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