Targeting The Stress-Induced Cytoprotective Chaperone, Clusterin, to Overcome Treatment Resistance in Advanced Prostate Cancer

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Disclosure of Conflicts of Interest
Patent - OGX-011
Founder - OncoGenex Technologies
Consultant - CSO, OncoGenex Technologies
Castration Resistance and Prostate Cancer

1. Androgen receptor (AR) related
   - Overamplification (hypersensitive)
   - Mutations (promiscuous)
   - Cross-talk - TK, PKA, AKT, STAT3 (phosphorylation, co-regulators)

2. Adaptation
   - Up-regulation of survival genes (Bcl-2, clusterin, Hsp27, YB-1)
   - Increased alternative GF pathways (her2/neu; IGF-1/IGFBP2&5; IL-6/STAT3)
High-Throughput Bioprofiling of Hormone-Treated Prostate Cancers to Identify Stress-Induced Targets

Hypothesis:
➢ Treatment-induced changes in gene expression after hormone therapy render cells resistant to castration- and chemotherapy.

Tissue Bank:
- Xenografts
- Post-NHT-treated human CaP
- pre-surgery NHT + Taxotere RP specimens
- rapid autopsy HRPC specimens

Gene expression profiling for Target I.D.

TMA profiling for Target Validation
Changes in Gene Expression After Castration and During AI Progression

Androgen-dependent
Hormone withdrawal

Tumour regression
Clonal selection
Adaptive responses

Androgen-independent

++ PSA
- Bcl-2
- EGFR
- clusterin
- IGFBP 2&5
- TGFβ
++IGFBP 3 & 4
-YB-1
++survivin
-Hsp27
+ PKC-α

- PSA
++ Bcl-2
++Bclx-L
- EGFR
+++ clusterin
++++IGFBP 5
IGFBP 3 & 4
+c-myc
+YB-1
-survivin
+Hsp27
+ PKC-α

++ PSA
++ Bcl-2
++Bclx-L
+ EGFR
+++ clusterin
++ IGFBP 2
++ IGFBP 5
++YB-1
++ survivin
+++Hsp27
The image contains a page from a document discussing the relationship between therapeutic stress and increased clusterin levels in CaP (prostate cancer) tumor models and human tissues. The main points are:

**Therapeutic Stress Increases Clusterin Levels in CaP Tumor Models and Human Tissues**

Transcriptionally activated by:
- Hormone withdrawal (a.k.a. TRPM-2)
- Chemotherapies
- Radiotherapy
- Targeted therapies (Herceptin, Velcade, OGX-225, etc)

**Androgen Ablation in Shionogi Tumors**

<table>
<thead>
<tr>
<th>Days Post-Castration</th>
<th>Clusterin</th>
<th>GAPDH</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td><img src="image1" alt="Clusterin" /></td>
<td><img src="image2" alt="GAPDH" /></td>
</tr>
<tr>
<td>3</td>
<td><img src="image3" alt="Clusterin" /></td>
<td><img src="image4" alt="GAPDH" /></td>
</tr>
<tr>
<td>7</td>
<td><img src="image5" alt="Clusterin" /></td>
<td><img src="image6" alt="GAPDH" /></td>
</tr>
<tr>
<td>21</td>
<td><img src="image7" alt="Clusterin" /></td>
<td><img src="image8" alt="GAPDH" /></td>
</tr>
</tbody>
</table>

*Cancer Research 60; 170, 2000*

**Docetaxel Rx in PC-3**

<table>
<thead>
<tr>
<th>Docetaxel Rx (nM)</th>
<th>0</th>
<th>10</th>
<th>25</th>
<th>50</th>
<th>100</th>
<th>500</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clusterin</td>
<td><img src="image9" alt="Clusterin" /></td>
<td><img src="image10" alt="Clusterin" /></td>
<td><img src="image11" alt="Clusterin" /></td>
<td><img src="image12" alt="Clusterin" /></td>
<td><img src="image13" alt="Clusterin" /></td>
<td><img src="image14" alt="Clusterin" /></td>
</tr>
<tr>
<td>Vinculin</td>
<td><img src="image15" alt="Vinculin" /></td>
<td><img src="image16" alt="Vinculin" /></td>
<td><img src="image17" alt="Vinculin" /></td>
<td><img src="image18" alt="Vinculin" /></td>
<td><img src="image19" alt="Vinculin" /></td>
<td><img src="image20" alt="Vinculin" /></td>
</tr>
</tbody>
</table>
SCLU-2: Stress-induced Cytoprotective Chaperone

1. Transcriptionally activated by HSF-1, repressed by p53
   a. Increased by diverse array of therapeutic triggers (HT, CT, RT, velcade, herceptin)
   b. Increased by cell survival factors like androgen, IGF-1
2. Intrinsically disordered and flexible protein
3. Potent inhibitors of aggregation of client proteins under stress conditions
4. Often associated with neurodegenerative diseases and cancer
5. Interact with and inhibit activated Bax; enhances NF-kB transcriptional activity
Clusterin: Cytoprotective Mechanisms

Clusterin inhibits apoptosis by interacting with activated Bax

Honglai Zhang, Jin Koo Kim, Chris A. Edwards, Zhaohui Xu, Russell Taichman and Cun-Yu Wang

nature cell biology

LETTERS
sCLU-2 is a COMMD1 and ubiquitin binding partner in cancer cells

1. sCLU-2 and COMMD1 co-localize in cytoplasm with a juxtanuclear aggregation

![Immunochemistry images showing co-localization of sCLU-2 and COMMD1]

2. sCLU-2 Levels Negatively Correlate with COMMD1 & Ik-B Levels

![Western blot images showing changes in protein levels after sCLU-2 knockdown]

Zoubeidi et al, 2007
sCLU-2 Enhances TNF-a induced NF-κB Nuclear Translocation and Transcriptional Activity

1. sClu-2 Overexpression ↑↑ NF-κB Activity

![Graph showing NF-κB luc activity with fold induction/control for LN_mock and LNCLU2 under no treatment and TNF-a conditions.]

2. sClu-2 Knockdown ↓↓ NF-κB Activity

![Graph showing NF-κB luc activity with fold induction/control for siRNA Scr and siRNA Clu under 0min and 10min TNF-α conditions.]

Zoubeidi et al, 2007
Clusterin Expression Levels Positively Correlate with NF-κB-regulated Genes

**Northern Analysis**

- Sema3c
- NGAL
- sPLA2
- MIP3α

**Western Analysis**

- clAP2: 66kDa
- MCP-1: 12kDa
- MCP-2: 7.5kDa

*Ettinger et al, 2006*
sClu-2 Enhances COMMD1 and I-κBα Degradation by the Proteasome

Zoubeidi et al, 2007
Functional Significance of sCLU-2 Over-expression in Prostate Cancer

- sCLU overexpression is antiapoptotic: confers broad spectrum treatment resistance including hormone, radiation, and chemo-therapy
Inhibition of Clusterin Expression Enhances Activity of Chemotherapy in Prostate Cancer Cells

CLU ASO (OGX-011) Suppress Clu Levels in PC-3 Cells

OGX-011 Chemosensitizes PC-3 Cells to Docetaxel

OGX-011 Enhances Taxol Activity in PC3 Tumours in vivo

Clin Cancer Res 6:1655, 2000
CLU ASO (OGX-011) Suppresses sCLU Levels and Chemosensitizes MCF-7 Xenografts to Paclitaxel in vivo

Clusterin is expressed in 65% of Primary Breast Cancers

In vitro

- Cell proliferation compared to control
- Log Paclitaxel concentration (nM)

In vivo

- Mean Tumor Size (mm³)
- Weeks

So et al, Mol Cancer Ther, 2005
From Bench to Bedside: Translational Research in Action

Don't worry, I had the same thing, and they cured me!
sCLU as a Therapeutic Target: Preclinical Studies For Proof of Principle

Of Mice and Men

Clusterin:
• Stress-induced survival response
• confers resistance
• knockdown enhances chemo & HT in many tumor models
Antisense Clusterin: OGX-011 Product Description

- Licensed from UBC for development by OncoGenex in collaboration with Isis
  - 2nd generation antisense molecule
  - 4-13-4 21-mer MOE gapmer oligonucleotide

- Advantages of 2'MOE analogues
  - Increased potency and resistance to degradation
  - Facilitates more convenient dosing regimen
    - once-weekly infusion
  - J Pharmacol Exp Ther. 298(3):934-40, 2001
NCIC IND.153: Phase I Pre-Surgery pk/pd Trial of OGX-011 - Tissue Pk data

• 25 men with localized CaP treated with 5 weeks of NHT + escalating doses of OGX-011
IND.153: Target Regulation Data: Dose-dependent suppression of clusterin in Regional Lymph Nodes

<table>
<thead>
<tr>
<th>Untreated Controls</th>
<th>6 weeks NHT + OGX-011 (dose escalation)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>80 mg</td>
</tr>
<tr>
<td></td>
<td>160 mg</td>
</tr>
<tr>
<td></td>
<td>320 mg</td>
</tr>
<tr>
<td></td>
<td>480 mg</td>
</tr>
<tr>
<td></td>
<td>640 mg</td>
</tr>
</tbody>
</table>

Chi et al, JNCI. 97:1287-96, 2005
Clinical Proof-of-Concept:
Dose-dependent Decreases in Clusterin Levels in RP Specimens using LCM and Real-Time PCR

Clinical Trial Development with Clusterin ASO (OGX-011)

Established 640 mg once weekly as phase II dose (OBD) based on tissue pk and pd criteria

Completed:
2 regimens found well tolerated -
• OGX-011 + weekly Taxotere
• OGX-011 + q 3 weekly Taxotere

• 30-60% decrease in serum CLU levels

NCIC IND.165:
First Line mHRPC Phase II RCT - Taxotere +/- OGX-011
• 82/80 pts enrolled
• ASCO May 2007

CUOG P-06a:
2nd line mHRPC Phase RCT Taxotere or mitoxantrone + OGX-011
• 42/40 pts enrolled

Phase II stage III/IV NSCLC
Gem/cis + OGX-011
• 10 pts Phase I
• 70 pts Phase II
• ASCO May 2007

NCIC IND.164:
Phase II Metastatic Breast- Taxotere + OGX-011
• 16 pts enrolled

Phase I
NHT Pre - surgery

Solid Tumors + docetaxel n = 35
Phase 2 Study in 1st Line NSCLC: Treatment Schema

• 81 pts with stage IIIB/IV NSCLC treated with gem/cis plus OGX-011

<table>
<thead>
<tr>
<th>Results as of May 24, 2007</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Median Follow-up</strong></td>
</tr>
<tr>
<td><strong>Number of Deaths</strong></td>
</tr>
<tr>
<td><strong>Median Progression-Free Survival (range)</strong></td>
</tr>
<tr>
<td><strong>Estimated Median Survival</strong></td>
</tr>
<tr>
<td><strong>Number of Patients Surviving ≥ 1 year</strong></td>
</tr>
<tr>
<td><strong>Number of Patients Surviving ≥ 18 months</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Historical Controls*</th>
<th>Phase 1 and 2 (n=81)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Median Survival</strong></td>
<td>8.0 – 10.8 months</td>
</tr>
</tbody>
</table>

• Data from five randomized clinical trials using gemcitabine plus platinum-based chemo in 1st line NSCLC (1260 patients)

Laskins et al, ASCO, 2007
**NCIC IND.165: Taxotere +/- OGX-011 in First-Line mHRPC**

- Randomized Open label, multicentre trial comparing docetaxel +/- OGX-011 in men with mHRPC (PI - K. Chi).

### PSA Response Rates

<table>
<thead>
<tr>
<th>BEST RESPONSE CRITERIA</th>
<th>Arm A (OGX-011 + Docetaxel) N=40</th>
<th>Arm B (Docetaxel) N=41</th>
<th>% Change in favor of OGX-011</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥ 50% PSA Decline at 12 weeks</td>
<td>45%</td>
<td>34%</td>
<td>32%</td>
</tr>
<tr>
<td>PSA Response (50% decline - confirmed)</td>
<td>50%</td>
<td>51%</td>
<td>NA</td>
</tr>
<tr>
<td>≥ 80% PSA Decline</td>
<td>38%</td>
<td>22%</td>
<td>73%</td>
</tr>
<tr>
<td>PSA Progression (PSAWG Criteria)</td>
<td>0%</td>
<td>10%</td>
<td>100%</td>
</tr>
<tr>
<td>PSA Non-Progression/Non-Response</td>
<td>45%</td>
<td>32%</td>
<td>41%</td>
</tr>
<tr>
<td>Inevaluable</td>
<td>3%</td>
<td>2%</td>
<td>NA</td>
</tr>
</tbody>
</table>

Chi et al, ASCO, 2007
## NCIC IND.165: Taxotere +/- OGX-011 in First-Line mHRPC

<table>
<thead>
<tr>
<th>RECIST CRITERIA</th>
<th>Arm A (OGX-011 + Docetaxel) n=26</th>
<th>Arm B (Docetaxel) n=23</th>
<th>% Change in favor of OGX-011</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disease Control (CR+PR+SD)</td>
<td>92%</td>
<td>74%</td>
<td>24%</td>
</tr>
<tr>
<td>Complete Response</td>
<td>0%</td>
<td>0%</td>
<td>N/A</td>
</tr>
<tr>
<td>Partial Response</td>
<td>19%</td>
<td>22%</td>
<td>-14%</td>
</tr>
<tr>
<td>Stable Disease</td>
<td>73% (9.7 months)</td>
<td>52% (7.6 months)</td>
<td>40% (28%)</td>
</tr>
<tr>
<td>Progressive Disease</td>
<td>4%</td>
<td>22%</td>
<td>82%</td>
</tr>
<tr>
<td>Inevaluable</td>
<td>4%</td>
<td>4%</td>
<td>N/A</td>
</tr>
<tr>
<td>Median PFS</td>
<td>7.3 months</td>
<td>5.9 months</td>
<td>24%</td>
</tr>
</tbody>
</table>

Chi et al, ASCO, 2007
NCIC IND.165: Taxotere +/- OGX-011 in First-Line mHRPC

**Progression Free Survival**

Median for Arm A (OGX-011 + Docetaxel): 7.26 months (95% CI 5.22-9.33)

Median for Arm B (Docetaxel): 5.85 months (95% CI 3.61-10.74)

Chi et al, ASCO, 2007
NCIC IND.165: Taxotere +/- OGX-011 in First-Line mHRPC

Indicators of Anti-cancer Activity

- Consistent trend in favor of OGX-011/docetaxel arm:
  - More patients with a 50% decline in PSA within the first 12 weeks
  - More pts (38% vs 22%) with >80% decline in PSA; less pts (0 vs 10%) with primary PSA progression as best response
  - Longer time on treatment and a greater median # of treatment cycles.
  - Higher frequency and longer duration of stable measurable disease.
  - Lower frequency of progressive disease as “best response”.
  - Longer time to progression
OGX-011 in docetaxel-refractory HRPC:
CLU knockdown chemosensitizes taxane-resistant PC3-dR cells to docetaxel
Phase II Feasibility Trial of OGX-011 in 2nd Line Therapy in HRPC:

**Study treatment ongoing in 11 (26%) of patients**

HRPC Patients Who Had Disease Progression During or Within 6 Months of 1st Line Docetaxel Treatment

- **Arm A**
  - Docetaxel (75 mg/M² IV) q 21 days and OGX-011 (640 mg IV) weekly plus Prednisone (5 mg po bid) daily

- **Arm B**
  - Mitoxantrone (12 mg/M² IV) day 1 and OGX-011 (640 mg IV) weekly plus Prednisone (5 mg po bid) daily.

Continue treatment until disease progression or unacceptable toxicity. If removed from treatment for any reason, follow until death.
OGX-011 in 2nd Line Therapy in HRPC:
Chemosensitizes taxane-resistant patients to docetaxel
Summary:
Clusterin as a Therapeutic Target in HRPC

- **sCLU** is a stress-activated cytoprotective chaperone that is highly expressed in HRPC.

- Over-expression of sCLU-2 confers broad spectrum treatment resistance:
  - Inhibits protein aggregation, facilitates proteasome degradation of ubiquitinated proteins.
  - Interacts and inhibits activated Bax, preventing cytochrome C release.
  - Increases NF-kB transcriptional activity.

- **CLU knockdown using OGX-011**:
  - Enhances treatment-induced apoptosis in vitro and in vivo.
  - Pre-clinical proof-of-principle in prostate, breast, lung, urothelial, melanoma, renal cell.

- **OGX-011**, a 2nd generation ASO potently suppresses target CLU levels >90% in human CaP tissues:
  - Anti-cancer activity observed in multi-centre Phase II trials in breast, HRPC, lung.
  - Phase III registration trial in second-line HRPC set to begin in 2008.
Changes in Gene Expression After Castration and During AI Progression

**Androgen-dependent**
- PSA
- Bcl-2
- EGFR
- clusterin
- IGFBP 2&5
- TGFβ
++IGFBP 3 & 4
-YB-1
++survivin
-Hsp27
+ PKC-α

**Hormone withdrawal**
- PSA
- Bcl-2
++Bclx-L
- EGFR
+++ clusterin
++++IGFBP 5
IGFBP 3 & 4
+c-myc
+YB-1
-survivin
+Hsp27
+ PKC-α

**Androgen-independent**
++ PSA
++ Bcl-2
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++ IGFBP 5
++YB-1
++ survivin
+++Hsp27

**Tumour regression**

**Progression**

**Clonal selection**

**Adaptive responses**
Thanks to...

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Richard Sowery

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Ladan Fazli
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Torsten Neilsen
Ted Jones

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Scott Cormack

**Isis Pharmaceuticals**
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U Montreal - F. Saad

**NCIC IND Group**
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Lesley Seymour

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OGX-011: Safety Profile in >270 Patients

- Well tolerated in all Phase 1 and Phase 2 studies to date
- Safety profile of OGX-011 in combination with docetaxel vs docetaxel alone
  - Increase in Grade 1 or 2 AE’s events (fever, rigors/chills and sweating during the loading-dose week and sensory neuropathy during therapy)
  - Lymphopenia was more prevalent in the OGX-011 + docetaxel arm (no clinical sequelae)
  - No increase in SAEs in the OGX-011 + docetaxel arm
- OGX-011 in combination with gemcitabine/platinum-based or mitoxantrone regimens
  - Safety profile similar to that expected for regimen (no increase in expected rate of Grade 3 or higher AEs)
OGX-011 Mechanism of Action

OGX-011

Alternative splicing of Clusterin mRNA

↓ sCLU  ↑ nCLU

↑ Apoptosis

↑ Bax  

↑ COMMD1  

↑ Protein Aggregation  

↓ UBP

↑ Cytochrome C  

↓ NF-κB  

↑ ER Stress  

↓ Proteasome Activity

↑ Apoptosis
Clusterin: Isoforms and Splice Variants

CLU Isoform 1
sCLU-1

CLU Isoform 2
sCLU-2

Nuclear splice variant 1
nCLU-v1

Nuclear splice variant 2
nCLU-v2

Clusterin is an androgen-regulated gene

CLU Isoform 1

CLU Isoform 2

Fold Change

CLU Isoform 1

CLU Isoform 2

Cochrane et al, JBC, 2006