**Conclusion:** Human FGFR1-IIIb-variant was shown to reduce tumor growth in vitro and in vivo and prolong overall survival.

doi:10.1016/j.ejcsup.2006.04.100

## P41. RELEVANCE OF THE PTEN AND p27$^{KIP1}$ EXPRESSION IN PROSTATE CANCER AFTER SHORT TERM ANTIHORMONAL THERAPY

K. Becker[a], C.G. Sauer[a], H. Zentgraf[b], R. Grobholz[a]. [a]*Pathologisches Institut, Universitätsklinikum Mannheim, Heidelberg, Germany;* [b]*Abt. ATV DKFZ, Heidelberg, Germany.*

**Background:** PTEN is an important phosphatase, suppressing the phosphatidylinositol-3-kinase/Akt pathway which induces apoptosis. p27$^{kip1}$ binds to cyclin-E/Cdk2 and prevents mitosis. The inactivation of the tumor suppressor genes has been associated with many different types of cancer including the prostatic carcinoma (PCa). In this study we investigated the influence of an antihormonal treatment on the expression of PTEN and p27$^{kip1}$ in PCa.
**Methods:** 82 prostate cancer patients treated with antiandrogens or LH-RH analogs or a combination therapy between 1 and 77 weeks (mean 9.7 ± 1.2) were included in this study. The expression of PTEN and p27$^{kip1}$ were analyzed by immunhistochemistry and an immunreactive score. The results were compared with 183 untreated cases of a previous study.
**Results:** PTEN expression levels correlated with the duration of antihormonal therapy: significantly more PTEN positive cases were found after 3 weeks antihormonal therapy ($p = 0.0003$). PTEN-expression compared to untreated tumors showed a significantly stronger PTEN-expression in treated tumors ($p = 0.03$). A nuclear expression of p27$^{kip1}$ was more frequent in tumors after 4 weeks of treatment compared to tumors treated less than 4 weeks ($p = 0.015$).
**Conclusions:** Both PTEN and p27$^{kip1}$ expression was increased after 4 weeks of antihormonal treatment. This fact might indicate an early stress reaction of the tumor cells due to the androgen-deprivation therapy.

doi:10.1016/j.ejcsup.2006.04.101

## P42. GLYCOGEN SYNTHASE KINASE 3BETA (GSK3BETA) AS A KEY COMPONENT OF ESTRADIOL SIGNALLING PATHWAY

Grisouard Jean, Medunjanin Senad, Strauth Stephanie, Hermani Alexander, Mayer Doris. *German Cancer Research Centre (DKFZ), Research Group Hormones and Signal Transduction, Heidelberg, Germany.*

**Background:** GSK3beta is involved in the control of gene expression via the regulation of transcription factors, including estrogen receptor alpha (ERalpha). Recently, we discovered involvement of GSK3beta in estrogen-independent and estrogen-dependent activation of ERalpha, respectively.[1,2] While phosphorylation of ERalpha appears to be crucial for its activation, the impact of GSK3beta on the estrogen-dependent regulation of ERalpha function and activity remains to be clarified.
**Methods:** Phosphorylation of ERalpha by GSK3beta was analysed by in vitro kinase assays. Thereafter, the effects of GSK3 inhibitors on ERalpha phosphorylation and activation were analysed in breast cancer cells using Western blot and luciferase reporter assays. Further experiments using siRNA technology and transfection of cells with GSK3beta mutants were performed to investigate the effects of GSK3beta regarding ERalpha signalling pathway.
**Results:** In vitro kinase assays first depicted that GSK3beta phosphorylated ERalpha at Ser-118. Moreover, the addition of a GSK3 inhibitor (LiCl) on MCF-7 cells in culture stimulated with estradiol (E2) led to a decrease in Ser-118 phosphorylation and to an inhibition of ERalpha-controlled luciferase activity. In agreement with the previous observations, the knock-down of GSK3 by use of siRNA resulted in decreased basal and E2-induced ERalpha phosphorylation at Ser-118 as well as in reduced luciferase activity.
**Conclusion:** We suggest that GSK3beta plays an important role in the estrogen-dependent regulation of ERalpha function and activity.

doi:10.1016/j.ejcsup.2006.04.102

## P43. ErbB-SIGNALLING IN MULTIPLE MYELOMA – FROM THE IDENTIFICATION AS POTENTIAL THERAPEUTIC TARGET BY GENE EXPRESSION ANALYSIS AND FUNCTIONAL TESTING TO CLINICAL TRIALS

Karène Mahtouk[b], Dirk Hose[a], John De Vos[b], Ulrike Klein[a], Jean-François Rossi[b,c,d], Thierry Rème[b], Thomas Möhler[a], Jérôme Moreaux[b], Marion Moos[a], Eric Jourdan[b], Friedrich W. Cremer[a], Hartmut Goldschmidt[a], Bernard Klein[b]. [a]*Medizinische Klinik V, Heidelberg, Germany;* [b]*INSERM U475, CHU Montpellier, France;* [c]*Abteilung für Biostatistik, DKFZ, Heidelberg, Germany;* [d]*Cellgen SA, Montpellier, France.*

**Background:** ErbB-receptors/ligands are involved in several cancers. Plasma cells expressing the heparin-sulphate proteoglycan (HSPG) syndecan-1 (CD138) attach heparin-binding growth factors.[1] The aim of this study is to identify new targets in the therapy of multiple myeloma (MM).
**Methods:** Samples of 65 patients (CD138-purified MM-cells (MMC) and bone-marrow-microenvironment (BMME)), 7 normal bone-marrow-plasma-cell-samples (BMPC) and 20 human-myeloma-cell-lines were studied. The expression of the 4 ErbB-receptors/ligands on MMC and BMME will be assessed by real-time RT-PCR and Affymetrix U133 A+B DNA-microarrays. BMME-cells from MM-patients were exposed to PD169540 (a pan-ErbB-inhibitor) and IRESSA (ErbB1-specific).
**Results:** ErbB1 and ErbB2 are expressed by BMPC, MMC and the BMME. ErbB3 and ErbB4 are expressed by a subgroup of MMC. 7/10 ErbB-ligands are expressed by MMC and/or the BBME. Myeloma cell growth is stimulated by the 3 ErbB-ligands that are able to attach HSPG (i.e amphiregulin, HB-EGF and neuregulin-1) via binding to syndecan-1. PD169540 and IRESSA induced apoptosis of primary MMC from 10/14 and 4/14 patients in vitro, respectively.[1,2]

**Conclusion:** ErbB-inhibitors represent a new therapeutic approach in MM. A clinical trial testing the ErbB1-inhibitor cetuximab and correlating response with the expression of ErbB-receptors/ligands is in preparation at the University Hospitals of Cologne, Heidelberg and Montpellier.

doi:10.1016/j.ejcsup.2006.04.103

### P44. MOUSE MODELS OF SPONTANEOUS MELANOMA AS A TOOL FOR DEVELOPMENT OF NEW IMMUNOTHERAPIES OF HUMAN MELANOMA

V. Umansky, O. Speicher, S. Kimpfler, A. Thölke, D. Schadendorf. *Skin Cancer Unit (D070), German Cancer Research Center (DKFZ), Heidelberg, Germany.*

**Background:** Malignant melanoma, notorious for its poor response to currently available therapeutics, is one of the fastest increasing cancers. Therefore, development of new alternative treatment strategies (including immunotherapeutic ones) is extremely important. This approach requires an establishment of the reliable animal melanoma model that resembles human melanoma with respect to etiology, tumor genetics, histopathology and clinical development. We use a recently developed mouse model of spontaneous skin melanoma, in which ret transgene (tg) is expressed in melanocytes under the control of metallothionein-I promoter (MT/Ret). Activity of the receptor tyrosine kinase, Ret, is upregulated during the disease progression.
**Methods:** Immunohistology, flow cytometry, ELISPOT, ELISA, tetramer staining and in vivo kill.
**Results:** After a short latency (2–4 months), around 30% of mice develop skin melanoma metastasizing to lymph nodes, lungs and brain. We found that tumors expressed melanoma associated antigens tyrosinase, tyrosinase related protein (TRP)-1, TRP-2 and gp100, which could be applied as targets for the immunotherapy. Ret-tg mice without tumors could mount both antigen-unspecific (stimulation with Con A or with CD3/CD28 antibodies) and antigen-specific (ovalbumin or TRP-2 derived peptide) T-cell reactions, which were downregulated in melanoma bearing Ret-tg mice. In addition, Ret-tg mice have more effector memory and regulatory T cells than healthy (wild type) mice.
**Conclusion:** New strategies of melanoma immunotherapy in this spontaneous melanoma mouse model (including therapy with memory T cells together with dendritic cells or depletion of regulatory T cells) will be discussed.

doi:10.1016/j.ejcsup.2006.04.104

### P45. PROTEIN KINASE INHIBITORS AS MODIFIERS OF RADIOSENSITIVITY IN GLIOBLASTOMA CELLS

M. Wang, D. Milanovic, F. Lohr, F. Wenz, C. Herskind. *Department of Radiation Oncology, Mannheim Medical Center, University of Heidelberg, Mannheim, Germany.*

**Background:** Protein kinase (PK) inhibitors are candidates for modifying the response of tumour cells to anticancer agents such as radiotherapy. The purpose of the present study was to compare inhibition of the PI3K/Akt survival pathway by specific concentrations of different PK inhibitors with changes in the radiosensitivity of glioblastoma cell lines in vitro.
**Methods:** Glioblastoma cell lines U343MG, U87MG and U251MG were used. PI3K inhibitors (Wortmannin; LY294002) and receptor tyrosine kinase (RTK) inhibitors (AG1296; AG1478; erlotinib) were added to cultures before or after irradiation. The phosphorylation state of Akt was detected by Western blotting. The cellular radiosensitivity was measured by the colony formation assay fitting survival curves with the linear-quadratic model.
**Results:** The inhibitory effect of Wortmannin and LY294002 on Akt phosphorylation depended on the cell line. However, whereas downregulation to a variable degree was observed with 50 nM Wortmannin, radiosensitization required micromolar concentrations. RTK inhibitors had little influence on Akt phosphorylation but moderately sensitised cells to radiation. However, the sensitising effect was similar whether the inhibitor was added before or after irradiation.
**Conclusions:** The results did not support a correlation between radiosensitisation and inhibition of the PI3K/Akt survival pathway. RTK inhibitors were not required to be present during irradiation in order to sensitise cells and thus cannot be considered classical radiosensitisers. Instead they may exert their inhibitory effect on clonogenicity. We further conclude that signal transduction differs between glioblastoma cell lines and propose that this might have prognostic value in vivo.

doi:10.1016/j.ejcsup.2006.04.105

### P46. IDENTIFICATION AND CHARACTERIZATION OF CENTROSOMAL CLUSTER-INHIBITORS AS NOVEL ANTI-CANCER AGENTS

Blanka Rebacz[a], Harald Löffler[a], Cihan Cetin[b], Rainer Pepperkok[b], Anthony D. Ho[c], Thomas O. Larsen[d], Alwin Krämer[a]. [a]*Clinical Cooperation Unit for Molecular Hematology/Oncology, German Cancer Research Center (DKFZ) and Department of Internal Medicine V, University of Heidelberg, Heidelberg, Germany;* [b]*Department of Cell Biology/Biophysics EMBL, Heidelberg, Germany;* [c]*Department of Internal Medicine V, University of Heidelberg, Heidelberg, Germany;* [d]*Center for Microbial Biotechnology, BioCentrum-DTU, Technical University of Denmark, Copenhagen, Denmark.*

**Introduction:** The centrosome is a small organelle which consists of two centrioles and the pericentriolar matrix. It functions as the microtubule-organizing center of eukaryotic cells and plays a central role in chromosome segregation and cytokinesis. Many human malignancies harbor centrosomal aberrations, which are caused by deregulation of centrosome duplication or cytokinesis failure. Cells with supernumerary centrosomes usually form multipolar spindles leading to aberrant mitoses with consecutive chromosome missegregation. To regain secondary karyotype stability after clonal selection, some tumor cells coalesce their extra centrosomes by a poorly defined mechanism into two spindles poles in order to divide properly.
**Method:** Here, we describe an automated screening strategy designed to identify small molecules – produced by hundreds of