Results: Administration of IL-2, IL-12 or IL-2 gene-modified MK16 tumour vaccines at the site of TC-1 or MK16 tumour residua after surgery reduced the percentage of tumour recurrences and the number of MK16 lung metastases. In contrast, administration of IL-2, IL-12, or IL-2/GM-CSF gene-modified MK16 tumour vaccines in mice with minimal residual TC-1 or MK16 tumour disease after chemotherapy with ifosfamide derivative revealed that significant tumour-inhibitory and anti-metastatic effects can be obtained exclusively in mice carrying TC-1 (MHC class I-positive), but not MK16 (MHC class I-negative) tumour residua. Spleen cells from MK16 or TC-1 tumour-immunized mice were not cytolytic when allowed to react with the MK16 (MHC class I-negative) target cells, although they efficiently lysed the MHC class I-positive TC-1 cells. However, when the MK16 cells were cultivated *in vitro* in the presence of IFN[3], they acquired, together with the expression of MHC class I molecules, the sensitivity to the cytolytic effect of spleen cells from the MK16 or TC-1 tumour-immunized mice.

Conclusions: These results indicate that both MHC class I-positive and class I-negative, HPV16-associated tumours are sensitive to the IL-2 and IL-12 therapy, as well as to IL-2 gene therapy in a clinically relevant setting of surgical minimal residual tumour disease; in the residual disease after chemotherapy, the therapeutic effects could only be obtained in mice carrying MHC class I-positive, HPV16-associated tumours.

961 POSTER

Phase I study of escalating doses of TroVax® in patients with advanced colorectal cancer (CRC)

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TroVax® consists of the highly attenuated vaccinia virus, modified vaccinia Ankara (MVA), used as a vector to deliver the oncofetal antigen 5T4 which is expressed on over 70% of colorectal tumours. Immunohistochemical analysis indicates that 5T4 expression is an indicator of poor prognosis in CRC and when tumour cells are transfected with cDNA encoding 5T4 they display increased motility suggesting that expression may induce metastatic properties. This study was designed to assess safety and immunogenicity.

3 groups of 4 patients with histologically proven advanced colorectal cancer (CRC) at least 10 weeks post completion of chemotherapy with a life expectancy of greater that 3 months are entered into an open label upward titration study. Three dosage levels were $2.5x10^7$pfu(1x), next a fivefold higher dose $2.5x10^8$pfu(5x), the final group a dose of $5x10^8$pfu(10x) as intramuscular injections into the deltoid muscle. Immunogenicity is assessed in terms of antibody and CTL/T-cell responses to both the vector and 5T4 surface antigen. If the patient remained well and mounted an immune response then 2 further vaccinations are permitted. All patients will be followed up for a total of 18 months to assess tolerability, induction of humoral and cellular immunity to 5T4 and immune response to the vector.

In all patients TroVax® was well tolerated with no adverse effects related to the vaccine reported. In the first group 3 patients had an antibody and cellular response to 5T4 and vaccinia. The fourth patient has not mounted an immune response to any antigen tested. One patient developed a fall in CEA levels corresponding to development of necrosis in the tumour mass and one showed disease stabilization at 3 months. 2 patients in the 5x group showed antibody and cellular response to 5T4 and vaccinia with one stable until 9 months and one remains stable at 18 months from treatment, 2 failed to make any response. In the 10x group all of the 4 evaluable patients who received at least 3 vaccinations developed both antibody and cellular responses to 5T4.

These results show TroVax® to be safe and well tolerated in patients with advanced CRC. Clear cellular and humoral responses have been demonstrated at all 3 dosage levels. Long-term follow up continues. The dose to be used for the planned Phase II trial will be $5x10^8$ pfu and future studies in CRC are planned to include combinations with chemotherapy.

962 POSTER

Dendritic cell vaccines targeting MUC1 against breast and lung cancer

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The use of dendritic cells (DCs) for cancer vaccination is effective in suppressing cancer progression. This is because the DCs play a crucial role in priming tumor-specific immunity efficiently as antigen-presenting cells. In this study, we analyzed the ability of DCs to elicit tumor-specific immunity and clinical effects of DC vaccine immunotherapy targeting MUC1 tumor antigens. DCs from 14 patients with advanced or metastatic breast or lung cancer (9 positive for MUC1 and 5 negative for MUC1) were loaded with MUC1 antigens or tumor lysate and used for therapeutic vaccination. After vaccination, all of the MUC1-positive patients acquired antigen-specific immunity whereas only one case with MUC1-negative cancer showed the specific immunity. Clinically, marked effects such as reduction in tumor sizes or tumor marker levels or disappearance of malignant pleural effusion were observed in 7 of the 9 MUC1-positive cases. However, MUC1-negative patients did not respond to DC vaccines, with the exception of one case with MAGE3-positive lung cancer. Survival of MUC1-positive patients was significantly prolonged in comparison with MUC1-negative patients (mean survival: 16.75 versus 3.80 months, p = 0.0101). These data suggest that MUC1 is sufficiently immunogenic to elicit strong anti-tumor immunity as a tumor antigen and that DC vaccines targeting MUC1 are useful for immunotherapy of cancer.

963 POSTER

Clinical trial of a peptide based vaccine targeting telomerase in patients with inoperable pancreatic cancer

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The reverse transcriptase subunit of human telomerase (hTERT) is a tumor associated antigen expressed in almost all tumors. By re-expressing hTERT, tumor cells escape cellular senescence to become immortal. This makes hTERT uniquely attractive as a target candidate for cancer vaccines. We have identified several new epitopes in hTERT, and designed vaccines aimed at generating both CD4+ and CD8+ tumor-reactive T cells. The present studies were performed to determine safety and immunogenicity of such dual specific peptide vaccines in patients with inoperable pancreatic cancer and to correlate immune responses with clinical responses observed in the patients. In a single center dose escalation study, 42 patients with newly diagnosed, histologically confirmed, non-resectable pancreatic cancer were included. None of the patients received prior or concomitant chemotherapy. The peptide was injected intradermally 8 times over a period of 10 weeks. Selected patients received monthly booster vaccinations thereafter. The vaccine was tested in 3 dose levels, using GM-CSF as an adjuvant. In this study more than 350 vaccine injections (up to 18 injections in one patient) have been administered to 42 patient and no serious adverse events related to the treatment were observed. Specific immune responses measured as DTH in vivo and T cell proliferation in vitro could be induced in a dose dependent fashion. CTL's specific for several epitopes and Th cells restricted by HLA-DR, -DP and -DQ were obtained from vaccinated patients. In one patient cloned T cells were shown to recognize autologous targets obtained by short term primary cultures from ascites fluid. In the study, which started in September 2000, a strong correlation between vaccine dose, number of responders and survival was observed. In the group of patients who received the low dose 3/10 patients responded compared to 13/17 patients at the intermediate dose level. Median survival of evaluable patients in the two groups were 3,5 months vs. 10,3 months. These results demonstrate that immunity to hTERT can be generated safely and effectively in patients and encourage further trials.

964 POSTER

p53-independent cdk1 induction in response to irinotecan in the HT29 human colon cancer cell line

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Background & Aims: Mutations in the tumor suppressor gene p53 have been associated with advanced colorectal cancer. Irinotecan (CPT-11), a DNA topoisomerase 1 inhibitor that induces DNA double-strand breaks, has been recently incorporated to the adjuvant therapy, which is crucial at advanced stages of the disease. Since the DNA-damage checkpoint depends on p53 activation, the status of p53 might critically influence the response to CPT-11.

Methods: We analyzed the sensitivity to CPT-11 in the human colon cancer cell line HT29 (mut p53) and its subclone HT29-A4 (wt p53).

Results: Cell cycle analysis after treatment with CPT-11 in G0/G1 synchronized cells demonstrated the activation of transfected wild-type p53 and a consequent $p21^{WAF1/CIP1}$-dependent cell cycle blockage in S phase. Activated wt-p53 also increased apoptosis, leading to enhanced sensitivity to CPT-11. DNA microarray analysis showed that, in p53-deficient cells, the cell cycle regulatory machinery did not respond to CPT-11, leading to the accumulation of the G2/M cdk1/cyclin B complex. We found subsequent p53-independent activation of the cdk-inhibitor $p21^{WAF1/CIP1}$, which prevented cell cycle progression. We further exploited cdk1 induction in p53-deficient cells to improve the sensitivity to CPT-11 by additional treatment with the cdk-inhibitor roscovitine.

Conclusions: We demonstrate a gain of sensitivity to CPT-11 in a p53 mutated colon cancer cell line both by restoring wild-type p53 function or by additional treatment with a cdk-inhibitor. Considering that mutations in p53 are among the most common genetic alterations in colorectal cancer, a therapeutic approach that specifically targets tumors with mutated p53 could greatly improve the treatment outcomes.

965 POSTER

Molecular therapy for peritoneal dissemination of gastric cancer with adenovirus-mediated Bax gene transfer

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Gene therapy is one of the candidates for an innovative therapeutic approach against cancer. An adenoviral vector expressing the tumor suppressor p53 gene (Ad/p53) is currently under clinical evaluation in various cancers. We have recently developed a binary adenoviral vector system that can express the strong apoptotic Bax gene (Ad/PGK-GV16+Ad/GT-Bax: Ad/Bax). To evaluate the potential of Bax gene therapy for gastric cancer, we assessed the antitumor effect of the Bax gene in comparison with the p53 gene. The responses of human gastric cancer cell lines, MKN-1, MKN-7, MKN-28 and MKN-45, to recombinant adenoviruses, Ad/Bax or Ad/p53 were assessed in vitro. Cell viability was measured by XTT assay; transgene expression and caspase activation were analyzed by western blotting; and morphological feature of apoptosis was observed by Hoechst staining. The treatment with Ad/Bax or Ad/p53 resulted in the marked Bax or p53 protein expression and effective apoptosis induction in MKN-1, MKN-7, and MKN-28 cells. In contrast, MKN-45 cells showed resistance to Ad/p53 and only the treatment with Ad/Bax resulted in massive apoptosis. To compare antitumor effects between the Ad/Bax and Ad/p53 treatment in vivo, MKN-45 subcutaneous tumors were generated by inoculation of 2×10^6 MKN-45 cells into the dorsal flank of nude mice. When tumor had reached a diameter of about 3-5mm, each mouse was given intratumoral injection of 100 μl of 2×10^{10} particles of each virus. Mean tumor volume of the Ad/p53 group was 515.2± 151.9 mm^3, while that of Ad/Bax was only 236.5± 83.8 mm^3, as of 35 days after inoculation. Furthermore, peritoneal dissemination of MKN-45 cells were generated in nude mice, and each mouse was treated by intraperitoneal injection of 200 μl of 2×10^{10} particles of each virus. Disseminated tumor numbers and weights were assessed 24 days after inoculation. Similarly, mean total tumor weight of the Ad/p53 group was 371.8± 44.0 μg, while that of Ad/Bax was 161.9± 96.9 μg. The treatment with Ad/Bax significantly inhibited the growth of p53-resistant gastric cancer in vitro and in vivo. Therefore, our results suggest that Adenovirus-mediated Bax gene transfer may be useful in gene therapy for gastric cancers.

966 POSTER

Activation of a plasma membrane-cationic channel and apoptosis in prostate cancer cells overexpressing Bax

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Background: Our group has previously identified and characterized a 23 pS non selective-cation channel (NSC channel) in prostatic cancer-LNCaP cells undergoing apoptosis (J Physiol, 1999, 517:95-107). The activation of the channel was only induced by pro-apoptotic stimuli including ionomycin, thapsigargin, staurosporine or serum depletion. Accordingly, channel activity was never registered in intact cells. Further studies using an anti-Bax antibody suggested that the channel opening could be mediated by Bax proteins (Biophys J 2001, 80:2764). To test this hypothesis, we have induced cell death in prostatic cancer LNCaP cells using an inducible Bax adenoviral vector generated by a Cre/loxp system.

Material and Methods: Adenoviruses were replicated, purified and tittered by plaque assay, as described elsewhere (). For overexpressing Bax protein, cells were coinfected with a Bax recombinant adenovirus (Ad/Bax) and the inducing adenovirus Ad/Cre in a 5:1 relation (kind gifts of Dr D. Curiel's group, Mol Ther 2000, 1:545-554). LNCaP cells (2×10^5/ well) were infected at an m.o.i. of 5. The transgene encodes a fusion protein containing Bax and a hemaglutinin (HA) tag. Patch clamp in the cell-attached configuration was used to evaluate the opening of the 23 pS NSC channel at 20 to 24 hours post infection. Protein extraction and Western Blot analysis were carried out at 24 hours for evaluating the expression of endogenous Bax and Bax-HA proteins. Cell death was studied by fluorescence activated cell sorter analysis of annexin 5/ iodide propidium and by crystal violet staining at 72 hrs.

Results: The 23 pS NSC channel was only registered in cells co-infected with AdBax/Ad Cre but neither in control cells nor in cells co-infected with AdBax/AdTK. Overexpression of the Bax-HA fusion protein (27 kDa) was confirmed by WB in the former cells. Cell death occurred in over 90% of Ad Bax/AdCre cells at 72 hours.

Conclusions: The sole overexpression of Bax induces apoptosis and activates a 23 pS non selective-cation channel in LNCaP cells. The significance of the opening of this channel in early stages of apoptosis is under study.

967 POSTER

The detection of metastatic cancer cells in peripheral blood using reverse transcriptase polymerase chain reaction for CK 19

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Metastatic cancer cells spread is not detectable by conventional staging methods, but the combination of immunomagnetic cell enrichment and reverse transcription (RT-PCR) is an efficient method to identify small numbers of disseminating tumour cells in blood or bone marrow of patients with cancer. Our aim is to determine whether RT-PCR for cytokeratin 19 (CK19) provides a sensitive method for the detection of a single metastatic cell in peripheral blood.

In several spiking experiments, cell-line (ZR-75) derived tumour cells (2-100 cells) were added to 5ml of human peripheral blood of a healthy donor. We designed a semi-junctional CK19 specific primer set. Human peripheral blood without added tumour cells was used as our specificity control. After enrichment, mRNA was extracted using Oligobead mRNA extraction and a silica-based total mRNA extraction method. Products were amplified by a single-enzyme RT-PCR for CK-19 mRNA. Amplicon were visualised on 2% agarose gel.

Total mRNA extraction from unspiked blood samples followed by RT-PCR yielded in falsepositive amplification due to circulating hemaptopoietic elements. Immunomagnetic enrichment drastically improved the specificity for the CK-19 RT-PCR assay. Both mRNA extraction methods demonstrated similar sensitivity levels. Our detection sensitivity for the combined method including cell enrichment, RNA extraction and subsequent RT-PCR is less than 3 cells.

Immunomagnetic enrichment combined with CK19 RT-PCR is a very sensitive and specific method to detect disseminating tumour cell in peripheral blood of breast cancer patients. Further evaluation by real time quantitative PCR and using other specific breast markers is needed.

968 POSTER

FCU1: a highly potent suicide gene therapy based on 5-FU

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Background: Direct transfer of pro-drug activation systems into tumours was demonstrated to be an attractive method for the selective in vivo elimination of tumour cells. Besides its local cytotoxic impact, this strategy was further demonstrated to enhance the host anti-tumour immune response through the local release of cellular debris that can be presented by the antigen presenting cells.

Material and methods: We describe a novel and highly potent suicide gene derived from the Saccharomyces cerevisiae cytosine deaminase (FCY1) and uracil phosphoribosyltransferase genes (FUR1). This suicide