and time-dependent cell cycle arrest at G2/M, with appearance of 8N peaks (polyploidy) observed at 48 h and 72 h after drug exposure. In the MDA-MB-231 *in vivo* model, there were not fully additive effects of AS703569 with SoC agents cisplatin or taxotere. In vivo, AS703569 significantly inhibited tumor growth in 8/10 human primary BrCa models. The anti-tumor effect was not dependent on the status of Rb or p53. An impressive decrease of pHH3 was observed 6h after a single administration of AS703569 in the 3 primary xenografts tested, indicating that the drug induced a strong and rapid inhibition of AK activity. In a basal-like primary breast xenograft model showing tumor relapse after anthracycline-based chemotherapy, AS703569 administration significantly inhibited tumor recurrence.
Conclusions: In summary, this study shows for the first time that Aurora kinase inhibitor AS703569 has a strong anti-tumoral activity on a large panel of *in vitro* and *in vivo* human primary TNBC models. When combined with anthracyclines, it inhibited tumor recurrence in a basal-like breast cancer xenograft, suggesting that Aki could be used both in monotherapy and combination settings.

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A phase I trial of SCH900776, a selective inhibitor of checkpoint kinase CHK-1, in combination with Gemcitabine in advanced solid tumors

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Background: In cells undergoing DNA synthesis, antimetabolite-induced replication arrest results in the induction of CHK1, halting the progression of cells through G1/S to allow for DNA damage repair. Inhibition of CHK1 by SCH 900776 is hypothesized to synergize with Gem to promote replication fork collapse and apoptosis, even in the setting of anti-metabolite resistance.
Methods: A dose escalation study of SCH 900776 alone and in combination with fixed doses of Gem was conducted in subjects with advanced solid tumors. Subjects were assessed for safety, tolerability, dose-limiting toxicity (DLT), and maximal administered dose (MAD). A recommended Phase 2 dose (RP2D) will be determined based on the safety profile at pharmacologically active exposures.
Results: Twenty-six subjects have been enrolled and treated with 10 (n = 3), 20 (n = 3), 40 (n = 7), 80 (n = 6), and 112 mg/m^2 (n = 7) of SCH 900776 administered alone and following Gem (800 mg/m^2) in Part A on Days 1 and 8 every 21 days. Four subjects at 80 mg/m^2 and 3 subjects at 112 mg/m^2 of SCH 900776 have been enrolled and treated with Gem (1000 mg/m^2) in Part B. No DLTs have been observed and one SAE (G3 hyperbilirubinemia) has been reported during SCH 900776 monotherapy lead-in. Three reversible DLTs have been observed for the combination; supraventricular tachycardia with pneumonia/pneumonitis at 40 mg/m^2 and atrial fibrillation and Grade 4 thrombocytopenia at 112 mg/m^2 (1 subject each) of SCH 900776. MAD is 112 mg/m^2. Clinical activity has been noted in 5 subjects: PR in melanoma and Cholangiocarcinoma, prolonged SD in spindle cell sarcoma and 2 SDs in pancreatic cancer previously treated with Gem. Mean t1/2 is 6.29–9.38 hrs. Cmax and AUC(I) increase dose-proportionally across the dose range of SCH 900776. Similar PK exposures exist between SCH 900776 monotherapy and in combination with Gem. Exposure threshold for preclinical activity ($\geqslant$0.5 μM Cmax) and PD evidence of target engagement were achieved in the first dose cohort (10 mg/m^2).
Conclusions: Pharmacologically active plasma concentrations of SCH 900776 associated with the modulation of the CHK1 mechanism have been safely achieved in combination with Gem with early evidence of clinical activity, including in tumors previously progressing on Gem.

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Pharmacological profile of the novel pan-CDK inhibitor BAY 1000394 in tumor models of human small cell lung cancer, breast and prostate cancer as monotherapy and combination treatment

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BAY 1000394 is a nanomolar pan CDK inhibitor based on an aminopyrimidine scaffold. It shows good solubility in water, high metabolic stability, low blood clearance and moderate oral bioavailability in rats. BAY 1000394 inhibits cell proliferation in vitro at low nanomolar concentration in a broad spectrum of human cancer cell lines and shows potent and dose-dependent inhibition of the growth of human cervical HeLa-MaTu xenograft tumors.
Here we present the pharmacological profile of BAY 1000394 in a series of xenograft models of human small cell lung cancer (SCLC), breast and prostate cancers. SCLC xenograft models were generated from either cultured cells (NCI-H146, NCI-H82, NCI-H209, NCI-H69) or patient explants propagated in SCID mice (LXFS 538, LXFS 650, Lu7530). With oral dosing at various dose levels and schedules (2 mg/kg QD; 2.5 mg/kg BID $\times$ 2 and 5 days off; 1.7 mg/kg BID $\times$ 3 and 4 days off), median tumor growth inhibition (TGI) was 86% (range 60−95%). In the NCI-H209 model BAY 1000394 was similarly efficacious as compared to cisplatin, whereas in all other SCLC models BAY 1000394 was more efficacious than cisplatin (median TGI 59%). In the NCI-H82 model, BAY 1000394 (at suboptimal doses and schedule of 0.75, 1.0, or 1.5 mg/kg BID x3 and 11 days off) in combination with either cisplatin (at optimal dose and schedule of 6 mg/kg once, 13 days off) or etoposide (at optimal dose and schedule of 12 mg/kg QD x3, 11 days off) or the combination of cisplatin and etoposide showed strong synergistic efficacy, achieving TGI in the range of 91% to 105%.
In the MDA-MB 231 xenograft model of human triple-negative breast cancer, BAY 1000394 showed strong synergy with taxanes in combination treatment. Combination of BAY 1000394 (1.5 mg/kg BID $\times$3 and 4 days off) with paclitaxel (18 mg/kg once and 13 days off) resulted in TGI of 122%, whereas monotherapies using the same doses and schedules achieved TGI of only 26% for paclitaxel and 39% for BAY 1000394. Similar synergistic activity was also observed for the combination of BAY 1000394 (1 mg/kg BID $\times$3 and 4 days off) and docetaxel (4 mg/kg Q2D $\times$5) in the PC3 xenograft model of human prostate cancer.
In conclusion, BAY 1000394 demonstrates significant antitumor activity in xenograft models of human SCLC, breast and prostate cancers, both as monotherapy and in combination with chemotherapy.

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A phase I dose-escalation study of BI 811283, an Aurora B inhibitor, administered day 1 and 15 every four weeks, in patients with advanced solid tumours

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Background: BI 811283 is a reversible, potent inhibitor of Aurora B kinase. It causes mitotic override, induction of polyploidy, apoptosis and senescence. In vivo studies showed broad anti-tumour activity in several mouse xenograft models.
Material and Methods: Patients with a variety of advanced/metastatic solid malignancies were randomised to two treatment schedules (4-week & 3-week) in a phase I dose-escalation study. This abstract reports the results of the 4-week schedule. BI 811283 was administered as a 24-hour continuous infusion via central venous access, on Days 1 and 15 every 4 weeks. All patients underwent pharmacokinetic sampling. Pre- and post-treatment skin biopsies were performed to measure levels of histone H3 phosphorylation by Western analysis and immunohistochemistry (IHC), as a marker of Aurora kinase inhibition.
Results: A total of 62 patients were treated at two centres: M/F = 29/33, median age: 60 (range: 23−76); ECOG PS: 0/1/2: 27/32/3. Median number of courses administered: 2 (range:1−16). Patients were treated at 12 dose levels: from 5 to140 mg (Days 1 and 15, q4w). The most common AEs included: fatigue, anorexia, nausea, alopecia, diarrhoea, neutropenia and leucopenia. Haematological toxicity was the main adverse event and was dose-limiting. Dose-limiting toxicities observed included: G4/G3 AST/ALT (n = 1), G3 thrombocytopenia (n = 1), G3 anaemia (n = 1) and G3 neutropenia (n = 3). Dose-limiting neutropenia was seen at higher dose levels. The maximum tolerated dose (MTD) was exceeded at 140 mg, therefore 125 mg was defined the MTD. The best response was stable disease in 16 of 51 patients (31%) with complete data set and who were evaluable. C_{max} and AUC exposure appeared to increase with dose in a linear fashion at all dose levels. Mean terminal -------$t_{1/2}$ ranged from 10−20 hours. The AUC and C_{max} values of total BI 811283 (bound to AGP + unbound) appeared to increase with increasing pre-dose levels of α-acid glycoprotein (AGP) in patients with high variability. IHC analysis of skin biopsies showed a reduction in histone H3 phosphorylation post-treatment particularly at higher doses, consistent with Aurora kinase inhibition. Western analysis was less conclusive.

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.

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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.

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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.

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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.
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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.