**321** POSTER

**FISH analysis revealed amplifications of genes in both BDII rat model for endometrial adenocarcinomas and human type I endometrial tumors**

E. Samuelson[1], K. Levan[2], G. Horvath[2], G. Levan[3], A. Behboudi[3]. *[1]Cell and Molecular Biology-Genetics, Göteborg University, Göteborg, Sweden; [2]Department of Oncology, Göteborg University, Göteborg, Sweden; [3]Department of Clinical Genetics, Göteborg University, Göteborg, Sweden*

Endometrial cancer is ranked fourth among invasive tumors in women. In Sweden, approximately 1300 women, i.e. 27 per 100,000 women, are diagnosed annually. Gene amplification is an important factor in tumor progression and is often correlated to progressive tumor growth and poor prognosis. Identification and characterization of genes involved in amplification can provide valuable molecular tools for prognosis and therapy of cancer. Investigations in the BDII rat model for hormone-dependent endometrial adenocarcinomas (EAC) led to the identification of several amplified genes on rat chromosome 4 (RNO4) and RNO6. Cdk6 and Met were situated at the peaks of two amplified regions on RNO4 and MycN was the most likely target gene on RNO6. Previous CGH analysis of 13 human type I EAC tumors showed recurrent gains in human chromosome (HSA) 7q21-q31 and 2p21-p25 in this material. These regions are homologous to the above-mentioned amplified segments in the rat model. In the present work, 15 cancer-related genes were selected as candidate targets for gene amplifications; 12 located in the HSA2 segment (RRM2, ODC1, DDX1, MYCN, SDC1, POMC, GCKR, PPP1CB, XDH, CYP1B1, SLC8A1 and PRKCE) and three in the region on HSA7 (CDK6, TAC1 and MET). Gene-specific PAC and BAC clones were obtained and used as probes for FISH hybridization on tissue imprints from 13 frozen human EAC tumors. We found clear evidence of amplification, 11 tumors showed aberrations in either region, and only two tumors showed a normal genotype. All 15 genes showed amplification in at least two tumors; in fact 14 of 15 genes were found to be amplified in 50−75% of the tumors. SDC1 was most frequently showing amplification/gain among those genes located on HSA2p, whereas on HSA7q all three genes showed amplification in 75−88% of the tumors. Our results are an indication that specific findings from genetically defined rodent cancer models, at least in some cases, may give guidance with respect to which genes are involved in molecular changes in the corresponding human tumors.

**322** POSTER

**Molecular determinants of radiation response modulation by TNF-related apoptosis inducing ligand (TRAIL): Role of the pro-apoptotic Bcl-2 protein Bak**

A. Ruebel, P. Marini, R. Handrick, C. Belka, V. Jendrossek. *University of Tübingen, Radiation Oncology, Tübingen, Germany*

**Background:** TRAIL is a promising agent for targeted therapies in combination with ionizing radiation (IR). Own experiments revealed increased efficacy of combined treatment in which the combination effect depended on an intact death-receptor-regulated apoptosis signaling pathway. Aim of the present work was to analyze the importance of the proapoptotic Bcl-2 protein Bak for efficacy of combined treatment (TRAIL+IR).
**Methods:** Efficacy of IR (2.5−10 Gy), TRAIL (2−10 ng/ml) and the combination was tested in a Jurkat T-Lymphoma model (Bax-negative, p53-negative). Cell lines with (Jurkat Bak-positive) and without expression of Bak (Jurkat Bak-negative) were used to define the role of the proapoptotic protein Bak for either treatment. Apoptosis induction and cell cycle distribution were quantified by FACS-analysis (mitochondrial membrane potential, DNA-content). Caspase-activation was tested by Western Blot analysis.
**Results:** IR-induced apoptosis turned out to be clearly dependent on Bak-expression: while 45% of Bak-positive cells underwent apoptosis after 24 h increasing to 70% after 72 h, Bak-negative cells showed only 2% apoptosis after 24 h and 40% after 72 h. In contrast, TRAIL induced comparable rates of apoptosis within 24 h in Bak-positive and Bak-negative cells. At later time points apoptosis levels even further increased in Bak-negative cells. The kinetics of apoptosis induction reflected the kinetics of treatment-induced accumulation of cells in the G2/M phase of cell cycle: after IR Bak-negative cells showed a massively enhanced arrest in G2/M compared to Bak-positive cells. The time dependent increase in apoptosis levels corresponded in both cell lines to the decrease in G2/M. TRAIL itself however had no influence on cell cycle distribution.
In both cell lines IR improved TRAIL efficacy in a time-dependent manner. However, while in Bak-positive cells a clear combination effect could already be noted after 24 h, this outcome was only achieved after 48−72 h in Bak-negative cells. At that time point the initial resistance (24h) towards IR was nearly balanced by the excellent effect of TRAIL.
**Conclusions:** While the proapoptotic effect of IR in Jurkat cells is clearly dependent on Bak, the combination with TRAIL is suited to overcome Bak-related treatment resistance at least at prolonged incubation times. This may be due to the abrogation of the IR-induced arrest of cells in the G2/M phase of cell cycle in the presence of TRAIL and/or the strong efficacy of TRAIL.

**323** POSTER

**Patupilone, the novel microtubule stabilizer (MTS), retains activity against human colon tumour cells over-expressing P-gp in vitro and in vivo; comparison with other MTS**

P. McSheehy[1], M.T. O'Reilly[2], M. Wartmann[2], M. Maira[2], P. Allegrini[3], J. Brueggen[2]. *[1]Novartis Pharma AG, Oncology ResearchWKL-125.2.05, Basel, Switzerland; [2]Novartis Pharma AG, Oncology Research WKL-125, Basel, Switzerland; [3]Novartis Pharma AG, Discovery Technologies WKL-125, Basel, Switzerland*

**Purpose:** Evaluation of the potency in vitro, anti-cancer activity in vivo and pharmacokinetics (PK) of the microtubule stabilizer (MTS) patupilone in nude mice bearing human tumor cells with low and high expression of the P-gp drug efflux pump.
**Experimental design:** The potency in vitro of patupilone and two other MTS, paclitaxel and ixabepilone, was determined using human colon carcinoma cell lines with low (HCT-116, HT29, RKO) and high (HCT-15) P-gp expression, as well as two multi-drug resistance models (MCF7/ADR and KB-8511 cells and their drug-sensitive parental counterparts). Using HCT-15, HCT-116 and HT29 carcinoma cells to establish subcutaneous tumor xenografts in nude mice, the pharmacokinetics (PK) of patupilone was investigated in small and large tumors, and its activity in vivo was compared to that of paclitaxel.
**Results:** Patupilone was highly potent in vitro against the colon carcinoma cell lines (median IC50 of 0.36 nM) and retained activity against HCT-15 cells (IC50 of 0.36 nM) as well as the the two multi-drug-resistant cell line pairs (resistance factor, RF of 0.8−2.4). In contrast, paclitaxel and ixabepilone displayed significantly reduced activity on HCT-15 cells (IC50 of 324 and 110 nM, respectively) as well as markedly increased RFs of 274–1630 and 47–685, respectively, in the two multi-drug resistance models. A single i.v. bolus injection of patupilone (1.5−4 mg/kg) was rapidly distributed from the plasma to all tissues and was slowly eliminated from muscle, liver and small intestine but showed even longer retention in tumor and brain with no apparent elimination over 24 hr. Patupilone showed significant activity against all tumor models, unlike paclitaxel, which only had activity against the low P-gp expressing tumors. In HT-29 tumors, patupilone activity and retention was independent of tumor size, despite the fact that non-invasive dynamic-contrast-enhanced MRI showed that large tumors (500 $mm^3$ versus 100 $mm^3$ for small) had significantly reduced tumor blood volume and blood flow.
**Conclusions:** The high potency of patupilone both in vivo and in vitro which was unaffected by P-gp expression levels, together with a favorable PK profile, suggest that this novel MTS could show significant activity in colorectal cancer and other indications where high P-gp expression may compromise taxane activity.

**324** POSTER

**The transcriptional regulator gene E2 of the human papillomavirus (HPV) 16 influences the radiosensitivity of cervical keratinocytes**

K. Lindel[1], S. Daffinger[1], K.J. Weber[1], J. Debus[1], P. Beard[2], E.M. de Villiers[3]. *[1]University of Heidelberg, Dept. of Radiation Oncology/Radiotherapy, Heidelberg, Germany; [2]Swiss Institute for Experimental Cancer Research (ISREC), Dept. of Basic Research, Epalinges, Switzerland; [3]Deutsches Krebsforschungszentrum, Division of Tumorvirus Characterisation, Heidelberg, Germany*

**Introduction:** Integration HPV into the host genome is a key event in cervical neoplastic progression. Integration is associated with deregulated expression of the viral oncogenes E6 and E7 and a loss of the transcriptional repressor function of the viral gene E2. There is clinical evidence that patients with HPV 16 positive cancer of the uterine cervix with an intact E2 gene have a better prognosis than those with a disrupted E2 gene. This might be due to a better response to radiation treatment. Purpose of this study was to investigate the role of the E2 gene for radiosensitivity of HPV 16 positive cervical keratinocytes using the W12 cell line model.
**Method and Material:** W12 cell line was derived from a low grade cervical lesion by Stanley MA et al. 1989, and is unique among HPV16-containing cell lines in carrying its HPV 16 genome as a multicopy episome. We made use of a pair of isogenic cell lines, W12 and S12 to compare the difference of survival after irradiation. W12 cells contain episomal HPV 16 genomes,