Mark R. Kelley, Ph.D.

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- Professor, Departments of Pediatrics, Biochemistry & Molecular Biology and Pharmacology & Toxicology
- Associate Director, Herman B Wells Center for Pediatric Research
- Associate Director of Basic Science Research, Indiana University Simon Cancer Center
- Director, Program in Pediatric Molecular Oncology & Experimental Therapeutics
Disclosures:

Chief Scientific Officer and Founder, Apexian Pharmaceuticals

Licensing agreements for antibodies and reagents to:

Novus Biologicals
Abcam
Millipore

Supported by:

The National Institutes of Health, National Cancer Institute CA167291, R21NS091667, Hyundai Hope on Wheels Foundation Grant, Betty and Earl Herr Chair in Pediatric Oncology Research, Hamer Foundation, Jeff Gordon Children’s Research Foundation and the Riley Children’s Foundation.
The Target
APE1/Ref-1 Overview

- APE1 (apurinic/apyrimidinic endonuclease), also called Ref-1 (redox effector factor 1), is a multifunctional cellular protein with at least two distinct and separate functions:

  - **APE1 Redox Function**: Redox regulation of transcription factors (TFs) effecting critical aspects of cancer cell survival and growth including HIF-1, STAT3, NF-KB, and others.

  - **DNA Repair Function**: DNA base repair caused by oxidative stress, alkylating agents, and ionizing radiation

  - **RNA Degradation and quality control**: Interaction with NPM1

- Various cancers, including treatment resistant tumors, have shown elevated expression of APE1 suggesting adaptation and unique survival mechanisms through this pathway.

- We can target multiple signaling pathways relevant to various cancers with one protein— as APE1 regulates transcription factors (TFs) HIF1a, STAT3, NFkB and others.

- APX3330 inhibits only the APE1 redox signaling activity.
APE1/Ref-1 functions:
DNA repair and Redox signaling regulation of TFs

C99 C65 C93

Redox Role

DNA Repair

DNA damage

AP-1
p53
NFkB
HIF-1α
CREB
PAX
STAT3

Redox control of
Transcription factors

Target gene expression:
Growth
Inflammation
Angiogenesis

Glycosylase (Ogg1, Nth1)

APE1

DNA Ligase

Ref-1

(reduced)

(oxidized)

Nuclear localization
Redox activity
AP endonuclease activity
Alteration of APE1/Ref-1 protein expression has been shown to be elevated in:

1. Non-small cell lung cancer
2. Colorectal cancer
3. Breast cancer
4. Prostate cancer
5. Gynecologic cancers (ovarian, cervical)
6. Pancreatic cancer
7. Glioblastoma multiforme, medulloblastoma
8. Renal cancer
9. Gastric cancer
10. Germ cell tumors
11. Head-and-neck cancers
12. Multiple myeloma (hematologic cancer)
13. Osteosarcoma and Rhabdomyosarcoma (pediatric)
The Drug

E3330 = APX3330

- APX3330 was originally developed by Eisai (E3330) as a NFkB-TNFa inhibitor for the treatment of inflammatory liver disease.

- Eisai ended APX3330 development after in-licensing Revovir® (clevudine) for the treatment of hepatitis B and Humira (adalimumab) for treatment of rheumatoid arthritis, IBD and other indications.
• The drug has a direct and selective interaction with APE1 as demonstrated by chemical footprinting, mass spectrometry, and other biochemical data.

• Although multiple pathways may be modulated, unacceptable toxicity following APE1 inhibition has not been observed in animal or human studies.

• Preclinical data supports the use of the drug as a single agent; future directions indicate partnering APX3330 with various clinical agents such as JAK2 inhibitors (Ruxolitinib, LY3009104, etc), STAT3 inhibitors, gemcitabine and Abraxane (nab-paclitaxel).
APX3330 inhibits APE1 Redox Function Blocking TF Activity

- Redox active site
- C93 and C65

APE1

APX3330

<table>
<thead>
<tr>
<th>Reduced APE1</th>
<th>Oxidized TF</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIF-1α</td>
<td>TF</td>
</tr>
<tr>
<td>NFkB</td>
<td>Oxidized APE1</td>
</tr>
<tr>
<td>STAT3</td>
<td>Reduced TF</td>
</tr>
<tr>
<td>AP-1</td>
<td>Oxidized APE1</td>
</tr>
<tr>
<td>NRF2</td>
<td>Reduced TF binds to target DNA</td>
</tr>
</tbody>
</table>

Target gene expression:
- Growth
- Inflammation
- Angiogenesis
APE1 cysteines involved in redox function


Disulfide bonds
65-93
65-99
93-99

Courtesy of Dr. Millie Georgiadis, collaborator
Evolution of the C65 redox center in APE1

Chicken Ape1 is also missing the redox C

<table>
<thead>
<tr>
<th>Species</th>
<th>C5555555</th>
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</table>

Chicken Ape1 is also missing the redox C
APX3330 blocks the activity of primary Cys residues required for APE1 redox function

<table>
<thead>
<tr>
<th></th>
<th>wtAPE1</th>
<th>C65/C93/C99</th>
<th>C65/C93</th>
<th>C65/C99</th>
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<tr>
<td>APX3330</td>
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<td>40</td>
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</table>

Labeling indicates only the Cys present
Mass spectra of APE1 after incubation without (A) and with APX3330 (B) in the presence of NEM for 30 min (left panel) and 6 h (right panel).

No 3330, NEM specifically modifies APE1 resulting in the formation of a +2 NEM = C99 C138 (solvent accessible). +3330 = 7NEM appearance = all cys now accessible

Using HDX mass spectrometry, APX3330 interacts with and inhibits the redox activity of APE1 at two regions.

These results suggest that APX3330 destabilizes APE1’s structure rather than stabilizing it.

Interaction of APX3330 with APE1 as detected by HDX mass spectrometry. (A) HDX data are shown for peptides with slower exchange rates in the presence of 1.6 mM E3330 (□) as compared to the exchange rates in the absence of compound (■). (B) The peptides that showed protection from deuterium exchange are shown highlighted on the structure of APE1. Residues 68–74 are colored orange and residues 266–273 magenta. Shown as stick models are R73 (orange) and R177 (blue), two Arg residues in the proximity of the regions of interaction identified by HDX mass spectrometry.

Published in: Jun Zhang; Meihua Luo; Daniela Marasco; Derek Logsdon; Kaice A. LaFavers; Qiujia Chen; April Reed; Mark R. Kelley; Michael L. Gross; Millie M. Georgiadis; *Biochemistry* 2013, 52, 2955-2966.
Supporting Drug Selectivity Data

% Survival

Vector cont
wt Ref-1

Cont
APX3330
Inhibition of APE1/Ref-1 with APX3330 Blocks TF Function and Downstream Factors

NFκB  AP-1 (fos/JUN)  HIF1α  STAT3

APX3330 uM  APX3330 uM  APX3330 uM

DNA
High Unmet Clinical Need for Pancreatic Cancer

- In 2015, 48,960 Americans will be diagnosed with pancreatic cancer and more than 40,560 will die from the disease. Pancreatic cancer 1-year survival rates are ~25% and 5-year survival rates are ~7%.

- Pancreatic cancer thrives in an inflammatory, hypoxic, and dense/stromal microenvironment making it hard to treat. Few patients are diagnosed at an early stage leading to an average life expectancy following diagnosis of 3 to 6 months.

- Currently approved chemotherapeutic treatments include combination and single-agent use of paclitaxel, cisplatin, gemcitabine (Gemzar®), and 5-flurouracil. Other approved treatments (Abraxane, FOLFIRINOX) have high toxicities limiting use to patients that can tolerate the side effects.

- Pancreatic cancer represents an area of high unmet clinical need with Breakthrough Therapy regulatory approval potential for even modest improvements in survival.

SOURCE: American Cancer Society website
APE1 Expression is Linked to Poor Survival in Pancreatic Patients

Early stage patients undergoing Whipple procedure

<table>
<thead>
<tr>
<th>TCGA/PDAC Cohort</th>
<th>Patients</th>
<th>Median Survival (p&lt;0.0003)</th>
</tr>
</thead>
<tbody>
<tr>
<td>High Ref-1/APE 1 (Red)</td>
<td>N = 9</td>
<td>4 months</td>
</tr>
<tr>
<td>Low Ref-1/APE1 (Blue)</td>
<td>N = 61</td>
<td>16.8 months</td>
</tr>
</tbody>
</table>
Pancreatic cancer patient tumors express APE1/Ref-1 in both tumor and stroma

Patient #  1  2  3

A

Pancreatic cancer patient tumors express APE1/Ref-1 in both tumor and stroma

B

Pancreatic cancer patient tumors express APE1/Ref-1 in both tumor and stroma

C

Pancreatic cancer patient tumors express APE1/Ref-1 in both tumor and stroma

Pancreatic cancer patient tumors express APE1/Ref-1 in both tumor and stroma

Pancreatic cancer patient tumors express APE1/Ref-1 in both tumor and stroma

B

Pancreatic cancer patient tumors express APE1/Ref-1 in both tumor and stroma

C

Pancreatic cancer patient tumors express APE1/Ref-1 in both tumor and stroma

Pancreatic cancer patient tumors express APE1/Ref-1 in both tumor and stroma

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Pancreatic cancer patient tumors express APE1/Ref-1 in both tumor and stroma
E3330

Tumor-assoc. macrophages
Stellate Cells Cancer Associated Fibroblasts (CAFs)

Tumor Microenvironment
(Angiogenesis/Invasion/Metastases)

Ref-1 expression in panc patient sample

Pro-Angiogenic Factors
VEGF IL8

Angiogenesis / Invasion / Metastases

Signaling

Ref-1

HIF-1α

AP-1

NFκB

Pancreatic cancer cells
Tumor + CAFs (1:4)

(Pa03C & 1301-63 hTERT-GFP)
Addition of CAFs to tumors accelerates tumor growth rate *in vivo*

![Graph showing tumor volume and weight over time](image)

- **Tumor only**
- **1:2**
- **1:4**

*Addition of CAFs to tumors accelerates tumor growth rate in vivo*
0               20              30          40

µM APX3330

10.05  Pa03

PDAC (Tumor) Cells
Pa03C

<table>
<thead>
<tr>
<th>DMSO</th>
<th>APX3330 20</th>
<th>APX3330 30</th>
<th>APX3330 40</th>
<th>µM APX3330</th>
</tr>
</thead>
</table>

Pa03C alone

Pa03C + CAF co-cultures

PDAC Cells + CAFs
**Gem + APX3330 in 3D model**

<table>
<thead>
<tr>
<th>Gem</th>
<th>Cntl</th>
<th>1.25</th>
<th>2.5</th>
<th>5</th>
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<tbody>
<tr>
<td>Tumor only</td>
<td>![Images](B8, B9, B10, B11)</td>
<td>![Images](B8, B9, B10, B11)</td>
<td>![Images](B8, B9, B10, B11)</td>
<td>![Images](B8, B9, B10, B11)</td>
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<tr>
<td>CAFs</td>
<td>![Images](B8, B9, B10, B11)</td>
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<td>![Images](B8, B9, B10, B11)</td>
<td>![Images](B8, B9, B10, B11)</td>
</tr>
</tbody>
</table>

**Tumor + CAFs**

<table>
<thead>
<tr>
<th>Gem + APX</th>
<th>Cntl</th>
<th>1.25</th>
<th>2.5</th>
<th>5</th>
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<tbody>
<tr>
<td>Tumor</td>
<td>![Images](G8, G9, G10, G11)</td>
<td>![Images](G8, G9, G10, G11)</td>
<td>![Images](G8, G9, G10, G11)</td>
<td>![Images](G8, G9, G10, G11)</td>
</tr>
<tr>
<td>CAFs</td>
<td>![Images](F8, F9, F10, F11)</td>
<td>![Images](F8, F9, F10, F11)</td>
<td>![Images](F8, F9, F10, F11)</td>
<td>![Images](F8, F9, F10, F11)</td>
</tr>
</tbody>
</table>

**Graphs**

- **Tumor Area**: Comparison of Tumor Area with and without Gem + APX at different concentrations.
- **CAF Area**: Comparison of CAF Area with and without Gem + APX at different concentrations.
APX3330 Reduces Tumor Growth and Metastasis

**PaCa-2**

![Graph showing tumor volume suppression with APX3330](image)

**Human PDAC Metastasis**

![Graph showing lymph node metastases](image)

Endothelial cell tumor growth is Ape/ref-1 dependent

A: MCP-1 reporter activity (FL/RL, % change)
B: MCP-1 (ng/mL)
C: MCP-1 reporter activity (FL/RL, % change)
D: MCP-1 reporter activity (FL/RL, % change)

Redox function of Apex-1 is required for MCP-1 activation and EC tumor growth in vivo. Apex-1 knockdown in EOMA cells resulted in significant decrease in MCP-1 reporter activity (A), and MCP-1 release in the media was measured by ELISA (B). C: MCP-1 reporter activity was significantly decreased in c-Jun knockdown EOMA cells and in E3330 (50 µM, 5 h)-treated cells (D). Redox changes of Apex-1 influences HE outcome in vivo. E: tumor growth rates were evaluated after 7 days of E3330 treatment (25 mg/kg ip twice daily) alone and in combination with CRT0044876 (10 mg/kg ip twice daily). F: tumor volume was quantified using calipers (length × width × height). Results are expressed as means ± SD; *P < 0.05.
Clinical Plans for APX3330

- Apexian will complete a two-part phase I oncology study:
  - Increasing doses in patients with treatment-refractory solid tumors
  - 20-40 patients

- Study endpoint:
  - Identify the RP2 dose of APX3330
  - Based upon
    - tolerability of the agent
    - evidence of anti-tumor effect
    - pharmacokinetic and pharmacodynamic

- IND accepted by the FDA:
  - All study documents are ready and sites identified
  - Contracts pending completion of the funding round

- Additional safety, tolerability and efficacy POC
**Bench to Bedside**

**Bench Findings: Inhibition of Ref-1 via APX3330 reduces tumor burden in mice**

- Ref-1 (reduced) [X] (oxidized)
- HIF-1α
- STAT3
- Redox control of Transcription factors
- Target gene: Growth, Inflammation, Angiogenesis

**Inhibitor:** APX3330

**Target APE1/Ref-1**

**A Phase I Clinical Trial Open-Label Dose Escalation Study of Oral APX3330 in Subjects with Advanced Solid Tumors**

- IND approved July 16, 2016
- IU IRB approved Aug 22, 2016
Schematic of APX3330. Groups to be further investigated include the

(A) Quinone series,
(B) 3-Position series,
(C) Alkyl Sidechain series and
(D) Carboxylic Acid/Amide series.
Pipeline and Indications

<table>
<thead>
<tr>
<th>CURRENT PIPELINE</th>
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<tbody>
<tr>
<td><strong>APX 3330</strong></td>
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<tr>
<td><strong>Solid Tumors – Pancreatic Cancer</strong></td>
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<tr>
<td><strong>APX 3330</strong></td>
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<tr>
<td><strong>Liquid Tumors – ALL</strong></td>
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<tr>
<td><strong>APX 3330</strong></td>
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<tr>
<td><strong>Chemo-Induced Peripheral Neuropathy</strong></td>
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<tr>
<td><strong>APX 3330</strong></td>
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<tr>
<td><strong>Age-Related Macular Degeneration</strong></td>
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<tr>
<td><strong>APX3330 + combinations</strong></td>
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<tr>
<td><strong>Cancer - Multiple</strong></td>
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<td><strong>APX2009</strong></td>
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<td><strong>Ape1/Ref-1 Diseases</strong></td>
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<td><strong>APX2014</strong></td>
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**Discovery** | **Preclinical Development** | **IND Filing** | **Phase 1**
Continuing to Follow the Science

APE1 “Repair” Function

“Redox” Function

APX3330

Cancer

Leukemia

Pancreatic

Ovarian

Prostate

Combinations

Other Diseases

New Molecules
Example pathways that are altered in low passage patient derived PDAC cells following APE1 knockdown and Fluidigm C1 single cell sorting-RNA seq analysis.

Table 1. Pathway affected by Ref-1 knockdown

<table>
<thead>
<tr>
<th>Pathway</th>
<th># of genes affected</th>
<th>p-value</th>
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<tbody>
<tr>
<td>STAT3 Pathway</td>
<td>9</td>
<td>0.006</td>
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<tr>
<td>HIF1 Signaling</td>
<td>17</td>
<td>0.002</td>
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<tr>
<td>ERK/MAPK Signaling</td>
<td>25</td>
<td>0.003</td>
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Validation of Single Cell KD & Potential Use for Drug Development/Specificity

A. Identification of Ref-1 biomarkers using Single Cell RNaseq:

<table>
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<tr>
<th>sc RNaseq</th>
<th>N</th>
<th>S</th>
<th>R</th>
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<tbody>
<tr>
<td>Fold change from SCR</td>
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<tr>
<td>p value</td>
<td>4.3x10^-8</td>
<td>2.78x10^-6</td>
<td>8.14x10^-7</td>
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Validation by:

B. Knockdown, n=2

C. APX treatment

- Vehicle
- 20μM APX3330
- 30μM APX3330
- 40μM APX3330
- 50μM APX3330
APE1 Complexes with HIF1α & STAT3 under Hypoxia

Endogenous APE1

A

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<td>N</td>
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<tr>
<td>N</td>
<td>H</td>
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WB: HIF1α

STAT3

APE1

NFKB

APE1

B

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WB: HIF1α

STAT3

APE1

NFKB

APE1
DNA

CA9

VEGF

JAK

STAT3 Inhibitors

STAT3

IL-6 targeted agents; Siltuximab, Toxilizumab

JAK2 inhibitors; Ruxolitinib, Pacritinib, etc

HIF1α

APX3330

NFKB

IL-6, TNFα

Bcl2, Survivin, TGF-B, Bcl-xL, HIF, etc.

pH Stabilization

VEGF

CA9

Bevacizumab

Stromal Cells

X

X

X

X

X

X

Siltuximab, Toxilizumab

Ruxolitinib, Pacritinib, etc

Bcl2, Survivin, TGF-B, Bcl-xL, HIF, etc.
STAT3 DNA binding is redox sensitive and can be stimulated by APE1/Ref-1... and inhibited by APX3330.
Cytokine / Growth factor Signaling
Hypoxia
Inflammation

Ref-1

APX3330

Ruxolitinib

DNA

JAK

STAT3

IL-6

Stromal Cells

Cytokine
Growth factor
Signaling
Hypoxia
Inflammation

STAT3

DNA
Pa03C cells in 3D: Combo Ref-1 inhibition + Jak2 inhibition
Cytokine / Growth factor Signaling
Hypoxia
Inflammation

CAIX
HIF1α

SLC-0111
HIF1α
APX3330

DNA
Hypoxia-Induced CA9 mRNA: Inhibition by Ref-1 KD and APX3330
<table>
<thead>
<tr>
<th>Pa03C alone</th>
<th>APX3330</th>
<th>PDAC Cells + CAFs</th>
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<tbody>
<tr>
<td>DMSO</td>
<td></td>
<td></td>
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<tr>
<td>20 µM</td>
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<tr>
<td>30 µM</td>
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<tr>
<td>40 µM</td>
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Pa03C + CAF co-cultures

APX3330

APX3330 + SLC-0111

APX3330

APX3330 + SLC-0111
Pa03C cells in 3D: Combo Ref-1 inhibition + CA9/12 inhibition

Area

Tumor area – Co-culture

CAF area – Co-culture

Intensity

Tumor area – Tumor alone

CAF area – Co-culture

Intensity

Tumor area – Tumor alone

CAF area – Co-culture

RFU

APX3330 (uM)

APX3330 (uM)
Bevacizumab
Cancer therapies that produce CIPN

Sensory neuropathy with symptoms such as:

- distal paraesthesias (tingling, numbness, burning sensations)
- altered proprioception (awareness of position of one’s body)
- coldness in extremities
- acute/chronic pain

Chemotherapy effects motor neurons less frequently than sensory neurons

Autonomic nervous system dysfunction (palpitations, orthostatic hypotension, impotence) is rarely seen

Drugs Associated with CIPN

- Platinum compounds (cisplatin, carboplatin, oxaliplatin)
- Vincristine
- Taxanes (docetaxel, paclitaxel)
- Epothilones (ixabepilone)
- Bortezomib (CIPN occurs in 37%-44% of patients with multiple myeloma)
- Thalidomide (CIPN develops in 20%-40% of patients)
- Lenalidomide

Overall, 40% of patients receiving cisplatin and taxol develop CIPN!

A cross-sectional study of patients with testicular cancer re-evaluated 23–33 years after finishing treatment showed that CIPN remains detectable in up to 20% of patients, being symptomatic in 10% of them.

The combination of 5-FU and oxaliplatin is frequently used in patients with gastrointestinal cancer, and 92% of patients develop sensory CIPN.
Patients used analogies to describe symptoms (Tanay et al. 2016)

- ‘Severe buring in fingertips’, ‘Like putting them (fingers) on hot stove’,
- ‘A strip of numbness across fingers’ (Boehmke & Dickerson 2005)

- ‘Like fingernails on a chalkboard’, ‘Pain like needle stuck in my toes’
- (Bakitas 2007)

- ‘Walking on hot coals’, ‘Walking on a rock on the bottom of your feet’,
  ‘Sandpaper at the bottom of your feet’, ‘Something crawling’
- (Tofthagen 2010b)

- ‘Getting a cast off’, ‘Blob of numbness’, ‘Feet are asleep’
- (Speck et al. 2012)
Estimates of the number of adult patients treated annually with either cisplatin, oxaliplatin or carboplatin are approximately > 200,000 a year:

- 50,000 patients with metastatic colorectal cancer
- 20,000 with stage III colon cancer
- 12,000 with pancreatic cancer
- 25,000 with gastroesophageal, and
- 10,000 with head and neck cancer.
- 4,000 with ovarian cancer
- etc

Uses of platinum agents in pediatric oncology:

Cisplatin and Carboplatin are used in:

- Neuroblastoma
- Germ cell tumors
- Osteosarcoma
- Hepatoblastoma
- Brain tumors
- Retinoblastoma

Oxaliplatin is not used.
Putative sites of neuronal dysfunction following specific anticancer drug treatments, indicated in italics.
DNA damage in neurons is repaired using these pathways:

**Nucleus**
- Direct Repair
- BER
- NER
- MMR
- HR / NHEJ

**Mitochondria**
- ????
- BER
- NER
- MMR
- ????

Oxidative DNA damage and crosslinks induced by the platinum drugs

<table>
<thead>
<tr>
<th></th>
<th>Cisplatin</th>
<th>Oxaliplatin</th>
<th>Carboplatin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxidative DNA Damage</td>
<td>Yes High</td>
<td>Yes Moderate</td>
<td>No</td>
</tr>
<tr>
<td>Type of Crosslink</td>
<td>Intra-strand</td>
<td>Inter-strand</td>
<td>Intra-strand</td>
</tr>
<tr>
<td></td>
<td>predominant</td>
<td>predominant</td>
<td>predominant</td>
</tr>
<tr>
<td></td>
<td>Pt-d(GpG) (1,2-intrastand) &gt;90%</td>
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</tr>
<tr>
<td></td>
<td>Pt-d(ApG) (1, 2-interstrand crosslink)</td>
<td>Pt-d(ApG) (1,2-interstrand crosslink) &gt;90%</td>
<td>Pt-d(ApG) (1,2-interstrand crosslink)</td>
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</tbody>
</table>
APE1 knockdown effect on cisplatin, oxaliplatin and carboplatin-induced iCGRP release in DRG cells
Effect of altered Ape1 levels on cisplatin-induced iCGRP release from sensory neuronal cells.

Effect of knocking Ape1 down on cisplatin, oxaliplatin and carboplatin-induced DNA damage (p-H2AX) in DRG cells

A. Cisplatin (50uM)

B. Oxaliplatin (300uM)

C. Carboplatin (500uM)

*p<0.05

N=3

*p<0.05

N=4

*p<0.05

N=3
8-oxoG levels in DRG Neuronal Cultures following APE1 KD and cisplatin, oxaliplatin or carboplatin treatments

A

<table>
<thead>
<tr>
<th></th>
<th>Cisplatin 50 µM</th>
<th>Oxaliplatin 300 µM</th>
<th>Carboplatin 500 µM</th>
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<tr>
<td>Media only</td>
<td><img src="media.png" alt="Image" /></td>
<td><img src="oxali.png" alt="Image" /></td>
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<tr>
<td>APEsiRNA</td>
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<td><img src="apesi.png" alt="Image" /></td>
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</table>

B

<table>
<thead>
<tr>
<th></th>
<th>Media only</th>
<th>100 nM SCsiRNA</th>
<th>100 nM APE1siRNA</th>
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<tbody>
<tr>
<td>50 µM Cisplatin</td>
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<td><img src="bar.png" alt="Bar" /></td>
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<tr>
<td>300 µM Oxaliplatin</td>
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<tr>
<td>500 µM Carboplatin</td>
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</tr>
</tbody>
</table>

N=3

* Indicates significant difference.
Assessment of Cisplatin-Induced DNA damage

Pt-GpG (1, 2-intrastrand crosslink) >90%
Pt-GpG (1,3-intrastrand crosslink) <5%
Pt-GpG (interstrand crosslink) <5%

DNA Fragmentation
mAb specific to 1,2-Pt-GG

DNA slot blot

cisplatin conc.
30 uM 10 uM

Mock

0 2 4 8 12 hours after cisplatin treatment

α-Pt-GpG SYBR-Gold staining

1,2-Pt-GG G-Pt-G
1,3-Pt-GG
Targeted inhibition of APE1 expression in rat neuronal cells significantly reduces removal of Pt-damage.
Add-back of wt-APE1 restores repair of 1,2-Pt-GpG damage in DRG cells treated with Ape1-siRNA
APX3330 enhances APE1 endonuclease DNA repair activity in DRG cells

Each column is the mean ± SEM of the percent increase in APE1 endonuclease activity using the established AP endonuclease assay. An asterisk indicates a statistically significant difference between cultures treated with vehicle and those treated with E3330 using Student's t-test.

Peripheral blood flow is regulated by CGRP; i.e. measuring iCGRP release in vitro or blood flow in vivo is indicator of DRG function.
APX3330 treatment (25 mg/kg) can reverse cisplatin neurotoxicity in R1 and R2.
APX3330 attenuates neurotoxicity induced by systemic administration of cisplatin to neuroblastoma tumor-bearing mice
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