expression, serum adenoviral antibody were done on all patients up to one year.

Results: All patients tolerated the prostate gene therapy well with minimal toxicities. None of the patients exhibited irreversible grade 3 and 4 toxicities directly related to the therapy. Six out 12 patients show varying degrees of tumor response in terms of serum PSA for duration from three weeks to one year. Histological evidence was obtained in selected patients for the transgene expression up to two weeks after the viral administration.

Conclusion: This prospective phase I study of double suicide gene therapy using E1B-attenuated replication competent adenoviral vector shows a therapeutic efficacy in patients with recurrent prostate cancer with minimal normal tissue toxicity.

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Enhancement of cisplatin-induced apoptosis by infection with adeno-associated virus type 2

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The non-pathogenic human adeno-associated virus, AAV has been shown to sensitize human cancer cells and experimental tumors towards chemotherapeutic agents, such as cisplatin. Since these drugs induce apoptosis, we investigated whether one mechanism of AAV-mediated sensitization of human tumor cells may result from an enhancement of cisplatin-induced apoptosis. In HeLa and A549 cells, infection with AAV type 2 (AAV-2) increased cisplatin-induced DNA fragmentation but had no cytotoxic effect by itself. This enhanced apoptosis appeared to be mediated by a component of the viral capsid since empty or UV-inactivated AAV-2 particles were also able to boost cisplatin-induced DNA fragmentation. The effects were AAV-2-specific since they were not observed after infection with AAV type 5 (AAV-5) or the autonomous parvovirus, H-1. AAV-2-mediated enhancement of apoptosis was not associated with a modification of the expression of CD95 ligand, CD95 receptor or other death receptors, as shown by RT-PCR and RNase protection assay. In contrast, using the mitochondrial fluorescent dye, JC-1 in flow cytometry, AAV-2 infection was found to further reduce the mitochondrial transmembrane potential after treatment with cisplatin in a caspase-independent manner, suggesting that increase of apoptosis by AAV-2 occurred at the mitochondrial level. In contrast, in cells of the small cell lung cancer line, P693, an enhancement of cisplatin-induced DNA fragmentation was not observed after infection with AAV-2. In these cells, sensitization to cisplatin-toxicity was associated with cell cycle arrest in G2/M. The data indicate that in the absence of viral gene expression, AAV-2-mediated sensitization to cisplatin involves multiple cellular pathways promoting cell death signals, in a cell type-dependent manner. The results further support that AAV-2 particles may be appropriate adjuvants for improving cancer chemotherapy, and may also have consequences regarding AAV-2-based vectors for gene therapy.

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Gene expression under control of the radiation-inducible Egr-1 promoter in an adenoviral vector: vector optimization for reduction of unspecific gene expression in the absence of irradiation

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Purpose: Construction of a replication defective adenoviral vector expressing a cytotoxic gene under the control of a radiation inducible promoter, and determination of susceptibility to low doses of irradiation.

Methods: A reporter gene encoding the EGFP or the HSV-TK cytotoxic gene were placed in the E1 region of the Ad-genome under the control of the mEgr-1 promoter/enhancer. Replication defective adenoviruses were used to deliver either of the transgenes to rhabdomyosarcoma R1H tumour cells in vitro.

Expression of the reporter gene (EGFP) was detected by fluorescence microscopy; cytotoxicity of Ad.Egr-1.TK+GCV (10-2 to 10-4 mg/ml) was determined by crystal violet staining. Doses of 0 to 8 Gy were given 4hrs post infection and 1hr post GCV. For vector optimization, either insulating sequences of BGHpA were introduced at the upstream or at the upstream and downstream regions of the expression cassette; or the expression cassette with/without the insulating sequences was placed in an anti-parallel position to the E1 region of the Ad genome.

Results: The Ad-Egr1-EGFP and Ad-Egr1-HSV.TK were successfully constructed and the viral DNA was analysed. R1H cells were infected with the constructed viral vectors at different MOIs. If IR was combined with Ad-Egr1-TK and GCV, a significant decrease ($P<0.0001$) in cell survival was found after 4Gy (mean 25%, 95% C.I 20-29%) as compared to 4Gy alone (74%; 61-87%). Irradiating cells at 6Gy was comparable to 4Gy+Ad-Egr.1-TK + GCV. However, Ad-Egr.1-TK + GCV without IR also significantly decreased cell survival (47%; 42, 52%) indicating considerable leakiness of the system. This is supposed to be due to activation of the Egr-1 promoter by viral enhancers in the absence of IR. Preliminary results with EGFP reporter gene suggest that leakiness of the system can be reduced by introduction of the BGHpA insulating sequences and/or inversion of the expression cassette.

Conclusions: Radiation induced gene expression under control of the Egr-1 promoter can be achieved with low doses of IR. The combination of gene therapy with radiotherapy is significantly more effective than IR alone. However, induction of gene expression without IR is considerable. Currently, we are introducing insulating sequences and/or invert the expression cassette with the aim of achieving a tighter temporal and spatial control of gene expression by low doses of IR.

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583 ORAL

Phase II trial of HLA-b7 plasmid dna/lipid (allovectin-7®) immunotherapy in patients with metastatic melanoma

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This phase II study evaluates the response rate, duration of response and toxicity of direct intratumoral injections of Allovectin-7® (a plasmid DNA-based therapy encoding the genes HLA-B7 and β2-microglobulin) in patients with metastatic melanoma.

Seventy-eight adult pts have been enrolled to date with 73 in the intent to treat (ITT) population (at least one dose of Allovectin-7®) and 54 pts evaluable for response (completed evaluation at week 10). Entered pts have stage III or IV disease with visceral metastases limited to the lung; good performance status (KPS * 80%); adequate organ function; and an injectable lesion * 1 x 1 cm. Treatment consists of 10 μg Allovectin-7® administered by intratumoral injection weekly x 6 followed by a 4-week observation period. Stable or responding patients may receive additional treatment cycles. Median age is 58 (range 33-82) with 45 males and 28 females. Mean Karnofsky performance status is 94%. All patients received prior systemic therapy. The overall response rate is 11% (8/73) among the ITT population and 14.8% (8/54) in the evaluable population. Responses include 2 CR's and 6 PR's with a median duration of response of 21 weeks (range 6 to 26+ weeks). Stable disease was seen in 19.2% (14/73) of the ITT population and 25.9% (14/54) of the evaluable population.

The most common side effects are mild to moderate injection site reactions and flu-like symptoms, all of which resolved rapidly and decreased in incidence after the first injection. Five Grade 3 treatment-related adverse events have been reported and include ascites, pain and dizziness. No injection-related serious adverse events have been noted. Three drug-related serious adverse events occurred. All 3 events were seen in a single patient (abdominal pain and two episodes of ascites).

Preliminary results of this ongoing trial indicate that Allovectin-7® is active and well-tolerated in patients with advanced malignant melanoma. Future studies will include an evaluation of higher Allovectin-7® doses and injection of multiple tumors.

584 ORAL

Induction of anti-tumor immunity in vivo using cytokines and an agonistic antibody against 4-1BB

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Several studies have shown that tumor-specific T cells can be isolated from cancer patients and that these cells are capable of recognizing and killing autologous tumor cells in vitro. Despite the presence of tumor-reactive T cells, tumors frequently grow and metastasize, indicating that anti-tumor immune responses are suppressed or not strong enough to eliminate cancer cells in vivo. In an effort to boost anti-tumor immunity, we treated tumor-bearing mice with cytokines or agonistic antibodies that act at the level of antigen-presenting cells and immune effector cells. To generate and

activate dendritic cells in vivo, we used Flt3L, IFN-a and CD40L. To activate T cells, we used IL-2, IL-15 and an agonistic antibody against 4-1BB, a member of the TNF receptor family. Mice were inoculated intradermally with tumor cells and treated with cytokines and the anti-4-1BB antibody either alone or in combination. Treatment of mice for 10-20 days with Flt3L inhibited tumor growth and significantly increased the number of tumor rejections. Flt3L induced both innate (NK cell-mediated) and adaptive (T cell-mediated) immune responses. Combining Flt3L with IL-2 or IL-15 did not result in any beneficial effects, but combining Flt3L with IFN-a or CD40L significantly improved anti-tumor immune responses. Neither IFN-a nor CD40L alone had any significant effects on tumor growth. The beneficial effect of combining Flt3L with CD40L correlates with an overall increase in the number of dendritic cells, but not T, B or NK cells. Re-challenge of the Flt3L+CD40L-treated mice with tumor cells resulted in complete blockage of tumor growth, indicating that these animals had developed long-term anti-tumor immunity. Significant improvements in the anti-tumor immune response were also observed in mice treated with the anti-4-1BB antibody. Two to four bolus injections of this antibody markedly boosted anti-tumor immunity. Depletion of CD8 T cells, but not NK or CD4 T cells, completely eliminated the anti-tumor immune response induced by 4-1BB. Combining Flt3L and anti-4-1BB resulted in enhanced anti-tumor immunity compared to treatments with either reagent alone. Our results show that a strong anti-tumor immune response can be generated in mice treated with cytokines (Flt3L, IFN-a, and CD40L) or an agonistic antibody against 4-1BB. This approach can potentially be used to treat tumors for which no specific antigens are known or to boost anti-tumor immunity in vaccination protocols.

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Models of active specific immuno therapy of human malignancy bone metastases

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The occurrence of bone metastases is a very common and detrimental event associated with human cancer. Epithelial malignancies give rise to bone metastases when they acquire the ability to produce the parathyroid hormone related protein (PTH-rP), a secreted protein, which is considered a critical factor for tumor cell survival and growth in bone tissue. Since PTH-rP in the adult life is mainly produced by several very common cancer cell lineages, such as lung, prostate and breast carcinomas, we have investigated whether it could be used as a TAA target for the active specific immunotherapy of tumors giving bone metastases. To generate a PTH-rP specific CTL response in vitro we have utilized a previously described protocol employing low dose IL-2 and autologous dendritic cells, pulsed with PTH-rP peptides having HLA-A2.1 binding motifs or infected with influenza virosomes containing PTH-rP plasmid genes (GC90V). This protocol allowed the generation of multiple PTH-rP peptide specific CTL lines from PBMC of normal HLA-A2.1+ donors or TIL derived from a HLA-A2.1+ patient with prostate carcinoma. These CTL lines recognized three different PTH-rP peptides and killed HLA-A2.1+ prostate and breast carcinoma cells that produced large amounts of PTH-rP. Intranasal administration of GC90V to BALBc mice resulted in a significant CTL immune response to PTH-rP without occurrence of side effects or autoimmunity as evaluated in post mortem study. Similar results were also obtained in HLA-A2.1 expressing HHD transgenic mice immunized with the three PTH-rP derived epitope peptides. These results demonstrate that PTH-rP is a tumor antigen, that an anti-tumor PTH-rP CTL response may be induced in vitro and in in vivo and that PTH-rP peptides and GC90V may be potential candidate for use in immunotherapy against prostate cancer and bone metastases from the most common epithelial malignancies. (Partially supported by National Council of research, CNR, Italy).

586 ORAL

Therapy of HPV16-induced carcinomas with IL-2 gene modified and dendritic cell (DC)-based tumour vaccines

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Purpose: To examine local and systemic effects of vaccines against HPV16-associated carcinomas.

Methods: Vaccination with IL-2 gene-modified and DC-based tumour vaccines; surgically induced minimal residual tumour disease (SMRTD).

Results: To examine local and systemic effects of dendritic cell (DC)-based vaccines in a mouse model resembling human papilloma virus (HPV) type 16-associated carcinomas, murine kidney cells were malignantly converted by *in vitro* co-transfection of activated H-*ras*, HPV16 E6/E7, and neomycin resistance gene DNA. The resulting MK16 neoplastic cells grew s.c. in syngeneic mice and metastasized to lungs and lymph nodes (Šmahel, Sobotková, Bubeník et al., Brit. J. Cancer, in press). Immunization with HPV16 E6/E7 and activated H-ras plasmid DNA could specifically inhibit growth of the HPV16-induced tumours in the immunized mice. Priming of the proliferative anti-MK16 responses was efficient when DC and DC lines were pulsed with lysates of MK16 cells or HPV16 E7 (aa 49–57 RAHYNIVTF) synthetic peptide and co-cultivated *in vitro* with syngeneic spleen cells. Local pretreatment with DC could substantially inhibit growth of a subsequent inoculum of the MK16 cells. MK16 tumours were injected peritumorally with irradiated and MK16 lysate-pulsed DC; significant differences were found between MK16 tumour growth and survival of mice in the DC vaccine-treated and control groups (Indrová, Bubeník, Šimová et al., Int. J. Mol. Med., in press). Immunotherapy of the MK16 carcinoma transplanted in syngeneic mice was accomplished using genetically modified, IL-2-producing tumour vaccines. Both, small MK16 tumours, 2–4 mm in diameter, and MK16 SMRTD could be completely cured with IL-2 gene-modified tumour vaccines.

Conclusion: Taken collectively, the results indicate that the activating, co-stimulatory, and mitogenic signals delivered by HPV16 oncoprotein-pulsed DC as well as insertion of the IL-2 gene should be considered for the construction of vaccines for treatment of primary human HPV16-associated carcinomas and SMRTD.

Psychosocial and economical aspects of cancer

587 ORAL

The guessing game: waiting for the results of diagnostic investigations for symptoms of breast disease

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Purpose: Over recent years there has been increasing concern with the psychological impact of undergoing diagnostic investigations for breast abnormalities. UK NHS Executive guidelines have stipulated that "minimising" delay in the diagnostic process is critical to reducing patient anxiety. This paper presents the results of a multi-method research study that assesses patient distress during the peri-diagnostic interval, and explores the ways in which women reached their own diagnoses during this time.

Methods: Participants (n=98) completed the State Trait Anxiety Inventory immediately following investigations at the out-patient clinic. That same evening and for the following two days, patients completed diaries including the Profile of Mood States and Daily Coping Scale, and repeated the state anxiety assessment upon return to clinic for results. A subset of 20 women were interviewed about their experience and these data were combined with interview data (n=20) from a concurrent project.

Results: 75% of the cohort recorded levels of anxiety, confusion, uncertainty and depression equivalent to those reported for psychiatric out-patients. This distress was sustained throughout the waiting period, and emotion-focused strategies dominated coping efforts. Interviewees explained how they were able to guess their diagnosis from various "cues" which could be categorised according to type: temporal, interpersonal, procedural and spatial. Inferences appeared to be underpinned by an urgent need to reduce uncertainty and enhance prediction.

Conclusion: Service structure does impact upon patient's psychological distress, and further research is critical to the development of an evidence-based service for all women undergoing diagnostic investigations.

*This work was completed when the author was a doctoral student at School of Nursing Studies, University of Wales College of Medicine, Heath Park, Cardiff, South Wales.