A Phase I Pharmacokinetic and Pharmacodynamic Study of OGX-011, a 2′-Methoxyethyl Antisense Oligonucleotide to Clusterin, in Patients With Localized Prostate Cancer

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Background: Clusterin is a cytoprotective chaperone protein that promotes cell survival and confers broad-spectrum treatment resistance. OGX-011 is a 2′-methoxyethyl modified phosphorothioate antisense oligonucleotide that is complementary to clusterin mRNA and has been reported to inhibit clusterin expression and enhance drug efficacy in xenograft models. The primary objective of this clinical study was to determine a biologically effective dose of OGX-011 that would inhibit clusterin expression in human cancer. Methods: Subjects (n = 25) with localized prostate cancer with high-risk features who were candidates for prostatectomy were treated with OGX-011 by 2-hour intravenous infusion on days 1, 3, and 5 and then weekly from days 8–29 combined with androgen blockade starting on day 1; prostatectomy was performed on days 30–36. Six different doses were tested, from 40 to 640 mg. OGX-011 plasma and prostate tissue concentrations were measured by an enzyme-linked immunosorbent assay method, and the pharmacokinetics of OGX-011 were determined from these data. Prostate cancer tissue, lymph nodes, and serial samples of peripheral blood mononuclear cells were assessed for clusterin expression using quantitative real-time polymerase chain reaction and immunohistochemistry. All statistical tests were two-sided. Results: Only grade 1 and 2 toxicities were observed. The plasma half-life of OGX-011 was approximately 2–3 hours, and the area under the concentration versus time curve and $C_{\text{MAX}}$ (peak plasma concentration) increased proportionally with dose ($P_{\text{trend}}<.001$). OGX-011 in prostate tissue increased with dose ($P_{\text{trend}}<.001$). Dose-dependent decreases in prostate cancer and lymph node clusterin expression were observed by polymerase chain reaction of greater than 90% ($P_{\text{trend}} = .008$ and $P_{\text{trend}}<.001$, respectively) and by immunohistochemistry ($P_{\text{trend}}<.001$ and $P_{\text{trend}} = .01$, respectively). Conclusions: OGX-011 is well tolerated and reduces clusterin expression in primary prostate tumors. The optimal biologic dose for OGX-011 at the schedule used is 640 mg. [J Natl Cancer Inst 2005;97:1287–96]

The clusterin gene on chromosome 8p21-p12 has been linked to numerous physiologic and pathologic processes due to the binding of the clusterin protein with a wide variety of client proteins (1). In cancer, clusterin has been defined as an anti-apoptotic protein that is activated after therapeutic stress (2–5). Clusterin functions as a cytoprotective chaperone, much like an ATP-independent small heat shock protein, and its transcription is promoted by heat shock factor 1 (6,7). The amino acid sequence of clusterin is highly conserved across species; in humans, clusterin exists as both an intracellular truncated 55-kDa form and a 75–80-kDa extracellular heterodimeric secreted glycoprotein, making clusterin the only known chaperone protein to be secreted (4). In xenograft models, clusterin expression increases in response to cell stress induced by hormones, radiation, and chemotherapy (4,5,8,9). Forced overexpression of clusterin in preclinical cancer models confers resistance to radiation, hormone, and chemotherapy, whereas inhibition of clusterin expression enhances apoptotic death from these treatment modalities (2,4,5,10). Clusterin is expressed in a variety of human cancers, including those of the breast, lung, bladder, pancreas, kidney, and prostate (11–16). In human prostate cancer, increased expression of clusterin has been associated with high Gleason scores and clusterin is expressed at high levels in tumor cells that survive androgen ablation therapy (15,16).

OGX-011 is a second-generation phosphorothioate antisense oligonucleotide that is complementary to the clusterin mRNA translation initiation site and strongly inhibits clusterin expression in vitro and in vivo (16). In addition to a phosphorothioate backbone, OGX-011 incorporates second-generation antisense technology in the form of a 2′-methoxyethyl modification to the ribose moiety on the flanking four nucleotides on either end of the molecule. Unmodified phosphorothioate antisense molecules have relatively short serum and tissue half-lives (less than 2 and 4 hours, respectively), and only small amounts of full-length antisense oligonucleotide can be detected in tissues after 24 hours (17,22). Consequently, continuous or frequent intravenous infusions of these agents have been used for clinical trials. By contrast, second-generation phosphorothioate antisense agents, such as OGX-011, have been shown to form duplexes with RNA with a higher affinity than unmodified phosphorothioate antisense oligonucleotides, which results in improved potency (23). In addition, second-generation antisense oligonucleotides are more resistant to nuclease than unmodified phosphorothioate oligonucleotides, resulting in prolonged tissue half-life in vivo, producing a longer duration of action, and therefore, potentially allowing for a more convenient intermittent dosing schedule (17). Finally, second-generation antisense oligonucleotides have been reported to be less toxic and to cause less nonspecific immune stimulation than unmodified phosphorothioate antisense oligonucleotides (24), potentially allowing for a delivery of higher doses.
In preclinical efficacy studies, OGX-011 was shown to substantially enhance the therapeutic effect of hormone therapy, chemotherapy, and radiation therapy in a variety of cancer models, including prostate, breast, non–small-cell lung, bladder, and kidney (18). No clinical signs of toxicity were observed in animal toxicity studies at doses of up to 50 mg/kg in mice or of up to 10 mg/kg in monkeys. The primary toxicities were alterations in liver function in the form of elevated transaminase at doses of 50 mg/kg in mice, immune stimulation in mice, and minor evidence of complement activation related to peak concentration in monkeys at 10 mg/kg.

In this phase I trial, our primary objective was to determine a biologically effective dose of OGX-011 that inhibited clusterin expression in human cancer. We used a neoadjuvant design, in which volunteer subjects with localized prostate cancer were treated with OGX-011 and androgen ablation therapy prior to radical prostatectomy. In this way, we were able to determine both the standard phase I parameters of toxicity and plasma pharmacokinetics and the ideal phase II dose, based on OGX-011 tissue concentrations and clusterin suppression data in target tissue.

**Patients and Methods**

**Subject Eligibility**

To be eligible, subjects had to have a histologic diagnosis of prostate cancer, to have clinically localized disease, to have had no prior therapy, and to be candidates for radical prostatectomy. Subjects also had to have a minimum of two positive biopsies and one of the following high-risk features: prostate-specific antigen (PSA) at >10 ng/mL, clinical stage T3, or a Gleason score of 7–10. Patients with Gleason score 6 disease were also eligible if they had a minimum of three positive biopsies. All subjects had to have an Eastern Cooperative Oncology Group performance status of 0 or 1 and adequate organ function, defined as a total white blood count of ≥3.0 × 10^9/L, hemoglobin level of ≥100 g/L, platelet concentration of ≥100 × 10^9/L, normal partial thromboplastin time (PTT) and international normalized ratio for prothrombin time (INR), and normal levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine, and bilirubin. All subjects provided written informed consent and were registered prior to treatment by facsimile or telephone at the National Cancer Institute of Canada (NCIC) Clinical Trials Group office in Kingston, Ontario. The study was approved by the University of British Columbia Research Ethics Board and the U.S. Army Surgeon General’s Human Subjects Research Review Board and was registered with http://www.ClinicalTrials.gov (identifier NCT00054106).

**Treatment Administration and Patient Assessments**

OGX-011 is the 20-sodium salt of a 3′-5′ linked phosphorothioate oligonucleotide 21-mer length (5′-CAGCAGCAGAGTCTTCATCAT-3′) that is complementary to the translation initiation site of human clusterin. Each of the 20 internucleotide linkages is an O,O-linked phosphorothioate with additional 2′-O-(2-methoxy) ethyl modification to the 2′ position of the ribose moiety on the four nucleotides at the 5′ and 3′ ends of the molecule. OGX-011 is manufactured by Isis Pharmaceuticals Inc. (ISIS 112989, Carlsbad, CA) and was provided by OncoGeneX Technologies, Inc. (Vancouver, British Columbia, Canada). OGX-011 was delivered as a peripheral intravenous infusion over 2 hours on days 1, 3, and 5 and then weekly on days 8, 15, 22, and 29. The doses of OGX-011 were not adjusted for body weight. The starting dose (40 mg) was based on one-sixth the no-effect dose in monkeys. Subsequent dose levels administered to successive cohorts of subjects were 80, 160, 320, 480, and 640 mg. Also on day 1, a single injection of Buserelin acetate at 6.3 mg (2-month depot supplied by Aventis Pharma) was given and flutamide (250 mg three times a day by mouth for 28 days) treatment was started. Radical prostatectomy was performed within 7 days of the last dose of OGX-011. One subject was assigned to the 40-mg dose level, and three subjects each were assigned to the 80- and 160-mg dose levels. Because it was predicted from xenograft mouse models that biologic effects could be detected at doses of 320 mg and higher (16), six subjects each were assigned to the 320-, 480-, and 640-mg dose levels to increase the number of pharmacodynamic observations. At baseline, all subjects had a history and physical examination; complete blood count; analyses of INR, PTT, serum creatinine, electrolytes, AST, ALT, bilirubin, testosterone, and PSA; transrectal ultrasound; urinalysis; and electrocardiogram performed. A bone scan and computed tomography scan were performed if appropriate for staging purposes as per local standard of practice guidelines (30). All subjects were assessed for toxicity weekly, prior to radical prostatectomy and at 7 days and 3 months postoperatively. Serum complement 3a was assessed by enzyme immunoassay (Quidel Corporation, San Diego, CA) before each infusion, after each infusion, and 30 minutes after the end of each infusion. Toxicity was graded according to the NCIC Common Toxicity Criteria, version 2. Dose-limiting toxicity was defined as the occurrence of one or more of the following: grade 3 or 4 nonhematologic toxicity (except unpremedicated nausea or vomiting), thrombocytepenia <25 × 10^9/L or grade 3 thrombocytepenia associated with bleeding, grade 4 neutropenia lasting for ≥3 days, febrile neutropenia, INR or PTT elevation of grade 3 or higher with associated bleeding, or missing two or more OGX-011 doses due to toxicity. Dose-limiting toxicity was determined during the treatment period and up to 1 week postsurgery for dose escalation to the next level.

**Pharmacokinetic Analyses**

Plasma samples were obtained to determine the single- and multiple-dose pharmacokinetic profile of full-length intact OGX-011. On days 1 and 29, plasma samples were collected before OGX-011 infusion and then at 1, 2, 2.5, 3, 4, 6, 9, and 24 hours after the start of infusion. Trough and/or peak plasma samples were collected predose and at the end of infusion on days 3, 5, 8, 15, and 22. Plasma samples were analyzed either undiluted or diluted 1:1000-fold with blank human plasma (Biochemed Pharmacologicals, Winchester, VA, and Bioreclamation Inc., Hicksville, NY) and analyzed for OGX-011 using a validated enzyme-linked immunosorbent assay (ELISA)/cutting method (CTBR Bio-Research Inc., Senneville, Quebec, Canada). In this method, 125 mL of standards, quality control (QC) samples, and test samples was first added to a poly styrene 96-well plate and then 125 μL of a 0.5 mM cutting probe solution (complementary sequence of the antisense oligonucleotide containing biotin at the 5′ end and digoxigenin (DIG) at the 3′ end) was added to all wells. The samples were hybridized at room temperature for
approximately 1 hour. The hybridization mixtures (200 μL) were then pipetted into wells of a neutravidin-coated 96-well plate and incubated at room temperature for approximately 1 hour, followed by a 2-hour incubation with 300 μL of a 50-U/mL solution of S1 nuclease at room temperature to cleave the unhybridized probe. Anti-DIG immunoglobulin G conjugated to alkaline phosphatase was then added to catalyze the formation of fluorescent AttoPhos (Promega, Madison, WI), and fluorescence of the hybridized DIG-labeled probe was measured using a Spectramax Gemini fluorescence plate reader (Molecular Devices Corporation, Sunnyvale, CA).

To determine tissue concentrations of OGX-011, approximately 1 g of tissue from the prostatectomy specimen was frozen immediately after surgery. Frozen prostate tissue samples were ground to a fine powder under liquid nitrogen. Calibration standards and QC samples were prepared by spiking human prostate tissue homogenate with appropriate amounts of reference standard. Ammonium hydroxide and phenol-chloroform-isooamyl alcohol (25:24:1) were added to the homogenized prostate samples (including the test samples), which were then mixed and centrifuged for 5 minutes at 21 000g at room temperature. The aqueous layer was collected and evaporated to dryness using a SpeedVac System (Fisher Scientific, Pittsburg, PA). Samples were analyzed using a validated ELISA/cutting method as described above (CTBR Bio-Research Inc., Senneville, Quebec, Canada). Calibration standards and QC samples were analyzed in duplicate and study samples in single replicate. On a 96-well plate, standards were loaded at the beginning of the plating, followed by a set of QC samples, study samples, and then an additional set of QC samples.

Noncompartamental pharmacokinetic analysis was performed on the plasma concentration data, including assessment of the TMAX (time to reach peak plasma concentration), CMAX (observed peak plasma concentration), AUC (area under the plasma concentration versus time curve), k (plasma distribution rate constant), t1/2 (plasma distribution half-life), Vz (apparent volume of distribution) and CL (total clearance from plasma) using WinNonlin (version 3.2; Pharsight Inc., Mountain View, CA). Prostate tissue concentrations are reported as the observed values.

**Pharmacodynamic Analyses**

Clusterin expression was determined in prostatectomy and lymph node specimens from study subjects using several complementary approaches, including chromogenic in situ hybridization (CISH), quantitative real-time polymerase chain reaction (QRT-PCR) of laser-captured microdissected prostate cancer cells, and immunohistochemistry (IHC). Tumor and normal lymph node samples from radical prostatectomy specimens taken from a prospectively collected tumor bank at the Vancouver General Hospital (samples from patients who had previously undergone radical prostatectomy at the Vancouver General Hospital matched for eligibility who were treated without prior neoadjuvant hormone therapy or treated with less than 2 months of neoadjuvant hormone therapy) were used as contemporarily treated historical controls to illustrate the previously described effect of androgen ablation on apoptosis and inducing clusterin expression (16). Clusterin expression was also determined from serial samples of peripheral blood mononuclear cells (PBMC) of patients treated with OGX-011. Three samples for PBMC were taken on separate occasions separated by at least 24 hours prior to the first OGX-011 infusion to define baseline variation, with subsequent samples taken on days 8, 15, 22, and 29 prior to treatment with OGX-011 and 2 weeks postoperatively.

**Chromogenic In Situ Hybridization**

Four-micron-thick sections were cut from paraffin blocks in an RNase-free environment and stored at 4 °C. Paraffin sections were dewaxed in xylene, rehydrated in a graded ethanol series, and then treated with 0.2 μg of proteinase K in 20 μL of Tris-EDTA buffer (pH 8.0) for 30 minutes. Sections were acetylated by two 5-minute incubations on a shaking platform with 0.1 M Tris-EDTA acetic acid buffer (pH 8.0) containing 0.25% [v/v] acetic anhydride. Sections were then transferred to prehybridization buffer (4× standard saline citrate [SSC] solution containing 50% [v/v] deionized formamide). Riboprobes (sense and antisense) were transcribed in vitro from an Xba-1- and Nde-1-digested pCR-CMV-TRPM2 plasmid (clusterin). Clusterin sense and antisense probes were labeled using a DIG RNA-labeling kit (Sp6/T7) (Roche Applied Science, Penzberg, Germany). Sections were hybridized overnight at 42 °C with probes in buffer containing 40% deionized formamide, 10% dextran sulfate, 1× Denhardt’s solution, 20× SSC, 1 M diethiothreitol, 1 mg/mL yeast tRNA, and 1 mg/mL denatured, sheared salmon sperm DNA. Sections were then washed three times for 15 minutes in 2× SSC at 37 °C and three times for 15 minutes in 1× SSC and incubated with a buffer solution consisting of 0.5 M NaCl, 0.01 M Tris, and 0.001 M EDTA buffer containing 20 μL RNase A. After two 30-minute washes in 1× SSC, sections were incubated in posthybridization buffer containing 0.1% Triton X-100 and 2% bovine serum albumin for 30 minutes in a humid chamber. Anti-DIG alkaline phosphatase-Fab fragment (Roche Applied Science, Penzberg, Germany) diluted 1:100 was applied, and after two 10-minute washes and incubation in posthybridization buffer (0.1 M Tris HCl [pH 7.5], 0.15 M NaCl) at room temperature, sections were transferred to a 1:50 dilution of nitroblue tetrazolium salts plus 5-bromo-4-chloro-3-indolylphosphate (Roche Applied Science, Penzberg, Germany), incubated overnight, and then washed and mounted on microscope slides. Three slides per patient and 10 fields per slide were evaluated for staining intensity from 0 to 3 (representing negative to strong staining) and graded independently by two pathologists (L.F. and E.C.J) at 200× magnification. The overall score was determined as follows: overall score = [(% cells with visual score 1) × 1] + [(% cells with visual score 2) × 2] + [(% cells with visual score 3) × 3].

**Laser Capture Microdissection**

Eight-micron sections were cut (P.A.L.M Microlaser Technologies, Bernried, Germany) in an RNase-free environment (microtome blade cleaned with diethylpyrocarbonate [DEPC]-treated H2O and water bath filled with DEPC H2O). The sections were mounted onto slides, air-dried at room temperature, and stored at 4 °C. For staining, slides were dewaxed with histologic grade xylene (Fisher Scientific, Fairlawn, NJ) and then rehydrated with a graded ethanol series and rinsed with DEPC H2O. Slides were stained with Gill-modified hematoxylin (EM science, Gibbstown, NJ), rinsed with DEPC H2O, and air-dried at room...
temperature overnight. Randomly chosen tumor cells with a least amount of surrounding stroma (approximately 100,000 cells) were laser captured and collected with 40 μL of lysis buffer (Stratagene, Cedar Creek, TX). From lymph node sections, randomly chosen germinal centers and histiocytes in sinuses (approximately 100,000 cells) were captured.

QRT-PCR

Total RNA was extracted from laser-captured cells using the Absolutely RNA Nanoprep Kit (Stratagene, Cedar Creek, TX). All RNA extracted from laser-captured cells and PBMC was reverse transcribed using random hexamers (PerkinElmer Applied Biosystems, Branchburg, NJ) and 20 units of Moloney murine leukemia virus reverse transcriptase (Invitrogen, Carlsbad, CA). The resulting first-strand cDNA was used as the template for the QRT-PCR. The Applied Biosystems 3700 sequence detection system (PerkinElmer Applied Biosystems, Foster City, CA) was used for real-time monitoring of PCR-amplified cDNA following the TaqMan Universal PCR Master Mix protocol. The amplification of clusterin cDNA was performed using forward primer 5′-GAGCAAGCTTAACGAGCAGTTT-3′, reverse primer 5′-CTTCCCCTTGGTGGAGT-3′, and TaqMan probe 5′-VIC-ACTGTTGTCGCCGCTGGCA-TAMRA-3′. Relative quantification of gene expression was performed using rRNA as a control. Ribosomal cDNA was amplified separately on a duplicate set of samples using standard primers and a standard TaqMan probe (PerkinElmer). The comparative cycle threshold method (ABI Applied Biosystems User Guide, ABI Applied Biosystems, Foster City, CA) was used for the relative quantification of clusterin mRNA. Two replications per sample were performed.

Immunohistochemistry

Sections were prepared as previously described (16). Goat anti-human clusterin antibody (Santa Cruz Biotechnologies, Inc., Santa Cruz, CA) was used at a working dilution of 1:600 in 1% bovine serum albumin, and the LSAB+ kit (Dako, Carpetseria, CA) was used as the detection system. The staining intensity was assessed as described above for CISH and also evaluated using an automated quantitative image analysis system (Image-Pro Plus version 4.5.1.22; MediaCybernetics, San Diego, CA). The Apoptosis Detection Kit (Chemicon, Temecula, CA) was used to evaluate apoptosis by a terminal deoxynucleotidyltransferase-mediated dUTP-biotin nick end labeling assay. The percentage of apoptotic cells and bodies per 10 high-power fields (apoptotic index) was independently counted by the two pathologists (L.F. and E.C.J.).

Statistical Analysis

The Jonckheere’s trend test (19) was used to test the statistical significance of the association between increasing dose level and the plasma pharmacokinetic parameters (separately for day 1 and day 29 values), prostate tissue concentrations, prostate and lymph node clusterin expression parameters, and apoptotic index. The Cochran-Armitage trend test (20) was used to test the statistical significance of association between increasing dose level and toxicity incidences. All P values were two-sided and were not adjusted for the number of parameters evaluated. P values of <.05 were considered to be statistically significant.

RESULTS

Patient Characteristics

A total of 25 subjects were enrolled in the study from December 2002 to March 2004. Their baseline characteristics are listed in Table 1. The Eastern Cooperative Oncology Group performance status was 0 in all subjects.

Administered Treatments

Six patient groups were evaluated with doses of OGX-011 ranging from 40 mg to 640 mg. As planned, one patient was entered at the 40-mg dose level, three patients to each of the 80- and 160-mg doses levels, and six patients to each of the 320-, 480-, and 640-mg dose level cohorts. All patients received combined androgen blockade therapy with buserelin acetate and flutamide beginning on day 1. Five patients were changed from flutamide to bicalutamide because of elevated hepatic transaminase levels at doses of OGX-011 of 320 mg and higher. The switch in antiandrogens occurred because flutamide administration has been associated with increases in transaminases (25); however, this toxicity was more likely related to OGX-011 administration (see below). There were no dose reductions or delays in the dosing schedule. All 25 subjects completed OGX-011 protocol therapy and had radical prostatectomy within a week after completing this treatment. Median time from last dose of OGX-011 to surgery was 3 days (range = 1–7 days).

Adverse Events

Dose-limiting toxicity was not observed at any of the dose levels evaluated, and adverse events were limited to grade 1 or 2. There were no intra- or postoperative complications attributable to protocol therapy. Toxicity appeared to be dose related, occurring mainly within the first week and diminishing with continued dosing. Grade 1 leukopenia and thrombocytopenia were observed, with thrombocytopenia increasing in frequency with higher dose (P = .04); three of the six patients at the 640-mg dose level experienced grade 1 thrombocytopenia, and two of the six experienced grade 1 leukopenia. Grade 1 anemia was seen in 19 of the 25 patients but was not dose dependent (P = .44). The most common nonhematologic adverse events were fever.

<table>
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<th>Table 1. Patient characteristics</th>
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<td>Characteristic*</td>
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*PSA = prostate-specific antigen. Tumors were staged according to International Union Against Cancer (UICC) TNM 1997 classification.
fatigue, and rigors, which usually occurred several hours after completion of the infusion and were self-limiting. Fever and rigors appeared to be dose related (P = .001 and P < .001, respectively), with five of six patients at the 640-mg dose level experiencing fatigue and fevers and all six patients experiencing rigors. The fever and rigors typically occurred with the day 1 and 3 infusions, were lessened with the day 5 infusion, and did not occur with the day 8 and subsequent infusions. Grade 1 and 2 elevations in hepatic transaminase levels were also observed. At the 640-mg dose level, four of the six subjects had increases in their AST and ALT and two of the six subjects experienced grade 2 AST and/or ALT elevation. Elevated hepatic transaminases were observed to occur in these patients by day 8, but these all resolved to grade 1 or less by days 15–22 despite continuation of OGX-011 therapy. There was no apparent dose-dependent induction of serum complement 3a. Toxicity from the androgen withdrawal therapy was typical, including loss of libido, erectile dysfunction, and hot flashes. Selected adverse events that were grade 2 or occurring in more than 10% of subjects or greater that were related to OGX-011 therapy are listed by worst grade observed to occur in these patients by day 8, but these all resolved to grade 1 or less by days 15–22 despite continuation of OGX-011 therapy. There was no apparent dose-dependent induction of serum complement 3a. Toxicity from the androgen withdrawal therapy was typical, including loss of libido, erectile dysfunction, and hot flashes. Selected adverse events that were grade 2 or occurring in more than 10% of subjects or greater that were related to OGX-011 therapy are listed by worst grade experienced by subject in Table 2.

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*AST = aspartate aminotransferase; ALT = alanine aminotransferase. Toxicity was graded according to the NCI Common Toxicity Criteria, version 2.

Plasma Pharmacokinetics and Prostate Tissue Concentrations of OGX-011

Plasma pharmacokinetic parameters for OGX-011 are presented in Table 3 from the day 1 and the day 29 infusions by dose level. Mean plasma distribution t1/2 ranged from 0.476 to 3.83 hours, with a trend to longer values with higher doses (P < .001 at both day 1 and day 29). Average peak concentrations and AUC were dose dependent and displayed proportional and predictable increases in a linear fashion. Mean CMAX at 640 mg was 61.1 µg/mL (95% confidence interval [CI] = 55.3 to 66.9) after the day 1 infusion and 69.9 µg/mL (95% CI = 64.8 to 74.9) after the day 29 infusion. CL was similar across all subjects and occasions. Overall, there was no sign of plasma accumulation from the repetitive dosing.

Tissue concentrations of OGX-011 are depicted in Fig. 1. Proportional increases in tissue concentrations with dose were observed (P < .001). No apparent effect of timing of surgery on tissue concentrations was observed. Mean tissue concentrations at the 320-mg, 480-mg, and 640-mg dose levels were 1.67 (95% CI = 1.07 to 2.26), 2.29 (95% CI = 1.31 to 3.27), and 4.82 (95% CI = 2.80 to 7.93) µg/g.

Table 3. Plasma pharmacokinetic parameters of OGX-011*

| Dose level, mg | No. of patients | Treatment day | CMAX, µg/mL | t1/2, h† | AUC0–24, µg·h/mL‡ | Vz, L§ | CL, L/h||
|---------------|----------------|--------------|-------------|---------|-------------------|--------|-------|
| 40            | 1              | 1            | 4.0         | 0.5     | 9.5               | 3.3    | 4.2   |
| 80            | 3              | 1            | 12.3 (9 to 23.7) | 0.8 (0.5 to 1.2) | 34.0 (4.3 to 63.7) | 3.2 (−5 to 6.8) | 2.6 (1 to 5.2) |
| 160           | 3              | 1            | 23.1 (16.4 to 29.9) | 1.1 (0.7 to 1.5) | 72.6 (25.7 to 119.4) | 3.7 (1.2 to 6.2) | 2.3 (1.0 to 3.6) |
| 320           | 6              | 1            | 22.6 (7.9 to 37.2) | 2.2 (1.4 to 5.7) | 70.5 (−107.0 to 248.0) | 7.6 (−23.2 to 38.5) | 2.4 (−3.6 to 8.3) |
| 480           | 6              | 1            | 39.5 (28.3 to 50.7) | 2.0 (1.3 to 2.5) | 138.4 (117.1 to 159.7) | 6.6 (5.9 to 7.4) | 2.4 (2.0 to 2.7) |
| 640           | 6              | 1            | 30.4 (22.8 to 38.1) | 2.7 (2.3 to 3.1) | 116.8 (85.2 to 148.4) | 11.5 (7.5 to 15.5) | 2.9 (2.2 to 3.6) |

*Values represent means (95% confidence intervals). TMAX = time to reach peak plasma concentration. CMAX = observed peak plasma concentration.
†t1/2 = plasma distribution half-life.
‡AUC0–24 = area under the plasma concentration versus time curve.
§Vz = apparent volume of distribution.
||CL = total clearance from plasma.
Prostate Clusterin Expression

Clusterin mRNA expression in prostate cancer tissue was analyzed using QRT-PCR and CISH. QRT-PCR analysis demonstrated a statistically significant dose-dependent decrease in clusterin RNA expression (Fig. 2, A, \( P_{\text{trend}} = .008 \)). Similar to the previously published data on increasing clusterin protein expression in prostate cancer after hormone therapy (16), expression of clusterin mRNA increased after neoadjuvant hormone therapy, as seen in the first two columns in Fig. 2A, which show the historical control samples. With increasing doses of OGX-011, clusterin mRNA expression at the time of prostatectomy decreased, such that the mean expression score in the 640-mg cohort was 7.1% (95% CI = 4.0% to 10.1%) that of untreated historical controls as well as the low-dose OGX-011-treated samples. A dose-response effect was not apparent with the semiquantitative CISH analysis (Fig. 2, B; \( P = .43 \)), in which overall staining intensity score was generally low across all doses.

Using IHC to assess clusterin protein in prostate cancer tissue (representative sections are depicted in Fig. 3, A) a dose-dependent decrease in expression of clusterin was also seen (Fig. 3, B, \( P_{\text{trend}} < .001 \)). As expected, the overall IHC score was higher in the historical controls treated with neoadjuvant hormone therapy than in untreated controls, but in the trial subjects, overall score decreased with increasing doses of OGX-011 plus neoadjuvant hormone therapy. Moreover, the percentage of cancer cells with a visual score of 0 increased (i.e., complete suppression of clusterin expression) with increasing dose of OGX-011, with an apparent plateau effect between the 480- and 640-mg dose levels (Fig. 3, C; \( P_{\text{trend}} < .001 \)). Mean number of prostate cancer cells that had a visual score of 0 was 14.2% (95% CI = 1.8% to 26.7%) in the untreated historical control group and 1.5% (95% CI = 0.2% to 2.6%) in the historical control group that had received prior neoadjuvant hormone therapy, again demonstrating the increase (and thus decrease in visual score 0 cells) in clusterin expression that occurs with the stress of neoadjuvant hormone therapy. In the subjects treated with 480 and 640 mg of OGX-011, the mean numbers of prostate cancer cells with a visual score of 0 were 51.2% (95% CI = 26.7% to 76.6%) and 56.7% (95% CI = 33.2% to 80.1%), respectively, indicating clusterin expression inhibition relative to subjects treated at lower doses and historical controls.

There was also a dose-dependent increase in the number of prostate cancer cells with a visual score of 0 in patients treated...
with higher doses of OGX-011 as compared with their baseline biopsies (Fig. 3, D; \(P_{\text{trend}} = .009\)). It should be noted that the OGX-011 biologic effect in this latter comparison is likely being underestimated due to the treatment with neoadjuvant hormone therapy, which increases clusterin expression severalfold (16) in the radical prostatectomy samples (and thus decreases the percentage of cells with a visual score of 0), as can be seen in the historical controls and in the subjects treated with lower doses of OGX-011 (Fig. 3, C). Results from the automated quantitative image analysis system were similar to the visual scoring results (data not shown).

Expression of Clusterin in Surrogate Tissues

Clusterin expression in lymph nodes was high, and data from IHC and RT-PCR showed dose-dependent decreases in clusterin expression (\(P_{\text{trend}} = .01\) for IHC and \(P_{\text{trend}} < .001\) for QRT-PCR) similar to those seen in prostatectomy specimens (Fig. 4). PBMC were also assessed for clusterin using QRT-PCR; however, there was a wide variation in the three intrasubject baseline values (data not shown) that would have made any posttreatment effect interpretation unreliable.

Apoptotic Index

To determine whether suppression of clusterin levels by OGX-011 treatment was associated with increased apoptosis, the apoptotic index was evaluated in the prostatectomy specimens. In the historical control specimens treated without and with neoadjuvant hormone therapy, the mean apoptotic indices were 7.0% (95% CI = 4.2% to 9.9%) and 9.0% (95% CI = 5.1% to 13.0%), respectively. The apoptotic index from subjects treated...
The plasma pharmacokinetic parameters of OGX-011 were similar to those of other antisense molecules (21, 22). The \( C_{\text{MAX}} \) that was achieved was approximately 1.5 times that shown to activate complement by first-generation antisense in preclinical toxicity studies (22). This finding was expected based on the improved tolerability profile of the second-generation molecules in toxicology studies which demonstrated complement activation only at a \( C_{\text{MAX}} \) of greater than 100 \( \mu \)g/mL. The fixed, unadjusted-for-weight dosing appeared to result in a consistent plasma profile of OGX-011 across subjects treated with the same dose level.

The design of this trial also allowed for us to determine the concentrations of OGX-011 in target tissue. At doses of 320 mg and higher, concentrations of full-length OGX-011 that are associated with a preclinical effect (17) were achieved in the prostate and a biologic effect—dose-dependent inhibition of clusterin expression in prostate cancer cells—was observed. Evidence of OGX-011 effect could also be seen when comparing intrapatient clusterin expression in the baseline core biopsies as compared to those in the post-OGX-011 and hormone therapy biopsies. It should be emphasized that any comparison of tissues before and after OGX-011 in the same patient will likely underestimate treatment effect because the use of hormone therapy itself increases clusterin expression severalfold above the baseline (16). However, in the subjects treated with the two highest dose levels of OGX-011, there was an increase in the number of prostate cancer cells that did not stain positive for clusterin, indicating the suppression of expression.

Surrogate tissues in the form of normal lymph nodes and PBMC were evaluated for clusterin expression. Clusterin expression in lymph nodes decreased with dose, as in prostate tissue that resulted in dose-dependent decreases in clusterin expression and an associated increase of the apoptotic index in lymph nodes decreased with dose, as in prostate tissue that resulted in dose-dependent decreases in clusterin expression and an associated increase of the apoptotic index. From these data, we were able to identify a biologically active dose for OGX-011 to carry forward into phase II clinical testing.

Qualitatively, the toxic effects associated with OGX-011 treatment were similar to those seen with other antisense molecules, i.e., thrombocytopenia, elevation in hepatic transaminases, fever, rigors, and fatigue (21, 22). These side effects are generally considered to be non-sequence-specific and presumably related to the polyanionic nature of these compounds (21, 26). The mild anemia that was observed may have been possibly due to OGX-011; however, it may also have been a result of the neoadjuvant hormone therapy, which is known to reduce hemoglobin levels by approximately 15% (27).

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Surrogate tissues in the form of normal lymph nodes and PBMC were evaluated for clusterin expression. Clusterin expression in lymph nodes decreased with dose, as in prostate
tissue. However, PBMC did not appear to be a helpful surrogate tissue to assess clusterin expression because a wide variation in expression at baseline made any interpretation of a post-treatment effect unreliable, a finding similar to other studies attempting to assess antisense effects in PBMC. Effects of variability in PBMC isolation and lack of sufficient uptake of antisense oligonucleotides by circulating PBMC are potential limiting factors of trying to assess antisense effects in PBMC. An ongoing phase II neoadjuvant study of OGX-011 at the recommended phase II dose, i.e., 640 mg, will allow us to evaluate in more detail the time profile of serum protein clusterin levels, duration of biologic effect in prostate and lymph node tissues, and OGX-011 tissue concentrations that occur 1 to 14 days after a course of OGX-011.

Given the known antiapoptotic effects of clusterin, apoptotic index was used as the indicator for the downstream cellular effects of clusterin expression inhibition. The apoptotic index in the historical control specimens was low, consistent with other studies (29); however, with increasing doses of the OGX-011, the apoptotic index increased by approximately two- to threefold compared with the historical control specimens.

A limitation of this study is that, because of the effect of neoadjuvant hormone therapy on increasing clusterin expression and the sampling error inherent to core biopsies, only an indirect evaluation of intrasubject biologic effect can be made. However, the neoadjuvant hormone therapy in this situation enriches for the target in question (i.e., it increases clusterin expression), thus making it more likely to be able to observe OGX-011 biologic activity on clusterin expression. Another limitation is the relatively small number of subjects; this is an inherent problem in phase I trials, and the previously mentioned ongoing phase II study will serve to confirm the results here with a larger group of subjects.

In conclusion, the second-generation antisense oligonucleotide OGX-011, targeted to the clusterin mRNA, can be safely given to humans in biologically active doses that inhibit clusterin expression in cancer and other tissues, resulting in an increased apoptotic effect. The recommended phase II dose of OGX-011 is 640 mg based on the optimal biologic activity in inhibiting clusterin expression and tolerability of this dose in humans. Phase I trials of OGX-011 in combination with chemotherapy in patients with advanced cancers are currently ongoing, and phase II trials are planned in patients with hormone-refractory prostate, breast, and lung cancer.

**REFERENCES**

NOTES

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S. Larry Goldenberg holds stock in OncoGenex Technologies, the maker of OGX-11. Martin Gleave is the scientific founder of and holds stock in Onco\-Genex Technologies.

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