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

stage III whereas 43% had serous and 25% mucinous adenocarcinoma. 30% of patients had an optimal operation with residual disease (RD) ≤ 1 cm. All patients received carboplatin AUC 7.5 1 h infusion q 3 wks for a maximum 6 cycles. GM-CSF was also given 5 mcg/kgr days 2–14 of each cycle. 44 (53%) women entered cCR and 17 (20%) cPR. 21 women out of 44 with cCR were randomized to receive IP IFN and 23 for follow-up without treatment. IFN was given IP through catheter 25 × 10^6 units q 2 wks for 12 doses. 12 (57%) patients of the IFN group are still alive with a median survival 41.64 (15.90–52.16) months whereas 14 (60%) of the control group are alive and the median survival has not been reached (P = 0.46). In the subgroup of RD ≤ 1 cm the median survival of the patients with IFN has not been reached whereas in the control group is 25.90 (19.38–51.38) months (P = 0.28). Fever grade 3 (9%) was the main toxicity of IFN whereas thrombocytopenia grade 3–4 11%–24% was for Carboplatin without toxic deaths. We conclude that IP IFN consolidation in cCR OC patients following Carboplatin CT in feasible, tolerable without survival advantage

386 POSTER

Epithelial cells in the bone marrow (BM) of colorectal carcinoma (CRC) patients: A tool to monitor immunotherapy?

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Purpose: Immunotherapy is effective as adjuvant treatment for CRC pts. The therapeutic effect in advanced disease is limited. The development of adjuvant therapy requires a large number of pts and a long follow-up period. Surrogate end-points might therefore aid in the evaluation of a new therapeutic approach. However, new treatment concepts are mostly evaluated primarily in advanced disease where a clinical effect might not frequently be seen. The presence of cytokeratin positive (CK+) epithelial cells in BM of pts with CRC correlates strongly to the prognosis. Analyses of such cells during immunotherapy might be a way to early evaluate the therapy.

Methods: A double immunohistochemistry technique has been developed and used on BM aspirate (BMA) from 47 CRC pts with advanced disease or treated in the adjuvant setting. The pts received various combinations of unconjugated MAb17-1A.

Results: The presence of CK+ cells were found in 20/42 (48%) pts BMA. In further 5 pts the BMA was inadequate. p53 was detected in the nucleus of CK+ cells in 11/20 pts. KI 67 was seen in CK+ cells in 9/20 pts. CK+ cells were noted in aggregates in 12/42 pts. In 6 pts analyses showed CK+ cells in BMA before but not after treatment. In one pt this was paralleled by clinical tumor response.

Conclusions: Routinely processed BMA can be used to evaluate CK+ cells in the BM. Preliminary results indicate that CK+ cells in BM might be used to monitor immunotherapy.

387 PUBLICATION

Adjuvant therapy of renal cell carcinoma (RCC) with a pure cell-lysate autologous tumorvaccine (aTm)

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Purpose: An adjuvant therapy for RCC after radical tumor nephrectomy is not available. Therefore we investigated for this indication the efficacy of a pure cell-lysate autologous tumorvaccine produced by macropharm GmbH (autologous-tumorvaccine-macropharm, aTm).

Methods: 169 patients with RCC have been treated with aTm after radical tumor nephrectomy. The progressive free survival probability of these patients was compared to a historical control group of 107 patients from the same hospital, which received identical surgical treatment but without any adjuvant therapy.

Results: According to identical in- and exclusion criteria and two independent biometrical analyses there was no statistical difference between the main epidemiological and clinical parameter of the two patient groups. As a consequence, any observed significant effects resulting from the treatment with aTm are based on assumptions to be most probably clinically relevant. Two years after nephrectomy, the first analyses provide evidence that there exists a difference of 22.8% in favor of the aTm group (pT2, 3a, 3b pNO/+ MO). Only two patients out of 169 (aTm-group) showed minor side effects not exceeding WHO-grade I.

Conclusion: The results presented here justify a prospective randomized controlled and multicenter phase III study, which is underway now.

388 PUBLICATION

Interleukin-2 (IL-2), interferon-α (IFN-α), 5-fluorouracil (5-FU) and vinblastine (VBL) for metastatic renal cell carcinoma (MRCC): A clinical and immunological study

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Patients: 12 pts, 7 males and 5 females, median age 62 with MRCC in lungs, bones, liver, lymph nodes or contralateral kidney were included in this study. 11 pts had previous nephrectomy with DFS of 0–200 mos.

Treatment: IL-2 10 MIU/m2, S.C., 3 ×/week, weeks 1–4, IFN-α 6 MIU/m2, S.C., 1 ×/week, weeks 1–4 and 10 MIU/m2, 3 ×/week, weeks 5–8, 5-FU 600 mg/m2 and VBL 6 mg/m2, I.V. bolus, weeks 5 and 7. Courses given every 2 weeks.

Results: 11 pts were evaluated for response and toxicity. CR: 2 pts (+10, +6 mos), PR: 3 pts (+3, +3, +10 mos), SD. 2 pts and PD: 3 pts. Treatment was stopped in 1 patient due to toxicity. Mean values of T-cells phenotypings before treatment compared to normal: CD3 73% ± 8.8 vs 66% ± 8.8 ($p < 0.002$), CD4/CD8 1.1 vs 1.5 (1.6 after treatment), CD69 CD4 21% ± 10.3 vs 40% ± 13.0 ($p < 0.001$) and 37% ± 16.1 after treatment ($p < 0.05$). sIL-2R 1,919 vs 500 u/ml ($p < 0.001$). Side effects were flu-like syndrome, nausea, vomiting and depression.

Conclusion: This treatment schedule is effective, safe with acceptable toxicity. The study is still ongoing, to confirm these clinical results.

389 PUBLICATION

Targeted delivery of esperamicine A1 by using oncofetal protein α-fetoprotein

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Purpose: Human oncofetal protein α-fetoprotein (AFP) was selected as a vector for tumor specific delivery of esperamicin A1 (Esp) to the target cells due to the overexpression of AFP-receptors on the surface of malignant cells. Because of the very high toxicity of free Esp it's possible to use the conjugate AFP-Esp in extremely low concentrations of AFP. The aim of this work was the study of antitumor activity of AFP-Esp conjugates.

Methods: The method used for Esp conjugation with AFP involved AFP thiolation by SPDP after reducing the S-S bonds with dithiothreitol. The therapeutic activity of AFP-Esp was estimated taking the increase in mean life-span (ILS) and the tumor size of treated animals as a criteria.

Results: The free Esp was about three times more toxic than it's conjugate with AFP for different human and mouse tumor cell lines in vitro. In vivo in the model experiments on DBA/2 mice with inoculated s.c. P388 tumor the ILS for treated by the conjugate mice was about 120% for two months period. 92% of treated animals didn't develop tumors and were alive over 6 months.

Conclusion: Conjugates of AFP with Esp possess a very high therapeutic activity against solid tumors in mice. The rationality of using AFP conjugates with antitumor drugs for the development of new chemotherapeutic approaches for cancer treatment is discussed.

390 PUBLICATION

The efficacy of interferon alpha in polycythemia vera

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Purpose: In chronic granulocyte leukemia (CGL), primary thrombocytosis and idiopathic myelofibrosis recombinant interferon alpha (rINF-α) used as myelosuppressive agent. Recently, there is some reports about the use of rINF-α in polycythemia vera (PV).

Methods: In our study therapeutic efficacy of rINF-α has been evaluated in 7 (6 male 1 female) patients with PV, diagnosed according to the criteria of Polycythemia Vera Study Group. Patients follow up was 5 years. Recombinant interferon-alpha 2b was started as 3 mU 3 times a week sc.