

included colorectal cancer (7), soft tissue sarcoma (3), neuroendocrine tumor (1), urothelial (1) and pancreatic cancer (2). Median no. of cycles (i.e. 3 weeks) was 2 (1–8). Based on previous experience the starting dose was 6 µg/kg. DLTs were observed in 2 pts at the starting dose and consisted of an increase in AST and alkaline phosphatase in 1 pt, and elevation of gamma-GT and ALT, hypokalemia and fatigue in 1 pt. The protocol was amended and re-opened at 4 µg/kg for another 3 pts. No DLTs were observed with this dose. 5 µg/kg were tolerated well in 6 of 8 pts. 2 patients, previously exposed to mistletoe extracts, had allergic reactions on day 1 of cycle 1 and were replaced. At doses below 6 µg/kg increases of gamma-GT >50 U/l > baseline were observed in 2 pts (already gr 3 at baseline). The only other toxicity > gr 1 in >1 pt was pollakisuria (4 pts). Best response (RECIST) was stable disease in 4 pts for 4–7 cycles. PK analysis revealed plasma levels in the theoretically active range (>2 ng/ml) during the 24 h infusion.

Conclusions: The prolonged i.v. administration of aviscumine was well tolerated and feasible. The recommended dose for further clinical trials is 5 µg/kg weekly for 24 h.

210 POSTER Induction of apoptosis in chronic lymphocytic leukaemia by inhibition of NF-κB and novel sulfasalazine analogues

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Chronic lymphocytic leukaemia (CLL) is caused by the relentless accumulation of B-cells through a failure to undergo apoptosis. However, the factors controlling cell survival in CLL are poorly defined. NF-κB is a transcription factor which plays a critical role in controlling cell survival in B-cells. Here we have used molecular and chemical biology approaches to investigate the role of NF-κB in CLL.

Using electrophoretic mobility shift assays, we confirmed that CLL cells contained NF-κB and demonstrated that 66% (n=18) of CLL samples underwent accelerated apoptosis following treatment with various small molecule inhibitors of NF-κB, including sulfasalazine, a drug used in the treatment of inflammatory diseases. Surprisingly, the expression of several "classical" NF-κB target genes was unaltered in cells treated with inhibitors and we used microarray analysis to identify novel candidate NF-κB target genes in CLL, including cytokines and genes involved in apoptosis control and regulation of NF-κB.

To further investigate the role of NF-κB in CLL, we have generated a series of novel derivatives of sulfasalazine. We have identified a number of compounds with significantly improved (up to 8-fold) ability to interfere with NF-κB activity, and demonstrated that these are more effective at inducing cell death in CLL cells. The compounds were also more effective at killing multiple myeloma cells, which are also dependant on NF-κB for survival. Therefore, NF-κB is required for cell survival in the majority of CLL, but the gene targets of NF-κB in CLL may be novel. NF-κB inhibitors, including newer derivatives of sulfasalazine, may be attractive therapeutic agents for CLL.

211 POSTER BRCA1 functions as a differential modulator of chemotherapy induced apoptosis

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The BRCA1 tumour suppressor gene is mutated in 5–10% of familial breast cancers and is down regulated in one third of sporadic breast cancers. We have data to suggest that BRCA1 acts as a differential transcriptional regulator of pro and anti apoptotic signalling pathways depending on the nature of cellular insult. Wildtype BRCA1 expression resulted in a 10–100 fold resistance to a range of DNA damaging agents including those that give rise to double strand breaks such as cisplatin, etoposide and bleomycin. In contrast BRCA1 induced a greater than 1000-fold increase in sensitivity to the spindle poisons paclitaxel and vinorelbine. BRCA1 had an anti apoptotic role in response to DNA damaging agents and induced apoptosis in response to spindle poisons.

In this study, three breast cancer cell models were used: the BRCA1 inducible MBR62-bcl2 cell line, the HCC1937 cell line stably reconstituted with a wildtype BRCA1 construct and the BRCA1 siRNA knockout T47D cell line model. Chemotherapy response was quantified using dose response curves, PARP and Caspase 3 apoptotic assays. In addition flow cytometry

demonstrated that BRCA1 mediated a G2/M cell cycle arrest in response to both spindle poisons and DNA damaging agents. In order to further investigate these differential BRCA1 effects in response to chemotherapy, we have carried out microarray expression profiling and present our preliminary data.

We believe that this study may have implications for the management of breast cancer in those who carry the BRCA1 mutation or in those with sporadic breast cancer exhibiting low BRCA1 expression.

212 POSTER Nuclear factor-κB activation in human gastric cancer: its correlation with clinicopathologic features and prognosis

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Background: Although gastric cancer is major cause mortality in Asia, genetic alterations of gastric cancer to understand the behaviors of malignant tumors are largely unknown. High level of basal nuclear factor-κB (NF-κB) activity has been suggested to be related to tumor progression in various cancers. However, little information is available on the biological significance of constitutive NF-κB activation in human gastric cancer. The purpose of this study is to clarify the clinical significance and prognostic value of NF-κB in human gastric cancer.

Material and Methods: With the nuclear staining of RelA as a marker of NF-κB activation, we sought to investigate clinicopathologic significance of NF-κB activation in 290 human gastric carcinomas placed on tissue array slides. In addition, the possible correlation of Akt activation, tumor suppressor gene expression, and Bcl-2 expression with NF-κB activation was analyzed.

Results: Increased nuclear expression of RelA was found in 18% of the tumors. The nuclear expression of RelA was higher in early stage pTNM ($P = 0.019$). We also found that there is a negative correlation between NF-κB activation and lymphatic invasion ($P = 0.034$) or lymph node metastasis ($P = 0.055$). NF-κB activation was positively correlated with overall survival rate of patients with gastric carcinomas. In addition, NF-κB activation was highly correlated with pAkt ($P = 0.047$), p16 ($P = 0.004$), APC (adenomatous polyposis coli; $P < 0.001$), Smad4 ($P = 0.002$), and KAI1 (kangai 1; $P < 0.001$) expression. A combined evaluation of nuclear RelA expression and pAkt expression revealed that the survival rate of patients with either a nuclear RelA-positive and/or pAkt-positive pattern was better than that of patients with a nuclear RelA-negative and pAkt-negative phenotype pattern ($P < 0.0001$).

Conclusions: NF-κB activation, which is frequently observed at the early stage of gastric carcinoma, strongly correlated with Akt activation and a better prognosis. These findings suggest that NF-κB activation is a valuable prognostic parameter in cases of gastric carcinoma.

213 POSTER von Hippel-Lindau (VHL) and p53 dependent cytotoxic effects of the proteasome inhibitor bortezomib (PS) in human renal cancer cells

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Renal carcinoma with a wild type (wt) or mutant (mt) VHL gene differ in their response to clinical treatment. Since VHL is the substrate recognition unit of a multiprotein E3 ubiquitin ligase complex, we determined the cytotoxic effect of PS, an inhibitor of the 26S proteasome, in renal cell carcinoma (RCC) cell lines with either wt VHL (ACHN and RC-13) or mt VHL (RC-26 and RC-28). Following treatment with 0.05–1 µM PS for 30 minutes and re-incubation in drug free medium for 7 days, RC-26 and RC-28 cell lines with mt VHL were significantly ($p < 0.05$) more susceptible to the cytotoxic effects of PS as compared to ACHN and RC-13 cell lines with wt VHL. The increased cytotoxic response of RC-26 cells to PS (0.25–1 µM) correlated with induction of apoptosis (4–19%), which in the non-responsive RC-13 cell line was minimal (0.5–2%). Cell cycle traverse analysis of RCC cells treated with PS revealed that PS led to a >3-fold increase in the accumulation of cells in the G₂ + M phase (40%) in RC-26 cells, but not in RC-13 cells (9%). The increased accumulation of cells in the G₂ + M phase of the cell cycle was correlated with increased expression of the stress-response protein, p21, at 24 h in RC-26 cells. No significant increase in p21 levels was observed in RC-13 cells. Since, the cellular levels of p21 are regulated by the tumor suppressor protein, p53, we next determined whether the apoptotic response of RC-26 cells to PS was mediated via a p53-dependent pathway. For these studies the effect of down regulation of p53 by stable expression of p53 targeted si-RNA on the apoptotic response of PS was examined in RC-13 and RC-26 cells. Although >80% down-regulation of p53 was achieved in both RC-13 and

RC-26 cells, significant ($p < 0.05$) attenuation of the apoptotic response of PS was observed only in RC-26 cells, but not in RC-13 cells expressing reduced p53. The p53-dependent induction of apoptosis by PS in the RC-26 cell line was accompanied by a >90% reduction in the mRNA and protein levels of the anti-apoptotic protein survivin, which is known to be regulated by p53. The results from these studies indicate that renal cell carcinomas with mt VHL are more responsive to the apoptotic and cytotoxic effects of bortezomib compared to those with wt VHL. The cytotoxic effects of PS are mediated via a p53-dependent pathway, likely involving down-regulation of the anti-apoptotic protein, survivin.

214 POSTER Deregulated expression of BARD1 in human tumors; a novel diagnostic and prognostic marker

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Understanding molecular pathways enlightens the way to treatment. We have previously identified the pathway of apoptosis induction by tumor suppressor BARD1, a sensor of genotoxic stress (1). BARD1 can trigger apoptosis by binding and stabilizing p53 (1). An increase of overall p53 protein concentration is an important mechanism of apoptosis induction. The majority of human cancers harbor mutations or deletions in p53, leading to loss of or aberrant expression. Mutations in tumor BARD1 have been found in cases of inherited and spontaneous breast, ovarian and uterine cancers.

DNA repair and apoptosis, both functions of BARD1 (2), are tumor suppressor functions that are often defective in cancer cells. To evaluate the role of BARD1 in tumorigenesis further we determined its expression in ovarian and lung cancers and correlated BARD1 levels with the expression profile of other proteins in the BARD1-apoptosis pathway.

BARD1 expression profile is co-linear with expression of p53, bax, and activated caspase 3, only in a subset of cancers, suggesting a deficiency of BARD1's apoptotic function. While in ovarian cancer, BARD1 expression is inversely correlated with the statistical 5 year survival chance, no correlation of BARD1 expression with either grade or stage can be determined for lung cancer.

Surprisingly our studies show that in both types of cancer the tumor cells express BARD1, but its expression is not associated with apoptotic capacity. The molecular cause of this functional alteration could be posttranslational modification of the protein or alternative splicing.

Our results indicate that BARD1 is a diagnostic, and potential prognostic and prospective marker for ovarian cancer.

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2) Irminger-Finger, I. and W.-C. Leung (2002). BRCA1-dependent and independent functions of BARD1. *International Journal of Biochemistry and Cell Biology*, 34, 582–587.

215 POSTER High quality gene expression microarray data from the EORTC 10994/BIG 00-01 breast cancer study

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Background: The EORTC 10994/BIG 00-01 study is a prospective randomized trial of neoadjuvant chemotherapy comparing anthracyclines with taxanes (FEC vs ET) in patients with either large operable or locally advanced/inflammatory breast cancer. The goal of the study is to predict the response to treatment using microarray data and to show an interaction between p53 status and treatment. Supervised analysis for these variables will not be reported at this meeting.

Material and Methods: Currently 920 patients out of a projected 1400 have been enrolled. Tumour samples are snap frozen before randomization.

Frozen sections are taken for histology and samples are excluded if there is less than 20% tumour. RNA is extracted from 4 × 25 µm sections of the biopsy. The Agilent Bioanalyzer is used to assess the quality and the yield of RNA. p53 status is tested by yeast functional assay. So far, 49 tumours have been tested on Affymetrix U133A arrays, all of which gave high quality array data, with no evidence of technical bias caused by differences in centre or RNA quality.

Results: Hierarchical clustering shows that biopsies from the same patient cluster together. There is a near perfect correlation between ER status assessed by immunohistochemistry in each institution and ER expression level on the chip. The major split in the tumour dendrogram is between basal and luminal tumours. The first two components in PCA identify three groups, which correspond to basal (33%), luminal (55%) and a third group (12%), which is ER negative but AR positive. GO, KS and histological analysis suggest that this third group contains apocrine tumours, a rare form of breast cancer not previously described in microarray studies. Analysis of other published breast cancer array data indicates that apocrine tumours are not restricted to our data set.

Conclusion: We have demonstrated that it is possible to obtain high quality microarray data from sections of biopsies collected in a phase III multicentre trial. Apocrine tumours are more common by molecular than histological criteria, have a distinctive gene expression profile and may respond better than basal or luminal tumours to treatments targeting the androgen receptor. Acknowledgements: The EORTC 10994/BIG 00-01 study is an intergroup collaboration including the EORTC Breast Cancer Group (EORTC-BCG), Anglo-Celtic Cooperative Oncology Group (ACCOG), Swiss Group (SAKK) and Swedish Breast Cancer Group (SweBCG).

216 POSTER SPC-2004 – a potent locked nucleic acid drug against Bcl-2 in cancer

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In our discovery research programmes we search for new drug candidates for treating malignancies. We have identified short antisense oligonucleotides with Locked Nucleic Acid (LNA) e.g. SPC-2004 as potent inhibitors of Bcl-2. LNA modifies the oligonucleotide backbone by bridging the 2' and 4' carbon in the ribose with an oxymethylene bridge, which locks the conformation of the sugar ring. This modification causes a significant increase in the affinity of LNA modified oligonucleotides towards complementary RNA.

Bcl-2 plays a pivotal role as a key regulator of the apoptosis in many cancers through regulation of the intrinsic (mitochondria-mediated) pathway of apoptosis by regulating the mitochondrial membrane potential and the release of apoptotic factors like cytochrome c. Bcl-2 has also been suggested to play a role in angiogenesis, suppression of tumour cell invasiveness and metastasis¹.

Overexpression or dysregulation of Bcl-2 is associated with tumor cell resistance to a panel of apoptotic stimuli including chemotherapy². Bcl-2 modulation is therefore speculated to be a good method for sensitising the responsiveness to conventional chemotherapy in treatment of a number of malignancies – i.e. non-Hodgkins lymphoma, acute myelogenous leukaemia, chronic lymphocytic leukaemia, multiple myeloma, malignant melanoma, prostate cancer and a number of others^{2,3}.

We have shown in different cellular systems that SPC-2004 is a potent down-regulator of Bcl-2 mRNA and protein, thus leading to increased apoptosis, monitored through caspase3/7 activation. SPC-2004 shows anti-proliferative activity, monitored by MTS assay, and leads to antitumor activity in mouse tumour models at doses that makes it interesting for clinical development. No apparent serious toxicity of SPC-2004 has been observed even when dosed intravenously at up to 300 mg/kg in mice. SPC-2004 is currently undergoing pre-clinical evaluation and is likely to progress into clinical testing against cancer in the nearby future.

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