as high-grade gliomas. Thus, AP 12009 is now applied in comparison to standard chemotherapy in an international phase II/III study with currently 26 study centers.

440 POSTER

Effects of bispecific antisense oligonucleotide targeting both insulin-like growth factor binding proteins 2 and 5 on cell survival and apoptosis in prostate cancer model

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Novel treatment modalities designed to prevent androgen-independent prostate cancer progression and metastasis are the subject of strong interest given the lack of success with currently available therapies to prevent or treat this lethal stage of disease. Antisense oligonucleotides (ASO), designed to a chosen cancer-relevant target gene, show enhanced specificity for malignant cells. Insulin-like growth factor binding proteins 2 and 5 (IGFBP2 and IGFBP5) are the members of IGF-I axis that is known to be critical in the regulation of neoplastic tumor progression and differentiation. IGFBP2 is a major binding protein in the prostate that is up regulated in prostate cancer during progression. IGFBP5 has been suggested to play a role in the metastasis of prostate cancer through its role in the bone microenvironment. Since both binding proteins are involved in prostate cancer development and progression, they provide potential targets for antisense strategies.
Methods: A prostate cancer tissue microarray spotted with 382 untreated and post hormonetherapy treated cancers was used to evaluate changes in IGFBP-2 and -5 after androgen ablation and in osseous metastases. Sequence similarity between the genes coding for IGFBP2 and IGFBP5 permits the design of bi-specific antisense oligonucleotide (bs-ASO) to target both IGFBP2 and IGBP5 mRNA. Dose-dependent sequence-specific effects of bs-ASO on mRNA level of IGFBP2 in LNCaP and C42 prostate cancer cell lines and IGFBP-5 in the SaOS-2 osteosarcoma cells were evaluated using Northern Blotting, while flow cytometry and MTT assay were performed to evaluate effects of bs-ASO treatment on cell cycle, cell growth, and apoptosis.
Results: Prostate cancer tissue microarray confirmed that IGFBP2 increased during prostate cancer progression to the androgen independent (AI) state. High level of IGFBP5 was found in prostate cancer metastasis. Northern blot showed dose-dependent sequence-specific down-regulation (up to 90%) of mRNA in cells expressing BP2 and BP5 respectively after bs-ASO treatment. bs-ASO treatment showed dose-dependent sequence-specific cell growth inhibition (from 50% to 90% depending on cell type), and 2-fold increase in subG0 apoptotic fraction and 3 fold G2/M arrest in prostate cancer cells. In order to identify the way by which bs-ASO may affect cell biological behavior, LNCaP and C42 cells were treated with the PI3K inhibitor LY294002. IGF-I is known to overcome LY toxicity, which was measured by AKT phosphorylation. bs-ASO completely inhibited the ability of IGF-I to overcome LY toxicity compare to control.
Conclusion: Bispecific antisense oligonucleotide targeting IGFBP-2 and IGF-BP5 could be seen as a potential therapeutic approach in prostate cancer patient, targeting both local disease and metastatic progression.

441 POSTER

Sensitizing NSCLC to chemotherapy by Bcl-2 siRNA – what is the optimal chemo combination?

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Background: Non-small cell lung cancer (NSCLC) is the leading cause of cancer-related death in men and women and adjuvant chemotherapy resulted so far in no major survival improvement. Defective apoptosis regulation is suspected to be a fundamental aspect of the treatment resistance of lung cancer. In NSCLC the anti-apoptotic Bcl-2 is expressed in up to 71% of all cases and has been associated with significant shorter survival. Abrogating the tumour-protective function of Bcl-2 and restoring chemosensitivity in NSCLC has been suggested a novel rationale for NSCLC therapy. Only recently antisense oligonucleotides (ASO) targeting Bcl-2 has been entered clinical trials and the concept of sensitizing NSCLC to taxotere will be currently studied in a Phase III trial.
Using an alternative strategy we evaluated in the present study synthetic small interfering RNA (siRNA) compounds targeting Bcl-2 to downregulate Bcl-2 expression in NSCLC.
Material and Methods: In A549 NSCLC bcl-2 regulation by siRNA was determined on mRNA and protein level by real time PCR and western blotting, respectively. For cell growth assays, cell numbers for single-agent and combination therapies were measured by cell counting. The number of apoptotic cells was examined by PI staining using FACS analysis and activated caspase 3 ELISA.
Results: Two Bcl-2 siRNAs were screened for their potency to specifically silence Bcl-2 expression in NSCLC. Treatment with Bcl-2 siRNA compounds at low nanomolar concentrations led to a dose and time dependent reduction of bcl-2 mRNA levels (up to 6-fold) and decreased Bcl-2 protein expression down to 30%. As a result, silencing of Bcl-2 in NSCLC cells by siRNA alone (25nM) led to a clear inhibition of cell growth and increase in apoptotic cell death ($p<0.05$). However, combinations of Bcl-2 siRNA and taxotere at equipotent doses surprisingly did not show any synergistic anti-tumour activity in NSCLC, whereas combinations with other anti-tumour agents (e.g. cisplatin) indicate more favourable combination results (analysis ongoing).
Conclusion: These findings highlight Bcl-2 as an attractive target for molecular targeted therapies in NSCLC. Bcl-2 siRNA alone show a highly efficient anti-tumour activity while combination with taxotere did not result in synergistic results. Therefore, the optimal apoptosis inducing drug for combination with Bcl-2 targeting strategies needs to be determined.

442 POSTER

Depletion of DNA methyltransferase (DNMT)1, and/or DNMT3b mediates growth arrest and apoptosis in lung and esophageal cancer cells

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Background: Aberrant DNMT activity perturbs gene expression via chromatin remodeling mechanisms during malignant transformation. Recently we have observed induction of cancer testis antigen and tumor suppressor gene expression in biopsy specimens from thoracic oncology patients following prolonged Decitabine infusion. The present study was undertaken to evaluate the effects of DNMT depletion in cultured lung and esophageal cancer cells by antisense oligos (ASOs) as a possible prelude to evaluation of these agents in thoracic oncology patients.
Methods: A549 and CALU-6 lung cancer cells, SKGT5 and BIC esophageal adenocarcinoma cells, and normal human bronchial epithelial (NHBE) cells were transfected with ASOs specifically targeting DNMT1 or DNMT3b, or mismatch oligos using lipofectamine techniques. Quantitative RT-PCR, western blot, trypan blue exclusion, and ApoBrdU techniques were used to evaluate DNMT expression, proliferation, and apoptosis following ASO transfections. Gene expression profiles were assessed by long-oligo arrays.
Results: ASOs mediated specific, dose-dependent depletion of DNMT1 and DNMT3b, which coincided with a pronounced (80%) inhibition of proliferation of lung and esophageal cancer cells. These effects were not observed following ASO transfection of NHBE cells. Depletion of DNMT1 and /or DNMT3b mediated dramatic, caspase-dependent apoptosis in A549 (p53 wt) and CALU-6 (p53 −/−) lung cancer cells. In contrast, minimal apoptosis was observed in SKGT5 and BIC esophageal carcinoma cells following ASO transfections despite comparable inhibition of DNMT expression and proliferation. The antiproliferative effects of the ASOs were not attributable to induction of tumor suppressor genes such as RASSF1A or p16, and did not coincide with demethylation of genes encoding cancer testis antigens. p21 expression was induced in all of the cancer lines following DNMT1 and/or DNMT3b depletion; however p21 expression levels did not appear to directly coincide with apoptosis following ASO transfections. Micro-array analysis of ASO-transfected A549 cells revealed pronounced induction of a variety of genes mediating response to genotoxic stress. Interestingly, gene expression profiles following DNMT1, DNMT3b, or combined DNMT1/3b knockdown were remarkably similar, yet distinctly different from expression profiles mediated by low dose deoxyazacytidine.
Conclusions: ASOs targeting DNMT1 and DNMT3b mediate potent growth inhibition in lung and esophageal cancer cells. Further studies are warranted to define the mechanisms by which these ASOs induce apoptosis in lung cancer cells, and to examine potential strategies to sensitize esophageal carcinoma cells to the proapoptotic effects of DMNT depletion.

443 POSTER

Decreased expression of DNMT1 at the mRNA level following 7 day infusion of the antisense compound MG98 in a phase I study

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Background: DNA methylation in the promoter region of genes regulates gene expression and is involved in the silencing of tumour suppressor

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