These KSP inhibitors function as allosteric inhibitors of KSP ATPase activity, which cause the rate of ADP release extremely slower, but do not interfere with the KSP-microtubule interaction. To discovery another types of cell-permeable KSP inhibitors, we have been screening synthetic chemical library through *in vitro* ATPase assays followed by cell-based analyses. 28,000 small chemical molecules were examined by measuring inhibition ability of microtubule-induced ATPase activity of KSP motor domain *in vitro*. And then, the positive chemical molecules were treated with synchronized and asynchronized HeLa cells to check their ability to perturb mitotic progression. As a result of our screening procedure for KSP inhibitors, we identified two cell-permeable small chemical molecules. Their treatment partially accumulated mitotic HeLa cells with characteristic mono-astral spindle phenotype that was same as a typical phenotype induced by KSP inhibition with monastrol. Although they could inhibit microtubule-induced ATPase activity of KSP motor domains *in vitro* (IC50=5 uM), they could not affect KSP ATPase activity in the absence of microtubules, suggesting that they might interfere with KSP-microtubule interaction. They could affect neither dynamics of microtubules, nor ATPase activity of other kinesin members we tested (CENP-E, MKLP1, Kid, KIF4) *in vitro*. Together, we found a new type of KSP inhibitors that affect KSP ATPase activity only in the presence of microtubules, which might serve as starting points for the development of new anticancer drugs with improved efficacy. And also this study suggests that the microtubule binding surface of kinesin KSP motor domain might be an attractive candidate for anticancer drug target.

312 POSTER

Preclinical evaluation of a rationally designed novel class of non-hydroxamate Histone Deacetylase inhibitors (HDACi)

J.H. Gill[1], C.W.G. Fishwick[2], V. Androutsopoulos[1], A. Mohanarajah[2], T. Khamnaen[2], L.H. Patterson[1], S.D. Shnyder[1], P.M. Loadman[1], R. Grigg[2]. *[1]Institute of Cancer Therapeutics, University of Bradford, Bradford, United Kingdom; [2]University of Leeds, Department of Chemistry, Leeds, United Kingdom*

Inhibitors of Histone Deacetylases (HDACs) are promising anti-cancer agents which are showing signs of activity in early clinical trials. Current HDACi are however of limited potency and many are hydroxamic acids with unattractive pharmacological properties. Furthermore, although current evidence strongly suggest class I (nuclear) HDACs, particularly HDAC1, as the key targets for anticancer activity, the majority of HDACi do not demonstrate class selectivity.

We have designed and synthesised a series of non-hydroxamate candidate molecules using novel cascade combinatorial synthesis. Molecular modelling/docking studies demonstrated localisation of our compounds within the HDAC active site, suggesting a potentially novel binding mode.

Compounds were compared against the hydroxamate HDACi, SAHA and the non-hydroxamate HDACi, MS-275, currently in phase II clinical trials. Our compounds demonstrate *in vitro* tumour cell cytotoxicity comparable to MS-275, with IC50 values of <1 μM. Inhibition of HDAC enzymatic activity measured using a cell-free assay identified several agents exhibiting IC50 values of <10 μM (compared to 3 μM for MS-275). In order to address HDAC activity inhibition in situ and activity against class I HDAC, we measured levels of hyperacetylated histone-H4 following in vitro drug treatment using flow cytometric analysis. MS-275 demonstrated a 2.8±0.7 fold increase in acetylation compared to control whereas our lead compound demonstrated a much greater 7.2±1.0 fold increase. In vivo administration of our lead compound resulted in a significant 3.7 day delay in growth of an ovarian xenograft tumour model.

These 'proof of principle' studies support these non-hydroxamate based agents as novel HDACi and suggest class selectivity, improved pharmacokinetics and potential as valid anticancer therapeutics.

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Pediatric preclinical testing program (PPTP) evaluation of the KSP inhibitor Ispinesib (SB-715992)

P.J. Houghton[1], J.M. Maris[2], H.S. Friedman[3], S.T. Keir[3], R.B. Lock[4], R. Gorlick[5], E.A. Kolb[5], C.P. Reynolds[6], C. Morton[1], M.A. Smith[7]. *[1]St. Jude Children's Research Hospital, Memphis, TN, USA; [2]Children's Hospital of Philadelphia, Philadelphia, PA, USA; [3]Duke University, Durham, NC, USA; [4]Children's Cancer Institute, Randwick, Australia; [5]Albert Einstein College of Medicine, New York, NY, USA; [6]Children's Hospital of Los Angeles, Los Angeles, CA, USA; [7]National Cancer Institute, Bethesda, MD, USA*

Background: Ispinesib is a novel small molecule inhibitor of kinesin spindle protein (Eg5), a mitotic kinesin required for separation of the spindle poles. Ispinesib inhibits growth of a broad range of cancer cell lines at low nanomolar concentrations and induces regressions or tumor growth delay against adult cancer xenografts. The COG Phase 1 Consortium is initiating a phase 1 trial of ispinesib for children with refractory solid tumors.

Methods: The PPTP includes an *in vitro* panel (23 lines) as well as panels of xenografts (n = 61) representing most of the common types of childhood solid tumors and childhood ALL. Ispinesib was administered IP (10 mg/kg) to a representative subset of xenografts on a q 4d × 3 schedule repeated once at day 21. Three measures of antitumor activity were used: 1) response criteria modeled after the clinical setting [e.g., partial response (PR), complete response (CR), etc.]; 2) treated to control (T/C) tumor volume at day 21; and 3) a time to event measure based on the median EFS of treated and control lines (intermediate activity required EFS T/C > 2, and high activity additionally required a net reduction in median tumor volume at the end of the experiment).

Results: Ispinesib induced significant tumor growth delay in 82% (14/17) of evaluable solid tumor xenografts. Using a time to event measure of efficacy, ispinesib had intermediate and high levels of activity against 6 (35%) and 3 (18%) of the 17 evaluable solid tumor xenografts, respectively. Intermediate or high activity for the EFS measure was observed for most diagnoses [e.g., Wilms tumor (WT), rhabdoid tumor (RT), Ewing sarcoma (ES), rhabdomyosarcoma (RMS), and GBM], but not for neuroblastoma. Ispinesib induced maintained CRs in 3 xenografts: 1 of 2 WT, 1 ES, and 1 of 2 RT, and it induced a CR in 1 of 4 GBM. Preliminary analysis of results from the ALL panel suggests substantial activity against several xenografts. Ispinesib induced excessive toxicity in mice bearing osteosarcoma xenografts, and excessive toxicity precluded analysis of 6 xenografts for other diagnoses.

Conclusions: Ispinesib demonstrated broad activity against the PPTP's solid tumor xenografts. Antitumor activity manifested primarily as tumor growth delay, though tumor regressions were also observed. Further preclinical work with ispinesib will include an evaluation against the PPTP *in vitro* panel and further testing against the ALL panel.

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Extra-vascular penetration of taxanes may limit their efficacy

A.H. Kyle, L.A. Huxham, A.I. Minchinton. *BC Cancer Research Centre, Vancouver, Canada*

The failure of many anticancer drugs to control the growth of solid cancers may stem in part from inadequate delivery to tumour regions distant from vasculature. We compared the tissue penetration of tritium labelled paclitaxel and docetaxel in tumour xenografts and in multilayered cell culture (MCC), a tissue-engineered model of the tumour extra-vascular compartment. Drug distributions in the xenografts were mapped relative to blood vessels to obtain profiles of drug as a function of distance from vasculature. In the MCCs, drug levels were determined as a function of distance in from the exposed edge of tissue. Results were compared with predictions from an *in vitro* effect-based assay.

Experiments were carried out in tumour xenografts and MCCs using human HCT-116 colon carcinoma cells. For paclitaxel the effect of the vehicles Cremophor EL versus Tween 80 was examined. Paclitaxel (10 mg/kg) and docetaxel (5 mg/kg, Tween 80) were administered by tail vein injection. Tumours were removed and frozen 2 and 8 hours after drug injection. For MCC experiments, cultures were exposed to 0.3 and 3 μM of each drug for 1 and 2 hours and then frozen. Following cryosectioning, slides were clamped against tritium sensitive film and exposed for a period of 3 months. Results from xenograft and MCC-based autoradiography studies found that both drugs penetrated poorly. Of the two, paclitaxel exhibited approximately 2-fold greater tissue penetration than docetaxel, with drug falling by half 55 μm ± 5 μm away from vessels for paclitaxel versus 28 μm ± 5 μm for docetaxel (2 hour time point). The effect of vehicle on paclitaxel distribution was surprising in that at the 2 hour time point paclitaxel in Tween 80 showed significantly higher peak tissue levels relative to Cremophor EL (55% increase) but by 8 hours both vehicles produced similar drug distributions. In MCCs, drug levels fell to half max by 28 μm ± 5 μm into the tissue for paclitaxel versus 17 μm ± 5 μm for docetaxel (0.3 μM drug, 1h). Results were consistent with previous data obtained using an *in vitro* screening assay in which paclitaxel showed significantly better tissue penetration than docetaxel.

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