Results: Zoledronic acid but not pamidronate has a cytotoxic potential even at pharmacological dosage. Zoledronic acid does not only induce apoptosis by inhibiting the Ras-pathway but has also an anti-metastatic effect. Freshly prepared γδ T cells consisting mainly of Vδ2 cells showed increased cytotoxicity against bisphosphonate-treated pancreatic carcinoma cells. γδ T cells could be expanded fourfold by use of anti-CD3 and IL-2. However, activated. γδ T cells do not respond to bisphosphonates and kill mainly in a Vδ1 dependent manner.
Results: Our results demonstrate that zoledronic acid has a direct apoptotic effect on pancreatic carcinoma cells and has anti-metastatic properties. Tumor cells treated with zoledronic acid are more susceptible against Vγ9 Vδ2 T cells, the most abundant population of γδ T cells in the peripheral blood. Treatment with zoledronic acid for patients with pancreatic carcinoma might be an option.

doi:10.1016/j.ejcsup.2006.04.109

P50. THE TETRASPANIN D6.1A INDUCES TUMOR ANGIOGENESIS

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Background: Tetraspanins are involved in cell activation, proliferation, adhesion, motility and cell fusion. Some members including D6.1A are known to promote metastasis formation. Overexpression of the tetraspanin D6.1A on a rat pancreatic adenocarcinoma line BSp73AS (BSp73AS-D6.1A) is associated with the formation of haemorrhagic ascites and can induce disseminated intravascular coagulation.
Methods: Angiogenesis was analysed by intravital microscopy of the rat mesentery 6 days after intraperitoneal tumor cell application and after co-culture of the mesentery with tumor cells, supernatant of the tumor cells and tumor cell derived exosomes.
Results: D6.1A expressing tumor cells induced strong angiogenesis with vessels covering roughly 25% of the tumor area as compared to 5% in BSp73AS tumors. Also mesenteric cells displayed strikingly increased branching in co-cultures with BSp73AS-D6.1A cells, supernatant thereof or tumor cell derived exosomes. A D6.1A-specific antibody completely inhibited BSp73AS-D6.1A-, but also BSp73AS-induced angiogenesis in vivo and in vitro. This finding suggested the existence of an additional antibody target that has been identified as proliferating endothelial cells, which strongly upregulate D6.1A expression.
Conclusion: Tumor derived D6.1A is a strong angiogenesis inducer, that indicates for an angiogenic loop due to the striking upregulation of D6.1A on endothelial cells. Because of the latter, the antibody-mediated suppression of the angiogenesis likely offers a very effective and selective drug.

doi:10.1016/j.ejcsup.2006.04.110

P51. DEFINING THE APOPTOTIC PATHWAYS UNDERLYING HISTONE DEACETYLASE INHIBITOR-MEDIATED TUMOR THERAPY

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Background: Histone deacetylase inhibitors (HDACi) are novel anti-tumor compounds currently being tested in clinical trials. Our laboratory has previously shown that in cultured cells HDACi-induced cell death was mediated by mitochrondrial damage, cytochrome C release and Bid cleavage. However, it is presently unclear which apoptotic pathways are utilized by HDACi in vivo, i.e. in a therapeutic setting. Moreover, it is poorly understood how molecular events during anti-cancer drug-mediated apoptosis relate to therapeutic outcome.
Methods: We have employed the murine Eμ-myc B-cell lymphoma model to directly compare HDACi-induced cell death in vitro with therapeutic efficacy in vivo. Our system comprises lymphomas with defined genetic alterations in the apoptotic machinery and the tumors can either be grown and treated in culture or transplated into immunocompetent animals for therapy studies. Using this system, we have identified key apoptotic molecules that not only control sensitivity of cultured lymphoma cells to HDACi, but also determine therapeutic outcome.
Results: Overexpression of Bcl2, previously linked to treatment failure in human cancers, conferred complete chemoresistance in vitro and in vivo. Strikingly, the HDACi SAHA eradicated Eμ-myc lymphomas in a p53-independent manner, resulting in prolonged survival after SAHA treatment of p53−/− lymphomas. Constraining the cellular apoptotic program by genetic targeting of Apaf-1, Caspase-9 and Bid impinged on in vitro sensitivity and we are currently investigating whether this is associated with tumor relapse and chemoresistance while animals are under therapy.

doi:10.1016/j.ejcsup.2006.04.111

P52. ASSOCIATION OF DNA-REPAIR POLYMORPHISMS WITH SURVIVAL IN LUNG CANCER PATIENTS

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Introduction: The X-ray cross-complementing gene *XRCC1* and the excision repair cross-complementing group 2 gene *ERCC2* (*XPD*) are involved in the repair of DNA modifications resulting from DNA-damaging agents used in cancer therapy. Functional

polymorphisms in *XRCC1* and *ERCC2* leading to reduced DNA repair capacity may be of benefit in cancer chemotherapy and may prolong patient's survival.

Methods: Overall survival (OS) was evaluated in 303 Caucasians with primary lung cancer who received anticancer treatment that include first-line chemotherapy using the statistical methods of Kaplan–Meier curves and Cox proportional hazards model with hazard ratios (HRs). DNA isolated from peripheral blood was genotyped for *XRCC1* (*Arg*^{280} *His* and *Arg*^{399}*Gln*) and *ERCC2* (*Asp*^{312}*Asn*) by fluorescence-based melting curve (LightCycler) analysis and for *XRCC1* (*Arg*^{194}*Trp*) and *ERCC2* (*Lys*^{751}*Gln*) by PCR-RFLP.

Results: Among all lung cancer cases, only small cell lung cancer (SCLC) patients carrying the *XRCC1* ^{399}*Arg* allele had a reduced overall survival (HR 2.13, 95%CI 0.92–4.92) compared to the homozygous ^{399}*Gln* genotype. When compared to *XRCC1 Arg*^{280}*His* heterozygotes, ^{280}*Arg* homozygotes showed increased survival being significant only for SCLC patients (HR 0.31, CI 0.14–0.69). A significantly increased HR was found for *ERCC2* ^{751}*Gln* homozygous carriers (HR 3.05, CI 1.20–7.73) for SCLC but not for non-SCLC.

Conclusion: The survival of lung cancer patients receiving chemotherapy seems to be modified by certain repair gene polymorphisms notably in the subgroup of SCLC patients.

doi:10.1016/j.ejcsup.2006.04.112

P53. IN VITRO HISTOCULTURE OF COLORECTAL CARCINOMAS AS A MODEL SYSTEM FOR THE ASSESSMENT OF THERAPEUTIC APPROACHES

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Introduction: A histoculture experimental system of colon cancer was studied to evaluate the reliability and utility of new biological approaches. The three-dimensional growth pattern of the tissue, preserved by this technique is essential for observing the oncogenic properties, differentiated functions and cellular heterogeneity of the tumors. Colon cancer as one of the most common neoplastic diseases worldwide was chosen as a model for remedial testing with viruses with enhanced oncoselective activities, as parvovirus H-1. Paralleled in vitro testing of sensitivity to the oncolytic H-1 parvovirus, using experimental systems of both cell lines and organotipic cultures of colon cancer, will support a better prediction of the in vivo response of patient with this type of cancer.

Methods: Samples from 30 patients with primary untreated colorectal carcinomas were set in histoculture. This technique has been reported as a reliable system, in which tumours can be cultivated with high efficiency by the transfer of human tumour samples to collagen sponges. H-1 virus in the treated tissue samples and the colon cancer cell lines was detected by IHC and RT-PCR. Immunofluorescence was used to characterize the protein patterns of cellular E-cadherin and viral proteins. Tissue growth inhibition was determined by calculating glucose consumption rates.

Results: The analyses revealed significant differences in the response of the five different human colon cancer cell lines to H-1 wt parvovirus infectivity and killing abilities. Increased permissiveness of the colon cancer cells to virus killing was correlated to the lower expression pattern of E-cadherin. Higher virus toxicity was measured in colon cancer cells, over-transformed with SV40 large T-antigen. Growth inhibition assays performed in histocultures showed slight reduction on tissue viability after H-1 wt infection, compared to the stronger effects after 5-FU treatment. RT-PCR and IHC revealed the presence of viral transcripts and viral proteins in the samples, which showed reduced tissue metabolism after infection.

Conclusion: All examined colon cancer cell lines showed a heterogenic response to H-1 parvovirus infectivity. The genetic background of the cell lines showed that cells characterized as aneuploid exert better permissivness to virus infectivity. Straightforward correlation was observed between the E-cadherin expression pattern and sensitivity to virus infection. The weak effects observed after tissue treatment with H-1 wt parvovirus, are due to the intrinsic cellular heterogeneity of the tumor tissues and confirm the higher virus oncotropism, with selective killing of the tumor cells in the original samples, but not their normal counterparts.

doi:10.1016/j.ejcsup.2006.04.113

P54. EXPRESSION ALTERATIONS OF MOLECULAR MARKERS IN DIFFERENT TYPES OF NSCLC AS A SIGNIFICANT PROGNOSTIC FACTOR

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Background: The genetic alterations and expression rates of the molecular markers, associated with prognosis of patients with lung cancer (LC) were investigated. Biomarker expression levels were compared in groups of patients with different histological types and survival rates.

Methods: Tumor samples from 54 patients with NSCLC were analyzed in a retrospective study of paraffin-embedded tissues by immunohistochemistry and in fresh frozen biopsies by RT-PCR. The expression of MMP2, MMP9, b-catenin, E-cadherin, CAV1, Ki67 were evaluated.

Results: Normal membrane-bound E-cadherin expression was absent in 80% of the squamous cell LC, 69.2% adenocarcinomas, 85.7% bronchoalveolar carcinomas The normal membrane bound form of b-catenin was absent almost in all bronchoalveolar carcinomas, but it preserved partly in 40% cases of squamous cell LC and in 53.8% of adenocarcinomas. The MMP2 expression was common for squamous cell and for bronchoalveolar cancer (60–71%), but was absent in SCLC. There was no correlation between the frequency and intensity of expression of adhesion molecules and proliferation index. The expression of caveolin and RALGDS was also compared with clinical parameters. The prognostic significance of each marker and their complexes was analysed.

Conclusions: Abnormalities in adhesion molecules is common for different lung carcinomas. Frequency and intensity of expres-