using the crystal violet method. Tumorigenicity studies were performed in athymia mice. Intratumor coinjection and injection of tumor cells (HaCa4, MSC11A5) and MD-E1a retrovirus-producing cells were also performed.

Results: The E1a gene induced marked sensitivity to cisplatin and to radiation in both cell models (80% greater than in control cells lacking E1a expression). *In vivo* assays showed that constitutive E1a expression increased latency and decreased tumorigenicity. The results with coinjection of MD-E1a virus-producing cells were identical to those obtained *in vitro* using transfected lines. Moreover, intratumor injection of producer cells partially blocked the growth of tumors generated by MSC11A5 cells.

Conclusions: The E1a gene induces chemosensitivity and radiosensitivity in epidermoid carcinoma cell lines regardless of the p53 status. Injection of E1a-producer cells may block tumorigenicity and results in a new approach for gene therapy in cancer.

376 POSTER

Employment of the mdr1 promoter for a conditionally active retroviral vector system in cancer gene therapy

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Purpose: Inducible vectors are attractive tools for the conditional expression of therapeutic genes in cancer gene therapy. Earlier studies demonstrated that the promoter of the multidrug resistance gene (mdr1) harbors responsive elements that are inducible by MDR-associated drugs. This points to the applicability of the mdr1 promoter for the construction of drug-inducible vectors. We linked an mdr1 promoter element to the human TNFα gene in a retroviral vector to evaluate expression efficacy and drug-inducibility of this system.

Methods: The retroviral constructs were transduced into MCF-7 human mammary carcinoma and HCT116 human colon carcinoma cells. For the induction experiments transduced cells were treated with doxorubicin, vincristine, VP-16 and taxol. The expression and induction studies were performed by using RT-PCR and TNFα-specific ELISA.

Results: Transduced MCF-7 and HCT116 cells showed measurable basal expression of TNFα. Treatment of the cells with the MDR-associated drugs led to a 2–3 fold increase in TNFα mRNA followed by an 3–13 fold increase in TNFα secretion. This induction was drug-concentration dependent.

Conclusion: The studies have shown that the mdr1 promoter carrying retroviral vector is suitable for the inducible expression of therapeutic genes and could be employed for gene therapy in the context of cancer chemotherapy.

377 POSTER

Cytosine Deaminase – A suicide system for tumor therapy

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Cytosine Deaminase (CD) is a bacterial and fungal enzyme which is not expressed in mammalian cells. This protein dominates the non-toxic prodrug 5-fluorocytosine (5-FC) to 5-fluorouracil (5-FU), which is used for chemotherapy of colorectal cancer. Therefore, CD can be used as a suicide system for transfecting tumor cells and subsequent selective killing of CD-transfectants by 5-FC. In order to allow the detection of CD expression at the protein level we generated antibodies against this enzyme. Furthermore, we used a syngeneic rat pancreatic tumor model to examine the CD/5-FC system in *vivo* in immunocompetent animals.

The generated antibodies specifically recognize the CD protein from *E. coli* in various test systems including western blots and immunohistochemistry on frozen tissue sections of rat AS/CD-tumors. For *in vivo* experiments we induced tumors in rats by injection of CD-expressing AS-tumor cells (AS/CD). In comparison to control tumors (AS/neo), which grew rapidly, CD-expressing tumors regressed after initial tumor growth, when exposed to 5-FC or PBS. After a second injection of AS/CD cells and the AS parental cells into these animals AS/CD cells were rejected immediately. Complete regression of the parental tumor was observed in a significant number of animals. A third injection of AS parental cells into the surviving rats showed an immediate rejection of the syngeneic AS tumor cells.

The Abs generated against CD from *E. coli* facilitate the fast and direct detection of the CD protein in transfected cells and tumors. Our preliminary data obtained in the rat tumor model suggest the induction of an immune response by CD – a bacterial antigen – which might modulate the tumor microenvironment such that the originally non-immunogenic parental tumor cells become immunogenic. The molecular basis of this phenomenon is currently under investigation.

378 POSTER

Generation and characterization of cytotoxic T lymphocytes (CTL) against mutated *ras* peptides

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Mutated *ras* genes have been implicated in the initiation and progression of cancers. Therefore, peptides encompassing *ras* mutations appear to represent an appealing target for active immunotherapy procedures. In this work peptides encompassing GLY→VAL, 61 GLN→LEU and 61 GLN→LYS *ras* mutations and displaying HLA-A2.1 binding motifs, selected by a computer program, were used to attempt the generation of specific CTL *in vitro*. Initially, peripheral blood mononuclear cells (PBMC) from five HLA-A2.1+ healthy donors were stimulated *in vitro* with a mixture of peptides. Weekly thereafter, PBMC were restimulated with irradiated peptide pulsed, autologous Epstein Barr virus (EBV) transformed B cells. After eight rounds of restimulation reproducible cytotoxic activity against peptide pulsed target cells was detectable in one donor. CTL recognized two nonamers encompassing *ras* 61 Gln→Leu mutation. Killing was mediated by $CD8^+$ T cells displaying $\alpha\beta$ T cell receptor (TCR) and was inhibited by anti-HLA-A2.1 monoclonal antibodies. None was able to exert effective cytotoxic activity aginst tumor cells expressing the specific mutation.

379 POSTER

Improving tumour targeting and decreasing normal tissue uptake by optimizing stoichiometry of a two-step biotinylated monoclonal antibody (Mab)/streptavidin (Strv) based targeting strategy: Studies in a nude mouse xenograft model

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Purpose: To assess the impact of relative protein stoichiometry of first and second step and biotinylation density of first step on the pharmacokinetics, biodistribution and tumour targeting of a two-step biot-MAb/Strv approach.

Methods: The HT-29 xenograft nude-mouse model was used. AUA1 Mab was biotinylated to various degrees (r. 0.8–25 biotins per IgG). Protein stoichiometry of the two steps was studied through a range of 2 logs. Both steps i.e. the biot-Mab (1st step) and Strv (2nd step) were radiolabelled (^{131}I & ^{125}I). A 24 h interval between 1st/2nd step was studied, animals were killed 24 h after the 2nd step.

Results: Strv excess led to a decrease in circulating levels of biot-Mab (7.6 ± 1.0 vs 11.2 ± 1.3% i.d./g) and decreased amounts of biot-Mab in the tumour (3.7 ± 0.7 vs 5.7 ± 0.6% i.d./g). Biot-Mab excess led to increase in circulating levels of Strv (3.6 ± 0.5 vs 7.5 ± 1.7% i.d./g), a decrease in renal uptake of Strv (66 ± 12 vs 24 ± 5.9% i.d./g) and increased targeting of Strv to tumour (6.9 ± 1.4 vs 5.3 ± 0.9% i.d./g). At a constant protein molar ratio of 1st to 2nd step (10:1 Mab excess), varying IgG biotin density resulted in: (i) The circulating levels of Strv increasing from 4.8 ± 0.6 to 21 ± 2.6% i.d./g (ii) Increase in tumour uptake of biot-MAb (6.2 ± 0.8 vs 24.1 ± 7.7% i.d./g) and (iii) Renal uptake falling from 69 ± 5 to 8.8 ± 3.8% i.d./g while liver uptake increased from 8 ± 1 to 40 ± 14% i.d/g.

Conclusion: Factors pertaining to protein stoichiometry and to biotinylation density of the MAb profoundly affect biodistribution, pharmacokinetics and tumour targetting in two-step based strategies.

380 POSTER

How to perform effective IL-2 therapy

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Locoregional cancer treatment with IL-2 induces good therapeutic effects

without the toxicities associated with systemic application. We report on the following aspects:

(1) *Protocol:* Intra/peritumoral application of IL-2 is most effective in the dose range of 7,000–33,000 IU/day, injected for 5 consecutive days.

(2) *Sensitive tumour types:* This therapy induced cures/complete remissions in mice with breast cancer, lymphosarcoma, fibrosarcoma, mastocytoma; rats with bladder carcinoma; guinea pigs with liver carcinoma; cattle with spontaneous ocular squamous cell carcinoma (OCC); horses with spontaneous sarcoids; human patients with T1/G1G2 marker lesions of superficial bladder carcinoma.

(3) *Potency of IL-2 therapy:* This therapy induces cures in mice with severely infiltrated and metastasized lymphosarcoma comprising at least 5% of the body weight and complete remissions of spontaneous OCC of up to cm^2, and spontaneous sarcoids of up to 20 cm^2 surface in horses.

381 POSTER

Long-term therapeutic efficacy and toxicity of recombinant interferon-alpha 2a in Polycythemia Vera

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Purpose: To assess long-term therapeutic efficacy and toxicity of recombinant interferon alpha 2-a (IFN) in a series of 38 patients with Polycythemia Vera (PV).

Methods: In all patients haematocrit (PCV) was first brought into the normal range by venesection; IFN was then begun at a starting weekly dose of 9,000,000 I.U. Complete response (CR) was defined as persistence of normal PCV without phlebotomies; partial response (PR) as >50% reduction of venesection requirement.

Results: Eleven patients (28.9%) achieved CR and 8 (21.0%) PR. Median duration of response was 40 months; 12 responsive patients are still under treatment after 13, 15, 25, 35, 40, 41, 43, 49, 50, 51, 52 and 52 months. Both in CR and PR patients IFN also mormalized leukocyte and platelet counts besides relieving symptoms as generalized pruritus. As far as late toxicity is concerned, 13.1% of patients experienced severe weakness leading to treatment discontinuation. No case of leukemia/solid tumours was observed in PV patients treated with IFN.

Conclusion: IFN is an effective and safe long-term treatment for PV.

382 POSTER

Application of a novel growth suppressing gene, *tob*, for gene therapy of pancreatic cancer *in vitro*

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Purpose: Recently, a novel gene, termed *tob*, encodes a 38-kDa protein with homologous to the growth suppressing protein Btg-1 was identified. Elevated expression of the Tob protein suppressed growth of the NIH3T3 cells. In this study, we evaluated the *tob* expression in the pancreatic cancer cell lines, and have presented to the conditions for the transfection of adeno-viral vector containing *tob* cDNA (Ad-tob vector).

Method: Human pancreatic cancer cell lines, AsPC-1, BxPC-3, SOJ, were used. RNA blot hybridization was performed on samples cell lines using the 1.0 kbp HindIII fragment of ^{32}P-labeled *tob* cDNA. Transfection of Ad-tob vector was performed in these cell lines.

Results: The *tob* mRNA was expressed in every pancreatic cancer cell line, and the level of the *tob* mRNA of AsPC-1 cells was strongest. The titer of the Ad-tob vector was 3.5×10^8 pfu/ml. Transfection of adeno-viral vector containing *lac*-Z gene to pancreatic cancer cells revealed that these cancer cells were able to be transfected with high MOI from 50 to 100 without adeno-viral toxicicity.

Conclusion: Exogeneously expressed Tob exhibits the suppression of cell growth, therefore it may be possible to apply Ad-tob vector in gene therapy.

383 POSTER

A phase I trial of escalating repeated doses of PNU-214565 in patients with advanced colorectal and other gastrointestinal adenocarcinomas

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A T-cell-based therapeutic modality for carcinomas of gastrointestinal origin was provided by generation of a fusion protein consisting of the superantigen staphylococcal enterotoxin A (SEA) and the Fab fragment of the monoclonal antibody C242, reacting with human colorectal (CRC), pancreatic carcinoma (PC) and other adenocarcinomas of gastrointestinal origin (AGO), independently of MHC class II interaction. Based on the results of prior single dose phase I studies with this fusion protein PNU-214565 (formerly designated LS 4565) the starting dose with repeated doses (four consequtive days) was determined to be 0.5 ng/kg. A total of 11 patients with CRC, 5 with PC and 4 with AGO were treated with doses ranging from 0.5 ng/kg to 4.0 ng/kg. Three patients treated at 0.5 ng/kg and 1.5 ng/kg respectively, had only mild adverse events. At 4.0 ng/kg, two patients experienced dose limiting toxicities (DLT): The first patient developed transient grade IV vomiting, thrombocyto- and leucopenia, hyperbilirubinemia together with a acute renal failure requiring 5 weeks of dialysis before normalisation. The second patient had grade IV hepatotoxicity and thrombocytopenia lasting for 5 days. Of 12 patients treated at the next lower dose, 2.75 ng, only one developed DLT, a grade IV hypotension easily managed with Dopamine. Accordingly, the maximum tolerated dose was 2.75 ng/kg. However, analysis of the compiled data from all previos trials with PNU-214565 has indicated a correlation between pretreatment anti-SEA antibodies and the dose of PNU-214565 needed to induce effects/side effects of the drug. Clinical trials are now being carried out to test this correlation.

384 POSTER

Bcl2 and p53 expression in platinum and irradiation sensitive and resistant human ovarian cancer cells

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Purpose: Apoptosis is regulated by different genes equally involved in cell cycle and cell death (eg., Bcl2, p53). Apoptotic cells observed in tumors may govern curability. Therefore, we evaluated the expression of Bcl2 and p53 in Cisplatin (CDDP) and ionizing radiation (IR) sensitive and resistant human ovarian cancer cells.

Methods: Tumor cells were cultivated in tissue culture flasks. Sensitive cells were made resistant to CDDP and IR by chronic exposure. The resistance factor at the 50% survival level was 3.6–5.1 for CDDP, and 1.7–2.0 for IR. The resistance was stable after withdrawal of the drug. The sensitive cells were diploid. The DNA index of the resistant cells was 1.76–1.84. Cell survival after cytotoxic exposure was evaluated by clonogenic assay. The expression of Bcl2 and p53 was analyzed by immunochemistry on paraffin-embedded cells.

Results: CDDP and IR sensitive and resistant cells were both associated with a positive Bcl2 and p53 expression. There was no significant difference between both.

Conclusion: The difference in sensitivity of the tumor cells to CDDP and IR did not correlate with any change in expression of Bcl2 or p53. Therefore, the different expression reported as predictor for the sensitivity of tumor cells to cytotoxicity needs to be further evaluated.

385 POSTER

Intraperitoneal (IP) interferon A2b (INF) consolidation in cCR ovarian cancer (OC) patients following carboplatin chemotherapy (CT)

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Purpose of this study was to assess the feasibility and tolerance of IP IFN consolidation treatment as well as the overall survival of OC patients following clinical complete remission (cCR) after Carboplatin CT. Since May 92, 83 women with median age 56, PS 1 entered the study. 74.4% had