of the diseased with this malign illness has been noticed. The aim of this paper is to evaluate the results of the radiotherapy for bladder carcinoma by Split-course method applied.

Methods: After the schematic treatment we applied individual treatment in that way that we determined the localization and a size of the bladder and localization of the tumor. The radiotherapy was applied according to the following protocol of Split-course method: a total dose of 6000 cGy from the two opposite fixed fields was applied, 3000 cGy in 10 fractions.

Results: There were 148 patients with bladder carcinoma subjected to radiotherapy of Split-course method from 1985 to 1993. If we examine the 5-year survival rate we can conclude that the 5-year survival rate was 87.5% in the first stage. In the second stage even of the total 37 treated survived 5 years, in the third stage 9 lived longer than 5 years out of 21 patients treated if we consider. The total number of patients who survived 5 years we can notice that it is a high number 71 (47.9%) and that the results of our treatment are better than the results found in the world literature.

Conclusion: The contribution of the Split-course method in the treatment of bladder carcinoma resulted is in the reduction of the total time of treatment, the reduced number of fractions and the preservation of radiobiological efficacy.

Biotherapy-gene therapy-vaccination

1429 ORAL

Peptide aptamers: A new generation of molecules for the specific inhibition of oncoproteins?

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Human papillomaviruses (HPVs) are closely associated with the development of several cancers in humans, including cervical cancer. The tumorigenicity of HPVs depends on the expression of the viral E6 and E7 oncogenes. The E6 protein has anti-apoptotic potential and may counteract the elimination of HPV-positive cells under the abnormal growth stimulation by E7. Molecules that can specifically inhibit E6 could therefore form a novel basis for the development of molecular strategies to fight HPV-positive dysplasias and cancers.

The "peptide aptamer system" allows an in vivo selection in yeast for small molecules specifically binding to and functionally inhibiting a given target protein. We here screened a randomized peptide expression library for conformationally restrained 20-mer peptides binding to the human papillomavirus type 16 E6 oncoprotein. We isolated several peptide aptamers that bound with high affinity to the viral oncoprotein in vivo. These interactions were highly specific for E6 and no binding was observed to heterologous control proteins. Some peptides also interacted with E6 proteins of other HPV types, indicating the existence of common E6-epitopes. The peptide aptamers are currently also tested for their effect on E6 activities in mammalian cells and their influence on the tumorigenic phenotype of HPV-positive cancer cells.

Inhibitory peptide aptamers can be used in basic research as experimental tools to investigate the function of the HPV E6 oncoprotein in human tumor cells and, under therapeutic aspects, may serve as lead structures for the development of novel drugs specifically targeting HPV-positive cells. In principle, this approach is also applicable for the identification of low molecular weight inhibitors of any given target protein of pathological significance.

1430 ORAL

In vitro evaluation of a tumor vaccine based on the xenogenization of tumor cells with tetanus toxoid molecules

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The goal of this research project was the design of strategies for anti-tumor immune therapy based on the application of the xenogenization concept. We extended earlier experiments by loading of teanus toxoid, as opposed to peptides comprising xenoepitopes, into human primary tumor cells including primary leukemia cells and culture adapted primary neuroblastoma cells. To mediate loading we used polyarginin (pA) molecules of various degrees of polymerization, cationic liposoms, or chimeric molecules of transferrin (Tf) and the polycation polyethylenimin (PEI). All human primary tumor cells and cell lines studied could be loaded with high efficiency by all procedures as determined by flow cytometric detection of fluorescein labeled TT. Trypsin treatment of loaded cells provided evidence that liposomes and Tf-PEI mediated internalization of TT. As fluorescence labeling introduces negative charges into TT, the findings obtained by flow cyometry were confirmed by western blot analysis of cells loaded with unlabeled TT. Release of IFNg from mononuclear cells (MNCs) loaded with TT by liposomes or pA was clearly higher compared to passively loaded cells. In a human in vitro tumor model MNCs were pre-incubated with TT-xenogenized autologous lymphoblastoid cells and challenged with unmodified lymphoblastoid cells. In these cultures increased IFNg secretion was observed compared to MNCs derived from not xenogenized pre-stimulation cultures. Together, these data indicate the functional utility of the xenogenization strategy for the treatment of human neoplasias.

1431 ORAL

Anti-idiotype (anti-ID) vaccination plus intensive therapy (IT) and autologous stem cell transplantation (ASCT) for patients (PTS) with metastatic breast cancer (MBC)

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Purpose: A small proportion of pts with MBC achieve durable progression-free survival (PFS) with IT + ASCT. We are studying the effect of TriAb, an anti-Id vaccine and surrogate antigen for an epitope of human milk fat globule expressed on breast cancer cells with IT + ASCT in chemosensitive pts.

Methods: TriAb was given pre-ASCT weekly × 3 beginning 1 wk after the last cycle of conventional chemotherapy and monthly from day 7 post-ASCT & continued for 24 mo. or until progression. This design was to generate a primary immune response prior to collection of stem cells and IT. At the time of ASCT, 15 pts were in PR and 1 in CR. Treatment consisted of SC collection with a cyclophosphamide priming regimen followed by IT + ASCT with STAMP V.

Results: TriAb-related toxicity was minimal, with local injection site reactions and mild flu-like symptoms. No ASCT-related deaths occurred. High-titer IgG anti-anti-id (Ab3) responses were seen in all 16 pts at a median of 5 doses of vaccine. Three pts had disease progression at a median of 6 mo. post-ASCT; the others remain alive, without progression, at a median of 7 mo. post-ASCT.

Conclusion: Pre- and post-ASCT vaccination induces rapid Ab3 responses despite diminished-immunocompetency post-ASCT with minimal toxicity.

1432 ORAL

Humoral immune responses of cancer patients against 'Cancer – Testis' antigen NY-ESO-1: Correlation with clinical events

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Humoral and cellular immune responses against the 'Cancer – Testis' (CT) antigen NY-ESO-1 are frequently observed in patients (pts) with NY-ESO1-1 + tumors. This is in contrast to other known tumor-associated antigens (TAA) defined by antibody (Ab) or cytotoxic T cell (CTL) reactivity, i.e. MAGE Melan A, and tyrosinase, which induce immune responses in <10% of cancer pts. We showed previously, that high-titered NY-ESO-1 Ab and strong CTL against NY-ESO-1 can occur simultaneously. In healthy controls and pts with NY-ESO-1 – tumors, NY-ESO-1 Ab was not detected. In this study we assessed the NY-ESO-1 serum Ab response in pts with different NY-ESO-1 + tumors using Western blotting and ELISA. 10/12 patients had NY-ESO-1 serum Ab. All pts were followed for the development of NY-ESO-1 Ab titers under tumor treatment and clinical evolution. In 4 pts, an increase of NY-ESO-1 Ab titer was observed with progression of disease or extensive tumor necrosis. 1 pt showed a stable NY-ESO-1 Ab titer over 3 years along with gradual regression of a large tumor mass. In 5 pts, a decrease of

NY-ESO-1 Ab was detected: in 1 pt after curative tumor resection, in 3 pts with PR of metastatic disease under therapy, and in 1 pt with a NY-ESO-1 – tumor relapse. Our results suggest that NY-ESO-1 Ab is dependent on the presence of NY-ESO-1 + tumors, reflecting the evolution of disease.

1433 ORAL

Clinical and immune responses in metastatic melanoma patients immunized with an anti-idiotype (anti-Id) monoclonal antibody (mAb) mimicking disialoganglioside gd21

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Purpose: We initiated a clinical trial for patients with metastatic melanoma treated with an anti-Id mAb (TriGem) that mimics GD2. The primary goal was to determine immune responses & toxicity; secondary goals were clinical responses & survival.

Patients & Methods: Forty-seven patients received either 1, 2, 4 or 8 mg doses of TriGem mixed with 100 μg of QS-21 adjuvant s.c. weekly × 4 then monthly until disease progression.

Results: Hyperimmune sera from 40 of 47 patients revealed an anti-anti-Id (Ab3) response. The 7 who did not generate an immune response progressed on study prior to the fifth injection. Patient Ab3 was truly Ab1 since it specifically bound to purified GD2. The Ab3 was predominantly IgG, with all IgG subclasses represented. One patient had a complete response, 17 patients are stable on study & 27 progressed and 20 have died. The Kaplan-Meier derived overall median survival has not been reached but at 16 months was 52%. Toxicity consisted of local reaction at the site of the injection & mild fever & chills.

Conclusion: TriGem has minimal toxicity, generates strong & specific IgG immune responses against GD2, & appears to have a major favorable impact on disease progression & survival.

1434 ORAL

Gene therapy for colon cancer using a novel deoxytidine kinase suicide gene together with cytosine arabinoside

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Purpose: Cytosine arabinoside (ara-C) is an effective drug for treatment of acute myeloid leukemia. However, it has only a limited validity against most GI tract tumors such as colon cancers. The reason includes relatively less amounts of deoxycytidine kinase (dCK) activity in non-myeloid cells. Here we hypothesized that solid tumors could be sensitized to ara-C if we successfully increased the cellular dCK activity. To verify the assumption, we transduced the dCK cDNA to mouse colon cancer cells and examined the efficacy of ara-C.

Methods: The MC38 mouse colon carcinoma cells were retrovirally transfected with dck cDNA. After selected with geneticin, expressors (MC38-dck) were isolated and comparedto the wild-type (MC38-wt) or control cells (MC38-neo). In order to examine the sensitivity, we performed cytotoxic assays. Next, we constructed an adenoviral vector containing dCK cDNA under the CMV promoter. Using this vector, we evaluated the in vivo efficacy of the dCK.

Results: Compared to other cells, MC38-dck cells were significantly sensitive to ara-C (IC50 = 488 pM). While MC38 cells transduced by control Ad.CMV-b-gal did not demonstrate differences in a sensitivity to ara-C, cells infected by Ad.CMV-dck exhibited an MOI-dependent increase of the sensitivity (MOI, 0 = 23.4 nM, MOI, 100 = 5.86 nM, MOI, 500 = 1.95 nM, respectively). When implanted subdermally, tumors of dCK-transduced MC38 cells were significantly smaller than of non-transduced cells after treatment of are-C ($p < 0.05$, two different measurments).

Conclusion: This system has numerous advantages for non-myeloid cancer gene therapy. First, since the dCK cDNA is human origin, it potentially limits the immunological responses. Next, the working concentration of the prodrug demonstrated in this study is easily achievable in the patients. Lastly, since ara-C is a classic agent, its pharmacokinetics is fully understood. Taken together, the dck/ara-C suicide system may be a potent approach for gene therapy of colon cancers.

1435 ORAL

Phase I-Study for patients with inoperable pancreatic carcinoma with encapsulated cells producing cytochrome P450 CYP2B1 that activates ifosfamide

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Background: Conventional chemotherapy of pancreatic carcinoma is only marginal effective. Substances such as ifosfamide, registered for the treatment of pancreatic cancer, have not been followed up due to a high toxicity at therapeutic doses.

Hypothesis: The local concersion of ifosfamide into its active components, phosphomustard and acrolein, should be feasable for treatment employing low systemic concentration of the drug.

Rationale: Transfection of CYP2B1 in cells with subsequent microencapsulation.

Experimntal Work: The enzyme activity (resorufin-essay) remains stable for weeks in vitro and in vivo within the microencapsulated CYP 2B1-expressing cells. We could demonstrate a significant antitumorous effect of the intratumorally injected capsules on xenotransplanted human pancreatic carcinomas on the nude mouse (Gene Therapy 1998, 5: 1070–1078). Angiographic experiments in pig assured the feasibility of an intraarterial placement of the capsules into the pancreas (Ann NY Acad Sci 1999, in press). A clinical protocol was established and approved (J Mol Med 1999, 77: in press).

Patients, Material and Methods: L293-cells were transfected with CYP2B1-gene, microencapsulated (diameter 0.5 cm) under GCP-conditions and packed sterile. Patients with confirmed inoperable adenocarcinoma of the pancreas underwent angiography and capsules were injected into a vessel leading to the tumor. The patients were monitored for 48 hrs to exclude allergic reactions or pancreatitis. A day later, ifosfamide was administered at 1000 mg/m^2 BS for three consecutive days to be repeated day 21–23. The patients were followed-up for 6 months.

Results: The study was opened 7/98. A total of 12 patients were enrolled; 5 of them finished the entire study period. In 10/12 patients the capsules could be administered as planned. In one patient, this was technically impossible. Another patient experienced an acute abdomen and had to be operated for an ileus. The 10 patients treated tolerated the procedure without any complications. No allergic reactions or pancreatitis was encountered. Chemotherapy was uneventful.

Discussion: The intraarterial application of microcapsules for targeted chemotherapyx was well tolerated. The antitumoral effects cannot be judged at present time.

1436 ORAL

Oral administration of chimeric MBO antisense-Protein Kinase A inhibits growth, angiogenesis and growth factors production and cooperates with cytotoxic drugs in human cancer xenografts

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Introduction: Protein kinase A type I (PKAI) plays a key role in neoplastic transformation and conveys mitogenic signals from different growth factors and oncogenes. Different pharmacologic tools developed to inhibit PKAI expression and function are able to inhibit cancer cell growth in vitro and in vivo. We have recently shown that a novel class of mixed-backbone oligonucleotides (MBOs) targeting the PKAI subunit RI, exhibit improved pharmacokinetic properties and antitumor activity in vitro and in vivo in several human cancer types.

Methods: We have administered orally HYB 165, a chimeric DNA/RNA MBO targeting the PKAI, alone and in combination with different cytotoxic drugs. We have evaluated the tissue distribution, the pharmacokinetic and the effect on tumor growth, angiogenesis and expression of several factors involved in the control of cell proliferation.

Results: We have demonstrated that the chimeric MBO HYB 165 has a good bioavailability and accumulates as intact oligo in the tumor after oral administration. As compared to a scramble MBO, oral HYB165 demonstrated a dose-dependent inhibition of the growth in human cancer xenografts in nude mice. Histochemical analysis showed that the antitu-