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.

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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.

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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.

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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.

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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