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 varing 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

expresses to monitor extraneous gene expression. The extraneous gene was detected by PCR. The p53 mutation protein was examined by immunohitochemical stain of p53 antibody. Colony formation assay and Tumor transplanted on nude mice were carry out.

Result: The transferring cell lines PEGFP-P53(RS)801D, PEGFP-p53(AS)801D, PEGFP801D were established. Extraneous p53 gene presence and expression in PEGFP-p53(RS)801D and PEGFP-p53(AS)801D twas found out.p53 mutation protein in PEGFP-P53(AS) was negative. Rate of colony formation was 11% for PEGFP-p53(RS) ,22% for PEGFP-p53(AS) ($P<0.001$). Tumor growth on nude mice for PEGFP-p53(RS)801D was more slower than rate of colony formation for PEGFP-p53(AS)801D.Results show inhibition effects of extraneous sence p53(RS) comparing with extraneous antisence p53(AS) on malignant growth of 801D was more appeared.

Conclusion: Human lung cancer cell line with p53 deletion appear more malignant growth.That indicate p53 deletion play a key role on malignant growth of human lung cancer.

Biotherapy

833 POSTER

PEG-intron is effective therapy for essential thrombocythemia

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Purpose: No therapy has been proven to alter the natural history of essential thrombocythemia (ET). Hydroxyurea and anagrelide may control symptoms. Interferon α has been shown to reduce the megakaryocyte mass and to maintain long-term control of platelet counts in ET. We are studying a long-acting interferon α, PEG-Intron in ET.

Methods: Dose = 4.5 mcg/kg/week SQ, or ↑ to 6 mcg/kg/week or ↓ as tolerated. Concurrent tapering anagrelide was permitted in pts 5, 9, 10.

Results: Age 25–70 yrs; median 57 yrs, 8 females.

Pt	Dose	Prior TX platelets	Baseline count FMT	Platelet	Max Toxicity Grade ≤ 2
1	4.5	I, A	885	394	
2	1.5	H, A	414	305	fatigue
3	3	I, A	478	351	weight loss
4	3	A, H	776	157	
5	4.5	A	990	297	
6	2	None	895	305	nausea, vomiting, fatigue
7	1.5	H, A	453		↑ transaminases
8	3	None	902	209	
9	3	H, A	761	583	nausea, vomiting, fatigue
10	4.5	I, A	479	390	

A = anagrelide, H = hydroxyurea, I = interferon; FMT = First monitoring time point (1–2 months post initial Peg-interferon), plat = k/μl.

Conclusion: Once weekly Peg-Intron rapidly controlled the platelet counts in ET with moderate and infrequent adverse events. Longer follow-up will define the optimal maintenance regimen for these patients.

834 POSTER

Modulation of CTL-activity by TNF-a during postoperative radiotherapy in colorectal cancer patients

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Purpose: It is well established that cancer patients have a defective immune system secondary to their disease. Cytoreductive therapy, including radiotherapy (RT), can modulate the activity of immunocompetent cells. This study analyses the development of the specific immune response to CEA in colorectal cancer patients before and after RT in combination with parenteral TNF-alpha administration.

Methods: 29 patients with colorectal adenocarcinoma were observed. 18 patients (st. II - 6, st. III - 10, st. IV - 2) received daily intravenous injections of TNF-alpha (10^6 IU/day) during standard RT (total dose of the postoperative irradiation: 60Gy). 11 patients (control group: st. II - 3, st. III - 7, st. IV - 1) were treated without cytokine administration. CTLs were isolated from the peripheral blood in cancer patients. Their activity was determined in vitro as percentage of killed SW1463 cells (colorectal adenocarcinoma cells) expressing CEA.

Results: CTL activity against CEA-expressing cells before treatment was determined to be 9,14%±5,3 and 57,3%±8,7 after RT combined with TNF-alpha. In contrast to this highly significant increase the activity of tumor specific lymphocytes derived from the control group (RT alone) did not show such correlation (10,6%±6,1 before RT; 24,1%±7,3 after RT).

Conclusion: TNF-alpha increases the specific immune response to CEA in colorectal cancer patients during postoperative radiotherapy. This fact may be fruitfully used to up-regulate the specific immune recognition in cancer patients.

835 POSTER

Hepatic arterial administration of autologous activated lymphocytes in patients with liver metastases

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Purpose: Liver is the most common site of metastatic disease. Hepatic arterial infusion (HAI) of cytotoxic drugs may achieve high objective response rate, but almost all patients with liver metastases will ultimately die of progressive disease. The aim of the present study was to evaluate the feasibility of HAI of activated autologous lymphocytes (AAL).

Methods: Peripheral blood mononuclear cells were obtained by leukapheresis after stimulation with subcutaneous interleukin-2 (IL-2) in 4 patients (2 patients with breast cancer, 1 patient with colon cancer and 1 patient with renal carcinoma) with non-resectable hepatic metastases not responsive to conventional regimens and incubated for 2 - 4 h with IL-2. The cells were then administered by HAI either alone, or after HAI of melphalan (50 mg) through a catheter inserted percutaneously into the hepatic artery. Cytotoxicity was evaluated by MTT test using MDA2774 cell line at different effector:target (E:T) ratios, and phenotype was assessed by flow cytometry.

Results: Mean number (standard deviation; SD) of 19.0 (SD 9.7) x 10 exp 9 mononuclear cells was obtained through leukapheresis. The relative and absolute numbers of lymphocytes obtained were 60 (SD 18) % and 9.9 (SD 2.7) x 10 exp 9 cells, respectively. An increase in the percentage of CD3C69 positive cells (5 SD 3 vs 10 SD 4%) was observed during the ex vivo culture. Cytotoxic activity of AAL increased after stimulation (mean increase 45 SD 9% at 50:1 E:T ratio). Significant cytotoxic activity was observed after activation even at E:T ratios of 1:1 and 1:10. The therapy was well tolerated, and a marked decrease in tumor markers was observed in 2 patients treated by combination of melphalan and AAL, including one patient with a partial response.

Conclusion: HAI is a technically feasible way of regional delivery of high number of activated lymphocytes with significant anti-tumor activity both in vitro and in vivo.

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836 POSTER

An anti-leukemic single chain Fv antibody selected from a synthetic human phage antibody library

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The display of human antibody repertoire on the cell surface of a filamentous bacteriophage has offered a novel strategy for selecting antibodies to a diverse range of purified targets. Aim: Our aim is to establish a method for selection of phage scFv antibodies with therapeutic potentials using whole cells as affinity matrix. Methods: A synthetic human scFv phage antibody library was panned on whole pre-myelocytic leukemia cell line (HL60). Phages binding to common receptors and undesirable phages were subtracted by incubating the library with human glioma cells. Phages with high binding affinity to HL60 cells were enriched by fluorescence-activated cell sorting. After the 6th round of selection, the selected phages were tested for their binding specificity to HL60, Nalm-6 (human pre B-cell line) and human glioma cells by flowcytometry. The possible biological effect of the selected phages was tested by incubating different concentrations of the