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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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.

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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