interaction was investigated by ELISA and measuring the inhibition of Tie2 phosphorylation on HEK293-Tie2 cells. Antitumoral efficacy was assessed in established s.c. Colo205 and orthotopic i.m.f.p. KPL-4 xenografts in SCID beige mice. Tumors were explanted for histological analysis. Inhibition of angiogenesis was assessed in the cornea micropocket assay.
Results: Two lead antibodies, LC06 and LC08, were selected by biochemical and cellular assays: LC06 is selective for Ang-2; and LC08 shows cross-reactivity for Ang-1. Selectively blocking Ang-2 by LC06 resulted in a very potent tumor growth inhibition in subcutaneous and orthotopic tumor models that was at least comparable to the tumor growth inhibition mediated by the Ang-1/Ang-2 cross-reactive antibody LC08. However, selectively blocking Ang-2 by LC06 appeared to result in larger necrotic areas compared to blocking both cytokines. These effects were attended with a reduction of intratumoral microvessel density indicating an anti-angiogenic mechanism. Remaining vessels were better perfused hypothesizing a normalization phenotype. Further more, Ang-2 neutralizing antibodies potently inhibited VEGF-induced angiogenesis in the mouse corneal angiogenesis assay.
Conclusions: Taken together, these data provide strong support for the application of Ang-2 selective antibodies for the treatment of cancer patients by affecting neovascularization as well as survival of tumor cells.

490 POSTER

MMP-9 as a stromal target in cancer

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Matrix metalloproteinase 9 (MMP-9) is a secreted zinc metalloprotease which influences tumor recurrence and invasiveness, and is associated with angiogenesis. MMP-9 is one of two major gelatinases in the MMP family, which in addition to efficiently and rapidly cleaving unfolded collagen has been reported to cleave other matrix and nonmatrix components. Some of the reported extracellular catalytic actions of MMP-9 include generation of tumstatin from type IV collagen and release of soluble Kit ligand or bioactive VEGF, the latter being a major contributor to tumor angiogenesis.
Utilizing our phage display technology, we have identified DX-2802, a selective human monoclonal antibody targeting MMP-9. DX-2802 (IgG1 Lambda) potently inhibits human and mouse MMP-9 (IC_{50} = 2−3 nM) but does not inhibit a panel of other metalloproteinases tested. This antibody displays potent anti-invasive activity *in vitro* and significantly attenuated outgrowth of metastatic foci in the MC38 experimental intra-splenic mouse model in part by reducing tumor angiogenesis. Interestingly, DX-2802 did not affect the number of lesions in the livers or primary tumor growth in the cecum demonstrating that the effect of the antibody is not on the tumor cells themselves but on the tumor microenvironment. Our results are consistent with those previously published in *mmp-9* knockout mice crossed to the MMTV- PyVMT model of breast cancer (Martin et al, Cancer Res, *68:* 6251–6259, 2008) and show that MMP-9 may be considered as a stromal target in cancer.

Cell-cycle-interactive agents

491 POSTER

NCIC CTG IND.177: Phase I study of AT7519M given as a short infusion twice weekly

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Background: AT7519M is a small molecule inhibitor of multiple cdks (1, 2, 4, 5, 9) with lower potency against 3, 6 and 7. A recent phase I trial examined a daily short infusion for 5 days every three weeks. Dose dependent QTc prolongation was noted on this schedule. This study examines safety and tolerability of AT7519 delivered on an alternative schedule.
Material and Methods: Patients with refractory solid tumours or lymphoma were eligible and received escalating doses of AT7519M on days 1,4,8,11 every 3 weeks. A protocol amendment in 2007 excluded patients at risk of QTc prolongation and instituted serial EKG evaluation. Pharmacokinetics (PK) were planned for all patients. Patients at the recommended phase II dose level (RP2D) were planned for Holter monitoring and serial tumour and tissue acquisition to examine pharmacodynamic (PD) effects.
Results: 29 patients were treated at 4 dose levels from 14.4 mg/m^2 to 32.4 mg/m^2. RP2D was 27 mg/m^2. Dose limiting toxicity included mucositis, rash, fatigue and muscle weakness, renal dysfunction and febrile neutropenia. The most common toxicities were fatigue (46%), mucositis (50%), nausea or vomiting (36%). Hematologic toxicity was mild other than 1 patient who had grade 4 neutropenia documented. There was no evidence of QTc prolongation, including in external review of EKGs. Nine patients have had stable disease (2.5–11.1 months). PK are dose proportional. Accrual continues to the expanded RP2D level and patients are undergoing Holter testing (QTc) and PDs.
Conclusions: AT7519M given in a short infusion appears to be tolerable and is not associated with QTc prolongation noted with other schedules. NCIC CTG plans phase II trials in mantle cell lymphoma and CLL.

492 POSTER

GNE-900, an orally bioavailable selective CHK1 inhibitor, illustrates that optimal chemosensitization is schedule and tumor type dependent

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Checkpoint kinase 1 (CHK1) is a serine/threonine kinase, which functions as a central mediator of the S-phase checkpoint, blocking the G2/M transition to allow for repair of DNA damage. Inhibition of CHK1 is a strategy for selectively potentiating the efficacy of chemotherapeutic agents in G1 checkpoint defective tumor cells while minimizing toxicity to normal, checkpoint competent cells. Here, we show that GNE-900 is an ATP-competitive, selective, and orally bioavailable CHK1 inhibitor optimized from an HTS lead using structure-based drug design. In combination with chemotherapeutic agents, GNE-900 sustains ATR signaling, enhances DNA damage and induces apoptotic cell death. Checkpoint abrogation correlates with defects in the p53 G1 checkpoint gene and results in premature mitotic entry and induction of cell death. Importantly, we demonstrate that this class of CHK1 inhibitor has little single agent activity in the absence of chemotherapy and does not strongly potentiate the cytotoxicity of chemotherapeutic agents in normal bone marrow cells. In vivo scheduling studies using BrdU incorporation demonstrate that optimal timing for administration of CHK1 inhibitors following treatment with gemcitabine is coincident with release from S-phase arrest. With this schedule, gemcitabine antitumor activity is significantly enhanced in combination with GNE-900 in both gemcitabine-sensitive and resistant tumors. In summary, we demonstrate that in vivo potentiation of gemcitabine activity is mechanism-based, with optimal efficacy observed when S-phase arrest is induced first, followed by checkpoint abrogation with CHK1 inhibitor. Evaluation of alternate dosing schedules following administration of chemotherapy will be critical to the clinical development of this class of kinase inhibitors.

493 POSTER

AS703569/R763, a pan Aurora kinase inhibitor, shows strong antitumor activity in vitro and in vivo in a panel of triple-negative breast cancer cell lines and xenografts

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Background: Triple-negative breast cancers (TNBC) represent of breast cancers (BrCa) that has a particularly aggressive phenotype and poor clinical outcomes. Although there are agents in development for this indication, to date, there is no approved targeted therapy for TNBC. Because Aurora kinases (AKs) play critical roles in chromosome segregation and cell division, we investigated, both *in vitro* and *in vivo*, the effects of AS703569, a small molecule inhibitor of AK, in TNBC relative to other types of breast cancers.
Material and Methods: AS703569 was evaluated for activity in proliferation and cell cycle assays of a panel of breast cancer cell lines. The efficacy of AS703569 was also determined *in vivo* in one TNBC cell line, both alone and in combination with standard of care (SoC) agents, as well as in 10 xenograft models of patient-derived primary human breast cancer. Immunohistochemical analysis of phospho histone H3 (pHH3) expression, the biological indicator of Aurora kinase B activity, was perfomed in 3 of the primary xenograft models.
Results: TNBC cell lines were more sensitive to AS703569 than were other types in a BrCa cell line panel. Cell cycle analyses showed a dose

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.

494 POSTER

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.

495 POSTER

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.

496 POSTER

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.