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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

82% (9/11) cell lines, including 4 primary and 7 commercial cell lines, after 4 days treatment ($p < 0.001$). The cell death resulted from suppression of PAX8 was profound in GBM harboring mutant p53 (mtp53), whilst it was delayed in U87MG, which has wtp53.
Conclusion: The current study represents the first extensive analysis of the expression of PAX2, 5, and 8 and their phosphorylated proteins in gliomas. Our results showed that it is possible to induce a potent cytocidal effect by silencing PAX8 expression in a significant proportion of GBMs. PAX8 serves a pro-survival function in GBM, and therefore a potential therapeutic target in GBM.

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Combination of proapoptotic gene and cisplatin for the treatment of resistant SCLC xenografts in nude mice

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Background: Small cell lung cancer (SCLC) metastasizes at an early stage, and most cases relapse and become resistant to current therapies. A systemic therapeutic approach with higher specificity and efficacy is needed.
Materials and Methods: Lipids: DPEPC, DPPC, and DSPE-PEG2000 were used to make a stable liposome formulation to encapsulate the plasmid. Plasmids: an activity-enhanced human telomerase reverse transcriptase promoter (hTRpG) was used to drive bik (Bcl-2 interacting killer) or luciferase gene. CMV promoter was used as comparison in the similar constructions. Cells: human SCLC cell lines H69, H82, H466, H345 (ATCC) and human normal bronchial epithelium cells were used in vitro and in vivo studies. Nude mice were used in the efficacy studies. The main method was to use liposome delivered tumor-specific proapoptotic gene combined with chemotherapy for systemic treatment of human SCLC xenografts in nude mice.
Results: The transfection efficiency of the liposome formulation in human SCLC cell lines was equivalent to Fu-gene 6. The hTRp-driven luc specifically expressed in human SCLC cell lines but not in the normal cells, the RLU in the cancer cells was 5- to 20-fold higher than that in normal cells ($p < 0.002$). The liposome delivered hTRp-bik could significantly sensitize the chemo-resistant human SCLC cell lines for cisplatin treatment (by increasing the response [%killing] by >5-fold, $p < 0.004$). In nude mice bearing orthotopic human SCLC, the reporter gene expression after IV injections of the liposome delivered hTRp-luc was significantly higher in tumor but not in other organs compared with the same formulation delivering CMV-luc. The combination therapy of liposome delivered hTRp-bik and cisplatin in human SCLC xenografts in nude mice was 2- to 4-fold higher than the chemotherapy alone ($P < 0.01$).
Conclusions: The nonviral gene delivery system is capable for the systemic gene delivery in animal. The combination strategy presented above is significantly more effective than the optimal chemotherapy alone in the resistant human SCLC xenografts models. The survival genes of Bcl-2 family may be used as targets for sensitizing the resistant SCLC.

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Long-term suppression of tumor growth by intermittent administration of oblimersen sodium in combination therapy with taxanes and kinase inhibitors

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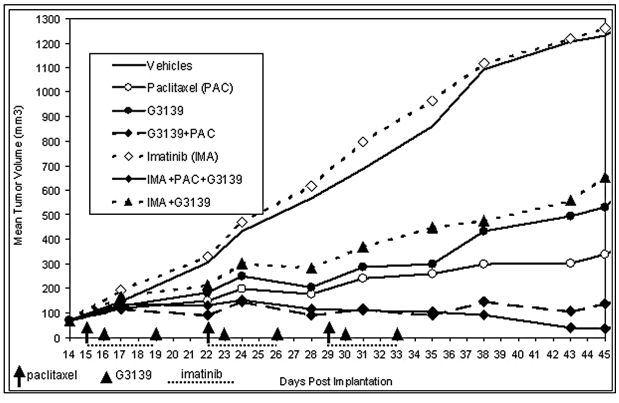
Background: Periodic dosing of the Bcl-2-targeted antisense oligonucleotide oblimersen sodium (G3139, Genasense®) has been shown to be as efficacious as or more efficacious than daily dosing against xenograft tumor models both as monotherapy and in combination with other agents. These results are highly consistent with the analysis of tumor sections taken from animals treated with fluorescently labeled oligonucleotide (FAM-G3139 or G4243). In these studies, intermittent dosing caused greater oligonucleotide uptake into xenograft tumor tissues, even when the total oligonucleotide dose was held constant or reduced by a periodic administration schedule. Finally, G3139 has been shown to increase the efficacy of other antitumor agents with distinct mechanisms of action, such as paclitaxel, DTIC, and kinase inhibitors gefitinib and erlotinib.
Materials and Methods: Xenograft tumors (A375 melanoma, A549 NSCLC, H460 NSCLC, HT29 colon carcinoma) grown in C.B-17/SCID mice were used to evaluate antitumor efficacy. G3139 was administered intravenously (i.v.) via bolus injections at levels ranging from 2.5 mg/kg/day daily to 20 mg/kg twice a week. Kinase inhibitors (erlotinib, imatinib, sunitinib, sorafenib) or temozolomide were administered orally (p.o.) daily. Taxanes (paclitaxel and paclitaxel albumin nanoparticles) were administered once a week i.v. Weight loss was used as a marker of overall toxicity.
Results: Intravenous administration of G3139 as infrequently as twice a week, in triplet combinations with a taxane and a kinase inhibitor was more efficacious than any doublet combination therapy. This result was most striking for imatinib in the A549 model, where single agent imatinib alone has no detectable activity at doses as high as 100 mg/kg/day. Weight loss was dependent on dosing schedules and drug sequencing. Highly effective therapeutic regimens were identified that were well tolerated and that could be administered repeatedly to completely suppress xenograft tumor growth. Growth delay and life span results will be presented.



A549 NSCLC treated with imatinib + paclitaxel + G3139.

Conclusions: G3139 does not require continuous administration to significantly inhibit tumor growth. On the basis of the preclinical efficacy of G3139 administered periodically via either subcutaneous bolus injections or short intravenous infusions, periodic dosing regimens will be incorporated into clinical trials combining oblimersen sodium with other agents such as alkylators, taxanes and kinase inhibitors.

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Carcinoma of the Ampulla of Vater with CIMP+ after treatment with pegylated liposomal based formulation of Ras siRNA combined with vinorelbine-tartrate exhibited inhibition of Raf/MEK/ERK, PI3K/AKT, DNA methylation and re-expression of tumor suppressor genes inducing type I, II, III PCD

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Background: Activated Ras signaling pathway due to mutations is common in carcinoma of the ampulla of Vater(CAV) causing upregulation of DNA and DNMT1 which leads to inactivation of tumor suppressor genes with subsequent chemoresistance due to inhibition of programmed cell death.
Materials and Methods: We treated chemoresistant CAV characterised by Ras codon 12 point mutations which activated the Ras/DNMT1/DNA methylation pathway with pegylated liposomal double stranded short interfering RNAs (siRNA) of synthetic 21 nucleotides specific to Ras combined with vinorelbine.
Results: Post-treatment, we observed Ras gene silencing after recognition of cognate mRNA through hydrogen bonding of the complementary short interfering RNA sequence leading to inhibition of DNA MeTase, DNMT1, Raf/MEK/ERK, PI3K/AKT, Wnt, p44/42 MAPK, cyclin D1, FGF-8, FGFR3, TGF-b, cyclin E-CDK2, RFC1, IL-8 and VEGF-R3 leading to reduced vessel density as assessed by CD31. Angiogenesis and lymphangiogenesis was inhibited. The complete degradation of Ras mRNA after the siRNA mediated RNA interference (RNAi) led to upregulation and re-expression due to epigenetic inactivation of PTEN, RB1, small GTPase RhoB, Par-4, GADD153, p16INK4a/p19Arf, p27Kip1, Skp2, thrombospondin-1, PKC-ä, CKIp21, HIC-1, p15, RARB, p53-MDM2, CHK2, RASSF2A, pcdc-25c and ATM. Vinorelbine-tartrate depolymerized microtubules at G2/M of tumor and endothelial cells and inhibited by phosphorylation the expression of anti-apoptotic and metastatic oncogenes bcl-2, bcl-xL, bcl-w and bcl-G, while it downregulated mcl-1, cIAP1, CAP2, XIAP, bfl-1/A1. Furthermore, vinorelbine upregulated 15-PGDH, ARHI, ICAD, Omi, Diablo, cyt-c, procaspase 7, bax, bak, bok, bad, bid, bcl-xs, bin, krk, Mtd, Nip3,