stability in human serum for up to 24h. The stability of these aptamers in mouse serum, however, is significantly lower, with substantial degradation occurring within 20 min. Flow cytometry studies, have shown that these aptamers are able to bind to various cancer cell lines proposed to express the biomarker. Furthermore, the truncated versions of each aptamer displayed better binding than their full length versions. Finally, the shortened aptamers have demonstrated the ability to effect direct cell kill by inhibiting vital cellular pathways leading to cell apoptosis.

Our data demonstrates the therapeutic potential for aptamers targeting a cell surface biomarker involved in tumour progression and further studies are underway to characterise fully the anti-tumour efficacy of these reagents.

531 Poster

5T4-specific antibody responses are associated with survival in a phase II trial of renal cell carcinoma patients vaccinated with modified vaccinia Ankara delivering the tumour antigen 5T4 in combination with low-dose IL-2

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Background: The tumour antigen 5T4 is highly expressed in over 90% of renal cell carcinoma (RCC). Modified vaccinia Ankara (MVA) engineered to deliver 5T4 (TroVax) is being evaluated alongside low-dose IL-2 in an open label phase II trial in patients with metastatic RCC. The primary endpoints of this study are safety and immunological efficacy.

Materials and methods: Twenty five patients with locally advanced or metastatic RCC eligible for first or second line treatment with low dose IL-2 were recruited. IL-2 was given for up to 6 cycles with the following schedule: 250,000 U/kg/dose for 5 days in week 1 followed by 125,000 U/kg/dose for 5 days in each of weeks 2 though 6 inclusive, followed by a two week recovery. TroVax was administered by intra-muscular injection every 3-4 weeks for the first 4 injections and every 8 to 12 weeks thereafter. 5T4-specific cellular and humoral responses were monitored and clinical responses assessed by CT scan according to RECIST criteria.

Results: TroVax was well tolerated with no serious adverse events attributed to vaccination. 21 (84%) of 25 intent to treat patients mounted 5T4-specific antibody responses. Three patients showed complete responses (2 for 24+ and 1 for 12+ months), 6 patients had disease stabilization (6 to 21+ months) and the remainder had progressive disease. Median progression free survival (PFS) and overall survival (OS) was 3.4+ months (1.5-24.8+) and 12.9+ months (1.9-24.8+) respectively. A statistically significant correlation was detected between the magnitude of 5T4-specific antibody responses and PFS and OS (both $P<0.05$).

Conclusions: The primary endpoints of safety and immunological efficacy were met. TroVax was shown to be safe and well tolerated in all patients in combination with IL-2. The high frequency of 5T4-specific immune responses, number of complete clinical responses and correlation with clinical benefit are encouraging and warrant further investigation. A Phase III study with TroVax is ongoing in this indication.

532 Poster

Search for a breakthrough sensitizer in photodynamic therapy - contribution to expand a tidy technique

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Background: Photodymanic therapy (PDT) is presently a well established way for the treatment of oncological and non-oncological diseases. It is a minimal invasive procedure based on the destruction of malignant cells by action of singlet oxygen ($^1O_2$) generated through the combined action of a molecule (sensitizer) and light. The sensitizer which is not a therapeutic agent becomes active only when irradiated with low power light, developing a reaction cascade that produces apoptotic pathways leading to cell death. In absence of light the sensitizer is not harmful for cells. PDT has attracted a lot of interest due to the selectivity shown by malignant tumours for the molecules of porphyrins as sensitizers relatively to healthy tissues.Photofrin®, one of the most used sensitizers for cancer treatment actually approved by the FDA, is a β-substituted porphyrin. To become a more widely used technique, PDT needs the development of more specific an efficient sensitizers but, above all, get the sensibility and the motivation of the clinics to dominate the technique in a broader type of situations.

Materials and Methods: The sensitizers 5,15-diarylporphyrins, (1-3) were sensitized as previously reported in Patent nº102721, WO 03/064427, PCT/EP03/00829.

For each experiment, cells were plated in 48 multiwells (Corning Costar Corp), in a concentration of 40 000 cells/mL and kept in the incubator overnight, in order to allow the attachment of the cells. The formulation of these sensitizers consisted in a 1 mg/mL solution in a ternary mixture of H_2O:PEG_{400}:EtOH (50/30/20, v/v/v), the desired concentrations being achieved by successive dilutions. The sensitizers were administered in several concentrations (50 nM, 250 nM, 500 nM, 1 μM, 5 μM, 10 μM) and cells were incubated for 24 hours. Cells were washed with PBS and new drug-free medium was added. Each plate was irradiated with a fluence rate of 7.5 mW/cm^2 until a total of 10 J or 5 J was reached. Cell viability was measured 24 hours after the photodynamic treatment.

Results: The IC_{50} values for dose/response curves for WiDr human colon adenocarcinoma cells and melanoma A375 irradiated with 10 J are reported in table 1 as well as the values obtained for Photofrin® as reference compound.

Table 1 (Poster 532)

Compound	IC50 - WiDr - 10J	IC50 - A375 - 10J
Photofrin®	666 nM	156 nM
Compound 1	38 nM	27 nM
Compound 2	27 nM	27 nM
Compound 3	88 nM	-

Using 5 J of energy the values of IC_{50} for the sensitizer 1 are 68 nM and 27 nM and for the sensitizer 2 are 32 nM and 27 nM for WiDr and A375 respectively.

Conclusion: In this study we determined the anti-tumoral activity of our new 5,15-diarylporphyrins (1-3) against WiDr and melanoma A-357 cancer cell lines. The IC_{50} values are compared with those for Photofrin® and show an activity of 20 times superior. The results for PDT action of compound 2 in the inhibition of tumor growth in implanted tumor in nude mice will be presented.

533 Poster

Evaluation of antiproliferative and molecular effects of vinorelbine and its active metabolite 4-O-deacetyl-vinorelbine on human endothelial cells in an in vitro simulation model of metronomic chemotherapy

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Background and Aim: Metronomic chemotherapy is a novel approach of cancer therapy, developed on the concept that activated vascular endothelial cells are selectively sensitive to protracted exposure to very low concentrations of cytotoxics. Microtubule-targeting drugs are most potent against endothelial cells. Thus availability of an oral formulation of vinorelbine (Navelbine®) prompted us to take into clinical investigation this antimitotic drug at a metronomic dosing schedule [NCT00278070]. In this context we investigated antiproliferative and molecular effects of vinorelbine (VRL) and its active metabolite 4-O-deacetyl-vinorelbine (DVRL) on proliferating endothelial cells in an in vitro simulation model of metronomic chemotherapy.

Methods: Human umbilical vein endothelial cells (HUVEC) were plated to sub-confluence in 96- or 6-well plates and treated with VRL and DVRL for 24 and 96h replacing medium every 24h. The effects of different concentrations of VRL and DVRL on cell proliferation and the expression of angiogenesis modulating molecules TSP-1, VEGF, VEGFr2 and IL8 were assessed. We employed cell proliferation (MTS) assay for growth inhibition and measured molecular biomarkers of angiogenesis at a transcript level (RT-PCR) and also as excreted proteins in cell medium (ELISA).

Results: The half-maximal inhibitory concentrations (IC50) obtained against HUVEC were four orders of magnitude lower at the 96h-exposure compared with the 24h-exposure (1.23 nM vs 32 μM for the VRL and 0.55 nM vs 78 μM for DVRL). Notably the IC50 observed at the 96h-exposure are close to the trough levels recorded in patients treated with metronomic oral vinorelbine (Briasoulis et al, 18th EORTC-NCI-AACR Symposium 2006). At molecular level concentrations of both compounds at the high nanomolar and low micromolar range, which are commonly achieved with the conventional dosing of vinorelbine, induced proangiogenic feedback effects on the exposed HUVEC: at concentrations above 100 nM we observed a dose-dependent increase of proangiogenic molecule IL-8 and a parallel decrease of antiangiogenic TSP-1 at mRNA and protein levels. Such molecular responses did not occur at low-range nanomolar