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Combination with a bcr-directed antisense oligonucleotide synergistically improves the antileukemic efficacy of erucylphospho-N,N,N-trimethylpropyl-ammonium in chronic myeloid leukemia cell lines

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The aim of this study was to enhance the antileukemic efficacy of the alkylphosphocholine erucylphospho-N,N,N-trimethylpropylammonium (ErPC3) in chronic myeloid leukemia (CML) derived cell lines by antisense oligonucleotides that reduce the BCR-ABL expression levels. Reduction of BCR-ABL expression levels was substantiated by Western blotting, and the efficacy by inhibition of colony formation. Electrotransfection of cells from a panel of five CML cell lines (BV173, CML-T1, LAMA-84, K-562 and AR-230) with an antisense oligonucleotide directed against the start codon of bcr (ASO-bcr) caused marked reductions in their BCR-ABL levels as evinced by Western Blot. The most resistant cell lines (K-562 and AR-230) showed reduced BCR-ABL expression only after repeated ASO transfection. ASOs directed against various junction sites of bcr-abl were effective only in those cell lines with the respective fusion protein. The clonogenicity of K-562 cells expressing high levels of p210 BCR-ABL was inhibited significantly by the ASO-bcr (T/C%: 30, $p< 0.05$), but not by ErPC3 (T/C%: 70). Combined sequential exposure to ErPC3 and the ASO-bcr, however, inhibited colony growth synergistically (T/C%: 3, $p< 0.01$). The colony growth of BV-173 cells expressing lower levels of p210 BCR-ABL than K562 cells was inhibited to a greater extend by the ASO-bcr (T/C%: 15, $p< 0.01$). AR-230 cells which express high levels of p230 BCR-ABL showed an intermediate decrease in colony formation in response to the ASO-bcr (T/C%: 20, $p< 0.05$). ErPC3 and the ASO-bcr did not reduce colony formation (CFU-GM) of normal mouse bone marrow cells from long term bone marrow cell cultures; instead, ErPC3 stimulated colony formation ($p< 0.05$) and did not induce chromosomal aberrations in mouse bone marrow. In conclusion, the ASO-bcr was effective in reducing BCR-ABL expression levels in cell lines with different types of fusion protein and this may be advantageous over more specific ASOs directed against the various junction sites. The combination of ErPC3 with a bcr-directed antisense oligonucleotide inhibited synergistically colony formation of CML cell lines without damaging normal cells and thus could be a useful tool for the purging of autologous hematopoietic transplants in CML patients.

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A novel human P450 reductase activated indolequinone prodrug for use in adenoviral mediated hypoxia selective gene therapy

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We have developed a novel synthetic series of indolequinone bioreductive pro-drugs similar in structure to the MMC to selectively target hypoxic cells that are refractive to other forms of therapy. From these we have selected 5-aziridinyl-3-hydroxymethyl-1-methylindole-4,7-dione (629) as our lead compound based upon its high hypoxic cytotoxicity ratio (HCR) compared with MMC in a panel of human tumour cell lines. Using transfected cell lines we have demonstrated that 629 exhibits selectivity for activation by the reducing enzyme cytochrome P450 reductase (P450R) particularly under hypoxic conditions resulting in an increased HCR compared to wild-type cells.
The MDA 468 cell lines were selected for *in vivo* evaluation of MMC and 629. Wild-type MDA 468 tumours were unresponsive to MMC (Treated/control [T/C] = 105%). However, although the reductase over expressing clone exhibited a 20-fold decrease in activity *in vivo* compared to *in vitro* (400 reduced to 15 nmol.cyt c.reduced min^{-1} mg^{-1}) these tumours responded to MMC (T/C = 57%, p=0.04). Similarly, wild-type MDA468 tumours transduced with an adenoviral vector encoding for P450R were sensitised to MMC treatment (T/C = 50.5%, p=0.008). In an on-going experiment we have witnessed a partial response of the P450R xenograft when 629 was administered in the same regime as that used with MMC (a single dose of 2mg/kg repeated after seven days) giving a T/C = 80%. Based upon the IC_{50} data it is likely that this response is driven by the specific cytotoxicity of 629 to the hypoxic cells in the P450R tumour. To expand on this work we have engineered an adenoviral vector encoding for P450R but with expression driven via a hypoxia responsive promoter. This vector has been used to infect a panel of human tumour cell lines (>60% transduction using a multiplicity of infection 100) resulting in a 5-10 fold increase in P450R levels within hypoxic cells. Using the virus and 629 to target the hypoxic tumour fraction we hope to modulate tumour responses to radiotherapy where the link between hypoxia and poor treatment outcome has been clinically established.

Cell Line	Clone	Drug	IC_{50} (μM)		HCR
			Air	Anoxia	
T47D	WT	629	11.2	0.57	19.6
		MMC	2.3	0.75	3.1
	P450R	629	0.49	0.0093	52.7
		MMC	0.13	0.13	1
MDA 468	WT	629	34.8	0.27	130
		MMC	2.6	0.6	4.3
	P450R	629	2.52	0.0041	619
		MMC	0.46	0.32	1.4
MDA 231	WT	629	25.6	2.56	10
		MMC	12.7	1.8	7.1
	P450R	629	2.15	0.036	59.7
		MMC	2.7	3.0	0.9

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A ribozyme-based gene therapy approach to target the survivin pathway in human prostate cancer cells

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Survivin is a structurally unique member of the inhibitors of apoptosis protein (IAP) family and is involved in the control of cell division and inhibition of apoptosis. Its anti-apoptotic function is related to the ability to inhibit caspases. The notion that survivin is expressed in most human tumors but absent in normal adult tissues with only a few exceptions has led to the proposal of survivin as a promising therapeutic target for novel anticancer therapies. In this context, we have constructed a Moloney-based retroviral vector expressing a ribozyme targeting the CUA110 triplet in survivin mRNA, encoded as a chimeric RNA within adenoviral VA1 RNA. In a cell-free system, the ribozyme was able to induce a dose-dependent cleavage of an *in vitro* transcribed RNA substrate corresponding to a portion of survivin mRNA. Androgen-independent DU145 human prostate cancer cells overexpressing survivin were infected with the retroviral vector, and a polyclonal cell population proven to endogenously express the ribozyme was selected. This population was characterized by a significant reduction of survivin expression in terms of mRNA (-83%, as detected by RT-PCR) and protein (-95% as detected by western blotting) compared to DU145 cells transduced with a control ribozyme. Survivin ribozyme-expressing cells underwent spontaneous apoptosis (20% TUNEL-positive cells) and showed processing of caspase-3 to its active subunits and enhanced caspase-3 catalytic activity. Moreover, DNA microarray analysis carried out in these cells revealed the modulation of several genes involved in the apoptotic pathways. Consistent with the role of survivin in the proper execution of mitosis, survivin ribozyme-expressing cells became polyploid and multinucleated. Survivin inhibition also affected the chemosensitivity profile of DU145; specifically, ribozyme-expressing cells displayed cisplatin-induced apoptosis threefold that of control cells. Finally, survivin inhibition completely prevented tumor formation upon s.c. injection of DU145 cells into athymic Swiss mice. Results from the study indicate that ribozyme-mediated survivin inhibition was able to reduce the proliferative potential of DU145 cells and to increase their response to chemotherapy and suggest that manipulation of the anti-apototic survivin pathway may provide a novel approach for treatment of androgen-independent prostate cancer.

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Phase I study of G3139, a bcl-2 antisense oligonucleotide, combined with carboplatin and etoposide in patients with previously untreated extensive stage small cell lung cancer

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The combination of carboplatin and etoposide is a commonly used regimen for newly diagnosed extensive stage small cell lung cancer (SCLC). The apoptotic inhibitor Bcl-2 is expressed in the majority of SCLC and its expression has been correlated with chemotherapeutic resistance in several

tumor models. G3139 (Genasense, oblimersen sodium; Genta Inc., Berkeley Heights, NJ) is a phosphorothioate oligonucleotide complementary to bcl-2 mRNA. Treatment with G3139 can reduce Bcl-2 protein levels in vivo. Since Bcl-2 overexpression is thought to contribute to chemotherapeutic resistance, Bcl-2 suppression by G3139 may enhance the anti-tumor efficacy of standard cytotoxic chemotherapy. We have previously reported that a combination of G3139 and paclitaxel led to prolonged stable disease in a small number of patients with advanced chemorefractory SCLC. Here we report initial results of a phase I study evaluating the combination of G3139, carboplatin, and etoposide in patients with previously untreated extensive stage SCLC. Eleven patients have been treated to date in 3 dose cohorts. The primary goals of this study are to assess toxicity and to determine a maximally tolerated dose for this combination. Cohort 1 initially received G3139 5 mg/kg/d IVCI days 1-8 on a 21 day cycle, with carboplatin AUC=6 on day 6 and etoposide 80 mg/m^2/d, days 6-8. Of the 4 patients evaluable for toxicity in cohort 1, 2 developed grade 4 neutropenia in cycle 1. Cohort 2 was initiated at identical G3139 and etoposide doses, with carboplatin dose reduced to AUC=5. One patient in each of the first two cohorts elected to discontinue therapy before completing cycle 1. All other patients in both dose cohorts have completed 6 cycles of therapy. In cohort 2, 0 of 4 patients experienced cycle 1 DLT. Several patients did require dose delays in later cycles due to hematologic toxicity. This may be due to the truncation of days from last chemotherapy to next cycle from 19 to 14 because of the 5-day lead-in period of G3139 administration. No non-hematologic toxicities > grade 2 have been attributed to therapy. Enrollment is continuing in cohort 3, with G3139 7 mg/kg/d, carboplatin AUC=5, and etoposide 80 mg/m^2 × 3; 2 patients in this cohort are in cycle 3 and 4, respectively. Overall, in 9 patients evaluable for response, we have documented PR in 7, and SD in 2. Final toxicity and clinical outcome data will be presented. The phase II dose will be tested in a randomized trial within the CALGB.

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G3139 (Genasense; Oblimersen) induces production of reactive oxygen species and hydrogen peroxide in human prostate and bladder carcinoma cells in a backbone and cpg-dependent manner

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PC3 and T24 cells, when treated with 400 nM G3139/Lipofectin or Oligofectin, produce reactive oxygen species (ROS) and H2O2, as measured flow cytometrically by hydrethidium-ethidium and fluorescence and The production of ROS and H2O2 were measured flow cytometrically by hydroethidine to ethidium oxidiation and 2'-7'-dichlorofluorescein fluorescence, respectively. The increase in ROS and H2O2 were is as much as 500% and 250% respectively, vs. untreated cells after 72 h incubation. Maximum generation of ROS and H2O2 was observed after 72 hours, and near maximal generation as long as 5 days after initial incubation, and at a 200 nM oligo concentration, but no lower. A control oligo, 4126, (a two base mismatch of G3139 in which the two CpG motifs are eliminated), produced only a small increase in ROS and H202 production (<50%). However, the mechanism of production was unlike that in immune cells because cells treated with naked oligo (5 microM) did not increase ROS or H2O2 production. Treatment of cells with 2006, a 24mer phosphorothioate triple tandem repeat of an optimized CpG motif (GTCGTT) did not downregulate bcl-2 expression, but did induce ROS and H2O2 production to the same extent as G3139, indicating that bcl-2 downregulation did not cause the increase in production. Confirming this observation, we found that oligo 2009, which is directed to the coding region of the bcl-2 mRNA, downregulated the expression of bcl-2 protein to the same extent and with identical kinetics as G3139, yet did not induce the production of ROS and H2O2. Elimination of the increase in production of ROS and H2O2 could be accomplished by either cytosine C5-methylation, or even more dramatically by C5-propynylation of both of the CpG motifs of G3139. The rate of growth of cells treated with either of these two oligomers (which do not induce the production of ROS and H2O2) was approximately identical to those cells treated with 2009 or 4126 (both of which do not), and much faster than those cells treated with either G3139 or 2006 (both of which do, although the latter does not downregulate the expression of bcl-2). However, 4126 could be transformed into an ROS and H202 producing oligo by modification of the backbone with five LNA (locked nucleic acid) linkages at the 3' and 5' positions. Thus, it appears that there is a CpG directed, non-bcl-2 dependent induction of production of ROS and H2O2 in PC3 and T24 cells that strongly affects the rate of cell growth.

Monoclonal antibodies and immunoconjugates

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A new Tc-99m labelled somatostatin analogue (Tc-99m EDDA-TRYCINE-HYNIC-TOC) for receptor imaging: first clinical results before and during radioreceptor therapy with Y-90 DOTATOC

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Aim: Radioreceptor therapy using radiolabelled peptides is a promising new approach for the treatment of neuroendocrine tumors. We evaluated Tc-99m EDDA-TRYCINE-HYNIC-TOC (TET-H-TOC) in patients with somatostatin receptor (SSTR)-positive tumours (staging, dosimetry and follow-up).
Methods: The Tc-99m labelled somatostatin analogue was synthesized in our pharmaceutical lab using lyophilized kits (radiochemical purity by HPLC, TLC >95%, product stability *in vitro* 4 to 6h). So far, 46 patients (53 examinations) were studied after injection of 580-890 MBq (median 673 MBq) TET-H-TOC. The histologically proven tumours were endocrine neoplasias, renal carcinomas, bronchial carcinoma, mesothelioma and malignant fibrous histiocytoma. The imaging protocol consisted of whole-body scans and planar images of the tumor region (15 min, 1h, 2h, 4h, 8h, 24h p.i.) and additionally SPECT-images (1h und 4h p.i.). For semi-quantitative assessment, individual regions of interest (ROI) were drawn in order to generate time-activity curves and to calculate tumour-to-tissue/background ratios. Pharmacokinetic analysis was carried out (radioactivity kinetics in plasma and urine). In some selected patients, image fusion of the whole-body scans was performed with CT and/or MRT and/or PET using a HERMES computer.
Results: 7 out of 46 patients showed an intense tracer accumulation in the SSTR-positive tumours (visual 3+, tumour / background ratio >2.5). In these patients, radioreceptor therapy was carried out using Y-90 DOTATOC (simultanous injection von 150 MBq In-111 DOTATOC). All pretherapeutic scans with the Tc-99m labelled ligand (4h p.i.) showed a similar overall pattern of biodistribution and tumour uptake in comparison to the therapy scans with In-111/Y-90 DOTATOC (24h p.i.). The Tc-99m EDDA-HYNIC-TOC scans (incl. SPECT) offered superior imaging properties with earlier tumour visualisation (all lesions were detected 1h p.i.) as compared to the In-111 labelled analogue. Hence, the receptor scintigraphy with Tc-99m EDDA-TRYCINE-HYNIC-TOC enables to select patients suitable for radioreceptor therapy with Y-90 DOTATOC.
Conclusion: Our results demonstrate the ability of Tc-99m EDDA-TRYCINE-HYNIC-TOC for receptor scintigraphy of SSTR-positive tumours with superior image performance as compared to In-111 labelled SST-analogues (staging), for an individual selection of patients suitable for a radioreceptor therapy with Y-90 DOTATOC (dosimetry) and for post-therapeutic control.

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Cetuximab (C225, Erbitux) in combination with irinotecan, infusional 5-fluorouracil (5-FU) and folinic acid (FA) is safe and active in patients (pts) with metastatic colorectal cancer (CRC) expressing epidermal growth factor-receptor (EGFR). Results of a phase I study

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C225 is a chimeric antibody targeted against EGFR with activity in refractory CRC. Our current phase I trial evaluates the feasibility and safety of C225 combined with irinotecan and weekly infusional 5-FU/FA (modified AIO-regimen) as 1st-line treatment for pts with CRC. 24/27 screened tumors were found positive for EGFR by immunhistochemistry, and 21 positive pts entered the trial. None of them had previous palliative chemotherapy or relevant organ dysfunction. After a loading dose of 400 mg/m^2 i.v., C225 was given weekly at a dose of 250 mg/m^2 i.v. Chemotherapy was administered weekly × 6, followed by 1 week rest, and consisted of irinotecan 80 mg/m^2, FA 500 mg/m^2 and 5-FU 1500 mg/m^2/24h (low dose group) or 2000 mg/m^2/24h (high dose group). Dose limiting toxicity (DLT) was defined as neutropenia or skin toxicity >grade 3, neutropenia/leukopenia with fever;