

PO301

**Analysis of single nucleotide polymorphisms in the promoter region of ICB-1 gene in women with breast cancer**Konwisorz A.<sup>1</sup>, Springwald A.<sup>1</sup>, Juhasz-Böss I.<sup>1</sup>, Ortmann O.<sup>1</sup>, Treeck O.<sup>1</sup><sup>1</sup>Universität Regensburg, Klinik für Frauenheilkunde und Geburtshilfe, Regensburg

Icb-1 (C1orf38) is a human gene initially described by our group to be involved in *in vitro* differentiation processes of tumor cells derived from gynecological malignancies. Icb-1 transcript levels associated with differentiation status also were shown to be upregulated by 17- $\beta$  estradiol in breast cancer cells. In this study we tested the hypothesis that polymorphisms in the promoter region of icb-1 gene may be associated with increased risk for breast cancer. We investigated two icb-1 promoter region polymorphisms, rs1467465 and rs12048235, for association to breast cancer risk. A total of 156 breast cancer cases and 148 controls were included in the study. DNA isolated from blood samples of these patients was analyzed by means of an allele specific tetra-primer PCR approach. Breast cancer patients more frequently carried the homozygous genotype AA of SNP rs1467465 (17.31 %) than healthy women (10.81 %) suggesting that this genotype confers an increased breast cancer risk (OR 2.079,  $p = 0.041$ ). Allele G of SNP rs1467465 was significantly less frequent in breast cancer patients than in controls and thus is suggested to have a weak protective effect on breast cancer development (OR 1.452,  $p = 0.027$ ). In contrast, no significant association to breast cancer risk was found for SNP rs12048235. In conclusion, our data suggest that single nucleotide polymorphism rs1467465 in the promoter region of icb-1 gene is able to affect breast cancer risk. Whether this association is due to altered expression levels of icb-1 gene resulting from this polymorphism has to be examined in further studies.

PO302

**Selective endothelin-A-receptor antagonist ZD4054 reduces breast cancer cell migration and invasion and exhibits additive effects with aromatase inhibitors and fulvestrant**Smollich M.<sup>1</sup>, Götte M.<sup>1</sup>, Fischgräbe J.<sup>1</sup>, Radke I.<sup>1</sup>, Chen S.<sup>2</sup>, Kiesel L.<sup>1</sup>, Wülfing P.<sup>1</sup><sup>1</sup>Universitätsklinikum Münster, Universitätsfrauenklinik, Münster; <sup>2</sup>Beckman Research Institute, Duarte, CA, USA

**Background:** In breast cancer, the endothelin axis (ET axis) with endothelin-1 (ET-1), endothelin-A-receptor (ET<sub>A</sub>R), and endothelin-converting enzyme-1 (ECE-1) is of major importance for tumour progression and individual prognosis. Overexpression of ET-1, ET<sub>A</sub>R, and ECE-1 is associated with aggressive disease, metastasis and decreased survival. By binding to ET<sub>A</sub>R, ET-1 induces cell proliferation, angiogenesis and anti-apoptosis. Therefore, selective ET<sub>A</sub>R antagonism, alone or in combination with endocrine therapies, represents an innovatively targeted therapy for breast cancer.

**Methods:** We characterised the effects of the selective ET<sub>A</sub>R antagonist ZD4054 on breast cancer cell gene expression, proliferation, migration, and invasion. Changes in gene expression were quantified by real-time PCR. Proliferation was analysed using bromodeoxyuridine and alamarBlue<sup>®</sup> assays. Migration and invasion assays were performed using modified Boyden chambers, which were coated with a Matrigel<sup>®</sup> membrane for the latter assays, to simulate the physiological basement membrane barrier.

**Results:** Investigating the effect on the ET axis expression, we found that ZD4054 significantly reduced ET-1, ET<sub>A</sub>R, and ECE-1 mRNA expression in different breast cancer cell lines (MCF-7, MDA-MB-231, MDA-MB-468) in a concentration-dependent manner. As expected from previous studies, proliferation of breast cancer cells was not affected by ZD4054. However, ZD4054 significantly reduced cellular migration by up to 26.7% (MDA-MB-468;  $P < 0.001$ ) and cellular invasion by up to 46.3% (MCF-7;  $P < 0.001$ ). In aromatase-overexpressing MCF-7aro cells, when either ZD4054 or aromatase inhibitors (anastrozole, letrozole) were administered alone, there were only minimal effects on cellular migration. However, combinations of ZD4054 with

either anastrozole or letrozole significantly reduced cellular migration ( $P < 0.05$ ). In MCF-7 cells, combination of ZD4054 with the estrogen receptor downregulator fulvestrant produced reduction of cellular migration and invasion by 36.0% ( $P = 0.027$ ) and 56.7% ( $P < 0.001$ ), respectively, with effects significantly exceeding those seen with either compound alone.

**Conclusions:** Our data are the first to indicate that selective antagonism of ET<sub>A</sub>R by ZD4054 may represent a promising approach in future breast cancer therapy. Additive anti-invasive effects of ZD4054, when co-administered with aromatase inhibitors or fulvestrant, support a rationale for the clinical use of ZD4054 in combination with endocrine therapies.

PO303

**AKAP12 / gravin – A candidate tumor suppressor gene in breast cancer is inactivated by epigenetic mechanism**Blankenburg M.<sup>1</sup>, Hofmann W.<sup>2</sup>, Scherneck S.<sup>1</sup><sup>1</sup>Max Delbrück Center for Molecular Medicine, Department of Tumor Genetics, Berlin; <sup>2</sup>Berufsverband Deutscher Humangenetiker e.V., Berlin

AKAP12 (A-kinase anchoring protein) is a candidate tumor suppressor gene in breast cancer. This gene, also known as gravin, has been mapped on chromosome 6q24-25.2, a hot spot region of loss of heterozygosity (LOH) in breast cancer.

AKAP12 acts as a scaffold protein that binds protein kinase A (PKA), protein kinase C (PKC) and protein phosphatases, associating reversibly with the  $\beta_2$ -adrenergic receptor.

Significant down regulation of AKAP12 expression in breast tumors was shown by cancer profiling and chip array experiments, northern blot analysis and semi-quantitative RT-PCR. In addition, the AKAP12 expression was investigated by Western Blot experiments and tissue microarray.

To correlate expression of AKAP12 mRNA and LOH at the AKAP12 locus in primary breast tumors we analysed tumor samples in comparison to matched normal genomic DNA by sequencing of different SNPs and by amplification of the internal gene marker D6S476.

To evaluate whether down regulation of AKAP12 expression is due to aberrant methylation we performed 5'-Aza-dC treatment, methylation-specific PCR and bisulfite sequencing.

Based on the expression data we investigated the effect of AKAP12 on breast cancer cell growth by transient and stable transfection of breast cancer cell lines. The tumor suppressor activity was examined in nude mice. Our data showing lowered AKAP12 expression in several breast tumor cell lines and tumor samples as well as its ability to retard cell growth support an important role of AKAP12/gravin in the development of breast cancer.

PO304

**Ki-67 as a prognostic molecular marker in breast cancer**Lux M.P.<sup>1</sup>, Bani M.R.<sup>1</sup>, Schrauder M.G.<sup>1</sup>, Löhberg C.<sup>1</sup>, Beckmann K.<sup>1</sup>, Wiesner F.G.<sup>2</sup>, Beckmann M.W.<sup>1</sup>, Fasching P.A.<sup>1</sup><sup>1</sup>Universitätsklinikum Erlangen, Universitäts-Brustzentrum Franken (UBF), Erlangen; <sup>2</sup>Gynäkologische Praxis, Erlangen

**Introduction:** Prognostic and predictive factors need to be identified in order to establish the indication for adjuvant treatment on an individual basis in patients with breast carcinoma (BC), and to allow adequate weighing up of the benefits and risks involved. Tumor size, estrogen receptor (ER)/progesterone receptor (PR) status, nodal status, tumor grade, and lymphatic and vascular invasion are recognized parameters. A change in the therapeutic approach is taking place, with increasing indications for preoperative chemotherapy. The criteria for the indication are difficult to establish, as only clinical parameters and punch biopsy findings with biological parameters are available. Specific prognostic parameters need to be evaluated. Ki-67 expression is an interesting candidate, as it allows assessment on the basis of a relatively small number of cells from a punch biopsy. However, there is a lack of relevant studies on this topic with sufficiently long follow-up periods.

**Materials and Methods:** In a large group of patients (n = 1564) at the University Breast Center Franconia (UBF), the present study correlated the Ki-67 proliferation rate with the traditional prognostic parameters. The prognostic efficacy of Ki-67 for assessing the disease-free survival (DFS) and overall survival (OS) was investigated on the basis of a long follow-up period.

**Results:** In the N- group, significant differences were observed for all of the characteristics studied ( $P < 0.001$ ): 85.7% of triple-negative, 82.7% of ER-negative, 70.3% of PR-negative, and 74.4% of Her2neu-positive BC showed Ki-67 expression  $\geq 10\%$ . In the N+ group, no significant differences in the tumor stage were observed ( $P = 0.247$ ): 85.7% of the triple-negative, 81.0% of the ER-negative, 53.0% of the PR-negative, and 72.9% of the Her2neu-positive BC showed a proliferation rate of  $\geq 10\%$  ( $P < 0.001$ ). Ki-67 expression was an independent prognostic parameter. In the multivariate analysis, Ki-67  $\geq 10\%$  was significantly associated with reduced DFS (HR 1.798; 95% CI, 1.302 to 2.482;  $P < 0.001$ ) and OS (HR 1.563; 95% CI, 1.010 to 2.418;  $P = 0.045$ ). Using a cut-off value of 10% led to the most reliable results.

**Conclusions:** The study confirms that Ki-67 is a reliable prognostic parameter. It correlates with the traditional parameters and is significantly associated with reduced DFS and OS. Increasing clinical application of Ki-67 as a prognostic parameter in the preoperative treatment situation appears to be useful.

PO305

### Mammographic density in breast cancer patients and polymorphisms in the estrogen pathway

Schrauder M.<sup>1</sup>, Weihbrecht S.<sup>1</sup>, Löhberg C.<sup>1</sup>, Heusinger K.<sup>1</sup>, Bani M.<sup>1</sup>, Strick R.<sup>1</sup>, Strissel P.<sup>1</sup>, Wenkel E.<sup>2</sup>, Magener A.<sup>3</sup>, Hartmann A.<sup>3</sup>, Schulz-Wendtland R.<sup>2</sup>, Beckmann M.<sup>1</sup>, Fasching P.<sup>1</sup>

<sup>1</sup>Universität, Frauenklinik, Erlangen; <sup>2</sup>Universität, Radiologie, Erlangen; <sup>3</sup>Universität, Pathologie, Erlangen

**Introduction:** Breast density is an important parameter in relation to risk factors for breast cancer. It is not only associated with an increased risk of breast cancer, but also directly influences the selection of patient groups for scientific studies, as it influences the sensitivity and specificity of mammography. Hormone replacement therapy has also been reported to influence the risk of breast cancer and mammographic density. Furthermore mammographic density seems to be inheritable. Therefore we wanted to correlate genetic polymorphisms that influence the estrogen pathway with mammographic density in a cohort of breast cancer patients.

**Patients and Methods:** A cohort of consecutive breast cancer cases from the University Breast Center Franken was taken for the study. Patients have been previously asked to take part in the Bavarian Breast Cancer and Control Trial (BBCC). Patients have been diagnosed with breast cancer between 2002 and 2005. A total of 490 patients out of 923 were assessable with both genotype information and mammographic density. Polymorphisms in the genes CYP19, ESR1, and PGR were analyzed with an TaqMan assay. Mammographic density was centrally reviewed according to the American College of Radiology BI-RADS™ atlas. Genotypes were correlated with phenotypes with chi-squared tests.

**Results:** None of the examined polymorphisms showed a strong correlation with the mammographic density. Only the polymorphism rs700519 in the Aromatase Gen CYP19. was associated with the mammographic density with a p-value of 0.046. Genotype frequencies for this polymorphism were for the homozygous wildtype, heterozygous and homozygous variant genotype 93.4%, 6.6% and 0% respectively. The wildtype genotype was more often correlated (54.6%) with a higher mammographic density of 3 or 4 and the heterozygous genotype only in 36.7% of the cases.

**Conclusions:** We have not been able to show strong associations between genotypes of the examined polymorphisms in the Genes CYP19, ESR1 and PGR and mammographic density. These findings are consistent with finding in another cohort, in which CYP19 was analyzed. Although some of the polymorphisms in CYP19 could be correlated with higher levels of circulating estrogen, there seems to be no effect on breast cancer risk or mammographic density.

PO306

### Reassessment of HER2 status in breast cancer patients with initially HER2 negative or HER2 unknown primary tumors at the time of metastatic disease by serum HER2 and HER2 status of circulating tumor cells

Fehm T.<sup>1</sup>, Solomayer E.<sup>1</sup>, Uhr J.<sup>2</sup>, Wallwiener D.<sup>1</sup>

<sup>1</sup>Universität Tübingen, Frauenheilkunde, Tübingen; <sup>2</sup>University of Dallas, Cancer Immunobiology Center, Dallas

**Introduction:** Several studies have indicated that predictive markers e.g. HER2, ER can change during course of disease in breast cancer patients. Therefore, reassessment of this markers after at the time of disease progression might help to optimize treatment decisions. Metastatic tissue may be difficult to obtain for repeated analysis. In this context, evaluation of HER2 status or other predictive markers in circulating tumor cells (CTCs) could be of relevance to optimize treatment decisions. Therefore, the aim of this study was to determine the serum HER2 status and the HER2 status of corresponding CTCs in metastatic breast cancer patients with initially HER2 negative or unknown HER2 status.

**Methods:** Blood samples were obtained from 77 metastatic breast cancer patients with negative (n = 44) or unknown (n = 33) HER2 status. Serum HER2 was determined using the commercial HER-2/neu ELISA-kit (Oncogene Science, MA, USA). CTCs were detected by a slide based assay using immunomagnetic enrichment and characterized by pheno- and genotyping. Alternatively, a commercial kit (Adnagen, Langenhagen, Germany) based on RT-PCR was used for CTC detection and characterization.

**Results:** Twenty of 77 metastatic patients had elevated serum HER 2 levels. Blood samples were analyzed for presence of CTC in 67 patients. 8 of 21 patients with CTCs showed HER2 amplification. 23 of 77 patients were HER2 positive by at least one method. Concordance between HER2 status of circulating tumor cells and serum HER2 was seen in 15 of 21 patients (71%). In 6 patients conflicting results were obtained. 3 patients with elevated serum HER2 status had HER2 negative CTCs whereas three patients with HER2 amplified CTC had normal serum HER2 levels.

**Conclusions:** Our study confirms that a subset of HER2 negative patients develop elevated serum HER2 levels and HER2 positive CTCs associated with metastatic disease. This is clearly of clinical relevance since these patients in current practice do not have access to HER2 targeted therapy. However, non-concordant results were obtained in 29% of patients using both methods. Hence, correlating clinical responses to HER2 targeted therapy based on each method should be further studied to help determine when such treatment should be given.

PO307

### CD 44+/CD 24- cancer stem cell in breast tissue distribution and prevalence

Swiatek J.<sup>1</sup>, Kärner A.<sup>1</sup>, Heil J.<sup>1</sup>, Aulmann S.<sup>2</sup>, Sinn H.<sup>2</sup>, Sohn C.<sup>1</sup>, Gebauer G.<sup>1</sup>

<sup>1</sup>Universitätsfrauenklinik, Gynäkologie, Heidelberg; <sup>2</sup>Universität, Pathologie, Heidelberg

**Introduction:** In breast cancer tumor formations there are different cell types. Some cells are able to induce tumor growth. There is one special cell group found in tumour formations, cancer stem cells, which is assumed to induce tumor proliferation. This assumption is supported by the well-known ability of stem cells to renew and proliferate into different cell types. Different tumor cells are distinguished by different cell surface markers. Cancer stem cells are characterized by the prevalence of CD 44+/CD24-.

The aim of this study was to detect the prevalence of cancer stem cells in different types of breast tissue. These findings will be associated to the clinical outcome and survival.

**Methods:** Tissues of 360 patients (breast cancer n = 260, carcinoma-in-situ n = 10, normal breast n = 97) were included in this study. Tissues were analyzed by micro-tissuearrays with double-staining immunohistochemistry for CD44+/CD24-. The results were evaluated by microscopic inspection.

**Results:** Preliminary results show that more than 90% of all tumor formations contain cells expressing CD 44+/CD24- surface markers. Of all tumor found in the inspected tumor samples, cancer stem cells accounted for less than 10%.

**Conclusion:** The presence of stem cells and their individual surface markers in tumor tissues has already been described by different research groups. Few studies have been published investigating these experimental findings in a clinical setting, all with a smaller number of evaluated case numbers. Further studies will be needed to focus on the association of cancer stem cells, the clinical outcome and survival rate.

PO308

**Early initiation of External Beam Radiotherapy (EBRT) increases the risk of long term toxicity in patients undergoing Intraoperative Radiotherapy (IORT) as a boost for breast cancer**

Blank E.<sup>1</sup>, Welzel G.<sup>1</sup>, Keller A.<sup>1</sup>, Vorodi F.<sup>1</sup>, Herskind C.<sup>1</sup>, Scheda A.<sup>1</sup>, Tomé O.<sup>2</sup>, Sütterlin M.<sup>2</sup>, Kraus-Tiefenbacher U.<sup>1</sup>, Wenz F.<sup>1</sup>

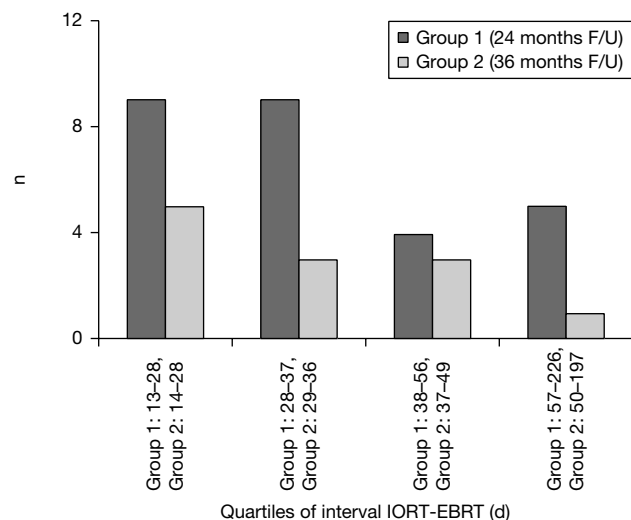
<sup>1</sup>Universitätsklinik Mannheim, Klinik für Strahlentherapie und Radioonkologie, Mannheim; <sup>2</sup>Universitätsklinik Mannheim, Klinik für Geburtshilfe und Frauenheilkunde, Mannheim

**Purpose:** Intraoperative radiotherapy (IORT) for breast cancer is a novel approach, which is increasingly used. When given as a boost during breast conserving surgery, the sequence of IORT, systemic therapy and external beam radiotherapy (EBRT) may play a decisive role as to local tumour control and normal tissue damage. We analyzed the influence of the interval between IORT und EBRT on the development of late toxicity in breast cancer patients receiving IORT as a boost before EBRT to the whole breast.

**Material and Methods:** 91 patients were followed for a median of 24 months (min. 21) and 48 patients were followed for a median of 36 months (min. 30) after IORT and EBRT. 20 Gy IORT were given with 50 kV x-rays (Intrabeam, Carl Zeiss Surgical, Oberkochen, Germany) followed by 46-50 Gy EBRT. Systemic therapy was prescribed according to the St. Gallen consensus. The median interval between IORT and EBRT in the group of patients having a 24 months-follow-up (group 1) was 37 days and in the other group with a 36-months-follow-up (group 2) 36 days. Toxicity was assessed with the modified LENT SOMA score.

**Results:** No higher grade toxicity was seen in 54 patients in group 1 and in 30 patients in group 2. There were 27 patients with fibrosis II-III<sup>o</sup>, 13 with retractions, 8 with pain II<sup>o</sup>, 8 with edema I<sup>o</sup>, 4 patients with teleangiectases, 1 with lymphedema II<sup>o</sup>, and 1 patient with hyperpigmentation IP<sup>o</sup> in group 1. In the second group 12 patients developed fibrosis II-III<sup>o</sup>, 6 had retractions, five had pain II<sup>o</sup>-III<sup>o</sup>, 4 hyperpigmentations, 3 patients showed teleangiectases, and 1 patient edema II<sup>o</sup>. Both in group 1 and group 2 most higher grade fibrosis (group 1: n = 9 (33%); group 2: n = 5 (42%)) were noticed in the first quartil (13-28 days) looking at the interval between IORT and EBRT (figure 1). Most indurations could be seen around the surgical scar. Any degree of induration was scored as fibrosis regardless of the contribution of surgery or radiotherapy. Finally the interval in patients with higher grade toxicity (group 1:

Fig. 1. Higher Grade Fibrosis/LENT SOMA scale



n = 37; group 2: n = 18) was significantly shorter in both groups than in the patients without chronic late effects (group 1: 34d vs. 40d, p = 0,044; group 2: 29,5d vs. 39,5d, p = 0,023; Mann-Whitney-U-Test).

**Conclusion:** Starting EBRT about five weeks after IORT appears safe. In both groups a shorter interval between IORT and EBRT was associated with an increased frequency of chronic late toxicity.

PO309

**Mammographic density and risk for reexcision after breast-conserving surgery for primary breast cancer**

Bani M.<sup>1</sup>, Heusinger K.<sup>1</sup>, Lux M.P.<sup>1</sup>, Wenkel E.<sup>2</sup>, Magener A.<sup>3</sup>, Hartmann A.<sup>3</sup>, Schulz-Wendtland R.<sup>2</sup>, Beckmann M.W.<sup>1</sup>, Fasching P.A.<sup>1</sup>

<sup>1</sup>Universitätsklinikum Erlangen, Universitäts-Brustzentrum Franken, Erlangen; <sup>2</sup>Universitätsklinikum Erlangen, Insitut für diagnostische Radiologie, Erlangen; <sup>3</sup>Universität Erlangen-Nürnberg, Institut für Pathologie, Erlangen

**Introduction:** Aim of this study was to evaluate the impact of mammographic density on the risk for reexcision following breast-conserving surgery. Positive tumor margins are critical in relation to local disease control following surgery for breast cancer. Several factors, including tumor size, multifocality, and the presence of an extensive in-situ component, may be associated with a higher rate of repeat operations due to positive margins.

**Methods:** A total of 565 breast cancer patients were considered eligible for breast-conserving therapy after a core biopsy had confirmed malignancy. The patients' mammographic findings were reviewed, and mammographic density was documented in addition to the histopathological features of the lesions. Possible associations between these factors and the risk for a second operation were analyzed using the chi-squared test, and a model was developed for multivariate analysis.

**Results:** 121 patients (21.4%) had to undergo at least one repeat operation, and mastectomy was ultimately necessary in 54 patients (9.6%). Tumor size, multifocality, and the presence of an in situ component were identified as risk factors. A mammographic density of category 4 was associated with a need for further surgery (OR 3.2; 95% CI, 1.2 to 11).

**Conclusions:** Mammographic density is associated with the risk for a second operation following breast-conserving procedures, and it may compromise the technical feasibility of radiographic and intraoperative localization of the tumor. Using mammographic density to define a group of patients who have a higher risk of reexcision might make it possible for these patients to benefit from more sophisticated methods of localization and margin assessment.

PO310

**Results of the German IPEP study evaluating the tolerability, efficacy, and acceptance of fulvestrant (FASLODEX®) under routine clinical conditions in advanced breast cancer**

Warm M.<sup>1</sup>, Thomas A.<sup>2</sup>, Ziegler K.<sup>1</sup>, Kates R.<sup>3</sup>, Overkamp F.<sup>4</sup>, Harbeck N.<sup>3</sup>

<sup>1</sup>Uniklinik, Brustzentrum, Köln; <sup>2</sup>Charite, Berlin; <sup>3</sup>Technische Universität, München; <sup>4</sup>Onkologische Praxis, Recklinghausen

**Material and Methods:** 848 patients were enrolled in this Fulvestrant In Practice Evaluation Programme (IPEP). Under clinical routine conditions in the participating centers regarding selection of subjects, diagnostic procedures or therapeutic decisions, all relevant data was documented over 9 months of fulvestrant therapy in postmenopausal women with advanced ER+ breast cancer who had relapsed during or after adjuvant anti-estrogen treatment, or with disease progression under palliative anti-estrogen therapy.

**Results:** Data of 597 (70 %) of patients was evaluable according to protocol. Median age was 64 years, 52 % of patients had a co-morbidity, 78 % one or more prior palliative therapies.

**Safety:** 15.6 % had one or more adverse events, mainly hot flushes, gastrointestinal, or musculo-skeletal symptoms, 27 patients had a serious adverse event, 20 died during observation.

**Efficacy:** after 3 months only 11.5 % of patients had progressed; 6 % had a complete remission, 16.5 % partial remission, 58 % stable disease as locally

assessed by the investigators. After 9 months, 15 % of evaluable patients had progressed, 13 % were in complete remission, 28 % in partial remission and 42 % had stable disease, 2% are not available.

**Tolerability:** Tolerability was judged as being good to very good by the majority of both investigators and patients with stable tolerability parameters reported at 3, 6 and 9 months (Patients "very good": 43,5 %, "good": 48 %. Specialists "very good": 48 %, as "good": 46 %).

**Conclusion:** Overall, fulvestrant showed very good efficacy with a reported clinical benefit rate of 83 % after 9 months, safety and tolerability in this observational study in a patient collective with advanced breast cancer.

PO311

### Comparable effectiveness of intravenous and oral ibandronate in metastatic bone pain reduction regardless of previous bisphosphonate treatment

Kühnle H.<sup>1</sup>, Seraphin J.<sup>2</sup>, Meden H.<sup>3</sup>, Ziegler J.<sup>4</sup>

<sup>1</sup>Medizinische Hochschule Hannover, Abteilung Gynäkologische Onkologie, Hannover; <sup>2</sup>Hämatologisch-onkologische Schwerpunktpraxis, Northeim; <sup>3</sup>Diakoniekrankenhaus Rotenburg (Wümme) gGmbH, Frauenklinik, Rotenburg; <sup>4</sup>Roche Pharma, Grenzach

**Background:** In phase III trials, intravenous and oral ibandronate reduced bone pain significantly below baseline for up to 2 years. An ongoing post-marketing surveillance study in Germany is currently assessing the ability of i.v. and oral ibandronate to reduce metastatic bone pain regardless of previous bisphosphonate treatment. An interim analysis is presented (n = 1704).

**Patients and Methods:** Breast cancer patients (age: 63.1 ± 12 years) were treated with i.v. ibandronate 6mg every 4 weeks (89%) or daily oral ibandronate 50mg (11%) for 24 weeks. Furthermore, patients were divided into three sub-groups according to previous treatment: bisphosphonate-naive (n = 1079), previous ibandronate treatment (n = 199), or previous treatment with other bisphosphonates (n = 426). Bone pain was assessed using a visual analog scale (VAS, range: 0 [no pain] to 10 [maximum pain]).

**Results:** 65% of patients experienced an overall improvement in pain scores after ibandronate treatment. The i.v. formulation and the oral formulation were comparably effective (i.v.: 64%, oral: 75%). Pain reduction was greatest in bisphosphonate-naive patients (70% reported improved bone pain scores). Benefit was also reported by patients who had received pre-treatment with either other bisphosphonates (58%) or ibandronate (56%). The mean maximum bone pain reduction in patients with pain relief was 2.3 ± 1.6 VAS in the i.v. group and 2.5 ± 1.6 VAS in the oral group respectively. 93% of the patients of the i.v. group and 95% of the oral group experienced no skeletal-related event during the study.

**Conclusions:** Ibandronate provided pain relief for patients who were bisphosphonate-naive and those who switched from other bisphosphonate therapy. Furthermore, patients who had received ibandronate pre-treatment gained continued benefit from this therapy. The study shows that in clinical practice intravenous as well as oral ibandronate is a valuable treatment for the prevention of skeletal-related events and causes relief of metastatic bone pain.

PO312

### Bisphosphonate-induced osteonecrosis of the jaw: Incidence and risk factors in patients with breast cancer and gynecological malignancies

Gall C.<sup>1</sup>, Bachmann C.<sup>1</sup>, Krämer B.<sup>1</sup>, Solomayer E.<sup>1</sup>, Wallwiener D.<sup>1</sup>, Fehm T.<sup>1</sup>

<sup>1</sup>Universität Tübingen, Frauenklinik, Tübingen

**Background:** Bisphosphonates are potent inhibitors of osteoclast-mediated bone resorption. They are successfully used in conditions of increased bone turnover such as osteoporosis or bone metastases. Since 2003 multiple cases of bisphosphonate-induced osteonecrosis of the jaw (ONJ) were reported. Our purpose was to describe the incidence and risk factors of ONJ in patients with breast cancer or gynecological malignancies.

**Patients and Methods:** ONJ was assessed retrospectively for all patients with breast cancer or gynecological malignancies treated with bisphosphonates at the Department of Gynecology and Obstetrics, University Hospital Tuebingen during April 1999 until May 2006.

**Results:** 10 of 310 (3%) patients with breast cancer or gynecological malignancies developed ONJ while receiving bisphosphonate therapy. All patients with ONJ were treated for bone metastases. Except one all patients with ONJ had a history of recent dental procedures. All patients had received zoledronic acid as part of their bisphosphonate regimen. In 4 of 10 patients this was the only bisphosphonate given. The remaining 6 patients had received at least one of the other bisphosphonates (alendronate, ibandronate, clodronate or pamidronate) before or after zoledronic acid therapy during their course of disease. Time of exposure to bisphosphonates and the number of treatment cycles were significant risk factors for the development of ONJ (p < 0.001). In patients diagnosed with ONJ the mean number of treatment cycles was 27 +/- 18 cycles (median: 21 cycles, range 6-62 cycles) and the mean duration of bisphosphonate therapy was 29 +/- 20 months (median: 22 months, range 1-67 months). In contrast, the mean number of treatment cycles in patients without manifestation of ONJ was 11 +/- 12 cycles (median: 6 cycles, range 1-90 cycles). The mean duration of therapy was 12 months (median: 7 months, range 1-81 months).

**Conclusion:** Osteonecrosis of the jaw is regarded a major side effect of bisphosphonate therapy. Length of exposure to bisphosphonates and the number of treatment cycles seem to be the most important risk factors for the development of ONJ. Recent dental procedures favours the ONJ.

PO313

### Is tumorbio logical risk assessment realistic in node-negative breast cancer? Study progress and quality assurance of the multicenter trial NNBC 3-Europe

Vetter, M.<sup>1</sup>, Geurts-Moespot, A.<sup>2</sup>, Persing, M.<sup>1</sup>, Paepke, D.<sup>3</sup>, Schmidt, M.<sup>4</sup>, Hoffmann, G.<sup>5</sup>, Augustin, D.<sup>6</sup>, Hanf, V.<sup>7</sup>, Meisner, C.<sup>6</sup>, Von Minckwitz, G.<sup>9</sup>, Sweep, F.<sup>2</sup>, Harbeck, N.<sup>3</sup>, Thomssen, C.<sup>1</sup>

<sup>1</sup>Frauenklinik der Martin-Luther-Universität, Halle (Saale); <sup>2</sup>Chem. Endocrinology, Radboud Universiteit, Nijmegen, Netherlands; <sup>3</sup>Frauenklinik der Technischen Universität, München; <sup>4</sup>Frauenklinik der Johannes-Gutenberg-Universität, Mainz; <sup>5</sup>St. Josephs-Hospital, Wiesbaden; <sup>6</sup>Frauenklinik des Klinikums Deggendorf; <sup>7</sup>Frauenklinik des Klinikums, Fürth; <sup>8</sup>MI Eberhard-Karls Universität, Tübingen, and <sup>9</sup>German Breast Group, Neu-Isenburg

**Background:** Various groups started clinical trials aimed at a feasible way to improve risk-assessment by testing biological parameters based on RNA or protein level. Current ASCO guidelines allow to use biological risk assessment by invasion factors uPA/PAI-1 (Harris et al. 2007). We launched the NNBC 3-Europe trial with the following questions:

1. Is risk-assessment by the invasion markers urokinase-type plasminogen activator (uPA) and its inhibitor PAI-1 more effective than by clinico-pathological factors (St. Gallen 2005) with regard to identification of low-risk patients?
2. Is adjuvant chemotherapy using an anthracycline-taxane sequence (FEC-Docetaxel) superior to standard FEC in high-risk patients?

**Study Design:** Risk assessment was performed either by St. Gallen 2005 or by the invasion markers uPA/PAI-1. In low-risk patients, no adjuvant chemotherapy is given. High-risk patients receive adjuvant chemotherapy according to randomisation: FEC-Doc versus FEC. Adjuvant endocrine therapy is given according to current AGO guidelines.

Use of fresh tissue sampling was necessary within this trial. Quality assurance for the testing procedure was prospectively performed and provided by the central QA laboratory (Nijmegen, Netherlands).

**Results:** To date, 103 centres participate, 2123 patients have been registered for that trial. Biological risk assessment was performed in 1630 patients. Using both, grading and uPA/PAI-1 results, the low-risk group comprised 43% of the patients, whilst 57% of the patients still had to receive adjuvant chemotherapy (FEC-Doc, n = 639, or FEC, n = 643).

The median values of the coefficient of variation (CV) between 6 labs were 15% for uPA and also for PAI-1, with a range from 6 to 22% and from 8 to 25% respectively. The median of the within-lab between runs CVs were 10% for uPA and 12% for PAI-1.

**Discussion:** The NNBC-3 Europe trial shows that risk assessment based on biological testing of fresh frozen tumor material is feasible. To date, the patient distribution is as expected. The quality assurance within this trial showed acceptable performance. The study is planned to recruit 5.700 patients and it is performed in cooperation with the EORTC Patho-Biology Group, the

## Cancer Metastasis

### Oral Presentation

OP185

#### Prognostic and diagnostic relevance of s100A4, a newly identified target gene of beta-catenin/TCF signalling, for colon cancer metastasis

Stein U.<sup>1</sup>, Artl F.<sup>1</sup>, Walther W.<sup>1</sup>, Harris E.D.<sup>2</sup>, Smith J.<sup>1</sup>, Fichtner I.<sup>1</sup>, Herrmann P.<sup>3</sup>, Birchmeier W.<sup>1</sup>, Schlag P.M.<sup>3</sup>, Shoemaker R.H.<sup>2</sup>

<sup>1</sup>Max-Delbrück-Center for Molecular Medicine, Berlin; <sup>2</sup>National Cancer Institute, Frederick, MD; <sup>3</sup>Robert-Rössle Cancer Clinic, Berlin

Colorectal cancer is one of the most frequent malignant tumors in Western countries. Although much has been learnt on mutations that control the initiation and progression of colorectal cancer, less is known about molecular events that are crucial in metastasis formation. Activation of the Wnt/beta-catenin pathway is frequently observed in colorectal cancers. Mutations of the beta-catenin gene have been described as early and critical steps in the genesis of the disease, and accumulation of beta-catenin in the nucleus has been associated with late stages of tumor progression. Our aim was to elucidate the impact of gain-of-function beta-catenin on the metastasis-associated gene S100A4 in human colon cancer cell lines and tumors. We identified S100A4 as the most regulated gene by gain-of-function beta-catenin using a 10K microarray. Cell lines with gain-of-function beta-catenin expressed up to 60-fold elevated S100A4 levels, displayed strongly increased cell migration and invasion in vitro, and developed distant metastases in mice. In contrast, knock-down of S100A4 by specific siRNA and by beta-catenin siRNA blocked these biological effects. Furthermore, we identified a TCF binding site within the S100A4 promoter and demonstrated the direct binding of heterodimeric beta-catenin/TCF complexes to the S100A4 promoter. Reporter assays confirmed the beta-catenin-induced S100A4 promoter activity. For human colon carcinomas, S100A4 mRNA expression was increased in primary tumors, which later developed distant metastases, compared to tumors which did not metastasize. Colon tumors heterozygous for gain-of-function beta-catenin showed concomitant nuclear beta-catenin localization, high S100A4 expression and distant metastases formation. Recently we found, that S100A4 transcript levels in blood of colon cancer patients were significantly higher than in healthy volunteers. Patients bearing the primary tumor together with a distant metastasis showed higher transcript levels than patients without metastases. Our results relate two previously unconnected molecular pathways, which play important roles in tumor progression and metastasis: the beta-catenin/TCF signaling pathway and S100A4, that controls motility and invasiveness. Moreover, we demonstrate first the prognostic and the diagnostic value of S100A4 transcripts in patients tumors and blood for colon cancer metastasis.

OP186

#### Sequential resection of liver- and pulmonary metastases of colorectal cancers: Results of 45 patients

Neeff H.<sup>1</sup>, Hoerth W.<sup>2</sup>, Makowiec F.<sup>1</sup>, Hopt U.T.<sup>1</sup>, Passlick B.<sup>2</sup>

<sup>1</sup>Chirurgische Universitätsklinik, Abt. Allgemein- und Viszeralchirurgie, Freiburg; <sup>2</sup>Chirurgische Universitätsklinik, Abt. für Thoraxchirurgie, Freiburg

Multimodal therapies (especially surgery of metastases and aggressive chemotherapy) in patients with metastases of colorectal cancers (CRC) are frequently performed and may even provide healing in selected patients with more than one location of metastases. In the current literature there are only few studies with relatively low patient numbers reporting on the outcome after resection of both, hepatic and pulmonary metastases of CRC. We, therefore, evaluated survival of patients who underwent sequential resection of hepatic and pulmonary metastases under potentially curative intention.

**Methods:** From 1989 until 2006 45 patients (31% female, median age 58 years) with (during the course of the disease) hepatic and pulmonary CRC-metastases

underwent sequential resections at both metastatic sites. In all patients further tumor locations were excluded preoperatively. Metastases occurred synchronous (regarding primary CRC) in 29%. In 82% liver resection was performed prior to pulmonary resection. In all 45 patients the first resection of metastases was performed a median of 16 months after resection of the primary CRC, the median interval to the second resection of metastases was 7 months. The primary CRCs were in 53% rectal and in 47% colon carcinomas (62% nodal positive, all with free resection margins). In both, the first and the second resection of metastases, free margins were achieved in 94%. Survival analysis was performed using the Kaplan-Meier-method.

**Results:** The 5-year survival (5-y SV) after initial diagnosis of CRC was 63%, after the first resection of metastases 43% and 29% after the last resection. Patients with synchronous metastases had a significant poorer survival compared to patients with metachronous metastases (37% 5-y SV after first metastectomy vs. 72% in patients with metachronous disease;  $p < 0.01$ ). The nodal status and the type of the primary CRC did not influence survival so far.

**Conclusions:** In the multimodal management of patients with metastasized CRC a resection of both, hepatic and pulmonary metastases may achieve acceptable survival rates or even healing in selected patients, especially in the presence of biologically less aggressive (= metachronous) disease. Survival rates in these selected patients are comparable to those of patients after isolated hepatic resection reported during the last decade.

OP187

#### Blockade of SDF-1 does not affect metastatic tumor growth due to activation of an alternative CXCR-4 – VEGF-dependent pathway after liver resection

Rupertus K., Scheuer C., Junker B., Tilton B., Schilling M.K., Menger M.D., Kollmar O.

Universität des Saarlandes, Homburg/Saar

**Background:** Previous studies demonstrated that the interaction of the CXCL12/CXCR-4 axis, the specific ligand CXCL12 for CXCR-4 is also known as stromal cell-derived factor (SDF)-1, is involved in the metastatic process of different types of carcinomas including breast and colorectal cancer. Whereas liver resection enhances intrahepatic engraftment of colorectal cancer metastasis, the main role of the CXCL12/CXCR-4 pathway influencing tumor engraftment is still unknown. Herein we studied how liver resection-associated SDF-1 affects extrahepatic tumor cell engraftment and whether SDF-1 also stimulates the growth of already established metastases.

**Material and Methods:** Green fluorescent protein (GFP)-transfected CT26.WT colorectal cancer cells were implanted into dorsal skinfold chambers of syngeneic BALB/c mice. Additionally, all animals underwent a 30% hepatectomy. To study SDF-1 in extrahepatic tumor cell engraftment, animals were treated with an anti-SDF-1 anti-body, starting at the day of tumor cell implantation. To study SDF-1 in established metastases, anti-SDF-1 treatment was initiated at day 5 after tumor cell implantation. Hepatectomized animals without neutralization of SDF-1 received control IgG antibody treatment and served as controls. Tumor vascularization and growth as well as tumor cell migration, proliferation, apoptosis and CXCR-4 expression were studied over 14 days using intravital fluorescence microscopy, histology, immunohistochemistry and western blot analysis.

**Results:** Functional inhibition of SDF-1 delayed extrahepatic tumor cell engraftment but not the growth of established metastases. The initial delay of engraftment was associated with a highly significant compensatory stimulation of vascularization and an increased microvascular permeability shown by a significant higher incidence of petechial bleeding. Accordingly, tumors treated with anti-SDF-1 directly after hepatectomy showed significant more VEGF expression than controls at day 5 and 9 after tumor cell implantation. Further, inhibition of tumor cell engraftment by initial anti-SDF-1 treatment was associated with a significant increase of tumor cell invasion, CXCR-4 expression and tumor cell apoptosis.

**Conclusion:** Our study indicates that SDF-1 is involved in extrahepatic engraftment of CT.26 colorectal cancer cells. Interestingly, blockade SDF-1 did not effect tumor growth by compensatory activation of an alternative CXCR-4 – VEGF dependent pathway. The SDF-1/CXCR-4 signaling pathway in combination with VEGF-dependent pathways may be a promising target for early anti-tumor therapy in patients undergoing liver resection.

**The value of local treatment in patients with Extrapulmonary Metastasized Ewing Tumours (EPMET)**

Dirksen U.<sup>1</sup>, Häusler J.<sup>1</sup>, Ranft A.<sup>1</sup>, Bölling T.<sup>2</sup>, Gebert C.<sup>3</sup>, Vieth V.<sup>4</sup>, Jürgens H.<sup>1</sup>

<sup>1</sup>Universitätsklinikum, Pädiatrische Hämatologie und Onkologie, Münster; <sup>2</sup>Universitätsklinikum, Radiotherapie und Radioonkologie, Münster; <sup>3</sup>Universitätsklinikum, Allgemeine Orthopädie, Münster; <sup>4</sup>Universitätsklinikum, Radiologie, Münster

**Background:** In contrast to patients diagnosed for localized Ewing tumours and/or pulmonary metastases only, extrapulmonary disseminated Ewing tumors (EPMET) have a poor prognosis. The value of local treatment in patients with EPMET was investigated.

**Methods:** We analyzed 120 German GPOH-EPMET patients (pts) registered from 1998-2006 into the EURO E.W.I.N.G. 99 trial. Median age was 16.2 years. 26 of 120 pts received surgery (OP), 21 pts OP and radiotherapy (RT), and 40 pts definite RT of the primary tumour (PT). Viewing the local treatment of metastases, 8 of 122 pts received OP, 7 pts OP and RT, and 37 pts definite RT. 6 pts received whole lungs RT. 49 pts (41%) received local treatment to the PT and EPMET, and 90 pts (75%) to the PT or EPMET .

**Results:** The event-free survival (EFS) in the whole group of patients was 0.24 [95% CI 0.16-0.33] at 3 years. Univariate analyses on the value of local treatment modalities showed the following favourable factors for local therapy of the PT (3-years EFS): OP 0.25, OP&RT 0.47, and RT 0.23 vs. no local therapy of PT 0.13 (p < .001). For local treatment of the metastases: OP 0.25, OP&RT 0.86 (N = 7!), and RT 0.27 vs. no local therapy of metastases 0.17 (p = .002). In pts who received any local treatment to PT and EPMET, EFS was 0.37 compared to other pts with 0.16 (p < .001), and 0.28 for pts with

any treatment to PT or EPMET compared to pts who received no local treatment 0.15 (p < .001). Multivariate analysis in patients with bbone metastases (n = 94), that more than one bony lesion (>1 EPMET; N = 83) is a major risk factor (Hazard Ratio [HR] = 2.9, p = .05; 3y-EFS = 0.18). Therefore, analyses on the value of local treatment in pts with bone metastases were performed. OP (with or without RT) of the PT was multivariate no longer a significant favourable factor (HR = 0.92; p = .756), but OP (with or without RT) of EPMET (HR = 0.37; p = .039).

**Conclusion:** These data indicate that also in patients with primary disseminated Ewing tumour, local therapy is essential. Radiotherapy and surgery both of the PT and metastases must complement systemic treatment whenever possible.

**Molecular detection of tumor cells in bone marrow: Quantification and expression analysis using the Embedded Tumor Cell (ETC) calibrator technique**

Aggarwal M.<sup>1</sup>, Fersis N.<sup>1</sup>, Zeifang F.<sup>2</sup>, Kaul S.<sup>1</sup>

<sup>1</sup>Universitätsfrauenklinik, Onkologisches Labor, Heidelberg; <sup>2</sup>Orthopedic Clinic, Orthopedic Surgery, Heidelberg

**Background:** Using criteria for classification of disseminated tumor cells in compliance with the publication of Fehm et al. (Cancer, Vol.107, 2006) we have reported a cytological DTC-positivity rate of 1.9% (39/2050) in patients with primary breast cancer (T1-4, N0/N+, M0/M1). To improve the DTC detection rate we have adopted the technique of immunomagnetic tumor cell separation in combination with molecular gene expression to Ficcoll-separated BM samples.

Table 1. Molecular tumor cell analysis in 104 bone marrow samples (for abstract OP189)

Material: 1x10 <sup>7</sup> BM-cells from Ficoll separation Method: Immunomagnetic selection in combination with real-time RT-PCR Selection markers: MUC1 (Mb BM7) and EpCAM (Mab VU1D9) Detection markers: Cytokeratin 19 (CK19) , Mammaglobin1 (MG1) and CD276 Standard cytology: A45 and 5D3 in combination with SA-AP (Ventana)									
PCR	ULP-Primer							PCR-Result	Cyto 5D3/A45
	ETC	β-Actin	Sur	CK 19	Mg	HER-2	CD 276		
2100	×	20.87	26.82	27.87	–	35.25	31.1	>100 cells	negative
2346	×	20.26	29.37	32.68	–	35.33	29.7	>20 cells	50 cells
1535	–	19.77	25.78	35.17	33.22	–	#	4 cells	negative
1984	×	17.47	26.25	36.46	–	35.19	28.9	1 cell	n.a.
2313	×	19.4	32.34	36.5	–	36.59	31.2	1 cell	n.d.
2439	×	21		36.6	–	35	26	1 cell	negative
1319	–	20.07	29.69	37.18	–	37.19	31.4	<1 cell	negative
1346	–	20.06	31.56	37.34	37.99	–	#	<1 cell	negative
1899	×	17.73	25.19	37.66	–	35.88	28.7	<1 cell	negative
1306	–	19.65	30.42	37.8	35.92	36.12	29.4	<1 cell	negative
2528	×	21.9	27.1	38	–	–	32.8	<1 cell	n.d.
2229	×	18.8	26.42	–	34.46	36.43	32	2 cells	negative
1688	–	19.38	24.45	–	35.17	–	29.8	1 cell	negative
1321	–	22.76	32.11	–	35.74	36.16	33.6	1 cell	negative
1308	–	19.76	30.62	–	36.9	37.64	32.2	<1 cell	negative

RT-PCR: 15/104 (14.4%) samples positiv based on CK19/MG1-analysis  
RT-PCR: 11/104 (10.5%) samples CK19 positive, 4/104 (3.8%) samples MG1 positive  
Cytology: 1/44 (2.3%) cytokeatin (A45 und 5D3) positive  
**ETC::** embedded tumor cell calibration (n=78) with mit 2, 4 and 8 und HD-ZE breast carcinoma cells

**Material and Methods:** Mononuclear cells from bone marrow of 104 patients with primary breast cancer and 21 normal bone marrow donors were analysed in parallel by standardized cytospin immunocytology techniques ( $2 \times 10^6$  cells) and by an immunobead-RT-PCR technique.  $1 \times 10^7$  BM cells were used for immunomagnetic enrichment of epithelial cell types using the antibodies BM7 and VU1D9 targeting the cell membrane glycoproteins MUC1 and EpCAM. Separated cells were lysed and used for mRNA isolation and c-DNA synthesis. The markers cytokeratin 19 (CK19), mammaglobin1 (MG1), Survivin (Sur), HER-2, CXCR4 and CD276 were analysed by real-time quantitative RT-PCR using primers and FAM-labeled TaqMan probes selected with the UniversalProbeLibrary system (Roche AG, Basel, CH). The newly developed technique of embedded tumor cell calibrator (ETC) was introduced for cell quantification and gene expression analysis (N. Fersis, DKK 2008). Samples were divided in native probes and matched calibrator probes containing 2, 4 and 8 breast carcinoma cells/ $10^7$  BM cells.

**Results:** One from 43 BM probes (2.3%) was positive in the standard immuno-cytochemistry screening of  $2 \times 10^6$  cells. In contrast 15 from 104 (14.4%) BM samples were positive based on the molecular analysis of CK19 and MG1 expression in samples of  $1 \times 10^7$  BM cells. Nine of these probes had elevated expression levels of the tumor-specific angiogenesis marker CD276. However, DTC quantification based on ETC revealed tumor cell numbers above 10 cells in only two samples, thereby confirming the low detection rates of the standard immunocytology.

**Discussion:** We have improved the detection rate of tumor cells in bone marrow significantly by a) increasing the sample size to  $1 \times 10^7$  BM cells, b) by the adaptation of an immunobead-RT-PCR and by c) introducing the embedded tumor cell calibration method. With these improvements DTC analysis may become a valuable tool for gene expression analysis in selected patients diagnosed with metastatic disease.

## Cancer Metastasis

### Poster Presentation

PO338

#### OMCC, a newly identified regulator of the met signaling pathway, is prognostic for colon cancer metastasis

Arlt F.<sup>1</sup>, Walther W.<sup>1</sup>, Smith J.<sup>1</sup>, Fleuter C.<sup>1</sup>, Birchmeier W.<sup>1</sup>, Schlag P.M.<sup>2</sup>, Stein U.<sup>1</sup>

<sup>1</sup>Max-Delbrück-Center for Molecular Medicine, Surgery/Surgical Oncology, Berlin; <sup>2</sup>Charité Berlin, Robert-Rössle-Cancer Clinic, Berlin

Metastasis determines a very poor prognosis for colon cancer patients. Once metastases occurred treatment options are very limited. Our focus is the identification of new molecular markers to assess the metastatic risk and give an early prognosis of the patient's clinical outcome. Identified high risk patients could be provided with an adapted, more intense monitoring and treatment.

We identified the gene Overexpressed in Metastatic Colon Cancer (OMCC) by differential display RT-PCR of colon cancer tissues. *In silico* analyzed OMCC structure is typical for proteins involved in receptor tyrosine kinase signaling, including a SH3 domain, a PXXP domain, and tyrosine phosphorylation sites. The aim of this project was the characterization of the cellular function of OMCC and the analysis of its prognostic potential regarding metastases formation.

Stable OMCC transfectants, but not mutants lacking the SH3 or PXXP domain displayed strongly enhanced proliferative, migratory, and invasive behaviour. All these biological effects could be reduced by transfecting OMCC-specific siRNA. Treatment of OMCC clones with the hepatocyte growth factor (HGF), ligand of the receptor tyrosine kinase Met, resulted in striking morphological changes in cells and nuclear accumulation of OMCC. Remarkably, OMCC clones showed a strong Met overexpression that could be knocked down by OMCC siRNA. To corroborate the hypothesis of an OMCC-induced Met expression we analyzed the Met promoter in CAT reporter- and chromatin immunoprecipitation assays and could confirm an interaction of OMCC with the Met promoter. To test the prognostic value of OMCC we analyzed OMCC expression in resected stage I-III primary colon tumors. OMCC expression was significantly higher in patients that later developed metastases.

Here we provide evidence, that the newly identified gene OMCC is a master regulator of Met signaling in colon cancer. Deregulated Met signaling affects many cellular mechanisms associated with metastasis and is correlated with a

poor prognosis. Therefore, OMCC could emerge as a new target for intervention strategies. Furthermore, we show that OMCC is a new molecular marker for the early prognosis of the patient's individual metastatic risk.

PO339

#### Ex vivo treatment of malignant pleural effusions with the bispecific antibody MT110 targeting EPCAM and CD3

Witthauer J.<sup>1,2</sup>, Schlereth B.<sup>2</sup>, Brischwein K.<sup>2</sup>, Jauch K.-W.<sup>1</sup>, Mayer B.<sup>1</sup>

<sup>1</sup>LMU, University of Munich, Department of Surgery, Großhadern, Munich, Germany; <sup>2</sup>Micromet AG, Munich, Germany

**Purpose of the Study:** Approximately half of the patients with metastatic breast cancer develop a malignant pleural effusion (MPE) that often becomes persistent and progressive despite therapeutic interventions. Mean survival of these patients is only a few months. Hence, current treatment options for breast cancer patients with MPE are insufficient. Promising drug candidates are bispecific single chain antibodies that simultaneously target the epithelial antigen EpCAM (CD326) and the T cell antigen CD3. In the present study, the *ex vivo* efficacy of the bispecific antibody MT110, was tested in pleural effusion samples from breast cancer patients.

**Patient and Methods:** Malignant pleural effusions were collected by chest drainage and cells isolated by fractionated centrifugation. Target antigens were detected using immunohistochemical methods. *Ex vivo* treatment of EpCAM<sup>+</sup> effusion samples was performed with MT110 (0.1, 1, 10 and 1000 ng/ml) for 48 h and 72 h. Redirected target cell lysis was determined by FACS analysis using double fluorescence labelling with 7-AAD and EpCAM-APC. Stimulation of endogenous T cells by MT110 was determined by the CD25 and granzyme B expression on CD4<sup>+</sup> and CD8<sup>+</sup> cells. Statistical significance was evaluated using the Wilcoxon matched-pairs signed-ranks test ( $p < 0.05$ ).

**Results:** Immunohistochemical target analysis revealed EpCAM<sup>+</sup> cells in 78% (14 out of 18) of MPE samples. The fraction of EpCAM<sup>+</sup> pleural carcinoma cells varied between 30% and 100% (median 78%), and that of CD3<sup>+</sup> leukocytes between 60% and 93% (median 80%). Subpopulation analysis showed a median fraction of 71% CD4<sup>+</sup> and 27% CD8<sup>+</sup> T cells (median ratio of CD4:CD8 = 2.6:1). The E:T ratio between endogenous CD3<sup>+</sup> effector (E) and EpCAM<sup>+</sup> target cells (T) varied between 1:4 and 620:1. *Ex vivo* treatment of seven MPE samples with MT110 revealed a dose-dependent lysis of EpCAM-expressing target cells. After 72 h,  $37\% \pm 27\%$  (median  $\pm$  SD) of EpCAM<sup>+</sup> cells were killed using 10 ng/ml MT110 ( $p = 0.03$ ), and  $57\% \pm 29.5\%$  with 1000 ng/ml MT110 ( $p = 0.016$ ). A high degree of lysis often correlated with a high fraction of CD3<sup>+</sup> leukocytes, i.e. an E:T ratio  $\geq 1$ . Accordingly, MT110 induced a dose-dependent increased expression of the activation marker CD25 on CD4<sup>+</sup> and CD8<sup>+</sup> cells, e.g. at 1000 ng/ml MT110 after 48 h ( $p = 0.03$ ), and 72 h ( $p = 0.016$ ). Furthermore, a high granzyme B-expression in CD8<sup>+</sup> cells, that is involved in apoptosis, correlated with a higher degree of specific lysis of EpCAM<sup>+</sup> cells.

**Conclusion:** Single-agent treatment with MT110 is capable of activating unstimulated T cells in pleural exudates for efficient and specific redirected lysis of EpCAM<sup>+</sup> tumor cells. The present *ex vivo* analysis supports the treatment of metastatic breast cancer patients, stratified with the bispecific antibody MT110. Further studies including a large number of patients with MPE need to be done clarifying the underlying mechanisms.

PO340

#### Stromal Cell-Derived Factor (SDF)-1 promotes cell migration and tumor growth of colorectal metastasis

Kollmar O., Rupertus K., Scheuer C., Junker B., Tilton B., Schilling M.K., Menger M.D.

Universität des Saarlandes, Homburg/Saar

In a mouse model of established extrahepatic colorectal metastasis, we analyzed whether the chemokine CXCL12, known as stromal cell-derived factor (SDF)-1, stimulates tumor cell migration in vitro and angiogenesis and tumor growth in vivo. Methods: Using chemotaxis chambers, CT26.WT colorectal tumor cell migration was studied under stimulation with different concentrations of SDF-1. To evaluate angiogenesis and tumor growth in vivo, green fluorescent

protein (GFP)-transfected CT26.WT cells were implanted in dorsal skinfold chambers of syngeneic BALB/c mice. After 5 days, the tumors were locally exposed to 100nM SDF-1. Cell proliferation and apoptosis as well as tumor microvascularization and growth were studied during a further 9-day period using intravital fluorescence microscopy, histology and immunohistochemistry. Tumors exposed to PBS only served as controls. Results: In vitro, >30% of unstimulated CT26.WT cells showed expression of the SDF-1 receptor CXCR4. In the chemotaxis assay, SDF-1 provoked a dose-dependent increase of cell migration. In vivo, SDF-1 accelerated neovascularization and induced a significant increase of tumor growth. Capillaries of SDF-1-treated tumors showed significant dilation, indicating an increased release of VEGF. Of interest, SDF-1 treatment was associated with a significantly increased expression of proliferating cell nuclear antigen (PCNA) and a downregulation of cleaved caspase-3 when compared with PBS-treated controls. Conclusion: Our study indicates that the CXC chemokine SDF-1 promotes tumor cell migration of colonic cancer cells in vitro and tumor growth of established extrahepatic metastasis in vivo. The stimulation of tumor growth is most probably due to an angiogenesis-dependent induction of tumor cell proliferation and inhibition of apoptotic cell death.

PO341

### Cathepsin B influences metastasis in genetically generated oral-esophageal cancer cells

Kunert H.<sup>1,2,3</sup>, Hauß S.<sup>1,2,3</sup>, Franzke C.<sup>4</sup>, Egenter E.<sup>1,2,3</sup>, Hirt N.<sup>1,2,3</sup>, Queisser A.<sup>1,2,3</sup>, Döbele M.<sup>1,2,3</sup>, Heeg S.<sup>1,2,3</sup>, Reinheckel T.<sup>1</sup>, Bruckner-Tudermann L.<sup>4</sup>, Peters C.<sup>1</sup>, Blum H.<sup>2</sup>, Opitz O.G.<sup>1,2,3</sup>

<sup>1</sup>Institut, Molekulare Medizin und Zellforschung, Freiburg;

<sup>2</sup>Universitätsklinik, Medizinische Klinik II, Freiburg; <sup>3</sup>Universitätsklinik, Comprehensive Cancer Center, Freiburg; <sup>4</sup>Universitätsklinik, Hautklinik, Freiburg

**Introduction:** The ability of a tumor cell to migrate across the extracellular matrix (ECM) is a prerequisite for metastasis. In our cellular model of human oral-esophageal carcinogenesis we were able to recapitulate tumor development in a stepwise fashion. Cyclin D1 overexpression and p53 inactivation led to immortalization, additional EGFR overexpression induced an *in vitro* transformed phenotype whereas additional c-myc overexpression resulted in invasive cancer cells. To study the ability of these cells to migrate, we were interested in the expression and localization of the lysosomal cysteine protease Cathepsin B thought to be involved in tumor invasion and metastasis. In malignant tumors Cathepsin B is secreted and becomes associated with the cell surface.

**Methods:** Cathepsin B expression was assayed in immortalized oral keratinocytes (OKF6D1/d.n.p53), in *in vitro* transformed OKF6D1/d.n.p53/EGFR and in OKF6D1/d.n.p53/EGFR/c-myc cancer cells by real-time PCR and western blot analysis. The cellular distribution of the protein was studied by immunofluorescence. The proteolytic activity of the enzyme on the cell surface was determined with active-site labelling. Furthermore, we analyzed the migration behavior in our cellular model using a matrigel assay.

**Results:** RT-PCR revealed that there were no differences in the expression of Cathepsin B in our cellular model. Western-blot analysis shows a decrease in the single chain expression in *in vitro* transformed and in cancer cells. Western-blot analysis of the cell culture medium revealed that Cathepsin B is released into the medium of the generated cancer cells overexpressing c-myc. Immunofluorescence demonstrated that the cellular localization of Cathepsin B is influenced by malignant transformation and it is translocated to the plasma membrane. Active-site labelling reveals proteolytically active Cathepsin B on the cell surface. Migration and invasion appears to be increased in the genetically generated cancer cells.

**Conclusions:** In our human cellular model of oral-esophageal carcinogenesis we demonstrated that the cellular localization of Cathepsin B is defined by the distinct steps of tumor development. Finally, the recruitment to the membrane and secretion of Cathepsin B leads to an increased degradation of the ECM potentially allowing fully transformed cells to migrate through the ECM and ultimately to metastasize.

PO342

### Analysis of progression patterns of malignant melanoma

Schmidt M., Hölzel D.

Tumorzentrum München, Tumorregister München, München

**Objectives:** In co-operation with all three dermatological centers the Munich Cancer Registry (MCR) has achieved an almost complete documentation of patients with malignant melanoma (MM) in Southern Bavaria/Germany. In addition to incidence data, the MCR processes informations on local and regional progressions and on the occurrence of metastases, respectively. This quality of data is a fundamental base on which the benefit of diagnostic and therapeutic strategies regarding the outcome of the disease can be discussed.

**Methods:** The database of the MCR currently contains profiles of about 350,000 cancer patients with numerous clinical and epidemiological parameters as well as detailed follow-up informations including complete life status. These parameters (e.g. tumor thickness, lymph node status, progression characteristics) were used for analyzing patients with regional or distant events and for discovering different types of progression. Relative survival curves illustrate tumor dependent prognosis in particular subgroups and Cox's Proportional Hazard Model was performed to simultaneously predict survival of patients with individual risk patterns.

**Results:** The data of 12,349 patients with MM were analyzed from a total of 18,586. 64% of these patients were diagnosed with a tumor thickness equal or less than 1 mm and about 5% were lymph node positive. The probability of relapse strongly depends on the tumor thickness as the main risk factor just like the length of survival from progression (< = 1 mm: median 28.5 mths, >4 mm: 12.3). The detection of initial distant metastasis in primary M0 melanomas is markedly correlated with the site of the lesion (for >4 mm: lymph node: median 11.0 mths, CNS: 21.7). The median survival time after relapse varies from 32.1 mths for local events to 8.7 mths for distant metastases. These results are strongly confirmed by multivariate analysis. The presented statistics are part of comprehensive evaluations of MM disease and are being prepared for all participating dermatological departments as feedback and support for the clinicians.

**Conclusion:** Countrywide and multidisciplinary documentation of cancer patients is able to provide sufficient results of survival outcome in melanoma patients. The biometric analysis of type and chronological distribution of the occurring events supply adequate knowledge to discuss established therapeutic strategies like lymph node dissection.

PO343

### High rate detection of gene mutations in preneoplastic and early neoplastic lesions of the human colon and rectum

Schneider M.<sup>1</sup>, Scholtka B.<sup>1</sup>, Friedrich D.<sup>2</sup>, Berghof-Jäger K.<sup>2</sup>, Steinberg P.<sup>1</sup>

<sup>1</sup>Chair of Nutritional Toxicology, Institute of Nutritional Science, University of Potsdam; <sup>2</sup>BIOTECON Diagnostics GmbH, Potsdam

**Introduction:** The mutation spectra of four genes, which are involved in two different pathways (WNT and MAPK) and which are mutated early during colon carcinogenesis, were analyzed in 165 human preneoplastic or neoplastic samples. The aim of this study was to estimate the mutation rate of both signalling pathways in the above-mentioned samples.

**Methods:** The mutation status of the *B-RAF*, *CTNNB1*, *K-RAS* and *APC* genes in 165 preneoplastic or neoplastic lesions from patients that had undergone a colonoscopy were investigated. The samples were divided into two groups, carcinomas (n = 73) and pre-stages (n = 92). PCR-SSCP was used to detect aberrations in the *B-RAF* and *CTNNB1* gene. *K-RAS* gene mutations were analyzed by PCR-RFLP and the mutation cluster region of the *APC* gene was analyzed by direct sequencing.

**Results:** The multitarget panel presented in this study is able to detect mutations in colorectal cancer pre-stages as hyperplastic polyps as well as in low-grade and high-grade dysplasia and in colorectal cancer tissues. Mutations in the *B-RAF* gene codon 600 were detected in 28% (44/159) of the lesions, the mutation rate being 35% in the case of the pre-stages and 19% in carcinoma.



A high frequency of *B-RAF* mutations was found in hyperplastic polyps (50%). Mutations in the codons 12 and 13 of the *K-RAS* gene were detected in 27% (40/150) of all tissues analyzed (in 25% of the pre-stages and 29% of the carcinomas). Ten percent (16/165) of the lesions showed mutations at the phosphorylation sites of the *CTNNB1* gene. In the mutation cluster region of the *APC* gene mutations were found in 35% (46/133) of all tissues analyzed (in 44% of the carcinomas and 25% of the pre-stages). Taken together the results show that 74% of all lesions analyzed carried at least one mutated gene. The mutation rate of the pre-stages was 72%, in the case of carcinomas it was 77%. **Conclusion:** Mutations in at least one out of four genes studied could be detected in about 74% of all colon tumors analyzed. Therefore this assay has a clear potential to detect mutations in preneoplastic and early neoplastic lesions of the colon.

PO344

### Proteasome inhibitors suppress angiogenesis by altering endothelial VEGFR-2 expression

Meissner M.<sup>1</sup>, Hrgovic I.<sup>1</sup>, Stein M.<sup>1</sup>, Kaufmann R.<sup>1</sup>, Gille J.<sup>1</sup>

<sup>1</sup>Universität Frankfurt, Zentrum für Dermatologie, Frankfurt

The ubiquitin-proteasome system is the major pathway for intracellular protein degradation in eukaryotic cells that controls a wide range of cellular regulatory proteins, including transcription factors and cell cycle regulatory proteins. Recent evidence established the importance of the proteasome also in tumor development, showing anti-tumor and anti-angiogenic actions by selective inhibitors *in vivo*. As signaling via the VEGFR2 pathway is critical for angiogenic responses to occur, we explored whether anti-angiogenic effects via proteasome inhibition were mediated in part through diminished endothelial VEGFR2 expression. Our studies show that different proteasome inhibitors (MG132, ALLN and lactacystin) all blocked VEGFR2 expression in a time- and concentration-dependent manner, which was paralleled by respective inhibition of capillary like structure formation and endothelial cell migration. In contrast, tie-2 or VEGFR-1 expression was not significantly affected by proteasome inhibitor treatment. The suppressive effects on VEGFR2 expression were neither conveyed by increased shedding nor by shortened protein half-life, suggesting that transcriptional mechanisms accounted for the observed effects. In line with this conclusion, proteasome inhibition significantly suppressed VEGFR2 mRNA accumulation. In addition, inhibitor treatment considerably decreased transcriptional activity of 5'-deletional VEGFR2 promoter gene constructs. Proteasome inhibition-mediated repression was conveyed by a GC-rich region, harboring one consensus Sp1 binding site. Subsequent EMSA analyses demonstrated diminished constitutive Sp1-dependent DNA binding in response to proteasome inhibition. Hence, VEGFR-2 expression may constitute a critical molecular target of proteasome inhibitors that may mediate their anti-angiogenic effects *in vivo*.

PO345

### Antiapoptotic ARC is overexpressed in renal cell carcinomas

Heikaus S.<sup>1</sup>, Kempf T.<sup>1</sup>, Mahotka C.<sup>1</sup>, Gabbert H.E.<sup>1</sup>, Ramp U.<sup>1</sup>

<sup>1</sup>Institut für Pathologie, Heinrich-Heine Universitätsklinikum, Düsseldorf

**Aims:** ARC is an important antiapoptotic regulator that can interact with and thereby inhibit multiple proapoptotic factors of the extrinsic and intrinsic apoptotic signalling pathways. One important of these genes is the initiator Caspase-8 transmitting the apoptotic signal of extrinsic and intrinsic apoptotic stimuli to more downstream pathways in the apoptotic cascade. Therefore, we analysed the expression of ARC and Caspase-8 in primary renal cell carcinomas (RCCs) of the clear cell type.

**Methods and Results:** ARC and Caspase-8 expression could be detected in primary tumour tissue of 48 moderately (G2) or poorly (G3) differentiated RCCs and in corresponding non-neoplastic renal tissue by real-time PCR. The relative ARC mRNA expression was increased in RCCs of all tumor stages when compared to non-neoplastic renal tissue without significant differences between the different tumor stages. In contrast, relative Caspase-8 mRNA expression was increased in pT1 and pT2 tumors when compared to non-neoplastic renal tissue. However, no significant difference in relative Caspase-8

expression between pT3 tumors and the corresponding non-neoplastic tissue became evident. These results were confirmed on the protein level by Western Blot analysis. Quite similar, immunohistochemistry of 5 arbitrarily selected G1 and 5 G3 RCCs revealed no differences in ARC protein expression. The relative mRNA expression ratio between ARC and Caspase-8 significantly increased from RCCs confined to the kidney (pT1 and pT2) to tumours infiltrating beyond the kidney (pT3).

**Conclusions:** The antiapoptotic regulator ARC is overexpressed in RCCs compared to non-neoplastic renal tissue. Furthermore, the delicate balance between antiapoptotic ARC and proapoptotic Caspase-8 is gradually disturbed during tumour progression, resulting in a relative increase of antiapoptotic ARC over Caspase-8 probably contributing to the marked apoptosis-resistance of RCCs.

PO346

### Defective vascular adaptation accounts for structural and functional heterogeneity of tumor microvascular networks

Cornelissen A.<sup>1,2</sup>, Höpfner M.<sup>3</sup>, Ulm L.<sup>3</sup>, Styp-Rekowska B.<sup>3</sup>, Secomb T.<sup>4</sup>, Dewhirst M.<sup>5</sup>, Pries A.R.<sup>3</sup>

<sup>1</sup>Université Paris 7 Denis Diderot, Laboratoire Matière et Systèmes Complexes (MSC), Paris, Frankreich; <sup>2</sup>Université Paris, Laboratoire Matière et Systèmes Complexes, CNRS-UMR 7057, Paris, Frankreich; <sup>3</sup>Charité – Universitätsmedizin Berlin, Institut f. Physiologie, Campus Benjamin Franklin, Berlin; <sup>4</sup>University of Arizona, Dept. of Physiology, Tucson, USA; <sup>5</sup>Duke University Medical Center, Durham, Dept. of Radiation Oncology, Durham, North Carolina, USA

**Background and Aim:** Vascular networks continuously adapt to the changing needs of the tissue. In healthy tissues angiadaptive mechanisms are optimally balanced to adequately distribute blood and oxygen. By contrast, tumor microvascular networks exhibit high structural and functional heterogeneity resulting in hypoxia and irregular distribution of blood flow. This has strong implications on tumor development and metastasis and severely limits chemotherapeutic treatment efficacy. Thus, improving the distribution of blood flow and oxygen by normalizing the tumor vasculature may be a promising approach to improve chemotherapeutic cancer treatment. However, the key players responsible for the degenerated formation of tumor microvascular networks remain to be deciphered. To address this question we investigated normal and tumor vascular networks to analyze possible alterations of vascular adaptation characteristics in tumor microvasculature.

**Methods and Results:** Normal and tumor microvascular networks were visualized by intravital microscopy, vascular morphology (length, diameter), connection pattern and red blood cell velocity was measured. Pressure (P), flow (Q) and shear stress ( $\tau$ ) of all vessel segments were estimated by a computer model based on the experimental network architecture. Structural vascular adaptation in response to hemodynamic (P, Q) and metabolic ( $pO_2$ ) stimuli including information transfer along the vessels was simulated. The adequacy of the assumed adaptation rules was quantified by calculating the heterogeneity of vessel diameters ( $H_D$ ) at branch points and the relative fraction of hypoxic vessels ( $F_O$ ). Measured  $H_D$  and  $F_O$  of normal networks were low relative to tumor networks. Performing simulated adaptation on normal networks assuming a reduced amount of information transfer, an increased growth tendency and an increased randomness in vascular reactions led to values of  $H_D$  and  $F_O$  that matched the values for  $H_D$  and  $F_O$  measured in tumor networks. In turn, tumor networks could be matched to measured  $H_D$ - and  $F_O$ - values of normal networks when normal adaptation parameters were applied.

**Conclusions:** Functional properties of tumor microvascular networks appear to result from specific deficiencies in vascular adaptation. Besides a generally increased biological randomness, an increased growth tendency and a reduced information transfer along the vessel seem to be the key parameters for the tumor-specific network development. Interfering with these key parameters may help to normalize tumor vasculature, rendering tumors more susceptible to chemotherapeutic treatment.

PO347

### Resveratrol sensitizes colorectal cancer cells to oxaliplatin-induced cell growth inhibition

Ulrich S., Kempf M., Stein J.

J.W.Goethe Universität, Med. Klinik I, Gastroenterologie, Frankfurt am Main

**Background:** Resveratrol is a natural occurring polyphenol found in grapes, peanuts and red wine. Previous results indicate, that resveratrol inhibits cell growth of colon carcinoma cells via PPAR $\gamma$  mediated activation of spermine/spermidine acetyltransferase (SSAT) [Cancer Res, 2006]. Conventional cancer therapies as single modalities have a limited but important role in the overall treatment of most solid tumors. Thus, the strategies of cancer treatment using combined therapies are considered more promising for higher efficacy. The objective of this study was to investigate whether resveratrol can potentiate the antitumor activities of the common chemotherapeutic oxaliplatin in a cell culture model of colorectal cancer.

**Materials and Methods:** Caco-2 cells expressing PPAR $\gamma$  were treated with increasing concentrations of resveratrol [10-200 $\mu$ M] and oxaliplatin [100nM-10 $\mu$ M]. For co-incubation the cells were pre-treated with resveratrol [10-50 $\mu$ M] for 24h. Cell growth was determined by BrdU incorporation and crystal-violet staining. Activity of spermine/spermidine acetyltransferase was assayed with a radiometric technique measuring the amount of synthesized [acetyl-<sup>14</sup>C]-spermidine. Apoptosis induction was detected by measuring DNA fragmentation. Caspase-3 induction was determined via an activity assay. Drug interactions were assessed using the combination-index (CI) method as defined by median-effect analysis. CI < 1 indicates synergism.

**Results:** Resveratrol [IC<sub>50</sub> = 75  $\mu$ M] and oxaliplatin [IC<sub>50</sub> = 7.5  $\mu$ M] significantly inhibit cell growth of Caco-2-cells in a dose dependent manner after 24 h (\*\*p < 0.001). These IC<sub>50</sub> values were reduced to [25  $\mu$ M] and [1  $\mu$ M] when the drugs were used in combination (CI = 0.466, \*\*\*p < 0.001). Furthermore, oxaliplatin synergistically increased SSAT activity in pre-treated Caco-2 cells up to 300% in a dose dependent manner after 24h (\*\*p < 0.001). This effect was strictly related to the ability to induce apoptosis, as Caspase-3-activity (~40% vs. resveratrol, \*p<0.05) as well as DNA-fragmentation (~40% vs. resveratrol, \*\*p < 0.01) were significantly increased in pre-treated Caco-2 cells after incubation with oxaliplatin [1  $\mu$ M] for 24h.

**Conclusions:** These data suggest that the polyphenol resveratrol sensitizes colon cancer cells to oxaliplatin-induced cell growth inhibition, which may be due to the conspicuous induction of apoptosis. These effects are mediated, at least partly, by an activation of SSAT, the catabolic key enzyme of polyamine metabolism, which is associated with intracellular apoptotic responses.

PO348

### An optimized protocol for laser capture micro-dissection for cytogenetic characterization of colon cancer specimen

Fritzsche B.<sup>1</sup>, Roblick U.J.<sup>1</sup>, Kalies K.<sup>2</sup>, Gheribi E.<sup>1</sup>, Gemoll T.<sup>1</sup>, Bader F.G.<sup>1</sup>, Westermann J.<sup>2</sup>, Bruch H.-P.<sup>1</sup>, Habermann J.K.<sup>1</sup>

<sup>1</sup>UKSH Campus Lübeck, Klinik für Chirurgie, Chirurgisches Forschungslabor, Lübeck; <sup>2</sup>UKSH Campus Lübeck, Institut für Anatomie, Lübeck

**Background:** A late diagnosis of colorectal cancer leads to a significant reduction of average survival time. In current screening programs most of the tumors are detected at advanced stages. Therefore, highly sensitive and specific biomarkers must be identified to detect colorectal malignancies at very early stages. Colon cancer specimens consist of heterogeneous tissue including different cell types such as tumor-, inflammatory- and stroma-cells. For a better understanding of cancer tumorigenesis and to dissect potential biomarkers of high sensitivity and specificity, it is thus crucial to dissect uncontaminated cell types for further analyses. The method of Laser Capture Microdissection (LCM) allows the selection and dissection of e.g., tumor cells independent and free of surrounding cells. The drawbacks of LCM however are the time-excessive workload, the interference of section staining with subsequent cytogenetic techniques and the low yield of DNA-, RNA- and protein-extraction of the microdissected cells.

**Methods:** In order to address these limitations of LCM we aimed at identifying an optimized workflow to gain a high DNA- and RNA-yield of high quality with decent workload and time investment. Thus, colorectal cancer samples and non aberrant, healthy colon samples were prepared for kryosectioning.

Under RNase- and Dnase-free conditions sections at different thickness (e.g. 12, 20 and 25 micrometer) were placed on special membrane slides for microdissection. After toluidin-blue-staining sections were microdissected. The dissected cells were further examined by different extraction methods for RNA and DNA (classical method vs column-assisted-method). We evaluated the necessary minimum amount of cells for additional experiments, without further amplification of the yield RNA/DNA.

**Results:** The isolated RNA and DNA are highly representative for the transcriptome and genome of the examined tumor. With this method we are able to detect characteristic chromosomal abbreviations, disease-specific mutations in the examined genome and specific expression pattern. These findings provide the basis for the development of new diagnostic and therapeutic approaches. Our findings will be of great impact for future understanding of colorectal tumorigenesis and for development of a specific early diagnosis and therapy.

PO349

### Enrichment of mutated K-RAS from human stool DNA by a combined magnetic capture hybridization and PCR-RFLP assay

Schneider M., Steinberg P., Scholtka B.

Universität Potsdam, Ernährungstoxikologie, Potsdam

**Introduction:** Although many techniques for DNA mutation analysis are available, most of them lack the sensitivity to detect alterations in human stool DNA at a comparable level to that present in tissues. In faeces usually one mutated DNA molecule among hundred to ten thousand wild-type DNA molecules does in fact exist. The aim of the project was to develop an assay to enrich the mutated *K-RAS* from a mixture of wild-type and mutant DNA in stool and to compare the achieved detection rate with that of an established PCR-RFLP method.

**Methods:** A combined magnetic capture hybridization and PCR-RFLP assay to detect *K-RAS* codon 12 or 13 mutations was developed. Briefly, two PCR reactions with an intermediate restriction digest followed by a hybridization reaction with a biotinylated single-strand DNA probe were performed. Positive controls were derived from human carcinoma cell lines. Serial dilutions of mutant and wild-type controls were prepared to determine the sensitivity of the assay. Furthermore, a few stool samples and tissues from patients with colon or rectum carcinomas or prestages were analyzed. All observed mutations were confirmed by sequencing.

**Results:** The combination of restriction digest and magnetic capture hybridization led to a 100-fold increased detection limit for *K-RAS* mutations in both, codons 12 and 13, when compared to the conventional PCR-RFLP assay. With the conventional PCR-RFLP assay it was not possible to detect alterations in stool samples of patients with known *K-RAS* gene mutations in their corresponding preneoplastic or neoplastic intestinal tissues. In contrast, by using the combined magnetic capture hybridization and PCR-RFLP assay *K-RAS* mutations could be found in stool samples as previously observed in the corresponding biopsies.

**Conclusion:** The combined magnetic capture hybridization and PCR-RFLP assay leads to an enrichment of mutated *K-RAS* and is sensitive enough to detect *K-RAS* codon 12 or 13 mutations in stool DNA from patients with preneoplastic and neoplastic intestinal lesions.

## Cancer Metastasis

### Poster Exhibition

PE564

### A new staging classification of colon cancer with peritoneal dissemination

Pelz J., Esquivel J.

St. Agnes Hospital, Peritoneal Surface Malignancy Program, Baltimore, MD

**Background:** Peritoneal carcinomatosis is associated with poor quality of life and limited survival. The benefit of cytotoxic and biological agents remains unproven in this group of patients as they were not included in recent trials. Cytoreductive surgery with Hyperthermic Intraperitoneal Chemotherapy

(HIPEC) has had good results in patients with complete resection of their peritoneal dissemination but offers limited benefit after incomplete resections. Staging systems that would allow individualized treatment for patients with carcinomatosis from colon cancer are lacking.

**Methods:** Based on an analysis of current therapeutic options for patients with peritoneal carcinomatosis, we constructed a staging system that would outline the primary treatment modality in a patient with newly diagnosed peritoneal dissemination from colon cancer and no hematogenous metastasis. Staging parameters scored on a 3-point scale according to severity included, 1. symptoms of peritoneal dissemination; 2. extent of peritoneal disease; and, 3. histology of the primary tumor. Final stage (I–IV) was based on stratification of summation of scores for the staging parameters. Staging system testing and treatment algorithm was based on 40 patients staged retrospectively and assessed for oncological outcome.

**Results:** There were 2, 9, 9, and 20 patients in Stages I–IV respectively. At a mean follow-up of 28 months, survival was 100, 88, 66, and 35 percent for Stages I–IV. Median survival for Stage IV was 25 months. Stage-defined treatment recommendations would suggest cytoreduction with HIPEC followed by best systemic therapy for Stage I, neoadjuvant therapy followed by re-staging and evaluation of resectability for Stage II, clinical trial enrolment for Stage III, and best supportive/palliative care for Stage IV.

**Conclusion:** The proposed staging system, based on clinically relevant factors determined pre-treatment, represents an important step towards selecting patients with peritoneal dissemination for appropriate therapy in an effort to maximize treatment-related benefit, while minimizing toxicity of multimodality therapy. Prospective evaluations are warranted to validate the proposed staging classification and clinical pathway.

PE565

### Sichere Metastasenresektion durch ein neues Konzept in der navigierten Leberchirurgie

Beller S., Hünerbein M., Eulenstein S., Lange T., Schlag P.M.  
Universität, Klinik f. Chirurgie u. Chir. Onkologie, Berlin

**Einleitung:** Computergestützte Ortungstechnologien können zu einer Präzisierung chirurgischer Eingriffe führen. Für die Anwendung von Navigationstechniken in der Viszeralchirurgie stellt die Form- und Lageveränderung von Organen jedoch ein zentrales Problem dar.

**Material und Methodik:** Mit einem Infrarot-basierten Navigationssystem (Polaris<sup>®</sup>) wird ein chirurgisches Dissektionsinstrument (CUSA<sup>®</sup> Ultraschallaspirator) im Rahmen von Leberresektionen geortet. Durch ein elektromagnetisches Navigationssystem (Aurora<sup>®</sup>) werden Organbewegungen registriert. Die Kombination dieser Navigationstechniken und ein spezieller Navigationsalgorithmus mit Fokussierung auf einen umschriebenen kritischen Dissektionsbereich wurden erstmals bei Leberresektionen angewendet.

**Ergebnisse:** Die neue Technik konnte rasch in den Algorithmus einer navigierten Leberresektion implementiert werden. Das chirurgische Instrument konnte kontinuierlich geortet werden. Durch die intraoperative Segmentierung von verbotenen Zonen und die Kombination mit einem Warnsignal oder einer Instrumenteninaktivierung („Navigated Control“) war eine wiederholte Kontrolle am Navigationsbildschirm nicht mehr nötig.

**Schlussfolgerung:** Die Kombination von direkter optischer und indirekter elektromagnetischer Trackingtechnologie ermöglicht die Visualisierung und Kontrolle chirurgischer Instrumente in 3D-Ultraschallabbildungen von Organ- und Tumorstrukturen bei gleichzeitiger Erfassung und Berücksichtigung von Organbewegungen.

PE566

### Das resezierende operative Vorgehen bei Patienten mit Skelettmastasen eines Nierenzellkarzinoms verbessert die Prognose signifikant

Dürr H.R.<sup>1</sup>, Staehler M.<sup>2</sup>, Szalantzy M.<sup>1</sup>, Baur-Melnyk A.<sup>3</sup>, Jansson V.<sup>4</sup>

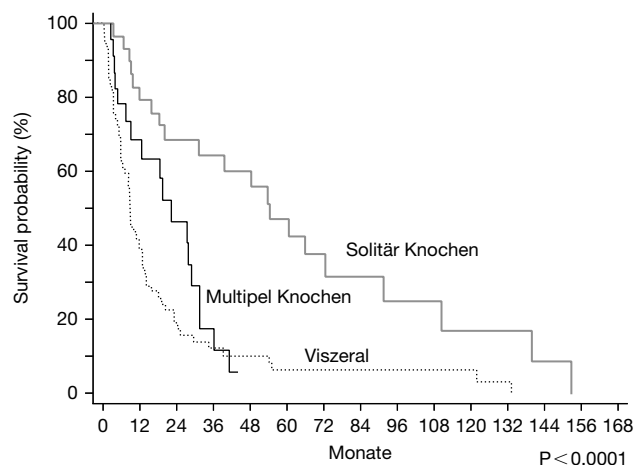
<sup>1</sup>Klinikum der LMU München, Standort Großhadern, Schwerpunkt Tumororthopädie, Orthopädische Klinik, München; <sup>2</sup>Klinikum der LMU München, Standort Großhadern, Klinik für Urologie, München; <sup>3</sup>Klinikum der LMU München, Standort Großhadern, Institut für Radiologie, München; <sup>4</sup>Klinikum der LMU München, Standort Großhadern, Orthopädische Klinik, München

**Fragestellung:** Aufgrund längerer Überlebenszeiten wird häufiger die skeletale Metastasierung zum die Lebensqualität bestimmenden Faktor bei Patienten mit metastasierten Karzinomen. Es konnte gezeigt werden, daß die Wahl des therapeutischen Vorgehens und die Prognose der Patienten dabei sehr vom Primärtumor abhängig ist. Beim Nierenzellkarzinom scheinen erste, auch eigene, Arbeiten einen prognostischen Vorteil bei einen resezierenden Ansatz zu zeigen. Auch lokale Komplikationen können damit besser vermieden werden. Ziel dieser Arbeit war es nun nach Änderung des vormals eher palliativen intraläsionalen Vorgehens den Einfluß auf Lokalrezidiv und Gesamtüberleben zu evaluieren.

**Material und Methoden:** In die Auswertung gingen 100 Patienten (116 Eingriffe) ein, die im Zeitraum 1980-2005 an unserer Klinik operativ versorgt wurden. Das Durchschnittsalter bei der Operation betrug 63 Jahre (28-84 Jahre). Dokumentiert wurden retrospektiv Lokalisation und Ausdehnung, Klinik, angewandtes operatives Verfahren, Metastasierungsstatus, Komplikationen, Rezidive und die sich in einer aktuellen Analyse ergebende Überlebenszeit.

**Ergebnisse:** Die endoprothetische Versorgung erfolgte nach Tumorresektion in 32%, ein Eingriff an der Wirbelsäule war in 24% notwendig, eine Verbundosteosynthese oder intraläsionale Versorgung mit einem Marknagel erfolgte in 22%. In 16% wurde eine Tumorresektion mit oder ohne biologische Rekonstruktion durchgeführt, eine ausschließliche Biopsie war nur bei 7 Patienten notwendig. Die Nachuntersuchung fand zwischen 1 und 17,5 Jahren nach dem Eingriff statt (Durchschnitt 62,6 Monate). Lokalrezidive traten in 15% auf. Die 30-Tage Letalität lag bei 3,4%. 26% der Patienten zeigten eine solitäre Knochenmetastasierung, 18% der Patienten waren multipel ossär metastasiert, 56% Patienten wiesen zusätzlich viszerale Filiae auf. Die mediane Überlebenszeit betrug dabei 12,5 Monate. Nach 5 Jahren waren noch 19,5% der Patienten am Leben. Die Überlebenszeit zwischen den einzelnen Subgruppen unterschied sich dabei signifikant (siehe Abb.). Die Rate an lokalen Komplikationen war in der Gruppe der intraläsional versorgten Patienten deutlich höher.

**Schlussfolgerung:** Patienten mit einer Skelettfilialisierung bei Nierenzellkarzinom zeigten mit nahezu 20% über 5 Jahre ein ausgesprochen günstiges Überleben für diese Erkrankung. Dies führte bei palliativer Versorgung zu einer Reihe erheblicher lokaler Probleme aufgrund von Implantatversagen. Intraläsionale Prozeduren zeigten eine höhere Rate an Lokalrezidiven. Insgesamt gelang es durch ein radikales Vorgehen auch im multipel ossär und viszeral metastasierten Status mit Metastasenresektionen wann immer möglich, die Prognose der multipel ossär und/oder auch viszeral metastasierten Patienten deutlich zu bessern und an jene der solitär ossär metastasierten Fälle anzugleichen.



PE567

### Bone marrow suppression enhances tumor growth of colorectal metastasis due to Stromal Cell-Derived Factor (SDF)-1 related stimulation of angiogenesis and tumor cell proliferation

Rupertus K.<sup>1</sup>, Haberl G.<sup>2</sup>, Scheuer C.<sup>3</sup>, Tilton B.<sup>2</sup>, Schilling M.<sup>2</sup>, Menger M.<sup>3</sup>, Kollmar O.<sup>2</sup>

<sup>1</sup>Universitätsklinikum des Saarlandes, Homburg; <sup>2</sup>Universitätsklinikum des Saarlandes, Homburg/Saar; <sup>3</sup>Universität des Saarlandes, Homburg/Saar

**Background:** Colorectal cancer is one of the leading causes of cancer-related mortality worldwide. Tumor progression and uncontrolled metastatic spread are closely related to tumor angiogenesis. Mobilization into the peripheral blood of c-kit+ bone marrow-derived hematopoietic stem cells (HPCs) can contribute to the formation of new blood vessels. Survival and proliferation of these HPCs are regulated by binding of the stem cell factor (SCF) to the c-kit receptor. Migration and 'homing' of these HPCs to sites of tumor growth is directed by chemotactic signaling in which binding of stromal cell-derived factor (SDF)-1 to its receptor CXCR4 plays an important role. Thus, targeting migration and proliferation of HPCs might provide a promising new strategy of anti-angiogenic tumor therapy. Therefore, we studied the influence of the chemokine SDF-1 on angiogenesis and tumor growth, related to c-kit+ HPCs.

**Material and Methods:** Evaluation of angiogenesis and tumor growth *in vivo* was realized using GFP-transfected CT26.WT colorectal cancer cells which were implanted into the dorsal skinfold chambers of syngeneic BALB/c-mice (n = 24). The animals were randomized into 3 groups. 16 Animals were pre-treated with a monoclonal anti-c-kit-antibody with treatment beginning 4 days before tumor cell implantation (c-kit). 8 of these animals received additional injections with a monoclonal anti-SDF-1 antibody. Controls were treated with a control IgG antibody (n = 8). Angiogenesis, tumor growth, tumor cell proliferation and apoptosis were studied using intravital fluorescence microscopy, histology and immunohistochemistry during the observation period of 14 days.

**Results:** Blockade of c-kit significantly increased tumor growth ( $3.02 \pm 0.26$  vs.  $4.25 \pm 0.58$  mm<sup>2</sup>) when compared to controls without markedly affecting angiogenesis and tumor cell migration. This enhancement of tumor growth was associated with a significantly increased tumor cell proliferation ( $44 \pm 5$  vs.  $58 \pm 2\%$ ) and a decreased rate of apoptotic cell death ( $0.28 \pm 0.05$  vs.  $0.06 \pm 0.04$ ) especially next to the tumor border. Neutralization of SDF-1 significantly decreased the enhanced tumor growth by anti-c-kit treatment ( $2.61 \pm 0.21$  mm<sup>2</sup>) comparable with values of the control group. These anti-c-kit / anti-SDF-1 treated tumors showed significantly decreased angiogenesis and capillary densities as well as a significant reduction of tumor cell infiltration, proliferation and apoptosis compared to the other groups.

**Conclusion:** The results of our study indicate that bone marrow suppression by blockade of c-kit receptor enhance tumor growth of extrahepatic colorectal metastasis. Interestingly, this stimulation of tumor growth by anti-c-kit treatment is mediated by SDF-1.

PE568

### Second liver resection for recurrent metastases of colorectal cancer: Perioperative complications and oncological results

Adam U.<sup>1,2</sup>, Makowiec F.<sup>1</sup>, Neeff H.<sup>1</sup>, Trepel M.<sup>3</sup>, Opitz O.G.<sup>3</sup>, Hopt U.T.<sup>1</sup>

<sup>1</sup>Chirurgische Universitätsklinik Freiburg, Freiburg; <sup>2</sup>Vivantes-Humboldt-Klinikum, Viszeral- und Gefäßchirurgie, Berlin; <sup>3</sup>Universitätsklinik Freiburg, Tumorzentrum Ludwig Heilmeyer, Freiburg

Resection of liver metastases of colorectal cancer (CRC) is performed with increasing frequency, due to advances in multimodal therapy, low mortality and relatively good outcomes. However, only few data have been reported regarding the results of repeat liver resections for recurrent metastases. We, therefore, analyzed our experience of those selected patients in whom a second liver resection could be performed.

**Methods:** From 2002 until 2006 36 patients had a second liver resection for recurrent hepatic metastases of CRC in our department (28% female, median age 64 years) a median of 16 months after first liver resection. In all patients other (extrahepatic) tumor manifestations were excluded during preoperative

staging (CT/MRI, PET). 84% of the patients had received chemotherapy prior to re-resection. A segmental or atypical resection was performed in 23 patients whereas the other 13 patients underwent hemihepatectomy. During 5 of the 36 re-resections an additional radiofrequency thermoablation was done. Survival analysis was performed using the Kaplan-Meier-method. The median follow-up after hepatic re-resection was 2 years (n = 34).

**Results:** Two of the 36 patients died postoperatively (mortality 5.6%). Perioperative complications (all) occurred in 50%, surgical complications in 25% and a hepatic insufficiency in two patients (5.6%). Blood transfusion were necessary in 37% of the re-resections. Free hepatic resection margins could be achieved in 78%. During the still relatively limited follow-up eight of 34 patients died of recurrent CRC. The cumulative 2. and 4-year survival rates in our series are currently 75% und 64%. The status of the resection margin showed a clear tendency to influence prognosis after hepatic re-resection: After a R-0-resection 4-year survival was 75% whereas it was only 36% in patients with positive resection margins (p = 0,12). No further potential prognostic factors could be identified up to now, also because of the limited follow-up time.

**Conclusions:** Repeat liver resections for recurrent metastases of CRC can be performed with acceptable morbidity and mortality in selected patients. Hepatic re-resections with free margins achieved (surprisingly) high survival rates in our series. In the context of modern multimodal treatment (especially chemotherapy) hepatic re-resection should always be considered in the absence of other tumor manifestations and in patients fit for major surgery.

PE569

### Adenocarcinoma of the ovary and transitional cell carcinoma of the bladder with metastases to the breast: Two case reports

Asciutto K.C., Bonatz G.

Augusta Krankenhaus, Klinik für Gynäkologie und Geburtshilfe, Bochum

**Introduction:** Metastasis of ovarian or transitional cell carcinoma to the breast and/or axillary lymph nodes are a rare event. Nevertheless, their recognition and distinction from mammary carcinoma are of great clinical importance because the treatment and prognosis differ significantly. We report two unusual cases of extramammary neoplastic diseases with metastases to the breasts

**Case Report:** A 53 year old woman with stage IV ovarian adenocarcinoma at primary presentation developed an isolated CA 15-3 elevation, 42 months after tumor reduction operation and palliative chemotherapy. Further diagnostic investigations were performed to rule out the existence of a breast carcinoma. Mammography detected multiple discrete lesions in the right breast.

In the other case a 57 year old woman presented with abdominal pain and impaired renal function. CT-scans revealed an pelvic mass infiltrating the uterus and the ureter. Histological confirmation was gained via cystoscopy detecting an transitional cell carcinoma of the bladder (stage IV). Besides disseminated metastatic lesions of the axial skeleton, the peritoneal and muscular tissue, CT scans also showed suspect masses in the left breast.

In both patients mammography confirmed the presence of well-circumscribed lesions with increased density but without speculations, calcifications or other typical radiological signs of primary breast cancer. Also breast ultrasound revealed circumscribed masses without the retrotumor acoustic shadowing typical for malignant masses. In the first patient additional MRT also revealed suspect transformation of lymph nodes in the right axilla.

Definitive histologic diagnosis of metastatic breast lesions was performed with the help of ultrasound guided High-Jet fine needle biopsy. In both women the obtained specimen were negative for estrogen and progesterone receptors and showed a negative expression of Her 2/neu supporting the diagnosis of a metastatic process.

**Conclusions:** Most patients with metastatic breast lesions suffer from an advanced malignant disease. Breast and/or axillary lymph node involvement can occur at initial presentation of the carcinoma but is rare. Ultrasound-guided biopsy seems to be an effective diagnostic tool to confirm the histological origin of tumors that either clinically nor radiographically are typical for primary breast cancer. Further histochemical investigations can be helpful in establishing the correct diagnosis.

PE570

### Morbidity and mortality of the cytoreduction with Hyperthermic Intraperitoneal Chemotherapy (HIPEC): Experience in 50 patients

Roitman M., Link K.-H.

Asklepios Paulinen Klinik, Chirurgisches Zentrum, Wiesbaden

**Background:** Intraperitoneal chemotherapy is effective in treatment concepts involving cytoreduction with peritonectomy in combination with hyperthermic abdominal perfusion and postoperative chemotherapy. Cytoreductive surgery with peritonectomy and hyperthermic abdominal perfusion may be indicated in selected patients with peritoneal carcinomatosis of colorectal cancer (evidence level 1), pseudomyxoma peritonei and mesothelioma (evidence level 2-3).

**Methods:** From February 2002 to February 2007 in our hospital cytoreduction with peritonectomy and hyperthermic intraperitoneal chemotherapy (HIPEC) has been used in 50 patients with intraperitoneal malignancy; mean age was 53 years; 28 patients were female and 22 male. 26 patients had a diagnosis of gastrointestinal cancer as primary (colon-15, appendix-8, stomach-2, small bowel-1), 9 had pseudomyxoma peritonei, 5- peritoneal mesothelioma, and 10 ovarian cancer. Multiorgan resection was necessary in 44 from 50 patients (88 %). Macroscopically complete cytoreduction (tumor rests < 2.5 mm) was performed in 42 from 50 patients (84 %). Hyperthermic intraperitoneal chemotherapy (HIPEC) was performed after all resections were completed and before the construction of anastomosis. 42 patients received intraperitoneal chemotherapy with mitomycin C 10 mg/l in 5 liter carrier solution, and 8 patients received CDDP 25 mg/m<sup>2</sup>/l in combination with mitomycin C 3.3 mg/m<sup>2</sup>/l. Perfusion was performed with open abdomen at 42° Celsius for one hour under strict safety conditions.

**Results:** The morbidity rate was 22 % (n = 11). Most frequent complications were intestinal fistula (4/50; 8 %) and anastomotic leakage (2/50; 4 %). In one patient (2 %) was postoperatively haematological toxicity WHO IV<sup>o</sup> observed. This patient had been preoperatively treated with intensive palliative CDDP-based chemotherapy protocols and died postoperatively from severe bone marrow suppression. Accordingly hospital mortality (30 days after treatment) was 2 %.

**Conclusions:** Cytoreductive surgery with peritonectomy and hyperthermic intraperitoneal chemotherapy is promising method of treatment for selected patients with limited peritoneal carcinomatosis. Cytoreductive surgery with HIPEC has been performed safe in our interdisciplinary oncologic unit with acceptable low morbidity and mortality by a specialized surgical oncological team.

PE571

### Side effects and toxic responses caused by 5 cytostatic substances in an experimental animal model of peritoneal carcinomatosis

Hribaschek A.<sup>1</sup>, Ridwelski K.<sup>1</sup>, Meyer F.<sup>2</sup>, Kuester D.<sup>3</sup>, Halangk W.<sup>2</sup>, Lippert H.<sup>2</sup>

<sup>1</sup>Municipal Hospital, Department of Surgery, Magdeburg; <sup>2</sup>University Hospital, Department of Surgery, Magdeburg; <sup>3</sup>University Hospital, Institute of Pathology, Magdeburg

The aim of this study was to investigate side effects & toxic responses of various cytostatic substances previously tested for their efficacy to prevent &/or treat peritoneal carcinomatosis in rats.

**Methods:** Established & novel antineoplastic drugs such as mitomycin (10 mg/m<sup>2</sup>), cisplatin (25 mg/m<sup>2</sup>), 5-FU (425 mg/m<sup>2</sup>), oxaliplatin (60 mg/m<sup>2</sup>) & CPT-11 (300 mg/m<sup>2</sup>) (dosages according to their LD50) were applied intraperitoneally (i.p.) after tumor cell transfer (n = 1,000,000) via laparotomy (groups of 8 animals/drug; 2 control groups [sham operation -/+ tumor cells]) in a systematic comparative study. Animals were sacrificed & autopsied on the 30th day. Side effects & toxic responses were characterized by occurrence of i) & ii) necrosis (yes/no) at the peritoneal & liver surface, respectively, iii) i.p. bleeding & iv) death (positive [therapeutic] control, cytostatic effects of the 5 agents indicated by tumor weight & “-/+” detectable tumor growth).

**Results:** Mitomycin & cisplatin were the most toxic substances (e.g., peritoneal necrosis in 5 & 7 animals out of 8, respectively) but this correlated with the most pronounced cytostatic effect (no detectable tumor growth). Though

oxaliplatin showed also a high rate of necrosis (n = 8/8) & death (n = 4/8), its therapeutic potential was only low (tumor detectable in each animal). It was not surprising that necrosis at the peritoneal surface was the most sensitive characteristic of side effects & toxic responses. In addition, bleeding was frequently associated with death prior to the 30th day after tumor cell transfer. Interestingly, CPT-11 provided the best compromise in decreasing i.p. tumor growth & an acceptable rate of side effects.

**Summary and conclusion:** Despite some favorable effects of novel & established cytostatic drugs in i.p. chemotherapy, side effects & toxic responses need to be simultaneously tested even in earlier stages of drug development & experimental as well as clinical studies for an appropriate dose adaptation & escalation. Further studies should also focus on other parameters & study characteristics such as i) combination & interaction of drugs, ii) various application time points & modes (e.g., i.p. versus i.v.) & iii) effects on wound & anastomosis healing as well as iv) induction of peritonitis.

PE572

### Monoisozentrisch, gleichzeitige IMRT-Radiochirurgie von bis zu 3 Hirnmetastasen mit dem System hyperion – Elekta-synergy-s – Eine Planungsstudie an 5 Patienten

Pütz M., Paulsen F., Ganswindt U., Alber M., Bamberg M., Belka C.

Universität, Radioonkologie, Tübingen

**Fragestellung:** Zielsetzung der Arbeit war es, im Rahmen einer Planungsstudie ein konventionell radiochirurgisches Vorgehen mit einem IMRT basierten Vorgehen (mit einem Isozentrum) bei Patienten mit bis zu drei Hirnmetastasen zu vergleichen. Hierbei war es von besonderem Interesse, zu bestimmen, inwieweit mehrere Metastasen gleichzeitig mit einem Isozentrum erfasst und, ob sehr kleine Metastasen adäquat umschlossen werden können.

**Methodik:** Für fünf Patienten die mit Rundlochkollimatoren (Radionics, Xknife) wegen Hirnmetastasen als Einzeistereotaxie bestrahlt wurden, sind Alternativpläne mit dem IMRT Planungssystem Hyperion für einen Linearbeschleuniger Elekta Synergy S (Leafbreite 4mm im Isozentrum) generiert worden.

Die ausgewählten Patienten hatten eine oder drei Hirnmetastasen mit einem Durchmesser zwischen 4 mm und 30 mm. Die Dosisverschreibung erfolgte auf die tumorumschließende Isodose. In den ausgewählten Fällen wurden 15 bis 20 Gy verschrieben.

**Ergebnis:** Die mit den Rundlochkollimatoren gewählten tumorumschließenden Isodosen lassen sich auch mit dem System “Hyperion – Elekta Synergy S” planen. Die Normalgewebisdosen sind vergleichbar. Im Fall von 3 Hirnmetastasen ist in den beiden ausgewählten Fällen die gleichzeitige Bestrahlung aller drei Metastasen (mit nur einem Isozentrum) möglich.

Bis zu fünf unterschiedliche Tischauswinkelungen wurden zur Planung benötigt. Der Abstand zwischen den Gantrypositionen bei gleicher Tischauswinkelung betrug in der Regel 15°. Zur Erzielung optimaler Dosisverteilungen waren bis zu 30 Gantrypositionen und bis zu 60 Segmente erforderlich. Als Constraints wurden für die intracraniellen Normalgewebe wie Hirnstamm, Innenohr usw. mittlere Höchstdosen festgelegt. Die Kontrolle des Dosisgradienten um das Planungszielvolumen erfolgte mittels spezifischer Zielvolumen-Constraints. Innerhalb des Tumorumfanges wurde die Maximaldosis begrenzt.

**Schlussfolgerung:** Die Planungsstudie zeigt, dass eine Radiochirurgie von Hirnmetastasen mit dem verwendeten System als IMRT mit guten Dosisverteilungen bei gleichzeitiger Erfassung von bis zu drei Herden möglich ist. Die erzielten Dosisverteilungen entsprechen denen einer etablierten Radiochirurgie mit Rundlochkollimatoren.

PE573

### Targeting cell death and survival pathways for the modulation of treatment resistance

Jendrossek V.<sup>1</sup>, Handrick R.<sup>1</sup>, Rübél A.<sup>2</sup>, Elsässer S.<sup>2</sup>, Rudner J.<sup>2</sup>, Müller A.-C.<sup>2</sup>, Belka C.<sup>2</sup>

<sup>1</sup>Universitätsklinikum Essen, Institut für Zellbiologie, Essen; <sup>2</sup>Universität Tübingen, Klinik für Radioonkologie, Tübingen

During the last two decades, extensive research aimed at the identification of compounds specifically targeting altered signalling pathways of tumour cells.

The first generation of these novel anticancer agents such as the tyrosine kinase inhibitor imatinib has already been successfully introduced into clinical application.

It turned out that most malignant tumours are characterized by deregulated pathways related to the regulation of cell growth, survival and apoptosis. In this regard, inactivation of apoptosis and/or over-activation of pro-survival pathways can contribute to resistance to classical anticancer drugs and ionizing radiation. Consequently, deregulated cell death pathways of tumour cells constitute an important target for the modulation of treatment resistance. From the complex cell death and survival signalling network several major targets emerged that may be of value for a biological modulation of treatment resistance. In this regard, small molecules targeting cell surface receptors, survival kinases, transcription factors and further components of the cellular death pathways are at present exploited for their use as single agents as well as in combination with chemo- or radiotherapy in preclinical and clinical investigations. A prerequisite for the rational use of cell death targeting agents is a detailed knowledge on the cellular cell death and survival signalling network, the molecular mechanisms of therapy-induced cell death and associated resistance mechanisms as well as specific alterations of cell death and survival signalling within a given tumour.

## Cancer Screening and Quality Assurance

### Oral Presentation

OP215

#### Digital medical documentation and communication: New software overcoming sectorial boundaries among different intrahospital and general practitioners" systems to assess the quality of cancer patients" outcome

Mohr M., Klinkhammer-Schalke M.

Tumorzentrum Regensburg e. V., Regensburg

**Introduction:** To improve the quality of cancer treatment for patients in Germany, standardized ways of medical documentation are necessary and must be legally regulated. With all historic software developments for oncologic data acquisition being mostly proprietary of data format, the need for a countrywide usable "harmonizing" common basic data set and new software applications making use thereof arises.

**Methods:** The Tumor Center Regensburg (TCR, founded in 1991) is equally composed of regional hospitals (43), general practitioners (1.200) in the Bavarian districts Oberpfalz and Niederbayern, and the University of Regensburg. The TCR's primary goal is the improvement of the quality of cancer care.

Both initial and especially follow-up diagnostic and treatment data (1992 to 2007: 124.000 patients) are processed by the TCR. After many steps to standardize oncologic data acquisition, the decision was made to develop a new software application depicting the entire process of data acquisition, data analysis, and data communication in concordance to the German data security regulations in one single suite of applications.

The new software integrates many aspects of modern data handling: Implementation of 51 tumor entities, bidirectional data exchange through various interfaces, selective data export to disease management programmes and quality assuring societies, internal and external benchmarking, automatic generation of discharge letters and tumor conferences, automatic readmission scheduling according to organ-based guidelines, and many others.

In the Regensburg context, physicians communicate every patient via a secured internet platform, both discuss and adopt therapies, and improve treatment with a mandatory interdisciplinary consensus.

**Results:** After three years of use of the new software, far better data quality is accomplished. Especially the new software's integrated data analysis portal as well as the automated readmission scheduler were widely used applications of internal and external (through TCR) quality control. In this fashion, the implementation of guidelines e.g. for breast-conserving therapy rose from 45 % in 2001 up to 72 % in 2006. Similar results were obtained for the control of e. g. chemotherapy in colon cancer stage UICC III (from 45 % in 1998 up to 80 % in 2006).

**Conclusion:** By the use of a modern software application integrating the most actual documentation standards, data exchange facilities, and data analysis, the documentation cleft between intrahospital and general practitioners can be reduced significantly.

OP216

#### Follow-up care for breast cancer – Guideline, study, practice and patient wishes: Results of a German follow-up treatment study and literature review

Heidemann E.<sup>1</sup>, Meisner C.<sup>2</sup>, Brinkmann F.<sup>1</sup>, Rössle S.<sup>1</sup>

<sup>1</sup>Onkologischer Schwerpunkt Stuttgart e.V., Stuttgart; <sup>2</sup>Universität Tübingen, Institut für Medizinische Biometrie, Tübingen

**Objects:** Comparison of symptom-oriented and apparatus-oriented follow-up care strategy over a follow-up observation period of five years following primary therapy in regard to the overall time of survival and the recurrence time, in Germany. Survey of the acceptance of and motivation for the follow-up care strategy.

**Methods:** Reviews of studies and literature, patient surveys regarding the choice of follow-up care form and prospective, comparative, multi-centre, non-randomised cohort study from 1995 to 2005 on 665 patients.

**Results:** The results of the study confirm that regularly performed imaging procedures and laboratory investigations have no significant influence on the survival of breast cancer patients following curative primary therapy and support the introduction of symptom-oriented follow-up treatment in routine care. The literature shows that symptom-oriented follow-up care has been incorporated into the S3 guideline and the disease management programme (DMP) for breast cancer on a national scale. Internationally, this has been the standard for some time. Discussions indicate that, at the present time, the persons affected continue to request, above all apparatus-oriented, follow-up care.

**Conclusion:** Symptom-oriented follow-up care has required a long time to gain acceptance in the form of guidelines and in practice and does not always conform to the need for reassurance of different patient characters, as shown by a specially developed patient survey. Practice and study at the OSP Stuttgart have shown that quality management of follow-up care offers both patients and doctors the required assurance and can be successfully integrated in the disease management programme for breast cancer.

OP217

#### Assessment of new avenues of screening for colorectal cancer based on novel molecular stool tests (BliTz-Study)

Hundt S., Haug U., Brenner H.

Deutsches Krebsforschungszentrum, Klinische Epidemiologie und Altersforschung, Heidelberg

**Background:** With more than 1 million new cases and about 530,000 deaths per year, colorectal cancer (CRC) is the third most common fatal malignancy in the world. Due to its slow development from curable precancerous lesions, a large proportion of CRC cases and CRC deaths may be prevented through the use of screening tests. However compliance with current screening recommendations is poor.

The success of a particular screening strategy strongly depends on the performance characteristics, but also on practicability and acceptability of the screening method. As stool-tests are non-invasive and require no cathartic bowel preparation or medication, they meet crucial criteria of acceptable and safe screening methods.

**Methods:** Starting in January 2006, we set up a study (BliTz, "Begleitende Evaluierung innovativer Testverfahren zur Darmkrebs-Früherkennung") in South-west Germany to assess the ability of novel stool tests to predict abnormal colonoscopic findings in a large sample of asymptomatic people undergoing screening colonoscopy. Participants are recruited through a network of 20 gastroenterological practices. Stool and blood samples are collected from participants prior to preparation for colonoscopy. Furthermore patients are asked to fill out a standardized questionnaire, and colonoscopy and histology reports are collected.

**First Results and Outlook:** By October 2007, 1800 screening participants have been recruited. As a first step, comparative evaluation of 6 different immunochemical office-tests for blood in stool (iFOBT), a laboratory-based immunochemical measurement of fecal human hemoglobin (ELISA) and guaiac-based fecal occult blood test (gFOBT) is currently ongoing. By this presentation, we would like to present preliminary results of this evaluation and to inform the scientific community about this new study, which is still open for collaboration.

OP218

### Stage-specific breast cancer incidence after implementation of the pilot project mammography screening in the weser-ems region (lower saxony)

Urbschat I., Kieschke J.

Epidemiologisches Krebsregister Niedersachsen, Registerstelle, Oldenburg

**Background:** Before introducing the centralized Mammography Screening into the German health care system, the pilot project Mammography Screening Weser-Ems (MSWE) had tested the organised screening in the Weser-Ems region in the period 2002 to 2005. At the start of the screening an increase of the breast cancer incidence is to be expected. A first sign of mortality reducing effects of the Mammography Screening would be a decrease of the incidence of advanced breast cancers in the second screening round. The aim of this study is to compare pre-screening and screening periods and to estimate the effects of the pilot project MSWE on the stage-specific breast cancer incidence in the Weser-Ems region.

**Methods:** In the MSWE pilot project (5/2002-3/2005) approximately 23.000 50 to 69 year old women were invited to a biennial population-based quality assured Mammography Screening. Approximately 65% of the invited women took part in the prevalent screening round. The age-standardized breast cancer incidence for the screening area will be estimated with data from the Epidemiological Cancer Registry of Lower Saxony (EKN). The completeness of breast cancer registration exceeds 90 percent in the EKN. All breast cancers diagnosed in 50 to 69 year old women between 2000 and 2005 were analysed (n = 504, data base July 2007). Additional stratified analyses take place for in-situ-carcinomas and small tumours < 20 mm (Tis + T1) and large tumours (T2-T4: size > = 20 mm).

**Results:** At the start of the screening, a strong increase of the age-standardized breast cancer incidence (invasive and in-situ-carcinomas) from 291/100.000 (2001) to 439 (2002) is observed. Afterwards, a steady decrease of the incidence takes place down to 255 (2005). The incidence of the large tumours increased from 125 (2001) to 153 (2002). Since 2003 a steady decrease is observed down to 74 in 2005. In comparison to 2001, the reduction of the incidence rate of large tumours amounts to approximately 40 percent.

**Discussion:** The results of this study confirm an increase of the breast cancer incidence after the start of the screening. Primarily this is founded in the increasing diagnosis of small tumours. In the second screening round, the decreasing incidence of great tumours is remarkable. Representative statements will be possible after inclusion of other screening area in the study. Furthermore the study confirms that cancer registries are important in monitoring the effect of screening programmes.

OP219

### The role of stereotactic biopsies in the German breast cancer screening program: Demand for structured cooperation

Junkermann H.<sup>1</sup>, Bauer L.<sup>2</sup>, Daniel H.<sup>3</sup>, Job H.<sup>4</sup>, Maleika A.<sup>5</sup>, Rahmfeld T.<sup>3</sup>, Schmidt D.<sup>6</sup>, Schütz F.<sup>5</sup>, Sinn H.-P.<sup>7</sup>, Sohn C.<sup>5</sup>

<sup>1</sup>Universität, Sektion Senologische Diagnostik, Heidelberg;

<sup>2</sup>Kreis Krankenhaus, Frauenklinik, Weinheim; <sup>3</sup>Radiologische Gemeinschaftspraxis, Mannheim; <sup>4</sup>Theresienklinik, Hedwigsklinik, Mannheim; <sup>5</sup>Universität, Frauenklinik, Heidelberg; <sup>6</sup>Gemeinschaftspraxis für Pathologie, Mannheim; <sup>7</sup>Universität, Sektion Gynäkopathologie, Heidelberg

**Purpose:** To analyze the role of delegated stereotactically guided biopsy in a German regional breast cancer screening program

**Method and Materials:** The local screening program covers a region of 1,1 million inhabitants comprising 137 000 women between 50 and 70 years of age. Mammography is carried out in 4 offices in the region. All stereotactically guided biopsies are performed in the section of breast diagnostics of the womens hospital at the regional university. The results of all biopsies are discussed in a weekly conference in the screening center between the program responsible radiologist, the pathologist, the physician performing the stereotactic biopsies and the gynaecological surgeons from the three participating hospitals. The results of the stereotactic biopsies have been compiled and compared to the total results of the screening program.

**Results:** The program started in march 2007. 13107 women have been invited until August 31th. 8780 women participated. A large proportion of these have been invitations on their own initiative. In 62 women were carcinomas detected. 83 women have been sent for stereotactic biopsy. 75 of these women had a vacuum assisted biopsy, 6 women could be biopsied under ultrasound guidance, one woman received a primary open resection because of allergy against local anaesthesia and one woman opted for short term recall as the lesion probably resulted from a hematoma. In the 82 biopsied patients 33 carcinomas were detected (22 in situ, 11 invasive). In the 75 stereotactically biopsied patients 24 carcinoma were found (17 in situ, 7 invasive) Two of these were detected at open surgery for B3 lesions ( 1 in situ, 1 invasive).- 90 % of the women chose one of the screening accredited surgeons for therapy.

**Conclusion:** Half of all detected carcinomas have been found in the women sent for stereotactic biopsy. Therefore a close cooperation of the physicians running screening program with regular participation of the colleague performing the stereotactic biopsies is mandatory in order to maintain the continuous treatment path necessary for optimal results according to the European guidelines.

## Cancer Screening and Quality Assurance

### Poster Exhibition

PE639

### Chances and limits of benchmarking for process- and outcome-quality new possibilities due to web based analysis of data derived from cancer registry

Brinkmann F.<sup>1</sup>, Stauch A.<sup>2</sup>, Meisner C.<sup>2</sup>, Dippon J.<sup>3</sup>, Junack C.<sup>1</sup>, Stumpp C.<sup>1</sup>, Klenk S.<sup>3</sup>, Heidemann E.<sup>1</sup>

<sup>1</sup>Onkologischer Schwerpunkt Stuttgart e.V., Stuttgart; <sup>2</sup>Universität Tübingen, Institut für Medizinische Biometrie, Tübingen; <sup>3</sup>Universität Stuttgart, Institut für Stochastik und Anwendungen, Stuttgart

**Objectives:** In the Stuttgart region benchmarking of outcome quality for the oncology has been established as a solid component of quality management for the hospitals in Stuttgart. The process quality is demonstrated by comparing the actual performed diagnostic and therapeutical procedures with the valid diagnosis and treatment guideline. The outcome quality is analyzed and compared based on the overall survival (OAS), the relapse free survival (RFS) and the quality of life in relation to prognostic relevant factors. Within this project the client data of the three entities breast, colon- and rectum carcinoma, from 13 hospitals in the region, will be evaluated.

#### Method:

1. Prospective study 2003 to 2006 on breast cancer (n = 1061), colon cancer (n = 434) and rectum cancer (n = 322). Entity related, multivariate data analysis, taking into consideration the heterogeneity of the individual population of the hospitals.
2. Utilization of web based tools for retrospective analysis of patient data from 1986 to 2006 for 14.367 breast cancer patients.

#### Results:

- Valid demonstration of outcome quality requires a complete integrity and correctness of the collected data. Methods and procedures were developed so that data quality could be increased.
- Web based analysis of registered cancer data for expedient orientation and for hospitals to compare. Intranet based comparison of OAS in breast cancer so that an individualised prognosis and control on quality result is achieved.
- The multivariate analysis of the prospective study showed differences in the outcome quality between hospitals, on which now an incentive analysis is performed taking clinical principles into consideration.

**Conclusion:** Benchmarking and web based tools are to be used to control the outcome quality

A maximum of prognosis relevant criteria are to be taken into consideration for an accurate comparison.

Limits show up while comparing the complete hospital profiles. Economic factors of data ascertainment and the necessary data quality must be accomodated.

PE640

### Blood markers for early detection of colorectal cancer. A systematic review

Hundt S., Haug U., Brenner H.

Deutsches Krebsforschungszentrum, Klinische Epidemiologie und Altersforschung, Heidelberg

**Background:** With more than 70.000 new cases per year, colorectal cancer is the most frequent cancer in Germany. Despite different available methods for colorectal cancer (CRC) screening and their proven benefits, morbidity and mortality of this malignancy are still high, partly due to low compliance with screening. Minimally invasive tests based on the analysis of blood specimens may overcome this problem.

**Method:** We searched the MEDLINE database for relevant studies published until August 2007. Only studies with more than 20 cases and more than 20 controls were included. Information on the markers under study, on the underlying study populations and on performance characteristics was extracted. Special attention was given to performance characteristics by tumor stage.

**Results:** Overall, 101 studies evaluating 75 different markers were included. Most studies have been done on protein markers (75 studies, 57 different markers) and RNA markers (20 studies, 13 different markers). Genetic and epigenetic markers were assessed in 7 studies and cytological assays were investigated in 3 studies. Performance characteristics varied widely between different markers, but also between different studies using the same marker. Promising results were reported for some novel assays, e.g. assays based on SELDI-TOF MS or MADLI-TOF MS, for some proteins (e.g. soluble CD26 and C3a-desarg) and also for some genetic assays (e.g. L6 mRNA), but evidence so far is restricted to single studies with limited sample size and without further external validation.

**Conclusion:** Many approaches have been reported to develop and evaluate new blood-based tests for the early detection of colorectal cancer. According to some recent reports some tests may have the potential to improve current screening, but phased validation strategies resulting in large-scale prospective evaluation of the most promising candidates and final cancer control studies are needed. In addition, future studies should pay increased attention to the potential of detecting precursor lesions.

**Reference:** Hundt et al. 2007 Cancer Epidemiology Biomarkers and Prevention 16(10):1935–1953

PE642

### Survival of cancer patients in Hamburg – Routinely clinical utilisation of cancer registry data

Hentschel S.<sup>1</sup>, Nennecke A.<sup>1</sup>

<sup>1</sup>Hamburgisches Krebsregister, Hamburg

**Objective:** The Hamburg Cancer Registry (HCR) implemented a computer based surveillance system serving both epidemiological and clinical interests in data on oncological outcome quality. The main aim of this project, which has been professionally and financially supported by the Hamburg Cancer Society, is to supply clinical oncologists with a routine feedback on their patient's long-term survival. Regarding epidemiological aspects the focus is on estimation of cancer specific survival and timely statements on trend alterations. In contrast the attending physician needs an appealing and clearly arranged presentation of his patients' survival versus an adequate comparison group.

**Method:** The HCR collects data on malignant neoplasms from various sources converting them to a 'best-of information' for each incident case according to international rules. If cases are attributable to a patient's address in Hamburg at the time of diagnosis they are followed up systematically by means of further notifications and residential registration offices' information on death and change of residence or names. In addition all death certificates issued in Hamburg are evaluated concerning unidentified cancer diagnoses and the decease of registered patients, thus enabling a further validation of data and an active investigation of not notified cases.

In collaboration with clinical oncologists a report structure covering the institution's notifying activity and the survival of its patients was developed. The routine analysis includes observed and relative survival rates of patient cohorts defined by period of diagnosis, sex and treating institution, compared to a Hamburg reference. Further stratifications by age, stage, etc. are available on request.

**Results:** In Hamburg a periodical feedback report on cancer patient survival for clinical oncologists has been established. A considerable degree of automation ensures performance at regular intervals even in view of limited registry staff. Population-based cancer survival in Hamburg ranges among that published by neighbouring German and European registries, while the rates relating to several treating institutions vary considerably.

**Conclusions:** Statements on oncological outcome quality are highly relevant in the medical and public context as pointed out by the current discussion of internationally published cancer survival data (e.g. EURO-CARE4). Apart from scientific aspects results the necessity of regional and institution-specific cancer survival information is obvious to detect time trends early, to generate hypotheses and to optimise intra-institutional quality management. The evaluation and interpretation of clinic specific cancer survival analysis requires a structured and long-ranging cooperation of population-based cancer registry and treating oncological institution to produce reliable and comparable results.

PE643

### Serumtest für C3A Anaphylatoxin ermöglicht minimal-invasives Screening für kolorektale Tumoren

Habermann J.K.<sup>1</sup>, Roblick U.<sup>1</sup>, Luke B.<sup>2</sup>, Prieto D.<sup>3</sup>, Finlay W.<sup>4</sup>, Oevermann E.<sup>1</sup>, Schiedeck T.<sup>1</sup>, Homann N.<sup>5</sup>, Conrads T.<sup>3</sup>, Veenstra T.<sup>3</sup>, Burt S.<sup>2</sup>, Bruch H.-P.<sup>1</sup>, Auer G.<sup>6</sup>, Ried T.<sup>7</sup>

<sup>1</sup>Universitätsklinikum Schleswig-Holstein, Campus Lübeck, Chirurgisches Forschungslabor, Klinik für Chirurgie, Lübeck; <sup>2</sup>National Cancer Institute Frederick, Advanced Biomedical Computing Center, Frederick; <sup>3</sup>National Cancer Institute Frederick, Laboratory of Proteomics and Analytical Technologies, Frederick; <sup>4</sup>Dublin City University, Biomedical Diagnostics Group, Dublin; <sup>5</sup>Universitätsklinikum Schleswig-Holstein, Campus Lübeck, Internal Medicine Department, Lübeck; <sup>6</sup>Karolinska Institute, Dept. of Oncology and Pathology, Stockholm; <sup>7</sup>National Cancer Institute, Genetics Branch, Bethesda

**Einleitung:** Eine späte Diagnose kolorektaler Malignome resultiert in einer signifikant verminderten Überlebenswahrscheinlichkeit. Trotz etablierter Screeningverfahren werden jedoch über 50% der kolorektalen Karzinome erst in einem späten Tumorstadium mit entsprechend schlechter Prognose diagnostiziert. Die Suche nach innovativen Biomarkern für eine verbesserte Frühdiagnose ist daher von äußerster klinischer Relevanz. Daher war es das Ziel dieser Studie, zu untersuchen, ob sich kolorektale Tumoren aufgrund tumor-spezifischer Serumprotein-Marker detektieren lassen und diese somit helfen könnten, maligne Entartungen bereits im Frühstadium einer präventiven oder therapeutischen Intervention zuzuführen.

**Methoden:** Untersucht wurden Serumproben von Patienten mit kolorektalen Karzinomen (n = 58) und von gesunden Kontrollpersonen (n = 32). Diese insgesamt 90 Serumproben wurden als Trainingset auf potentielle Unterschiede in ihrer Serumproteinexpression hin mittels Surface-enhanced Laser Desorption/Ionization Time-of-Flight Massspektrometrie (SELDI-TOF MS) analysiert. Potentielle Markerproteine wurden identifiziert und ihr Expressionsniveau mittels spezifischer Immunassays (ELISA) in einem unabhängigen Testset (n = 59) validiert.

**Ergebnisse:** Die Expressionsmuster der Trainingset-Serumproben wurden mittels verschiedener statistischer Analysetechniken ausgewertet. Potentielle Serummarker, die sich in ihrer Expression zwischen der Gruppe der Erkrankten und den Kontrollpersonen unterschieden und in der Mehrzahl der Analysen gleichermaßen ein hohes Diskriminierungspotential zeigten, wurden nachfolgend identifiziert. Der viel versprechendste Marker wurde als Teil des Komplementsystems, und zwar als stabile Form von C3a Anaphylatoxin, d.h., C3a-desArg identifiziert. Die Validierung dieser Serumproteins mit Hilfe eines speziellen ELISAs in einem unabhängigen und geblindeten Testset (n = 59) erbrachte eine Sensitivität und Spezifität von über 96%. Hierbei wurden keine signifikanten Unterschiede in der Detektierung früher (UICC I + II) und fortgeschrittener Karzinome (UICC II + IV) offenbar. Erhöhte Serumspiegel waren auch in 86.1% der Seren von Patienten mit kolorektalen Adenomen zu beobachten (n = 36).

**Zusammenfassung:** Im Vergleich zu gesunden Kontrollpersonen weisen Patienten mit kolorektalen Adenomen (p < 0.0001) und mit kolorektalen Karzinomen (p < 0.0001) signifikant höhere C3a-desArg Serumspiegel auf. Die Quantifizierung des C3a-desArg Serumlevels, z.B. mittels ELISA-Test, könnte somit das Ensemble bislang etablierter Screeningmethoden für kolorektale Tumore bezüglich Sensitivität und Spezifität verstärken.



## Ultrasound as a sensitive approach to breast cancer in BRCA1/2 mutation carriers

Bosse K.<sup>1</sup>, Wimber I.<sup>2</sup>, Rhiem K.<sup>2</sup>, Warm M.<sup>2</sup>, Mallmann P.<sup>2</sup>, Schmutzler R.<sup>1</sup>

<sup>1</sup>Centre for Hereditary Breast and Ovarian Cancer, Department of Gynecology and Obstetrics, University hospital of Cologne, Cologne;

<sup>2</sup>Department of Gynecology and Obstetrics, University hospital of Cologne, Cologne

**Objective:** BRCA1/2 mutations confer a significantly elevated lifetime risk for breast cancer reaching up to 80%. The currently performed intensified surveillance programme include semi-annual ultrasound (US) and annual mammography (MG) and MRI beginning at the age of 25. We evaluated the efficacy of sonography for the early detection of breast tumour in women with a genetic predisposition.

**Patients:** Data were collected in a prospective study from 137 BRCA1 and 86 BRCA2 mutation carriers who participated in the surveillance programme from 1997-2007. Surveillance consisted of ultrasonography every six months and mammography and MRI scan once a year. All three imaging modalities were coded according to the American College of Radiology Breast Imaging and Reporting Data System (ACR BI-RADS). The overall assessment was performed according to a five-point scale as indicated in the ACR BI-RADS lexicon.

**Results:** We detected 12 primary and 9 secondary contralateral BRCA-associated tumours in 19 patients (17 BRCA1 and 2 BRCA2 mutation carriers). Age at diagnosis ranged from 29 to 55 years (median 37). In 5 cases we discovered prevalent tumours in the first screening round. Mean tumour size measured 1.2 cm and 17 patients (81%) were diagnosed with stage I disease. According to BI-RADS classification IV and V mammography enabled detection of 7 (33%) cancers, US of 14 (66%); and MRI of 20 (95%). With regard to the sonomorphological characteristics of BRCA1-related tumours (clear cut margins, syncytial growth pattern etc.) resembling fibroadenomas we further upgraded 4 lesions from BI-RADS III to BIRADS IV. This led to an increased sensitivity for US to 85%. Moreover, we detected a considerably elevated tumour doubling time in BRCA1 mutation carriers compared to sporadic carcinomas (45 days versus 80 days). Two BRCA1 mutation carriers presented with palpable interval tumours before the next appointment was scheduled. This means an interval cancer rate of 10% (2 out of 19 BRCA1-associated cases).

**Discussion:** Taking into consideration the specific tumour morphology and the fast growth rate in BRCA1 mutation carriers the addition of semi-annual ultrasound may offer a sensitive and cost-effective method especially in this subgroup of mutation carriers in addition to annual mammography and MRI.

## Cancer Surgery

### Oral Presentation

OP205

#### Outcome according to local therapy modality in localized osseous ewing tumors

Jürgens H.<sup>1</sup>, Dirksen U.<sup>1</sup>, Paulussen M.<sup>2</sup>, Gebert C.<sup>3</sup>, Gosheger G.<sup>3</sup>, Winkelmann W.<sup>3</sup>, Bölling T.<sup>4</sup>, Willich N.<sup>4</sup>, Ranft A.<sup>1</sup>

<sup>1</sup>Universitätsklinikum, Pädiatrische Hämatologie und Onkologie, Münster;

<sup>2</sup>Universitätsklinikum (UKBB), Pädiatrische Hämatologie/Onkologie, Basel;

<sup>3</sup>Universitätsklinikum, Allgemeine Orthopädie, Münster;

<sup>4</sup>Universitätsklinikum, Radiotherapie und Radioonkologie, Münster

**Background:** Local therapy plays an essential role in Ewing tumor treatment. The specific impact of timing and type of local therapy modality and its interaction with known risk factors such as size, response and margins are still under debate.

**Cohort and Methods:** 645 osseous Ewing tumor patients, treated from 1991-2006 according to the appropriate GPOH studies, were analysed with univariate and multivariate survival analyses. The patients had localized disease of pelvis (30.1%), long bones (48.6%) or spine (11.3%). All received adjuvant chemotherapy according to the EICESS 92 or EURO-E.W.I.N.G. 99 protocol. Local control was obtained with surgery alone (29.9%), surgery and radiotherapy (51.9%) or radiotherapy alone (18.1%). Median follow up was 4.5 years.

**Results:** Long bone tumors showed superior outcome with 3-y EFS of 0.75, followed by spinal tumors with 0.70, and pelvic tumors with 0.51 ( $p < .001$ ). For all patients radiotherapy alone was inferior in outcome ( $p = .009$ ), but site dependent no significant differences were found with respect to local therapy modality. Pelvic and spine tumor patients received more often radiotherapy alone (31% and 45%), long bone patients more surgery alone (43%,  $p < .001$ ). In fine-tuned analyses a significant inferior outcome was found for radiotherapy alone in large tumors ( $>200\text{ml}$ ), especially in pelvic sites ( $p < .001$ ). Surgery patients with poor histological response (17.9%) or bad margins (21.2%) appeared to benefit from radiotherapy in contrast to patients without risk factors.

**Conclusions:** Surgery is essential for large volumes and additional radiotherapy reduces the poor outcome of patients with other risk factors. The inferior outcome for radiotherapy alone is size dependent, and, due to selection in this cohort, site dependent. More large pelvic tumors received radiotherapy alone while spinal tumors were mainly small and large long bone tumors generally received surgery. Definitive radiotherapy has its place in small tumors, especially where surgery is difficult or mutilating.

OP206

#### Portal branch ligation reduces initial outgrowth of colorectal metastasis followed by a compensatory angiogenic response and cell proliferation

Kollmar O.<sup>1</sup>, Corsten M.<sup>1</sup>, Scheuer C.<sup>1</sup>, Vollmar B.<sup>2</sup>, Schilling M.K.<sup>1</sup>, Menger M.D.<sup>1</sup>

<sup>1</sup>Universität des Saarlandes, Homburg/Saar; <sup>2</sup>Universität Rostock, Rostock

**Background:** Surgery is the only curative option for patients with liver metastasis from colorectal cancer. Whereas portal branch ligation (PBL) prior to resection may prevent liver failure after extended hepatic resection, clinical studies indicate that tumors within the ligated lobe develop accelerated growth. However, the effect of liver regeneration after PBL on tumor growth and tumor microvascularization is unknown in the literature. Therefore, we studied in a mouse model the time-dependent effect of PBL on angiogenesis and tumor growth of colorectal metastasis. **Material and Methods:** According to an established liver metastasis model  $5 \times 10^5$  CT-26 colon cancer cells were implanted in the left liver lobe of syngeneic BALB/c mice. Animals were randomized to PBL of the left liver lobe or control group. Microcirculatory responses and microvascular remodeling of the normal liver as well as angiogenesis, tumor cell proliferation, apoptosis and growth were studied 3d, 7d and 14d after PBL ( $n = 8$  each) using intravital multicolor fluorescence microscopy, laser Doppler fluxmetry, immunohistochemistry and biochemical techniques. **Results:** After 14 days tumor volume was significantly reduced by PBL ( $<20\%$  of controls) when compared to controls. During the first 14d PBL induced a reduction of left hilar blood flow by  $\sim 50\%$ , resulting in a delayed development of an angiogenic front of the tumors, a reduced density of draining tumor venules and reduced functional sinusoidal density in the normal liver. Sinusoidal dilation at the tumor border was associated by a significant increase of VEGF expression. PBL was associated with a higher leukocyte response in the tumor and normal liver. Immunohistological analyses demonstrated that PBL significantly induced tumor cell and hepatocyte proliferation after 14 days as well as apoptosis over the 14 days observation period. **Conclusions:** Liver regeneration after PBL induces a late compensatory angiogenic response resulting in a delayed outgrowth of colorectal liver metastases. Microvascular remodeling within the ligated lobe and hepatocellular proliferation may explain the late accelerated tumor progression observed in patients after PBL.

OP207

#### Fascia-preserving radical prostatectomy – A prospective clinical validation

Baumunk D., Miller K., Jentzmk F., Kempkensteffen C., Hinz S., Schrader M., Schostak M.

Charité-Universitätsmedizin Berlin, Klinik für Urologie, Berlin

**Introduction:** Recent publications have shown that the fascia of the levator ani can be preserved during radical retropubic prostatectomy (RPX). It thus seems possible to more effectively spare neural plexus controlling continence and potency. First clinical reports were promising. This is a prospective clinical validation study of the procedure.

**Materials and Methods:** The technique was adapted at our hospital in November 2005. Learning curve effects and interoperator variations were ruled out by including only interventions performed by one very experienced operator (KM) between June 1, 2006, and August 30, 2007. Apart from clinical parameters, functional results (continence and potency) were obtained after 3, 6 and 12 months using standardized questionnaires.

**Results:** Ninety-one interventions were included. Patients had a median surgery duration of 134 minutes (118-175), a median age of 65 years (43-74 years), a median preoperative PSA of 9.1 ng/ml (2.6-99 ng/ml), and a mean hospitalization of 6.6 days (2-19 days). The postoperative cystogram on day 3 (median) documented a watertight anastomosis in 96.8%. After consecutive removal of the catheter, 76.8% of the patients showed complete primary continence. An increase to 93.7% was seen in the further clinical course. The incontinence rate was 6.3% for grade 1 (0% for both grade 2 and 3). Urinary retention developed in 6.5% and was treated by recatheterization for one day. The potency results are preliminary due to the short follow-up in most cases. Seventy-five percent of the assessable preoperatively potent patients regained full potency after one year (IIEF >20). The great majority of patients had a locally confined stage; only one patient (1.09 %) had an R1 resection in stage pT2.

**Conclusion:** Pelvic-floor-preserving RPX achieves excellent functional results with constant oncological effectiveness.

OP208

### Evaluation of quality indicators in the surgical therapy of rectal cancer after implementation of total mesorectal excision

Vallböhmer D.<sup>1</sup>, Schneider P.M.<sup>1,2</sup>, Ploenes Y.<sup>1</sup>, Metzger R.<sup>1</sup>, Ling F.<sup>1</sup>, Brabender J.<sup>1</sup>, Baldus S.<sup>3</sup>, Drebber U.<sup>4</sup>, Hölscher A.H.<sup>1</sup>

<sup>1</sup>Universität zu Köln, Klinik und Poliklinik für Visceral- und Gefäßchirurgie, Köln; <sup>2</sup>Universitätsspital Zürich, Klinik für Visceral- u. Transplantationschirurgie, Zürich; <sup>3</sup>Universität Düsseldorf, Institut für Pathologie, Düsseldorf; <sup>4</sup>Universität zu Köln, Institut für Pathologie, Köln

**Background:** In recent years, improvements have been achieved in the outcome of patients with rectal cancer with advances in surgical techniques and (neo-)adjuvant therapy. An important breakthrough in the surgical management of rectal cancer was the advent of total mesorectal excision (TME) to improve local tumor control. Aim of this study was to evaluate the surgical and oncological outcome of primary resected rectal cancer patients by potential quality indicators after the implementation of TME.

**Patients and Methods:** Data of 164 consecutive patients (103 men, 61 women; median age: 64 years) with rectal cancer that underwent primary surgical resection between 1997 and 2004 were reviewed. The following potential quality indicators were evaluated: 1. number of resected lymph nodes; 2. selection of operative technique depending on tumor localisation; 3. primary construction of a protective loop ileostomy to prevent complications due to an anastomotic leakage; 4. frequency of local recurrence; 5. frequency of adjuvant therapy.

**Results:** According to the quality indicators, the following results were detected: 1. Patients with a higher number of lymph nodes resected were significantly associated with a higher nodal metastasis rate ( $p < 0.02$ ). 2. Patients with low rectal cancer underwent significantly more often an abdominoperineal rectum amputation compared with patients having a tumor in the upper rectum ( $p < 0.0001$ ). 3. Clinical symptoms but not the rate of anastomotic leakage (9.5%) was influenced by the primary construction of a protective loop ileostomy: 6 of 7 patients without a protective ileostomy became symptomatic due to a leakage and surgical revision was needed, where as none of the patients with a primary ileostomy needed a relaparotomy. 4. The frequency of local recurrence was 8.5%, whereby a higher T-stage predicted for a higher recurrence rate ( $p < 0.02$ ). 5. Forty-two (29.7%) patients received an adjuvant therapy, most of them with UICC stage III (61.9%). Interestingly, the local recurrence rate was higher in patients receiving adjuvant therapy compared with patients obtaining no postoperative treatment (14.2 vs. 6.1%).

**Conclusion:** The number of resected lymph nodes influences the N-stage, thus especially important regarding the indication for adjuvant therapy. The primary construction of a protective loop ileostomy significantly decreases the need for a surgical revision if an anastomotic leakage occurs. The local recurrence rate of 8.5% demonstrates that through the implementation of TME excellent oncological results can be achieved. However, in contrast to neoadjuvant treatment, the value of adjuvant therapy after the implementation of TME

remains highly controversial, because it is questionable if postoperative therapy additionally reduces the local recurrence rate.

OP209

### Postoperative complications after liver resections for colorectal metastases: Analysis of risk factors and influence of preoperative chemotherapy

Makowiec F.<sup>1</sup>, Neeff H.<sup>1</sup>, Adam U.<sup>1,2</sup>, Opitz O.G.<sup>3</sup>, Trepel M.<sup>3</sup>, Hopt U.T.<sup>1</sup>

<sup>1</sup>Chirurgische Universitätsklinik Freiburg, Freiburg; <sup>2</sup>Vivantes-Humboldt-Klinikum, Viszeral- und Gefäßchirurgie, Berlin; <sup>3</sup>Universitätsklinik Freiburg, Tumorzentrum Ludwig Heilmeyer, Freiburg

In the context of modern multimodal therapy liver resections for metastases of colorectal cancer (CRC) are frequently performed after chemotherapy (CTx). Several CTx-medications result in steatohepatitis and other liver changes up to fibrosis. Some initial larger studies recently reported conflicting results regarding complication rate after liver resection in correlation to prior CTx. We, therefore, analyzed our experience with postoperative complications after hepatic resection for CRC metastases.

**Methods:** 199 primary liver resections were performed at our institution for CRC metastases (wedge resections 14%, segmental 32% and hemihepatectomy/extended hemihepatectomy 54%). After prior CTx liver resection was performed not earlier than four weeks after the end of CT. A Ctx was performed in 62% before liver resection either as adjuvant CTx or as neoadjuvant therapy for metastases. During the analyses we subclassified also between the first and second part of the study period (P1 and P2) and between 'limited' (wedge and segmental) and 'extended' (at least hemihepatectomy) resection. In P2, under new direction of the hepatobiliary team, refined techniques and perioperative management strategies were applied. In P2 the annual numbers of liver resections increased 3-fold compared to P1.

Seven clinical and multiple preoperative laboratory parameters as well as preoperative CTx were evaluated on their prognostic influence on complications. Multivariate risk factors were analyzed by logistic regression.

**Results:** Any complication occurred in 48%, surgical complications in 32% and a hepatic insufficiency in 6.5%. mortality was 2.5%. In P1 four patients died perioperatively, in P2 only one (mortality < 1% in P2). Four of those five patients were diabetic (frequency of diabetes only nine percent in all 199 patients). Multivariate risk factors for any complication were a diabetes ( $p < 0.05$ ; relative risk RR 3,1) and extended resections. Risk factors for surgical complications were extended resections ( $p < 0.001$ ; RR 3,3) and male gender ( $p < 0.01$ ; RR 3,2). Independent risk factors for hepatic insufficiency were a diabetes ( $p < 0.02$ ; RR 2,0) and extended resections ( $p < 0.02$ ; RR 2,3). A diabetes ( $p < 0.01$ ; RR 3) and resections performed during P1 ( $p < 0.01$ ; RR 2,6) were independent risk factors for mortality. Age, BMI, laboratory values and blood transfusions did not influence any complication rate. A preoperative CTx was not correlated with perioperative complications (even tendency of fewer complications after CTx)

**Conclusions:** The presence of a diabetes and extended resections are relevant risk factors for complications following hepatic resection for CRC metastases. An increasing center experience/hospital volume including improved perioperative management clearly decrease perioperative mortality. In our experience a preoperative CTx did not increase postoperative morbidity.

## Cancer Surgery

### Poster Exhibition

PE649

### Surgery is the most important prognostic factor for thymic epithelial tumors

Stremmel C., Siemel W., Dango S., Passlick B.  
Universität Freiburg, Abt. Thoraxchirurgie, Freiburg

Thymoma is the most common tumor of the anterior mediastinum. Thorough its sporadic occurrence clinical treatment is challenging and has been based on observation of only a few patients. Here we present a 20 year experience in

clinical treatment of thymomas as a result of a retrospective single centre analysis done within an European university setting.

Between 1984 and 2004 84 patients with thymoma underwent operation and clinical data were collected. In this population 14 (20%) patients were classified in WHO subgroup C (thymus carcinoma) and 5 (4%) patients presented with a thymic carcinoid. There were 71 (84%) complete resections, 5 (6%) patients with R1- and 8 (10%) patients with R2-resection. R-Status showed up to depend on Masaoka stage. 42 (50%) patients were classified in Masaoka stage I, 19 (22%) in stage II, 9 (11%) in stage III and 14 (17%) patients in stage IV. Overall survival was 88.1% after 5 years. A local recurrence was seen in 5%. A pericard resection was necessary in 19 (21%) and appeared to depend on WHO classification. Further more, vessel infiltration as well as Masaoka stage, WHO classification, R-status and an encapsulated tumor were of prognostic significance. In multivariate analysis only R-status and Masaoka stage appeared to be of independent prognostic significance. Adjuvant radiation was seen to have a positive influence in Masaoka stage III.

Summarized, complete resection is the most important factor in treatment of thymoma. Masaoka stage has a stronger relevance than WHO classification to evaluate prognosis. Both, R-status and recurrence rate depended on Masaoka stage. Generally, further studies including a larger population are essential to define subgroups that must be taken to neoadjuvant or adjuvant treatment.

PE650

### Prognostic value of the lymph node ratio in pancreatic adenocarcinoma following resection

Bruns C., Christians S., Kleespies A., Eichhorn M., Angele M., Jauch K.-W.

Universität, Chirurgie, München

**Introduction:** The prognosis of pancreatic adenocarcinoma (PC) of the pancreas remains poor. Recent studies reported the ratio of metastatic/examined lymph nodes (LNR) as an important prognostic factor in patients undergoing resection of PC. The aim of this retrospective single-center study was to re-evaluate the prognostic relevance of the LNR compared to other histopathological factors for PC following resection.

**Material and Methods:** At the University of Munich-Großhadern altogether 341 pancreatic resections were performed with increasing frequency between January 2001 and December 2006, thereof in 87 patients a pancreatic left and in 254 patients a classical (PPD) or pylorus preserving partial pancreato-duodenectomy (PPPD). In 81% pancreatic cancer was the underlying disease. The R0-resection rate was 86%, the mortality rate 2,3%.

**Results:** Altogether in 149 patients (71 female, 78 male, median age of 64 (32–84) years) with PC the overall survival was correlated to histopathological factors of microinvasion (lymphangiosis, hemangiosis, perineural invasion), the LNR, tumor size and resection margins in consideration of the UICC-Klassifikation (2002). Type of surgery was PPD (51%), PPPD (24,2%), total pancreatectomy (9,4 %) or pancreatic left resection (15,4 %). Portal vein resection was performed in 22 cases (14,7%). The median survival following resection was 16 months (CI: 12,09 – 19,91). The UICC staging was distributed as follows: 2% UICC IA, 0% UICC IB, 32,9% UICC IIA, 40,3 % UICC IIB, 7,4 % UICC III und 16,8 % UICC IV. Lymphangiosis was found in 19,5%, hemangiosis in 8,1% and perineural invasion in 50,3 %. Lymph node metastasis occurred in 59,1 %, distant metastasis in 16,8 %. The median number of harvested lymph nodes was 11 (1 – 40), the median number of metastasized lymph nodes was 2 (1–19). There was no significant difference in the overall survival in patients with PC (R0M0) following resection with an LNR of 0.01 versus >0.04.

**Conclusion:** In contrast to recent studies our data analysis suggests that the LNR had no prognostic value in patients with PC undergoing resection. Our data showed prognostic strength for hemangiosis, significant in uni- and multivariate analysis, with respect to patients' overall survival. For accurate staging it seems to be necessary to harvest more than 12 lymph nodes. Patients with infiltration of paraortic lymph nodes show survival rates as poor as those with distant metastasis or peritoneal carcinosis.

PE651

### Fast track vs. conventional management in the perioperative care of radical cystectomy and urinary diversion. Results of a prospective randomized monocentric trial

Olbert P.J., Hegele A., Schrader A.J., Hofmann R.

Universitätsklinikum Giessen und Marburg GmbH, Klinik für Urologie und Kinderurologie, Marburg

Fast track (FT) regimens in abdominal surgery are widely accepted as safe and beneficial in terms of patient (pt.) satisfaction, earlier recovery and improvement of the perioperative immunological status of the pt.. Renunciation of bowel preparation (bp) and nasogastric tubes (NGT), shortening of drainage dwelling times and early postoperative enteralization are decisive elements of FT surgery. Objective of this trial was the prospective, monocentric, randomised assessment of perioperative safety of fast track surgery in major abdominal surgery for urogenital cancer.

For a period of 1 year, 57 consecutive pts. scheduled for radical cystoprostatectomy/pelvic exenteration and urinary diversion were randomly assigned in a 1:1 ratio to 2 groups of perioperative management: Group I (n = 28, conventional) underwent preoperative bp, postoperative parenteral nutrition until first bowel passage and intravenous bowel stimulation from POD 4. Group II (n = 29 FT) received no bp, NGT was removed in the OR and enteralization was started on POD 1. Data acquisition was performed at least until day 10 or the first day of complete enteral food intake. The trial was statistically designed to prove non inferiority of FT in terms of duration of postoperative bowel passage and enteralization and the frequency of bowel associated complications. The two groups (I vs. II) were comparable for age (68 vs. 69 y), BMI (28 vs. 26.2), preoperative ASA score (17 pts. vs. 16 pts. ASA III and IV) and history of cardiovascular disease (50 vs. 43%). There were more males (73 vs. 90%) in group II and more diabetic subjects in group I (27 vs. 7%). Ileal neobladder was performed in 23 and 53 % of pts.. Pts. in group II had a significantly shorter stay on the intermediate care unit (8 vs. 5 days p = 0.05) and significantly earlier return to complete enteral nutrition (11 vs. 7 days, p = 0.002). Bowel passage was faster as well (7 vs. 5 days, n.s.). Peri- and postoperative complications, especially bowel associated problems did not occur more frequently in group II. The surgeons did not observe intraoperative problems in those pts. without bowel preparation.

FT concepts in major urological surgery including bowel anastomoses are safe, support earlier return to enteral nutrition and improve cost-efficiency of postoperative management.

PE652

### Outcome after surgical treatment of malignant fibrous histiocytomas in 140 patients

Lehnhardt M., Daigeler A., Goertz O., Homann H., Hauser J., Steintraesser L., Steinau H.U.

Ruhr Universität Bochum, Klinik für Plastische Chirurgie und Schwerbrandverletzte, Handchirurgiezentrum, Referenzzentrum für Gliedmaßen-tumore, BG-Universitätskliniken Bergmannsheil Bochum

MFH/NOS-sarcoma is the second most common malignant soft tissue tumor in adults. The objective of this study is to determine prognostic factors and 5y-survival. From 1995 to 2005, 140 MFH/NOS-sarcoma out of 1200 soft tissue sarcomas were admitted and treated at our institution and recorded in a prospective database. Overall survival (OS) and isolated local recurrence (ILR) were determined by Kaplan-Meier analysis. All tumors were retrospectively analyzed regarding prognostic factors of the disease, which included: primary or recurrent, histological grade, adjuvant chemotherapy, size (T1-2), adjuvant radiotherapy, surgical margin and location of surgery (author's institute-others). In 123 patients a wide complete resection was performed (limb sparing surgery). The overall 5-year survival rate was 77% (median follow-up 52 months). There was a significant difference between the group of primary tumors (5y survival: 85%, p < 0.05) and recurrent tumors (5y survival: 68%, p < 0.05). Isolated local recurrence occurred in 36 patients. In terms of OS and ILR, primary or recurrency, negative surgical margins, size, grading and adjuvant radiotherapy have a highly significant impact whereas, location of surgery and adjuvant chemotherapy have not. Prognosis for patients with malignant fibrous histiocytoma depends predominantly from adequate wide resection at the time of primary tumor presentation.

PE653

### Operating procedure of the retroperitoneal liposarcoma – 26 years of clinical experience

Guthoff I., Sommer P., Anthuber M.

Zentralklinikum Augsburg, Klinik für Allgemein-, Viszeral- und Transplantationschirurgie, Augsburg

**Background:** Among the heterogeneous group of adult soft tissue sarcomas, liposarcomas represent the largest entity. Retroperitoneal liposarcomas are often remarkable because of their huge size. In general, three major subtypes of liposarcoma can be distinguished in terms of pathomorphology: well-differentiated/dedifferentiated liposarcoma, myxoid/round cell liposarcoma, and pleomorphic liposarcoma. When a local relapse has occurred, the liposarcoma may show dedifferentiation and may metastasize.

**Patients:** 60 cases of operations in case of retroperitoneal liposarcoma were analyzed regarding gender, age, recurrence rate, weight, survival rates, and histopathology retrospectively from 1981 until 2007.

**Results:** 34 patients (pts) underwent surgery because of retroperitoneal liposarcoma. From 1981 to 2007, 17 women and 17 men. Mean age was 60 years (y) of all pts at time of first operation (range 31 to 88), of women 62 y (range 36 to 88), and of men 58 y (range 31 to 83). We removed surgically primary tumour, recurrent sarcoma, and metastases, whenever possible, partly doing multivisceral resection. 7 pts were free of recurrence, 17 pts presented with one recurrence, and 6 pts with two recurrences. The liposarcomas' weight was between 80 g and 20.4 kg, mean weight was 5618 g. Survival rate from date of first operation lasted from 2 months up to 20 y. Survival rate was 84 % after 3 months (28 of 33 pts.), 81 % after 1 y (27 of 33 pts.), 58 % after 3 y (17 of 29 pts.), 54 % after 5 y (12 of 21 pts.), 50 % after 20 y (1 of 2 pts.), and 0% after 25 y (0 of 1 pt.). Mixed type liposarcomas of all grades were most common, followed by highly differentiated liposarcomas, then highly differentiated and partly myxoid liposarcomas and last dedifferentiated liposarcomas grade 3.

**Discussion:** Liposarcomas are rare malignant tumours with an aggressive course of disease and high local recurrence rate. Local disease is the main cause of death. Clinically symptoms of liposarcoma often progress rapidly and severely disable the affected patient. Until there are no other reliable treatment modalities, aggressive surgery for liposarcoma is recommended. Proper diagnosis and appropriate management can significantly reduce morbidity and mortality. The hands of an experienced surgeon is needed in cases like this more than ever.

PE654

### Cytoreductive surgery and hyperthermic intraperitoneal chemotherapy in patients with malignant peritoneal mesothelioma

Glockzin G., Lang S.A., Ghali N., Schlitt H.J., Piso P.

Klinikum der Universität Regensburg, Klinik und Poliklinik für Chirurgie, Regensburg

**Introduction:** Malignant peritoneal mesothelioma (PMP) is a relatively rare disease with poor prognosis. The median survival of untreated patients is less than 1 year. Cytoreductive surgery (CRS) and hyperthermic intraperitoneal chemotherapy (HIPEC) represent an innovative therapeutic option for a highly selected part of patients with PMP leading to prolonged survival rates. In the present study we evaluated morbidity, mortality and first follow-up data of 7 patients operated on for PMP.

**Patients and Methods:** Between 2003 and 2007 more than 175 patients underwent cytoreductive surgery and HIPEC at the University of Regensburg Medical Center. Seven patients were operated on for PMP. The mean age of patients at the time of surgery was 57 years. Two patients were female (29 %) and five male (71 %). The mean follow-up time was 22 months.

**Results:** Six of seven patients underwent complete macroscopic cytoreduction. One patient had residual tumor mass after surgery. The mean operative time was 365 minutes. Four patients had previous surgery. One patient was operated on for recurrent PMP. Three patients developed postoperative complications (43 %). Postoperative complications were anastomotic leakage, biliary leakage, postoperative bleeding, renal insufficiency, pneumonia and arrhythmia. There was no operative mortality in our series. Two patients developed

tumor recurrence after 8 and 20 months. Two patients died 12 and 13 months after initial surgery, respectively. One of these patients showed incomplete tumor resection. The other patient developed severe postoperative cardiac, pulmonary and renal complications requiring prolonged intensive care therapy.

**Conclusion:** Cytoreductive surgery and HIPEC are an innovative treatment strategy for selected patients with PMP and can be performed with acceptable mortality and morbidity in specialized centers.

PE655

### Free tissue transfer in surgical management of malignant tumors

Penna, V., Bannasch, H., Momeni, A., Stark, G.B.

Universitätsklinikum, Chirurgische Klinik, Tübingen

Multimodal therapy of malignant tumors such as soft-tissue sarcomas and sarcomas of the bone consists of radiation either preoperatively or postoperatively, surgery and often chemotherapy. The standard surgical approach claiming wide tumor excision and limb salvage has increased the demand for coverage of large composite defects. The present study retrospectively reviews records of a series of patients (n = 26) who underwent free tissue transfer for reconstruction of various tissue defects due to malignant tumors from 1995 to 2006.

26 patients with an age range from 22 to 85 years (mean: 62) received a total of 27 free flaps. The following flap types were used: radial forearm free flap (n = 2), fibula osteocutaneous free flap (n = 3), musculocutaneous latissimus dorsi free flap (n = 8), rectus abdominis free flap (n = 4), lateral arm fasciocutaneous free flap (n = 5), free anterolateral thigh flap (n = 4) and parascapular free flap (n = 1). 25 patients had tissue defects related to malignant tumor resection, 1 patient underwent surgery under sarcoma suspicion, postoperative histological tissue examination showed myxoma.

Complete flap survival was observed in 22 cases (81,5%), partial flap loss in two cases (7,4%) and complete flap loss in three cases (11,1%). Early revision of the five partial or failed flaps included debridement and split-thickness skin graft (n = 4) as well as repeated performed free tissue transfer (n = 1). The overall complication rate including major (partial and complete flap loss) and minor complications (infection, wound healing complications) was 40,9%. Extremity salvage could be achieved in all patients with extremity tumors (n = 20). Complete tumor resection was obtained in 92,3%.

Microsurgical reconstruction using free tissue transfer allows larger resection, provides vascularized bone and functioning muscle replacement with an independent blood supply. Thus, free tissue transfer has become a reliable and versatile surgical option in management of malignant tumors.

### Cell Cycle, Apoptosis, Angiogenesis

#### Oral Presentation

OP116

### Defining the apoptotic pathways underlying HDAC inhibitor-mediated tumor therapy in vivo

Lindemann R.<sup>1,2</sup>, Newbold A.<sup>2</sup>, Whitecross K.<sup>2</sup>, Cluse L.<sup>2</sup>, Frew A.<sup>2</sup>, Ellis L.<sup>2</sup>, Williams S.<sup>2</sup>, Wiegman A.<sup>2</sup>, Dear A.<sup>3</sup>, Scott C.<sup>4</sup>, Pelligrini M.<sup>4</sup>, Wei A.<sup>4</sup>, Richon V.<sup>5</sup>, Marks P.<sup>6</sup>, Lowe S.<sup>7</sup>, Smyth M.<sup>2</sup>, Johnstone R.<sup>2</sup>

<sup>1</sup>Nationales Zentrum für Tumorerkrankungen, Translationale Onkologie, Heidelberg; <sup>2</sup>The Peter MacCallum Cancer Centre, Cancer Immunology Program, Melbourne; <sup>3</sup>Australian Center for Blood Diseases, Melbourne; <sup>4</sup>The Walter and Eliza Hall Institute, Melbourne; <sup>5</sup>Merck & Co., Boston; <sup>6</sup>Memorial Sloan-Kettering Cancer Center, New York; <sup>7</sup>Cold Spring Harbor Laboratories, Cold Spring Harbor

Histone deacetylase inhibitors (HDACi) can elicit a range of biological responses that affect tumor growth and survival including inhibition of tumor cell cycle progression, induction of tumor cell-selective apoptosis, suppression of angiogenesis and modulation of immune responses and show promising activity against hematological malignancies in clinical trials. However, it is unclear which of these pathways mediates the anti-cancer activities of HDACi *in vivo*. Using the Eμ-myc model of B-cell lymphoma we performed an *in vivo*

candidate screen for molecules that confer therapeutic activity to the HDACi Vorinostat. Our system comprises lymphomas with defined genetic alterations in the apoptotic machinery, which can be translated into immunocompetent animals for therapy studies. Using this model, we have identified key apoptotic molecules that not only control sensitivity of lymphoma cells to HDACi, but also determine therapeutic outcome. Furthermore, we demonstrated a direct correlation between induction of tumor cell apoptosis *in vivo* and therapeutic efficacy. Vorinostat did not require p53 activity or a functional death receptor pathway, but depended on apoptosis mediated by the intrinsic apoptotic pathway with the pro-apoptotic BH3-only proteins Bid and Bim playing an important role. Our results provide important information regarding the mechanisms of action of HDACi that may have broader implications regarding future stratification of patients receiving HDACi therapy and the use of these compounds in combination with other anti-cancer agents.

OP117

### Hypoxia-inducible-factor 2 alpha mediates tumor progression in the lung adenocarcinoma cell line A549

Kamlah F.<sup>1</sup>, Eul B.<sup>2</sup>, Lang N.<sup>2</sup>, Kwapiszewska G.<sup>1</sup>, Marsh L.<sup>1</sup>, Seeger W.<sup>1</sup>, Grimminger F.<sup>2</sup>, Rose F.<sup>3</sup>, Hänze J.<sup>1</sup>

<sup>1</sup>Universitätsklinikum Giessen, Innere Medizin, Medizinische Klinik II, Giessen; <sup>2</sup>Universitätsklinikum Giessen, Innere Medizin, Medizinische Klinik V, Giessen; <sup>3</sup>Universitätsklinikum Marburg, Klinik für Strahlentherapie, Marburg

Tumor progression is dependent of adequate oxygen and metabolite supply to provide the rapid proliferating cells. Therefore a sufficient angiogenesis is essential. Since tumors expand, diffusion distances from the existing vascular supply increases resulting in hypoxia. The hypoxia inducible factors (HIF-1 $\alpha$  and HIF-2 $\alpha$ ) are key players in the response to hypoxia affecting angiogenesis, cell metabolism, proliferation and apoptosis. Here we describe the differential role of HIF-1 $\alpha$  and HIF-2 $\alpha$  in tumor progression and the HIF dependency of cellular interaction in A549 tumors. To define the role of HIF-1 $\alpha$  and HIF-2 $\alpha$  in tumor progression, specific inhibition of either HIF-1 $\alpha$  or HIF-2 $\alpha$  *in vivo* was achieved by RNA interference technology. Continuous knock down of HIF-2 $\alpha$  abolished the growth of subcutaneous A549 tumors. This was in line with HIF-2 $\alpha$  dependent increased apoptosis and decreased proliferation. However, cellular proliferation of A549 cells was increased in the presence of macrophage conditioned media. This was in line with an increased monocyte attraction in the presence of A549 cells. Interestingly a reduced number of macrophages were observed in the HIF-2 $\alpha$  knock out tumors. This was due to a HIF-2 $\alpha$  dependent reduced angiogenesis and microvessel density. As an essential mechanism increased VEGF secretion was observed in hypoxic A549 cells. This caused a higher, HIF-2 $\alpha$  dependent, HUVEC migration in the presence of hypoxic A549 cells. In conclusion these data showed the HIF-2 $\alpha$  dependent angiogenesis as a major factor for tumor progression and emphasizes HIF-2 $\alpha$  as an important target for tumor therapy.

OP118

### Angiostatic immune reaction in colorectal carcinoma: Impact on survival and antiangiogenic therapy

Naschberger E.<sup>1</sup>, Croner R.<sup>2</sup>, Merkel S.<sup>2</sup>, Dimmler A.<sup>3</sup>, Tripal P.<sup>1</sup>, Amann K.<sup>3</sup>, Brueckl W.<sup>4</sup>, Papadopoulos T.<sup>3</sup>, Hohenberger W.<sup>2</sup>, Stürzl M.<sup>1</sup>

<sup>1</sup>Universitätsklinikum Erlangen, Chirurgische Klinik, Abteilung Molekulare und Experimentelle Chirurgie, Erlangen; <sup>2</sup>Universitätsklinikum Erlangen, Chirurgische Klinik, Erlangen; <sup>3</sup>Universitätsklinikum Erlangen, Institut für Pathologie, Erlangen; <sup>4</sup>Universitätsklinikum Erlangen, Innere Medizin I, Erlangen

Angiogenesis and inflammation are the two major stroma reactions in colorectal carcinoma (CRC). Guanylate binding protein-1 (GBP-1) is a key mediator of angiostatic effects of inflammation. Therefore, we hypothesized that GBP-1 may be a biomarker of intrinsic angiostasis associated with an improved outcome in CRC patients.

GBP-1 was strongly expressed in endothelial cells and immune cells in the desmoplastic stroma of 32% of CRC as determined by immunohistochemical investigation of 388 sporadic CRC. Cancer-related 5-year survival was highly

significant ( $p < 0.001$ ) increased (16.2%) in patients with GBP-1-positive CRC. Multivariate analysis showed that GBP-1 is an independent prognostic factor indicating a reduction of the relative risk of cancer-related death by the half ( $p = 0.032$ ). A comparative transcriptome analysis (22,215 probe sets) of GBP-1-positive ( $n = 12$ ) and -negative ( $n = 12$ ) tumors showed that particularly IFN- $\gamma$ -induced genes including the major antiangiogenic chemokines CXCL9, CXCL10 and CXCL11 were coexpressed with GBP-1. Coregulation of CXCL9-11 with GBP-1 was verified in an independent cohort by multiplex RT-PCR and by *in situ* hybridization.

Altogether our findings indicated that GBP-1 may be a novel biomarker and an active component of a Th-1-like angiostatic immune reaction (IAR) in CRC. IAR may affect patient's response to antiangiogenic therapy and the identification of tumors with IAR may provide a novel criterion for patient selection. Induction of IAR may be a promising approach for the clinical treatment of CRC.

OP119

### Expression of EFEMP1 mediates tumor progression and angiogenesis in human pancreatic adenocarcinoma

Seeliger H.<sup>1</sup>, Camaj P.<sup>1</sup>, Ischenko I.<sup>1</sup>, Thieme S.<sup>2</sup>, Blum H.<sup>2</sup>, Jauch K.-W.<sup>1</sup>, Bruns C.J.<sup>1</sup>

<sup>1</sup>Ludwig-Maximilians-Universität München, Chirurgische Klinik und Poliklinik, München; <sup>2</sup>Ludwig-Maximilians-Universität München, Genzentrum, München

**Background:** Pancreatic cancer progression is dependent on local tumor growth and invasion, angiogenesis and metastasis. The role of EFEMP1, a recently discovered member of the fibulin family of extracellular matrix proteins, in cancer and metastasis, is unknown. Here, we show that EFEMP1 is upregulated in a metastatic form of pancreatic adenocarcinoma and exerts protumorigenic effects *in vitro* and *in vivo*.

**Materials and Methods:** Differential expression of EFEMP1 was assessed by mRNA microarray hybridization in FG human pancreatic adenocarcinoma cells and L3.6pl cells, a highly metastatic variant of FG. *In vivo* orthotopic tumor growth of EFEMP1 transfected FG cells was examined in BALB/c nu/nu mice. To evaluate the influence of EFEMP1 expression on angiogenic events, VEGF expression in transfected cells and microvessel density in tumor xenografts were assessed. Finally, EFEMP1 expression was analyzed by RT-PCR in human pancreatic adenocarcinoma samples and corresponding normal pancreatic tissue.

**Results:** In microarray hybridization, EFEMP1 was shown to be significantly upregulated in L3.6pl cells, compared to FG cells. Orthotopic tumors arising from EFEMP1 transfected FG cells *in vivo* were significantly larger, compared to nontransfected cells. VEGF production was significantly increased in EFEMP1 transfected cells, resulting in an increased microvessel density in tumor specimens. Consistent with the experimental results, clinical tumor specimens showed an overexpression of EFEMP1, compared to normal pancreatic tissue.

**Conclusion:** For the first time, we showed that EFEMP1 has protumorigenic effects on pancreatic cancer *in vivo* and *in vitro* mediated by angiogenic mechanisms. Hence, EFEMP1 might be a promising candidate for assessing prognosis of human pancreatic cancer in a clinical tumor setting.

OP180

**Phase II trial of radiochemotherapy with daily concomitant and adjuvant intensified (one week on/one week off) temozolomide and indomethacin therapy in newly diagnosed glioblastoma: UKT-05**

Weiler M.<sup>1,2</sup>, Wiewrodt D.<sup>3</sup>, Bähr O.<sup>2</sup>, Hartmann C.<sup>4</sup>, Herrlinger U.<sup>5</sup>, Meyermann R.<sup>6</sup>, Bamberg M.<sup>7</sup>, Tatagiba M.<sup>8</sup>, von Deimling A.<sup>9</sup>, Weller M.<sup>2</sup>, Wick W.<sup>1,2</sup>

<sup>1</sup>Universitätsklinikum, Abteilung Neuroonkologie, Heidelberg;

<sup>2</sup>Universitätsklinikum, Abteilung Allgemeine Neurologie, Tübingen;

<sup>3</sup>Universitätsklinikum, Neurochirurgische Klinik, Mainz; <sup>4</sup>Deutsches Krebsforschungszentrum, Klinische Kooperationseinheit Neuropathologie, Heidelberg; <sup>5</sup>Universitätsklinikum, Neurologische Klinik, Bonn;

<sup>6</sup>Universitätsklinikum, Institut für Hirnforschung, Tübingen;

<sup>7</sup>Universitätsklinikum, Klinik für Radioonkologie, Tübingen;

<sup>8</sup>Universitätsklinikum, Klinik für Neurochirurgie, Tübingen;

<sup>9</sup>Universitätsklinikum, Abteilung Neuropathologie, Heidelberg

**Background:** To evaluate toxicity and efficacy of a (i) pre-irradiation one week on/one week off schedule of temozolomide (TMZ), (ii) concomitant low-dose and (iii) maintenance one week on/one week off schedule of TMZ chemotherapy and indomethacin (INDO) in addition to involved-field radiotherapy (RT) in patients with newly diagnosed glioblastoma.

**Methods:** 41 patients (pts) (median Karnofsky performance status 90%; median age, 56.0 years) after tumor resection (37/41, 90%) or biopsy (4/41, 10%) were treated with pre-irradiation TMZ at 150 mg/m<sup>2</sup> (one week on/one week off), involved-field radiotherapy combined with concomitant low-dose TMZ (50 mg/m<sup>2</sup>), maintenance TMZ starting at 150 mg/m<sup>2</sup> according to an intensified one week on/one week off schedule, plus maintenance INDO (25 mg bid) treatment. The primary endpoint was to achieve a median progression-free survival (PFS) of 10.4 months. O<sup>6</sup>-methylguanine-DNA methyltransferase (*MGMT*) gene promoter methylation status was assessed in 39 (95.1%) pts.

**Results:** At the cutoff date (August 1, 2007), the median follow-up interval was 15.3 months (minimum, 9.4 months). Hematologic grade 3/4 toxicities were: anemia (3/41, 7%), leukopenia (9/41, 22%), lymphopenia (26/41, 63%), neutropenia (6/41, 15%), and thrombopenia (8/41, 20%). Treatment-related non-hematologic grade 3 through 5 toxicities were reported for n = 6/41 (15%) patients: bleeding in tumor cavity (grade 3, n = 1) during radiotherapy; toxic hepatitis/pancreatitis (grade 4, n = 1); intestinal perforation (grade 3, n = 1); febrile gastroenteritis with subileus and acute renal failure (grade 3, n = 1); generalized necrotizing zoster infection (grade 3, n = 1); pulmonary infection with *Pneumocystis carinii* and *cytomegalovirus* (grade 5, n = 1). In total, the median PFS was 7.6 months [95% CI, 6.0-9.9 months]. The progression-free survival rate at 6 months (PFS-6) was 68.8% [95% CI, 56.3-79.0%]. The censored one year survival rate was estimated at 69.9% [95% CI, 56.8-80.5%]. Median PFS, PFS-6 and median survival time (MST) in the subgroup of pts with *MGMT* gene promoter methylation were significantly larger than in the subgroup of pts without epigenetic *MGMT* gene silencing (median PFS: 15.8 vs. 6.2 months, *P* = .001; PFS-6: 85.8% vs. 59.3%; MST: 24.1+ vs. 12.9+ months, *P* = .027).

**Conclusions:** The dose-dense regimen of TMZ administered in a one week on/one week off schedule resulted in acceptable toxicity. Although the primary endpoint of the trial was not reached, PFS-6 is encouraging in pts both with methylated and unmethylated *MGMT* gene promoter status. However, compared to data from the EORTC trial 26981/22981, pts with unmethylated *MGMT* gene promoter appeared not to benefit from intensifying the TMZ schedule regarding median PFS or MST. This study provides first toxicity and efficacy data on TMZ administered in a dose-intensive regimen in the first-line treatment of glioblastoma.

OP181

**An advanced technique for analysis of *MGMT* promoter methylation: Methylation specific quantitative PCR**

Hattermann K.<sup>1</sup>, Mentlein R.<sup>1</sup>, Mehdorn H.M.<sup>2</sup>, Held-Feindt J.<sup>2</sup>

<sup>1</sup>Universität Kiel, Anatomisches Institut, Kiel; <sup>2</sup>UK S-H, Campus Kiel, Neurochirurgie, Kiel

The O<sup>6</sup>-methylguanine DNA methyltransferase (*MGMT*) gene encodes a DNA repair enzyme, whose activity is a major mechanism of resistance to alkylating drugs in treatment of glioblastoma patients. Hypermethylation of the *MGMT* promoter is associated with chemosensitivity as it reduces *MGMT* activity. Therefore, techniques to detect and measure DNA methylation status in research and diagnosis are required. In this study we present a novel method named MSQP combining methylation-specific (MS) and SYBR<sup>®</sup> Green based quantitative PCR (QP) for *MGMT* promoter methylation analysis. With MSQP technique we describe a highly specific, sensitive and reproducible method, which allows the quantitative determination of not only fully methylated, but also of fully unmethylated bisulfite converted *MGMT* DNA species in parallel. Values are calculated in relation to standard curves, normalized for input of DNA by an internal standard, and calculated in relation to fully methylated and unmethylated control DNAs. Finally, values are normalized to total fully methylated or unmethylated *MGMT* DNA sample amount for getting PMR (percentage of methylated reference) and PUR (percentage of unmethylated reference) results. We have used this novel technical approach to investigate *MGMT* gene promoter methylation status in relation to *MGMT* mRNA expression in nine different tumour cell lines and 15 different primary glioblastoma patients. Presented data confirm that this assay is useful for detection of low amounts of both methylated and unmethylated *MGMT* promoter DNA within glioblastoma samples. Carefully validated quantitative MSQP assays will be useful in both research and clinical molecular diagnosis.

OP182

**Rechallenge of malignant gliomas with temozolomide – Can it be effective?**

Strik H.<sup>1</sup>, Buhk J.<sup>2</sup>, Nitsche M.<sup>3</sup>, Hoffmann A.<sup>1</sup>, Giese A.<sup>4</sup>, Bock C.<sup>4</sup>, Bähr M.<sup>1</sup>

<sup>1</sup>Universität, Neurologie, Göttingen; <sup>2</sup>Universität, Neuroradiologie, Göttingen; <sup>3</sup>Universität, Strahlentherapie, Göttingen; <sup>4</sup>Universität, Neurochirurgie, Göttingen

**Background:** No standard treatment of recurrent malignant glioma is established. Moreover, it is unclear if a rechallenge with temozolomide is effective. We present here feasibility and first treatment results of a high-dose, individually dose adapted 21-day regimen with temozolomide for recurrent malignant gliomas.

**Methods:** 21 consecutive patients with recurrent malignant gliomas (18 glioblastomas [GBM], 2 anaplastic astrocytomas, 1 anaplastic oligodendroglioma) were treated. The basic data for GBM are: median time to first relapse 8.2 months; median Karnofsky Performance Status 60%; median age 54.4 years; 11 males, 7 females. All patients were pretreated with concomitant and/ or adjuvant temozolomide in first line. In 14 of the 21 patients, therapy was switched without or with less than 3 months delay from the conventional 5/28-day regimen to the dose-dense schedule: temozolomide 100mg/m<sup>2</sup> day 121/28. Dosage was adapted individually, aiming towards total leukocyte counts of approximately 4,000/μL. Patients with critical blood counts were switched to a 5/7-day regimen.

**Results:** The 21/28 days regimen was better tolerated than the 5/28 days schedule regarding nausea and fatigue. Blood counts decreased continuously, enabling a gradual dose adaptation. The dosage was reduced to 50-75 mg/m<sup>2</sup> in 11 and gradually increased to up to 130 mg/m<sup>2</sup> in 3 patients. WHO grade 3/4 toxicity was: hematotoxicity 4 patients; infection 1; gastrointestinal 1 patient. In GBM (n = 18), response after ≥3 months was: 2 partial and 2 complete remissions (22%), 7 stable (39%), 7 progressive diseases (39%). Progression free survival at 6 months (PFS 6M) was 39%, median survival after relapse 7.0 months, median overall survival 17.7 months. All 3 patients with anaplastic glioma were progression-free after 6 months (1 CR, 2 PR).

**Conclusions:** rechallenge with high-dose temozolomide at 100 mg/m<sup>2</sup> day 1-21/28 and individual dose adaptation is feasible also in patients with critical

blood counts. Objective responses can be achieved even after relapse during conventional 5/28 regimen. In spite of relatively unfavourable prognostic criteria, the treatment results compare well with the most successful series in the literature. The results will be controlled in a prospective study.

OP183

### Long term stabilization of leptomeningeal and solid CNS metastases from breast cancer with combined intrathecal liposomal ARA-C and systemic temozolomide

Hoffmann A.<sup>1</sup>, Buhk J.<sup>2</sup>, Nitsche M.<sup>3</sup>, Strik H.<sup>1</sup>

<sup>1</sup>Universität, Neurologie, Göttingen; <sup>2</sup>Universität, Neuroradiologie, Göttingen; <sup>3</sup>Universität, Strahlentherapie, Göttingen

**Objective:** CSF dissemination of breast cancer is dismal with a median survival of 8-12 weeks. We present here long-term stabilisation with combined intrathecal liposomal Ara-C, dose-dense oral temozolomide and radiotherapy in two consecutive patients with leptomeningeal and solid CNS metastases from breast cancer.

**Case Reports:** two 42- and 43- year-old females presented with CSF-spread and disseminated solid spinal and in one case also cerebellar metastases from intensely pretreated Her2/neu positive breast cancer. After irradiation of the symptomatic solid tumours, combined treatment with intrathecal liposomal Ara-C biweekly and dose-dense temozolomide 100mg/m<sup>2</sup> day 1-5 every week were initiated. With this continuous therapy, the neurological symptoms of both patients improved. The spinal metastases remained stable for 6 months, the cerebellar metastases decreased by approx. 50%. CSF cell counts normalised and only single malignant cells remained. Both patients are alive 8 months after diagnosis of CNS dissemination. One is progression-free, the other developed disease progression after 6 months.

**Discussion:** the unfavourable prognosis of patients with CSF dissemination and solid central nervous system metastases from breast cancer is caused by most often intense pre-treatment and the lack of active CNS-penetrating systemic drugs. The value of intrathecal chemotherapy is unclear. The data available to date indicate that a combination of systemic and intrathecal chemotherapy may achieve better results than either treatment option alone. Intrathecal liposomal Ara-C is active in meningeal disease especially from hematopoietic cancer. CSF-spread from solid tumours can be improved in terms of cytological and sometimes short-term clinical response, but long-term stabilization or improvement is difficult to achieve.

Temozolomide is an orally available alkylating drug with favourable toxicity profile and good penetration into the central nervous system. In CNS metastases from breast cancer, however, conventionally applied temozolomide (200mg/m<sup>2</sup> day 1-5/28) had only limited activity.

Based on good results with recurrent gliomas, we treated our patients with dose-dense temozolomide. This systemic therapy was combined with intrathecal liposomal Ara-C which appears to be the most promising drug for chemotherapy into the CSF to date. The long-term stabilisation of both patients indicates activity of this combined intrathecal and systemic approach. The regimen will be tested prospectively in a larger series.

OP184

### Genetic profiling of brain- and bone-seeking clones of MDA-MB-231 breast cancer cells

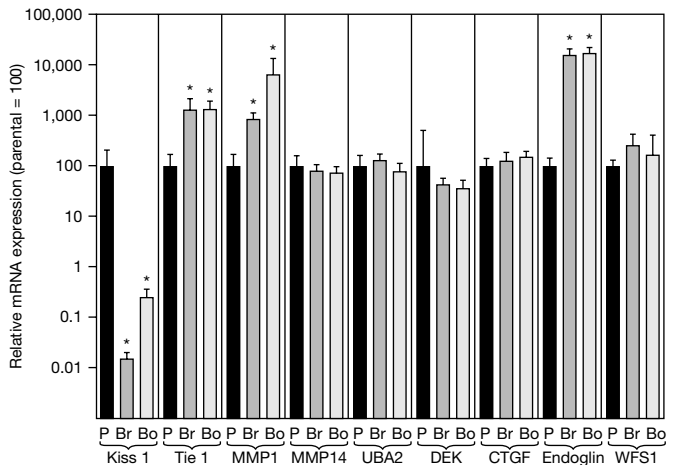
Stark A.M.<sup>1</sup>, Held-Feindt J.<sup>1</sup>, Mehdorn H.M.<sup>1</sup>, Mentlein R.<sup>2</sup>

<sup>1</sup>Klinik für Neurochirurgie UKSH Kiel, Kiel; <sup>2</sup>Anatomisches Institut, CAU Kiel, Kiel

**Introduction:** Haematogenous metastasis to the brain and bone is a major source of morbidity and mortality in cancer patients. We have performed genetic profiling of brain- and bone-selective metastatic MDA-MB-231 breast cancer cells using cDNA Array technology. The selective clones were compared to 231-parental cells.

**Material and Methods:** Gene profiling was performed using the 16K gene chip of the National Human Genome Research Institute, NIH, USA. Results were verified by real-time RT-PCR. Protein expression was examined using enzyme-linked immunosorbent assays (ELISA) and western blotting. Migration and invasion assays were performed with the QCM 96-well Migration/Invasion Assay +/- specific MMP inhibitors.

Figure 1. Real-Time PCR demonstrates mRNA expression differences in 231-Parental (P), -Brain (Br) and -bone-seeking clones (Bo). \*Significant.



**Results:** cDNA array analysis revealed significant over- or underexpression in brain- and / or bone-seeking cells of 113 genes. 9 gene sequences were chosen for data validation using real-time RT-PCR. Herein, significant changes in both selective clones were found for: (1) matrix-metallo-proteinase 1 (MMP-1), (3) the metastasis suppressor gene KISS-1 and (2) vascular factors endoglin and TIE-1. These genes were chosen for further analysis. Functional experiments determined the potential role for breast cancer metastasis to the brain and bone. In vitro experiments were supplemented by examinations on human tumor tissue. Herein, the expression of MMP-1 and -9 were increased in brain-metastatic cells (mRNA and -protein level). We found significantly decreased of several metastases suppressor genes in human breast cancer brain metastatic tissues: KISS-1, BRMS-1, MKK4, KAI-1 and Maspin (SERPINB5), a promising candidate for future treatment options.

**Conclusion:** (1) MMP-1 and -9 were overexpressed in brain-seeking 231-clones. MMP-1 and -9 inhibitors lead to decreased invasive and migratory potential in vitro. (2) several metastasis-suppressor genes, including Maspin (Serp B5) were decreased in human breast cancer brain metastases.

OP195

### Rezidivtherapie bei vorbestrahlten malignen gliomen

Schäfer U.<sup>1</sup>, Micke O.<sup>2</sup>

<sup>1</sup>Klinikum Lippe GmbH, Lemgo; <sup>2</sup>Franziskus Hospital Bielefeld, Klinik für Strahlentherapie, Bielefeld

**Fragestellung:** Die Rezidivbehandlung maligner Gliome ist problematisch und beinhaltet häufig eine alleinige systemische Therapie mit nur limitiertem Behandlungserfolg. Eine erneute Bestrahlung wird dagegen selten erwogen aufgrund des erhöhten Risikos von radiogenen Nebenwirkungen. Das Ziel dieser Phase II-Studie war, die Durchführung einer Kombinationsbehandlung bestehend aus einer Wiederholungsbestrahlung mit moderaten Dosen und Temozolomide zu untersuchen.

**Methodik:** 16 Patienten (7 Frauen, 9 Männer, med. Alter 60 Jahre) wurden mit einem inoperablen In-field Rezidiv eines malignen Glioms (9/16 anaplastisches Astrozytom, 7/16 Glioblastom) einer Rezidivbehandlung unterzogen. Das Protokoll bestand aus einer sequentiellen Radiochemotherapie. Die Chemotherapie (Temozolomid) wurde an 5 aufeinander folgenden Tagen (Mo.-Fr.) mit 200 mg/m<sup>2</sup> durchgeführt. Diese Zyklen wiederholten sich in Abständen von 4 Wochen (Tag 1, Tag 29, Tag 57, etc.) im Sinne einer Erhaltungschemotherapie bis zum klinischen Progress. Zwischen den ersten beiden Zyklen wurde die Strahlentherapie mit 30 Gy (5 x 2 Gy) über 3 Wochen interponiert (Tag 8 bis Tag 26). Alle Patienten wurden bis zum Progress / Tod verfolgt und das Überleben mit der Kaplan-Meier-Methode errechnet.

**Ergebnis:** Bei 14/16 Pat. konnte die Strahlentherapie plangemäß beendet werden und bei 2/16 Pat. erfolgte nach 20 bzw. 28 Gy ein Therapieabbruch wegen klinischem Progress der Erkrankung. Ein weiterer Pat. erlitt eine akute

Thrombozytopenie ( $6000 \times 10^6/l$ ) und Leukozytopenie ( $0,6 \times 10^6/l$ ). Im Vergleich zum Therapiebeginn war bei Therapieende der Allgemeinzustand / Neurologie bei 12/16 Pat. unverändert und bei 4/16 schlechter, nach 6 Wochen bei 1/16 verbessert, bei 7/16 stabil und bei 8/16 verschlechtert. Im Median zeigte sich nach 4.5 Monaten klinisch ein Progress der Erkrankung. Alle Pat. verstarben tumorbedingt. Das mediane Überleben betrug 6 (1 bis 24) Monate. 4 Pat. lebten allerdings über 11 Monate. Strahlenbedingte akute wie chronische Nebenwirkungen > Grad II konnten nicht eruiert werden. Schlussfolgerung: Innerhalb eines therapeutisch schwer zu behandelnden Patientengutes ist das durchgeführte Protokoll ohne wesentliche Steigerung der Nebenwirkungen einsetzbar und kann im Einzelfall zu einer deutlichen Stabilisierung der Erkrankung über mehrere Monate führen.

OP196

### Spinal re-irradiation for in-field recurrence of metastatic spinal cord compression

Rades D.<sup>1</sup>, Veninga T.<sup>2</sup>, Stalpers L.<sup>3</sup>, Schild S.E.<sup>4</sup>

<sup>1</sup>UK-SH, Radiation Oncology, Luebeck; <sup>2</sup>Dr. Bernard Verbeeten Institute, Radiotherapy, Tilburg; <sup>3</sup>Academic Medical Center, Radiotherapy, Amsterdam; <sup>4</sup>Mayo Clinic, Radiation Oncology, Scottsdale

**Background:** Radiation myelopathy is a serious late toxicity after radiotherapy (RT) of metastatic spinal cord compression (MSCC). The risk of myelopathy depends on the Equivalent Dose in 2 Gy Fractions (EQD2), which takes into account both total radiation dose and dose per fraction. Many radiation oncologists are concerned about spinal re-irradiation (Re-RT), because it may result in a high cumulative EQD2. This analysis includes the largest series of patients re-irradiated for MSCC ever reported. It investigates effectiveness and feasibility of Re-RT for in-field recurrence (recurrence in the previously irradiated part of the spine) of MSCC.

**Methods:** A total of 123 patients, initially irradiated between 1/95 and 12/06 for MSCC, were re-irradiated for in-field recurrence of MSCC. Primary RT was performed with 1x8 Gy (n = 47), 5x4 Gy (n = 59), 10x3 Gy (n = 8), 15x2.5 Gy (n = 5), or 20x2 Gy (n = 4). Recurrence occurred after median 6 (2-62) months. Re-RT was performed with 1x8 Gy (n = 48), 5x3 Gy (n = 29), 5x4 Gy (n = 30), 7x2-3 Gy (n = 3), 10-12x2 Gy (n = 11), or 17x1.8 Gy (n = 2). The cumulative (primary RT plus Re-RT) EQD2 was 39-45 Gy in 28 patients, 49-55 Gy in 68 patients, 56-60 Gy in 19 patients, and >60 Gy in 7 patients. The maximum EQD2 was 71.3 Gy. The median follow up after Re-RT was 6 (1-52) months. The a/b-ratio chosen for myelopathy was 2 Gy.

**Results:** Re-RT resulted in improvement of motor function in 46/123 (37%) patients, in no further progression of motor deficits in 60/123 (49%) patients, and in deterioration of motor function in 17/123 (14%) patients. Radiation myelopathy was not observed in the entire cohort.

**Conclusions:** Spinal Re-RT appears to be an effective treatment for an in-field recurrence of MSCC, as it prevents further progression of motor deficits in 86% of patients. If the cumulative EQD2 is  $\leq 60$ Gy, spinal Re-RT appears safe, and radiation myelopathy appears unlikely. Regarding an EQD2 of >60 Gy, further data are required to evaluate efficacy and safety of spinal Re-RT.

	at 12 months	at 24 months	at 36 months	at 48 months	at 60 months	at 72 months
Entire cohort, amifostine	1.69	1.36	1.07	0.91	0.89	0.95
Entire cohort, no amifostine	1.88	1.82	1.85	1.79	1.71	1.75
RT alone, amifostine	1.71	1.38	1.05	0.94	0.93	1.00
RT alone, no amifostine	1.87	1.88	1.85	1.88	1.71	1.76
RCT, amifostine	1.63	1.31	1.10	0.90	0.88	0.86
RCT, no amifostine	1.80	1.89	1.86	1.71	1.71	1.80

OP197

### Leptomeningeal metastasis from breast cancer: Response and long-term stabilization upon intrathecal therapy with liposomal cytarabine (DEPOCYTE) in combination with radiotherapy

Glas M., Stuplich M., Rasch K., Herrlinger U.

University Bonn, Clinical Neurooncology Unit, Dept. of Neurology, Bonn

Leptomeningeal metastasis (LM) is a late and devastating complication of breast cancer. Although intrathecal chemotherapy with MTX may be moderately effective extending median survival to 7 months, the prognosis is much shorter in patients with negative prognostic factors such as higher age, elevated lumbar cerebrospinal fluid (CSF) protein and lactate, lung metastasis and cranial nerve involvement. For these patients, novel and more effective therapeutic options are warranted. We describe the case of a 55 year old female with an inflammatory carcinoma of the left (1994: pT4d, PN2a, M0, G3) and the right breast (2003: pT4, PN2a, LI, R1, G3, Her 2/neu -, ER 1/12, PR-, bone and periclavicular metastasis). She presented to our department with a paresis of the VII and Xth cranial nerve. Cytological analysis revealed malignant cells in the CSF. MRI showed multiple CNS parenchymal metastases and leptomeningeal metastasis with predominant involvement of the cerebellum. Progressive extra-CNS disease was excluded. With an elevated CSF protein and lactate, an age of 55 years and the presentation with cranial nerve involvement the patient had several negative prognostic factors. She received whole brain radiation therapy and intrathecal therapy with depocyt 50 mg biweekly for the first six cycles and monthly lumbar injections for cycle 7 to 9. Upon therapy, the neurological symptoms improved, the CSF was cleared from malignant cells and MRI showed partial regression of contrast-enhancing lesions. The response to depocyt therapy lasted until 12 months after initial diagnosis of LM when the patient presented with new contrast-enhancing brainstem lesions. So far, no neurotoxic side-effects have been observed. In conclusion, this patient showed an encouraging long-term stabilization upon combined radiotherapy and depocyt therapy despite multiple negative prognostic factors. Thus, this therapy deserves further investigation in patients with LM from breast cancer.

OP198

### Tumor cells with stem cell features in glioblastomas: Markers, differentiation and sensitivity towards irradiation and chemotherapeutic drugs

Lemke D.<sup>1,2</sup>, Tabatabai G.<sup>2</sup>, Tatagiba M.<sup>3</sup>, Hopf N.<sup>4</sup>, Weller M.<sup>2</sup>, Wick W.<sup>1,2</sup>

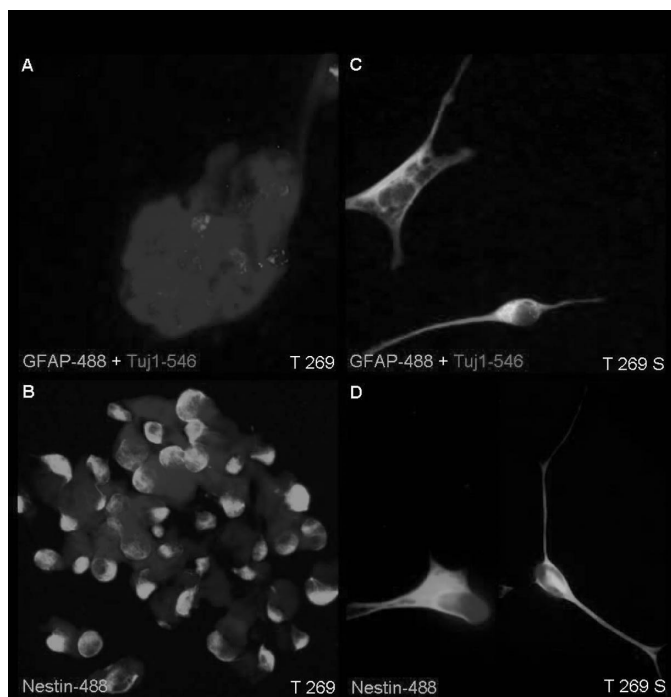
<sup>1</sup>Clinical Cooperation Unit Neurooncology, German Cancer Research Center, Heidelberg; <sup>2</sup>Laboratory of Molecular Neuro-Oncology, Department of General Neurology and Hertie Institute for Clinical Brain Research, University of Tuebingen; <sup>3</sup>Department of Neurosurgery, University of Tuebingen; <sup>4</sup>Department of Neurosurgery, Klinikum Stuttgart

Recent evidence suggests that glioblastomas are organised hierarchically. A little number of multipotent tumor initiating cells have the exquisite potential to proliferate, self renew, resist against radio-chemotherapy and differentiate, while the rest of the cells form the phenotypically distinct main tumor mass. According to Singh *et al.*, these brain tumor initiating cells can be isolated based on the expression of CD133.

We demonstrate that specimens from human glioblastomas contain cells, which form neurospheres and display characteristic stem cell features *in vitro*. These features are multipotency with evidence of astroglial and neuronal differentiation, self-renewal capacity at single-cell level, as well as a stable proliferation potential. Most importantly, these cells are tumorigenic *in vivo*: 50 cells suffice to establish tumors after orthotopic intracerebral implantation and serial orthotopic transplantations *in vivo* resembling the histological features of the original human glioblastomas. In contrast to published evidence, CD133 is not discriminating in the 9 tumour specimens analyzed in this project, as we find stem cell characteristics in CD133<sup>+</sup> as well as CD133<sup>-</sup> cells.

The differentiation of neurospheres by the use of serum-containing medium is not specific as stem cell properties persisted in some tumors after this procedure. Moreover, there was no significant difference between the more differentiated cells and the neurospheres in respect to their resistance towards radio-chemotherapy *in vitro*.





Thus, human glioblastomas contain cells with features typical of neural stem cells. These cells are identified by a characteristic sphere formation *in vitro* when kept in serum free medium supplemented with epidermal growth factor and fibroblast growth factor. Tumor cells with stem cell features kept in stem cell medium are not differentially resistant towards radio-chemotherapy *in vitro* compared to more differentiated cells kept in serum containing medium. CD 133 was neither sensitive nor predictive as a stem cell marker. Probably the capacity to resist undergoing differentiation accounts for the exquisite tumor forming features of brain tumor cells with stem cell features.

OP199

### The role of matrix metalloproteinase-2 in transforming growth factor-beta2 mediated gliomacell migration

Arslan F.<sup>1</sup>, Bosserhoff A.-K.<sup>2</sup>, Doerfelt A.<sup>1</sup>, Beier D.<sup>1</sup>, Leukel P.<sup>1</sup>, Nickl-Jockschat T.<sup>3</sup>, Bogdahn U.<sup>1</sup>, Hau P.<sup>1</sup>

<sup>1</sup>Universität Regensburg, Neurologie, Regensburg; <sup>2</sup>Universität Regensburg, Pathologie, Regensburg; <sup>3</sup>RWTH Aachen, Psychiatrie, Aachen

Transforming growth factor-beta2 (TGF-beta2) is known to contribute to a malignant course in gliomas partially by inducing a mesenchymal phenotype and by modulating the microenvironment including the extracellular matrix. Matrix metalloproteinase 2 (MMP-2) also correlates with the malignant progression of human gliomas together with cell surface adhesion receptors like integrin alpha v beta3. We have described elsewhere that the V0/V1-isoforms of the proteoglycan versican interact with TGF-beta2 and enhance glioma migration. In this study, we aimed to investigate possible interactions of TGF-beta2 with MMP-2 and integrin alpha v beta3 that might contribute to glioma migration. Using real-time qPCR to screen for expressional changes in response to TGF-beta2, we showed that TGF-beta2 enhances the expression of MMP-2 and of integrin alpha v beta3. Additionally, TGF-beta2 increased the MMP-2 secretion detected by Enzyme Linked-Immuno-Sorbent Assay (ELISA) and the activation of MMP-2 in glioma cells as measured by gelatin zymography. *In vitro* migration assays demonstrated that the inhibition of MMP-2 activity using a MMP-2 inhibitor suppresses TGF-beta2 stimulated glioma cell-migration. This effect can be enhanced by additional blockade of integrin alpha v beta3. We also found that MMP-2 interacts with and cleaves versican V0/V1. Taken together, we outline a new set of interactions involving TGF-beta2, MMP-2, integrin alpha v beta3 and the proteoglycan versican which might constitute a crucial

network for glioma migration. This molecular cascade may further elucidate the diffuse parenchyma-infiltrating character of glioma invasion and thus be a target for new approaches to anti-tumor therapy.

## Central Nervous System Tumors

### Poster Presentation

PO397

### Silencing of 06-methylguanine DNA methyltransferase (MGMT) increases temozolomide sensitivity in human malignant glioma cell lines

Adams B., Hermisson M., Naumann U., Weller M.

Hertie-Institut, Neuroonkologie, Tübingen

Temozolomide (TMZ) is an orally applied methylating agent used for the treatment of glioblastoma multiforme. Recent studies show that concomitant and adjuvant temozolomide chemotherapy combined with radiotherapy significantly improves progression-free and overall survival in glioblastoma patients. The response to TMZ depends on the expression of MGMT, a DNA repair enzyme which attenuates the cytotoxic effects of TMZ. Using a panel of 12 different glioma cell lines, we found a direct correlation between the MGMT status and the response to TMZ. We also find that the forced overexpression of MGMT desensitizes glioma cells to TMZ in acute cytotoxicity as well as in clonogenic assays. Conversely, silencing MGMT expression via RNA interference technology could be a promising approach to overcome TMZ resistance. We engineered stable clones from two different MGMT-positive glioma cell lines expressing MGMT shRNA. Both in acute and in clonogenic survival assays, we observed a striking increase of the sensitivity to TMZ. With the aim to complement our *in vitro* data with corresponding *in vivo* data, we produced non-replicative MGMT shRNA-expressing adenoviruses (AdHPMGMT1shRNA and AdHPMGMT2shRNA). A significant MGMT depletion was already detected 24 h after infection. Consequently, we consider viral gene therapy targeting MGMT a seminal tool to resensitize MGMT-positive glioma cells and tumors to TMZ.

PO398

### Adenoviral gene therapy of malignant glioma with soluble transforming growth factor-beta receptors ii and iii

Naumann U., Maass P., Gleske A.-K., Aulwurm S., Weller M., Eisele G.

Hertie-Institut für Klinische Hirnforschung, Allgemeine Neurologie, Tübingen

The cytokine transforming growth factor-beta (TGF-beta) is abundantly expressed in malignant gliomas and crucial for the tumor microenvironment. TGF-beta not only enhances migration and invasion of glioma cells but also inhibits an effective anti-glioma immune response. TGF-beta mediates its biologic effect through interactions with specific TGF-beta receptors I to III (TbetaR I-III). Binding of TGF-beta leads to activation of an intracellular signalling cascade and subsequent phosphorylation of Smad and MAD related proteins (SMAD). Several experimental approaches like the use of soluble TbetaRs (sTbetaR) aim at abrogating the TGF-beta effect. sTbetaRs bind TGF-beta isoforms, compete for the binding of the ligand to its receptor on the cell surface and therefore block TGF-beta-induced autocrine and paracrine effects on cancer and immune cells. Genetically engineered sTbetaR II contains the extracellular TGF-beta binding domain and the signal peptide necessary for secretion whereas the intracellular kinase domain and transmembrane region of the protein are deleted. sTbetaR III is a truncated protein that contains the extracellular TGF-beta binding domain but lacks the domain of membrane anchorage and the intracellular domain. Here we used adenoviral gene transfer to produce sTbetaR II and III in human glioma cell lines LN-308, LNT-229 and U87MG. Tumor cells were transduced with adenovirus encoding sTbetaR II or III. Soluble receptor protein was detectable in supernatants of transduced cells on immunoblot analysis. sTbetaR inhibited the activation of the intracellular signalling cascade in glioma cells upon addition of TGF-beta2. The phosphorylation of SMAD proteins was reduced, as well as the activity of TGF-beta dependent reporter genes. The combined action of both sTbetaR II and III was superior to that of sTbetaR II alone,

whereas sTBR III as a single agent had no significant effect. The combination of sTBR II and III also showed functional activity: the anti-proliferative and pro-apoptotic effects of TGF $\beta_2$  on the sensitive cell line CCL-64 were abrogated. Incubation of natural killer (NK) cells with TGF $\beta_2$  attenuated their lytic capability. This effect was reversed by addition of sTBR II and III. This indicates a possible virtue of sTBR in mounting an effective immune response against glioma cells. Moreover, transduction of LN-308 cells with sTBR markedly delayed growth of intracerebral xenografts in nude mice *in vivo*. In summary, these data commend sTBR as an effective experimental treatment of gliomas by counteracting TGF- $\beta$ .

PO399

### Selenium in the glioblastoma multiforme treated with concomitant radiotherapy and temozolomide

Micke O.<sup>1</sup>, Bruns F.<sup>2</sup>, Mücke R.<sup>3</sup>, Schäfer U.<sup>4</sup>

<sup>1</sup>Franziskus Hospital, Klinik für Strahlentherapie und Radioonkologie, Bielefeld; <sup>2</sup>Medizinische Hochschule Hannover, Abteilung Strahlentherapie und spezielle Onkologie, Hannover; <sup>3</sup>St. Josefs-Hospital, Strahlentherapie, Wiesbaden; <sup>4</sup>Klinikum Lippe, Klinik für Strahlentherapie, Lemgo

The overall prognosis of patients with resected or inoperable glioblastoma multiforme (GBM) is generally very poor. The establishment of the concomitant application of radiotherapy and temozolomide in the primary or postoperative treatment of GBM lead to a marked increase of progression-free and overall survival for the first time. One of the major toxicities compromising patients' quality of life in combined radiotherapy and temozolomide chemotherapy is the development of radiation-associated brain edema.

The anti-edematous effect of selenium is well known from early historical studies. The results of an own exploratory study (MICKE et al. 2003) involving 48 patients suggest that selenium has a positive effect on radiation-associated secondary lymphedema in patients with limb edemas as well as in the head and neck region, including endolaryngeal edema. The majority of patients showed a reduction in edema characteristics. We also found that 65% of patients with interstitial grade III or IV endolaryngeal edema, who normally would require tracheotomy for treatment, could avoid surgical intervention.

Based on these clinical results we initiated an exploratory study combining sodium selenite with combined radiotherapy and temozolomide chemotherapy to improve the tolerability of treatment.

A total 43 patients with newly diagnosed GBM received Temozolomide (75 mg/m<sup>2</sup>/day) concurrent with 60 Gy of conventional radiotherapy according to the EORTC study scheme. As a supportive treatment 350 microgramm sodium selenite/m<sup>2</sup> body surface were applied during the radiation treatment and one week before and after radiotherapy.

Number, type and grade of treatment-associated side effects as well as episodes of symptomatic brain edema and need of corticosteroids were recorded. Patients quality of life (QoL) was evaluated by 10 point visual analogue scale (VAS).

Overall the treatment was well tolerated. No episodes of symptomatic brain edema were observed. In all patients the steroid doses were reduced. No steroid dependency was observed. The overall toxicity was limited to hematotoxicity, some mild episodes of nausea were observed. QoL improved in all patients during selenium treatment.

This exploratory results show that selenium addition to combined radiochemotherapy with oral temozolomide is well tolerated and has high patients compliance. The data suggest that selenium may improve the QoL of patients and the tolerability of the treatment scheme.

PO400

### Brain tumor following head injury?

Stendel R.<sup>1</sup>, Stoltenburg G.<sup>2</sup>, Brock M.<sup>1</sup>

<sup>1</sup>Charité-Universitätsmedizin Berlin, Campus Benjamin Franklin, Neurochirurgische Klinik, Berlin; <sup>2</sup>Charité-Universitätsmedizin Berlin, Institut für Neuropathologie, Berlin

Is there a relationship between head injury and the development of a brain tumor?

This question has been the object of controversial discussions since the origins of neurosurgery and neurology. Already in the past century reference was

made to the possible relation between head injury and subsequent brain tumor development. Since then, numerous cases have been reported in which the connection between head injury and brain tumor is recognized according to the criteria established by Zülch. Despite a great variety of approaches, however, it has not been possible as yet to verify or disprove such a connection. The cases of two patients fulfilling the criteria of Zülch, prompts a revival of this discussion.

We report on two cases of brain tumor and discuss the possible relationship to a previous brain trauma. The first patient, a 67-years-old male patient developed a glioblastoma at the same site of an open shell-splinter injury of the brain after a latency of 48 years. The second patient, a 55 years old male, had a malignant anaplastic astrocytoma in the right frontal lobe 10 years after clipping of an aneurysm of the anterior communicating artery. Both cases fulfill the criteria of Zülch for the correlation between trauma and tumor.

The development of a brain tumor following head injury is very rare, although possible. Probably the patient must display some form of predisposing genetic alteration as a preassumption for tumor genesis following head injury.

PO401

### Imatinib plus hydroxyurea in pretreated non-progressive glioblastoma (GBM) – A single center phase II study

Dresemann G.<sup>1</sup>, Hosius C.<sup>2</sup>, Nikolova Z.<sup>3</sup>, Schleyer E.<sup>4</sup>

<sup>1</sup>Franz-Hospital Dülmen, Onkologische Abteilung, Dülmen; <sup>2</sup>Novartis Pharma, Oncology, Nürnberg; <sup>3</sup>Novartis Pharma, Oncology, Basel; <sup>4</sup>Krankenhaus Merseburg, Medizinische Klinik, Merseburg

**Introduction:** GBM is a highly malignant brain tumor. Median survival of about 15 months. Dysregulated signalling of platelet derived growth factor receptors (PDGF-Rs) is implicated in pathogenesis. The combination therapy of Imatinib (I) plus Hydroxyurea (HU) showed interesting efficacy and tolerability in patients (pts) with recurrent progressing GBM. In a pilot group of 30 pts with recurrent GBM the progression free survival (PFS) at 6 and 24 months was 32 % and 16 % respectively. Disease stabilisation (SD) was achieved in 37%. Prolonged disease stabilisation for more than 2 years was possible. Despite the aggressive course of GBM, short periods of SD after primary treatment or effective treatment of relapse are observed. The current Phase II study was conducted to analyze the efficacy of I plus HU treatment in GBM pts with documented disease stabilisation for at least 6 weeks as maintenance treatment. **Methods:** From December 2003 up to June 2005 30 non-progressive GBM pts were included, all of them with SD for more than 6 weeks following effective treatment, including surgery, radiotherapy and at least one chemotherapeutic regimen. No enzyme-inducing anticonvulsive drugs were allowed. I at the dose of 600 mg od and 1000 mg of HU (500mg bid) were given as a continuous daily treatment, all pts were followed up by blood cell count weekly and magnetic resonance imaging every 6 weeks.

**Results:** All 30 pts are eligible for safety and for 6, 12 and 24 months PFS and overall survival (OS); 25 pts are male, 5 pts female, the median age is 44 years (32 to 71), 24 pts had primary and 6 pts secondary GBM. All 30 pts had prior radiotherapy, 21 pts had temozolomide containing chemotherapy and 9 pts non-temozolomide containing regimens only. 8 pts were free from relapse, 17 pts after first and 5 pts after second relapse. The median observation time is 36 months. 6, 12 and 24 months PFS is 60% (18/30), 40% (12/30) and 17% (5/30) respectively. 6, 12 and 24 months OS is 90 % (27/30), 67% (20/30) and 37% (11/30) so far. PFS for more than 24 months occurred in 3/6 pts with secondary and in 2/24 pts with primary GBM. Hematotoxicity grade 2 and 3 occurred in 11 out of 30 pts (anemia grade 3: 2 pt; anemia grade 2: 4 pts; leucocytopenia grade 3: 2 pts; leucocytopenia grade 2: 7 pts; thrombocytopenia grade 2: 4 pts) and required dose reduction of HU in 8 pts, dose reduction of I in 1 patient and G-CSF subcutaneously in 8 pts. No febrile neutropenia, no interruption of the treatment due to toxicity and no treatment related death occurred.

**Conclusion:** The combination of I (600 mg/d) and HU (1000 mg/d) was well tolerated and effective as maintenance treatment in this study with 17% PFS and 20% OS after 3 years. Pts with secondary GBM where PDGF-R signaling plays an important role seem to be more likely to benefit.

PO402

### Bevacizumab (B) plus irinotecan (I) in progressive temozolomide (T) resistant Glioblastoma Multiforme (GBM) – A single center experience

Dresemann G.

Franz-Hospital Dülmen, Onkologische Abteilung, Dülmen

**Background:** Although a standard treatment consisting of surgery, irradiation plus concomitant and following T is established median survival of patients (pts) with GBM still is 15.6 months, indicating the need for further effective treatment. In a small single center study Imatinib plus Hydroxyurea showed efficacy as maintenance treatment with progression free survival (PFS) of 17% and overall survival of 20% at 3 years, the same regimen was less effective in progressive disease (PD). In several phase II studies conducted in the United States B plus I showed impressive objective response rates of more than 50% with acceptable toxicity duration of response was moderate. Therefore B plus I might be an induction regimen.

**Methods:** From 2006, December, to 2007, October, 44 pts with progressive T resistant GBM were treated with B 4 mg/kg body weight intravenously (iv) followed by I 80 mg/m<sup>2</sup> iv repeated every 2 weeks. MRI scans were performed at baseline, after 4 weeks (before the third treatment application), afterwards every 6 weeks. Treatment was given until disease progression or intolerable toxicity occurred. Every 2 weeks clinical examination and blood cell count were performed.

**Results:** All 44 pts are eligible for toxicity and efficacy. 33 pts were male, 11 female, median age was 45 years (21 – 79), 32 pts had primary GBM 7 pts preceding astrocytoma grade II and III, 4 pts had 4 prior chemotherapy regimens, 11 pts 3, 22 pts 2 and 7 pts T only. All pts had prior irradiation (56 – 60 Gy). ECOG-performance status (PS) was 0 – 3. The patient with ECOG 3 developed grade IV leucocytopenia and died of clostridien sepsis after the first treatment, there was another patient with grade III leucocytopenia and 2 pts with grade III thrombocytopenia, 1 patient with a grade III pneumonia, 2 pts with asymptomatic intracerebral bleeds requiring treatment delay and 1 patient developed grade III fatigue. In 21 pts a partial response (PR) was achieved, in 15 pts disease stabilisation for at least 2 months, 7 pts showed primary PD. Median duration of PR was 3 months (2 – 8), best MRI response usually could be demonstrated after 4 to 8 weeks of treatment.

**Conclusion:** B plus I seems to be the most effective regimen for induction of objective response in T resistant GBM with acceptable toxicity profile. Pts with ECOG-PS 3 should not be considered for treatment. These results should be confirmed in further studies restricted to ECOG-PS 0 to 2. A following maintenance treatment should be considered.

PO403

### Clinical characteristics of newly diagnosed primary glioblastoma

Fiss I., Stendel R.

Charite – Universitätsmedizin Berlin, Klinik für Neurochirurgie, Berlin

**Objective:** Glioblastoma multiforme (GBM) is the most common and most malignant primary brain tumor in adults. We present 71 cases treated at a single institution and discuss clinical characteristics and prognostic factors with regard to the neurosurgical literature.

**Methods:** Included in this study were 71 patients who underwent craniotomy for newly diagnosed GBM between 2000 and 2003 at our department. Demographic data were recorded, as well as symptoms at onset, entity of tumour resection, post-surgical Karnofsky Performance Score, radio- and chemotherapy, presence/absence of venous thrombosis, type of antiepileptic treatment, time to tumour progression and survival time (ST).

**Results:** Median patient age was 61 years (mean, 61.4; range, 27-90 years), the male-female ratio was 1.03:1. In 10 cases (14.1%) the tumor was multicentric. During follow-up, 15 patients (21.2%) underwent re-craniotomy for GBM recurrence, either once (12 patients; 16.9%) or twice (3 patients; 4.3%). Initial Symptoms leading to diagnosis were cephalgia in 14 patients (19.7%), dysphasia in 13 patients (18.3%), sensible deficits in 1 patient (1.4%), motoric deficits in 7 patients (9.9%), seizures in 14 patients (19.7%), mnestic deficits in 4 patients (5.6%), visual deficits in 7 patients (9.9%) and psychomotoric impairment in 6 (8.5%) patients. Complete resection was performed in 32 patients (45.1%), subtotal resection in 5 patients (7.0%), partial resection in 3 patients

(4.2%), open biopsy in 2 patients (2.8%), and stereotactic biopsy in 22 patients (31.0%). In 37 patients (52.1%), the tumour involved 1 brain lobe at diagnosis. Diagnosis of GBM was confirmed histologically in all patients. Initial diagnosis was made by cranial computertomography in 36 patients (50.7%), by MRI in 22 patients (31.0%), and in 13 patients (18.3%) histologically. 45 Patients (63.3%) presented with an initial Karnofsky performance scale of 70 or more.

**Conclusions:** Glioblastoma multiforme remains an important cause of morbidity and mortality from intracranial tumors. The overall prognosis is dismal, although interdisciplinary therapy can significantly prolong survival. Data in newly diagnosed glioblastoma patients in Berlin are in line with other case series reported in other populations.

PO404

### Re-challenge with Temozolomide (TMZ) at Recurrence in high-grade gliomas

Hau P.; Pascher C.; Beier C.; Beier D.; Dudel C.; Grauer O.; Hirschmann B.; Müller C.; Reinert C.; Wismeth C.; Bogdahn U.

Universität Regensburg, Neurologie, Regensburg

**Background:** TMZ is standard therapy for patients with high-grade gliomas (HGG). Recent data suggest potentially enhanced efficacy of alternative schedules of TMZ administration based on optimizing depletion of MGMT or alternative mechanisms as antiangiogenesis. However, no prospective data have been published on the efficacy of TMZ regimens after failure of first-line TMZ. We therefore assessed the outcome of rechallenge with TMZ in pts with HGG.

**Methods:** A retrospective review of pt with recurrent HGG who were rechallenged with TMZ between Jan 2000 and June 2007 was conducted. Standard criteria for rechallenge were as follows: if pts relapsed  $\geq 8$  wks after discontinuation of first-line TMZ, they were treated with the same or an alternative regimen of TMZ; however, if they progressed while still receiving TMZ, they were treated with an alternative regimen.

**Results:** Of a total 81 patients identified, 54 intra-institutional pts were evaluable, 23 glioblastoma (GBM), 22 anaplastic glioma (AG). Median age was 44 years with median KPS of 80. The response rate (RR) to first-line TMZ was 65% in GBM and 95% in AG using Macdonald criteria. At rechallenge, patients received one of four TMZ regimens: 150-200mg/m<sup>2</sup>/d for 5/28 days (n = 35), 150mg/m<sup>2</sup>/d for 7/14 days (n = 7), 75mg/m<sup>2</sup>/d for 21/28 days (n = 4), or continuous 40mg/d (n = 7). All patients had progressed during first line TMZ or after initial discontinuation of TMZ. Discontinuation during rechallenge TMZ was due to progressive disease in 36 patients and patient request in 6 patients. None of the patients discontinued due to toxicity. The response rates were 66% and 70% in GBM and AG, respectively. 8/12 non-responders to first-line TMZ responded to alternate regimens of TMZ. Median time to progression was 20 weeks and 22 weeks, respectively. Six-month progression-free survival was 41% in GBM and 43% in AG.

**Conclusions:** TMZ was well tolerated without major toxicities and had a good response rate in pts who had previously failed TMZ. Durable responses were observed in patients who had not initially responded to front-line TMZ. These data suggest that rechallenge with TMZ in previous responders and non-responders warrants further investigation in a prospective study.

## Central Nervous System Tumors

### Poster Exhibition

PE748

### Postoperative treatment of primary glioblastoma multiforme with radiation and concomitant temozolomide in elderly patients

Combs S.E., Wagner J., Bischof M., Welzel T., Debus J., Schulz-Ertner D.

Universitätsklinikum Heidelberg, RadioOnkologie, Heidelberg

**Background:** To evaluate efficacy and toxicity in elderly patients with glioblastoma multiforme (GBM) treated with postoperative radio-chemotherapy (RCHT) with temozolomide (TMZ).

**Patients and Methods:** Forty-three patients aged 65 or older were treated with postoperative with RCHT using TMZ for primary GBM.

Median age at primary diagnosis was 67 years, fourteen patients were female, and 29 were male. A complete surgical resection was performed in 12 patients, subtotal resection in 17 patients and a biopsy only in 14 patients.

RT was applied with a median dose of 60 Gy, in a median fractionation of 5x2Gy/week. Thirty-five patients received concomitant TMZ at 50mg/m<sup>2</sup>, and in eight patients 75mg/m<sup>2</sup> of TMZ was applied. Adjuvant cycles of TMZ were prescribed in 5 patients only.

**Results:** Median overall survival (OS) was 11 months in all patients, the actuarial overall survival rates were 48% at 1 year and 8% at 2 years. Median overall survival was 18 months after complete resection, 16 months after subtotal resection, and 6 months after biopsy only.

Median progression-free survival (PFS) was 4 months, actuarial progression-free survival rate was 41% at 6 months and 18% at 12 months.

RCRT was well tolerated in most patients and could be completed without interruption in 38 out of 43 patients. Four patients developed hematologic side effects > CTC grade II, which led to early discontinuation of TMZ in 1 patient.

**Conclusion:** RCRT is safe and effective in a subgroup of elderly patients with GBM and should be considered in patients without major comorbidities.

PE749

## Tumor and hydrocephalus

Stark A.M., Mehdorn H.M.

Klinik für Neurochirurgie UKSH Kiel, Kiel

**Introduction:** The co-occurrence of tumor and hydrocephalus in cancer patients is relatively frequent. It may cause severe clinical problems in terms of diagnosis and treatment. Noteworthy, this issue is underestimated in the literature.

**Material and Methods:** We report our experience with this issue. We present cases from our department in the light of the literature and draw conclusions for diagnostic and therapeutic pathways.

Figure 1. 57-year-old patient with chronic recurrent subarachnoid hemorrhage from a trigeminal nerve malignant peripheral nerve sheath tumor (arrow) causing hydrocephalus



**Results:** Cases can be classified by the impact of tumor and hydrocephalus on treatment and prognosis: (1) surgical tumor removal can massively reduce the risk of persistent hydrocephalus (as in infratentorial brain metastases, heman-gioblastoma, pilocytic astrocytoma, medulloblastoma, choroid plexus papil-loma). (2) tumor-induced hydrocephalus might be treated by CSF diversion procedures (shunt/endoscopic third ventriculostomy) while the tumor itself does not require surgical treatment (as in tectal glioma, pineal region germinoma). (3) the tumor may complicate CSF diversion by raised protein content (as in a case of congenital diffuse anaplastic astrocytoma, vestibular schwannoma). In some cases, hydrocephalus is temporary, as observed in a case of subarachnoid bleed-ing from a trigeminal nerve malignant peripheral nerve sheath tumor and a pedi-atric astrocytoma with leptomeningeal spread. The histological diagnosis of meningeal spreading from primary brain tumors may be hardened when repeated cytological diagnosis is negative. Open meningeal biopsy may be necessary.

**Conclusion:** The co-existence of tumor and hydrocephalus is a frequent event in neurooncology. Differential diagnostic and therapeutic approaches are required including high resolution magnetic resonance imaging (MRI), microneuro-surgery, neuroendoscopy, serial CSF puncture and current shunt valve technology.

## Gastrointestinal Cancer including Liver

### Oral Presentation

OP130

### Efficacy and safety of sunitinib in patients with gastrointestinal stromal tumour resistant or intolerant of prior imatinib therapy: Results from a worldwide treatment-use study

Schütte J.<sup>1</sup>, Reichardt P.<sup>2</sup>, Schlemmer M.<sup>3</sup>, Wendtner C.-M.<sup>4</sup>, Demetri G.D.<sup>5</sup>

<sup>1</sup>Marien Hospital, Klinik für Onkologie und Hämatologie, Düsseldorf; <sup>2</sup>Helios Klinikum, Bad Saarow; <sup>3</sup>Klinikum der Universität München-Großhadern, München; <sup>4</sup>Klinikum der Universität Köln, Köln; <sup>5</sup>Dana-Farber Cancer Institute, Boston

**Background:** Sunitinib malate (SUTENT<sup>®</sup>) is an oral, multitargeted tyrosine kinase inhibitor approved for imatinib-resistant or -intolerant gastrointestinal stromal tumour (GIST). This ongoing study is providing access to sunitinib for patients with GIST who were ineligible for sunitinib clinical trials or had no access to sunitinib prior to regulatory approval in their country.

**Methods:** In this multicentre, open-label study, patients with advanced GIST who had failed prior imatinib therapy receive sunitinib 50 mg/day in 6-week cycles (4 weeks on treatment, 2 weeks off treatment; 4/2 schedule). Antitumour response (as per local standard of care), overall survival (OS), safety and tolerability are assessed.

**Results:** A total of 1,097 patients had been enrolled in 33 countries as of April 2007 with an intention-to-treat (ITT) population of 1,091. Patients (median age 59 years; range 10-92) received a median of 4 cycles (range 1-23). Estimated median time to tumour progression was 37 weeks (95% CI: 35-44). At time of data cut-off, 697 (64%) patients were alive, with an estimated median OS of 73 weeks (95% CI: 66-92). The median OS for patients <65 years and ≥65 years was 91 weeks (95% CI: 72-NA; n = 703) vs 64 weeks (95% CI: 59-73; n = 387), respectively. Patients who previously received imatinib ≤400 mg had median OS of 93 weeks (95% CI: 72-100; n = 339) vs 68 weeks (95% CI: 63-85; n = 749) for those receiving imatinib >400 mg. Median OS was 93 weeks (95% CI: 76-122; n = 915) in patients with an ECOG PS of 0/1 vs 27 weeks (95% CI: 16-40; n = 130) for patients with an ECOG PS of 2. Sunitinib dose reductions (for any reason) occurred in 39% of patients. Lack of efficacy accounted for 34% of patients discontinuing treatment and 17% stopped due to adverse events (AEs). The most common AEs of any cause were fatigue (48%), diarrhoea (45%) and nausea (35%). The most common grade 3/4 AEs were fatigue (10%), hand-foot syndrome (9%) and abdominal pain (10%). Haematological AEs (total, grade 3/4) included anaemia (22%, 8%), thrombocytopenia (18%, 5%) and neutropenia (17%, 8%).

**Conclusions:** Sunitinib is an effective treatment for patients with advanced GIST after imatinib failure, corroborating previous studies. The safety profile seen here is similar to that observed with sunitinib in other GIST trials, with most AEs mild to moderate in severity. Age, prior imatinib dose and ECOG PS may be important prognostic factors in this population; further studies are needed to confirm this.

### Development and independent validation of a genomic profile for the prognosis of colorectal cancer patients

Simon I.<sup>1</sup>, van Laar R.<sup>1</sup>, Salazar R.<sup>2</sup>, Westerga J.<sup>3</sup>, Capella G.<sup>2</sup>, Marijnen C.<sup>4</sup>, Floore A.<sup>1</sup>, Glas A.<sup>1</sup>, Tollenaar R.<sup>5</sup>, Van't Veer L.<sup>1,4</sup>

<sup>1</sup>Agendia BV, Amsterdam; <sup>2</sup>Institut Catala d'Oncologia, Barcelona; <sup>3</sup>Slotervaart Hospital, Department of Pathology, Amsterdam; <sup>4</sup>Netherlands Cancer Institute, Department of Radiotherapy and Molecular Pathology, Amsterdam; <sup>5</sup>LUMC, Department of Surgery, Leiden

**Background:** It is commonly accepted that colon cancer patients with increased risk of recurrent disease are those most likely to benefit from adjuvant treatment following surgery. However, the current standard for determining prognosis, the clinical staging system, is suboptimal. Using microarray technology and tumor classification methods, we identified a subset of 320 genes that are predictive for the prognosis of recurrence of stage II and III colon cancer patients.

**Patients and Methods:** Tumor RNA from cohort of 128 predominantly stage II and III CC patients was analyzed using whole-genome microarrays. By integrating staging and outcome variables with the gene expression data, a set of 320 significant genes was identified ( $p < 0.001$ ) and used to train a Support Vector Machine classifier. Performance of the classifier was assessed using cross validation and an independent series of 58 tumors, not used for gene selection. The signature was converted into a robust diagnostic tool (ColoPrint) and control systems were established to monitor reproducibility and accuracy. This classifier was further validated in a second independent validation cohort from patients with stage II and III CRC collected at a Spanish hospital.

**Results:** The SVM classifier was observed to correctly classify 70% of patients in both training and validation sets into their correct classes, at time of last follow-up. Applying the prognostic profile in the first validation cohort (6 stage I, 27 stage II, 26 stage III), approximately 61% of patients were classified as low risk and 39% as high risk; 3 stage I, 10 stage II and 9 stage III. Patients with a high risk score had a significant higher risk of developing distant metastasis (HR 4.2,  $p = 0.01$ ). In the multivariate analysis, including profile, number of assessed lymph nodes and stage, the profile was identified as the strongest independent prognostic factor ( $p = 0.001$ ). The performance of this test is now validated in a second independent patient set.

**Conclusion:** Microarray gene expression profiling is able to identify subsets of stage II and III colon cancer patients most likely to benefit from adjuvant chemotherapy and adds significant value to the currently used clinicopathologic risk stratification.

### The dual EGF/VEGF-receptor tyrosine kinase inhibitor AEE-788 inhibits growth of human hepatocellular carcinoma xenografts in mice

Okamoto K.<sup>1</sup>, Neureiter D.<sup>2</sup>, Alinger B.<sup>2</sup>, Sass G.<sup>3</sup>, Schmitz V.<sup>4</sup>, Di Fazio P.<sup>1</sup>, Wissniowski T.<sup>1</sup>, Hahn E.G.<sup>1</sup>, Ocker M.<sup>1</sup>

<sup>1</sup>Universitätsklinikum Erlangen, Medizin 1, Erlangen; <sup>2</sup>Salzburger Landeskliniken, Institut für Pathologie, Salzburg; <sup>3</sup>Friedrich-Alexander-Universität Erlangen-Nürnberg, Institut für Pharmakologie, Erlangen; <sup>4</sup>Universitätsklinik Bonn, Medizinische Klinik I, Bonn

**Background:** Treatment for advanced stages of hepatocellular carcinoma (HCC) remains unsatisfactory. HCC is a highly vascularised tumor and also shows enhanced expression of epidermal growth factor receptors. We therefore investigated the effect of AEE-788, a novel inhibitor of the receptor tyrosine kinase activity of the EGF and the VEGF receptor, for HCC treatment in vitro and in a subcutaneous xenograft model in vivo.

**Methods:** The human HCC cell lines HepG2 and Hep1B were cultured under standard conditions and incubated with different concentrations of AEE-788 (0.1 to 100  $\mu$ M) for 24 to 120 h. Cell viability was assessed after trypan blue staining in a Neubauer chamber. Apoptosis was quantified by flow cytometry after propidium iodide staining. For the xenograft model,  $5 \times 10^6$  cells were injected subcutaneously into the flank region of male NMRI nude mice ( $n/\text{group} = 8$ ). Oral treatment with 25 or 50 mg/kg AEE-788 three times per week was started when tumors reached a diameter of 7 mm. At the end of

treatment, tumor specimens were obtained for further analysis (immunohistochemistry, western blotting).

**Results:** In vitro, the number of viable cells was rapidly reduced in both cell lines at concentrations of AEE-788 of 10  $\mu$ M or higher, while lower concentrations remained ineffective. Apoptosis induction was observed in a time-dependent manner at concentrations of 10  $\mu$ M or higher. In HepG2, apoptosis rose from 8.8% at 48 h to a maximum of 58.4% at 120 h at 10  $\mu$ M, and from 24.2% at 24 h to 96.3% at 120 h at 100  $\mu$ M. This was accompanied by a loss of the mitochondrial transmembrane potential and the cleavage of cyokeratin 18 fragments. Westernblotting showed an inhibition of the MAPK pathway by AEE-788. In vivo, untreated control tumors doubled their size in 14 days, while AEE-788 treated tumors showed a delayed growth with nearly stable tumor size over the treatment period and a significant reduction in blood vessel size and distribution. All animals survived until the treatment endpoints. Treated animals showed a reversible skin reaction after AEE-788 application. **Conclusions:** AEE-788 is a promising novel small molecule inhibitor of the EGF- and the VEGF-pathway and can contribute to treatment of HCC.

### Sorafenib improves survival in patients with hepatocellular carcinoma: Results of a large multi-center, randomized, placebo-controlled phase III trial

Hilgard P.<sup>1</sup>, Trojan J.<sup>2</sup>, Galle P.R.<sup>3</sup>, Greten T.<sup>4</sup>, Erhardt A.<sup>5</sup>, Shan M.<sup>6</sup>, Moscovici M.<sup>7</sup>, Voliotis D.<sup>8</sup>, Häussinger D.<sup>5</sup>

<sup>1</sup>Universitätsklinikum Essen, Gastroenterologie und Hepatologie, Essen; <sup>2</sup>Klinikum der Johann Wolfgang Goethe Universität, Zentrum der Inneren Medizin, Frankfurt/Main; <sup>3</sup>Johannes Gutenberg-Universität, I. Medizinische Klinik und Poliklinik, Mainz; <sup>4</sup>Kliniken der medizinischen Hochschule Hannover, Abt. Gastroenterologie, Hepatologie und Endokrinologie, Hannover; <sup>5</sup>Heinrich-Heine-Universität, Gastroenterologie, Düsseldorf; <sup>6</sup>Bayer Pharmaceutical Corporation, Westhaven, Connecticut (CT) /US; <sup>7</sup>Bayer Healthcare, Milano; <sup>8</sup>Bayer Healthcare AG, Pharma research Center, Wuppertal

**Background:** Hepatocellular carcinoma (HCC) is the fifth most common malignancy worldwide and very difficult to treat. Patients with HCC have a poor prognosis as there is currently no effective treatment for advanced stages. Therefore, effective therapy options are urgently needed. Sorafenib (Sor), a multikinase targeted agent with Raf and VEGF-R inhibitory properties, has demonstrated activity in advanced HCC in a phase II trial. We report the findings of a large, multicenter, randomized phase III trial evaluating the efficacy and safety of sorafenib in patients with HCC against placebo (P).

**Methods:** Patients with advanced measurable HCC, no prior systemic treatment, ECOG PS 0–2 and Child-Pugh status A received Sor 400 mg bid or P. Primary efficacy endpoints were overall survival (OS) and time to symptomatic progression (TTSP). Secondary endpoints were time to progression (TTP) and disease control rate (DCR; CR+PR+SD for at least 2 cycles). Treatment arms were compared for OS and TTSP using a 1-sided log-rank test [overall  $\alpha$  of 0.02 (OS) and 0.005 (TTSP)] stratified by region, ECOG PS and tumor burden. An O'Brien-Fleming-type error spending function determined criteria for early stopping for efficacy.

**Results:** 602 patients (Sor  $n = 299$ ; P  $n = 303$ ) were randomized. Baseline characteristics were similar for Sor vs P: median age (65 vs 66 y), male (87% vs 87%), ECOG PS 0 (54% vs 54%), Child-Pugh A (95% vs 98%), and BCLC stage C (82% vs 83%). Based on 321 deaths (Sor  $n = 143$ ; P  $n = 178$ ), the hazard ratio (HR) for OS (Sor/P) was 0.69 (95% CI: 0.55, 0.87;  $p = 0.00058$ ), representing a 44% improvement in OS vs P which met early stopping criteria. Median OS was 10.7 vs 7.9 months (Sor vs P). Primary TTSP analysis demonstrated no statistically significant difference for Sor versus P. HR for TTP (independent assessment) was 0.58 (95% CI: 0.45, 0.74;  $p = 0.000007$ ). Median TTP was longer (5.5 vs 2.8 months) and DCR was higher (43% vs 32%) with Sor versus P. Incidence of serious adverse events was similar for Sor versus P (52% vs 54%). The most frequent drug-related grade 3/4 events were diarrhea (8% vs 2%), hand-foot skin reaction (8% vs <1%), pain (2% vs <1%) and weight loss (2% vs 0%) for Sor versus P.

**Conclusions:** Sorafenib is the first agent to show a statistically significant improvement in OS for patients with advanced HCC. This effect is clinically meaningful and establishes sorafenib as first-line treatment for these patients. Sorafenib therapy was well tolerated.

### Clinical significance and therapeutic potential of programmed death 1 ligand-1 and programmed death-1 ligand-2 expression in human colorectal cancer

Grimm M.<sup>1</sup>, Gasser M.<sup>1</sup>, Königshausen M.<sup>1</sup>, Stein C.<sup>1</sup>, Lutz J.<sup>2</sup>, Krol S.<sup>1</sup>, Nichiporuk E.<sup>1</sup>, Thiede A.<sup>1</sup>, Heemann U.<sup>2</sup>, Waaga-Gasser A.M.<sup>1</sup>

<sup>1</sup>Chirurgische Universitätsklinik, Molecular Onko-Immunologie, Würzburg;

<sup>2</sup>Klinikum rechts der Isar, Abtl. Nephrologie, München

**Purpose:** The negative regulatory programmed death-1/programmed death-1 ligand (PD-1/PD-L) pathway in T-cell activation has been suggested to play an important role in tumor evasion from host immunity. Levels of immune cells expressing PD-1 in clinical colorectal carcinoma (CRC) tumors have not been evaluated. Thus, we investigated whether PD-1 positive T cells were expressed within CRC tumors and the expression of PD-L1 and PD-L2 in human CRC to define their clinical significance in patients' prognosis after surgery.

**Experimental Design:** Tissue samples from 116 patients operated between 2001 and 2003 were collected in our institution and histologically confirmed CRC were evaluated retrospectively for this study. PD-L1 and PD-L2 gene expression was evaluated by real time quantitative PCR and the samples were immunostained and anti-PD-1 Outcome analyses were performed.

**Results:** The protein and the mRNA levels of determination by immunohistochemistry and real time quantitative PCR were closely correlated. PD-L expression was inversely correlated with tumor-infiltrating T lymphocytes, particularly CD8+ T cells. T cell infiltration was observed in 105 (90.5%) specimens. 93 (80.2%) specimens were PD-1+ T cells. Intratumoral PD-1+ T cells were associated with advanced tumor stage ( $p = 0.002$ ). Patients with PD-1+ T cells had significantly more PD-L1 tumor cell expression. Multivariate analysis indicated that PD-L positive patients and those with PD-1+ T cells had a significantly poorer prognosis than the negative patients. This was more pronounced in the advanced stage of tumor than in the early stage.

**Conclusions:** These data suggest that interactions of T cells expressing PD-1 and PD-L may promote cancer progression. PD-L1 and PD-L2 status may be a new predictor of prognosis for patients with CRC.

## Gastrointestinal Cancer including Liver

### Poster Presentation

PO373

### Preoperative FDG-PET-CT correlates with intraoperative findings in patients with peritoneal carcinomatosis

Königsrainer I.<sup>1</sup>, Aschoff P.<sup>2</sup>, Beckert S.<sup>1</sup>, Zieker D.<sup>1</sup>, Hartmann J.<sup>3</sup>, Pfannenbergl C.<sup>2</sup>, Miller S.<sup>2</sup>, Coerper S.<sup>1</sup>, Brücher B.<sup>1</sup>, Königsrainer A.<sup>1</sup>

<sup>1</sup>Universität, Allgemeinchirurgie, Tübingen; <sup>2</sup>Universität, Radiologie, Tübingen; <sup>3</sup>Universität, Onkologie, Tübingen

**Background:** Peritoneal carcinomatosis until recently were considered incurable. Interdisciplinary multimodal therapy including extensive cytoreductive surgery followed by hyperthermic intraperitoneal chemotherapy (HIPEC) offers significant survival benefit. So far, no radiomorphological imaging can predict the intraoperative tumor load. Combined FDG-PET-CT-scan might offer an impact on patient selection, because its findings might influence making the decision for peritonectomy and HIPEC. We evaluated the results of preoperative FDG-PET-CT-scan by correlating the findings of metabolic imaging with the intraoperative findings of patients who underwent peritonectomy and HIPEC.

**Patients and Methods:** Eleven consecutive patients (mean age: 61,45years) underwent preoperative FDG-PET-scans, followed by cytoreductive surgery and HIPEC. The intraperitoneal tumor load was assessed preoperatively by FDG-PET-CT-scan according to the Peritoneal-Carcinomatosis-Index (PCI-PET-CT) according to the intraoperative PCI reported by Sugarbaker (PCI-Sugarbaker). Both indices (PCI-PET-CT and PCI-Sugarbaker) underwent correlation analysis. Moreover we divided the abdominal quadrants into 4 regions (1: upper abdomen, 2: middle abdomen, 3: lower abdomen-pelvis, 4: intestine including jejunum and ileum) and correlated again the different regions.

<!--[if !supportEmptyParas]--> <!--[endif]-->

**Results:** The PCI-PET-CT (mean: 20,54+/- 12,18) was statistically significant correlated with the PCI-Sugarbaker (mean: 21,09+/- 12,8) ( $r^2 = 0.929$ ;  $p < 0.0001$ ). In addition, this correlation could be found in respect to the 4 PCI subgroups (upper abdomen:  $r^2 = 0.765$ ;  $p = 0.006$ ; middle abdomen:  $r^2 = 0.779$ ;  $p = 0.005$ ; lower abdomen-pelvis:  $r^2 = 0.746$ ;  $p = 0.008$  and intestine:  $r^2 = 0.862$ ;  $p = 0.001$ ).

<!--[if !supportEmptyParas]--> <!--[endif]-->

**Conclusion:** The PCI-FDG-PET-CT correlates well with the intraoperative Peritoneal-Carcinomatosis-Index by Sugarbaker. Further studies are warranted investigating the potential impact of FDG-PET-CT on preoperative patient selection for peritonectomy and HIPEC in patients suffering from peritoneal carcinomatosis.

PO374

### Sorafenib in the treatment of advanced Hepatocellular Carcinoma (HCC) – Experiences beyond the sharp trial

Wörms M.-A.<sup>1</sup>, Schulze-Bergkamen H.<sup>1</sup>, Schuchmann M.<sup>1</sup>, Otto G.<sup>2</sup>, Galle P.R.<sup>1</sup>, Weinmann A.<sup>1</sup>

<sup>1</sup>Johannes Gutenberg-Universitätsklinik, I. Medizinische Klinik und Poliklinik, Mainz; <sup>2</sup>Johannes Gutenberg-Universitätsklinik, Transplantationschirurgie, Chirurgie von Leber, Pankreas, Gallenwegen, Mainz

**Background:** Sorafenib (Nexavar®, Bayer Vital, Leverkusen, Germany), a novel multikinase inhibitor blocking tumor cell proliferation and angiogenesis by targeting Raf/MEK/ERK signaling pathway and VEGFR-2/3, PDGFR- $\beta$  tyrosine kinases, was recently approved for the treatment of patients (pts) with hepatocellular carcinoma (HCC) due to positive data from the phase III (SHARP) trial. Here, we report about our experiences with Sorafenib beyond the SHARP trial in pts with advanced liver cirrhosis (Child Pugh B/C) and after orthotopic liver transplantation (OLT).

**Patients and Methods:** From January to October 2007, 21 pts with advanced HCC were treated with the initial standard dose of 400 mg Sorafenib bid independent of liver function and local/systemic pre-treatment. Pts with recurrence of HCC after OLT were also included. Side effects were graded according to CTCAE v3.0. In the beginning, cost coverage from the health insurance for the off-label use was requested.

**Results:** 21 pts (19 m/2 f, mean age 63 (35-81) yrs) were treated over a period of 1591 days (mean 76 days) as of October 31th. 13 pts had underlying liver cirrhosis (LCI), mainly due to chronic alcohol consumption ( $n = 7$ ). 2/9/2 pts had LCI Child-Pugh A/B/C, BCLC stage was C/D in 17/3 pts. 7 pts presented with ascites, 11 pts had distant metastases (lung, adrenal gland, bones). 8 pts had portal vein thrombosis and 3 pts presented with pulmonary recurrence after OLT. Most common adverse events were diarrhea ( $n = 14$ , CTCAE grade 3 in 4 pts), dry skin ( $n = 8$ ), fatigue ( $n = 6$ ), hand-foot skin reaction ( $n = 6$ , CTCAE grade 3 in 1 pts) and weight loss ( $n = 6$ ). Dose modifications were necessary in 12 pts, mainly due to diarrhea or hand-foot skin reaction. Hyperbilirubinaemia or liver dysfunction related to Sorafenib could not be ruled out in 6 pts. Side effects improved under symptomatic therapy or intermittent dose modifications. Therapy was terminated in 6 pts due to symptomatic/radiological tumor progression. 8 pts died during the observation period (1888 days).

**Conclusions:** Sorafenib may be safely administered in patients with advanced LCI or after OLT under periodic surveillance. Management of side effects is feasible and advanced LCI or history of OLT may not be a limiting factor for Sorafenib therapy in advanced HCC.

PO375

### The benefit of adjuvant chemotherapy in colon carcinoma UICC stage III depends on surgical quality. Results of the intact I trial

Meyer T.<sup>1,2</sup>, Merkel S.<sup>1</sup>, Reingruber B.<sup>1</sup>, Papadopoulos T.<sup>3</sup>, Wein A.<sup>2</sup>, Hahn E.<sup>2</sup>, Hohenberger W.<sup>1</sup>

<sup>1</sup>Universitätsklinikum, Chirurgische Klinik, Erlangen;

<sup>2</sup>Universitätsklinikum, Medizinische Klinik I, Erlangen

<sup>3</sup>Universität, Pathologie, Erlangen

**Background:** Meanwhile, adjuvant chemotherapy is established after curative resection of colon carcinoma, UICC stage III. One of the aims of the prospectively

randomised, multicentric Interdisciplinary Trial for Adjuvant Colon Cancer Treatment (InTACT I) was to investigate in how far adjuvant chemotherapy can improve the results after optimised surgical resection of colon carcinomas, UICC stage III.

**Patients and Methods:** Primary end-point of the study was disease-free survival (DFS). Standardised surgical resection and follow-up (S, arm A) were compared to surgery and adjuvant chemotherapy (S+Ctx, arm B). Chemotherapy consisted of 3 cycles of folinic acid (500 mg/m<sup>2</sup> iv/24hrs) and 5-FU (2000 mg/m<sup>2</sup> iv/24hrs) once weekly for six weeks. Between 02/1996 und 09/2001 146 patients (pat.) in 14 active centers were included (1-58 pat./center, 5 centers with more than 12 pat.). 5 years' follow-up data are presented.

**Results:** Demographic and clinical data were equally distributed in the participating centers. pT3 tumors were most common (75%), 68% were classified as pN1. 53% of the pat. were in UICC stage IIIB. After 5 years, there was a statistically significant difference for DFS (S+Ctx: 75% vs. S: 54%, p = 0.0057) and a tendential benefit for overall survival (OS, S+Ctx: 81% vs. S: 72%, p = 0.1628) in favour of adjuvant chemotherapy for the total of study patients. However, the most favourable results concerning DFS as well as OS were achieved in center 2 (best recruiting, n = 58) with no statistical difference in both treatment arms (DFS, S+Ctx: 75% vs. S: 62%, p = 0.3316; OS: S+Ctx: 85% vs. S: 91%, p = 0.5532). The median number of dissected and examined lymph nodes in this center was significantly higher compared to other centers (n = 35 vs. n = 25, p < 0.001).

**Conclusion:** The prognostic benefit of adjuvant chemotherapy with an improvement of DFS of 20 % was evident for the total of study patients. Despite the specification of surgical (and pathological) guidelines in the study protocol, there were apparent differences between the participating centers that influenced the additional benefit of adjuvant chemotherapy. The results of the InTACT I study point out to the necessity of quality control in clinical investigations, mainly focussing on surgery.

PO376

### Direct and natural killer cell-mediated anti-tumor effects of low-dose bortezomib in hepatocellular carcinoma

Salih H., Armeanu S., Krusch M., Lauer U., Steinle A., Kanz L., Bítzer M.

Eberhard-Karls Universität, Department für Innere Medizin, Tuebingen

**Background and Aims:** Hepatocellular carcinoma (HCC) displays a particular resistance to conventional cytostatic agents. Therefore, alternative treatment strategies focus on novel substances exhibiting anti-neoplastic and/or immunomodulatory activity enhancing for example Natural Killer (NK) cell anti-tumor reactivity. However, the tumor-associated ligands engaging activating NK cell receptors are largely unknown. Exceptions are the NKG2D ligands (NKG2DL) of the MIC and ULBP protein families, which potently stimulate NK cell responses. We studied the consequences of proteasome inhibition with regard to direct and NK cell-mediated effects against HCC. **Methods:** Primary human hepatocytes (PHH) from different donors, hepatoma cell lines and NK cells were exposed to Bortezomib. Growth and viability of hepatoma cells and PHH as well as immunomodulatory effects including alterations of NKG2DL expression on hepatoma cells, specific induction of NK cell cytotoxicity and IFN- $\gamma$  production were investigated. **Results:** Bortezomib treatment inhibited hepatoma cell growth with IC<sub>50</sub> values between 2.4 and 7.7 nanomol/liter. These low doses increased MICA/B mRNA levels and total and cell surface protein expression in hepatoma cells, which stimulated cytotoxicity and IFN- $\gamma$  production of cocultured NK cells. Importantly, while IFN- $\gamma$  production of NK cells was concentration-dependently reduced, low-dose Bortezomib neither induced NKG2DL expression or cell death in PHH nor altered NK cell cytotoxicity. **Conclusion:** Low-dose Bortezomib mediates a specific dual anti-tumor effect in HCC by inhibiting tumor cell proliferation and by priming hepatoma cells for NK cell anti-tumor reactivity. Our data suggest the clinical evaluation of Bortezomib treatment in HCC, especially in combination with immunotherapeutic approaches like adoptive NK cell transfer.

PO377

### The novel HDAC-inhibitor panobinostat inhibits growth of human hepatocellular carcinoma xenografts in nude mice

Di Fazio P.<sup>1</sup>, Schneider-Stock R.<sup>2</sup>, Okamoto K.<sup>1</sup>, Neureiter D.<sup>3</sup>, Wissniowski T.<sup>1</sup>, Sass G.<sup>4</sup>, Hahn E.G.<sup>1</sup>, Ocker M.<sup>1</sup>

<sup>1</sup>Universitätsklinikum Erlangen, Medizin 1, Erlangen; <sup>2</sup>Otto-von-Guericke-Universität Magdeburg, Institut für Pathologie, Magdeburg; <sup>3</sup>Salzburger Landeskliniken, Institut für Pathologie, Salzburg; <sup>4</sup>Friedrich-Alexander-Universität Erlangen-Nürnberg, Institut für Pharmakologie, Erlangen

**Background:** Liver cancer (hepatocellular carcinoma, HCC) represents an unmet medical need. Despite its rising incidence, the overall prognosis of HCC is poor as systemic chemotherapy is of low efficacy. Histone deacetylase inhibitors (HDACi) have demonstrated anticancer activity in various cancers. We therefore investigated the effect of the novel HDACi Panobinostat (LBH589) on human hepatocellular carcinoma cell lines in vitro and in a subcutaneous xenograft model in vivo.

**Methods:** HepG2 (p53<sup>wt</sup>) and Hep3B (p53<sup>-/-</sup>) cells were cultured under standard conditions and incubated with various concentrations of Panobinostat for 6 to 120 h. Cell viability was determined by trypan blue staining, apoptosis was quantified by flow cytometry after propidium iodide staining. Mitochondrial transmembrane potentials (DY<sub>m</sub>) were determined by JC-1 and DiOC<sub>6</sub> staining. Quantitative RT-PCR and western blotting was used to investigate signalling pathways involved in Panobinostat mediated apoptosis. ChIP was performed to investigate p53-dependent transcriptional regulations of Panobinostat target genes.

In vivo, HepG2 cells were xenografted to male NMRI mice (n/group = 8) and treated with daily intraperitoneal injections of 10 mg/kg Panobinostat. Tumor size and animal weight were determined daily. Tumor samples were obtained for further analysis (immunohistochemistry, RT-PCR, western blotting). Liver transaminases were determined from blood samples as a surrogate marker for toxicity.

**Results:** In vitro studies showed a pronounced growth inhibitory and pro-apoptotic effect of LBH589 on both HCC cell lines at low micromolar concentrations (IC<sub>50</sub> approx. 0.1  $\mu$ M). Interestingly, the pro-apoptotic effect of Panobinostat was not paralleled by a breakdown of DY<sub>m</sub>. p53<sup>wt</sup> HepG2 cells were more sensitive than the p53<sup>-/-</sup> Hep3B cells. Quantitative PCR and western blotting showed an involvement of the cell cycle regulators p21<sup>cip1/waf1</sup> and Chk1 but not the bax/bcl-2 system. Panobinostat regulated the expression of p21<sup>cip1/waf1</sup> via a transcriptional upregulation as evidenced by ChIP.

In vivo, the daily application of Panobinostat significantly reduced the growth of HepG2 xenografts (mean tumor diameter: 12 mm vs. 16 mm in untreated controls) and prolonged the overall survival of animals (100% vs. 43% in controls). Macroscopically, a marked reduction of tumor angiogenesis was observed and the PCR results confirmed the in vitro findings, too. No signs of toxicity or elevation of liver transaminases was observed (ALT: 238 U/l vs. 320 U/l in controls).

**Conclusion:** Panobinostat is a potent novel HDACi for the treatment of human HCC.

PO378

### Neoadjuvant irinotecan-based combination chemoradiotherapy in locally advanced rectal cancer: Long-term results of 140 patients treated in two academic hospitals within clinical trials

Hofheinz R.-D.<sup>1</sup>, Klautke G.<sup>2</sup>, Wenz F.<sup>3</sup>, Willeke F.<sup>4</sup>, Klar E.<sup>5</sup>, Horisberger K.<sup>6</sup>, Mai S.<sup>3</sup>, Post S.<sup>6</sup>, Hochhaus A.<sup>1</sup>, Fietkau R.<sup>2</sup>

<sup>1</sup>Universität Heidelberg, Onkologisches Zentrum, III. Medizinische Klinik, Mannheim; <sup>2</sup>Universität Rostock, Strahlentherapie, Rostock; <sup>3</sup>Universität Heidelberg, Klinik für Strahlentherapie, Mannheim; <sup>4</sup>St. Marienkrankenhaus, Chirurgische Klinik, Siegen; <sup>5</sup>Universität Rostock, Chirurgische Klinik, Rostock; <sup>6</sup>Universität Heidelberg, Chirurgische Klinik, Mannheim

**Background:** In patients with localized rectal cancer preoperative radiochemotherapy with 5-fluorouracil (5-FU) has been shown to reduce local failure rates and to improve early and late toxicities in comparison with post-operative chemoradiation. Major or complete histopathological responses predict improved survival. Thus, in an attempt to increase the patholocal



response rates, combination therapies using oxaliplatin or irinotecan in conjunction with 5-FU (applied i.v. or as an oral pro-drug) are currently under intense investigation.

The current analysis comprises patients undergoing neoadjuvant chemoradiotherapy with locally advanced rectal cancer (cT3/4 or cTxN+). Patients were included in five clinical studies on the combination of irinotecan with capecitabine (CapIri, n = 84), capecitabine and cetuximab (Cet-CapIri, n = 20) or 5-FU (FU-Iri, n = 36) in two academic hospitals. Radiotherapy dose was 45-54 Gy. Surgery was scheduled 4-6 weeks after the completion of chemoradiation. We herein report long-term results of local control, disease-free (DFS) and overall survival.

**Results:** A total of 140 patients (m/f 100/40) with a median age of 61 years (range 34-82) were analysed. Tumor localisation was as follows: 0-5cm: n = 56, 5-10cm n = 67, >10cm n = 17. Clinical T-stages: T2 n = 13, T3 n = 97, T4 n = 30. 123 patients had clinically nodal-positive tumors (88%). The rate of diarrhea was as follows (NCI-CTC grades): grade 0/1: 26%; grade 2: 38%; grade 3 23%; grade 4: 3%. 135/140 patients underwent surgery (anterior resection n = 96 [71%]; Hartmann's procedure n = 4 [3%], abdominoperineal resection n = 35 [26%]). R0-resection was accomplished in 129 patients (96%). Pathological staging was as follows: pT0 n = 26 (19%), pT1 n = 8 (6%); pT2 n = 36 (26%), pT3 n = 62 (46%), pT4 n = 4 (3%), pN0 n = 89 (65%), pN1 n = 33 (24%), pN2 n = 14 (10%). 24 patients had complete pathological remission (pCR; 17%) and another 37 patients (26%) had microfoci of tumor only (near pCR). During a median follow-up of 29 months, eight patients had a local relapse (6%), and 22 patients developed metastases (16%). Overall 3-year DFS was 77% (85% for patients with pCR or near pCR, 71% for patients with minor remission). 3- and 4-year overall survival for the whole group is 81% and 77%, respectively.

**Conclusion:** Irinotecan-based preoperative chemoradiation is safe with diarrhea being the main toxicity. Early efficacy parameters are very promising (pCR and near pCR in 43% of patients). The local relapse and metastases rate are comparably low in this high-risk group (cT3/4 91%, cN+ 88%), although the median follow thus far is only 29 months. Mature data will be presented. Patients exhibiting a pCR or near pCR tend to have improved DFS. Clearly, randomised trials are needed to define the role of intensified perioperative treatment in locally advanced rectal cancer.

PO379

### Neoadjuvant chemotherapy in cancer of the esophageal-gastric junction with early pet response evaluation

Möbius C.<sup>1</sup>, Staab H.<sup>1</sup>, Wuttke P.<sup>1</sup>, Kassahun W.<sup>1</sup>, Witzigmann H.<sup>2</sup>, Hauss J.<sup>1</sup>

<sup>1</sup>Universität, Chirurgie II, Leipzig; <sup>2</sup>Krankenhaus Dresden Friedrichstadt, Allgemein und Viszeralchirurgie, Dresden

**Introduction:** Neoadjuvant chemotherapy in locally advanced cancer of the esophageal-gastric junction followed by surgery has become a standard treatment. But only 30% of the patients will profit from this therapy. The aim of the present study was to evaluate the possible role of positron emissions tomography in discriminating between chemo-sensible and non-sensible tumors.

**Patients:** Between 01/2005 and 06/2007 23 patients with locally advanced adenocarcinoma type I and II were included. PET was conducted before and after the first and second administration of induction chemotherapy (platinum and fluorouracil based). Metabolic responders – defined as a decrease of SUV > 35% in PET – completed 12 weeks and proceeded to surgery. Metabolic non-responders discontinued the chemotherapy and were operated. Results: From the 23 patients 4 had to be excluded because of chemo toxicity, progression beside metabolic response and distant metastasis were diagnosed in PET. 11 patients were classified as metabolic responders, SUV decreased by 44% after the first cause of CTx and by 50% after the second cause, 67% in total. In the non-responder group the values were 34%, 4% and 29%. Endoscopically, the tumor length downsized about 4 cm (2 to 7 cm) in the responder group. Postoperative TNM classifications in the responder group were: ypT0N0:1; ypT2N:7; ypT3N0: 2; ypT3N1:1; in the non-responder group ypT3N1:7.

**Conclusion:** The present study shows the feasibility and usefulness of the PET in the neoadjuvant treatment of the adenocarcinoma of the esophageal-gastric junction. It allows to discriminate between chemosensible and non-chemosensible tumors and correlates with the downsizing of the histomorphological classification.

PO380

### Disseminated tumor cells in bone marrow in adjuvant dukes B and C colon cancer – A frequent event with prognostic and therapeutic relevance ?

Dresemann G.<sup>1</sup>, Gerhards R.<sup>2</sup>

<sup>1</sup>Franz-Hospital Dülmen, Abteilung für Onkologie, Dülmen; <sup>2</sup>Marienhospital Herne, Labor für angewandte und experimentelle Onkologie, Herne

**Background:** Stage dependant Colon Cancer (CC) potentially disseminates building distant metastases although local treatment is sufficient. Adjuvant therapies have been established to reduce risk of metastasis especially in stage Dukes C disease. With improved laboratory techniques including magneto bead accumulation and advanced immunocytochemistry disseminated tumor cells (DC) can be detected in bone marrow (BM). If the model in which DC can be regarded as potential seeds for metastasis is true it should be useful to search for DC by BM examination (BME) especially after adjuvant treatment as a prognostic tool. If DC can be detected in BM these cells can be tested for surface antigens like EGF-Receptor (R) or 17-1A antigen. As bisphosphonates (BP's) seem to be able to block activity of farnesyltransferase a signal transduction enzyme for EGF-R. Therefore BP's might prevent EGF-R positive DC from induction of proliferation.

**Methods:** From 1996, November to 2005, November all patients (pts) with stage Dukes B and C CC were divided into two groups, one underwent BM examination after adjuvant treatment was finished in stage C CC or about 6 months after surgery in stage B CC. In case of EGF-R positive DC pts were treated with BP's until BM was DC negative in yearly examination or progression was detected. The other group was treated according to consensus recommendation. All pts were followed up for progression free survival (DFS) and overall survival (OS).

**Results:** 103 pts with stage B CC and 112 pts with stage C CC were included in this examination. In stage B CC 45 pts (44%) underwent BME, 58 pts did not. 29 pts (64%) had DC and were treated with BP's, 16 pts (36%) were DC negative and received no further treatment. In stage C CC 60 pts (54%) underwent BME, 52 pts did not. 49 pts (82%) were DC positive and were treated with BP's, 11 pts were DC negative. Median follow up was 7 years. For stage B pts PFS was 84% for pts without BME, 90% for DC positive pts with BP's and 94% for DC negative pts in total 91% for the BME group. For stage C pts PFS was 69% for pts without BME, 84% for DC positive pts with BP's and 82% for DC negative pts in total 83% for the BME group.

**Conclusion:** PFS data of stage B and C CC pts without BME are comparable to those published in literature. Improved PFS data for DC negative pts seem to indicate that absence of DC is of significant prognostic value, number of pts, however, is small. Data for DC positive pts with BP's seem to indicate that BP's are able to improve outcome as sequential treatment for DC positive stage B and C CC. Controlled studies are required to confirm these data and the underlying model.

PO381

### Pretherapeutic gene expression profiling in prediction of disease free survival after preoperative chemoradiotherapy in locally advanced rectal cancer (UICC stages II and III)

Liersch T.<sup>1</sup>, Grade M.<sup>1</sup>, Langer C.<sup>1</sup>, Becker H.<sup>1</sup>, Varma S.<sup>2</sup>, Ried T.<sup>3</sup>, Ghadimi M.<sup>1</sup>

<sup>1</sup>Universitätsmedizin, Allgemein- und Viszeralchirurgie, Göttingen; <sup>2</sup>NCI, NIH, Biometrics Research Branch, Bethesda, Maryland, USA; <sup>3</sup>NCI, NIH, Genetics Branch, Bethesda, Maryland, USA

**Purpose:** Preoperative 5-FU based chemoradiotherapy (CT/RT) is recommended for locally advanced rectal cancer (UICC stage II/III). We recently demonstrated that differential expression (p<0.001) of 54 genes could distinguish between CT/RT responsive and non-responsive rectal cancers [1]. In this follow-up study, we analyzed the influence of CT/RT-induced tumor response on survival and explored the value of gene expression profiling for individualized prognostication.

**Patients and Methods:** Pretherapeutic biopsies from 23 participants of the CAO-JARO-AIO-94 trial, all of whom received preoperative CT/RT, were analyzed for gene expression signatures using 10K cDNA microarrays.



CT/RT-induced local tumor shrinkage (T-level downsizing) was correlated with disease free survival (DFS) and overall survival (OS). Leave-one-out cross-validation (LOOCV), Principal Component Analysis (PCA) and Diagonal Linear Discriminant (DLD) classifier analyses were performed to explore the predictive value of the identified 54 gene-set.

**Results:** After 44 months (median) of follow-up, DFS was 77% and OS 87%. The metastatic recurrence rate was 20%. All five patients with metastatic disease belonged to the group of non-responders ( $p = 0.0135$ ). A significant difference of DFS ( $p = 0.018$ ), but not of OS ( $p = 0.18$ ), was observed for responders measured by T-level downsizing. LOOCV resulted in correct prediction of all five patients with recurrence. Furthermore, all 11 patients who were predicted to remain cancer free showed no evidence of recurrence. Analyses utilizing both PCA and DLD identified a risk gradient from response to non-response, and to actual recurrence.

**Conclusion:** Our results suggest that gene expression profiling may assist both in the prediction of response to preoperative CT/RT and to DFS.

1. Ghadimi BM, Grade M, Difiippantonio MJ, Varma S, Simon R, Montagna C, Füzesi L, Langer C, Becker H, Liersch T, Ried T: Effectiveness of gene expression profiling for response prediction of rectal adenocarcinomas to preoperative chemoradiotherapy. *J Clin Oncol* 2005;23(9):1826–1838

PO382

### Gene expression of members of different apoptotic pathways is differentially regulated by taurolidine and trail leading to synergistic induction of apoptosis in human esophageal cancer cells

Daigeler A.<sup>1</sup>, Geisler A.<sup>2</sup>, Bulut D.<sup>3</sup>, Chromik A.<sup>2</sup>, Hilgert C.<sup>2</sup>, Lehnhardt M.<sup>1</sup>, Klein-Hitpass L.<sup>4</sup>, Mittelkötter U.<sup>2</sup>, Uhl W.<sup>2</sup>

<sup>1</sup>BG-Universitätsklinik Bergmannsheil, Universitätsklinik für Plastische Chirurgie, Bochum; <sup>2</sup>St. Josef Hospital, Chirurgische Klinik, Bochum; <sup>3</sup>St. Josef Hospital, Medizinische Klinik II, Bochum; <sup>4</sup>Universität Duisburg Essen, Institut für Zellbiologie, Tumorforschung, Essen

**Background:** The treatment of choice for esophageal cancer is considered surgical resection, but a median survival of around 20 months after treatment is still discouraging. The value of adjuvant or neoadjuvant radiation or chemotherapy is limited and to date benefits have only been described for certain tumor stages. Therefore new therapeutic options are to be found.

**Methods:** As alternative chemotherapeutics, we tested the antibiotic Taurolidine (TRD) on KYSE 270 human esophageal carcinoma cells alone and in combination with rhTRAIL (TNF related apoptosis-inducing ligand). Viability, apoptosis and necrosis were visualized by TUNEL-Assay and quantitated by FACS analysis. Gene expression was analysed by RNA-Microarray. **Results:** The most effective concentration of TRD as single substance (250  $\mu$ mol/l) induced apoptosis to a maximum of 40% after 12h dose dependently, leaving 4% viable cells after 48h; by comparison, rhTRAIL did not have a significant effect. The combination of both substances could double the effect of TRD alone.

In the cells treated with Taurolidine alone, we found TNFSF10/TRAIL, TNFRSF1A, TRADD, TNFRSF1B, TNFRSF21, FADD, MAP2K4/c-Jun N-terminal kinase kinase 1, JAK2, Bcl-2, Bcl2l1/BclxL, APAF1, and Caspase 3 “downregulated”.

Caspase 1, 8, 9, Cytochrome-c, PARP1, TNFRSF25, NFKBIA, GADD45A, and JUN/AP-1 were “upregulated”.

Cells exposed to TRAIL “downregulated” TNFSF10 (TRAIL), Bcl2l1, and Caspase 1. BIRC2, BIRC3, TNFAIP3, and NFKBIA were “upregulated”.

When the TRD/TRAIL incubated cells were compared to the cells only receiving TRD, DFFA, TRAF3 were “downregulated”. TNFSF10 (TRAIL), NFKBIA, and JUN/AP-1 were “upregulated”.

**Conclusion:** Synergistic effects are dependent on the same and on distinct apoptotic pathways which, jointly triggered, result in an amplified response. Several apoptotic pathways, including the TNF-receptor associated and the mitochondrial pathway, were differentially regulated by the substances. Additionally transcription factors like NFKB were influenced. We conclude, that TRAIL and Taurolidine qualify for further studies on human neoplasias.

PO383

### Inhibition of insulin-like growth factor-I receptor using NVP-AEW541, a small molecule kinase inhibitor, reduces orthotopic pancreatic cancer growth and angiogenesis

Moser C.<sup>1</sup>, Schachtschneider P.<sup>1</sup>, Lang S.A.<sup>1</sup>, Schlitt H.-J.<sup>1</sup>, Geissler E.K.<sup>1</sup>, Kaufmann B.<sup>2</sup>, Zimmermann J.<sup>3</sup>, Stoeltzing O.<sup>1</sup>

<sup>1</sup>University, Surgery and Surgical Oncology, Regensburg; <sup>2</sup>Novartis Pharma AG, Oncology, Nürnberg; <sup>3</sup>Novartis Pharma AG, BioMedical Research, Basel

The insulin-like growth factor-I receptor (IGF-IR) has emerged as a promising target for cancer therapy. Importantly, IGF-IR is frequently overexpressed and constitutively activated in pancreatic cancer. In this study we investigate the impact of a novel small molecule inhibitor to IGF-IR (NVP-AEW541) on cell signaling and growth of pancreatic cancer. Human pancreatic cancer cells (HPAF-II, L3.6pl, BxPC-3) and endothelial cells (HUVEC) were employed. Effects of NVP-AEW541 on IGF-I-mediated signaling pathways were investigated by Western blotting. Cytotoxic effects were determined by MTT and cell death ELISA. The impact on cell motility was determined in migration assays. Effects on tumor growth were assessed in an orthotopic model of pancreatic cancer. NVP-AEW541 diminished the activation of IGF-IR, IRS-1 and Erk, Akt and STAT3. Furthermore, NVP-AEW541 significantly reduced cancer cell proliferation and abrogated migratory effects of IGF-I ( $P < 0.05$  for both). NVP-AEW541 also elicited a direct effect on endothelial cells in terms of reducing endothelial cell migration ( $P < 0.01$ ). Treatment of mice with NVP-AEW541 significantly reduced orthotopic tumor growth, vascularization, and VEGF expression ( $P < 0.05$  for all). Interestingly, NVP-AEW541 lowered serum levels of IGF-binding-protein-3 (IGFBP-3) ( $P < 0.01$ ). In conclusion, the IGF-IR inhibitor NVP-AEW541 effectively disrupts IGF-I signaling and reduces pancreatic tumor growth. Hence, targeting IGF-IR could prove to be valuable for molecular therapy concepts for treating pancreatic cancer.

PO384

### Impact of Endoscopic Ultrasonography (EUS)-guided Fine Needle Aspiration (FNA) to the diagnostic of suspicious tumor lesions of the pancreas and peripancreatic lymph nodes

Will U.<sup>1</sup>, Mueller A.-K.<sup>1</sup>, Meyer F.<sup>2</sup>

<sup>1</sup>Municipal Hospital, Department of Gastroenterology, Gera; <sup>2</sup>University Hospital, Department of Surgery, Magdeburg

The aim of the study was to investigate i) the value of EUS-guided FNA in the diagnostic of pancreatic tumor lesions & peripancreatic lymph nodes, & ii) its impact to the decision-making for the therapeutic management. In addition, results of the cytologic & histologic investigation were compared with the detection rates of various imaging procedures.

**Methods:** Through a defined time period, all consecutive patients with suspicious or definitive pancreatic tumor lesions revealed by any imaging procedure who subsequently underwent EUS-guided FNA were enrolled in this observational uncenter study (case series). Diagnostic-, treatment- & patient-associated characteristics were prospectively documented in a computer-based registry & were retrospectively evaluated. Depending on the finding in EUS-guided FNA & subsequent cytologic & if done, histologic investigations, patients were kept under surveillance through a follow-up time period of minimally 6months. The diagnostic value of EUS-guided FNA was characterized by detection rate, sensitivity, specificity, positive & negative predictive values (PPV/NPV) as well as diagnostic accuracy.

**Results:** Overall, 153patients (mean age, 56.9years) underwent EUS-guided FNA from I/2000-III/2003 (3years). Comparing CT scan (80%), MRI (57.1%) & abdominal ultrasound (88.8%), EUS achieved the highest diagnostic accuracy: 100%. For EUS-based T staging in 26patients with surgical intervention, there was a sensitivity of 73.3% (specificity, 85.9%; PPV, 69.2%; NPV, 84.4%) whereas the parameters for N staging ( $n = 25$ ) were as follows: Sensitivity, 61.5%; specificity, 75%; NPV, 64.3%; PPV, 72.7%. While the sensitivity of EUS-guided FNA in the group of patients who underwent surgical intervention ( $n = 55$ ) was 81.4% (specificity, 75%; PPV, 92.1%; NPV, 52.9%), the parameters were in the subgroup of individuals with chronic pancreatitis ( $n = 50$ )

as follows: Sensitivity in detecting a malignant pancreatic tumor lesion, 50%; specificity, 91.7%; PPV, 60%; NPV, 88%. Based on preoperative characteristics such as TNM stage & tumor entity, a futile surgical intervention could be thus avoided in 29 patients (19%).

**Conclusion:** EUS-guided FNA allows to clarify definitive diagnosis, in particular, malignant tumor growth & type of pancreatic tumor-like lesion, in a high percentage. Therefore, it provides a substantial impact to the diagnostic accuracy. In addition, reliable diagnosis may assist a sufficient decision-making for an appropriate therapeutic approach.

PO385

### Neoadjuvant chemoradiation: New hope for pancreatic carcinoma?

Meyer T.<sup>1</sup>, Golcher H.<sup>1</sup>, Merkel S.<sup>1</sup>, Brunner T.<sup>2</sup>, Grabenbauer G.<sup>2</sup>, Hohenberger W.<sup>1</sup>

<sup>1</sup>Universitätsklinikum, Chirurgische Klinik, Erlangen; <sup>2</sup>Universitätsklinikum, Strahlentherapie, Erlangen

**Background:** Ductal adenocarcinoma of the pancreas can only be cured by curative resection of the tumor. However, after curative surgery 5-year survival is in the range of 20-25%, even in specialised centers. Neoadjuvant chemoradiation (nCRT) might contribute to a better control of local tumor growth to increase the rate of actual R0 resections.

**Patients and Methods:** 376 patients (pat.) with ductal adenocarcinoma of the pancreatic head were treated in our institution between 1995 and 2005 (other pancreatic tumor sites and malignancies excluded). In 99 out of 253 operated pat., the tumor was resected (R0 n = 81, 92%). 25 of these pat. received nCRT for locally advanced or borderline resectable tumors. The regimen consisted of 55.8Gy (28x1.8Gy, single fraction, five times/week + 3x1.8 Gy boost) to the primary tumor site and the locoregional lymph nodes, together with a simultaneous chemotherapy (5-FU/MMC bzw. cisplatin/gemcitabine).

**Results:** Median survival for curatively resected patients was 24 months (all resections, 23m) and was not statistically different between pretreated and non-pretreated pat., however with a tendency in favour of the nCRT-group (27m vs. 21m). 2- and 5-year survival was 52% and 45%, respectively, for pretreated pat. and 46% and 29%, respectively, for non-pretreated pat. (p = 0.191). R0-resections were more frequent in the nCRT-group (23/25 = 92% vs. 58/74 = 74%) as well as the proportion of node-negative findings (17/25 = 68% vs. 22/74 = 30%).

**Conclusion:** Although neoadjuvant chemoradiation was performed in more advanced tumors, prognosis of these tumors did not differ significantly from that of primarily resectable tumors. The rate of R0 resections and node-negative findings was markedly higher after pre-treatment. This clinical experience prompted us to launch a study on the value of neoadjuvant chemoradiation in resectable ductal adenocarcinoma of the pancreatic head (Brunner et al., BMC Cancer 2007). In contrast to adjuvant therapy a neoadjuvant approach could be delivered a greater proportion of patients.

PO386

### Randomized first line therapy with gemcitabine plus lipid complexed paclitaxel (ENDOTAG-1) compared with gemcitabine monotherapy in patients with locally advanced or metastatic adenocarcinoma of the pancreas. A phase II study

Löhr M.<sup>1,2</sup>, Bechstein W.<sup>3</sup>, Bodoky G.<sup>4</sup>, Gog C.<sup>3</sup>, Haas S.<sup>1</sup>, Karrasch M.<sup>5</sup>, Lilla C.<sup>5</sup>, Lutz M.<sup>6</sup>, Mayer I.<sup>5</sup>, Myasoedov D.<sup>6</sup>, Singer M.V.<sup>1</sup>, Osynsky D.<sup>7</sup>, Schulze S.<sup>5</sup>, Mescheder A.<sup>5</sup>, Szanto J.<sup>8</sup>, Fölsch U.<sup>9</sup>

<sup>1</sup>Universitätsklinikum Mannheim, II. Medizinische Klinik/KKE MolGa (DKFZ G350), Mannheim; <sup>2</sup>Karolinska Institutet, CLINTEC, Stockholm;

<sup>3</sup>Universitätsklinikum Frankfurt, Chirurgische Klinik, Frankfurt/Main;

<sup>4</sup>Universität Budapest, Szent Laszlo Hospital, Budapest; <sup>5</sup>MediGene AG, Martinsried; <sup>6</sup>Caritasklinik St. Theresia, Saarbrücken; <sup>7</sup>Institute of Oncology AMS of Ukraine, Kiev; <sup>8</sup>Kiev City Oncology Hospital, Kiev;

<sup>9</sup>Universitätsklinikum Schleswig-Holstein, I. Medizinische Klinik, Kiel

**Introduction:** Cationic Lipid Complexed Paclitaxel (EndoTAG-1) represents a novel anti-neovascular therapy designed to target newly formed blood

vessels in tumors. It has been demonstrated in non-clinical studies that this modification of paclitaxel delivery has a significant impact on drug disposition and cellular presentation of the active substance as compared to the administration of Taxol® or generic paclitaxel formulations. In contrast to conventional interventions targeting tumors at the cellular level, EndoTAG-1 has been shown to target activated endothelial cells of tumor blood vessels (neovascular targeting) due to its positively charged carrier liposomes binding preferentially to negatively charged cells of the neo-vasculature of tumors.

**Study Design:** Study CT4001 is a controlled, randomized, open-label phase II trial to evaluate safety and efficacy of a 1st line combination treatment with weekly infusions of Gemcitabine and twice weekly administration of EndoTAG-1 at 3 different dose levels (11 mg/m<sup>2</sup>, 22 mg/m<sup>2</sup>, 44 mg/m<sup>2</sup>) compared to Gemcitabine monotherapy (1000 mg/m<sup>2</sup>) in patients with measurable locally advanced and/or metastatic adenocarcinoma of the pancreas. In brief, patients were planned to receive treatment for at least 7 weeks with the option to continue treatment in case of response, and are followed up for survival.

**Summary of Safety Results:** In this trial comprising 213 patients, 124 Serious Adverse Events (SAEs) have been reported by Oct 15, 2007, 33 of which were assessed as possibly or probably related to EndoTAG-1. The most frequently reported SAEs attributable to treatment with EndoTAG-1 were pyrexia and infusion related reactions (13 patients), and blood and lymphatic system disorders (4 patients). None of the 124 deaths reported to date was related to study medication. Since the follow-up of patients is still ongoing, results on all adverse event data are not available yet.

**Conclusion:** Preliminary data from study CT4001 corroborate the favorable safety profile of EndoTAG-1 observed in previous clinical phase I studies. There is a trend towards a superior safety profile to the established safety profile of commercially available paclitaxel. Final data from this study will provide a more accurate safety profile of EndoTAG-1 and will allow to compare the frequency and severity of the side effects as observed under EndoTAG-1 with those of Taxol®. The efficacy analysis of this study is currently ongoing.

## Gastrointestinal Cancer including Liver

### Poster Exhibition

PE703

### Proliferation inhibition of colon cancer cells by UDCA: Cellular response mechanisms

Peiro-Jordan R.<sup>1</sup>, Krishna-Subramanian S.<sup>1</sup>, Hanski M.-L.<sup>1</sup>, Behrens J.<sup>2</sup>, Zeitz M.<sup>1</sup>, Hanski C.<sup>1</sup>

<sup>1</sup>Charité Campus Benjamin Franklin, Gastroenterologie, Berlin; <sup>2</sup>Fr.-Alexander-Universität Erlangen-Nürnberg, Erlangen

**Introduction:** Ursodeoxycholic acid (UDCA) treatment of patients with primary sclerosing cholangitis appears to reduce the risk of colon cancer. UDCA prevents colon cancer in animal models; the mechanism of the chemopreventive action is still unknown. We investigated the effects of UDCA on proliferation of colon cancer cells *in vitro*.

**Materials and Methods:** Nine established human carcinoma cell lines were treated with UDCA (0-400 µM) for 3 days (short-term) or 6 days (long-term). The short-term effect on proliferation was determined by MTT test and bromodeoxyuridine (BrdU) incorporation, and the long-term by cell count. The cell cycle was studied by FACS using Nocodazol-synchronized or non-synchronized cells. Cell-cycle markers were analysed by western blot, senescence by b-galactosidase staining and apoptosis by DAPI staining. The influence on the *wnt* pathway was investigated in an isogenic pair of *wnt*-proficient and *wnt*-deficient cell lines.

**Results:** UDCA inhibited the proliferation of all cell lines in short- and long-term assay. This effect was partially reversible and its extent reversely correlated with the cell doubling time. UDCA decreased BrdU incorporation and slowed down the S-phase. Decrease in S-phase was mediated by p53 protein while G1 arrest was dependent on p53 and p21 proteins. UDCA inhibited Rb phosphorylation at Ser780 and Ser807/811. Slight induction of senescence, but neither apoptosis nor effect of the *wnt* pathway status on UDCA response were observed.

**Conclusions:** Our results indicate that UDCA decreases the proliferation of colon cancer cells in a partially reversible manner, by decreasing S-phase population (in a p53 dependent way), causing G1 arrest (which is p53 and p21 dependent), and that the decrease of phosphorylation of the Rb protein could play a role in this effect.

PE704

### Genotype analysis of glutathione S-transferase polymorphisms in patients with colorectal cancer

Merkel U.<sup>1</sup>, Farker K.<sup>1</sup>, Wedding U.<sup>2</sup>, Gruca D.<sup>1</sup>, Höffken K.<sup>2</sup>, Hippus M.<sup>1</sup>

<sup>1</sup>Universitätsklinikum Jena, Klinische Pharmakologie, Jena;

<sup>2</sup>Universitätsklinikum Jena, Klinik für Innere Medizin II, Jena

Glutathione S-transferase (GST) genes have been found to show functional polymorphisms that are frequently present in the general population. Genetic variations in carcinogen metabolizing enzymes like GSTP1, GSTM1 and GSTT1 have been proposed as susceptibility marker for colorectal cancer. Homozygous deletion of GSTM1 and GSTT1 genes has been associated with a loss of enzyme activity. In the current study, we examined GSTP1, GSTM1 and GSTT1 polymorphisms in 60 patients with colon cancer and 43 patients with rectum cancer.

There were no deviations from Hardy-Weinberg equilibrium for the GSTP1 genotype frequencies. Investigated genotype distributions of colon and rectum cancer patients were consistent with reported data of healthy Caucasian populations. Similarly, we did not find significant differences compared to reported data of colorectal patients. Gender-specific effects in genotype frequencies were not detected. Considering interaction of GST polymorphisms with the risk factor cigarette smoking, no disparities were observed in genotype distributions.

Our results suggest that studied GSTT1, M1 or P1 polymorphisms are not independent risk factors for colorectal cancer because genotype frequencies did not differ in relation to reported data in healthy Caucasians. The results of genotyping were in accordance with other data in colorectal cancer patients.

PE705

### Glutathione S-transferase polymorphisms in relation to clinical parameters of colorectal cancer patients

Farker K.<sup>1</sup>, Merkel U.<sup>1</sup>, Wedding U.<sup>2</sup>, Gruca D.<sup>1</sup>, Höffken K.<sup>2</sup>, Hippus M.<sup>1</sup>

<sup>1</sup>Universitätsklinikum Jena, Klinische Pharmakologie, Jena;

<sup>2</sup>Universitätsklinikum Jena, Klinik für Innere Medizin II, Jena

Glutathione S-transferases (GST) have been extensively examined in association with genetic susceptibility and clinical outcomes of several types of cancer. We examined associations between different GST polymorphisms (P1, M1, T1) and tumour site, age at diagnosis as well as survival of patients. In the current study, we included 60 patients with colon cancer and 43 patients with rectum cancer.

We found no differences in the distribution of genotypes according to right- or left-sided colorectal cancer. We identified no significant association between deletion polymorphisms in GSTM1 and GSTT1 as well as determined GSTP1 polymorphisms and patients' age at diagnosis. Survival analyses in relation to genotypes were performed using multivariate Cox regression models and survival curves were constructed using Kaplan-Meier method. Neither GSTP1 nor GSTT1 polymorphisms were significantly associated with survival, but significant association was demonstrated with GSTM1. In patients with GSTM1 gene deletion we observed a longer survival than in patients with GSTM1 present. Our results suggest that in patients with colorectal cancer GSTM1 null genotype confers a survival advantage. Considering additional clinical parameters, in prospective studies it should be further investigated if evaluation of GST functional polymorphisms may help to predict colorectal cancer prognosis.

PE706

### Prognostic factors of rectal carcinoma after conventional and optimized surgery

Trinh T.T.<sup>1</sup>, Oberholzer K.<sup>2</sup>, Junginger T.<sup>1</sup>

<sup>1</sup>Johannes Gutenberg-Universität, Klinik und Poliklinik für Allgemein- und Abdominalchirurgie, Mainz; <sup>2</sup>Johannes Gutenberg-Universität, Klinik und Poliklinik für diagnostische und interventionelle Radiologie, Mainz

After conventional surgery of rectal cancer, pT and pN are risk factors of local recurrent tumor and prognosis. By optimized surgery (TME, Heald 1982) the

local recurrence rate could be reduced to about 10%. The aim of a study of patients operated between 1985 and 2006 in the department of general and abdominal surgery of university Mainz was to analyse the importance of pT and pN for the local recurrent rate after optimized surgery.

**Patients and Methods:** 439 patients (1985-1996, conventional operated) were followed retrospectively, 495 (1996-2006, optimized surgery) were followed prospectively. The end of follow up was December 31<sup>st</sup> 2006. The incidence of a local recurrent tumor (with or without distant metastasis) was determined.

**Results:** The rate of local recurrence was 14/80 (17,5%) after conventional surgery and 6/124 (4,8%) after optimized surgery (R0-resection). Median follow up was 6 years (conventional surgery) and 4 years (optimized surgery). The 5 year rates of local recurrent rate after conventional vs optimized surgery were in pT1-tumors 33% vs 0% in pT2-tumors 9,1 vs. 2,4% in T3 tumors 18,8% vs 7,9% and in pN0 tumors 9,8% vs 4,9%, in pN1 tumors 26,3% vs 3,7% and in pN2 tumors 40% vs. 7,1%. After optimized surgery the T and N category had no significant influence on the local recurrent rate.

**Conclusion:** The criteria for adjuvant radiochemotherapy after conventional surgery are not suited for neoadjuvant multimodal therapy in case of optimized surgery. In this case the preoperative RCT should be based in the distance of tumor from the circumferential resection margin (CRM)

PE707

### A distance of 1 or 2 mm as indication of neoadjuvant radiochemotherapy for rectal carcinoma?

Trinh T.T.<sup>1</sup>, Oberholzer K.<sup>2</sup>, Junginger T.<sup>1</sup>

<sup>1</sup>Johannes Gutenberg-Universität, Klinik und Poliklinik für Allgemein- und Abdominalchirurgie, Mainz; <sup>2</sup>Johannes Gutenberg-Universität, Klinik und Poliklinik für diagnostische und interventionelle Radiologie, Mainz

The minimal distance of the tumour from the Circumferential Resection Margin (CRM) is an independent prognostic factor of the occurrence of a local recurrence after total excision of the mesorectum, apart from the pT and pN stage. So far it is assumed that patients with a distance of 1mm and less have a higher risk of a local recrudescence, but it is possible that even patients with a tumour distance to 2mm show a high risk.

The aim of a prospective study was to determine the reliability of the magnetic resonance tomography (MRT) on the CRM-status  $\leq 1\text{mm}$  and  $\leq 2\text{mm}$ .

34 patients with biologically proven rectal carcinoma were examined by a 1,5 T MR-system. A correlation with pathohistological findings after total or partial excision of the mesorectum followed.

**Results:** A high risk of a local recurrence (pCRM  $\leq 1\text{mm}$  9/34) could be predicted or excluded with high certainty (sensitivity: 89%, specificity: 96%, positive and negative predictive value: 89% respectively 96%). Concerning pCRM  $\leq 2\text{mm}$  the sensitivity of the MRT increased to 100%, the specificity decreased to 92%, the positive predictive value declined to 83%, the negative predictive value was 100%.

**Conclusion:** By establishing a distance of 2mm instead of 1mm between tumour resection margin in the MRT, all patients who are not in need of a radiochemotherapy could be identified correctly, however the part of "sub-treatments" would increase.

Regarding to the less efficacy of the adjuvant radiochemotherapy it is recommendable to determine a distance of  $\leq 1\text{mm}$  for the selection of patients receiving a neoadjuvant radiochemotherapy.

PE708

### Prognostic factors after liver resection for colorectal metastases: Multivariate analysis and comparison with the fong-score

Adam U.<sup>1,2</sup>, Makowiec F.<sup>2</sup>, Obermaier R.<sup>2</sup>, Fischer E.<sup>2</sup>, Opitz O.<sup>3</sup>, Hopt U.T.<sup>2</sup>

<sup>1</sup>Vivantes-Humboldt-Klinikum, Viszeral- und Gefäßchirurgie, Berlin;

<sup>2</sup>Chirurgische Universitätsklinik, Freiburg; <sup>3</sup>Universität Freiburg, Tumorzentrum Ludwig Heilmeyer, Freiburg

Because of a low mortality and morbidity and partially good survival rates the resection of liver metastases of colorectal cancer (CRC) plays an important

role in the multimodal management of patients with metastasized CRC. The knowledge of prognostic factors (as for example the FONG-criteria) is crucial in the planning of different treatment options. We here evaluated outcome after liver resection for CRC metastases and analyzed potential prognostic factors.

**Methods:** Long-term follow-up was available in 186 patients (32% female, median age 63 years) who underwent liver resection for CRC metastases between 1996 and 2006. Initially 57% had colon and 43% rectal cancer. 66% of the primary tumors were lymph node positive. The median time interval between resection of the primary and of liver metastases was 12 (range 0-140) months. For further analyses interval was classified as < 12 months or  $\geq$  12 months. Survival analysis was performed by the Kaplan-Meier- and Cox-methods. The FONG-criteria as well as age, gender, blood transfusions and extent of resection were evaluated for potential prognostic influence.

**Results:** An atypical or segmental resection was performed in 47%, whereas 53% had at least a hemihepatectomy. Free resection margins were achieved in 88%. The five year survival (5-y SV) of all patients after liver resection was 45% (median survival 4.1 years). Significant univariate risk factors for poorer survival were a positive margin (5-y SV 36% vs 47% with R-0;  $p = 0.001$ ) and size of metastases  $\geq$  5cm (5-y SV 33% vs 50% < 5cm;  $p = 0.009$ ). A trend to poorer survival was found for patients with node-positive primaries, female gender, more than one metastasis and elevated CEA-levels.

In multivariate analysis the resection margin ( $p = 0.003$ ), size of metastases ( $p = 0.002$ ) and gender ( $p < 0.05$ ) were factors independently influencing survival. After classifying patients according to the FONG-criteria (0 to 6 positive) univariate survival analysis showed a clear correlation of survival with this FONG-score ( $p < 0.001$ ): patients with a score of 0 or 1 had a 5-y SV of more than 50%, whereas the 3-year survival of patients with a score of five was only 20%. No patient with a score of six was alive after more than two years following liver resection.

**Conclusions:** Prognosis after resection of CRC liver metastases is relatively good especially in the case of small metastases and free resection margins. Identified prognostic factors (e. g. FONG-criteria) should be considered in the planning of multimodal therapy in order to achieve optimal treatment results in the individual patient, possibly even without (primary) resectional therapy in high-risk patients.

PE709

### Prognostic value of eicosanoid pathways in extrahepatic cholangiocarcinoma

Möbius C.<sup>1</sup>, Aust G.<sup>1</sup>, Wiedmann M.<sup>2</sup>, Hauss J.<sup>1</sup>, Witzigmann H.<sup>3</sup>

<sup>1</sup>Universität, Chirurgie II, Leipzig; <sup>2</sup>Universität, Medizin II, Leipzig;

<sup>3</sup>Krankenhaus Dresden Friedrichstadt, Allgemein und Viszeralchirurgie, Dresden

**Background:** Chronic inflammation of the bile duct is linked to an increased risk for the development of cholangiocarcinoma. Arachidonic acid and linoleic acid oxidation through cyclooxygenase and lipoxygenase – two major pro-inflammatory pathways – have rarely been investigated in extrahepatic cholangiocarcinoma.

**Materials and Methods:** Paraffin-embedded specimens from 51 resected adenocarcinomas of the extrahepatic bile duct were immunostained for cyclooxygenase 2 and 5-lipoxygenase to evaluate their intracellular distribution and prognostic value.

**Results:** Cholangiocarcinoma had significantly higher levels of 5-lipoxygenase and cyclooxygenase 2 expression compared with normal tissue ( $p = 0.015$ ). High expression of nucleus-located 5-lipoxygenase was significantly associated with intensive staining for cyclooxygenase 2, ( $p = 0.023$ ). Median disease free survival in patients with low expression of 5-lipoxygenase was significantly better than in patients with high expression of 5-lipoxygenase (log rank  $p = 0.046$ ). DFS in patients with low cyclooxygenase 2 expression was also significantly better than DFS in patients with high cyclooxygenase 2 expression (log rank  $p = 0.0187$ ).

**Conclusions:** The present study demonstrate that 5-LOX and COX 2 protein expression is increased in cholangiocarcinoma suggesting that these two enzymes might be of prognostic value and offer a potential additional adjuvant therapeutic approach in this dreadful disease.

PE710

### Comparative gene expression of CC531 rat colorectal cancer cells in rat liver versus hepatocyte-based co-culture models

Georges R., Adwan H., Zhivkova M., Eyol E., Berger M.

DKFZ, G401, Heidelberg

Colorectal cancer accounts for 11% of all cancers and is the second cause of cancer related death, with the majority of deaths attributable to hepatic metastasis. Since not all disseminated colorectal cancer cells develop into macro-metastases it was hypothesized that sub-populations of these cells evolve a genetic advantage to adhere to certain structures of the extra-cellular matrix, escape the immune system, and survive in their new hepatic environment. The aim of this study was to investigate changes in gene expression which occur in CC531 rat colon adenocarcinoma cells and are instrumental to the metastatic phenotype for homing into the liver. These genes which are thought to be intimately linked to colorectal metastasis included osteopontin (OPN), osteonectin (SPARC), runx2, homeobox C8 (Hoxc8), matrix metalloproteinase-7 (MMP-7), matrix-metalloprotease-9 (MMP-9), and bone sialoprotein II (BSP II). For assessing their specific role, two models of co-culturing hepatocytes with CC531 cells were established. OPN, SPARC, Runx2 and MMP-7 were found to be highly expressed in CC531 metastases explanted from the liver but subsequently were down-regulated and/or disappeared in cell culture. Concomitantly with the down regulation of OPN and Runx2, Hoxc8 was up-regulated in vitro. This inverse regulation suggests that these genes may be regulated in a feed-back loop manner. Interestingly, SPARC levels increased with time in CC531 cells cultured in the presence of hepatocytes. Thus, it seems that hepatocytes play a key role in inducing the expression of SPARC in CC531 cells, but they did not induce a similar induction of OPN and Runx2. Moreover, addition of TGF- $\beta$ 1 to the co-culture of CC531 cells and hepatocytes caused over-expression of SPARC in hepatocytes whereas OPN and Runx2 were not affected, suggesting that hepatocytes are unlikely to stimulate OPN or Runx2 expression in vivo in CC531 cells in response to TGF- $\beta$ 1 induction. Furthermore, MMP-9 mRNA and active MMP-7 protein were expressed by the CC531 tumor cells themselves and were only partially down regulated under cell culture conditions. In conclusion, the up-regulation of SPARC but not of OPN is likely to be caused by the presence of hepatocytes. Future studies will focus on the role of other liver cell types, as well.

PE711

### Immunohistochemical peculiarities of gastric carcinomas in patients younger than 50 years

Schildberg C.<sup>1</sup>, Littwin T.<sup>1</sup>, Hohenberger W.<sup>1</sup>, Schlabrakowski A.<sup>2</sup>, Merkel S.<sup>1</sup>, Horbach T.<sup>3</sup>

<sup>1</sup>Universität Erlangen/Nürnberg, Chirurgie, Erlangen; <sup>2</sup>Pathologisches Institut, Phatologie, Erlangen; <sup>3</sup>Stadtkrankenhaus Schwabach, Chirurgie, Schwabach

**Background:** Young patients (<50 years) comprise 7%-15% of all gastric carcinomas. Therefore, immunohistochemical peculiarities were analyzed in our facility.

**Materials and Methods:** The examined group had 40 patients. The median age of the group was 46 years (28–49 years), the ratio male/female was 2.1/1. Tumor tissue, which was embedded in paraffin, was initially marked, so that it could be further examined using the Tissue Array technique and consequently immunohistochemically stained. Following this, the following markers were analyzed: COX2, EGFR, E-Cadherin, p53, TFF1 and CDX 2. After semi-quantitative representation, a link to data of the tumor register was performed.

**Results:** In the younger patients, the diffuse Type (Lauren-Classification) was overwhelmingly represented with 77%. Early tumor stages (I and II) were distributed similarly with 52% as advanced stage carcinomas with 48%. The 5-year survival rate was 57%. Notable was that Stage IIIA had a distinctly better 5-year survival rate with 65% than those patients with Stage II (55%). In our evaluation of the immunohistochemical stains, it showed that younger patients with the diffuse type showed significantly more down-regulation of COX 2. This is particularly noticeable when one compares tumor stages II and IIIA (16% vs. 0%). With TFF1, there was a notable over-expression shown ( $p > 0.05$ )

in Stage II and IIIa (16% vs 3%). CDX 2 and E-Cadherin were significantly more frequently extracted for the diffuse type.

**Conclusion:** It is known that younger patients with worse histological results (diffuse vs. Intestinal 77% / 23%) display a better 5-year survival rate. In particular, there seems to be a difference between Stages II and IIIa. This could be contributed to and explained by a down-regulation or an over-expression of COX 2 or TFF1.

PE712

### Incidental gallbladder cancer after laparoscopic cholecystectomy and the problem of a practical classification

Goetze T., Paolucci V.

Ketteler- Krankenhaus, Chirurgische Klinik, Offenbach

**Introduction:** Incidental gallbladder carcinoma (IGBC) is a carcinoma first detected by the pathologist. The indication for the cholecystectomy was a benign disease. It is often supposed that the laparoscopic technique could deteriorate the prognosis of gallbladder cancer. In the literature there is a big variety of different 5 year survival curves for the different T- stages and operation methods, so it seems to be very difficult for the surgeons to compare the different results. These assumptions are going to be verified by German- Registry of incidental gallbladder carcinoma.

**Material and Method:** To obtain data we are using the German- Register, which is an institution of the German Society of Surgery.

We have calculated the data for the T- stages, UICC/AJCC- stages and the modified Nevin-classification, which seems to be a very practical kind of classification.

**Results:** 550 cases of incidental gallbladder cancer have been recorded so far. There are 307 patients treated by the laparoscopic procedure, 145 by the open one, 96 with an intraoperative conversion from the laparoscopic to the open technique for non oncological reasons.

After analyzing the T- stages the Kaplan- Meier graphs show a significant advantage for the laparoscopic procedure compared with the open surgery for the entire patients (n = 550) (p < 0,05).

According to the T- stages calculated with the Kaplan Meier method the laparoscopy seems not to worsen the prognosis. In 285 cases it was possible to make a complete staging, regarding the N and M- status. After analyzing the UICC/ AJCC- stages and the Nevin stages for 285 there also seems to be no disadvantage for the laparoscopic treated patients.

**Discussion:** The access technique open or laparoscopic does not seem to influence the prognosis of incidental gallbladder carcinoma.

The problem in the literature is, that often many authors only classify the tumors according to the T- stages without regarding the "n" and "m" status, so a variety of different 5 year survival curves exists. A possible explanation could be that nodal status is not known, or that the UICC/ AJCC- system is very complex and difficult, so according to the authors the mod. Nevin classification is in such cases a very good additional system to divide local, local advanced and metastatic tumors, as a system added to T- system so that every reader can see and compare the different data.

PE713

### The immediate re-resection of T1b incidental gallbladder carcinoma-A controversy debate about the possible improvement of the prognosis and about getting a definite staging

Paolucci V., Goetze T.

Ketteler- Krankenhaus, Chirurgische Klinik, Offenbach

**Introduction:** The indication for an immediate re-resection (IRR) in T1b incidental gallbladder carcinoma (IGBC) is debated in the literature, and different recommendations are often drawn based on data collected from only small groups. But the management of IGBC is difficult, because there are no established guidelines.

A IRR is recommended in cases of T2 tumours and more advanced stages according to many authors and the effective guidelines in of Germany. For a T1 tumour a simple cholecystectomy is enough.

The IRR includes a liver resection and a loco regional lymphadenectomy. In which T- stage such a reoperation should be undertaken is debated in the international literature.

Some authors recommend a reoperation in case of T1b- stage and improve the 5 year survival from 60 to 100%, because the rate of positive lymph nodes is up to 16% and the lymphatic, venous and perineural infiltration is up to 50% according to the literature.

Others recommend a reoperation only when the margins are positive or when there is subserosal invasion > 2mm.

The question is, if T1b tumours do profit from a reoperation or if this operation is only an additional lethality, the same question for T3 and T4 tumours.

**Material and Method:** To obtain data we use the German- Registry of incidental gallbladder carcinoma, which is institution of the German Society of Surgery.

Within a period of 3 months we are actualizing the data.

**Results:** 550 cases of incidental gallbladder carcinomas are registered.

In 79 patients with T1- tumour there was no IRR.

In 31 patients with T1- tumour there was an IRR.

Graph 1 shows survival according to Kaplan- Meier for T1- tumours.

There is a significant prognostic advantage for T1- tumours with an IRR.

In 138 patients with T2- tumours there was no IRR.

In 117 patients with T2- tumour there was an IRR.

Graph 2 shows survival according to Kaplan- Meier for T2- tumours with a significant prognostic advantage for T2- tumours with IRR.

The Kaplan- Meier graphs for T3 and T4- tumours indicate no survival after IRR.

**Discussion:** There is a significant survival benefit for the T2 tumours and T1b- tumours after an IRR (log- rank < 0,05). The analysis shows no advantage for T1a and T3/4 carcinomas after IRR. An IRR should be highly recommended for patients with IGBC in the T1b stage. An extended resection is also necessary in order to exactly determine the nodal status, to make an exact definite staging for these patients, and to separate nodal negative patients (Stage Ia, IIb) from those with positive lymph-nodes.

PE714

### The perforation of the gallbladder in cases of incidental gallbladder cancer- and the use of isolation bags

Goetze T., Paolucci V.

Ketteler- Krankenhaus, Chirurgische Klinik, Offenbach

**Introduction:** The accidental intraoperative perforation of the gallbladder is a problem of the laparoscopic surgery, if an incidental gallbladder carcinoma exists at the time of operation. According to the literature this complication comes up to 30% of the laparoscopic operations. In order to prevent the dissemination of tumourcells the use of an isolation bag is proclaimed. The question is if the intraoperative perforation of gallbladder carcinoma really leads to a prognostic deterioration and if the patients which have been treated with an isolation bag have a prognostic advantage.

**Material and Method:** To obtain data we are using the data of the German-registry of incidental gallbladder carcinoma, which is supported by the German Society of Surgery. We are collecting our data with a standardized questionnaire, which has been sent to all german and now to all austrian surgical clinics as well. In a period of 3 months we are actualizing the data.

**Results:** 550 cases of incidental gallbladder carcinomas are registered. 307 were operated laparoscopically, 97(32%) of them get a relapse of the tumour. 158 patients were treated with the support of an isolation bag, the rate of a relapse was 35% (n = 55). 149 of the laparoscopic group have treated without a isolation bag, the rate of an relapse was 19% (n = 28). In 68 of 307 laparoscopic treated patients there was an intraoperative accidental opening of the organ, the rate of a relapse was 41% (n = 28). In 46 of 68 cases an isolation bag was used, the rate of a relapse was 43% (n = 20). The other 22 of 68 patients who were operated without an isolation bag have a rate of relapse of 36%(n = 8). The group without an intraoperative perforation (n = 239) have 29% (n = 69) of tumour recurrence. 112 of this 239 were treated with the use of an isolation bag, 35 (31%) of them had a tumour recurrence, the other 127 of the 239 patients treated without a bag had a recurrence rate of 27% (n = 34).

**Discussion:** In our register, the intraoperative perforation leads to a significant prognostic disadvantage(p = 0.046 Fisher's exact test). The patients treated with an isolation bag have a tendency of a higher rate of tumour recurrences (p = 0,055 Fisher's exact test), but the isolation bag was used significant

more often in cases of gallbladder perforation, nevertheless the recurrence rate is higher in patients without an intraoperative perforation treated with the use of an isolation bag compared with without bag. Maybe microperforations are a possible explanation therefore.

PE715

### **Patient selection in a prospective randomised multicenter study (CAO, ARO, AIO 04) at the university hospital of Erlangen**

*Besendörfer M., Hohenberger W.*

Chirurgische Universitätsklinik Erlangen, Chirurgie, Erlangen

**Introduction:** 448 patients with the diagnosis of a non metastasized carcinoma of the rectum were treated in Erlangen between 1995 and 2002. After discussion of the patient in our interdisciplinary tumor conference we could randomize 139 patients for the multicenter study (CAO, ARO, AIO 94). The remaining 309 patients received a follow up examination regarding to the inclusion and exclusion criteria of the study to check out a negative or positive patient selection.

**Patients and Methods:** Between 1995 and 2002 we could evaluate 309 patients with a carcinoma of the rectum, which could fit potentially in the study due to data of our tumor documentation.

The inclusion criteria were the distance of the tumor to the anal verge (until 16cm above anal verge), the age of the patient.

**Results:** The preoperative staging in 127 cases was too low. 25 patients had a synchronic or former malignoma. 33 patients had a distal carcinoma of the sigma. In 7 cases the patients were too sick for chemo- radiotherapy. One patient had a Crohns Disease. Distant metastasis led to exclusion in 9 patients. 107 patients were primary suitable for the study.

Reasons for non recruitment.

45 patients were treated by chemo- radiotherapy to reach a R0 resection.

26 patients with lower rectal cancer got a chemo- radiotherapy with the aim of an anal sphincter preservation.

36 patients denied a randomisation for the study.

**Conclusion:** Despite of structured examination of a certain patient group, just 54% of the patients could be recruited for the study.

Because 2 third of the non-randomised patients had negative prognostic factors, we can say that there was a positive patient selection in the study.

PE716

### **Treatment of colorectal cancer and other tumours with a simultaneous application of 5-fluorouracil (5-FU)/ sodium folinate (ONCOFOLIC®): Results of a large observation study**

*Hofheinz R.-D.<sup>1</sup>, Hutschenreuther U.<sup>2</sup>, Sauer U.<sup>2</sup>, Eschenburg H.<sup>3</sup>, Rudolph R.<sup>4</sup>, Tummes D.<sup>5</sup>, Weinberg R.<sup>5</sup>, Guggenberger D.<sup>5</sup>*

<sup>1</sup>Universität Heidelberg, Onkologisches Zentrum, III. Medizinische Klinik, Mannheim; <sup>2</sup>Onkologie, Nordhorn; <sup>3</sup>Onkologie, Güstrow; <sup>4</sup>Onkologie, Essen; <sup>5</sup>Onkologie, Aachen

**Background:** A multi centre, open labelled observation study assessed the safety and efficacy of 5-FU/sodium folinate containing combination regimen in various cancers, especially gastrointestinal cancers. The main focus of this study was to assess the safety and feasibility of a simultaneous infusion of 5-FU and sodium folinate (Oncofolic®). We report the first results of this study.

**Methods:** All patients with colorectal cancer or other tumours were included, who were scheduled to receive a 5-FU and sodium folinate containing (combination) therapy.

**Results:** Between June 2001 and June 2007, 1136 patients were included in this study. 52.9% suffered from colon cancer, 31.5% rectum cancer and 7.6% gastric cancer. 8% had other tumours.

Of all colorectal cancers patients (947 pts, median age 65.9 years, 62% male and 38% female) 29.0% were treated with 5-FU/sodium folinate, 26.8% with 5-FU/sodium folinate plus irinotecan and 34.6% with 5-FU/sodium folinate plus oxaliplatin. 9.5% were treated with 5-FU/sodium folinate plus other substances (cytostatic drugs or targeted therapies, which were used in 6.6%).

Overall, 3123 chemotherapy cycles were applied. The vast majority of the chemotherapy applications used a mixture of 5-FU and sodium folinate. No calcium carbonate precipitation caused by 5-FU and sodium folinate was observed. The compatibility of 5-FU with sodium folinate is clearly shown. No unexpected side effects were observed

**Conclusions:** These results demonstrate that sodium folinate can easily be mixed with 5-FU. The treatment regimen were well-tolerated, and no unexpected side effects or major port problems due to the mixture of 5-FU/sodium folinate were observed. Detailed efficacy and safety data for the whole patient population will be presented. Moreover, comprehensive analysis of subgroups (e.g. elderly patients and patients with comorbidity) will be shown at the meeting.

PE717

### **Single-center surveillance study evaluating the impact of treatment with epoetin beta (NEORECORMON®) on anaemia and quality of life in patients with gastrointestinal tumors receiving palliative chemotherapy**

*Hilbig A., Goerke A., Sinn M., Pelzer U., Stieler J., Roll L., Nicolaou A., Oettle H., Dörken B., Riess H.*

Charité, Campus Virchow-Klinikum, Universitätsmedizin Berlin, Medizinische Klinik m.S. Hämatologie und Onkologie, Berlin

**Background:** Anaemia has a high prevalence among cancer patients, either in terms of anaemia of chronic disease or as adverse side effect of antineoplastic treatment. Anaemia is associated with a higher risk of fatigue syndrome, enhanced toxicity of antineoplastic therapy and inferior prognosis and outcome of therapy. To examine the impact of the erythropoietic growth hormone epoetin beta (Neorecormon®) on anaemia, outcome and quality of life of patients we conducted a single-center surveillance study including patients with gastrointestinal tumors receiving palliative chemotherapy.

**Patients and Methods:** 1g/dl from baseline during first four weeks of chemotherapy. Epoetin beta (Neorecormon®)  $\geq 11$  g/dl at treatment start or a decrease of serum hemoglobin level  $\leq 21$  patients (10/11; f/m; median age 64 years) with different gastrointestinal tumors receiving palliative chemotherapy were included in this single-center surveillance study. Further inclusion criteria was serum hemoglobin level  $\geq 10$  g/dl. Measurement of serum erythropoietin, hemoglobin and other parameters like transferrin saturation were performed every second week. A questionnaire concerning quality of life and fatigue score was performed at treatment start and after 8 weeks of application of epoetin beta. If serum hemoglobin was not increased by 2 g/dl from baseline after 8 weeks, treatment with epoetin beta was stopped.

**Results:** Median serum hemoglobin at treatment start was 10,2 g/dl. After 8 weeks of treatment with epoetin beta (Neorecormon®) median serum hemoglobin was 11 g/dl. In 3 patients out of 21 serum hemoglobin level was improved by  $> 2$  g/dl, 4 patients did not respond in terms of increase of serum hemoglobin. The majority of patients (76 %) showed a modest increase of serum hemoglobin (median 1 g/dl) after 8 weeks. Toxicity in general was low, no occurrence of grade 3/4 toxicities according to NCI-CTC was observed. One patient withdrew consent after 3 weeks of treatment due to occurrence of headache, but it could not be excluded that the symptoms were evoked by the underlying disease (neuroendocrine pancreatic tumor).

**Conclusion:** Epoetin beta (Neorecormon®) improves serum hemoglobin level in patients with gastrointestinal tumors undergoing palliative chemotherapy. The application is feasible, no grade 3/4 toxicities according to NCI-CTC were observed. Further data concerning the impact on outcome and quality of life will be reported at the meeting.

PE718

### Treatment results with fermented mistletoe (*Viscum Album* L.) extract as a part of long-term supportive care in patients with primary non-metastatic colorectal carcinoma

Friedel E.<sup>1</sup>, Matthes H.<sup>2</sup>, Bock P.<sup>3</sup>

<sup>1</sup>Hospital Bad Bocklet, Internal Medicine and Oncology, Bad Bocklet, Germany; <sup>2</sup>Hospital Havelhöhe, Oncology Clinic, Berlin, Germany; <sup>3</sup>FAG Basel AG, Basel, Switzerland

**Objectives:** To evaluate efficacy and safety of the fermented mistletoe extract Iscador® (ISC) in supportive care of surgically treated patients with primary non-metastatic colorectal carcinoma in comparison with a parallel control group without ISC.

**Methods:** In a multicenter, comparative, non-interventional cohort study in Germany and Switzerland, ISC was given in addition to conventional adjuvant chemo- and radiotherapy, while the control was treated with conventional therapy only. Endpoints were surrogates of quality of life and survival, adjusted to baseline, therapy regimen and other confounders.

**Results:** In 804 (429 ISC and 375 control) evaluable patients from 26 centers, the majority of the baseline characteristics, prognostic criteria, and therapy was sufficiently balanced between the therapy groups. After a median follow-up of 58 vs. 51 months, and a median ISC therapy duration of 52 months, significantly fewer ISC (19.1%) than control patients (48.3%) developed ADRs (adverse drug reactions) related to the conventional therapy ( $p < 0.001$ ), had fewer symptoms, mainly gastrointestinal and CNS, during the therapy ( $p < 0.001$ ), and had an on average one week shorter hospitalization. ISC vs. control patients showed a longer tumor-free survival ( $p = 0.013$ ). Systemic ADRs by ISC developed 2.3% of the patients, and local ADRs 23.3%. Severe ISC-related ADRs or tumor enhancement were not observed.

**Conclusions:** The ISC-group showed significantly fewer ADRs of the conventional therapy, fewer disease- and therapy-related symptoms, and longer tumor-free survival than the parallel control group. The ISC-treatment was well tolerated and appears beneficial in supportive care in primary non-metastatic colorectal carcinoma.

PE719

### Colorectal cancer in patients below 50 years of age

Schellerer V.<sup>1</sup>, Schumann S.<sup>1</sup>, Schlabrakowski A.<sup>2</sup>, Hohenberger W.<sup>1</sup>, Croner R.<sup>1</sup>

<sup>1</sup>Universität, Chirurgie, Erlangen; <sup>2</sup>Universität, Pathologie, Erlangen

Colorectal cancer is typically diagnosed in the 6<sup>th</sup> decade of life and is one of the three leading cancers in both genders. Screening for non-symptomatic patients usually starts not before the age of 50.

Analyzing our patient population between the years 2000 and 2005 we found that about 10% of patients diagnosed for CRC were below the age of 50. The median age of these patients was 44 years (range: 21–50 years). About 5% suffered from chronic inflammatory bowel disease. Tumors presented in 62% ( $n = 76$ ) in the rectum, in 17% ( $n = 21$ ) in the sigmoid, in 7% ( $n = 8$ ) in the left colon, in 4% ( $n = 4$ ) in the transverse colon and in 10% ( $n = 13$ ) in the right colon. Most patients presented in stage UICC I (27%) and UICC III (26%), followed by UICC IV (23%) and UICC II (17%). In 4% no tumor (T0) was detectable in resected specimens and in 3% UICC stage was unclear. In 69% of cases a R0 resection was possible. This encouraging result is mandatory not to rule out a colorectal malignancy in younger people.

PE720

### Ist die Pfortaderresektion bei malignen Pankreastumoren gerechtfertigt?

Demir R., Alvarez P.M., Merkel S., Hohenberger W., Klein P.

Universitätsklinik, Chirurgie, Erlangen

Die vorliegende retrospektive Untersuchung des Patientengutes der Chirurgischen Universitätsklinik Erlangen vom 01.01.1978 bis 31.12.1996 überprüft, unter welchen Umständen die Resektion der Pfortader im Rahmen

der partiellen, subtotalen oder totalen Duodenopancreatektomie beim Pankreaskarzinom eine sinnvolle Operationserweiterung darstellt. Die Patienten wurden bis zu ihrem Tod bzw. bis zum 01.01.1999 nachbeobachtet. Von insgesamt 263 Patienten hatten 218 ein Adenokarzinom. 15% der Pankreaskarzinome waren gut differenziert (G1), 42% mäßig differenziert (G2) und 31% schlecht differenziert (G3). Der Rest war undifferenziert (11%) oder unbestimmt (1%).

Durch die erweiterte Duodeno-Pankreatektomie mit Pfortaderresektion bei 52 Patienten konnte die RO-Resektionsrate erhöht werden: 31 (61%) Patienten wurden kurativ reseziert, bei 16 Patienten (30%) verblieb histologisch ein Tumorrest, bei 4 Patienten (8%) verblieb makroskopisch ein Residualtumor. Bezüglich der Stadieneinteilung befanden sich von den 52 Patienten, die sich einer Pfortaderresektion unterzogen, 8% (4 Patienten) im Stadium II, 21% (11 Patienten) im Stadium III und 69% (36 Patienten) im Stadium IVA. Bei 20 der 52 Patienten (38%) die sich einer Pfortaderresektion unterzogen traten Komplikationen auf, dagegen nur bei 64 Patienten (30%) von den 211 Patienten die mit einer Standard Duodeno-Pankreatektomie ohne Pfortaderresektion operiert wurden. 31% der Patienten erlitten nach kurativer Resektion ein Tumorrezidiv an welchem sie schließlich verstarben. Dabei gab es keinen signifikanten Unterschied, ob die Pfortader mitreseziert war oder nicht. Des Weiteren konnte beobachtet werden dass sowohl bei allen kurativ, als auch bei den nicht kurativ resezierten Patienten eine signifikante schlechtere 2 Jahre-Überlebensrate, wenn sich diese einer Pfortaderresektion unterzogen hatten. Ebenso war die mediane Überlebenszeit signifikant geringer bei den 31 kurativ resezierten Patienten mit Pfortaderresektion (11 Monate) im Gegensatz mit den 152 Patienten, die mit einer Standardresektion operiert wurden (15 Monate). Dabei gab es keinen signifikanten Unterschied, ob die Pfortader infiltriert war oder nicht. Eine Resektion der Pfortader erscheint nur beim Vorliegen günstiger Kriterien gerechtfertigt: wenn eine kurative Resektion möglich erscheint, höchstens ein regionärer Lymphknoten metastatisch befallen ist (NO/Nla), der Tumor gut oder mäßig differenziert ist (G1/G2) und die Infiltration durch den Tumor nicht bis an die Intima der Pfortader heranreicht. Der wichtigste prädiktive Faktor für die Prognose und das Langzeitüberleben ist der Beweis von Tumorbefall und die Tiefe der Infiltration in die Wand der Pfortader. Auch wenn die erweiterte Lymphknoten-dissektionen das Langzeitüberleben verbessert, entwickeln die meisten Patienten auch nach kurativer Resektion Rezidive.

PE721

### Protecting families against intestinal cancer

Beck H., Hecke T., Burkhardt M.

Techniker Krankenkasse, Hamburg

**Background:** Colorectal carcinoma belong to the most common types of tumor, both among women and men. In Germany, every year, almost 30,000 people die of intestinal cancer and 71,400 people develop intestinal cancer. Approximately 25% of all intestinal cancer cases are based on familial risk factors, i.e., there is a family history of intestinal cancer or polyposis. This increases the risk for other members of the family, especially for first-degree relatives, to also develop intestinal cancer. The most common type of hereditary intestinal cancer is hereditary nonpolyposis colorectal cancer (HNPCC). In 5–6% of all cases of colorectal cancer a genetic mutation was identifiable.

**Goals:** In most cases, at-risk groups with a familial predisposition do not know about their particular disposition. Due to the early age at which they develop intestinal cancer, this category of people does not benefit from the statutory early detection program. These deficiencies need to be corrected. In addition, the quality of the medical care provided through this project shall be optimized by ensuring cross-stage and cross-sector care, and by developing an evidence-based and controlled diagnostic and therapeutic plan.

**Approach:** The health insurance provider Techniker Krankenkasse (TK) has, based on the legal framework of Paragraph 140 of the SGB V (Social Code V), signed a contract with the Berufsverband Deutscher Internisten e.V. (German Association of Internists) regarding “further diagnostic testing and optimized aftercare with respect to colorectal carcinomas for at-risk persons.” This involves supplementary diagnostic services to TK-members who run an elevated risk for developing familial or hereditary colorectal cancer. They are offered the option of undergoing a diagnostic test with a gastroenterologist or a human geneticist earlier than the current regulations allow.

**Results:** The project “Protecting Families against Intestinal Cancer” is an important contribution to optimizing the secondary prevention of cancer, and

to ensuring the quality of early cancer detection. First results are expected at the end of 2008. During the current stage of the project it is critical that as many medical care providers as possible, as well as more health insurance providers, participate in this project.

PE722

### Diminished expression of the MUC1 splice variant MUC1/Y in carcinomas of the stomach and the oesophago-gastric junction

Brandenburg A.K.<sup>1</sup>, Engelmann K.<sup>2</sup>, Schneider P.M.<sup>3</sup>, Hanisch F.-G.<sup>2</sup>, Hölscher A.H.<sup>4</sup>, Dienes H.P.<sup>1</sup>, Baldus S.E.<sup>5</sup>

<sup>1</sup>Universität, Pathologie, Köln; <sup>2</sup>Universität, Biochemie II, Köln; <sup>3</sup>Universität, Chirurgie, Zürich; <sup>4</sup>Universität, Viszeral- und Gefäßchirurgie, Köln; <sup>5</sup>Universität, Pathologie, Düsseldorf

Aberrant expression of MUC1 mucin has been described in most adenocarcinomas, including breast, pancreatic, colorectal and gastric carcinomas. In a number of studies a correlation between MUC1 overexpression and a worse prognosis could be demonstrated. In addition, during the last years several MUC1 isoforms have been discovered and the expression of some of them has been examined in few tissues. In breast as well as ovarian cancer, an overexpression of the MUC1/Y isoform, an alternative splice variant of the common MUC1 gene, was shown. In the present study we investigated the clinical significance and reliability of MUC1/Y in gastric carcinomas as well as carcinomas of the oesophago-gastric junction.

Although this membrane-tethered isoform lacks the VNTR domain, MUC1/Y is involved in signal transduction, and thus it can elicit cellular responses. Messenger RNA of tumor and corresponding normal gastric (n = 42) or oesophago-gastric tissues (n = 24) was subjected to conventional RT-PCR or quantitative real-time PCR to amplify MUC1/Y.

Our results indicate no differences in MUC1/Y expression between normal and tumor tissue, as determined by semi-quantitative RT-PCR. However, expression of MUC1/Y mRNA in tumors was significantly lower compared to normal tissues using real-time PCR. Statistically significant differences concerning tumor localization or pTNM staging were not observed. Kaplan-Meier analysis indicated that up-regulation of MUC1/Y mRNA in carcinomas might support a favourable prognosis. However, these correlations were not statistically significant.

In conclusion, the mRNA expression in gastric and oesophago-gastric carcinomas does not provide a valuable clinical marker for tumor progression or prognosis.

PE723

### Inhibition of telomerase by mutant template telomerase rna and anti-telomerase sirna induces alt in immortalized human esophageal epithelial cells and in generated human squamous cancer cells

Queisser A.<sup>1</sup>, Doebele M.<sup>1</sup>, von Werder A.<sup>1</sup>, Egenter E.-M.<sup>1</sup>, Heeg S.<sup>1</sup>, Hirt N.<sup>1</sup>, Kunert H.<sup>1</sup>, Hauss S.<sup>1</sup>, Harada H.<sup>2</sup>, Nakagawa H.<sup>2</sup>, Li S.<sup>3</sup>, Blackburn E.H.<sup>3</sup>, Blum H.E.<sup>4</sup>, Opitz O.G.<sup>1</sup>

<sup>1</sup>Universitätsklinikum, Institut für Molekulare Medizin und Zellforschung, Comprehensive Cancer Center, Freiburg; <sup>2</sup>Universitätsklinikum, Gastroenterologie, Philadelphia; <sup>3</sup>Universität, Biochemie und Biophysik, San Francisco; <sup>4</sup>Universitätsklinikum, Medizinische Klinik II, Freiburg

**Introduction:** Immortalization is important for malignant transformation of human cells depending on telomere maintenance. This is either mediated through activation of the enzyme telomerase, which contains a template RNA (hTER) and the core protein (hTERT) or through a recombination based alternative mechanism (ALT). Little is known about the regulation of these two mechanisms in a single cell. We investigated, whether ALT can be induced in genetically defined immortalized or malignant transformed cells by specific genetic telomerase inhibitors.

**Methods:** We generated immortalized human esophageal epithelial cells overexpressing Cyclin-D1 or hTERT (EPC-D1 and EPC-hTERT) as well as human oral squamous cancer cells by overexpression of Cyclin-D1, d.n.p53, EGFR, c-myc (OKF6-D1/d.n.p53/EGFR/c-myc) by retroviral transduction. To

genetically inhibit telomerase, all cell types were transduced with mutated versions of hTER, anti-hTER, siRNA or a combination of both by lentiviral mediated gene transfer. Transduced cells were sorted by GFP-coexpression, telomerase activity (TRAP-assay), telomere length (PFGE-TRF, Q-FISH) and indirect immunofluorescence (APBs) were assayed.

**Results:** Overexpression of MT-hTER, anti-hTER siRNA and the combination of both showed a reduction of telomerase activity in all cell types, whereas TRF analysis revealed telomere elongation, characteristic for ALT. Control cells displayed a robust telomerase activity and a shorter telomere length in immortalized as well as in malignant transformed cells. Additionally, in indirect immunofluorescence ALT-associated PML bodies (APBs) were observed in a higher frequency in immortalized hTER-inhibited cells.

**Summary:** Genetically defined immortalized human esophageal epithelial cells and the generated cancer cells can elongate their telomeres using both telomere maintenance mechanisms. ALT can be induced by the inhibition of telomerase using a lentiviral delivery system of mutant template telomerase RNA and anti-telomerase siRNA. These findings suggest that eventually all telomerase positive cancer cells treated with telomerase inhibitors as a potential anti-cancer strategy might find alternative ways to maintain their telomeres.

PE724

### The influence of age in colon carcinoma

Merkel S., Göhl J., Meyer T., Hohenberger W.  
Universitätsklinikum, Chirurgie, Erlangen

**Introduction:** About 10–15% of patients with primary colon carcinoma are older than 80 years at time of diagnosis. With modern anaesthetic techniques also in older patients a resection is usually possible.

**Patients:** Between 1990–2006 1531 patients with primary colon carcinoma were resected at our department. The patients were divided into three groups according to their age: younger than 60 years (n = 410; 26.8%), 60–79 years (n = 951; 62.1%) and 80 years or older (n = 170; 11.1%). These three groups were compared with respect to pathology, postoperative outcome and prognosis.

**Results:** There were not found significant differences in pT-classification, grade, venous invasion, distant metastases and R-classification. But there were found significant differences in lymphatic invasion (p<0.001), number of regional lymph nodes examined (p = 0.007), pN-status (p = 0.014), postoperative complications and mortality (p<0.001). During follow-up the occurrence of locoregional recurrence and distant metastasis did not reach significant differences as did cancer-related survival. However, observed survival significantly depended on age (p<0.001).

**Conclusion:** In older patients beyond 80 years with colon carcinoma the number of regional lymph nodes examined is reduced and lymph node metastases are less frequent. Postoperative course is affected by higher rates of postoperative complications, observed survival is decreased according to age.

PE725

### Neoadjuvant radiochemotherapy in patients with rectal carcinoma and synchronous liver metastasis?

Merkel S.<sup>1</sup>, Göhl J.<sup>1</sup>, Matzel K.<sup>1</sup>, Meyer T.<sup>1</sup>, Rödel C.<sup>2</sup>, Sauer R.<sup>3</sup>, Hohenberger W.<sup>1</sup>

<sup>1</sup>Universitätsklinikum, Chirurgie, Erlangen; <sup>2</sup>Universitätsklinikum, Strahlentherapie, Frankfurt/Main; <sup>3</sup>Universitätsklinikum, Strahlentherapie, Erlangen

**Introduction:** About 15 – 20% of all patients with primary rectal carcinoma suffer from liver metastases at time of diagnosis. It is discussed controversially if neoadjuvant radiochemotherapy (RCT) is indicated in these patients.

**Patients:** Between 1995 and 2004 129 patients with primarily hepatic metastasised rectal carcinoma were resected at our department. 32 patients (25%) received neoadjuvant RCT. There were 25 men and 7 women aged 45 to 82 years (median 58). 23 carcinomas were localised in the lower third of the rectum, 9 in the middle third. 12 patients suffered from extrahepatic metastases beside their liver metastases. In 17 patients distant metastases were diagnosed before RCT, in 10 patients during restaging after RCT and in 5 patients primary diagnosis was intraoperatively. Median follow-up time was 18 months (range 4 – 143).



**Results:** 15 patients (47%) underwent sphincter sparing procedures, in 17 patients (53%) an abdomino-perineal resection was performed. Locally curative resection was achieved in 27 patients (84%). In 6 patients (19%) also distant metastases were removed completely. During follow-up a locoregional recurrence was observed in 5 patients. The observed 2-year survival-rate of all 32 patients was 37.5%, the 3-year survival-rate was 18.2% (median survival 18 months). Survival was independent from primary pT- or pN-category, grade or venous invasion. Significant differences were found with respect to R-classification, regarding local situation (median 21 versus 11 months,  $p = 0,001$ ) as well as distant situation (median 31 versus 16 months,  $p = 0,033$ ). Younger patients ( $< = 58$  years) had a clearly better survival than older patients ( $> 58$  years) (median 21 versus 15 months,  $p = 0,059$ ).

**Conclusion:** The intention of neoadjuvant RCT in rectal carcinoma with distant metastasis is to perform a sphincter preserving resection and to reduce the risk of locoregional recurrence. These requirements could not be fulfilled in all patients.

PE726

### **Addition of the monoclonal Epidermal Growth Factor (EGF) receptor antibody cetuximab (E) to cisplatin/5-FU (CF) versus CF in 1st line Metastatic Squamous Cell Carcinoma of the esophagus (MESCC): First results of the phase II oesotux trial**

Lorenzen S.<sup>1</sup>, Porschen R.<sup>2</sup>, Al-Batran S.-E.<sup>3</sup>, Hofheinz R.<sup>4</sup>, Thuss-Patience P.<sup>5</sup>, Möhler M.<sup>6</sup>, Grabowski P.<sup>7</sup>, Arnold D.<sup>8</sup>, Greten T.<sup>9</sup>, Müller L.<sup>10</sup>, Gerecke U.<sup>11</sup>, Röthling N.<sup>12</sup>, Peschster T.<sup>1</sup>, Peschel C.<sup>1</sup>, Lordick F.<sup>13</sup>

<sup>1</sup>Klinikum rechts der Isar, Technische Universität, München; <sup>2</sup>Klinikum Bremen Ost, Bremen; <sup>3</sup>Krankenhaus Nordwest, Frankfurt; <sup>4</sup>Universitätsklinikum, Mannheim; <sup>5</sup>Charité, Campus Virchow Klinikum, Berlin; <sup>6</sup>Klinikum der Johannes Gutenberg-Universität, Mainz; <sup>7</sup>Charité, Campus Benjamin Franklin, Berlin; <sup>8</sup>Klinikum der Martin Luther Universität, Halle/Saale; <sup>9</sup>Medizinische Hochschule, Hannover; <sup>10</sup>Onkologische Schwerpunktpraxis, Leer; <sup>11</sup>Hanse-Klinikum, Stralsund; <sup>12</sup>Studienzentrum der Technischen Universität, München; <sup>13</sup>Nationales Zentrum für Tumorerkrankungen (NCT) der Ruprecht-Karls-Universität, Heidelberg

**Background:** Combining the EGF receptor antibody cetuximab (Erbix, E) to platinum-based chemotherapy has shown increased tumor response and survival compared to chemotherapy alone in various types of cancer. This trial was conducted to assess the activity of E in combination with CF compared to CF alone in MESCC.

**Methods:** Patients (pts) who had not been pretreated for metastatic disease received E 400mg/m<sup>2</sup> at first infusion followed by weekly 250mg/m<sup>2</sup> in combination with C 100mg/m<sup>2</sup> d1 plus 5FU 1000mg/m<sup>2</sup> d1-5 qd 29 (E-CF) or CF alone. The primary endpoint was tumor response rate according to RECIST criteria. Patients who progressed on CF were allowed to cross-over to CF plus E or E alone.

**Results:** From 12/04 until 12/06 66 pts with MESCC were included: male:female = 54:12, median age 61 (range 40-76), ECOG PS 0:1 = 33:33. 2 pts died and 2 pts had a deterioration of their performance status prior to therapy start and were excluded from the outcome analyses. In 62 pts (30 CF, 32 E-CF) the median duration of treatment was 11 vs 16 weeks for CF vs E-CF (range 1-35 CF; 1-30 E-CF). Main NCI-CTC grade III/IV toxicities for 62 pts treated with CF vs E-CF were (%): leukopenia 10 vs. 19, thrombocytopenia 7 vs 0, nausea 3 vs 13, emesis 0 vs 3, diarrhea 0 vs 16, fatigue 10 vs 3, sensory neuropathy 0 vs 6, skin rash 0 vs 6. In the intent-to-treat efficacy analysis population the confirmed overall response rate (PR+CR) was 13% vs 19% [CI 95%: 4-31 vs 7-36] and the disease control rate (CR+PR+SD) was 57% vs 75% [CI 95%: 37-75 vs 57-89] for the CF vs E-CF arm, respectively. 3 of 5 pts, who crossed over to E after disease progression during CF, showed a disease control, including 2 PR (1pt with E alone; 1pt with E-CF) and 1 SD (with E alone). With a median follow up of 19.5 months (mos), the median progression free survival was 3.6 [1.0-6.2] vs 5.9 [3.8-8.0] mos and the median overall survival was 5.5 [1.9-9.1] vs 9.5 [8.4-10.6] mos in CF vs E-CF. The median survival in pts with grade II or III skin reactions compared to those without or only grade I skin reactions were 9.3 and 7 mos, respectively.

**Conclusions:** E can be combined safely to standard doses of CF chemotherapy. Although this study was not powered to demonstrate a significant difference

in survival, results suggest that the addition of E may increase the efficacy of standard CF chemotherapy in MESCC.

PE727

### **Lebensqualität (LQ) als Prädiktor für das Überleben von Patienten mit kolorektalen Karzinomen**

Bestmann B.<sup>1</sup>, Egberts J.-H.<sup>2</sup>, Schafmayer C.<sup>2</sup>, Leins A.<sup>2</sup>, Tepel J.<sup>2</sup>, Schmidt C.<sup>3</sup>

<sup>1</sup>Universitätsklinikum Schleswig-Holstein, Campus Kiel, Referenzzentrum Lebensqualität in der Onkologie, Kiel; <sup>2</sup>Universitätsklinikum Schleswig-Holstein, Campus Kiel, Allgemeine und Thoraxchirurgie, Kiel; <sup>3</sup>Mühlenkreiskliniken AöR, Vorstand, Minden

**Einleitung:** Kolorektale Karzinome gehören sowohl bei Männern als auch bei Frauen zu den häufigsten Krebserkrankungen in Europa und den USA. Insgesamt steigt die Inzidenz kolorektaler Karzinome in der Bundesrepublik leicht an, der Inzidenzspitzen liegt derzeit im 7. und 8. Dezennium

**Ziele:** Den zunehmenden Stellenwert von Lebensqualitätserhebungen in der kolorektalen Chirurgie untermauern verschiedene in letzter Zeit veröffentlichte Untersuchungen. In der Literatur finden sich darüber hinaus Hinweise, dass neben den „typischen“ medizinischen Faktoren wie TNM-Stadium oder Resektionsstatus auch Faktoren wie Lebensqualität, Familienstand und soziale Unterstützung einen deutlichen Einfluss auf das Überleben von Karzinompatienten haben können. Der Forschungsstand hierzu ist jedoch verhältnismäßig heterogen. Im Rahmen der vorliegenden Studie sollte daher der Frage nachgegangen werden, ob die prä-operative Lebensqualität einen Prädiktor für das Überleben von Patienten mit Rektum- und Sigmakarzinomen darstellt.

**Methodik:** Insgesamt wurden 368 Patienten in die Untersuchung aufgenommen. Die Lebensqualität wurde erfasst mit dem EORTC QLQ-C30 und einem diagnose-spezifischen Zusatzmodul. Zur Analyse des Einflusses auf die Überlebenszeit wurden neben Kaplan-Meier-Kurven zusätzlich multivariate Analysen mittels Cox-Regressionen durchgeführt. In einer schrittweisen Selektion wurden hierbei die Prädiktoren mit dem größten Einfluss in das Regressionsmodell aufgenommen.

**Ergebnisse:** Im ersten Schritt wurden nur Lebensqualitätsparameter (EORTC QLQ-C30 Kernfragebogen) in die Analyse aufgenommen. Hier zeigte sich, dass die (prä-operativen) Werte der Skalen „Physical Functioning“, „Emotional Functioning“, „Nausea/ Vomiting“ und „Constipation“ den größten Einfluss auf das Überleben haben. Patienten mit schlechten LQ-Werten auf diesen Skalen haben ein doppelt so hohes Risiko zu versterben wie Patienten mit guter Lebensqualität. Dieser Effekt bleibt auch bestehen, wenn klinische Variablen wie TNM-Stadium, Alter oder AP Status (temporär/ endständig/ keiner) in das Modell aufnimmt, wobei in diesem Vorhersage-Modell das N-Stadium den größten Einfluss auf das Überleben hat.

**Schlussfolgerung:** Die neben den „harten“ klinischen Parametern besitzt auch prä-operative Lebensqualität prognostischen Wert für die Abschätzung der Überlebenszeit von Patienten mit Rektum- und Sigmakarzinomen. Dieses auf den ersten Blick erstaunliche Ergebnis hält in unserer Untersuchung allen kritischen statistischen Analysen stand. Daher sollte in Zukunft die prä-operative Lebensqualitätserfassung systematischer Bestandteil des Gesamtbehandlungskonzeptes von Patienten mit kolorektalen Karzinomen sein.

PE728

### **Expression of beta-catenin, C-Met and MUC1 in diffuse-type-gastric carcinomas: Correlations with tumor progression and prognosis**

Retterspitz M.F.<sup>1</sup>, Mönig S.P.<sup>2</sup>, Hölscher A.H.<sup>2</sup>, Dienes H.P.<sup>1</sup>, Baldus S.E.<sup>3</sup>

<sup>1</sup>Universität, Pathologie, Köln; <sup>2</sup>Universität, Viszeral- und Gefäßchirurgie, Köln; <sup>3</sup>Universität, Pathologie, Düsseldorf

Cell adhesion as well as cell scattering represent important features in tumor biology, especially invasion and metastasis of tumor cells. Beta-catenin maintains cell-to-cell adhesion and mediates the Wnt signal transduction. Beta-catenin/Tcf complexes facilitate transcription of target genes encoding

activators of cell proliferation, invasion and inhibition of apoptosis. C-Met represents the receptor for hepatocyte growth factor/ scatter factor (HGF/SF) and is involved in cell scattering, invasion and metastasis. As demonstrated recently, HGF stimulation can prompt beta-catenin tyrosine phosphorylation and dissociation from c-Met. Furthermore, beta-catenin and c-Met can cooperate in promoting entry into the cell cycle and in protecting colorectal cancer cells from apoptosis. In addition, interactions between beta-catenin and the cytosolic part of the MUC1 mucin molecule were described. Thereby, MUC1 may function as a transforming protein by coactivating transcription of Wnt target genes. In the present study, we characterized the expression of beta-catenin, c-Met and MUC1 in gastric carcinomas belonging to the diffuse type according to the Laurén classification.

Formalin-fixed and paraffin-embedded tumor specimens from 105 patients were investigated immunohistochemically. Tumor center and invasion front were separately evaluated applying a semiquantitative scoring system. With regard to beta-catenin, membranous/ cytoplasmic and nuclear immunoreactivity were scored.

Membranous/ cytoplasmic beta-catenin at the invasion front correlated with pN and pTNM stages in subcardial carcinomas and c-Met expression was associated with the presence of lymph node metastasis. A strong MUC1 immunoreactivity in the tumor center was significantly more frequent in higher pN and pTNM stages. In uni- as well as multivariate survival analysis, patients with a strong membranous/ cytoplasmic beta-catenin expression at the invasion front exhibited a better prognosis. Finally, a strong MUC1 reactivity at the tumor invasion front revealed as an independent predictor of a worse survival probability in multivariate analysis.

In conclusion, beta-catenin, c-Met as well as MUC1 contribute to the progression of diffuse-type gastric cancer. Their role should be more extensively investigated with special regard to their downstream effectors as well as the response to targeted therapies.

PE729

### Repeated anti-cea-radioimmunotherapy (RAIT) with 131iodine-labetuzumab (phase-II-study) versus a single dose rait post-salvage resection of Colorectal Liver Metastases (CRC-LM)

Liersch T.<sup>1</sup>, Sahlmann C.O.<sup>2</sup>, Becker H.<sup>1</sup>, Goldenberg D.M.<sup>3</sup>, Meller J.<sup>2</sup>

<sup>1</sup>Universitätsmedizin, Allgemein- und Viszeralchirurgie, Göttingen;

<sup>2</sup>Universitätsmedizin, Nuklearmedizin, Göttingen; <sup>3</sup>Garden State Cancer Center, Center for Molecular Medicine and Immunology, Belleville, NJ, USA

**Aim:** Within a phase-II study a single dose RAIT (40-60 mCi/m<sup>2</sup> 131Iodine-Labetuzumab, Immunomedics, Inc., Morris Plains, NJ/USA) after salvage resection of CRC-LM has been well tolerated. Compared with a control group (OP +/- CTX) the RAIT-patients showed an improved overall survival (1). The aim of the ongoing phase-II-study was to evaluate the toxicity and efficacy (DFS, OS) in patients who received a repeated RAIT with 50 mCi/m<sup>2</sup> 131Iodine-Labetuzumab after R0-resection of CRC-LM.

**Methods:** 32 consecutive patients (age 61 years; 8x rectal cancer, 24x colon cancer; hepatic metastases: 16x synchronous, 16x metachronous) were re-staged with CT/MRT and FDG-PET after R0-resection of extended CRC-LM prior to RAIT. On account of established clinically and histopathologically risk scores (Nordlinger-, Fong- and Gayowski-Scores) 87% of the patients had an intermediate or high risk of recurrence of liver metastases within the next 2 years (after resection of CRC-LM). During staging procedures 16 patients showed no suspect lesions ("adjuvant"), while 16 patients showed lesions suspicious of residual or new malignant disease ("non-adjuvant"). All patients were treated with 40-50mCi/m<sup>2</sup> 131Iodine-Labetuzumab (humanized monoclonal antibody against CEA). 24 patients were treated a second time after re-staging (CT/MRT, FDG-PET) prior to the second RAIT.

**Results:** 8/32 (25%) of the patients developed a hematologic toxicity WHO-grade 4 (leukocytes or thrombocytes). After the second cycle of RAIT no cumulative toxicity could be observed. Complete bone marrow regeneration in all cases was noted. The patients' compliance was 100% during a median follow-up of 21 months. The "adjuvant" patients had an enhanced median DFS of 18 months in comparison to the "non-adjuvant" patients (DFS: 6 months) and the control group (DFS: 12 months). The OS in patients with repeated RAIT is 96% until now.

**Conclusion:** Repeated Anti-CEA-RAIT with 40-50mCi/m<sup>2</sup> 131Iodine-Labetuzumab is safe and feasible. The side effects are acceptable and the patients' compliance is high. The DFS- and OS-data of the "adjuvant" patients are very encouraging considering their bad prognosis. The efficacy of 2xRAIT will be evaluated versus a combination of RAIT + CTX within the prospectively randomized, multicentric ARC-08 trial which will be initiated in 2008.

1. Liersch T, Meller J, Bittrich M, Kulle B, Becker H, Goldenberg DM: Update of Carcinoembryonic Antigen Radioimmunotherapy with 131I-Labetuzumab after salvage resection of colorectal liver metastases: Comparison of outcome to a contemporaneous control group. *Ann Surg Oncol* 2007, 14 (9): 2577-2590

PE730

### Preoperative chemoradiotherapy in locally advanced rectal cancer (UICC stages II and III): Influence of postsurgical tumor response parameters on prognosis

Liersch T.<sup>1</sup>, Sprenger T.<sup>1</sup>, Rothe H.<sup>2</sup>, Ghadimi M.<sup>1</sup>, Hess C.F.<sup>3</sup>, Becker H.<sup>1</sup>, Langer C.<sup>1</sup>

<sup>1</sup>Universitätsmedizin, Allgemein- und Viszeralchirurgie, Göttingen;

<sup>2</sup>Universitätsmedizin, Pathologie, Göttingen; <sup>3</sup>Universitätsmedizin, Strahlentherapie/Radioonkologie, Göttingen

**Aims:** Preoperative chemoradiotherapy (CT/RT) is recommended for UICC stage II/III rectal cancer. In order to verify prognostic tumor response parameters we assessed the impact of CT/RT-induced tumor (T-level-) downsizing, UICC-downstaging, and morphological tumor regression on disease free- (DFS) and overall survival (OS).

**Patients and Methods:** 78 patients with UICC stage II/III rectal cancer received 5-FU-based preoperative CT/RT approximately six weeks before standardized surgery including total mesorectal excision. CT/RT-induced tumor regression was assessed based on a five-point semi-quantitative tumor regression grading (TRG) system. Tumors were considered as non-responsive when assigned to TRG 0-1 (none or minor regression) and as responsive when assigned to TRG 2-4 (moderate or complete regression). All results were compared with DFS and OS, and the prognostic value of each factor was determined by univariate (logrank-test) and multivariate (cox-regression) analyses.

**Results:** After (R0) resection, the ypUICC stages were stage 0 in 10/78 [12.8%] cases, stage I in 19/78 [24.4%] cases, stage II in 25/78 [32.1%] cases, stage III in 21/78 [26.9%] cases and stage IV in 3/78 [3.8%] cases. During follow-up over 43 months (CI: 37 - 49 months) the local recurrence rate was 3.8% (n = 3), and distant cancer relapse occurred in 23% (n = 18). In univariate analyses T-level downsizing, UICC-downstaging and TRG indicated a significant influence of preoperative CT/RT on DFS, but concerning OS only UICC-downstaging remained a prognostic parameter. Multivariate cox-regression analysis confirmed only UICC-downstaging to correlate with DFS (p = 0.002) and OS (p = 0.007).

**Conclusion:** Neoadjuvant CT/RT-induced UICC-downstaging significantly prolonged DFS and OS in patients with UICC stage II/III rectal cancer.

PE731

### Preoperative radioimmunotherapy with cetuximab and 5-FU in patients with advanced rectal cancer – First results of a phase-I/II-trial

Semrau R.<sup>1</sup>, Vallböhmer D.<sup>2</sup>, Kocher M.<sup>1</sup>, Lux S.<sup>1</sup>, Metzger R.<sup>2</sup>, Schneider P.<sup>3</sup>, Hölscher A.H.<sup>2</sup>, Müller R.-P.<sup>1</sup>

<sup>1</sup>Klinikum der Universität zu Köln, Klinik und Poliklinik für Strahlentherapie, Köln; <sup>2</sup>Klinikum der Universität zu Köln, Klinik und Poliklinik für Visceral- und Gefäßchirurgie, Köln; <sup>3</sup>Universitätsspital Zürich, Klinik für Viszeral- und Transplantationschirurgie, Zürich

**Background:** Preoperative radiochemotherapy in advanced rectal cancer reduces local recurrence rates and therefore is regarded as standard therapy. Nevertheless, histopathological response rates remain poor. This study tested the feasibility of cetuximab (CET) added to a standard 5-FU-based radiochemotherapy protocol.

**Patients and Methods:** Patients with potentially resectable and locally advanced tumors (cT<sub>3-4</sub>, cN<sub>0-2</sub>) received 50,4Gy to primary tumor and lymphatic

drainage, 250mg/m<sup>2</sup> cetuximab (loading dose 400mg/m<sup>2</sup>) day 1, 8, 15, 22, 29, and 36. 5-FU was administered according to dose levels (level 1: 750mg/m<sup>2</sup> d1-5 and 29-33; level 2: 1000mg/m<sup>2</sup> d1-5 and 29-33).

**Results:** Nine patients were treated in phase I (3 in dose level I and 6 in dose level II). All patients received 50.4Gy. All 3 patients in dose level I received 6 courses of CET and completed 5-FU. Four patients in dose level II received all 6 courses of CET, 2 patients only received 5 courses. One patient in level II did not receive second 5-FU course due to severe thrombopenia, most probably caused by genetic DPD mutation. All patients underwent a R0-resection with total mesorectal excision. The postoperative recovery was uneventful. There were 2 dose limiting toxicities (DLTs) in dose level 2: thrombopenia grade IV and diarrhoea grade III (NCI-CTCAE v.2). There were no other haematological grade III/IV toxicities. Five patients had grade I diarrhoea, 3 patients grade II and 1 patient grade III diarrhoea (DLT). Typical acneiform rash was present in all patients (1 patient grade I, 5 patients grade II, 3 patients grade III) but resolved completely after treatment.

**Conclusion:** Neoadjuvant radiotherapy with cetuximab and 5-FU is feasible. Recommended 5-FU dose in combination with cetuximab and radiotherapy is 750mg/m<sup>2</sup> d1-5 and 29-33. There were 2 DLTs at 5-FU dose level of 1000mg/m<sup>2</sup>. Thrombopenia in 1 patient was most probably caused by DPD gene mutation.

PE732

### Thermotherapy of oesophageal cancer with paramagnetic nanoparticles

Rau B.<sup>1</sup>, Steinbach M.<sup>2</sup>, Koswig S.<sup>3</sup>, Dresel S.<sup>2</sup>, Ollek J.-T.<sup>4</sup>, Schlag P.M.<sup>1</sup>, Jordan A.<sup>4</sup>

<sup>1</sup>Charité – Campus Buch, Klinik für Chirurgie und Chir. Onkologie, Berlin; <sup>2</sup>HELIOS Klinikum Berlin-Buch, Klinik für Chirurgie und Chir. Onkologie, Berlin; <sup>3</sup>HELIOS Klinikum Berlin-Buch, Chefarzt / Klinik für Radioonkologie und Strahlenheilkunde, Bad Saarow; <sup>4</sup>Charité – Campus Virchow-Klinikum, Institut für Radiologie mit Abt. Neuroradiol. und Kinderradiologie CCM/CVK, Berlin

**Background:** Treatment of oesophageal cancer with surgery alone or in combination with an interdisciplinary approach leads to poor outcome according to overall and disease free survival. Thermotherapy using magnetic nanoparticles is a therapeutic option to improve treatment. The objective of this study is to show that this new approach, which is basically an interstitial hyperthermia, can be applied safely and effectively in case of incurable oesophagus carcinoma. The method comprises direct injection of a magnetic fluid into the tumor and its subsequent heating in an alternating magnetic field.

**Methods:** Altogether 7 patients with a squamous cell carcinoma and 2 with an adenocarcinoma were involved in the study. Of these 9 patients 2 received thermotherapy as monotherapy due to previous chemoradiotherapy. 7 patients received combined therapy with chemoradiotherapy (GHD 45 Gy; 5-FU 225 mg/m<sup>2</sup>/d continuously and Cisplatin 30 mg/m<sup>2</sup> as bolus). Hyperthermia was applied synchronous to Cisplatin infusion once a week. The nanoparticles were placed pretherapeutically via endoscope and controlled by CT scan. Temperature was measured by a probe placed inside the oesophagus. Therapy associated toxicities were recorded according to the WHO-guidelines.

**Results:** Altogether 11 therapy associated toxicities of grade 1 and 2 were detected during treatment. Grad 4 toxicities were registered in two cases each with a lobar pneumonia which lead to abort treatment. One patient left the study on his own wish. In all patients energy levels were limited due to heat sensations caused by the magnetic field. A partial reduction of these sensations could be achieved by applying cooling devices or giving analgetics. Successful endoscopic placement of the nanoparticles depended on morphology and structure of the tumour. Measured treatment temperature inside the oesophagus was in average 40°C. Calculated temperatures according to the distribution of the nanoparticles measured by post instillation CT were 41.5°C on average.

4 of the patients reported of subjective improvement of the oesophageal passage after finishing therapy. Decrease in tumour volume was 46% after monotherapy and 93% after combined therapy. Activity of the tumour in PET-examination decreased 38% after monotherapy and 74% after combined therapy.

**Conclusion:** With magnetic nanoparticles induced thermotherapy in advanced oesophageal cancer both, monotherapy or combination with chemo- and/or radiotherapy is feasible and regression of tumour size was observed with reasonable toxicity. To improve quality of temperature measurement and to proof validity of treatment further investigations are necessary.

PE733

### Quality control indicated by perioperative characteristics and short-term outcome in daily surgical treatment of cardia carcinoma (CA) – Results of a prospective observational multicentre study

Jannasch O.<sup>1</sup>, Meyer F.<sup>1</sup>, Meyer L.<sup>2</sup>, Gastinger I.<sup>3</sup>, Lippert H.<sup>1</sup>

<sup>1</sup>University Hospital, Department of Surgery, Magdeburg; <sup>2</sup>Municipal Hospital, Department of Surgery, Plauen; <sup>3</sup>Municipal Hospital, Department of Surgery, Cottbus

The *aim* of the study was to investigate diagnostic, therapeutic and outcome measures of cardia carcinoma(Ca) in daily surgical practice.

**Methods:** Using a prospective observational multicentre study over a defined time period, all consecutive patients with cardia Ca out of a pool of patients with histologically confirmed gastric Ca who were treated in surgical departments were registered, in particular, detailed patient, diagnostic & treatment characteristics, in a computer-based format for analysis on quality issues. Short-term outcome was characterized by hospital stay, complication rate, morbidity & hospital mortality.

**Results:** Overall, 198 subjects (17.4%) with cardia Ca out of 1,139 patients with gastric Ca from 80 surgical departments of each level of care were documented from 01/01-12/31/2002. Tumor site (*n* = 186) according to Siewert's classification: Type I, 22.2% (*n* = 44); type II, 40.4% (*n* = 80); type III, 31.3% (*n* = 62). 172 patients underwent surgical intervention (operation rate, 86.9% [*n* = 62]) out of whom 145 individuals underwent resection (rate, 84.3%). A potentially curative resection was achieved in 111 patients (R0 resection rate, 56.1% *versus* 82.3% in all gastric Ca). Fresh frozen section was only used in 72 resections (rate, 49.7%). Of 142 standard resections (distal esophagectomy with proximal or total gastrectomy), systematic D1, D2 & D3 lymphadenectomy was performed in 81.0%, 67.6% & 7.7% , resp. Histologic investigation revealed UICC stage I/II in 39.5% of all operated patients: III/IV, 54%; not classified, 6.5%. Distant metastases occurred most frequently at the peritoneal site (15.2%), liver (10.6%) & non-regional lymph nodes (7.1%). Postoperative morbidity was 33.7%. Anastomotic leakage occurred in 13 patients (9.1% *versus* 5.8% in gastric Ca) out of whom 8 subjects (5.6%) underwent surgical reintervention. Hospital mortality was 8.6% (*n* = 17) compared to 8.0% in gastric Ca.

**Conclusion:** More than 50% of patients with cardia Ca show an advanced tumor growth when they are diagnosed. Not all resections assessed as potentially curative were accompanied by D2 lymphadenectomy. It is suggested to treat patients with cardia Ca at surgical centres for optimal outcome (5-year survival still under investigation) i) to further improve hospital volume & R0 resection rate, ii) to consequently use intraoperative fresh frozen section for the detection of an adequate tumor-free resection margin & iii) to lower the rate of anastomotic insufficiency.

PE734

### Cetuximab with irinotecan/NA-FA/5-FU as first-line treatment in advanced gastric cancer: Preliminary results of a non-randomised multi-centre AIO phase II study

Moehler M.<sup>1</sup>, Trarbach T.<sup>2</sup>, Seufferlein T.<sup>3</sup>, Kubicka S.<sup>4</sup>, Lordick F.<sup>5</sup>, Geissler M.<sup>6</sup>, Daum S.<sup>7</sup>, Wilke J.<sup>8</sup>, Höhler T.<sup>9</sup>, Kanzler S.<sup>1</sup>, Galle P.<sup>1</sup>

<sup>1</sup>Uniklinik, Mainz; <sup>2</sup>Uniklinik, Essen; <sup>3</sup>Uniklinik, Ulm; <sup>4</sup>Uniklinik, Hannover; <sup>5</sup>Uniklinik, München; <sup>6</sup>Klinik, Esslingen; <sup>7</sup>Uniklinik Charite, Berlin; <sup>8</sup>Klinik, Essen; <sup>9</sup>Prosper Hospital, Recklinghausen

**Background:** Since cetuximab showed promise in irinotecan-based therapies in human colorectal cancer, and irinotecan was effective with FA/5-FU in advanced or metastatic gastric cancer, we evaluated tolerability and efficacy of cetuximab with irinotecan/5-FU/FA as first-line treatment in patients with initially locally unresectable advanced or metastatic gastric cancer .

**Methods:** Patients (pts) with untreated adenocarcinoma of the stomach or the oesophagogastric junction, with ECOG < 2, measurable lesions and adequate organ functions were eligible. Pts received cetuximab at an initial dose of 400 mg/m<sup>2</sup> followed by 250 mg/m<sup>2</sup> weekly, irinotecan 80 mg/m<sup>2</sup> + 24 hours' continuous infusion of sodium folinate (Na-FA) 200 mg/m<sup>2</sup> and 5-FU 1500 mg/m<sup>2</sup>, on days 1, 8, 15, 22, 29, 36. Cycles were repeated on day 50.

Treatment was continued until disease progression. Tumour assessments were performed every other cycle, i.e. every 14 weeks.

**Results:** From August 2006 to September 2007, 49/50 enrolled pts were 71% males, median age 63 years (range 33–77); median PS 0 (65%); 69% gastric and 31% oesophagogastric carcinomas. Median treatment time was 13 weeks (range 0–43). Grade 3/4 toxicities were diarrhoea 16%, skin toxicities 14%, allergic reactions, anaemia and leucopenia 4%. For all other toxicities, see table below. No early death occurred. Among 29 response-evaluable patients, an overall response rate (CR + PR) was 55% and a tumour control rate for at least 7 weeks was 100%. No early death occurred.

**Conclusion:** In nearly all cases, chemotherapy with irinotecan/Na-FA/5-FU plus cetuximab was very well tolerated. All response data are intriguing. Cetuximab combined with chemotherapy in gastric cancer should clearly be analysed in an upcoming phase III trial. Updated results will be presented at the meeting.

PE735

### Alternative EUS-guided transluminal drainage of bile and pancreatic duct obstructions

Will U.<sup>1</sup>, Wanzar J.<sup>1</sup>, Gerlach R.<sup>1</sup>, Fueldner F.<sup>1</sup>, Thieme A.<sup>1</sup>, Meyer F.<sup>2</sup>

<sup>1</sup>Municipal Hospital, Department of Gastroenterology, Gera; <sup>2</sup>University Hospital, Department of Surgery, Magdeburg

ERCP-guided stent implantation into the bile or pancreatic duct is considered an established treatment for obstructions. However, there are cases with not introducible catheter into the papilla or not reachable papilla because of both pyloric/duodenal stenosis or previous GI surgery (BII gastric resection, Whipple procedure, gastrectomy with Roux-en-Y reconstruction).

The aim of the study was to investigate feasibility & outcome of the EUS-guided transluminal drainage of the i) bile duct in cases with failure of PTCO or in patients who decline it as the only previous therapeutic option, & ii) pancreatic duct.

**Methods:** All consecutive patients were enrolled in this ongoing prospective observational unicenter study (case series) through a 4-year time period. Patient- & intervention-related specifics were documented. Feasibility was characterized by success rate (e.g., regressive cholestasis, improvement of clinical symptoms) & outcome by complication rate (frequency of bleeding or perforation), mortality & short-term follow-up.

**Results:** From XI/2002–X/2006, 30 patients underwent attempts for transluminal drainage into the i) bile duct (main indication, cholestasis because of advanced tumor growth),  $n = 15$ ; ii) pancreatic duct (chronic pancreatitis),  $n = 15$ . After transluminal ductal puncture, cholangiography was successful 15x out of 16 attempts (93.8%) versus pancreaticography in each case of 19 attempts (100%). While cholangiodrainage was achieved in 12 of 15 subjects (technical success rate, 80%), drain into the pancreatic duct was correctly placed in 11 of 15 individuals (73.3%) using this novel transluminal route. Though slight postinterventional pain was observed in each of the 30 cases, only cholangitis ( $n = 1$ ) & hemobilia ( $n = 1$ ) occurred after cholangiodrainage whereas after transluminal drainage of the pancreatic duct, perforation, pancreatitis ( $n = 1$  each) & bleeding ( $n = 2$ ) were documented as severe postinterventional complications resulting in a periinterventional morbidity of 12.5% & 21.1%, resp. Endoscopic reintervention rate was 25% & 18.2%, resp. (main cause, stent dislocation; intervention-related mortality, 0).

**Conclusion:** In selected patients, EUS-guided transluminal drainage of the bile & pancreatic duct is a reasonable, feasible & promising endoscopic approach, with a low periinterventional risk. It broadens the spectrum of therapeutic options but still needs further evaluation on the indication & the advantageous impact as well as long-term follow-up investigation.

PE736

### Down-regulation of osteopontin and osteonectin RNA levels causes reduced proliferation of asml rat pancreatic carcinoma cells

Zhivkova M., Adwan H., Naydenova V., Berger M.

DKFZ, G401, Heidelberg

Uncontrolled cell proliferation and formation of metastasis are among the main problems related to cancer growth. Two of the proteins suspected to be

involved in these processes are osteopontin (OPN) and osteonectin (ON). Transfections with an antisense oligonucleotide against OPN as well as with four si-RNAs directed against ON, pooled or individually, were used to modulate the levels of OPN and ON RNA in AsML rat pancreatic carcinoma cells. Treated cells were harvested after 24, 48 and 72 hours and their RNA was isolated for RT-PCR analysis with primers derived from OPN, ON and tubulin sequences. Cells treated in the same manner were investigated by MTT assay or methylcellulose colony formation assay for their proliferation rate and their capability to form colonies from single cells. The strongest antiproliferative activity was observed after transfecting AsML cells with anti-ON si-RNAs which reduced not only the level of ON- but also that of OPN-RNA. This was valid for the si-RNA pool as well as for one of the four individual si-RNAs. The other three si-RNAs decreased only ON levels and had no negative effect on the proliferation rate. The si-RNA pool against ON caused also a decreased colony formation of transfected cells as compared to cells transfected with control siRNA. As could be expected from these results, reduction of the OPN-RNA level by a specific antisense oligonucleotide inhibited also the proliferation of AsML cells. In conclusion, the modulation of the ON RNA level alone was not sufficient to reduce the proliferation of AsML rat pancreatic carcinoma cells. However, reduction of OPN levels, alone or concomitant with that of ON, was more effective regarding an anti-proliferative or an anti-clonogenic effect against these cells. Future experiments will include the protein analysis of AsML cells that have been treated as described above. It is hoped that the understanding of these mechanisms will add to the therapeutic arsenal against pancreatic carcinoma.

PE737

### Effects of differentiation, origin and mesothelial adhesive potential of pancreatic cancers

Mees S., Schleicher C., Colombo-Benkmann M., Senninger N., Haier J.

Universitätsklinik Münster, Klinik für Allgemeine Chirurgie, Münster

**Background:** Adenocarcinomas of the exocrine pancreas belong to the most aggressive malignancies with an overall 5-year survival rate of still less than 5%. The early development of distant metastases, such as peritoneal carcinomatosis, is a specific characteristic of this particular tumor entity, but their mechanisms are poorly understood. In a series of newly established orthotopic models for pancreatic carcinomas their potential for mesothelial adhesion as one of the first steps of metastasis formation was investigated.

**Methods:** 12 cell lines of well- to undifferentiated pancreatic cancers from different tumor sites (primary or metastatic lesions) were used to implant subcutaneous donor tumors and subsequent orthotopic transplantation in nude mice. After 12 weeks primary tumor volume, local infiltration, and patterns of systemic metastases were assessed using a standardized dissemination score. This in vivo behaviour was compared with in vitro tumor cell adhesion to mesothelial cells.

**Results:** In vivo experiments resulted in a tumor take rate in the implantation group of 100%. Differences with regard to tumor size, infiltration and metastatic spread were found depending on the grade of differentiation of the cell lines used. Less differentiated cells of primary tumors caused higher dissemination scores and metastasis formation (liver, lymph nodes) than better-differentiated cells ( $p < 0.05$ ). A significant increase ( $p < 0.05$ ) of tumor growth and infiltration was also seen for cells originating from metastases compared to those from primary tumors. Adhesion assays revealed an adhesive potential at mesothelium of all pancreatic cell lines (24–91%), while significant differences in adhesion rates depending from their origin [primary tumors (mean 64.6%) vs. metastases (mean 48.6%), t-test] or grade of differentiation were not observed. The maximum adhesion rates were found in average 76 min. after addition of pancreatic cells to mesothelial monolayers (observation period 90min).

**Conclusion:** The grade of differentiation and the origin of pancreatic tumors can be reproduced in a number of animal models with comparable tumor growth and metastatic spread making these experimental systems an interesting tool for the investigation of mechanisms or pancreatic cancer progression. The observed adhesion of all pancreatic cells at the mesothelium suggests that this adhesive potential is a required, but not rate-limiting step for the formation of peritoneal carcinomatosis.

PE738

### Development of rat models for pancreatic cancer liver metastasis

Eyol E.<sup>1</sup>, Adwan H.<sup>1</sup>, Zhivkova M.<sup>1</sup>, Zepp M.<sup>1</sup>, Djandji D.<sup>2</sup>, Kleeff J.<sup>3</sup>, Berger M.<sup>1</sup>

<sup>1</sup>DKFZ, G401, Heidelberg; <sup>2</sup>DKFZ, D050, Heidelberg; <sup>3</sup>Technical University Munich, Department of Surgery, München

Pancreatic cancer (PDAC) is the third-most frequent neoplasia of the GI-tract and the fourth to fifth leading cause of cancer-related mortality in the Western world. The overall 5-year survival rate of PDAC is approximately 1% and the median survival time after diagnosis is less than 6 months.

To help overcoming this situation a model would be desirable which mimics in a relevant way the characteristics of PDAC outgrowth and metastasis. Such a model could be used for identifying new markers for early diagnosis as well as potential targets suited for therapy. The aim of this study was to establish an orthotopic liver metastasis model in male nude rats based on pancreatic cancer cell lines which carry a marker suited for monitoring their growth *in vivo*. Therefore, 15 pancreatic cancer cell lines (AsPC-1, BXPC3, CAPAN 1, Colo357, DANG MiaPaca, PANC 1, PANC 89, PATU 390, S2-013, Su8686, T3M4, AS, ASML, and C. 8.18) were investigated for their take rate in the liver of RNU rats. Logarithmically growing cells ( $4 \cdot 10^6$ – $2 \cdot 10^7$ ) were suspended in 0.25 ml PBS and 0.15 ml matrigel and were injected via the portal vein or under the liver capsule of anaesthetized rats. For monitoring the subsequent tumor growth all animals were subjected to relaparotomy with the time interval pending on clinical signs as well as on information from earlier passages.

Only two of the 15 cell lines (S2-013 and ASML) were suited with regard to stable and efficient tumor growth in the liver. Three other cell lines (Aspc1, Colo357, Panc 89) grew primarily in the liver but spontaneously regressed thereafter.

Subsequently ASML cells isolated from the fifth animal passage were transfected with the plasmid pBudCE4.1 containing both, the GFP and luciferase genes to facilitate their detection in the intact animal. Prior to imaging the animals were injected *i.p.* with D-Luciferin, the substrate of luciferase, at a dose of 150 mg/kg body weight and subsequently imaged using the IVIS100 imaging system (Living Image software v2.5). To confirm the location of the respective signals obtained from imaging living rats the animals were sacrificed and the liver was removed. *Ex vivo* imaging of the excised livers was carried out immediately and led to show that the signals obtained *in vivo* matched those *in vitro*.

In conclusion, two models of orthotopic pancreatic liver metastasis were established in nude rats and it is envisaged that they will have application for various therapeutic strategies.

PE739

### CD154 gene transfer in an orthotopic pancreatic adenocarcinoma mouse model

Serba S., Schmidt J., Ryschich E., Märten A.  
Universität, Chirurgie, Heidelberg

**Background:** Patients with adenocarcinoma of the pancreas have only limited promising therapy options. Therefore, immunotherapeutic approaches might be considered a promising therapy that has gained in importance over the last years. Several studies and own experiments have shown that activation of CD154 leads to tumor regression. CD40/CD154 interactions play a key role in maturation of dendritic cells and in the activation of cytotoxic T cells. In this study, CD154 gene transfer was tested as potent immunotherapy in an orthotopic pancreatic carcinoma mouse model. Animals are investigated for tumor growth and immune response. **METHODS:** The efficacy of CD154 gene transfer in initiating anti-Tumor immune response was investigated in a pancreatic ductal adenocarcinoma orthotopic syngeneic mouse model and the role of dendritic cells was determined, as well. Male C57Bl6 mice were injected with syngeneic tumor cells; treatment with different doses started five days later. Tumor volume was measured on day 25 after tumor cell injection. For adoptive cell transfer studies donor splenic or tumor infiltrating lymphocytes were isolated from tumor bearing treated mice and injected in the tail vein of untreated tumor-bearing animals. Tumor and spleen were investigated for lymphocyte infiltration.

**Results:** Significant slower Tumor growth rate and less metastasis were observed following administration of CD154 plasmid. Meanwhile, such an effect of the plasmid was not observed in immunodeficient mice. Tumors of treated mice were found to be infiltrated with T cells and dendritic cells. Tumor-infiltrating lymphocytes were Tumor-specific as shown in IFN-gamma ELISpot assays. Using intravital microscopy it was possible to exhibit the significant induction of leukocytes sticking to the Tumor endothelium after CD154 treatment. By performing adoptive cell transfer experiments, it has revealed that Tumor-derived dendritic cells and CD8 cells from CD154-treated donor mice either harbour anti-Tumor activity or induce it in the recipients. Distinctly, CD8 cells from donor-spleens were found to migrate directly into the recipients Tumor.

**Conclusions:** The induction of anti-Tumor activity initiated after treating mice with CD154 plasmid was realised. Further investigations showed that this is mediated by mature myeloid dendritic cells which activate CD8 cells. Clinical trials investigating CD154-based therapies should be extended.

PE740

### Whole blood transcriptomic and proteomic profiling in pancreatic cancer patients treated with radiotherapy, gemcitabine and cetuximab

Timke C.<sup>1</sup>, Muenter M.<sup>2</sup>, Wagner M.<sup>1</sup>, Schwager C.<sup>1</sup>, Wirkner U.<sup>1</sup>, Robert K.<sup>2</sup>, Heeger S.<sup>3</sup>, Kleef J.<sup>4</sup>, Buechler M.<sup>4</sup>, Friess H.<sup>4</sup>, Debus J.<sup>2</sup>, Abdollahi A.<sup>1</sup>, Huber P.<sup>1</sup>

<sup>1</sup>Deutsches Krebsforschungszentrum, Radioonkologie, Heidelberg; <sup>2</sup>Universität, Klinik für Radioonkologie, Heidelberg; <sup>3</sup>Merck-Pharma, Darmstadt; <sup>4</sup>Universität, Klinik für Chirurgie, Heidelberg

**Introduction:** Pancreatic cancer continues to be one of the cancers with the worst prognosis. Multimodal therapy approaches integrating targeted drugs such as EGFR signaling inhibitors (e.g. Cetuximab) into radiochemotherapy regimens are promising. The goal of this study was to identify genomic and proteomic fingerprints correlating with therapy response in locally advanced pancreatic cancer patients undergoing combined radio-, chemo-, and Cetuximab therapy.

**Methods:** Advanced pancreatic cancer patients were treated with neoadjuvant intent within a prospective phase I/II study (PARC) using a trimodal combination of IMRT radiotherapy (54 Gy), Gemcitabine and Erbitux [A: day 1 (400mg/m<sup>2</sup>), day 8, 15, 22, 29, 36 (each 250 mg/m<sup>2</sup>), B: for another 3 months] starting one week prior to the concurrent radiochemotherapy. At various time points before, during and after therapy, whole blood RNA and serum protein was collected from 26 patients and whole blood transcriptomics was performed using Agilent's human genome wide microarray platform. RNA was also analyzed from tumor samples of 15 pts undergoing actual surgery after neoadjuvant treatment, and from > 200 pts undergoing surgery without prior therapy. Quantitative serum protein analysis was performed using the 'FastQuant' (sandwich antibody) system on 20 selected proteins (i.e., cytokines and angiogenesis related proteins).

**Results:** Whole blood transcriptomics resulted in therapy specific expression signature. We identified genes collectively up- or down- regulated based on inhibition of EGFR signaling (day 8 post Cetuximab therapy alone) and after combined trimodal therapy (e.g. days 24, 72). Blood RNA expression profiling data were correlated with expression profiling from tumor samples and clinical parameters including CA19-9 response, local control, survival, or appearance and degree of "predictive" side effects (e.g. acne grading). Serum proteomics showed that Cetuximab increased e.g. PDGF, MCP-1, Rantes, IL-6, Angiopoietin, and downregulated TIMP-1, IL-8, and Angiogenin levels while radiochemotherapy had the respective opposite effects.

**Conclusion:** Whole blood genome wide transcriptomics combined with serum proteomics analysis provide novel tools to monitor pancreatic cancer patients undergoing multimodal therapy strategies. The protein and gene expression "signatures" from blood and tissue can be correlated with clinical endpoints. The predictive value of these markers needs to be validated in larger studies.

PE741

### The adhesion molecule L1CAM mediates myofibroblasts induced malignant transformation of pancreatic ductal epithelial cells by enhancing their chemoresistance and migratory abilities

Geismann C.<sup>1</sup>, Koch D.<sup>1</sup>, Ungefroren H.<sup>2</sup>, Tsao M.-S.<sup>3</sup>, Bachem M.G.<sup>4</sup>, Fölsch U.R.<sup>1</sup>, Altevogt P.<sup>5</sup>, Schäfer H.<sup>1</sup>, Sebens S.<sup>1</sup>

<sup>1</sup>Clinic for General Internal Medicine, Laboratory of Molecular Gastroenterology & Hepatology, Kiel; <sup>2</sup>Clinic of General Surgery and Thoracic Surgery, Kiel; <sup>3</sup>Ontario Cancer Institute (OCI), Division of Applied Molecular Oncology, Toronto; <sup>4</sup>University of Ulm, Department of Clinical Chemistry and Pathobiochemistry, Ulm; <sup>5</sup>German Cancer Research Center, Tumor Immunology Programme D010, Heidelberg

Pancreatic ductal adenocarcinoma (PDAC) is characterized by an early metastasis and a profound chemoresistance, both accounting for its highly malignant phenotype. Pancreatic myofibroblasts (PMFs), representing the main cellular compartment of fibrosis in chronic pancreatitis and of the tumoral stroma in PDAC, essentially contribute to carcinogenesis and tumor progression either by secretion of humoral factors or by direct cell-cell contact. In this context, adhesion molecules are of pivotal importance, including L1CAM (CD171) that could be detected in various tumors, e.g. in PDAC. Recently, we demonstrated that L1CAM is involved in chemoresistance of PDAC cells and that it contributes to enhanced PDAC cell migration. Based on these findings, we intended to study the impact of PMFs on malignant transformation of pancreatic ductal epithelial cells supposed to be the origin of PDAC carcinogenesis and to elucidate the role of L1CAM in this process. When co-cultured together with PMFs, the immortalized human pancreatic duct cell line H6c7 – inherently exhibiting an epithelial duct cell phenotype – underwent epithelial-mesenchymal-transition (EMT) which was characterised by changes in cell morphology and elevated expression of mesenchymal marker proteins such as vimentin and N-cadherin. This process involved also an increased secretion of TGFβ1 and an elevated expression of L1CAM in co-cultured H6c7 cells. Accordingly, treatment of mono-cultured H6c7 cells with TGFβ1 resulted in upregulation of L1CAM expression and the EMT. Accordingly, TGFβ1 blockade diminished L1CAM expression and the EMT in co-cultured H6c7 cells. Moreover, H6c7 cells gained a stronger migratory phenotype as well as protection from anti-cancer drug induced apoptosis when treated with TGFβ1 or co-cultured with PMFs. Both events involved L1CAM expression, as shown by the prevention of TGFβ1 or co-culture induced chemoresistance and cell migration of H6c7 cells subjected to siRNA mediated L1CAM knock down. The TGFβ1 or co-culture induced L1CAM expression in H6c7 cells required the activation of JNKs, as shown by the blocking effect of JNK siRNA or the JNK inhibitor SP600125, whereas the activation of SMAD2/3 was obviously not involved.

PE742

### Supportive care in pancreatic carcinoma UICC stages I–IV patients treated with fermented mistletoe (*Viscum Album* L.) extract

Illert B.<sup>1</sup>, Matthes H.<sup>2</sup>, Friedel W.E.<sup>3</sup>, Bock P.<sup>4</sup>

<sup>1</sup>University Wuerzburg, Surgical Clinic, Wuerzburg, Germany; <sup>2</sup>Hospital Havelhöhe, Oncology Clinic, Berlin, Germany; <sup>3</sup>Hospital Bad Bocklet, Internal Medicine and Oncology, Bad Bocklet, Germany; <sup>4</sup>IFAG Basel AG, Basel, Switzerland

**Objectives:** To evaluate efficacy and safety of the fermented mistletoe extract Iscador® (ISC) in supportive care of surgically treated or inoperable patients with pancreatic carcinoma in comparison to a parallel control group without ISC.

**Methods:** In a multicenter comparative non-interventional cohort study, ISC was given in addition to conventional adjuvant chemo- and/or radiotherapy, while the control was treated with conventional therapy only (gemcitabine and/or 5-fluorouracil and/or radiotherapy). Unselected standardized pseudonymized data from medical records meeting the eligibility criteria were followed-up until last visit or death. The endpoints were ADRs (Adverse Drug Reactions) related to conventional adjuvant chemo- and/or radiotherapy, disease- and treatment-associated symptoms, hospitalization and overall survival. All endpoints were adjusted to confounders.

**Results:** 396 (201 ISC and 195 control) evaluable patients from 17 centers were documented. After a median follow-up of 15.2 vs. 10.1 months, and a median ISC therapy duration of 15.0 months, significantly fewer ISC (13.7 %) than control patients (48.9 %) developed ADRs by the conventional adjuvant therapy (p = 0.001) and had fewer persistent symptoms, mainly gastrointestinal, CNS, and back pain, during the therapy (p = 0.006). The ISC-group had an on average 14.1 days shorter hospitalization (p < 0.001), and showed a 42% hazard ratio reduction in overall survival (HR = 0.58, p = 0.001). No severe ISC-related ADRs or tumor enhancement were observed.

**Conclusion:** As compared to a parallel control group the ISC-treated group showed significantly fewer ADRs by the conventional therapy, fewer disease- and therapy-related symptoms and longer overall survival. The ISC-treatment was well tolerated and appears beneficial in the supportive care in patients with pancreatic carcinoma of all stages.

PE743

### Cytotoxic effect of riproximin in a panel of pancreatic cancer cell lines and investigation of some apoptotic pathways induced by this lectin

Murtaja A., Adwan H., Georges R., Berger M.

DKFZ, G401, Heidelberg

Pancreatic ductal adenocarcinoma (PDAC) is considered one of the most lethal neoplasias. It is the fifth leading cause of cancer deaths and shows a 5-year survival rate of less than 5% in the Western world. In contrast to clearly improved treatment options in the therapy of various types of cancer that have been achieved in the past two decades, PDAC is still a fatal disease which is barely affected by any therapeutic regimen. It is therefore obvious, that new effective treatment strategies are desirable.

Riproximin is a newly detected, highly promising antineoplastic protein which has been isolated from the plant *Ximenia americana* and found to be a new type II ribosome inhibiting protein (lectin; Voss et al, FASEB J. 20, 1194-96, 2006). The aim of this study was to investigate the cytotoxic effect of riproximin on several pancreatic cancer cell lines and to evaluate its apoptosis pathway. The anti-proliferative activity of riproximin was tested in 13 human and two rat pancreatic cancer cell lines (AsPC-1, Colo357, BXPC3, CAPAN 1, MiaPaca, DANG, PANC 1, PANC 89, PATU 390, S2-013, Su8686, T3M4, C. 8.18, AS, and ASML) by MTT assay.

The cells were treated with different concentrations of riproximin (0,1pM-1nM) for up to 96h. Generally the cytotoxic effect of riproximin increased over time and in proportion with the dose. The average IC50 over all cell lines was 13 pM after 72h (range: 1.3 pM – 43 pM). Panc 89 and T3M4 were the most sensitive cell lines (1.3 and 1.7 pM, respectively) whereas AS and Colo-357 were least sensitive (28 and 43 pM, respectively).

For determining the apoptosis pathway induced by riproximin, ASML cells were exposed to riproximin at low (3-30 pM) concentrations 72h or at high concentrations (30-100 pM) for 16h. Thereafter, the cells were harvested, lysed and checked for some apoptosis proteins by Western blot. The exposure of ASML cells to riproximin led to the activation of the caspases 8, 3, and 9, as well to the down regulation of the anti-apoptotic protein Bcl-2.

This suggests that the mechanism of action inducing apoptosis could involve activation of both, the death receptor and the mitochondrial pathways.

In conclusion, the observation that riproximin shows a distinct cytotoxic activity already at very low concentrations encourages further investigation of this lectin and its development as antineoplastic compound.

PE744

### Identification of human pancreatic tumor antigens by serological analysis of a recombinant cDNA expression library (SEREX)

Müller T.<sup>1</sup>, Giese N.<sup>2</sup>, Seil I.<sup>1</sup>, Frei C.<sup>1</sup>, Jäger D.<sup>1</sup>

<sup>1</sup>Nationales Centrum für Tumorerkrankungen, Heidelberg; <sup>2</sup>Universität Heidelberg, Abteilung für Allgemeine, Viszerale und Unfallchirurgie, Heidelberg

Pancreatic cancer is the fourth most frequent cause of cancer death and there are 13000 new cases each year in Germany. The five year survival rate for patients with pancreatic cancer is 5 %. Thus, novel therapeutic strategies, such

as immunotherapy, are urgently needed. Immunogenic tumor-associated antigens (TAA) that are selectively or abundantly expressed in cancer cells, represent attractive targets for antigen specific cancer immunotherapy.

The ability of immune recognition of cancer cells by the host's immune system provides the basis for the SEREX (serological analysis of recombinant cDNA expression libraries) approach. This method is a multistep process for TAA identification, that are detected by IgG antibodies and involves the immunoscreening of cDNA libraries prepared from human tumor specimens with sera from cancer patients. Subsequently, clones identified by SEREX can directly be analysed in terms of structure, expression patterns, frequency of seroreactivity, and T-lymphocyte reactivity.

Here, we used this method to identify tumor markers and novel target antigens for vaccine-based cancer therapy approaches in pancreatic cancer. We extracted mRNA from a patient's pancreatic tumor to generate a lambda bacteriophage cDNA expression library which was prokaryotically expressed. These recombinant proteins were transferred to nitrocellulose membranes and screened with autologous serum. In two rounds of immunoscreening 42 clones could be verified as being seroreactive. After isolation and sequence analysis, the number of seroreactive clones could be narrowed down to 18.

Currently, we are performing RT-PCR and immunohistochemical analysis to determine the expression patterns of these 18 candidate genes in a panel of normal tissues and additional tumor samples. Furthermore, we are determining the frequency of antibody responses in normal individuals and patients with neoplastic and non-neoplastic disease by ELISA assays with purified proteins. From these 18 genes, 83% (15/18) are already described in the literature showing association to cancer and 22% (4/18) of these genes are related to pancreatic cancer progression.

In summary, according to our present data, this screen identified 3 antigens representing interesting candidates for immunotherapy approaches in pancreatic cancer.

PE745

### Results of the conko 003 trial. A randomized second line trial in patients with gemcitabine refractory advanced pancreatic cancer

*Pelzer U.<sup>1</sup>, Stieler J.<sup>1</sup>, Schwaner I.<sup>2</sup>, Heil G.<sup>3</sup>, Seraphin J.<sup>4</sup>, Gömer M.<sup>5</sup>, Mölle M.<sup>6</sup>, Greten T.<sup>7</sup>, Lakner V.<sup>8</sup>, Kubica K.<sup>1</sup>, Hilbig A.<sup>1</sup>, Roll L.<sup>1</sup>, Goerke A.<sup>1</sup>, Sinn M.<sup>1</sup>, Dörken B.<sup>1</sup>, Riess H.<sup>1</sup>, Oettle H.<sup>1</sup>*

<sup>1</sup>Universitätsmedizin Berlin, CharitéCentrum für Tumormedizin, Berlin; <sup>2</sup>Ambulantes Therapiezentrum, Hämatologie/ Onkologie, Berlin; <sup>3</sup>Krankenhaus, Medizinische Klinik, Lüdenscheid; <sup>4</sup>Ambulantes Therapiezentrum, Hämatologie/ Onkologie, Northeim; <sup>5</sup>Krankenhaus, Medizinische Klinik, Bielefeld; <sup>6</sup>Ambulantes Therapiezentrum, Hämatologie/ Onkologie, Dresden; <sup>7</sup>Krankenhaus, Medizinische Klinik, Hannover; <sup>8</sup>Ambulantes Therapiezentrum, Hämatologie/ Onkologie, Rostock

**Introduction:** For nearly ten years gemcitabine (G) was standard first line therapy for patients (pts) with advanced pancreatic cancer (APC). There is no consensus about second line therapy after disease progression while receiving G, but 5-FU-based regimens are considered. Results about randomized second line studies in APC are very rare. Our phase II study showed activity of the OFF (Oxaliplatin/Folinic Acid (FA)/5-Fluorouracil (FU) [24h] ) regimen in 23 pts. To examine the impact and the side effects of oxaliplatin in this schedule we initiated a multicenter phase III study to compare OFF and FF in pts with G refractory APC.

**Patients and Methods:** Pts with CT/ MRT confirmed failure with G in first line therapy, Karnofsky Performance Status (KPS) >60%, controlled pain, adequate hematological, renal and liver functions were eligible. Pts were stratified according to duration of first line therapy, KPS and tumor stage. We randomized pts dynamically to outpatient treatment with FF (FU 2 g/m<sup>2</sup> (24h)/ FA 200 mg/m<sup>2</sup> (30min) on d1, d8, d15 and d22) vs. OFF (FF + Oxaliplatin 85 mg/m<sup>2</sup>, d8, d22). In both arms the next cycle started on day 43. Pts were followed with regular staging every 3 months or at any signs of disease progression.

**Results:** We achieved the planned number of pts in May 2007. We are able to present results regarding PFS and survival at this meeting. This is an GCP-required ITT-Analysis.

PE747

### A prospective, randomized trial of simultaneous pancreatic cancer treatment with enoxaparin and chemotherapy (prospect – CONKO-004). Results of the second interim analysis

*Pelzer U.<sup>1</sup>, Hilbig A.<sup>1</sup>, Scholten T.<sup>2</sup>, Opitz B.<sup>3</sup>, Stauch M.<sup>4</sup>, Hahnfeld S.<sup>5</sup>, Knigge O.<sup>6</sup>, Stieler J.<sup>1</sup>, Roll L.<sup>1</sup>, Sinn M.<sup>1</sup>, Goerke A.<sup>1</sup>, Dörken B.<sup>1</sup>, Riess H.<sup>1</sup>, Oettle H.<sup>1</sup>*

<sup>1</sup>Universitätsmedizin Berlin, CharitéCentrum für Tumormedizin, Berlin; <sup>2</sup>Krankenhaus, Medizinische Klinik, Hagen; <sup>3</sup>Krankenhaus St. Elisabeth/ Barbara, Medizinische Klinik, Halle; <sup>4</sup>Ambulantes Therapiezentrum, Hämatologie/ Onkologie, Kronach; <sup>5</sup>Ambulantes Therapiezentrum, Hämatologie/ Onkologie, Jena; <sup>6</sup>Krankenhaus, Medizinische Klinik, Frankfurt/ Oder

**Introduction:** Up to 20 % of patients (pts) diagnosed with pancreatic adenocarcinoma (PA) develop venous thromboembolism. This may contribute to the dismal prognosis of PA. The addition of low molecular weight heparin (LMWH) to chemotherapy suggested an improved survival. But there is no significant result about the clinical impact. We conducted a small pilot study which indicated that the addition of Enoxaparin to chemotherapy (GFFC) is safe and feasible in pts with advanced PA. Furthermore, results of several phase III studies suggest that pts with good performance status may benefit from more intensive chemotherapy regimen (Riess et al; Heinemann et al; ASCO 2005). Based on these considerations we started the randomized multicenter study CONKO-004.

**Patients and Methods:** 540 patients are to be recruited into this study. Primary stratification takes place according to Karnofsky performance status and kidney function. Patients with KPS > 80 % and normal kidney function receive GFFC ± LMWH (Gemcitabine 1 g/m<sup>2</sup> (30 min), Cisplatin 30mg/m<sup>2</sup> (90 min), 5-Fluorouracil 750 mg/m<sup>2</sup> (24 h), Folinic Acid 200 mg/m<sup>2</sup> (30 min), d1, 8; q3w ± Enoxaparin 1 mg/kg daily s.c.). Pts with KPS < 80 % and increased creatinin plasma levels (> 1.3 mg/dl) receive the current standard therapy (Gemcitabine 1 g/m<sup>2</sup> (30min), d1, 8, 15; q4w) ± Enoxaparin 1 mg/kg daily s.c. After 12 weeks of initial chemotherapy all pts who have not progressed received the standard therapy (Gemcitabine mono) ± Enoxaparin 40 mg/d s.c.

**Results:** From April 2004 to April 2007 240 pts have been recruited in this study. Until now no unexpected serious adverse events concerning severe bleedings were observed in the Enoxaparin treatment group. No heparin induced thrombocytopenia (HIT II) was documented. According to protocol we conducted this second interim analysis after 24 thromboembolic events. This interim analysis has been completed in Sept 2007.

**Conclusions:** There is no significant difference in the incidence of bleeding due to the addition of Enoxaparin in this setting. So we have no objections against further treatment with this protocol. This study is open to recruitment.

## Gene Therapy / Molecular Pathology

### Oral Presentation

OP225

### Homing of circulating mesenchymal stem cells in colon carcinoma liver metastases following liver resection

*Bruns C.<sup>1</sup>, Conrad C.<sup>1</sup>, Valesky M.<sup>1</sup>, Ischenko I.<sup>1</sup>, Niess H.<sup>1</sup>, Huss R.<sup>2</sup>, Jahn B.<sup>1</sup>, Mojaat A.<sup>2</sup>, Nelson P.<sup>3</sup>, Jauch K.-W.<sup>1</sup>*

<sup>1</sup>Universität, Chirurgie, München; <sup>2</sup>Universität, Pathologie, München; <sup>3</sup>Universität, Medizinische Poliklinik, München

**Background:** 50% of all patients with the diagnosis of colorectal cancer develop liver metastases. Surgical liver resection not only results in regeneration of healthy liver tissue but also in re-growth of metastases based on growth factor expression and angiogenesis. The aim of the project was to investigate, if adult mesenchymal stem cells (MSC) participate at the growth of liver metastases and if MSCs could be used as vehicle for combined suicide gene-stem cell therapy.

**Methods:** Balb/c SV-40 immortalized mesenchymal stem cells were stably transfected with red fluorescent protein (RFP) under the control of the



tie2-promotor. RFP is only expressed when tek/tie2-receptors are activated. Murine CT26 colon cancer cells were injected into the spleen of balb/c mice. All animals underwent splenectomy 8 days later, in groups of animals a 2/3-hepatectomy was performed. Starting at day 14, MSCs were i.v. injected twice the week. 30 days after cancer cell injection all animals were sacrificed, liver weight and -volume were assessed, the macroscopically visible liver metastases were counted and harvested for further immunohistochemical studies (Ki67, Anti-RFP, SV40, CD31).

**Results:** Following intrasplenic injection of CT26 colon cancer cells repetitive intravenous injections of MSCs led to an increase of liver weight and volume as compared to control animals without MSC injection (3,4g/10,25cm vs. 2,1g/4,25cm, respectively). Following 2/3-hepatectomy the liver weight increased up to 5,1g in the presence of MSCs. Moreover, we detected an increase of the median amount of macroscopic visible metastases after 2/3-hepatectomy + MSC treatment (n = 21) as compared to animals which did neither receive 2/3-hepatectomy nor MSC-treatment. The median amount of Ki67 positive cells in liver metastases following 2/3-hepatectomy and MSC injection was markedly higher as compared to groups without MSC treatment (43 + 7 vs. 35 + 5, respectively).

**Conclusion:** During the process of liver regeneration after 2/3-hepatectomy treatment with MSCs led to an increase of the total liver weight and the amount of macroscopically visible liver metastases. MSCs and their differentiation status still has to be examined in detail. In the future MSCs might be used as vehicle for combined suicide gene-stem cell therapy.

OP226

### Nonviral jet-injection phase I clinical trial for local gene transfer into metastases from melanoma and breast cancer

Walther W.<sup>1</sup>, Siegel R.<sup>2</sup>, Kobelt D.<sup>1</sup>, Aumann J.<sup>2</sup>, Knösel T.<sup>3</sup>, Dietel M.<sup>3</sup>, Stein U.<sup>1</sup>, Schlag P.M.<sup>2</sup>

<sup>1</sup>Max-Delbrück-Centrum, Berlin; <sup>2</sup>Robert-Rössle-Klinik, Charité Berlin, Berlin; <sup>3</sup>Institut für Pathologie, Charité Berlin, Berlin

Nonviral gene transfer, including physical transfer technologies such as jet-injection are gaining importance particularly for transfer of naked DNA. The jet-injection technology is based on high-speed jets possessing deep tissue penetration, leading to the efficient gene transfer of naked DNA. We conducted a phase I clinical trial (DeReGe 62) to apply the beta-galactosidase (LacZ)-expressing pCMVbeta reporter-plasmid by intratumoral jet-injection to evaluate safety, efficacy and feasibility of this nonviral gene transfer. In this trial we used the low-volume jet-injector prototype (EMS Medical Systems, SA Nyon, Switzerland) to apply the GMP-grade plasmid-DNA (PlasmidFactory, Bielefeld, Germany). Between September 2005 and December 2006 seventeen patients with metastatic melanoma or metastatic breast cancer were treated. All patients received 5 injections of 10µL plasmid-DNA into one single metastatic lesion. Two to six days post jet-injection tumor lesions were surgically removed for analyses. Clinical and laboratory safety monitoring was performed. In the resected tumor specimens plasmid-load was determined by real-time PCR and LacZ mRNA-expression was analyzed by real-time RT-PCR. LacZ-protein expression was assessed by X-Gal-staining and Western-blot. Plasmid-clearance in the blood was analyzed 0,5, 3, 6, 24, 48 and 72 hours after jet-injection. All seventeen patients tolerated the treatment well. Apart from minor bleeding at the injection sites, no adverse events or complications were observed. Within all tumors plasmid-DNA and LacZ-expression at mRNA- and protein-level was detected. Intratumoral plasmid-DNA load correlated with LacZ-expression at mRNA- and protein-level. The real-time PCR-analysis of patient-blood revealed timely restricted, low-level peaking of plasmid-DNA 30 minutes after jet-injection, which rapidly declined. The results from this phase I trial demonstrate safety, efficiency and clinical applicability of the nonviral jet-injection gene transfer. A new phase-I/II-trial for therapeutic application of this nonviral transfer technology will be initiated.

OP227

### The role of HIF-1α and HIF-2α in cisplatin and doxorubicin-resistance in the lung adenocarcinoma cell line A549

Eul B.<sup>1,2</sup>, Kamlah F.<sup>1,3</sup>, Kwapiszewska G.<sup>3</sup>, Lang N.<sup>2</sup>, Marsh L.<sup>3</sup>, Seeger W.<sup>3</sup>, Grimminger F.<sup>2</sup>, Hänze J.<sup>3</sup>, Rose F.<sup>4</sup>

<sup>1</sup>Universitätsklinikum Giessen, Innere Medizin, Giessen;

<sup>2</sup>Universitätsklinikum Giessen, Innere Medizin, Medizinische Klinik V, Giessen;

<sup>3</sup>Universitätsklinikum Giessen, Innere Medizin, Medizinische Klinik II, Giessen;

<sup>4</sup>Universitätsklinikum Marburg, Klinik für Strahlentherapie, Marburg

Hypoxia in solid tumors is associated with cancer progression, metastasis and resistance to therapy. Hypoxia inducible factor (HIF)-1α and -2α are key players in the response to hypoxia, affecting angiogenesis, proliferation, apoptosis and tumor cell metabolism. These adaptive mechanisms influence resistance to chemotherapy, and thus influence clinical outcome. We describe here the role of HIF-1α and HIF-2α in cellular resistance to cisplatin and doxorubicin in non small cell lung cancer cells (A549), and the impact of these cytotoxic drugs on the expression of both HIF isoforms. Hypoxia reduced the efficiency of cisplatin and doxorubicin treatment in A549 cells. Both drugs increased HIF-dependent target gene expression. Cisplatin induced HIF-2α under normoxic and hypoxic conditions, but decreased the expression of HIF-1α. Doxorubicin induced both isoforms. To define the role of HIF-1α and HIF-2α in resistance to treatment, specific inhibition of either HIF-1α or HIF-2α was achieved by RNA interference technology. Employing this approach, suppression of HIF-2α enhanced the sensitivity to cisplatin treatment *in vitro* and *in vivo*, while, sensitivity to doxorubicin treatment was increased by the suppression of both HIF-1α and HIF-2α. The HIF-2α-dependent expression of drug resistance proteins such as MRP1 and LRP was identified as an underlying mechanism of hypoxia-induced chemotherapy resistance. This study identified HIF-2α as a key player in hypoxia-induced resistance to cisplatin, and demonstrated at the molecular level that HIF is a credible target for therapeutic strategies.

OP228

### Drug resistance gene therapy by simultaneous lentiviral overexpression of MDR1 in combination with the MGMT<sup>P140K</sup> mutant in human hematopoietic stem cells

Spier I.<sup>1</sup>, Maier P.<sup>2</sup>, Laufs S.<sup>3</sup>, Veldwijk M.R.<sup>2,1</sup>, Wenz F.<sup>2</sup>, Fruehauf S.<sup>4</sup>, Zeller W.J.<sup>1</sup>

<sup>1</sup>German Cancer Research Center, Research Group Pharmacology of Cancer Treatment, Heidelberg; <sup>2</sup>Mannheim Medical Center, Department of Radiation Oncology, Mannheim; <sup>3</sup>German Cancer Research Center, Cooperation Unit Molecular Gastroenterology, Heidelberg; <sup>4</sup>Paracelsus Klinik, Center for Tumor Diagnostics and Therapy, Osnabrueck

**Introduction:** Myelotoxicity is a dose-limiting effect of many chemotherapeutic regimens. Thus, there is great interest to protect human hematopoietic stem cells (CD34<sup>+</sup>) by transfer of drug resistance genes. In this study we cloned a vector based on a self-inactivating lentiviral backbone containing MDR1 connected by an EMCV-IRES-element with MGMT<sup>P140K</sup> (O<sup>6</sup>-BG-resistant mutant). This combination vector (HR'SIN-MDR-IRES-MGMT) was compared to single vectors (HR'SIN-MDR, HR'SIN-MGMT), regarding capability to convey chemoprotection.

**Methods:** HL60 (AML cell line) and human CD34<sup>+</sup> cells were transduced with the various lentiviral vectors. After chemotherapeutic treatment MTT assays were used to detect chemoresistance levels in HL60 cells, CD34<sup>+</sup> cells were held in liquid culture under differentiation conditions and analysed by FACS.

**Results:** HL60 cells transduced with the combination vector showed significant chemoresistance to O<sup>6</sup>-BG/ACNU (IC<sub>50</sub> 13-fold higher), the IC<sub>50</sub> of cells transduced with HR'SIN-MGMT was 35-fold higher compared to untransduced control. Furthermore the IC<sub>50</sub> of paclitaxel (MDR1 substrate) was 24-fold higher in cells transduced with HR'SIN-MDR and 25-fold higher with HR'SIN-MDR-IRES-MGMT compared to untransduced control. Combined exposure of cells to O<sup>6</sup>-BG/ACNU and paclitaxel showed a survival advantage of cells transduced with the combination vector (IC<sub>50</sub> 6.25-fold higher), for the single vectors the IC<sub>50</sub> was 1.63-fold higher (MDR1) and



2.08-fold higher (MGMT) compared to untransduced control. Treatment of CD34<sup>+</sup> cells with increasing concentrations of doxorubicin (up to 0.8 μM) resulted in a higher fraction of MDR1-transduced cells either with HR'SIN-MDR (26.6-fold) or with HR'SIN-MDR-IRES-MGMT (30.6-fold) compared to untreated cells. After combination treatment (20 μM O<sup>6</sup>-BG/16 μM BCNU and 0.4 μM doxorubicin) the fraction of MDR1-positive cells was higher for HR'SIN-MDR-IRES-MGMT (14-fold) than HR'SIN-MDR (8-fold) transduced cells.

**Conclusions:** The protective effect of the combination vector is comparable with that of the single vectors for monotherapy and superior for combined therapy. The combination vector presents simultaneous protective effects of two drug resistance genes, whilst only one transduction process is required, thus reducing the risk of insertional mutagenesis.

Consequently our results might help to improve chemotherapy regimens by reducing myelotoxic side effects and increasing the therapeutic efficiency.

## Gene Therapy/Molecular Pathology

### Poster Exhibition

PE645

#### Expression and immune recognition of tumor associated antigens p33<sup>ING1b</sup> and p29<sup>ING4</sup> in patients with renal cell carcinoma

Nichiporuk E.<sup>1</sup>, Gasser M.<sup>1</sup>, Grimm M.<sup>1</sup>, Lutz J.<sup>2</sup>, Hillig F.<sup>3</sup>, Thiede A.<sup>1</sup>, Heemann U.<sup>2</sup>, Riedmiller H.<sup>3</sup>, Waaga-Gasser A.M.<sup>1</sup>

<sup>1</sup>Chirurgische Universitätsklinik, Molecular Onko-Immunologie, Würzburg;

<sup>2</sup>Klinikum rechts der Isar, Abtl. Nephrologie, München;

<sup>3</sup>Universitätsklinikum Würzburg, Urologische klinik und Poliklinik, Würzburg

The inhibitor of growth (ING) genes as type II suppressors are important in cell cycle arrest and apoptosis through modulating of p53. Defective function of these genes promotes further tumor growth in melanoma and as tumor associated antigens they are used for immune targeted strategies in breast cancer. Here we investigate the expression of ING isoforms p33<sup>ING1b</sup> and p29<sup>ING4</sup> in patients with renal cell carcinoma (RCC), the local and systemic immune environment and the recognition of ING isoforms as tumor associated antigens by T cells. While early stage RCC showed significantly upregulated ING-isoform mRNA, protein levels late tumor stages revealed a reduction in the overall ING gene expression. We demonstrated that p33<sup>ING1b</sup> and p29<sup>ING4</sup> tumor specific T effector cell responses can be induced. The peptide sequences p33<sup>ING1b</sup> aa 259-268 and p29<sup>ING4</sup> aa 149-158 elicited *in vitro* a significant IFN-γ but not IL-10 production indicating natural anti-tumor immune responses. Lymphocytic infiltration in early stage of RCC was characterized by increased CD8 and CD4, Foxp3 regulatory cells as well as higher IL-10 and IFN-γ levels but all the latter except CD8 cells showed lower than normal levels in advanced tumor stages. Analysis of systemic IL-10 cytokine levels, CD4+CD25+ and CD4+ T cells expressing IL-10 was consistent with the local measures. The amount of CD8s expressing IL-2 or IFN-γ was increased in tumors and diminished in the peripheral blood in patients compared to controls. RCCs show a tumor stage dependent expression of p33<sup>ING1b</sup> and p29<sup>ING4</sup> isoforms (type II suppressor genes modulating p53). Natural immune responses against ING peptides were detected in the PBL of patients, with p29<sup>ING4</sup> peptide aa 149-158 characterized by both IFN-γ and IL-2 responses supporting use as a vaccine candidate capable of inducing anti-tumor directed Th1 immunity *in vivo*.

PE646

#### Peptide ligands targeting integrins on acute myeloid leukemia cells

Jäger S.<sup>1</sup>, Jahnke A.<sup>1</sup>, Adebar S.<sup>1</sup>, Vögtle F.-N.<sup>1</sup>, Delima-Hahn E.<sup>1</sup>, Pfeifer D.<sup>1</sup>, Berg T.<sup>1</sup>, Lübbert M.<sup>1</sup>, Trepel M.<sup>1,2</sup>

<sup>1</sup>Department of Hematology and Oncology, <sup>2</sup>Institute for Molecular Medicine and Cell Research, University of Freiburg Medical Center, Freiburg

**Introduction:** leukemia cell binding ligands could be used for drug targeting, thereby enhancing efficiency and reducing side effects of currently available cytotoxic drugs. Knowledge on receptors specifically expressed on acute

myeloid leukemia (AML) cells or ligands thereof is limited. Here, we selected random phage display peptide libraries on Kasumi-1 AML cells to obtain leukemia-specific ligands.

**Methods:** A CX7C random phage display peptide library was used to select peptides binding to Kasumi-1 AML cells. Leukemia cell-bound phages were separated from unbound phage by differential centrifugation and subsequently recovered and amplified by bacterial infection. The peptide inserts enriched on leukemia cells were analyzed indirectly by DNA sequencing, revealing several peptide motifs. Single clone binding assays were performed on various cell lines including Kasumi-1. Receptor identification of the Kasumi-1-binding peptide was done using differential gene expression profiling using Affymetrix whole genome expression arrays as indicated below.

**Results:** A peptide with the sequence CPLDIDFYC was enriched from the library selection. Phage displaying this peptide strongly bound to Kasumi-1 and SKNO-1 cells. The binding could be blocked by the cognate peptide suggesting that it is specific and peptide-mediated. Kasumi-1 and SKNO-1 cells both carry the chromosomal translocation 8;21, resulting in aberrant expression of the AML1/ETO fusion protein, the most frequently expressed aberrant fusion protein in AML. These results suggested that the CPLDIDFYC receptor may be upregulated upon AML1/ETO expression. In line with this hypothesis, CPLDIDFYC also strongly and specifically bound primary AML1/ETO-positive AML blasts from clinical samples as well as U-937 cells with forced AML1/ETO expression. Gene expression profiling comparing a panel of CPLDIDFYC-binding and CPLDIDFYC-non-binding cell lines revealed a set of potential receptors for the CPLDIDFYC peptide. Further experiments identified alpha4-beta1 integrin (VLA-4) as the CPLDIDFYC receptor. In addition, we showed that the CPLDIDFYC-phage is internalized upon receptor binding, suggesting that the CPLDIDFYC-receptor-ligand interaction may be exploitable for targeting drugs or gene therapy vectors to leukemia cells carrying the suitable receptor.

PE647

#### Novel recombinant Adeno-Associated Viral (AAV) vectors for efficient gene transfer into primary human Peripheral Blood Progenitor Cells (PBPC)

Veldwijk M.R.<sup>1</sup>, Sellner L.<sup>1</sup>, Stiefelhagen M.<sup>2</sup>, Kleinschmidt J.<sup>2</sup>, Laufs S.<sup>3</sup>, Wenz F.<sup>4</sup>, Zeller W.J.<sup>1</sup>, Fruehauf S.<sup>5</sup>

<sup>1</sup>Deutsches Krebsforschungszentrum, G402, Heidelberg; <sup>2</sup>Deutsches Krebsforschungszentrum, F242, Heidelberg; <sup>3</sup>Deutsches Krebsforschungszentrum, G360, Heidelberg; <sup>4</sup>Universitätsklinikum Mannheim, Universitätsklinik für Strahlentherapie und Radioonkologie, Mannheim; <sup>5</sup>Paracelsus-Klinik, Zentrum für Tumordiagnostik und -therapie, Osnabrück

**Introduction:** Mobilized PBPCs are excellent targets for the cure of many hematopoietic disorders; recombinant AAV2 (rAAV2) vectors have an advantageous safety profile, due to low immunogenicity, lack of any associated diseases and their episomal localization. Unfortunately, standard rAAV2 vectors lack the required efficiency for gene transfer into human blood progenitor cells. To increase the gene transfer efficiency on these cells, AAV random peptide library was applied on primary human CD34<sup>+</sup> PBPC.

**Materials and Methods:** The AAV random peptide library was applied on primary human CD34<sup>+</sup> PBPC samples from two patients (3 selection rounds), several mutant blood progenitor-targeted rAAV capsid clones were obtained and sequenced and 5 new vectors with the most efficient clones produced. To determine efficiency and specificity, a panel of leukemia (CML and AML) and non-leukemia cell lines, as well as primary human CD34<sup>+</sup> PBPC were transduced and GFP expression was determined.

**Results:** On a panel of leukemia cell lines at least one capsid mutant was significantly more efficient (p<0.01) than standard rAAV2. On solid tumor cell lines rAAV2 was more efficient, suggesting the increased specificity of our capsid mutants for hematopoietic cells. Significantly higher gene transfer efficiency (up to 16% GFP<sup>+</sup> cells) could be obtained with the newly generated vectors compared to standard rAAV2 (≤9-fold higher; p<0.01). Further (preliminary) data revealed even >50% gene transfer.

**Conclusion:** These novel vectors may finally enable efficient (>20%) gene transfer using rAAV-based vectors into primary human PBPC and may offer a safe alternative to other vector systems. Currently further optimizations and developments are ongoing with the ultimate goal of realizing a clinical PBPC-based gene targeting/correction study.