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.

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

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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.

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

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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.

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

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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

different fungal species – that inhibit centrosomal clustering and thus force tumor cells with supernumerary centrosomes to undergo multipolar mitoses and consequently apoptosis.
Results: This approach led to the identification of several substances which are currently characterized in more detail. One of these substances is the well-known antifungal drug griseofulvin, which, in addition to its inhibition of centrosomal clustering described here, has recently been shown to suppress microtubule dynamic instability.
Conclusion: Taken together, this screening may help identify new potential anti-cancer drugs.

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P47. RADIOPROTECTION OF NORMAL TISSUE CELLS BY TRANSFER OF THE HUMAN SUPEROXIDE-DISMUTASE GENE

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Background: Protection of normal tissue against radiation-induced damage would increase the therapeutic ratio of radiotherapy. A promising strategy for this approach is gene therapy-mediated overexpression of copper–zinc (CuZnSOD) and manganese superoxide-dismutase (MnSOD). Recombinant adeno-associated virus 2 (rAAV2) are attractive vectors owing to their ability to infect non-dividing cells and a very low risk of insertional mutagenesis. The purpose was to test the radiomodulating effects of SOD on human primary lung fibroblasts (HPLF).
Methods: Low passage HPLF (MRC5) cells were transduced with the rAAV2-SOD vectors, harvested on day 3, irradiated (1–8 Gy) and analysed using FACS, Western blot, SOD-activity and colony formation assays.
Results: High transduction rates were obtained with >80% of the HPLF cells expressing the respective SOD. Compared to transduction controls, CuZnSOD did not exhibit any radioprotective effects, whereas for MnSOD-transduced HPLF an increase of approximately 30% in the survival of colony-forming cells was observed (1–4 Gy).
Conclusion: An increase in clonogenic survival (1.3-fold) of HPLF cells after transfer of MnSOD and subsequent irradiation was shown. Earlier, we have shown lack of protection in tumour cells (HeLa), thus supporting that MnSOD may increase the therapeutic ratio. rAAV2 vectors are promising tools for the delivery of radioprotective genes in normal tissue such as the lung for pulmonary or intestine cells for prostate irradiation.

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P48. GONADOTROPHIN RELEASING HORMONE BASED VACCINE (GnRHm1-TT), AN EFFECTIVE CANDIDATE FOR HORMONEDEPENDENT CANCER IMMUNOTHERAPY

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Background: The normal development and functioning of the prostate gland, as well as its benign and neoplastic growth is dependent of androgen. Previous studies with Gonadotrophin Realeasing Hormone (GnRH/LHRH) vaccines, have shown the usefulness of immunization against this hormone in prostate and breast cancer.
Methods: In this work we have designed a vaccine candidate called GnRHm1-TT based on a completely synthetic immunogen. The peptide was formulated as a white semiviscous water in oil preparation and injected to animals.
Results: In healthy animals, this vaccine candidate showed to be very immunogenic, resulting in high anti-GnRH antibodies titers, testosterone reduction and significant decrease of the prostate and testicle weight. In tumor implanted rats the vaccine candidate had demonstrated to produce significant tumor growth inhibition of Dunning R3327-H androgen responsive prostate tumor in rats $P = 0.025$ and survival increase, $P = 0.001$.
Conclusion: GnRHm1-TT have demonstrated to be highly immunogenic and safe, causing prostate and testicle atrophy and significantly tumor growth inhibition. These results make our vaccine candidate useful as an effective androgen deprivation therapy, and possible application to prostate cancer and other hormone-dependent malignancies therapy.

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P49. ZOLEDRONIC ACID HAS DIRECT ANTI-PROLIFERATIVE AND ANTI-METASTATIC EFFECT ON PANCREATIC CARCINOMA CELLS AND ACTS AS AN ANTIGEN FOR δ2 γ/δ T CELLS

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Background: Beside their use as anti resorptive drug, bisphosphonates are well known to stimulate γδ T cells and to have direct effects on tumor growth.
Methods: We determined the direct cytotoxic effect of pamidronate and zoledronic acid, the induction of apoptosis and their anti-metastatic potential. Next, we analyzed how bisphosphonates act on γδ T cells propagated with our recently published protocol. The susceptibility of pancreatic carcinoma cells pretreated with bisphosphonates against γδ T cells was tested in cytotoxicity assays and the subgroup involved in killing was investigated.