caused accumulation of G2/M cells and increased pH3 in human HT29 colorectal cancer cell line. TAK-960 inhibited proliferation of multiple cancer cell lines, with mean EC_{50} (concentration resulting in 50% efficacy) values ranging from 8.4 to 46.9 nM, but did not affect viability of quiescent human lung normal fibroblast (MRC5) cells