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ABSTRACT

The clusterin gene encodes a cytoprotective chaperone protein that promotes cell survival. Clusterin is expressed in a variety of cancers including prostate, increases in response to apoptotic stimuli, and confers a resistant phenotype. OGX-011 is a 2nd generation antisense complementary to clusterin mRNA that inhibits expression of clusterin in xenograft models and thereby increases sensitivity to therapy. To evaluate OGX-011 as a potential treatment in humans, we have undertaken this Phase I/II study to evaluate the clinical, pathologic and biologic effects of OGX-011, in combination with neoadjuvant hormone therapy (NHT) in patients with prostate cancer and high risk features prior to radical prostatectomy. The primary objective of the phase I study was to determine phase II dose based on target regulation effect. The phase II component of this trial will assess the effects of combined NHT and OGX-011 on pathologic complete response. Progress: 25 patients were enrolled to 6 cohorts with doses of OGX-011 up to 640mg delivered. Toxicity was limited to grade 1/2, including fevers, rigors, fatigue and transient AST and ALT elevations and no dose-limiting toxicities. Plasma PK analysis showed dose proportional increases in AUC and Cmax with a t_{1/2} of approximately 2h. Prostate tissue concentrations of OGX-011 increased with dose, and tissue concentrations associated with preclinical effect could be achieved. Dose dependent decreases in prostate cancer cell clusterin expression were observed by QRT-PCR and immunohistochemistry (IHC). At 640mg dosing, clusterin mRNA was decreased to a mean of 8% (SD=4%) compared with lower dose levels and historical controls as assessed by QRT-PCR on laser captured microdissected cancer cells. By IHC, mean % cancer cells staining 0 intensity for clusterin protein at 640mg dosing was 54% (SD=24%). Dose-dependent changes in serum clusterin were also apparent. Conclusions: OGX-011 is well tolerated and can inhibit clusterin expression in prostate cancers. The recommended phase II dose for OGX-011 is 640mg based on target regulation results. The Phase II portion of this study, evaluating a 3-month neoadjuvant treatment with OGX-011 at the recommended phase II dose, enrolled the first patient by mid-June 2005 and 11 patients were enrolled at a rate of 4-5 per month. However, because of two episodes of unexpected toxicity with increased liver enzyme tests, enrolment was held until protocol revisions could be made. This required local research ethics board approval, Health Canada regulatory approval, and the U.S. Army Medical Research and Materiel Command's Human Subjects Research Review Board for activation. Enrolment was able to recommence in June 2006, and to date 5 patients have been registered (16 in total).

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INTRODUCTION

The *clusterin* gene on chromosome 8 encodes a chaperone protein which has been implicated in a variety of physiologic processes. Also known as *Testosterone repressed prostate message-2* [TRPM-2], or *sulfated glycoprotein-2*, *clusterin* is associated with numerous tumors including prostate [1], neuroblastoma [2], breast [3], lymphoma [4], urothelial [5] and renal cell carcinoma [6], and with various pathologic conditions including Alzheimer's [7] and nephrotoxic injury [8]. Clusterin levels increase dramatically during castration-induced apoptosis in rat prostate epithelial cells [9], in androgen dependent Shionogi tumors [10], and human prostate cancer CRW22 [11] and PC82 [12] xenografts. In human prostate cancer, clusterin levels are low or absent in most untreated hormone-naive tissues, but increase significantly within weeks after neoadjuvant hormone therapy [13]. Because clusterin binds to a wide variety of biological ligands [14,15], and is regulated by transcription factor HSF1 (heat shock factor 1) [16], the emerging view suggests that clusterin functions similarly to heat shock protein to chaperone and stabilize conformations of proteins at time of cell stress. Indeed, clusterin is substantially more potent than other HSP's at inhibiting stress-induced protein precipitation [17]. Significant differences exist, however, in amino acid sequence analysis which suggests that clusterin is a unique protein without any closely related family members yet identified. More recently, clusterin has been shown to inhibit the apoptosis inducing protein Bax, thus rendering cells more resistant to cell death [22].

Experimental and clinical studies in prostate cancer implicate clusterin with AI progression and with playing a protective role against apoptotic cell death from androgen withdrawal, chemotherapy and radiation [10,18,19,20]. OGX-011 is an ASO complementary to the *clusterin* mRNA. OGX-011 incorporates a phosphorothioate backbone with second-generation chemistry in the form of 2'-O-Methoxyethyl modifications to the 4 bases on either end of the 21-mer molecule. Such "gap-mer" modifications maintain the improved tissue pharmacokinetic profile of the second-generation chemistry but preserves high affinity for target mRNA and recruitment of RNase H necessary for activity. In pre-clinical models, OGX-011 improves the efficacy of chemotherapy, radiation, and androgen withdrawal by inhibiting expression of clusterin and enhancing the apoptotic response [10,19,20,21]. Furthermore, because of the second-generation chemistry and enhanced tissue half-life of OGX-011, more relaxed dosing schedules are possible while maintaining biologic efficacy of target inhibition. Rather than the prolonged continuous infusions of first generation phosphorothioate molecules that are usually employed, pre-clinical studies suggest that only weekly infusional dosing or less is required to maintain tissue levels of OGX-011 and target inhibition of clusterin [21], which is much more acceptable for patients in terms of tolerance and repeated administration.

To evaluate OGX-011 as a potential treatment in humans, we have undertaken this Phase I/II study to evaluate the clinical, pathologic and biologic effects of OGX-011, in combination with neoadjuvant hormone therapy in patients with prostate cancer and high risk features prior to radical prostatectomy. This primary objective of the phase I component of this trial is to define a recommended phase II dose of OGX-011 based on toxicity and maximal biologic effect. Secondary aims are to determine toxicity, the serum and tissue pharmacokinetic profile and measure evidence of OGX-011 effect on clusterin expression in tumor and peripheral blood mononuclear cells, and clusterin serum levels. The primary objective of the phase II component of this trial will assess the effects of combined neoadjuvant hormone therapy and OGX-011 for 3 months prior to radical prostatectomy on pathologic complete response.

A significant difficulty in the development of targeted therapy agents like OGX-011 is the determination of a biologically effective dose. The biologically effective dose can often be significantly different from that of the maximally tolerated dose, the usual endpoint in classically designed phase I trials. This study's phase I design allowed for a determination of an optimal biologically effective dose based on the target of interest (i.e. clusterin) within target tissue itself (i.e. prostate cancer) which has allowed for confidence in moving forward in phase II trials of the agent. The phase II portion of this study will serve to further define toxicity, confirm our observations of biological activity, and determine clinical activity of the recommended phase II dose of OGX-011 (i.e. 640 mg) in a larger group of patients.

BODY

TASK 1. STUDY INFRASTRUCTURE PREPARATION

- *Health Canada (Therapeutic Products Program) Investigational New Drug Submission*
- *Case Report Forms*
- *Medical and data monitoring*
- *Institutional Review Board*

All preparatory steps have been completed. Federal regulatory approval was given on 4 October 2002 (File Number 9427-N0711-98C). Initial University of British Columbia Research Ethics Board approval was granted October 24, 2002 (Number C02-0430), and HSRRB approval granted December 2002 (Number A-11279). Medical and Data monitoring and Case Report Form creation services were contracted with the National Cancer Institute of Canada - Clinical Trials Group.

TASK 2. PHASE I TRIAL

- *Patient enrollment*
- *Protocol treatment and dose escalation with OGX-011*
- *Define recommend phase II dose based on toxicity, serum and tissue pharmacokinetic and pharmacodynamic data*

TASK 4. SUPPORTING AND TRANSLATIONAL STUDIES

- *Serum pharmacokinetics*
- *Tissue pharmacokinetics*
- *Clusterin expression – prostate/tumor, mononuclear cells, serum*
- *Comparative molecular marker analysis in pathologic specimens*

As previously described in the 2005 Annual Report, these tasks have been completed for the phase I trial. To summarize, 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. 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 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 $< .001$, respectively) and by immunohistochemistry ($P_{\text{trend}} < .001$ and $= .01$, respectively). We concluded that OGX-011 was well tolerated and reduces clusterin expression in primary prostate tumors and that the optimal biologic dose for OGX-011 at the schedule used was 640 mg.

The manuscript was published in the Journal of the National Cancer Institute (J Natl Cancer Inst 2005;97:1287–96) which has one of the highest impact factors for cancer journals. A reprint of the manuscript is included in the appendices.

TASK 3. PHASE II TRIAL

- *Patient enrollment*
- *Phase II protocol treatment with OGX-011 (estimate 300g total drug)*
- *Efficacy determination*
- *Pathologic complete response rate*
- *Characterize clusterin expression*
- *PSA nadir and recurrence*

All preparatory steps have been completed for the phase II trial. University of British Columbia – British Columbia Cancer Agency Research Ethics Board (UBC-BCCA REB) approval was granted 3 November, 2004 (Number R04-0092). Federal regulatory approval was given on 17 December 2004 (File Number 9427-B0877-32C). However, HSRRB final approval notification was not received until May 4, 2005 (Number A-11279.2) which was a longer approval process than initially anticipated. Medical and Data monitoring and Case Report Form creation services were contracted out to private groups.

The first subject was enrolled and received their first protocol treatment on 15 June 2005. Eleven subjects were initially enrolled at a rate of 4-5 per month. However, in this first set of subjects, there were 2 subjects who experienced serious adverse events in the form of increased liver enzyme test elevations (Grade 3-4) requiring holding of protocol therapy. The subjects were otherwise asymptomatic with no other toxicities. After withdrawal from protocol therapy, the subjects liver enzyme elevations had resolved and both patients went on to complete their prostatectomies without incident.

Two possibilities exist for the liver enzyme abnormalities/liver toxicity that has been observed:

- 1) Flutamide induced hepatotoxicity. This is a known side effect of flutamide occurring in less than 5% of patients. The incidence of hepatotoxicity seen is higher than what would have been normally expected and the timeline (increases in liver enzyme tests after flutamide had been stopped) is also atypical. However, in the present trial and the preceding phase I trial of the same regimen, patients developing Grade 1 or 2 liver enzyme test elevations on flutamide were changed to bicalutamide, with normalization of their liver enzyme tests. This suggests that OGX-011 may potentiate the liver toxicity of flutamide, and that bicalutamide is a safer alternative when a non-steroidal, anti-androgen agent is being used with OGX-011.
- 2) OGX-011 induced hepatotoxicity. Grade 3 and 4 elevations in transaminases are a well described effect of phosphorothioate antisense molecules like OGX-011, likely secondary to non-sequence specific inflammation. Elevations to the degree seen in the current trial however, have not been seen in previous Phase 1 trials of OGX-011 in combination of hormone therapy (but given over a shorter duration than in OGX 011-04) or with chemotherapy (e.g. docetaxel, given with steroid prophylaxis). The latter might suggest that chemotherapy or corticosteroids associated with chemotherapy delivery may have a preventative effect on OGX-011 associated hepatotoxicity.

Because of the unexpected and severe nature of the toxicity, enrolment was suspended on the trial until the protocol was amended for safety considerations to take into account these two possibilities:

- 1) Flutamide was replaced with bicalutamide, given its lower incidence of toxicity and the phase I experience of successfully changing from flutamide to bicalutamide in the face of Grade 1 or 2 elevated liver enzymes.
- 2) Patients that developed AST, ALT or bilirubin elevations were to have OGX-011 dose modifications and be prescribed a short course of dexamethasone.
- 3) Biochemistry lab testing was to be performed on a weekly basis in cycle 1, 2 and 3 (instead of every 2 weeks during cycle 2 and 3) to more closely monitor liver enzymes and other lab tests.

Approval was granted by Health Canada and the local REB by January 2006. Approval from the HSRRB was granted in June of 2006. Since HSRRB approval, 5 patients have been accrued to date. Because of delays in start-up and approval for the phase I and II trials, and the amendment, an additional no-cost one-year time extension was requested and provided. Enrolment is expected to proceed at 1-2 patients per week and that rate the project should be able to finish on schedule in 2007.

KEY RESEARCH ACCOMPLISHMENTS:

- Completion of the first clinical trial of a second generation phosphorothioate antisense oligonucleotide in patients with cancer
- Novel study design using neoadjuvant therapy prior to radical prostatectomy. This design is now being used to evaluate pharmacodynamic effect of a number of other targeted agents.
- Proof of principal demonstration of biologic effect
- Determination of recommended phase II dose of OGX-011 based on biological efficacy. This dose is the basis for other trials involving OGX-011 in patients with lung, breast and hormone refractory prostate cancer.
- Phase II neoadjuvant trial initiated

REPORTABLE OUTCOMES:

Manuscripts

1. Chi KN, Eisenhauer E, Fazil L, Jones EC, Goldenberg SL, Powers J, Tu D, Gleave ME. A Phase 1 Pharmacokinetic and Pharmacodynamic Study of OGX-011, a 2'-Methoxyethyl Antisense Oligonucleotide to Clusterin in Patients with Localized Prostate Cancer. *Journal of the National Cancer Institute*, 97(17):1287-96, 2005.
2. Gleave M, Chi KN. Knock-down of the cytoprotective gene, clusterin, to enhance hormone and chemosensitivity in prostate and other cancers. *Ann N Y Acad Sci*. 2005; 1058:1-15.
3. Gleave M, Miyake H, Chi K. Beyond simple castration: targeting the molecular basis of treatment resistance in advanced prostate cancer. *Cancer Chemother Pharmacol*. 2005; 56 Suppl 1:47-57.

Abstracts

1. Chi KN, Eisenhauer E, Fazli L, Jones EC, Powers J, Hurtado-Coll A, Goldenberg SL, Gleave ME. A phase I pharmacokinetic (PK) and pharmacodynamic (PD) study of OGX-011, a 2'-methoxyethyl phosphorothioate antisense to *clusterin*, in patients with prostate cancer prior to radical prostatectomy. 16th EORTC-NCI-AACR Symposium on Molecular Targets and Cancer Therapeutics. Oral Presentation at the Proffered Papers Plenary Session, Geneva, September 2004. *European Journal of Cancer Supplements*. Volume 2, No. 8, September 2004.

Presentations

1. "Clusterin Antisense". 4th International Congress on Targeted Therapies in Cancer. Washington, DC. August 26-28, 2005.

CONCLUSIONS

This phase I trial provides proof of principal evidence that OGX-011 can inhibit expression of clusterin in prostate cancer cells in humans. This is the first demonstration of dose dependent inhibition of a target, within target tissue by an antisense targeted therapeutic. Because of the successful determination of the biologically effective dose, phase II clinical trials with OGX-011 can move forward with confidence in the dosing regimen and schedule.

This trial has renewed interest in the antisense therapeutic platform, and several trials are moving forward with the second generation chemistry including antisense targeted against the cell survival proteins surviving and X-linked inhibitor of apoptosis using the data from the phase I trial of OGX-011 to support dosing.

Phase II trials using the recommended phase II dose of OGX-011 are now proceeding. In addition to the neoadjuvant phase II trial discussed above, four other trials using OGX-011 are currently enrolling patients: a randomized phase II trial of OGX-011 and docetaxel for patients with hormone refractory prostate cancer (grant funded by the National Cancer Institute of Canada), a randomized phase II trial of OGX-011 and mitoxantrone as second line therapy for patients with hormone refractory prostate cancer, a phase II trial of OGX-011 and docetaxel for patients with metastatic breast cancer, and a phase II trial of OGX-011 and Cisplatin-Gemciabine for patients with advanced lung cancer.

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APPENDIX: MANUSCRIPT REPRINT

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

Kim N. Chi, Elizabeth Eisenhauer, Ladan Fazli, Edward C. Jones, S. Larry Goldenberg, Jean Powers, Dongsheng Tu, Martin E. Gleave

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_{MAX} (peak plasma concentration) increased proportionally with dose ($P_{trend} < .001$). OGX-011 in prostate tissue increased with dose ($P_{trend} < .001$). Dose-dependent decreases in prostate cancer and lymph node clusterin expression were observed by polymerase chain reaction of greater than 90% ($P_{trend} = .008$ and $P_{trend} < .001$, respectively) and by immunohistochemistry ($P_{trend} < .001$ and $P_{trend} = .01$, 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 nucleases 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.

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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 $\geq 3.0 \times 10^9/L$, hemoglobin level of ≥ 100 g/L, platelet concentration of $\geq 100 \times 10^9/L$, 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'-CAGCAGCAGAGTCTTC ATCAT-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), thrombocytopenia $<25 \times 10^9/L$ or grade 3 thrombocytopenia 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, 2.25, 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 2- to 8000-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 polystyrene 96-well plate and then 125 μ L 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 μ L) 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 μ L 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-isoamyl 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.

Noncompartmental pharmacokinetic analysis was performed on the plasma concentration data, including assessment of the T_{MAX} (time to reach peak plasma concentration), C_{MAX} (observed peak plasma concentration), AUC (area under the plasma concentration versus time curve), k (plasma distribution rate constant), $t_{1/2}$ (plasma distribution half-life), V_z (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 μ g of proteinase K in 20 μ L 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 \times 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 pRc-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 \times Denhardt's solution, 20 \times SSC, 1 M dithiothreitol, 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 \times SSC at 37 °C and three times for 15 minutes in 1 \times 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 μ L RNase A. After two 30-minute washes in 1 \times 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 \times magnification. The overall score was determined as follows: overall score = [(% cells with visual score 1) \times 1] + [(% cells with visual score 2) \times 2] + [(% cells with visual score 3) \times 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 μ L 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 5700 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'-GAGCAGCTGAACGAGCAGTTT-3', reverse primer 5'-CTTCGCCTTGCGTGAGGT-3', and TaqMan probe 5'-VIC-ACTGGGTGTCCCGGCTGGCA-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 replicates 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, Carpinteria, 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 1. Patient characteristics

| Characteristic* | N |
|-----------------------|------------|
| Median age (range), y | 63 (45–71) |
| Gleason score | |
| 6 | 5 |
| 7 | 14 |
| 8–10 | 6 |
| Baseline PSA, ng/mL | |
| ≤10 | 16 |
| >10–20 | 6 |
| >20 | 3 |
| Clinical stage | |
| 1c | 12 |
| 2a | 9 |
| 2b | 3 |
| 3a | 1 |

*PSA = prostate-specific antigen. Tumors were staged according to International Union Against Cancer (UICC) TNM 1997 classification.

Table 2. Selected adverse effects of the phosphothionate oligonucleotide OGX-011 by dose level*

| Toxicity | Grades for the following dose levels (mg) and no. of subjects: | | | | | | | | | |
|------------------|--|---|------------|---|------------|---|------------|---|------------|---|
| | 80, N = 3 | | 160, N = 3 | | 320, N = 6 | | 480, N = 6 | | 640, N = 6 | |
| | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 |
| Leukopenia | 1 | 0 | 0 | 0 | 4 | 0 | 2 | 0 | 2 | 0 |
| Anemia | 2 | 0 | 3 | 0 | 4 | 0 | 5 | 0 | 5 | 0 |
| Thrombocytopenia | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 3 | 0 |
| AST | 1 | 1 | 0 | 0 | 2 | 0 | 1 | 0 | 2 | 2 |
| ALT | 0 | 1 | 0 | 0 | 2 | 0 | 1 | 0 | 3 | 1 |
| Fever | 0 | 0 | 0 | 0 | 3 | 0 | 4 | 1 | 4 | 1 |
| Fatigue | 0 | 0 | 2 | 0 | 5 | 0 | 2 | 1 | 5 | 0 |
| Rigors | 0 | 0 | 1 | 0 | 4 | 0 | 6 | 0 | 6 | 0 |
| Creatinine | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 |
| Anorexia | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| Rigors | 0 | 0 | 1 | 0 | 4 | 0 | 6 | 0 | 6 | 0 |
| Arthralgias | 0 | 0 | 0 | 0 | 2 | 0 | 1 | 0 | 0 | 0 |
| Myalgias | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 1 | 0 |

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

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 experienced by subject in Table 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 $t_{1/2}$ 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 C_{MAX} at 640 mg was 61.1 $\mu\text{g/mL}$ (95% confidence interval [CI] = 55.3 to 66.9) after the day 1 infusion and 69.9 $\mu\text{g/mL}$ (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%

Table 3. Plasma pharmacokinetic parameters of OGX-011*

| Dose level, mg | No. of patients | Treatment day | C_{MAX} , $\mu\text{g/mL}$ | $t_{1/2}$, h† | $AUC_{0-\infty}$, $\mu\text{g} \cdot \text{h/mL} \ddagger$ | V_z , L§ | CL, L/h |
|----------------|-----------------|---------------|------------------------------|-------------------|---|---------------------|---------------------|
| 40 | 1 | 1 | 4.0 | 0.5 | 9.5 | 3.3 | 4.2 |
| | | 29 | 3.6 | 0.5 | 8.1 | 3.4 | 4.9 |
| 80 | 3 | 1 | 12.3 (.9 to 23.7) | 0.8 (.5 to 1.2) | 34.0 (4.3 to 63.7) | 3.2 (-.5 to 6.8) | 2.6 (.1 to 5.2) |
| | | 29 | 11.2 (-.5 to 22.9) | 0.8 (.3 to 1.3) | 23.1 (-74.1 to 120.4) | 4.4 (-13.8 to 22.7) | 3.9 (-12.5 to 20.3) |
| 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) |
| | | 29 | 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) |
| 320 | 6 | 1 | 39.5 (28.3 to 50.7) | 2.0 (1.5 to 2.5) | 138.4 (117.1 to 159.7) | 6.6 (5.9 to 7.4) | 2.4 (2.0 to 2.7) |
| | | 29 | 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) |
| 480 | 6 | 1 | 55.4 (37.9 to 72.9) | 2.1 (1.4 to 2.8) | 217.6 (147.8 to 287.5) | 7.0 (3.7 to 10.3) | 2.3 (1.7 to 2.9) |
| | | 29 | 48.0 (30.8 to 65.2) | 3.2 (2.8 to 3.7) | 199.7 (128.3 to 271.1) | 12.2 (8.1 to 16.4) | 2.6 (1.9 to 3.3) |
| 640 | 6 | 1 | 61.1 (55.3 to 66.9) | 2.4 (1.8 to 2.9) | 251.9 (225.9 to 278.0) | 8.8 (6.8 to 10.7) | 2.6 (2.3 to 2.9) |
| | | 29 | 69.9 (64.8 to 74.9) | 3.3 (2.9 to 3.8) | 284.3 (221.1 to 347.4) | 11.0 (9.4 to 12.6) | 2.3 (1.8 to 2.8) |

*Values represent means (95% confidence intervals). T_{MAX} = time to reach peak plasma concentration. C_{MAX} = observed peak plasma concentration.

† $t_{1/2}$ = plasma distribution half-life.

‡AUC = area under the plasma concentration versus time curve.

§ V_z = apparent volume of distribution.

||CL = total clearance from plasma.

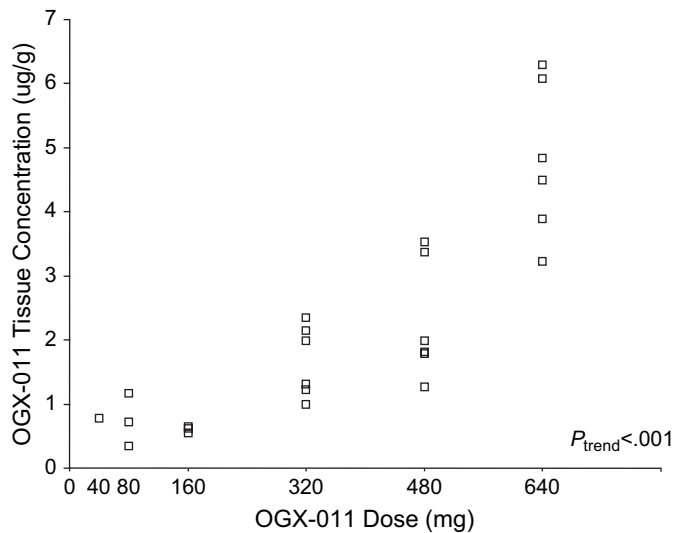


Fig. 1. OGX-011 concentration in prostate tissue from men with prostate cancer who were treated with OGX-011 prior to prostatectomy. Men were treated with six different doses. Concentration was measured in single replicates using a validated ELISA. Each point represents an individual subject.

CI = 3.54 to 6.10) $\mu\text{g/g}$ of prostate tissue, respectively, corresponding roughly to concentrations of 223 nM, 306 nM, and 644 nM.

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 protein 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

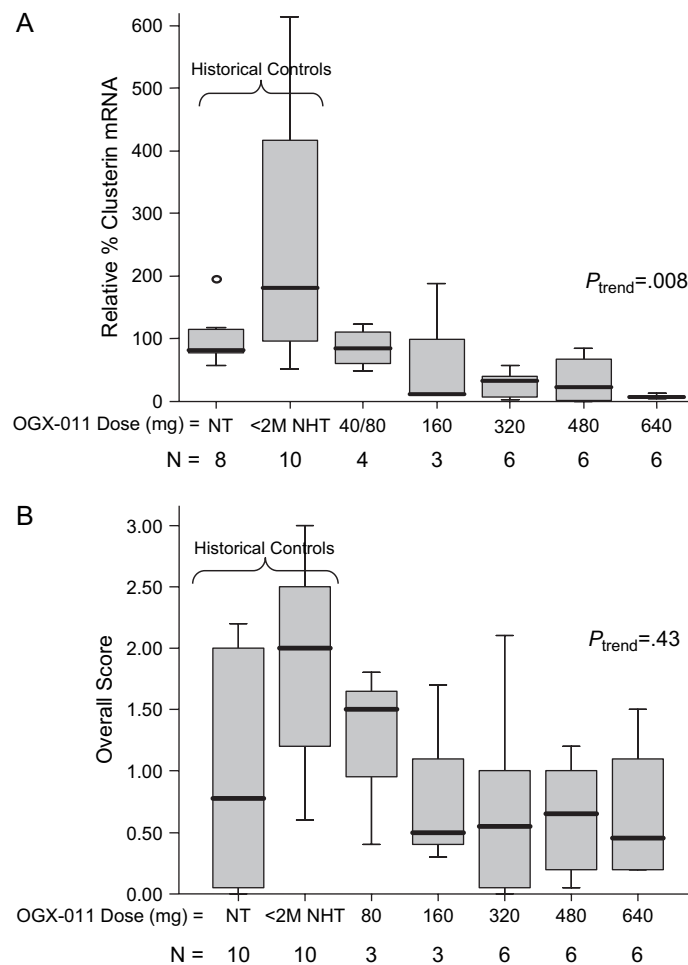


Fig. 2. Clusterin mRNA expression in prostate cancer cells of men with prostate cancer who were treated with OGX-011 prior to prostatectomy and in historical control subjects. In both panels, the boxes represent the interquartile range, lines extending from the boxes represent the closer of either 1.5 times the interquartile range or the most extreme observation, the line across the box indicates the median, and circles represent outliers (values between 1.5 and 3 box lengths from the upper or lower edge of the box). NT = no prior treatment; <2M NHT = less than 2 months of neoadjuvant hormone therapy historical controls. **A)** Clusterin mRNA expression as assayed by quantitative real-time polymerase chain reaction (QRT-PCR) from laser-captured microdissected prostate cancer cells. Levels in untreated historical control samples were set at 100%, and levels in other samples are shown relative to the control. Total RNA was extracted from approximately 100000 laser-captured cells, and RNA was reverse transcribed, with the resulting first-strand cDNA used as the template for QRT-PCR. Two replicates per sample were performed and both shown. **B)** Clusterin mRNA expression by in situ hybridization. Sections were prepared and analyzed as described in Materials and Methods. Three slides per patient and 10 fields per slide were evaluated for staining intensity (0 to 3, representing negative to strong staining, respectively) and graded independently by two pathologists (L.F. and E.C.J.). The overall score was determined as follows: overall score = [(% cells with visual score 1) \times 1] + [(% cells with visual score 2) \times 2] + [(% cells with visual score 3) \times 3].

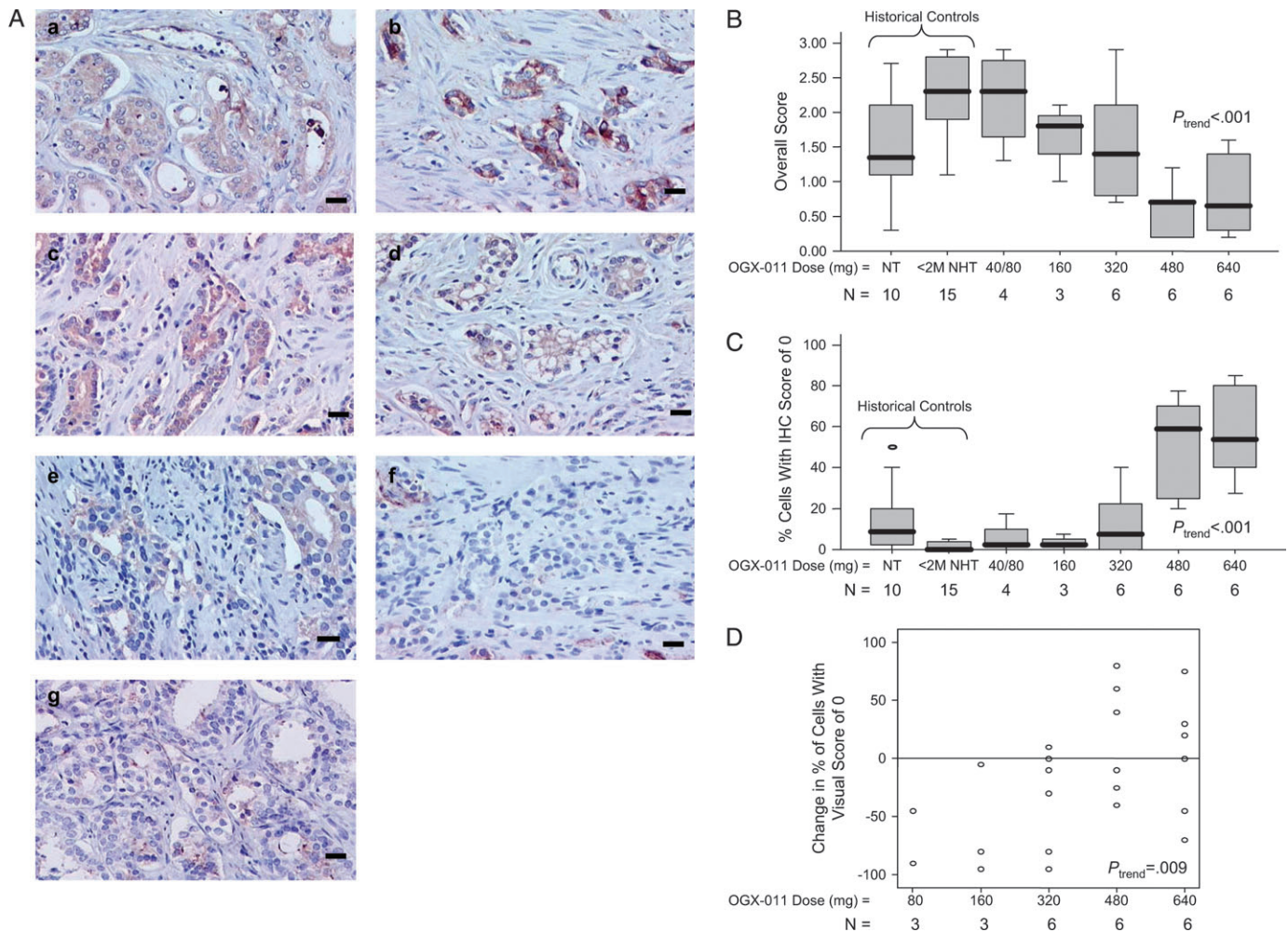


Fig. 3. Clusterin protein expression in prostate cancer samples from men who had a prostatectomy following treatment with OGX-011 and in untreated historical control subjects. **A)** Representative sections from prostatectomy specimens stained for clusterin protein expression by immunohistochemistry. Panels a and b are from contemporarily treated historical control specimens with no neoadjuvant therapy prior to surgery and less than 2 months of neoadjuvant hormone therapy, respectively. Panels c through g are from trial subjects treated with OGX-011 at doses of 80 mg (c), 160 mg (d), 320 mg (e), 480 mg (f), and 640 mg (g). Goat anti-human clusterin antibody was used at a working dilution of 1:600 in 1% bovine serum albumin, and the staining kit was detected by use of a biotinylated secondary antibody. Bar = 100 μ m. **B)** Box plot of the overall score of immunohistochemistry staining. Three slides per patient and 10 fields per slide were evaluated for staining intensity from 0 to 3 (representing negative to strong staining, respectively) and

graded independently by two pathologists (L.F. and E.C.J.). The overall score was determined as follows: overall score = [(% cells with visual score 1) \times 1] + [(% cells with visual score 2) \times 2] + [(% cells with visual score 3) \times 3]. **C)** Box plot of percentage of prostate cancer cells with a visual score of 0 (i.e. negative clusterin expression by immunohistochemistry). The boxes in B and C represent the interquartile ranges, lines extending from the box represent the closer of either 1.5 times the interquartile range or the most extreme observation, the line across the box indicates the median, and circles represent outliers. NT = no prior treatment; <2M NHT = less than 2 months of neoadjuvant hormone therapy historical controls. **D)** Change from initial biopsy to prostatectomy in percentage of prostate cancer cells that have a visual score of 0. Open circles represent individual patients, with scores above the 0 line indicating an increase in the number of cells that score 0 and thus do not express clusterin.

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

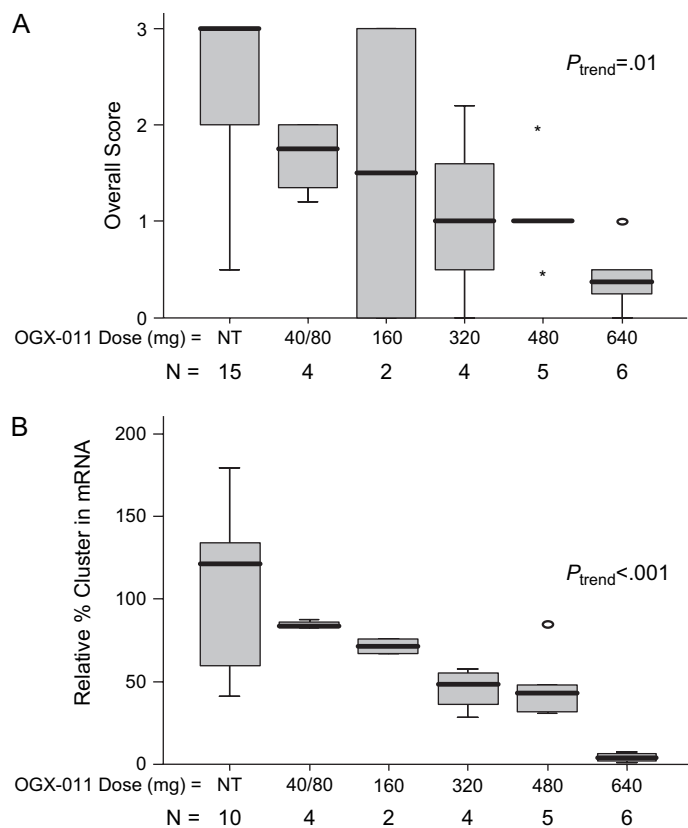


Fig. 4. Box plot of clusterin expression in normal lymph nodes of men with prostate cancer who were treated with OGX-011 prior to prostatectomy and in untreated historical control subjects. Clusterin expression was assayed by **A**) immunohistochemistry and **B**) quantitative real-time polymerase chain reaction. The boxes represent the interquartile ranges, lines extending from the boxes represent the closer of either 1.5 times the interquartile range or the most extreme observation, the lines across the boxes indicate the median, circles indicate outliers, and stars show extreme outliers (values more than three box lengths from the upper or lower edge of the box). NT = no prior treatment historical control. Values from 40- and 80-mg treatment groups were combined.

at the lower two dose levels of OGX-011 was 7.1% (95% CI = 2.4% to 11.8%), but at the 640-mg dose level, the mean apoptotic index was 21.2% (95% CI = 18.1% to 24.2%) (Fig. 5; $P_{\text{trend}} < .001$).

DISCUSSION

In this first-in-man trial, we found that OGX-011 antisense oligonucleotides to clusterin were well tolerated and had a predictable plasma pharmacokinetic profile. More important, however, because of the design of this trial—that is, as OGX-011 treatment was followed by radical prostatectomy, we were able to demonstrate that concentrations of OGX-011 could be achieved 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).

The plasma pharmacokinetic parameters of OGX-011 were similar to those of other antisense molecules (21,22). The C_{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_{MAX} of greater than 100 $\mu\text{g/mL}$. 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 intrapatent 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

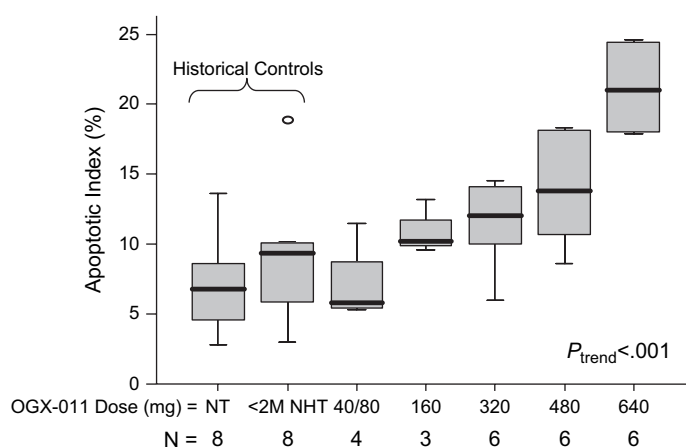


Fig. 5. Box plot of apoptotic indexes in prostatectomy specimens. Apoptosis was evaluated with the terminal deoxynucleotidyltransferase-mediated dUTP-biotin nick end labeling assay. The percentage of apoptotic cells and bodies per 10 high-power fields (Apoptotic Index) were independently counted by the two pathologists (L.F. and E.C.J.). The boxes represent the interquartile range, lines extending from the box represent the closer of either 1.5 times the interquartile range or the most extreme observation, lines across the boxes indicate the median, and circles indicate outliers. NT = no prior treatment; <2M NHT = less than 2 months of neoadjuvant hormone therapy. Values from 40- and 80-mg treatment groups were combined.

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 (28). 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.

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