increments) to define a maximum non-toxic metronomic dose (MNMD). In phase-beta, patients allocated randomly to one of three dose levels (low-medium-MNMD) and underwent blood sampling throughout the treatment to analyze for kinetics of surrogate biomarkers of angiogenesis (soluble VEGF, VEGFr2, TSP1, bFGF and circulating progenitor endothelial cells) and twice for pharmacokinetics. OMD will be defined by considering kinetic profile of biomarkers in association with antitumor activity and pharmacokinetics.
Results: Forty six patients [21 female and 25 male; median age 58 range 38–85 years; median PS 1, median prior treatments 2 range 0–7] were enrolled between June 2004 and May 2005. They had a variety of progressive refractory solid cancers and were treated at the dose range of 20 to 50 mg. All patients received continuous non-stop therapy without overt clinical toxicity. Median time of treatment failure was 17 weeks (range 6 to 44+ weeks) that also corresponded to time to progression, since no withdrawals from therapy were due to toxicity. MNMD has not been reached and dose is presently escalated to 60 mg. Objective tumor response (confirmed partial remission) was documented in four cases (renal cancer, NSCLC, unknown primary and sarcoma Kaposi) and 32% of treated patients had stable disease for more than 6 smonths. The trial is ongoing to define the OMD.
Conclusions: Continuous administration of metronomic oral vinorelbine, given TIW is feasible and exceptionally well tolerated at doses up to 50 mg. Early results show activity against refractory tumors and provide evidence towards clinical proof of principle for metronomic chemotherapy. Data on biomarker analysis and pharmacokinetics will be presented at the meeting.

1461 POSTER

Circulating endothelial cells and monocytes as markers of sunitinib malate (SU11248) activity in patients with imatinib mesylate-resistant GIST

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Background: This study evaluated circulating endothelial cells (CECs), monocytes (MCs), circulating VEGF and soluble VEGFR-2 (sVEGFR-2) as potential pharmacodynamic markers of sunitinib malate (SU) activity in GIST patients (pts).
Materials and methods: SU is an oral multitargeted tyrosine kinase inhibitor of VEGFR, PDGFR, KIT, RET and FLT3 with antiangiogenic and antitumor activities. The majority of pts enrolled in a phase I/II trial of SU for metastatic GIST resistant to imatinib mesylate (IM) therapy received repeating cycles of 50 mg SU daily for 4 wks followed by 2 wks off treatment. CBC and differential WBC counts were taken pre- and post-treatment in 73 pts enrolled in the study. In 16 pts, CECs were assessed serially by 4-color flow cytometry. Changes in mature CEC and MC counts were correlated with clinical outcome, classified as either clinical benefit (CB: PFS >6 mos) or PD. Plasma levels of soluble proteins were analyzed using validated sandwich ELISA assays.
Results: MC levels decreased 54% after 2 wks of SU therapy ($P < 0.001$) and rebounded (96%) after 2 wks off therapy ($P < 0.001$). This pattern of decrease and increase in MC levels during periods on and off SU therapy was observed across multiple drug cycles, while no such pattern was observed with lymphocytes. After 2 wks of SU therapy, mean MC count decreased 59% in the PD group, but only 48% in the CB group ($P = 0.03$). CEC counts increased significantly early after initiation of SU therapy (first follow-up: 6–20 d), but not at subsequent timepoints. Changes in CEC count following therapy distinguished pts based on clinical outcome: all 7 pts with SU-related CB also exhibited a rise in CEC count between baseline and first follow-up, while only 3 of 9 pts with PD had a rise in CEC count. The rate of change per day in CECs was significantly different between pts with CB and PD (median: 0.52 vs. –0.01 cells/μL; $P = 0.03$). After active treatment in each cycle, mean plasma VEGF levels increased by an average of 2.9-fold, while mean sVEGFR-2 levels decreased by an average of 1.7-fold, with both returning to near-baseline levels during treatment breaks. These changes did not strongly correlate with clinical outcome.
Conclusions: Percent drop in MC counts and rate of change per day in mature CECs distinguished pts with metastatic IM-resistant GIST who experienced CB during SU therapy from those who did not. CEC and MC counts may be useful markers for identifying pts who may ultimately benefit from SU therapy.

1462 POSTER

A multicenter Phase II trial of gefitinib 500 mg/day in 192 patients with advanced epidermal growth factor receptor-positive solid tumors who had failed previous chemotherapy.

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Background: 15 Belgian oncology centers participated in an open-label Phase II trial of gefitinib (IRESSA) 500 mg/day in patients (pts) with advanced epidermal growth factor receptor-positive solid tumors who had failed >1 previous chemotherapy regimen and had no further chemotherapy treatment options.
Methods: EGFR expression was assessed using DakoCytomation's EGFR pharmDx™ kit. Pts with EGFR expression of >1 received gefitinib 500 mg/day until disease progression. Objective tumor assessments, by RECIST, were made every 8 weeks and confirmed by repeat assessments >4 weeks later. Disease control rate (DCR) was defined as objective response (confirmed complete response or partial response [PR]) plus stable disease (SD) for >8 weeks. All adverse events (AEs) were reported and assessed by NCI-CTC version 2.0.
Results: 192 pts have been enrolled with the following tumors: pancreatic cancer (PC) [n = 39], cervical cancer (CC) [n = 36], ovarian cancer (OC) [n = 33], sarcoma (S) [n = 23], cancer of unknown primary origin (U) [n = 19], hepatocellular carcinoma (HC) [n = 18], bladder cancer (BC) [n = 14], and endometrial cancer (EC) [n = 10]. Mean time between first tumor diagnosis and the start of gefitinib treatment was 33.5 months (±44.8). 18 pts (HC only) had received no previous chemotherapy, 74 pts 1 line, 58 pts 2 lines and 42 pts >3 lines. DCR was 34%: 7 pts had a PR (3 CC, 3 OC, 1 PC) and 58 pts had SD at 8 weeks (16 CC, 9 OC, 8 S, 6 PC, 9 HC, 4 EC, 3 BC, 3 U). Mean duration of disease control was 27.2 weeks (±2.2). 84% of pts had at least one drug-related AE. In 16% of patients (n = 31), this drug-related AE was CTC grade 3, in 0.5% (n = 1) of patients CTC grade 4. Most of these grade 3–4 adverse events were of cutaneous or gastrointestinal origin. Gefitinib dose reduction to 250 mg/day was required in 21% of pts. In 7% of patients gefitinib treatment was withdrawn because of drug-related AEs.
Conclusions: Gefitinib showed promising activity and acceptable tolerability in patients with EGFR-positive pancreatic cancer, cervical cancer and ovarian cancer. Our data support findings from previous gefitinib Phase I/II trials in patients with advanced cervical cancer[1] and ovarian cancer [2].
IRESSA is a trademark of the AstraZeneca group of companies

References

[1] Viens et al. ASCO 2003; abstract 1833.
[2] Ranson et al. J Clin Oncol 2002;20:2240–50.

1463 POSTER

The urinary excretion of l-carnitine and its short-chain ester acetyl-l-carnitine in patients undergoing carboplatin treatment

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Background: During chemo- and radio-therapy cancer patients experience fatigue symptoms. Dietary and nutritional factors are among postulated to be involved in the multifactorial aetiology of fatigue. In fact, when L-carnitine (LC), a compound necessary for energy production through mitocondrial fatty acid oxidation, is reduced in the body or its endogenous system is altered, fatigue symptoms appear and are improved after its administration. Some anticancer drugs, such as cisplatin, cause excessive urinary LC elimination through a possible inhibition of its renal reabsorption. Other platinum-derivatives, such as carboplatin, could have a similar effect. This study investigated the influence of carboplatin treatment on plasma concentration and urinary excretion of LC and its main ester, acetyl-L-carnitine (ALC) in tumoral patients.

Material and Methods: Plasma and urine concentrations of LC and ALC from eleven patients under carboplatin chemotherapy (1 hr intravenous infusion; AUC dose of 4.8±1.1 mg ml^{-1} min^{-1}) were determined before, during and after treatment using a high performance liquid chromatography method.
Results: Before carboplatin chemotherapy, the plasma concentration (mean±SD) of LC and ALC was 47.8±10.9 and 7.0±1.0 nmol/ml, respectively, and remained constant for the entire period of plasma collection. In contrast, the urinary excretion of LC and ALC, increased significantly during the chemotherapy from 115±105 to 480±348 μmoles/day ($p < 0.01$; One-way repeated measures ANOVA) and from 41±41 to 89±52 μmoles/day ($p < 0.05$) for LC and ALC, respectively. It normalised six days after the end of chemotherapy. Similarly, the renal clearance of LC and ALC increased substantially during the chemotherapy from 1.67±1.43 to 9.05±9.52 ml/min ($p < 0.05$) and from 4.02±4.51 to 7.97±5.05 ml/min (p = not significant) for LC and ALC, respectively, reaching normal values six days after the end of chemotherapy. However, plasma concentration and urinary excretion of glucose, phosphate and nitrogen and the creatinine clearance were not affect by carboplatin treatment indicating no impaired function of the kidney.
Conclusions: Treatment with carboplatin was associated with a marked urinary loss of LC and ALC, most likely due to inhibition of LC (and ALC) reabsorption in the kidney.

1464 POSTER

Enhancement of gemcitabine antitumor effects by pretreatment of S-1, a novel oral derivative of 5-fluorouracil, in pancreatic cancer

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Background: Although Gemcitabine (GEM) has been accepted as a key drug for treatment of pancreatic cancer patients, its efficacy as a single agent remains to be unsatisfied. On the other hand, S-1 is a novel oral derivative of 5-FU prodrug tegafur combined with two modulators, 5-chloro-2, 4-dihydropridine and potassium oxonate, and S-1 has the promising antitumor effect against various malignant tumors including pancreatic cancer. It was recently reported that cellular uptake of GEM was mediated by the equilibrative nucleoside transporter 1 (ENT1) and ENT1 expression was enhanced by TS inhibitor such as 5-fluorouracil (5-FU). In this study, to enhance the effect of GEM by S-1, we evaluated the antitumor effects and treatment schedules of combination therapy with GEM and S-1 in *in vivo* pancreatic cancer model.
Material and Methods: Expression of ENT1 was determined by quantitative RT-PCR. GEM cellular uptake was determined using [3H] GEM. Seven pancreatic cancer cell lines (AsPC1, BxPC3, MiaPaCa-2, PSN1, Panc1, PCI6, and KMP4) were treated *in vitro* with 5-FU either before or following exposure to GEM. Growth inhibitory effects *in vitro* were determined by MTT assay. Human pancreatic cancer xenografts (BALB/c nu/nu mice) were prepared with subcutaneous injection of MiaPaCa-2 cells, and divided into the following six groups (5 mice /group): no treatment; weekly intraperitoneal injections of GEM (240 mg/kg); daily oral administrations of S-1 (10 mg/kg/day) for 5 consecutive days a week; sequential combination treatment with GEM prior to S-1; coadministrations of GEM and S-1; sequential combination treatment with S-1 prior to GEM. The antitumor effects were evaluated with the tumor volume at the day 18.
Results: Significant increases in ENT1 expression and GEM cellular uptake were observed after 5-FU treatment *in vitro* and S-1 treatment *in vivo*. The *in vitro* growth inhibitory effect was significantly greater in the sequential treatment of 5-FU followed by GEM in all cell lines except for Panc1. Furthermore, the significant tumor growth inhibition *in vivo* was observed in the mice treated with S-1 followed by GEM compared with either untreated mice or the mice treated with gemcitabine followed by S-1.
Conclusions: The administration of gemcitabine followed by S-1 provides greater inhibitory effects than the other GEM/ S-1 schedules. These data suggest new effective combination treatment for patients with pancreatic cancer.

1465 POSTER

A heparan-mimetic protects mice from radiation-induced mucositis

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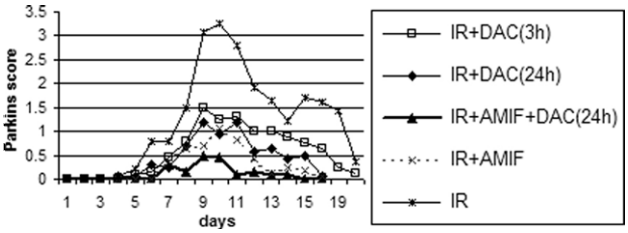
Background: The purpose was to evaluate the effect of RGTA-DAC (ReGeneraTing Agent), a synthetic heparan-mimetic, on radiation-induced mucositis in a murine model and on tumor growth *in vivo* and *in vitro*.
Material and methods: Reagents: RGTA-DAC 1mg/kg for intra-peritoneal (IP) injection; 10 μg/ml in spray solution; 10 μg/ml for *in vitro* assay. Amifostine (Ethyol®) 200 mg/kg for IP injection.
Radiation-induced mucositis: the oral region of C57 black female mice was selectively irradiated with a single dose of 16.5 Gy; mucosal reactions were evaluated daily for 21 days and scored by the Parkins scoring system (Parkins et al, 1983).
Tumor growth: 3×10^6 HEP2 cells (human pharynx cancer cell line) were implanted subcutaneously into nude Balb/c mice. Animals were irradiated with a single dose of 15 Gy selectively to the tumor when tumor diameter reached a mean of 5 mm. The tumor volume was evaluated for 21 days by the formula: length × $width^2/2$ and tumoral response was estimated versus initial volume.
Clonogenic survival: survival fraction of HEP2 cells was evaluated using the Park model; cells were exposed to irradiation (IR) at 0, 2, 4, 6 Gy and to RGTA-DAC 2 h after IR.
Results: Radiation-induced mucositis scored after IR plus RGTA-DAC spray followed by IP injections 3 h after, at day 1 and every 3 days, was significantly lower than after IR alone ($p < 0.001$). A marked decrease of severity and duration of mucositis was observed with administration of RGTA-DAC 3 h after IR ($p = 0.0006$) and 24 h after IR ($p = 0.001$) compared to IR alone. Association of amifostine 10 min before IR with RGTA-DAC 24 h after IR evidenced a major and better protection than RGTA-DAC 3 h or 24 h after IR ($p = 0.002$; $p = 0.001$) and than amifostine 10 min before IR ($p = 0.005$).
Tumor growth: administration of RGTA-DAC IP 3h after IR, then at day 1 and every 3 days didn't evidence significant interference on tumor growth associated (p = NS) or not (p = NS) to IR.
Clonogenic survival: the assay of radio-sensitivity *in vitro* didn't show a significative activity of RGTA-DAC on HEP2 cells clonogenicity (p = NS).



Mucositis

Conclusions: RGTA-DAC demonstrated a protective activity in radiation-induced mucositis in mice, without interference on tumor growth; RGTA-DAC associated with amifostine gave an almost total protection. Further investigations are neeed to understand the selective protective activity on healthy tissues.

1466 POSTER

EGFR and C-KIT/CD117 gene mutational screening and oncoprotein expression in patients with cancer of unknown primary: Implications for molecular pathophysiology and therapy

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Background: Cancer of unknown primary site (CUP) is a heterogeneous group of malignancies that often follow an aggressive clinical course. In order to elucidate its biology, we studied the expression of two transmembrane receptor genes with tyrosine kinase activity, C-KIT/CD117