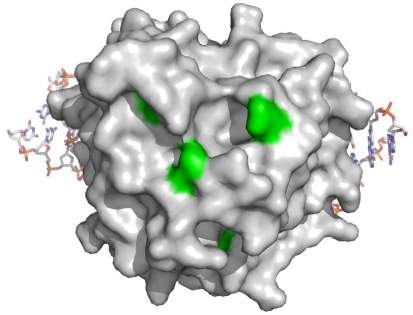
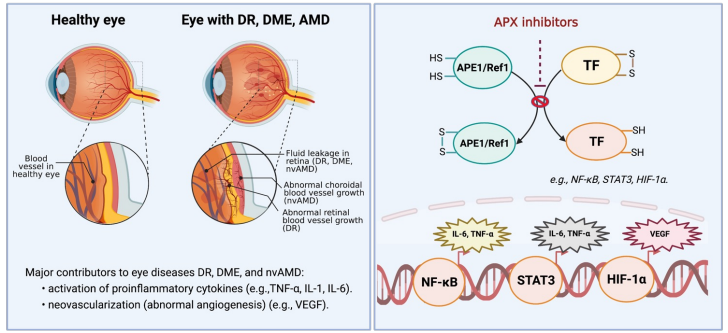
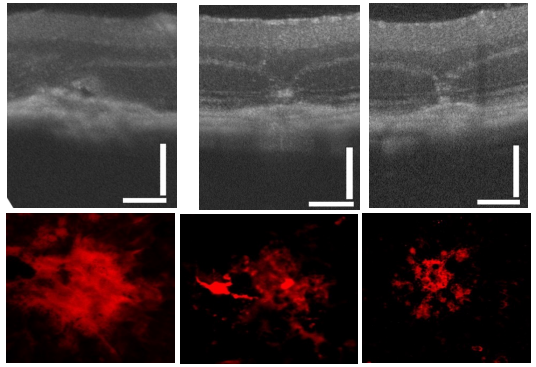
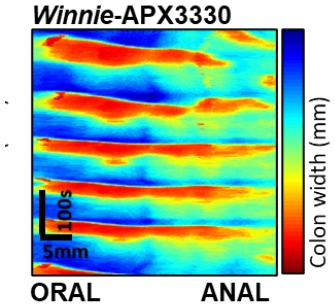


What do Cancer, Retinal Diseases and Inflammatory Bowel Disease have in common?
One target multiple diseases: A trip from the bench to biotech startup to clinical trials.







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- 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
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- Glenn W. Irwin, Jr. M.D. Research Scholar
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- Co-leader, Cancer Drug Discovery and Development Accelerator (C3DA) Program IUSCCC
- AAAS Fellow

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Disclosures

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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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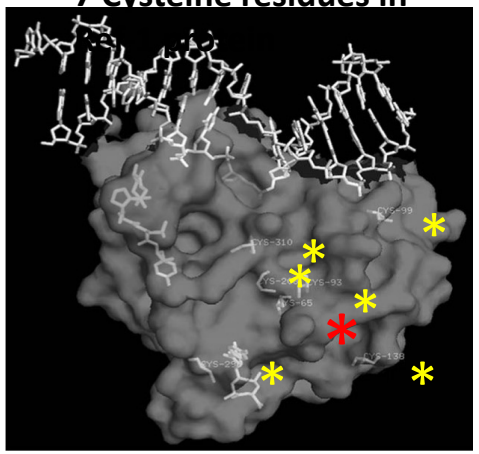
Ocuphire Pharma

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

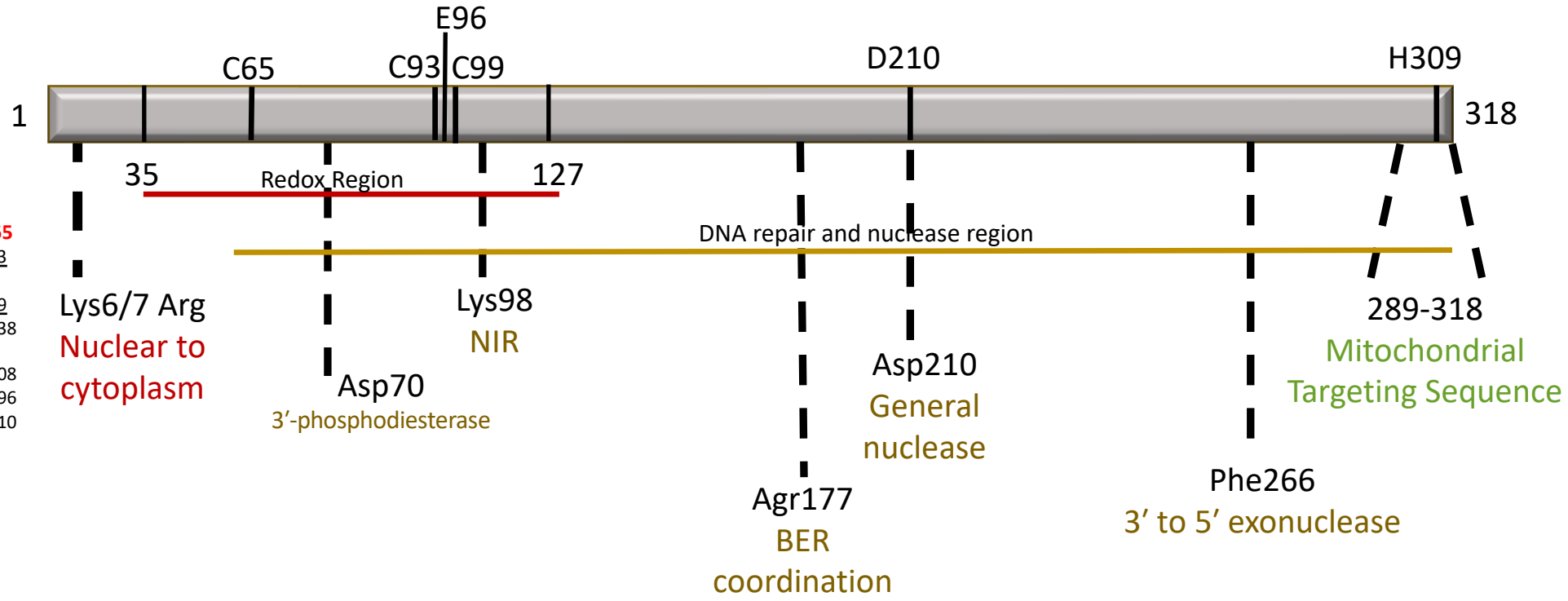
Regions

7 Cysteine residues in



- **Cys65**
- Cys93
- Cys99
- Cys138
- Cys208
- Cys296
- Cys310

(Bhakat et al., 2009 Antioxidant & Redox Signaling)



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)

Ref-1/APE1 is a dual functional enzyme

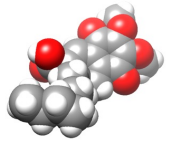
Cell membrane

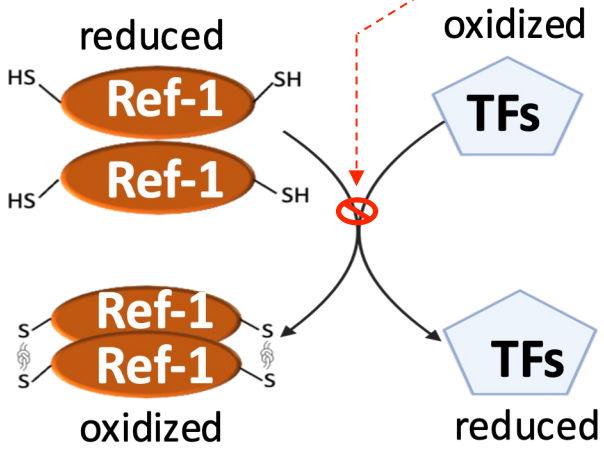
Cytoplasm

➢ Redox function

Ref-1 redox inhibitors

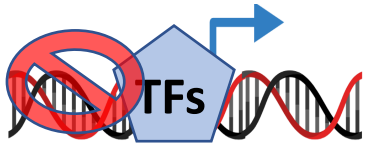
- APX3330
- APX2009.....



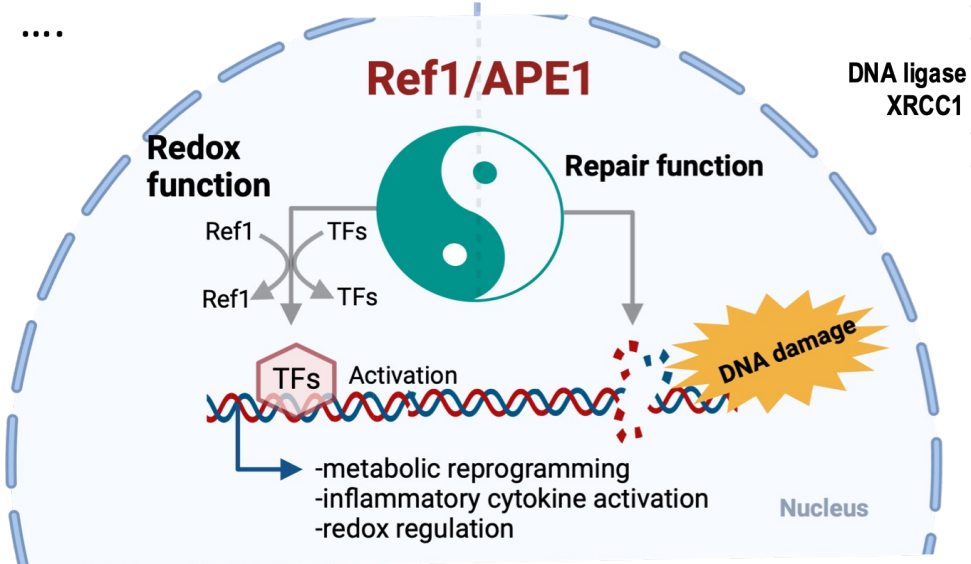


e.g., NF-κB
AP-1
STAT3
HIF1-α
p53
....

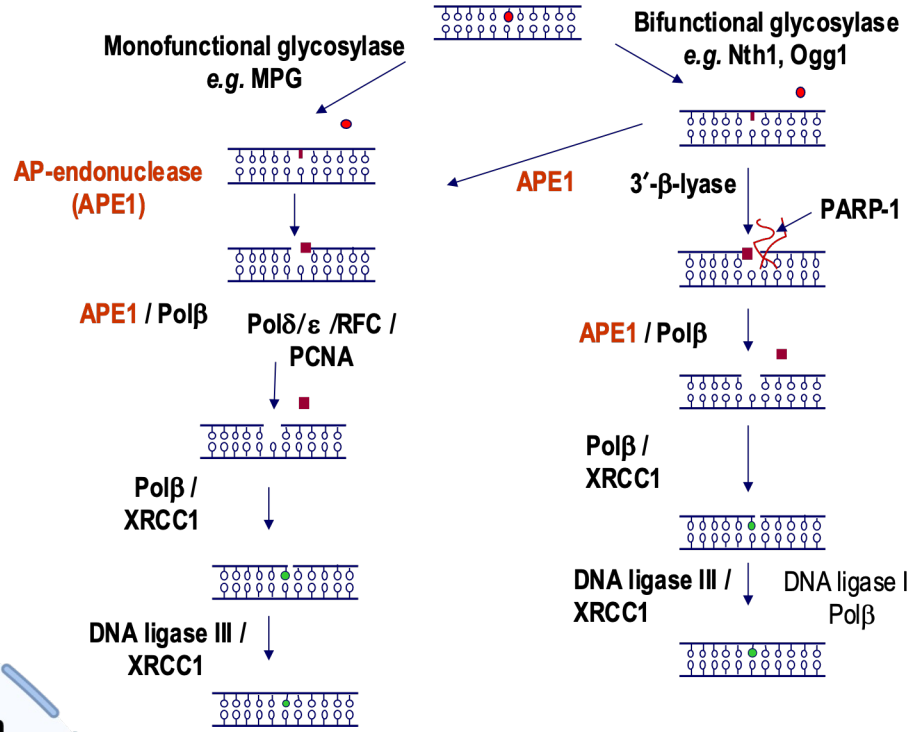
Biological functions

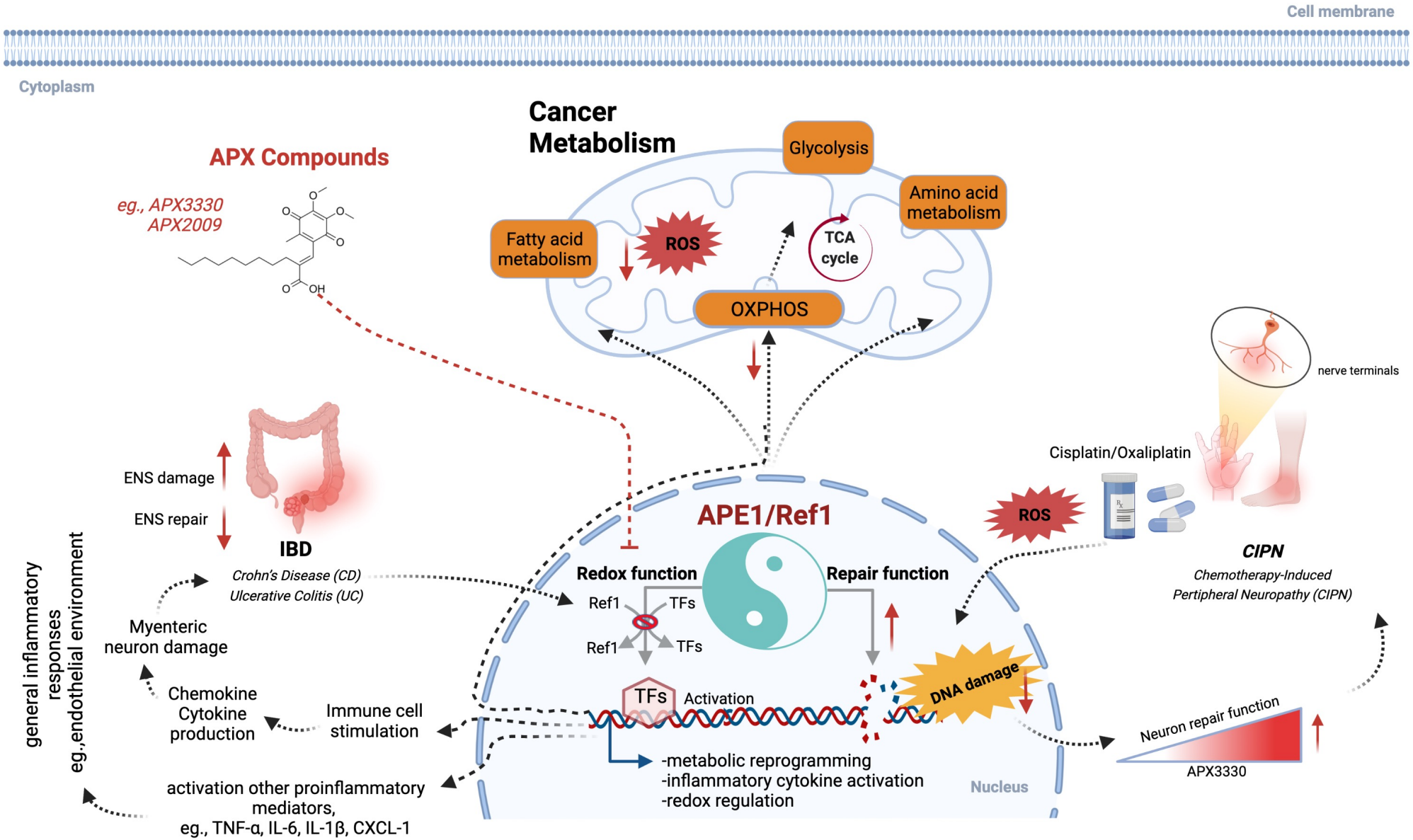


reduced

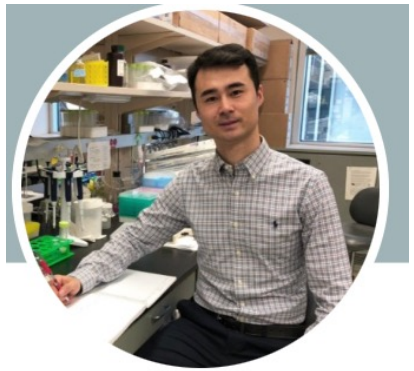


➢ BER Repair function

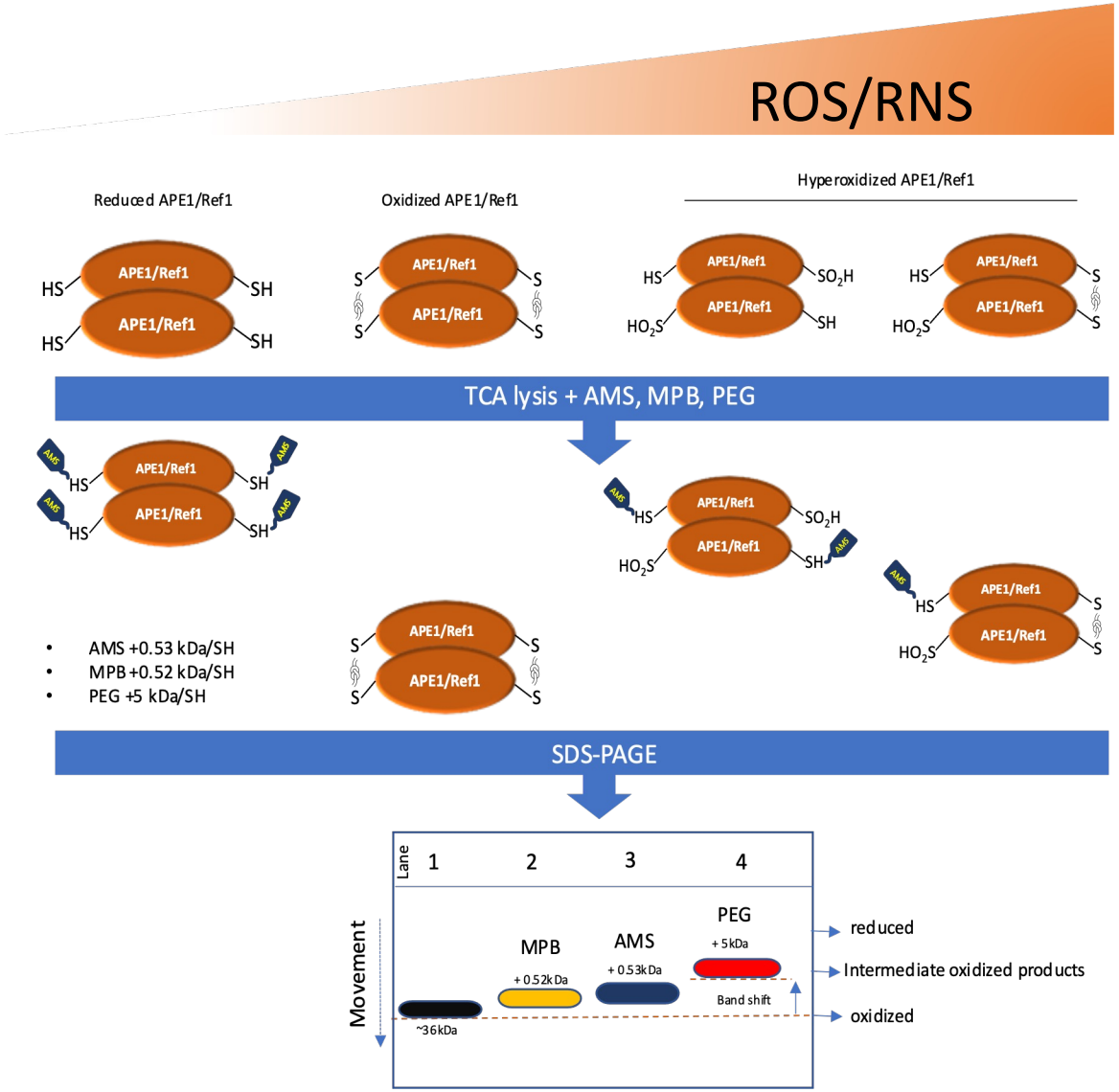



Cell membrane
Cytoplasm
APX Compounds
eg., APX3330
APX2009
Cancer Metabolism
Glycolysis
Amino acid metabolism
Fatty acid metabolism
ROS
TCA cycle
OXPHOS
ENS damage
ENS repair
IBD
Crohn's Disease (CD)
Ulcerative Colitis (UC)
APE1/Ref1
Redox function
Repair function
Ref1
TFs
Ref1
TFs
TFs
Activation
DNA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

Cysteine 65 in Ref-1 is critical for Ref-1 redox function and transcriptional regulation in human PDAC cells

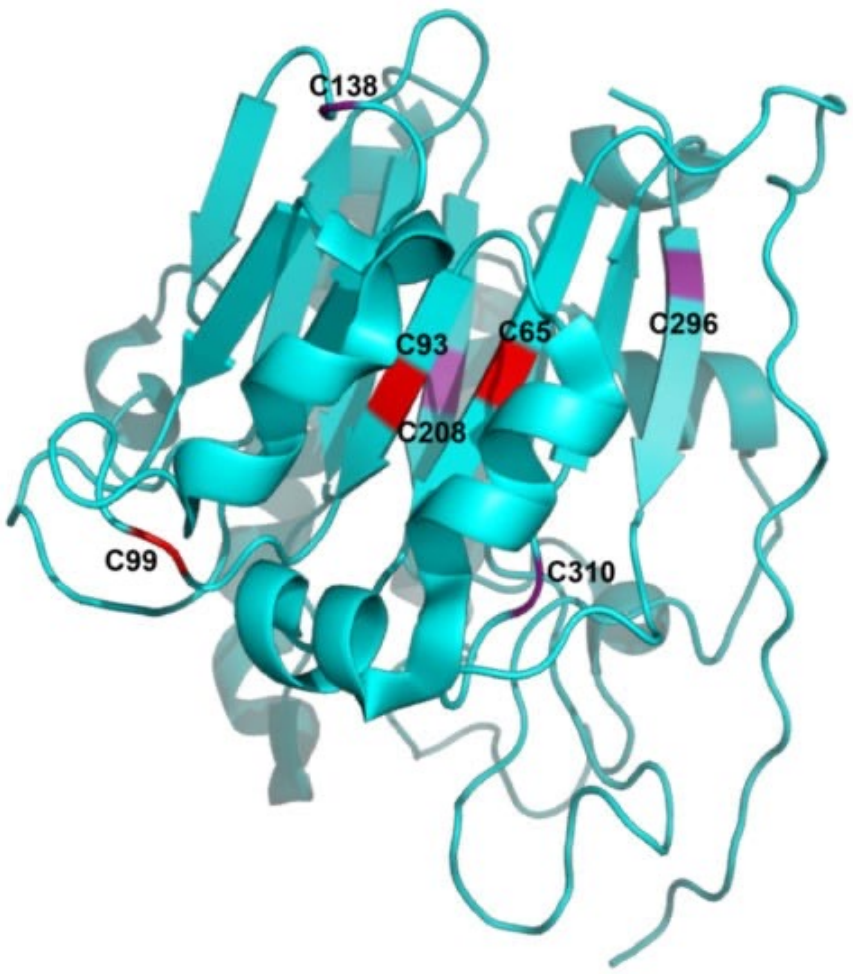


Mahmut Mijit, PhD
(Former Postdoctoral Fellow)

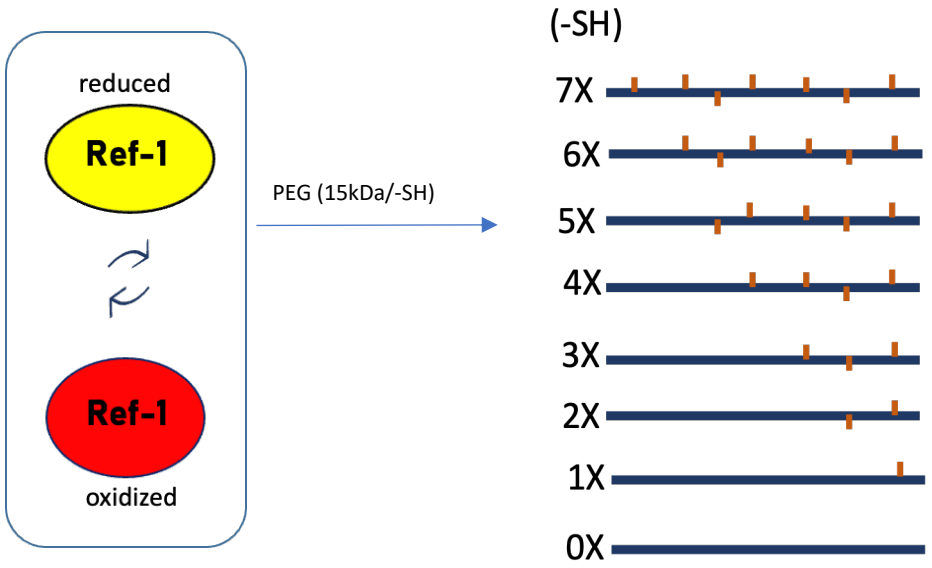


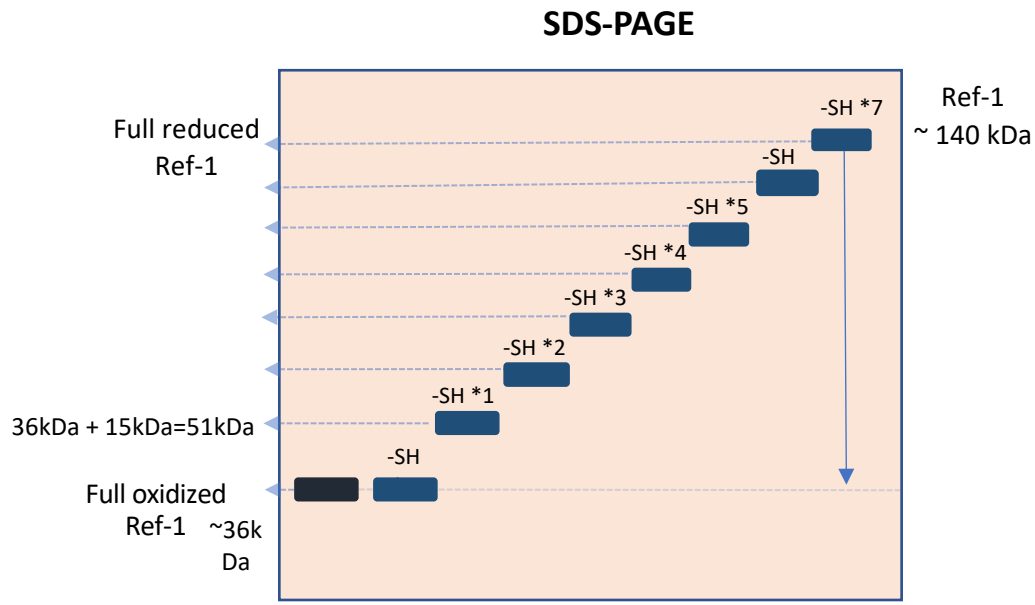
Schematic model for determination of Ref-1 redox state in cells

A



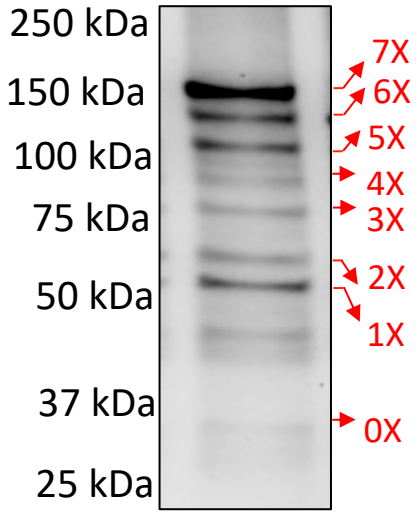
B





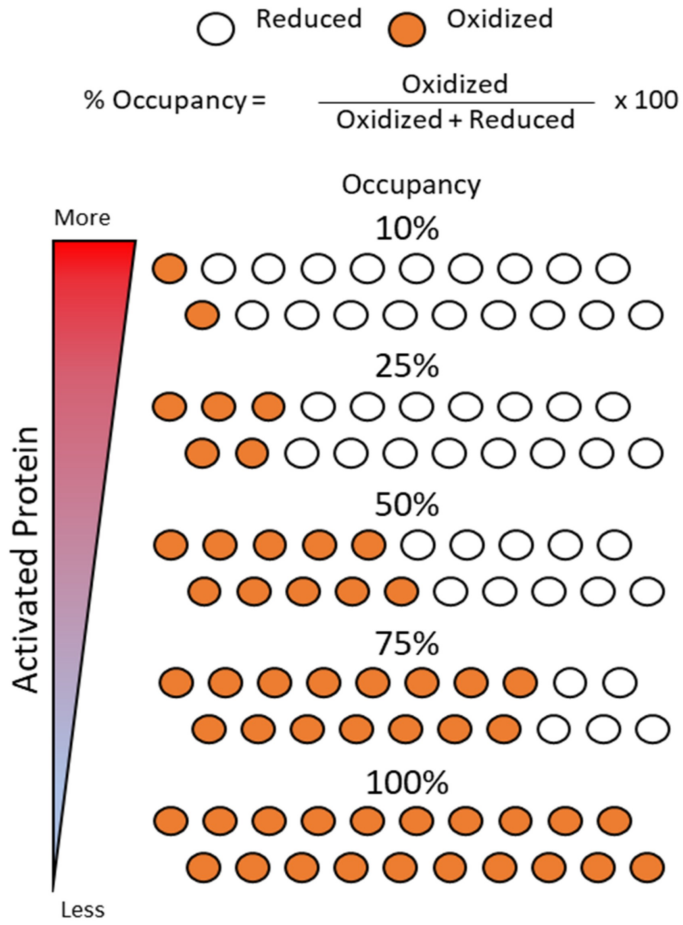
How we quantify the redox status of Ref-1?

Ref-1 Redox status

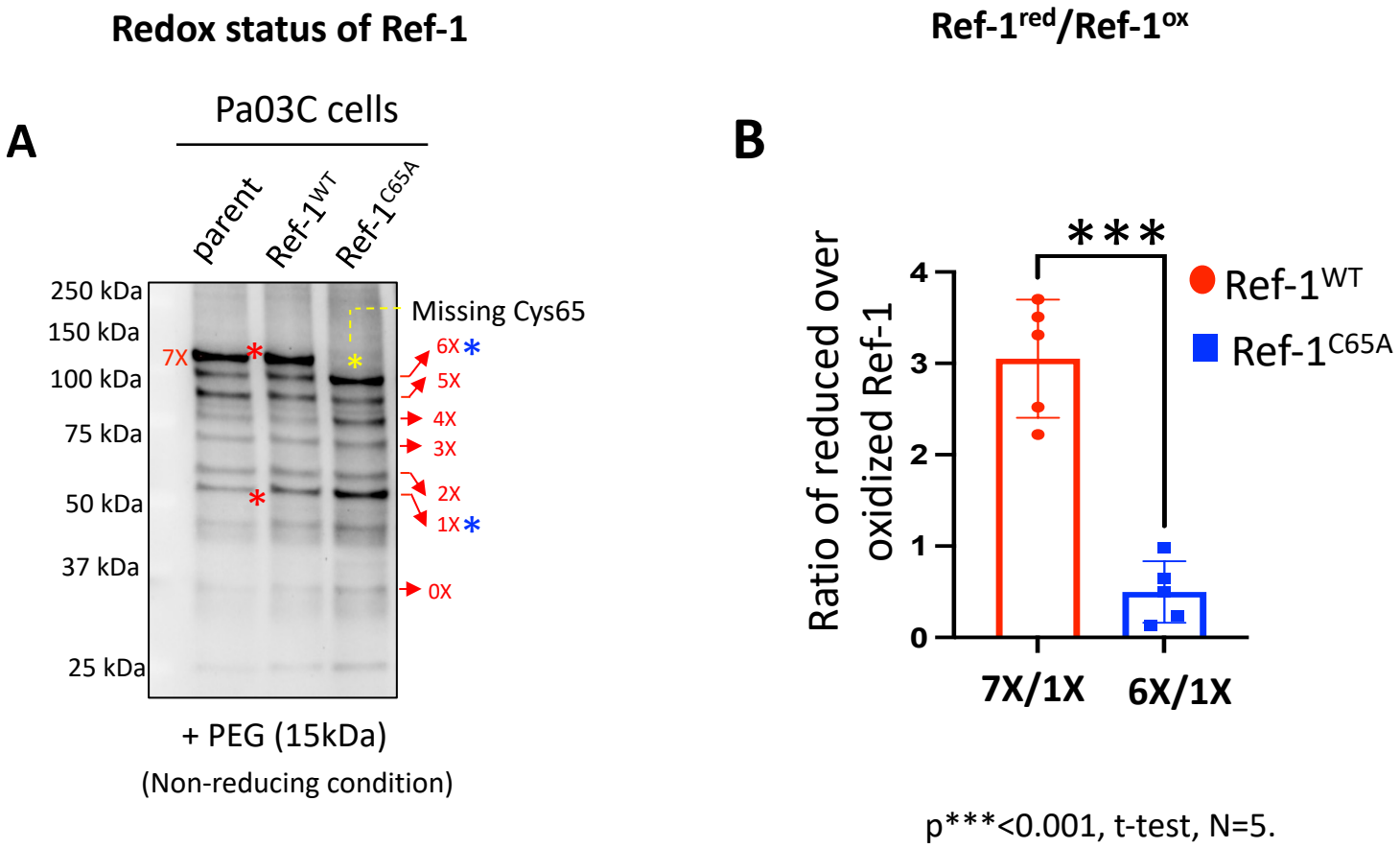


+ PEG (15kDa)

$$\text{Max. Ref-1}_{red}\ (\%) = \frac{\text{Single band intensity (e.g., 7X)}}{\text{Total band intensity (7X+6X+5X+4X+3X+2X+1X)}} \times 100\%$$



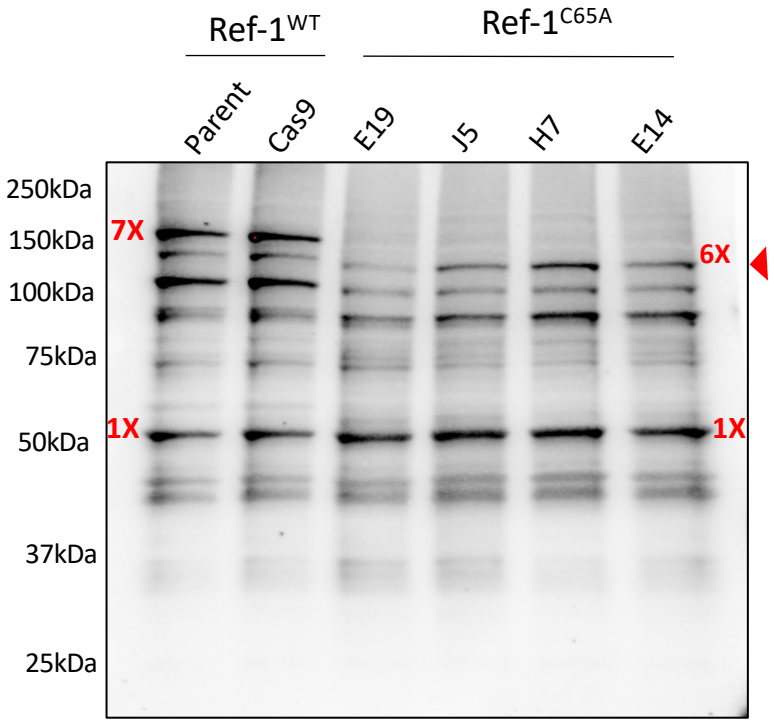
Effects of Cys65 (C65) on Ref-1 redox status in PDAC Pa03C cells



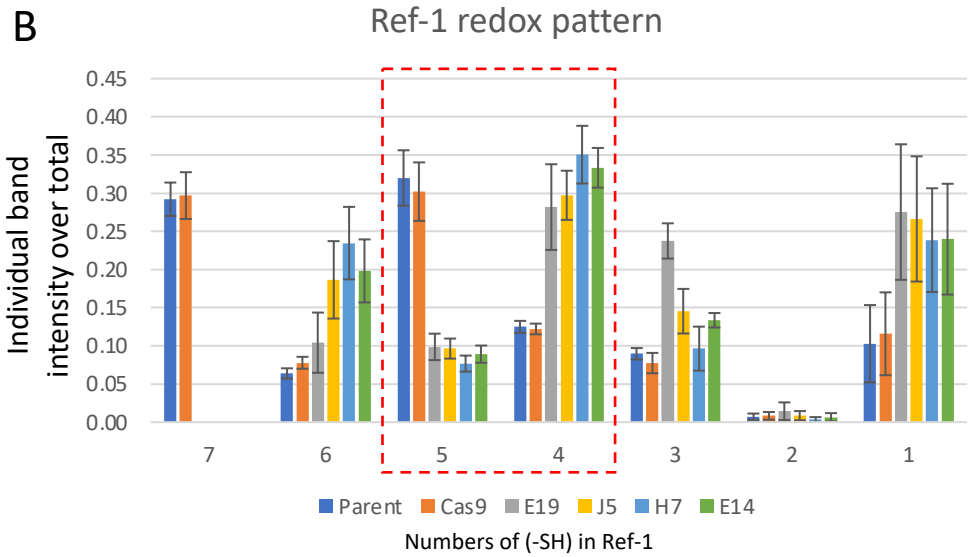
p***<0.001, t-test, N=5.

Cys65 is critical for maintaining a reduced state of Ref-1 in cells, which is the active form of the protein.

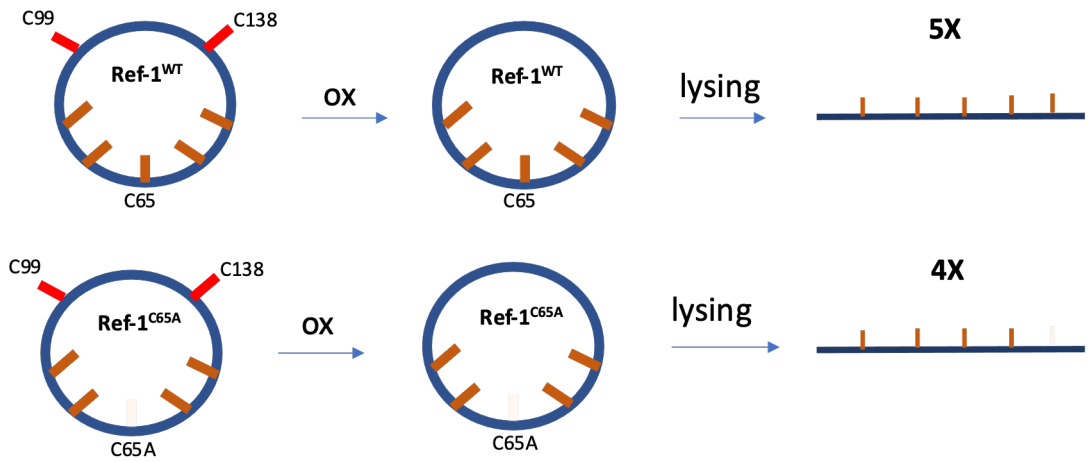
A


Ref-1WT
Ref-1C65A
Parent
Cas9
E19
J5
H7
E14
250kDa
150kDa
100kDa
75kDa
50kDa
37kDa
25kDa
7X
6X
1X
1X

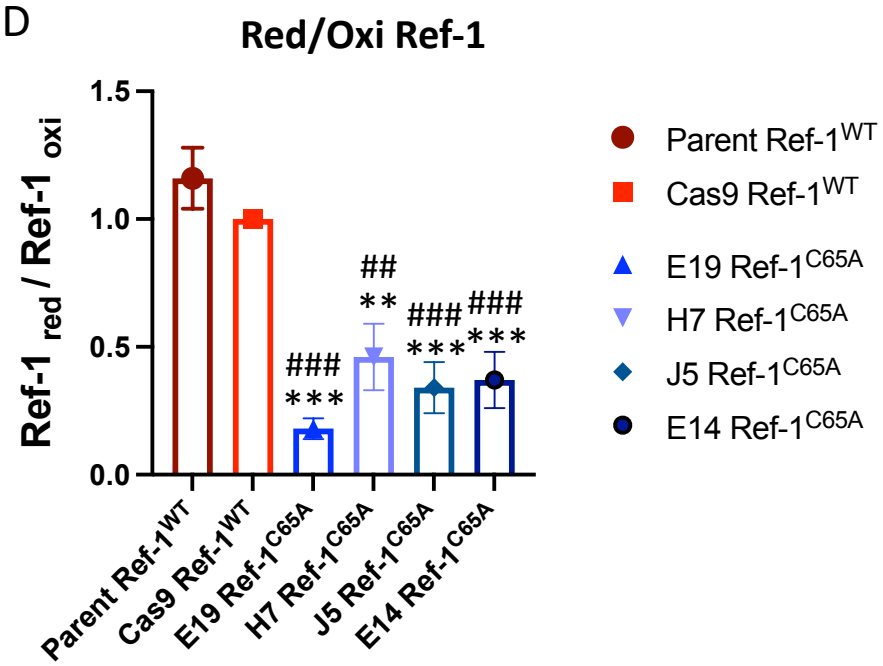
B


Ref-1 redox pattern
Individual band intensity over total
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
7
6
5
4
3
2
1
Parent
Cas9
E19
J5
H7
E14
Numbers of (-SH) in Ref-1

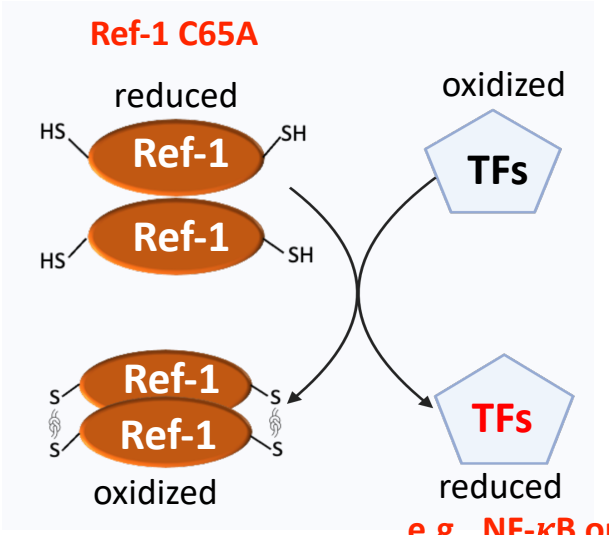
C


C99
C138
Ref-1WT
C65
OX
Ref-1WT
C65
lysing
5X
C99
C138
Ref-1C65A
C65A
OX
Ref-1C65A
C65A
lysing
4X

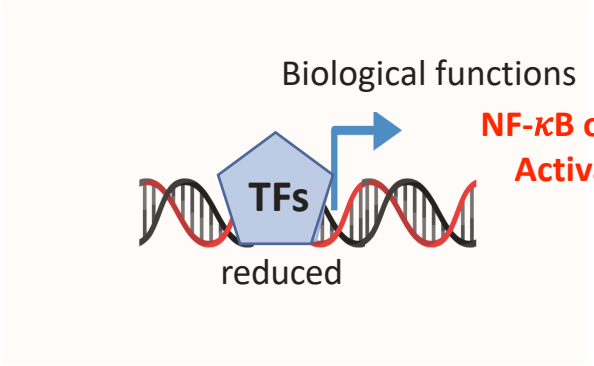
D


Red/Oxi Ref-1
Ref-1 red / Ref-1 oxi
1.5
1.0
0.5
0.0
###
***
##
**
###
***
###
***
Parent Ref-1WT
Cas9 Ref-1WT
E19 Ref-1C65A
H7 Ref-1C65A
J5 Ref-1C65A
E14 Ref-1C65A

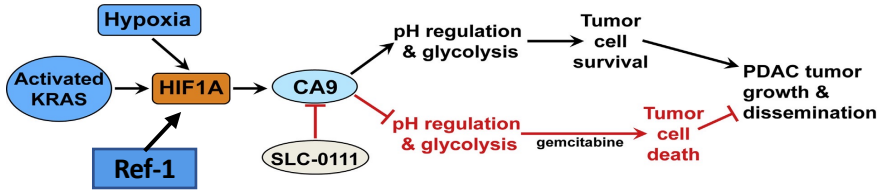
Ref-1 regulation of HIF activity is critical for full activation of CA9

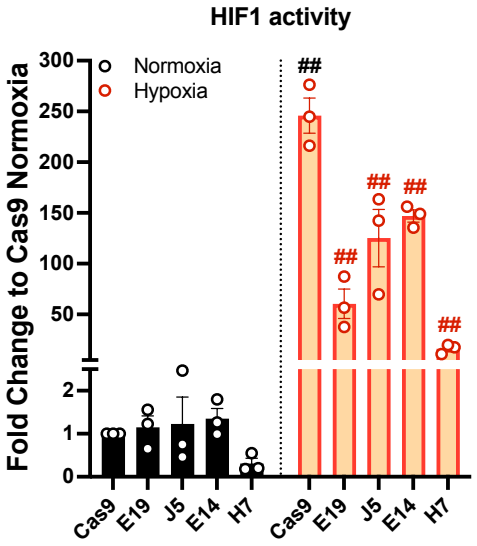


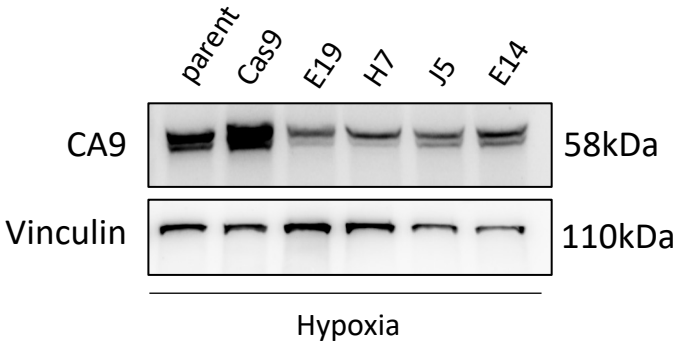
e.g., NF-κB or HIF-1a

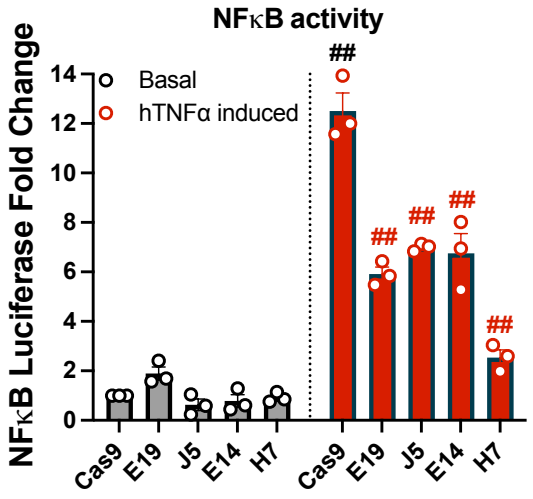


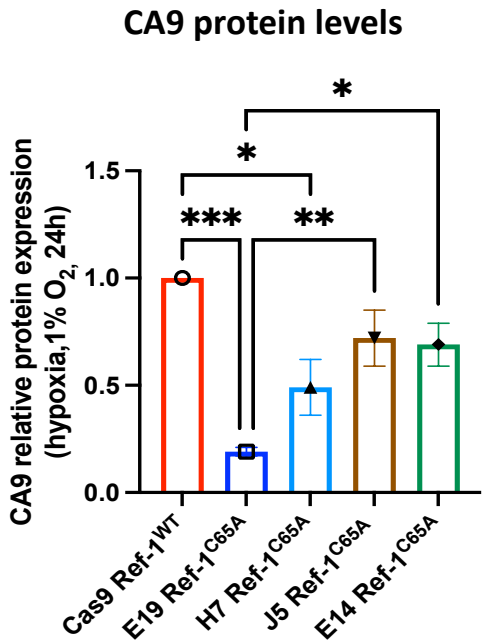
NF-κB or HIF1-a Activation?



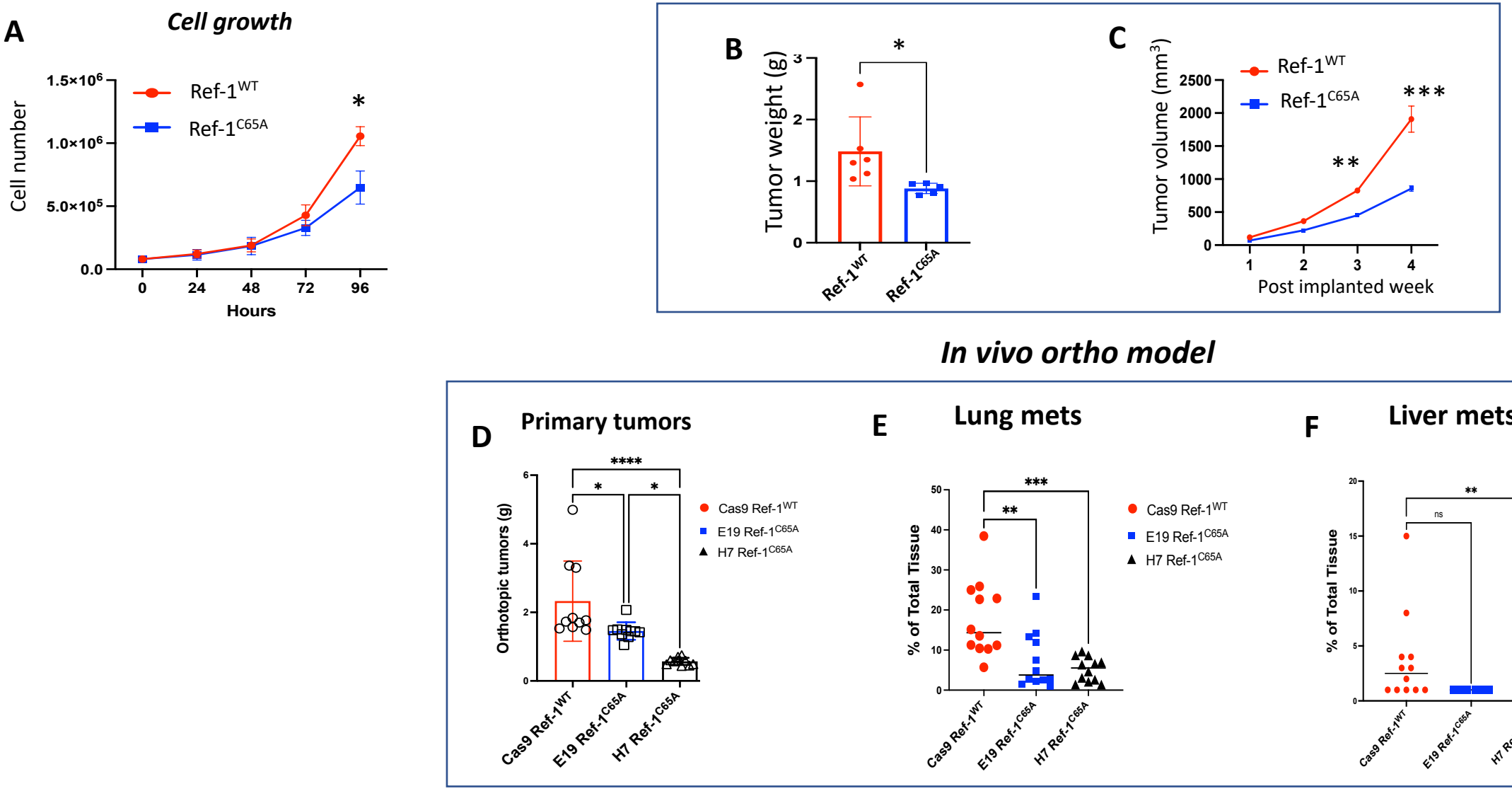






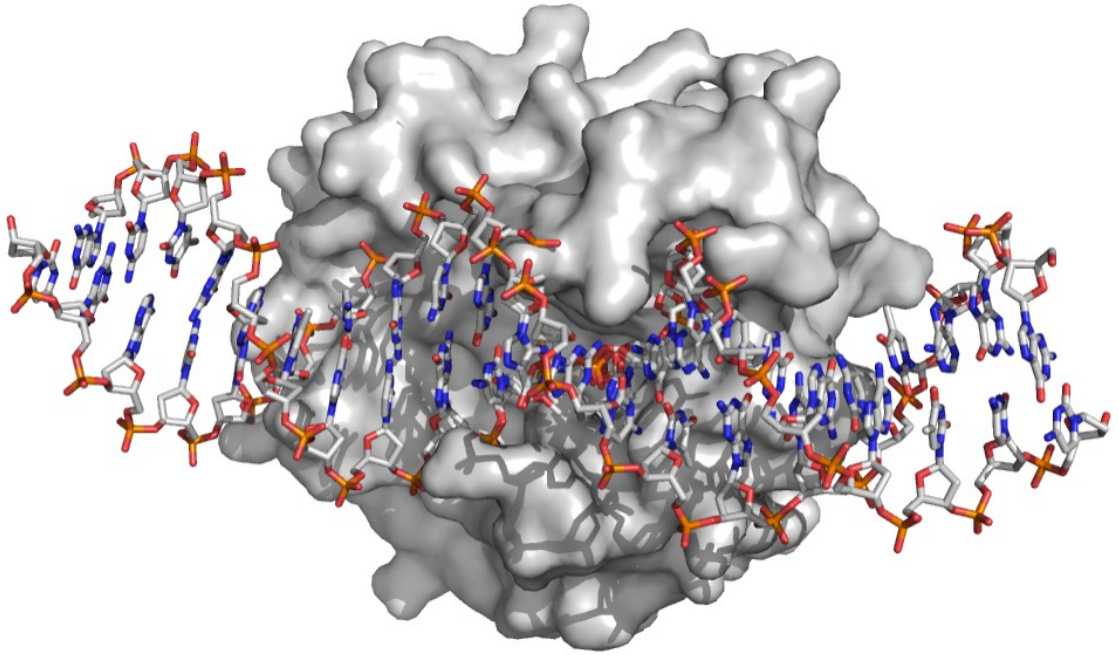


Mutation of Cys 65 in Ref-1 protein affects PDAC cell phenotype both *in vivo* and *in vitro*



HSQC measurements showing APC3330 binding to Ref-1

Data & figure generated
By Dr. Millie Georgiadis



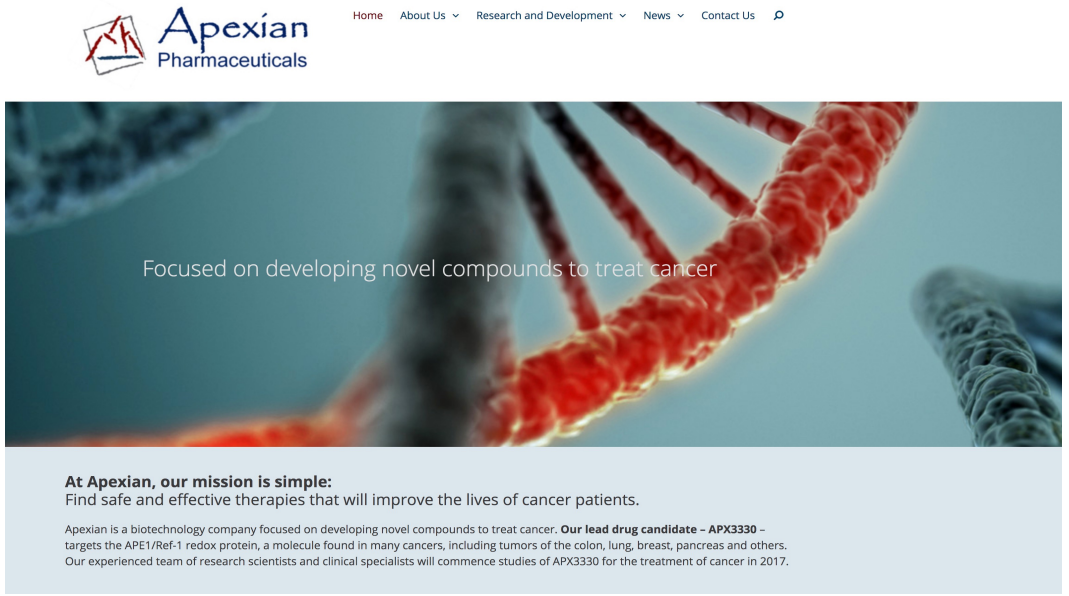
Heteronuclear Single-Quantum Correlations Spectroscopy HSQC measurements showing APX3330 binding to Ref-1 (4HDQ)

- Ref-1 produces a well resolved ^{1}H-^{15}N HSQC/TROSY spectrum.
- Addition of APX3330 to ^{15}N-labelled Ref-1 produced specific shifts in the HSQC spectrum of residues defining a binding site (Green, left panel).
- This binding site is located on the opposite face from the Ref-1 endonuclease binding site, which binds DNA shown in a stick model by superimposing the structure of Ref-1 (5DFI) with bound DNA (right panel).
- We find no evidence for binding of APX3330 to the endonuclease active site as previously reported.

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



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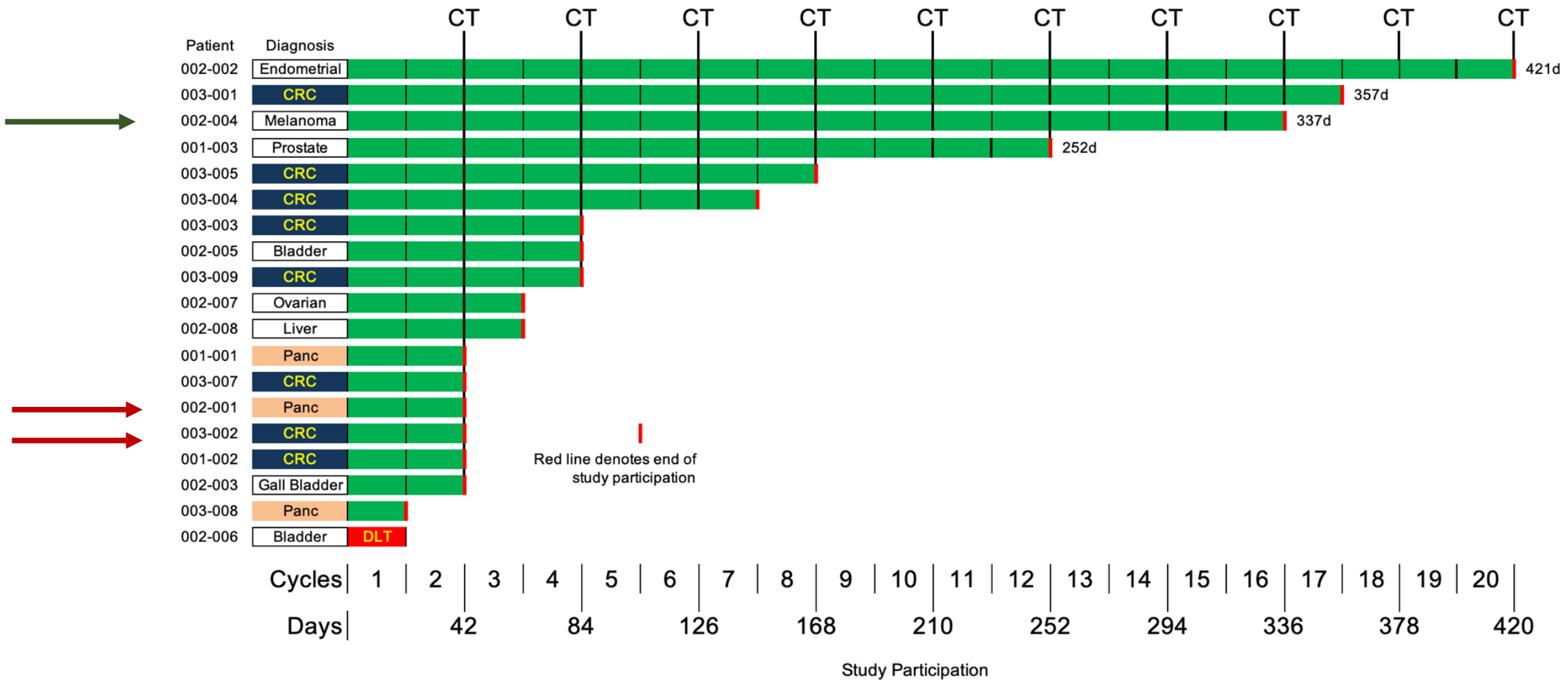
INDIANA UNIVERSITY Department of Pediatrics

INDIANA UNIVERSITY
MELVIN AND BREN SIMON COMPREHENSIVE CANCER CENTER

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

Approximately 30% Response Rate



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.

Ocuphire PHARMA

Restore Vision & Clarity

APX3330 and Diabetic Retinopathy/Diabetic Macular Edema

Diabetic Patients Usually Present with Complex Co-Morbidities

Diabetic Patients are Young and Face Life-long Systemic and Ocular Complications

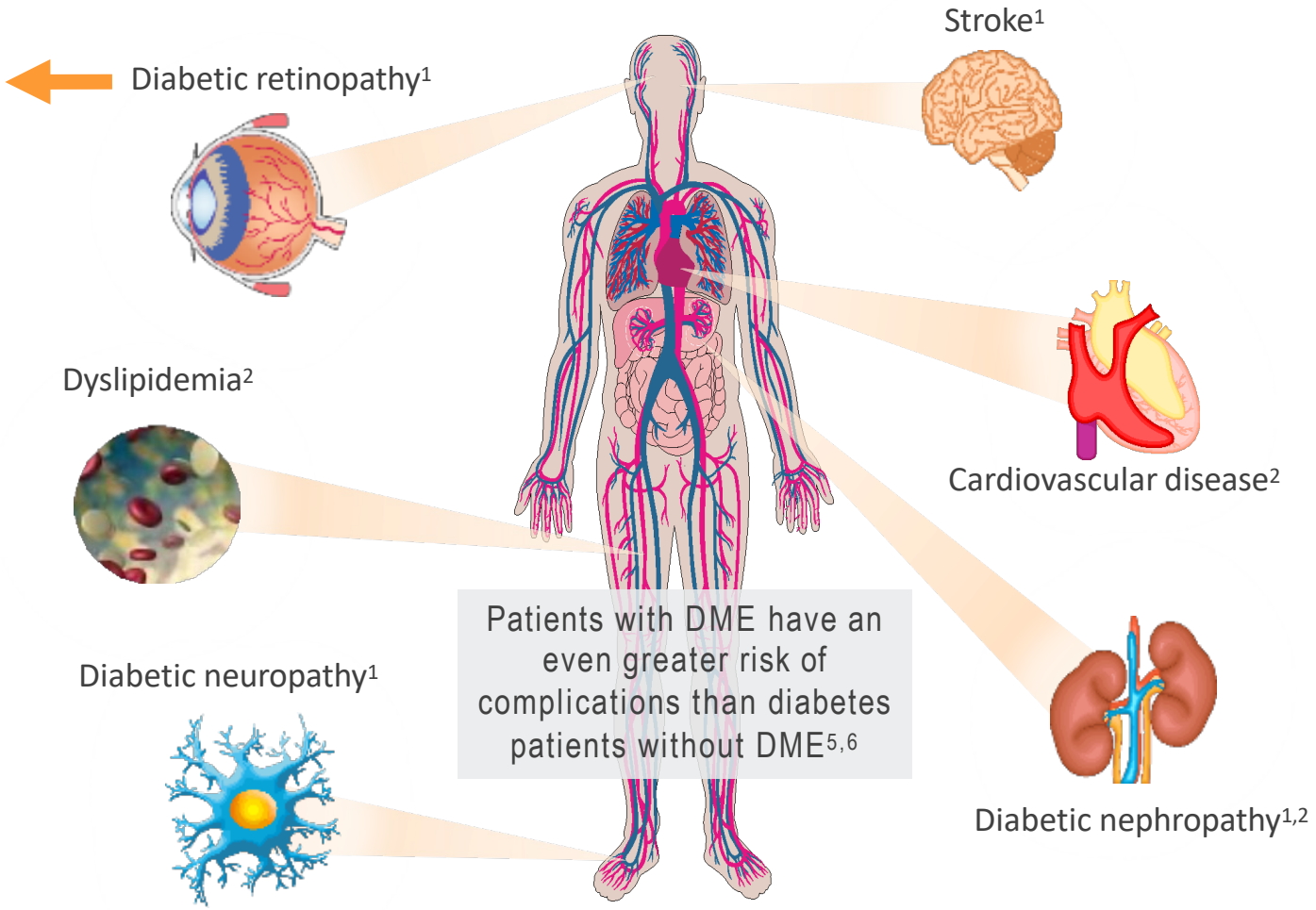
DR is the most common cause of vision loss or blindness in working-age adults, usually affecting both eyes

↓

DME is a vision threatening complication caused by DR where excess fluid leaks near fovea and triggers swelling of the macula

↓

Treating DR leads to control of DME



Oral options have the potential to reach other vascular beds to **treat kidney and neuropathic co-morbidities**

1. Petrella RJ, et al. J Ophthalmol 2012;159167; 2. International Diabetes Federation, Diabetes Atlas 6th Edition, http://www.idf.org/diabetesatlas; 3. National Diabetes Fact Sheet, 2011 http://www.cdc.gov/diabetes/pubs/pdf/ndfs_2011.pdf; 4. Rodbard HW, et al. Endocr Pract 2007;13:4-69; 5. Wong TY, et al. JAMA 2002;288:67-74; 6. Nguyen-Khoa B, et al. BMC Ophthalmol 2012;12:11

Diabetic Eye Disease is Common Cause of Blindness

Diabetes and Diabetic Retinopathy (DR)

Diabetic retinopathy (DR) occurs when fluctuations or instability in blood glucose levels damages blood vessels in the retina

Two Types of DR

Non-Proliferative Diabetic Retinopathy (**NPDR**) – most common form of DR – early stages of edema and exudates, blurred central vision

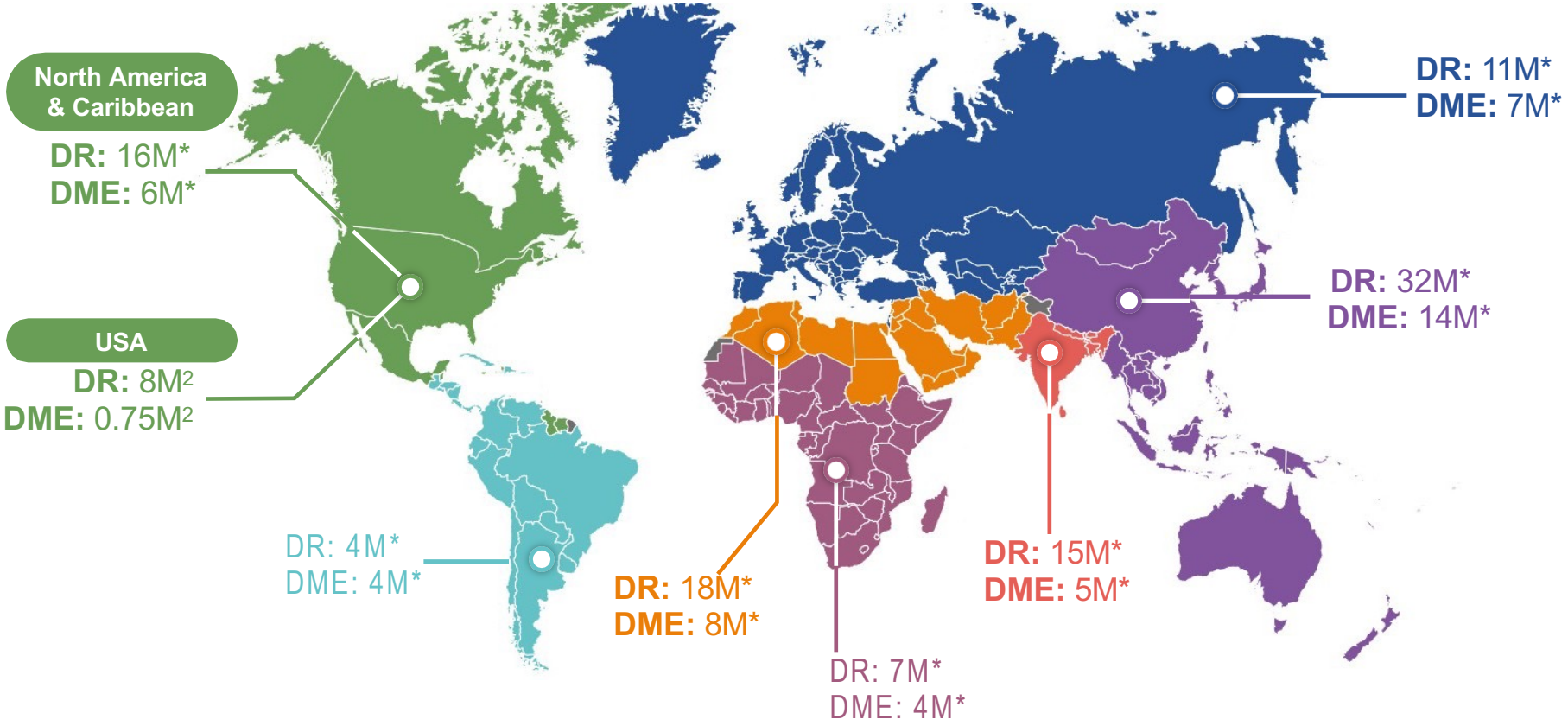
Proliferative Diabetic Retinopathy (**PDR**) – later stage of DR, marked by abnormal blood vessels and scar tissue on retina

Diabetic Macular Edema (DME) can occur at any stage of DR

https://webeye.ophth.uiowa.edu/eyeforum/tutorials/diabetic-retinopathy-med-students/Classification.htm
https://www.mayoclinic.org/diseases-conditions/type-1-diabetes/symptoms-causes/syc-20353011
https://www.mayoclinic.org/diseases-conditions/type-2-diabetes/symptoms-causes/syc-20351193

Global Prevalence of Diabetes-Associated Retinal Disease

DR Affects 1 in 3 People with Diabetes; DME Affects 1 in 13 People with Diabetes1



*Global estimates are provided by the National Eye Institute, FactSheet, Global Data, Research and Markets, American Academy of Ophthalmology, and PLOS One

1. Holekamp N. M. (2016). Overview of diabetic macular edema. The American journal of managed care, 22(10 Suppl), s284–s291.
2. American Diabetes Association; American Journal of Managed Care, International Diabetes Federation; Healthline; Ocuphire internal analysis and assumptions

Multiple Targets in DME/DR Treatment Landscape

Anti-VEGF Therapy is Mainstay, but Under/Non-Responders Remain, and Early Treatment is Limited

Available Commercialized Therapies:

Anti-VEGF IVT:

Aflibercept (Eylea®)

Ranibizumab (Lucentis®)

Bevacizumab (Avastin®)

Faricimab (Vabysmo®)

IVT Steroids:

Dexamethasone (Ozurdex®)

Emerging therapies that could shape industry:

Longer Duration IVTs

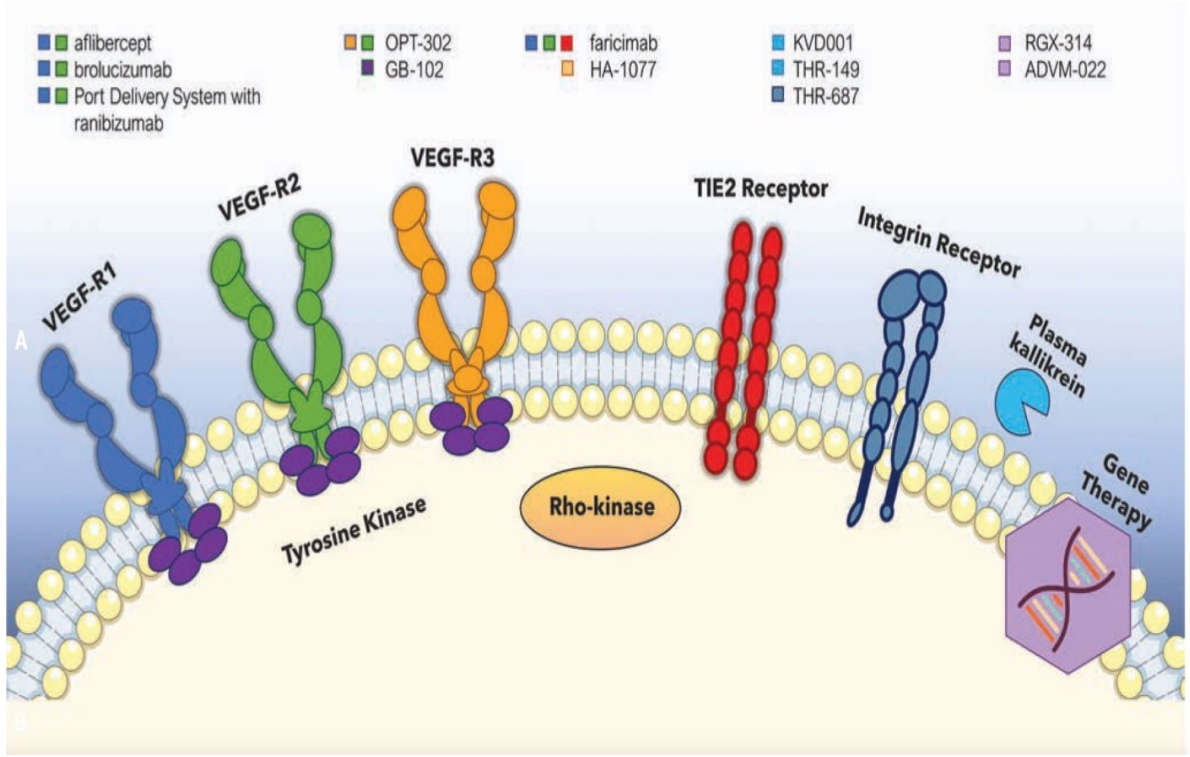
Extended Release

Combination Therapies

Oral Therapies

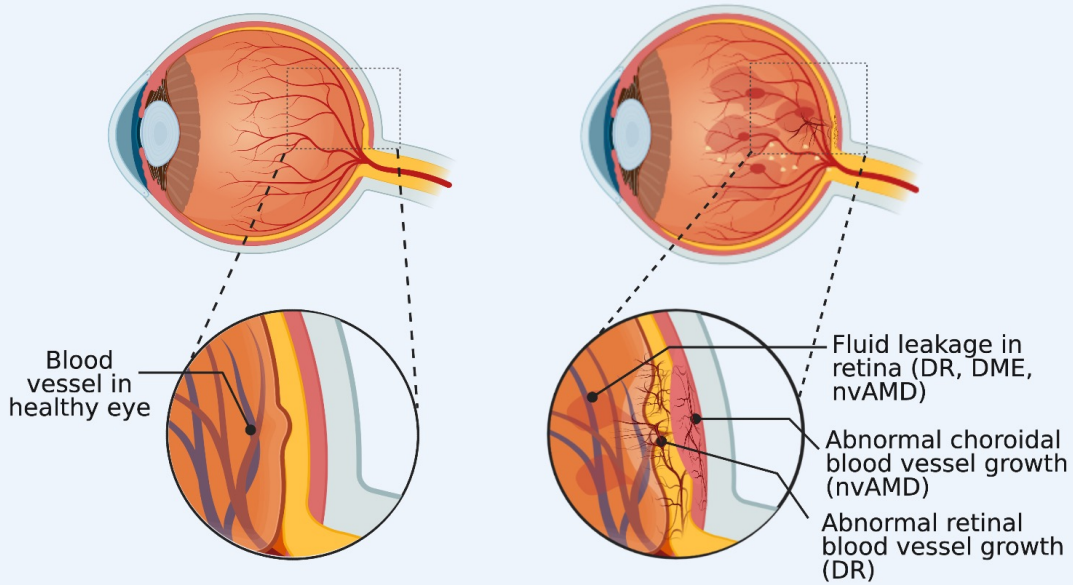
Topical

Gene Therapies



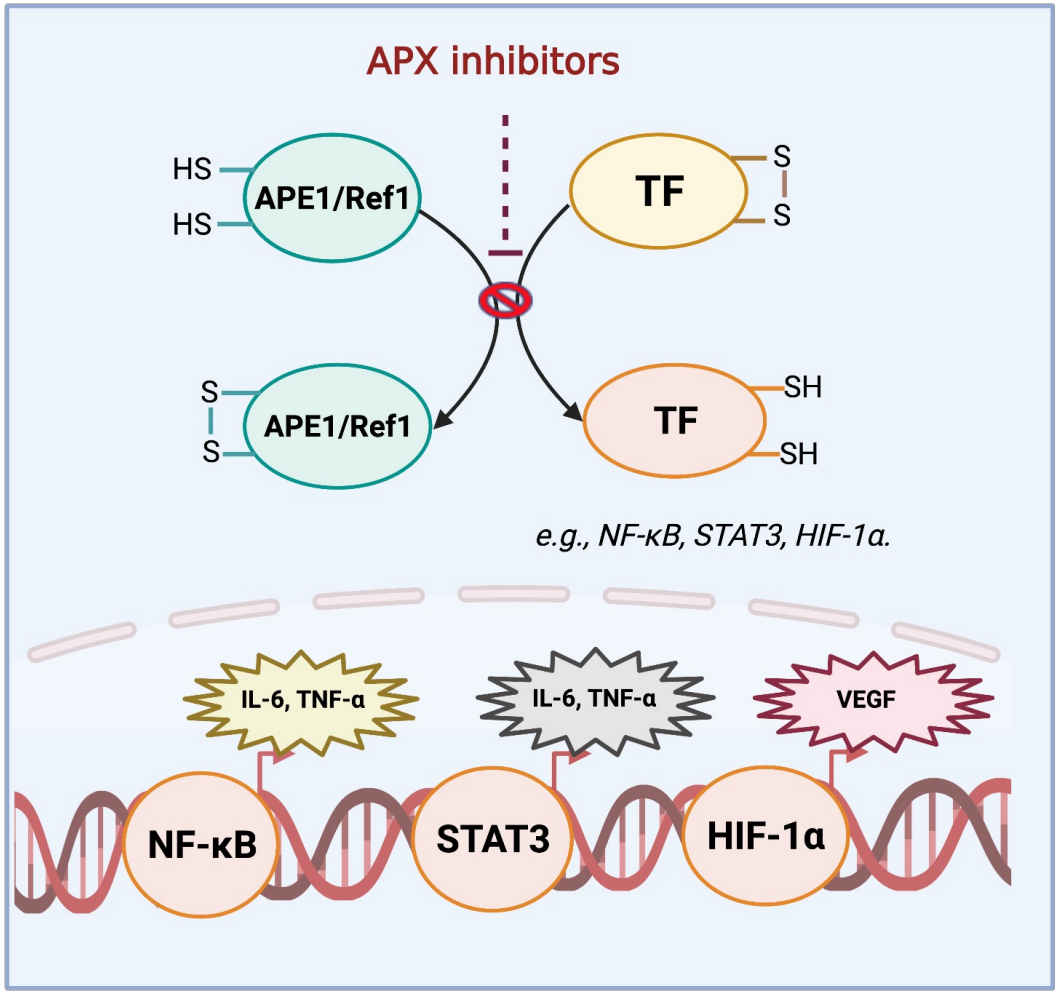
https://www.reviewofophthalmology.com/article/a-peek-into-the-diabetic-retinopathy-pipeline

Role of Ref-1 in ocular diseases DR, DME and nvAMD (wet AMD)



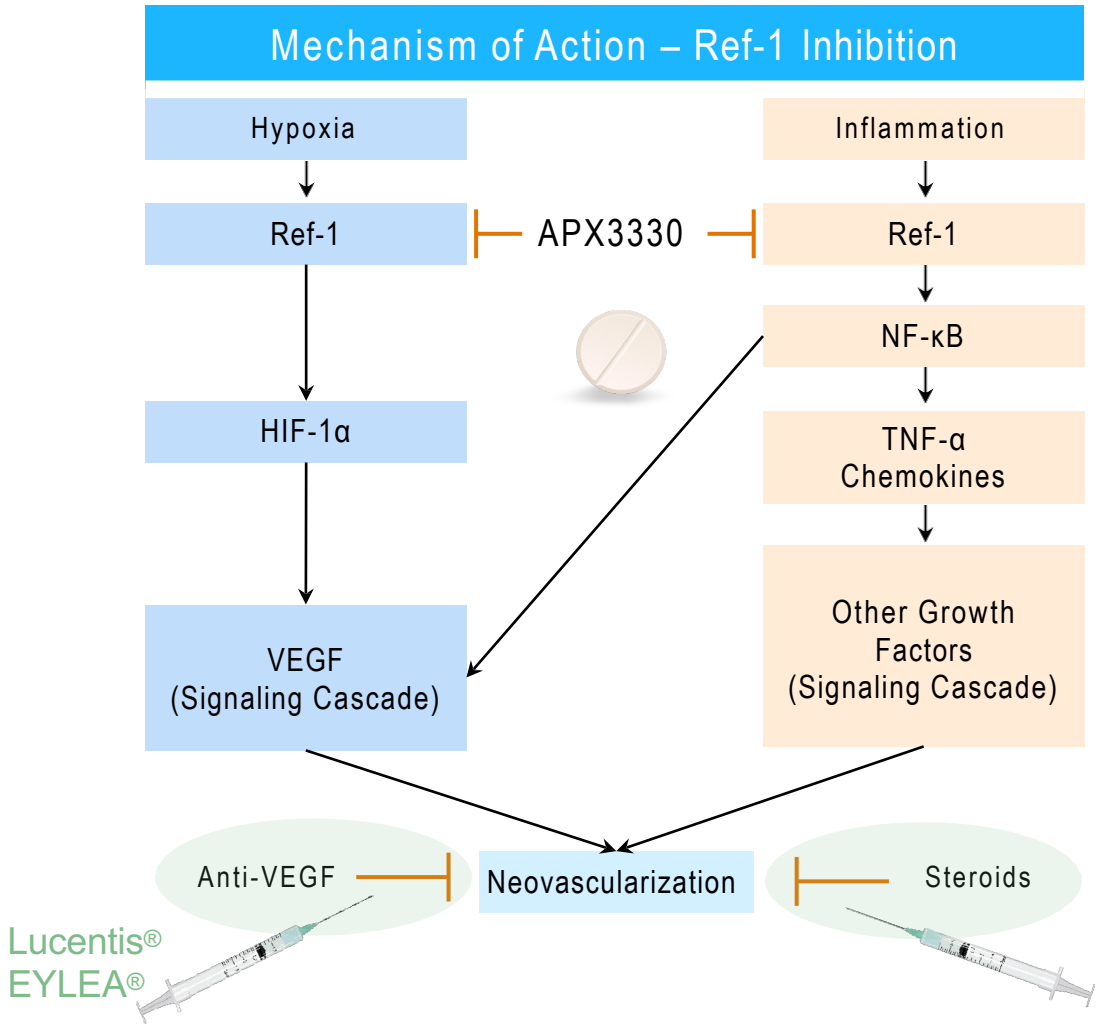
Major contributors to eye diseases DR, DME, and nvAMD:

- activation of proinflammatory cytokines (e.g.,TNF-α, IL-1, IL-6).
- neovascularization (abnormal angiogenesis) (e.g., VEGF).



APX3330 – Novel and Dual-Acting MOA in an Oral Pill

Ref-1 Involved in Multiple Key Pathways that Contribute to Diabetic Retinopathy and DME



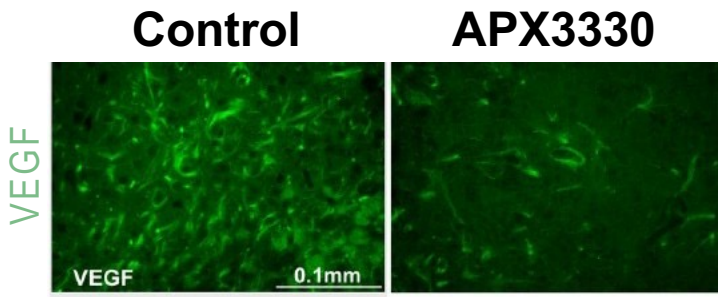
- Ref-1 (reduction-oxidation effector factor-1) is a novel target
- APX3330 is a small molecule oral drug candidate and a first-in-class inhibitor of Ref-1
- APX3330 developed by Apexian for advanced solid tumors
  - Similar oncology origin as approved anti-VEGFs
- MOA uniquely decreases both abnormal angiogenesis and inflammation by blocking pathways downstream of Ref-1

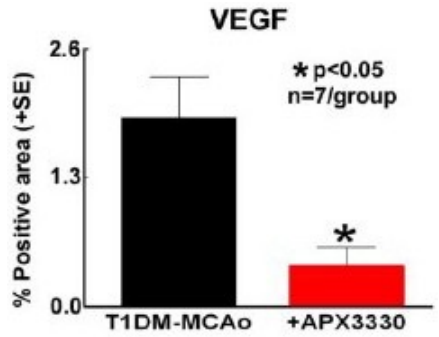
Logsdon et al (2018), Li et al (2014).
Rangasamy S, McGuire PG, Das A. Middle East Afr J Ophthalmol 2012;19:52–59; Sohn HJ et al. Am J Ophthalmol 2011;152:686–694.

In Vitro Validation of APX3330 Mechanism of Action

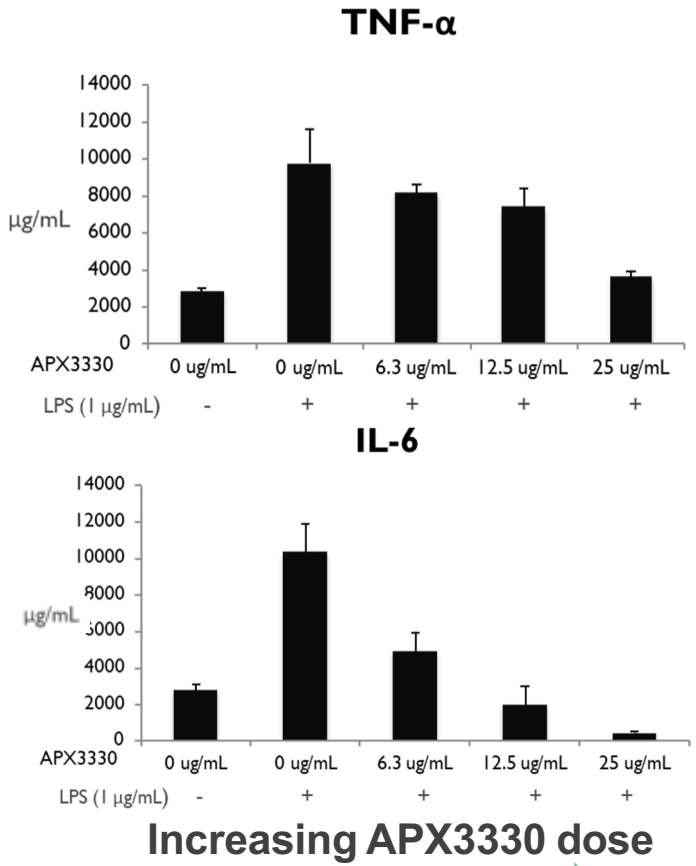
APX3330 Reduces VEGF levels and Inflammatory Cytokines; Provides Neuronal Protection

APX3330 reduces VEGF protein expression in preclinical stroke model

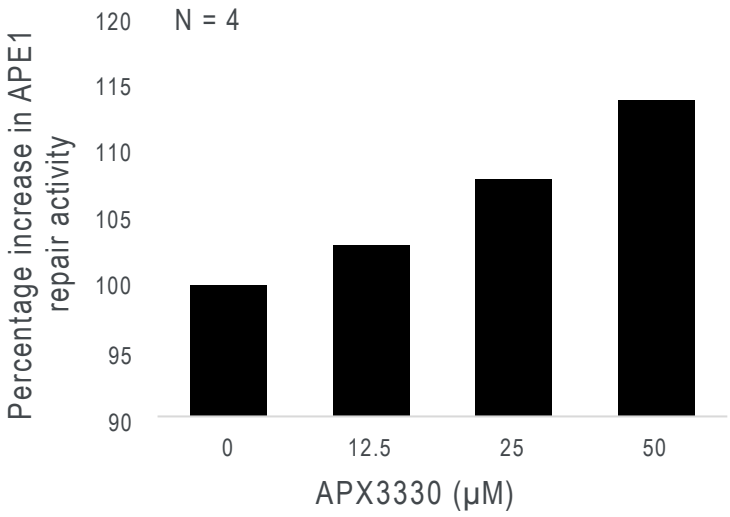




APX3330 reduces pro-inflammatory cytokines in LPS stimulated macrophages



APX3330 increases DNA oxidative repair and neuronal protection



APX3330 enhances Ref-1 endonuclease activity in dorsal root ganglion neurons

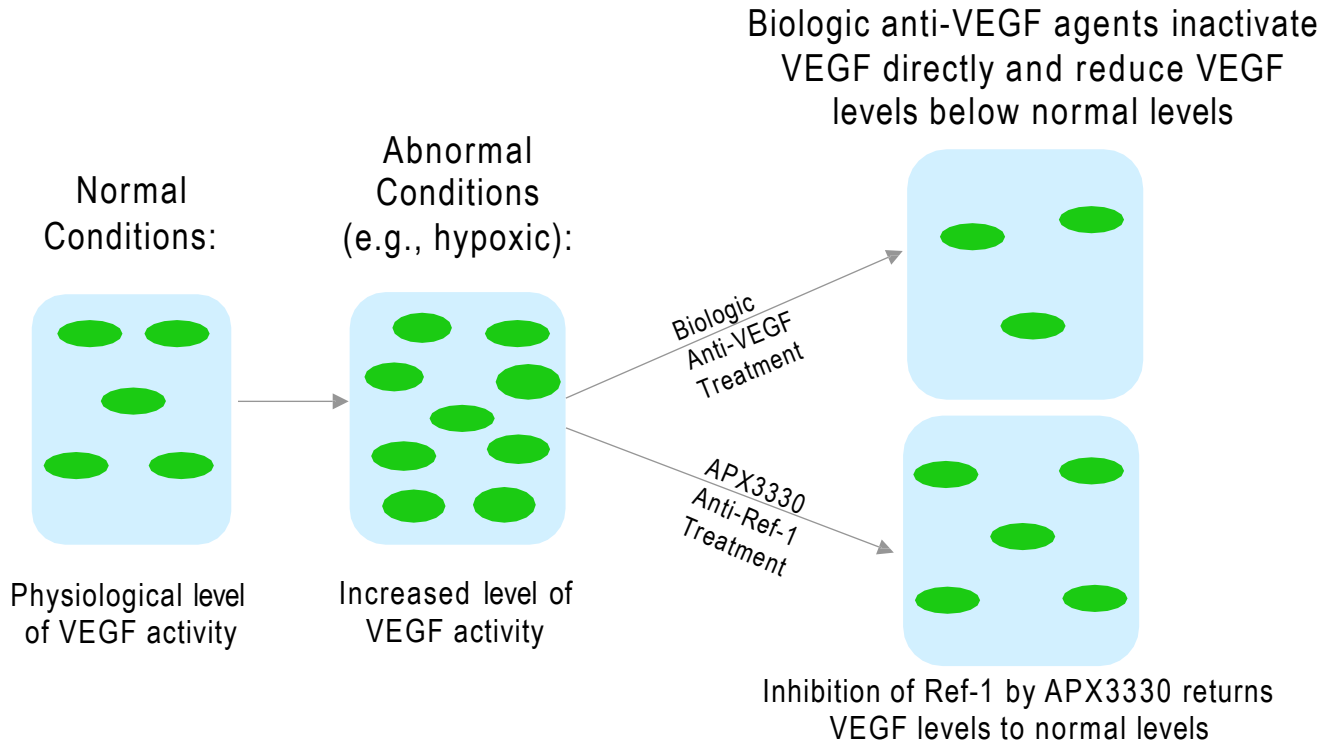
Tao Yan et al. APX3330 Promotes Neurorestorative effects after stroke in type one diabetic rats. Aging and Disease. Vol 9, Oct 2018

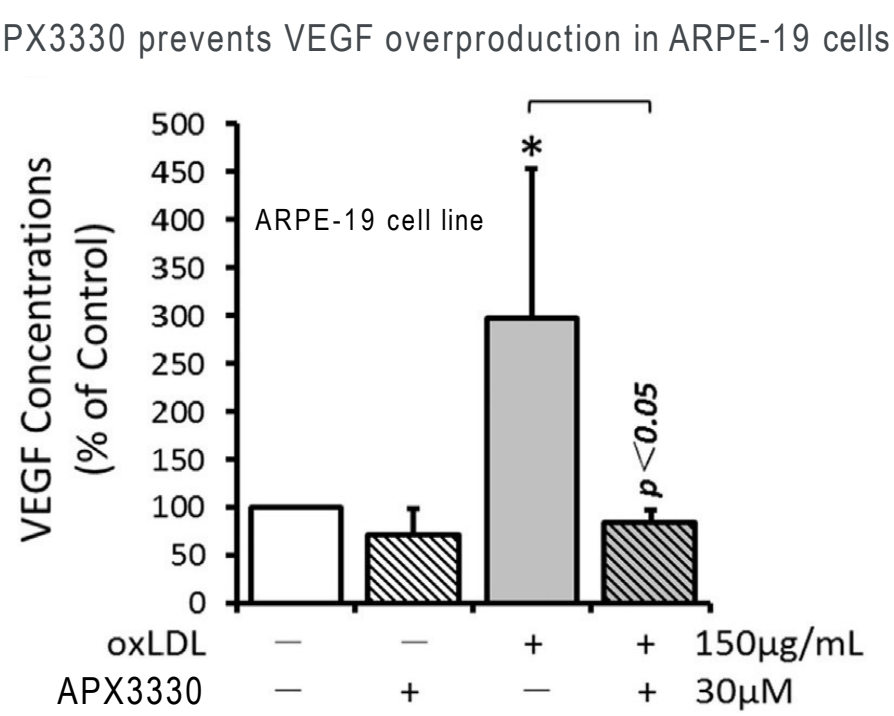
Apurinic/Apyrimidinic endonuclease 1 regulates inflammatory response in macrophages. Jedinak A, Dudhgaonkar S, Kelley MR, Sliva D. Anticancer Res. 2011 Feb;31(2):379-85. PMID: 21378315

Fehrenbacher, J. C., Guo, C., Kelley, M. R. & Vasko, M. R. DNA damage mediates changes in neuronal sensitivity induced by the inflammatory mediators, MCP-1 and LPS, and can be reversed by enhancing the DNA repair function of APE1. Neuroscience 366, 23-35, doi:10.1016/j.neuroscience.2017.09.039 (2017).

APX3330 VEGF Effects in Normal Cells

APX3330 Restores Normal Levels Unlike Biologic Anti-VEGFs that Reduce VEGF Below Normal





- VEGF is a growth factor that is necessary for normal function of multiple cell types including vascular endothelium and neurons ➔ By returning VEGF levels to normal, APX3330 can reduce neovascularization, vascular leakage and the inflammatory response without adverse systemic effects
- The safety profile of APX3330 to date in over 300 subjects has not shown any of the adverse effects that has been seen with systemic administration of anti-VEGF biologics such as cardiovascular pathology, hypertension, arteriothrombotic events, or renal dysfunction

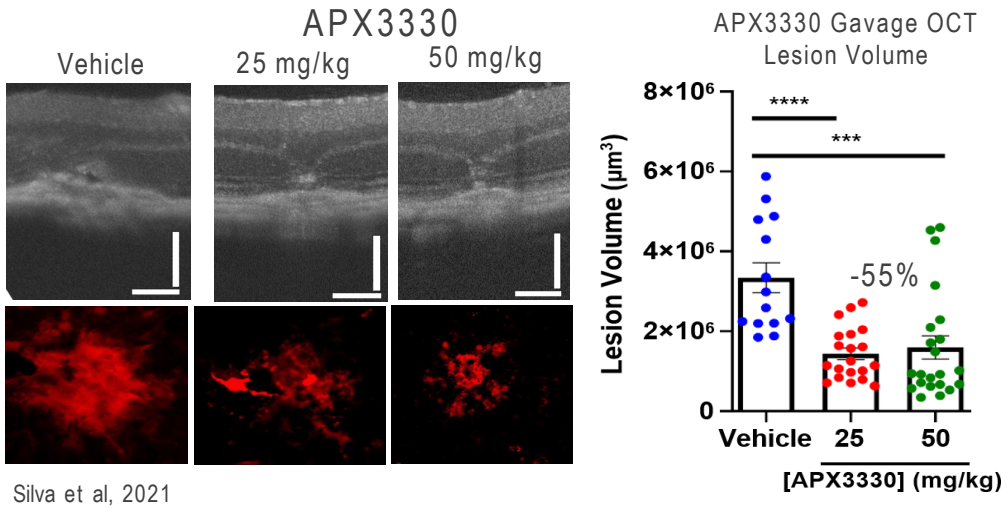
Kamba 2007; Girardi 2010; Li 2014; APX3330 Investigator Brochure

Preclinical Data: Oral APX3330 Blocks Neovascularization

Lesion Volume Decrease with Oral APX3330 in Murine Laser CNV Model Similar to EYLEA® Data

L-CNV Mouse Retina Model

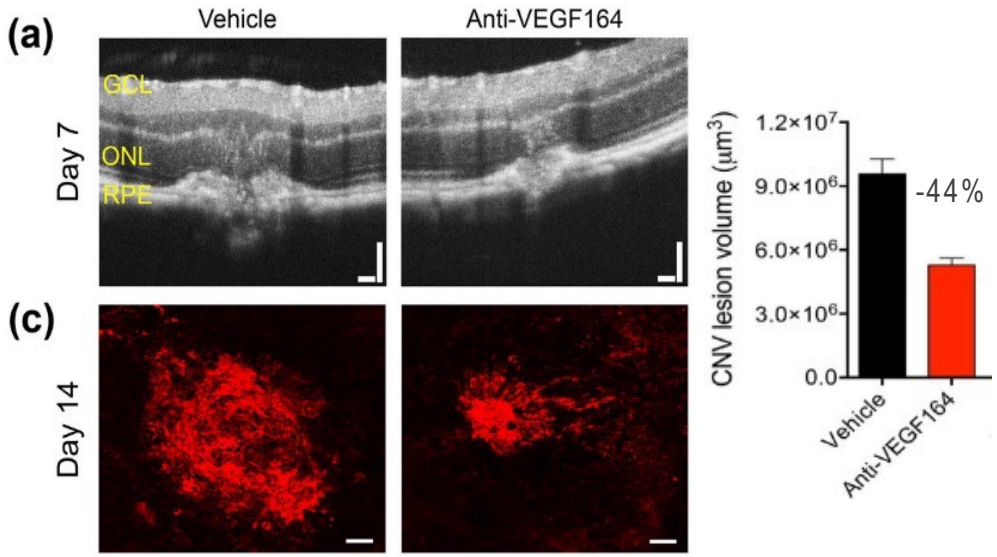
Lesion Size and Corresponding Fluorescent Stains in L-CNV Models Treated with APX3330 at 25 mg/kg oral gavage



Silva et al, 2021

L-CNV Mouse Retina Model

*EYLEA



- ✓ Efficacy was also seen after single intravitreal injection of 20 µM APX3330 in mouse L-CNV model**
- ✓ Efficacy was also seen after dosing intraperitoneal injection of 50 mg/kg twice daily, 5 days on/2 days off, for 2 weeks in mouse L-CNV model***
- ✓ Efficacy was also seen after single intravitreal injection of 20 µM APX3330 in Vldlr $^{-/-}$ mice model****

Silva et al. ARVO 2021 Annual Meeting
*Published data on EYLEA. This study was performed independently from APX3330 study and is a cross-study comparison.
Li 2014; * Pasha 2018; ****Jiang 2011 (Vldlr -/- : Very Low-Density Lipoprotein receptor knock-out mice)

DR/DME ZETA-1 Phase 2 Design

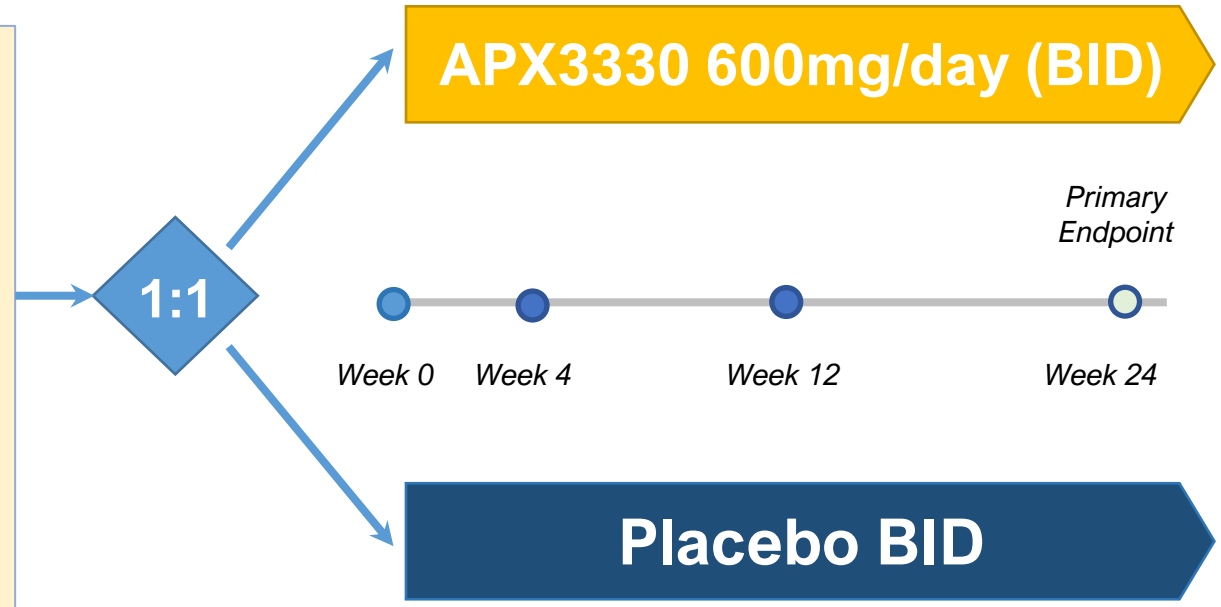
Randomized, Double-Masked, Placebo-Controlled 24-Week Trial (Similar To Eylea P3 DR Trial)

ZETA-1

25 US sites

90-100 participants with moderately severe-to-severe NPDR or mild PDR

Noncentral DME is permitted in study eye and central DME allowed in fellow eye



Eligibility Screening

Randomization

NPDR = non-proliferative diabetic retinopathy
PDR = proliferative diabetic retinopathy

103 Subjects Enrolled (FPFV Apr 2021- LPLV Aug 2022)
Top Line Announced in Early 2023

Endpoints

Primary: % subjects with ≥ 2 step improvement on DRSS (Diabetic Retinopathy Severity Scale) at wk 24

Secondary:

- DRSS worsening*
- DRSS improvement*
- Progression to vision threatening complications
- Central subfield thickness (CST)
- Best Corrected Distance Visual Acuity (BCDVA)
- Rescue subjects
- DME fellow eye status
- Safety and tolerability

Exploratory:

- Labs / PK

**Potential Phase 3 approvable endpoints*

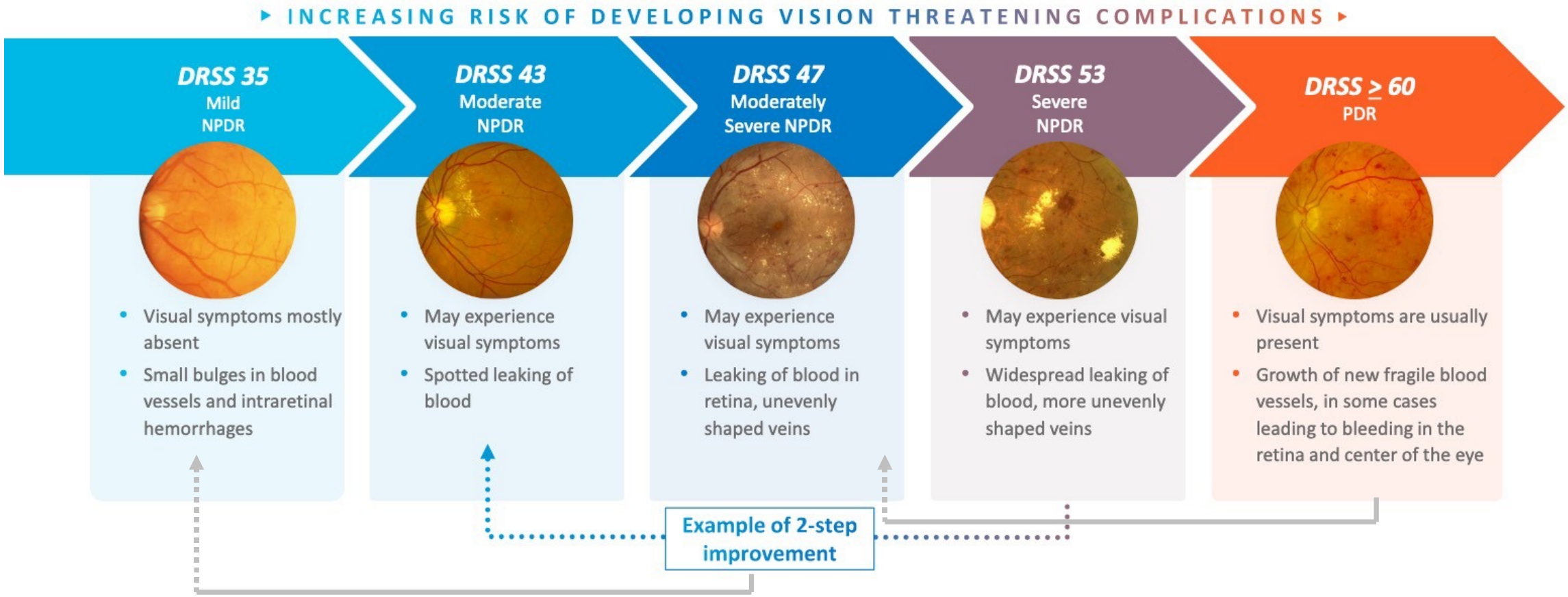
NPDR = non-proliferative diabetic retinopathy; PDR = proliferative diabetic retinopathy

ZETA-1 Clinical Trial is Sponsored by Ocuphire Pharma

Why DRSS is an Important Endpoint?

FDA Accepted Endpoint for EYLEA® in PANORAMA Pivotal DR Trial - 2 Step Improvement on the DRSS Score

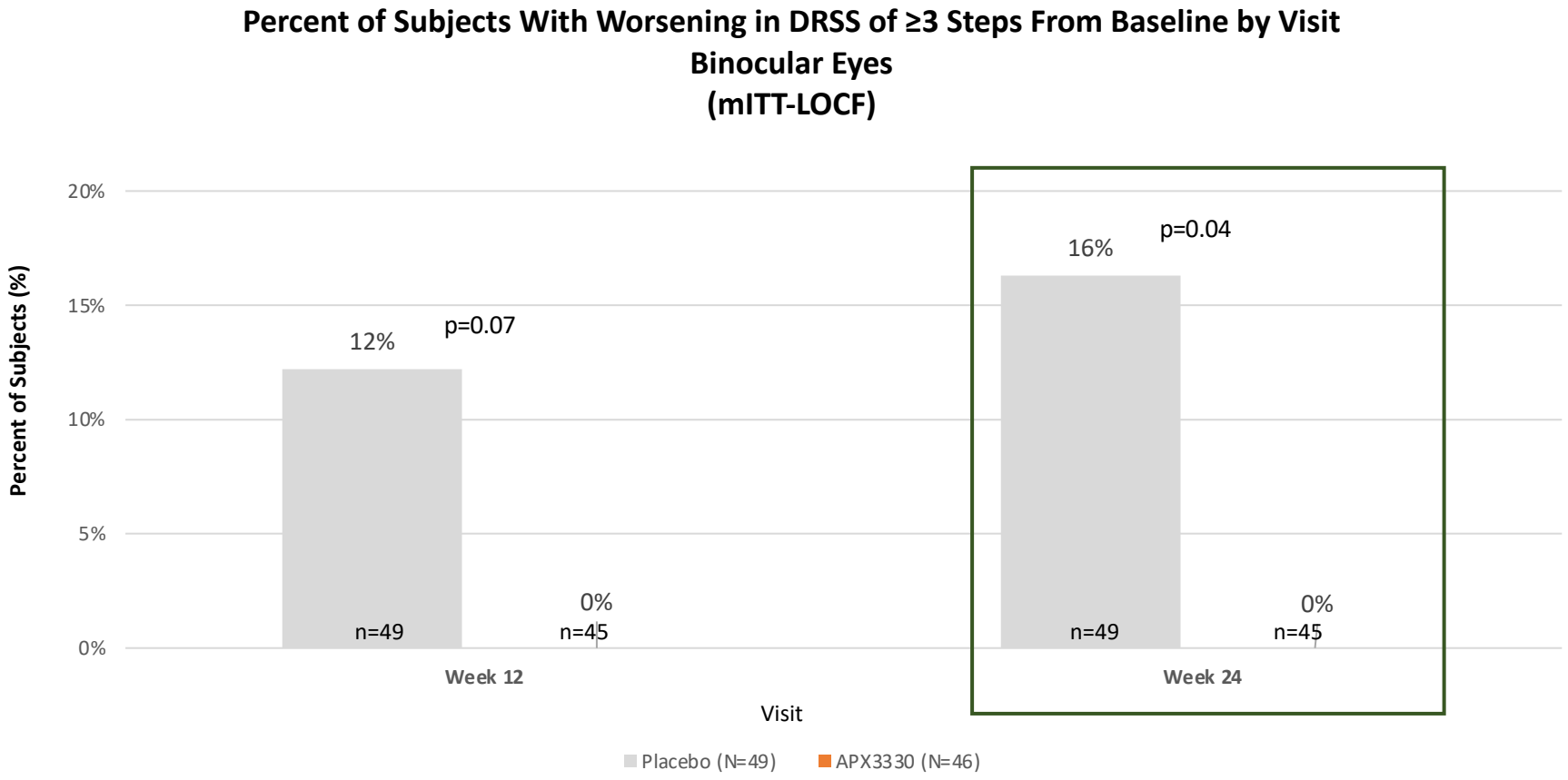
Diabetic Retinopathy Severity Scale (DRSS)



Eylea® Panorama study

Percent of Subjects With Binocular Worsening in DRSS of ≥ 3-Step

Selected Primary Registration Endpoint for Phase 3, To Be Formally Confirmed at EOP2 FDA Meeting

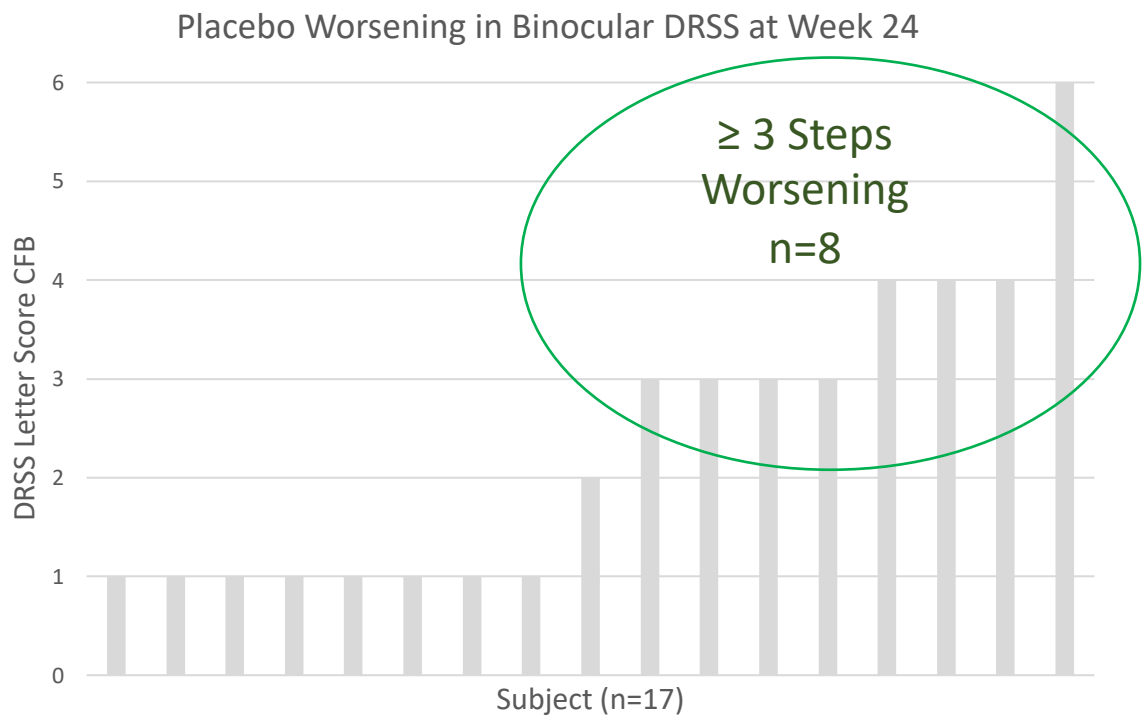


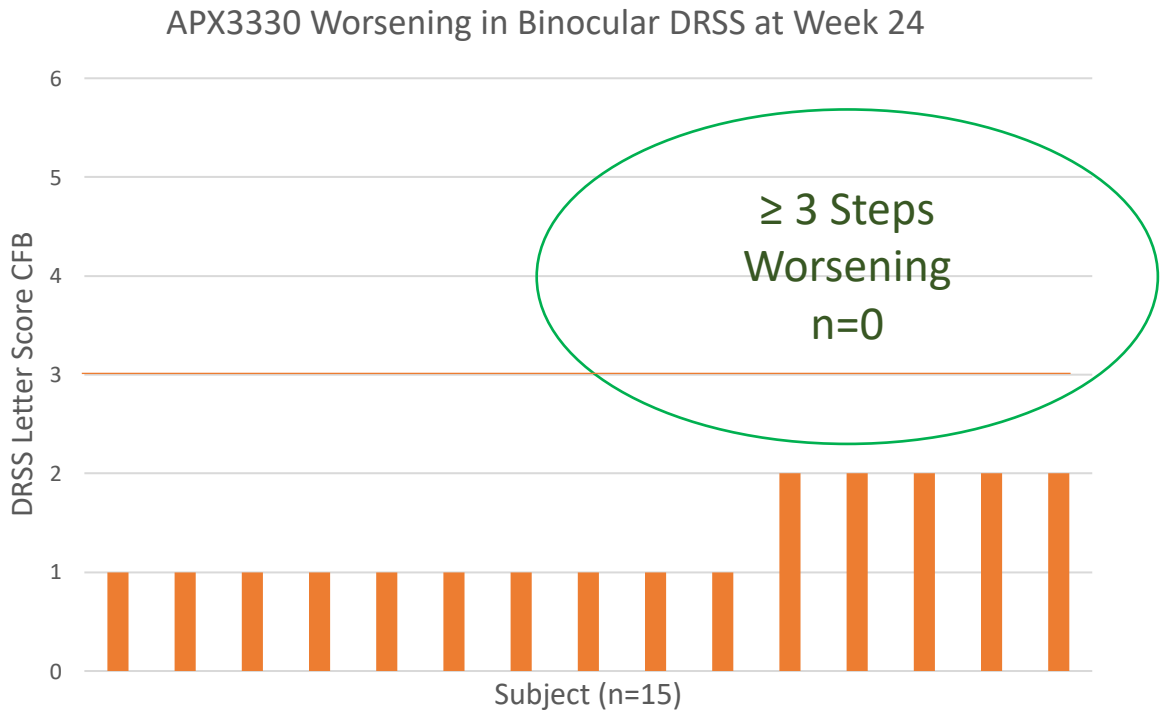
Based on extrapolation from ZETA-1, ~25% of patients may progress by ≥ 3 steps in binocular DRSS over 1 year if untreated

Source: ZETA-1 Clinical Trial
Note: Images from Central Reading Center will be reviewed prior to EOP2 FDA meeting
Note: Large "N" indicates total number of participants within each arm for the mITT-LOCF population. Small "n" indicates total number of evaluable eyes for each respective endpoint and arm.

Waterfall by Subject Binocular Change in DRSS at Week 24

8 Subjects in Placebo and 0 in APX3330 had a 3-Step DRSS Worsening at Week 24





Waterfall plots show subjects with worsening

Source: ZETA-1 Clinical Trial
Note: Images from Central Reading Center will be reviewed prior to EOP2 FDA meeting

Treatment Emergent Adverse Events

APX3330 Safety Similar To or Better Than Placebo

103
Subjects Enrolled

91
Subjects completed thru week 24

211 Treatment Emergent AEs (64 Subjects)
91 (29 Subjects) APX3330, 120 (35 Subjects) Placebo

31
Treatment-Related AEs (in 21 Subjects)

APX3330
14 AEs in 10 subjects
(10 mild, 4 moderate, 0 severe)

Placebo
17 AEs in 11 subjects
(8 mild, 9 moderate, 0 severe)

4 withdrew due to an AE	**5** lost to follow-up	**3** withdrew consent or site closure
2 APX, 2 PBO	2 APX, 3 PBO	2 APX, 1 PBO

0
Treatment-Related AEs involving liver, heart, kidney, brain, lung, or vital signs

14 SAEs (in 11 Subjects)

3 unrelated SAEs in APX3330

11 unrelated SAEs in Placebo

Oral APX3330 safety profile consistent with that seen in prior trials

ZETA-1 Clinical Trial Safety Population (subject to final review)

Landscape of Systemic Therapies for Diabetic Retinopathy

Ocuphire's APX3330 is the Most Advanced Oral Drug Moving into EOP2 Mtg and Phase 3

Company	Drug	Target/MOA	Indication	Route of Administration	Phase 1	Phase 2	Phase 3	Primary Endpoint/ Secondary Endpoints
Lilly	LY333531	Protein Kinase C inhibitor	DR	Oral	✓	✓	✗ 2006	2002: BCVA 3-line
aerpio pharmaceuticals	çAKB-9778	Tie2	DR	Subcutaneous	✓	✗ 2019		2017: 2-step DRSS @wk24
Ocuphire PHARMA	APX3330	Ref-1 inhibitor (Anti-VEGF and Anti-inflammatory)	DR	Oral	✓	✓		2020: 2-step DRSS @wk24
BAYER	BAY1101042	Guanylate Cyclase activator	DR	Oral	✓	◌		2021: 2-step DRSS @wk24
ALKAHEST	AKST4290	CCR3 Eotaxin inhibitor	DR	Oral	✓	✗ 2022		2021: 2-step DRSS @wk24
Roche	RG7774	CB2 receptor (cannabinoid)	DR	Oral	✓	◌		2020: 2-step DRSS @wk36
Boehringer Ingelheim	BI 1467335	AOC3	DR	Oral	✓	✗ 2021		2017: Primary:safety@wk12 Secondary: 2-step DRSS@wk12
Valo	OPL-0401	ROCK 1/2 inhibitor	DR	Oral	✓	◌		2021: 2-step DRSS @wk24

Note Two Tyrosine Kinase and a Plasma Kallikrein Inhibitors failed as orals in Phase 2 due to dose limiting adverse events (e.g., liver and cardiovascular)

✓ Completed	◌ Ongoing	✗ Discontinued

www.clinicaltrials.gov

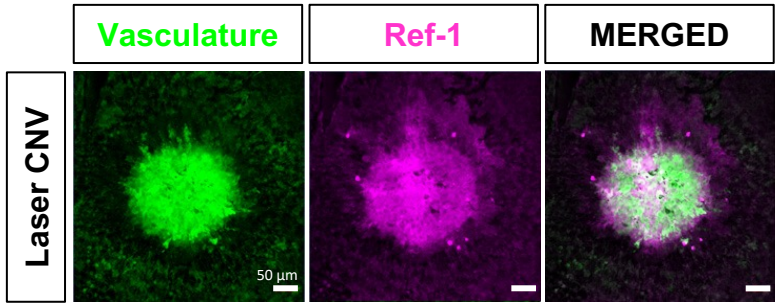
ZETA-1 Trial: Key Takeaways

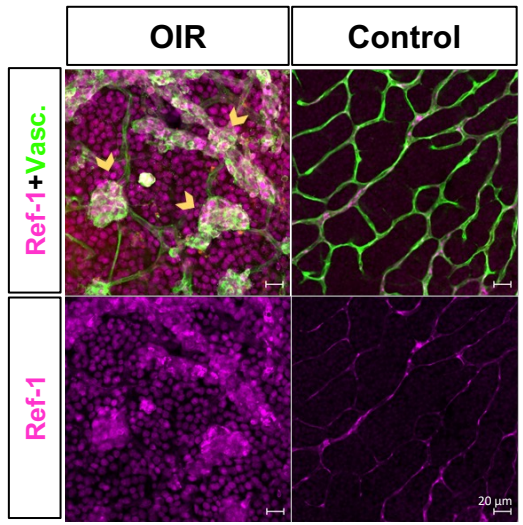
- APX3330 achieved statistical significance on a key pre-specified secondary endpoint of preventing clinically meaningful progression of diabetic retinopathy (as defined by binocular 3 or more steps worsening on the DRSS[1]) after 24 weeks of treatment
    - Trend toward more efficacy at 24 weeks vs 12 weeks, suggests that the 52-week Phase 3 trial may generate a larger signal due to an increase in % of placebo subjects who progress
- Prevention of 3-step worsening (binocular) is a suitable endpoint for an oral, systemically drug ➔ Ocuphire plans to go forward with this potential registration endpoint in Phase 3 following confirmation with the FDA in EOP2 meeting
- Oral APX3330 demonstrated favorable safety and tolerability
- Retinal KOLs feedback suggest that slowing of DR progression with an oral agent would be a useful treatment in patients with background DR and good visual function
- If approved, APX3330 could be an important new primary preventative therapeutic option that could be used in a large number of diabetic patients who are earlier in their disease

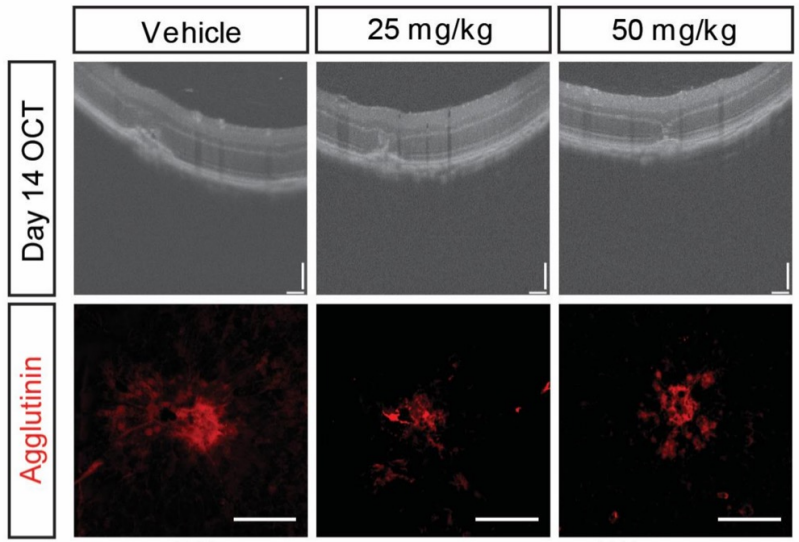
1. diabetic retinopathy severity score
Source: ZETA-1 Clinical Trial

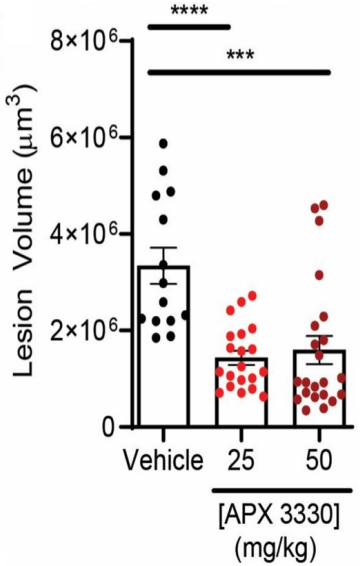
Role of APE1/Ref-1 in Neovascular Eye Disease

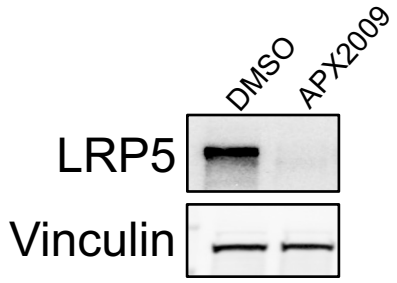
- Overexpressed in models of retinal and choroidal neovascularization (relevant to ROP, PDR, nAMD) and human nAMD
- First and second-generation redox inhibitors decrease neovascularization in vivo
- Wnt signaling downregulated by redox inhibitors in human retinal endothelial cells, including receptors and β-catenin translocation
- Underway: dissection of Wnt pathway effects, efficacy in genetic neovascularization models





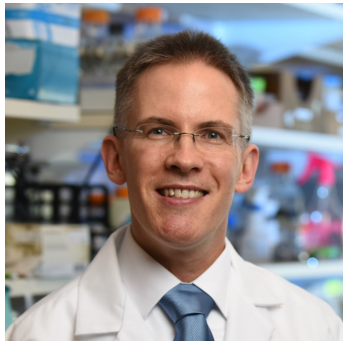






Acknowledgements: Corson Lab Ref-1 Team

- Tim Corson, PhD
- Gabriella Hartman
- Anbukkarasi Muniyandi, PhD
- Kamakshi Sishtla
- Nathan Lambert-Cheatham, MD (former)



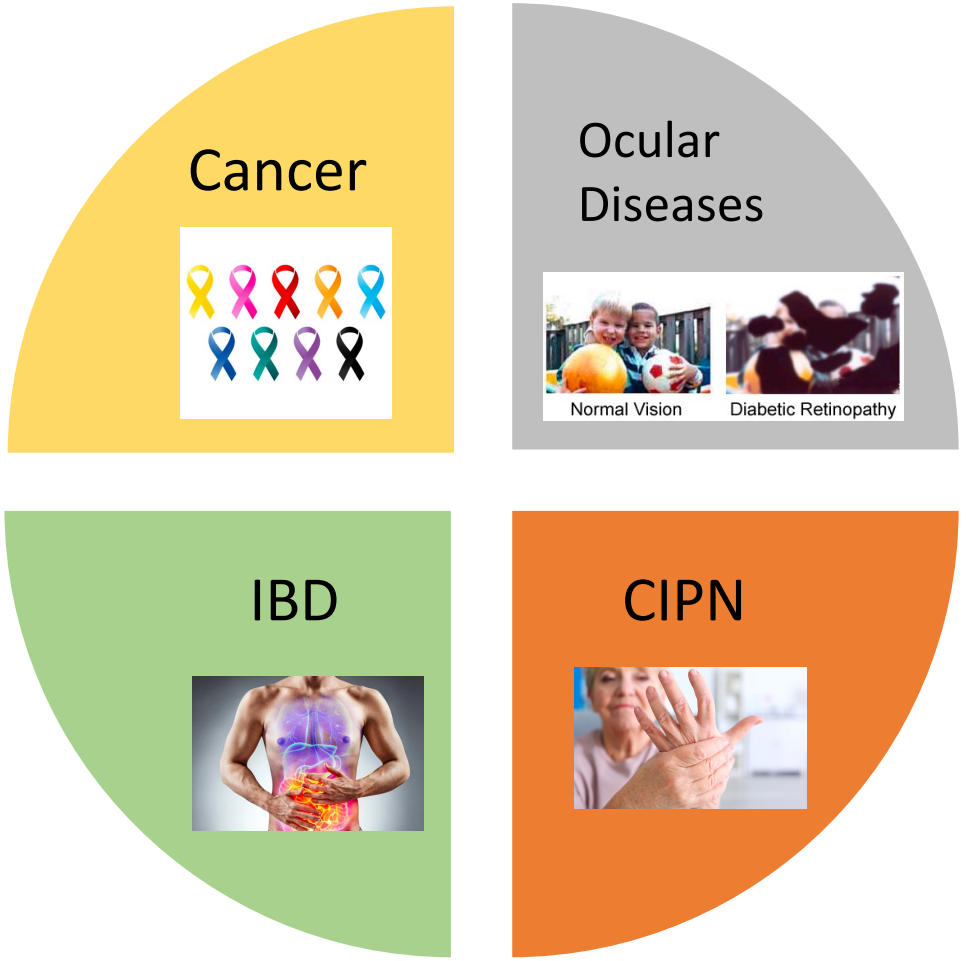
One target: Multiple Indications

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
- **Positive registration endpoints achieved in recent ph 2 trial**

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)

Inflammatory Bowel Disease (IBD)

>6.8 million
Prevalence and incidence worldwide

>700,000
Office visits
>100,000
Hospitalizations

119,000
Disabled patients (US)

>$6.3 billion
Annual healthcare costs (US)

Potential Causes IBD

Muscle contraction dysfunction in the intestine

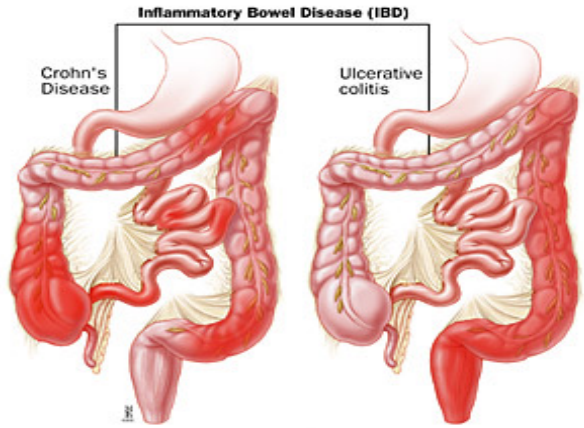
Nervous system

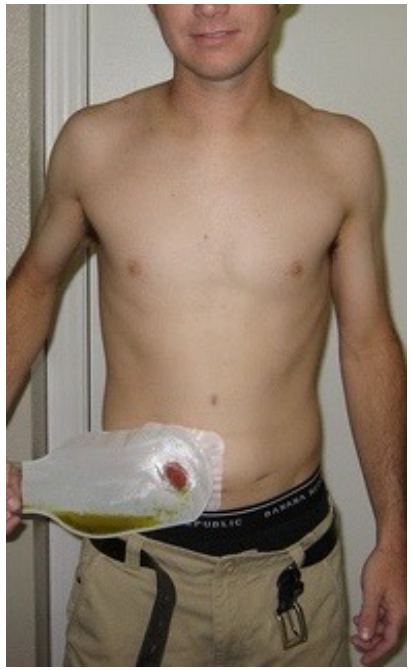
Severe infection

Early life stress

Changes in gut microbiome

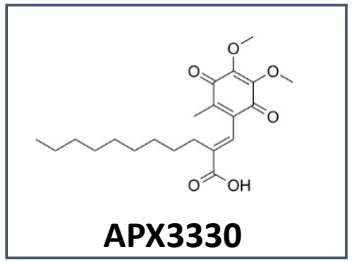
Key Diseases

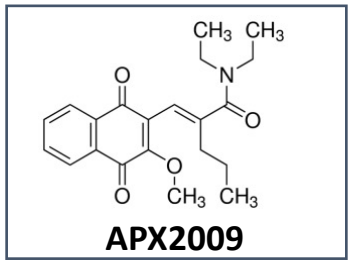


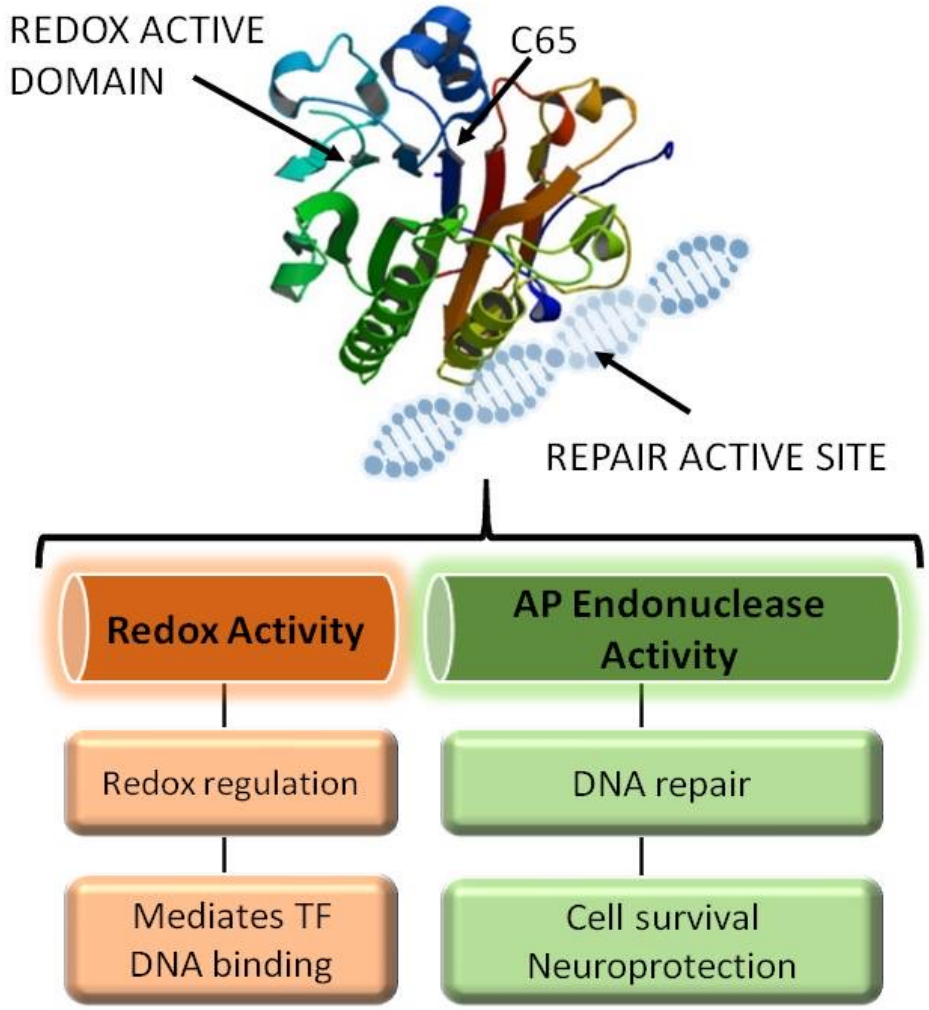


Apexian Pharmaceuticals

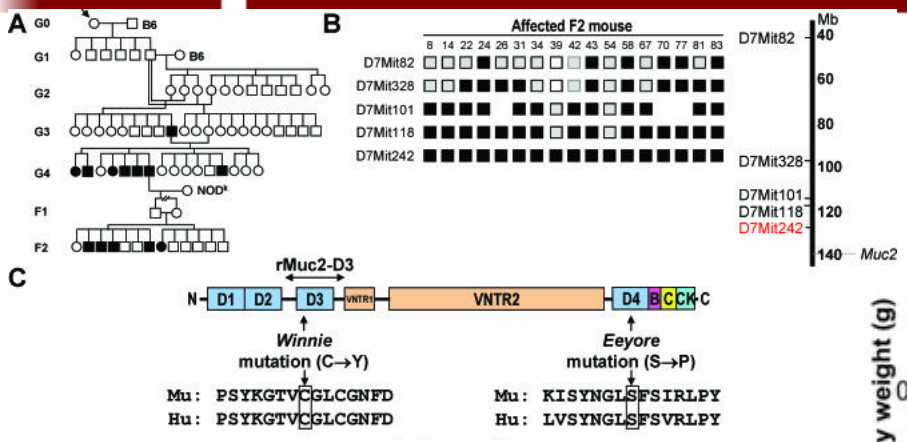
TARGET: Apurinic/Apyrimidinic Endonuclease/ Redox-factor 1 (APE1/Ref-1)

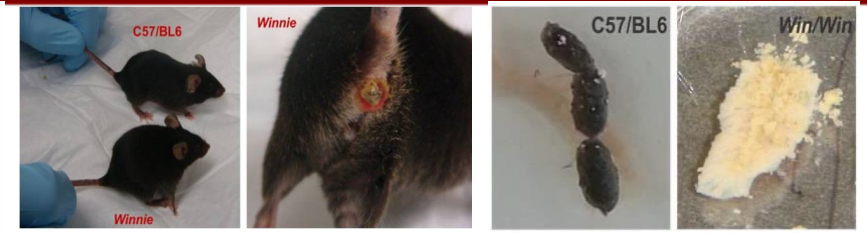


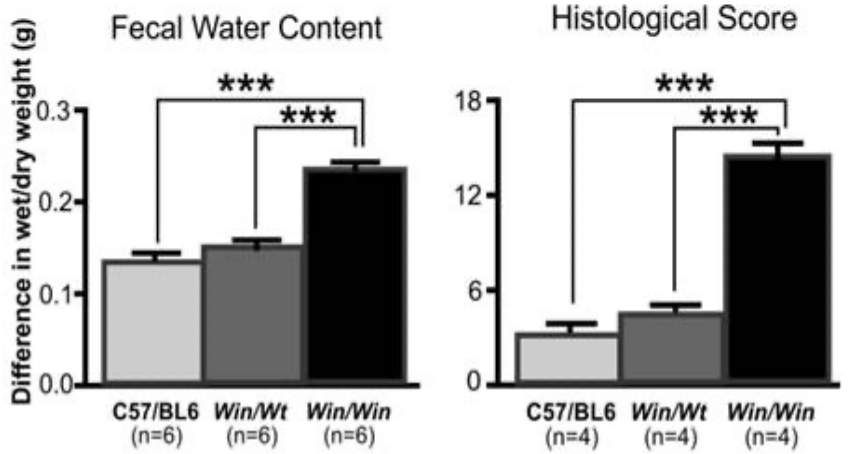


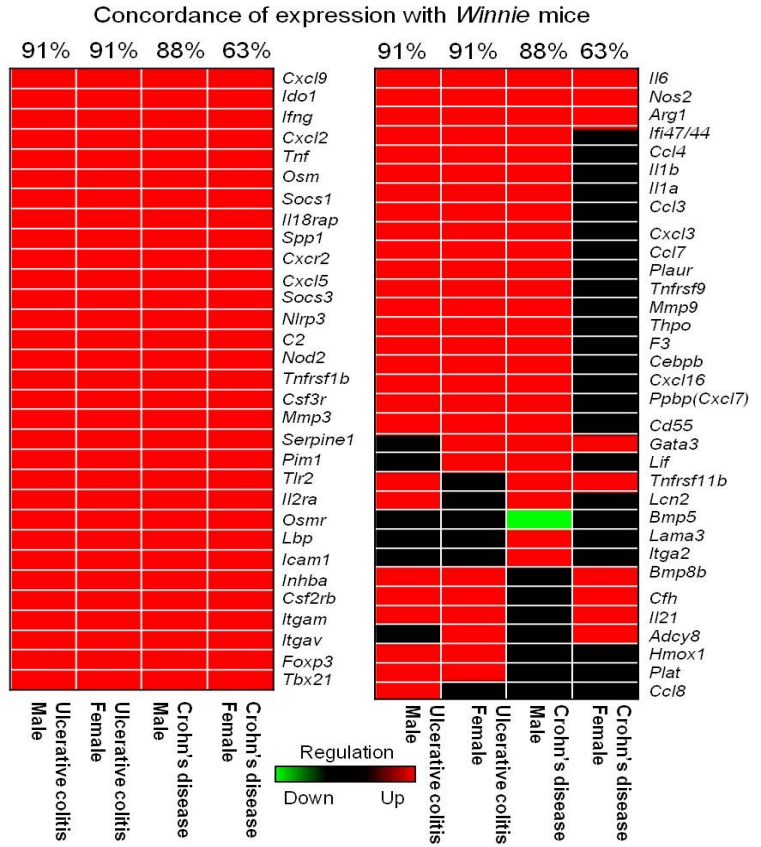


Winnie mouse model of spontaneous chronic colitis









Chronic colitis in Winnie is caused by a primary epithelial cell defect due to **a point mutation in the *Muc2* gene** resulting in aberrant mucin-2 biosynthesis leading to reduced secretion of mucus, a thinner mucosal layer and increased intestinal permeability, which is very similar to active ulcerative colitis in humans. Extensive studies done in Winnie has proven it to be the best available murine model to study human chronic colitis and its pathogenesis.

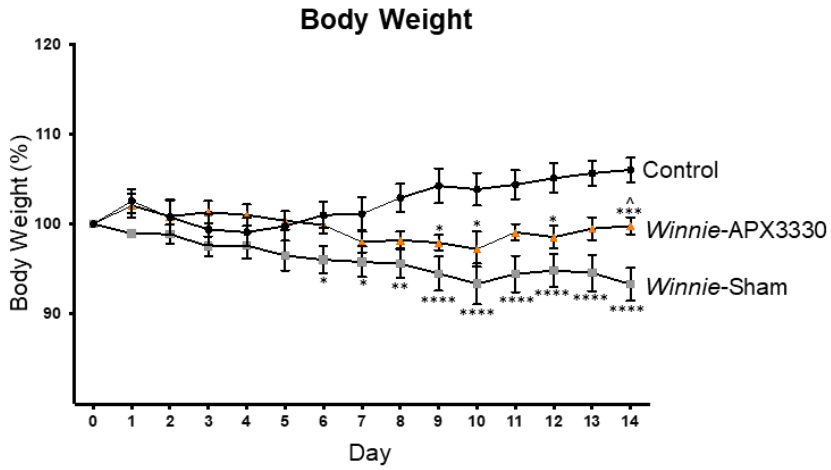
Heazlewood CK, Cook MC, Eri R, Price GR, Tauro SB, Taupin D, Thornton DJ, Png CW, Crockford TL, Cornall RJ, Adams R, Kato M, Nelms KA, Hong NA, Florin TH, Goodnow CC, McGuckin MA. Aberrant mucin assembly in mice causes endoplasmic reticulum stress and spontaneous inflammation resembling ulcerative colitis. *PLoS Med*. 2008 4;5(3):e54.

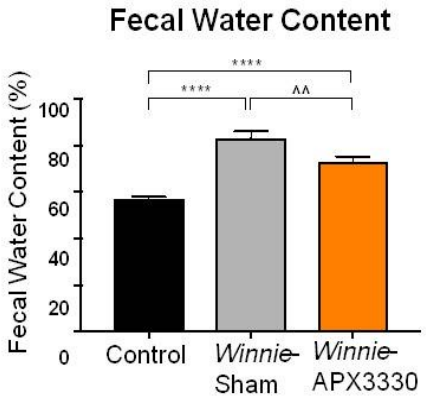
Winnie mice develop spontaneous chronic colitis at 6 weeks of age in pathogen-free conditions and progresses over time to severe colitis by 12-16 weeks. **A.** *Winnie* mice display symptoms of pain, rectal bleeding, and chronic diarrhea. **B.** Fecal water content and histological score in the distal colon of *Winnie (Win/Win)* mice vs C57BL/6 and heterozygous *Win/Wt* mice.

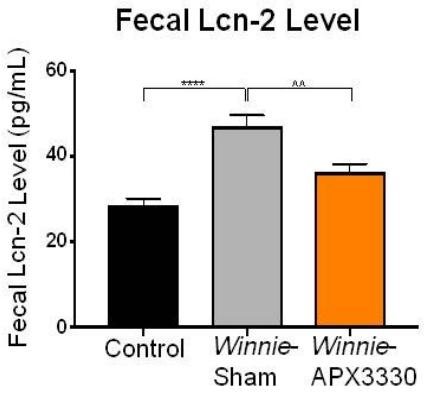
Rahman AA, Robinson AM, Jovanovska V, Eri R, Nurgali K. Alterations in the distal colon innervation in Winnie mouse model of spontaneous chronic colitis. *Cell Tissue Res.* 2015 Dec;362(3):497-512.

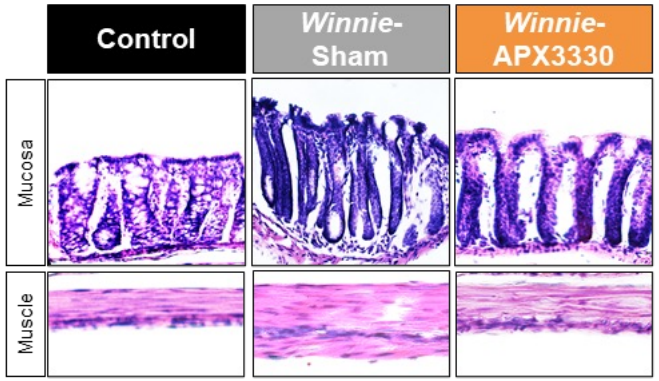
Heat map representation of upregulated (red), downregulated (green), unchanged (black) genes associated with colonic inflammation determined by RNAseq (unpublished). ***Winnie* mice show up to 91% similarity in the expression of inflammation-associated genes in male and female UC patients.** In comparison, only 16.1% of genes in DSS-treated mice, 12.5% in TNBS-treated rats, 68.5% in piroxicam-accelerated colitis in IL-10$^{-/-}$ mice similarity to human IBD.

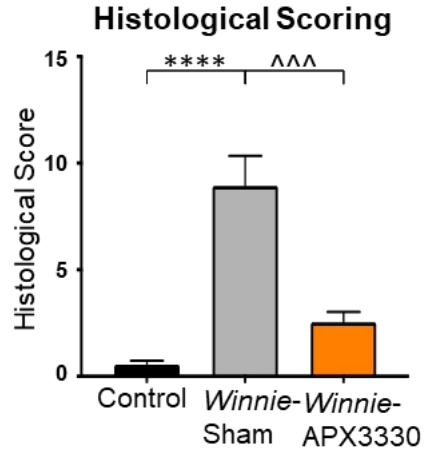
APX3330 attenuates intestinal inflammation in *Winnie* mice with chronic colitis









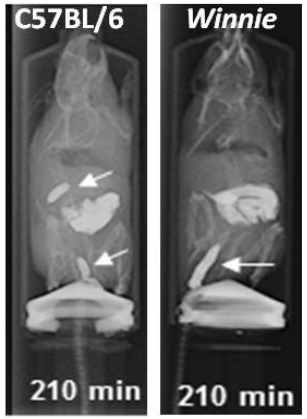


APX3330 (25 mg kg^{-1}) x2/day with 8h interval x14days, n=8/group

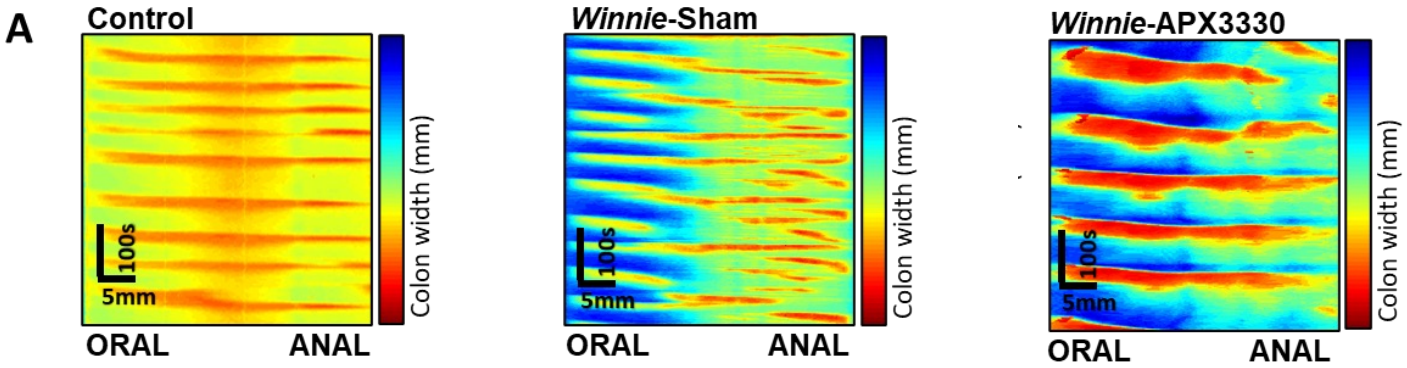
(Sahakian, PIs, Co-Is, *Inflam Bowel Dis*, 2021)

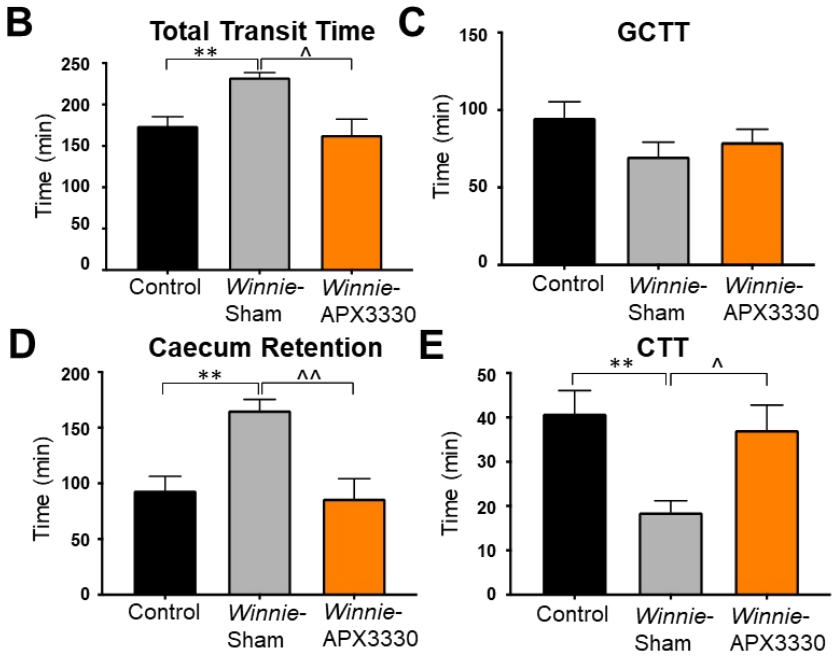
Data expressed as mean ± SEM, **P*<0.05, *****P*<0.0001 compared with C57BL/6 control mice; ^*P*<0.05, ^^^^*P*<0.0001 compared with *Winnie* sham-treated mice.

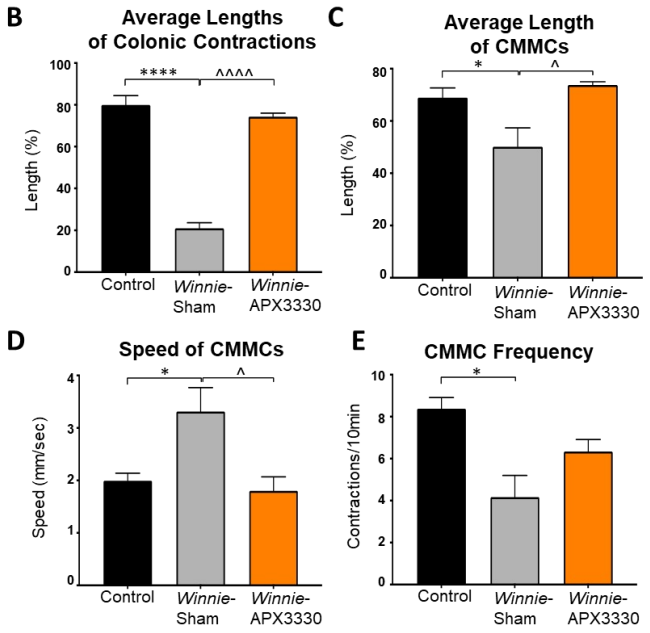
APX3330 treatment improves intestinal function in *Winnie* mice with chronic colitis



Radiographic images captured movement of the contrast agent and barium sulfate from the stomach to the expulsion of the first pellet (n = 7/group)







Video recordings from ex vivo whole colon samples were transposed into spatiotemporal maps. Contractions were distinguished as red and relaxation as blue (n = 6/group).

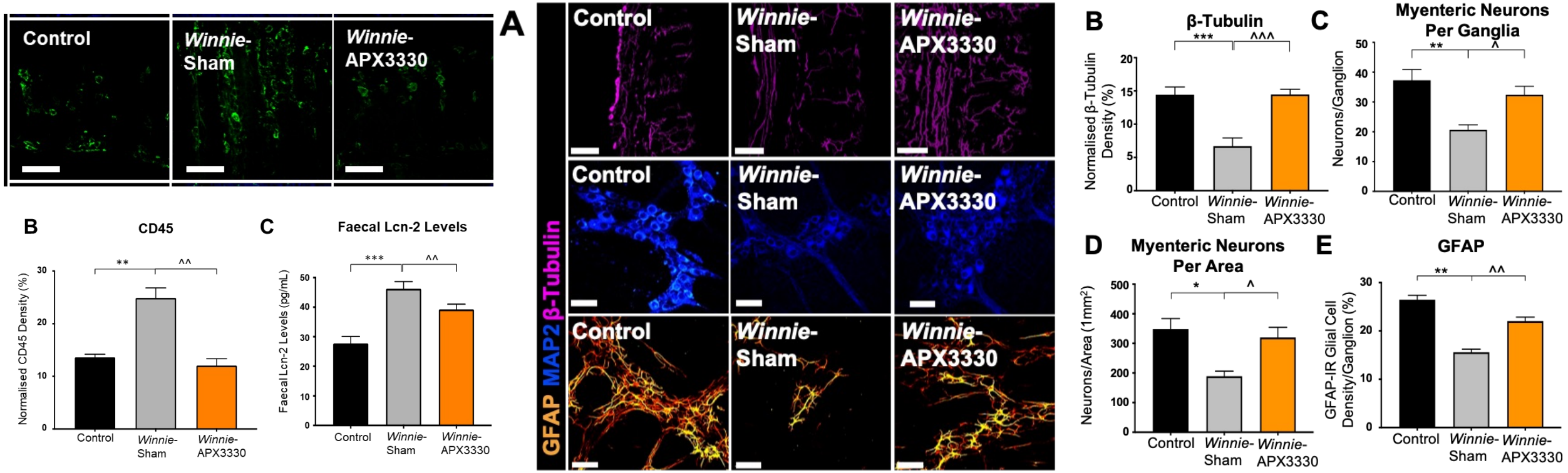
(Sahakian, PIs, Co-Is, *Inflam Bowel Dis*, 2021)

Data expressed as mean ± SEM, **P*<0.05, *****P*<0.0001 compared with C57BL/6 control mice; ^*P*<0.05, ^^^^*P*<0.0001 compared with *Winnie* sham-treated mice.

APX3330 provides both anti-inflammatory and neuroprotective effects

Reduced number of CD45+ leukocytes in the mucosa and level of faecal lipocalin-2 after APX3330 treatment

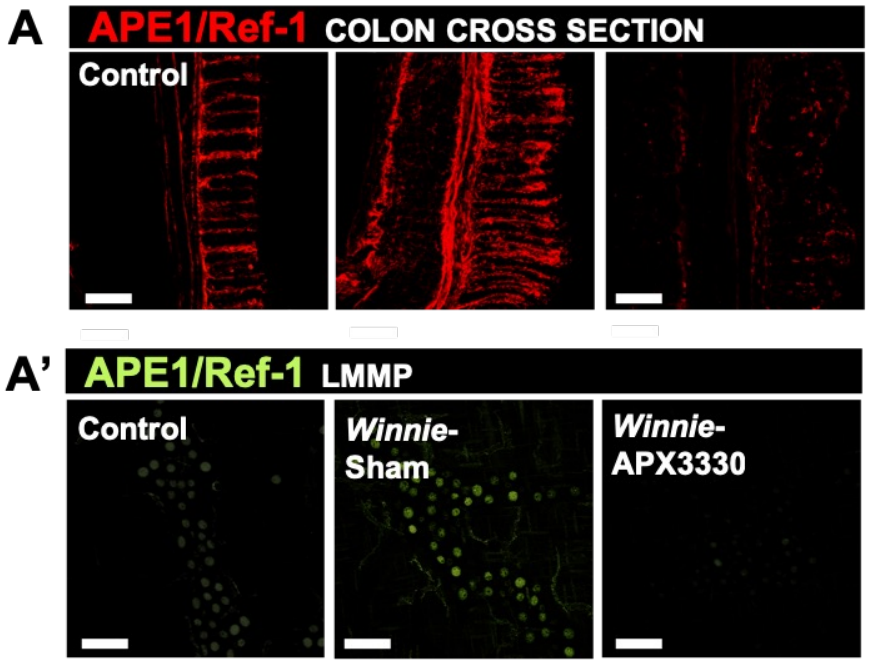
Increased density of nerve fibers, number of myenteric neurons and glial cells after APX3330 treatment

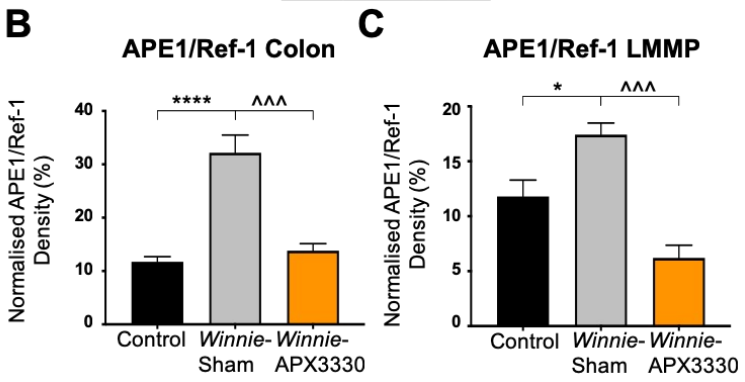


Data expressed as mean ± SEM, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with C57BL/6 control mice; ^^$P < 0.01$ compared with Winnie sham-treated mice, n=5/group

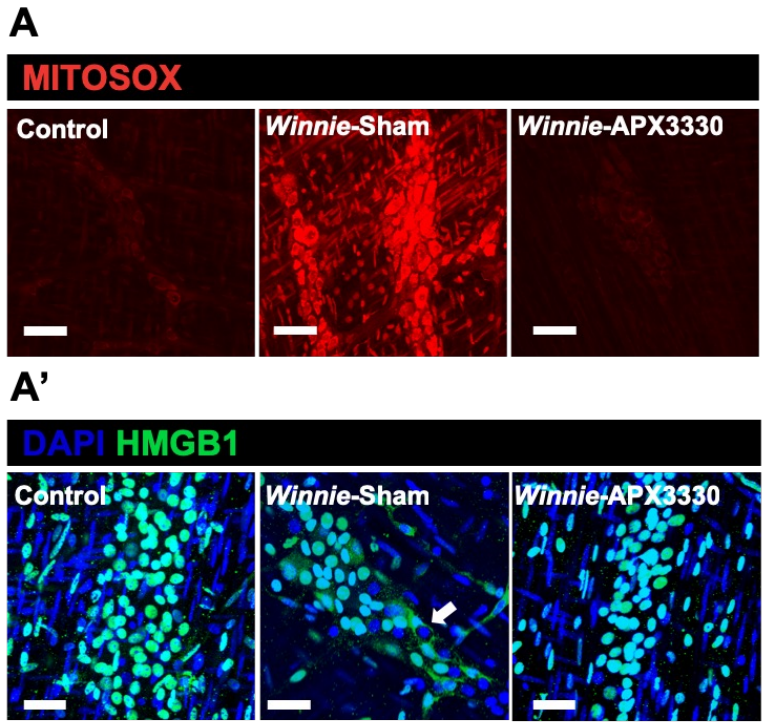
Anti-oxidative and DNA repair mechanisms of APX3330 treatment

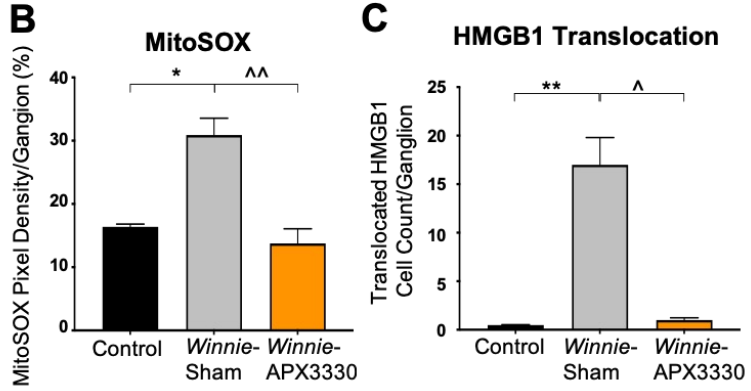
Inhibition of APE1/Ref-1 overexpression



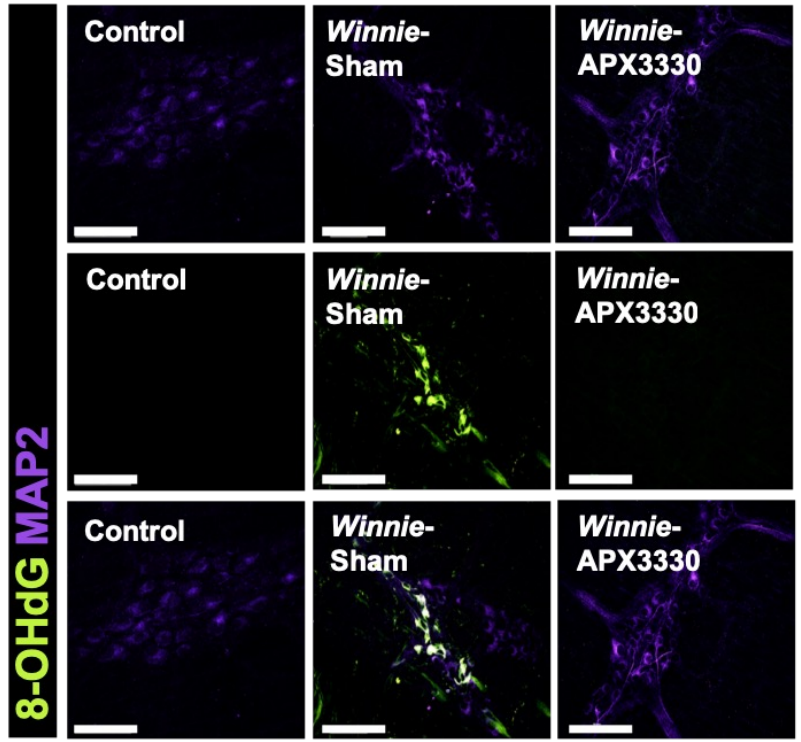


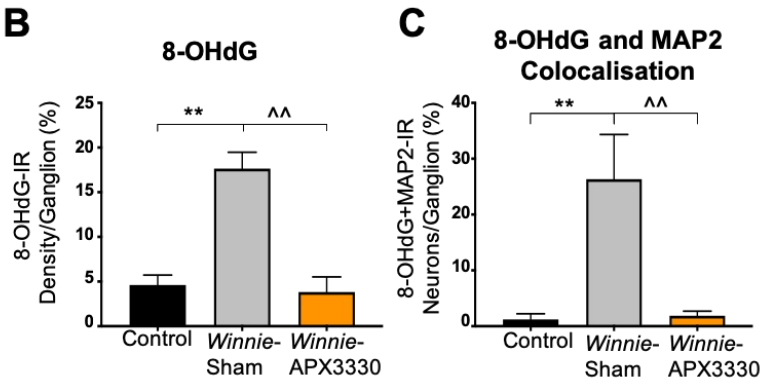
Inhibition of ROS and HMGB1 translocation





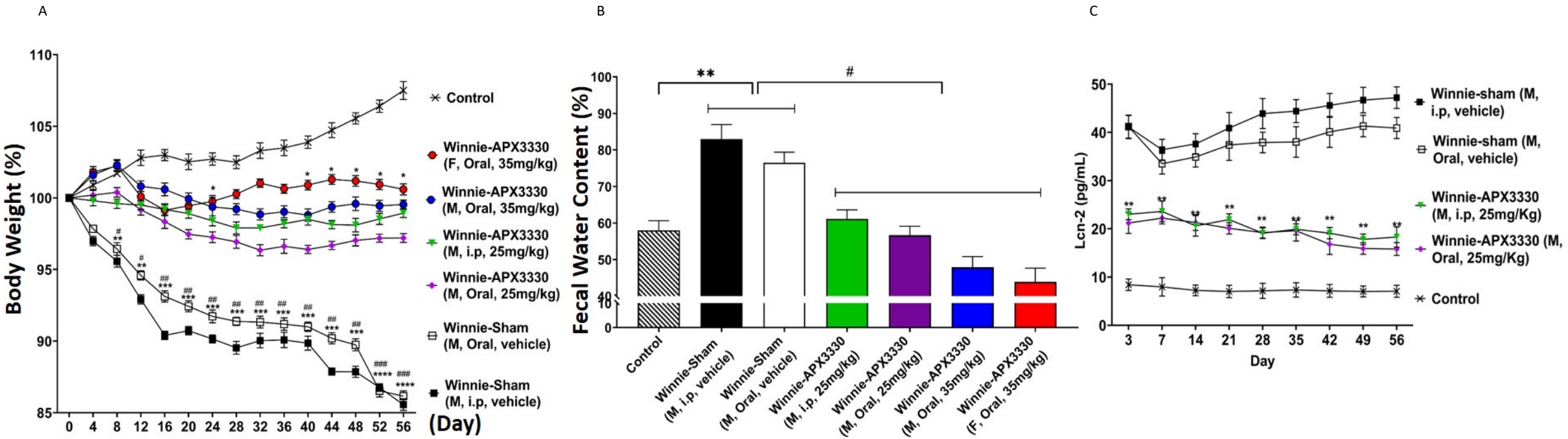
Inhibition of DNA damage





Data expressed as mean ± SEM, *$P < 0.05$, **$P < 0.01$ compared with C57BL/6 control mice; ^$P < 0.05$, ^^$P < 0.01$ compared with Winnie sham-treated mice, n=5/group.

Long-term effects of APX3330 treatment in *Winnie* mice with chronic colitis



Effects of APX3330 administered x2/day for 14 days via oral gavage (25 and 35 mg kg^{-1}) and intraperitoneally (i.p., 25 mg kg^{-1}) on the body weight up to 56 days (**A**) and fecal water content at day 56 (**B**). Fecal lipocalin-2 was measured weekly for up to 56 days (25 mg kg^{-1}, i.p. and oral gavage) (**C**). N=6/group, M=males, F=females (unpublished).

APX3330 & Inflammatory Bowel Disease

APX3330 in the IBD mouse models…

- Blocks inflammation
- Reduces clinical symptoms: decreased body weight, rectal prolapse, edema and observed bleeding
- Corrects colonic contractile activity and GI transit
- Regeneration of nerve fibers and glial cells in colon
- Prevents oxidative stress and DNA damage in myenteric neurons
- Corrects intestinal permeability
- Normalizes GI anti-bacterial defense

Proposed APX3330 Phase 1b trials
Crohn's Disease (CD) & Ulcerative Colitis (UC)

A Phase 1b, Placebo-Controlled, Double-Blind, Single Ascending Dose Study to Assess Safety, Pharmacokinetics, Pharmacodynamics and Efficacy of APX3330 in Patients with a Diagnosis of Crohn's Disease.

- Study Population: 12-16 Patients diagnosed with CD with a Crohn's Disease Activity Index (CDAI) between 220- 450
- Study Period: Each subject will be followed for 57 days (8 weeks) after receiving study drug. Total study conduct (including all cohorts) will require approximately 8-12 months.
- The Objectives of this study are to:
  - Evaluate the safety and tolerability of APX3330 in patients with moderate to severe CD.
  - Evaluate the pharmacokinetics (PK) and pharmacodynamics (PD) of APX3330.
  - Evaluate exploratory efficacy endpoints in patients with CD.

A Phase 1b, Placebo-Controlled, Double-Blind, Single Ascending Dose Study to Assess Safety, Pharmacokinetics, Pharmacodynamics and Efficacy of APX3330 in Patients with a Diagnosis of Ulcerative Colitis Disease.

- Study Population: 12-16 Patients diagnosed with UC with a modified Mayo score between 5-9.
- Study Period: Each subject will be followed for 57 days (8 weeks) after receiving study drug. Total study conduct (including all cohorts) will require approximately 8-12 months.
- The Objectives of this study are to:
  - Evaluate the safety and tolerability of APX3330 in patients with moderate to severe UC.
  - Evaluate the pharmacokinetics (PK) and pharmacodynamics (PD) of APX3330.
  - Evaluate exploratory efficacy endpoints in patients with UC.

Apexian Pharmaceuticals

IBD Team

Professor Kulmira Nurgali

University of Melbourne & Victoria University

- Enteric Neuropathy
- GI Pathology
- Preclinical models

Dr Rhian Stavely

Harvard Medical School

- Enteric Neuropathy
- Stem Cells
- 3D Organoids, cell culture, preclinical models

Co-Investigators

Professor Allan Goldstein

Harvard Medical School

- Chief Surgeon
- Paediatric GI Disorders, including IBD

Professor Joel Bornstein

University of Melbourne

- Enteric Neuropathy
- GI Physiology
- Functional studies

Professor Raj Eri

University of Tasmania

- Immunology
- Animal models of IBD and IBD-induced cancer
- 3D organoids
- Clinical trials

Acknowledgements:

- ✓ **Randy Wireman – Research Analyst**
- ✓ **Mahmut Mijiti – former postdoctorate**
- ✓ **Eyram Kpenu – MD/PhD student**
- ✓ **Silpa Gampala – postdoctorate in Fishel lab**
- ✓ **Megan Boner - Irish exchange MS student**

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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 and the Riley Children's Foundation.

Disclosure:

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

- **Collaborators**
  - **Dr. Melissa Fishel (cancer)**
    - **Dr. Silpa Gampala**
  - **Dr. Millie Georgiadis (structure/function)**
  - **Dr. Tim Corson (eye)**
    - **Gabriella Hartman**
    - **Anbakkarasi Muniyandi, PhD**
    - **Kamakshi Sishtla**
    - **Natham Lambert-Cheatham, MD**
  - **Dr. Jun Wan and Dr. Sheng Liu (C3B bioinformatics)**
  - **Dr. Chi Zhang - bioinformatics**
  - **Dr. Kulmira Nurgali (Australia - IBD)**
  - Dr. Reuben Kapur (Heme) - DSS
  - Dr. Karen Pollok (in vivo therapeutics)
  - Dr. Jill Fehrenbacher (CIPN)

Apexian Pharmaceuticals

Ocuphire PHARMA

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.

2009: Funded by Indiana VC Group

Sept 2009 Received **Phase 1 STTR**

2010: Private Investors

Oct 2012 Private Investors & BioCrossroads

Series A

July 2013 Received **Phase 1 SBIR**

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**

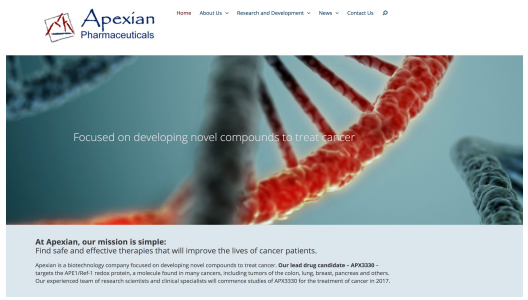
Efficacy of APE1 inhibitor vs panc and other cancers

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**

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 2b trial in DR/DME opens- completed 2022

Ocuphire completes Phase 2b trial in DR/DME. Data reported out in Q1 2023

2014 2015 2016 2017 2018 2019 2020 2021 2022 2023

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.