synthase and incorporated into the DNA molecule, resulting in interruption of DNA synthesis. However, orally administered FTD is rapidly degraded to an inactive form by thymidine phosphorylase. TPI increases the concentration of FTD by preventing its degradation. In the U.S., 5 phase I studies were conducted at different schedules. Those studies showed divided daily dosing of TAS-102 maintained stable disease (SD) and a twice daily schedule was more feasible than a three times a day schedule. Accordingly, we conducted a phase I study with twice daily administration of TAS-102 to Japanese pts with advanced solid tumors.
Materials and Methods: Pts with advanced solid tumors, ECOG PS of 0 to 2, and adequate organ functions were eligible. TAS-102 was orally administered twice daily for days 1 to 5 and 8 to 12, repeated every four weeks. The objectives were to determine the maximum tolerated dose (MTD) and dose limiting toxicity (DLT), to assess anti-tumor activity, pharmacokinetics and pharmacodynamics.
Results: A total of 21 pts (14 males, median age 59 yrs, median prior therapy 3 regimens) were enrolled into 5 dose levels (at 30, 40, 50, 60, and 70 mg/m^2/day). Eighteen pts had colorectal cancer. Two pts experienced DLTs during cycle 1; one pt developed grade 4 neutropenia, leucopenia and thrombocytopenia at 30 mg/m^2/day, and another developed grade 4 neutropenia and leucopenia at 70 mg/m^2/day. The most common grade 3 and 4 toxicities in cycle 1 were hematological toxicities. Although the MTD was not reached, the frequency of grade 3 and 4 neutropenia tended to increase in a dose-dependent manner. Therefore, dosage was not escalated more than 70 mg/m^2/day. Although there were no objective responses, 11 pts (52%) maintained SD by RECIST. One pt with colon cancer showed partial response at one assessment. SD persisting longer than 12 wks was observed in 8 pts (38%). The pharmacokinetics in Japanese pts was comparable with the results of the U.S. study.
Conclusions: Twice daily administration of TAS-102 is well tolerated with manageable hematological toxicities in Japanese pts with advanced solid tumors. The recommended dose for phase II trial of TAS-102 administered twice daily was determined to be 70 mg/m^2/day. We are currently planning to conduct studies of TAS-102 alone and in combination with other cancer drugs.

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Phase 1 study of food effects on pharmacokinetics of brivanib alaninate in patients with advanced or metastatic solid tumors

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Background: Brivanib alaninate is the prodrug of brivanib (BMS-540215), a dual inhibitor of vascular endothelial growth factor receptor (VEGFR) and fibroblast growth factor receptor (FGFR) signaling. In a previous pilot study (n = 5) conducted with an earlier formulation of brivanib, a high-fat meal slightly reduced C_{max} by 24% without affecting AUC. The aim of this study was to assess the effect of a high-fat meal on the PK of brivanib in patients with advanced or metastatic solid tumors.
Material and Methods: This was a phase 1, open-label, randomized, 2-treatment, 2-period, crossover study evaluating the effect of a high-fat meal or fasting on the PK of brivanib. Patients were assigned to either fasting or a high-fat meal after 10 h of fasting and received a single 800-mg oral dose of brivanib on Day 1. After a 7-day washout period, patients received a single 800-mg oral dose of brivanib on Day 8 and were allocated to the reverse meal content. PK samples were collected up to 48 h post-dose. Patients were monitored for adverse events (AEs) throughout the study. Physical examination, vital signs, and clinical laboratory tests were also assessed throughout the study.

Table: Geometric mean (CV%) of PK parameters of brivanib in fasting and high-fat meal groups

Parameter	Fasting (n = 19)	High-fat meal (n = 19)
T_{max} (h), median (range)	4.0 (1.0–9.8)	3.1 (1.0–10.0)
C_{max} (ng/mL)	2847 (40%)	2877 (46%)
AUC_{0-T} (ng·h/mL)	44,610 (32%)	39,503 (39%)
AUC_{inf} (ng·h/mL)	53,685 (36%)	48,823 (40%)
$T_{1/2}$ (h), mean (SD)	18.3 (6.4)	17.7 (5.8)

Results: A total of 29 patients were enrolled; 21 completed both parts of the study having ingested a minimum of 800 calories, while 19 were evaluable for PK. There was no effect of food on the PK of brivanib. The geometric mean PK parameters are shown in the Table. The geometric mean ratio (90% CI) of high-fat meal/fasting of C_{max} and AUC_{inf} were 1.00 (0.86 to 1.18) and 0.92 (0.82 to 1.02), respectively. The incidences of most frequently reported AEs – constipation, fatigue, hypertension, and nausea were similar in the high-fat and fasting treatment groups. Furthermore, the changes in laboratory values were similar in the 2 treatment groups.
Conclusions: The systemic exposure (C_{max} and AUC_{inf}) of brivanib, following a single oral 800 mg dose of brivanib alaninate, was unaffected by a high-fat meal compared with fasting in patients with advanced or metastatic solid tumors, confirming that brivanib can be given with or without food.

Polo kinases

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Characterization of BI 6727, a novel Polo-like kinase inhibitor with a distinct pharmacokinetic profile and efficacy in a model of taxane-resistant colon cancer

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Background: Plk1 is a key regulator of multiple steps in mitosis and an attractive target for cancer drug discovery. We have previously presented data on BI 2536, a dihydropteridinone inhibitor of Plk1 currently in Phase II clinical studies. To further explore the potential of Plk1 inhibition in oncology, we have synthesized and profiled additional derivatives and now describe BI 6727, a novel clinical candidate with distinct pharmacological and pharmacokinetic characteristics.
Material and Methods: Inhibition of Plks and other kinases was assessed in enzyme assays. The anti-proliferative activity of BI 6727 was determined using AlamarBlue assays. PK profiles were determined in mice and rats. Nude mice bearing subcutaneous xenografts derived from lung (NCI-H460) or colon cancer (HCT-116, CxB1) were treated i.v. (weekly doses, 40−50 mg/kg) or p.o. (50−70 mg/kg) using various schedules.
Results: BI 6727 is a potent and selective Plk1 inhibitor (IC50 = 0.87 nM) that blocks proliferation of multiple cancer cell lines with EC50 values in the range of 10–40 nM, inducing a distinct prometaphase arrest phenotype ("Polo-arrest") and apoptosis. The pharmacokinetic profile indicates sustained tissue exposure with a high volume of distribution and a long terminal half-life in mice (Vss = 7.6 L/kg, $t\frac{1}{2}$ = 46 h) and rats (Vss = 22 L/kg, $t\frac{1}{2}$ = 54 h). The physicochemical and pharmacokinetic properties of the compound allow in vivo testing of intravenous as well as oral (F = 40–55%) formulations. BI 6727 shows efficacy in multiple models of human cancer, including a model of taxane-resistant colorectal cancer, independent of route of administration or treatment schedule.
Conclusion: BI 6727 is a potent and selective Plk inhibitor with sustained tissue exposure that shows efficacy in multiple human cancer xenograft models using oral and intravenous dosing schedules. The compound has been advanced into clinical phase I testing.

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A phase I first-in-human study of the polo-like kinase 1-selective inhibitor, GSK461364, in patients with advanced solid tumors

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Background: Polo-like kinase 1(Plk1) plays multiple roles during mitotic progression. Plk1 over expression is present in a broad range of cancers and is associated with poor prognosis in some tumor types. GSK461364 is potent inhibitor of Plk1 (~400-fold more selective for Plk1 vs. Plk 2 or 3) and has demonstrated anti-proliferative activity against a large panel of cancer lines as well as efficacy against multiple xenograft tumor models.
Methods: Pts with advanced solid tumors, ECOG PS 0−2, and adequate organ function were included in this study. Sequential cohorts of 2−3 pts received escalating doses of GSK461364 administered as a 4-hr IV infusion on different schedules. The primary objectives of the study were to determine the MTD and PK of GSK461364. Secondary objectives included preliminary evaluation of anti-tumor activity.
Results: 12 pts (10M/2F), median age 60.5, were evaluated on two schedules at 5 dose levels [D1, 8, 15 q28: 50 mg(n = 2); 100 mg(n = 3); 150 mg(n = 3)] [D1, 2, 8, 9, 15, 16 q28: 25 mg(n = 2); 50 mg(n = 2)]. Data are available for 10 pts. A median of 2 cycles were administered for a total of 17 cycles. The most common adverse events, regardless of attribution,

included abdominal or pelvic pain/discomfort [7 events; Gr1/2 (4), Gr3 (3)], infusion site reaction/thrombophlebitis [5 events; Gr1/2], malaise/fatigue [5 events; Gr1/2], and nausea [4 events; Gr1/2]. One Gr4 and eight Gr3 AEs, all attributed to underlying cancer, were observed and included pulmonary embolus (Gr4), abdominal/pelvic pain (3), ascites (2), pleuritic chest pain, vomiting, and constipation. The most common lab abnormalities observed during treatment included hyperbilirubinemia [4 pts; Gr1/2 (3), Gr3 (1)], hyperglycemia (4 pts; Gr1), and anemia [4 pts; Gr1/2 (4)]. Two Gr3 lab abnormalities were noted and included hypermagnesemia and hyperbilirubinemia that occurred in the presence of disease progression involving the liver. No DLTs were observed. To date, no consistent effects on hematologic parameters were observed. Preliminary PK data indicate that AUC and Cmax were proportional across doses; mean values were CL (~75 L/hr), Vss (~900 L), and t1/2 (~13hr). At the doses administered thus far, there is no evidence of anti-tumor activity.
Conclusions: No consistent mechanism-related toxicity has been observed and an MTD has yet to be defined. Dose escalation continues.

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A small molecule allosteric inhibitor of Polo-like kinase 3 induces apoptosis and disrupts the integrity of the mitotic spindle apparatus in cancer cells

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Proper functioning of the mitotic spindle apparatus is paramount for normal cellular division and maintenance of genomic integrity. Amplification and/or over-expression of proteins within the Aurora/centrosome signaling pathway, which include members of the Aurora and Polo like kinase families, lead to mitotic instability and malignant progression in many solid tumors. For these reasons, many pharmaceutical companies have created programs to identify inhibitors of the Aurora and Polo kinases. Utilizing a gene expression biomarker based platform (AvalonRx) we identified small molecule compounds that disrupt the centrosome signaling pathway. One compound series, LC-445, were predicted to be novel kinase inhibitors based upon gene expression biomarker profiling and comparison to a database of gene expression profiles from hundreds of small molecule drugs with known mechanisms. To identify the kinase targets of the compound series, we tested a panel of 220 kinases using a Fluorescent Resonance Energy Transfer-based assay. The compound was found to be highly selective with activity against only a few kinases, most notably of which was Polo-like Kinase 3. Polo-like Kinase 3 has been reported to mediate multiple mitotic process including bipolar spindle formation, activation of CDC25C, centrosome maturation and activation of the DNA damage response. LC-445 compounds induce cell cycle arrest and apoptosis and are broadly active against a large panel of malignant cell lines. We found that LC-445 causes a decrease in phosphorylation of Ser-191 of CDC25C (the activation site of CDC25C by PLK3) in a concentration dependent manner. Kinetic studies show that LC-445 is a non-ATP competitive, allosteric inhibitor of PLK3. Furthermore, LC-445 compounds affect not only PLK3 enzymatic activity but decrease levels of PLK3 protein both in cell culture and in xenograft animal models. Importantly, treatment of cancer cells with LC-445 induced formation of monopolar and tripolar spindles, abnormal chromosome alignment and a disruption of spindle structure reminiscent of the known spindle check point inhibitors. These data show that LC-445 compound series is a novel, specific allosteric inhibitor of PLK3 that inhibits kinase activity and PLK3 protein levels leading to mitotic catastrophe and subsequent cell death in cancer cells.

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Crystal structures of Plk1 kinase domain in complex with ATP-competitive inhibitors

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Background: Polo-like kinase 1 (Plk1) is a serine/threonine protein kinase involved in several processes during mitosis. It belongs to the Polo-like-kinase family, comprising the structurally related Plk1, 2, 3 and 4 proteins. All of these proteins are characterized by an N-terminal kinase domain and a C-terminal “polo-box” domain. Pkl1 is ubiquitously expressed in normal tissues and is over-expressed in a wide variety of human tumours, where it also correlates with poor prognosis. Inhibition of Plk-1 expression by siRNA or DNA antisense oligonucleotides further validates Plk1−1 as an attractive target for anticancer drug therapy.
Material and Methods: In order to help in the design of Plk1 small-molecule inhibitors, we initiated a program to determine the crystal structure of the Plk1 catalytic domain. A total of 33 protein constructs bearing different combinations of the N- and C-termini of the catalytic domain were cloned, expressed and purified for crystallization trials.
Results: In the end we succeeded in growing crystals by co-crystallization of the methylated construct Plk1(36–345) with the ATP analog AMP-PNP. The crystals belong to space group P3221 and have one molecule in the asymmetric unit. The resulting structure was solved at a resolution of 2.0 Å and comprises the residues 39–328. Interestingly, the crystal contains a dimer of symmetry-related molecules tethered by zinc ions, a feature that was also observed by other groups (1). Subsequently we solved the structure of Plk1(36–345) in complex with different classes of Plk1 inhibitors and the analysis of these complexes allowed us to identify key structural features of the investigated inhibitors. This information proved helpful in guiding the chemical expansion and obtaining potent and selective Plk1 inhibitors.
Conclusions: The crystal structure of Plk1(36–345) construct with different inhibitors will be shown and key features within the different chemical classes will be discussed in the poster.

References

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Antitumoral activity of pyrazoloquinazoline derivatives as potent oral Plk-1 specific inhibitors

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Background: Polo-like kinase 1 (Plk-1) is a serine / threonine protein kinase involved in different stages of mitosis with roles in centrosome maturation, bi-polar spindle formation, chromosome separation and cytokinesis. The expression, activity and localization of Plk-1 is dynamically regulated during the cell cycle and PLK-1 protein levels increase from the late S phase to mitosis. Plk-1 is over-expressed in a variety of human tumours including lung, colon, stomach, breast, ovary, head and neck, and melanomas where often correlates with poor prognosis. Inhibition of Plk-1 expression by siRNA or DNA antisense oligonucleotides further validates PLK-1 as an attractive target for anticancer drug therapy.
Material and Methods: From HTS screening several small molecules belonging to the pyrazoloquinazoline class emerged as interesting hits to target Plk-1 kinase. Chemical modifications at the R1, R2 and R3 residues of the pyrazoloquinazoline core scaffold reported in the figure resulted in new compounds with favourable drug-like properties. The Co-crystal structure of methylated construct Plk-1 (36–345) with some of the most interesting compounds was also determined.
Results: These compounds posses sub-nanomolar Ki in a Plk-1 biochemical assay, accompanied by a very high selectivity towards a panel of more than 250 kinases and no cross-reactivity with the other PLK family members. The compounds exhibit high potency in an antiproliferation assay having IC50 < 100 nM on a large number of cell lines, both from solid and haematological tumors, while demonstrating excellent PK properties and good oral bioavailability in rodent and non-rodent species. Oral administration of compounds causes tumor stabilization or regression in a variety of tumor xenograft models using flexible schedules. Notably also prolonged daily administration is well tolerated.
Conclusions: In summary, the data suggest that some members of this chemical class are very potent antiproliferative agents suitable for further development as oral and selective Plk-1 inhibitors for anticancer therapy.