Drug Evaluation
Future Oncology

Custirsen (OGX-011): a second-generation antisense inhibitor of clusterin in development for the treatment of prostate cancer

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Clusterin is a stress-induced cytoprotective chaperone that confers broad-spectrum treatment resistance and is overexpressed across a number of cancers. custirsen (OGX-011) is a promising novel second-generation antisense inhibitor of clusterin in clinical development. This article describes the mechanism of action and safety profile of OGX-011 and details the Phase I and II results in human solid organ malignancies. Two Phase III registration trials are currently under recruitment evaluating OGX-011 in combination with chemotherapy in patients with metastatic castration-resistant prostate cancer. These studies not only have the potential to significantly alter the standard of care in prostate cancer, but would also endorse a new class of targets and targeted therapy approach for cancer.

Custirsen (OGX-011, OncoGenex Pharmaceuticals Inc., WA, USA and Teva Pharmaceuticals Ltd, Petach Tikva, Israel) is a novel antisense oligonucleotide compound that targets expression of the clusterin gene. This article describes clusterin's structure, function and influential role in the development of treatment-resistant cancers. The rationale for employing antisense technology, and in particular OGX-011, to target gene overexpression is discussed. Finally, detailed efficacy and tolerability data are presented that establish the safety of OGX-011 in combination with chemotherapy and the potential role of OGX-011 in overcoming treatment resistance in many malignancies.

Clusterin is a secretable cytoprotective protein that is upregulated in response to cellular stress, which includes standard cancer treatments such as hormone-, radiation- and chemo-therapy, and is implicated in treatment resistance. Clusterin is expressed in many malignancies including prostate, breast, ovarian, non-small-cell lung, colon, renal, urothelial and pancreatic cancers, and anaplastic large-cell lymphoma and melanoma [1–7]. Since clusterin binds to a wide variety of client proteins involved in a diverse array of biological processes and regulated by HSF-1 it is viewed as a heat shock-like protein that chaperones and stabilizes proteins at times of cellular stress to promote cell survival. As clusterin isoforms are also secreted extracellularly, some investigators suggest that clusterin may be the first identified secreted mammalian chaperone [8].

OGX-011 is a phosphorothioate antisense oligonucleotide (ASO) inhibitor of *clusterin* expression that incorporates second-generation chemistry in the form of a 2′-methoxyethyl (2′-MOE) modifications to increase tissue half-life, enhance potency and decrease nonsequence-specific toxicity of the molecule. The *clusterin* gene was screened with a series of ASO sequences, and the most potent at inhibiting expression was the OGX-011 sequence targeting the translation initiation site. Preclinical studies have demonstrated the potency of OGX-011 at inhibiting clusterin expression *in vitro* and *in vivo*, resulting in the therapeutic enhancement of standard anticancer treatments including hormone-, radiation- and chemo-therapy [9].

Phase I and II clinical trials with OGX-011 have demonstrated biologically active dosing and successful delivery of the drug to malignant tissues with inhibition of clusterin expression in both tissue and serum. A randomized Phase II study of OGX-011 in combination with docetaxel in patients with metastatic castration-resistant prostate cancer (CRPC) demonstrated a survival advantage for patients receiving the combination [10]. This led to the current registration pathway for OGX-011 in combination with standard therapies in patients with metastatic CRPC.

For prostate cancer in 2012, standard treatments for the metastatic CRPC patient include chemotherapeutics (docetaxel [11], mitoxantrone [12] and cabazitaxel [13]), bone-targeting agents (zoledronic acid [14] and denosumab [15]),

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immunotherapy (sipuleucel-T [16]) and a potent inhibitor of androgen biosynthesis (abiraterone acetate [17]). A novel, next-generation androgen receptor inhibitor, MDV3100 (enzalutamide) [18], and the infusional radioisotope radium-223 [19], have also demonstrated improved overall survival in patients after docetaxel or in docetaxel-ineligible patients (in the case of radium-223). Both agents remain investigational and are expected to enter the CRPC market in 2012–2013. Despite these therapeutic advancements, treatment for metastatic CRPC remains palliative with a median overall survival of approximately 18 months [11]. Due to the role of clusterin in cell survival across multiple mechanisms and broad-spectrum treatment resistance, inhibition of clusterin with OGX-011 has the potential to improve outcomes across all these therapeutic modalities.

Overview of the market

Prostate cancer is the most frequently diagnosed cancer other than skin cancer and the second-leading cause of death from cancer in men in North America [20]. In 2011, prostate cancer was diagnosed in 240,000 men and led to nearly 33,000 deaths in the USA. Worldwide, approximately 910,000 cases of prostate cancer were recorded in 2008, accounting for approximately 14% of all new cancer cases in men [101]. CRPC is the lethal phenotype of the disease that may emerge after standard androgen ablation therapy for advanced disease. The term CRPC is commonly used when a patient's cancer progresses despite castrate levels of testosterone (<50 ng/ml). The therapeutic landscape has recently become enriched with multiple new agents that are either US FDA approved or recently reported in Phase III trials with positive results in terms of overall survival, progression-free survival (PFS) and symptomatic outcomes. Docetaxel was the first chemotherapeutic agent to demonstrate a survival advantage in CRPC [11], whereas mitoxantrone was only approved for palliation of symptoms in a similar patient group [12]. More recently cabazitaxel, a third-generation taxane, gained FDA approval by establishing a 2.4-month survival advantage in patients progressing after docetaxel [13]. Sipuleucel-T, the first FDA-approved vaccine-based therapy for any malignancy, established a 4.1-month survival advantage in patients with asymptomatic or minimally symptomatic metastatic CRPC [16]. New agents that target persistent androgen receptor signaling in CRPC through either inhibition of extragonadal steroidogenesis or direct inhibition of the androgen receptor have also been developed. Abiraterone acetate is currently the first approved drug in this class after establishing a 3.8-month median overall survival advantage in the postdocetaxel setting [17]. MDV3100 is a novel oral anti-androgen that directly inhibits the androgen receptor, which has also shown a survival advantage in the same patient group [18]. Lastly, the bone-targeting agents zoledronic acid and denosumab are also employed in the CRPC treatment model after demonstrating reduction in skeletal-related events and postponing development of bone metastases [14,15]. **Table 1** summarizes both the currently approved agents and promising agents with unpublished positive Phase III results.

The current approach to managing metastatic CRPC is under major transformation as the

Table 1. Agents that are currently US FDA approved or with positive Phase III study data in castration-resistant prostate cancer.

Agent	Mode of action	Benefit	Ref.
Enzalutamide (MDV3100)	Anti-androgen	4.8-month median OS	[18]
Sipuleucel-T	Immunotherapy	4.1-month median OS	[16]
Abiraterone acetate	Anti-androgen	3.9-month median OS	[17]
Alpharadin	Radiopharmaceutical	2.8-month medial OS	[19]
Docetaxel	Chemotherapeutic	2.4-month median OS	[11]
Cabazitaxel	Chemotherapeutic	2.4-month median OS	[13]
Mitoxantrone	Chemotherapeutic	Pain palliation	[12]
Denosumab	RANK ligand inhibitor	4.2-month bone metastasis-free survival	[15]
Zoledronic acid	Inhibitor of osteoblasts	Delayed time to SRE†	[14]

†Pathologic bone fractures, spinal cord compression, surgery to bone, radiation therapy to bone (including the use of radioisotopes) or a change of antineoplastic therapy to treat bone pain.
OS: Overall survival; SRE: Skeletal-related event.

optimal sequencing of these new agents continues to evolve. Yet perhaps the greater challenge is how best to manage the emergence of universal treatment resistance. Targeting the fundamental effectors of treatment resistance is an appealing approach; inhibition of heat-shock and cellular chaperone proteins fit into this category.

The target: clusterin

Clusterin is a single-copy gene, organized into nine exons (eight introns) and a 5′-untranslated region, located on chromosome 8p21–p12 and extending more than 16 kb [21,22]. It is highly conserved across species and constitutively expressed in almost all mammalian tissues. In humans, the clusterin gene codes for two secretory isoforms (sCLU-1 and sCLU-2), originating from transcriptional start sites in exons 1 and 2, respectively; only sCLU-2 is expressed in subprimates. Secreted clusterin (sCLU) is an endoplasmic reticulum-targeted, 449-amino acid polypeptide that represents the predominant translation product of the human gene. Although sCLU is cytoprotective and anti-apoptotic, a pro-apoptotic activity ~55-kDa nuclear (nCLU) splice variant lacking exon 2 and the endoplasmic reticulum signal peptide has been described [23,24]. Analysis of the promoter has revealed numerous transcription factor-binding sites and also a conserved clusterin element, recognized by HSF-1/HSF-2 heterocomplexes [25]. Clusterin is transcriptionally activated by HSF-1 in response to cellular stress [26,27].

In malignancy, clusterin has largely been defined as an inhibitor of apoptosis [28–31]. Its anti-apoptotic actions have been described as functioning through a variety of mechanisms including inhibition of activated Bax [32] and enhanced survival signaling through upregulation of PI3k/Akt pathway signaling [33]. Clusterin has also been implicated in tumorigenesis through loss of the tumor suppressor gene Nkx3.1 [34], and its overexpression in response to hormonal ablation, chemotherapy and radiotherapy contributes to treatment resistance [6,29,35]. This clusterin-induced treatment-resistant phenotype has been much studied in prostate cancer and docetaxel-resistant prostate tumor cells have been resensitized to docetaxel following exposure to OGX-011 [36,37]. However, drug resistance induced by clusterin has been observed within other tumor types and with chemotherapy agents including doxorubicin, camptothecin, cisplatin, 5-fluorouracil, gemcitabine, dacarbazine and etoposide [2,32,38–42].

Introduction to the compound

OGX-011 is a novel second-generation 2′-MOE-modified phosphorothioate ASO complementary to clusterin mRNA. OGX-011 is a potent inhibitor of clusterin expression *in vitro*, *in vivo* and in humans in clinical trials. As a single agent and in combination with chemotherapy, OGX-011 has been well-tolerated in Phase I trials and with promising activity in Phase II clinical trials. Registration Phase III trials are underway with OGX-011 in combination with chemotherapy in patients with metastatic CRPC.

Chemistry

ASO therapy is one strategy to specifically target functionally relevant genes. ASOs are chemically modified stretches of single-strand DNA complementary to mRNA regions of a target gene that inhibit translation by forming RNA/DNA duplexes, thereby reducing mRNA and protein levels of the target gene [43]. OGX-011 is a 21-nucleoside ASO complementary to the clusterin exon 2 mRNA AUG translation initiation site, with one CpG motif. The custirsen sequence was identified as the most potent to inhibit clusterin expression after the gene was 'walked' with a series of antisense sequences. OGX-011 is a second-generation phosphorothioate and incorporates the 2′-*O*-2′-MOE modification with four 2′-MOE-modified nucleosides at the 3′ side, four 2′-MOE-modified nucleosides at the 5′ side and 13 2′-deoxyribonucleosides in between (referred to as a 4-13-4 MOE gapmer).

Phosphorothioate ASOs are water-soluble, stable agents resistant to nuclease digestion through substitution of a nonbridging phosphoryl oxygen of DNA with sulfur [44]. In clinical trials, a major technical limitation with the first generation of phosphorothioate ASOs was the requirement for continuous or frequent intravenous infusions owing to the short tissue half-life of these agents. The approach to overcome this has been by a 2'-MOE modification to the 2′-position of the carbohydrate moiety. This forms ASO–RNA duplexes with a significantly higher affinity relative to unmodified phosphorothioate ASOs. This increased affinity has been shown to result in improved antisense potency *in vitro* and *in vivo*. In addition, 2′-MOE second-generation ASOs display significantly improved resistance against nuclease-mediated metabolism resulting in an improved tissue half-life *in vivo*, which produces a longer duration of action and allows for an intermittent dosing regimen [45]. Finally, these second-generation phosphorothioate

ASOs have the potential for an improved safety profile relative to unmodified phosphorothioate ASOs [46].

Pharmacokinetics

The disposition and metabolism of OGX-011 was measured in mice and monkeys. Consistent with pharmacokinetic (PK) studies with other ASOs, plasma was rapidly cleared of OGX-011 in both species [47]. Plasma concentrations of OGX-011 generally peaked at the end of the 60-min intravenous infusion period and then decreased in an apparent biexponential fashion that included two distinct half-lives. Mean concentrations increased with dose in all tissues examined, except for brain where no measurable drug could be detected. Approximately 90% of the compound was found in the parent form in all tissues at all time points. PK parameters were similar at the beginning and end of the treatment period in both species, suggesting no plasma accumulation or changes in plasma kinetics after multiple dosing in either species.

In humans, plasma PK parameters for OGX-011 have followed predictions from preclinical studies. The first-in-man Phase I trial [48] was uniquely designed to permit a dose-dependent analysis of OGX-011 levels in the plasma and prostatic tissue. The mean plasma distribution half-life was 3.3 h at the 640-mg dose. Average peak concentrations and area under the curve were dose dependent and displayed proportional and predictable increases in a linear fashion. This study also demonstrated concentrations in prostate cancer cells of full-length OGX-011 at levels sufficient for inhibition of clusterin expression. Furthermore, the prostate tissue concentrations were proven to be dose dependent: 320 mg delivered 223 nM tissue concentration and 640 mg delivered 644 nM into the prostate tissue. PK plasma parameters for humans are detailed in Table 2. As ASOs are degraded by both serum and intracellular nucleases [45] and only 7% of the drug is excreted unchanged in the urine, tissue distribution of OGX-011 dominates plasma clearance.

Pharmacodynamics

OGX-011 is a potent inhibitor of clusterin expression in *in vitro* and *in vivo* laboratory models [45]. Furthermore, because of the targeting to exon 2 of clusterin RNA there is a specificity for OGX-011 to inhibit only the expression of the anti-apoptotic secreted form of clusterin, with no effect on the pro-apoptotic nuclear form [49].

In vitro activity of ASOs and documented overexpression of tissue clusterin has been demonstrated in multiple chemotherapy resistant tumor cell lines [35,50]. In prostate cancer, a docetaxel-resistant human prostate cancer cell line (PC-3dR) had significantly higher secreted clusterin levels compared with controls [37]. Clusterin protein expression was significantly decreased upon exposure to OGX-011 compared with a control oligonucleotide. Chemotherapy resensitization was demonstrated by combining the docetaxel-resistant PC-3dR cells with OGX-011 resulting in a fall of the IC_{50} of docetaxel from >1000 nM to 125 nM.

Downregulation of sCLU was established in multiple *in vivo* mice models (lung [35], bladder [50] and prostate [37,45]). In prostate cancer, treatment with OGX-011 decreased sCLU expression in both PC-3 and PC-3dR xenografts. Western blot analysis of protein harvested from tumors obtained from mice at the end of the study indicated that OGX-011 decreased sCLU expression levels to 32 and 27% of the sham ASO-treated controls in PC-3 and PC-3dR tumors, respectively. Resembling the *in vitro* data, baseline sCLU expression were 2.25-fold higher in the PC-3dR tumors compared with the wild-type PC-3 tumors ($p < 0.001$).

Antitumor effect was also confirmed across several mouse models. In the prostate cancer mice xenografts, OGX-011 treatment significantly enhanced the antitumor effects of paclitaxel compared with the sham ASO control, reducing the mean PC-3 tumor volume to 33% of controls at week 9 ($p < 0.01$). More importantly, OGX-011 was also able to significantly enhance cytotoxicity of paclitaxel in PC-3dR xenografts, decreasing mean tumor volume by 76% compared with the MOE gapmer mismatch control

Table 2. Plasma and tissue pharmacokinetic data for OGX-011.

Dose	Plasma				Tissue
	AUC (µg/h/ml)	C_{max} (µg/ml)	Clearance (ml/h/kg)	Half-life (h)	Mean prostate tissue concentration (µg/g)
640 mg[†]	290.15	69.85	32.9	7.63	4.82

[†]*Recommended dose in humans.*
AUC: Area under the curve.

at week 9 ($p < 0.01$). Collectively, these results show that sCLU knockdown using OGX-011 not only sensitizes PC-3 tumors to chemotherapy *in vivo*, but also reverses resistance and enhances chemosensitivity in PC-3dR xenografts.

The first-in-man study combined OGX-011 with neoadjuvant hormone ablation for patients with localized prostate carcinoma prior to radical prostatectomy (RP). Prostate specimens and historical controls were then evaluated for clusterin expression. Changes in expression of tissue clusterin were correlated to the administered plasma dose and the dose delivered to the prostate. OGX-011 produced statistically significant dose-dependent suppression of clusterin mRNA and protein expression in normal and tumor tissue. Approximately double the amount of tumor cell death (as determined by apoptotic index) occurred in the prostate of patients receiving the highest dose of OGX-011 (640 mg) compared with hormone ablation therapy alone. With this novel design and the use of PK and pharmacodynamic end points, an effective biologic dose of 640 mg was established for OGX-011 based on its ability to suppress clusterin mRNA by >90% in prostate cancer tissue (Figure 1) [51].

In the historical control specimens treated with and without neoadjuvant hormone therapy (NHT), the mean apoptotic indices were 9.0% (95% CI: 5.1–13.0) and 7.0% (95% CI: 4.2–9.9), respectively. The apoptotic index from patients treated at the lower two dose levels of OGX-011 was 7.1% (95% CI: 2.4–11.8), but at the 640-mg dose level, the mean apoptotic index was 21.2% (95% CI: 18.1–24.2). These data provide proof-of-principle for the biologic activity of OGX-011 and reinforce the dose–response effect initially demonstrated in preclinical testing.

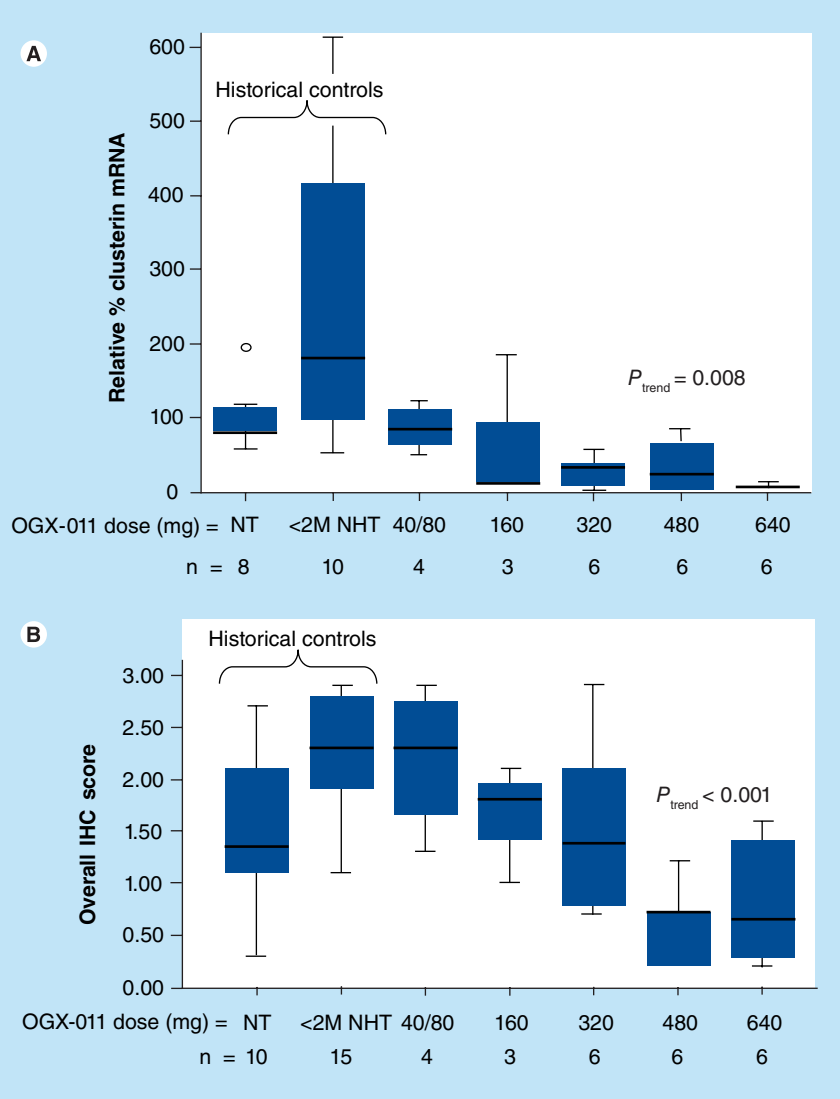

Figure 1. OGX-011 inhibits clusterin expression in human prostate tissue. **(A)** Box-plot of clusterin mRNA expression in prostate cancer cells of men with prostate cancer who were treated with OGX-011 prior to prostatectomy and in historical control subjects. **(B)** Box-plot of the overall score of immunohistochemistry staining. Three slides per patient and ten fields per slide were evaluated for staining intensity from 0 to 3 (representing negative to strong staining, respectively) and graded independently by two pathologists.
<2M NHT: Less than 2 months of neoadjuvant hormone therapy historical controls; IHC: Immunohistochemistry; NT: No treatment.
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Clinical efficacy

To date, two Phase I and five Phase II trials have been completed with a total of 313 patients enrolled: 213 with prostate cancer and 95 in lung and breast cancer. In prostate cancer, OGX-011 has been evaluated in multiple settings and in combination with both hormonal and chemotherapy agents (see Table 3). In advanced lung and breast malignancies, OGX-011 has been combined with chemotherapy agents (platinums, gemcitabine and docetaxel). Three Phase III trials have been initiated and two are currently recruiting patients with CRPC. The SYNERGY study is randomizing chemotherapy-naive patients with metastatic CRPC to receive standard docetaxel and prednisone with or without OGX-011 [102]. The study commenced in 2010 and is due for completion in December 2013 with a planned enrollment of 1000 men and a primary outcome of overall survival. The trial has been designed for 90% power to detect a hazard ratio of 0.75 assuming an 18-month expected overall survival in the control arm. A second Phase III trial in patients with metastatic CRPC previously treated with docetaxel, SATURN [103], aimed to assess pain response as a primary end point with OGX-011 in combination with docetaxel–prednisone retreatment versus placebo and docetaxel–prednisone. The study was

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