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.

126 POSTER

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

127 POSTER

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.