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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