time course experiments were performed. Both types of nanodiamonds were efficiently internalized, as shown by optical, fluorescence and transmission electron microscopy and by flow cytometry. Internalized nanodiamonds did not produce cytotoxic effects (MTT assay), at doses lower than 100 μg/ml, and did not affect microtubular cytoskeleton and cell morphology. In particular, transmission electron microscopy showed that nanodiamonds were internalized by endothelial cells at higher extent than glioblastoma U87-MG cells. Internalized nanodiamonds accumulated in specific intracellular compartments. Further experiments are needed to identify these compartments and to better characterize the specific route of nanodiamonds.

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125 POSTER

The antineoplastic activities of a novel oral formulation of interleukin-2 (IL-2)

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Background: A novel oral (mucosal) formulation for cytokine delivery is being developed for human administration: the tumour growth inhibition efficacy of oral mucosally (muc) administrated recombinant human (rh) IL-2 in mice implanted with melanoma B16, murine renal cancer RENCA, or H22 liver cancer was evaluated.

Materials and Methods: rhIL-2 for subcutaneous (sc) injection and in microencapsulated form (in solution) for oral muc administration were prepared by Kambridge Life Sciences (Melbourne, Australia). Well-grown H22 liver cancer, B16 melanoma or renal cancer clumps were isolated into single cell suspension in isotonic saline and 2×10^6 cancer cells (0.2 mL) inoculated sc dorsally into female Kunming, male C57BL/6 or male Balb/C mice, respectively. Mouse weight ranged 18−22 g. Animals were then randomly allocated (10 per group) to receive: no treatment; isotonic saline; 100 international units (IU) of rhIL-2 sc; 1, 10, 100 or 500IU oral mucosal rhIL-2 for 10 days (melanoma and liver) or 15 days (renal). Post sacrifice, body and extracted tumor weights were recorded. The studies were conducted in duplicate. Inhibition rate (IR) was mean tumour weight reduction compared to the respective control (%). Data were analysed by ANOVA. Significance level was $p < 0.05$ (denoted as *) or $p < 0.001$ (**) compared to control.

Results: Significant tumour growth inhibition of muc rhIL-2 occurred in a dose dependent manner (plateau between 10 and 100 IU) and was similar to sc rhIL-2 for all 3 cancer models. For H22 liver cancer, IR of 45.2**-47.5**% (results from duplicate studies) for 10 IU muc rhIL-2, 50.5**-59.5**% for 100 IU muc rhIL-2 while 100 IU sc rhIL-2 IR was 40.6**-42.8**%. For melanoma, IR of 10 IU muc rhIL-2 was 44.4**-71.6**% and 100 IU muc rhIL-2 was 67.0**-74.4**%. 100 IU rhIL-2 sc IR was 34.0**-58.5**%. In the renal cancer model, IR of 10 IU and 100 IU muc rhIL-2 were 37.0*-40.9**% and 44.7**-47.7**%, respectively. IR of 100 IU rhIL-2 sc was 32.3*-34.5**%. There was no evidence of toxicity in any animal.

Conclusions: rhIL-2 muc was well tolerated and resulted in significant growth inhibition of renal, melanoma and liver cancers in the murine model.

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Novel phage display-derived peptides for tumor- and vascular-targeted therapies against neuroblastoma

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Background: Disseminated neuroblastoma (NB) is refractory to most current therapeutic regimens. We showed that the therapeutic index of anticancer drugs is increased by liposome encapsulation and further improvements have been obtained by coupling tumor-specific ligands to the surface of the lipidic envelop. Phage display technology are used as a powerful tool in discovering novel ligands specific to receptor on the surface of tumor and tumor endothelial cells. The targeting of therapeutics to tumor blood vessels, using probes that bind to specific molecular addresses in the vasculature, combines blood vessel destruction with the expected anti-tumor activities of the drug, resulting in increased efficacy and reduced toxicity.

Methods and Results: In vivo selection of phage display libraries was used to isolate peptides binding specifically to the tumor blood vessel addresses aminopeptidase N (APN) and A (APA), expressed on endothelial and perivascular tumor cells, respectively. APN-targeted, doxorubicin (DXR)-entrapped liposomes displayed enhanced anti-tumor effects and prolonged survival in NB-bearing mice. In preliminary results APA-targeted, liposomal DXR displayed in vitro specific binding to APA-transfected cells and in vivo tumor growth delay in clinically relevant animal models of human NB. APN- and APA-targeted combination therapies are under investigation for their synergistic effectiveness on inducing NB tumor regression. To find more specific NB-targeting moieties, we established a protocol for the isolation of heterogeneous cell populations by tissue fractionation of primary tumors and metastases from orthotopic NB-bearing mice. By screening these mouse tissues with phage-displayed peptide libraries, we globally isolated 135 NB-binding peptides. Of these, 31 were selected for binding to the primary tumor mass, 16 to the metastatic mass, 63 to tumor endothelial cells, and 25 to endothelial cells of metastases. The binding enrichment in these experiments raised from 1.80 to 3.90 compared to healthy tissues and tumor cells. Based on their sequence homologies and conserved motifs, 3 peptides for each specific setting will be further validated.

Conclusions: The availability of novel ligands binding to additional tumor-associated antigens and to targets on both endothelial and perivascular tumor cells will allow to design more sophisticated liposomal targeted anticancer strategies that exhibit high levels of selective toxicity for the cancer cells.

Drug screening

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Development of potent water-soluble inhibitors of the DNA-dependent protein kinase (DNA-PK)

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The cellular response to DNA double-strand break (DSB) formation is an essential component of normal cell survival, following exposure to DNA-damaging chemicals (*e.g.* cisplatin and doxorubicin) and ionising radiation [1]. The serine/threonine kinase DNA-dependent protein kinase (DNA-PK) is a member of the phosphatidylinositol (PI) 3-kinase related kinase (PIKK) family of enzymes, and plays an important role in DNA DSB repair *via* the non-homologous end-joining (NHEJ) pathway [2]. DNA-PK inhibitors may, therefore, be useful as agents to improve the activity of radio- and chemo-therapy in the treatment of cancer [3]. Identification of the lead benzo[*h*]chromen-4-one DNA-PK inhibitor NU7026 ($IC_{50} = 0.23$ mM), guided the subsequent development of the potent and selective ATP-competitive chromenone NU7441 (DNA-PK $IC_{50} = 30$ nM) [4]. Although proof-of-principle studies with NU7441 confirmed promising activity *in vitro* as a chemo- and radio-potentiator in a range of human tumour cell lines [5], further biological studies with NU7441 were hampered by sub-optimal pharmaceutical properties.

Structure–activity relationship studies for DNA-PK inhibition by chromen-one-derivatives were conducted in conjunction with homology modelling. This approach predicted several positions on the pendant dibenzothiophen-4-yl substituent of NU7441 as tolerant to substitution, without detriment to DNA-PK inhibitory activity. The introduction of suitable functionality (*e.g.* OH, NH_2 CO_2H *etc*) at these positions provided a platform for the synthesis of focussed libraries of compounds bearing water-solubilising amine substituents. Interestingly, substitution with a methyl or allyl group (R) at the 3-position of the dibenzothiophen-4-yl ring enabled the separation by chiral hplc of atropisomers, as a consequence of restricted rotation about the dibenzothiophene-chromenone bond, albeit with a marked loss of potency (R = 3-Me, $IC_{50} = 2.5$ mM).

Library synthesis was undertaken employing a solution multiple-parallel approach, by *O*-alkylation or *N*-acylation of the appropriately substituted NU7441 derivatives, respectively, followed by reaction with a range of amines to afford the target compounds. These studies resulted in the identification of compounds that combined potent DNA-PK inhibition with excellent aqueous solubility (20−40 mg/mL as acid salts), without compromising cellular activity. Prominent amongst these derivatives is KU-0060648 (DNA-PK $IC_{50} = 8.6$ nM), which exhibits 20–1000 fold selectivity for DNA-PK over related PIKK enzymes and PI3K family members. The development of KU-0060648 and related water-soluble DNA-PK inhibitors will be described.

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128 POSTER

Unraveling therapeutic bio-signatures through pathway mapping at the single cell level using an analysis platform for simplified interrogation of complex data sets

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Solid tumors comprise genetically heterogeneous cell populations whose growth and survival depends on the complex interplay of distinct, yet overlapping, signaling networks. A major challenge in developing a course of therapy is determining which signaling nodes to target for a specific malignancy. Profiles from siRNA gene silencing are integral to mapping disease-specific signaling cascade(s) and provide insight to key targets for therapeutic intervention. Successful siRNA screening relies not solely upon optimizing transfection, but also cell analysis systems capable of high content screening (HCS) at the single cell level, within overall populations (sample well), and across multiple data sets. The Guava EasyCyte™ Plus flow cytometer, with integrated Guava® Simplicity software, provides a revolutionary new platform for cell-based analysis. The software's intuitive architecture and ease of use facilitates the comparison of multiple experimental conditions or disease states through heat-map visualization. To demonstrate, a mini-drug screening was performed. Following exposure to a panel of 80 cytoactive compounds, cells were assayed for multiple parameters of apoptotic induction as well as monitoring alterations in mitotic state. Parallel to screening, siRNA silencing was performed to identify genes that impact Camptothecin (CPT)-induced apoptosis. Knockdown efficiency of each gene target was examined via intracellular staining and optimized for each cell line prior to functional screening. "Hit" compounds were further tested in combination with siRNA knockdown. For this study, changes in the phosphorylation state of multiple proteins were also measured. Briefly, apoptotic assays following gene silencing identified enhancers (PTEN) and inhibitors (GSK3a) of CAM-induced cell death as well as modulators of cell cycle progression (MDM2, CHK1). Moreover, it was evident that certain genes are functionally linked and thus part of the same or overlapping networks. "Hit" screening identified cytochalasin as a potential therapeutic with similar biological profiles as CPT. However, phospho-mapping suggests their specific mechanisms of action are quite different. In summary, this experimental methodology, when used in concert with Guava Technologies' cell analysis platform and Simplicity software, significantly expedites the drug discovery process by providing a means for extraction of key biological findings from complex experimental results.

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Design, synthesis and evaluation of bivalent conformationally constrained Smac mimetics as a new class of anticancer agents

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Second mitochondria-derived activator of caspase (Smac) is a protein released from mitochondria in response to apoptotic stimuli. Smac promotes apoptosis, at least in part, by effectively antagonizing several members of inhibitor of apoptotic proteins (IAPs), including XIAP, cIAP-1 and cIAP-2, by targeting one or more BIR domains in IAPs. We designed and synthesized a series of non-peptidic, cell-permeable, bivalent small-molecules which mimic the dimeric Smac protein for targeting IAPs (bivalent Smac mimetics). We performed extensive evaluations of these bivalent Smac mimetics for their interaction with IAP proteins and their activity and mechanism in cancer cells. Our studies show that these Smac mimetics bind to XIAP, cIAP-1 and cIAP-2 with low nano-molar affinities. They function as extremely potent XIAP antagonists by concurrently targeting both the BIR2 and BIR3 domains. Consistent with their potent binding affinities to cIAP-1, these bivalent Smac mimetics potently and effectively induce rapid degradation of cIAP-1 protein in cancer cells. Our data showed that the lengths of the linker in these bivalent Smac mimetics have a significant effect on their ability in induction of cIAP-1 degradation. These bivalent Smac mimetics potently inhibit cell growth with IC_{50} values between low nanomolar and sub-micromolar and effectively induce apoptosis in a subset of cancer cell lines. Their potencies in inhibition of cell growth and induction of apoptosis nicely correlate with their ability in induction of cIAP-1 degradation. The most potent bivalent Smac mimetic SM-164 is capable of inducing of robust apoptosis in cancer cells at concentrations as low as 1 nM and effectively inhibits tumor growth in the MDA-MB-231 xenograft model. Importantly, SM-164 shows a minimal toxicity to normal cells *in vitro* and to mouse tissues *in vivo*. Taken together, our data provide strong evidence that bivalent Smac mimetics may have a great therapeutic potential for the treatment of human cancer by induction of apoptosis through targeting multiple IAP proteins.

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Structure–activity relationships for a library of C2-aryl substituted monomeric pyrrolo[2,1-c][1,4]benzodiazepines (PBD) antitumour agents

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There is growing interest in pyrrolo[2,1-c][1,4]benzodiazepine (PBD) antitumour agents as one example of this class, the synthetic DNA-interactive sequence-selective PBD dimer SJG-136, is likely to move into Phase II clinical trials later this year. Unlike this synthetic agent which contains two PBD units and works by cross-linking DNA, the naturally-occurring PBDs isolated from various *Streptomyces* species are monomeric compounds that form single covalent bonds to the N2 of guanine in the DNA minor groove in a similar manner to the recently licensed marine-derived anticancer agent trabectidin. We have previously reported that insertion of an aryl group at the C2-position of the C-ring of monomeric PBDs can dramatically enhance their overall in vitro cytotoxicity and their selectivity towards particular cell lines (especially melanoma). Novel C2-aryl PBDs of this type have not been observed in nature, and the precise role of the C2-aryl substituent in enhancing their activity was not understood. We report here the use of combinatorial technologies to synthesize a library of over 110 C2-aryl substituted monomeric PBD analogues via palladium-catalyzed cross-coupling. Each library member retains the C2/C3-endo unsaturation observed in the naturally occurring compounds, and the C2-aryl substituents contain a diverse array of ring types (including mono-, bi- and tricyclic systems) and heteroatoms (O, N and S). In addition, for comparative purposes, some library members contain C2-aliphatic alkenyl substituents (as found in the most potent natural product sibiromycin) or a combination of aryl and alkenyl substituents (i.e., C2-styryl derivatives). Biological evaluation of library members has confirmed that introduction of a C2-aryl group significantly enhances overall in vitro cytotoxicity and DNA-binding affinity with a good correlation between the two. The most active library members include the C2-aryl analogue SG2897 which has remarkable DNA-binding affinity (i.e., ?Tm = 20.8ºC) and cytotoxicity (e.g., IC50 = 0.62 nM in SK-MEL-5 melanoma; 1.45 nM in K562 leukaemia) and is significantly more potent than the best natural product sibiromycin (i.e., ?Tm = 16.3ºC, IC50 = 10 nM in SK-MEL-5; 1.40 nM in K562) which lacked efficacy in clinical trials and, unlike SG2897, is problematic to synthesize. These data will be reported in full along with the results of preclinical studies on SG2897 presently underway.

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Specific induction of the p53 pathways by low dose cytotoxic drugs assessed by gene expression pattern analysis

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Background: Induction of the p53 pathway is seen as a potentially exciting new therapeutic approach in human tumor therapy. Recently small molecule activators of the pathway have been identified by both biochemical and cell-based screens. Nutlin, a MDM2 inhibitor, has shown pre-clinical efficacy. Using expression arrays as a powerful method to determine small molecule specificity, we noted potent p53 activation by several known cytotoxic drugs at very low doses. Actinomycin D (ActD, a known DNA-interacting transcription inhibitor) and Leptomycin B (LMB, an inhibitor of exportin I driving nuclear protein export) both showed p53 activation in our reporter assay. LMB is too toxic to be used clinically while ActD is one of the older chemotherapy drugs which have been used in treatment of a variety of cancers. Potentially beneficial therapeutic use could be achieved if these compounds were shown to activate p53 pathways without the corresponding toxic effect. Literature reviews have demonstrated that at