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)

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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.

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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.

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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