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