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.

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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*.

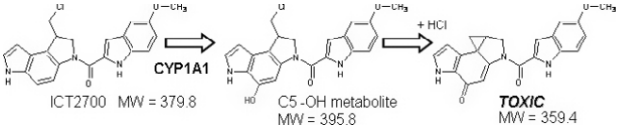
New molecular targets

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.

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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,