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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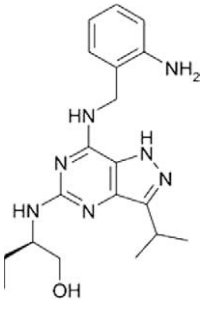

LGR1492

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

(EC_{50} > 1000 nM). The mutation status of *p53* or *KRAS* did not correlate with the potency of TAK-960 in the cell lines tested in this study. In addition, EC_{50} values in MDR1-overexpressing cell lines were similar to those in cell lines that do not express MDR1.
Conclusions: TAK-960 is a potent, selective PLK1 inhibitor with broad range proliferation inhibition activities including MDR1-expressing tumors. TAK-960 is currently being investigated in phase I clinical trials.

504 POSTER

The role of interferon-gamma- and TNF-induced cell cycle arrest in insulinoma

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Even though most established tumor immunotherapies are based on tumor cell destruction by cytotoxic cells, an increasing number of data shows that successful cancer immunotherapy depends on interferon γ (IFNγ)-producing T cells, i.e. T helper 1 (Th1) cells. RIP1Tag2 mice, where the tumor promoter T-antigen (Tag2) is specifically expressed in β cells, develop well-characterized carcinomas of the pancreatic islets that follow well described multistage carcinogenesis. In a previous study, Tag-specific Th1 cells doubled the lifespan of RIP1Tag2 mice by decreasing the proliferation rate of tumor cells and by inhibiting tumor angiogenesis without causing either tissue destruction or apoptosis in vivo. The therapeutic effect of the Tag-specificTh1 cells was critically dependent on IFNγ and TNF signalling. To unravel the underlying mechanisms, we investigated the direct effects of IFNγ and TNF on malignant β cells from RIP1Tag2 mice and measured in vitro proliferation by the BrdU-proliferation assay and Ki67, analysed the cell cycle progression by flow cytometry and PCR arrays concerning cell cycle genes, and determined the apoptosis rate by TUNEL staining and subG1 analysis. To specify the signalling pathways, we further examined insulinoma from RIP1Tag2xTNFR1$^{-/-}$ (TNF-pathway) and RIP1Tag2xSTAT1$^{-/-}$ (IFNγ-pathway) using the same assays as described above.
We found a significant suppression of the proliferation rate of the isolated RIP1Tag2 tumor cells *in vitro* by IFNγ and TNF that was accompanied by a decrease of the cells in the G2 phase. On the other hand, IFNγ and TNF didn't cause apoptosis (no increase of subG1 cells and negative TUNEL staining). The effects of both cytokines were specific: IFNγ did not block the proliferation of RIP1Tag2xSTAT1$^{-/-}$ cancer cells, and TNF did not block the proliferation of RIP1Tag2xTNFR1$^{-/-}$ tumor cells. Using PCR arrays we found that IFNγ strongly affects the expression of specific cell cycle regulating genes.
Taken together, our data suggest that Tag-Th1-mediated immunotherapy is based on IFNγ- and TNF-dependent repression of insulinoma proliferation by inducing cell cycle arrest in the absence of cell destruction.

505 POSTER

Dual Cdc7/Cdk9 kinase inhibitor, PHA-767491, targets both quiescent and proliferating CLL cells

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Proliferation rate has been recognized as an important factor in the outcome of patients with chronic lymphocytic leukemia (CLL). Proliferation centers, containing dividing CLL cells can be identified in lymph nodes. In this report we show that proliferating CLL cells express active Cdc7 kinase, an S-phase specific kinase essential for DNA replication. Since specific knockdown of Cdc7 induces apoptosis in cancer cells independent of TP53, we decided to evaluate the potential of Cdc7 inhibition in CLL. PHA-767491 is a first in class, prototype Cdc7 inhibitor, which also has cyclin dependent kinase 9 (Cdk9) inhibitory activity.
In this study we assess the activity of PHA-767491 against both quiescent and cells that have been prompted into the proliferative programme using a cellular co-culture system that leads to CD40 stimulation and that mimics lymph node microenvironment.
We find that PHA-767491 is highly active as a single agent in CLL cells purified from peripheral blood of patients regardless of recognized risk factors including TP53 inactivation. PHA-767491 activates Bax leading to mitochondrial dependent apoptosis by decreasing the levels of Mcl-1 at the transcriptional level through inhibition of Cdk9.
We also find that PHA-767491 inhibits replication in proliferating CLL cells following stimulation by CD154 and interleukin-4 (IL-4), with clear evidence of Cdc7 inhibition.
These data show that dual Cdc7/Cdk9 inhibition has the potential to target quiescent and actively proliferating CLL cells and may be a new therapeutic strategy in CLL.

Radiation interactive agents

506 POSTER

Darinaparsin (ZIO-101) is a novel cytotoxic and radiosensitizing agent for prostate cancer

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Background: Darinaparsin (DAR) is a novel organic arsenical (dimethylated arsenic linked to glutathione) with promising anticancer activity. Unlike other arsenicals, DAR appears to have broad spectrum activity in hematologic and solid tumors. Given that DAR appears to have multiple mechanisms of action, including generation of reactive oxygen species (ROS) and arrest of cells in G2/M, we hypothesized that DAR would have significant radiosensitizing effects and efficacy against prostate cancer under both normoxic (NO) and hypoxic (HO) conditions.
Materials and Methods: Experiments were performed in the hormone-independent (HI) and radio-resistant prostate cancer cell line LAPC-4. Cells were treated with DAR at concentrations ranging from 0.01 to 10 uM under either NO or HO (0.5% O2) conditions and irradiated with doses of 0−5 Gy. Viability, proliferation and colony formation were assessed. Mechanistic studies were performed to assess the role of apoptosis, mitochondrial damage, DNA damage, ROS generation, androgen receptor expression, signal transduction pathway activation, and endoplasmic reticulum (ER) stress on cytotoxicity under both NO and HO conditions.
Results: DAR had significant cytotoxicity against prostate cancer cells in vitro under both NO and HO conditions, with approximately twice as much cell killing under HO than NO conditions. DAR was a significantly more potent cytotoxin than ATO. Significant radiosensitization was observed in clonogenic assays at clinically relevant doses of radiation under both NO and HO, with the greatest magnitude of sensitization observed under HO. Mechanistic studies to date demonstrate that apoptosis is an important mechanism of DAR-induced cell death, with a greater induction of apoptosis under HO than NO conditions. Interestingly, DAR increased cellular ROS and ER stress under NO, but not HO, suggesting under HO, DAR-mediated cytotoxicity may be independent of ROS. In addition, while unrepaired DNA damage could be demonstrated in cells treated with DAR under NO conditions, DNA damage was not detectable in cell treated under HO. Addition of exogenous GSH completely inhibited DAR-induced cell death in both NO and HO, which could be secondary to either replacement of depleted glutathione (GSH) and/or effects on the membrane transporter of DAR. JNK activation occurred under both HO and NO conditions, but occurred earlier and to a greater extent under the NO conditions tested. Experiments are ongoing to better elucidate the mechanism of action of DAR under HO conditions.
Conclusions: DAR has significant cytotoxic and radiosensitizing effects against HI LAPC-4 prostate cancer cells, with the greatest effect under HO conditions. In vivo experiments will be initiated shortly to further study these effects in clinically relevant murine models of HI prostate cancer. These results could have broad potential applicability for the treatment of prostate cancer, with near term translational potential.

507 POSTER

Sensitization of hypoxic cells to ionising radiation by a hypoxia activated inhibitor of DNA dependent protein kinase

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Tumour hypoxia is a negative prognostic marker for patients undergoing radiation therapy. Radiation therapy acts by inducing DNA damage and DNA double strand breaks (DSB) are the primary lethal lesion caused by ionizing radiation (IR). DNA dependent protein kinase (DNA-PK) is a key holoenzyme in the non-homologous end joining (NHEJ) repair pathway which is the predominant mechanism used to repair IR induced DSBs. We have synthesized prodrugs of DNA-PK inhibitors that are bioreductively activated in hypoxic conditions and have demonstrated that these compounds can selectively sensitize hypoxic cells to IR.