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Use of low molecular weight polyethyleneimine conjugated to transferrin for siRNA delivery

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Background: Since transferrin-conjugated polyethyleneimine (PEI) has previously been shown to mediate delivery of DNA in vivo, providing shielding against serum nucleases and uptake by rapidly dividing cells, we investigated its utility for delivery of siRNA to gastrointestinal cancer cells.
Material and Methods: siRNA or siRNA was complexed with linear PEI alone or with a mixture of linear PEI and transferrin-conjugated PEI (TfPEI), and transfected into colonic and gastric gastrointestinal (GI) cancer cells. Particle size and zetapotential were measured and correlated with transfection efficiency and knockdown in vitro over 72 hours. Transfection efficiencies were investigated by immunofluorescent microscopy of cells transfected with fluorescent siRNAs or a GFP-expressing plasmid and knockdown was quantified in cells expressing the luciferase reporter gene following transfection with luciferase-specific siRNAs. The most effective formulation was also investigated in a bioluminescent xenograft model.
Results: In vitro up to 93% knockdown was achieved. The ratio of PEI:nucleic acid was found to be the most important factor for determining efficacy of uptake for both siRNA and DNA, with optimal delivery achieved at intermediate ratios, correlating with intermediate particle size and charge. siRNAs were more sensitive than DNA to use of a low N:P ratio. At higher N:P ratios, whilst knockdown at 24 hrs was reduced, increased knockdown was observed at later time-points. The siRNA formulation which gave sustained knockdown in vitro was also effective in knocking down luciferase activity in the in vivo model with a 42.7% lower luminescence in the treated group compared with the control group 48 hours after treatment.
Conclusions: This study provides proof-of-principle that peptide-conjugated low molecular weight PEI has potential as a method for therapeutic delivery of siRNA provided that complex formulation is carefully optimised. This approach provides the possibility of specifically targeting siRNA to cancer cells as well as protecting siRNA from degradation by serum nucleases, and thus has potential to reduce toxicicity in normal cells as well as improving the efficacy of siRNA delivery.

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Identification of novel and potent RNA inhibitors of ErbB3, based on Locked Nucleic Acid (LNA) technology

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Background: As part of a drug discovery program, the aim of this study was to design, synthesize and screen a library of short single stranded Locked Nucleic Acid (LNA)-antisense oligonucleotides targeting human ErbB3 in order to identify potent RNA inhibitors as drug candidates for subsequent *in vivo* studies. LNA is a nucleic acid analogue that displays much increased binding affinity towards both DNA and RNA compared to other second and third generation nucleotide analogues and increases the resistance to nuclease degradation when incorporated into an oligonucleotide. We and others have previously shown that LNA enhances the potency of single stranded mRNA inhibitors.
Materials and Methods: A library of approximately 20 short LNA-oligonucleotides targeting human ErbB3 was designed. The library of ErbB3 oligonucleotides was screened at different concentrations in the cancer cell lines 15PC3 and HUH7 by lipofection. Target mRNA downregulation was measured by quantitative RT-PCR. The most potent oligonucleotides were evaluated in relation to IC50 values, plasma stability, thermal stability (Tm), and functional tumor responses of apoptosis (caspase 3/7 activity) and anti-proliferation (MTS assays).
Results: Several ErbB3 mRNA inhibitors were identified with IC50 values below 4 nM, with respect to ErbB3 mRNA downregulation measured 24 h after transfection. Apoptosis assays showed that the ErbB3 modulation led to apoptosis induction in prostate (15PC3) and hepatoma (HUH7) cancer cells after transfection with 5−25 nM of ErbB3 inhibitor. In addition, the ErbB3 oligonucleotides showed anti-proliferative activity in the cancer cell lines at 5−25 nM concentrations. A scrambled control LNA-oligonucleotide included in the screens displayed no effect on proliferation and showed no induction of caspase 3/7 activity. All LNA oligonucleotides had a Tm above 60 ºC against complementary RNA and showed high plasma stability, whereby more than 90% of the oligonucleotides remained intact after 24 h incubation in mouse plasma.
Conclusions: Based on the results from the *in vitro* screenings using a library of ErbB3 LNA oligonucleotides, several very potent new ErbB3 mRNA inhibitors were identified. These ErbB3 inhibitors downregulate ErbB3 at low nanomolar concentrations, inhibit proliferation of cancer cells and induce apoptosis. One of these LNA oligonucleotides, the 16-mer SPC3920, is currently being evaluated in pre-clinical pharmacology by Enzon Pharmaceuticals.

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Enhanced efficacy of therapy of anti-CD20 antibody with Locked Nucleic Acid antisense oligonucleotide targeting Bcl-2 in human Burkitt's lymphoma xenografts

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Background: Cell survival by abolishing programmed cell death in cancer cells has been closely linked to high Bcl-2 expression. The therapeutic potential of reducing Bcl-2 in cancer cells has been documented and resistance to existing cancer therapies have been linked to Bcl-2.
Materials and Methods: The RNA antagonist, SPC2996, is a 16-mer oligonucleotide incorporating Locked Nucleic Acid (LNA) with unique high-affinity binding to Bcl-2 mRNA and enhanced resistance to nuclease digestion. SPC2996 has completed a phase I/II trial in CLL where a dose response effect of SPC2996 was observed with higher doses giving improved effects on lymphocyte counts, lymph nodes, time to progression and overall responses
Results: Here we report on the anti-tumour activity of SPC2996 alone and in combination with Rituximab in SCID mice bearing disseminated Raji or Namalwa human Burkitt's lymphoma. SPC2996 was administered IV daily at 5 mg/kg for 14 days while Rituximab was dosed IV twice weekly for 3 weeks. A scrambled oligonucleotide was used as a negative control. In the Raji model the combination of SPC2996 plus Rituximab showed synergistic effect with significant longer survival than either treatment alone and a T/C value of 245 compared to Rituximab plus the scrambled control oligonucleotide.
Analysis of the bone marrow at day 18 after tumor cell injection showed a significant reduction in the percentage of human tumor cells from 27.7% in mice treated with the scrambled control oligonucleotide to 1.3% with SPC2996 alone and no signal above background level with the combination of SPC2996 plus Rituximab.
In the Namalwa model Rituximab alone had no significant effect on survival while SPC2996 alone showed significant prolonged survival with a T/C value of 143 compared to Rituximab and 195 compared to saline. The combination of SPC2996 plus Rituximab significantly prolonged the survival even further.
The percentage of human tumor cells in the bone marrow at day 14 showed a reduction from 14.5% in mice treated with the scrambled control oligonucleotide to 6.6% in the Rituximab treated mice while only background staining was observed with SPC2996 alone and in combination with Rituximab.
Conclusion: We have here presented data on the LNA containing RNA antagonist SPC2996 targeting Bcl-2 in two different human Burkitt's lymphoma xenograft models. SPC2996 shows highly significant prolonged survival in combination with Rituximab compared to Rituximab as monotherapy.

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Combination of a fusogenic glycoprotein, pro-drug activation and oncolytic HSV as an intravesical therapy for superficial bladder cancer

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Background: There are still no treatments for superficial bladder cancer (SBC) which alter its natural history where 20% of patients develop metastatic disease. SBC is often multifocal, has high recurrence rates after surgical resection and recurs after intravesical live BCG (bacillus Calmette-Guerin therapy). OncoVexGALV/CD, an oncolytic herpes simplex virus 1 (HSV-1), has shown enhanced local tumour control by combining oncolysis with the expression of a highly potent pro-drug activating gene (yeast cytosine deaminase/uracil phospho-ribosyltransferase fusion [Fcy::Fur]) and the fusogenic glycoprotein from gibbon ape leukemia virus (GALV). Previous studies with OncoVexGALV/CD have shown enhanced cell killing and tumour shrinkage (in vitro and in vivo) within tumours derived from