

expressed in a cervical tissue derived from transgenic mouse, when compared with a cervix derived from normal mouse. From these results, we conclude that the E7 transgene expression inactivates the transactivation function of IRF-1 *in vivo*, which might be important for the elucidation of the E7-mediated immune evading mechanism that is frequently found in cervical cancer.

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Tumor necrosis alpha (TNF α) blockade as an adjunct to dose intense chemotherapy

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TNF α can be responsible for the fatigue/cachexia associated with malignancies. Docetaxel(D) when given weekly produces asthenia/fatigue in most patients(pts). We evaluated the ability of etanercept(soluble TNF receptor that neutralizes bioactive TNF α) to maintain the intensity of weekly D chemotherapy. 12 pts with refractory solid malignancies were randomized to receive D 43mg/m²/wk \times 6, every 8 wks) or D(same dose-schedule)plus etanercept(E)25mg SQ twice weekly. Escalations in the weekly doses of D in additional pts receiving E were planned in the event that dose intensity was maintained at the 43mg/m² dose. Plasma/serum samples for pharmacokinetics (PK) and unbound-TNF α and peripheral blood cells lysates for evaluation of the TNF-downstream transcription factor NF-kB and the relative expression of cytokines IL-1B, TNF α , IFN γ and IL-6(TaqMan real time RT-PCR) were obtained. 12 pts received 111 doses of D(alone, 32 doses; D/E, 79 doses). Three pts, all in the D/E arm, achieved partial antitumor responses and received at least 3 cycles (6 months). During the 1st cycle, 9 doses (38%) of D were missed due to toxicity on the D arm, whereas none were missed on the D/E group. D PK parameters(AUC₀₋₆, ng*hr/mL) were similar between days 1 and 29(717 \pm 372 and 817 \pm 470, respectively(n=12) and between single agent D(648 \pm 297,d1; 985 \pm 121,d29) and D/E (787 \pm 453,d1; 678 \pm 268,d29). Serum concentrations of unbound TNF were undetectable by bioassay in both arms. However, NF-kB relative activity (to HeLa+ cells)(9 pts) decreased on day 29(23.9 \pm 7) compared to baseline (36.9 \pm 6) in patients receiving D/E(mean % decrease, 30), and not in pts receiving D(29, 35.2 \pm 12; baseline 32.1 \pm 12)(mean % increase, 17). Mean relative expression of IL-1B, TNF α , IFN γ and IL-6 decreased on day 29 compared to baseline by 81, 59, 83 and 82%, respectively in pts receiving D/E (n=4) but increased with D alone (n=1). Three additional pts received D/E at 52mg/m²/wk D. At this dose, myelosuppression (grade 3 WBC) but not fatigue was the major factor limiting dose intensity (4/18 doses missed). In summary, the current study indicates that D PKs are similar after 4 wks of administration with or without E and that the D/E combination is well tolerated. The improved compliance in this preliminary study with this dose-intense chemotherapy regimen would suggest a role for TNF α in the associated asthenia/fatigue and offers a rationale for randomized studies focused in quality of life at conventional doses of weekly D. The cytokine and NF-kB regulated-gene expression patterns observed provide rationale for hypothesis driven explorations of this combination in man.

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A phase II trial of etanercept, a tumour necrosis factor- α inhibitor in recurrent ovarian cancer

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Purpose: There is preclinical data to support the role of TNF- α in the pathogenesis of ovarian cancer. We have initiated a phase II trial with etanercept (ENBR) in patients with recurrent ovarian cancer.

Methods: 17 patients received etanercept at a dose of 25 mg SC twice weekly. All patients were planned to receive a minimum of 3 months of treatment up to a maximum of 1 year, with disease evaluation at 3 monthly intervals. Surrogate end points for biological effect of etanercept included sE-Selectin, TNF- α , TNF-R1, MCP-1 and MMP-3 which were measured in the plasma (13 patients). A whole blood cytokine release inhibition assay was performed for IL-6 and MCP-1. The time points were pre treatment, 24 hours, 7 days, 28 days and 4 weeks thereafter.

Results: Median age was 54 years (range 34-75). 15/17 patients had optimal surgery, and 2/17 had suboptimal surgery. The histological subtypes were serous (13), clear cell (1), endometrioid (2) and mixed tumour (1). Previous chemotherapy included carboplatin and/or Taxol (12 platinum sensitive and 5 resistant), 11/17 patients had second line and 7/17 had third line chemotherapy. 11/17 patients completed 3 months or more of treatment. There was no significant toxicity. Two patients achieved stabilisation of disease for 6.3 and 10 months respectively with improvements in quality of life. Median overall survival was 9.6 months. There was a significant reduction in IL-6 levels in 9/13 patients (24 hours) which was maintained until 12 weeks (6/8 patients). MCP-1 levels declined in 8/12 patients on day 1 and by 3 months was inhibited by 50% (6 patients). All other surrogate markers did not change significantly with treatment.

Conclusions: Definite biological effect is seen at 3 months of etanercept therapy. A new cohort of patients are being treated with a dose of etanercept at 25mg thrice weekly.

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Modulation of cytokine production by SRIK-NKL, a Rare CD8+ NK cell line, by hormones: implications for carcinogenesis and treatment

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Natural killer (NK) lymphocytes are non-MHC restricted T cells involved in host defense against infection and malignancy, and can contribute to chronic inflammation via cytokine and defensin production. Tight regulation of NK function is critical, but mechanisms not fully defined. Modulation of NK activity is a potential strategy for cancer prevention and treatment. We established a unique CD8+ NK cell line (SRIK-NKL) from a patient with ALL and previously reported its constitutive cytokine profile (MIP-1 α , MIP-1 β , RANTES, GRO- α , IL-10, IL-4, TNF- α , TNF- β , IFN- γ , LIF, GM-CSF positive; IL-8, IL-6, IL-7, SCF negative). SRIK-NKL allowed us to further investigate the behavior of this unusual CD8+ subset of NK cells, specifically hormone responsiveness. Cells were incubated at 0.5 \times 10⁶ cells/ml in RPMI 1640 5%FCS/no antibiotics in CO2 incubator at 37 degrees C for up to 24 hours with and without hormone (tamoxifen or mifepristone 0.05-0.5 mM; progesterone, estradiol, or testosterone 0.5-5 mM; retinoic acid 1-10 mM; epinephrine 1 mcg/ml; substance P, VIP, somatostatin, or gastrin 1-10 mM) and supernatants quantitated for cytokines by ELISA (R&D). RANTES was significantly inhibited to 35-59% control by mifepristone and tamoxifen; MIP-1 α was decreased by retinoic acid to 52% control; but substance P and VIP greatly increased TNF- α to 166-226% control. Thus, cytokine production by CD8+ NK cells is altered by the hormonal milieu. This may be an important regulatory mechanism to consider when designing NK-dependent biologic treatments for cancer, or during the use of hormonal agents such as tamoxifen for established disease.

Gene therapy and antisense approaches

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Phase II study of ONYX-015 in patients with hepatobiliary tumors with p53 correlative studies

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ONYX-015 is a genetically modified adenovirus, which was designed to replicate preferentially in p53 mutated cells. To test the efficacy of ONYX-015, a phase II trial of intravesical ONYX-015 was conducted in patients with hepatobiliary tumors. All patients had biopsy-proven, measurable tumors of the liver, gall bladder or bile ducts which were beyond the scope of surgical resection. Patients received intravesical injections of ONYX-015 at either 6 \times 10⁹ or 1 \times 10¹⁰ pfu/lesion up to a total dose of 3 \times 10¹⁰ pfu. The status of p53 was assessed by immunohistochemistry (IHC) or Affymetrix GeneChip microarray analysis. Studies were conducted for viral shedding and for the presence of anti-adenoviral antibodies prior to and

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/m²/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 TNFα, 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 × 10⁹ - 4 × 10¹¹ 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-TNFα 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