form an antiparallel homodimer based on the characteristics of high affinity interaction between the amino (N) and carboxyl (C) termini of the receptor. Recently, it is suggested that AR N-to-C interaction is critical for the ability of this receptor to up-regulate the transcription of androgen-responsive genes, and may be a new target for treatment of prostate cancer. In this study, we investigated the effect of N-terminal (1-34) peptide of AR (ARN34) on androgen-dependent function in prostate cancer cell.

**Material and methods:** We constructed a plasmid, pTriARN34, expressing ARN34 by cytomegalovirus promoter. To measure the *in vivo* interaction of the amino terminal domain and ligand-binding domain of AR, we used the mammalian two-hybrid system. Stable clones of LNCaP cells expressing ARN34 were selected with medium containing of G418.

**Results:** Transfection of pTriARN34 suppressed dihydrotestosterone (DHT)-dependent N-to-C interaction of AR in a dose-dependent manner. On AR-mediated reporter gene assay, the expression of ARN34 suppressed DHT-dependent prostate specific antigen transcription. ARN34 also suppressed AR nuclear translocation induced by DHT. Stable expression of ARN34 suppressed androgen-dependent cell growth of LNCaP cells. Moreover, this inhibitory effect of ARN was also confirmed in hydroxyflutamide-induced mutated AR transactivation and cell growth. Treatment of LNCaP cells with 1 nM DHT drove transition of cells from G1 to S-phase. On the other hand, the ectopic expression ARN34 led to cell cycle arrest by inhibiting the entry into S phase in LNCaP cells.

**Conclusions:** Our results demonstrate that disruption of AR N-to-C interaction caused by ARN34 leads to AR dysfunction and inhibition of AR-mediated prostate cancer cell growth. This approach is thus considered to provide a useful therapeutic opinion for blocking AR-mediated prostate cancer growth.

958 POSTER

## Adenoviral transfer of a natural antisense to survivin mRNA down-regulates survivin expression and promotes apoptosis in breast cancer

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**Background:** Survivin, a member of the inhibitor of apoptosis (IAP) family of proteins, is mostly expressed in malignant cells in adult and recognized as a good target for cancer gene therapy. Previously we demonstrated that induction of a natural antisense of survivin, effector cell protease receptor-1 (EPR-1) down-regulated survivin expression with decrease of cell proliferation, increase of apoptosis, and increase of sensitivity to anticancer agent (Yamamoto et al. European Journal of Cancer, 2002; 38:2316). In this study, we constructed a replication defective adenoviral vector encoding the same antisense sequence to survivin and attempted to enhance the efficacy of previous study. By demonstrating an effect of survivin modulation, we ultimately would like to explore a strategy of gene therapy only toxic to malignant cells expressing survivin.

**Material and methods:** Breast cancer BSMZ cell line was established by one of the authors (Watanabe et al. Cancer Research, 1992; 52:5178). An adenoviral vector encoding antisense RNA to survivin was constructed by homologous recombination of adenovirus type 5-derived pJM17 and shuttle plasmid, pCMV-EPR-1 in HEK 293 cells.

**Results:** We infected the vector to BSMZ cells with multiplicity of infection (MOI) of 0, 1, or 5. Cells were harvested, then transcription and expression of survivin were monitored. Northern blot demonstrated that signals of transduced EPR-1 increased MOI-dependently. Correspondingly, cellular levels of survivin decreased 72-hours after viral infection. In cell cycle analysis, down-regulation of survivin caused increased population in the fraction of apoptotic cells (sub-G1 peak) (MOI=0: 4.88%, MOI=1: 5.05%, MOI=5: 11.54%) with decrease in the S phase population (8.78, 9.94, 10.28%, respectively). Cytotoxic assay revealed that transduction of antisense conferred MOI-dependent sensitivity of docetaxel, a chemotherapeutic agent to BSMZ cells.

**Conclusions:** Current study demonstrated that adenoviral transduction of antisense sequence to survivin mRNA down-regulated survivin expression and increased apoptotic fraction. Moreover, it sensitized cells to docetaxel. Since survivin is expressed primarily in malignant cells, our results suggest possible cancer gene therapy with no adverse effect on normal tissues which do not express survivin. Enhancement of chemosensitivity by modulation of survivin may also have a roll for further development of therapies to drug-refractory malignant tumors.

959 POSTER

## Expression of functional CXCR4 on colorectal human cancer.

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**Background:** The chemokines are small proteins known to direct hematopoietic cells to home-specific anatomical sites. The chemokine receptor for SDF1-alfa chemokine, CXCR4, has been implicated in cancer metastasis. Emerging data suggest that it has a key role in determining the metastatic destination of tumor cells as demonstrated in breast, melanoma, ovary, and lung cancer. Since the expression of the CXCR4 receptor may be of prognostic value we studied the expression of CXCR4 on human colorectal cancer.

**Methods:** CXCR4 expression was examined by immunohistochemical staining on paraffin-embedded sections of normal colorectal mucosa (14), iperplastic polypi (6), dysplastic polypi (27), 16 primary carcinomas and 5 hepatic metastasis. CXCR4 expression was also studied by flow cytometry on Caco2, GEO, SW480, SW48, Lovo and SW620 human colorectal cancer cell lines. The effect of SDF1-alfa and liver-derived proteins on migration of cell lines was measured using transwell inserts (8 1/4 m diameter) and 24-well plates. The inhibitory effect of anti-CXCR4 antibody (10 1/4 g/ml) on migration was also studied.

**Results:** CXCR4 staining resulted weakly positive in 6 and strongly positive in 1 (infiltrated by melanoma) out of 14 samples of normal mucosa, clearly positive in 19 out 27 dysplastic lesions with higher staining intensity for moderate/poorly differentiated lesions (13/17, moderate/poorly vs 6/10, well differentiated), and dramatically positive in 16 out of 16 carcinomas.

SW480, SW48 and SW620 human colon cancer cell lines showed the highest levels of the CXCR4 (60-80% of positive cells), 30-60% for Caco2 cells, 20% Lovo cells and 5-10% GEO cells compared to the 50% of the MDA231 human breast cancer cell line considered to be an ephitelial cell line overexpressing CXCR4 and to the 8% of the HT1080 human fibrosarcoma cell line.

In order to verify the functional status of CXCR4, the ability to migrate versus its natural ligand was assayed on SW480 human colon cancer cells. Preliminary results showed that SW480 migrate in response to SDF1-alfa chemokine relatively to the expression of CXCR4. Furthermore the neutralization of CXCR4 by antibodies inhibits *in vitro* the migratory response to purified SDF1-alfa as well as to liver-derived proteins. Thus the overexpressed CXCR4 is functional.

**Conclusions:** These preliminary results showed CXCR4 overexpression on human colon cancer tissue compared to normal mucosa and benign lesions. Experiments on human colon cancer cell lines suggest a functional activity of CXCR4. Studies on the possible prognostic role of the CXCR4 expression in patients bearing colorectal cancer and its eventual role for targeted therapy are warranted.

960 POSTER

## Therapy of MHC class I+ and class I- HPV16-associated tumours with IL-2, IL-12, and genetically modified tumour vaccines

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**Purpose of the study:** To examine local and systemic effects of IL-2, IL-12 and genetically modified tumour cell-based vaccines directed against HPV16-associated neoplasms in experimental model systems.

**Experimental models:** Moderately immunogenic, MHC class I-negative MK16/1/IIIABC (MK16) cells were previously established by co-transfection of HPV16 E6/E7 and activated Ha-ras DNA into C57 BL/6 murine kidney cells. The MK16 cells formed s.c. tumours in syngeneic mice and metastasized to lungs and lymph nodes (Smahel, Sobotkova, Bubenik et al., Br. J. Cancer 84:374-380, 2001). For comparison, MHC class I-positive, non-metastasizing TC-1 cells, established by co-transfection of C57BL/6 murine lung cells with E6/E7 HPV16 and activated Ha-ras DNA (Lin, Guarnieri, Staveley-O'Carroll et al., Cancer Res. 56:21-26, 1996) were utilized.

**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).