strated at least additivity in vivo. Based on these data, we conduct a study of capecitabine combined with CPT-11 in patients (pts) with GI tumors. The aim of this study was to define the dose-limiting toxicities (DLTs) of the combination, the doses of CPT11 and Capecitabine for further phase II studies and the pharmacokinetic behavior of both drugs.

CPT-11 was administered as a 90 minute intravenous infusion at doses of 200 to 350 mg/m* on day 1 every 3 weeks, followed by Capecitabine 700 to 1250 mg/m* twice daily for 14 days followed by a one week rest period.

Seven dose levels are planned (see table). No intra-patient dose escalation was allowed. We defined DLTs as toxicities occurring during the first two cycles.

So far, 18 pts with GI malignancies have been included. 15 patients were evaluable for safety with diarrhea as the main side effect. One patient (out of seven) at level 4 (250, 1000) experienced a DLT. Other toxicities were mild: nausea (grade 1/2) in 10 patients, neutropenia (grade 1/2) in 7 patients, hand-foot syndrome (grade 1/2) in 2 patients. The study is currently ongoing at level 5.

Conclusions: These preliminary data suggest that oral capecitabine and CPT-11 present a favorable toxicity profile and can be combined in a three-weekly regimen. An update of toxicity profile, dose escalation, pharmacokinetic data and efficacy will be presented at the congress.

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Phase I study of ZD0473 and liposomal doxorubicin in advanced refractory solid tumor malignancies

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The prognosis for patients with advanced solid tumors remains poor. This has been the major drive in the search of new modalities of therapy. ZD0473 is a new generation platinum drug that appears to differ from cisplatin in its specificity toward DNA. In addition, preclinical studies show it circumvents cellular changes in drug uptake and retention, DNA repair, and glutathione uptake associated with acquired platinum resistance. Liposomal doxorubicin is a new formulation of doxorubicin which confers extended pharmacokinetics and differing toxicity profile compared with the intravenous formulation. We conducted a phase I trial of l-doxorubicin followed by ZD0473 administered once every 4 weeks in patients with advanced solid tumor malignancies. The objective of the study was to determine the recommended doses and toxicity profile of ZD0473 in combination with l-doxorubicin. Dose-limiting toxicity was defined as one of the following: febrile neutropenia, Grade 4 hematologic toxicity, or ≥ Grade 3 non-hematologic toxicity excluding alopecia. To date, nine patients with advanced solid tumor malignancies have been enrolled on 3 dose levels: l-doxorubicin (mg/m2)/ZD0473 (mg/m2) 20/100, 30/100, 40/100. The malignancies represented are ovarian (4), bladder (2), melanoma (1), head and neck (1), and lymphoepithelioma (1). The median number of treatments prior to enrollment was 1.6 (range, 0-3). Two patients underwent definitive radiation therapy. Of the evaluable patients, there has been no DLT reported to date. One mixed response in a patient with lymphoepithelioma was noted. Two patients have had stabilization of disease with one ovarian carcinoma patient normalizing her CA-125. We are actively accruing patients to this trial. Updated data will be presented on our cohort.

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Phase I trial of ZD0473 in combination with vinorelbine for patients with advanced cancer

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Aims: ZD0473 is a new generation platinum drug designed to have an extended spectrum of antitumour activity and overcome platinum resistance mechanisms. Single-agent Phase I evaluation of ZD0473 has demonstrated a manageable safety profile. This abstract outlines the interim results of a Phase I open-label, dose-escalation trial, which was designed to assess maximum tolerated doses of ZD0473 and vinorelbine when used in combination, in patients with advanced cancers.

Methods: Each patient received 15 mg/m2 vinorelbine as a 6- to 10-min iv infusion on days 1 and 8, followed 30 min later by either a 60 mg/m2 or 90 mg/m2 1-h iv infusion of ZD0473, on day 1 only; this cycle was repeated every 21 days. Six dose levels of the combination are planned, with the doses of ZD0473 ranging from 60 to 120 mg/m2 (day 1), and the doses of vinorelbine ranging from 15 to 30 mg/m2 (day 1 and day 8).

Results: To date, six patients (M:F, 3:3; median age 57 years [range 51-75]) have been recruited into the study. Patients had a range of tumour types: non-small cell lung (1 patient), colorectal (1), prostate (1), carcinomatosis (1), hepatocellular carcinoma (1) and neoplasm of the bladder (1). Five patients had received prior chemotherapy/immunotherapy or hormonal therapy with radiotherapy or surgery, including four who had received previous platinum drugs. One patient had undergone only prior surgery. Three patients received a dose of 60/15 mg/m2 (ZD0473/vinorelbine) and three received 90/15 mg/m2, with one patient increasing from 60/15 mg/m2 to 90/15 mg/m2 after 3 cycles. The median number of 60/15 mg/m2 and 90/15 mg/m2 cycles received were 2 (range 2-3) and 1.5 (range 1-2), respectively. Patients did not require dose reductions or delays and, so far, no dose-limiting toxicity has been observed. Haematological toxicities rated as grade 3/4 were neutropenia (2) and thrombocytopenia (1). There were no grade 3/4 haematological toxicities in patients receiving a dose of 60/15 mg/m2. Non-haematological toxicity was mild to moderate and included nausea and vomiting, which was easily controlled. No drug-related deaths occurred and no adverse events led to withdrawal.

Conclusion: The combination of ZD0473 and vinorelbine in this schedule is well tolerated and no dose-limiting toxicity was observed. Patients are currently being treated at dose level 3 (120/15 mg/m2 [ZD0473/vinorelbine]). Further results are awaited and will be presented at this meeting.

Preclinical drug development

275 POSTER

In vitro methods for the validation of pet tracers for oncology

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Purpose: New chemical entities are labelled with positron emitting radionuclides, with the purpose of being used in PET examinations. Such studies aim at defining drug pharmacokinetics, drug interaction, improved diagnosis or characterisation of tumor biology.

Before application into man, a preclinical assessment is needed to exclude candidates with limited chance of success in vivo.

Methods: Tumour cell culture, preferably as multicellular aggregates is used for studies of drug interaction and secondary physiology to screen among surrogate PET tracers. Frozen section autoradiography helps in defining a tracers binding characteristics and to screen among cancer types for the expression of a specific target. Small animal tumour models are used for drug distribution, target validation and assessment of surrogate marker PET methods. Animal PET camera makes animal experiments more efficient.

Results: The development of PET methods via preclinical assessment are illustrated with the development of a specific imaging tracer for adrenocortical cancer: 11C-metomidate, a surrogate PET method for the assessment of effect of a farnesyl-transferase inhibitor, a labelled drug for pharmacokinetic studies: 11C-alpha-amino-buturic acid, the development of a method for the assessment of antiproliferative effects, using 76Br-bromo-fluoro-deoxyuridine, and attempts to develop labelled anti-sense oligonuclides for the recording of gene expression.

Conclusion: In vitro methods are essential for the development of new PET tracers and allow rejection of candidates or new routes of development. These methods are additionally easy to use and give possibilities for oncology researchers to probe the PET methodology under cheaper and easier conditions.

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Differencial effects of choline kinase inhibitors in tumoral and primary human cells

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Purpose: Lipid metabolic pathways are frequently altered during carcinogenesis. Some of them play an important role in mitogenic signalling such as diacylglycerol and phosphoinositoides. Phosphoscholine (PCho) is generated by choline kinase (ChoK) after mitogenic stimulation by growth factors, and it is found elevated in human tumors. We have investigated the requirement of PCho in the regulation of cell cycle progression and the

effects of inhibition of its production by direct inhibition of ChoK in normal and tumoral proliferation. Methods: Cell culture assays were performed in 4 non solid tumoral cell lines, K562 (Human erythroleukemia), IM-9 (Human multiple myeloma), Jurkat (Human lymphoma), U-937 (Human histocytic lymphoma), in comparison with a non solid primary cell line (Human lymphocytes). On the other hand, we have compared 2 solid tumoral cell lines, HT-29 (Human adenocarcinoma of colon grade II) and Hela (Human Epitheloid cervix carcinoma) in contrast to 2 solid primary cell lines, CCD986 sk (Human skin fibroblast) and IMR-90 (Human lung fibroblast). We have also compared NIH with LP8-3 (NIH transformed by H-ras). Flow-cytometry (apoptosis and cycle analysis), radiolabeling (synthesis of DNA, RNA and lipids metabolites), crystal violet (sensibility and recovery from treatment) and westernblotting (expression and phosphorylation analysis) were used. ChoK inhibitors developed by our group were used to inhibit PCho production. Results: A differential effect by ChoK inhibitors among non tumoral and tumoral cell lines is reported. All tumoral cell lines tested were very sensitive to the antiproliferative effect of these drugs, and were promoted to apoptosis. By contrast, under similar conditions, non tumoral cell lines were arrested but recovered normal proliferation rates after withdrawal of the drug. These results imply the absence of an unespecific toxic effect derived from ChoK inhibition that is corroborated by the observed bypass of arrest when the cultured medium is saturated with growth factors. Furthermore, no alteration in mitogenic signalling pathways like MAPK, PI-3K or lipid stress was observed. Conclusions: Phosphocholine production is required for normal cellular progression in normal human cells. Inhibition of PCho production may be a new element for the development of a strategy against abnormal cell proliferation of human tumors.

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Significant experimental decrease of the hepatocarcinoma (HPC) incidence in C3H/SY mice after administration of EB1089, a vitamin D analogue

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EB1089, a Vitamin D analogue, without the acute side effects of the original Vitamin, exerts strong antiproliferative activities in malignant cells, including hepatocytes, in vitro and in experimental HPCs in animals, as well. It also induces cell cycle arrest and apoptosis, a fact suggesting its application in chemopreventive trials.

We examined the possible chemopreventive effect of EB1089 on the incidence of HPCs in C3H/Sy virgin female mice, a strain developing 58% incidence of spontaneous HPCs. A total of 95 mice, 4 months old, were used. EB1089 injections of 0.5 g/ml/kg of BW were given i.p. every other day for 2, 4 and 6 months to 18, 19 and 14 mice respectively. The rest 44 mice were divided into three control groups accordingly and injected with the vehicle solution. The mice which developed disease were sacrificed just before they died. The rest of the mice were sacrificed at the age of 18th months. A full autopsy was performed and liver tissue was processed for histological examination. The results obtained are shown

Treatment Period (months)	Experimental groups		Control groups	
	HPC mice/Total mice	%	HPC mice/Total mice	%
2	2/18	11.1	7/18	38.9
4	0/19	0	7/18	38.9
6	0/14	0	2/8	12.5
Total	2/51	3.9*	16/44	36.4*

*P<0.0001 vs. control for all groups

Our results show that the chemopreventive administration of EB1089 causes a very statistically significant inhibitory effect on the incidence of hepatocellular carcinomas on C3H/Sy. These data suggest a potential application of EB1089 in the chemopreventive control of hepatocarcinomas.

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Inhibition of growth of human breast cancer cell lines with the combination of zoledronic acid and a COX-2 inhibitor

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Purpose: Cyclo-oxygenase (COX) is prostaglandin H synthase which is the principal enzyme mediating the formation of prostanoids (a collective term for prostacyclins, prostaglandins and thromboxanes). COX-2 is up-regulated in a high percentage of common human cancers and is associated with invasive and metastatic tumor behavior. COX-2 inhibitors suppress colon cancer growth in vitro by inducing apoptosis. Zoledronic acid, a new generation bisphosphonate used in the treatment of breast cancer-induced bone disease, significantly reduces cell number and induces apoptosis in human breast cancer cells. The purpose of this study was to assess the effect of combining a COX-2 inhibitor with zoledronic acid on breast cancer cell growth.

Methods: The effect of combining the COX-2 inhibitor (SC236) and zoledronic acid compared to either agent alone was tested in a HER-2/neu transfected human breast cancer cell line (MCF/18) and the control vector transfected line (MCF/neo). Cell number was determined after a 3 day incubation using the MTT tetrazolium dye assay.

Results: Treatment of the HER-2/neu transfected MCF/18 and control MCF/neo cell lines with the SC236 COX-2 inhibitor (1-10 uM) resulted in dose-dependent growth inhibition (15-41% inhibition and 18-53% inhibition, respectively). Treatment with zoledronic acid (1-10 uM) also gave dose-dependent growth inhibition. The HER-2/neu overexpressing MCF/18 cells, however, were less sensitive to zoledronic acid (11-56% inhibition) than the MCF/neo cells (16-70% inhibition). The combination of zoledronic acid (5 uM) and SC236 (5 uM) appeared to have an enhanced inhibitory effect on the MCF/neo cells and a synergistic effect on the MCF/18 cells.

Conclusion: The bisphosphonate, zoledronic acid, gave dose-dependent growth inhibition in both a HER-2/neu transfected human breast cancer cell line (MCF/18) and a control vector transfected line (MCF/neo). The MCF/18 line, however, was less sensitive to zoledronic acid. The combination of zoledronic acid with the SC236 COX-2 inhibitor gave an enhanced inhibitory effect on the control MCF/neo breast cancer cells and a synergistic effect on the HER-2/neu transfected MCF/18 cells compared to either agent alone.

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Basic HGF-like peptides have anti-angiogenic and anti-metastatic effects

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Purpose: The majority of cytokines, responsible for auto- or paracrine regulation of normal and transformed cells are characterized by heparin-binding properties. We have postulated, that the peptide domain(s) of the heparin-binding cytokine(s) might have biological activity which theoretically could be exploited for modulation of the biological behavior of cancer cells. Furthermore, the major angiogenic factors are also heparin-binding proteins.

Methods: We have used HGF as model heparin-binding cytokine and synthesized two HGF b-chain domains, HHRGK (HGP1) and RYRNKH (HGP2) as well as four scrambled variants. As target cells, we have used three cancer cell lines (HT25 human colonic carcinoma, M1/9 human melanoma and 3LL-HH murine lung carcinoma), all characterized by high liver metastatic potentials, as well as normal (HBE) and transformed (KS-IMM) human endothelial cells. For liver metastasis assay, we used SCID mice and intraspenic injection of tumor cells while chicken CAM assay served as angiogenesis model.

Results: All the basic penta- or hexapeptides exhibited similar antiproliferative effects in vitro on cancer cells in a dose range of 0.1-1 mg/ml. None of the HGP peptide exhibited significant antitumoral effect on the primary tumors in form of systemic treatment but HGP1, but not HGP2, had inhibitory effect on liver metastatisation of all the tumor lines studied. Furthermore, one out of the four scrambled hexapeptides, BP4 (KRKRKR), had similar activity. Interestingly, HGP1,2 and BP4 all inhibited the growth of normal human endothelial cells in vitro and angiogenesis in vivo in the chicken CAM assay. Local treatment of HT25 human colon carcinoma in SCID mice with HGP1 resulted in significant inhibition of tumor growth and maturation of intratumoral vessels.