signaling by phosphatidylinositol-3-kinase (PI3K) or Akt, two of its important upstream regulators. Considering this, mTOR is a very attractive target for pharmacological inhibition in cancer therapy and several mTOR inhibitors are at various stages in development. One important consequence of mTOR inhibition is the abrogation of HIF1α translation, which leads to the shutdown of the VEGFR and PDGFR signaling cascades and the disruption of angiogenesis. Since HIF1α stabilization frequently occurs in renal cancer, the mTOR inhibitor temsirolimus has exhibited promising anticancer activity for the treatment of this malignancy. Despite this, drug resistance continues to be a major obstacle and there is a major focus on the identification of novel therapeutic strategies to improve clinical outcomes. Here we demonstrate that the histone deacetylase (HDAC) inhibitor vorinostat significantly enhances the anticancer activity of temsirolimus in vitro and in xenograft models of renal cancer.
Materials and Methods: The anticancer efficacy of the temsirolimus and vorinostat combination was determined by MTT and clonogenic assays in a panel of nine renal cancer cell lines. We further investigated the antitumor activity of this therapeutic combination in vivo in two xenograft models of renal cancer. Immunohistochemistry was conducted to evaluate the effects of the drug combination on angiogenesis.
Results: Temsirolimus exhibited varying degrees of in vitro efficacy in the nine renal cancer cell lines tested. In spite of this, vorinostat sensitized all nine renal cancer cell lines to temsirolimus-induced death. Further investigation of a "sensitive" and "resistant" cell line in vivo demonstrated that both tumors were equally sensitive to temsirolimus. This indicates that in vitro models may not best predict the in vivo anticancer activity of this agent. Importantly, vorinostat significantly increased the anticancer activity of temsirolimus in both xenograft models evaluated. The combination regimen potently inhibited tumor cell proliferation and angiogenesis suggesting that these are two key mechanisms of action that underlie the antitumor effects of these agents.
Conclusions: Temsirolimus possessed strong anticancer activity in two different xenograft models of renal cancer. Importantly, vorinostat significantly augmented the efficacy of this agent by blocking angiogenesis and inhibiting tumor cell proliferation. A clinical trial to further investigate the therapeutic potential of this combination regimen for the treatment of renal cancer is planned.

330 POSTER

Preclinical evidence for the effectiveness of mTOR inhibitor, nanoparticle albumin-bound (nab®) rapamycin as an anticancer agent

V. Trieu[1], T. De[1], A. Yang[1], J. Cordia[1], B. Grim[1], S. Ci[1], P. Nguyen[1], N. Desai[1]. [1]*Abraxis BioScience LLC., R & D, Los Angeles, CA, USA*

Background: The mammalian target of rapamycin (mTOR) is involved in the control of cellular growth and proliferation and is an important target in tumor therapy. Use of rapamycin as an anticancer agent has been hampered because existing oral formulations have previously shown poor solubility, low oral bioavailability, and dose-limiting-intestinal toxicity. We developed a novel albumin-bound nanoparticle form of rapamycin (*nab*-rapamycin) for intravenous administration and describe its preclinical pharmacokinetic (PK) properties and antitumor activity *in vivo*.
Material and Methods: A nanoparticle form of rapamycin was prepared using Abraxis' proprietary *nab*-technology. Repeated-dose toxicity of *nab*-rapamycin was also determined in Sprague-Dawley rats with dose levels of 0 (vehicle), 20, 40, 90, 120, and 180 mg/kg (N = 5M/5F per group) on a q4dx3 schedule. Pharmacokinetics (PK) of *nab*-rapamycin was investigated in Sprague-Dawley rats at dose levels of 1, 15, 30, and 45 mg/kg. Antitumor activity of *nab*-rapamycin was examined against breast (MX-1, N = 4) and colon (HCT-116, N = 10; HT29, N = 8) tumor models in athymic mice at a dose level of 40 mg/kg with a 3× weekly/4 week or 2x weekly/3−4 week schedule respectively.
Results: Intravenous administration of *nab*-rapamycin was well tolerated in rats at dose levels up to 90 mg/kg/dose on a q4dx3 schedule, with no significant clinical signs of toxicity, and no observed hypercholesterolemia and hypertriglyceridemia. *Nab*-rapamycin exhibited linear pharmacokinetics with respect to dose and rapid tissue distribution and was effective against all tumor models tested ($P < 0.005$), achieving a tumor growth inhibition of 71%, 81%, and 88% against HCT-116, HT29, and MX-1 xenografts respectively.
Conclusions: *Nab*-rapamycin was well tolerated at repeated doses up to 90 mg/kg in rats (540 mg/m^2) with no remarkable toxicity, displayed dose-linear PK and demonstrated effective antitumor activity *in vivo*.

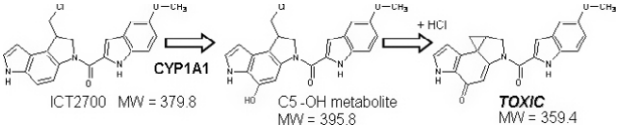
New molecular targets

331 POSTER

CYP1A1 activation and pharmacokinetics of a novel chloromethylpyrrollolindoline with potential as a tumour selective prodrug

A.D. Race[1], K. Pors[1], S.D. Shnyder[1], T.C. Cooper[1], H.M. Sheldrake[1], M. Searcey[2], L.H. Patterson[1], P.M. Loadman[1]. [1]*University of Bradford, Institute of Cancer Therapeutics, Bradford, United Kingdom;* [2]*University of East Anglia, School of Chemical Sciences and Pharmacy, Norwich, United Kingdom*

Introduction: The expression in a wide range of cancers of selected isoforms of Cytochrome P450 (CYP) that have drug metabolising activity has important implications for CYP-mediated tumour selective chemotherapy. We are exploring the use of a novel chloromethylpyrolloindoline (ICT2700) that is inactive until metabolised into a highly potent (IC50 < 1 nM) antitumour agent by CYP1A1. Here we describe the activation and pharmacokinetics of ICT2700 using CHO cell transfected with human CYP1A1 and grown as xenografts.
Materials and Methods: Female Balb/C nude mice bearing s.c. CHO xenografts overexpressing hu CYP1A1, were administered ICT2700 at a non-toxic dose of 150 mg/kg (i.p.). The pharmacokinetics of ICT2700 and formation of the active C5 hydroxy metabolite were studied in plasma and major organs including lungs, liver and tumour. Sensitive and specific analytical LC/MS methodology was developed for the analysis of ICT2700 (m/z 379.8) and the C5 hydroxy metabolite (m/z 395.8).



Results: Greater than 95% of ICT2700 was present as parent compound in tissues and plasma indicating the systemic stability of this potential prodrug in normal tissue. The remaining 5% was a complex mixture of metabolites which are non toxic in vitro. ICT2700 AUCs (0−24 h)) and Cmax was 662.8 uM h, 51.3 uM (plasma), 2209 uM h, 72.1 uM (liver), 981.3 uM h, (lung) and 221.5 uM h, 17.2 uM (tumour) respectively demonstrating excellent distribution throughout the host tissue. The C5 hydroxy active metabolite was only detected in xenograft tissue. C5 hydroxylation facilitates conversion of ICT2700 to a cyclopropyl derivative, which is the active species responsible for alkylating DNA and a potent cytotoxin. AUC and Cmax for the C5 metabolite in CHO xenografts were 2.3 uM h and 1.0 uM and are consistent with the concentrations required to produce cytotoxicity in vitro.
Conclusions: The biological stability and CYP1A1 expressing xenograft-selective activation of ICT2700 demonstrates the potential of the chloromethylpyrroloindolines as tumour activated therapies. Structural variants are being explored for activation by a variety of different CYP expressing tumours. In principle these agents could also be used as a biomarkers of CYP functional activity in clinical tumours.

332 POSTER

In vivo activity of SGI-1776, an orally active Pim kinase inhibitor

D. Bearss[1], E. Gourley[1], X.H. Liu[1], J. Bearss[1], C. Jones[1], C. Olsen[1], D. Vollmer[1], S. Warner[1], H. Vankayalapati[1], P. Severson[1]. [1]*SuperGen Inc., Drug Discovery, Dublin, USA*

A small family of serine/threonine kinases known as Pim-1, Pim-2, and Pim-3 are involved in various signaling pathways in which they act as downstream effectors and potent inhibitors of apoptosis. The Pim kinases are unique in that they are expressed as active kinases and therefore gene expression levels directly correlate to their activity in cells. Pim-1 and Pim-2 are expressed in cells of hematopoietic lineage and Pim-3 appears to be more important in cells of epithelial origin. In concordance with these different patterns of expression, Pim-1 and Pim-2 are commonly overexpressed in hematological malignancies such as leukemias and lymphomas, while Pim-3 overexpression has been noted in melanoma, pancreatic adenocarcinoma, gastric, and other epithelial tumors. Thus, the Pim kinases are interesting targets for drug development, which offer promising potential in the treatment of hematological and solid malignancies.
Utilizing the published Pim-1 crystal structure and our proprietary CLIMB™ process, we identified a subset of leads from a large, virtual library from which a series of optimal analogs were synthesized to produce SGI-1776. The IC50 of this compound in a biochemical enzyme-based assay was 7 nM for Pim-1, 69 nM for Pim-3, and 363 nM for Pim-2. Cell-based activity,

determined by an anti-proliferative assay using hematological and solid tumor cell lines shows IC50 values as low as 54 nM. MV-4−11 leukemia cells treated with SGI-1776 shows a dramatic decrease in phospho-BAD levels (a direct substrate of the Pim kinases) as determined by western blot with an EC50 value of <10 nM. In conjunction with the anti-proliferative and phospho-BAD data, cell death via apoptosis was observed in cells treated with SGI-1776. Pharmacokinetic analysis of SGI-1776 in rats has demonstrated good oral bioavailability of the drug. Treatment of xenograft tumor models showed decrease tumor growth rates and in some models complete regression of the tumors at doses as low as 67 mg/kg. To determine if SGI-1776 was modulating Pim-1 activity in xenograft tumor in vivo pharmacodynamic studies were performed. Bad phosphorylation levels were determined in xenograft tumors by western blot and IHC. These studies showed that oral delivery of SGI-1776 modulated PD markers and could do so in a dose dependent manner. SuperGen's SGI-1776 exhibits potent inhibition of Pim kinase activity, translating into potent inhibition of cellular signaling pathways, cancer cell proliferation, and in vivo tumor progression in non-clinical models.

333 POSTER

Effectiveness of 6-(7-nitro-2,1,3-benzoxadiazol-4-ylthio)hexanol (NBDHEX) on human osteosarcoma and melanoma tumours

M. Pasello[1], F. Michelacci[1], F. Pellizzari Tregno[2], S. Pezzola[2], G. Filomeni[3], C. Geroni[4], M. Serra[1], G. Federici[5], C. Lapenta[6], A.M. Caccuri[2]. *[1]Istituti Ortopedici Rizzoli, Laboratorio di Ricerca Oncologica, Bologna, Italy; [2]University Tor Vergata, Chemical Sciences and Technologies, Roma, Italy; [3]University Tor Vergata, Biology, Roma, Italy; [4]Nerviano Medical Sciences, Oncology, Nerviano, Italy; [5]University Tor Vergata, Internal Medicine, Roma, Italy; [6]Istituto Superiore di Sanita', Cell Biology and Neuroscience, Roma, Italy*

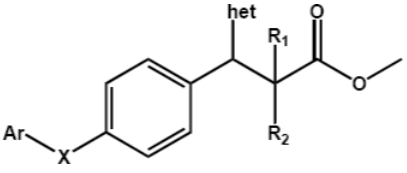
Background: NBDHEX is a promising anticancer drug which activates the c-Jun N-terminal kinase (JNK) pathway through the binding to glutathione S-transferase P1−1 (GSTP1−1). The disruption of the GSTP1−1-JNK complex is followed by a remarkable pro-apoptotic effect in tumor cells. In the present work we assessed the in vitro and in vivo effectiveness of NBDHEX on poorly responsive/resistant human osteosarcoma (OS) and melanoma models.
Material and Methods: NBDHEX was tested on 10 human OS cell lines and on 20 U-2OS or Saos-2 variants resistant to cisplatin (CDDP), doxorubicin or methotrexate and on two human melanoma cell lines (A375 and Me501 cells). Apoptosis was evaluated by chromatin fragmentation and caspase activation tests. GSTP1−1 levels and activation of the JNK/cJun pathway were tested by Western blot. The activity of the NBDHEX-CDDP combination was evaluated in OS cell lines sequentially exposed to equitoxic concentrations of the two drugs. In vivo experiments were performed on SCID mice implanted with A375 or Me501 tumors. Mice were treated orally with different doses of NBDHEX. Tumor growth inhibition (TI) was monitored three times a week. Toxicity was evaluated on the basis of weight loss and the autopsy findings. The mitotic index (MI) was determined by microscopy.
Results: NBDHEX was very active on both OS and melanoma cells with IC50 values in the micromolar range, independently of GSTP1−1 levels. In these cell lines NBDHEX induced the activation of the JNK/cJun pathway and a strong pro-apoptotic effect. Drug combination studies on OS cells, showed that NBDHEX can be used in association with CDDP. Anti-tumor efficacy was observed in vivo against Me501 human melanoma. NBDHEX showed 70% TI after oral (daily $\times$25) administration. Tolerability was good at all tested doses with no significant changes in body weight. The tumor MI value was decreased by 50% after NBDHEX treatment. Similar results were obtained on advanced human melanoma, A375 model, with 63% TI after oral (daily $\times$10) treatments. Tests on in vivo efficacy of NBDHEX on OS models are still ongoing.
Conclusions: The low responsive/resistant OS and melanoma tumor cells are efficiently committed to death by NBDHEX. This drug is effective and well tolerated in in vivo tumor models. These findings indicate that activation of JNK/cJun pathway, through a selective GSTP1−1 targeting, could prove a promising new strategy for treating tumors that respond poorly to conventional therapies.

334 POSTER

Molecular modelling and synthesis of novel CYP26A1 inhibitors

A.S. Mohamed Aboraia[1], M.S. Gomaa[1], C. Simons[1]. *[1]Cardiff UniversityWelsh School of Pharmacy, Medicinal chemistry, Cardiff, United Kingdom*

Cancer is one of the major causes of death in the world nowadays. The treatment of cancer could be accomplished through surgery or radiation followed by application of chemotherapy, however the main drawback of most chemotherapeutic agents is the side effects which accompany their use, many of which arise owing to the inability of chemotherapeutic agents to differentiate between the malignant cells and the fast dividing normal cells. One of the major targets of the scientist is to find drugs which can affect cancer cells without impairing the normal cells. One approach to fulfill this criterion is through drugs which act upon Cytochrome P450, such as CYP19A1, CYP24A1 and CYP26A1.
Our main target is CYP26A1, which is the enzyme responsible for the metabolism of retinoic acid. Retinoic acid (RA), the active metabolite of vitamin A, binds with nuclear receptors RAR/RXR to regulate cell growth and differentiation in a variety of cell types, and can reverse malignant growth in vitro and in vivo. These properties have led to RA being used effectively in a number of clinical situations including APL and neuroblastoma. However, in both cases resistance to RA can limit the therapeutic benefits observed. Evidence is growing that this resistance is related to up-regulation of CYP26 resulting in accelerated metabolism of RA. Inhibition of CYP26A1 results in the increase of the tissue level of retinoic acid and/or maintenance of a high therapeutic level of retinoic acid. Liarozole is the most studied and first CYP26A1 inhibitor to undergo clinical investigation for the treatment of ichthyosis. Liarozole also showed promising results in the treatment of prostate cancer compared with cyproterone acetate, however it lacked CYP selectivity. A model for CYP26A1 enzyme, developed within our group, has been used for docking of our compounds. Also, we have generated a pharmacophore model for CYP26A1 inhibitors. The docked compounds have been synthesized using different chemical methods, and analyzed for their CYP26A1 inhibitory activity within our laboratory using MCF-7 breast cancer cells. The tested compounds have shown good to moderate activity against CYP26A1, with nanomolar to low micromolar activities and good selectivity for CYP26A1.



General structure formula of the tested compounds. Ar = naphthyl, phenyl; X = O, NH; R_1 = H, CH_3; R_2 = H, CH_3.

335 POSTER

Identification of potent, selective sphingosine-1-phosphate 1 receptor (S1P1R) antagonists with antitumor activity

G. Shankar[1]. *[1]Exelixis Inc, Clinical Development, South San Francisco, CA, USA*

Background: Bioactive lipids, including sphingosine-1-phosphate (S1P), have been shown to play important roles as signaling and regulatory molecules. Many, if not all, of the effects of S1P are mediated by five distinct but related G-protein coupled receptors, S1P1−5. Despite the large body of evidence for the role of S1P1R in angiogenesis, proliferation, and cell migration, the development and application of potent, selective, small molecule S1P1R antagonists is lacking. The goal of this study was to identify and characterize selective S1P1R antagonists using in vitro and in vivo angiogenesis and tumor models.
Methods: High-throughput screening (HTS) of approximately 4.5 million compounds was conducted using HEK-293 cells overexpressing S1P1R and a modified cyclic nucleotide gated channel that served as a biosensor for intracellular cAMP. Compounds identified from screening were further optimized by medicinal chemistry and characterized using a variety of bioassays. Migration assays were conducted with SK-Hep-1 cells using a Boyden chamber assay. S1P1R selectivity assays were performed using β-arrestin recruitment assays in cell lines that expressed S1P1R, S1P2R or S1P3R. Tube formation assays were conducted using conditioned media from DU145 cells in a co-culture system consisting of primary human fibroblasts and endothelial cells. The effects of S1P1R antagonists were assessed in vivo using human tumor xenograft models in nude mice.
Results: Several potent, selective compounds were identified from HTS. Optimization by medicinal chemistry yielded S1P1R antagonists with in vitro IC50 values <10 nM in the cAMP biosensor assay that were highly selective for S1P1R compared to the related S1P2R and S1P3R receptors. Moreover, compounds were equally potent in inhibiting migration of SK-HEP-1 cells and blocking in vitro tube formation. Selected compounds had high oral bioavailability in rodents, and administration to nude mice bearing MDA-MB-231 tumor xenografts produced significant changes in tumor microvessel structure and evidence of antitumor activity.