scan appears to underestimate the true response rate as indicated by the patients who went on to surgery. The 3 patients who demonstrated complete pathologic responses at surgery, only showed MR (1 patient) or SD (2 patients) on CT scan.

Conclusion: TNFerade + radiation was well tolerated without DLTs or SAEs. The treatment appears to be very active as all patients showed dramatic tumor necrosis. Consequently, TNFerade + radiation could represent a new paradigm in the treatment of soft tissue sarcoma, either as neoadjuvant treatment or for palliation. Final dataset including all patients at all dose levels will be presented at the meeting.

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Phase I clinical trials with direct intratumoural injection of an adenovirus-nitroreductase (Ad-NTR) vector, CTL102, in liver and prostate tumour patients

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A gene-directed enzyme prodrug therapy approach, using bacterial nitroreductase (NTR) to convert the prodrug CB1954 to a toxic bifunctional alkylating agent, has demonstrated activity in a range of preclinical models. We have constructed an adenovirus coding for NTR (CTL102) and are conducting phase I clinical trials in patients with liver (1° hepatocellular / colorectal 2°) or prostate tumours with a view to defining safe doses which give sufficient NTR expression to provide activation of co-administered CB1954. Escalating CTL102 doses were directly injected into tumours, using ultrasound guidance, with subsequent monitoring for overt toxicity, virus shedding, viral dissemination and immune response. In addition, following tumour resection, NTR expression was assessed by immunohistochemistry. 14 patients have entered the liver trial with a 4-log escalation of CTL102 dose (10e8 - 10e11 particles). Toxicity has been minimal, 1 patient developed transient pyrexia and flu-like symptoms at a low dose. No shedding of intact virus has been detected, although some viral DNA was detected in whole blood up to 24 hours after dosing. All patients showed an increased neutralising anti-adenovirus antibody titre, although there was significant interpatient variation in the isotype and kinetics of these elevated levels. NTR expression was detectable at all dose levels and showed an increasing dose-response relationship. Tumour architecture appeared to influence NTR expression, which was evident in both tumour and non-tumour (stroma, fibroblast and lymphocyte) cells, but not in associated normal liver from the resection margin. The top dose of 10e11 particles produced a level of NTR expression considered adequate to initiate a further arm of the study, co-administering CTL102/CB1954 to patients with inoperable tumours. 3 patients have entered the prostate trial at the initial dose of 10e10 particles. No toxicity was seen and no evidence of virus shedding was detected. Like the liver trial, some viral DNA was detected in whole blood, and also urine, up to 24 hours after dosing. NTR expression was detectable in both tumour and normal epithelial cells of the prostatic ducts in all 3 patients. NTR expression was localised to the peripheral zone, where the majority of prostatic tumours arise, and correlated well to the injection site. Multiple injections may thus be required to maximise the spread of virus throughout the prostate. Dose escalation in this trial continues.

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Delivery of a c-raf Antisense Oligodeoxynucleotide (LErafAON) by intermittent bolus dosing (Weekly Infusions) in patients with advanced solid tumors: a phase I study

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Rapid cleavage *in vivo* and inefficient cellular uptake limit the clinical utility of antisense oligonucleotides (AON). The delivery of AON agents has required continuous infusion and large doses. Liposomal encapsulation of an AON to the c-raf proto-oncogene mRNA (LErafAON) using a novel cationic lipid results in prolonged circulation, inhibition of target protein and delayed growth of tumor xenografts after bolus intermittent dosing in pre-clinical studies. Safety and dose-limiting toxicities of LErafAON, administered by weekly 90-180 minute intravenous infusions for 8 weeks, were evaluated in patients with advanced solid tumors. To date, 19 patients have received 139 doses of LErafAON (median 6 doses; range 1-32 doses): 4 at 1 mg/kg/week; 3 at 2 mg/kg/week; 4 at 4 mg/kg/week; and 8 at 6 mg/kg/week. Age range was 29-77 years; M:F ratio was 9:10. Acute infusion-related reactions (IRR, as with other liposomal preparations), including chills, fever, flushing, chest tightness, dyspnea, hypoxemia, back or flank pain, hypertension or hypotension, occurred in 15 patients and required discontinuation in 5. Transient complement activation was observed; IRR were not evidently dose-related. In successive cohorts, increased infusion duration and pre-treatment with corticosteroids, H1- and H2-antagonists reduced the frequency and severity of IRR. Progressive dose-related decline in platelet count, potentially related to c-raf inhibition, was observed. At 4 mg/kg/week, platelet declines of 65% were observed by week 5 with subsequent plateau. Of 6 patients who received at least 3 doses at 6 mg/kg/week, 3 had Grade 2 and 2 had Grade 3 (dose-limiting) thrombocytopenia prior to the next weekly dose; suppression persisted for 2-3 weeks. With pre-treatment, the maximum tolerated dose appears to be 4 mg/kg/week. Plasma levels indicate dose proportionality with end of infusion rafAON levels of 0.3 to 0.9 μg/mL after 1 to 6 mg/kg. RafAON was detectable (sensitivity *10 ng/mL) for up to 24 hours post-infusion. At 4 mg/kg/week, 2 of 4 patients had treatment extended beyond the planned 8 weeks (16 and 32 weeks). Pharmacodynamic studies to assess intracellular c-raf mRNA and Raf-1 protein levels are in progress. Alternative formulation of LErafAON that may reduce IRR is underway. Patient enrollment continues at 4 mg/kg/week.

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Antisense oligonucleotides targeting ceramide glycosylation overcome multidrug resistance in cancer cells

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Glucosylceramide synthase (GCS) catalyzes ceramide glycosylation, disrupts ceramide-induced apoptosis elicited by chemotherapy, and appears be a major cause of multidrug resistance (MDR) in cancer. Previous studies pinpoint GCS as a therapeutic target for MDR [Liu, Y. Y., Han, T. Y., Giuliano, A. E., and Cabot, M. C. FASEB J. 15, 719-730, (2001)]. In this work, we have synthesized antisense GCS oligodeoxyribonucleotides (asGCS ODNs) to block GCS mRNA transcription, and tested several of the oligos for chemotherapy-enhancing properties in drug resistant cancer cell models. Of the eleven reagents generated, asGCS ODN-7 at low concentrations (EC_{50} 0.3 μM) displayed a dramatic inhibitory influence on cell growth. Antisense GCS ODN-7 suppressed GCS mRNA expression (RT-PCR) by 80%, and GCS protein (Western blot) by 40%. Consistent with down-regulation of GCS and the ceramide mode of anthracycline action, asGCS ODN-7 affected 30- and 10-fold increases in sensitivity to Adriamycin in drug resistant breast cancer MCF-7-AdrR (EC_{50} 0.25 vs. 7.8 μM), and in drug resistant ovarian cancer A2780-AD cells (EC50 0.6 vs. 6.0 mM), respectively. Further, asGCS ODN-7 increased MCF-7-AdrR cell sensitivity to Taxol, Vinblastine, and Actinomycin D by 3-, 9- and 11-fold, respectively. Compared to asGCS ODN-7, the GCS chemical inhibitor, PDMP (D-threo-1-phenyl-2-decanoylamino-3-morpholino-1-propanol), was less efficient and increased Adriamycin sensitivity approximately 4-fold. Subsequent studies revealed that asGCS ODN-7 overcomes drug resistance by enhancing ceramide-induced apoptosis and drug uptake. In conclusion, antisense GCS oligonucleotides effectively depress GCS expression, enhance apoptosis and drug uptake, and increase chemotherapy sensitivity, making them promising agents for cancer therapy.

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bcl-2 specific siRNA molecules inhibit growth of pancreatic cancer *in vitro* and *in vivo*

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Aim: Double-stranded oligoribonucleotides (siRNAs) effectively suppress gene expression via the RNA interference (RNAi) mechanism. In cancer cells, a variety of growth promoting and anti-apoptotic genes is overexpressed. Inhibition of bcl-2 expression should shift the bax/bcl-2 ratio towards pro-apoptotic bax and induce apoptosis. Anti-sense approaches have shown that inhibition of bcl-2 induces apoptosis in different tumor cells and enhances sensitivity for chemotherapy.

Methods: Pancreatic carcinoma cell lines YAP C and DAN G as well as human foreskin fibroblasts were transfected with siRNAs against bcl-2 in serum-free media for 4 h using Oligofectamine. The bacterial Neomycin-resistance gene served as control. Final concentrations of siRNAs ranged from 1 to 100 nM. After 24 to 120 h incubation, the number of vital cells was determined by trypan blue exclusion test and apoptosis was quantified by flow cytometry after propidium iodide staining. NMRI-mice transplanted with human pancreatic cancer xenografts were used as an *in vivo* model. Animals received daily intraperitoneal injections of 200 μg/kg siRNAs dissolved in physiologic saline for 28 days. Tumor diameters were determined by daily measurements. After sacrificing the animals, tumor specimens were subjected to conventional histology, immunohistology (bcl-2, Ki-67) and TUNEL staining. Total protein and RNA was extracted for Western and Northern Blot analysis.
Results: siRNA directed against bcl-2 induced apoptosis and reduced the number of viable cells in both tumor cell lines in a time- and dose-dependent manner, while mock-transfected cells or cells receiving control-siRNA remained unaffected. siRNA against bcl-2 delayed growth of pancreatic carcinoma xenografts *in vivo*. Western-Blot analysis revealed a down-regulation of target molecules in responsive cells as well as in tumor specimens.
Conclusions: 1) siRNA against bcl-2 induces apoptosis in human pancreatic cancer cells *in vitro*. 2) Induction of apoptosis is specific, control-siRNA has no effect. 3) siRNAs against bcl-2 is effective *in vivo*. 4) Application of oncogene-specific siRNAs may contribute to therapy of pancreatic carcinoma.

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Specific Inhibition of bcr-abl Gene Expression by small interfering RNA in bcr-abl+ Cells

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RNA interference (RNAi) as a protecting mechanism against invasion by foreign genes was first described in Caenorhabditis elegans and has subsequently been demonstrated in diverse organisms such as protozoa, insects, plants, fungi, and mammalian cells. RNAi is the mechanism of sequence-specific, post-transcriptional gene silencing initiated by double-stranded RNAs (dsRNA) homologoues to the gene being suppressed. dsRNAs are cleaved by cellular RNAses such as Dicer, to generate duplexes of 21 nt with 3'-overhangs (short interfering RNA, siRNA) that induce degradation of mRNAs. mRNAs derived as fusion transcripts from chromosomal translocations often found in human leukemias that encode oncogenic proteins are attractive targets for molecular defined therapeutical approaches. As a model system we focused on the bcr-abl mRNA, the fusion transcript arising from the Philadelphia translocation t(9;22)(q34;q11) expressed in about 95% of patients with chronic myelogenous leukemia (CML) and in about 20% of adult patients with acute lymphoblastic leukemia (ALL). In cotransfection assays using a recombinant reporter gene consisting of the bcr-abl-fusion sequence linked to the EGFP-gene (sb3a2-d4EGFP), several siRNAs reduced the number of sb3a2-d4EGFP+ cells by 90% and the fluorescence intensity per cell up to one hundred-fold. Electroporation of K562 cells expressing bcr-abl with anti-laminA/C siRNA inhibited laminA/C protein expression in up to 80% of the cells. Transfection with anti-bcr-abl siRNAs specifically reduced bcr-abl-mRNA expression up to 75% in K562 cells, while c-bcr- and c-abl mRNA expression were unaffected as quantified by real-time RT-PCR. Anti-bcr-abl- siRNA also inhibited proliferation of K562 cells up to 75% after 4 days. To analyze the effects of anti-bcr-abl siRNAs on cell proliferation in more detail we used the murine TonB cell line derived from IL-3 dependent BaF3-cells. In TonB cells that can be induced to express the b3a2-bcr-abl variant under control of a doxycycline-inducible promoter, anti-bcr-abl siRNA reduced bcr-abl mRNA expression by 70% independent of IL-3. Anti bcr-abl siRNA inhibited factor-independent, but not cytokine-dependent proliferation of TonB cells to a similar extent as the tyrosine kinase inhibitor STI571. These data demonstrate that siRNAs can specifically and efficiently interfere with expression of an oncogenic fusion gene and may revert malignant transformation in bcr-abl positive cells.

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Antitumor activity and I-124 pet imaging based on tumor-localized salmonella

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Genetically modified Salmonella typhimurium (TAPET-TK) expressing herpes virus thymidine kinase (HSV1-tk) display the ability to replicate in tumors, cause tumor necrosis and to accumulate I-124 labeled 2'-fluoro-1-beta-D-arabino-furanosyl-5-iodo-uracil (FIAU). Therapy with TAPET-TK Salmonella treatment is safe due to genetically stable attenuation of bacterial virulence. The parental strain of bacteria VNP20009 is currently being studied in phase I human clinical trials. Here, we demonstrate selective targeting and replication of TAPET-TK Salmonella in B16-F10 melanoma and its ability to be imaged using positron emission tomography (PET). C57BL/6 mice bearing B16-F10 melanomas were injected i.v. with 10e6 TAPET-TK Salmonella typhimurium or saline (control group). On the fourth day after bacterial injection, the animals received 200 uCi of I-124 labeled FIAU. Twenty four hours after FIAU injection, MicroPet acquisition was obtained. After imaging, tumors and normal tissues were removed and radioactivity and bacterial count assays performed. Immunohistochemistry with anti-Salmonella antibodies and H & E staining were also performed. Mean values of cfu/g for tumors varied from $8.4 \times 10e8 \pm 5.2 \times 10e8$ (sd) to $9.9 \times 10e8 \pm 4.8 \times 10e7$, and were 380, 720, 290 and 690 fold higher than that in muscle, liver, spleen and kidney, respectively. Tumor radioactivity was higher in the treated group than the non-treated control. Tissue radioactivity (% dose/g) correlated with tissue bacterial number in treated animals (cfu./g) and did not vary with tumor size. Tumor mass was less in the treated animals, compared to that in non-treated animals. A linear relationship was found between level of radioactivity and bacterial count in all the tissues assayed. Differences in tumor mass did not appear to alter the bacterial or radioactivity concentration. A difference in tumor mass and level of radioactivity between treated and control groups was observed consistent with treatment response. These results demonstrate that selective targeting of TAPET-TK Salmonella to tumors can be imaged non-invasively with [124I]-FIAU and PET.

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Antitumor activity and bystander effects of the tumor necrosis factor-related apoptosis-inducing ligand (trail) gene

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Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) has been reported to specifically kill malignant cells but to be nontoxic to normal cells. To evaluate the antitumor activity and therapeutic value of the TRAIL gene, we constructed adenoviral vectors expressing the human TRAIL gene. The *in vitro* transfer elicited apoptosis, as demonstrated by the quantification of viable or apoptotic cells and by the analysis of activation of pro-caspase-8 and cleavage of poly(ADP-ribose) polymerase. The intratumoral delivery elicited tumor cell apoptosis and suppressed tumor growth. In comparison with Bax gene treatment, which is toxic to normal cells, TRAIL gene treatment caused no detectable toxicity in cultured normal fibroblasts nor in mouse hepatocytes after systemic gene delivery. Furthermore, coculture of cancer cells expressing TRAIL with those expressing green fluorescent protein (GFP) resulted in apoptosis of both cells, whereas coculture of Bax-expressing cells with GFP-expressing cells resulted in the cell death of the Bax-expressing cells only, which suggested that the transfer of the TRAIL gene resulted in bystander effects. Transfection of the GFP-TRAIL gene into cancer cells resulted in the death of GFP-positive cells and their neighbors, whereas GFP-TRAIL genes, transfected into normal human fibroblasts or bronchial epithelial cells, did not kill such cells. Thus, the direct transfer of the TRAIL gene led to selective killing of malignant cells with bystander effect, which suggests that the TRAIL gene could be valuable for treatment for cancers.