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

phage or the purified scFv with HL60 cells for four days and measuring the cell proliferation by pulsing with 3H-thymidine. Results: Approximately 50% of the selected phage antibodies showed significant binding to HL60 cells, whereas non of the analyzed phage antibodies bound to human glioma and Nalm-6 cells. In addition to the specific binding, one of the phage antibodies and its purified scFv antibody had an inhibitory effect on HL60 cell proliferation as compared to irrelevant scFv antibodies. The purified scFv antibody inhibited HL60 cell proliferation in a dose dependent manner. Conclusion: We have characterized a scFv antibody with specificity for the HL60 leukemic cell line. This antibody inhibited the cell proliferation in a dose dependent manner Taken together the data demonstrate that specific scFv antibodies with biological functions can be isolated by using whole cells as affinity matrix.

837 POSTER

Combined administration of IL-2 and mistletoe lectin for treatment of eNOS-positive transplanted adenocarcinoma in C3H/HeJ mice

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Purpose: The present study was designed to test whether a biochemically purified galactoside-specific lectin from Viscum album L. (VAA) given at nontoxic immunomodulatory dosage provided additional therapeutic benefits in combination with IL-2 therapy of a highly metastatic eNOS expressing C3L5 mammary adenocarcinoma in C3H/HeJ female mice bearing one week s.c. transplants or could be stimulatory for tumor growth and spread.

Methods: Immunomodulators were applied i.p. (IL-2) or s.c. (VAA) according to the standard regimen and the following parameters were measured after one or two weeks of treatment: primary tumor growth, spontaneous lung metastasis, capillary leakage, NO production (nitrate+nitrite) in vivo, and the presence of immunoreactive nitrotyrosine in the kidney.

Results: Biweekly injections of VAA (1 ng/kg) alone promoted the growth of primary tumors as well as the incidence of spontaneous lung metastases. This occurred in spite of the inability of VAA to induce additional NO production by C3L5 cells in vitro, unlike that noted with LPS+IFN-gamma. IL-2 therapy alone had antitumor and antimetastatic effects, but also induced capillary leakage and nitrotyrosine deposition in the kidneys. There was a rise in NO levels in the serum, pleural fluid and organs (kidneys and lungs) after the first round, but a decline after the second round of IL-2 therapy. Addition of VAA to IL-2 therapy provided no additional benefit nor detriment to IL-2 therapy as indicated by a lack of change in any of the above parameters.

Conclusion: Although animal studies may not always predict the clinical outcome in the human, our observations suggest that the use of VAA alone or as an adjuvant for IL-2 based immunotherapy of cancer should be considered with caution.

Immunobiology

838 POSTER

Engineering the magic bullet for targeted therapy in bladder cancer

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Bladder cancer is one of the most common male cancers. Currently, around 250,000 new cases are diagnosed worldwide every year. The incidence is higher in males (with a ratio of 3:1) and in the elderly. On average, the use of conventional chemotherapy has only achieved a response rate of up to 50%, and few cures. In addition, death rates from the disease have remained largely unchanged over the last 50 years. These facts highlight the limited effectiveness of the current therapeutic regimens and novel treatment strategies are urgently required.

Our research effort to tackle this problem involves the use of the monoclonal antibody C595 to specifically target cytotoxic moieties to the bladder tumour. C595 targets the epithelial mucin MUC1 that is frequently upregulated and aberrantly expressed in bladder cancer. The exquisite binding specificity of C595 enables the tumour-selective delivery of large doses of cytotoxic agents while sparing healthy tissue the debilitating side effects. C595 is one of the most well-characterised anti-MUC1 antibodies for use in bladder cancer, especially in the targeting of radioactive isotopes. A pioneering phase I clinical trial has recently been initiated with the use of a copper-67-C595 conjugate in the intravesical radioimmunotherapy of bladder cancer. Success in this proof of concept study will open the application of the antibody to a much wider setting such as disease staging and treatment of metastatic tumours. Before these avenues can be fully explored, however, C595 must be humanised. Unlike intravesical therapy where the murine antibody is confined within the bladder, multiple systemic administrations of the antibody are required for these extended applications. The intravenous administration of C595 may consequently trigger the human anti-murine antibody response – an immunological reaction that, in serious cases, can lead to anaphylaxis and death. The use of a humanised antibody reduces this likelihood to a minimum and allows the full potential of C595 to be realised.

The humanised C595 antibody (BLC595) was produced by complementarity-determining region (CDR) grafting. This involves the intricate transfer of antigen binding specificity from C595 onto a human antibody, resulting in an antibody that is 95% human. A combination of molecular modelling, bioinformatics analysis and site-directed mutagenesis allowed the rational optimisation of antigen-binding properties of BLC595. The initial characterisation of the CDR-grafted antibody has shown favourable binding characteristics to the MUC1 mucin. It has also demonstrated the ability to specifically target radioisotopes to an *ex vivo* bladder cancer model. With further successful clinical testing, it is envisaged that BLC595 will be the first of its kind to be used in the targeted therapy of bladder cancer. This novel reagent, when conjugated to suitable diagnostic and cytotoxic moieties, will offer an attractive strategy from the initial diagnosis and staging to the final treatment of localised and metastatic diseases. It is hoped that BLC595 will complement existing chemotherapeutic regimens in the management of bladder cancer, with the ultimate aim of significantly improving therapeutic outcome.

839 POSTER

Prognostic role of ebstein barr virus latent membrane protein-1 and interleukin-10 expression in patients with nasopharyngeal carcinoma

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Purpose: We aimed to investigate Ebtein Barr Virus (EBV) Latent Membran Protein-1 (LMP-1) and Interleukin-10 (IL-10) expression in 74 nasopharyngeal carcinoma (NPC) patients and to evaluate their prognostic significance.

Material and Methods: Between 1993 and 1999, 144 patients were treated with the diagnosis of non-metastatic NPC at our department. The expression of LMP-1 and IL-10 was investigated by using immunohistochemical approach in 74 (53 male, 21 female) patients whose paraffin embedded tissue samples were obtained. A detailed histopathological analysis including degree of apoptosis and lymphocyte infiltration was made and all patients were reclassified according to the WHO classification. Univariate and multivariate regression analysis were performed using all clinical and pathological prognostic factors. All patients were treated with radiotherapy +/- chemotherapy. Follow-up time is ranged between 12-80 months (Mean:32.2 months).

Results: The histopathological diagnosis was WHO-I in one (1.3%), WHO-II in 15 (20.2%) and WHO-III in 58 (79.5%) patients. There were 38 (51%) patients with IL-10 expression and 44 patients with (61%) LMP-1 expression. Twenty-seven (36.4%) patients were found to be both IL-10 and LMP-1 positive. There were significantly more N0 disease in patients without LMP-1 expression compared to LMP-1 positive patients (65% vs. 35%, p=0.01). The logistic regression analysis showed that advanced nodal involvement to be the major parameter effecting the expression of IL-10 (p=0.03). Three year overall survival (OS), locoregional relapse free survival (LRRFS) and distant metastasis free survival (DMFS) rates were 67.8%, 84.4% and 74.3%, respectively, for whole group. On univariate analysis, LRRFS was significantly lower in WHO-III patients, DMFS was significantly lower in advanced nodal disease and IL-10 negative patients and OS is significantly lower in WHO-III patients. Multivariate analysis showed that WHO-III and T2 patients were significantly associated with lower OS and N3 patients were significantly associated with lower DMFS.

Conclusion: As a conclusion, we observed a high rate of (61%) EBV and NPC association in our patients. LMP-1 negative tumors were found to be less prone to invade lymph nodes. Patients without IL-10 expression has more advanced N disease. We did not find prognostic significant role of IL-10 and EBV LMP-1 on survival in multivariate analysis.