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

L. Devy[1], B. Fingleton[2], M. Pham[1], Q. Chang[1], K.J. Carter[2], A. Dozier[2], D.T. Dransfield[3]. *[1]Dyax, Cell Biology and Translational Research, Cambridge, USA; [2]Vanderbilt, Vanderbilt University Medical Center, Nashville, USA; [3]Dyax, 300 Technology Square, Cambridge, USA*

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

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

S.J. Hotte[1], E.X. Chen[2], L. McIntosh[3], H.W. Hirte[1], S. Turner[1], A. Jarvi[2], M.S. Squires[4], L. Seymour[3]. *[1]Juravinski Cancer Centre, Oncology, Hamilton, Canada; [2]Princess Margaret Hospital, Medicine/Medical Oncology, Toronto, Canada; [3]NCIC Clinical Trials Group, Cancer Research Institute, Kingston, Canada; [4]Astex Therapeutics Inc., Translational Biology, Cambridge, United Kingdom*

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

S. Malek[1], E. Blackwood[2], T. O'Brien[3], J. Epler[2], I. Yen[1], J. Ramiscal[2], S. Schmidt[1], Y. Xiao[3], J. Choi[3], P. Jackson[3]. *[1]Genentech Inc., Biochemical Pharmacology, South San Francisco CA, USA; [2]Genentech Inc., Translational Oncology, South San Francisco CA, USA; [3]Genentech Inc., Cell Regulation, South San Francisco CA, USA*

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.

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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

E. Marangoni[1], A. Clark[2], F. Assayag[1], S. Chateau-Joubert[3], A. Hamini[1], M.F. Poupon[1], E. Liu[2], A. Spooner[2], A. Romanelli[2], D. Decaudin[1]. *[1]Institut Curie, Translational Department, Paris, France; [2]EMD Serono, Research Institute, Rockland, USA; [3]National Veterinary School, Pathology Department, Maisons-Alfort, France*

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