after the injection of ONYX-015. Twenty patients were enrolled, 19 were eligible. Serious toxicities (> grade 2) were uncommon, and included hepatic (3), anemia (1), infection (1), and cardiac (1, atrial fibrillation). Sixteen patients were evaluable for response. Among these evaluable patients, 1/16 (6.3%) had a partial response, 1/16 (6.3%) had prolonged disease stabilization (49 weeks), and 8/16 (50%) had a >50% reduction in tumor markers. Among the 19 eligible patients,18 (94.7%) had specimens available for p53 analysis. Fifteen/18 (83.3%) had evidence of p53 mutation by one or both methods, although the methods correlated poorly. Viral shedding was confined to bile (2/2) and ascites (4/4). Pretreatment adenoviral antibodies were present in 14/14 patients and increased by 33.2% after ONYX-015 treatment. In the course of the trial, a patient with paired abdominal wall implants from a primary gall bladder carcinoma was injected with ONYX-015, followed by sequential excision of the lesions at 37 h and 7 days. Tissue sections were analyzed for evidence of viral replication using a novel assay, in situ RT-PCR to measure expression of hexon, a viral gene, which is expressed late during viral replication. Strong signals were obtained in gland-forming tumor cells both at 37 h and at 7 days, indicating that the virus was both present and replicating at those time points. Of interest, signal was also observed in adjacent normal stromal cells which were presumably wild-type for p53. Intralesional treatment with ONYX-015 in patients with hepatobiliary tumors is safe and well-tolerated, and some patients had evidence of an anticancer effect.

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Phase II Study of the DNA Methyltransferase I (DNMT1) Inhibitor MG98 in Patients (Pts) with Renal Cell Carcinoma (RCC). A Trial of the National Cancer Institute of Canada Clinical Trials Group (NCIC CTG)

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MG98, a 2nd generation phosphorothioate antisense oligodeoxynucleotide, is a specific inhibitor of human DNMT1 mRNA. Hypermethylation by DNMT1 is postulated to inactivate tumor suppressor genes leading to neoplastic transformation. Thus, inhibition of this enzyme might restore normal growth control. Two phase I trials of MG98 have been conducted by the NCIC CTG: 21-day continuous IV and a 2-hour twice weekly 3 out of 4 weeks schedule. The latter was selected for phase II evaluation because it was well tolerated up to 360 mg/m^2/dose and a pt with RCC had an objective response. This 2-stage phase II study of MG98 evaluating this dose and schedule was conducted in pts with recurrent, unidimensionally measurable RCC. Accrual to the second stage of this trial would occur if 1/15 pts had an objective response or if 11 or more pts showed stable disease for a minimum of 8 weeks. Pharmacokinetic (PK) evaluation and assessments of DNMT1 mRNA in PBMCs were done in all patients. 17 eligible pts were entered with the following characteristics: prior nephrectomy: 12; prior chemo- or immunotherapy: 0; ECOG PS 0/1: 6/11; male: 11. All pts were evaluable for toxicity and 15 for response. Toxic effects graded by CTC v 2.0 were: fatigue 16 pts (4 gr 3, 1 gr 4), nausea 13 pts (1 gr 3), anorexia 12 pts (1 gr 3), fever 9 pts (1 gr 3), neurosensory changes 6 pts (0 gr 3). No grade 3 or 4 neutropenia or thrombocytopenia was seen. Elevations in serum biochemistry were noted as follows: creatinine: 9 (0 gr 3), ALT: 13 (6 gr 3, 1 gr 4), AST 13 (1 gr 3). All creatinine changes and all grade 3 ALT and AST elevations were in the subset of patients (n=12) with prior nephrectomy. Pharmacokinetic evaluation is currently ongoing and will be available for presentation. Best response was stable disease in 7 pts and progressive disease in 8 pts. PBMC data are awaited. The trial closed to accrual after the first stage. Study supported by Grants from the National Cancer Institute of Canada and MethylGene Inc.

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Molecular imaging in the development of efficient gene therapy for human glioma

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Objective: To non-invasively evaluate the safety and efficiency of vector delivery and gene transduction after gene therapy of patients with recurrent glioblastoma by positron emission tomography (PET) and magnetic resonance imaging (MRI).
Methods: 8 patients (age: 49-67) received a stereotactically guided Gd-DTPA infusion with subsequent MRI and intratumoral convection-enhanced delivery (CED; max. flow: 0.6 ml/h, volume 30-60 ml) of a liposome-gene-complex (LGC; DAC-Chol/DOPE [w:w; 30:70]) transducing herpes simplex virus type 1 thymidine kinase (HSV-1-TK) as part of a Phase I/II clinical trial. To determine the transduction efficiency, PET was performed after injection of [124I]-2-fluoro-2-deoxy-1-β-D-arabinofuranosyl-5-iodo-uracil ([124I]FIAU), a specific marker substrate for HSV-1-TK. Ganciclovir (GCV) treatment (2 × 5 mg/kg/bw; 14 days) was started four days after LGC-infusion. Treatment response was recorded by means of MRI, [18F]-2-fluoro-2-deoxy-D-glucose (FDG) and [11C]-methionine (MET) PET.
Results: Infusion of LGC was tolerated well. In 1/8 patient specific [124I]FIAU-accumulation was observed as indication for HSV-1-TK expression in coregistration to signs of necrosis after GCV treatment as determined by FDG- and MET-PET. In 4/8 patients [18F]FDG- and [11C]MET-uptake was focally decreased in areas coregistering to the distribution volume of Gd-DTPA. Two patients showed transient reduction of the methionine positive tumor-volume by more than 50 %. All patients developed tumor relapses outside areas with reduced tracer activity.
Conclusions: Intratumoral convection-enhanced delivery of LGC is safe and leads to focal alterations of tumor activity. However, overall therapeutic efficacy is low indicating that more efficient vectors have to be engineered. Non-invasive imaging of vector distribution and vector-mediated gene expression by PET and MRI shall contribute to the development of standardized gene therapy protocols and improve efficiency and safety of vector applications in humans.

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TNFerade, an adenovector encoding the human tumor necrosis factor alpha gene, in soft tissue sarcoma in the extremity. safety and early efficacy data

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Background: TNFerade is a second-generation replication deficient adenovector, carrying the transgene encoding for human TNFa, regulated by the radiation-inducible promoter Egr-1. Purpose: The purpose of the study was to identify the maximum tolerated dose (MTD) of TNFerade in combination with radiation and to assess potential biologic activity in patients with extremity soft tissue sarcoma, receiving radiation pre-operatively or for palliation.
Method: Standard Phase Ib study, exploring in a dose-escalating fashion 3 dose levels of TNFerade (4×10^9 - 4×10^{11} pu), given in 1 log increments by intra-tumoral injections, twice weekly during week 1, then once weekly during weeks 2-5, concurrent with radiation (~50Gy).
Results: Accrual into cohorts 1-2 has completed with 7 patients enrolled. TNFerade + radiation was well tolerated with no dose limiting toxicities (DLTs) and no drug-related serious adverse events (SAEs). Plasma-TNFa levels remained low in all patients (<5pg/ml); no patients had virus detected in cultures from blood or urine. Of the 6 patients evaluable for response assessments, 4/6 received TNFerade + radiation pre-operatively, 2/6 received TNFerade + radiation for palliation. Of the pre-operative patients, 3/4 showed a complete pathologic response and 1/4 showed >95% necrosis (PR). This is remarkable as these patients had very large tumors (baseline volumes of 837, 2985, 4056 and 2142cm3, respectively). One palliation patient demonstrated radiologic PR, the other stable disease. However, CT

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.