

# **The importance of dose response for the biology of synthetic triterpenoids**

**Karen T. Liby**

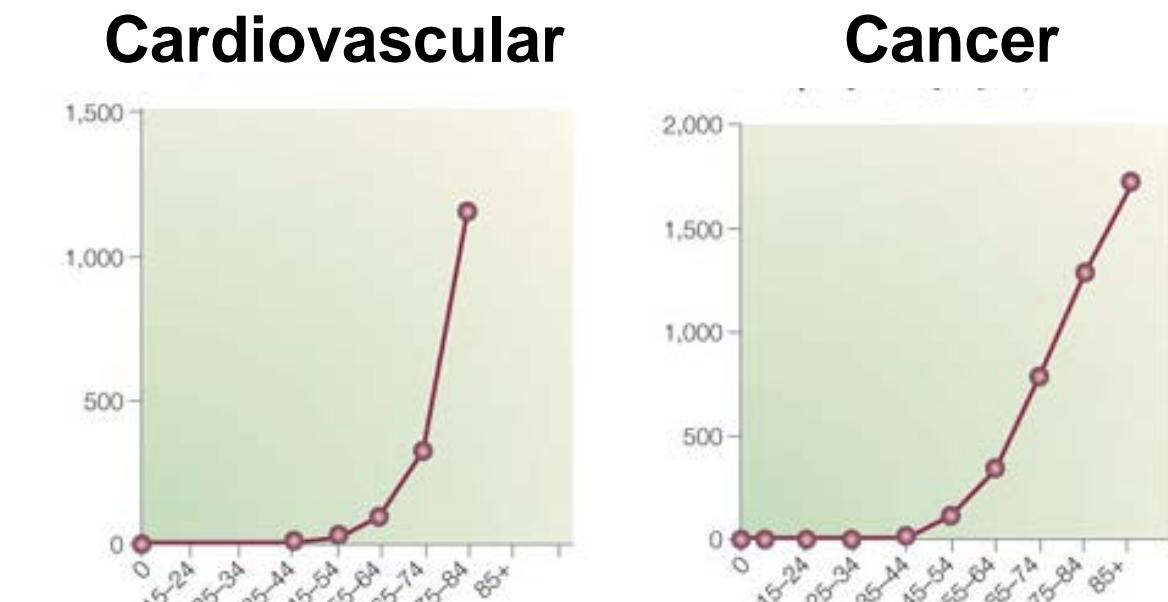
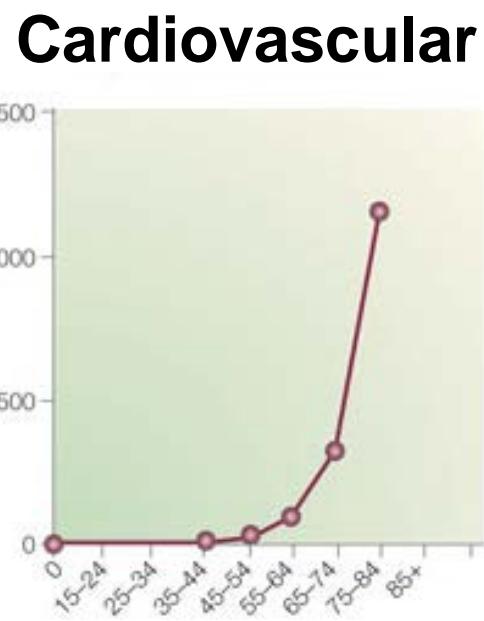
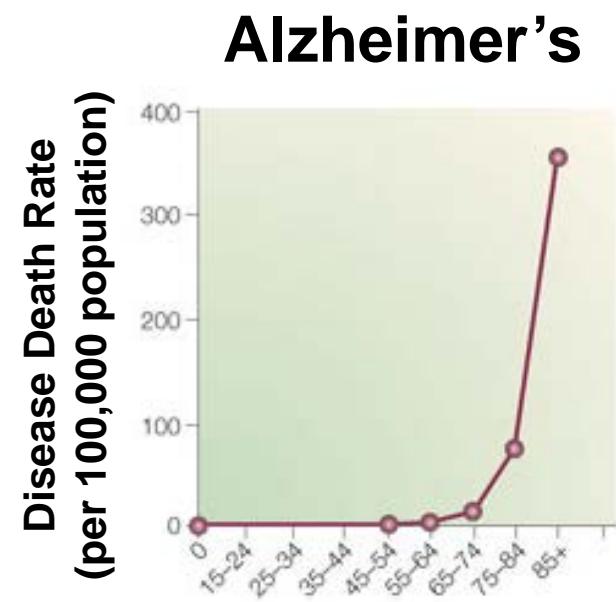
**Geisel School of Medicine at Dartmouth**

**Dose-Response 2013**

**Amherst, MA**

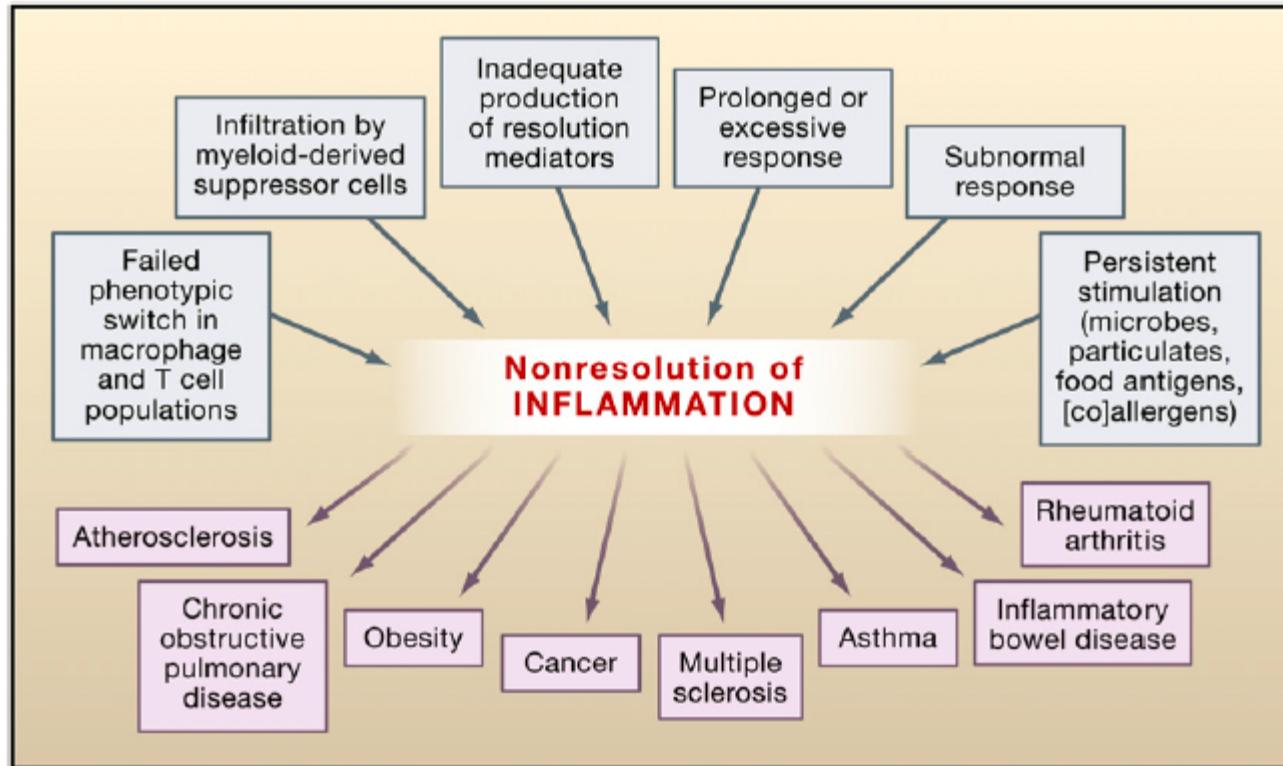
**April 24, 2013**

# Disease-specific death rates as a function of time



Age Range (years)

# Non-resolving inflammation



# Triterpenoids

Exist widely in nature, especially in higher plants

> 6,000 triterpenoids reported

Medicinal uses in Asian countries

Anti-inflammatory

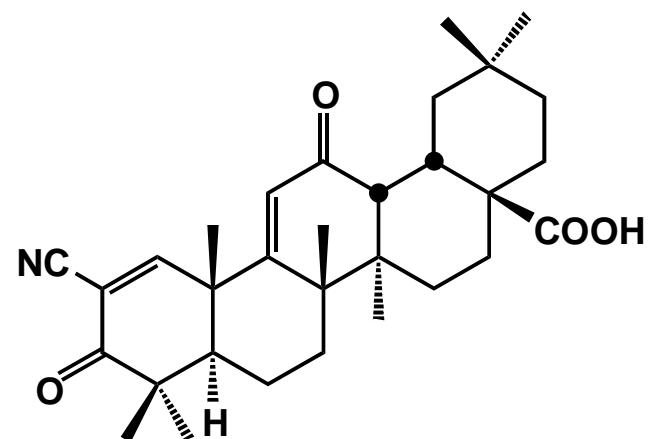
Anti-tumorigenic

Poor potency

Resemble steroids

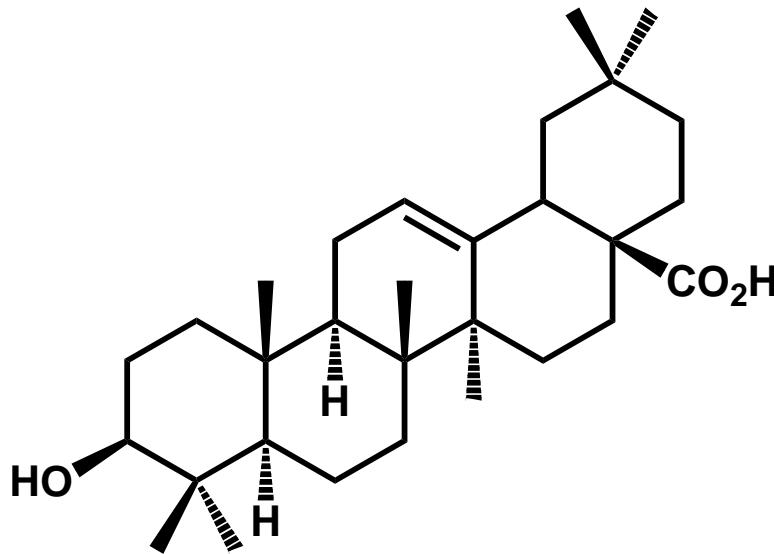
Biosynthesis

Pleiotropic activities



Synthesized by cyclization of squalene

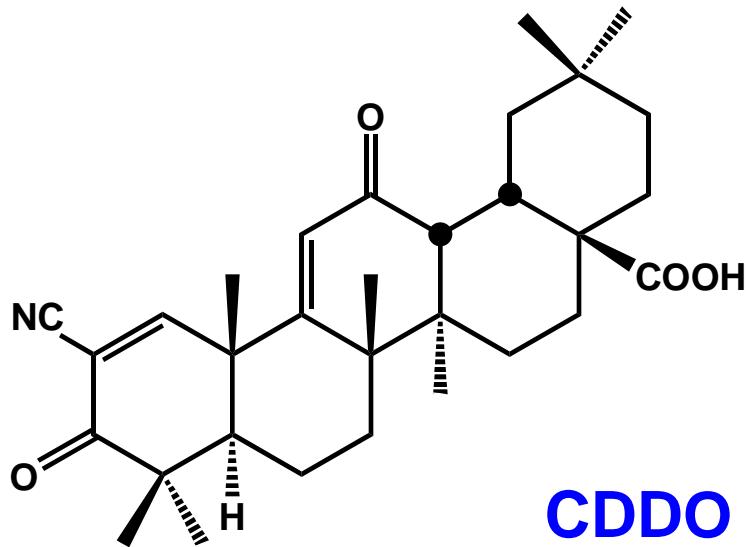
# Oleanolic acid



Oleanolic Acid

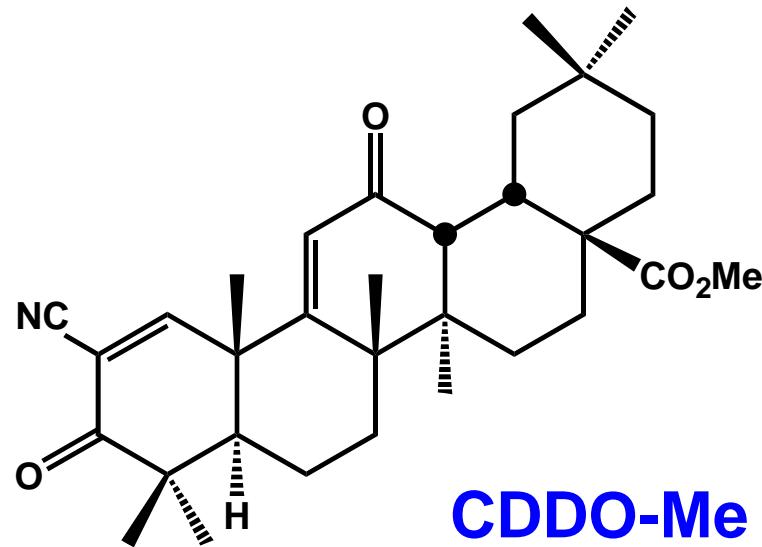
- Weak anti-inflammatory agent
- Weak anti-carcinogenic agent
- Readily, cheaply available in kilogram quantities from natural sources (e.g., olives, olive leaves)

# Triterpenoids



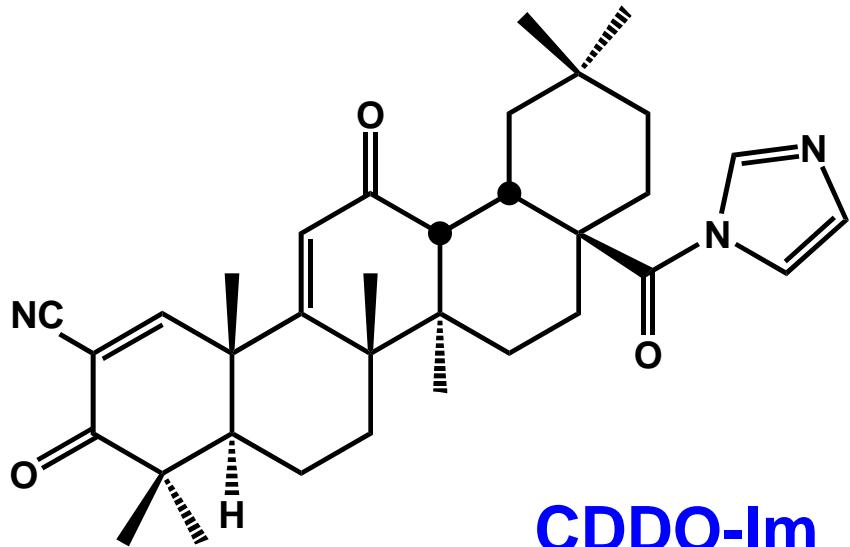
**CDDO**

Phase I trial terminated



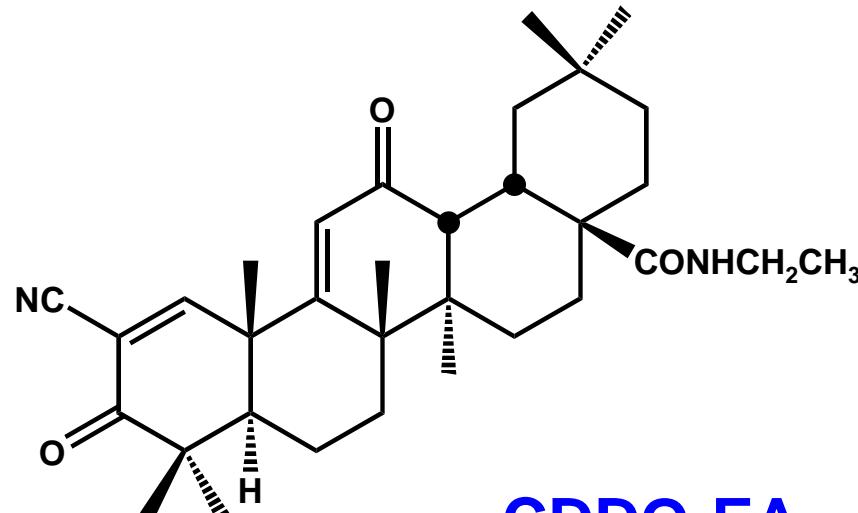
**CDDO-Me**

CDDO-Methyl ester  
Phase III trial terminated



**CDDO-Im**

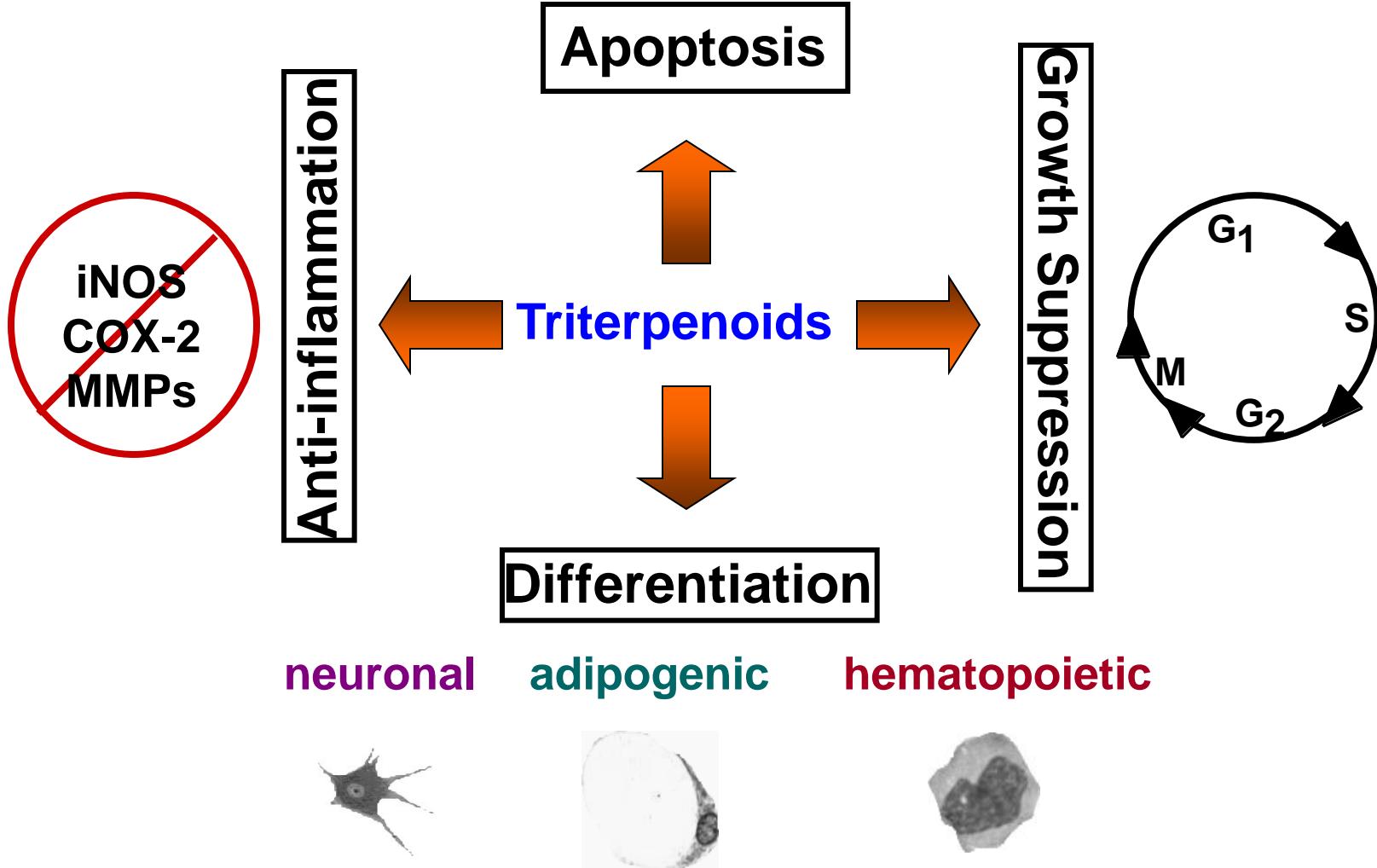
CDDO-Imidazolide



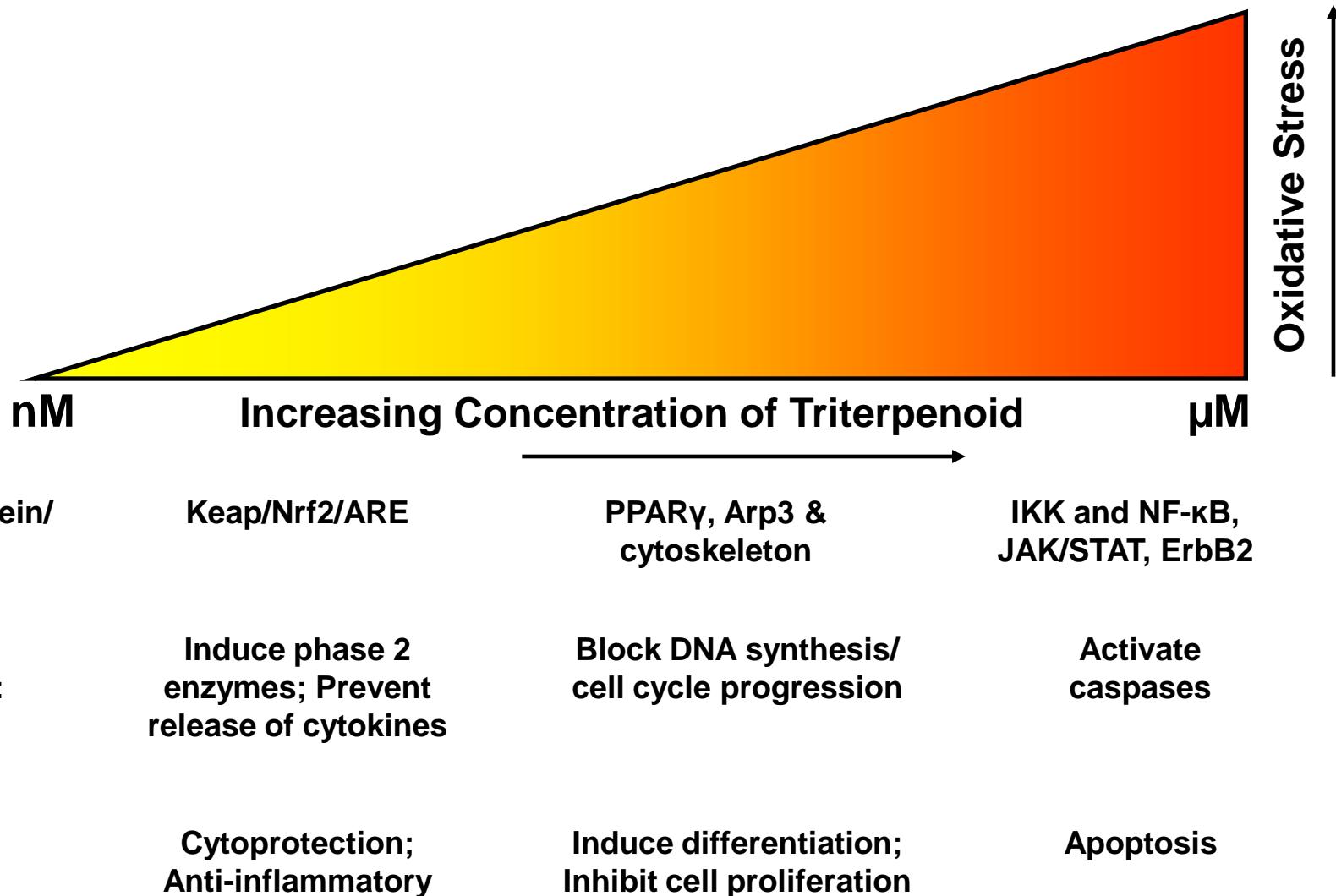
**CDDO-EA**

CDDO-Ethyl amide

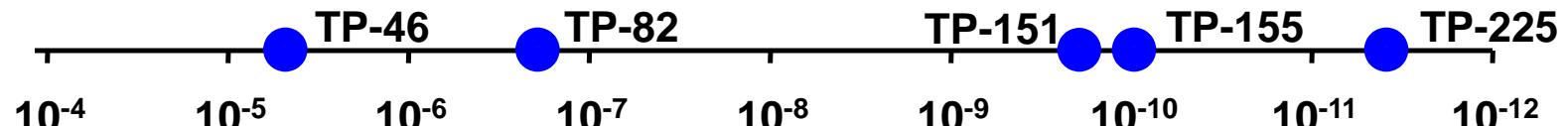
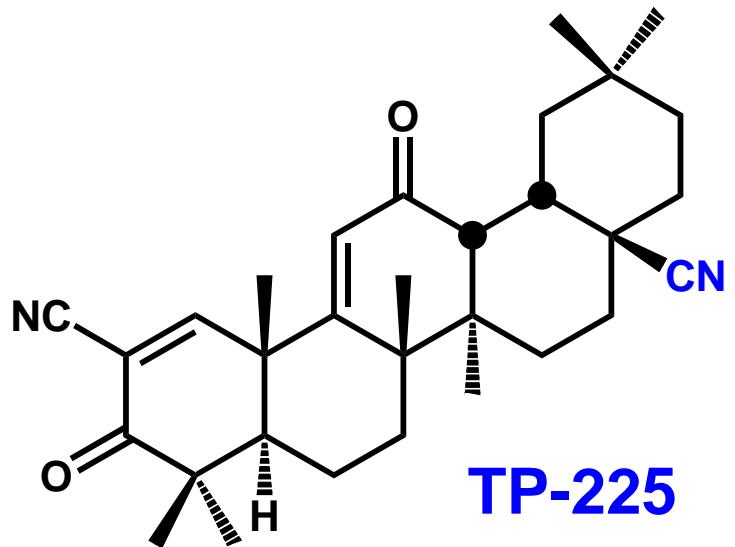
# Synthetic triterpenoids are multifunctional molecules



# Biological responses to triterpenoids are strongly dependent on dose



**Low nM concentrations  
of triterpenoids activate the  
Nrf2 cytoprotective pathway**

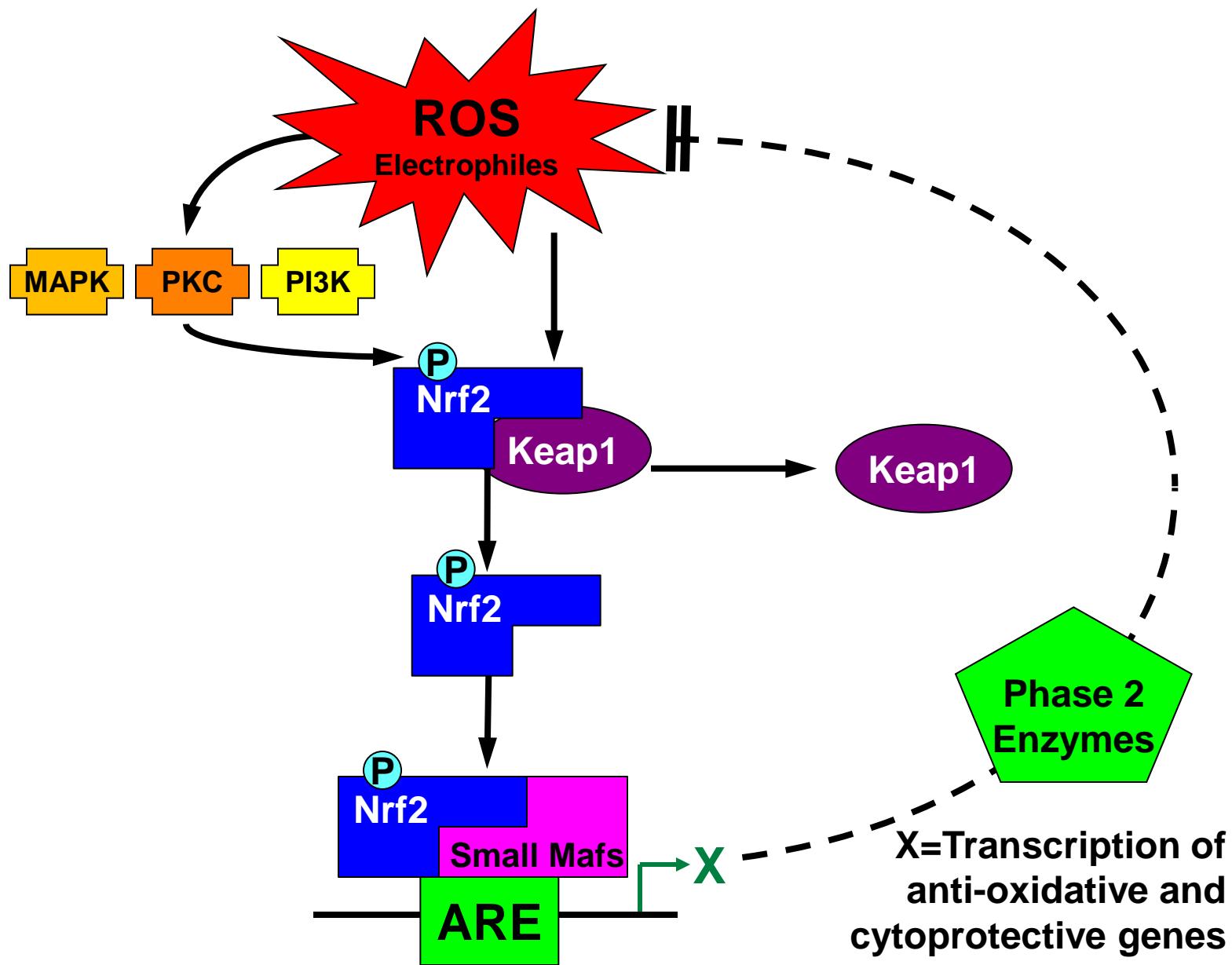


**IC-50, Suppression of Induction of iNOS**

# CDDO-Im increases expression of genes regulated by Nrf2

<u>Gene</u>	Fold Increase			
	CDDO		CDDO-Im	
	<u>4 hrs</u>	<u>12 hrs</u>	<u>4 hrs</u>	<u>12 hrs</u>
<b>heme oxygenase (decycling) 1</b>	<b>19.70</b>	<b>17.15</b>	<b>90.51</b>	<b>111.43</b>
ferritin, heavy polypeptide 1	2.64	6.06	2.64	6.96
NAD(P)H dehydrogenase, quinone 1	2.00	4.59	1.87	4.59
gamma-glutamylcysteine synthetase, regulatory	2.83	4.00	2.30	4.29
epoxide hydrolase 1, microsomal (xenobiotic)	1.41	2.64	1.87	4.00
NAD(P)H dehydrogenase, quinone 2	1.15	1.41	1.52	4.00
thioredoxin reductase 1	2.46	3.48	2.30	3.48
UDP-glucose dehydrogenase	1.23	1.62	1.41	2.30
glutathione reductase	1.41	2.30	1.52	2.14
crystallin, zeta (quinone reductase)	1.00	1.15	1.32	2.14
gamma-glutamylcysteine synthetase, catalytic	1.32	1.32	1.52	2.00
glutathione S-transferase A4	1.15	1.23	1.62	2.00

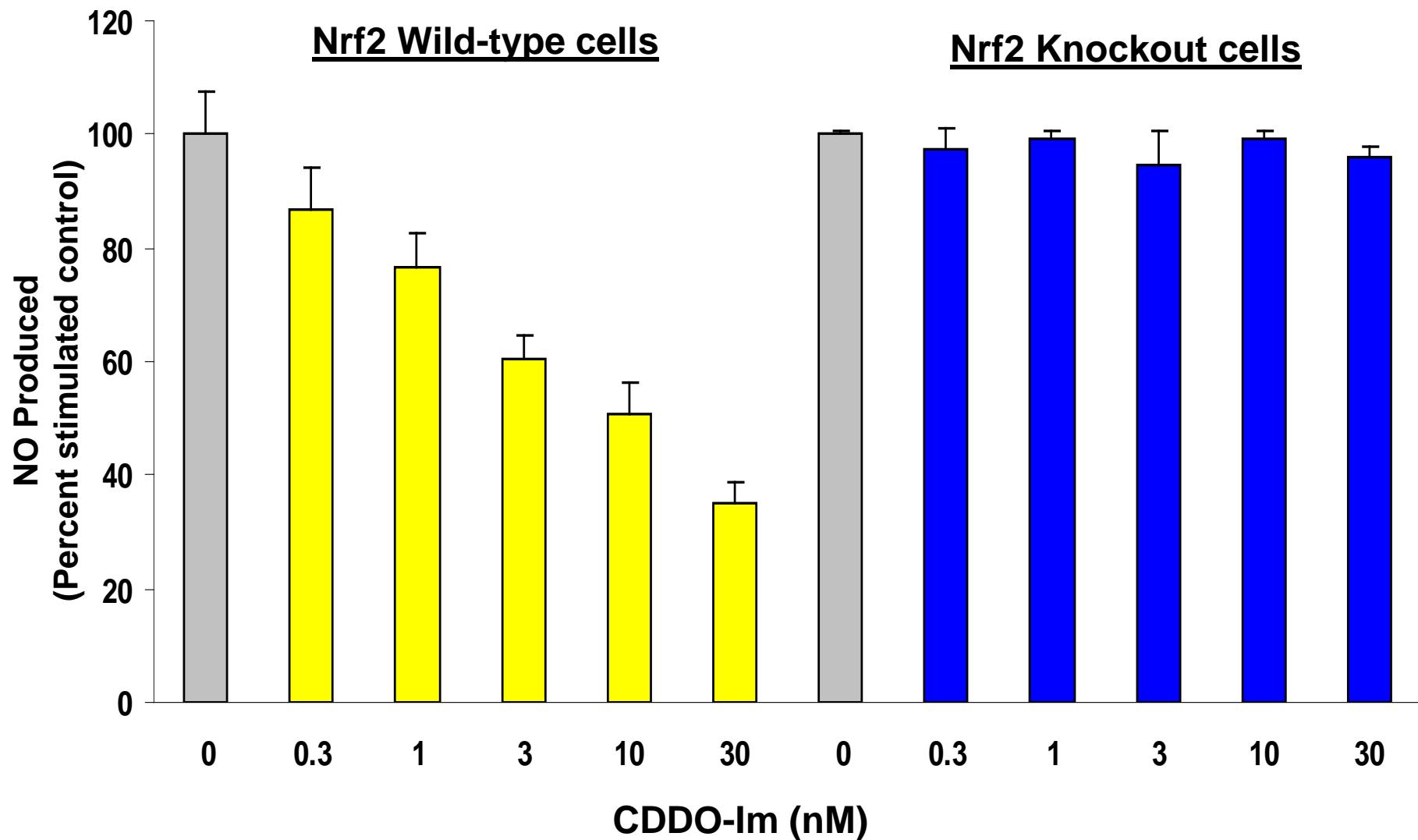
# The transcription factor Nrf2 activates a network of genes that protect against oxidative and electrophilic stress



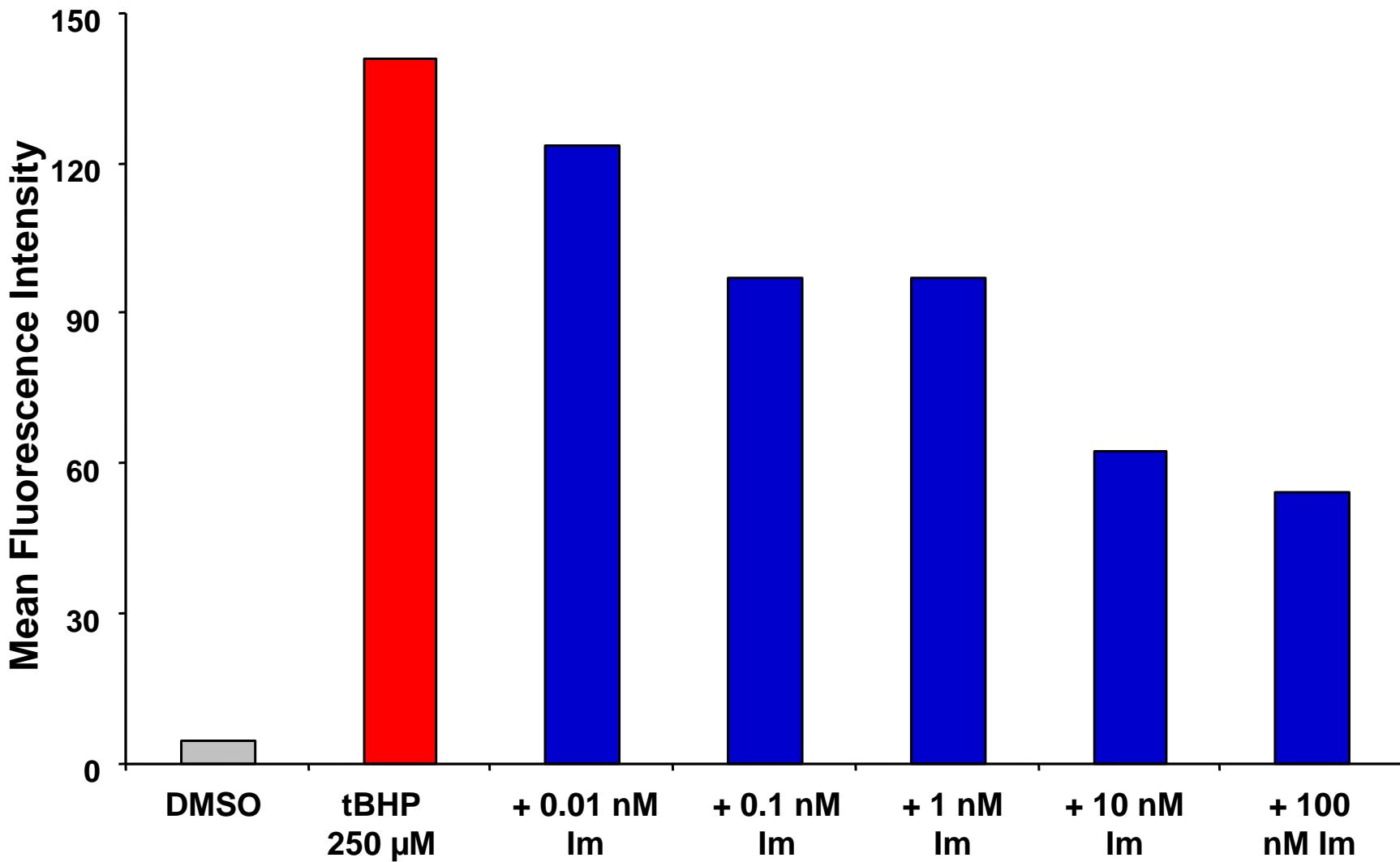
# **Target gene functions of the Nrf2-ARE pathway**

- Direct antioxidants
- Free radical metabolism
- Electrophile detoxification
- Glutathione homeostasis
- Generation of reducing equivalents
- Solute transport
- Inhibition of inflammation
- DNA damage recognition
- Proteasome function

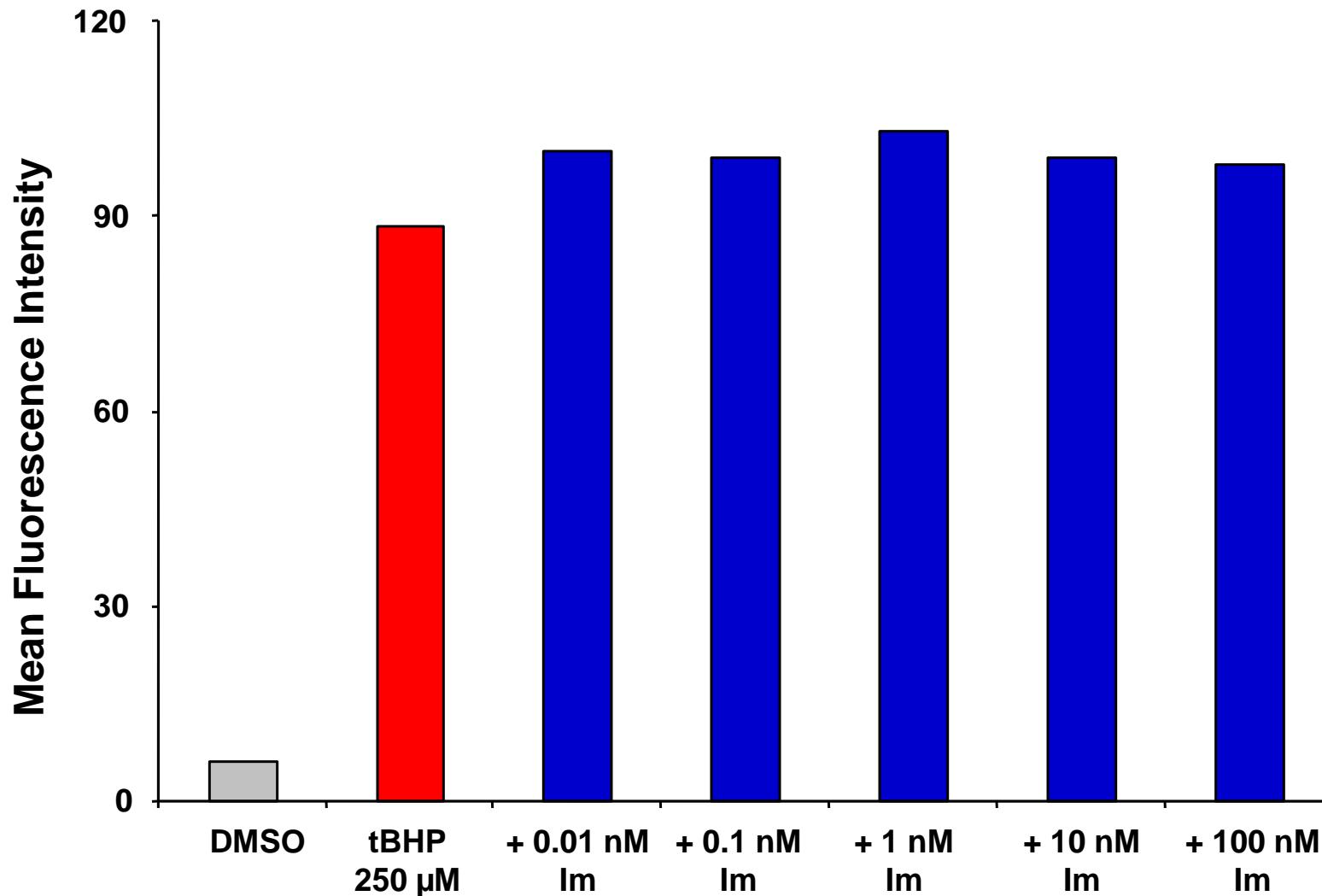
# CDDO-Im inhibits NO production in Nrf2 WT cells but not in Nrf2 KO cells



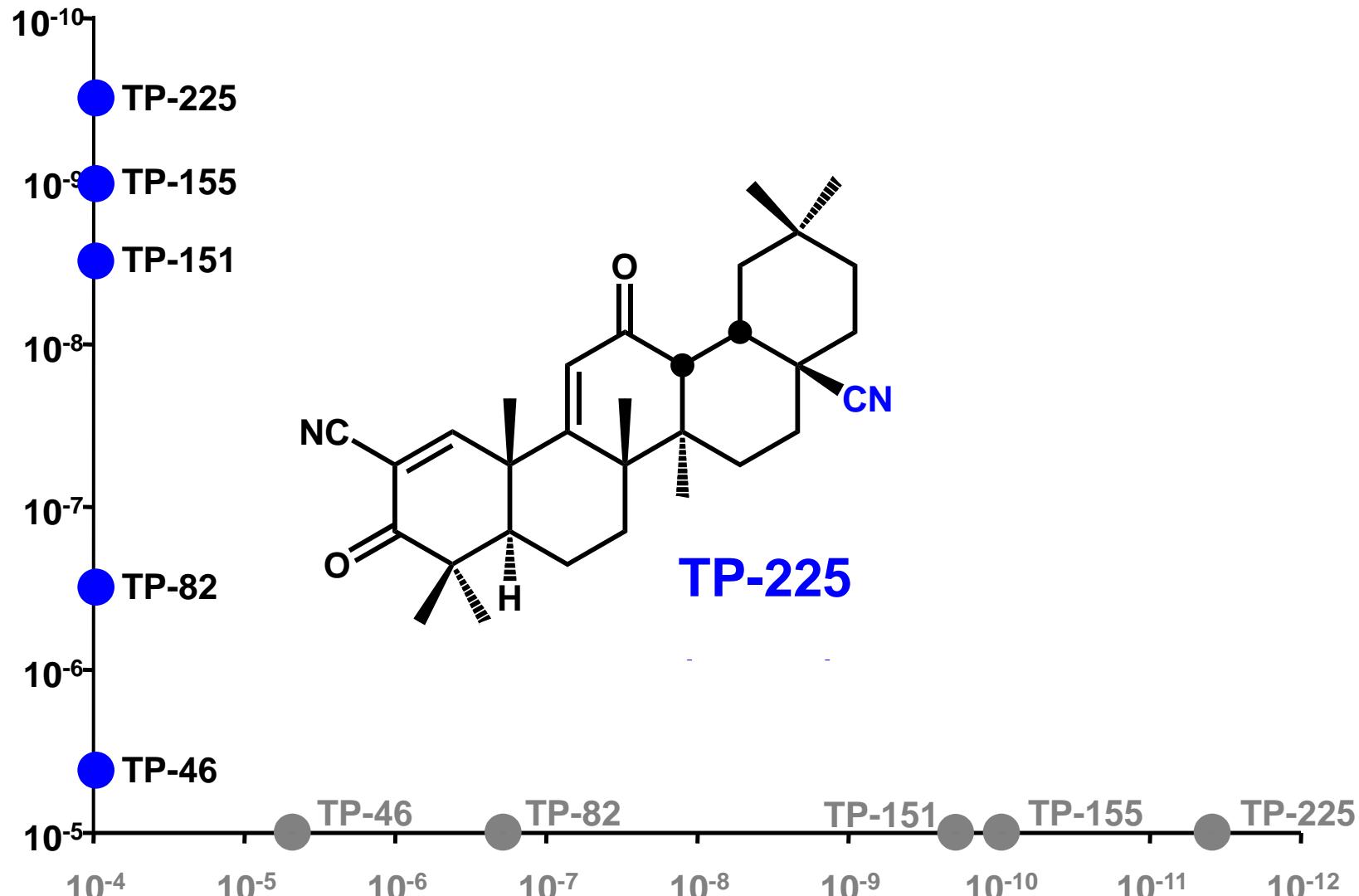
# CDDO-Imidazolidine *protects* against ROS in *Nrf2 wild-type cells* challenged with tBHP



# CDDO-Imidazolidide does *NOT* protect against ROS in *Nrf2 knockout* cells

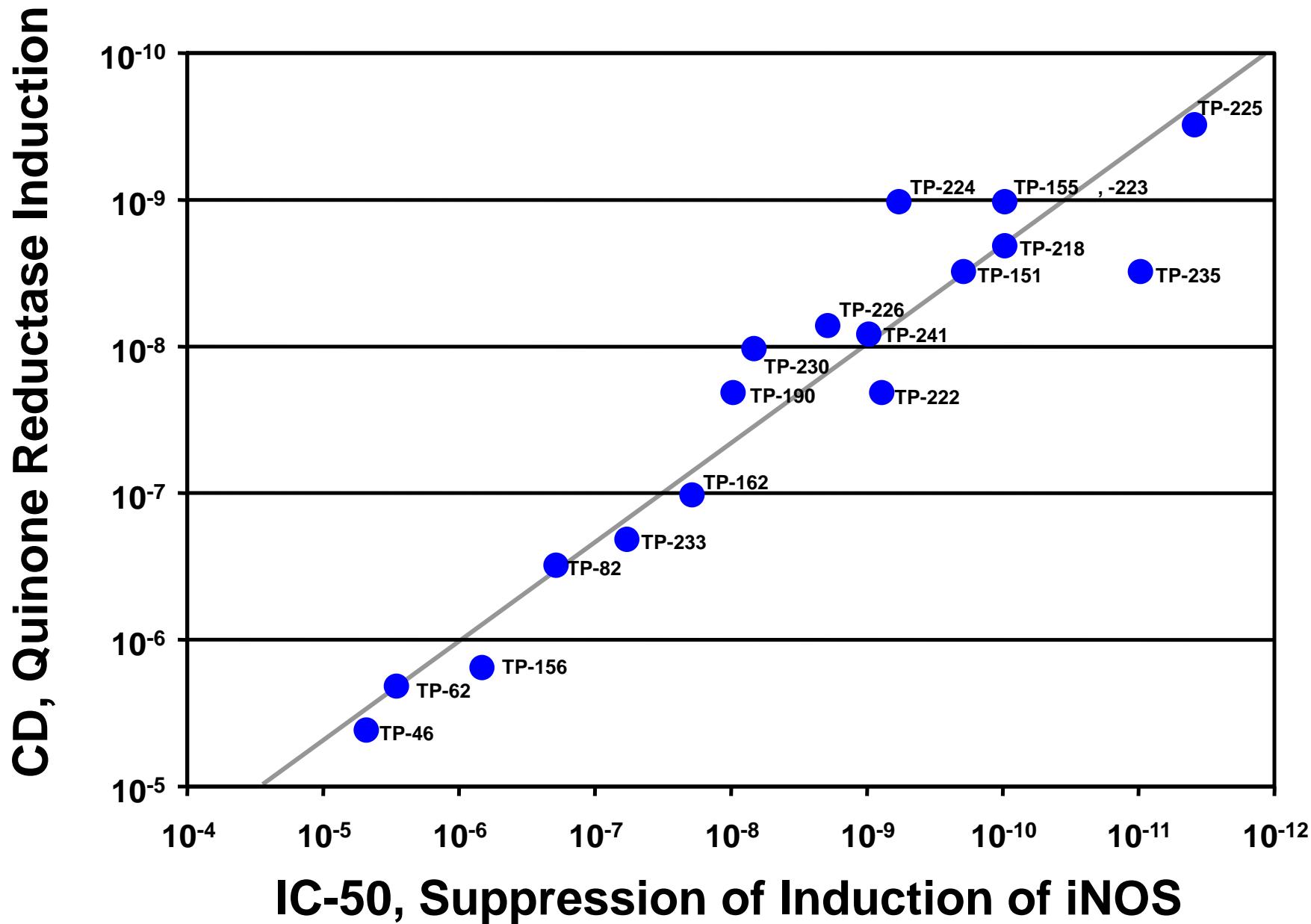


CD, Quinone Reductase Induction

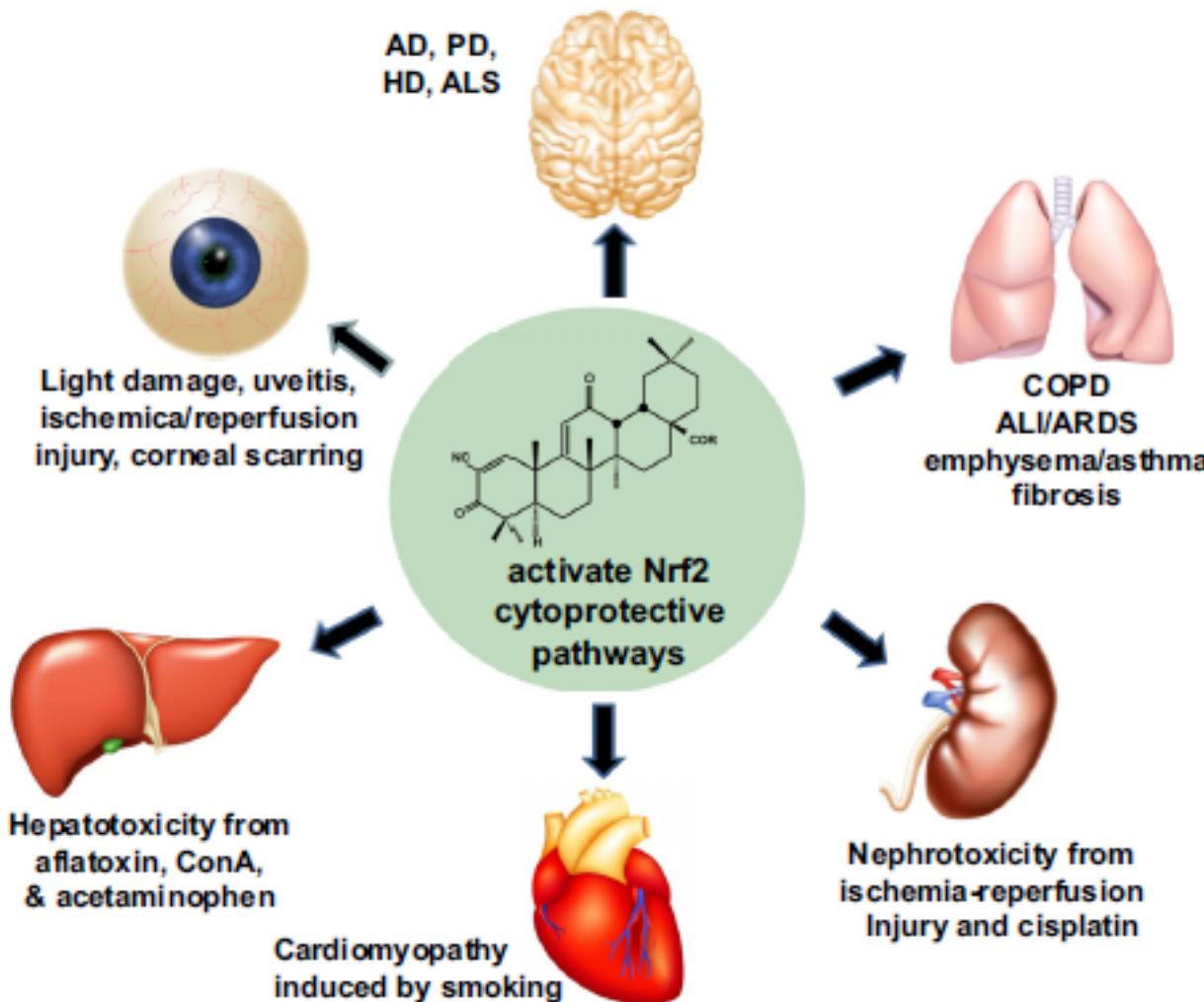


IC-50, Suppression of Induction of iNOS

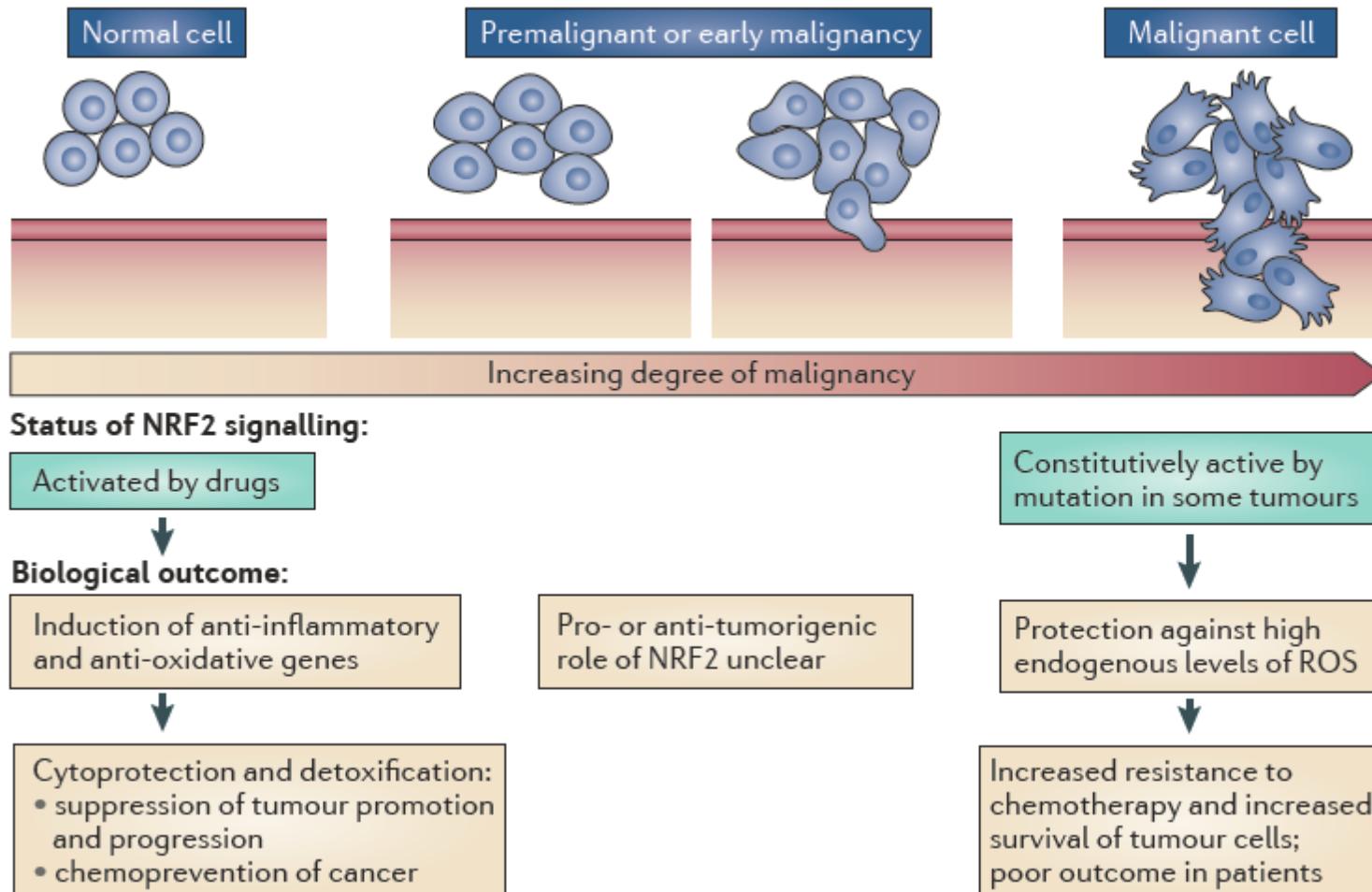
# Activation of the phase 2 cytoprotective enzyme NQO1 and inhibition of the inflammatory enzyme iNOS are tightly correlated



# Activation of the Keap/Nrf2/ARE pathway by the triterpenoids is cytoprotective

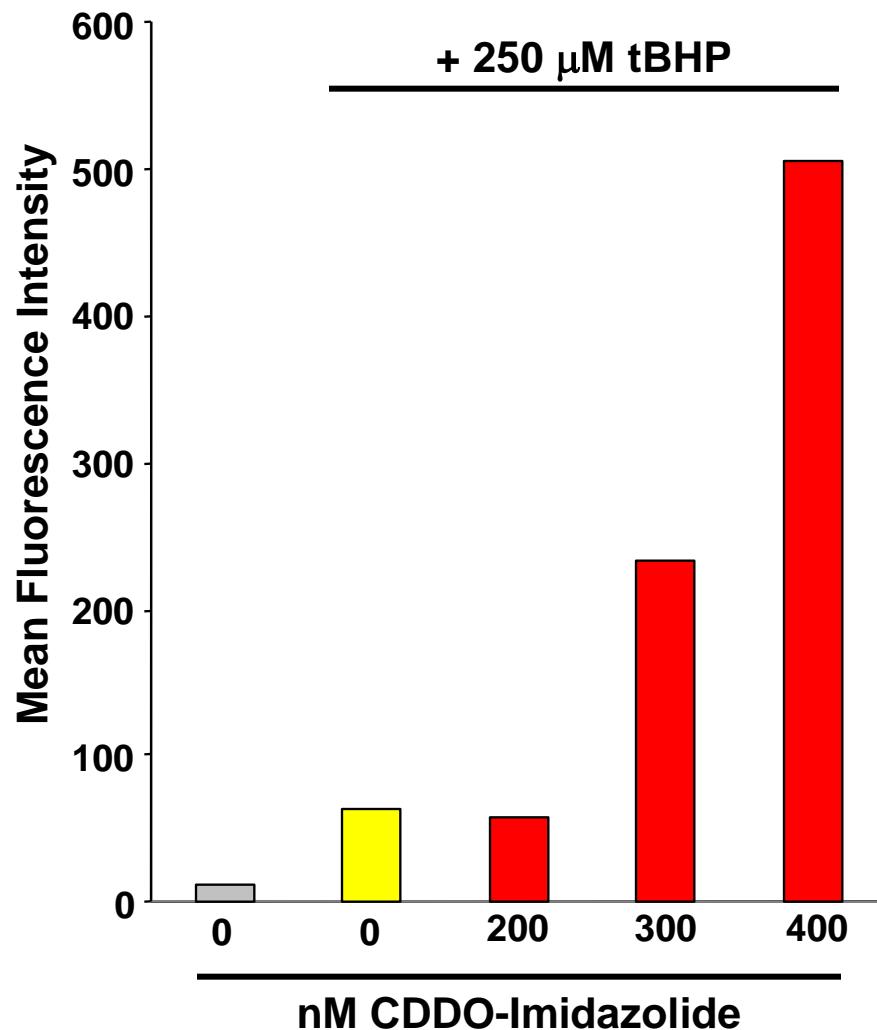
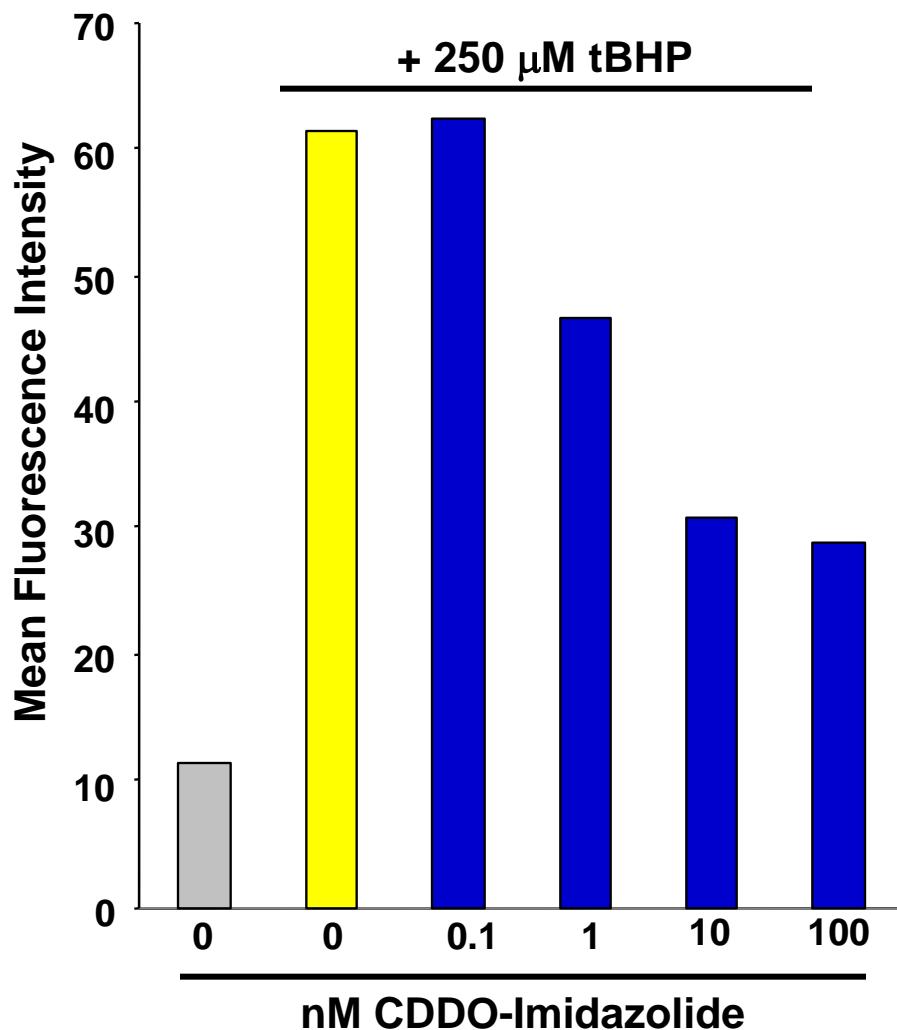


# Consequences of Nrf2 activation in cancer



**Higher concentrations (low  $\mu\text{M}$ ) of  
triterpenoids increase ROS and  
induce apoptosis in cancer cells**

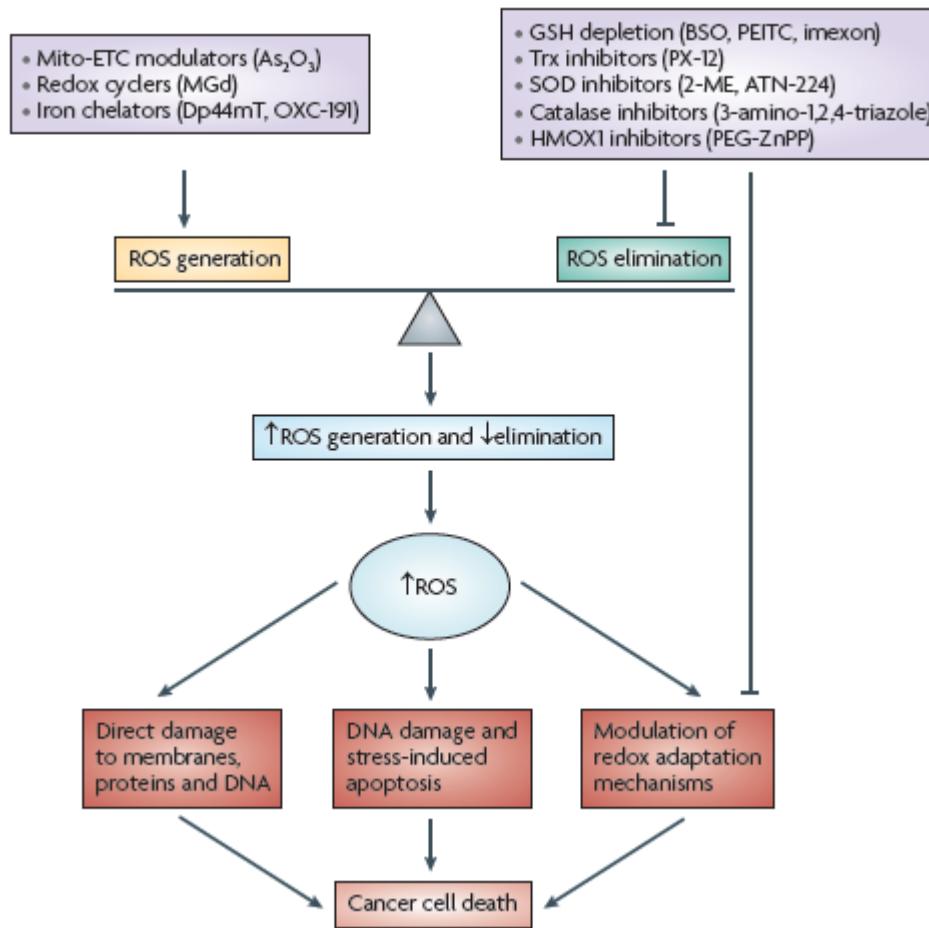
**Low concentrations of triterpenoids *suppress* formation of ROS, but *high concentrations increase* ROS**



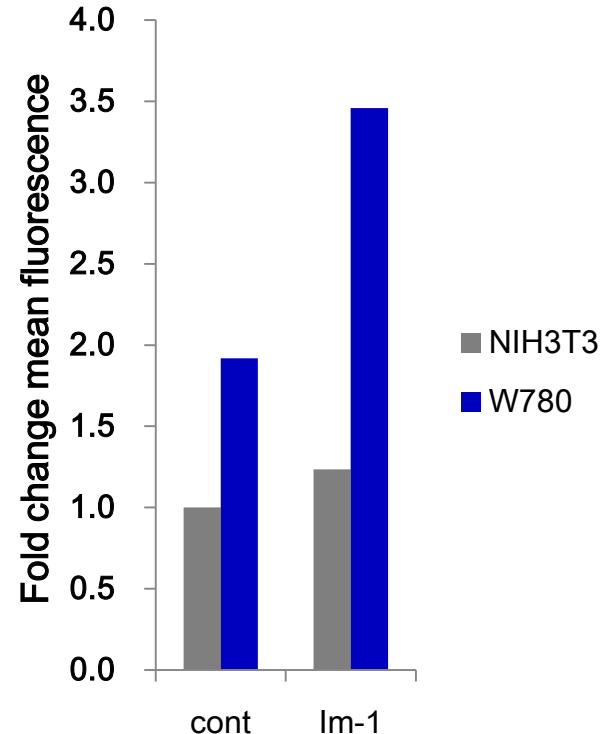
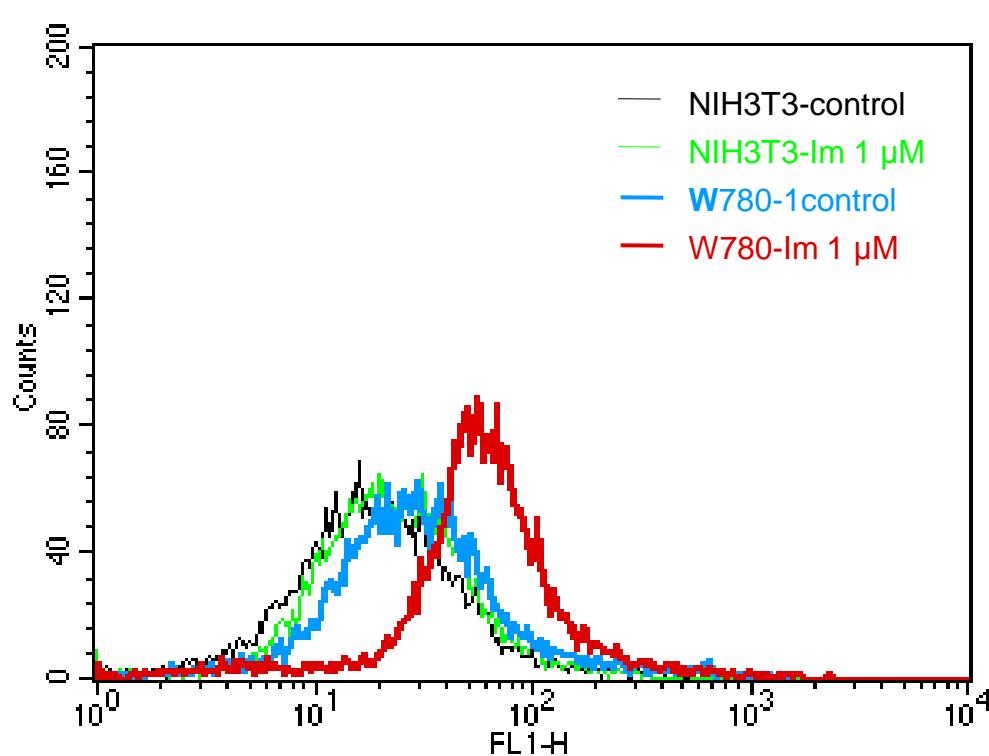
# **Targeting the altered redox status of cancer cells to preferentially kill malignant cells**

- Cancer Cell 10:241, 2006
- Nature Reviews Drug Discovery 8:579, 2009

# Targeting cancer cells via ROS

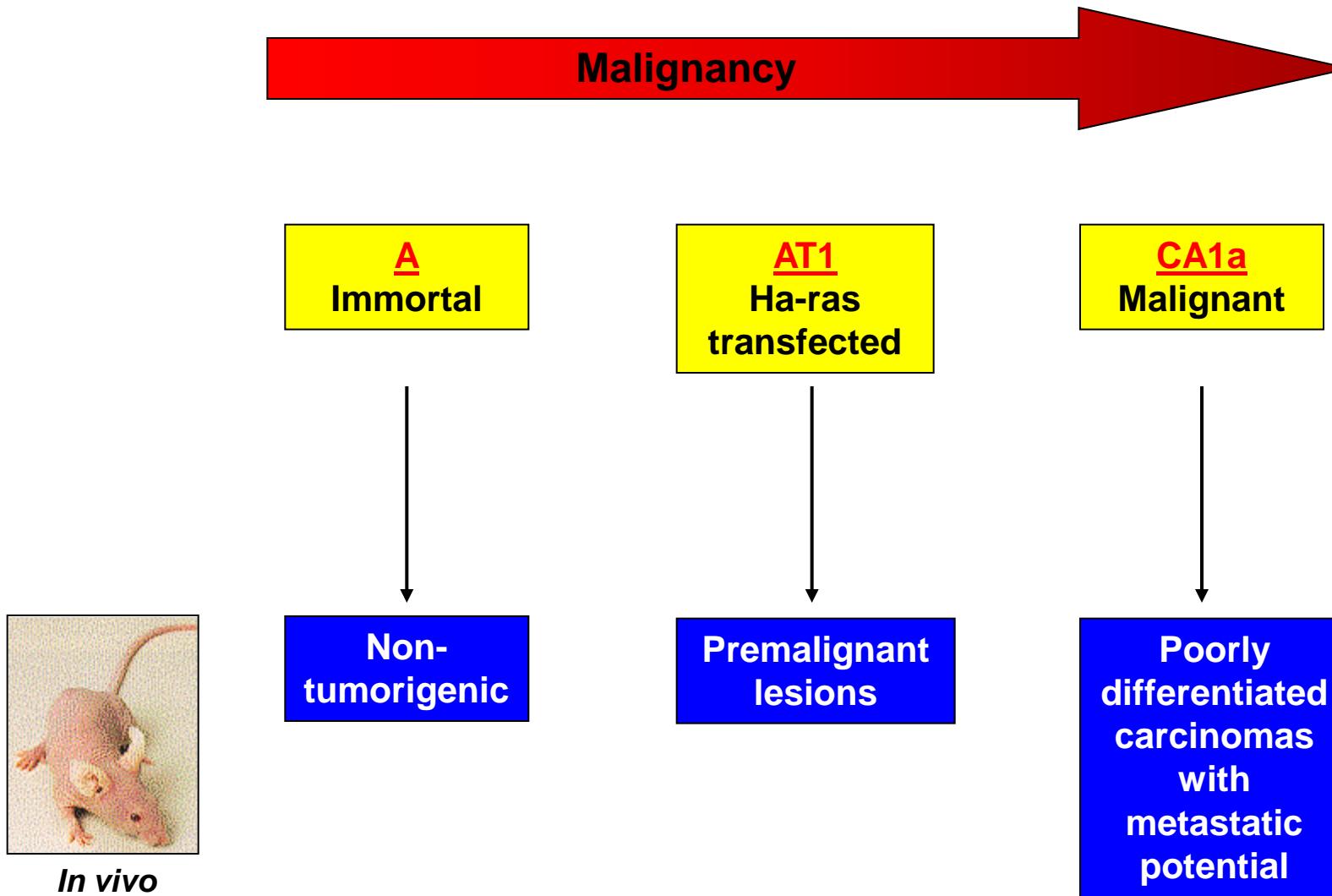


# CDDO-Im induces higher levels of ROS in *Brca-1* defective breast cancer cells than in normal 3T3 cells

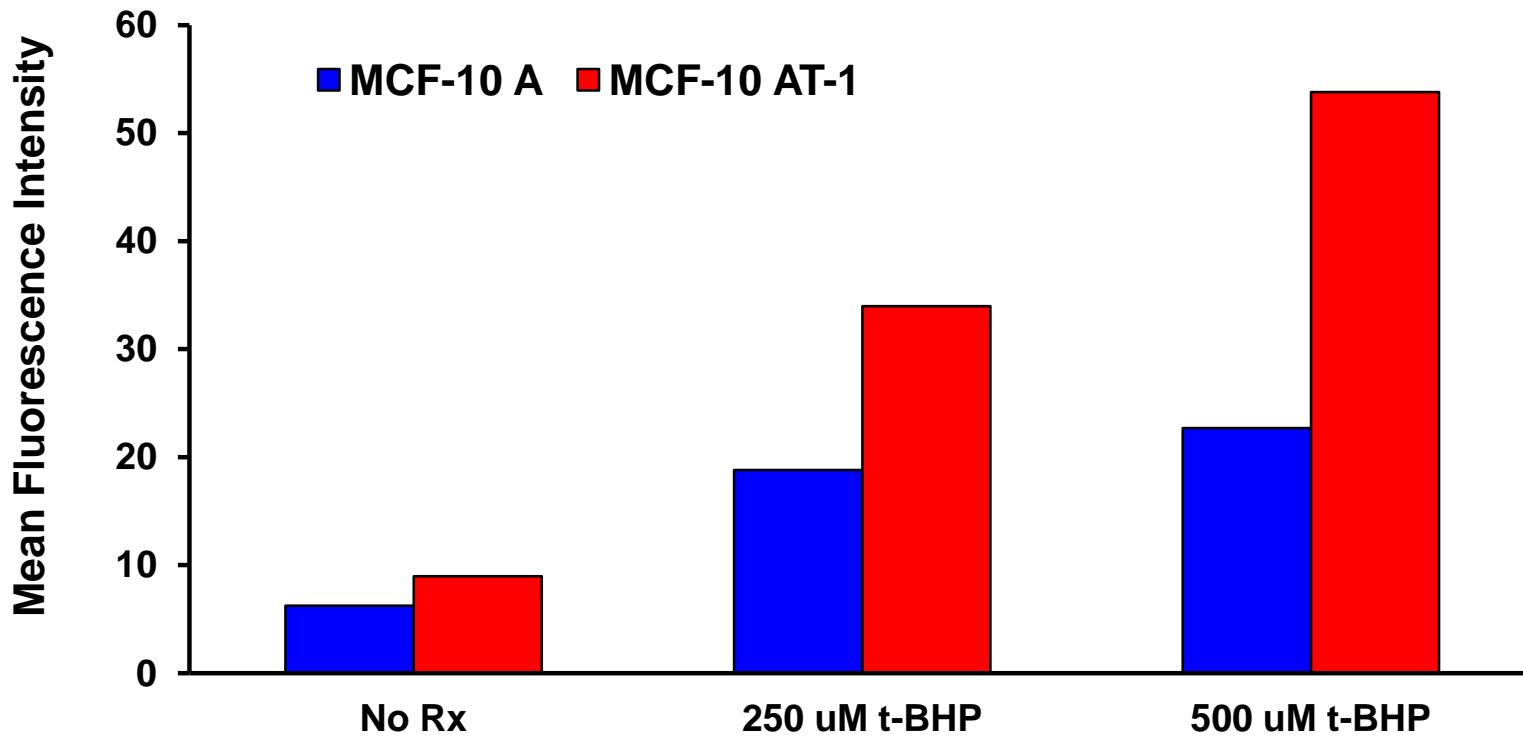


CDDO-Im, 1 h treatment

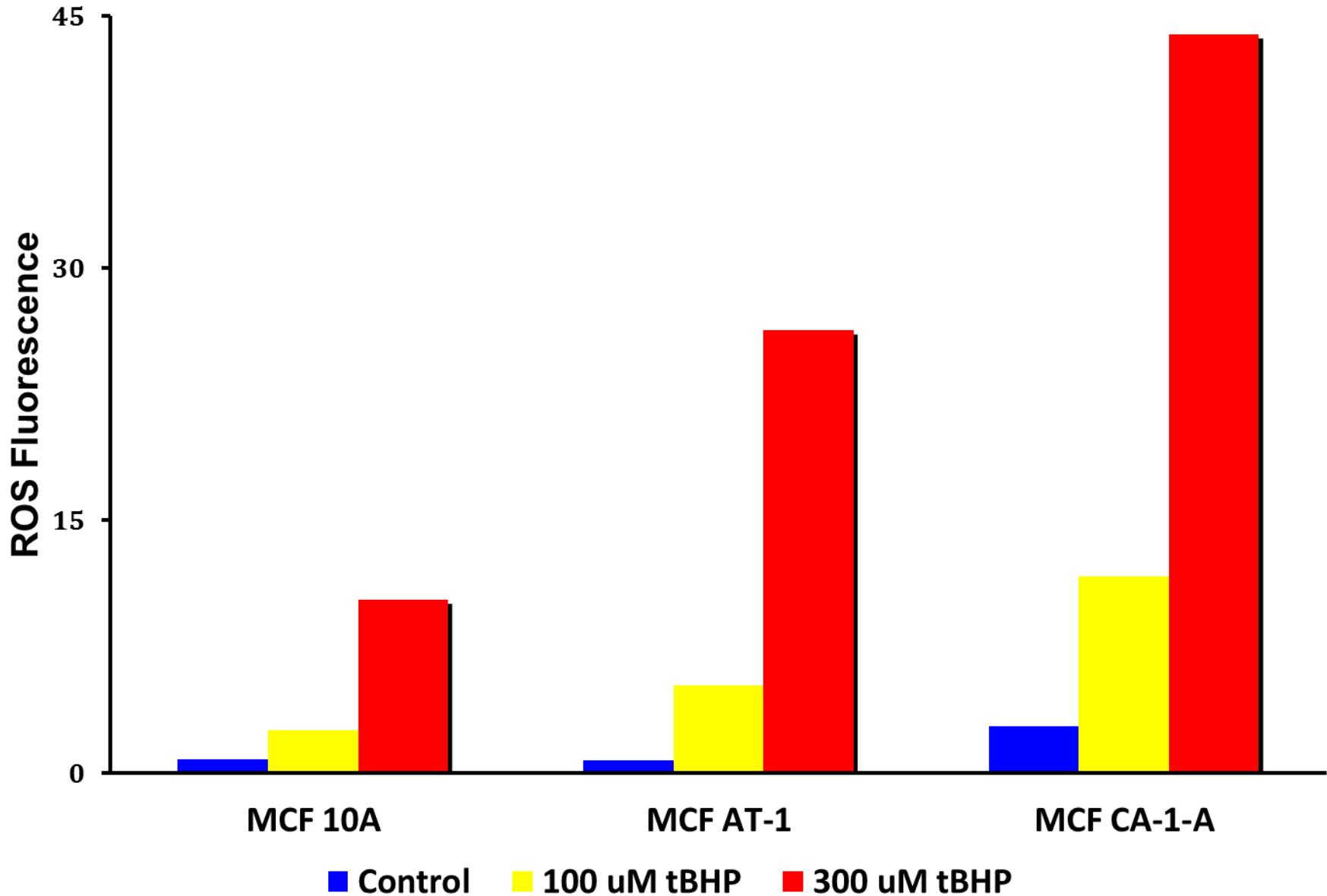
# MCF10 model of progressive breast disease



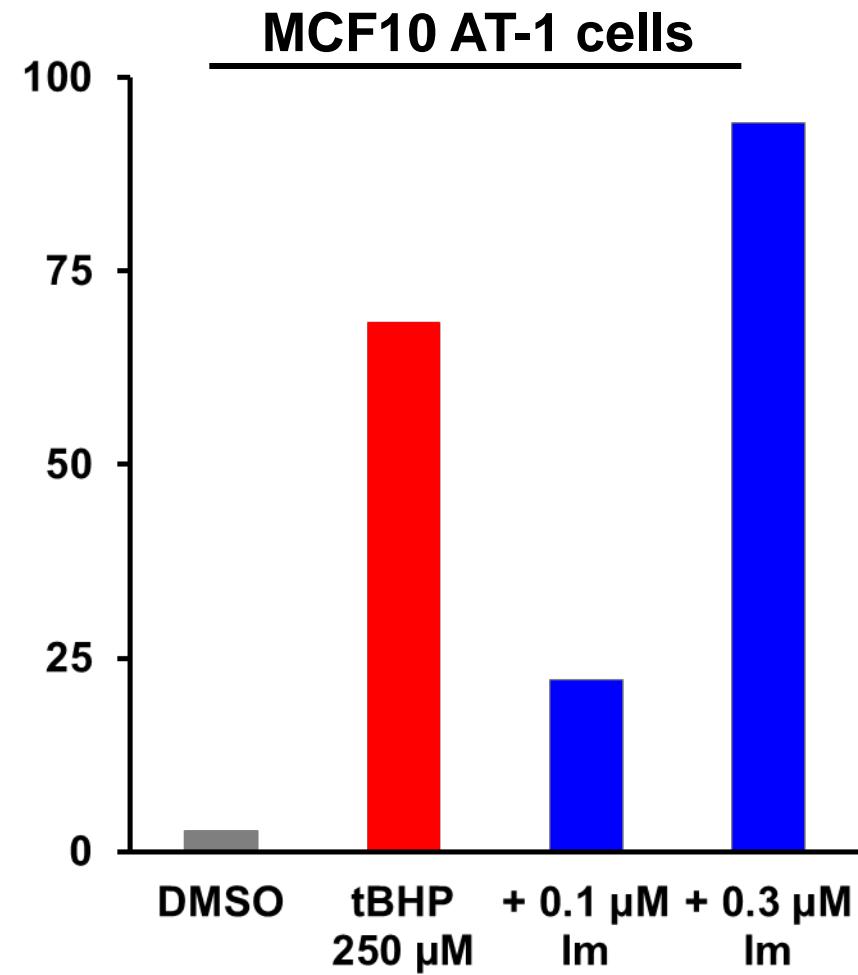
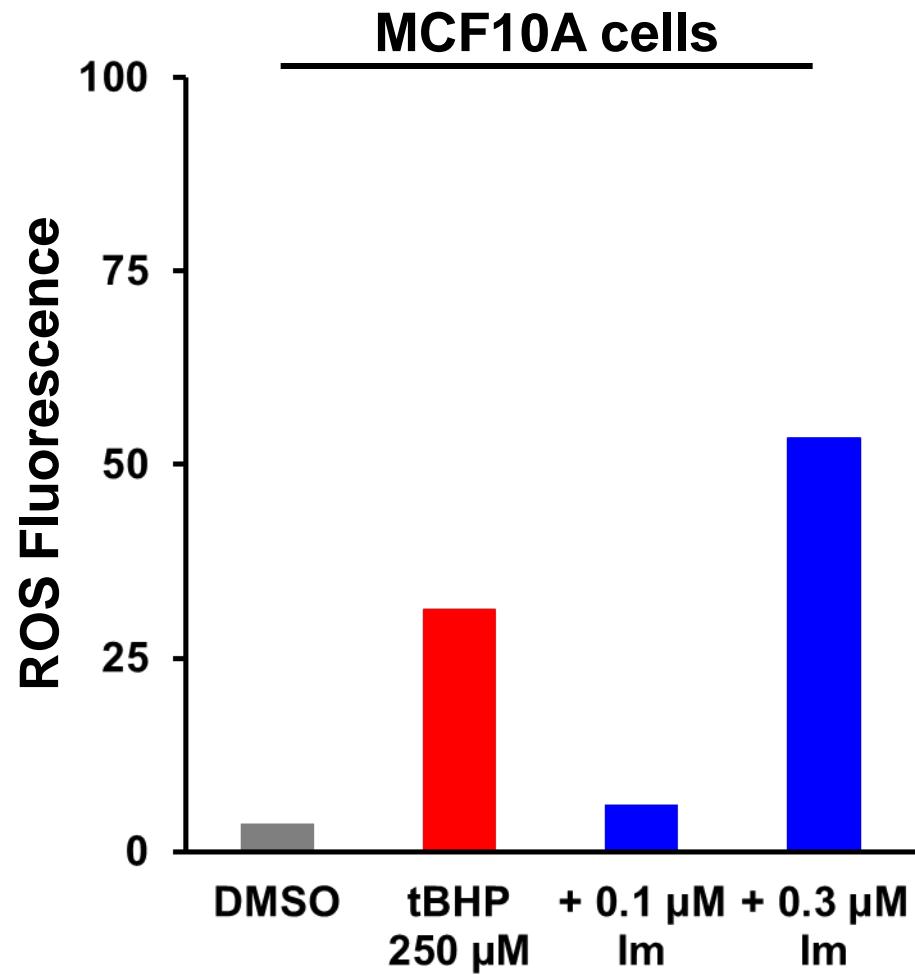
# Cells transformed with activated Ras generate more ROS when challenged with tBHP



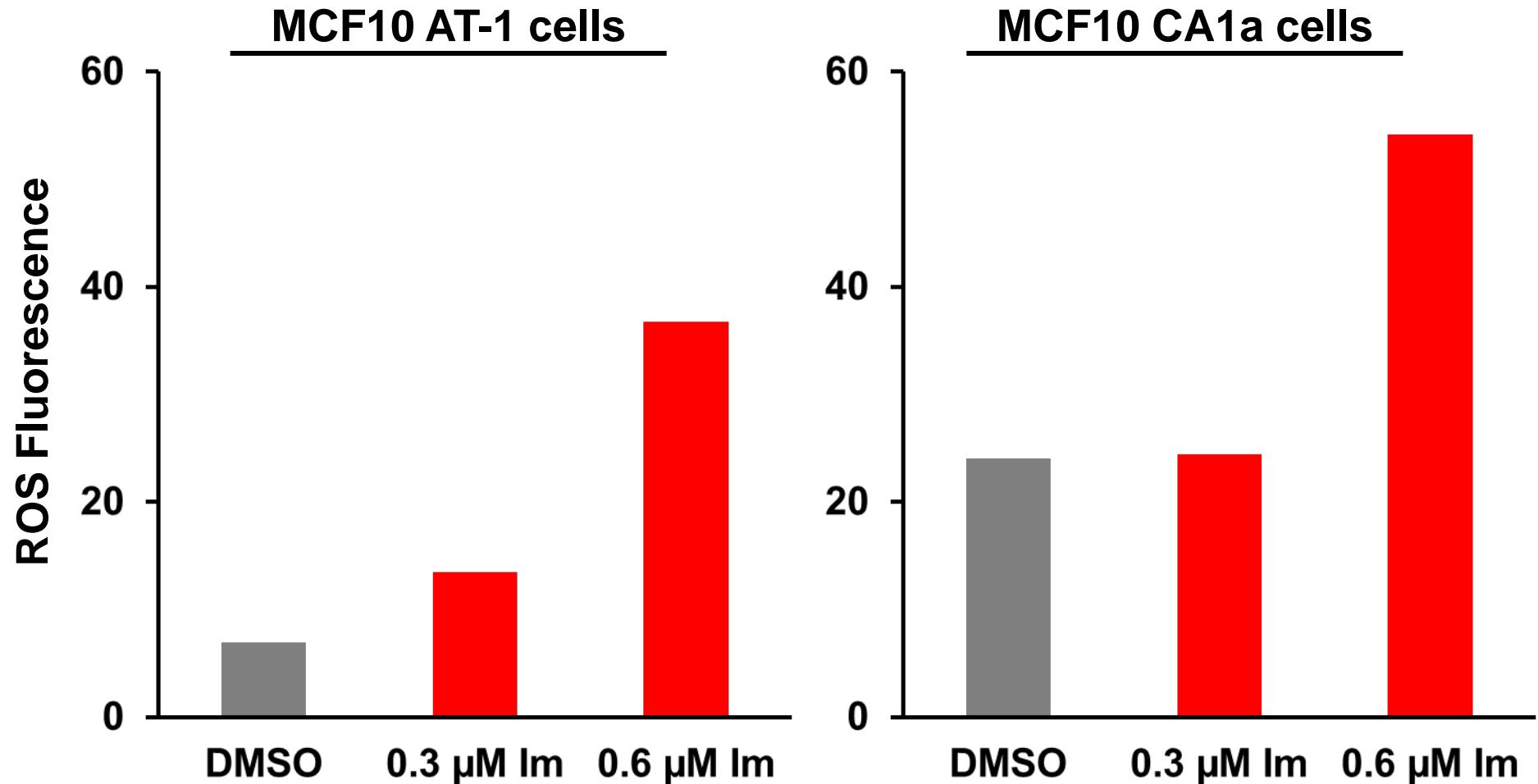
# The production of ROS is dose-dependent



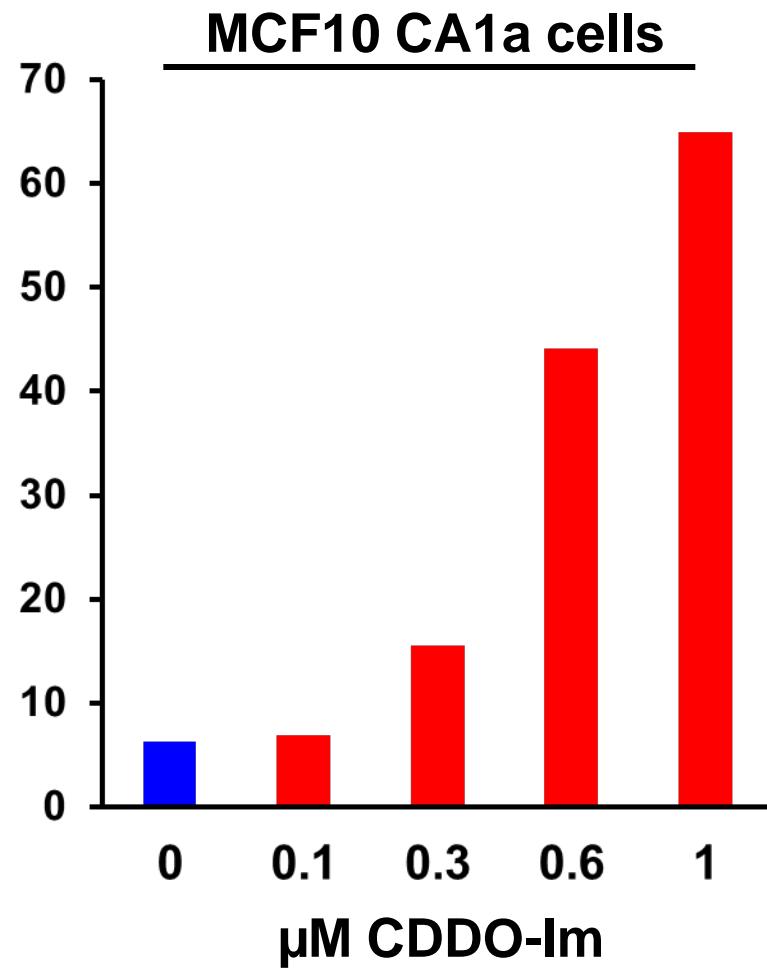
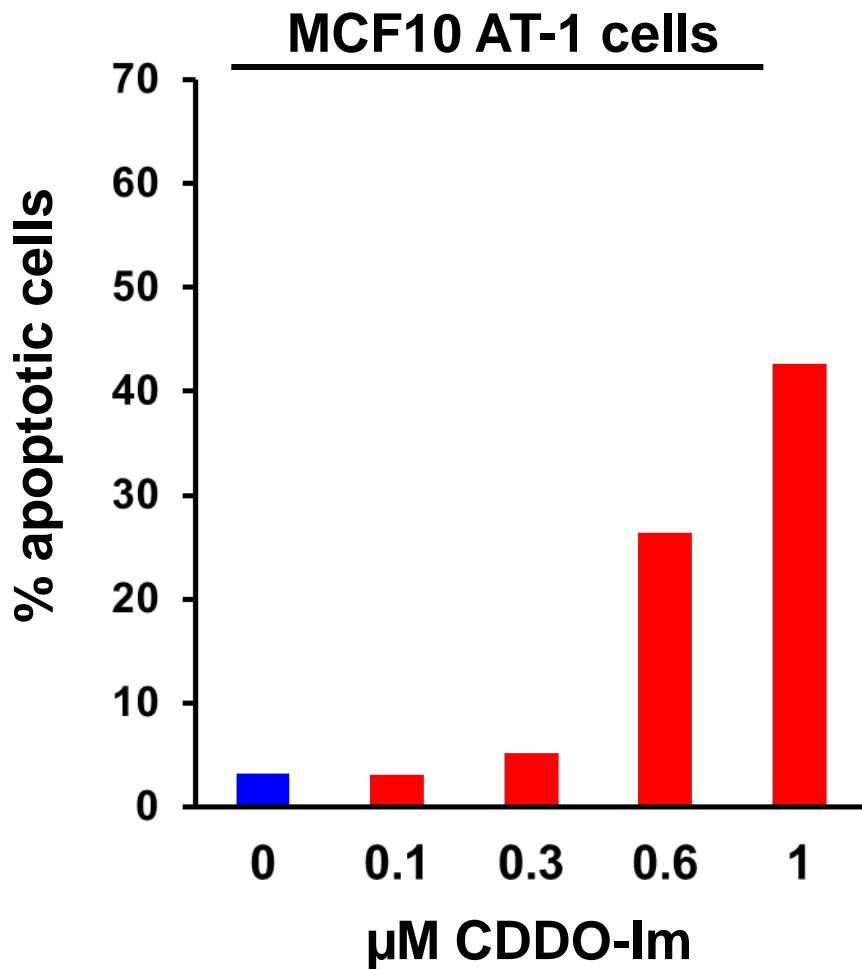
# ROS levels in response to tBHP challenge is dependent on triterpenoid dose and cell type



# Triterpenoids increase ROS

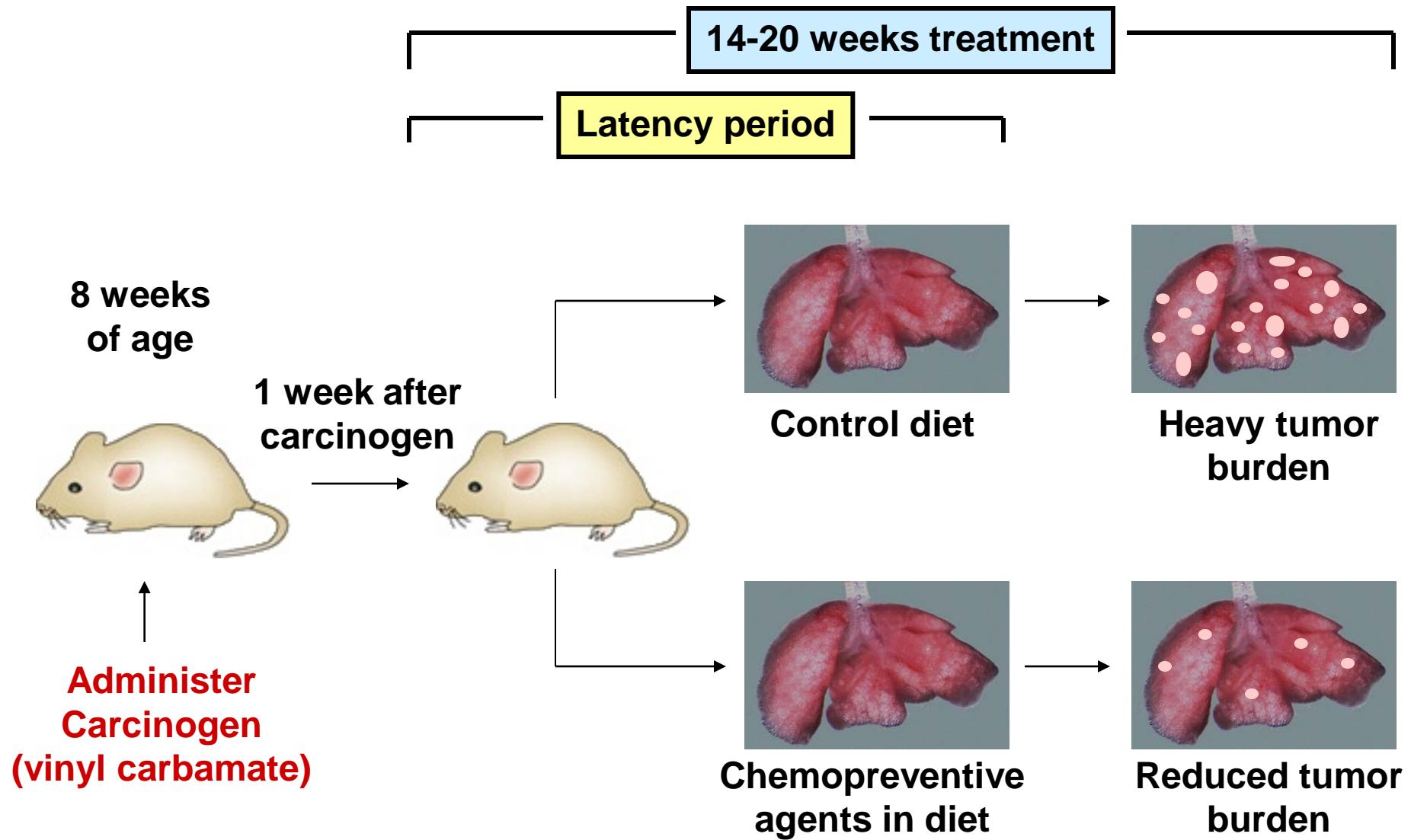


# Triterpenoids induce apoptosis

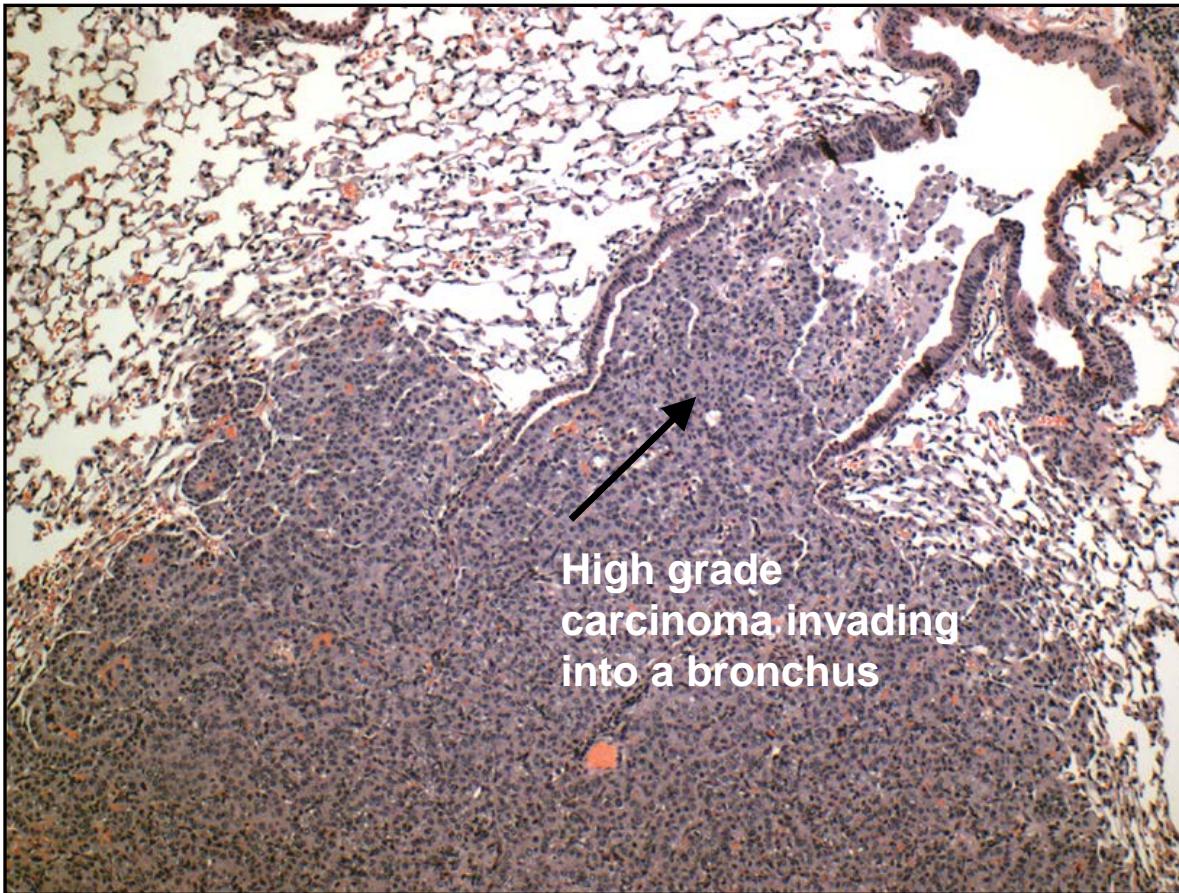


# **Prevention and treatment of lung carcinogenesis by triterpenoids**

# Prevention of lung carcinogenesis - A/J mouse model

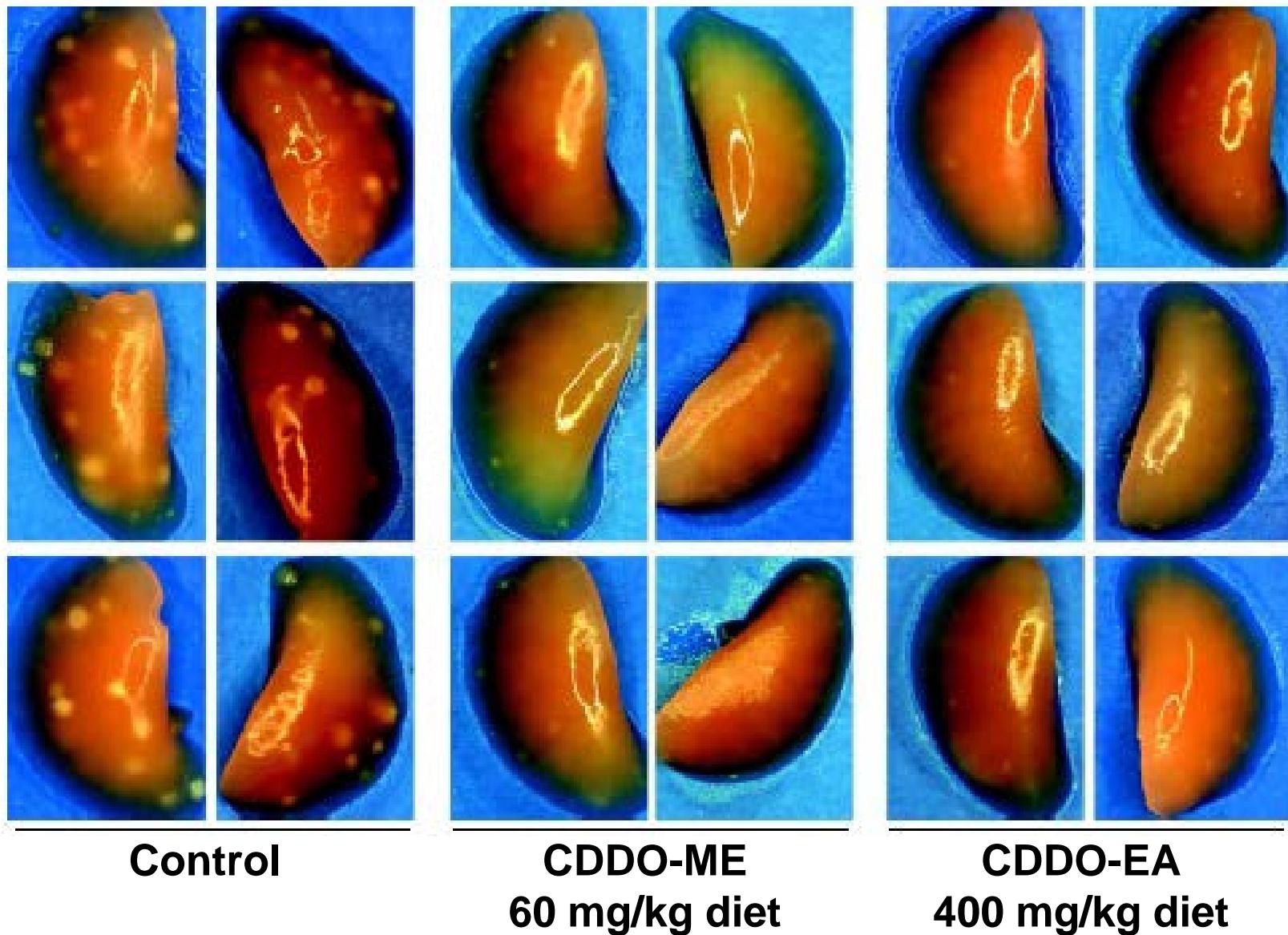


# Vinyl carbamate induces highly invasive carcinomas



High grade  
carcinoma invading  
into a bronchus

# CDDO-methyl ester and CDDO-ethyl amide prevent lung carcinogenesis

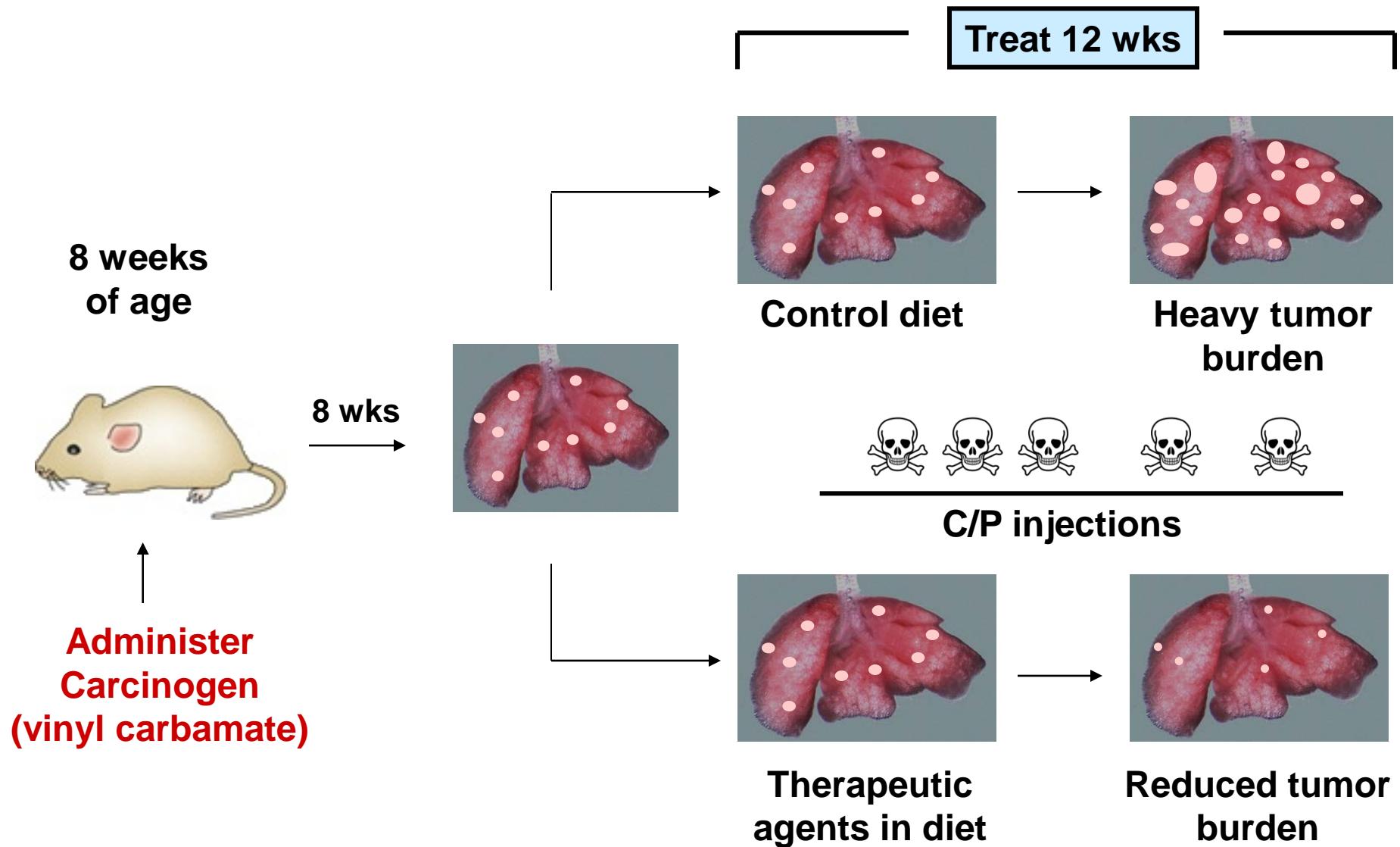


# **CDDO-methyl ester and CDDO-ethyl amide prevent lung cancer**

	<b>Control</b>	<b>CDDO-Me</b>	<b>CDDO-EA</b>
<b>Ave tumor size, mm<sup>3</sup></b>	<b>2.2</b>	<b>0.2 *</b>	<b>0.2 *</b>
(% control)	(100%)	(9%)	(9%)
<b>Ave tumor burden, mm<sup>3</sup></b>	<b>7.2</b>	<b>0.1 *</b>	<b>0.2 *</b>
(% control)	(100%)	(2%)	(2%)

**\*, P < 0.05 vs. control**

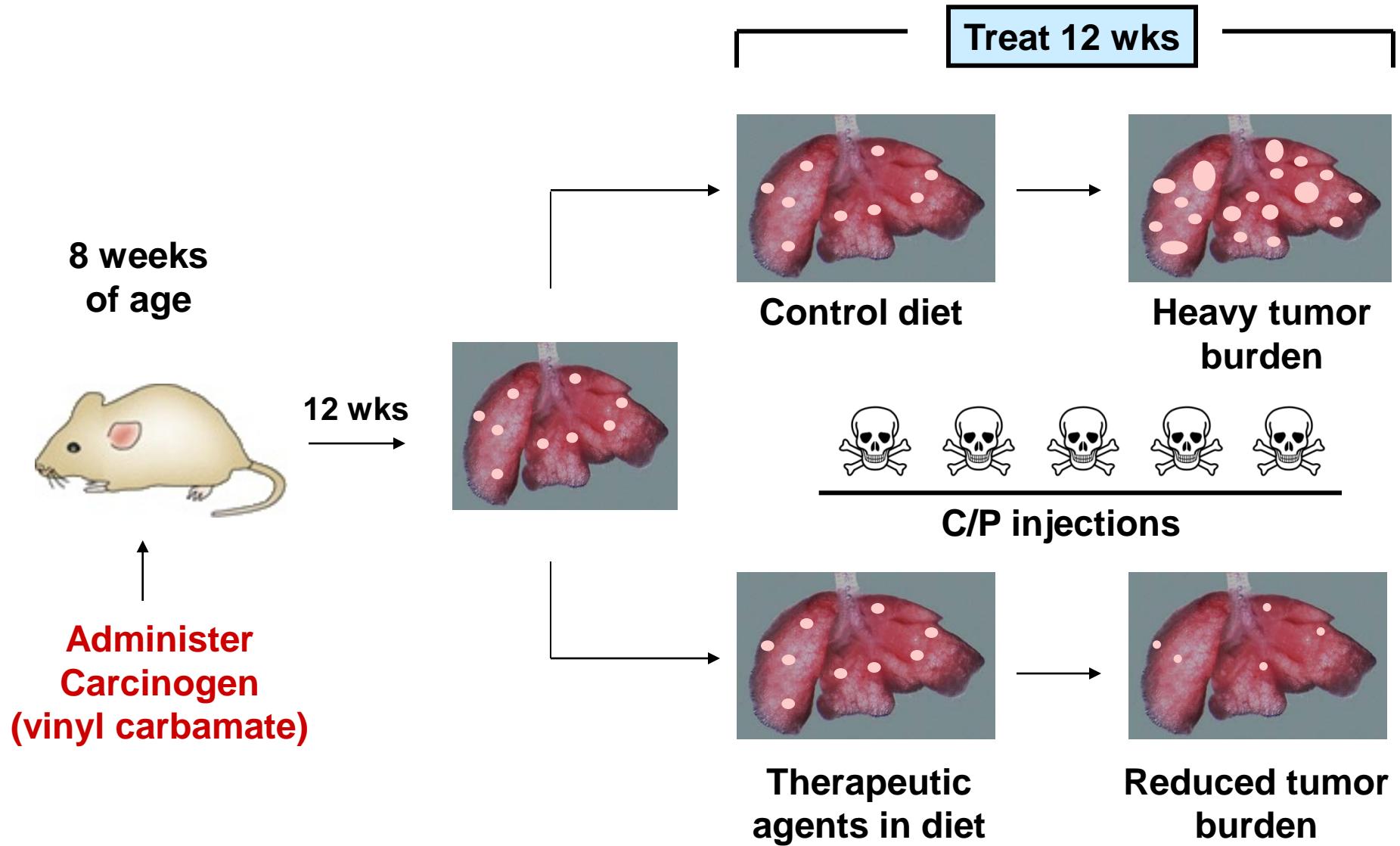
# Treatment of lung carcinogenesis - A/J mouse model



# **Triterpenoids protect against carboplatin/paclitaxel toxicity – Survivors after 5 C/P injections**

Treatment	Survivors
C/P <b>WITHOUT</b> triterpenoid	3/8 (38%)
C/P <b>WITH</b> triterpenoid	14/16 (88%)

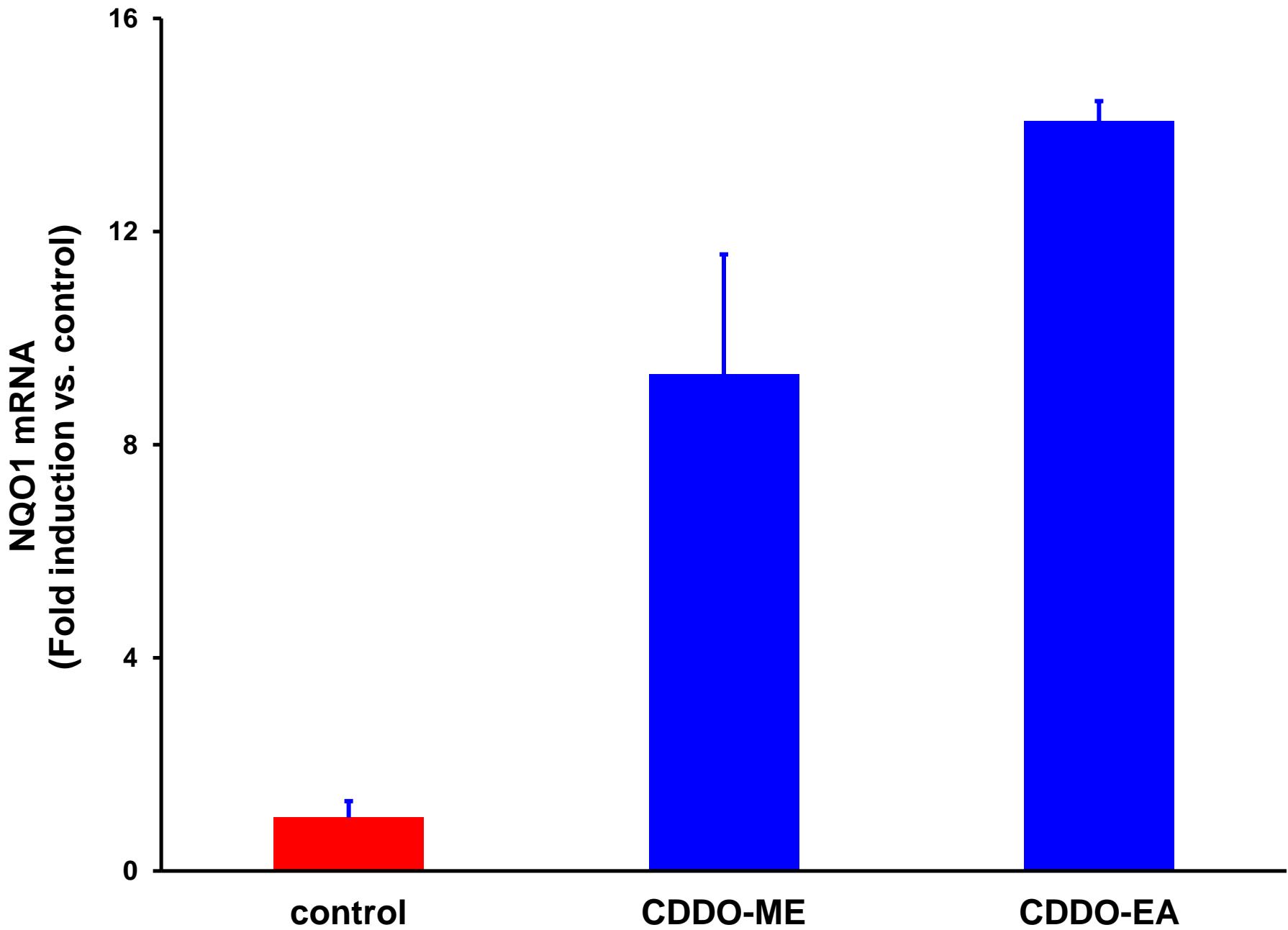
# Treatment of lung carcinogenesis - A/J mouse model



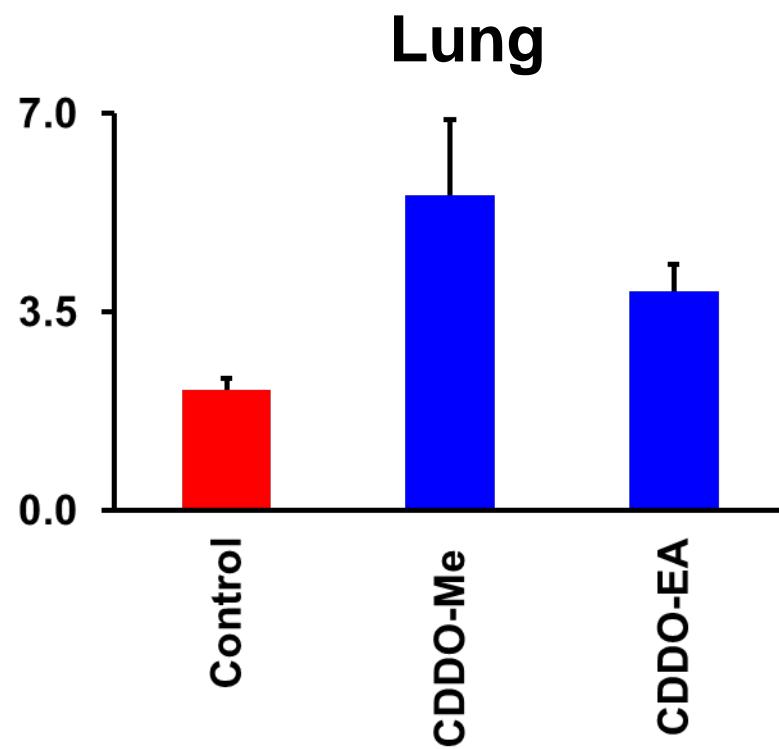
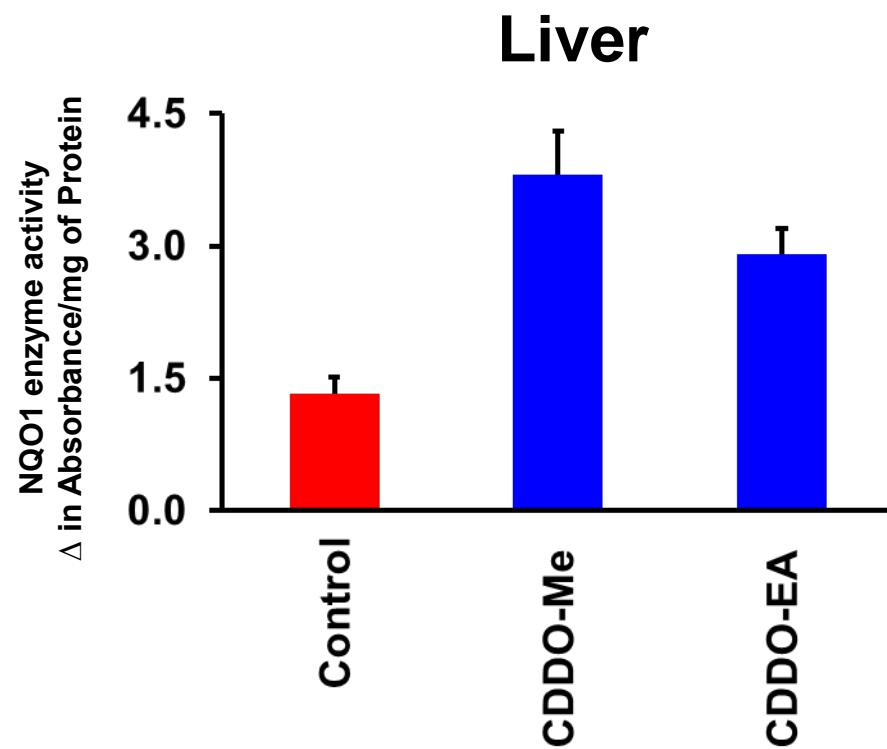
# **Triterpenoids protect against carboplatin/paclitaxel toxicity – Survivors after 5 C/P injections**

<b>Treatment</b>	<b>Survivors</b>
<b>Carboplatin/paclitaxel alone</b>	<b>21/23 (91%)</b>
<b>Triterpenoids + C/P</b>	<b>31/32 (97%)</b>

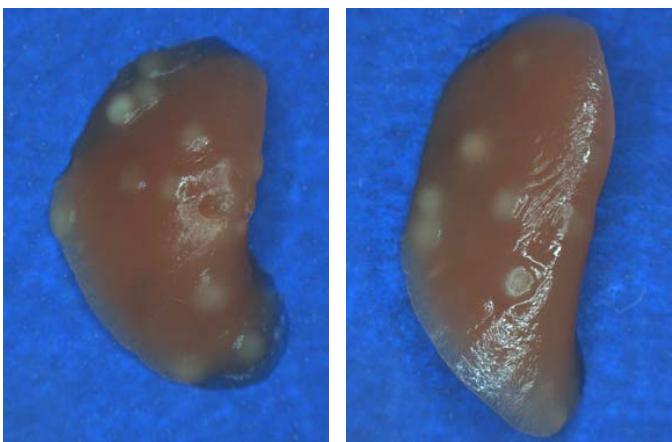
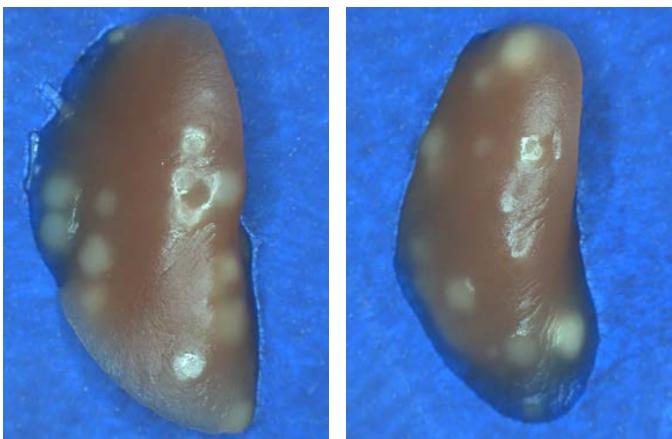
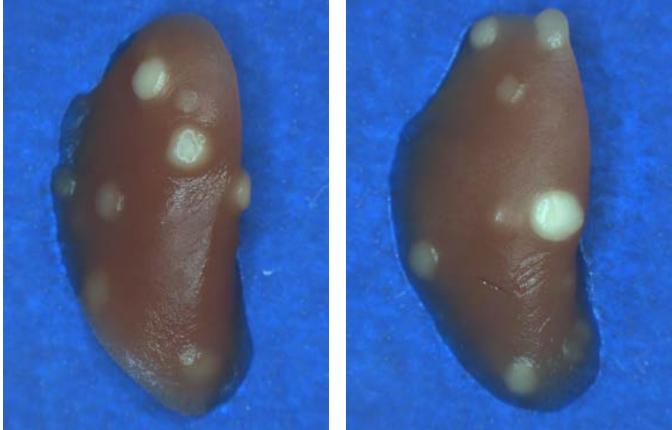
# Triterpenoids increase NQO1 mRNA in PBMCs



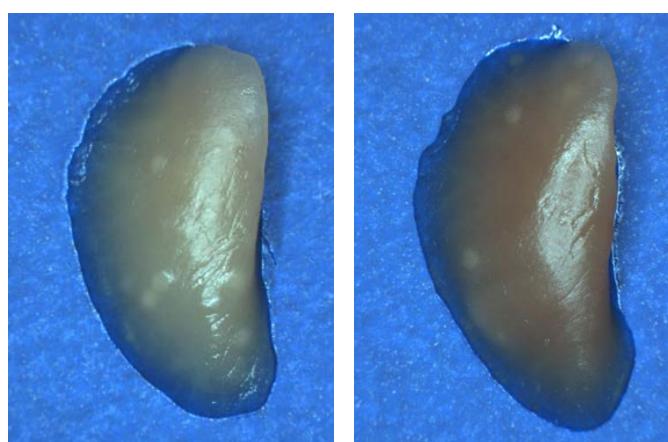
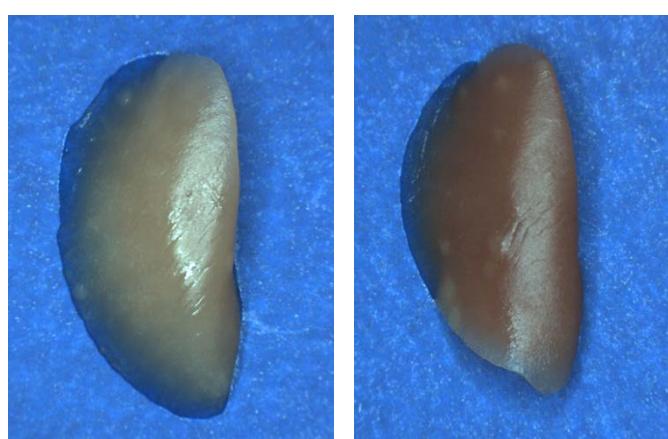
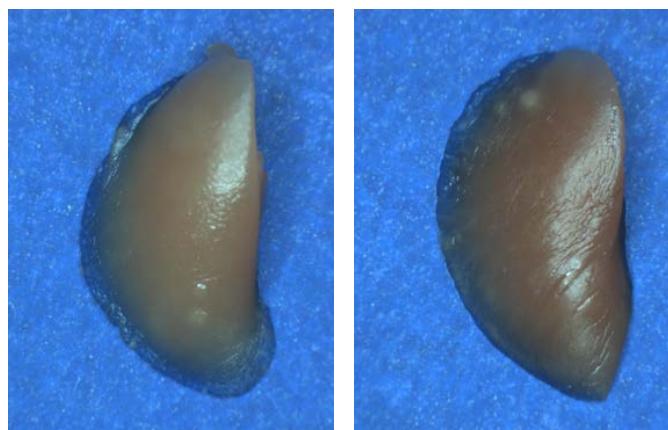
# Triterpenoids increase NQO1 enzyme activity in A/J mice



# Control lungs



# CDDO-Me + C/P



# The combination of CDDO-Me and C/P inhibits lung carcinogenesis in A/J mice

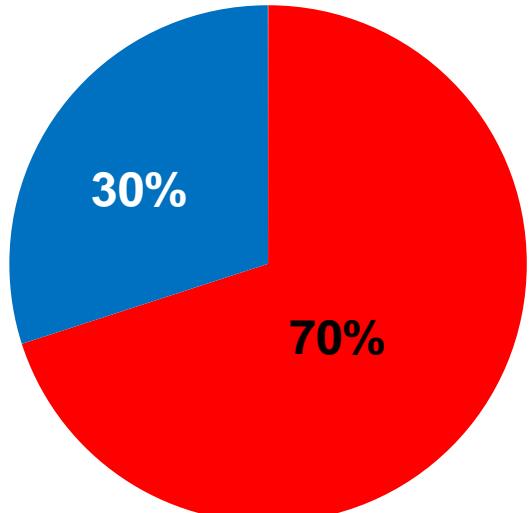
	Control	CDDO-ME 80 mg/kg diet	Carboplatin & Paclitaxel	CDDO-Me + C/P
Ave # of tumors/slide (% control)	3.5 (100%)	2.5 (71%)	2.1 * (62%)	1.5 * (43%)
Ave tumor volume (mm <sup>3</sup> ) per tumor (% control)	4.6 (100%)	1.6 * (34%)	1.5 * (33%)	1.0 * (21%)
Ave tumor volume (mm <sup>3</sup> ) per slide (% control)	15.9 (100%)	3.9 * (25%)	3.3 * (21%)	1.4 ** (10%)

\* , P < 0.05 vs. control

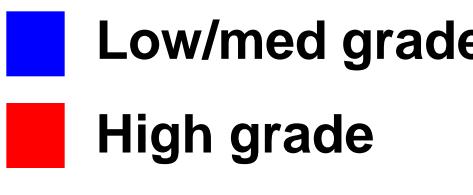
\*\*, P < 0.05 vs. Me and C/P

# Histopathology of tumors in A/J Mice

Control

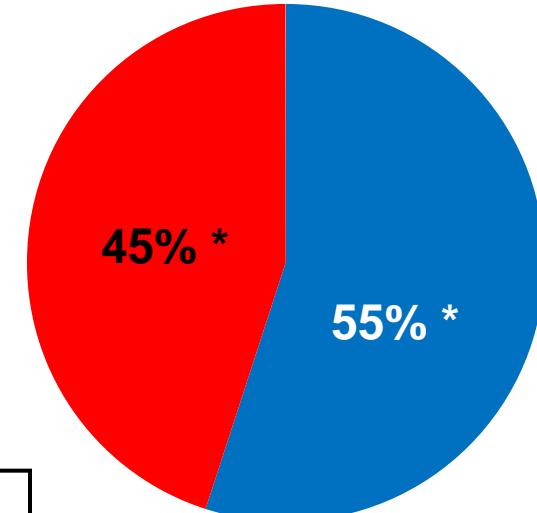


n = 52 slides, 180 tumors



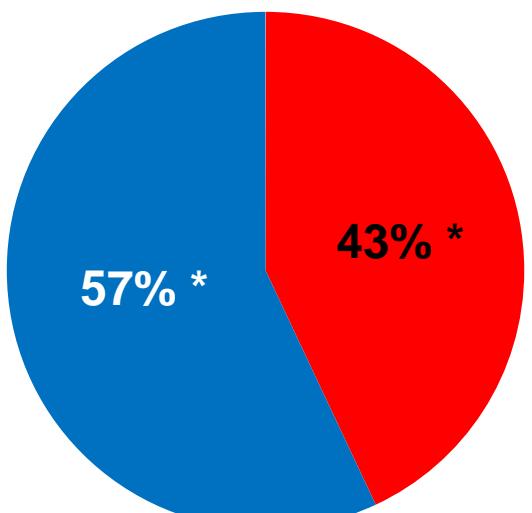
, p < 0.001 vs control

CDDO-Me



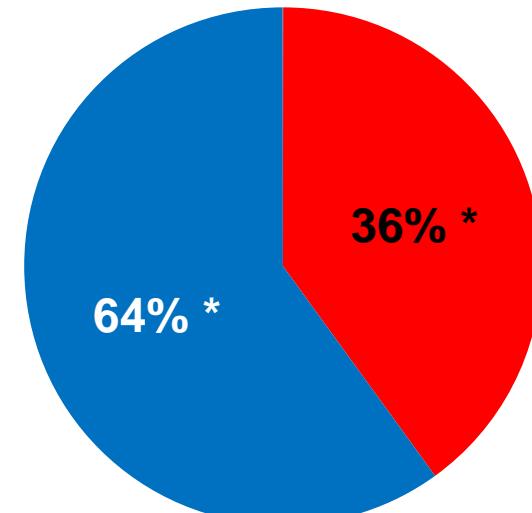
n = 22 slides, 55 tumors

Carboplatin/Paclitaxel



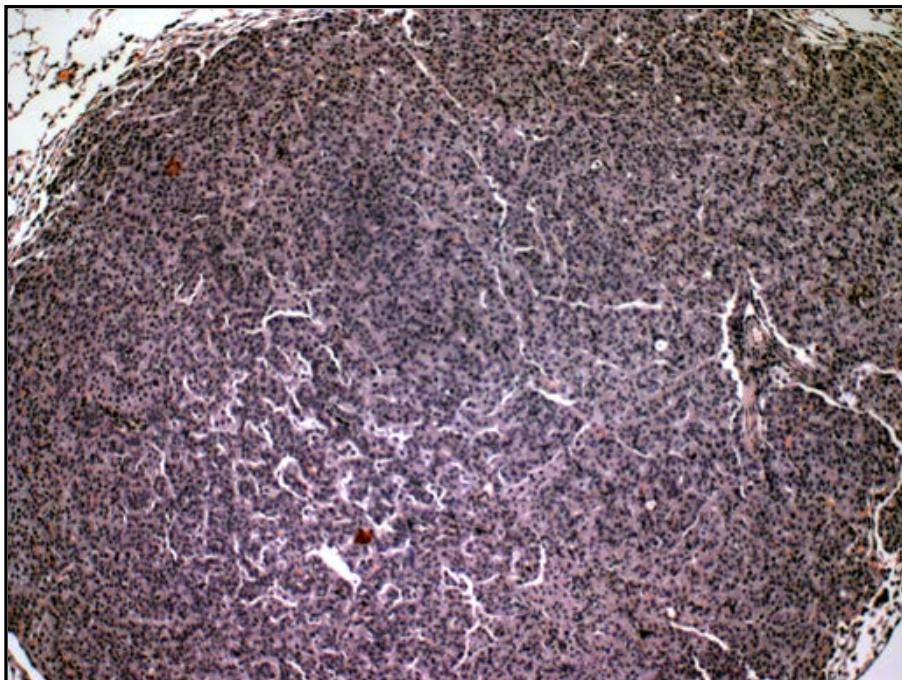
n = 42 slides, 90 tumors

CDDO-Me + C/P

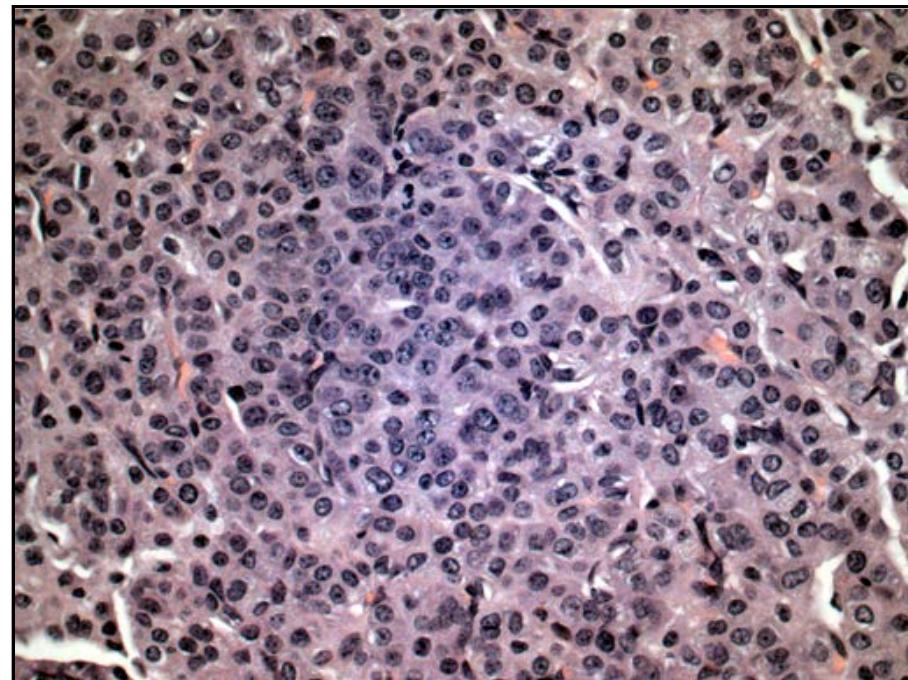


n = 32 slides, 45 tumors

# High grade tumors in control group

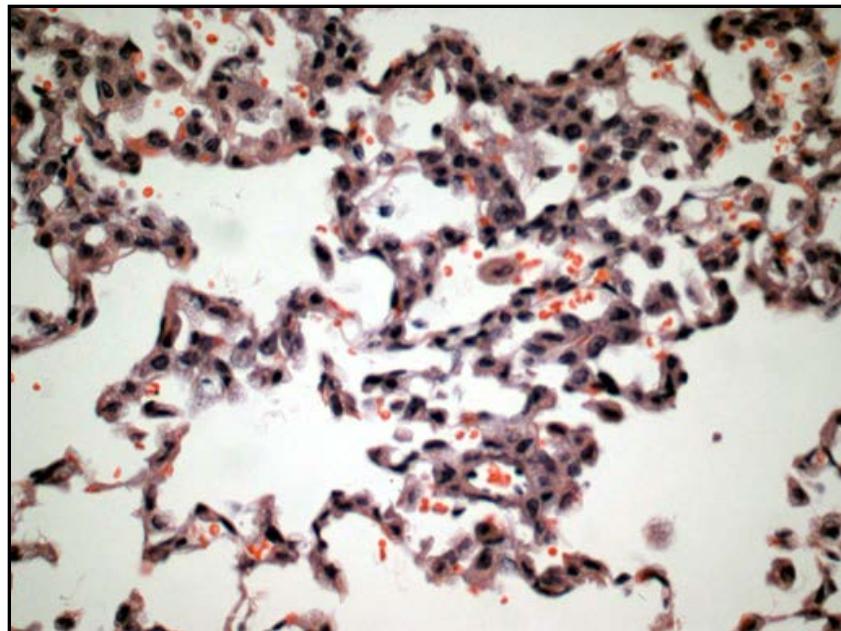
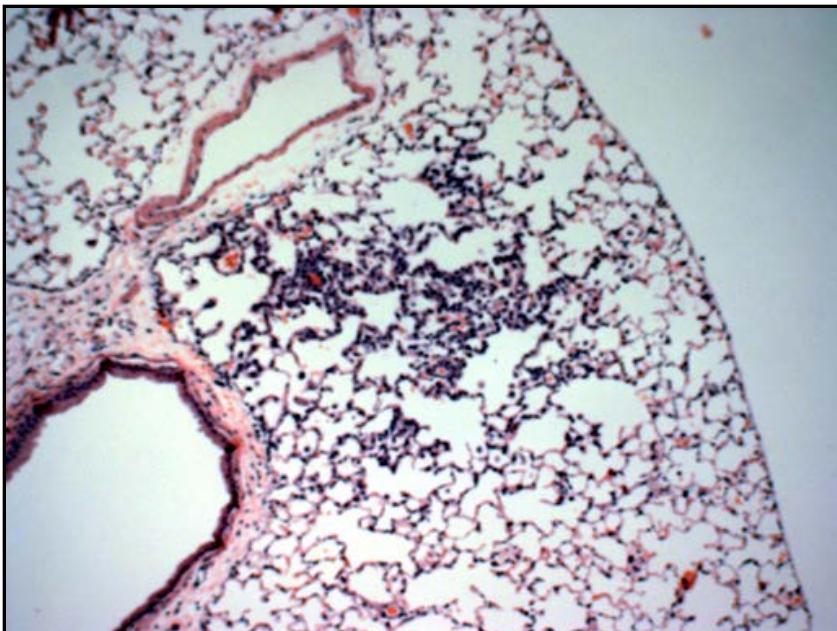
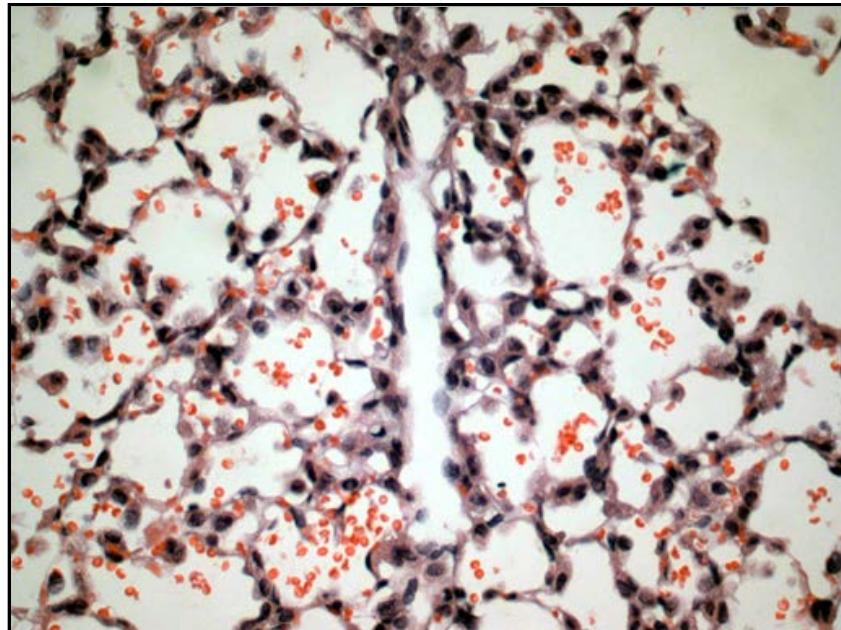
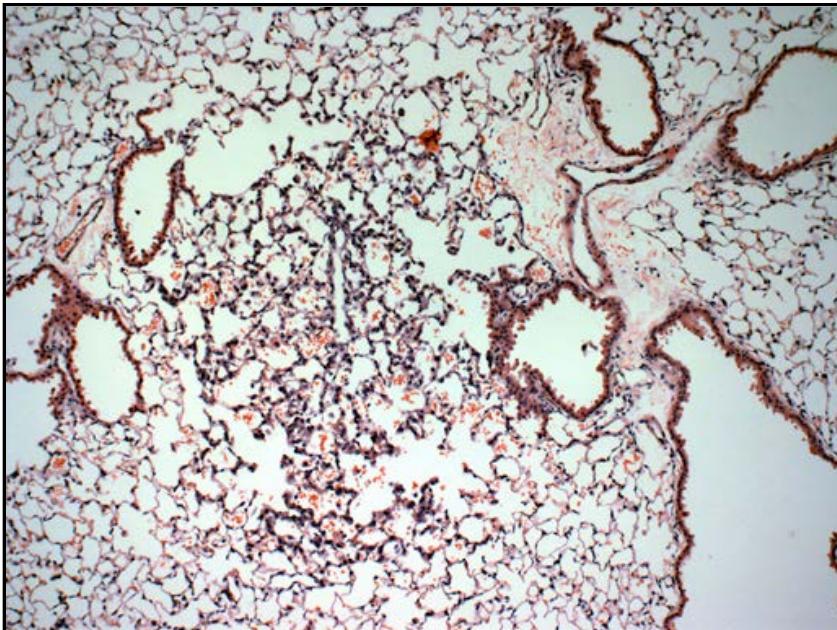


100X



400X

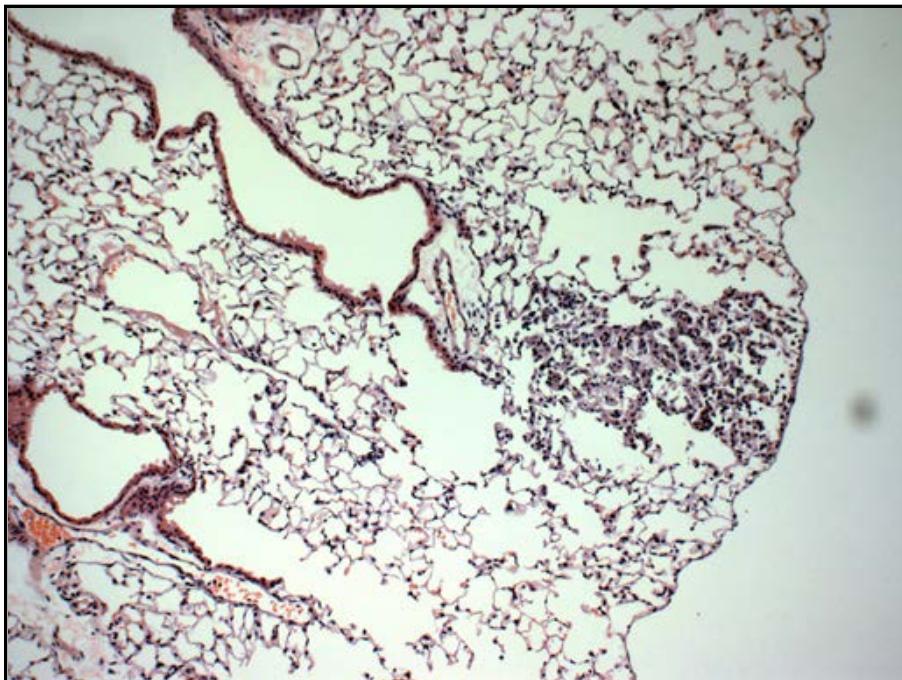
# Treatment – Carboplatin/Paclitaxel



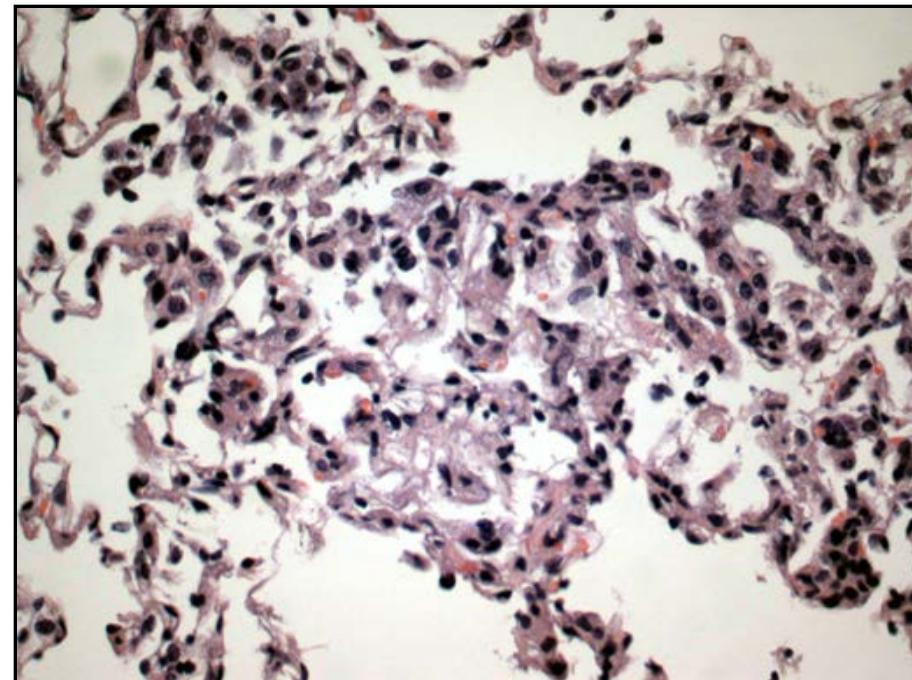
100X

400X

## Treatment – Triterpenoid + Carboplatin/Paclitaxel



100X



400X

# Summary

- Low doses of triterpenoids activate the Nrf2 cytoprotective pathway and reduce inflammation and ROS
- High doses of triterpenoids increase ROS and induce apoptosis in cancer cells
- Triterpenoids also reduce tumor burden and enhance treatment with carboplatin and paclitaxel in experimental lung cancer

# Acknowledgements

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Candice Black  
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## Johns Hopkins

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Albena Dinkova-Kostova  
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Melinda Yates

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