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

concentrations which are obtained with metronomic vinorelbine in patients. The effects on VEGF and VEGFr2 were insignificant.

Conclusion: This data provide experimental support to the rationale of metronomic dosing protocols of oral vinorelbine as a therapeutic strategy in cancer patients.

534 Poster

Mutation analysis of the genes coding for fluoropyrimidines' catabolizing enzymes in prediction of fluoropyrimidines-associated toxicity in cancer patients

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Fluoropyrimidines (FPs) are widely used for therapy of GIT, breast, and H&N cancers, however, in approximately 5-15 % of patients occur symptoms of severe FPs-related toxicity (mucositis and hematological toxicity), leading to life-threatening complications in about 1% of patients. We performed mutation analysis of the genes coding for three FPs-catabolizing enzymes: dihydropyrimidine dehydrogenase (DPD), dihydropyrimidinase (HPYS) and β-ureidopropionase (BUP1) to test their influence for development of FPs-related toxicity in Czech patients.

Mutation analysis was performed on the panel of cancer patients treated by FPs-containing regimes consisting of 73 patients with severe FPs-related toxicity (grade III-IV) and 40 patients with excellent tolerance of FPs treatment. Analysis of coding sequences of DPD was performed by sequencing of DPD mRNA (cDNA), mutation analysis of HPYS and BUP1 was performed using DHPLC analysis of PCR-amplified exons. Frequencies of characterized mutations were estimated by analysis of population controls.

Nine different alterations were found in DPD. The most frequent alterations/polymorphisms C29R; K63E; M166V; S534N; I543V; F632F; V732I; E412E, were scored in 72% of patients with toxicity and 77 % patients without toxicity. These frequencies were similar to that found in population controls. Disease-predisposing mutation IVS14+1G>A (e14 skipping) was presented in 4% of toxicity patients. We have found correlation of several DPD alterations in development of site-related toxicity: the positive correlation was scored for leucopenia and V732I (OR=6.8; p=0.0036), thrombocytopenia and V732I and C29R, and negative correlation was found for mucositis and I543V.

Mutation analysis of HPYS revealed seven different genetic changes: c.-1 T>C; R481W; S5S; L35L; F72F; IVS1-58T>C and IVS4+11G>T. Similarly to DPD, the frequencies of HPYS alterations did not differed significantly between toxicity and non-toxicity patients. Analysis of exons 1 and 2 of BUP1 lead to characterization of genetic alterations –29G>A; -6C>T and previously described SNPs (42C>G; 105A>T; 4764A>G). Frequencies of all these variants are similar in both groups of patients.

Despite we have failed to found association between occurrence of DPD, HPYS and BUP1 sequence variants and development of overall FPs-associated toxicity the analysis of DPD mutations/polymorphisms can be useful for site-related toxicity prediction.

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Nanoparticle of cholesterol-bearing pullulan as a carrier of anticancer drugs

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Background: Recently, Macromolecular assembly systems of polymer amphiphiles have attracted much attention as a vehicle for drug delivery systems (DDS). Cholesterol-bearing hydrophobized pullulan (CHP) modified with amino groups (CHPNH2) is a newly developed drug delivery vehicle that can be used to formulate nanoparticles (diameter 20–30 nm) including drugs. The complexed nanoparticles thus obtained formed a very stable colloid. The purpose of this study is to investigated whether and how effectively CHPNH2 nanogel could be used as a DDS of anticancer drug.

Materials and Methods: Docetaxel (DOC) was prepared by simple mixing with CHPNH2 (CHPNH2-DOC). Cytotoxicity of DOC and CHPNH2-DOC against five non small cell lung cancer cell lines were examined by WST-1 assay. Half-maximal inhibition constants (IC50) were determined using the non-linear regression program CalcuSyn (Biosoft, Cambridge, UK).

Results: The IC50 values of CHPNH2-DOC were significantly lower than that of DOC alone. In vivo efficacy of CHPNH2-DOC and DOC were also investigated preliminarily by pleural dissemination mouse model, in which H1299 cells were implanted into the murine pleural space. Tumor growth was monitored using an in vivo imaging system. Pleural tumor cell growth treated with CHPNH2-DOC was lower than DOC alone mice, and CHPNH2-DOC also was prolonged the survival of mice inoculated with non small cell lung cancer cells.

Conclusions: These findings showed that CHPNH2-DOC can be achived stronger effect than DOC alone in vitro and in vivo. Furthermore, CHPNH2 may be a promising efficient drug delivery vehicle.

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Synthesis and cytotoxic activity of novel imidazo[1,2-a]pyridines and quinolines

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Cancer is a leading cause of death only after cardiovascular diseases. The incidence of cancer has not dropped over the last decades and the most complicated cases are presented in developing countries. For that reasons, it is important to develop new compounds with antitumoral activity.

In recent years, cyclin dependent kinases (CDKs) have been proposed as a plausible target against cancer. As a part of our search for new antitumoral compounds, a new series of 2-aminopyrimidines-substituted imidazo[1,2-a]pyridines and quinolines, compounds 1-6, have been synthesized and tested against the following human cell lines: U251, PC-3, K-562, HCT-15, MCF-7 and SKLU-1.

Coupling between different aryl halides and the amine group of the pyrimidine ring was successfully achieved under the Buchwald-Hartig conditions using 4,5-Bis(diphenylphosphino)-9,9-dimethylxanthene as ligand, bis(dibenzylideneacetone)palladium (0) as catalyst and cesium carbonate in toluene.

Cell viability was measured using the sulforhodamine assay.

Compounds 1-3 diminished the metabolic ability of all cell lines tested at 50 μM. Compounds 1-3 exhibited remarkable cytotoxicity in K562 and HCT-15; SKLU-1 presented 100% growth inhibition at 50 μM. The results for compounds 4-6 will be presented. The IC_{50} values for cell lines growth inhibition and the CDK inhibition of selected compounds will be shown

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Treatment results with fermented mistletoe (Viscum album L.) extract as part of long-term supportive care in patients with primary non-metastatic colorectal carcinoma

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Objectives: To evaluate efficacy and safety of a fermented mistletoe extract (Iscador®, ISC) in supportive care of surgically treated patients with primary non-metastatic colorectal carcinoma in comparison with a parallel control group without ISC.

Methods: In a multicenter, comparative, non-interventional cohort study in Germany and Switzerland, ISC was given in addition to conventional adjuvant chemo- and radiotherapy, while the control was treated with conventional therapy only. Endpoints were surrogates of quality of life and survival, adjusted to baseline, therapy regimen and other confounders.

Results: In 804 (429 ISC and 375 control) evaluable patients from 26 centers, the majority of the baseline characteristics, prognostic criteria, and therapy was sufficiently balanced between the therapy groups. After a median follow-up of 58 vs. 51 months, and a median ISC therapy duration of 52 months, significantly fewer ISC (19.1%) than control patients (48.3%) developed ADRs related to the conventional therapy ($p < 0.001$), had fewer symptoms during the therapy, mainly gastrointestinal and CNS ($p < 0.001$), and had an on average one week shorter hospitalization. ISC vs. control patients showed a longer tumor-free survival (p=0.013). 2.3% of the patients developed systemic ADRs related to ISC, and 23.3% local ADRs. Severe ISC-related ADRs or tumor enhancement were not observed.

Conclusions: The ISC-group showed significantly fewer ADRs of the conventional therapy, fewer disease- and therapy-related symptoms, and longer tumor-free survival than the parallel control group without ISC. The ISC-treatment was well tolerated and appears beneficial in supportive care in primary non-metastatic colorectal carcinoma.