telomeric chromatin in xenografted tumor models. Here we studied the therapeutic index of RHPS4 and its integration with chemotherapeutics in preclinical model of solid tumors.
Material and Methods: The antitumoral activity of RHPS4 was evaluated on human xenografts of different histotypes and compared to that of standard antineoplastic agents. Moreover, the effect of RHPS4/chemotherapeutics combinations on cell survival was studied and the most favorable combination evaluated on tumor-bearing mice.
Results: RHPS4 was active in vivo as single agent and exhibited a high therapeutic efficacy when compared to conventional drugs. RHPS4 also showed a strong synergistic interaction with camptothecins and this effect was strictly dependent on the drug sequence employed. Treatment of mice with irinotecan followed by RHPS4 was able to inhibit and delay tumor growth and to increase mice survival. Interestingly, no evidence of toxicity was noted in all treated mice, thus demonstrating the favorable tolerability of this new antineoplastic strategy. Immunohystochemical analysis performed in tumors sections showed that the highest therapeutic efficacy of the irinotecan/RHPS4 combination resulted from the activation of apoptosis and damage response, identifying gH2AX as surrogate marker of tumor response.
Conclusions: Our data demonstrate that RHPS4 has a good pharmacodynamic profile and in combination therapy produces a strong antitumoral activity, identifying this drug as promising agent for clinical development. Finally, this study provides a compelling argument to suggest that the telomere pathway is a well-validated target at the preclinical level and encourage the development and evaluation of therapeutic combined option in future clinical protocols.

442 POSTER

Novel small molecule inhibitors of telomerase

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The ends of linear chromosomes are capped by DNA-protein structures known as telomeres. Telomerase is an enzyme complex responsible for telomere maintenance and this provides malignant tumours with unlimited replicative potential. Telomerase activity has been found in the majority of cancer cells but not in most normal human cells. Inhibition of telomerase activity therefore offers a potential for specific anti-cancer therapy.
Aim: The aim of this study was to screen a series of small molecule biaryl heterocycle compounds that were designed to target the telomeric G-quadruplex DNA, making it inaccessible to telomerase and thereby inhibiting telomerase activity. The anti-telomerase and anti-tumour properties of the compounds were investigated in the A459 human lung adenocarcinoma cell line.
Methodology: Telomerase activity was determined in a panel of tumour cell lines using the TRAP assay. The A549 cell line was selected as it showed consistently high telomerase activity. Berberine chloride, a known moderate inhibitor of telomerase was selected as a control drug. Specificity of the compounds for telomerase rather than other polymerases (Taq polymerase) was determined by amplification of β actin gene from cDNA obtained from cell lysates exposed to compounds. PCR products were separated by agarose gel electrophoresis. Acute cytotoxicity of the compounds was determined using the MTT assay.
Results: Of the 32 compounds screened, 16 appeared to inhibit telomerase in a cell free assay. Of these, 12 also inhibited the PCR reaction and were therefore excluded from further study. The remaining 4 compounds were explored and showed 50% inhibition of telomerase activity occurring at concentrations in the range of 80–170 μM, after 2 hour exposure. Short term cytotoxicity of the compounds at 2 hour exposure revealed IC_{50} concentrations ranging from 60 to 170 μM.
Conclusion: All 4 compounds inhibited telomerase in cell free assays at concentrations similar to that required to cause short term cell toxicity at 2 hours. Since a key goal in the development of telomerase inhibitors is the selection of compounds which have a lower IC_{50} in a cell free assay than that required for acute cell kill, it would seem that the compounds may have potential as therapeutic agents.

Tubulin-interacting agents

443 POSTER

Reduced expression of the epithelial specific ETS factor ESE-3 is associated with resistance to taxanes in prostate cancer cells

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Background: Prostate cancer (PCa) is the most common cancer and a leading cause of cancer death in Europe and North America. Resistance to anticancer drugs is the major reason of treatment failure for androgen-independent PCa. Deregulated expression of ETS factors has emerged as an important event in PCa pathogenesis. We have recently shown that expression of the epithelial-specific ETS factor ESE-3 is reduced ⩾4-fold in about 50% of PCa compared to normal prostate. ESE-3 was epigenetically silenced in established PCa cell lines (PC3 and DU145) and in Ras-transformed prostate epithelial (LHSR) cells, while it was expressed in immortalized prostate epithelial (LH) cells. Consistent with a tumor suppressor function, ESE-3 reduced clonogenic growth and survival of PCa cells. In this study, we used gain and loss of function experiments to determine whether reduced ESE-3 expression was associated with increased resistance of PCa cells to taxanes.
Methods: Cells were transiently transfected with full length and truncated ESE-3 expression vectors. Stable ESE-3 knockdown cell lines were established by transfection of shRNAs and selection with G418. Expression of ESE-3 was determined by RT-PCR and Western blot. Cells were exposed to taxol (5 to 50 nM) for 24−72 h. Caspase-3 activation and PARP cleavage were evaluated by Western blot. Cell growth and survival were assessed by cell viability (MTT) assay.
Results: Taxol induced caspase-3 activation and PARP cleavage only in ESE-3 expressing (ESE3+) LNCaP and 22rv1 cells and not in non-ESE3 expressing (ESE3−) PC3 and DU145 cells. Similarly, only the immortalized ESE-3+ LH cells and not the ESE3− LHSR cells underwent apoptosis in response to taxol. To confirm the relationship between ESE-3 expression and sensitivity to taxol-induced apoptosis, PC3 cells were transfected with ESE-3 or control vector and then treated with taxol. Taxol at 5 nM induced PARP cleavage in ESE3+ PC3 cells, while PARP cleavage was undetected in ESE3− PC3 cells at doses up to 50 nM. ESE-3 transfected PC3 cells were more sensitive to taxol than control cells in cell viability assays. Truncated forms of ESE-3 were unable to sensitize PC3 cells to taxol indicating that the full length protein was necessary for the effect. Consistently, stable knockdown of ESE-3 in LNCaP and 22rv1 cells by shRNA reduced sensitivity to taxol.
Conclusions: Our study demonstrates that loss of ESE-3 expression in PCa cells reduced apoptosis and growth inhibition in response to taxol. Thus, PCa with reduced ESE-3 levels might be less sensitive to taxanes and in these cases alternative drugs might be considered. On the other hand, reactivation of ESE-3 expression by epigenetic drugs might represent a therapeutic strategy to increase the efficacy of taxanes in ESE-3− PCa.

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BAL27862: a novel tubulin interacting agent with activity in multidrug resistant tumors and potential as a vascular disruption agent

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Background: BAL27862 is a synthetic small molecule which potently induces apoptosis in cancer cells due to inhibition of tubulin polymerization via a potentially new binding site. BAL27862 has a broad *in vitro* anti-proliferative activity against a diverse range of human tumor lines (low nM IC50s), eliciting significant antitumor responses in a range of animal models of human cancer when administered orally (p.o.) or intravenously (i.v.).
Materials and Methods: Anti-proliferative activity was analyzed using a monolayer (crystal violet) or soft agar (clonogenic) assay. Effects on microtubules (MTs) were assessed by immunofluorescence (IF) or immunoblotting (IB) for alpha-tubulin, and activity on endothelial cell organization with an *in vitro* 3D matrix model of angiogenesis. Efficacy was assessed in a multidrug resistant mammary tumor mouse model.
Results: BAL27862 showed potent anti-proliferative activity in 21 patient-derived tumor lines (clonogenic assay: IC50 < 30 nM in 12 [approx. 60%] lines), including cells resistant to paclitaxel. Human stem cell controls were relatively insensitive. Using monolayer assays, activity was retained against six Pgp-overexpressing tumor lines, which were up to several thousand-fold resistant to paclitaxel and vinblastine. BAL27862 administered p.o and i.v. elicited significant antitumor activity in Pgp-overexpressing MT-3/ADR xenografts, where taxol and doxorubicin were ineffective. *In vitro* BAL27862 (25−50 nM) caused MT disorganization in a manner distinct from paclitaxel, vinblastine and colchicine: in interphase cells the MT network was partially collapsed with an absence of peripheral MTs; in dividing cells tiny asters

were scattered within the nuclear region. Strikingly, BAL27862 abrogated both vinblastine- and colchicine-induced aster formation (assessed by IF) and disaggregated paclitaxel- and epothilone B-stabilized MTs (assessed by IB). MT destabilization occurred in isolated human peripheral blood mononucleocytes treated with BAL27862 *ex vivo*, suggesting a potential for a blood-based pharmacodynamic assay. Following a single 1 h pulse treatment, BAL27862 inhibited the formation of endothelial cell (HUVEC) tubular structures (maximal at 100 nM), while disrupting established tubules at 30 nM. Compared with its anti-proliferative activity against HUVECs, the theoretical 'therapeutic index' for vascular disruption activity (VDA) *in vitro* was 18−25; higher than observed for combretastatin A-4 (index: 6−7), an agent with known VDA.
Conclusions: BAL27862 is a new tubulin-interacting agent with an apparently novel mechanism of action. A broad antitumor activity, also in drug resistant tumor models, and potential vascular disruption activity strongly support further development of BAL27862 as a novel anticancer agent with a possibility for both i.v. and p.o. administration.

445 POSTER

Class III beta-tubulin overexpression in non-small cell lung, breast and prostate carcinoma xenografts confers innate or acquired resistance to taxanes and sensitivity to ixabepilone

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Background: Taxane type microtubule (MT) inhibitors are active agents but their therapeutic benefits are limited by multifactorial drug resistance. Substantial recent evidence suggests that suboptimal clinical response to taxanes in a variety of tumor types may be related to overexpression of class III beta-tubulin (TUBB3). Compared to other tubulin isoforms, TUBB3 has a lower affinity for paclitaxel (PTX) and is less susceptible to PTX-induced disruption of MT dynamics, the main mode of action of taxanes. Ixabepilone (IXA), an analog of epothilone B is the first of a new class of MT inhibitors designed to have reduced susceptibility to multiple mechanisms of drug resistance including MDR1, BCRP, MRP1 and tubulin mutation. In contrast to PTX, IXA is effective in disrupting the dynamicity of purified TUBB3 in vitro. We tested if IXA retains efficacy in a broad spectrum of TUBB3 overexpressing tumors, and whether TUBB3 overexpression can be induced during the development of acquired resistance to a taxane in vivo.
Methods: TUBB3 expression was determined by Western blot and immunohistochemistry with a TUBB3 specific antibody. Sensitivity to docetaxel (DTX), IXA and vinorelbine (VRB) was determined in mice administered each agent at its maximum tolerated dose (MTD). Sensitivity is defined as tumor response $\geqslant$1 log cell kill (LCK). Acquired resistance to DTX was developed over the course of 2 years (7 treatment courses) in the CWR22 prostate cancer xenograft by repeat cycles of treatment and re-transplantation of a regrown tumor at each relapse.
Results: All 5 tumors overexpressing TUBB3 were resistant to DTX and VRB, yielding activity ranging 0.2–0.9 and 0.1–0.3 LCK, respectively. IXA was active in all 5 tumors, yielding 1.6–4.2 LCK (Table 1) at its MTD. The parent CWR22 has equal sensitivity to DTX and IXA. Of clinical relevance, high TUBB3 staining was observed in breast cancer samples from taxane-resistant patients enrolled in a phase III clinical trials of IXA.
Conclusion: IXA exhibits reduced susceptibility to multiple drug resistance mechanisms and has robust activity in tumors overexpressing TUBB3. A randomized trial of IXA- versus PTX-containing regimens in NSCLC patients is planned.

Efficacy of IXA, DTX and VRB in 6 human tumors including 5 overexpressing TUBB3

Tumor	Histology	Activity (LCK)		
		IXA	DTX	VRB
H1155	NSCLC	4.2	0.2	0.1
DU4475	Breast	2.6	0.9	0.2
Pat-21	Breast	1.6	0.3	0.3
LX-1	NSCLC	2.6	0.5	0.1
CWR22/R	Prostate	1.6	0.7	ND
CWR22	Prostate	1.5	1.8	ND

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A phase I study of eribulin mesylate (E7389) in patients with refractory cancers

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Background: Eribulin mesylate (E7389), a structurally simplified, synthetic analogue of halichondrin B, shows anticancer activity against various types of tumors by inhibiting microtubule dynamics. A phase I study of eribulin was conducted to determine a recommended phase II dose and to assess safety and pharmacokinetics.
Methods: Patients with advanced solid cancers were enrolled. Eribulin mesylate was administered intravenously over 5 minutes on days 1 and 8 every 21 days. Cohorts of three patients were treated at 0.7, 1.0, 1.4 and 2.0 mg/m^2. Tumor measurements were performed at baseline and every 6 weeks. Pharmacokinetics were investigated on days 1 and 8 of the first cycle.
Results: A total of 15 patients with various cancers were treated (3, 3, 6 and 3 patients at 0.7, 1.0, 1.4 and 2.0 mg/m^2, respectively). The number of cycles ranged from 1 to 15, and 7 patients received >4 cycles. Dose-limiting toxicities were observed in 2 of 6 patients treated at 1.4 mg/m^2, and in all 3 patients at 2.0 mg/m^2. Of these patients, one patient treated at 1.4 mg/m^2 experienced grade 4 neutropenia for 5 days, and the other patient had febrile neutropenia and skipped day 8 administration. At 2.0 mg/m^2 one patient each developed either grade 4 neutropenia lasting 5 days or febrile neutropenia; neither patient received the day 8 administration. Administration on day 8 was also skipped in the third patient at 2.0 mg/m^2. Thus, administration on day 8 was omitted in 1 of 6 patients at 1.4 mg/m^2 and in all 3 patients at 2.0 mg/m^2. All omissions were because of grade 3 neutropenia on day 8. Other frequently observed non-hematological toxicities included fatigue, alopecia, nausea, anorexia, neuropathy, liver enzyme elevations, hyperglycemia, and increased CRP levels. However, these were generally mild, and grade 3 toxicities were fatigue (2 patients) and elevation of γ-glutamyltransferase (1 patient). No differences were observed between day 1 and day 8 in pharmacokinetic profiles. The systemic clearance on day 1 was 1.50–2.69 L/hr/m^2, and the volume of distribution was 93.4–106.8 L/m^2. Partial responses were achieved in 3 patients (two with non-small cell lung cancer and one with head & neck cancer) at 1.4 mg/m^2. Stable disease >12 weeks was observed in 3 patients (two with breast and one with cervical cancer).
Conclusions: The main toxicity of eribulin mesylate is neutropenia and easily managed. A dose of 1.4 mg/m^2 administered on days 1 and 8 every 3 weeks is recommended for phase II studies. Major responses observed warranted further clinical study.

447 POSTER

ARRY-520, a KSP inhibitor with potent in vitro and in vivo efficacy and pharmacodynamic activity in models of multiple myeloma

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Background: Kinesin spindle protein (KSP) plays a key role in spindle pole separation and production of the bipolar spindle. Inhibition of KSP causes cells to arrest at the prophase-metaphase transition with formation of monopolar spindles. Maintenance of this arrest leads to cell death. The KSP inhibitor ARRY-520 is currently in phase I testing for solid tumors and acute myeloid leukemia. We report here the characterization of the *in vitro* and *in vivo* activity of ARRY-520 in preclinical models of multiple myeloma.
Materials and Methods: The *in vitro* antiproliferative activity of ARRY-520 was determined using logarithmically growing cells. *In vivo* antitumor activity was determined using human multiple myeloma xenografts grown subcutaneously in SCID-beige mice. *In vivo* pharmacodynamic activity (accumulation of monopolar spindles and apoptotic cells) was evaluated by immunohistochemical analysis of tumor xenograft tissue harvested from mice after treatment with ARRY-520.
Results: ARRY-520 inhibited proliferation and induced mitotic arrest and apoptosis in the human multiple myeloma cell lines RPMI8226, JJN3 and H929, with EC_{50}s for proliferation of 1.5–2.5 nM. *In vivo*, treatment of mice bearing established subcutaneous tumors with the compound at 20 mg/kg IP, q4dx3 caused significant regression, including a 100%