and EGFR, both coding for proteins currently recognized as potential targets of tyrosine kinase inhibitors (TKI).
Methods: Fifty archival tissue specimens from CUP patients were screened for C-KIT and EGFR mutations. All samples were screened for the presence of small deletions and/or insertions in exon 11 coding sequences for the juxtamembrane domain of the C-KIT protein and for polymorphisms of a CA dinucleotide small sequence repeat (SSR) in the untranslated regulatory sequence in intron 1 of the EGFR gene by means of polymerase chain reaction-based single strand conformational polylmorphism (PCR-SSCP). Thirty-six out of 50 samples were stained by immunohistochemistry for EGFR (K1494 DAKO) and C-KIT/CD117 (A 4502 Dako) protein expression.
Results: Among the 35 immunostained specimens, 26 (74%) showed EGFR expression, 4(12%) strong, 15 moderate and 7 weak. In view of previous studies showing an association of decreasing EGFR transcriptional activity with increasing numbers of intron 1 repeats, we determined the SSR length of EGFR intron 1. We detected five alleles with CA repeat numbers 16 to 20. Allele 16 showed the highest frequency (39%) followed by allele 18 (34%), 19 (11%), 20 (11%) and 17 (5%). All samples were heterozygous, the commonest genotype consisting of allele lengths of 16/18 dinucleotides (78%). Seven samples revealed an unexpected genotype of 3 CA –length alleles, probably due to genetic instability, and were associated with EGFR overexpression in 40% of cases. Samples with alleles of $\leqslant$18 and >18 CA repeats showed EGFR overexpression in only 8% and 0% of cases respectively. C-KIT overexpression was found in 2 specimens (6%) while 7 showed moderate and 22 weak staining. No gain of function mutations nor any polymorphism were found by PCR-SSCP in C-KIT exon 11.
Conclusions: Positive EGFR and C-KIT/CD117 expression is seen in the majority of CUP patients, while overexpression less often. Our findings indicate the presence of an association between EGFR protein overexpression and the allelic length of EGFR intron 1 CA SSR. This observation may provide clues for the molecular biology of the disease and holds promise for TKI-based therapeutic interventions. C-KIT oncoprotein expression is not associated with activating mutations in exon 11 of the gene, indicating that such mutations are not implicated in CUP molecular pathophysiology.

1467 POSTER

RAD51: A DNA repair target for increasing the therapeutic potential in bladder cancer

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Background: Organ preservation using combination chemoradiation with gemcitabine or cisplatin has become an increasingly important strategy in the treatment of bladder cancer with complete response rates of ~70% in selected patients. Chemoresistance has been linked to the overexpression of RAD51, a pivotal protein in homologous recombination (HR), a DNA double strand (DNA-dsb) repair pathway. Therapeutic manipulation of RAD51 expression, or RAD51 signaling pathways, may allow increased tumour cell kill following ionizing radiation (IR) within a favorable therapeutic ratio.
We hypothesize that manipulation of the HR-RAD51 pathway could lead to a therapeutic gain for bladder cancers treated with XRT-cisplatin or XRT-gemcitabine.
Materials and Methods: Using the RT112 bladder-TCC cell model, clonogenic survival is being determined for cell exposure to IR, cisplatin(cDDP), gemcitabine, and mitomycin C (MMC; the latter used to measure relative HR). Imatinib, an inhibitor of c-ABL/RAD51 signaling, was used at a variety of doses from 7.5–50 μM alone, and in combination, with IR, gemcitabine and cDDP to look for supra-additive effects. Protein expression for c-ABL and DNA-dsb-related protein expression is determined using Western blots. Use of a plasmid-based HR assay (DR-GFP construct) within a RT112 clone will enable functional assessment of HR following imatinib treatment.
Results: Decreased RAD51, but not KU70, protein expression was observed at 24hours following 50 μM imatinib in RT112 cells. The ICD50 for gemcitabine is 13 nM and for MMC is 75 nM for RT112 cells. SF2 Gy following IR for RT112 cells is 0.68. Addition of imatinib resulted in an 8-fold increase in cell kill at 2 Gy (ratio cell kill 2 Gy: 2 Gy+gleevec = 1: 8.4). Ongoing experiments are testing for similar chemosensitization. Transfection of DR-GFP into RT112 cells was successful and will be used to correlate HR levels pre- and post-imatinib to cell toxicity in both RT112 cells and normal fibroblasts.
Conclusions: RAD51 inhibition may be a novel strategy to increase the radiosensitization of bladder cancer if it translates to maximal sensitization of bladder tumour cells over that of normal tissues. This may allow for novel treatment strategies that increase organ-preservation rates and quality of life for bladder cancer patients.

1468 POSTER

A phase 1B, open-label, dose-escalation study of bortezomib (Btz) in combination with gemcitabine (G) and cisplatin (C) in the first-line treatment of patients with advanced solid tumors: preliminary results

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Background: Bortezomib (VELCADE®) is a reversible, specific inhibitor of the 26S proteasome. G/C is a first-line treatment for several advanced solid tumors. MTD and tolerability of a weekly and twice-weekly schedule of Btz in combination with G/C are investigated.
Methods: Chemonaïve patients (pts) (KPS $\geqslant$ 70%) with advanced solid tumors received G 1000 mg/m^2 days 1&8, C 70 mg/m^2 day 1, on a 21-day cycle. In the weekly schedule pts received Btz days 1&8; dose levels (DL): 1.0?1.3?1.6 mg/m^2. In the twice-weekly schedule, pts received Btz days 1, 4, 8&11; DL: 0.7?1.0?1.3 mg/m^2. Safety assessments included CTC 3.0, audiometry and FACT/GOG-NTX. Limited PK analysis is carried out at MTD. Serum is collected for proteomics.
Results: So far, 31 pts (34 needed) were treated for at least 1 cycle: 19 in the weekly schedule (12 pts at 1.0 mg/m^2, 7 pts at 1.3 mg/m^2) and 12 in the twice-weekly schedule (3 pts at 0.7 mg/m^2 and 9 pts at 1.0 mg/m^2). Pt characteristics: Median age: 55; Sex M/F: 21/10; KPS < 80%/$\geqslant$80% = 5/26; tumor types: 24 NSCLC, 4 urothelial cell ca, 1 unknown primary, 1 pancreas ca, 1 HCC. First-cycle DLTs were observed in the weekly schedule at Btz 1.3 mg/m^2: GR3 diarrhea (1 pt), GR4 platelets (plts) (3 pts), GR4 ANC > 5 days (2 pts). In the twice-weekly schedule at Btz 1.0 mg/m^2, 1 pt with DLT was seen: GR4 plts with bleeding, GR4 febrile neutropenia. The MTD of Btz was 1.0 mg/m^2 in both schedules. At MTD, most common GR3/4 toxicities: plts 71%/14% (twice-weekly), 42%/17% (weekly), neutropenia 43%/14% (twice-weekly), 25%/8% (weekly). Treatment was generally well tolerated. No $\geqslant$GR2 drug-induced sensory neuropathies have been observed. Addition of Btz did not appear to lead to additional N/V, or diarrhea compared to G /C alone. C was reduced in 2 pts because of GR2 ototoxicity. There was one case of GR4 left ventricular dysfunction. Subsequent follow-up of following pts by MUGA revealed no marked EF decrease. In the first 25 evaluable pts, there were 11 partial responses (NSCLC, UCC, ACUP; 2 to be confirmed), 12 pts with stable disease and 2 pts with progressive disease. 7 out of 9 confirmed responses were seen in the weekly schedule.
Conclusion: The MTD of Btz combined with G 1000 mg/m^2 and C 70 mg/m^2 is equal in the two schedules at 1.0 mg/m^2. The combination has been well tolerated with less (myelo) toxicity being observed in the weekly regimen. In general, Btz may increase myelosuppression of G/C chemotherapy. Updated information (including PK) at the meeting.

1469 POSTER

Molecular mechanisms of cis-4-hydroxy-L-proline – induced antiproliferative effects in colon and pancreatic tumor cell lines in vitro

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Background: Proline analogs have been supposed to target collagenous proteins in cancer cells ultimately disturbing the formation of extracellular matrix, which directly or indirectly has important functions in the growth and metastasis of solid tumors. The aim of the present study was to investigate the cellular mechanisms affected by cis-4-hydroxy-L-proline (CHP), leading to antiproliferative activity in a panel of tumor cell lines.
Material and methods: Proliferation was assessed using a formazane assay (MTT), EGF receptor expression and cell cycle distribution (propidium iodide) by FACS analysis. Cellular ultrastructure was studied in transmission electron microscopy (TEM).
Results: Application of CHP in concentrations ranging from 50–400 μg/ml resulted in dose-dependent inhibition of proliferation and cell death in a panel of tumor cell lines including lines derived from colon, pancreatic, prostate, breast and other cancers. Tritiated CHP showed uptake by the MeAIB-sensitive proline transporter and was incorporated into cellular proteins. TEM data of CHP-treated cells (Colo 205 and Caco-2 colon lines and BxPC3 and MIAPaCa2 pancreatic cancer lines respectively) revealed the induction of extensive intracellular vacuolization and cell death. CHP-induced ultrastructural alterations were absent in normal fibroblasts and colonocytes. Furthermore CHP was found to induce a pronounced extracellular acidification (0.1–0.4 pH units), not observable upon treatment

with other analogues like trans-4-hydroxy-L-proline or cis-4-hydroxy-D-proline. The anti-tumor activity of CHP could be antagonized by the Na^+/K^+-ATPase inhibitor ouabain (1 mM) and by addition of high extracellular potassium (>70 mM), however not by the vacuolar H^+-ATPase inhibitor bafilomycin. Additionally CHP was found to decrease significantly the expression of the EGF receptor.
Conclusions: The results of the present study demonstrate that CHP effects extensive intracellular vacuolization in colon and pancreatic tumor cell lines by modulating intracellular ion concentrations of K^+, Na^+ and H^+involving Na^+/K^+-ATPase, which is differentially expressed in tumor cells. Tumor cell proliferation may be further limited by the generation of a CHP-induced acidic extracellular environment and downregulation of the EGF receptor. Therefore CHP may constitute an antitumor agent with broad activity/low toxicity targeting a unique intracellular and tumor-associated mechanism of Na^+/K^+ homeostasis.

1470 POSTER

Reduction and activation of RH1 by NADPH cytochrome P450 reductase

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Background: RH1 is a novel diaziridinylbenzoquinone bioreductive agent that is in clinical trials. RH1 is a very efficient substrate for the two-electron reducing enzyme NAD(P)H quinone oxidoreductase 1 (NQO1; DT-diaphorase). Reduction by NQO1 results in activation of the aziridine groups and DNA alkylation with interstrand cross-link formation. Because RH1 is a very good substrate for NQO1, this agent was considered ideal for use in an enzyme-directed tumor targeting strategy to treat tumors with high levels of NQO1. However, studies have shown that RH1 can be a substrate for the one-electron reducing enzyme NADPH cytochrome P450 reductase (P450 Red), and that leukemia and lymphoma cell lines with low or absent NQO1 expression can have a high sensitivity to RH1. Thus, it is not clear if P450 Red can contribute to activation of RH1. In this study, we investigated the role of P450 Red in the reduction and activation of RH1.
Materials and methods: We compared reduction and activation of RH1 by P450 Red and NQO1. Reduction was studied by spectroscopic analysis, and production of RH1 semiquinone was monitored by EPR. DNA damage produced by reduced RH1 was measured by gel assays, and cytotoxicity of RH1 in T47D human breast cancer cells and T47D cells transfected with P450 Red (T47D-P450) was compared by MTT assays.
Results: Under hypoxia, reduction was faster with NQO1 than with P450 Red. Under aerobic conditions redox cycling was slower after reduction by NQO1 compared with P450 Red. RH1 reduction by P450 Red gave a very strong semiquinone EPR signal while NQO1 gave only a weak signal. Reduction of RH1 with NQO1 produced significantly more DNA strand breaks than reduction with P450 Red. P450 Red activity was 20-fold higher in T47D-P450 cells than in T47D cells while the levels of NQO1 were similar. Despite this, the cytotoxicity of RH1 in the two cell lines was similar. The P450 Red inhibitor, diphenyliodonium chloride (DPIC), did not inhibit RH1 cytotoxicity in T47D-P450, while the NQO1 inhibitor, dicoumarol, significantly inhibited RH1 cytotoxicity. However, if the transfected cells were treated with both inhibitors there was additional inhibition of RH1 cytotoxicity compared with dicoumarol alone. A similar study in the T47D cells showed that treatment with both enzyme inhibitors did not result in greater inhibition of cytotoxicity than treatment with dicoumarol alone.
Conclusions: These results confirm that P450 Red can reduce RH1, and in the absence of NQO1 activity high levels of P450 Red can contribute to RH1 activation and cytotoxicity. However, NQO1 appears to be the major activating enzyme for RH1, and P450 Red likely does not play a role in RH1 activation at normal cellular levels of this enzyme.
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1471 POSTER

Therapeutic benefit of combining AQ4N with radiation

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Background: AQ4N, a bioreductive drug currently in clinical evaluation, is effective at killing the hypoxic tumours cells that have a negative impact on radiation therapy. The aim of this study was to investigate the effect of combining AQ4N with radiation in a murine tumour model, and early and late responding mouse normal tissues.
Methods: Female CDF1 mice with or without a C3H mammary carcinoma in the right rear foot were used. AQ4N was dissolved in saline and intraperitoneally injected at 0.02 ml/g body weight. Radiation (240 kV X-rays) was given locally to either the right rear foot of normal or tumour bearing mice, or the lungs, of restrained non-anaesthetised animals. Response was assessed as the percentage of animals at each radiation dose showing either local tumour control at 90 days; development of moist desquamation of the foot between 11–23 days; or a 20% increase in lung ventilation rate within 9 months, after treatment. Following logit analysis of the radiation dose response curves the radiation dose producing a response in 50% of treated animals (RD50) was calculated. A Chi-squared test was used for statistical analysis ($p < 0.05$).
Results: The RD50 value (±95% confidence intervals) for control-irradiated tumours was 53 Gy (51–55). Injecting AQ4N (60 mg/kg) 0, 2 or 4 hours prior to irradiating significantly reduced the RD50 to similar values of 44 Gy (40–49), 47 Gy (44–50) and 45 Gy (42–47), respectively. Using a 2-hour interval, the respective RD50 values obtained with AQ4N doses of 30 and 120 mg/kg were 50 Gy (46–54) and 43 Gy (39–47). For skin the RD50 value for radiation alone was 32 Gy (30–33) and a small yet significant enhancement was obtained with radiation given 2 hours after injecting 60 mg/kg AQ4N; the RD50 value being 30 Gy (29–31). With lung the RD50 value was 14 Gy (11–17) regardless of whether radiation was given alone or 2-hours after AQ4N (60 mg/kg).
Conclusions: AQ4N significantly enhanced the radiation response of this C3H mammary carcinoma and did so in a dose dependent, yet time independent, fashion. Using a clinically relevant 60 mg/kg dose and a 2-hour interval gave rise to a 1.12 fold enhancement of radiation response. In early responding skin the enhancement was only 1.06 and in late responding lung absolutely no enhancement was found, resulting in a clear therapeutic benefit.
This study was financed by KuDOS Pharmaceuticals with additional support from the Danish Cancer Society.

1472 POSTER

A Phase I study with Fosfluridine tidoxil, a novel oral fluoropyrimidine, in patients with advanced solid tumors

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Methods: Sequentially, two dosing schedules were explored: 1. p.o. once qd for 14 days q 3 wks and 2. p.o. once qd for 7 days q 2 wks. For PK assessment, a single dose was given to all pts 7 d before daily dosing. An adapted Fibonacci '3+3' dose escalation design was used. 23 pts (14 women/9 men, med age 61 yrs, range 37–80 yrs) were treated with schedule 1, 19 (9 women/10 men, med age 62 yrs, range 41–73 yrs) with schedule 2. Standard Phase I eligibility criteria were required for study entry. DLT observed during the first 2 cycles with schedule 1 and the first three cycles with schedule 2 were taken into account for MTD assessment.
Results Schedule 1: Explored DL's: 50, 100, 200 and 300 mg with 3 (8), 6 (35), 10 (27) and 4 (4) pts (cycles) per DL. MTD: 200 mg with gastrointestinal (GI) DLT (diarrhea, abdominal pain, colitis, vomiting, nausea) at the end of or shortly after the first 14 treatment days in 5/12 pts (42%) and 5/27 cycles (19%). Several pts were dose-reduced due to toxicity. No DLT was observed at 100 mg.
Results Schedule 2: Explored DL's: 100, 150 and 200 mg with 3 (23), 10 (32) and 6 (22) pts (cycles) per DL. MTD: 200 mg with GI DLT (vomiting, nausea, anorexia) in 2/6 pts (33%) and 2/22 cycles (9%). No DLT was observed at 150 mg.
Tumor activity: No OR was observed. Long-lasting SD was seen in 3 pts treated with schedule 1 (RCC: 100 mg, 12 cycles; Pancreatic neuro-endocrine Ca: 100 mg, 10 cycles; Thyroid Ca: 200 mg, 11 cycles) and 2 pts treated with schedule 2 (Pancreas Ca: 100 mg, 9 cycles; RCC: 100 mg, 8 cycles).
PK: With a mean of 17.75 h (range 13–23) T1/2 is much longer than that of other fluoropyrimidines. The PK profile of Cmax and AUC is linear over the studied DLs. There is no measurable accumulation of FT in the plasma over multiple cycles of schedule 2.
Conclusions: The principal DLT of FT is similar to that of other fluoropyrimidines. Dosing schedule 2 with a recommended dose of 150 mg orally once-a-day was chosen for Ph II "proof-of-concept" studies in selected solid malignancies.