Conclusions: BI 811283 was well tolerated overall; dose-limiting neutropenia was the most common high grade AE observed. 125 mg was defined the MTD. There was some evidence of pharmacodynamic effect as demonstrated by a reduction of histone H3 phosphorylation at higher doses, consistent with inhibition of Aurora B kinase.

497 POSTER

Combination therapy with an Aurora B kinase inhibitor AZD1152 and AraC, shows enhanced tumouricidal activity in a preclinical model of acute myeloid leukaemia (AML)

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Abstract: Acute myeloid leukaemia (AML) is characterized by an overproduction of immature, abnormal hematopoietic cells in the bone marrow and peripheral blood. Intrinsic resistance or treatment-induced acquired resistance is one of the major obstacles to the effective treatment of patients with AML, and underlies the continuing need to develop new treatments for AML. The Aurora kinases (AK) play a critical role in mitosis and have been suggested as promising targets for cancer therapy due to their frequent overexpression in a variety of tumours. Several AK inhibitors are advancing in various stages of development including AZD1152, a selective Aurora B kinase inhibitor, with a novel anti-tumour mechanism of action, inducing endoreduplication, apoptosis and inhibition of cytokinesis, leading to prolonged anti-tumour activity in solid and haematological preclinical cancer models (Wilkinson et al. Clin Can Res. 2007; Oke et al. Can Res. 2009). Cytarabine (cytosine arabinoside, Ara-C) is widely used as a therapy in clinical management of AML to induce remission and also for post remission therapy.
In the present study, we treated SCID mice bearing subcutaneous human AML tumour (HL60) xenografts with AZD1152 (25 mg/kg once daily i.p. for 4 consecutive days) or AraC (25 mg/kg twice daily i.p. for 2 consecutive days) as monotherapies or together in two overlapping combination schedules [either AZD1152 (Day 1−4) plus AraC (Day 1−2) (SCHEDULE 1) or AZD1152 (Day 1−4) plus AraC (Day 3−4) (SCHEDULE 2)]. Both treatments, when dosed as monotherapy, produced significant tumour growth inhibition (TGI) compared to vehicle-control animals (Maximum TGI of 31.7% & 48.3% for AraC & AZD1152 respectively, both $p < 0.05$). When dosed in combination, both sequences of dosing produced enhanced anti-tumour activity compared to vehicle-control (Maximum TGI of 110.9% for SCHEDULE 1 & 76.2% for SCHEDULE 2, both $p < 0.05$), as well as the monotherapy groups. Additionally, the data suggest that the combination SCHEDULE 1 was more effective in inhibiting tumour growth compared to combination SCHEDULE 2. Histological analysis of tumour sections showed a decrease in mitotic cells and an increase in apoptotic cells in drug treated tumours compared to vehicle-control treated tumours. Additionally, there was an increased level of apoptosis in tumours treated with SCHEDULE 1 compared to tumours treated with SCHEDULE 2, in concordance with the effects on tumour growth.
These data indicate a promising therapeutic strategy of combining AZD1152 and AraC for the treatment of AML, and suggest that the schedule of drug administration may have a consequence on the overall anti-tumour efficacy. AZD1152 is currently in phase II trials.

498 POSTER

In vivo evaluation of TAK-960, a novel, orally bioavailable inhibitor of Polo-like kinase 1

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Background: Polo-like kinase 1 (PLK1) plays an essential role in mitosis, including chromosome segregation, centrosome maturation, bipolar spindle formation, regulation of anaphase-promoting complex, and execution of cytokinesis. Human PLK1 has been shown to be overexpressed in various human cancers, and has been associated with poor prognosis. TAK-960 is a novel, highly selective inhibitor of PLK1 that demonstrates nanomolar activity *in vitro*. TAK-960 is currently being investigated in phase I clinical trials.
Materials and Methods: Nude mice or SCID mice ($n = 5$) were inoculated subcutaneously with human cancer cell lines and treated PO using various dosing schedules. Antitumor activity was evaluated by the ratio of treated to control (T/C) tumor volume on day 14 or 21 and response criteria modeled after the clinical standards. In PK/PD studies, mitotic index (pHistone H3 ELISA) and TAK-960 concentrations in tumor and plasma were evaluated in HT-29 xenograft tumor tissues after a single PO or IV administration.
Results: Once daily (QD) administration of TAK-960 potently inhibited the tumor growth of HT-29 colorectal xenograft model in a dose-dependent manner with T/C values of −7.59, −20.2 and −20.3% at 6.25, 10 and 12.5 mg/kg, respectively. Complete regression (CR) was observed in 4/5 mice in 10 and 12.5 mg/kg groups. TAK-960 also resulted in regression in two hematological malignancy models, MV4−11 (AML, 10 mg/kg of TAK-960 QD for 2 weeks, 4 partial responses (PRs) in 5 mice) and KARPAS299 (NHL, 10 mg/kg of TAK-960 QD for 3 weeks, 1CR and 2PRs in 5 mice). In addition, 10 mg/kg of TAK-960 QD $\times$ 6/week for 2 weeks resulted in a significant T/C of 4.7% against K562ADR xenograft model, which was established as doxorubicin-resistant cell line from K562 (CML). In the PK/PD studies, TAK-960 is distributed preferentially into tumor tissue compared to the circulating plasma levels, irrespective of the dosing routes. AUE (area under the effect-versus-time curve) for pHistone H3 appears to have a linear correlation with exposure of TAK-960 in HT-29 tumor xenografts.
Conclusions: TAK-960 showed the potent antitumor activity against various xenograft models including MDR1-expressing tumors, by oral administration. TAK-960 induced PD responses, which correlated with preferential retention of TAK-960 in tumor tissues. Taken together, these preclinical data indicate the therapeutic potential of TAK- 960 in the treatment of diverse human malignancies.

499 POSTER

Metastatic lung cancer proliferation is inhibited by Caveolin-1 silencing

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Background: Caveolin-1 (cav-1) is an essential structural constituent of caveolae implicated in mitogenic signalling, oncogenesis, angiogenesis, neurodegenerative diseases and senescence. Its role as an oncogene or as a tumour suppressor gene seems to strictly depend on cell type and tumour stage/grade. The high expression of caveolin-1 in some tumours *in vivo,* amongst which lung adenocarcinoma, is associated with increased tumour aggressiveness, metastatic potential and suppression of apoptosis. The aim of the present study was to investigate the role of caveolin-1 in metastatic lung cancer proliferation.
Materials and Methods: Human cell lines RAL and SCLC-R1 were obtained by us from metastatic lesions of lung adenocarcinoma and of small cell lung carcinoma respectively and grown in H/H medium supplemented with 10% foetal bovine serum (FBS). Inhibition of Cav-1 expression was performed by the use of small interfering RNA (siRNA). Cell growth inhibition was determined by Trypan Blue Dye Exclusion test and protein expression by Western Blotting analysis.
Results: Results indicate that lung RAL and SCLC-R1 metastatic cells express high levels of cav-1 protein; a siRNA-mediated down-regulation of cav-1 expression is evident in SCLC-R1 (100%) and RAL (80%) cells; cav-1 knockdown causes arrest of cell growth in both cell lines, maintained up to 72 h after transfection; cav-1 inhibition affects the expression of cell cycle regulatory proteins (cyclin-D1, Cdk2, Cdk4, phosphoRb) and thereby cell cycle progression, by a novel molecular pathway that we describe here.
Conclusions: A growing body of evidence links elevated cav-1 expression to an aggressive malignant and metastatic phenotype in several tumors. This has been recently reported in lung adenocarcinoma. The present data indicate for the first time that lung RAL and SCLC-R1 cell lines express high levels of cav-1 and demonstrate that cav-1 knock-down arrests metastatic growth either in small cell lung carcinoma or in adenocarcinoma in vitro by a novel molecular pathway.
Grants from MIUR (RFO, PRIN), Pallotti's Legacy for Cancer Research, University of Bologna, Italy.

500 POSTER

A novel pyrazolo[4,3-d]pyrimidine inhibitor of cyclin-dependent kinases: antiproliferative and proapoptotic effects

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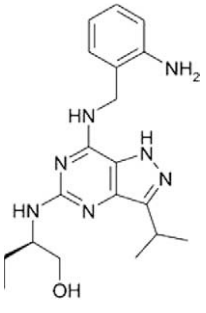
Background: Cyclin-dependent kinases (CDK) are a group of enzymes involved in many cellular processes including regulation of the cell cycle and transcription. Deregulation of the cell cycle connected with CDK hyperactivity is a common feature of tumor cells and provides a rationale for the development of specific CDK inhibitors. We have recently prepared a novel class of purine bioisostere CDK inhibitors based on the pyrazolo[4,3-*d*]pyrimidine skeleton. This work is focused on the biological and biochemical characterization of a new 3,5,7-trisubstituted pyrazolo[4,3-*d*]pyrimidine, LGR1492.

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.
The work was supported by grants from MSM (6198959216) and GACR (204/08/0511).



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