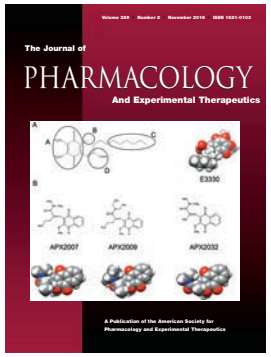
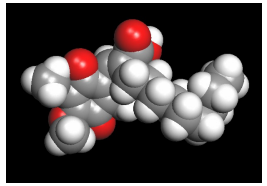


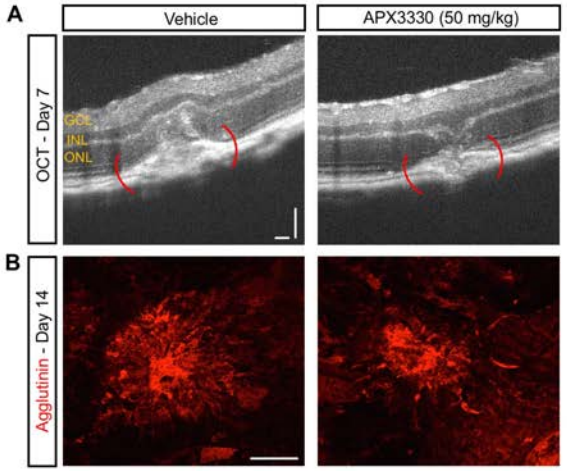
Translating basic science discoveries for patients: Use of bioinformatics to discover new pathways for targeting APE1/Ref-1 for cancer treatments



June 17, 2021

Mark R. Kelley, Ph.D.

- Associate Director of Basic Science Research, Indiana University Simon Comprehensive Cancer Center
- Betty and Earl Herr Professor in Pediatric Oncology Research and
- Professor, Departments of Pediatrics, Biochemistry & Molecular Biology, Pharmacology & Toxicology and Ophthalmology
- Adjunct Professor, Eugene and Marilyn Glick Eye Institute
- Director, Program in Pediatric Molecular Oncology & Experimental Therapeutics
- Glenn W. Irwin, Jr. M.D. Research Scholar
- Bantz-Petrino Translating Research into Practice Scholar
- Chair, Indiana University Conflict of Interest Committee
- Co-leader, Experimental and Development Therapeutics Program, IUSCCC



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- Chief Scientific Founder and CSO of Apexian Pharmaceuticals
- Ocuphire Medical Advisory Board member and consultant, Ocuphire Pharma

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Betty and Earl Herr Chair in Pediatric Oncology Research
Tom Wood Foundation
Tom Wood Cares
Jeff Gordon Children's Research Foundation
Riley Children's Foundation

Today, I am going to talk about:

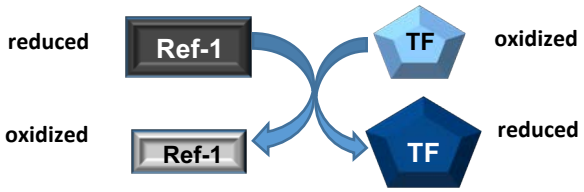
- A little science
- How big data/bioinformatics has led to new avenues of research
- Pathway from bench to clinical trial
- New directions based on data analysis, target and drug development

The Target & Drug

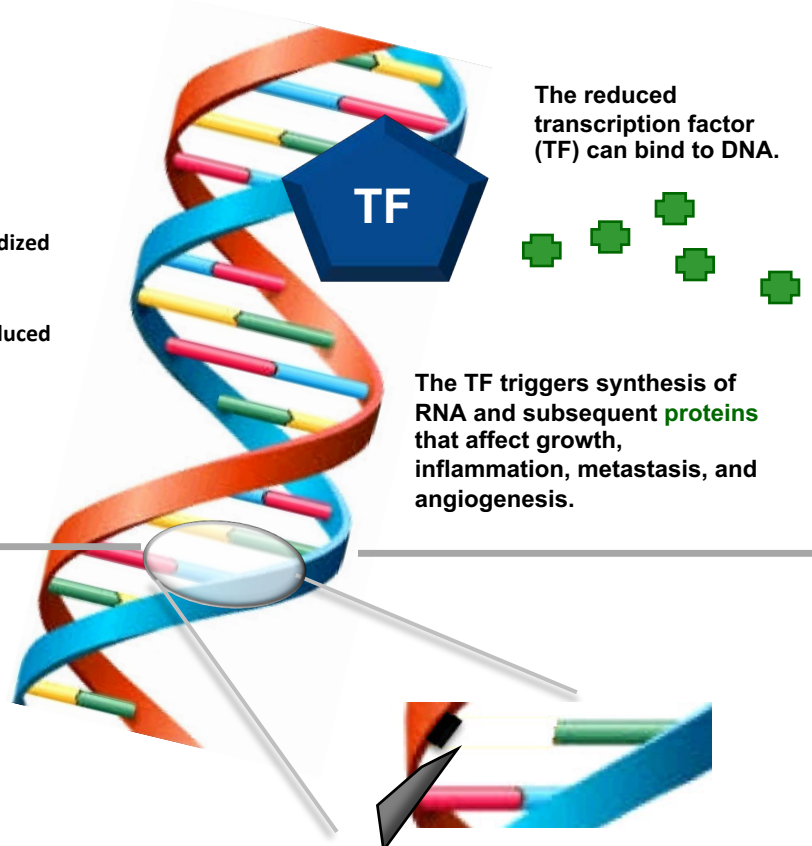
The Target

APE1/Ref-1

Ref-1 function: oxidation-reduction



In a thiol/sulfide exchange on its cysteine residue, Ref-1/APE1 reduces a transcription factor to its active form. Ref-1/APE1 becomes oxidized in the process.



The reduced transcription factor (TF) can bind to DNA.

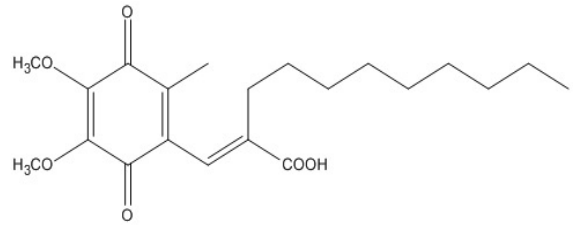
The TF triggers synthesis of RNA and subsequent proteins that affect growth, inflammation, metastasis, and angiogenesis.

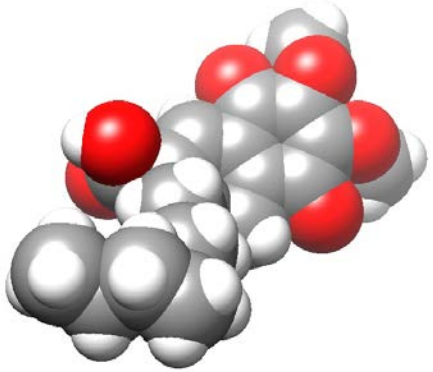
APE1 function: AP endonuclease

After a glycosylase removes the damaged base, Ref-1/APE1 nicks

The Drug

APX3330

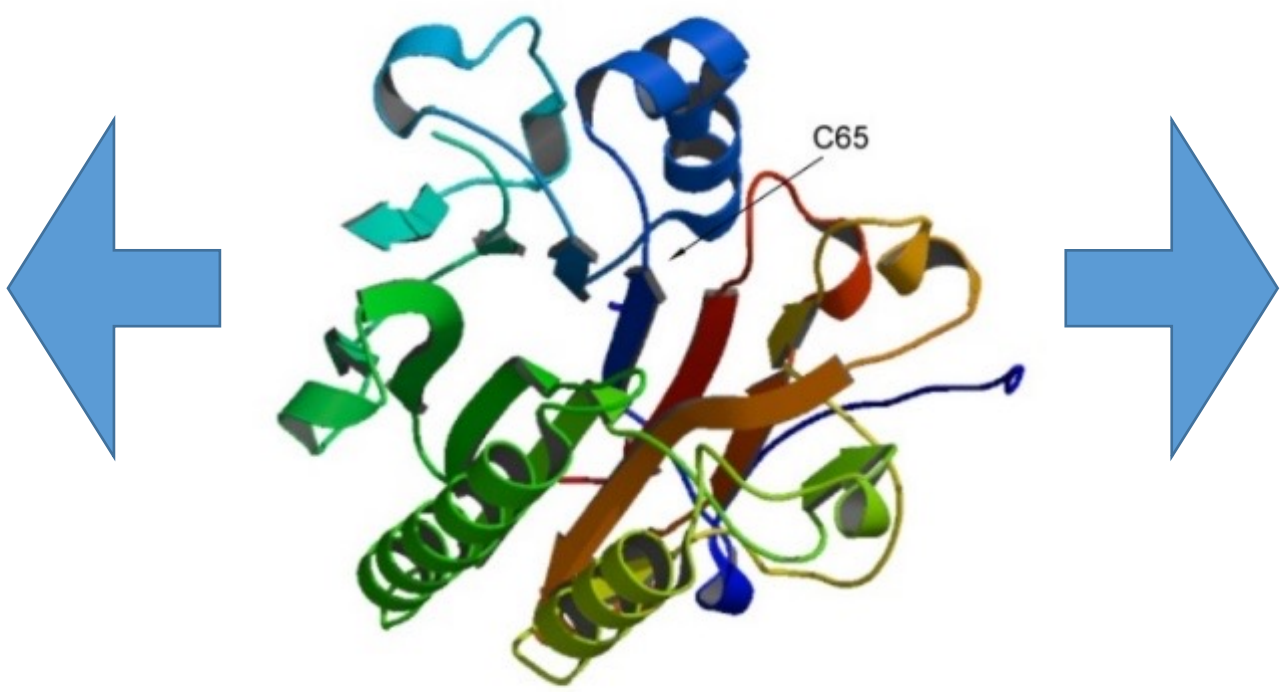




APE1/Ref-1 is a dual function protein

Function #1:

DNA Repair: repairs the DNA single-strand breaks (SSB) induced by ROS (i.e., oxidative damage)

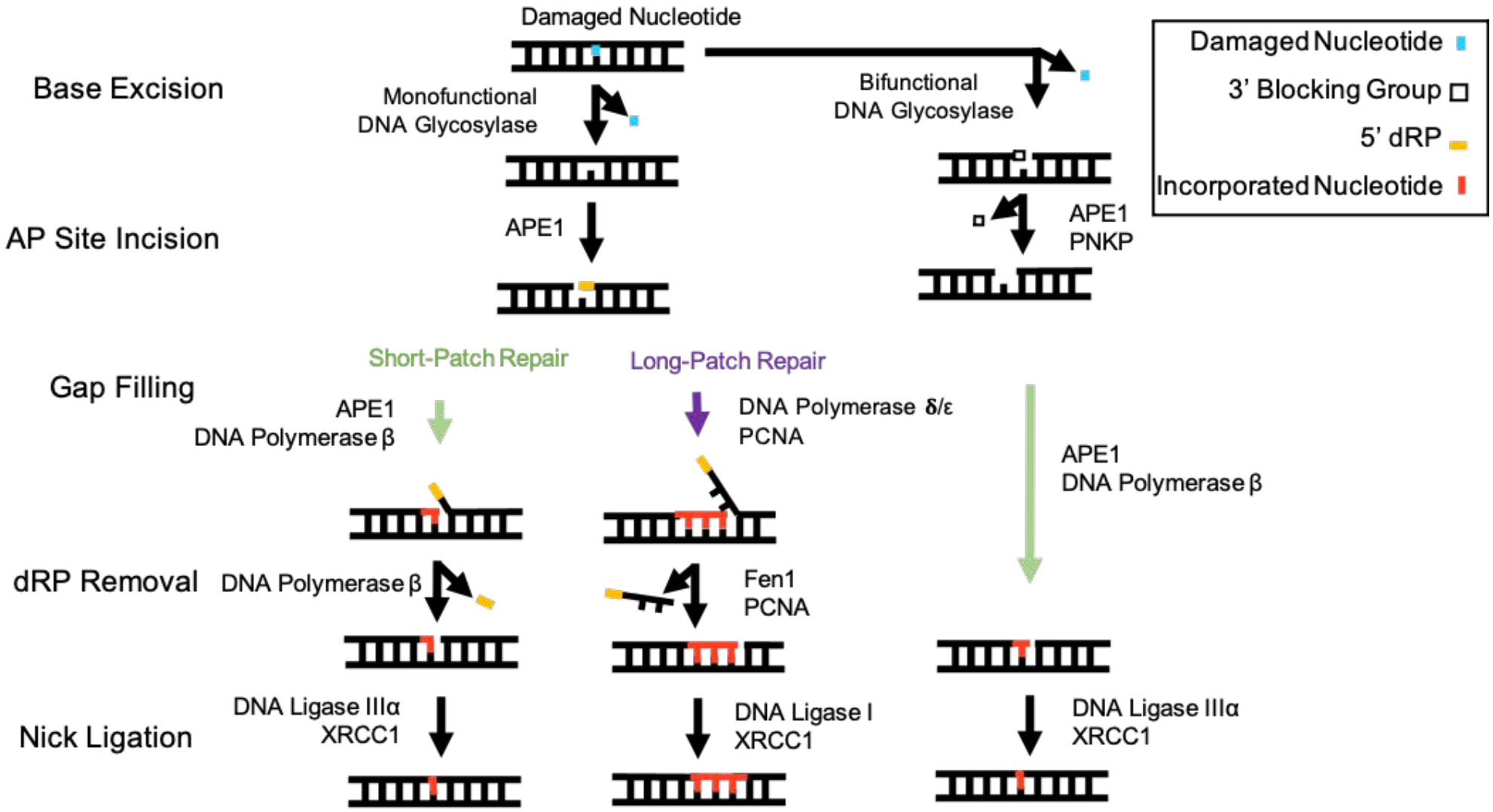


APE1/Ref-1

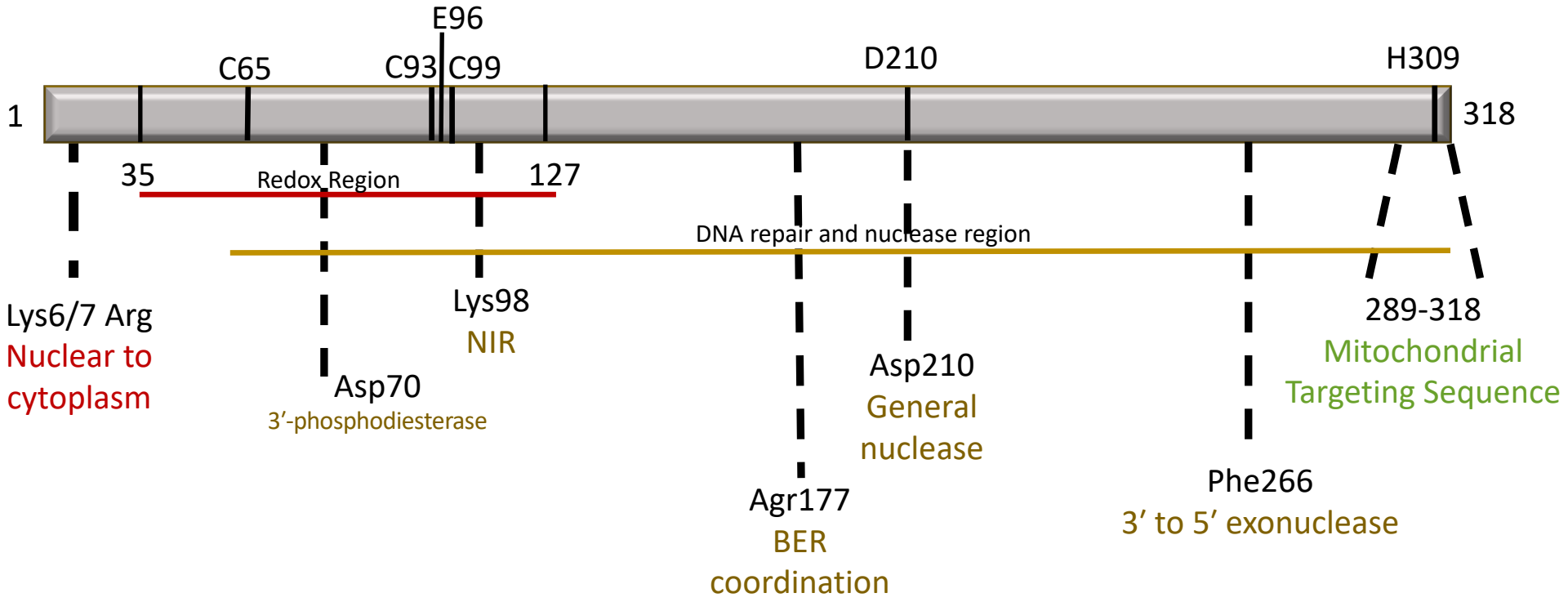
Function #2:

APE1 Redox: regulates transcription factors (TFs) critical to cell signaling and DNA duplication

Summary Publication: F. Shah, D. Logsdon, R.A. Messmann, J.C. Fehrenbacher, M.L. Fishel, and M.R. Kelley. (2017) Exploiting the APE1-Ref-1 node in cancer signaling and other diseases: from bench to clinic. ***Nature Precision Oncology*** June 2017; 1:19.


Damaged Nucleotide
Base Excision
Monofunctional DNA Glycosylase
Bifunctional DNA Glycosylase
AP Site Incision
APE1
APE1 PNKP
Damaged Nucleotide
3' Blocking Group
5' dRP
Incorporated Nucleotide
Short-Patch Repair
Long-Patch Repair
Gap Filling
APE1 DNA Polymerase β
DNA Polymerase δ/ε PCNA
APE1 DNA Polymerase β
dRP Removal
DNA Polymerase β
Fen1 PCNA
Nick Ligation
DNA Ligase IIIα XRCC1
DNA Ligase I XRCC1
DNA Ligase IIIα XRCC1

Regions



FUNCTIONS

Ref-1 Redox Signaling Regulation

NF-κB, HIF1α, STAT3, AP-1, p53

APE1 Repair & Nuclease Functions

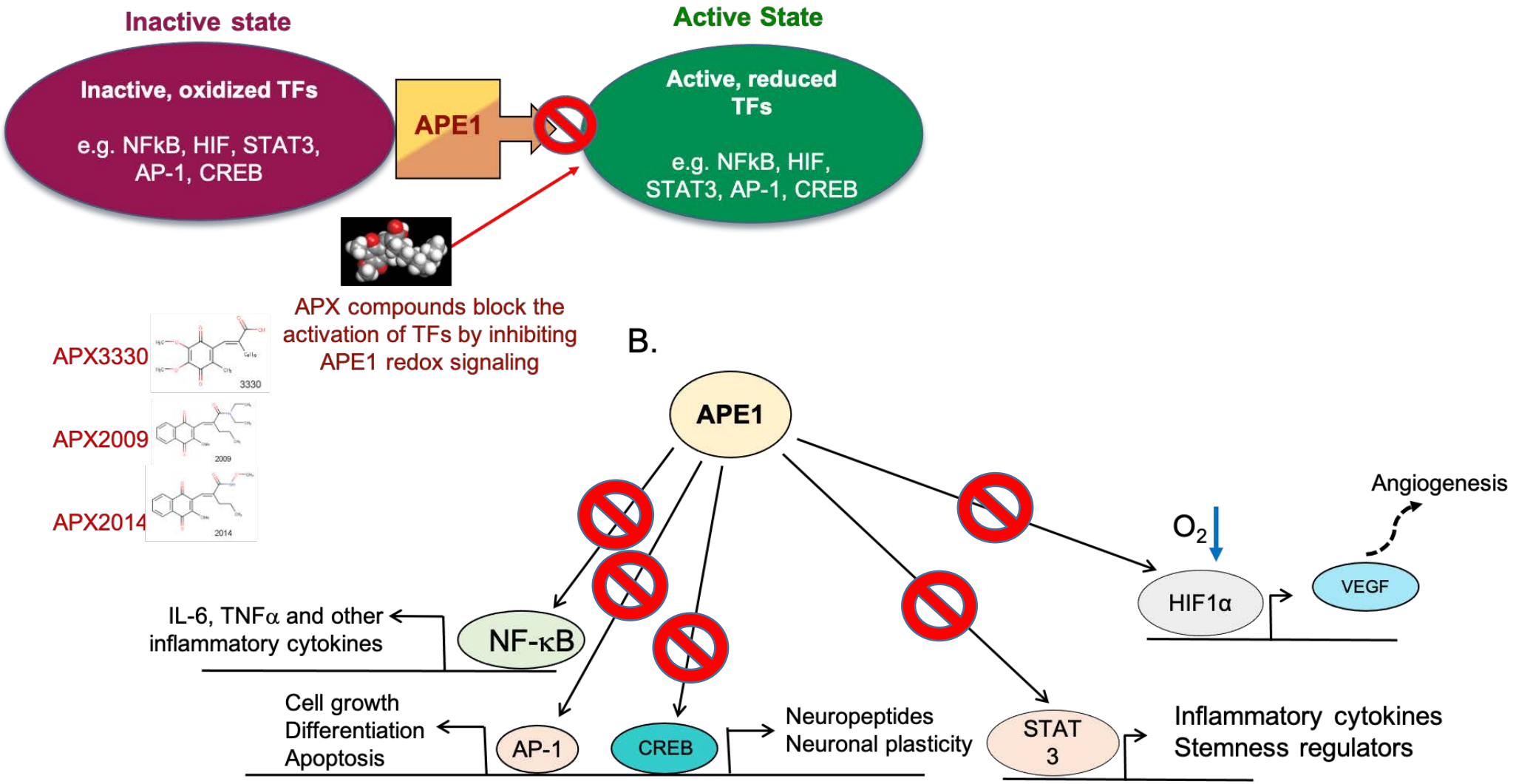
- BER:
  - Endonuclease, exonuclease
  - 3' phosphodiesterase
  - 3' to 5' exonuclease
- NIR: processes oxidative damage not repaired by BER
- RNA metabolism

APPLICATIONS

Anti-inflammation, anti-neovascularization for cancers, CIPN ocular diseases (AMD, DME, DR) and IBD

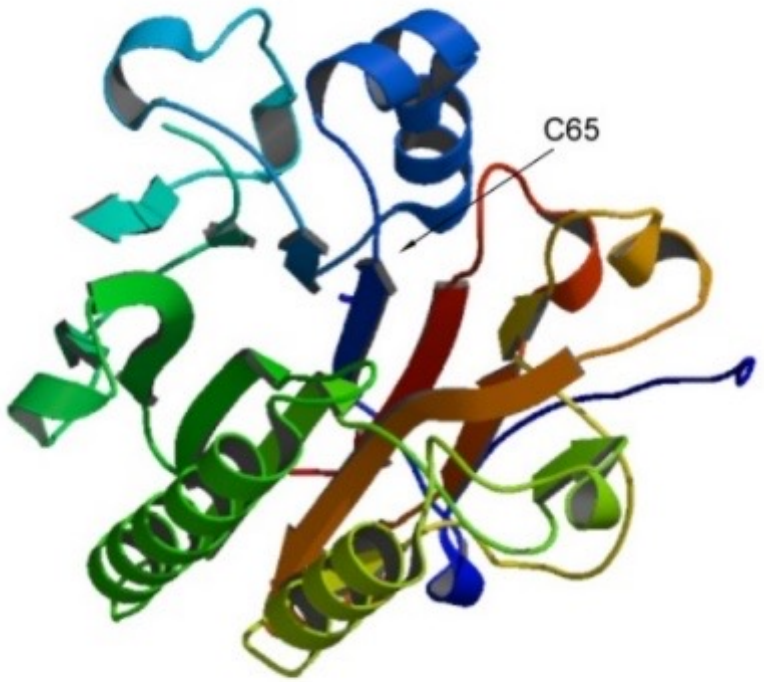
Inhibition of DNA repair for anti-cancer treatment (including reversal of drug resistance)

Diagnosis based on secretory levels (Serum and Urine)


Inactive state
Active State
Inactive, oxidized TFs
e.g. NFkB, HIF, STAT3, AP-1, CREB
APE1
Active, reduced TFs
e.g. NFkB, HIF, STAT3, AP-1, CREB
APX compounds block the activation of TFs by inhibiting APE1 redox signaling
APX3330
APX2009
APX2014
B.
APE1
IL-6, TNFα and other inflammatory cytokines
NF-κB
Cell growth
Differentiation
Apoptosis
AP-1
CREB
Neuropeptides
Neuronal plasticity
O2
HIF1α
VEGF
Angiogenesis
STAT 3
Inflammatory cytokines
Stemness regulators

The Importance of the APE1 Protein: *Cancer Cells*

Cancer cells “hijack” APE1 Redox Function to control transcription factors (TFs) and increase tumor proliferation, survival, angiogenesis, inflammation, and migration

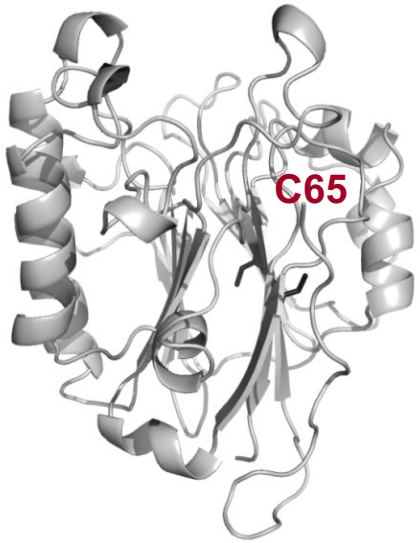


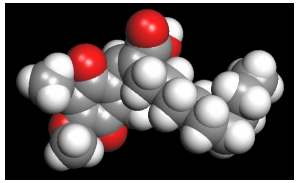
Redox control of TFs HIF-1α, STAT3, NF-KB, and others

Tumor aggressiveness

How does APX3330 work?

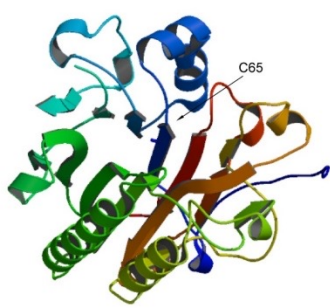
APX3330 knocks out only the **APE1/Ref-1 Redox function** of the APE1 protein. Keeping transcription factors in the "off" position

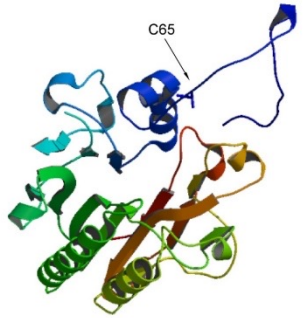


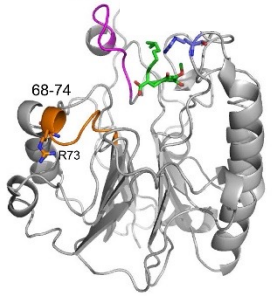


APX3330

APX3330 inhibits only the APE1 redox signaling activity.



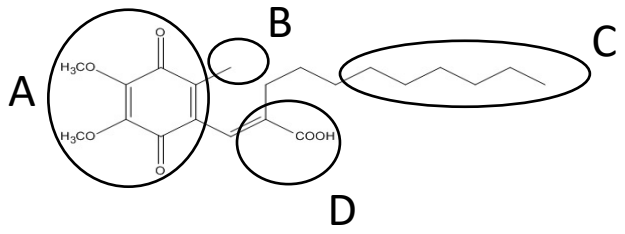




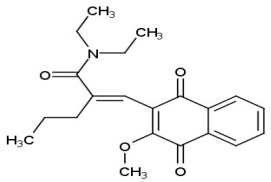
The drug has a direct and selective interaction with APE1/Ref-1 as demonstrated by chemical footprinting, mass spectrometry, Thermal Shift Assay (TSA) and other biochemical data.

Zhang et al. *Biochemistry* 2013, 52, 2955-2966. Luo et al. Biochemistry. 2012 Jan 17;51(2):695-705. Epub 2012 Jan 4 Zhang et al. *Biochemistry*. Apr 30;52(17):2955-66 PMCID: PMC3706204

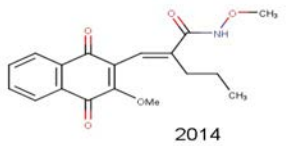
Next generation APX compounds: some examples

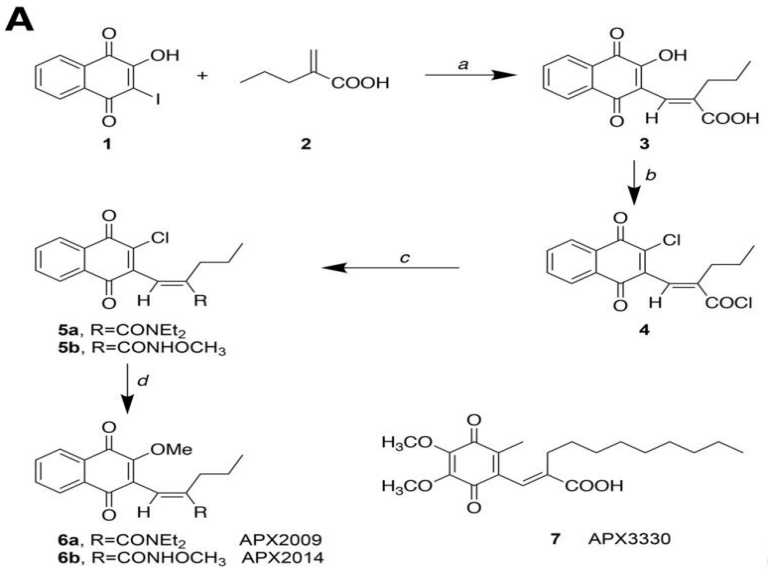


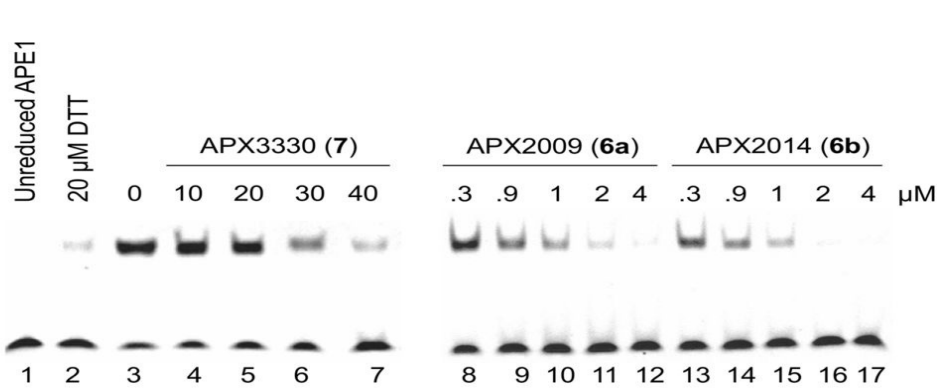
APX2009

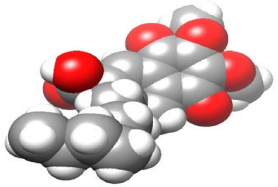


APX2014



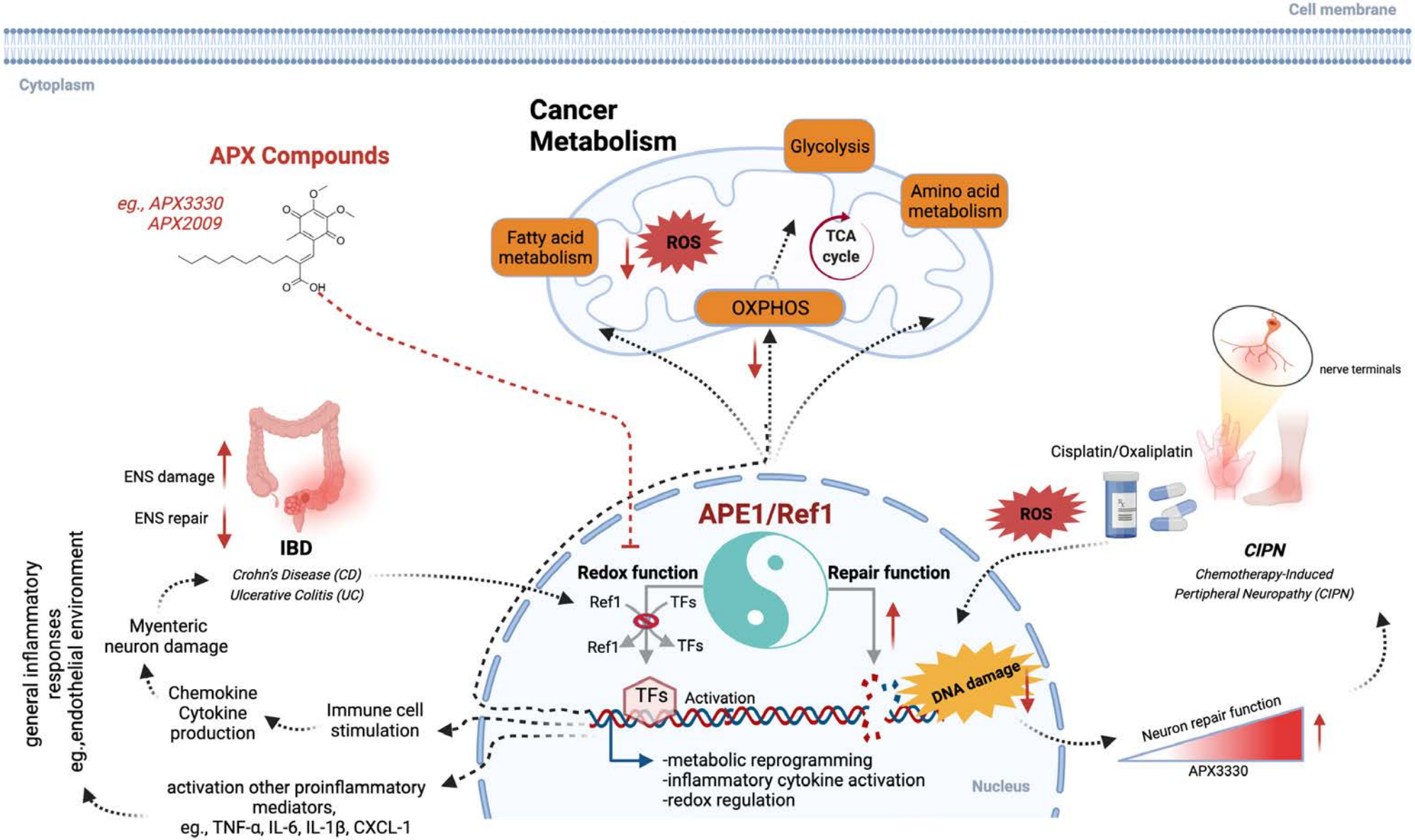






APX3330

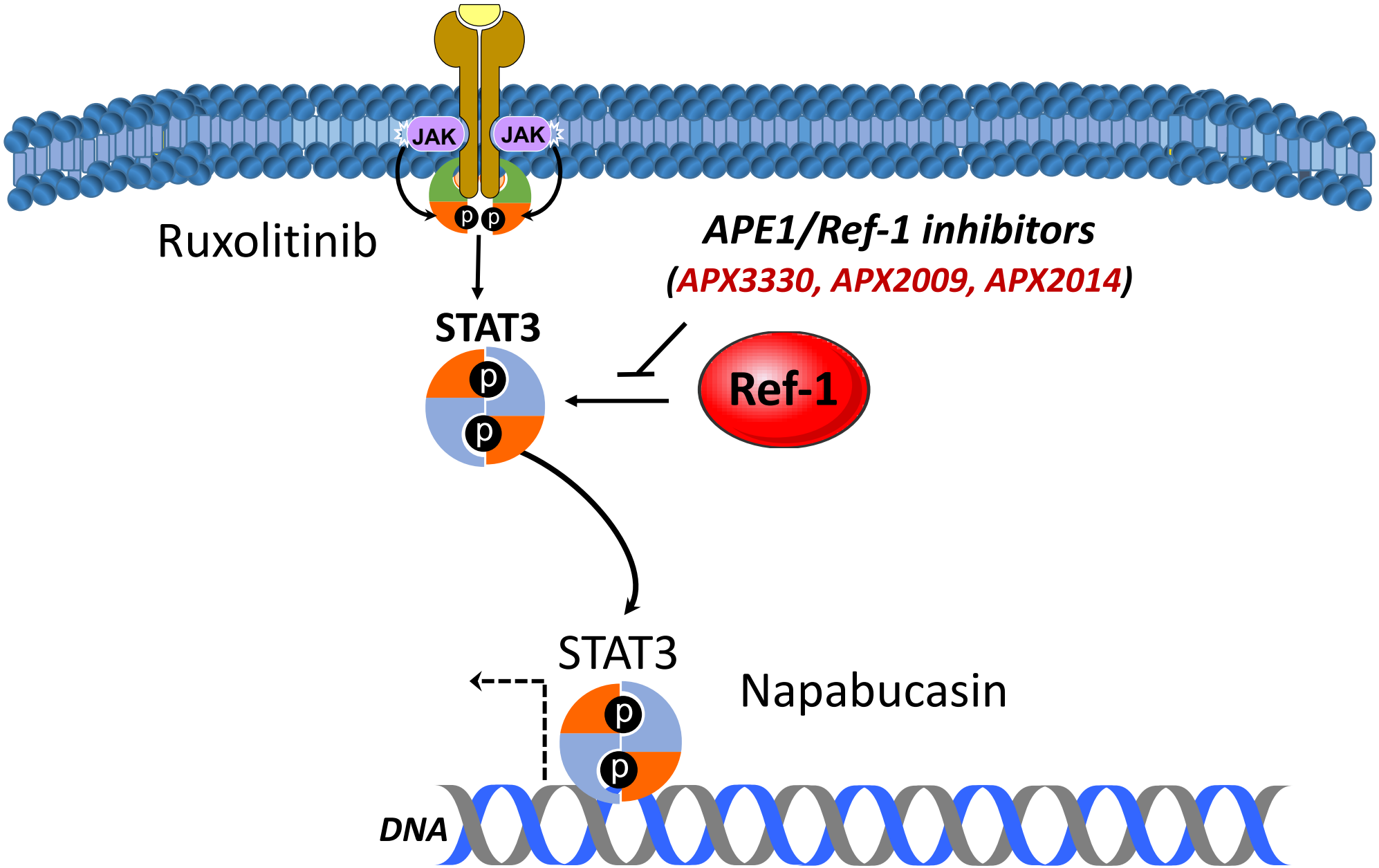
Sardar Pasha SPB, Sishtla K, Sulaiman RS, Park B, Shetty T, Shah F, Fishel ML, Wikel JH, Kelley MR, Corson TW. (2018) Ref-1/APE1 Inhibition with Novel Small Molecules Blocks Ocular Neovascularization. *J Pharmacol Exp Ther.* Oct;367(1):108-118. PMID:30076264


Cell membrane
Cytoplasm
Cancer Metabolism
Glycolysis
Amino acid metabolism
Fatty acid metabolism
ROS
TCA cycle
OXPHOS
APX Compounds
eg., APX3330
APX2009
ENS damage
ENS repair
IBD
Crohn's Disease (CD)
Ulcerative Colitis (UC)
Myenteric neuron damage
Chemokine Cytokine production
Immune cell stimulation
general inflammatory responses eg.,endothelial environment
activation other proinflammatory mediators, eg., TNF-α, IL-6, IL-1β, CXCL-1
APE1/Ref1
Redox function
Repair function
Ref1
TFs
Ref1
TFs
TFs
Activation
-metabolic reprogramming
-inflammatory cytokine activation
-redox regulation
Nucleus
DNA damage
ROS
Cisplatin/Oxaliplatin
nerve terminals
CIPN
Chemotherapy-Induced Pertipheral Neuropathy (CIPN)
Neuron repair function
APX3330

Example Cancer Data

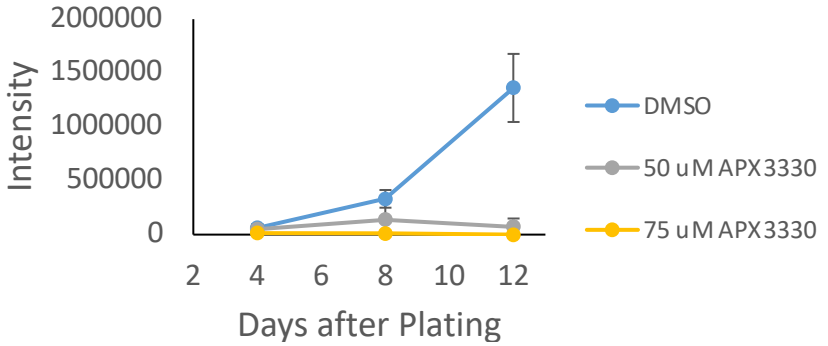
- Pancreatic cancer
- Bladder
- MPNST
  - (Malignant peripheral nerve sheath tumor)
- Leukemia
  - AML
  - T-cell ALL
- Chemotherapy-induced peripheral neuropathy
  - (CIPN)
- Apexian Phase I Clinical Trial

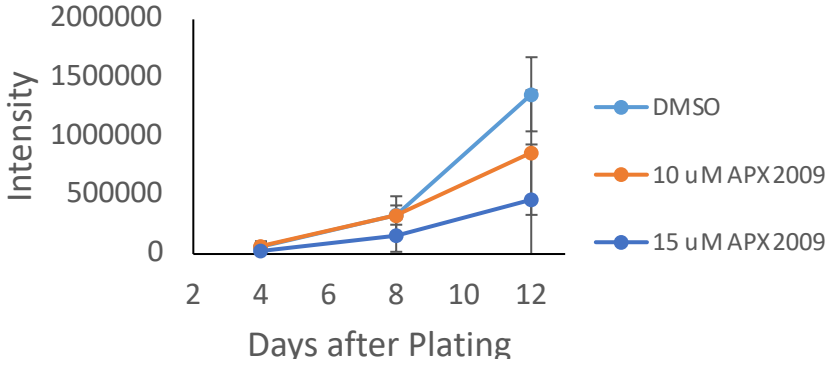
STAT3 regulates key signaling pathways involved in proliferation, survival, migration/invasion, and hypoxia response and is under Ref-1 redox signaling control

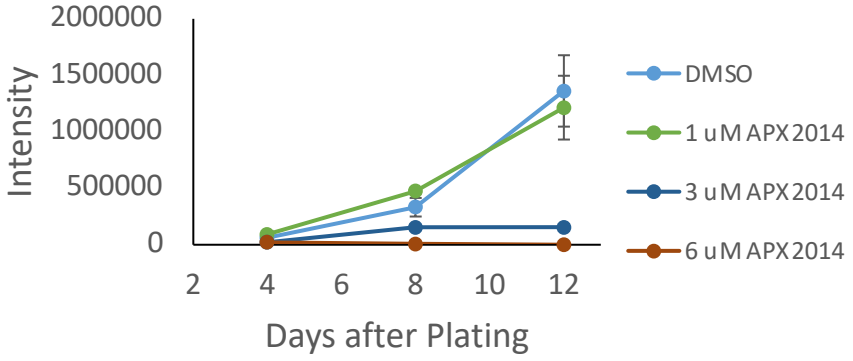


Single agent efficacy of parent compound APX3330 and next-generation compounds APX2009 & APX2014 in pancreatic 3D spheroid models

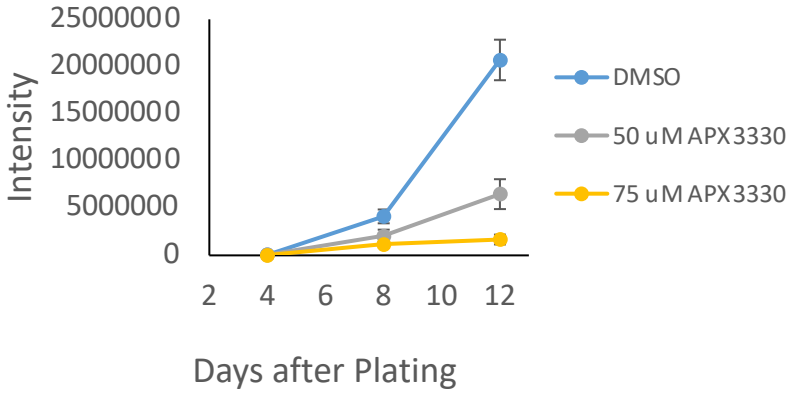
Tumor Alone

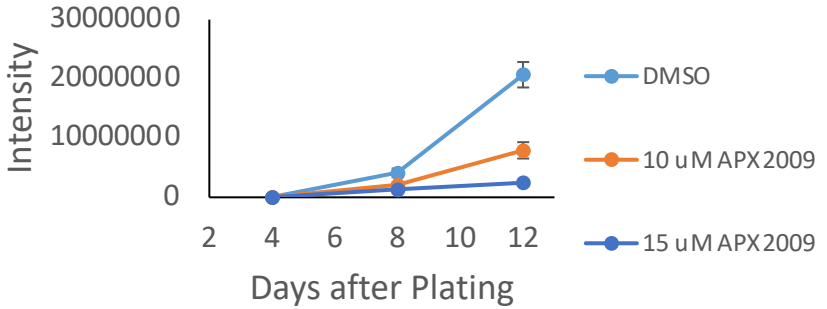


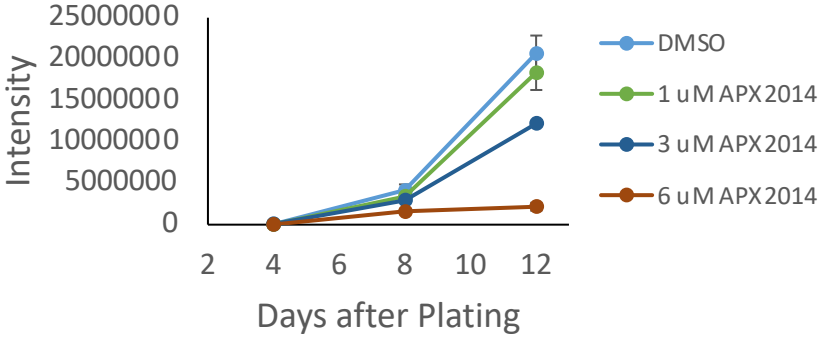




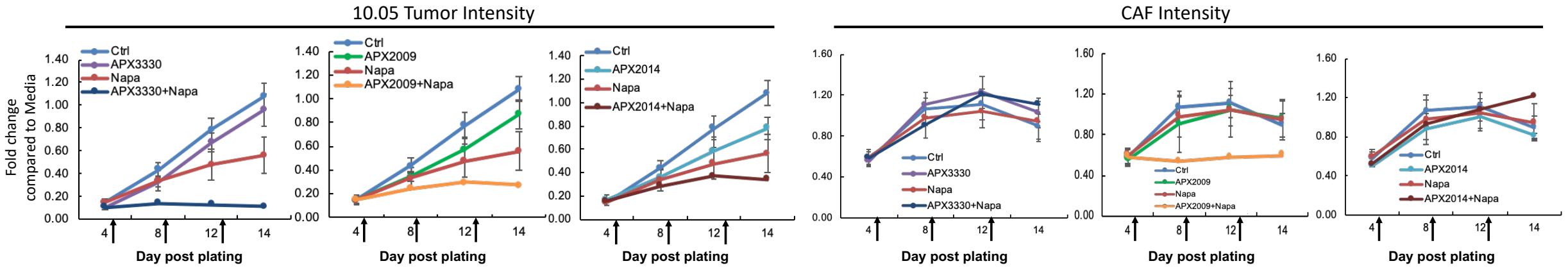
Tumor + CAFs

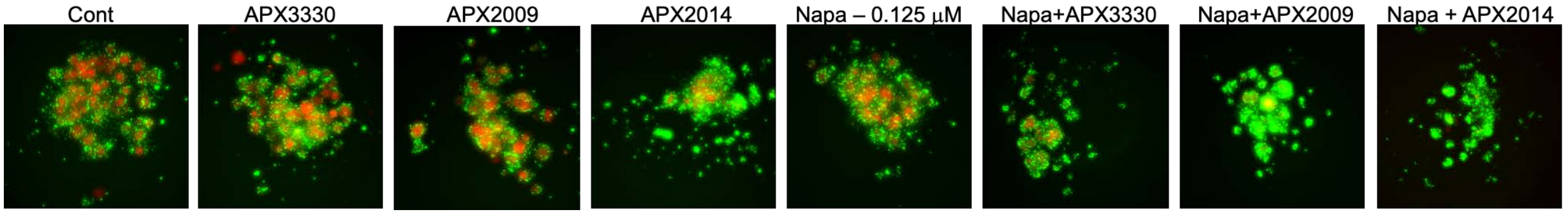






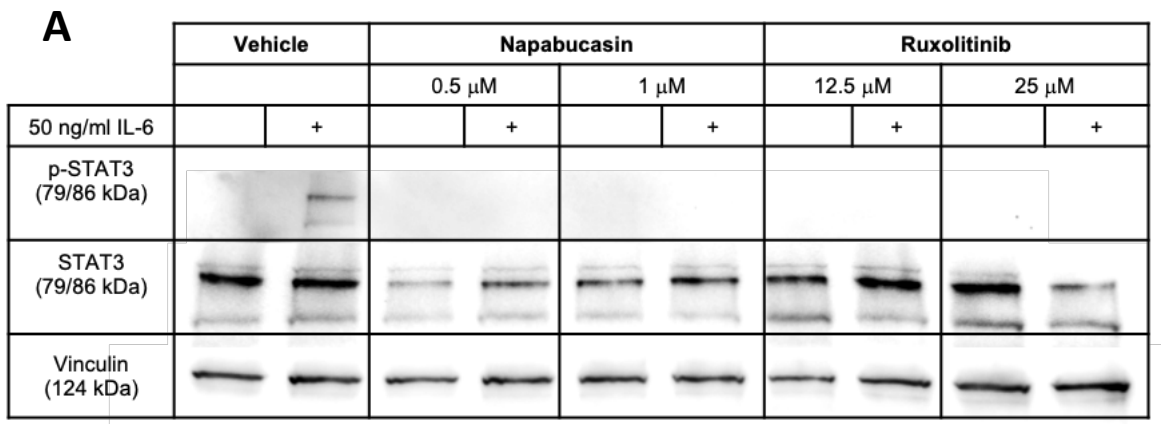
Napabucasin in combination with Ref-1 inhibition in 3D co-culture models of pancreatic cancer dramatically effect spheroid growth and signaling- tumor killing, not CAFS

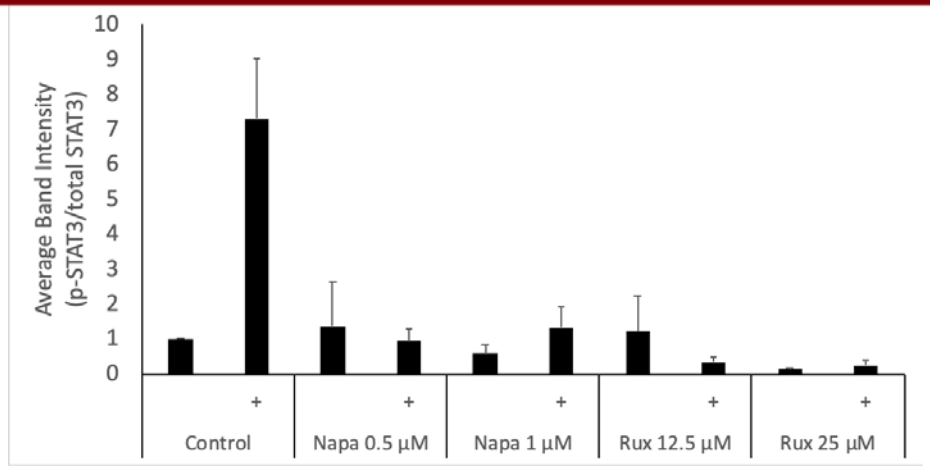




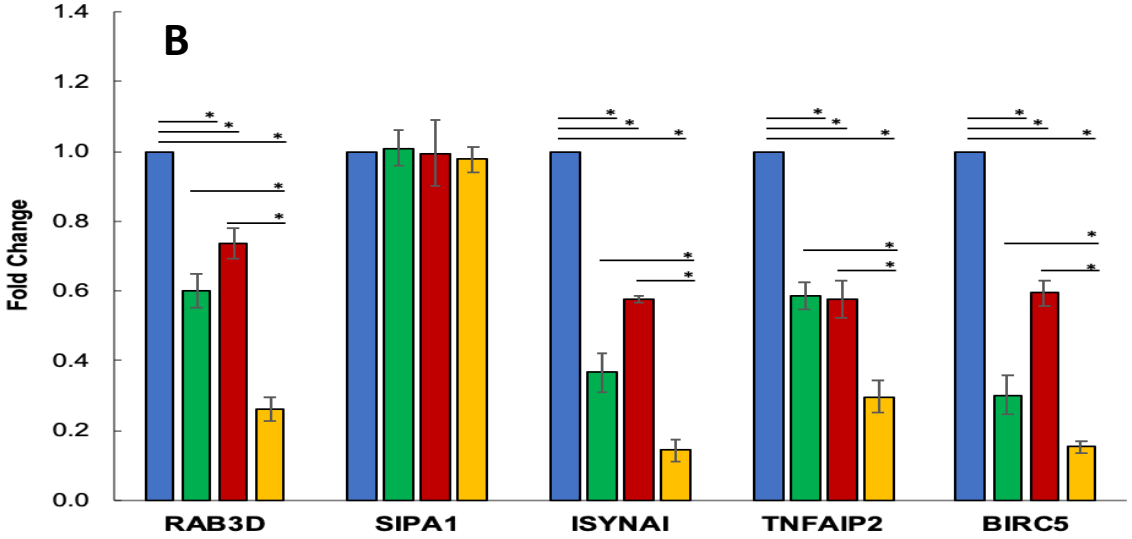
Journal of Cellular and Molecular Medicine, Volume: 25, Issue: 2, Pages: 784-800, First published: 03 December 2020, DOI: (10.1111/jcmm.16132)

Napabucasin in combination with Ref-1 inhibition in 3D co-culture models of pancreatic cancer dramatically effects spheroid growth and signaling

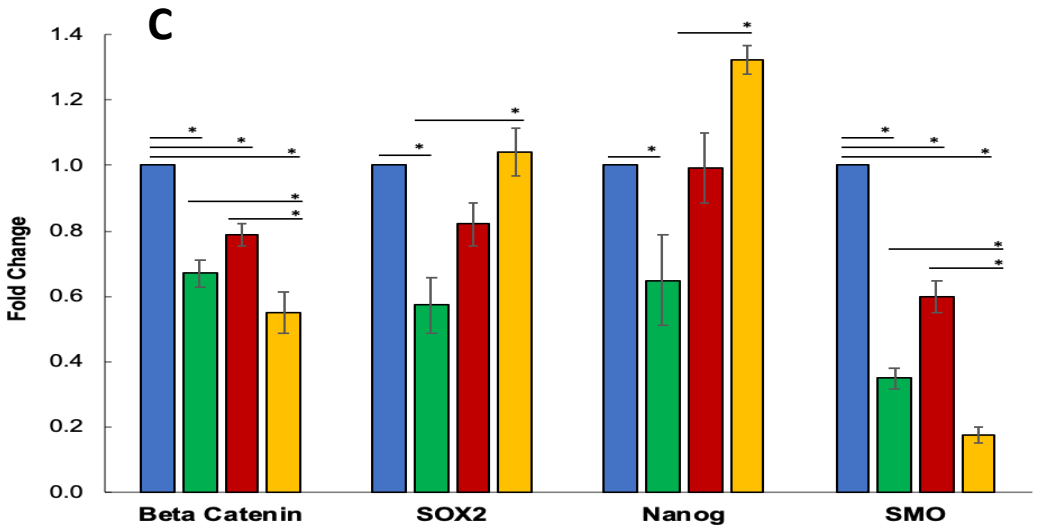




Cont APX2009 Napa APX2009+Napa



Biomarkers of Ref-1 Inhibition



Biomarkers of Napa treatment

Journal of Cellular and Molecular Medicine, Volume: 25, Issue: 2, Pages: 784-800, First published: 03 December 2020, DOI: (10.1111/jcmm.16132)

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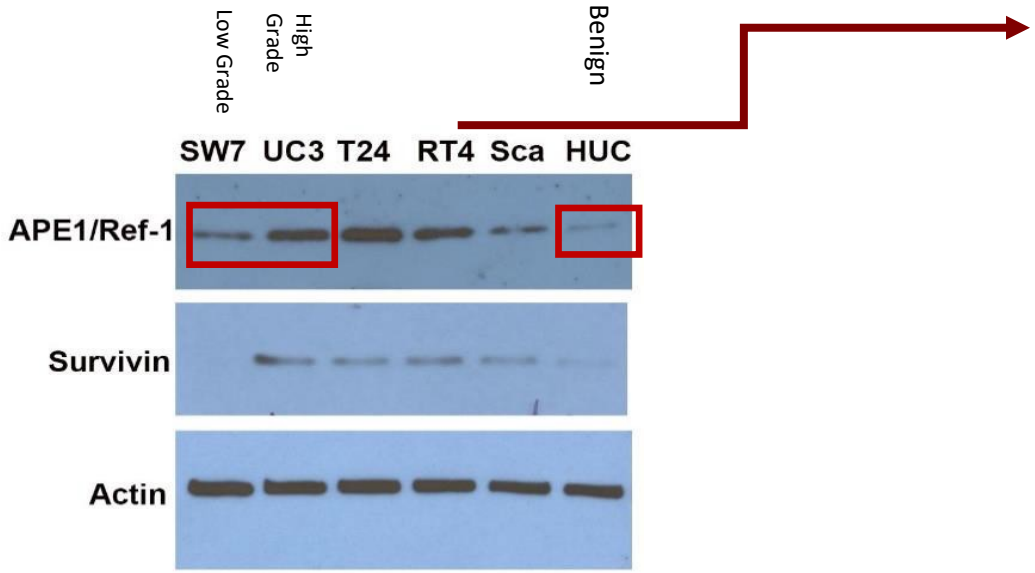
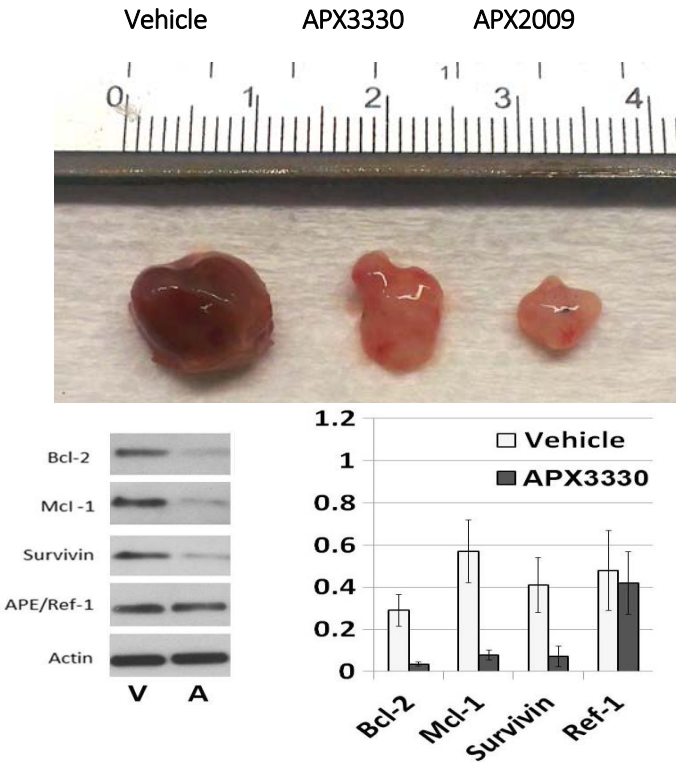
- Pancreatic cancer
- Bladder
- MPNST
  - (Malignant peripheral nerve sheath tumor)
- Colon
- Leukemia
  - AML
  - T-cell ALL
- Chemotherapy-induced peripheral neuropathy
  - (CIPN)
- Apexian Phase I Clinical Trial

Bladder Tumor Aggressiveness Correlates with APE1/Ref-1 Expression: APX compounds reduce tumor growth and angiogenesis



- SW7 (SW-780) **low grade** TCC
- UC3 **high grade** TCC
- T24 high grade papillary
- RT4 low grade papillary
- Sca (SCaBER) squamous variant
- HUC, an immortalized **benign** urothelial cell line.

In vivo treatment of T24 tumor cells

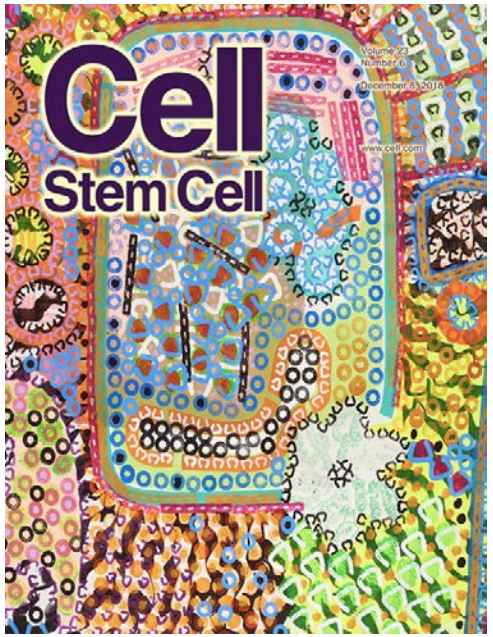


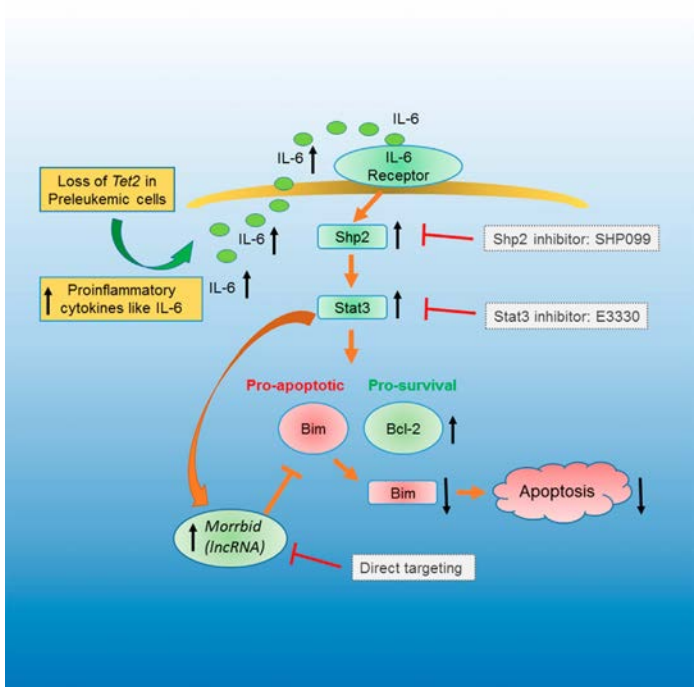
APX3330 blocks transcription factors that regulate **Bcl-2, Mcl-1** and **Survivin**. These proteins are critical for bladder cancer tumor growth.

Example Cancer Data

- Pancreatic cancer
- Bladder
- MPNST
  - (Malignant peripheral nerve sheath tumor)
- Colon
- Leukemia
  - AML
  - T-cell ALL
- Chemotherapy-induced peripheral neuropathy
  - (CIPN)
- Apexian Phase I Clinical Trial

Inhibition of STAT3 through APE1/Ref-1 inhibition by APX3330 blocked leukemia cancer progression





Inhibition of Inflammatory Signaling in Tet2 Mutant Preleukemic Cells Mitigates Stress-Induced Abnormalities and Clonal Hematopoiesis
Cell Stem Cell, 2018 Dec., 23(6), 773-90

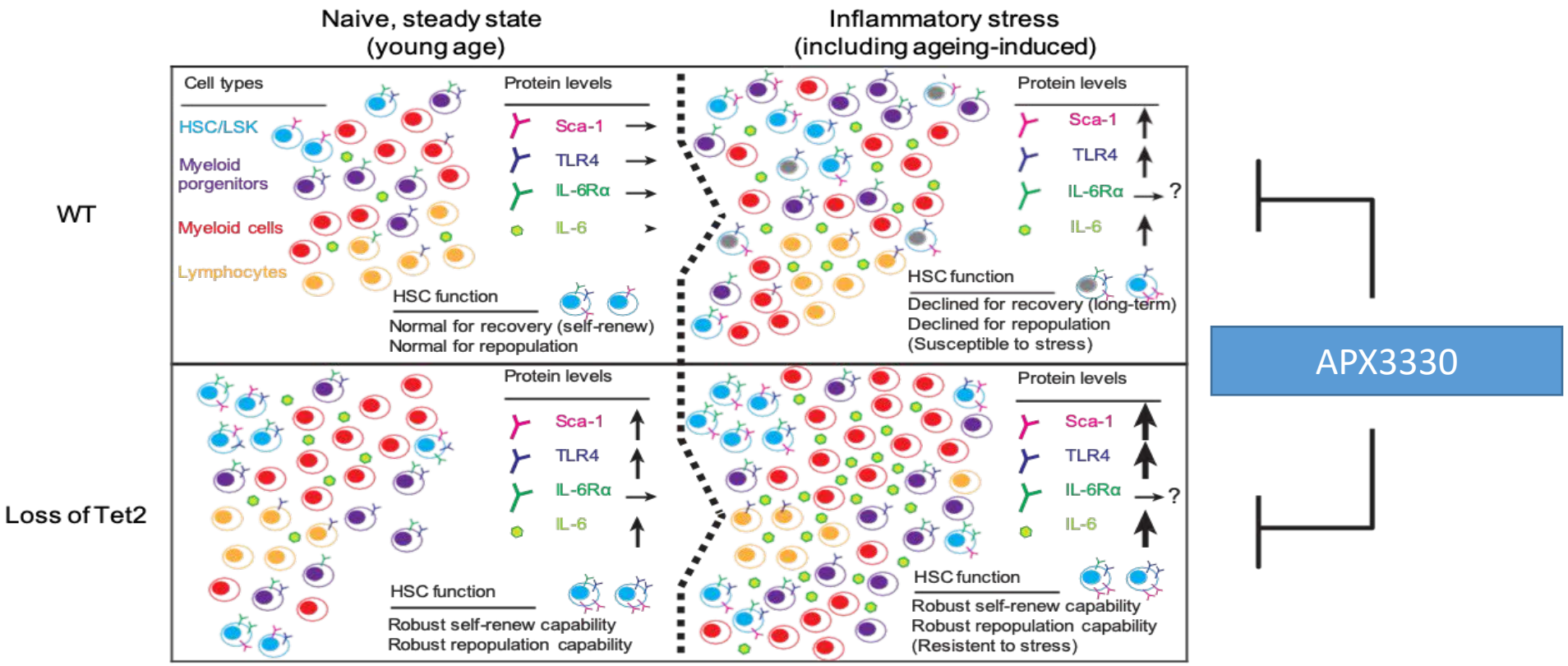
Contributing IU Authors: Reuben Kapur, PhD; Zhigang Cai, MD; Baskar Ramdas, PhD; Sisi Chen, PhD; Lakshmi Reddy Palam, PhD; Ruchi Pandey, PhD; Yan Liu, PhD; **Mark R. Kelley, PhD**; and George Sandusky, PhD

Summary: Inflammation is a risk factor for cancer development. People with preleukemic TET2 mutations develop clonal hematopoiesis and are at a higher risk of developing leukemia. This study showed a rapid increase of Tet2-KO mature myeloid cells and HSPCs in response to inflammatory stress, which results in enhanced production of inflammatory cytokines, including IL-6, and resistance to apoptosis. IL-6 induces hyperactivation of the Shp2-Stat3 signaling axis and increased expression of a novel anti-apoptotic long non-coding RNA, *Morrbid*, in Tet2-KO myeloid cells and HSPCs. In vivo, pharmacologic inhibition of Shp2 or Stat3 (through blocking APE1 activation with APX3330) or genetic loss of Morrbid in Tet2 mutant mice rescues inflammatory-stress-induced abnormalities in HSPCs and mature myeloid cells, including clonal hematopoiesis.

Studies in this paper used APX3330 to block cancer progression

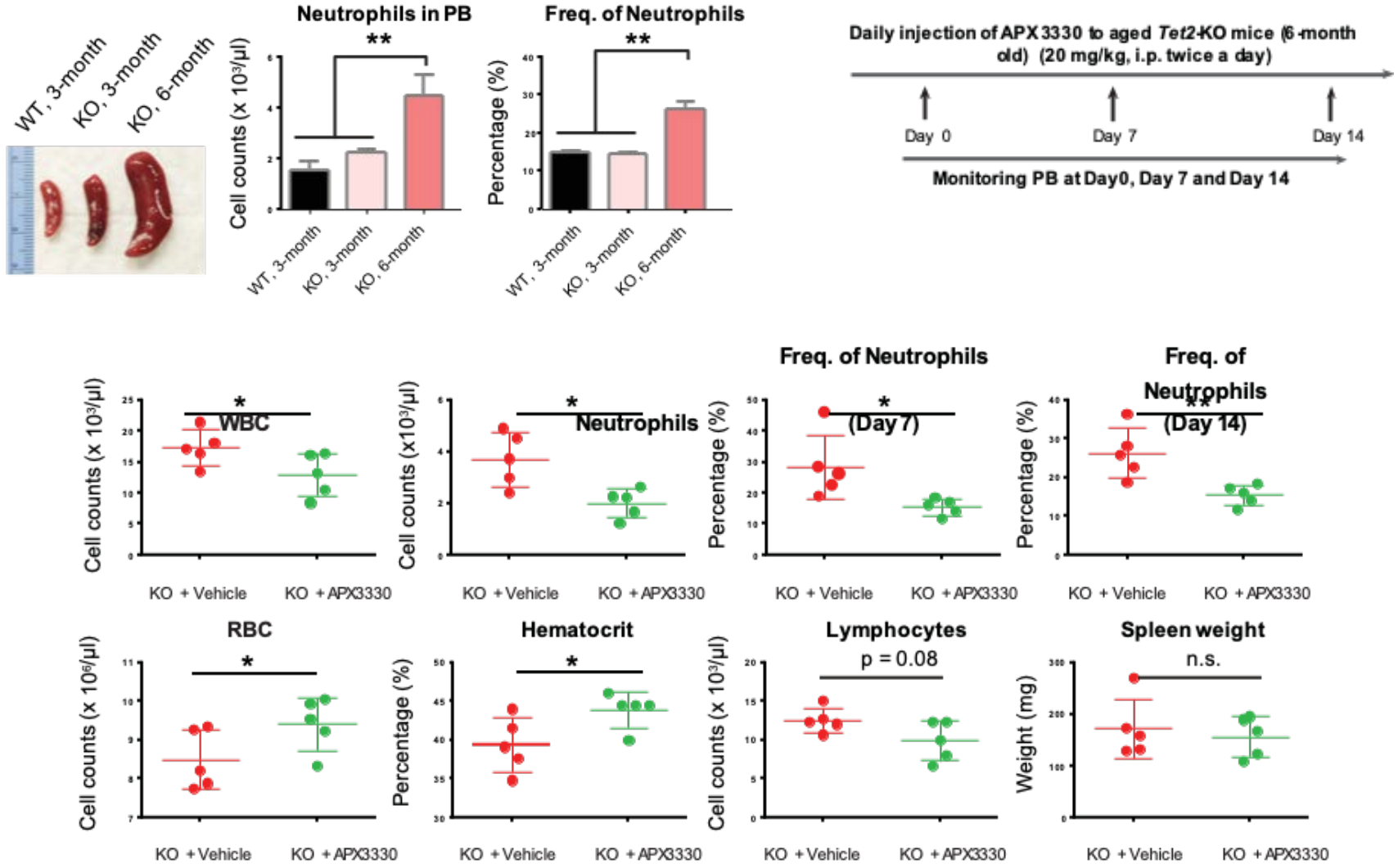
Model for myeloid skewing and altered HSC activity induced by Tet2 deficiency and/or inflammatory stress

Inflammation induced functional changes in pre-leukemic stem and progenitor cells lacking Tet2 can be modulated by targeting the NFκB pathway



Loss of Tet2 in the pre-leukemic mice maintains increased basal levels of TLR4, IL-6 and Sca-1 protein compared to normal mice. Upon inflammatory stress, Tet2-deficient mice show enhanced emergency granulopoiesis and hematopoiesis (myeloid skewing), in part by regulating the expression of TLR4, IL-6 and Sca-1. While wild type HSCs are susceptible to inflammatory stress, Tet2-deficient HSCs are resistant to such form of stress and maintain self-renewal and repopulating advantage compared to wildtype cells.

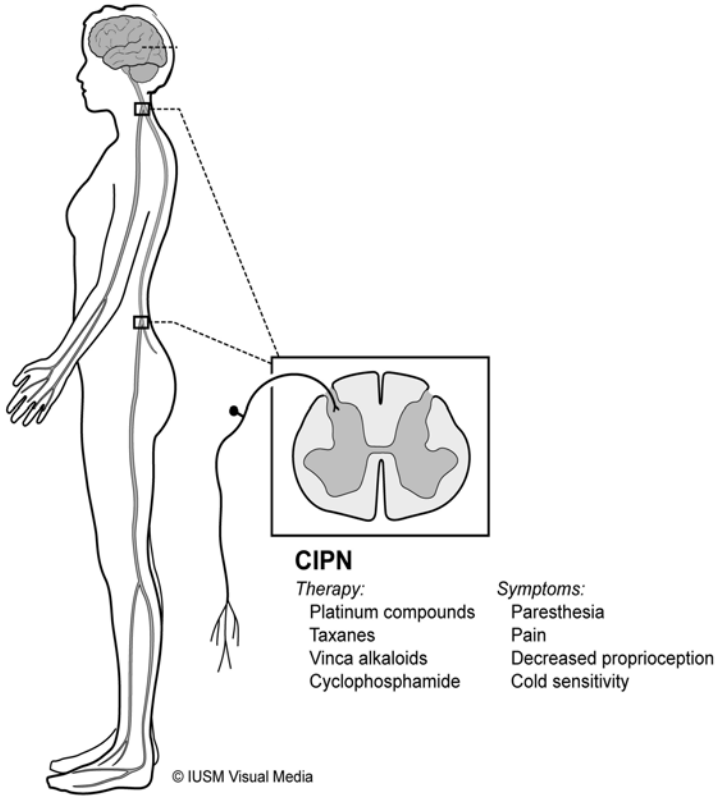
APX3330 reverses early signs of myeloproliferative neoplasms (MPN) in aged *Tet2*-KO mice



Example Cancer Data

- Pancreatic cancer
- Bladder
- MPNST
  - (Malignant peripheral nerve sheath tumor)
- Colon
- Leukemia
  - AML
  - T-cell ALL
- **Chemotherapy-induced peripheral neuropathy**
  - **(CIPN)**
- Apexian Phase I Clinical Trial

CIPN Affects ~20 million Cancer Patients

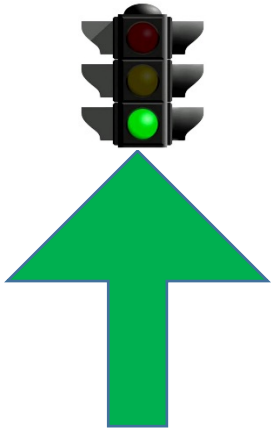


- Pain (which may be there all the time or come and go, like shooting or stabbing pain)
- Burning
- Tingling ("pins and needles" feeling) or electric/shock-like pain
- Loss of feeling (which can be numbness or just less ability to sense pressure, touch, heat, or cold)
- Trouble using your fingers to pick up or hold things; dropping things
- Balance problems
- Trouble with tripping or stumbling while walking
- Being more sensitive to cold or heat
- Being more sensitive to touch or pressure
- Muscle weakness
- Trouble swallowing
- Constipation
- Blood pressure changes
- Decreased or no reflexes

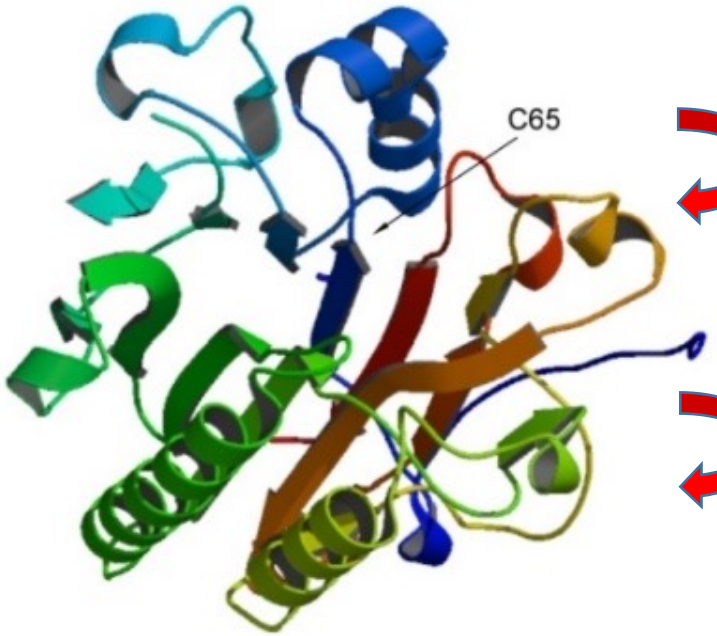
Limits administration of chemotherapy and survival benefit
ASCO Guidance Statement: "No clinically effective treatment against CIPN"

J Clin Oncol. 2020 Oct 1;38(28):3325-3348.

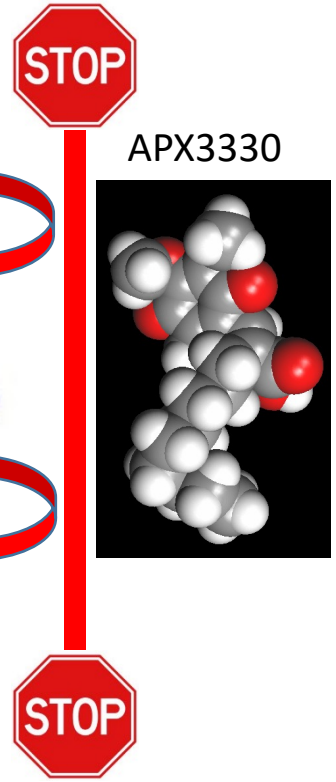
APX3330 Protects Neurons/Inhibits Cancer Cells



Increased DNA oxidative repair **and neuronal protection**



APE1/Ref-1



Decreased redox function and tumor aggressiveness

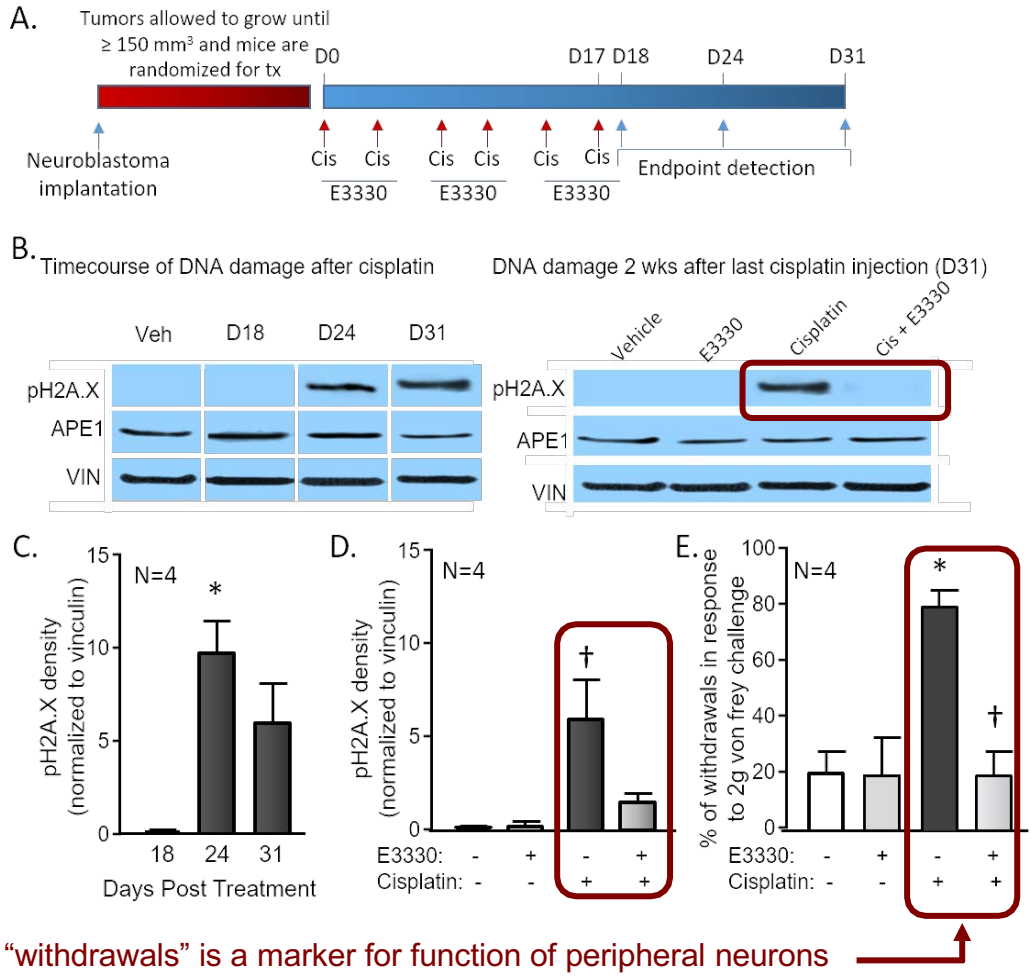
By blocking the redox function of APE1/Ref-1,
APX3330 shifts APE1 activity ***toward*** oxidative damage repair
and neuron protection

APX3330 Prevents CIPN When Given With Cisplatin

Overview of studies completed:

- Mouse models implanted with human tumors (neuroblastomas)
- Cisplatin + APX3330 administered after tumor growth, through Day 17
- A biomarker called pHA2.X is measured as a marker for DNA damage in nerves
- “withdrawals” is a marker for function of peripheral neurons
- Results show change in pHA2.X levels (i.e., decreased DNA damage) and decreased number of withdrawals (increased normal neuronal function).
- **APX3330 prevents DNA damage in the neurons**

APX3330 given **with** Cisplatin treatment **prevents** CIPN



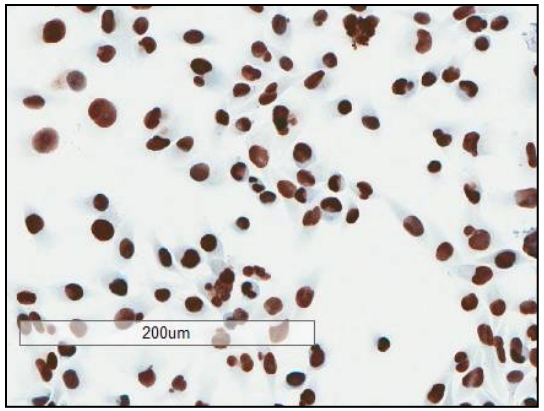
“withdrawals” is a marker for function of peripheral neurons

Today, I am going to talk about:

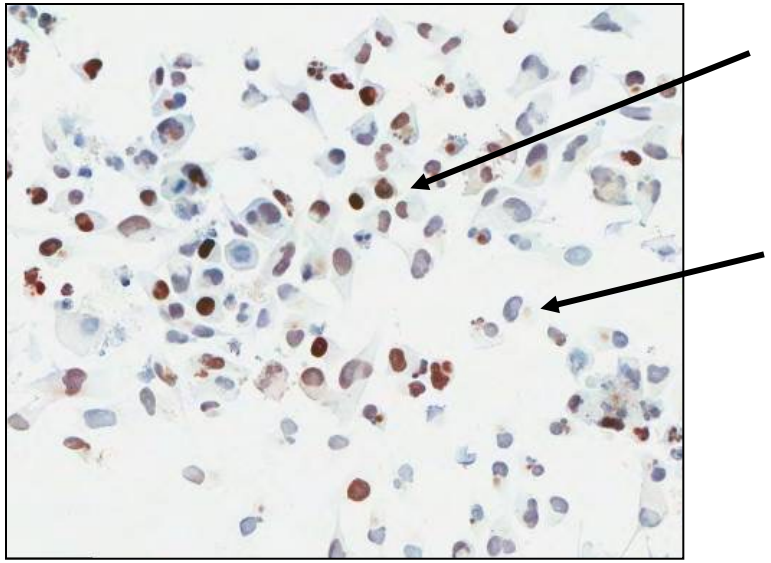
- A little science
- **How big data/bioinformatics has led to new avenues of research**
- Pathway from bench to clinical trial
- New directions based on data analysis, target and drug development

Why do single-cell sequencing?

10nM SCR



10nM siAPE1

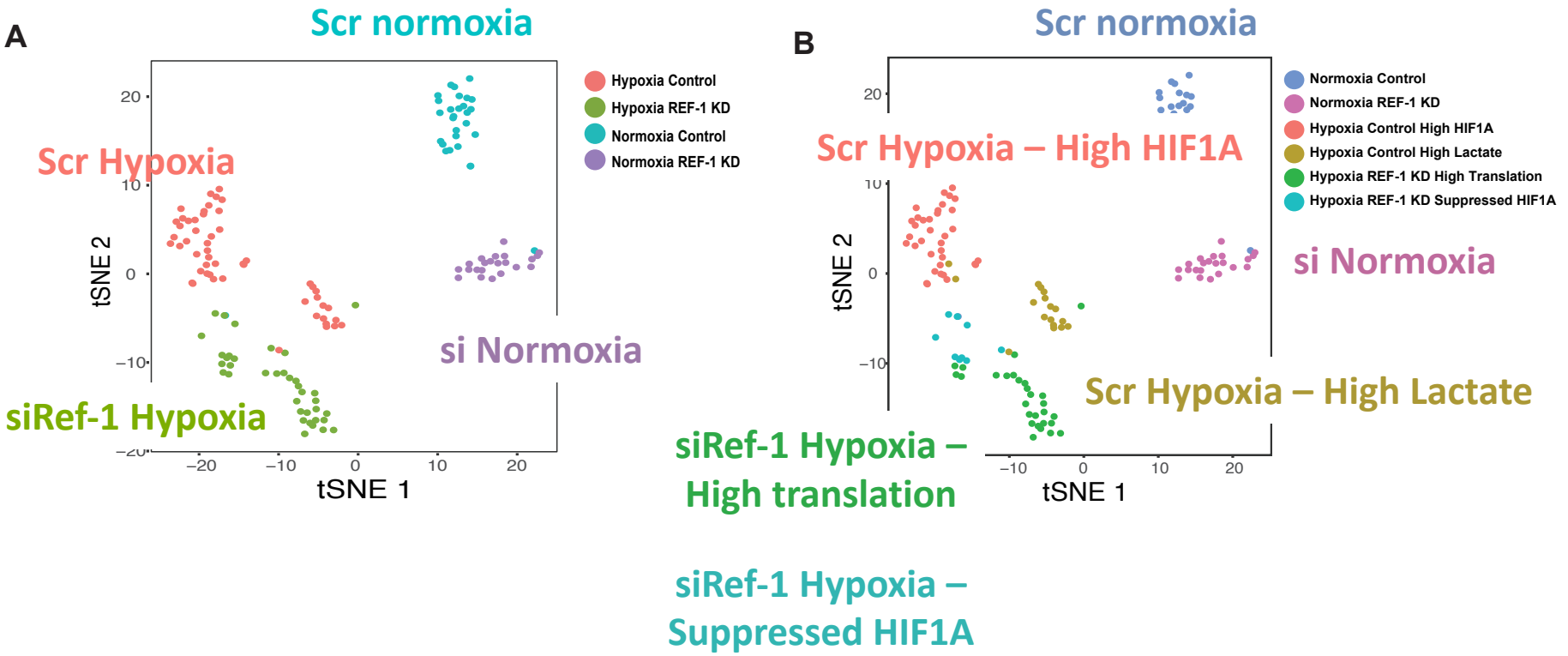


Allows us to compare the transcriptional changes between these two cells which have both been treated with siAPE1, but clearly have different levels of APE1

- To observe heterogeneity and subpopulations
- Look at lineages and subsets
- Compare cells with undetectable APE1 to a cells with reduced levels of APE1

Left truncated mixture Gaussian model was used to define cell clusters under the various conditions:

Normoxia vs Hypoxia; Scrambled vs siRef-1



The “usual suspects” are changed as expected.

Pathway affected by Ref-1 knockdown	p-value
STAT3 Pathway	1.2e-6
HIF1 Signaling	8e-4
NFκB Signaling	3e-3

1855 DEGs identified between Ref-1 knock-down and control cells in normal O_2, 2114 DEGs in hypoxia

>100 different canonical pathways found to be overrepresented amongst differentially expressed genes

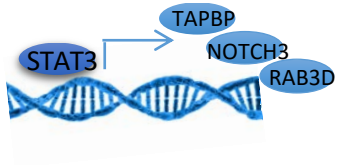
Genes Selected for scRNA-seq Validation

Gene affected	Pathway/Role	Fold change	Significance (Adj. p-value)
BCRP	ATP-binding cassette transport	12.9	0.011
CIRBP	Cold Shock response	0.20	7.92E-07
ITGA1	Virus Entry via Endocytic Pathways	0.15	0.015
NOTCH3	Notch Signaling	0.09	4.87E-09
PPIF	Mitochondrial Protein folding and permeability	4.48	0.008
PRDX5	Mitochondrial Dysfunction pathway	0.28	6.86E-13
RAB3D	Intracellular transport	0.11	1.29E-07
SIPA1	GTPase Activation	0.17	1.33E-04
TAPBP	Peptide Loading complex, antigen presentation pathway	0.25	1.81E-10

	SCR vs siAPE1		siAPE1 non-zero vs siAPE1 zero		SCR vs siAPE1 non-zero vs siAPE zero
Genes	Fold change	Significance (Adj. p-value)	Fold change	Significance (Adj. p-value)	Significance (Adj. p-value)
COMMD7	0.16	0.003	0.06	0.038	6.18E-06
ISYNA1	0.2	0.013	0.019	0.046	0.0005
TNFAIP2	0.09	0.004	0.01	0.034	5.15E-08

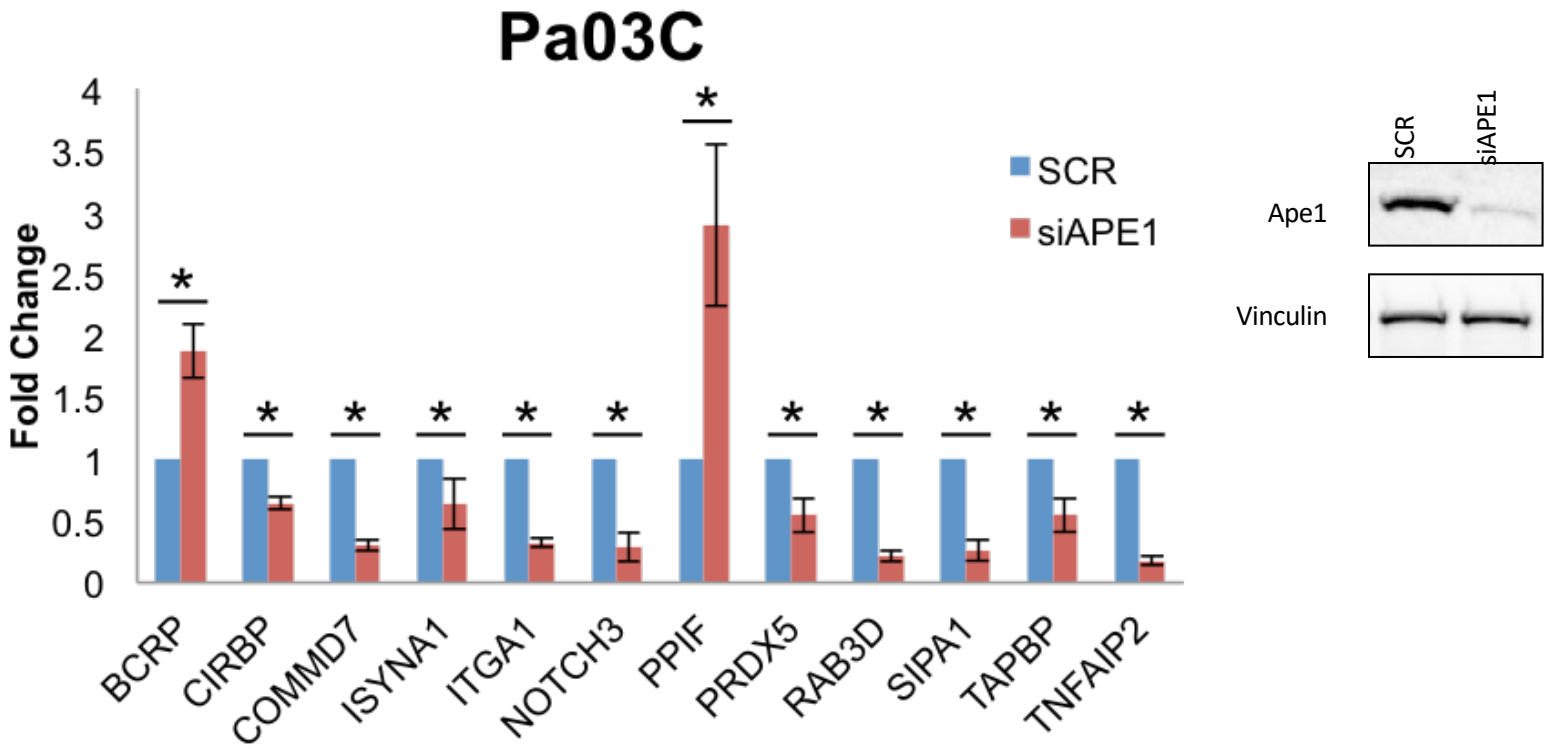
Rationale for selection

- p-value
- Fold change
- Regulation by Transcription Factors that are regulated by APE1



Shah, Goosens, Atallah, Grimard, Kelley and Fishel (2017) APE1/Ref-1 knockdown in pancreatic ductal adenocarcinoma: Characterizing gene expression changes and identifying novel pathways using single-cell RNA sequencing. *Molecular Oncology, In Press.*

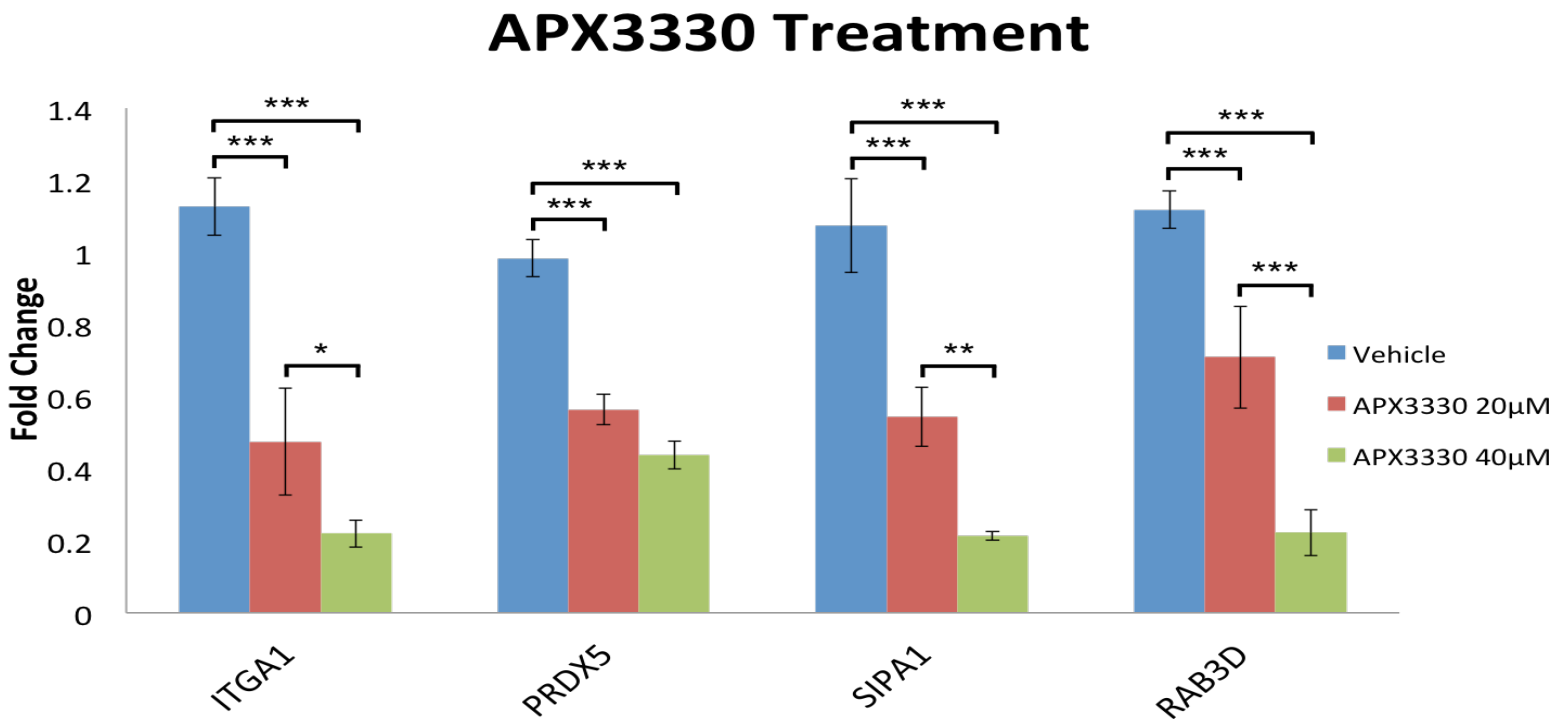
Validation via qPCR demonstrates all 12 genes change expression as in scRNA-seq



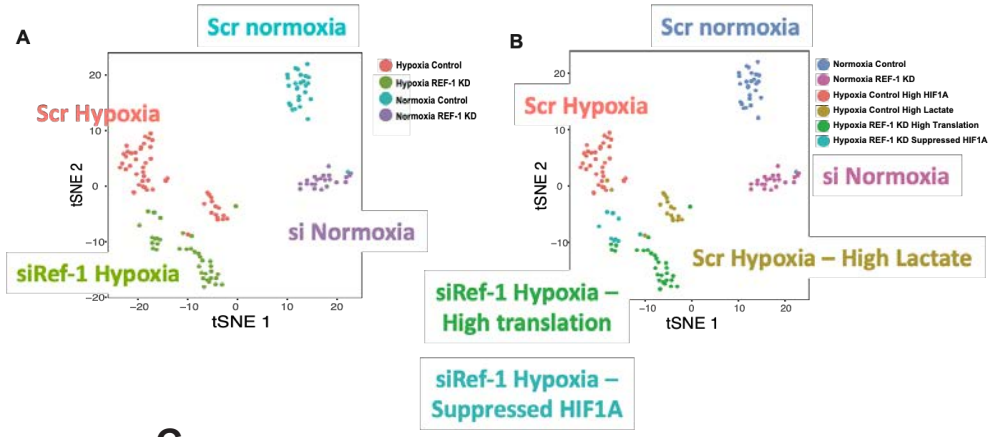
Shah, Goosens, Atallah, Grimard, Kelley and Fishel (2017) APE1/Ref-1 knockdown in pancreatic ductal adenocarcinoma: Characterizing gene expression changes and identifying novel pathways using single-cell RNA sequencing. Mol Oncol. 2017 Dec;11(12):1711-1732.

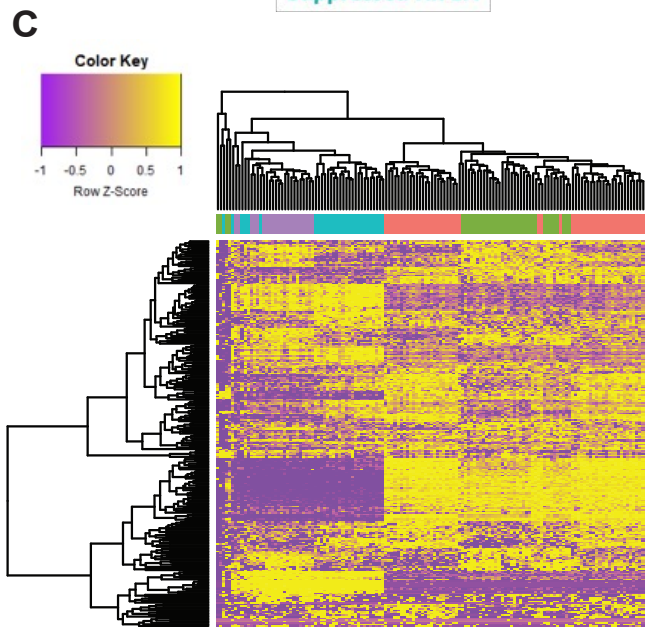
Confirmation that the redox function of APE1 is responsible for the decrease in expression

sc RNAseq	ITGA1	PRDX5	SIPA1	RAB3D
Fold change from SCR	0.01	0.26	0.01	0.10
p value	0.001	1.02×10^{-15}	2.78×10^{-6}	8.14×10^{-7}

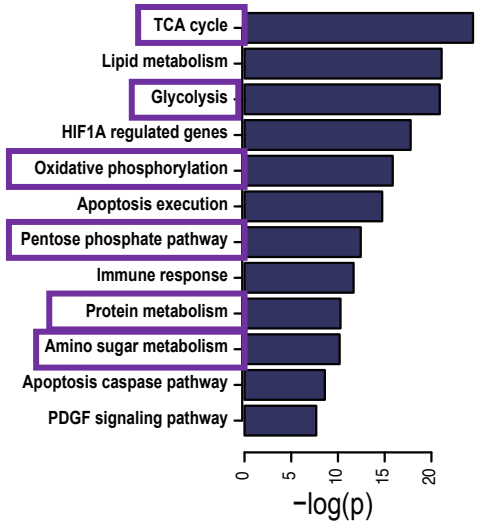


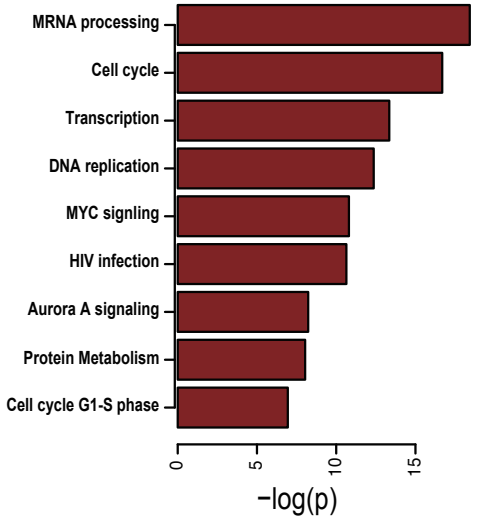
DEG analysis and Pathway Enrichment for the scRNA-seq data





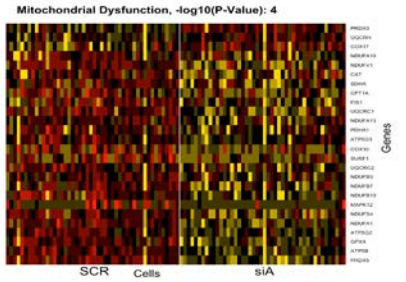
Pathway Enrichment

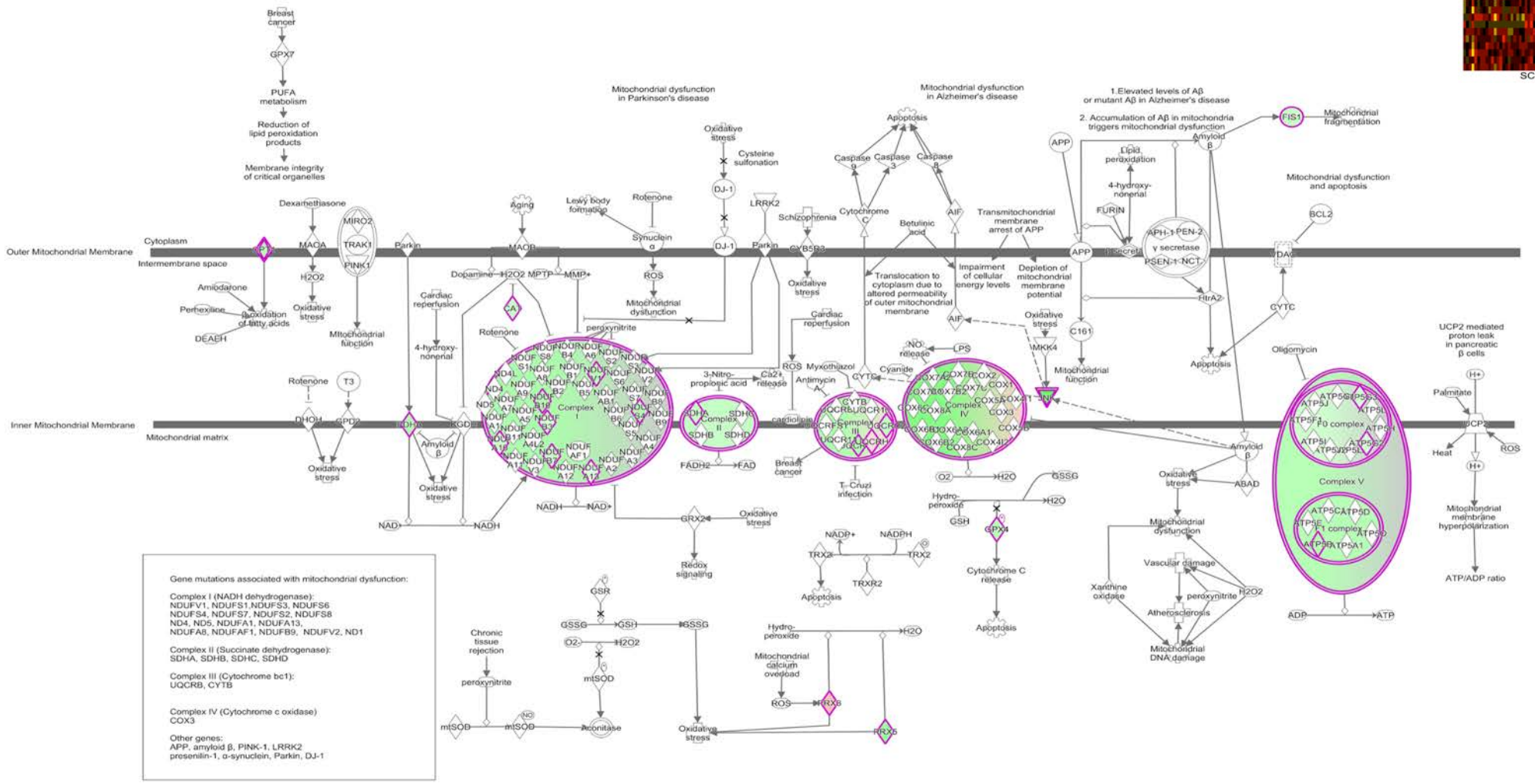




normal O_2

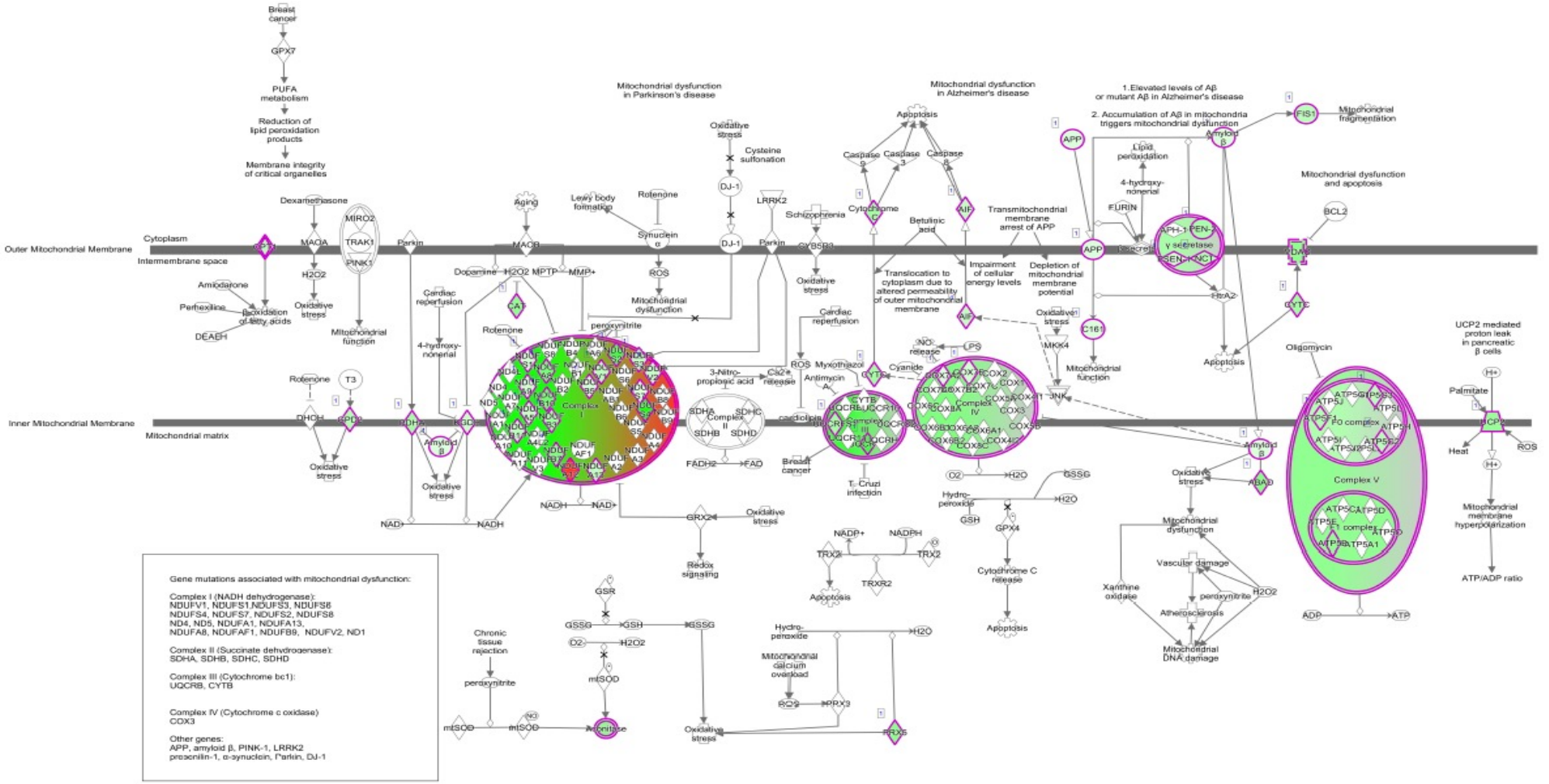
Mitochondrial dysfunction





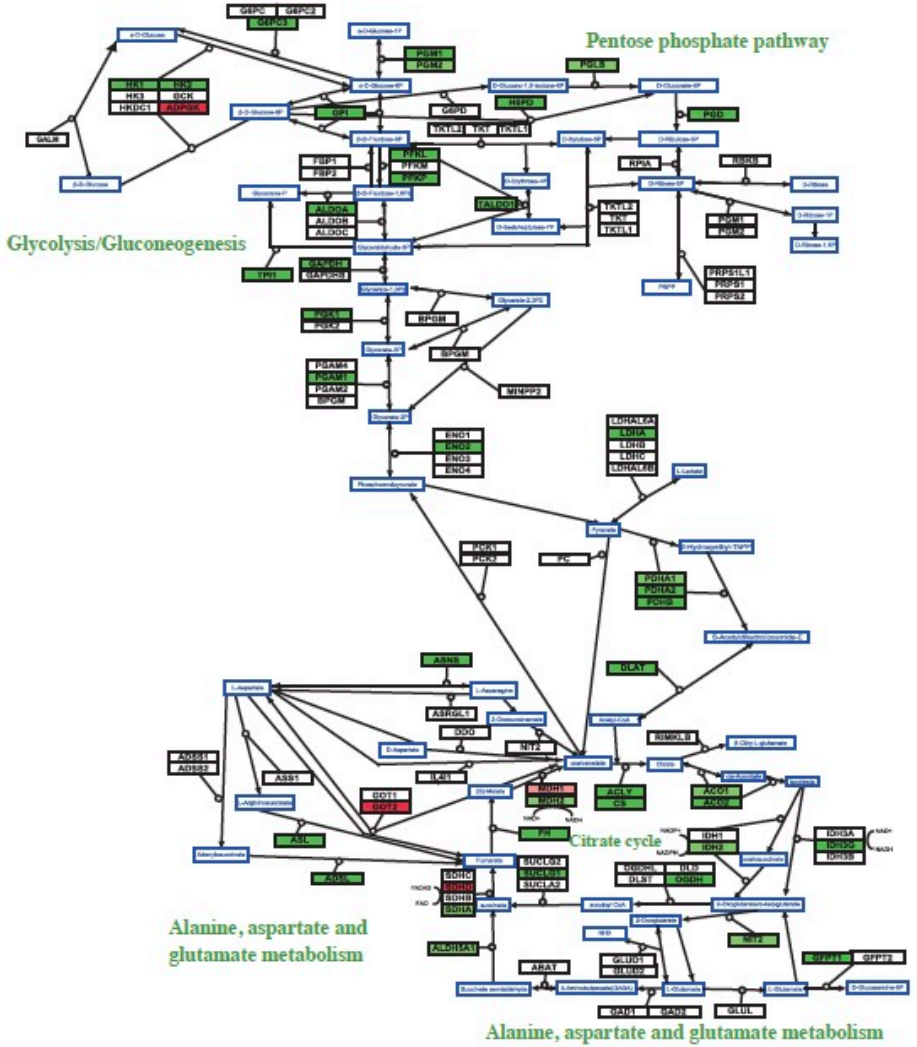
1% O_2

Mitochondrial dysfunction

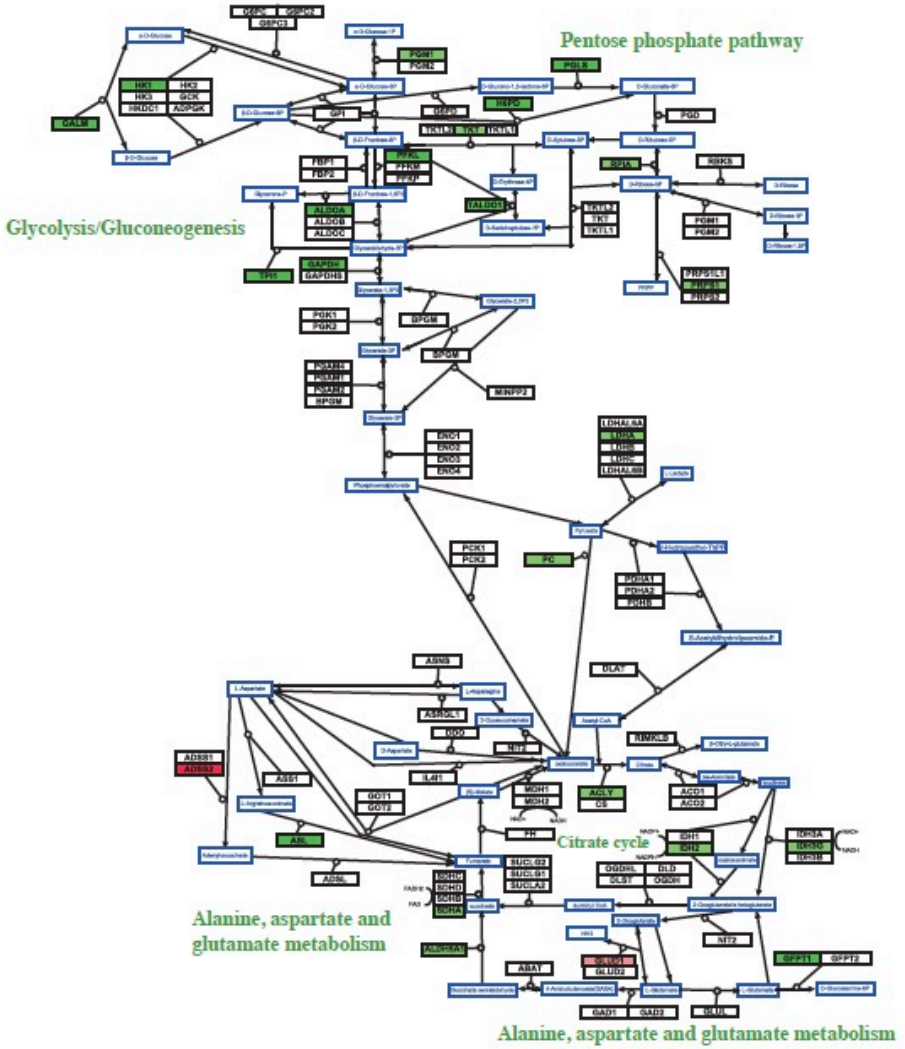


Ref-1 inhibition downregulates mitochondrial complex genes as well as 819 Ref-1 PD marker genes

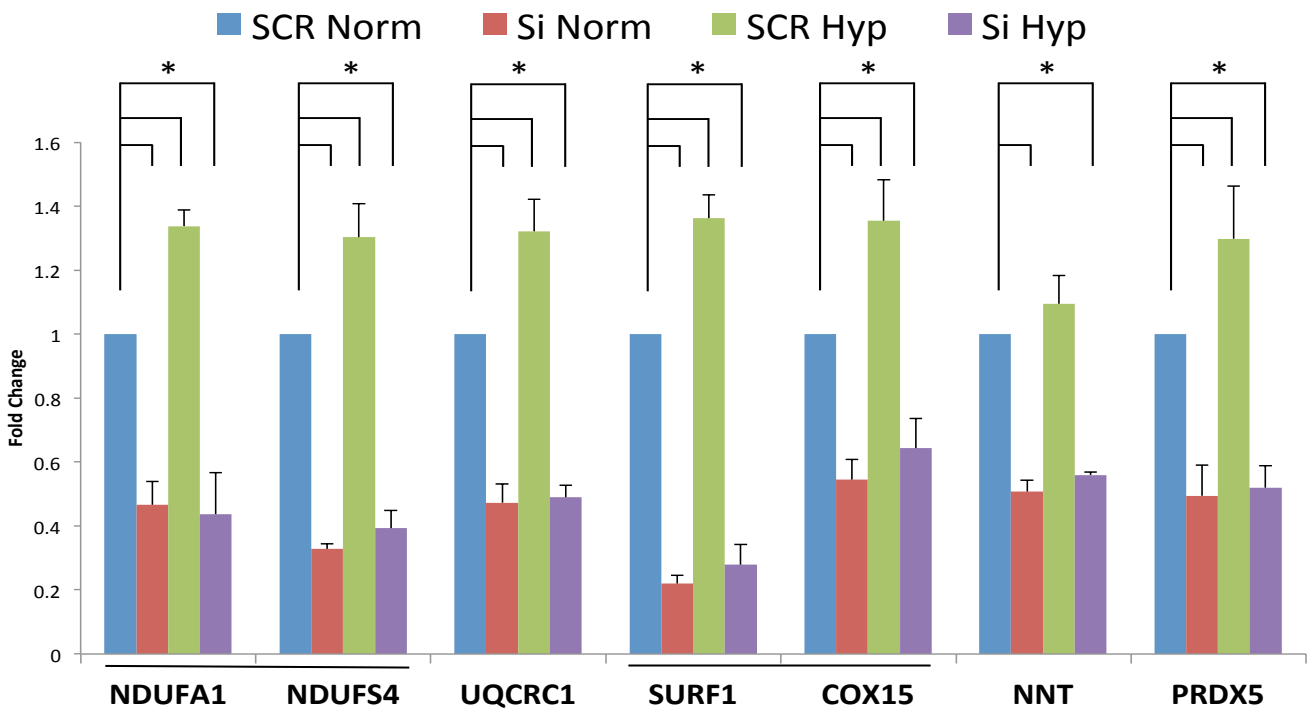
Hypoxia



Normoxia



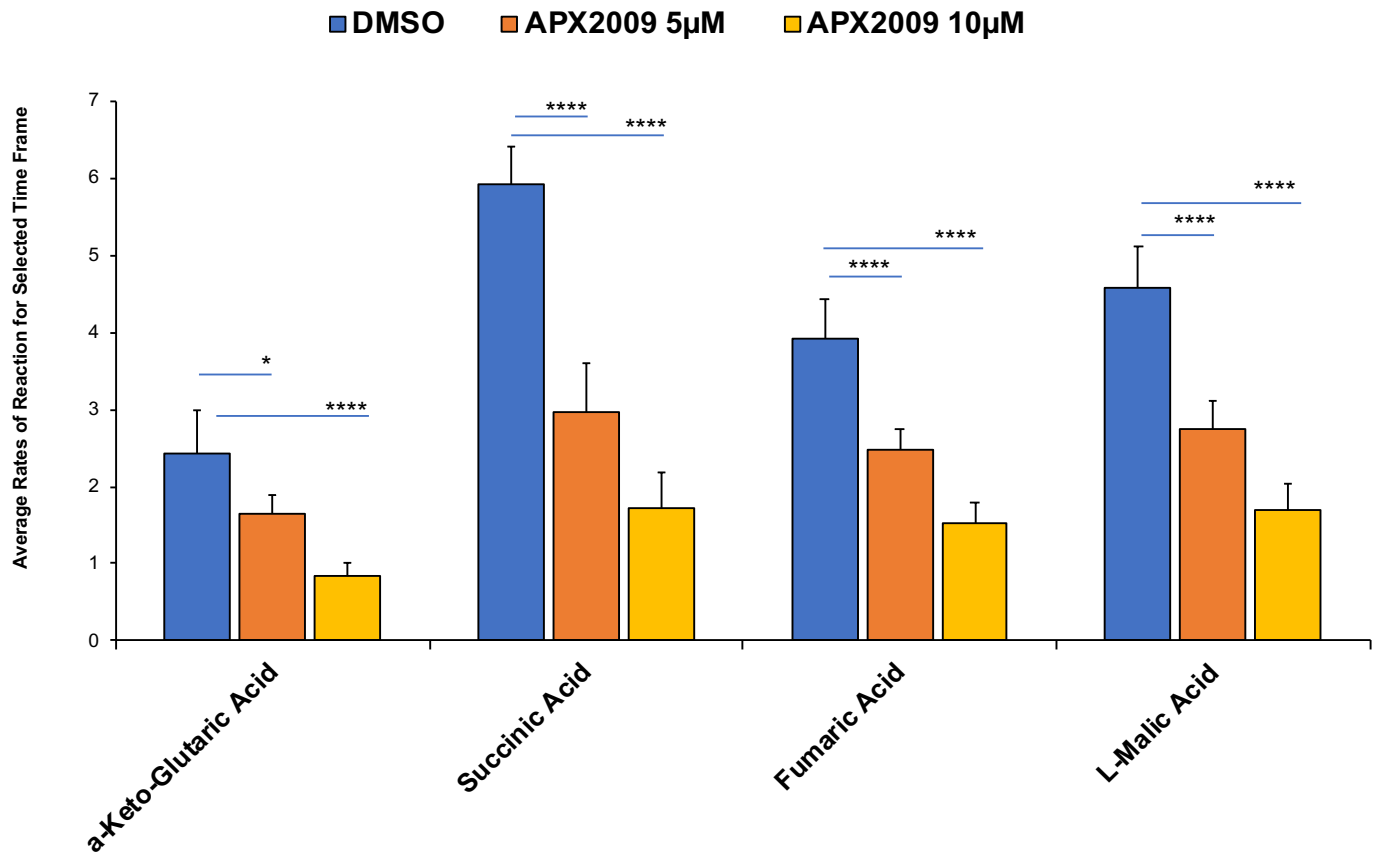
Metabolic genes implicated in APE1 signaling following hypoxia using single cell RNA-seq



Complex 1 Complex 3 Complex 4 Membrane Mito Redox

Functional effects of APE1 redox inhibition on tumor cell growth and metabolism

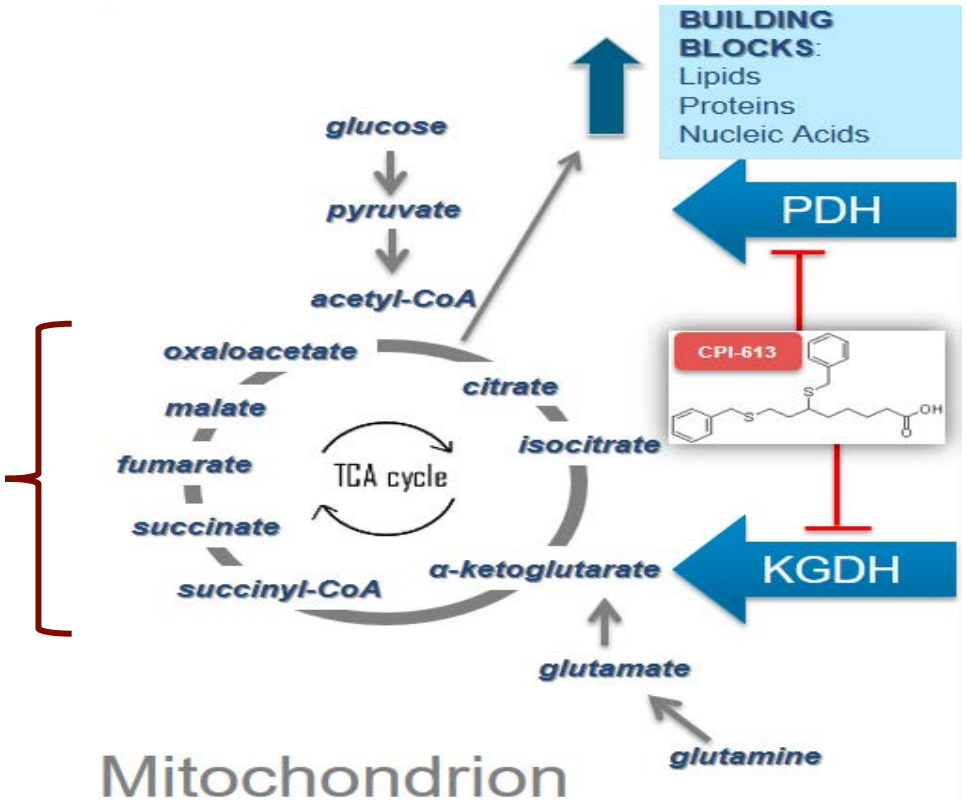
Pa03C
2009



Symbol	Meaning
ns	P > 0.1234
*	P ≤ 0.0332
**	P ≤ 0.0021
***	P ≤ 0.0002
****	P ≤ 0.0001

CPI-613 (Devimistat) : metabolic inhibitor in Phase 2 clinical trials.

APX compounds

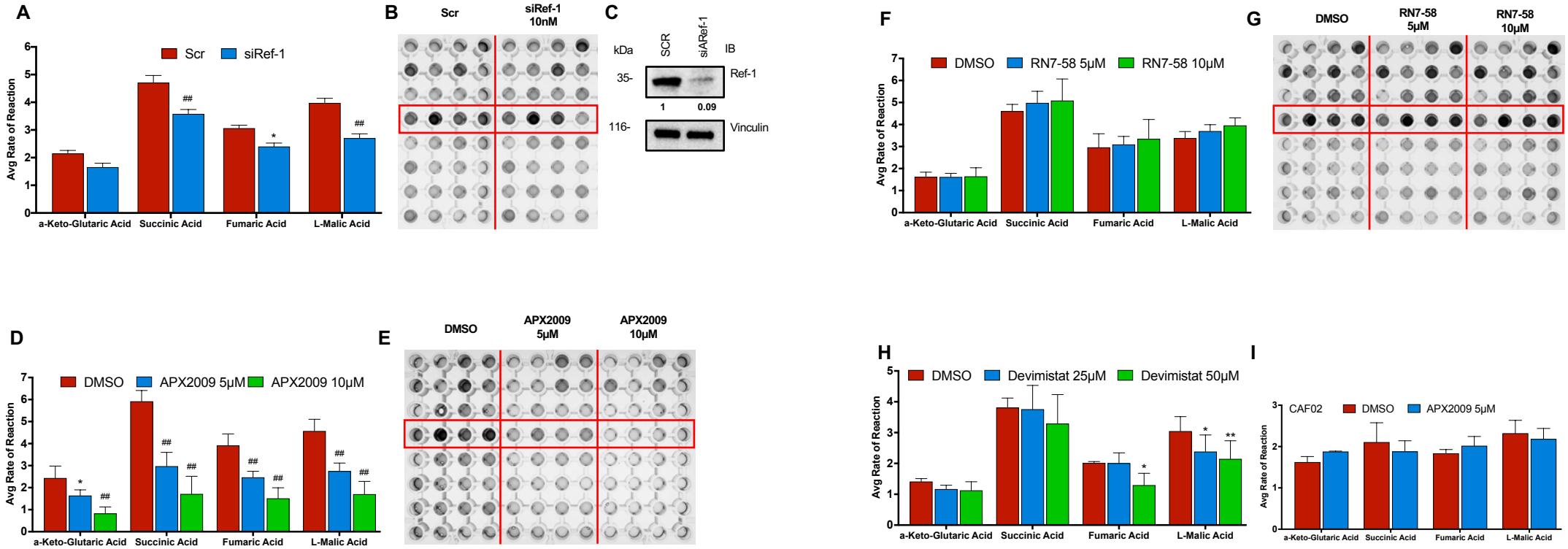


CPI-613 - Devimistat

- **Inhibits PDH** by causing hyper-activation of its regulatory kinases (extensive activation of PDK phosphorylation of the PDH E1)
- **Inhibits α-ketoglutarate dehydrogenase (a-KGDH)** downstream of Glutaminase by causing a hyper-activation of its redox autoregulatory/feedback circuitry

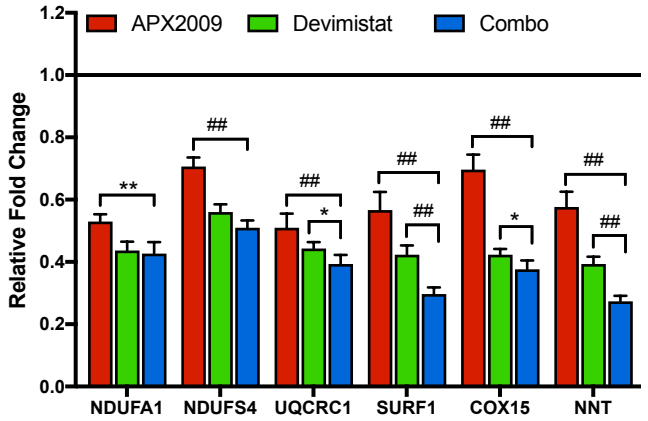
Ref-1 genetic or pharmacological inhibition reduces TCA cycle substrates

Mitochondrial functional assay



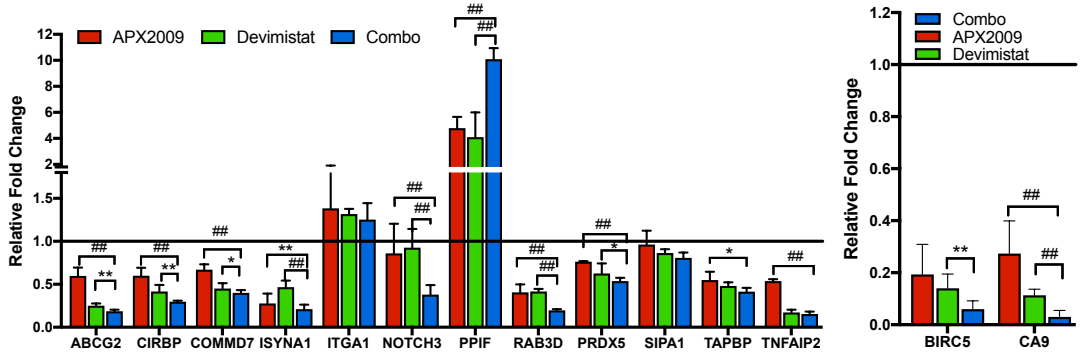
Ref-1 inhibition in combination with Devimistat shifts metabolism significantly compared to single agents.

A



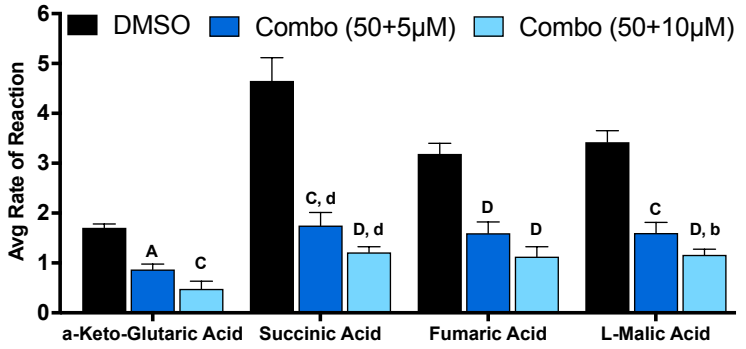
qPCR for mitochondrial genes

B

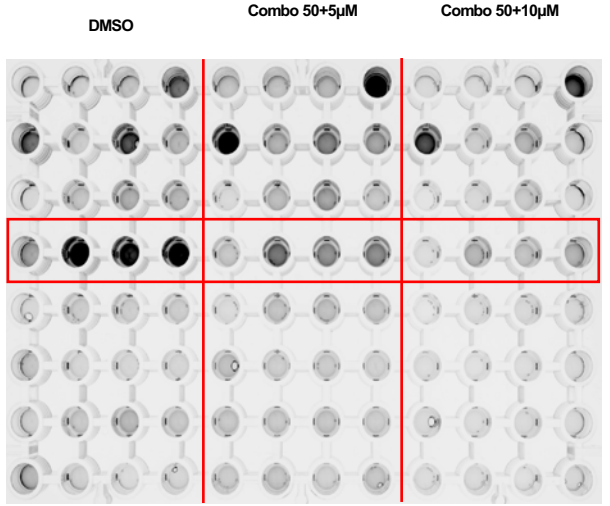


qPCR for Ref-1 regulated genes

C

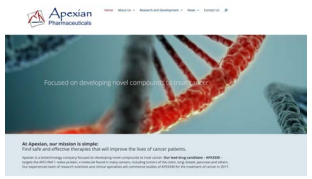


D



Trajectory of APE1/Ref-1 studies into the Clinic…

Trajectory of APE1/Ref-1 studies into the Clinic…



Market crash; recession begins

April 2008: ApeX formed based on APE1/Ref-1 assets.

Sept 2009 Received **Phase 1 STTR**

July 2013 Received **Phase 1 SBIR**

2009: Funded by Indiana VC Group

2010: Private Investors

Oct 2012 Private Investors & BioCrossroads

Series A

ApeX

1989 1993 1995 2000 2005 2008 2010 2012 2013

Kelley Lab & Collaborators

Kelley develops specific antibody for APE1

First antibody sold By Novus Biological

Kelley joins Dept of Pediatrics and IUSM

Publishes APE1 expression level data for normal & malignant tissue

Shows specific inhibition of TF activation of APE1 by 3330

Work initiated to discover & develop novel, potent APE1 inhibitor

First grant studying APE1 in sensory neurons funded: **Vasko**

Efficacy of APE1 inhibitor vs panc and other cancers

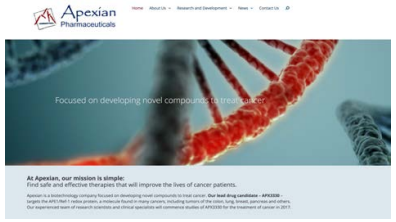
First **in vivo eye** study with APX3330 and APE1 as target published

Biochemical mechanism of APE1 inhibitors described

NCI grant on APE1 in pancreatic cancer Funded with **Melissa Fishel**

BIG GRE 2021

Trajectory of APE1/Ref-1 studies into the Clinic…continued…….



Apexian Pharmaceuticals

Kelley Lab & Collaborators

2015 - Additional funding through various individual

2015: ApeX renamed Apexian Pharmaceuticals

2015: Rich Messmann joins as CMO

IND accepted by FDA

2017: Anti-CIPN indication accepted by FDA for inclusion into current IND

Phase I trial opens

Phase I trial completed

IND DME/DR indication accepted by FDA

Licensing agreement with Ocuphire for eye indication

Ph II trial in DR/DME opens

2014 2015 2016 2017 2018 2019 2020 2021 2022

Various new indications identified using APX3330

Patents granted for numerous uses of APX3330 and new COM

NCI Provocative Question grant on CIPN funded with **Jill Fehrenbacher, Millie Georgiadis**

APE1 pancreatic cancer grant renewed with **Melissa Fishel**

Additional patents submitted and granted for various non-cancer and cancer indications

Collaboration with **Reuben Kapur** in leukemia models- published in Cell Stem Cell

Collaboration with **Tim Corson on eye** studies continues – 2nd gen cpds; JPET publication.

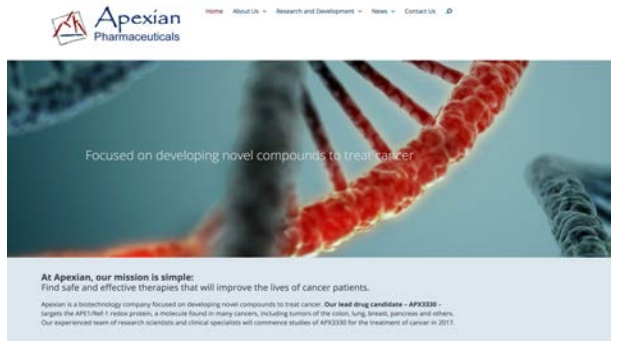
Collaboration with Kulmira Nurgali (Australia) on **IBD** indications and models.

https://www.ocuphire.com/

A phase I study targeting the APE1/Ref-1 DNA repair-redox signaling protein with the APX3330 inhibitor

Mark R. Kelley[1,4,5], Safi Shahda[5], Nehal J. Lakhani[2], Bert O'Neil[5], Lincy Chu[3], Amanda K. Anderson[3], Jun Wan[5], Amber L Mosley[5], Hao Liu[5], Richard A. Messmann[4]

[1]Wells Center for Pediatric Research
[5]Indiana University Simon Cancer Center
[2]START-Midwest, Grand Rapids, MI
[3]Epic Sciences, Inc., San Diego, CA
[4]Apexian Pharmaceuticals, Indianapolis, IN



HERMAN B WELLS CENTER FOR PEDIATRIC RESEARCH
INDIANA UNIVERSITY
Department of Pediatrics

INDIANA UNIVERSITY
MELVIN AND BREN SIMON COMPREHENSIVE CANCER CENTER

NCI Comprehensive Cancer Center
A Cancer Center Designated by the National Cancer Institute

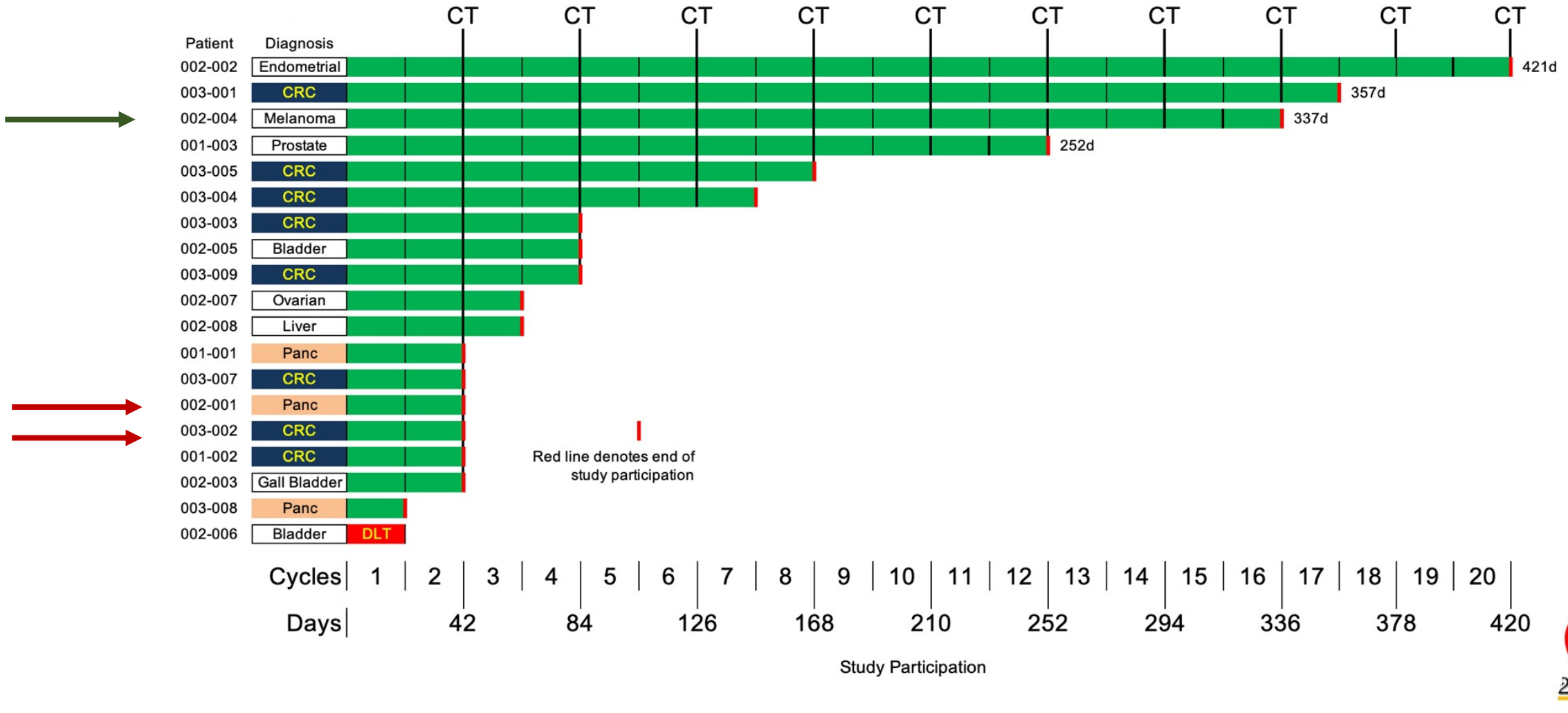
BIG GR 2021

Completed Successful Phase 1 Oncology Study

APX3330:

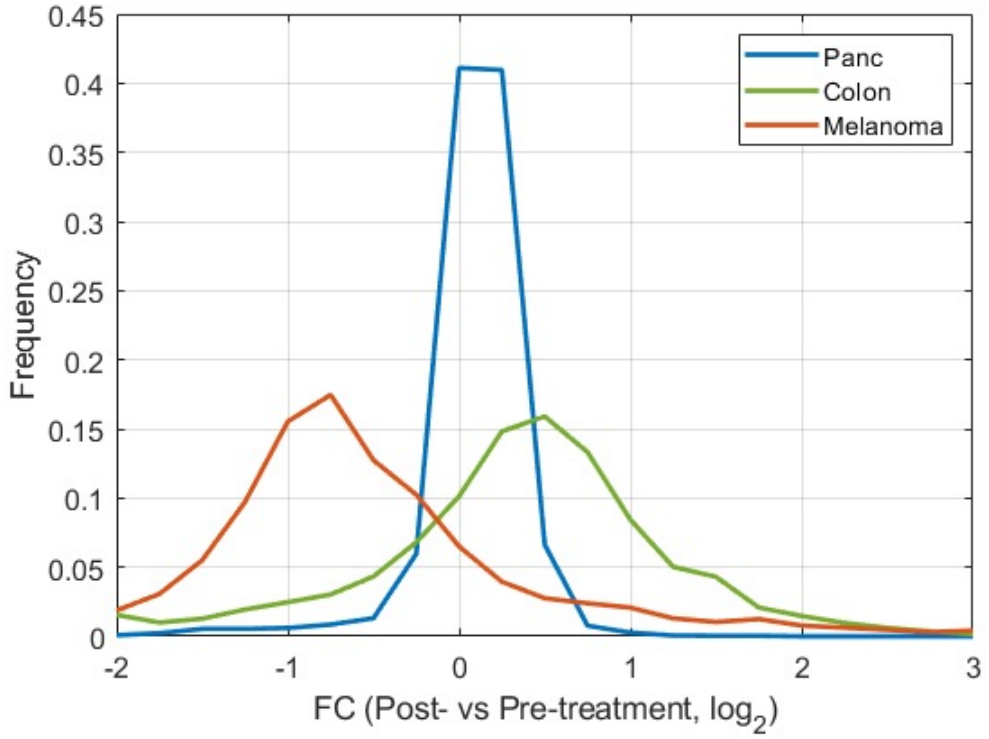
- Was well tolerated at dose levels from 240-600 mg/d
- Is safe for chronic **oral** dosing at 600 mg/d
- Patients on drug for extended period of time:
    - **Six** subjects had disease stabilization for **> 4 cycles**, and of these, **four** subjects with the following diagnosis, RECIST response and days on study included: (CRC, PR, **357d**), (Endometrial, SD, **421d**), (Melanoma, SD, **337d**), (Prostate, SD, **252d**).
    - The most frequent treatment-related adverse events (all grades) included G1 nausea (16%) and fatigue (16%). A G3 rash occurred in two subjects at the 720 mg level defining 600 mg/d as the RP2D for further development
- Provides clinical benefit to patients with a variety of tumor types - **30% Response Rate**
- Patient biopsy evaluation indicates APX3330-mediated effect upon cancer cells, including **decrease in transcription factor activity regulated by the APE1/Ref-1 protein**
- Circulating tumor cell analysis indicates APX3330-mediated decrease in tumor cells
- All results consistently show that APX3330 mediates activity of APE1/Ref-1 target as expected.

Approximately 30% Response Rate



Overall protein expression of genes downstream of APE1 are decreased in SD patient vs. PD patients

Paired biopsy analysis of pre-treatment and while on-treatment: Melanoma patient with disease stabilization > 1 year (green arrow) with lower APE1-regulated protein expression than patients with mPCa and mCRC.
FCs in the scale of $\log_2$.



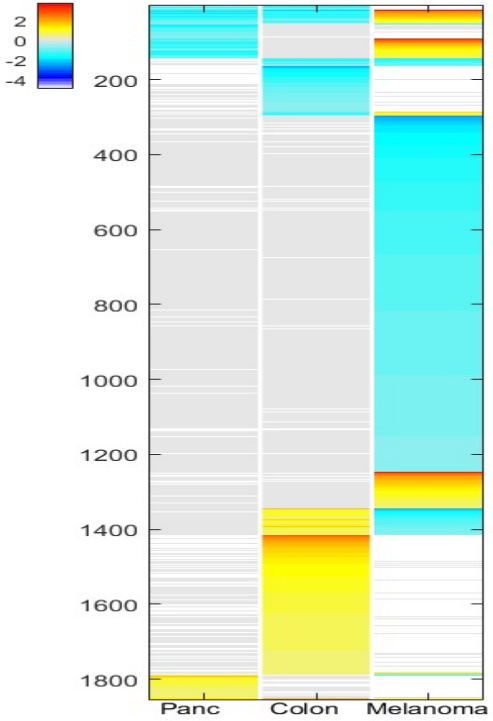
Heatmap of differentially-expressed proteins (DEPs) obtained by comparing pre-treatment and on-treatment tumor biopsies from 3 patients receiving APX3330.

Patient diagnoses:

Panc
Colon
Melanoma

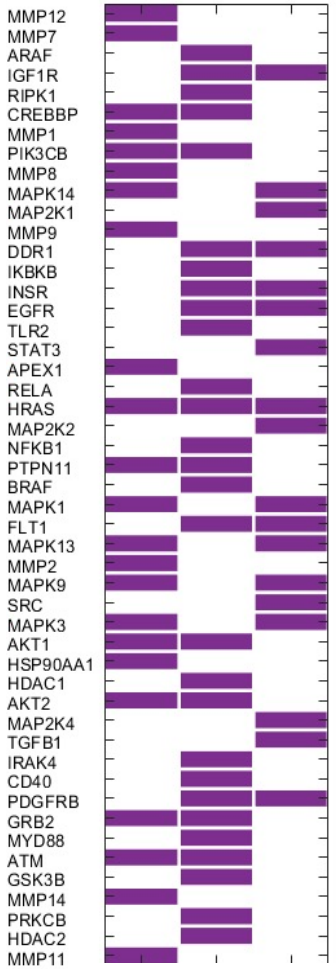
DEPs with <1.5-fold change (FC) are shown as grey bars.

White bars represent proteins not detected.



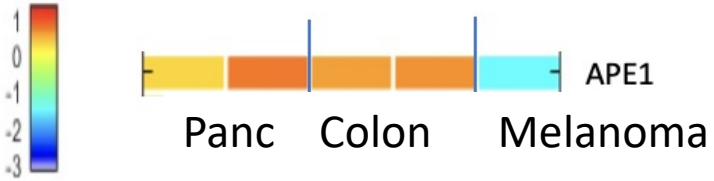
Confirmed target engagement: Proteins altered downstream of APE1 regulated transcription factors

Proteins altered downstream of APE1/Ref-1 regulated transcription factors HIF1α, NFκB and STAT3 by APX3330 in melanoma patient



HIF1 NFkB STAT3

Heatmap of APE1 protein levels which were reduced following APX3330 treatment in the melanoma patient with SD > 1 year.

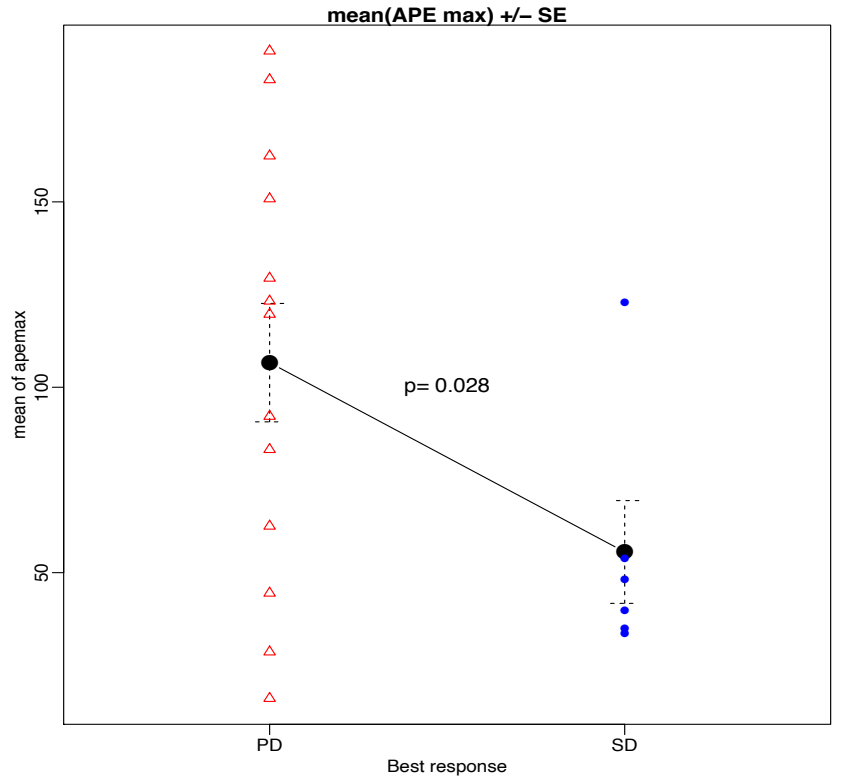


APE1 serum levels decreased in SD vs PD patients

APE1 serum levels were determined using a standard ELISA assay.

Statistical comparisons were done between two groups (patients with SD vs PD) using two-sample t-test. p-value 0.028 is statistically significant.

SD patients are defined as those on treatment past 4 cycles.



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- Richard A. Messmann – CMO, Apexian Pharmaceuticals
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- Bert O'Neil - IU Cancer Center (now at Eli Lilly)
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- Amanda K. Anderson – Epic Sciences
- **Jun Wan** – Medical Genetics, Bioinformatics, IUSM
- **Amber L Mosley** – Biochemistry and Molecular Biology, Director Proteomics Core
- **Hao Liu** – Biostatistics, IUSM
- Randy Wireman – Research Analyst, IUSM
- **Robert Stratford** – IUSM

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Disclosure:

- Subcontract funding from Apexian Pharmaceuticals.
- Chief Scientific Founder and CSO of Apexian Pharmaceuticals.

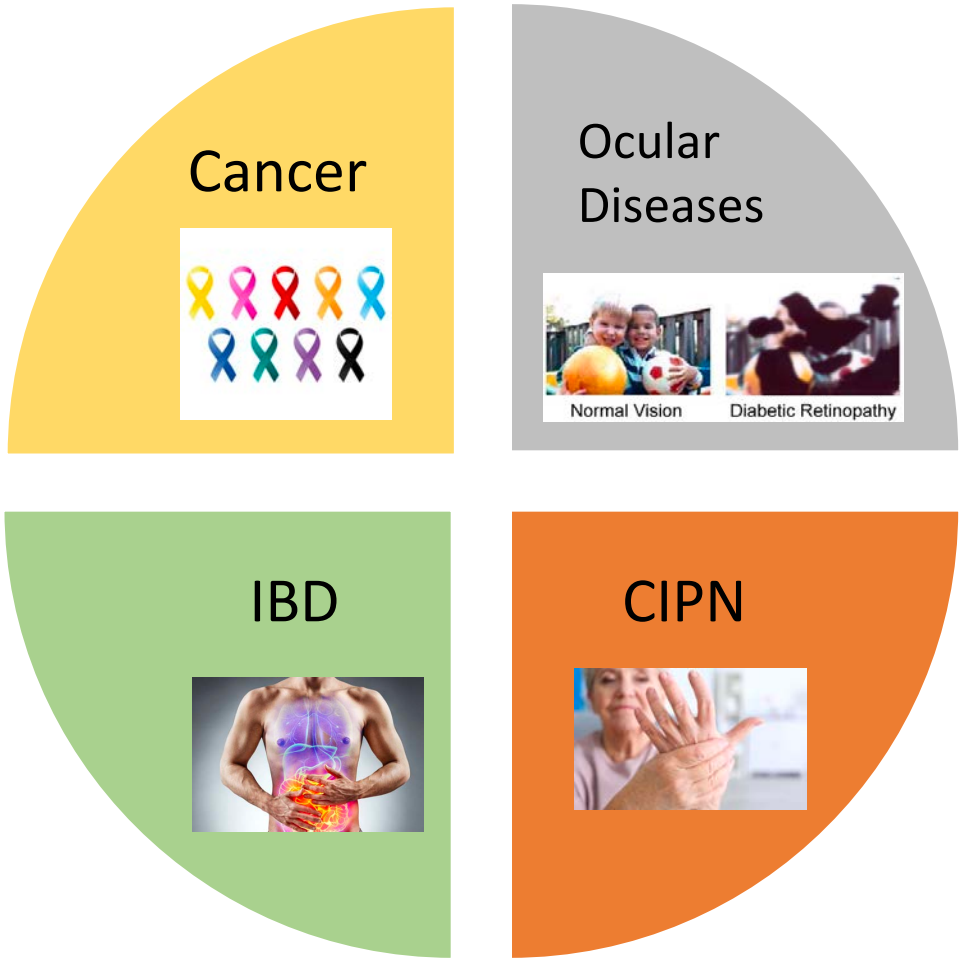
One target: Multiple Indications

Substantiated by

- APE1 protein overexpression in multiple tumors
- APX inhibition of APE1 redox signaling decreases tumor growth
- Inhibition of APE1 decreases activity of downstream transcription factors
- APX Phase I clinical trial results confirm tumor growth suppression, molecular target engagement and exceptional tolerability
- Pipeline of anticancer agents

Substantiated by

- APX blocks inflammatory process
- Minimizes weight loss, reduces rectal prolapse, edema and bleeding.
- Corrects colonic contractility and intestinal permeability, protecting colonic nerve fibers and glial cells Protects against DNA damage in myenteric neurons
- In vivo activity in animal models of IBD.



Substantiated by

- APE1 protein is a molecular target in retinal diseases
- Dual MOA of APX compounds decreases both abnormal angiogenesis and inflammation
- In vivo POC animal models demonstrate efficacy given systemically and locally
- Proven target engagement of inhibitors
- Confirmed retinal exposure

Substantiated by

- APE1 inhibition prevents CIPN in animal models
- Inhibitors protect neurons from oxidative DNA damage and inflammation caused by chemo agents

Retinal Diseases: Diabetic Retinopathey (DR), Diabetic Macular Edema (DME), Age-related Macular Degeneration (AMD); IBD: Inflammatory Bowel Disease (Crohn's/Colitis); Chemotherapy Induced Peripheral Neuropathy (CIPN)

Today, I am going to talk about:

- A little science
- How big data/bioinformatics has led to new avenues of research
- Pathway from bench to clinical trial
- **New directions based on data analysis, target and drug development**

Diabetic Retinopathy & Macular Edema

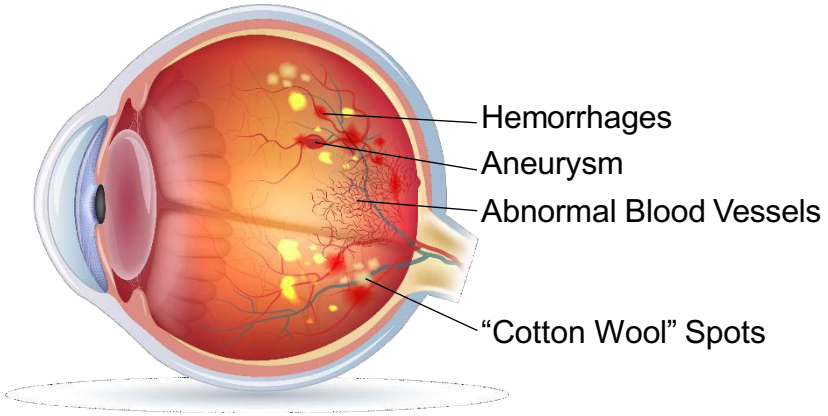
Non-Injectable Alternative Therapies are Needed For Earlier Stages of Disease

The Problem

- Diabetic retinopathy (DR) and diabetic macular edema (DME) are a leading cause of vision loss worldwide, especially in working age adults in developed countries
- Diabetes damages small blood vessels within the eye causing leakage, oxygen starvation, and abnormal vessel growth, which can obstruct vision
- DR patients are not commonly treated with approved injectable anti-VEGF drugs given earlier stage of retinal disease and many are asymptomatic
- DR progresses in steps and may result in vision loss if left untreated
- Current treatment for DME: 25% non-responders and 50% partial responders to anti-VEGF drugs

Injectable Anti-VEGF Approved Therapies Not Commonly Used for NPDR

Diabetic Eye



Diabetic Eye Opportunity	
DR	~7.7M Patients
DME	~750K Patients

Sources: Global Market Insights Report 2019-2025, Market Watch 2019 Report, Gene.com Retinal Diseases Fact Sheet

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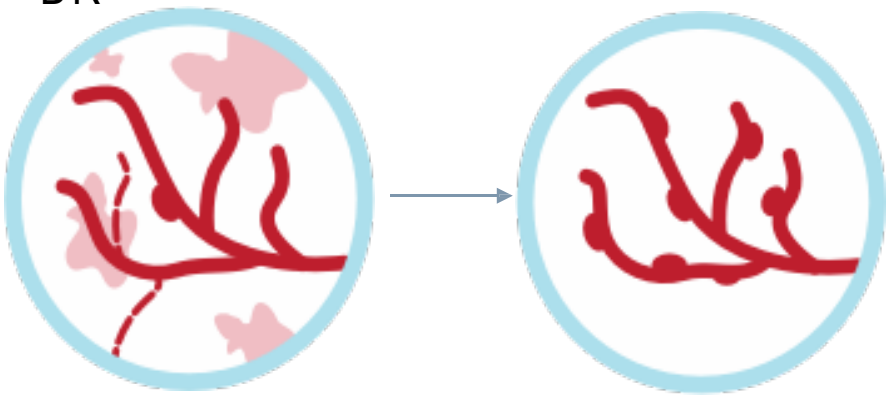
Diabetic Retinopathy & Macular Edema

APX3330 to Treat Patients Before Vision Loss Occurs

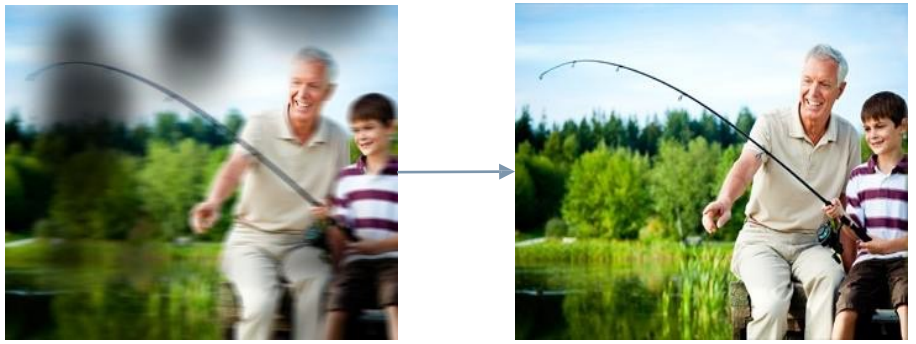
APX3330's Potential Differentiated Solution

- **Potential First Oral Therapy** to be used as an earlier intervention for the diabetic eye before vision symptoms appear or as add-on therapy to current anti-VEGF treatment
- **Proven Novel Mechanism** that may decrease both inflammation and VEGF activity
- **Convenient** option for patients to potentially alleviate the burden of injections and increase compliance
- **Tolerable** as seen in 11 completed Phase 1 and Phase 2 clinical trials

DR



DME



EYLEA Product Pamphlet

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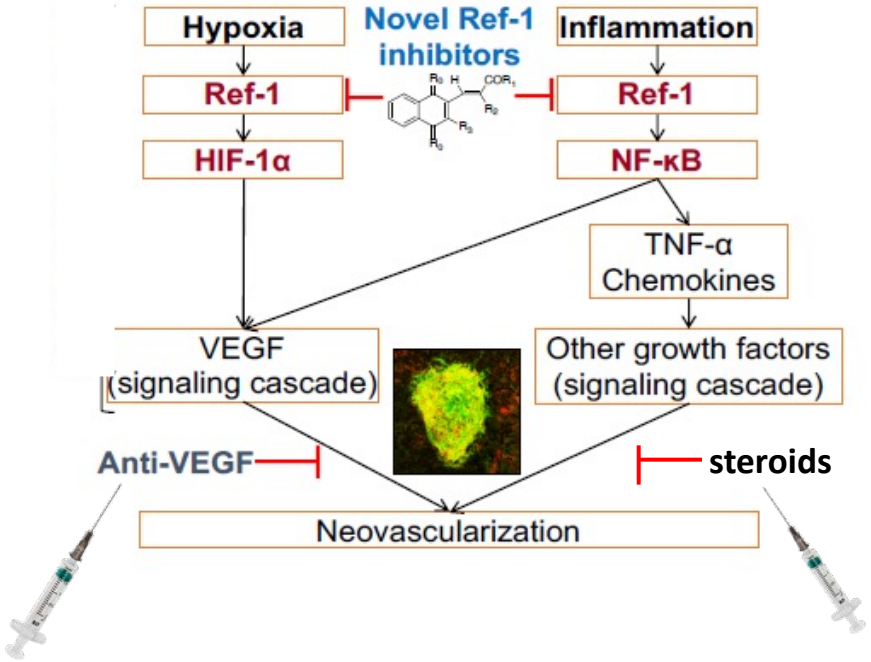
APX3330 Unique Dual Action MOA

Rationale for Potential Efficacy in Diabetic Retinopathy and DME

- APX3330 is a small molecule oral tablet drug candidate and a first-in-class inhibitor of the Ref-1 protein
- Ref-1 is a novel target in ocular disease
- Dual MOA uniquely decreases both abnormal angiogenesis and inflammation by blocking pathways downstream of Ref-1
  - Blocks HIF-1a to reduce VEGF signaling
  - Blocks NF-kB to modulate VEGF, TNF-α and other inflammatory cytokines



Avastin and similar compounds

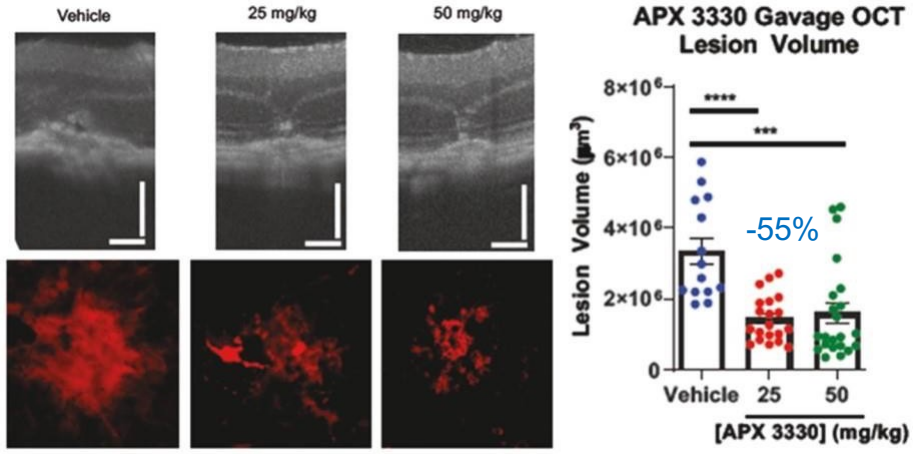
APX3330 Generally Well Tolerated with Clinical Signals

Observations from Pre-Clinical Studies and 11 Clinical Trials of APX3330

L-CNV Mouse Retina Model

APX3330 Reduces Neovascularization Similar to Eylea in Preclinical Models

Lesion Size and Corresponding Fluorescent Stains in L-CNV Models Treated with APX3330 at 25 mg/kg oral gavage

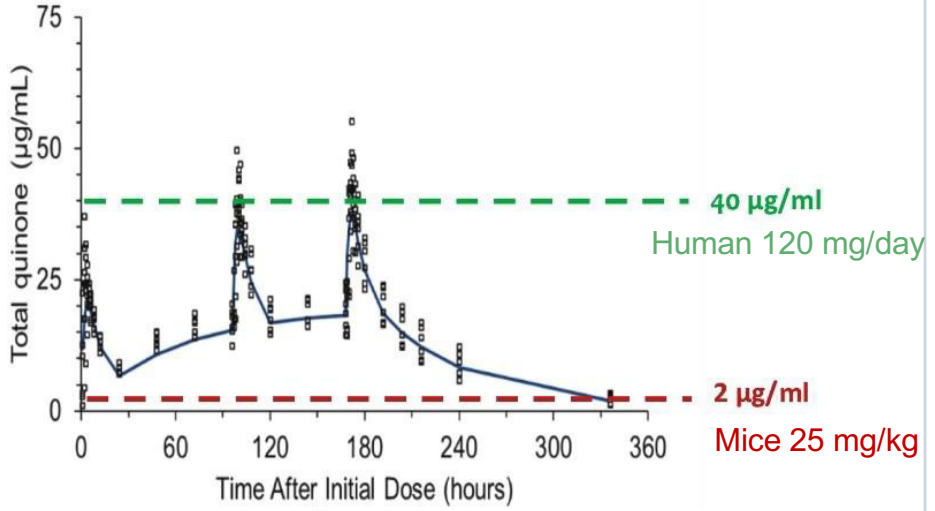


Source: Unpublished Data Dec 2019

Phase 1 Clinical Trials

Human Drug Exposure Multiple Times Higher than Mouse Efficacious Levels

Human Pharmacokinetics of APX3330 at 120 mg/day



Source: Unpublished Data Dec 2019

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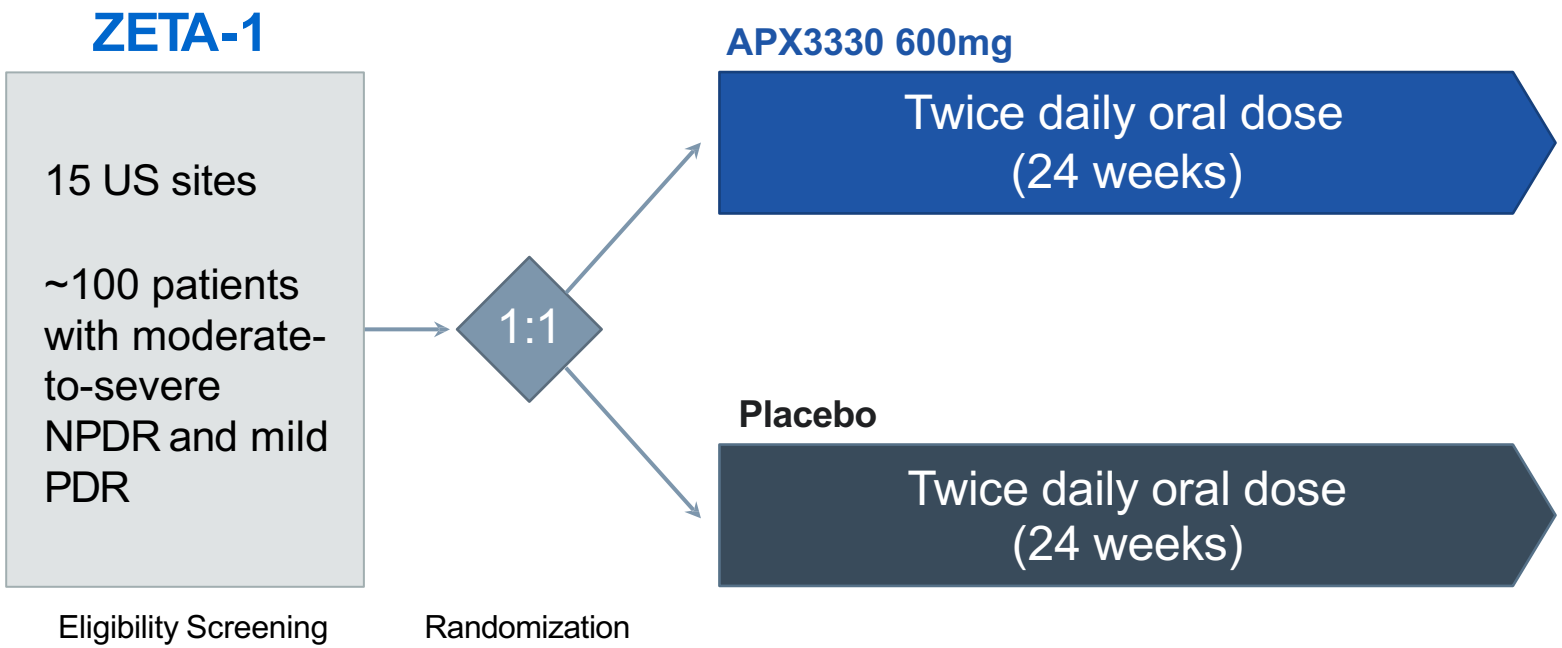
DR/DME ZETA-1 Phase 2 Proposed Design

Randomized, Double-Masked, Placebo-Controlled 24-Week Trial (Similar to Eylea's P3 for Approval)



Phase 2 Start April 2021

Top Line Expected Early 2022

Endpoints

Primary: % of subjects with a ≥ 2 step improvement on the DRSS (Diabetic Retinopathy Severity Scale) score at week 24

Secondary:

- Central subfield thickness (CST)
- BCDVA (ETDRS)
- Safety and tolerability

Exploratory:

- Labs / PK

NPDR = non-proliferative diabetic retinopathy (which includes non centrally involved diabetic macular edema)
PDR = proliferative diabetic retinopathy (which includes non centrally involved diabetic macular edema)

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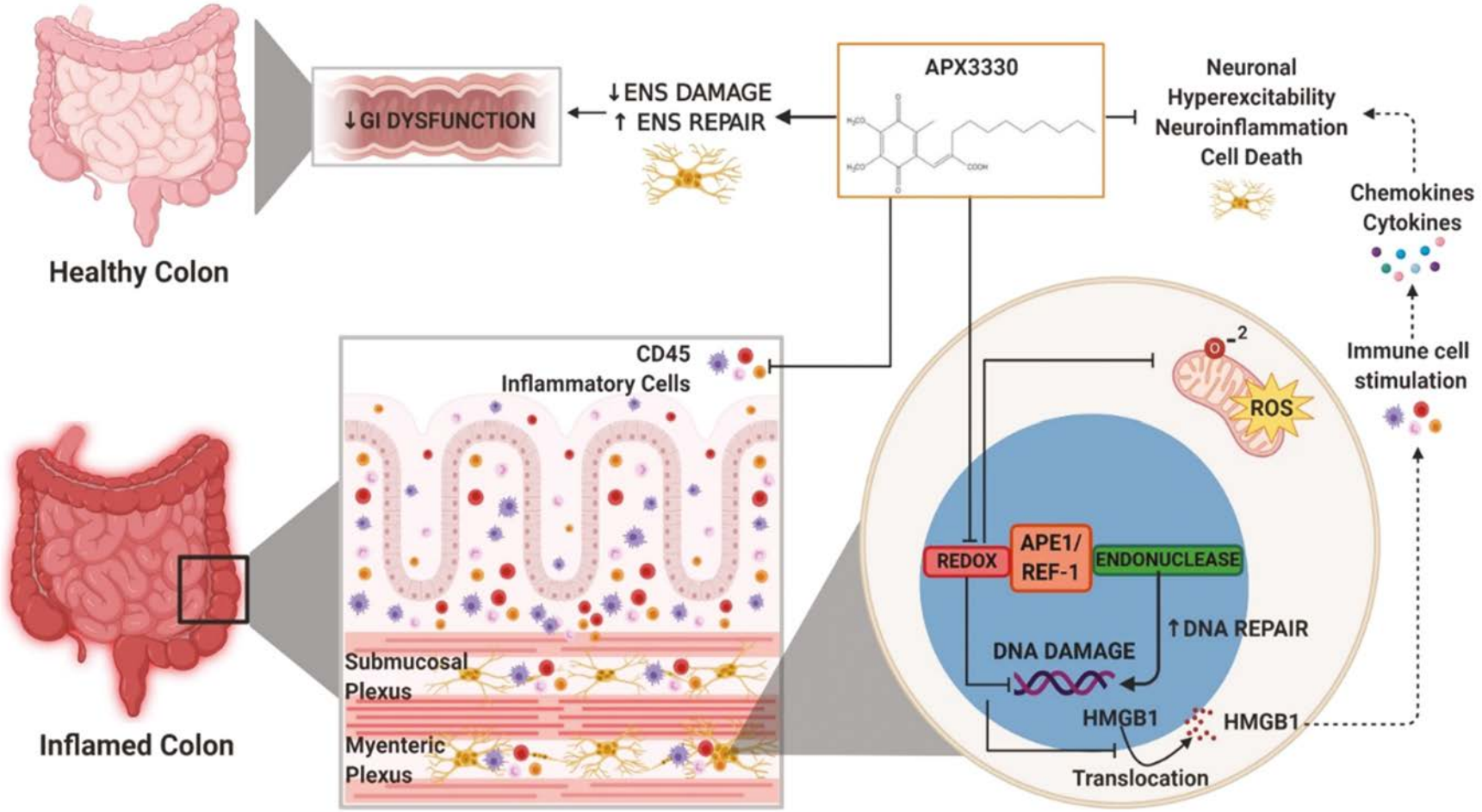
Inflammatory Bowel Disease (IBD)

- Inflammatory bowel disease (IBD) is chronic debilitating condition affecting more than **3 million people in US.**
- Current US market is **$22.4 Billion** (includes Crohn's and ulcerative colitis).
- Current treatments are **ineffective** long-term with serious side effects (immunosuppression, neutropenia and liver toxicity resulting in disease).
- Over time, IBD patients become less responsive to medical therapies and most of IBD patients undergo multiple surgeries during the course of the disease and have **high risk of cancer development**.
- IBD is the cause of 700,000 doctor visits, 100,000 hospitalizations and 119,000 patients who are disabled by the condition in the US.

APX3330 treatment in the animal model of chronic spontaneous colitis inhibits Ref-1 redox signaling, mitochondrial superoxide production, and oxidative DNA damage

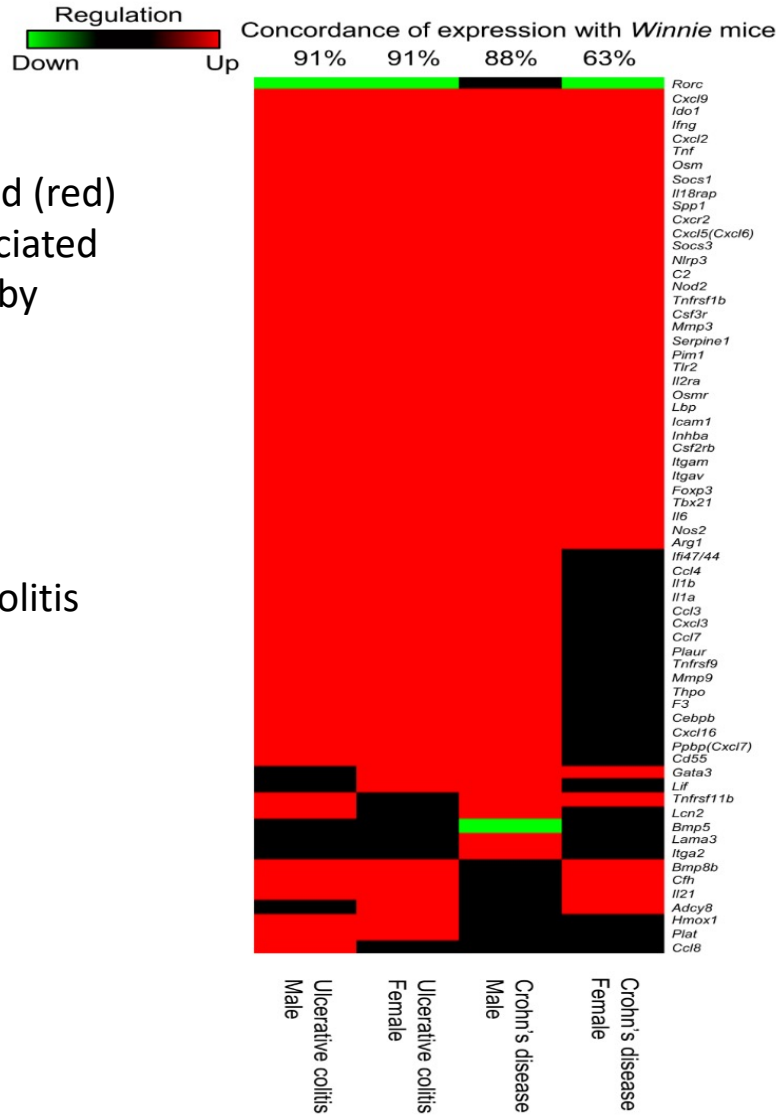


Inflamm Bowel Dis, izaa161, https://doi.org/10.1093/ibd/izaa161

Homology of genes in Winnie mice and IBD patients in colonic inflammation-associated genes is significant

Heat map representation of upregulated (red) and downregulated (green) genes associated with colonic inflammation determined by RNA-Seq.

Target genes of BM-MSC therapy in the *Winnie* mouse model of spontaneous colitis were identified.



The homology in expression of these genes was determined in *Winnie* mice and IBD patients compared to their respective uninflamed controls.

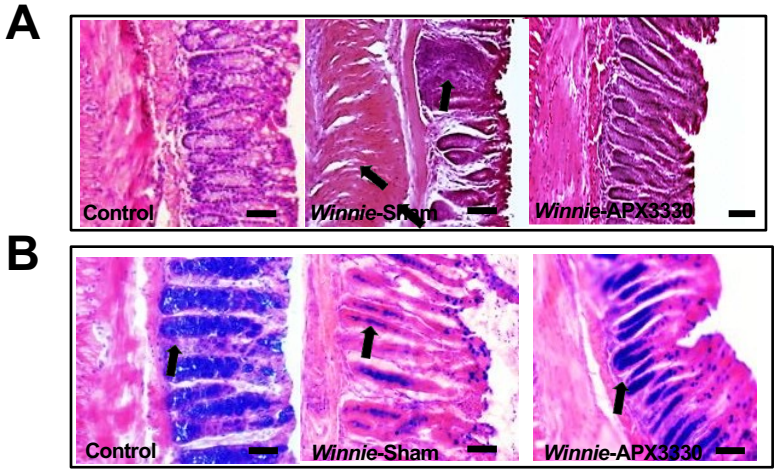
Genes failing the initial cut off between ±0.5logFC are represented as black (no change).

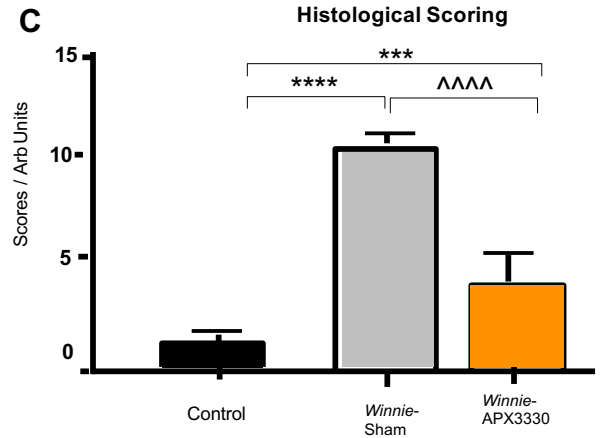
APX3330 corrects severity of inflammation in Winnie IBD mouse model

The Winnie mouse, carrying a missense mutation in Muc2, is a model for chronic intestinal inflammation demonstrating symptoms closely resembling inflammatory bowel disease (IBD).

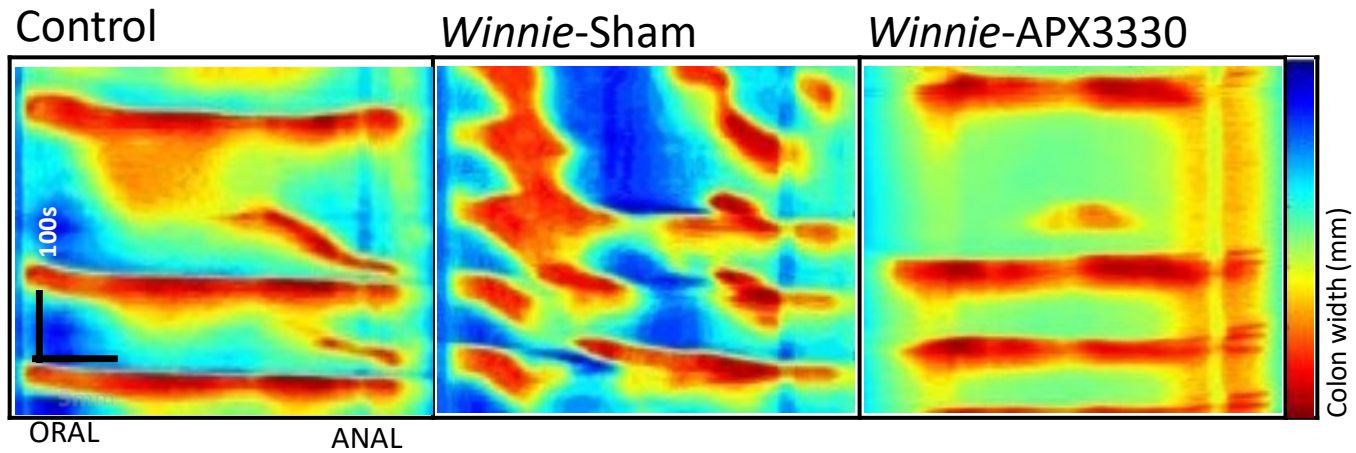
Severity of intestinal inflammation indicated by presence of rectal prolapse with blood vessel proliferation and oedema.

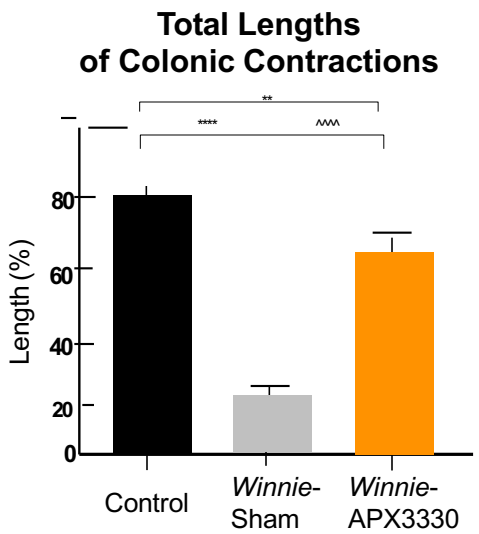
Images taken at day 14 of treatment in control, *Winnie*-Sham and *Winnie*-APX3330 treated mice.





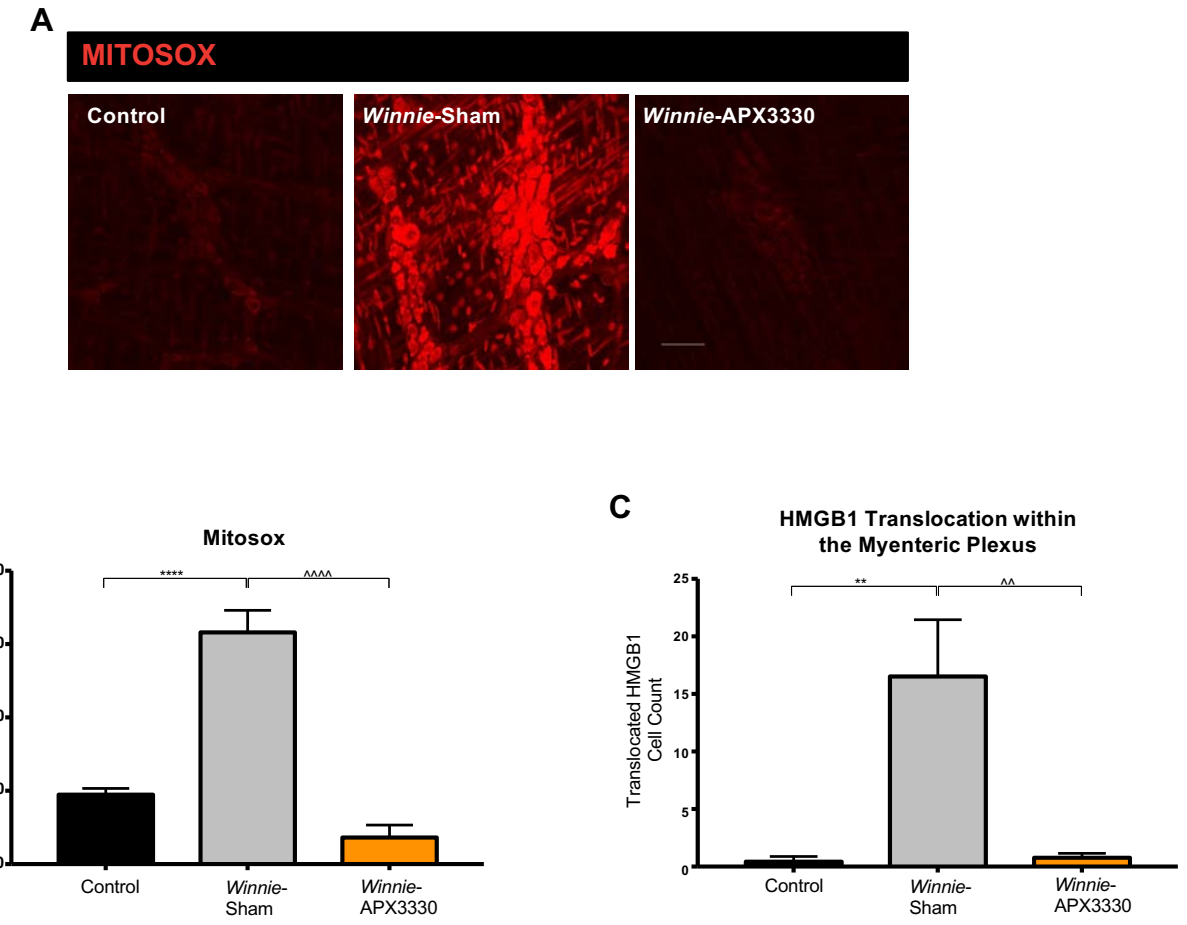
APX3330 corrects colonic contractile activity in IBD mice





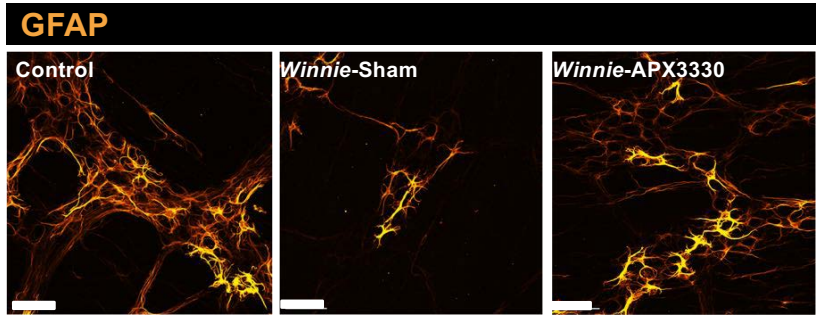
Sahakian L, Filippone R, Stavely R, Robinson A, Yan X, Abalo R, Eri R, Bornstein J, Kelley MR, Nurgali K. (2020) Inhibition of APE1/Ref-1 redox signalling alleviates intestinal dysfunction and enteric nervous system damage in a mouse model of chronic colitis, *Inflammatory Bowel Disease,*

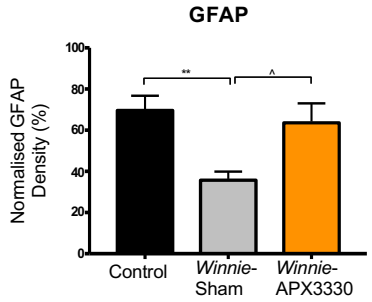
APX3330 reduces oxidative stress levels back to control levels in the Myenteric Plexus

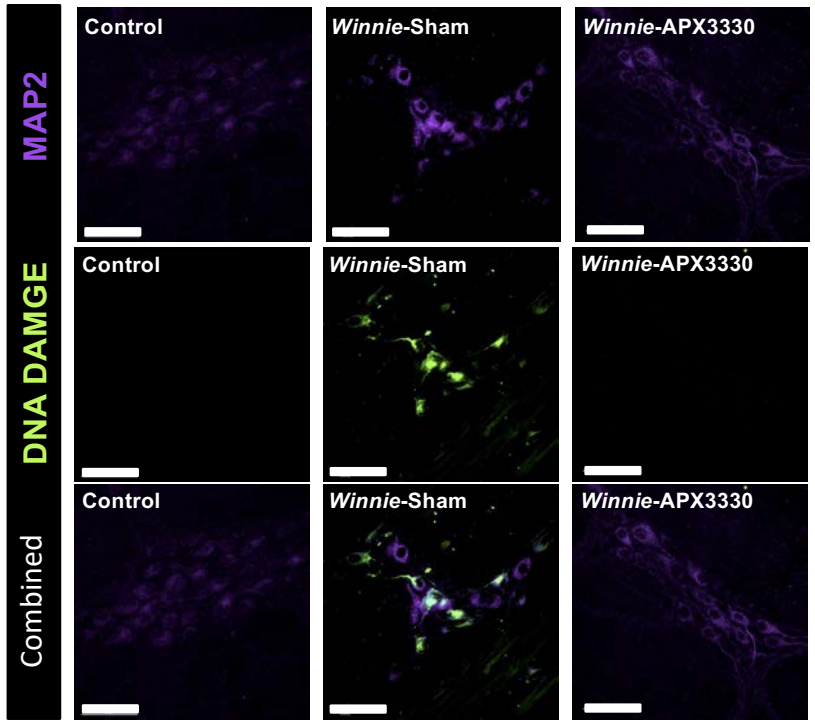


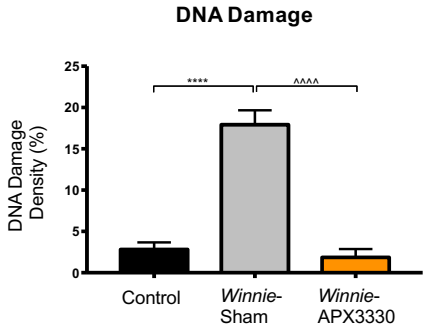
Sahakian L, Filippone R, Stavely R, Robinson A, Yan X, Abalo R, Eri R, Bornstein J, Kelley MR, Nurgali K. (2020) Inhibition of APE1/Ref-1 redox signalling alleviates intestinal dysfunction and enteric nervous system damage in a mouse model of chronic colitis, *Inflammatory Bowel Disease.*

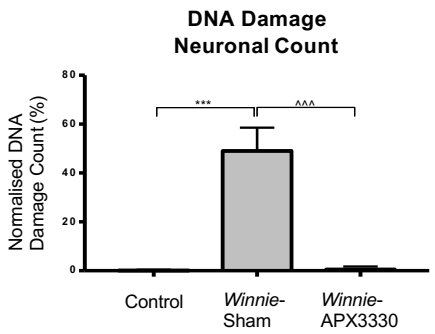
APX3330 protects myenteric neurons from DNA damage in IBD colon model







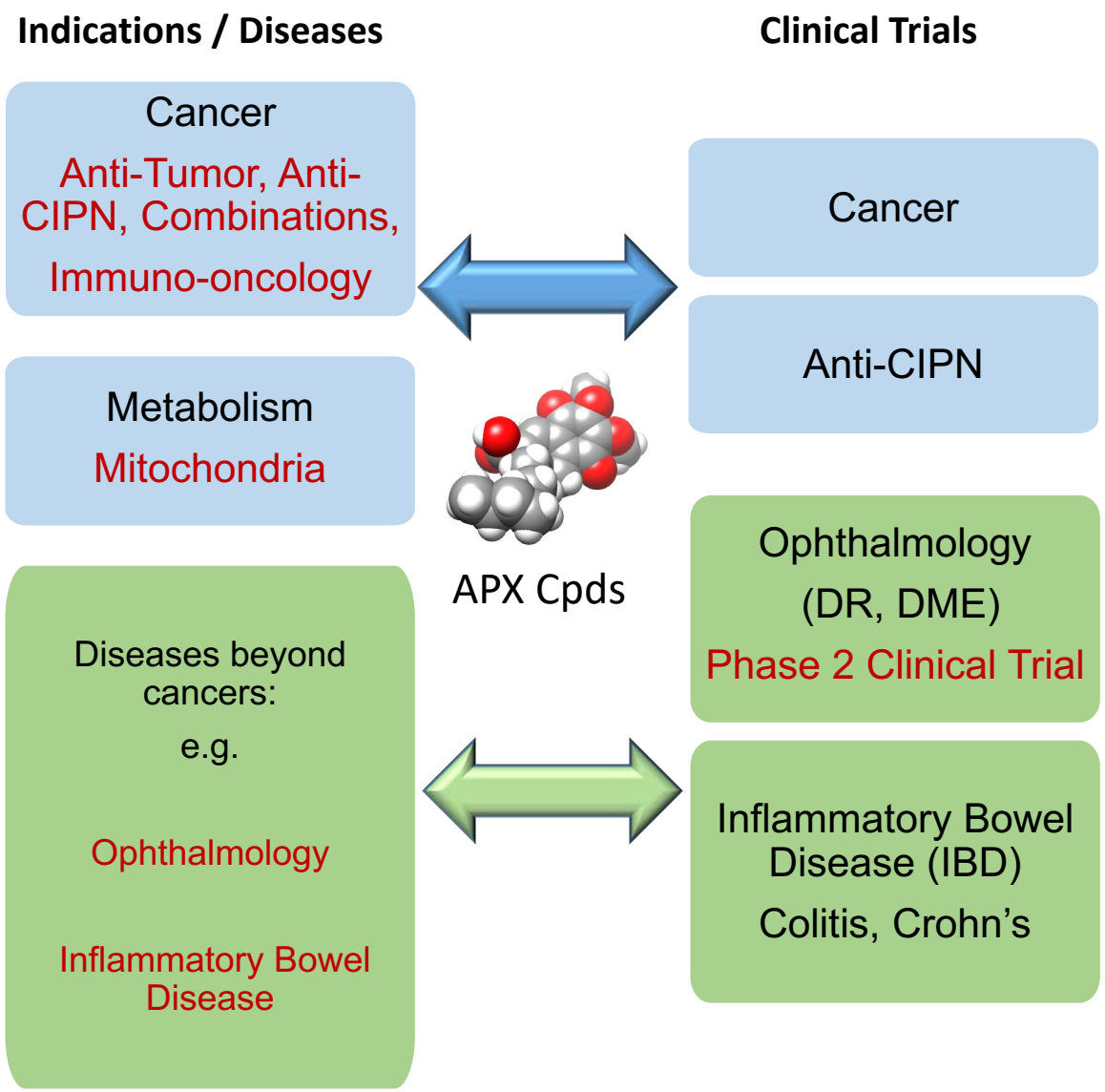




Similar APX neuronal protection observed in CIPN models:

Neural Regen Res. 2017 Jan;12(1):72-74.
J Pharmacol Exp Ther. 2016 Nov;359(2):300-309. Mutat Res. 2015 Sep;779:96-104.
PLoS One. 2014 Sep 4;9(9):e106485.

Future Directions for Inhibitors of Ref-1/APE1: Indications & Trials



Big Data Training for Cancer Research

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- Chief Scientific Founder and CSO of Apexian Pharmaceuticals.
- Ocuphire Medical Advisory Board member and consultant, Ocuphire Pharma

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    - **Olivia Babb**
    - **Dr. Silpa Gampala**
  - Dr. Millie Georgiadis (biochemistry)
  - Dr. Tim Corson (**eye**)
  - Dr. Jill Fehrenbacher (**CIPN**)
  - Dr. Kulmira Nurgali (Australia - **IBD**)
  - Dr. Travis Jerde (prostate/bladder)
  - Dr. Reuben Kapur (AML)
  - Dr. Karen Pollok (in vivo therapeutics)
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  - Dr. Amber Mosley (proteomics)
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