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Survivin mRNA antagonists using Locked Nucleic Acid, potential for molecular cancer therapy

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We have identified different mRNA antagonists against Survivin. These antagonists are a class of antisense oligonucleotides modified with Locked Nucleic Acid (LNA).
We and others have previously shown that LNA enhance the potency of single stranded mRNA antagonists. Currently, an mRNA antagonist targeting Bcl-2 has commenced a clinical study of safety and efficacy in patients with Chronic Lymphocytic Leukaemia.
Survivin is selectively expressed in most cancers and an elevated expression of Survivin is often associated with poor prognosis for the patient. In addition, several studies show that Survivin downregulation sensitises cancer cells to radiation and chemotherapy, which makes it a prominent molecular target for cancer therapy using mRNA antagonists.
We studied the effects of different Survivin mRNA antagonists in a prostate cancer model. Survivin mRNA and protein levels were analysed by qPCR and ELISA, respectively. The effect on cell division were studied by cell cycle arrest and apoptosis induction assays. The most potent Survivin antagonists were combined with taxol in order to synergise with this chemotherapeutic.
Our findings demonstrate the inhibitory potential of LNA modified mRNA antagonists against Survivin. These antagonists were found to be potent inhibitors of Survivin at low nanomolar concentrations. The synergistic effects of combining mRNA antagonists against Survivin with taxol were pronounced at concentrations of antagonists far lower than any other single stranded oligonucleotide ever used. Further characterisations *in vivo* are ongoing.

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Oncolytic adenovirus armed with artificial transcription factor as a highly potent agent for anti-cancer gene therapy

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Pathological neovascularization which is induced by complex angiogenic factors constitutes essential element in growth of many tumors. Among them is Vascular endothelial growth factor (VEGF), one of the most potent angiogenic factors.
We developed TG102, a humanized artificial zinc finger transcription factor (hZF) that binds specifically VEGF-A promoter and suppress its expression. To develop an anti-angiogenic cancer therapy using hZF, we used two prong approaches which use a membrane permeable-hZF protein, and an oncolytic adenovirus armed with hZF. The two methods were tested independently.
Treatment of purified PTD-TG102 suppressed VEGF strongly in various cancer lines, showing potential as a novel protein drug. When administered into nude mice transplanted with human cancer cells, PTD-TG102 attenuated tumor growth by 43%. While the anti-cancer effect was synergistically increased by combination with 5-FU, therapeutic effect has been shown limited possibly due to insufficient transduction into solid tumor tissues.
Use of oncolytic virus offers great opportunity to hZF drug development because the virus transduces high percentage of cells, and it replicates only in tumor cells, providing both anti-angionic and oncolytic dual activities. Replication-incompetent adenovirus Ad-dE1GFP (deletion of whole E1, E3) and oncolytic adenovirus Ad-dB7 (mutation on E10A, deletion of E1B 19kD/55kD and E3) have been served as controls, and compared their effect with each of those encoding TG102.
The VEGF expression was strongly suppressed in tumor cells infected with oncolytic adenoviruses armed with TG102 in-vitro. This anti-angiogenic effect was confirmed functionally by tube formation with HUVEC and aorta ring assays.
In-vivo studies using human cancer-xenograft nude mice showed that the TG102 delivered by replication-incompetent virus inhibited tumor growth by ~50% compared to controls. The oncolytic Ad-dB7 alone suppressed ~60% of tumor growth indicating oncolytic effect. Inhibition of tumor growth was dramatically enhanced by treatment of oncolytic Ad-dB7 armed with TG102.
Survival rate of xenograft mice resulted still striking outcome, showing 100% survival with Ad-dB7 armed with TG102 at the end point of 50 day-followup, compared to 50%-survival rates shown with different treatment (22day for PBS or Ad-dE1GFP, 40day for Ad-dB7).
Taken together, our results indicate that oncolytic virus armed with TG102 is a highly potent cellular- and gene-specific anti-tumor agent.

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Combination of c-myc and bcl-2 antisense oligonucleotides with docetaxel is highly effective in vitro and in vivo on hormone-refractory prostate cancer

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Background: The response of hormone-refractory prostate cancer (HPRC) to chemotherapy continue to be modest and the exploration of novel modalities of treatment is therefore essential to improve the prognosis. Based on the observation that transiton to HPRC is accompained by an increased expression of different oncogenes, including bcl-2 and c-myc, we evaluated if the concomitant down-regulation of these oncogenes by antisense oligonucleotides (ODNs) was able to sensitize HPRC to chemotherapy.
Materials and Methods: HPRC PC3 and DU145 lines were treated in vitro with ODNs against bcl-2 (G3139) and c-myc (INX-6295) alone and in combination with docetaxel. The efficacy of the different combinations was evaluated by analysis of cell proliferation, survival, cell cycle and apoptosis. The therapeutic efficacy of this multi-targeted therapy in combination with standard chemotherapy has been also assessed in xenografts, by giving repeated cycle of administrations, in terms of tumor weight inhibition, tumor growth delay and and increase mice survival.
Results: We showed that the triple combination given in the sequence G3139/docetaxel/INX-6295 is highly active in reducing the cell survival of HPRC lines to about 2% compared to 30% and 60% elicited by the combination of G3139/Docetaxel and by the inverse sequence INX-6295/docetaxel/G3139, respectively. The higher efficacy of G3139/docetaxel/INX-6295 combination was correlated to cell cycle perturbations leading to apoptosis induction. The results obtained in vivo demonstrated that the administration of G3139/docetaxel/INX-6295 completely inhibited the growth of PC3 xenografts for about 2 months after the start of treatment and resulted in 113% increase in overall survival of mice with 25% of mice being cured. Improved results were obtained in DU145 xenograts since the triple G3139/docetaxel/INX-6295 combination produced cures in 40% of treated mice. This combination exhibited efficacy also when the treatment started at a very late stage of tumor growth (about 500 mg of tumor mass) producing about 70% of tumor weight inhibition and a stable disease persisting for more than 60 days.
Conclusions: These data indicate that a multi-targeted approach based on the combination of ODNs able to down-regulated genes involved in uncontrolled proliferation and evasion of apoptosis is able to sensitize HPRC to chemotherapy and identify this as a promising strategy for the clinical management of this neoplasia.

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Silencing of pax8 transcription factor reduced the viability of glioma cells

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Background: The overall survival for patients with glioblastoma multiforme (GBM) has not improved over the last two decades. Understanding the molecular basis of GBM formation will facilitate the development of new therapeutics. We have previously shown that TP53 status correlates with telomere maintenance mechanisms (TMMs) and survival for patients with GBM. Paired box-containing transcription factors (PAX) have been shown to inhibit p53 function. We therefore studied PAX expression in relation to p53 status, TMM and survival in GBM.
Material and Methods: PAX 2, 5 and 8 expression was determined in 9 glioma cell lines and in 29 GBM biopsies by real time PCR. Results were confirmed by immunohistochemistry using phosphorylated PAX2 antibody recognizes the active form of PAX2, 5 and 8. The role of PAX expression in gliomas was determined by siRNA knockdown. The viability of gliomas treated with siRNA was measured by trypan blue exclusion assay.
Results: Relatively high levels of PAX8 message compared to PAX2 and PAX5 were detected in 66% (19/29) of GBM specimens and 44% (4/9) of glioma cell lines by real time PCR. Sixty six percent (21/32) of GBMs demonstrated more than 50% cells immunopositive for the active form of PAX2, 5, and 8 by IHC. High levels of PAX8 expression were associated with gliomas with wild type TP53 (wtp53) ($p = 0.0075$). PAX 2, 5, and 8 expression associated with poor outcome ($p = 0.02$). PAX8 knockdown with siRNA induced glioma cell death accompanied by an increased sub-G1 peak in the cell cycle analysis. Total cell number of PAX8 siRNA treated cells was reduced by more than 75% compared to the mock control in