of an exponential growth of treated tumors in comparison to control tumors (T-C). Toxicity was evaluated on the basis of the body weight reduction. The results show that on tumor cell lines brostallicin can be combined effectively with imatinib, gefitinib or erlotinib producing a synergistic effect, as indicated by combination indices of <1 according to Chou and Talalay's equation. In vivo, brostallicin combined with kinase inhibitors showed strong synergism or additivity depending on the drug combined with brostallicin or on the tested tumor model. No increased toxicity was observed when brostallicin is co-administered with molecular-targeted drugs. In conclusion, although the precise molecular mechanism of the interaction has not yet been identified for the tested combination protocols, results further support the value of brostallicin in cancer combination therapy.

305 POSTER

DNA cross-linking and in vivo antitumour activity of the extended pyrrolo[2,1-c][1,4]benzodiazepine dimer SG2057 (DRG-16)

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The minor groove cross-linking C2/C2′-exo-unsaturated pyrrolo[2,1-c][1,4]-benzodiazepine (PBD) dimer SJG-136 (SG2000) is currently undergoing Phase I evaluation against solid tumours and haematological malignancies in the USA and UK. This compound contains two PBD units joined through their aromatic A- ring phenol positions by an inert propyldioxy linkage and spans six base pairs in the minor groove, actively recognising and crosslinking a 5′-GATC sequence. The corresponding dimer containing a pentyldioxy linkage (DRG-16, SG2057) has been synthesised and has the potential to span and crosslink across an additional DNA base pair between the covalently-bound guanine bases. SG2057 has previously been shown to have increased in vitro cytotoxicity compared to SG2000 across a range of human tumour cell lines and to cross-link naked plasmid DNA with >10-fold efficiency. Cross-links can be detected using the single cell gel electrophoresis (comet) assay in human leukaemic K562 cells at doses of SG2057 as low as 0.5 nM following a 1 hour exposure. After removal of drug cross-links continue to form, reaching a peak within two hours, and persist with no evidence of unhooking over a 48 hour period. DNA footprinting and polymerase stop assays revealed different sites of sequence specific DNA interaction for SG2057 compared to SG2000. SG2057 has been evaluated in three human tumour xenograft models. Against LOX-IMVI melanoma, single dose i.v. treatment at the MTD (0.075 mg/kg) of early stage tumours resulted in 5 out of 8 animals being tumour free at day 68 (when the experiment was terminated). Three different administration schedules of SG2057 (0.01 mg/kg qd$\times$5, 0.04 mg/kg qwk$\times$5, 0.02 mg/kg q4d$\times$3) against the human leukaemia HL-60 model gave significantly greater tumour growth delays than the standard therapy of doxorubicin (6 mg/kg qwkx3). In the SK-OV-3 ovarian xenograft SG2057 was as effective as the standard therapy paclitaxel (30 mg/kg qodx5) when given at 0.02 mg/kg qd$\times$5 or 0.04 mg/kg q4d$\times$3 and was superior when administered at 0.06 mg/kg qwk$\times$3.

306 POSTER

Aryl hydrocarbon receptor ligands 2-(3,4-dimethoxyphenyl)-fluorobenzothiazoles elicit potent and selective in vitro antitumor activity, inducing DNA damage that is independent of CYP1A1 bioactivation

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In vitro screening of a series of 2-phenylbenzothiazoles bearing oxygenated substituents has led to identification of the fluorinated 2-(3,4-dimethoxy-phenyl)benzothiazole structure as a novel antitumor pharmacophore. 2-(3,4-Dimethoxyphenyl)-5-fluorobenzothiazole (**1a**) exacts exquisitely selective and potent growth inhibitory activity against colon, lung and breast carcinoma cells (GI_{50} < 10 nM). Structural modifications of **1a** are poorly tolerated: removal of the fluorine atom produced a molecule (**1b**) devoid of activity. The 4-fluoro- (**1c**) and 6-fluoro- (**1d**) regioisomers retain potency against lung and breast cancer cell lines. The dimethoxy alignment in positions 3 and 4 of the phenyl ring is essential for activity; a methylenedioxy bridge (**1e**) renders the molecule inactive.
Growth inhibition by **1a** was accompanied by G_2/M cell cycle arrest followed by accumulation of events pre-G_1. A distinct profile of co-eluting **1a**-generated DNA adducts formed in sensitive cell lines exclusively. Dimethoxyphenylbenzothiazole-derived adduct numbers correlated with growth inhibitory potency ($R^2 > 0.7$). Nuclear γH2AX foci were detected in sensitive cells following **1a** exposure periods $\geqslant$2 h; the number and intensity of γH2AX foci correlated with cell line sensitivity ($R^2 > 0.8$). Irrespective of antitumor potency, these small benzothiazole molecules (**1a**–**1e**; Mw < 300) are potent aryl hydrocarbon receptor ligands (nM K_i values), inducing CYP1A1 protein expression. However, growth inhibitory activity of **1a** was not abrogated by the CYP1A1 inhibitor resveratrol (10 μM), moreover equipotent activity was observed in colon cell lines (e.g. HCC2998) expressing neither inducible nor constitutive CYP1A1.
The data are consistent with the hypothesis that fluorinated 2-(3,4-dimethoxyphenyl)benzothiazole analogs elicit selective anticancer profiles *in vitro* by generating selective lethal DNA damage which is independent of CYP1A1-catalyzed bioactivation.

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Phase I study of arsenic trioxide in subjects with hepatocellular cancer and varying levels of hepatic dysfunction

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Background: The medical therapy for hepatocellular cancer remains a critically unmet medical need. Arsenic trioxide (Trisenox®) is an agent with proven efficacy in relapsed acute promyelocytic leukemia, with the promise of activity in solid malignancies. This dose escalation study evaluated a twice weekly intravenous regimen in subjects with surgically unresectable, and/or metastatic, hepatocellular cancers with varying levels of hepatic dysfunction.
Materials and Methods: Twenty-three subjects have been enrolled to date: male/female 18/5, mean age 57 years (range 24 – 87), 21 of whom had underlying cirrhosis [Child-Pugh grade A (15), B (5) and C (1)]. Treatment was given twice weekly (Mon, Thur or Tues, Fri) intravenously, at a starting dose of 0.25 mg/kg for six weeks, every eight weeks. The dose escalation scheme is outlined in the table below. Oral and parenteral supplementation of potassium and magnesium were given prior to arsenic trioxide to those subjects whose serum potassium levels were below 4.0 mEq/L and magnesium below 2.0 mg/dL. Occurrence of a dose limiting toxicity (DLT) was monitored during the eight week period; subjects who progressed during this period were replaced.
Results: Therapy was well tolerated, with minimal gastrointestinal and hematologic toxicity observed; no cardiac toxicity was encountered. The first DLT, a grade 3 diffuse angioedematous rash, was observed at the 0.5 mg/kg dose level. No partial responses were observed; three patients had stable disease for greater than 12 weeks. One subject, with radiographic evidence of disease progression prior to study entry, received arsenic trioxide at the 0.3 mg/kg dose level, experienced a durable incomplete partial response (meeting RECIST criteria for stable disease); the subject has received greater than 18 months of therapy and remains on study with no cumulative toxicity observed to date. Expansion of the 0.5 mg/kg dose cohort is ongoing: updated results will be presented at the meeting.

Dose level[a]	Number subjects enrolled	Child–Pugh cirrhosis[a]	Outcome
0.25	3	A (2) B (1)	Progression (3)
0.3	4	A (4)	Progression (3), Stable disease (1)
0.35	4	A (1), B (2), NP (1)	Stable disease (1), Progression (3)
0.4	4	A (3), NP (1)	Progression (2), Death from cirrhosis (2)
0.45	4	A (2), B(1), C (1)	Progression (3), Death from cirrhosis (1)
0.5	4	A (3), B (1)	Progression (2), Dose limiting toxicity [rash] (1), Inevaluable to date (1)

[a]mg/kg intravenously twice weekly. [b]NP: cirrhosis not present.

Conclusion: In this dose escalation study in subjects with hepatocellular cancer, the majority arising on a background of cirrhosis, arsenic trioxide was well tolerated. Therapeutic activity was modest, with three out of 22 subjects achieving stable disease, one durable.