

# Antitumor Activity of Antisense Clusterin Oligonucleotides Is Improved in Vitro and in Vivo by Incorporation of 2'-O-(2-Methoxy)Ethyl Chemistry

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## ABSTRACT

Phosphorothioate (P=S) antisense oligonucleotides (ASO) targeting the cell survival gene clusterin synergistically enhance castration- and chemotherapy-induced apoptosis in prostate cancer xenografts. This study compares efficacy, tissue half-lives, and toxicity of P=S clusterin ASO to third-generation backbone 2'-O-(2-methoxy)ethyl (2'-MOE) ribose-modified clusterin ASO. Northern analysis quantified changes in clusterin mRNA levels in human PC-3 cells and tumors. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay measured effects of combined clusterin ASO plus paclitaxel on PC-3 cell growth. Athymic mice bearing PC-3 tumors were treated with paclitaxel plus either P=S clusterin ASO, 2'-MOE clusterin ASO, or mismatch control oligonucleotides for 28 days. Weekly body weights and serum parameters were measured to assess toxicity. Tissue half-life of P=S and 2'-MOE ASO in PC-3 tumors was assessed using capillary gel electro-

phoresis (CGE). Both 2'-MOE and P=S ASO decreased clusterin mRNA levels in a dose-dependent and sequence-specific manner. 2'-MOE ASO more potently suppressed clusterin mRNA (80 versus 40% at 500 nM) compared with P=S ASO. IC<sub>50</sub> of paclitaxel was equally reduced (50–75%) by both compounds. In vivo tissue half-life was significantly longer for 2'-MOE-modified ASO than for P=S ASO (5 versus 0.5 days). Using CGE, >90% of detected 2'-MOE ASO in tumor tissue was full length. Weekly administration of 2'-MOE clusterin ASO was equivalent to daily P=S clusterin ASO in enhancing paclitaxel efficacy in vivo. 2'-MOE-modified ASO potently suppressed clusterin expression and prolonged tissue half-lives with no additional side effects. These results support the use of 2'-MOE-modified ASO over conventional P=S ASO by potentially increasing potency and allowing longer dosing intervals in clinical trials.

Clusterin, also known as testosterone-repressed prostate message-2 or sulfated glycoprotein-2 (TRPM-2), was first isolated from ram rete testes fluid (Montpetit et al., 1986) and has been implicated in a wide variety of physiological and pathological processes, including tissue remodeling, reproduction, lipid transport, membrane protection, complement defense, and apoptotic cell death (Sensibar et al., 1995). Since clusterin expression is increased in various benign and malignant tissues undergoing apoptosis, it has been associated with cell death (May et al., 1990; Connor et al., 1991; Kyprianou et al., 1991). Recent observations suggest that clusterin acts in a chaperone-like manner similar to that of small heat shock proteins, potentially inhibiting stress-induced protein

precipitation in vitro and appears to improve cell survival in vivo (Humphreys et al., 1999). In prostate cancer, experimental and clinical studies suggest that clusterin increases after therapeutic cell death signals, such as androgen ablation or chemotherapy, and functions to protect cells against apoptotic cell death (Miyake et al., 2000c, 2001). When overexpressed, clusterin confers a hormone- and chemoresistant phenotype, marking it as a target for therapy (Miyake et al., 2000c, 2001).

More than 20 years ago, Zamecnik and Stephenson (1978) first proposed using synthetic antisense oligonucleotide (ASO) analogs as a new class of rationally designed therapeutics capable of specifically inhibiting the synthesis of a chosen target protein. Since then, intense efforts have been mounted to improve ASO activity. Two important factors influencing efficacy of an ASO are the affinity for targeted mRNA to bind with a high degree of specificity

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**ABBREVIATIONS:** ASO, antisense oligonucleotide; P=O, phosphodiester; P=S, phosphorothioate; 2'-MOE, 2'-O-(2-methoxy)ethyl; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; CGE, capillary gel electrophoresis; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.

and its ability to resist degradation by intracellular nucleases. First-generation phosphodiester (P=O) ASO is highly unstable to nucleases, largely precluding their use to efficiently inhibit the expression of a targeted mRNA. In a first step to increase nuclease resistance, an equatorial oxygen atom in the phosphate backbone of P=O ASO was replaced by a sulfur atom. This phosphorothioate (P=S) modification provided considerable stability to both exo- and endonucleases (Wagner, 1994). However, each incorporation of a P=S generates a chiral center and reduces binding affinity for target mRNA. Furthermore, although P=S ASOs are less sensitive to nucleases, they will degrade in cells over time (McKay et al., 1996). To overcome these drawbacks, ongoing research has focused on backbone modifications that provide a more attractive pharmacological profile than P=S ASO. Among a number of different modifications at the 2'-sugar position, the 2'-*O*-(2-methoxy)ethyl (2'-MOE) incorporation was identified as enhancing both binding affinity and further resisting degradation by intracellular nucleases (Altmann et al., 1996). The 2'-MOE modification resulted in decreased binding affinity to RNase H, the principal nuclease that cleaves ASO-bound mRNA. This problem was overcome by the use of "gapped" ASO such that the 5' and 3' ends of the molecule contained 2'-MOE-modified sugar residues and the central portion of the ASO contained 2'-deoxy sugar residues that support RNase H activity (Monia et al., 1993; Baker et al., 1997). This chemical design is usually accompanied with a uniformly modified P=S backbone. The incorporation of 2'-MOE modifications into 20-mer P=S ASO showed a dramatic effect on the ability of the sequence to hybridize to a target mRNA as a result of the conformation of the sugar and the backbone. Furthermore, 2'-MOE incorporation into P=S ASO exhibited substantially increased resistance to intracellular nucleases, compared with conventional P=S ASO. Both increased hybridizing affinity toward the targeted mRNA and enhanced resistance toward both serum and intracellular nucleases resulted in a 20-fold increase in activity of 2'-MOE-modified ASO (McKay et al., 1999). The enhanced potency of this new class of ASO did not lead to any decrease in specificity.

We recently reported that inhibition of clusterin mRNA expression using conventional P=S ASO enhances chemotherapy-mediated apoptosis in prostate cancer (Miyake et al., 2000a). In this study, we compared the *in vitro* and *in vivo* efficacy, tissue half-lives, and toxicity of a conventional P=S and a 2'-MOE-modified ASO targeted against clusterin mRNA. All *in vitro* and *in vivo* experiments were conducted using the human prostate cancer cell line PC-3.

## Materials and Methods

**Tumor Cell Line.** PC-3, derived from hormone-refractory human prostate cancer, was purchased from the American Type Culture Collection (Rockville, MD). Cells were maintained in Dulbecco's modified Eagle's medium (Life Technologies, Gaithersburg, MD), supplemented with 5% heat-inactivated fetal calf serum and routinely passaged when 90% confluent.

**Antisense Clusterin Oligonucleotides (Clusterin ASO).** P=S and 2'-MOE-modified ASO used in this study were synthesized as described previously (Monia et al., 1993; Dean et al., 1994). The sequence of the clusterin ASO used corresponded to the human clusterin translation initiation site (5'-CAGCAGCA-

GAGTCTTCATCAT-3'). A two-base clusterin mismatch oligonucleotide (5'-CAGCAGCAGAGTATTATCAT-3') was used as a control. Conventional P=S clusterin ASO was previously demonstrated to significantly inhibit clusterin mRNA expression in a dose-dependent and sequence-specific manner (Miyake et al., 2000a). The sequence of the P=S and 2'-MOE antisense analogs, and their controls, were identical. The design of the 2'-MOE analogs was CAGCAGCAGAGTCTTCATCAT in which the underline bases represent 2'-MOE residues.

**Treatment of Cells with ASO.** Lipofectin, a cationic lipid (Life Technologies) was used to increase the ASO uptake of cells. PC-3 cells were treated with various concentrations of ASO after they have been preincubated for 20 min with 10  $\mu$ g/ml lipofectin in serum free OPTI-MEM (Life Technologies). Four hours after the beginning of the incubation, the medium containing ASO and lipofectin was replaced with standard culture medium described above.

**Northern Blot Analysis.** Total RNA was isolated from cultured PC-3 cells and PC-3 tumor tissues using the acid-guanidinium thiocyanate-phenol-chloroform method. Electrophoresis, hybridization, and washing conditions were carried out as previously reported (Miyake et al., 1998). Human clusterin and GAPDH cDNA probes were generated by reverse transcription-polymerase chain reaction from total RNA of human kidney using primers 5'-AAGGAAAT-TCAAATGCTGTCAA-3' (sense) and 5'-ACAGACAAGATCTCCCG-GCACTT-3' (antisense) for clusterin, and 5'-TGCTTT-TAACTCTG-GTAAAGT-3' (sense) and 5'-ATATTTGGCAGGTTTTTCTGA-3' (antisense) for GAPDH. Density of bands for clusterin was normalized against that of GAPDH by densitometric analysis.

**Capillary Gel Electrophoresis (CGE).** CGE (PACE 5000 System; Beckman, Fullerton, CA) was used to determine the fraction of full-length ASO in PC-3 tumors and confirmed by 20% denaturing polyacrylamide gel electrophoresis and laser scanning densitometry (Molecular Dynamics, Sunnyvale, CA). For CGE, a 100- $\mu$ l solution of ASO, at a concentration of  $\sim$ 0.1 AU<sub>260</sub>, was used for electrokinetic injection at -5 kV into a 45-cm polyacrylamide filled capillary column using a 100 mM Tris-borate (pH 8.0) running buffer. Separation was performed at -10 kV over 30 min with peak detection measured via UV absorption at 260 nm.

**MTT Assay.** The *in vitro* growth inhibitory effects of conventional P=S plus paclitaxel or docetaxel versus 2'-MOE-modified clusterin ASO plus paclitaxel or docetaxel on PC-3 cells were compared using the MTT assay as previously described (Miyake et al., 1998). Briefly,  $1 \times 10^4$  cells were seeded in each well of 96-well microtiter plates and allowed to attach overnight. Cells were then treated once daily with 500 nM either clusterin ASO or mismatch control oligonucleotides for 2 days. Following ASO treatment, cells were treated with various concentrations of paclitaxel or docetaxel. After 48 h of incubation, 20  $\mu$ l of 5 mg/ml MTT (Sigma Chemical Co., St. Louis, MO) in phosphate-buffered saline was added to each well, followed by incubation for 4 h at 37°C. The formazan crystals were dissolved in dimethyl sulfoxide. The optical density was determined with a microculture plate reader (Becton Dickinson Labware, Lincoln Park, NJ) at 540 nm. Absorbance values were normalized to the values obtained for the vehicle-treated cells to determine the percentage of survival. Each assay was performed in triplicate.

**In Vivo Treatments.** Approximately  $1 \times 10^6$  human PC-3 cells were inoculated s.c. with 0.1 ml of Matrigel (Becton Dickinson Labware, Bedford, MA) on the flank of 6- to 8-week-old male athymic mice under halothane anesthesia (5% induction and 1.5% maintenance concentration). When PC-3 tumors grew to 10 mm in diameter, usually 4 to 6 weeks after injection, treatment of animals was started.

In a first experiment, mice were randomized to one of three arms for treatment with conventional P=S clusterin ASO plus paclitaxel, 2'-MOE-modified clusterin ASO plus paclitaxel, or P=S mismatch control oligonucleotides plus paclitaxel. Each experimental group consisted of 10 mice. After randomization, 12.5 mg/kg of either type of clusterin ASO or mismatch control oligonucleotides was injected

i.p. once daily into each mouse for 28 days. From days 10 to 14, and from days 24 to 28, 0.5 mg of polymeric micellar paclitaxel (Leung et al., 2000) was administered once daily by i.v. injection. Tumor volume was measured once weekly and calculated by the formula length  $\times$  width  $\times$  depth  $\times$  0.5236. Data points were reported as mean tumor volumes  $\pm$  S.D. In each of the three treatment arms, three mice were designated immediately after randomization to be harvested 1 week after the last oligonucleotide/paclitaxel treatment (day 35) to determine multiple serum parameters for comparison of *in vivo* ASO toxicity.

In a second set of experiments, mice were randomized to one of four arms for treatment with P=S clusterin ASO once daily, P=S clusterin ASO once weekly, 2'-MOE-modified clusterin ASO once weekly, or P=S mismatch control oligonucleotides once weekly. Each experimental group consisted of eight mice. After randomization, 12.5 mg/kg clusterin ASO or mismatch control oligonucleotides was injected i.p. once daily or once weekly into each mouse over 4 weeks. Animals in all four treatment arms additionally received polymeric micellar paclitaxel as described above. Tumor volume was measured and data points were reported as described above.

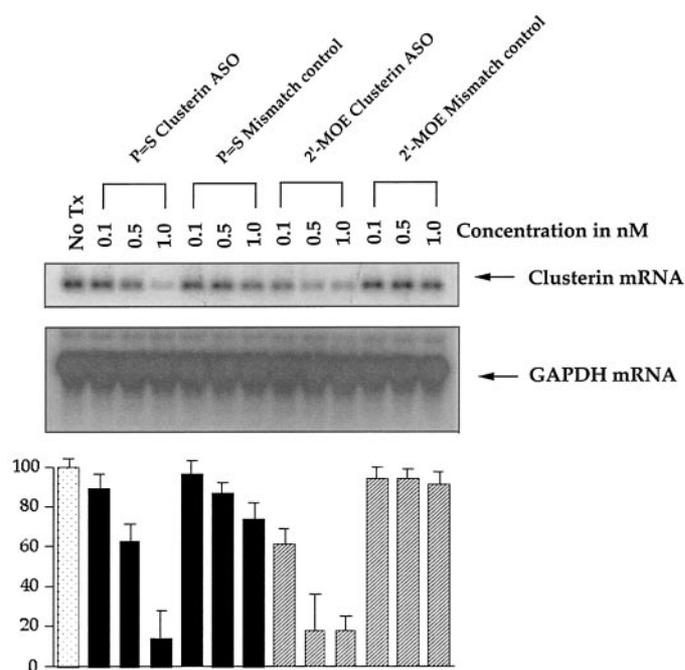
In a third *in vivo* experiment, mice were randomized to one of two arms for treatment with either P=S clusterin ASO or 2'-MOE-modified clusterin ASO. Each experimental group consisted of 12 mice. Clusterin ASO (12.5 mg/kg) was injected i.p. once daily into each mouse for 5 days. PC-3 tumors were harvested 1, 3, 5, and 7 days after the last ASO-injection for Northern blot and CGE analysis of clusterin. All animal procedures were performed according to the guidelines of the Canadian Council on Animal Care and with appropriate institutional certification.

## Results

**Enhanced Inhibition of Clusterin mRNA Using 2'-MOE-Modified ASO in PC-3 Cells.** Northern blot analysis was used to compare the effects of treatment with conventional P=S and 2'-MOE-modified ASO on clusterin mRNA expression in PC-3 cells. As shown in Fig. 1, both P=S and 2'-MOE-modified ASO decreased clusterin mRNA levels in a dose-dependent and sequence-specific manner. Using an ASO concentration of 500 nM, 2'-MOE-modified ASO was more potent than conventional P=S ASO, decreasing clusterin mRNA levels in PC-3 cells by 80 versus 40%.

**Conventional P=S and 2'-MOE-Modified Clusterin ASO Equally Enhance Chemosensitivity of PC-3 Cells *In Vitro*.** To compare the efficacy of conventional P=S and 2'-MOE-modified clusterin ASO to enhance cytotoxicity *in vitro*, PC-3 cells were treated with either type of clusterin ASO once daily for 2 days and then incubated with medium containing various concentrations of either paclitaxel or docetaxel. After 48 h of incubation, cell viability was determined by the MTT assay. As shown in Fig. 2, A and B, both types of clusterin ASO equally enhanced chemosensitivity of paclitaxel and docetaxel by more than 70 and 50%, respectively.

**Enhanced Tissue Half-Life of ASO by 2'-MOE Modification.** CGE was used to analyze time-dependent ASO metabolism in PC-3 tumors. As shown in Fig. 3A, *in vivo* tissue half-life of ASO was increased by more than 5-fold with the 2'-MOE modification, compared with conventional P=S ASO (>5 versus <1 day). Ninety percent of 2'-MOE-modified ASO was detectable as full-length material at 1 week, whereas only 10% of P=S ASO was found as full-length material at 1 day (Fig. 3B) following cessation

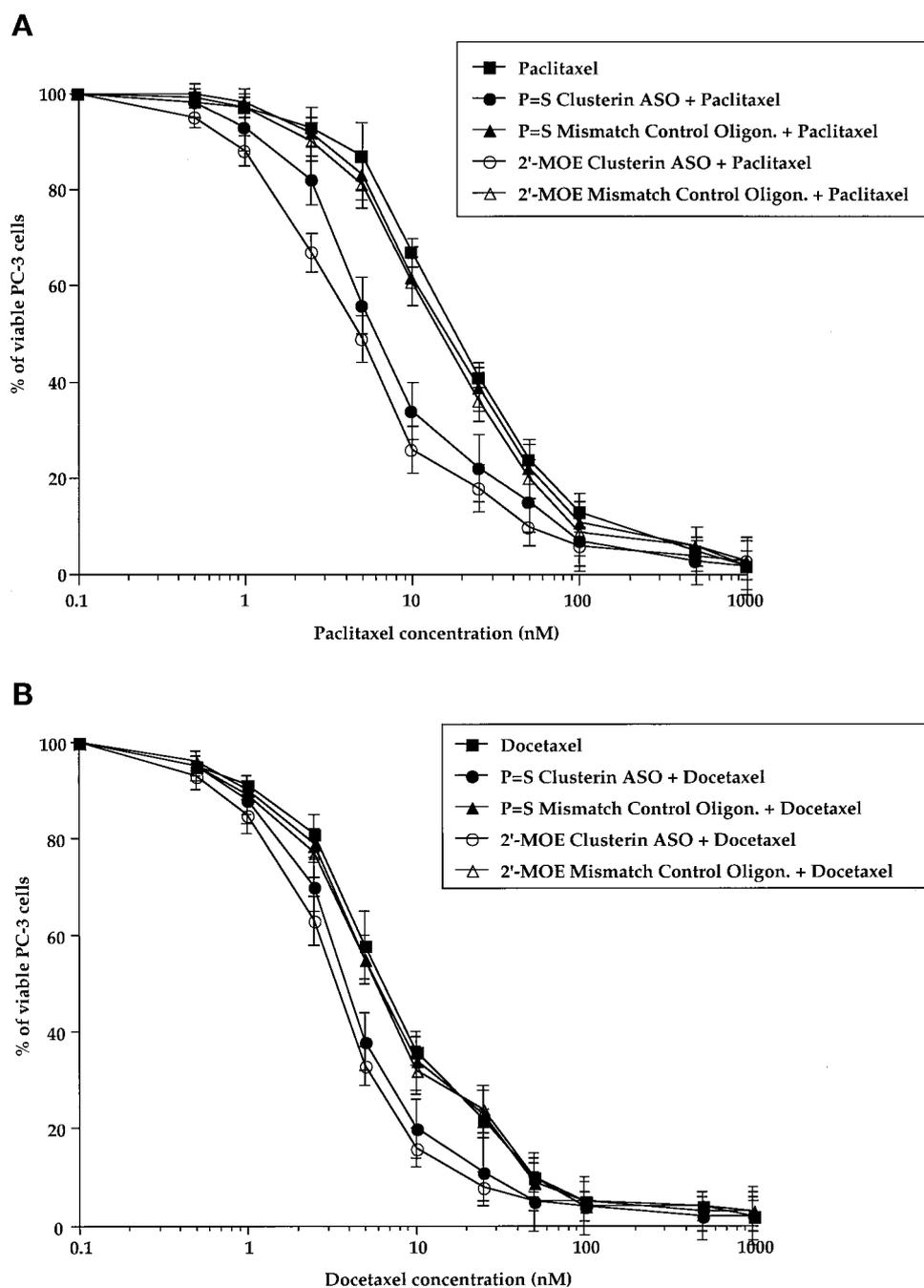


**Fig. 1.** Inhibition of clusterin mRNA is enhanced in PC-3 cells using 2'-MOE-modified ASO. Northern blot analysis was used to compare the effects of 3 days of treatment with either conventional P=S or 2'-MOE-modified clusterin ASO on clusterin mRNA expression in PC-3 cells. No Tx indicates untreated cells (control). Both P=S and 2'-MOE-modified ASO decreased clusterin mRNA levels in a dose-dependent and sequence-specific manner. Clusterin mRNA levels were quantified (after normalization to GAPDH mRNA levels) by laser densitometry. Each column represents the mean value of triplicate analysis with standard deviation. Numbers on the y-axis represent arbitrary densitometric units.

of dosing. Five and 7 days following the last ASO treatment, no full-length P=S ASO was detectable in tumor tissue. Furthermore, *in vivo* clusterin mRNA expression was more efficiently inhibited over this time period using 2'-MOE-modified ASO compared with P=S Clusterin ASO (Fig. 3C).

**2'-MOE-Modified Clusterin ASO Enhances the Potency of Paclitaxel *In Vivo*.** To compare the efficacy of conventional P=S versus 2'-MOE-modified clusterin ASO to enhance the cytotoxicity of paclitaxel *in vivo*, athymic mice bearing PC-3 tumors were treated with either type of clusterin ASO or mismatch control oligonucleotide over 28 days. From days 10 to 14, and from days 24 to 28, 0.5 mg of polymeric micellar paclitaxel was administered once daily by i.v. injection. As shown in Fig. 4, both types of clusterin ASO enhanced paclitaxel chemosensitivity in PC-3 tumors by 7 weeks following initiation of treatment. Treatment with 2'-MOE-modified clusterin ASO was significantly more potent in reducing mean tumor volume (over 80%) than conventional P=S clusterin ASO (40%), compared with treatment with mismatch control oligonucleotides. No side effects were observed for either compound.

**Weekly Administration of 2'-MOE-Modified Clusterin ASO Is Equivalent to Daily Administration of Conventional P=S Clusterin ASO *In Vivo*.** To assess whether increased stability and longer tissue half-life of 2'-MOE-modified ASO would permit longer dosing inter-

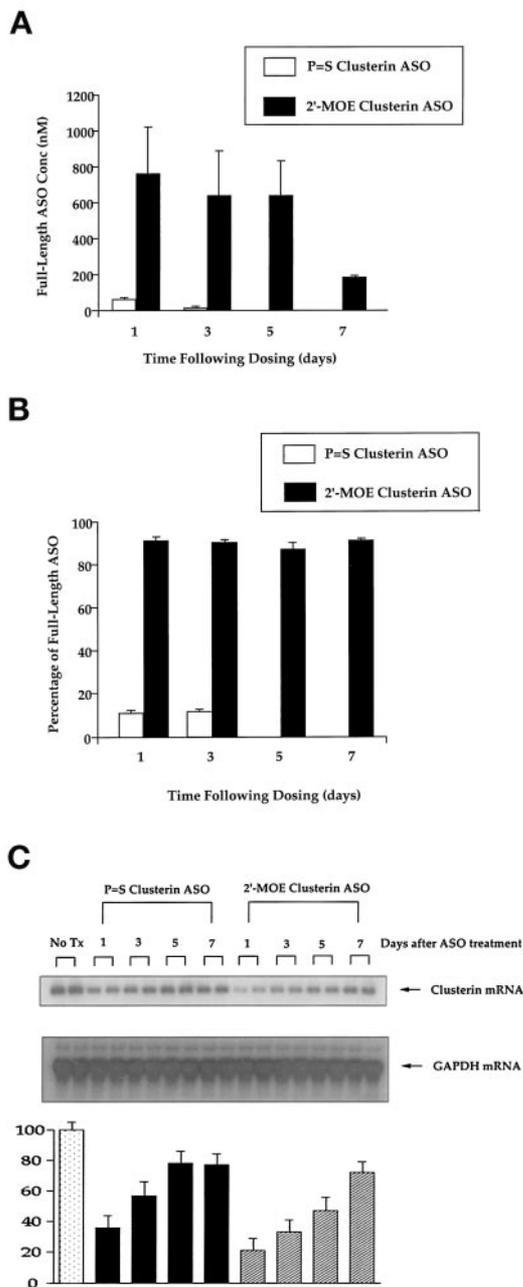


**Fig. 2.** Conventional P=S and 2'-MOE-modified clusterin ASO equally enhance chemosensitivity of PC-3 cells in vitro. To compare the efficacy of conventional P=S and 2'-MOE-modified clusterin ASO to enhance cytotoxicity in vitro, PC-3 cells were treated with both types of clusterin ASO (500 nM) once daily for 2 days and then incubated with medium containing various concentrations of either paclitaxel or docetaxel. After 48 h of incubation, cell viability was determined by the MTT assay. A and B, each data point represents the mean of triplicate analysis with standard deviation.

vals without loss of efficiency, athymic mice bearing PC-3 tumors were treated with either type of clusterin ASO or mismatch control oligonucleotides once weekly compared with conventional P=S clusterin ASO once daily. From days 10 to 14, and from days 24 to 28, 0.5 mg of polymeric micellar paclitaxel (Leung et al., 2000) was administered once daily by i.v. injection. In combination with paclitaxel, weekly administration of 2'-MOE-modified clusterin ASO was equivalent to daily administration of conventional P=S clusterin ASO, reducing mean tumor volumes by 31% compared with weekly administration of mismatch control oligonucleotides and by 21% compared with weekly administration of conventional P=S clusterin ASO, following 6 weeks after initiation of treatment (Fig. 5).

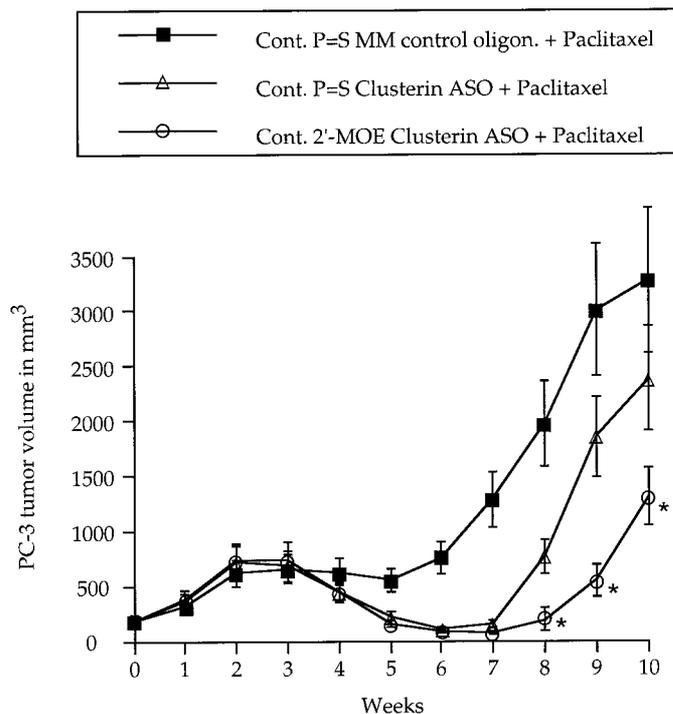
## Discussion

Hormonal and chemoresistance in advanced prostate cancer develops, in part, from alterations in the apoptotic machinery, due to increased activity of antiapoptotic pathways or expression of antiapoptotic genes. Research during the past decade has identified several proteins that may promote progression and resistance by inhibiting apoptosis. Of special relevance to development of androgen-independent progression and hormone refractory prostate cancer are those survival proteins that are up-regulated after apoptotic triggers, like androgen ablation, that function to inhibit cell death. Proteins fulfilling these criteria include antiapoptotic members of the Bcl-2 protein family and



**Fig. 3.** Tissue half-life of ASO is prolonged by 2'-MOE modification. CGE was used to analyze time-dependent ASO metabolism in PC-3 tumors. A, in vivo tissue half-life of ASO was increased >5-fold by the 2'-MOE modification, compared with conventional P=S ASO (>5 versus <1 day). B, 90% of 2'-MOE-modified ASO was detectable as full-length material 1 week following dosing, whereas only 10% of P=S ASO was found as full-length sequence 1 day following dosing. C, Northern blotting was used to analyze clusterin mRNA expression in PC-3 tumors after treatment of mice with either type of clusterin ASO for 5 days. Over a period of 7 days after the last ASO treatment, in vivo clusterin mRNA expression was more efficiently inhibited using 2'-MOE-modified compared with conventional P=S clusterin ASO. Clusterin mRNA levels were quantified (after normalization to GAPDH mRNA levels) by laser densitometry. Each column represents the mean value of triplicate analysis with standard deviation. Numbers on the y-axis represent arbitrary densitometric units.

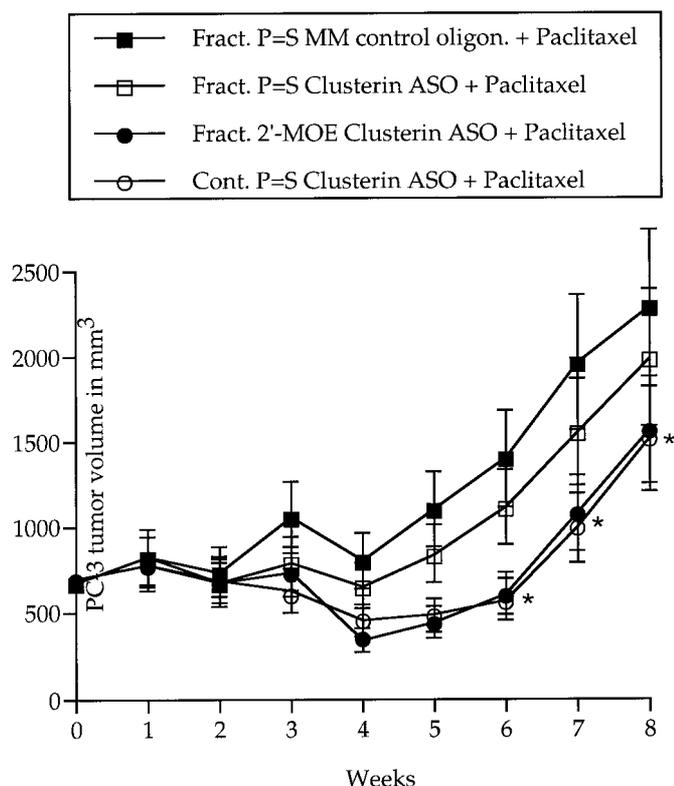
clusterin. We (Paterson et al., 1999) and others (McDonnell et al., 1992; Colombel et al., 1993; Raffo et al., 1995) have reported that Bcl-2 levels increase after androgen withdrawal and during androgen-independent progres-



**Fig. 4.** 2'-MOE modified clusterin ASO enhances the potency of paclitaxel in vivo. To compare the efficacy of conventional P=S versus 2'-MOE-modified clusterin ASO to enhance the cytotoxicity of paclitaxel in vivo, athymic mice bearing PC-3 tumors were treated with either type of clusterin ASO or mismatch control oligonucleotide over 28 days. From days 10 to 14, and from days 24 to 28, 0.5 mg of polymeric micellar paclitaxel was administered once daily by i.v. injection. Both types of clusterin ASO enhanced paclitaxel chemosensitivity in PC-3 tumors by 7 weeks following initiation of treatment. Treatment with 2'-MOE-modified clusterin ASO plus paclitaxel was significantly more potent in reducing mean tumor volumes (over 80%) than conventional P=S clusterin ASO plus paclitaxel (40%), compared with treatment with mismatch control oligonucleotides plus paclitaxel. Tumor volume was measured once weekly and calculated by the formula length × width × depth × 0.5236. Each data point represents the mean tumor volume from 10 mice with S.D. \*, differ from mismatch control ( $P < 0.05$ ) by Student's *t* test. No side effects were observed for either compound.

sion, and that Bcl-2 ASOs enhance cancer cell death after treatment with androgen withdrawal or chemotherapy (Gleave et al., 1999; Miyake et al., 1999; Miyake et al., 2000d). Similarly, clusterin levels increase in prostate cancer cells after androgen ablation or chemotherapy, where it functions like a heat shock protein to chaperone and stabilize protein conformation at times of cell stress (Humphreys et al., 1999; Wilson and Easterbrook-Smith, 2000; Miyake et al., 2000b,c). Forced overexpression of clusterin in human prostate LNCaP cells results in an androgen-independent and chemoresistant phenotype, while P=S clusterin ASOs enhanced cancer cell death after treatment with androgen withdrawal or chemotherapy (Miyake et al., 2000a,b,c).

In clinical trials, continuous intravenous infusions are required to administer conventional P=S ASOs because of their short tissue lives, which remains a major technical limitation. Therefore, over the past 10 years considerable effort has been made by numerous groups to improve the stability and efficacy of ASO by modifications of the phosphodiester linkage, the heterocycle, or the sugar. 2'-MOE ASOs form duplexes with RNA with a significantly higher affinity relative to phosphorothioate oligodeoxynucleotides.



**Fig. 5.** Weekly administration of 2'-MOE-modified clusterin ASO is equivalent to daily administration of conventional P=S clusterin ASO in vivo. To assess whether increased stability and longer tissue half-life of 2'-MOE-modified ASO would permit longer dosing intervals without loss of efficacy athymic mice bearing PC-3 tumors were treated with either type of clusterin ASO or mismatch control oligonucleotides once weekly compared with conventional P=S clusterin ASO once daily. From days 10 to 14, and from days 24 to 28, 0.5 mg of polymeric micellar paclitaxel (20) was administered once daily by i.v. injection. In combination with paclitaxel, weekly administration of 2'-MOE-modified clusterin ASO was equivalent to daily administration of conventional P=S clusterin ASO, reducing mean tumor volumes by 31% compared with weekly administration of mismatch control oligonucleotides and by 21% compared with weekly administration of conventional P=S clusterin ASO, following 6 weeks after initiation of treatment. \*, differ from mismatch control ( $P < 0.05$ ) by Student's  $t$  test. Tumor volumes were measured once weekly and calculated by the formula length  $\times$  width  $\times$  depth  $\times$  0.5236. Each data point represents the mean tumor volume from eight mice with S.D.

This increased affinity has been shown to result in improved antisense potency both in cell culture systems as well as in animals (Dean et al., 2001). In addition, 2'-MOE ASOs display significantly improved resistance against nuclease-mediated metabolism relative to phosphorothioate ASOs. This property results in significantly improved tissue half-life in vivo, which produces a longer duration of action and allows for more relaxed dosing regimens. Finally, 2'-MOE ASOs have been reported to display a more attractive safety profile relative to P=S ASOs (Henry et al., 2000).

In the present study, 2'-MOE-modified ASO targeted against clusterin more potently inhibited clusterin mRNA expression in human PC-3 cells compared with conventional P=S ASO. Furthermore, the efficacy of clusterin ASO to enhance the cytotoxic effect of paclitaxel and docetaxel in PC-3 cells was not affected by the 2'-MOE modification compared with conventional P=S, as demonstrated using the MTT assay.

In vivo, 2'-MOE-modified clusterin ASO more effectively reduced mean PC-3 tumor volumes after combined treatment

with paclitaxel compared with conventional P=S clusterin ASO. Analysis of body weights and multiple serum parameters after treatment with either compound did not reveal any sign of in vivo toxicity compared with untreated animals. Tissue half-lives in PC-3 tumors increased >5-fold using 2'-MOE-modified clusterin ASO, resulting in a more efficient inhibition of clusterin mRNA expression over 7 days after treatment. Ninety percent of 2'-MOE-modified ASO was detectable as full-length material at 1 week following dosing, whereas only 10% of P=S ASO was found as full-length sequence at 1 day following dosing. These CGE results were consistent with the findings demonstrating equivalently increased in vivo chemosensitivity toward paclitaxel by both daily administration of conventional P=S clusterin ASO and weekly administration of 2'-MOE-modified clusterin ASO.

Collectively, in vitro and in vivo results from this study in the human PC-3 tumor model confirm an improved efficacy for 2'-MOE-modified ASO over conventional P=S ASO to suppress clusterin and to enhance the chemosensitivity of paclitaxel. Furthermore, significantly increased ASO stability by incorporation of 2'-MOE chemistry will permit more convenient dosing regimens in clinical trials.

#### References

- Altmann KH, Dean NM, Fabbro D, Freier SM, Geiger T, Haener R, Huesken D, Martin P, Monia BP, Mueller M, et al. (1996) Second generation of antisense oligonucleotides: from nuclease resistance to biological efficacy in animals. *Chimia* **50**:168–176.
- Baker BF, Lot SS, Condon TP, Cheng-Flournoy S, Lesnik ES, Sasmor HM and Bennett CF (1997) 2'-O-(2-Methoxy)ethyl-modified anti-intercellular adhesion molecule 1 (ICAM-1) oligonucleotides selectively increase the ICAM-1 mRNA level and inhibit formation of the ICAM-1 translation initiation complex in human umbilical vein endothelial cells. *J Biol Chem* **272**:11994–12000.
- Colombel M, Symmans F, Gil S, O'Toole KM, Choplin D, Benson M, Olsson CA, Korsmeyer S and Buttyan R (1993) Detection of the apoptosis-suppressing oncoprotein Bcl-2 in hormone-refractory human prostate cancers. *Am J Pathol* **143**:390–400.
- Connor J, Buttyan R, Olsson CA, D'Agati V, O'Toole K and Sawczuk IS (1991) SGP-2 expression as a genetic marker of progressive cellular pathology in experimental hydronephrosis. *Kidney Int* **39**:1098–1103.
- Dean NM, Butler M, Monia BP and Manoharan M (2001) Pharmacology of 2'-O-(2-methoxy)ethyl modified antisense oligonucleotides, in *Antisense Technology: Principles, Strategies and Applications* (Crooke ST ed), in press.
- Dean NM, McKay R, Condon TP and Bennett CF (1994) Inhibition of protein kinase C- $\alpha$  expression in human A549 cells by antisense oligonucleotides inhibits induction of intercellular adhesion molecule 1 (ICAM-1) mRNA by phorbol esters. *J Biol Chem* **269**:16416–16424.
- Gleave ME, Tolcher A, Miyake H, Beraldi E and Goldie J (1999) Progression to Androgen-Independence is delayed by antisense Bcl-2 oligodeoxynucleotides after castration in the LNCaP prostate tumor model. *Clin Cancer Res* **5**:2891–2898.
- Henry S, Stecker K, Brooks D, Monteith D, Conklin B and Bennett CF (2000) Chemically modified oligonucleotides exhibit decreased immune stimulation in mice. *J Pharmacol Exp Ther* **292**:468–479.
- Humphreys DT, Carver JA, Easterbrook-Smith SB and Wilson MR (1999) Clusterin has chaperone-like activity similar to that of small heat shock proteins. *J Biol Chem* **274**:6875–6881.
- Kyprianou N, English HF, Davidson NE and Isaacs JT (1991) Programmed cell death during regression of the MCF-7 human breast cancer following estrogen ablation. *Cancer Res* **51**:162–166.
- Leung SY, Jackson J, Miyake H, Burt H and Gleave ME (2000) Polymeric micellar paclitaxel induces tumor regression in androgen-independent prostate cancer in the LNCaP tumor model. *Prostate* **44**:156–163.
- May PC, Lampert-Etchells M, Johnson SA, Poirier J, Masters JN and Finch CE (1990) Dynamics of gene expression for a hippocampal glycoprotein elevated in Alzheimer's disease and in response to experimental lesions in rat. *Neuron* **5**:831–839.
- McDonnell TJ, Troncoso P, Brisby SM, Logothetis CL, Chung LWK, Hsieh JT, et al. (1992) Expression of the protooncogene Bcl-2 in the prostate and its association with emergence of androgen-independent prostate cancer. *Cancer Res* **52**:6940–6944.
- McKay RA, Cummins LL, Graham MJ, Lesnik EA, Owens SR, Winniman M and Dean NM (1996) Enhanced activity of an antisense oligonucleotide targeting murine protein kinase C- $\alpha$  by the incorporation of 2'-O-propyl modifications. *Nucleic Acids Res* **24**:411–417.
- McKay RA, Miraglia LJ, Cummins LL, Owens RS, Sasmor H and Dean NM (1999) Characterization of a potent and specific class of antisense oligonucleotide inhibitor of human protein kinase C- $\alpha$  expression. *J Biol Chem* **274**:1715–1722.
- Miyake H, Chi KN and Gleave ME (2000a) Antisense testosterone-repressed prostate message-2 oligodeoxynucleotides chemosensitize human androgen-

- independent PC-3 prostate cancer cells both in vitro and in vivo. *Clin Cancer Res* **6**:1655–1666.
- Miyake H, Hanada N, Nakamura H, Kagawa S, Fujiwara T, Hara I, Eto H, Gohji K, Arakawa S, Kamidono S and Saya H (1998) Overexpression of bcl-2 in bladder cancer cells inhibits apoptosis induced by cisplatin and adenoviral-mediated p53 gene transfer. *Oncogene* **16**:933–943.
- Miyake H, Nelson C, Rennie PS and Gleave ME (2000b) Testosterone-repressed prostate message-2 is an antiapoptotic gene involved in progression to androgen-independence in prostate cancer. *Cancer Res* **60**:170–176.
- Miyake H, Nelson C, Rennie PS and Gleave ME (2000c) Acquisition of chemoresistant phenotype by overexpression of the antiapoptotic gene, testosterone-repressed prostate message-2 in prostate cancer xenograft models. *Cancer Res* **60**:2547–2554.
- Miyake H, Tolcher A and Gleave ME (1999) Antisense Bcl-2 oligodeoxynucleotides delay progression to androgen-independence after castration in the androgen dependent Shionogi tumor model. *Cancer Res* **59**:4030–4034.
- Miyake H, Tolcher A and Gleave ME (2000d) Antisense Bcl-2 oligodeoxynucleotides enhance taxol chemosensitivity and synergistically delays progression to androgen-independence after castration in the androgen dependent Shionogi tumor model. *J Natl Cancer Inst* **92**:34–41.
- Momia BP, Lesnik EA, Gonzalez C, Lima WF, McGee D, Guinosso CJ, Kawasaki AM, Cook PD and Freier SM (1993) Evaluation of 2'-modified oligonucleotides containing 2'-deoxy gaps as antisense inhibitors of gene expression. *J Biol Chem* **268**:14514–14522.
- Montpetit ML, Lawless KR and Tenniswood M (1986) Androgen-repressed messages in the rat ventral prostate. *Prostate* **8**:25–36.
- Paterson R, Gleave M, Jone E, Zubovits J, Goldenberg SL and Sullivan LD (1999) Immunohistochemical analysis of radical prostatectomy specimens after 8 months of neoadjuvant hormone therapy. *Mol Urol* **3**:277–286.
- Raffo AJ, Periman H, Chen MW, Streitman JS and Buttyan R (1995) Overexpression of bcl-2 protects prostate cancer cells from apoptosis in vitro and confers resistance to androgen depletion in vivo. *Cancer Res* **55**:4438–4445.
- Sensibar JA, Sutkowski DM, Raffo A, Buttyan R, Griswold MD, Sylvester SR, Kozlowski JM and Lee C (1995) Prevention of cell death induced by tumor necrosis factor  $\alpha$  in LNCaP cells by overexpression of sulfated glycoprotein-2 (clusterin). *Cancer Res* **55**:2431–2437.
- Wagner RW (1994) Gene inhibition using antisense oligodeoxynucleotides. *Nature (Lond)* **372**:333–335.
- Wilson MR and Easterbrook-Smith SB (2000) Clusterin is a secreted mammalian chaperone. *Trends Biochem Sci* **25**:95–98.
- Zamecnik PC and Stephenson ML (1978) Inhibition of Rous sarcoma virus replication and cell transformation by a specific oligodeoxynucleotide. *Proc Natl Acad Sci USA* **75**:280–284.

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