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