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