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.

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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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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 um 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).

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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 angiogenisis, suppression of tumour cell invasiveness and metastasis[1].

Overexpression or dysregulation of Bcl-2 is associated with tumor cell resistance to a panel of apoptotic stimuli including chemotherapy[2]. 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 intravenous 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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