range of morning testosterone in healthy men. Mean level of testosterone was 3,46 nmol/L(SD:1,98 nmol/L), high level of FSH (mean 58,2 IU/L) and LH (18,75 IU/L) were measured. The difference to the lower limit of testosterone reference range is statistically significant (P-value: 0.0000). In the total group of patients with oral testosteron substitution 20 displayed with serum oestradiol below the normal range. Only 6 patients of the group showed normal BMD. The average of BMD values were significantly low, with a mean of 61%, compared to age matched control). DHEA levels were statistically significant low in patients with low BMD values.

Conclusion: Oral testosterone undecanoate substitution therapy is not optimal for long term substitution treatment to maintain normal hormone level and BMD value in these patients.

825 POSTER

Long-term fatigue and quality of life (QL) after cure for testicular cancer (TC): a comparison with survivors of Hodgkins disease (hd) and the general population (GenPop)

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Aim: To assess the prevalence of long-term fatigue in TC survivors.

Methods: A mailed questionnaire with SF-36, HADS and a validated Fatigue questionnaire was answered by 791 TC survivors (mean age: 45 years), 12 years (mean) after primary diagnosis. Among these, 660 pts. had an out-patient examination and blood sampling.

Results: 16% of the TC pts. had chronic fatigue as compared to 24% after HD and 11% in the GenPop. Type of previous treatment (surgery only, radiotherapy only, chemotherapy +/-), duration of follow-up and age at survey were not associated with fatigue, whereas this was the case for current co-morbidity, educational level, and current and previous psychological distress. Depression was more correlated with fatigue than anxiety. Chemotherapy given to those aged ≥40 years and ≤20 years at diagnosis was a risk factor for post-treatment fatigue. In general, the mental health of TC survivors was superior to that of HD survivors and of the GenPop. TC survivors displayed higher levels of anxiety but lower depression scores than the GenPop. Most QL parameters (SF-36) of TC survivors were more favourable than those of HD survivors. Except for pain, the scores of the QL dimensions were similar to those of the GenPop. In patients <50 years at survey, subclinical gonadal insufficiency was associated with chronic fatigue. For all patients, the HADS-Depression score remained an independent predictor of chronic fatigue together with the Mental and Physical Component Summaries of the SF-36.

Conclusion: Chronic fatigue and decreased QL represent a lesser problem in middle-aged TC survivors than in HD survivors, but remain a larger problem than in the GenPop. Mental health, in particular depression, seems to be an important predictor of fatigue together with somatic health. Patients aged ≤20 or ≥40 years at chemotherapy appear to represent risk groups for chronic fatigue.

826 POSTER

Clinical characteristics, treatment and outcome of patients (pts) with bilateral testicular germ cell tumors (BTGCT)

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Purpose: The clinical characteristics, treatment and outcome of BTGCT in connection with the widespread use of cisplatin based chemotherapy was analyzed in a retrospective study.

Methods: The study involved 2386 pts treated between 1988 and 1998 with testicular cancer in our Department at the National Institute of Oncology.

Results: 72 pts had BTGCT; 19 cases (0.8%) were synchronous and 53 cases (2.2%) metachronous. Of the 19 synchronous BTGCT pts (median age 37.7 years, range 19-71) 13 had concordant seminoma (70%) and 7 discordant histology. The clinical stages were: 8 I/A, 5 I/B, 1 II/A, 2 II/B, 1 III/A, 2 III/B. The 5-year overall survival was 85%, three pts died, 2 due to tumor progression. The median follow-up is 93 months, range 31-150). In 53 pts with metachronous BTGCT median age at the diagnosis of the 1st tumor was 28 years (range 16-41), median time to second tumor was 76 months (range 18-203). Nine had concordant seminoma, and 9 concordant nonseminoma. Among the 53 pts 2 had a family history of TGCT, 5 (13%) had testicular maldescent (in 2 cases bilateral), 7 testicular atrophy, 1 azoospermia. 68% of the pts were younger than 30 years at the 1st tumor diagnosis. At the 1st TGCT diagnosis the following clinical stages were detected: 14 I/A, 21 I/B, 15 II/A, 2 II/B, 1 III/B. 22 pts were treated with chemotherapy. At the 2nd TGCT diagnosis 26 I/A, 16 I/B, 3 II/A, 1 II/B and 7 III/B stages were registered. In 38 cases chemotherapy was used. No relapse occurred between the two tumors. The 5-year overall survival was 95% (median follow up 42 months, range 27-121). Two relapses occurred after primary therapy, 1 patient died due to tumor progression.

Conclusion: The overall incidence of BTGCT is low, the majority of patients have a good prognosis. These results argue against the introduction of systemic contralateral biopsy at the 1st TGCT diagnosis in all pts in Hungary. Better identification of pts at risk for a 2nd TGCT is not possible by the proposed clinical risk factors, that is why education and long term follow up are important in the early detection of a second TGCT.

Immunobiology and biological therapies

827 POSTER

Immunotherapy for stomach cancer with the apoptosis-inducing human monoclonal antibody SC-1

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Purpose: Stomach carcinoma belongs to most dangerous malignant diseases worldwide. Treatment is mostly limited to radical gastrectomy, lymphadenomectomy and in cases of irresectable tumors to chemotherapeutical approaches. But even then, according to the number of people killed worldwide by this cancer, the prognosis is very poor and there is a big need for additional adjuvant therapies.

Methods: We have recently described the human monoclonal antibody SC-1, which was isolated from a patient with gastric cancer by hybridization of lymphnode cells with a heteromyeloma cell.

The moderately affinity-maturated IgM antibody (DP49) SC-1 binds to a novel modified form of membrane-bound CD55 (DAF-B, decay-accelerating factor), that is specifically overexpressed on stomach carcinoma cells and absent on other tumor cells or healthy tissue. DAF-B therefore exists in two different glycosylated forms on stomach carcinoma cells, in addition to the ubiquitously distributed 70 kD isoform, which protects cells from lysis through autologous complement, a specific modified 82 kD DAF-B is coexpressed. A tyrosine phosphorylation of 60, 75 and 100 kD proteins and a serine dephosphorylation of a 35 kD protein is observed shortly after SC-1 induced apoptosis. SC-1 apoptosis involves activity of caspases 6, other investigated caspases like caspases 3, 8 and 9 seem not to be involved in this process. In addition SC-1 apoptosis is independent of p53 and bcl-2.

Results: The antibody binds to 25% of tested intestinal-type and 70% of diffuse-type stomach adenocarcinoma. The antibody induces specific apoptosis in vitro and in vivo in animal studies. Used in a clinical trial with 44 stomach carcinoma patients, significant apoptotic and regressive effects on tumor cell proliferation in primary tumors and metastases could be observed without any toxic side effects.

Conclusion: Human cancer patients are the best source for tumor-specific and tumor-reactive reagents (cells, factors, antibodies) and the human hybridoma technology offers the only and unique technique for identification of new targets on tumor cells, new tumor-cell related mechanisms and complete human antibodies for diagnostic and therapeutical purposes. Human antibodies like SC-1 give hope for more effective and less harmful treatment of carcinoma and for the understanding of tumor-related mechanisms.

828 POSTER

Combined maintenance treatment of cancer patients responders to previous chemotherapy with immunotherapy (recombinant interleukin 2), hormone therapy (medroxyprogesterone acetate) and antioxidant agents: clinical outcome, effects on cachexia symptoms, on proinflammatory cytokines and evaluation of quality of life

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An open, non-randomized phase II study was carried out including all patients treated with whatever chemotherapy or combined modality regimen

for whatever cancer who were in clinical objective response or stable disease (SD) since more than three months, to receive a maintenance treatment with recombinant Interleukin-2 (rIL-2) plus medroxyprogesterone acetate (MPA) plus antioxidant agents Alpha-Lipoic Acid (ALA) and N-Acetyl Cysteine (NAC). This treatment was planned to be continued until disease progression or appearance of toxicity. The first study endpoints were clinical outcome and toxicity. The secondary endpoints were effects of treatment on cancer-related anorexia/cachexia syndrome (CACS) symptoms, on serum levels of proinflammatory cytokines, IL-2, C-reactive protein (CRP) and leptin as well as the evaluation of the patient quality of life (QL). rIL-2 was administered at a dose of 1.8 MIU subcutaneously three times/week on alternate days for the first two weeks of every month and MPA was given orally at a dose of 500 mg once a day at alternate days without interruption. ALA 300 mg/day orally and NAC 1800 mg/day orally were also administered. The treatment was administered until progression of disease or appearance of toxicity. From July 1998 to May 2000, 16 patients were enrolled in the study (M/F ratio: 15/1; mean age: 62 years, range 45-71). The median duration of maintenance treatment was 10 months (range 5-22).The response to maintenance treatment at September 2000 was: CR (persistent throughout all treatment) 4 patients (25%); SD 1 patient (6.2%);PD 11 patients (68.8%). The median duration of response was 9.8 months (range: 5-22). The median follow-up duration was 19 months (range: 8-102). The median OS was not reached. The median PFS was 14 months (range 1-29). The 1-year survival rate was 25%. At September 2000, 9 patients are still surviving. No grade 3/4 toxicity was observed. One Grade 2 skin toxicity was observed and Grade 1: 2 fever, 2 thrombocytopenia, 1 neutropenia and 1 skin were observed. The ECOG PS did worsen significantly, the body weight and BMI increased significantly after treatment, whereas the appetite did not change significantly. The QL evaluation showed a significant amelioration of cognitive functions and a borderline significant amelioration of emotional functions after treatment, whereas a borderline worsening of dyspnea was observed.Work supported by M.U.R.S.T,Rome,Italy,National Research Project No.9906041835

Gene therapy

829 POSTER

Cancer gene therapy: facs and real-time pcr analysis of lipofection

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Cationic lipids are widely used for gene transfer in vitro and show promise as a vector for in vivo gene therapy. However, there is a limited understanding of the cellular and molecular mechanisms involved.

We have developed a method combining FACS and Real-time PCR-technology to analyze single steps of lipofection in more detail. The technique allows to quantify binding and internalization of lipoplexes, and to follow-up the stability of internalized plasmids and transcribed mRNA. Various cells (e.g. tumor or dendritic cells) were transfected with reporter plasmid pEGF-PLUC and different cationic lipids at variing DNA/lipid ratios using a high troughput robot-supported screening system. Final transfection rate and efficacy were determined by the expression of GFP-luciferase fusion protein. The results were standardized by total protein amount (lipid toxicity) and compared to FACS and PCR data.

We could demonstrate striking differences in binding or internalization of lipoplexes between various cells. Additionally, binding of individual lipids was found not to be directly correlated to internalization in the cells or to transfection rate and efficacy. Furthermore, the stability of internalized reporter plasmid or of mRNA strongly varied in different cells and was also dependent on the lipid(s) used for lipofection. Our findings confirm the idea that different steps during transfection process might be critical and optimized gene transfer needs a complex analysis of cellular, lipid and DNA parameters.

Our new method will allow to do such a complex analysis of the lipofection process step by step. This might help to find more optimal transfection conditions enhancing effectiveness of gene transfer by lipofection for various cultured and primary cells, respectively.Thus, we have developed a very useful way to analyze new gene therapeutic tools and protocols, to enhance their potential efficiancy and it also might be used as quality control of such gene therapy tools.

830 POSTER

Pan-I-a peptides augmented antigen-specific humoral immunity elicited by vaccination with DNA encoding antigen proteins in mice

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Purpose: DNA vaccines are thought to be beneficial for maintaining high levels of tumor antigens and for eliciting anti-tumor immunity in vivo. However, the induced immunity has not been reported to be sufficiently strong to eradicate cancer in cancer-bearing hosts. To enhance specific immunity by DNA vaccination, syngeneic dendritic cells (DC) loaded with Pan-I-A peptides were co-vaccinated with DNA encoding target antigens.

Methods: BALB/c mice were vaccinated intramuscularly with expression vectors containing LacZ DNA. Some of the mice were inoculated simultaneously with syngeneic DCs loaded with synthetic peptides capable of binding to mouse I-A molecules with any allele at the vaccination site. Sera from the immunized mice were examined for antibodies to the target antigen by ELISA.

Results: Reactivity of sera from mice vaccinated with both LacZ DNA and peptide-loaded DCs to beta-galactosidase was significantly stronger than those from mice vaccinated with LacZ DNA and naive DCs, or with control DNA and peptide-loaded DCs.

Conclusion: Pan-I-A peptides were suggested to augment humoral immunity to target antigens by DNA vaccination. This animal model is useful for the development of a DNA vaccine in therapeutic immunotherapy for cancer.

831 POSTER

Local tumor irradiation augments the anti-tumor effect of cytokine producing autologous cancer cell vaccines in a murine glioma model

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The combined therapeutic effect of cytokine producing cancer cell vaccines and local radiotherapy was studied in a mouse glioma 261 (Gl261) brain tumor model. Brain tumor bearing mice were treated with cytokine (IL-2, IL-4, IL-6, IL-7, IL-12, GM-CSF, TNFalpha, LIF, LT) producing vaccines made by in vitro transduction of Gl261 cells with corresponding adenoviral vectors. Vaccines producing either IL-2, IL-4, IL-12 or GM-CSF cured about 20-40% of mice. The anti-tumor effect strongly depended on the secreted cytokine level. Vaccination therapy induced specific activation of cytotoxic T lymphocytes measured by cytotoxicity assay. Brain tumors were heavily infiltrated by CD4+ lymphocytes after treatment with IL-2, IL-4, IL-12 or GM-CSF secreting cells. GM-CSF vaccination induced moderate CD8+ infiltration, as well. Depleting either CD4+ or CD8+ lymphocyte subsets abolished the anticancer effect of GM-CSF expressing cells. Strong synergism was observed by combining cytokine vaccination with local tumor irradiation: about 80-100% of glioma bearing mice was cured. The high efficiency of combined treatment was maintained even under sub-optimal conditions when neither of the modalities alone cured any of the mice. This suggests that vaccination therapy might open a new potential on the clinical treatment of high-grade gliomas when applied as adjuvant to existing treatment modalities.

832 POSTER

The effect of p53 gene deletion and mutation on malignant phenotype of human lung cancer cell line

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Purpose: To study the inhibition effect of both extraneous sence p53 and antisence p53 on malignant phenotype of human lung cancer cell line.

Methods: The named 801D cell line with p53 deletion and mutation was selected as a model in vitro. The recombined plasmid PEGFP-p53(RS),PEGFP-p53(AS) were constructed at which GFP gene