genes in human cancer MG98 is a 20 nucleic acid phosphorothioate antisense oligonucleotide, targeted to the mRNA of DNA methyltransferase DNMT1. In vitro studies have shown that MG98 decreases expression of DNMT1, thus increasing the expression of tumour suppressor and cell-cycle regulating genes such as $p16^{ink4A}$. Previous Phase I studies of MG98 have indicated that a prolonged infusion of this compound may increase efficacy and provide sustained reductions in DNMT1 expression.
Methods: In the current study, patients received MG98 as a continuous infusion over 7 days, with a break of 7 days between cycles. The starting dose was 100mg/m^2/day. Samples of whole blood for the determination of DNMT1 expression in peripheral blood lymphocytes (PBL) were collected before, during and for up to 14 days after each of the first two cycles of administration. mRNA levels for DNMT1 were measured by a validated reverse transcriptase real-time PCR method, using beta-actin as a control for each sample. All analyses were performed in triplicate.
Results: Based on comparison with pre-treatment levels, DNMT1 expression in PBL decreased by between 17 and 69% on cycle one, and between 33 and 85% on cycle 2. The range of pre-treatment DNMT1 expression varied from 0.024 to 2.19 (arbitrary units). In some patients, an apparent rebound effect occurred, with increased DNMT1 expression at the start of cycle 2, compared with the pre-treatment value for cycle 1.
Conclusions: These data indicate that expression of DNMT1 is consistently decreased following MG98 infusion. Further investigations with this compound should include suppression of DNMT1 protein expression and measurement of subsequent changes in DNA methylation in clinical samples. This work was supported by the Vernalis Group of Companies" as well, that would be great (again, late request from our collaborators)

444 POSTER

A phase I/II study of oblimersen sodium in combination with oxaliplatin, 5-FU and leucovorin (FOLFOX4 regimen) in patients with advanced colorectal cancer

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Bcl-2 overexpression has been demonstrated in 30–94% of human colorectal cancer (CRC), appears to be an early event in CRC tumorigenesis and correlates with a negative prognosis in Dukes C CRC. In addition, bcl-2 overexpression confers a multi-drug resistant phenotype to tumor cells, including resistance to platinum derivatives. Oblimersen is an 18-mer phosphorothioate antisense oligonucleotide targeting the first 6 codons of the bcl-2 mRNA, and has demonstrated bcl-2 protein inhibition *in vitro* and *in vivo*. Oblimersen has been shown to enhance the effectiveness of apoptotic-inducing agents such as platinum derivatives. The objectives of this phase **I-II** trial were: to determine the maximum tolerated dose (MTD), to characterize the main toxicities, to assess plasma pharmacokinetics (PKs), to determine relevant predictive biomarkers and to document antitumor activity in colorectal patients treated with escalating doses of oblimersen, in combination with fixed doses of 5-FU, oxaliplatin and leucovorin (FOLFOX4 regimen). Oblimersen was given as a continuous IV infusion (CIVI) on days 1–7 and 15–21, and the FOLFOX4 regimen was administered on days 6–7 and 20–21 of a 28-day cycle. The protocol was subsequently amended to shorten the duration of oblimersen CIVI to 5 days (days 1–5 and 15–20), while maintaining the same schedule for FOLFOX4. To date, 16 patients [7 male/9 female, median age 52 (range 37–76)] have received 35 cycles of the combination over 3 dose levels of oblimersen: 5 mg/kg/day × 7 days, 7 mg/kg/day x 7 days, and 7 mg/kg/day × 5 days. Prolonged neutropenia resulting in dose delay > 14 days in cycle 1 was dose limiting in two patients, one at the 5 mg/kg/day and one at the 7 mg/kg/day × 7 days oblimersen dose levels, respectively. Additionally, one patient treated at the 7 mg/kg/day × 7 days oblimersen dose level experienced a treatment-related grade 3 fatigue. Other toxicities were mild to moderate (grade 1–2) and included vomiting (3 patients), diarrhea (5 patients), oral mucositis (4 patients) and proteinuria (5 patients). One patient experienced a complete response after cycle 2, and one patient had a partial response. PK and pharmacodynamic results will be presented at the meeting. Accrual continues at 7 mg/kg/day × 5 days oblimersen dose-level with FOLFOX4 standard dose.

445 POSTER

Genasense (G3139) causes apoptosis in melanoma cells by multiple mechanisms

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Genasense (G3139), an 18mer phosphorothioate oligonucleotide targeted to the bcl-2 mRNA, is an active agent in the treatment of stage IV melanoma. We have demonstrated that as a single agent, this molecule causes extensive apoptosis of 518A2 melanoma cells in tissue culture, characterized by decreased bcl-2 expression, mitochondrial membrane depolarization, caspase-3 activation, and bid cleavage. However, chemosensitization to a variety of drugs (taxotere, gemcitabine, thapsigargin) was not observed either with G3139 or with D6, an siRNA also targeted to bcl-2. Examination of the temporal progression of apoptosis subsequent to G3139 transfection demonstrated activation of caspase-3 (both by Western blotting and by measurement of DEVDase activity) by two hours after the five hour transfection. No activation of caspase-8 was observed. These changes could be completely reversed by the pan-caspase inhibitor zVADfmk, but not by the caspase-3/7 inhibitor DEVDfmk. However, cleavage of bid to tbid was reversible by DEVDfmk, demonstrating that here the activation of bid was downstream of caspase-3. In contrast, significant mitochondrial membrane depolarization (JC-1 staining) was not observed until 15 hours after the transfection, and could be correlated with the cytoplasmic appearance of cytochrome c both by Western blotting and immunohistochemistry. However, despite a dramatic decrease in cytosolic bcl-2 expression, mitochondrial bcl-2 expression did not change. As opposed to what we observed after G3139 administration, treatment of cells by cytotoxic chemotherapy led to synchronous mitochondrial membrane depolarization and the appearance of cytoplasmic cytochrome c 15 hours after the transfection. However, upregulation of bcl-2 protein expression by >1000 fold did not significantly increase chemoresistance nor change the temporal characteristics of caspase-3 activation, mitochondrial membrane depolarization, or apoptosis (as measured by Annexin V cell surface expression) after G3139 treatment. The emergence of capase-3 activation before mitochondrial membrane depolarization was not due to either lysosomal cathepsin or serine protease activation, as respective inhibitors (E64, pefabloc) were ineffective at altering the kinetics of caspase-3 activation. Rather, preliminary data suggests that intracellular acidification, induced by G3139, may produce the observed kinetics. Our data suggests that the lack of chemosensitization after G3139 treatment may occur because of early caspase-3 activation in a process that initially bypasses the mitochondrion.

446 POSTER

Evaluation of a cancer-specific Ad vector (Ad5-Id-1-luc) in the detection and monitoring of breast cancer

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Introduction: The goal was to develop a cancer specific vector and non-invasive imaging methods to detect and treat micro-sized non-palpable breast cancer.
Materials and Method: Id1 (Inhibitors of differentiation or Inhibitors of DNA binding) is a cancer specific promoter. Id1 protein, as a positive regulator for cell growth and negative regulator for cell differentiation, is highly expressed in malignant tumor cells, indicating Id1 promoter is highly active in these tumor cells. including in breast cancer cells. Using Id1 promoter (kindly supplied by Dr. Derperez) we developed a cancer-specific adenoviral vector (Ad5-Id1-luc) encoded for luciferase gene under the control of Id1 promoter. In vitro Studies; Human breast cancer cells (2LMP, T47D), ovarian cancer cells (Ovcar, SKOV3), prostate cancer cells (PC3, LNCap), non-small cell lung cancer cells (A427), and normal control cells (HUVEC) were grown in triplicate in 24-well plate (5x104cells/well). After 24 hours, cells were transfected with Ad5-Id1-luc (1x107 vp/well). Wells were imaged using a Xenogen IVIS 100 bioluminescence system. Luciferase quantity was measured as photons emission/sec. In vivo studies: Athymic nude mice (4/ group) were subcutaneously implanted with each of the cancer cell lines (4x106 cells/ site) at four different sites on the back. After three weeks, Ad5-Id1-luc 1x1010 vp/site) was injected directly into the tumor sites. In a separate set, a non-specific CMV promoter-controlled Ad vector was used to study the specificity of the vectors both in vitro and in vivo. Bioluminescence images were collected from the live mice after 24 hours and repeated on day 4 after Ad injection. Live images were collected with Xenogen system.
Results: CMV promoter driven Ad vector expressed reporter genes in all cancer cells in vitro and in vivo. However Id1 promoter driven Ad vector expressed luciferase only in cancer cells at different quantity. Reporter genes were expressed maximum in breast cancer cells. Ovarian cancer cells expressed at lower rate and prostate cancer cells expressed the minimum. More importantly, the normal cells (HUVEC) did not express the reporter genes in vivo. Nor did it express the reporter gene in the normal sc sites.
Conclusions: The present study indicates that Id1 promoter could be an effective promoter to detect breast cancer in live animal. Bioluminescence

imaging was highly sensitive to detect only a non-palpable cluster of cancer cells in vivo.

447 POSTER

Retargeting adenoviral gene therapy vectors to bladder tumours over-expressing EGFR and other cell surface markers

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The efficacy of adenovirus-mediated cancer gene therapy is potentially compromised by relatively low levels of expression of the human coxsackie-adenovirus receptor (hCAR) in a variety of tumour biopsy panels and cell lines. Several reports have described methods to by-pass the natural tropism of the adenovirus for hCAR, including genetic modification of adenoviral fibre-knob domains, adenobodies, diabodies and bi-specific fusion proteins (Dmitriev *et al*, 2000; *J. Virol.* **15**, 6875–84). Relative over-expression of epidermal growth factor receptor (EGFR) in human bladder tumour specimens compared with normal bladder urothelium correlates with poor prognosis. Retargeting of adenoviral cell-surface binding from hCAR to EGFR therefore represents an attractive means of achieving tumour-selective entry of adenoviral gene therapy vectors. In this report we describe the production of a fusion protein composed of the extracellular domain of hCAR fused with a 53 amino acid peptide encoding the mature form of human epidermal growth factor (EGF) (sCAR-EGF_{53}). Using a *lacZ*-expressing replication-defective adenovirus, we show that this protein preferentially targets tumour cell lines expressing low levels of hCAR and high levels of EGFR. Pre-incubation of the fusion protein with the adenovirus improves the infectivity of low-hCAR/high-EGFR cells from 2–5% to 95–100%. Reversal by anti-EGFR neutralising antibody confirms the specificity of the retargeting. Of equal significance, our fusion protein also hinders entry into high hCAR-expressing cells. We show that the sCAR-EGF_{53} protein increases marker gene expression in a variety of human bladder tumour cell lines, and that the improvement correlates well with CAR/EGFR expression status of the cells as determined by Western blotting. The design of our fusion protein construct permits substitution of EGF_{53} by other ligands, allowing the straightforward production of a number of fusion proteins designed to target cell surface molecules that are over-expressed in tumours. We demonstrate selective targeting of bladder tumour cell lines based upon knowledge of their expression profiles, using a panel of bi-specific fusion proteins.

448 POSTER

Targeting protein kinase CK2 for induction of apoptosis as an approach to cancer therapy

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Protein kinase CK2 (formerly casein kinase 2 or II) has been found to be dysregulated in cancers. Its role in cell growth and proliferation has long been known. However, besides its function in cell proliferation, CK2 can also exert a potent suppression of apoptosis mediated by agents such as etoposide, heat shock, and removal of survival factors (e.g., Ahmed et al., Trends in Cell Biology, 12: 226–230, 2002). We have now found that CK2 can block death receptor-mediated apoptosis induced by factors such as TNF-α, TRAIL, and Fas-ligand in responsive cells. Because of the extensive role of CK2 in cell growth and suppression of apoptosis, we examined the effects mediated by its downregulation. Various prostate cancer cells and squamous cell carcinoma of the head neck were treated with antisense CK2α. This resulted in extensive induction of apoptosis in a dose and time-dependent manner. Extension of these studies to xenograft models showed that prostate cancer tumors were eradicated when treated with antisense CK2α in vivo. Under similar experimental conditions the normal cells and tissue were relatively resistant to the effect of the antisense. We are also developing an approach to targeted delivery of the antisense to the tumors in vivo by encapsulating the antisense in sub-50 nm Tenascin nanocapsules which are taken up by the tumor cells via the caveolar route. Preliminary results suggest the feasibility of this approach for eradication of the xenograft tumor. These observations provide an initial "proof of the principle" for the potential feasibility of targeting CK2 for cancer therapy. [Supported by N.C.I. and V.A. Medical Research funds].

449 POSTER

Treatment of pancreatic cancer by TGF-beta2 suppression mediated by the antisense oligonucleotide AP 12009: Preclinical efficacy data

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Background: With a 5-year-survival rate of less than 1% pancreatic cancer is one of the most aggressive human cancers. Overexpression of TGF-beta2 in malignancies such as pancreatic cancer and glioma is associated with advanced tumor stage due to its pivotal role in malignant progression by inducing key mechanisms including immunosuppression, metastasis, angiogenesis and tumor cell proliferation. In pancreatic cancer patients TGF-beta2 levels were more than 3-fold elevated as compared to healthy controls.
Methods: Aiming at a highly specific anti-tumor therapy, AP 12009, a phosphorothioate antisense oligonucleotide specific for the human TGF-beta2 mRNA, has been developed and tested for its anti-tumor activity in a variety of preclinical studies with pancreatic cancer cells.
Results: AP 12009 significantly reduced the TGF-beta2 secretion in several human pancreatic cancer cell lines (Hup-T3, Hup-T4, PA-TU 8902). In functional assays AP 12009 inhibited pancreatic tumor cell proliferation in a dose-dependent manner by up to 76%. Migration of PA-TU 8902 cells was completely blocked as compared to untreated controls in a spheroid migration model in contrast to a TGF-beta2 antibody which had no effect. Additionally, AP 12009 reversed TGF-beta2 mediated immunosuppression in an allogenic system with pancreatic cancer cells targeted by IL-2 activated PBMC (LAK cells) derived from healthy donors. After AP 12009 treatment LAK cell cytotoxicity was increased in a donor-dependent manner up to 401% of the untreated control (effector/target ratio 20:1).
Conclusions: AP 12009 has already shown efficacy in phase I/II clinical studies as therapy for high-grade glioma. Based on this successful application of AP 12009 in clinical studies in glioma patients and the presented preclinical efficacy in tumor proliferation, migration and inhibition of escape from immunosurveillance in pancreatic cancer cells a multi-site dose-escalation trial with AP 12009 in pancreatic carcinoma has been started.

450 POSTER

The TGF-beta1 antisense oligonucleotide AP 11014 for the treatment of non-small cell lung, colorectal and prostate cancer: preclinical studies

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Background: Tumor derived transforming growth factor beta (TGF-beta) is a pivotal factor for malignant progression by inducing metastasis, angiogenesis and tumor cell proliferation and by mediating the tumors' escape from immunosurveillance. In colorectal, non-small cell lung and prostate cancer in particular expression of the TGF-beta1 isoform has been correlated with tumor progression and poor clinical prognosis. Significantly elevated TGF-beta1 plasma levels to more than 3-fold in colon cancer and NSCLC and to more than 4-fold in prostate cancer further support the role of TGF-beta1 as a key tumor promoter.
Methods: The in vitro effects of AP 11014, a TGF-beta1 specific phosphorothioate antisense oligonucleotide, on TGF-beta1 secretion, proliferation, migration of and immunosuppression by various cancer cell lines were determined.
Results: AP 11014 significantly reduced TGF-beta1 secretion by 43–100% in different NSCLC (A549, NCI-H661, SW 900), colon cancer (HCT-116) and prostate cancer (DU-145, PC-3) cell lines. Tumor cell proliferation was inhibited in a dose-dependent manner in all cell lines. In a scratch assay AP 11014 reduced migration of a NSCLC (SW 900) and prostate cancer (PC-3) cell line by max. 65% after 24h. Additionally, AP 11014 reversed immunosuppression mediated by NSCLC (NCI-H661, A-549), colon (HCT-116) and prostate cancer (PC-3) cell derived TGF-beta1 by increasing LAK cell cytotoxicity up to 368% of the untreated control (effector/target ratio 5:1).
Conclusion: These preclinical data clearly indicate antisense mediated suppression of TGF-beta1 by AP 11014 as a highly promising approach for the therapy of non-small cell lung, colorectal and prostate cancer in humans. Based on these data a clinical trial with AP 11014 in TGF-beta1 overexpressing tumors is in preparation.