that higher ER (p = 0.019), lower erbB2 (p = 0.046) and higher EGF receptor (p = 0.033) were associated with CA125 stable/responsive disease.

Conclusion: These results imply that letrozole treatment can produce disease stabilisation and CA125 responses which in turn are linked to higher levels of ER expression. These data suggest the presence of an "endocrine-sensitive" group which could be targeted in future studies.

1021 ORAL

Ovarian cancer: comparison of F-18-FDG-PET imaging technique versus computed tomography scan and serum CA-125 level for diagnosis of recurrent disease

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Purpose: To evaluate the sensitivity of whole body FDG-Positron Emission Tomography study in detecting recurrence of ovarian cancer.

Methods: 18 consecutive stage III and IV ovarian cancer patients (pts) previously treated with surgery and chemotherapy with suspicion of relapse, were evaluated with FDG-PET imaging scan. Recurrence disease was suspected by abnormal CA 125 levels and/or by CT scan. The images corrected by attenuation of thoracic, abdominal and pelvic regions were obtained 45 minutes after the iv injection of 370 MBq of F-18-FDG with an ECAT EXACT HR+ scanner. Ovarian cancer recurrence was confirmed by histopathologic analysis (9 pts) or follow up (9 pts).The sensitivity value of the functional imaging technique has been compared with the CA 125 levels and the CT scans.

Results: The sensitivity for CT scan, CA 125 and F-18-FDG-PET were 44%(8/18 pts), 83%(15/18 pts) and 100%(18/18 pts) respectively. PET has successfully detected recurrent disease in 3 pts with normal CA 125 levels and in 10 pts with non suspicious CT scan. There was significant difference between PET and CT in regard to sensitivity (The p value for the McNemar test was < 0,01).

Conclusion: In this small series of 18 pts with suspicion of relapsed ovarian cancer, PET has proven to have more sensitivity than CT scan in detecting recurrent disease. Updated results with more pts will be presented.

1022 ORAL

In vivo induction of HPV 16 specific cytotoxic ctl and T-helper immunity in patients with advanced cervical cancer using autologous dendritic cells (dc) pulsed with tumour lysate as a potential anti-cancer vaccine

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The feasibility, and safety of inducing specific class 1 and 2 specific CTL response to HPV E6 and E7 antigens using autologous dendritic cells primed with HPV +ve tumour lysate as an anti cancer vaccine has been tested in a phase 1B clinical trial.

Patients and Methods: 9 patients with advanced cervical cancer (8 recurrent with distant metastases) have been vaccinated. Monocyte derived DCs were cultured from 10 9 PBMC obtained at leucophoresis using GMCSF and IL 4 for 7 days. CD1 a + CD14 - immature DC were pulsed with sonicated HPV +ve tumour lysate (5 autologous and 4 allogeneic lysate) and the frozen in aliquots for 6 weekly subcutaneous vaccinations of 107 DC. Immunological endpoints were DTH skin reactions to recall antigens and lysate, tetramer CTL response and ELISPOT CTL and T-helper response in 5 evaluable patients. Tumour response was assessed clinically and radiologically.

Results: Toxicity was mild with occasional fever and malaise but one patient developed a capillary leak syndrome which was successfully treated with steroids. Only 2/9 patients reacted to recall antigens on skin testing. Specific HPV specific CTL response was demonstrated in peripheral blood in 2/3 evaluable (HPV16 + HLA 002*) patients after vaccination. In these patients the frequency of HPV16E7 [11-20] rose to 2.2% as detected by class1 tetramers and the IFN gamma ELISPOT assay -revealed a specific - response to 4 HPV 16 E6 and 7 derived CTL epitopes, 1 week and 2 months respectively after vaccination. In 1/4 evaluable HPV 16 + patients a specific T-helper response was also observed. T cell immunity as detected by ELISPOT correlated with the DTH response to tumour lysate and these patients followed a favourable clinical outcome (NED of disease 18mo + after resection of lung metastasis, stable disease for 3+ mo after progression.

Conclusion It is feasible to induce in vivo HPV specific class 1 and 2 T cell specific response in cervical cancer patients even with advanced disease using autologous DC primed with tumour lysate. However the optimum strategy may require IL 12 producing mature DC which is being currently investigated.

1023 ORAL

Survival after relapse in patients with endometrial cancer: results from a randomized trial

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Purpose: To determine the rates of local control and survival after relapse in patients with stage I endometrial cancer treated in the multicenter randomized PORTEC-trial with surgery and pelvic radiotherapy (RT) or surgery alone.

Materials & Methods: The PORTEC trial included patients with FIGO stages IC grade 1 or 2 and IB grade 2 or 3 endometrial cancer. In all cases an abdominal hysterectomy was performed, without lymphadenectomy. After surgery, patients were randomized to receive pelvic RT (46 Gy), or no further treatment. 715 patients were randomized.

Results: The analysis was done by intention-to-treat. 714 patients could be evaluated. At a median follow-up duration of 60 months, 5-year actuarial locoregional recurrence rates were 4% in the RT group, and 14% in the control group (p<0.001). The 5-year overall survival rates were 81% (RT group) and 85% (control group, p=0.31). The majority of the locoregional relapses were located in the vagina, mostly in the vaginal vault. At 5 years, 7 vaginal and 5 pelvic recurrences were recorded in the RT group, and 32 vaginal and 13 pelvic recurrences in the control group. Five-year rates of vaginal, pelvic and distant failures as first failure were 2%, 1.4% and 6.3% in the RT group, and 9%, 4% and 3.2% in the control group. Five-year rates of distant metastases were 8.4% in the RT group and 6.1% in the control group. Most patients with an isolated locoregional relapse could be treated with curative intent, usually with external RT and brachytherapy, and/or surgery in some. A complete remission was obtained in 85%. At the time of the analysis, only 8 out of the 52 patients with a locoregional relapse had died due to the relapse, while 39 of the 48 patients with distant metastases had died from the metastases. Patients with a vaginal recurrence had 2- and 3-year post-relapse survival rates of 79% and 71%, in contrast to 22% and 9% 2- and 3-year survival rates after pelvic relapse and/or distant metastases (p<0.001). The 3-year survival after first relapse was significantly better for patients in the control group (51%) than for patients in the RT group (19%, p=0.02).

Conclusion: Pelvic RT in stage I endometrial cancer reduces the risk of locoregional relapse, but without a survival benefit. Treatment for vaginal relapse is often successful in patients not previously irradiated, leading to a significantly better post-relapse survival for patients in the control group. Updated results will be presented.

Cell biology/Genetics II

1024 ORAL

A microcell hybrid based approach identifies human chromosome 3p genes that are silenced following tumor growth, at four distinct regions

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Purpose: We had previously shown (Imreh et al., 1994; 1997) that inoculation of human chr3/A9 mouse fibrosarcoma microcell hybrids (MCHs) into SCID mice was followed by the regular elimination of some 3p regions. Using this approach, referred to as the elimination test (Et), we have defined a

common eliminated region (CER) at 3p21.3 and an eliminated region (ER2) at 3p21.1-p14.2 (Kholodnyuk et al., 1997). ER2 borders at but does not include the FHIT gene, considered as a putativeTSG. We have found that FHIT was deleted at the DNA level in 17 of 21 tumors. The remaining 4 of 21 had no FHIT transcript. Later we have generated new SCID tumors that remained PCR-positive for all of the chr3-markers tested ("PCR+" tumors). FISH chromosome painting showed normal intact chr3 in 65-98% of MCHs cells and in 16-75% of 'PCR+' tumor cells. FHIT was expressed in vitro in 5 out of 7 MCH lines. All 'PCR+' tumors had no FHIT transcript. Our compiled data have shown that FHIT was either physically or functionally impaired in all 34 of the 34 analyzed tumors (Kholodnyuk et al., 2000).

The purpose of the present work was to examine the "PCR+" tumors by RT-PCR for the expression of 30 human chr3p genes located within CER1, ER2, and the regions that were homozygously deleted (HD) in a variety of carcinomas.

Results: FISH-RP has indicated losses over 3p26-3p25, 3p24, 3p22, 3p21 and 3p14 in 6 'PCR+'tumors derived from two MCHs, but not in 3 'PCR+' tumors derived from the third MCH. We have examined the expression of 30 human chr3p genes: among them 6 genes located within CER1 (LIMD1, CCR1, CCR2, CCR3, CCR5, LTF), and 12 genes located within regions that were homozygously deleted in a variety of carcinomas. We have found that the majority of the genes analyzed, including VHL, TGFBR2, MLH1, ITGA4L, SEMA4, SEMA5, BLU, LUCA1, PTPRG and DUTT1, were expressed in the MCH lines in vitro and in the derived SCID tumors. No transcripts of the four CCR genes, MYL3 or TGM4 have been detected in any of the MCH lines. Comparative duplex RT-PCR revealed the significant reduction of the amount of GNAI2 (3p21.3) transcript and DLEC1 (3p22) transcript in SCID tumors versus the MCH lines in vitro. In the addition to the FHIT gene, LTF (CER1), DRR1 (ER2) and LUCA2 (SCLC HD) have lost their expression in SCID tumors. These genes may function as tumor suppressor genes. Further studies of the biological activity of these genes are needed to clarify the role of these genes in tumorigenesis.

1025 ORAL

A new variant of cystein-rich FGF receptor (CFR-1) as target for mitotic antibody with possible diagnostic value in gastric cancer

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Purpose: In several human diseases autoantibodies are discussed to play a crucial role in inition and maintenance. In development of gastric carcinoma there is evidence that they are involved in inducing or enhancing proliferative changes of epithelial cells in the stomach mucosa. Here we describe the identification of the receptor of the mitogenic antibody 103/51 as a new variant of CFR-1 (cystein-rich fibroblast growth factor receptor). Expression of the CFR-1 variant was tested by immunochemistry on various tissues and cancerous lesions.

Method: The receptor of antibody 103/51 was purified by chromatographic methods and idenfied by MALDI-analysis. Binding of antibody 103/51 to CFR-1 was proved by transfection of stomach carcinoma cell lines with a CFR-1-antisense vector and the antigenic site to be a carbohydrate residue by specific protein deglycosylation. A murine monoclonal antibody against CFR-1 was prepared, which has identical immunohistochemical and stimulating properties. Expression pattern of CFR-1 was shown by immunohistochemical staining. Physiological in vitro effects were investigated by Western blot analysis and MTT-proliferation assay

Results: The antibody 103/51 enhances profileration of stomach cancer cell lines in vitro by binding to a carbohydrate residue of a CFR-1 isoform. The stimulation is dose-dependent and results in the phosphorylation of various proteins. The expression pattern of the described CFR-1 isoform is very restricted on normal tissues while it is widely expressed on cancerous tissues. Most interestingly, the receptor is also present in Helicobacter pylori gastritis and gastric dysplasia, where it is expressed on proliferating cells, while it is absent on non inflammed stomach mucosa.

Conclusion: Autoantibodies like antibody 103/51 can serve as ligands for receptors and can influence cell cycle control of the epithelial cell, and might be involved in the cancerogenesis of stomach carcinoma. The antibody 103/51 is also of diagnostic value, since the tissue distribution shows that the CFR-1 molecule is correlated with cellular activation and proliferation demonstrated by staining with antibody Ki67. The data show that human antibodies are a helpful tool for understanding of tumor-related mechanisms and for the discovery of new diagnostic targets.

1026 ORAL

Role of the tyrosine kinase lck for the induction of apoptosis in response to ionizing radiation

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Introduction: Preliminary studies revealed that the tyrosine kinase p56/lck is involved in caspase-8 activation and apoptosis in response to ionizing radiation. However, the definite role of tyrosine kinases for apoptosis regulation remains unclear. Our purpose was to define the relative position of p56/lck within the apoptotic signalling pathway.

Materials/Methods: The induction of apoptosis 12, 24, 36, 48 and 72h after irradiation with 10 Gy was quantified in Jurkat T cells, JCaM1.6(without lck), JCaM1.6lck+ and Jurkat/Bcl-2 employing FACS analysis and fluorescence microscopy (Hoe33342). In parallel, apoptosis-induction via the CD95 Death-receptor was determined after 6, 12, 24, 36 and 48 h. Activation of caspase-9, -8, -3 and PARP-cleavage was analysed by western blotting. The integrity of the mitochondrial function (DYm) was determined by TMRE-staining and flow cytometry.

Results: Induction of apoptosis after irradiation was almost completely blocked in p56/lck-negative JCaM1.6 cells, retransfection of lck restored the capacity to undergo apoptosis. The kinetics of CD95-receptor-mediated apoptosis was delayed. There was no difference in the baseline expression of CD95, FADD, caspase-8,caspase-3, Bcl-2, MCL-1 and Bcl-x, as determined by western blotting. Analysis of DYm revealed an delayed breakdown of DYm after CD95-stimulation; after irradiation, no breakdown of DYm occurred. The comparison with Bcl-2-overexpressing cells showed marked differences regarding CD95-mediated apoptosis which remains nearly unaffected by Bcl-2.

Conclusion: The lack of p56/lck causes a generalized apoptosis defect. The absence of lck most probably influences the signal transduction at the level of the mitochondria.

1027 ORAL

Expression, function and clinical implications of the estrogen receptor (ER) beta in human lung cancers

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Purpose: The higher frequency of human lung adenocarcinoma in females than in males, strongly suggests the involvement of gender dependent factors such as sex hormones in the etiology of this disease. Although there have been several reports of expression of ER-alpha in lung cancer, the results are inconsistent and controversial. Here, we assessed the expression of ER-beta in various human lung tissues and address the question of its physiological functions.

Methods & Results: Immunohistochemistry using an ER-beta polyclonal antibody revealed ER-beta protein expression in normal bronchiolar epithelium and all foci of atypical adenomatous hyperplasia (AAH), considered as a pre-cancerous lesion for adenocarcinomas. Adenocarcinomas showed significantly higher expression of ER-beta than squamous cell carcinomas, and especially the hobnail type, which tends to occur in females, was consistently positive. On the other hand, ER-alpha expression was not detected in any of the cases we tested. The functional integrity of ER-beta in lung cancer cells was confirmed using a RERF-LC-OK human lung cancer cell line, in which ER-beta protein expression was the highest of all 16 lung cancer cell lines examined. Binding ability to estrogen responsive elements (ERE) was observed in electrophoretic mobility shift assay. Transcriptional activity was assessed by transient transfection of an ERE-luciferase reporter plasmid, shown to be slightly but significantly stimulated by 17beta-estradiol, and suppressed by a pure antiestrogen, ICI 182,780 (ICI). Colony formation was significantly reduced in the presence of ICI, both anchorage dependent and independent growth.

Conclusion: ER-beta but not ER-alpha is present in lung tissues with an important physiological function in normal lung. Furthermore, ER-beta may play a role in growth and development of adenocarcinoma. Finally, we propose that pure antiestrogen may be useful for hormone therapy of certain types of lung cancer.