Preliminary PD data suggest that MGCD0103 can inhibit HDAC activity in a dose-dependent manner, and induce histone acetylation in peripheral blood mononuclear cells. Both these effects have been shown to persist for 72 hours following dosing.

301 POSTER

The design, synthesis and biological evaluation of a set of C2-aryl substituted pyrrolo[2,1-c][1,4]benzodiazepine dimers

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The pyrrolobenzodiazepines (PBDs) are a family of naturally occurring antitumour antibiotics. Many of the most potent family members contain an *endo-exo* unsaturated motif associated with the pyrrolo C-ring of the PBD.

We have previously reported the synthesis and potent *in vitro* and *in vivo* antitumour activity of SG2285, a PBD dimer which retains the *endo-exo* unsaturation motif in the form of a C2-aryl substituent conjugated to a 2,3 double bond. We now report the synthesis and biological evaluation of a set of 10 analogs with diverse C2-aryl substituents. These C2-aryl substituents were selected on the basis of the biological evaluation of approximately 80 C2-aryl PBD monomers.

The novel dimers were prepared from a key enol triflate PBD intermediate by Pd(0) catalysed coupling with the appropriate aryl boronic acids. Removal of the N10 Troc protecting group afforded the free PBD imines, which were converted to their bisulphite adducts in order to improve water solubility and modulate the DNA-reactivity of the PBD unit.

The C2-aryl PBD dimers were evaluated in the K562 human leukaemia cell line (96 hrs continuous exposure) with IC_{50} values in the range 0.02−43 nM. The 3-methoxyphenyl analog (SG2965) and the 3,4-benzodioxole analogs (SG2962 and 2965) were particularly potent with IC_{50} values of 20, 80 and 50 pM, respectively. These molecules were also found to be efficient DNA interstrand cross-linking agents in both plasmid and cellular DNA. In the case of the bisulphite adducts, the cross-links were found to form more slowly in cells reaching a maximum at approximately 24 hrs.

These studies confirm the potent activity of C2-aryl PBD dimers of this type, and SG2285 is currently undergoing preclinical development.

302 POSTER

Processing of 1-nitroacridine-induced DNA-DNA cross-links by topoisomerase I is associated with enhanced cellular survival: a possible role of topoisomerase I in the removal of DNA cross-links

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1-nitroacridines like nitracrine (Ledakrin) are potent antitumor agents with clinical activity toward ovary, lung and breast cancers. These compounds are activated by bioreduction and bind covalently to DNA. This is accompanied by formation of DNA cross-links and covalent DNA-protein complexes. Earlier studies showed that 1-nitroacridines could be activated by thiols *in vitro* to reactive species which bind DNA and proteins. On the other hand, DNA topoisomerases have been indicated in the processing of DNA adducts. We here show that modification of plasmid DNA with nitracrine and, to a lesser degree, the nitracrine derivative C-857 inhibited the catalytic activity of purified topoisomerase I in a dose-dependent manner. The inhibition is associated with the induction of DNA single strand breaks and the formation of covalent DNA-topoisomerase I complexes. In contrast, no detectable effects were observed for purified human topoisomerase IIα. Further studies revealed that both nitracrine and C-857 form unusual DNA cross-links between different DNA molecules. Unexpectedly, the high molecular weight cross-linked DNA formed in the presence of nitracrine, but not by C-857, was completely resolved after further incubation with topoisomerase I. Accordingly, the In Vivo link assay revealed the formation of covalent DNA-topoisomerase I complexes in LNCaP cells after treatment with nitracrine but not with C-857. DNA cross-linking was accompanied by the formation of double stranded DNA breaks that were particularly pronounced for cells treated by C-857 suggesting that the topoisomerase I-mediated processing of the cross-linked DNA may play a role in DNA repair. In agreement, cells with decreased topoisomerase I levels showed increased sensitivity to nitracrine but unchanged sensitivity to C-857. In conclusion, our results suggest a novel role for topoisomerase I in the removal of DNA-DNA cross-links which is accompanied by increased cellular survival.

303 POSTER

Additive and synergistic effects of irofulven and capecitabine in human prostate cancer cells

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Background: Irofulven (6-hydroxymethylacylfulvene, MGI 114) is a novel DNA-interacting anticancer drug derived from the mushroom natural product, illudin S. Irofulven displays a broad range of activity against human tumors in vitro and in vivo and is currently under study in clinical trials as a single agent and in combination with several other anticancer drugs. To optimize the clinical use of irofulven, the present study examined the cytotoxic effects of combining irofulven with 5′-DFUR or 5-FU, the active metabolite of capecitabine, in human prostate cancer cells.

Materials and Methods: Antiproliferative effects of irofulven, 5′-DFUR and 5-FU were evaluated in two prostate cancer cell lines, PC3 and DU145, with different expression levels of thymidine phosphorylase (TP), a key enzyme for capecitabine metabolism. Drug interaction studies were performed using isobolograms according to the method of Chou & Talalay.

Results: Single agent irofulven produced cytotoxic effects against human PC3 and DU145 prostate cancer cells with IC50s of 4.2+0.9 μM and 1.4+0.6 μM, respectively. Sensitivity to 5′-DFUR was directly correlated with TP expression level. PC3 cells expressed less TP and were less sensitive to 5′-DFUR than DU145 (IC50s of 62 and 33 μM, respectively). Combination of irofulven with 5′-DFUR produced additive/synergistic activity over a broad range of concentrations in both PC3 and DU145 cells, and similar effects were observed when irofulven was combined with 5-FU. While there was no clear schedule dependency for the tested combinations, both cell lines showed a trend favoring 5-FU/5′-DFUR exposure prior to irofulven. Cell cycle analysis showed consistent outcomes for 5′-DFUR and 5-FU with accumulation of cells in the S-phase of cell cycle, while combinations with irofulven were associated with cell cycle blockage in G1/S. Irofulven induced cellular apoptosis as a single agent, however, in combination with either 5′-DFUR or 5-FU the observed apoptosis was markedly increased.

Conclusion: Irofulven displays additive/synergistic anti-proliferative effects when combined with 5′-DFUR and 5-FU over a broad range of concentrations in human prostate cancer cells. Cell cycle arrest in S-phase and apoptosis appear as primary mechanisms of cytotoxicity of irofulven-based combinations. Based on these data, the irofulven-capecitabine combination should be further explored using a schedule that preferably gives capecitabine prior to irofulven.

304 POSTER

Combination treatment of new molecular-targeted therapies and the DNA minor groove binder brostallicin

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Brostallicin is a DNA minor groove binder, currently in Phase I/II trials as single agent or in combination. It has antitumor effect and strong pro-apoptotic activity in experimental tumors. The mechanism of DNA interaction is novel, since it binds covalently to DNA only in the presence of glutathione and glutathione S-transferase (GSH/GST). As a consequence, brostallicin is more effective on tumors expressing relatively high levels of GSH/GST. Multiple combinations of brostallicin with “classical” antitumor drugs have been previously studied; synergy was observed with cisplatin, gemcitabine and irinotecan. Based on the role of combination chemotherapy in cancer treatment, and its importance for the efficacy of newer therapies, we evaluated whether brostallicin could synergize with novel molecular-targeted dugs. In this study we examined the effects of combining brostallicin and different molecular-targeted agents (such as erlotinib or gefitinib or imatinib) on different experimental tumors. In vitro and in vivo studies were performed on tumors cells sensitive to brostallicin; for each combination, tumor cells sensitive to the kinase inhibitor were selected. In vitro cells were treated with increasing doses of brostallicin and/or the kinase inhibitor for 72 h. At the end of treatment, cell proliferation of treated and control cells was determined by a cellular ATP monitoring system. In vivo, DU145 human prostate carcinoma, A549 human lung carcinoma, HCT-116 human colon carcinoma and K562 human AML models, transplanted in nude mice were used to determine the effect of brostallicin/molecular targeted agents combinations. Drugs were administered at their best schedule and route and the simultaneous treatment was used for all the tested combinations. The effect of the antitumor treatment was determined as the delay, in days, in the onset

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.

307 POSTER

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.