World Congress on Breast Cancer 2015
Birmingham, UK

Role of Dietary Additives in Suppressing the PhIP Induced Cytotoxicity

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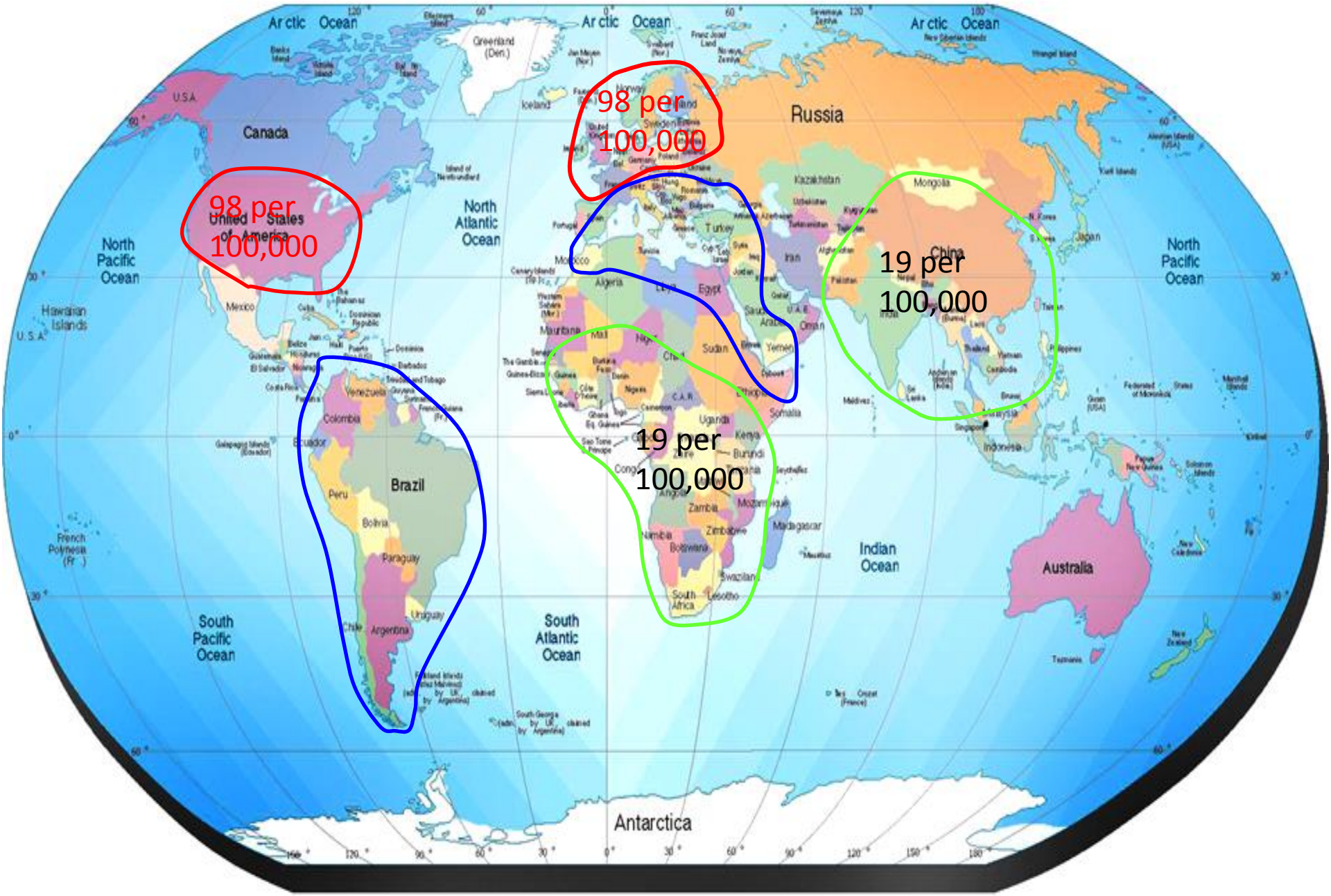
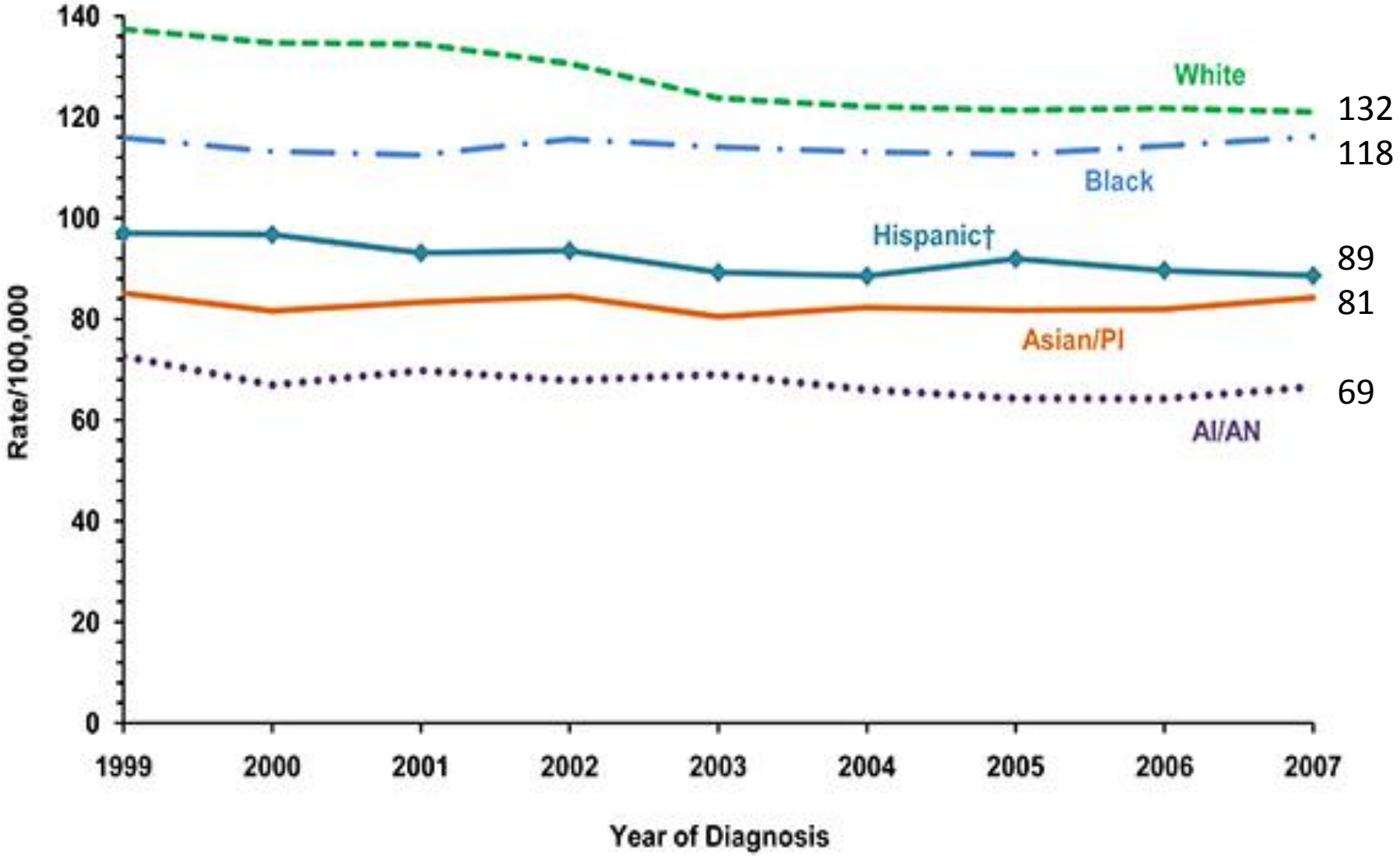
Breast Cancer Incidence – Worldwide

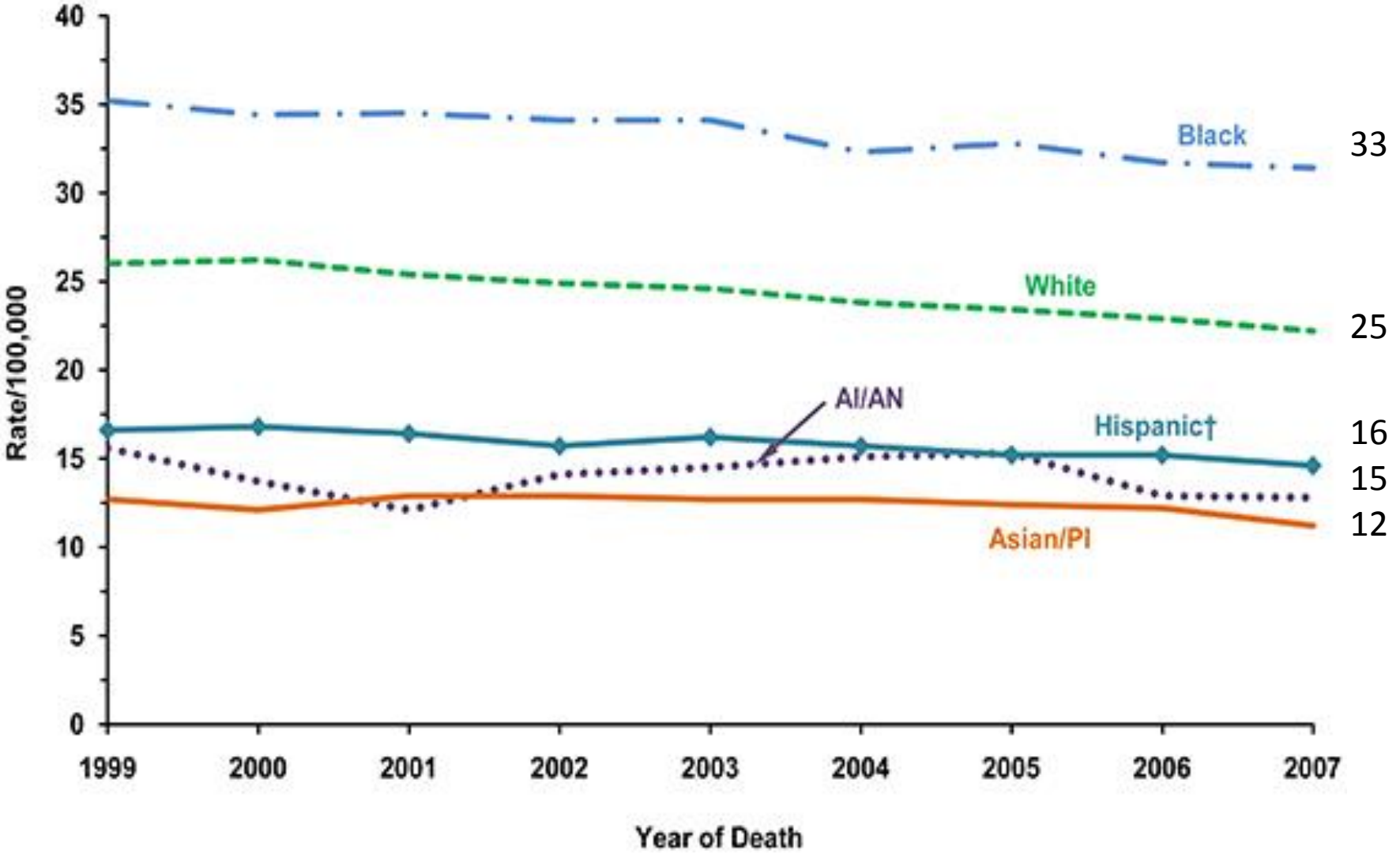


Female Breast Cancer
Incidence Rates* by Race and Ethnicity, U.S., 1999–2007



SOURCE: National Cancer Institute

Female Breast Cancer
Death Rates* by Race and Ethnicity, U.S., 1999–2007



SOURCE: National Cancer Institute

FACTS ABOUT BREAST CANCER

Breast Cancer Incidence **2000 data**

In developed countries: 98 per 100,000

In less developed countries: 20 per 100,000

The incidence (number of new cancers) is steadily increasing.

In USA about 184,000 new cases detected annually.

The National Cancer Institute estimates

Probability of developing Breast Cancer within 10 years	
By age 20	1 out of 1,760
By age 30	1 out of 229
By age 40	1 out of 69
By age 50	1 out of 42
By age 60	1 out of 29
By age 70	1 out of 27
Lifetime	1 out of 8

In other word::

- Every 3 minutes, one woman is diagnosed with breast cancer (USA)
- Every 11 minutes, one woman dies from breast cancer (USA)
- Every year, 30,000 women and 200 men are diagnosed with breast cancer (UK)

Troubling Meaty 'Estrogen'

High temperature cooking can imbue meats with a chemical that acts like a hormone

Depending on the temperature at which this burger was grilled—especially how hot its outer surface got—it may have hosted chemical reactions that created PhIP, a carcinogen that has a potent hormonal alter-ego. It can mimic the biological activity of estrogen, the primary female sex hormone.

Formation of HCAs (food mutagen)

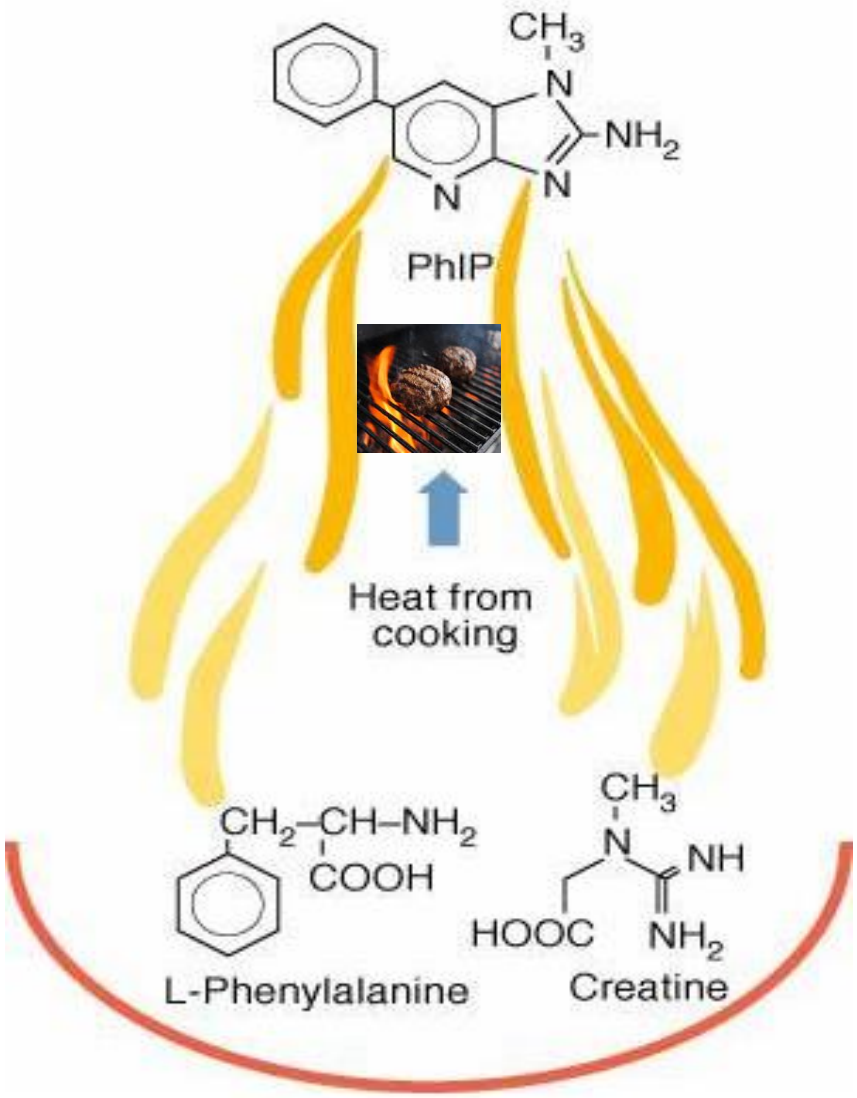


Illustration from E.G. Snyderwine (www.envimed.com)

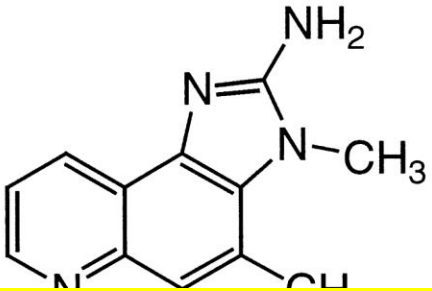
Heterocyclic amines (HCA): PhIP, MeIQ, IQ, 8 MeIQ etc

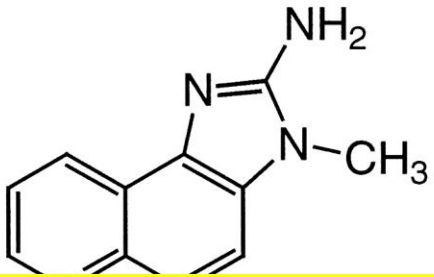
HIGH TEMP (~150 °C)

React with the free amino acids or proteins and, in some cases sugars (glucose)

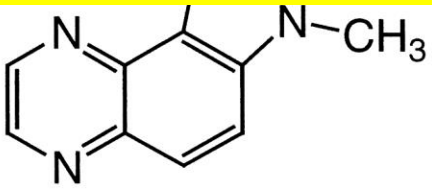
Muscle meats contain precursors: creatine and creatinine

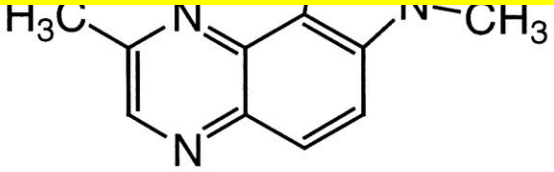
Nearly two dozen different heterocyclic amines that can form in cooked food - Polycyclic aromatic hydrocarbons





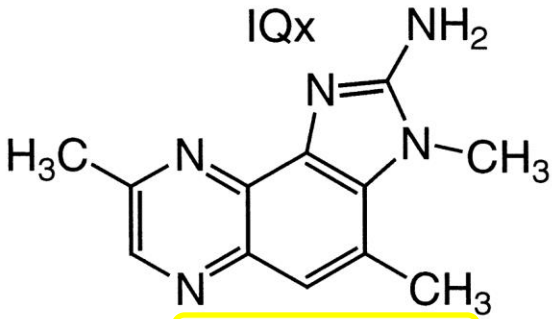
-2-amino-1-methyl-6-phenylimidazo[4,5-b] pyridine (PhIP) as most potent HCA



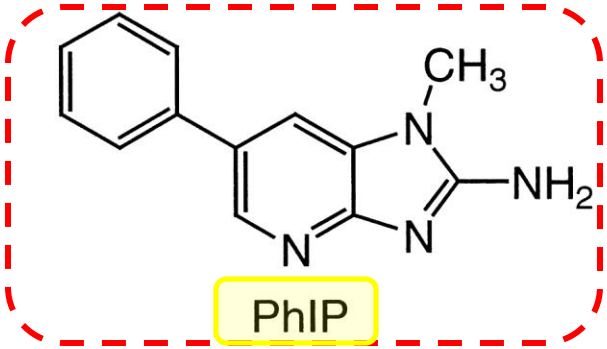


8-MeIQx

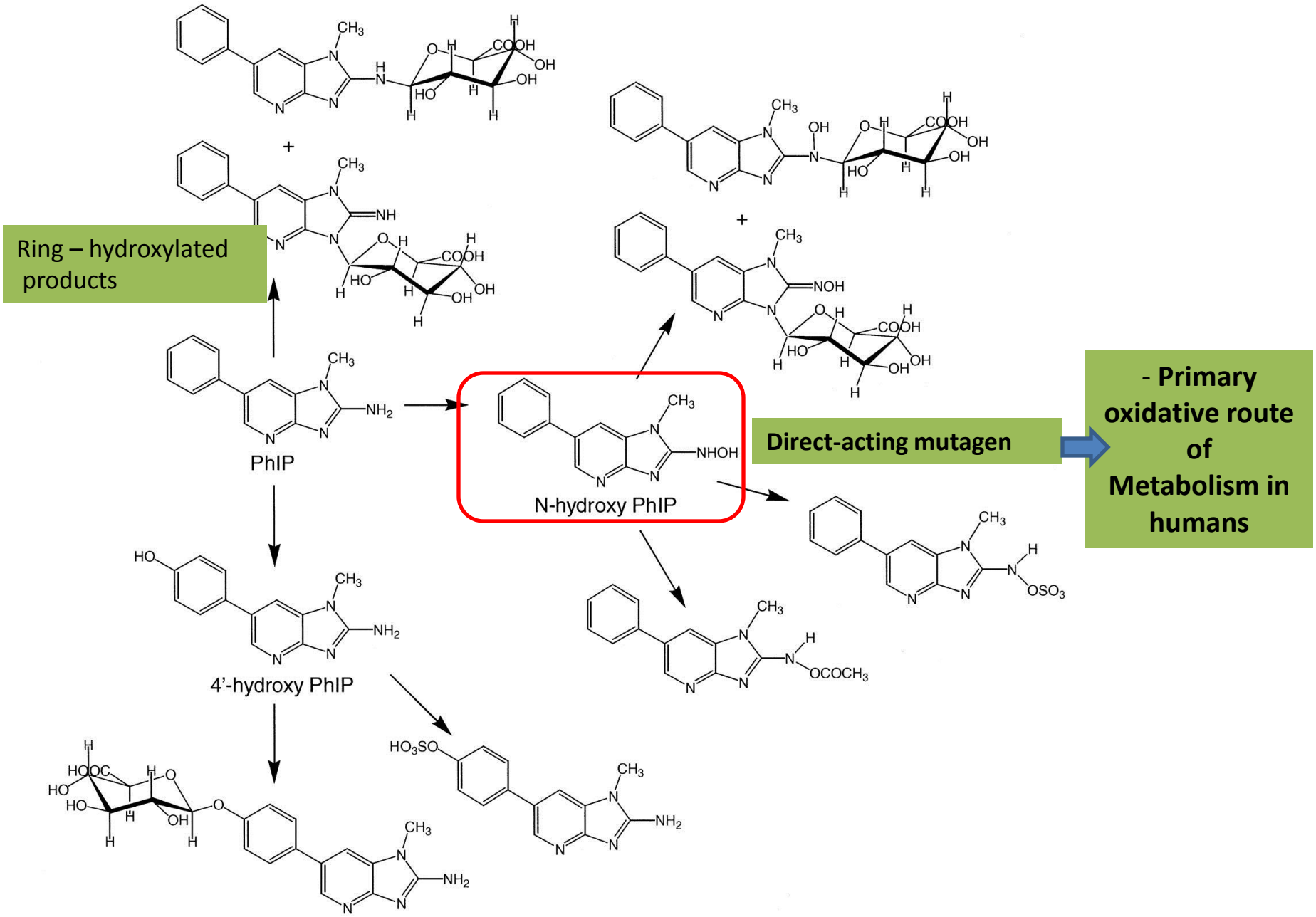
IQx



4,8-DiMeIQx

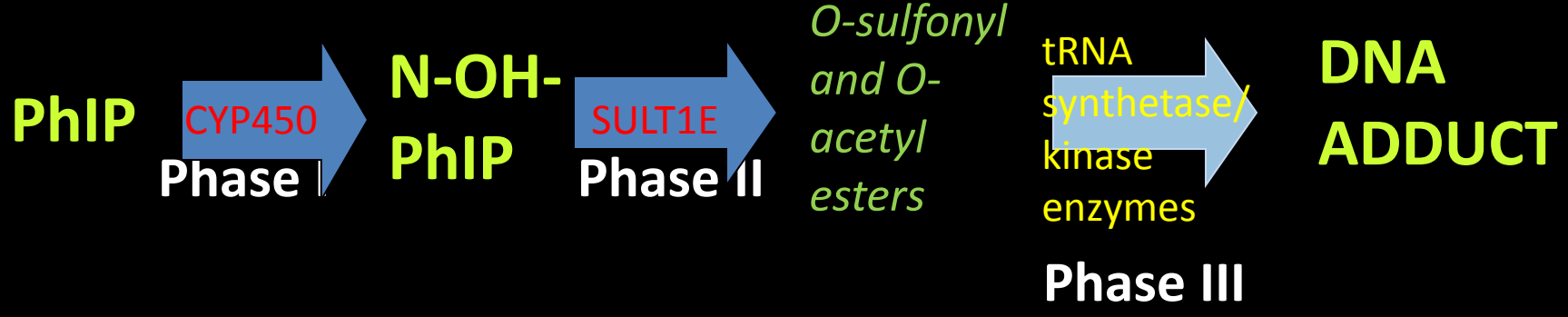


PhIP


Ring – hydroxylated products
PhIP
N-hydroxy PhIP
4'-hydroxy PhIP
Direct-acting mutagen
- Primary oxidative route of Metabolism in humans

- **PhIP is metabolized to N-OH-PhIP by cytochromes P450 (CYP1A1, CYP1A2, CYP2B6, CYP3A4) and then sulfated to a reactive sulfate ester by sulfotransferase (SULT1E)**

Schematic representation



- **The hypothesis is that breast epithelial cells contain all of the essential enzymatic machinery to bioactivate environmental carcinogens such as PhIP**

*** Most importantly DIET PLAY CRITICAL ROLE**

Hypothesis:

We hypothesize that the Phytochemical (or a combination of two or more) available in these dietary constituents is essential and effective to inhibit HCA induced DNA strand breaks. The inhibitions also protect cells from carcinogenicity of HCA and override chemoresistance.

Or - **the right combination of antioxidants/phytochemicals (naturally present in fruits, vegetables and spices) along with grilled meat can suppress the HCA induced cytotoxicity (breast cancer).**

Goal

Understand the biological mechanism of the protective effects of Phytochemicals (antioxidants) and their interaction with carcinogenic heterocyclic amines (found in cooked meat).

- **Specific aims:**

(i) Identify Phytochemical/s effective in inhibiting the HCA (PhIP) induced cytotoxicity.

(ii) Investigate the interaction of PhIP and phytochemical/s at gene level and understand the molecular mechanism of chemoprevention.

PhIP dose Curve on normal breast epithelial cells (MCF 10A)

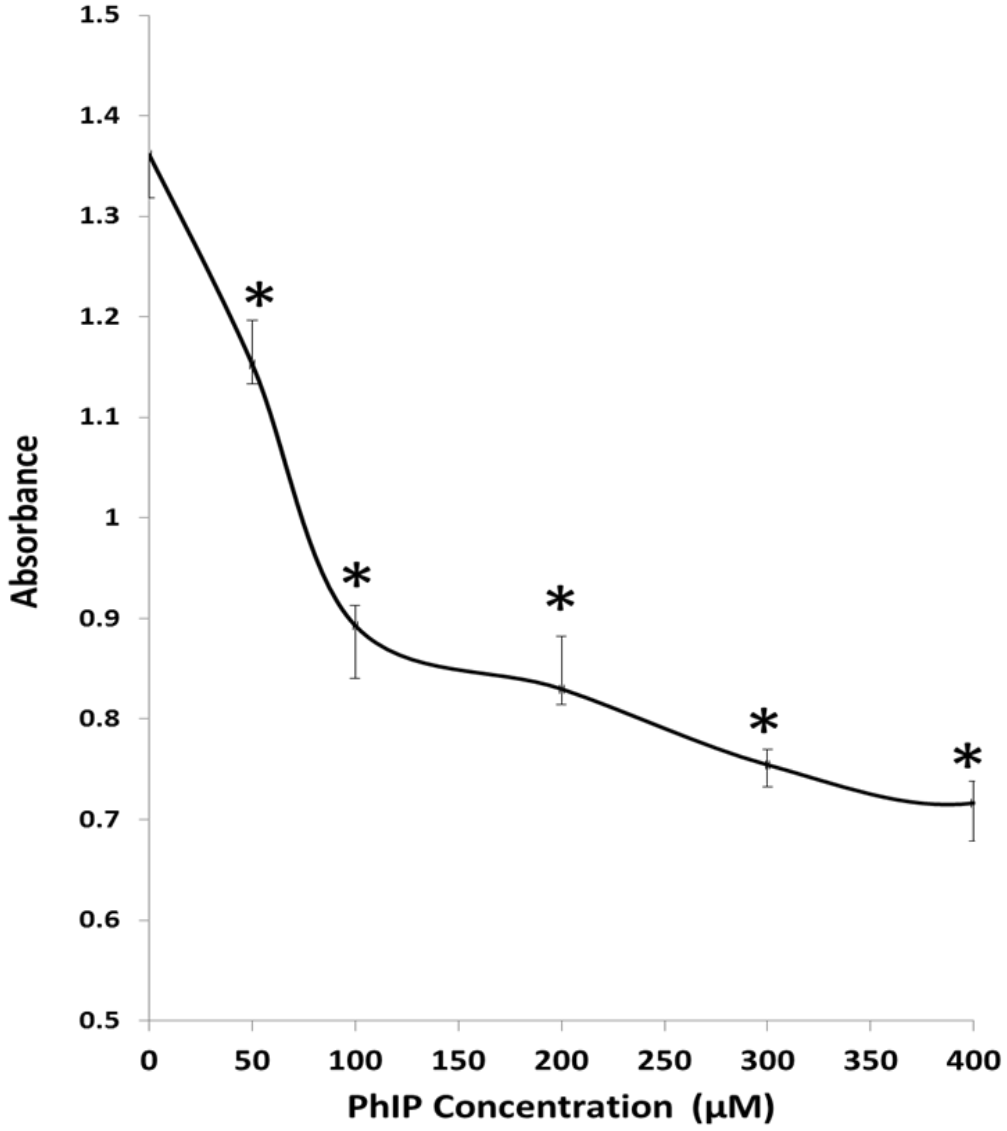


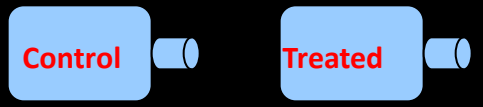
Figure-1

Phytochemicals tested in combination with PhIP (50 µM and 250 µM)

Phytochemical	Concentrations
N-Acetyl-L-Cysteine (NAC)	25 µM to 1 mM
Glutathione (GSH)	25 µM to 1 mM
Ascorbic acid (Vit C)	25 µM to 1 mM
Lycopene	1 µM, 5 µM
Gingerol [10]	2.5 µM 100 µM
Gingerol [6]	2.5 µM 100 µM
Vitamin K3	5µM to 500 µM
Vitamin D3	0.65 nM to 26 nM
Vitamin E	1µM to 100 µM
Curcumin	10 µM 1mM
Piperine	5µM to 500 µM

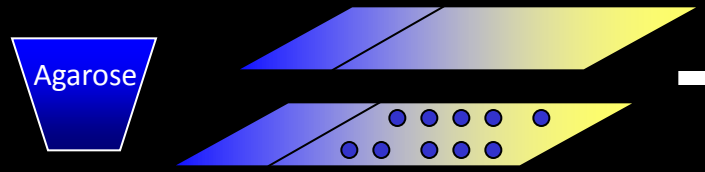
COMET ASSAY

1. **Culture cells and treat with an compound (toxin/carcinogen)**



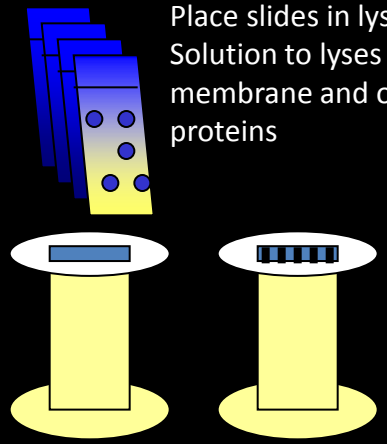
Harvest cells in to PBS or HBSS
To give 1X106 cells/ml

2. **Pre-cote slides and prepare cells sandwich**



Pore Agarose onto the slides and cast the cells

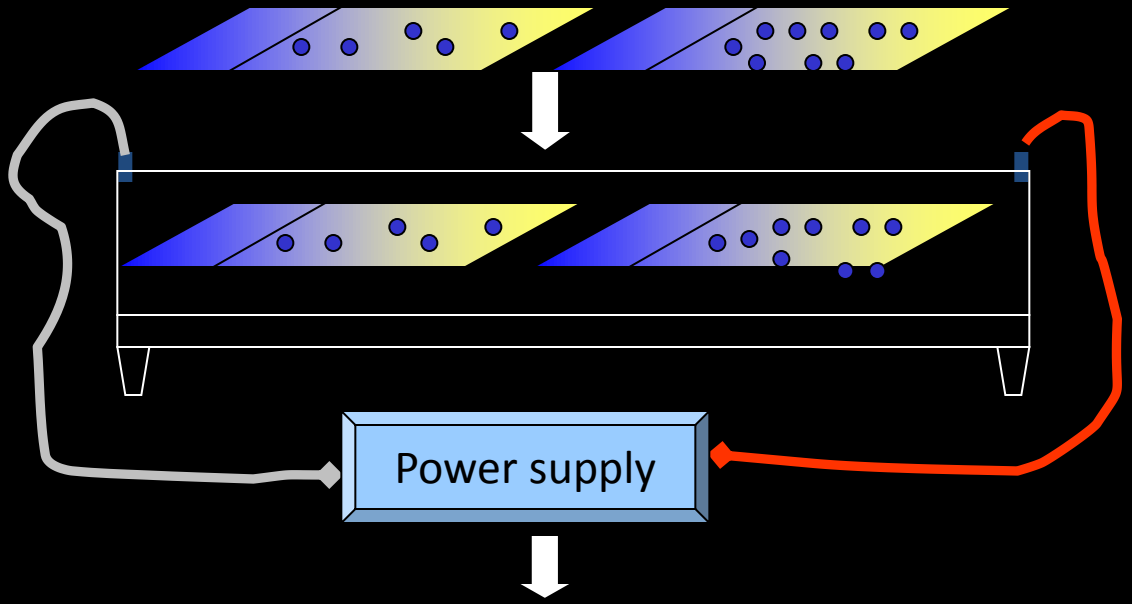
3. **Lysis –**



Place slides in lysis Solution to lyses cell membrane and other proteins

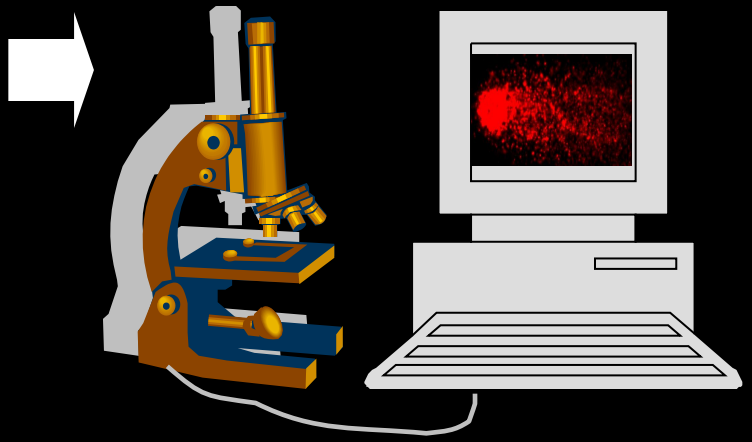
4. **Unwinding and electrophoresis**

Place slides in alkaline (pH>13) buffer to allow unwinding of DNA

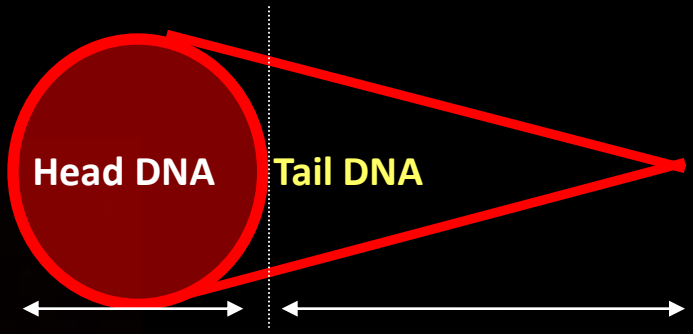


5. **Neutralization – Neutralizes excess alkali**

6. **Stain and Score of cells**



Determining DNA Strand Breaks using COMET ASSAY



Head DNA

Tail DNA

Control

Treated – 50 μM

Treated - 250 μM

Comet Tail

Olive Tail Moment (μM)
(Estimate of DNA Damaged)

$$= \frac{\text{Tail length X \% Tail DNA}}{100}$$

Developed a model system to demonstrate that antioxidants prevent PhIP cytotoxicity

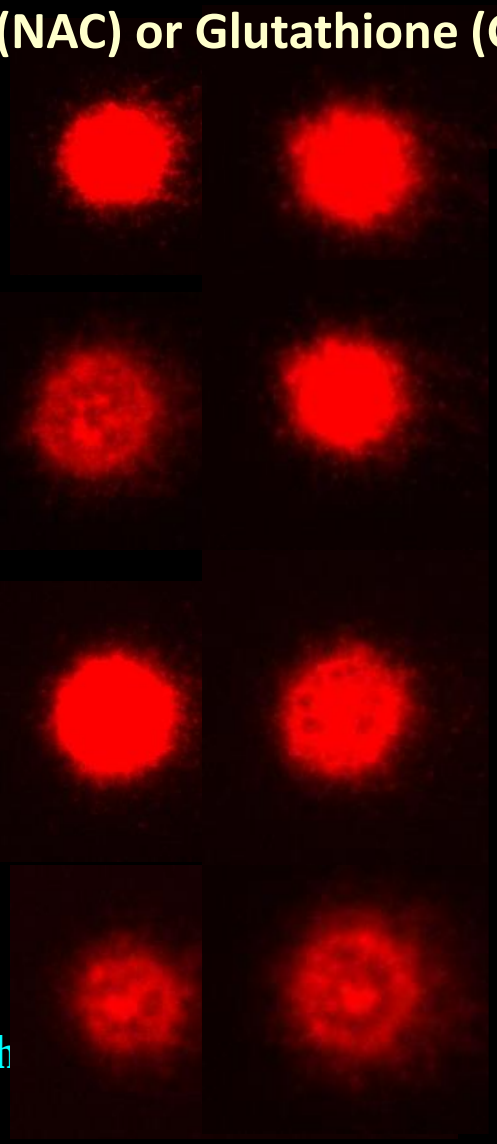
Protection of PhiP mediated DNA damage in presence of known antioxidant like n-acetyl cystine (NAC) or Glutathione (GSH)

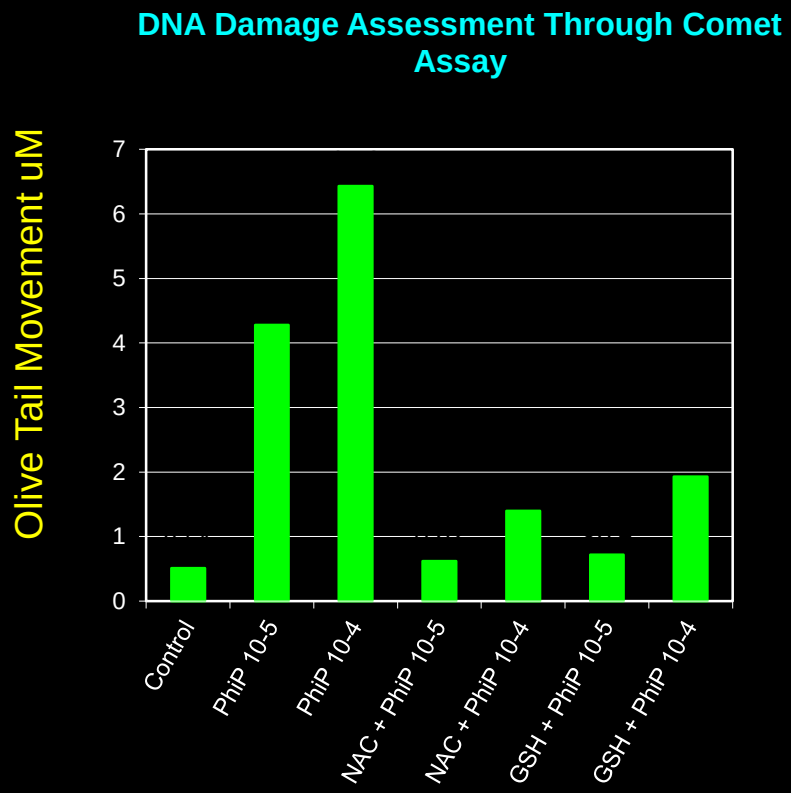
4. 10 mM NAC + PhIP 10^{-5} μM) - 24 h

5. 10 mM NAC + PhIP 10^{-4} μM) -24 h

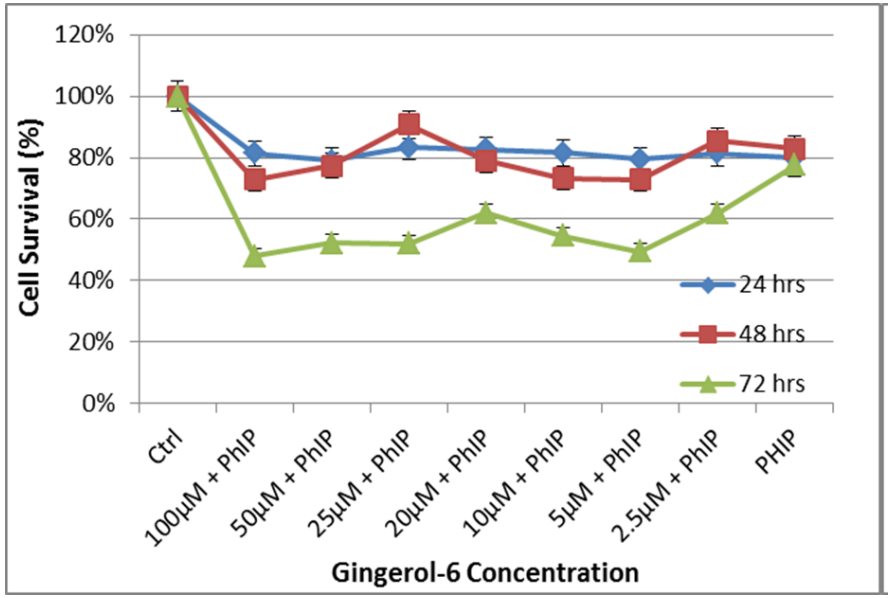
6. 10 mM GSH + PhiP (10^{-5} μM) – 24h

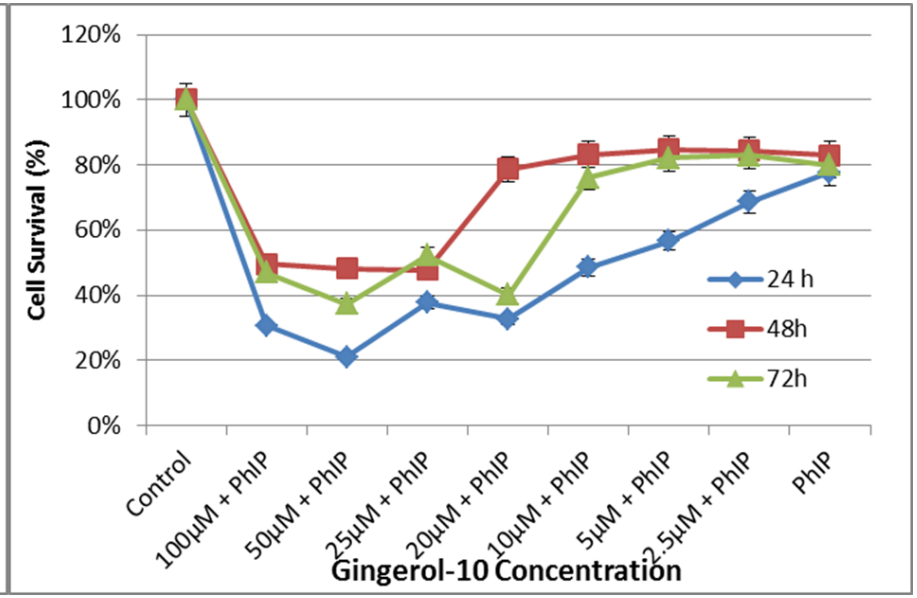
7. 10 mM GSH + PhiP (10^{-4} μM) – 24h





Screening based on cell survival and DNA Damage data (Comet assay)





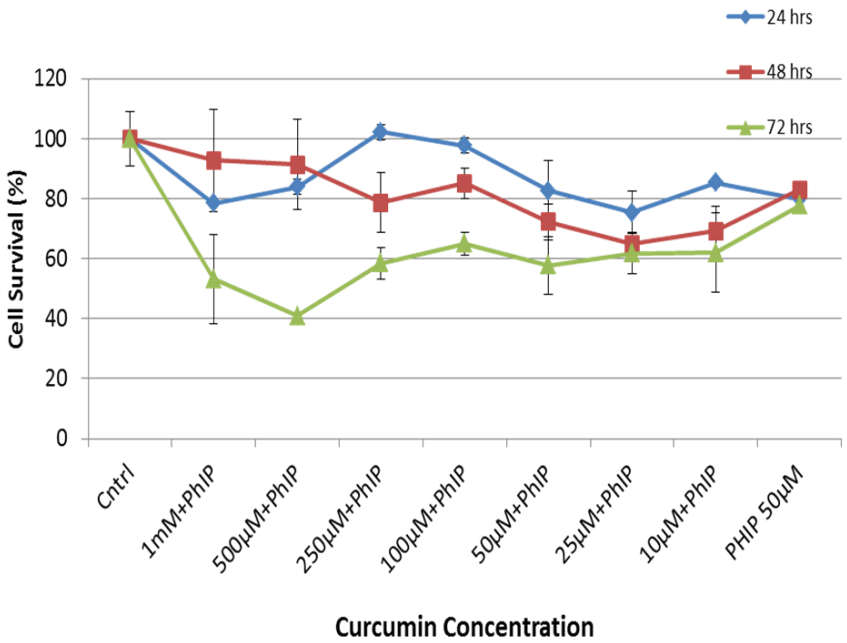
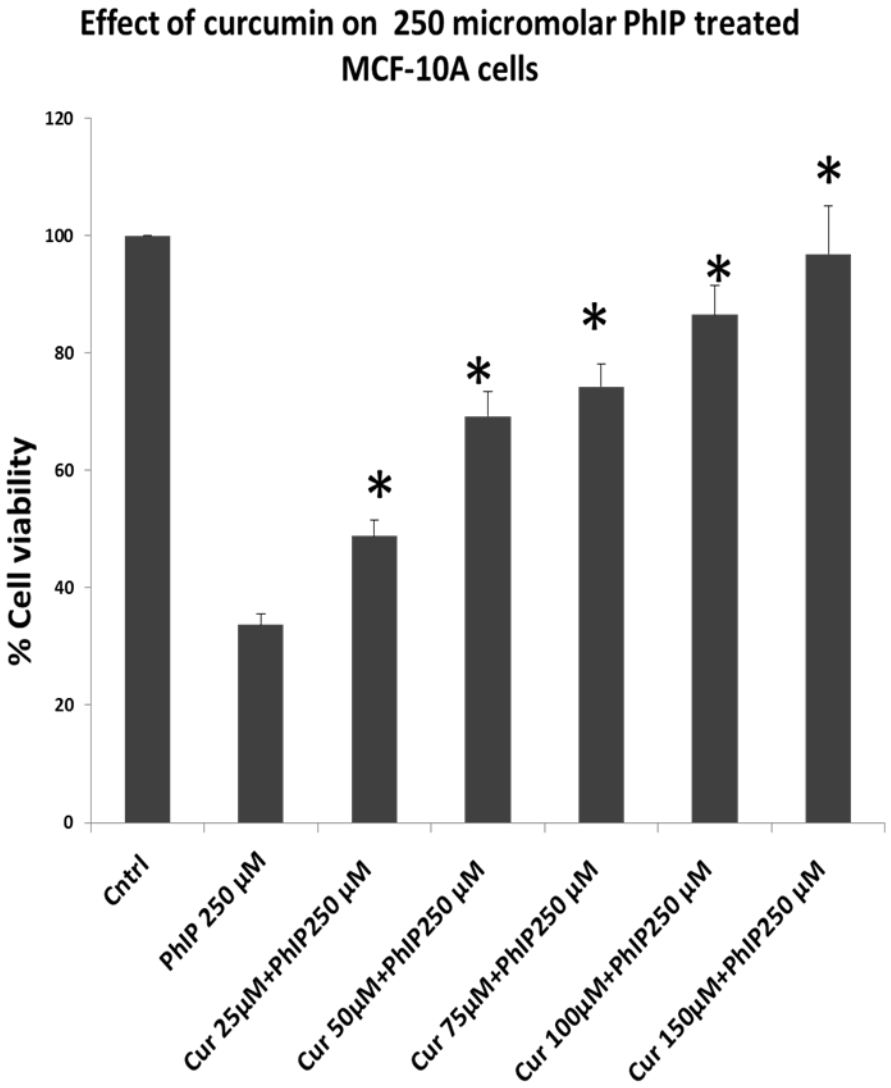


Table summarizing response of phytochemical based on cell survival and DNA damage (Comet assay)

- N-acetyl-L-Cysteine (NAC) - 500 µM ***
- Glutathione (GSH) - 500 µM ***
- Ascorbic acid (Vitamin C) - 500 µM***
- Vitamin E - 50 µM***
- Vitamin K3 - 10 µM or 25 µM*
- Vitamin D3 - 100 µM***
- Lycopene - 5 µM**
- 10-Gingerol - 20 µM or 40 µM**
- 6-Gingerol - 100 µM or 200 µM*
- **Curcumin - 150 µM****
- Piperine - 5 µM or 10 µM***+

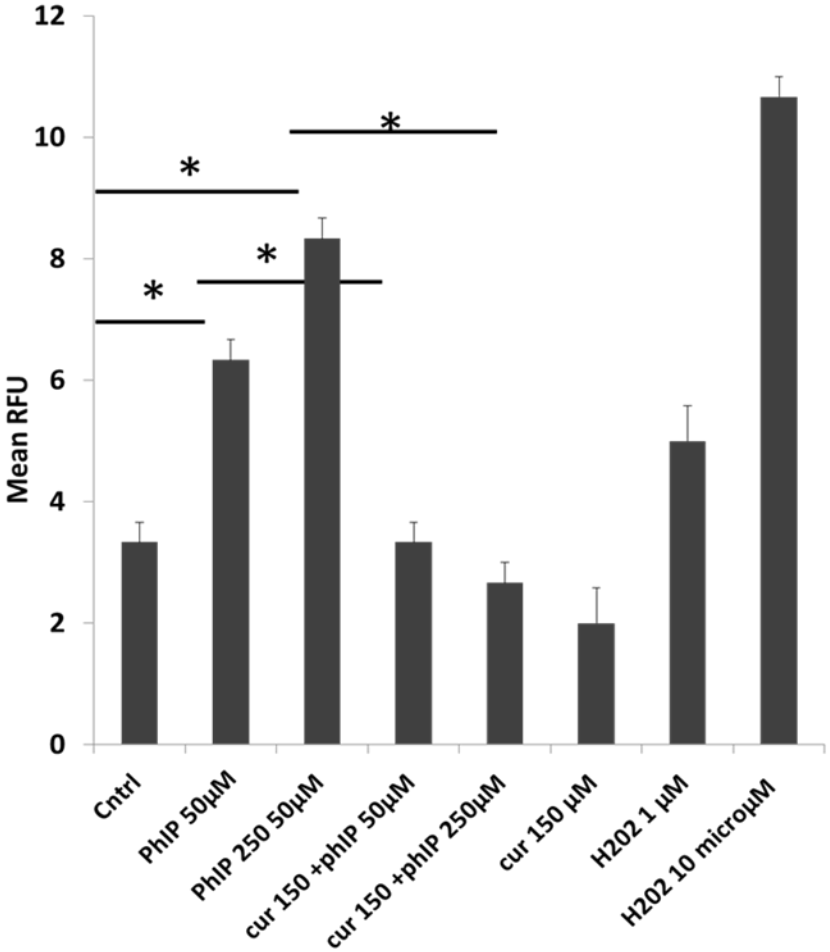
******= Highly effective;** ***+= Very effective; ***= Quite effective;
**= Moderately effective; *= Least effective

Curcumin and PhIP interaction

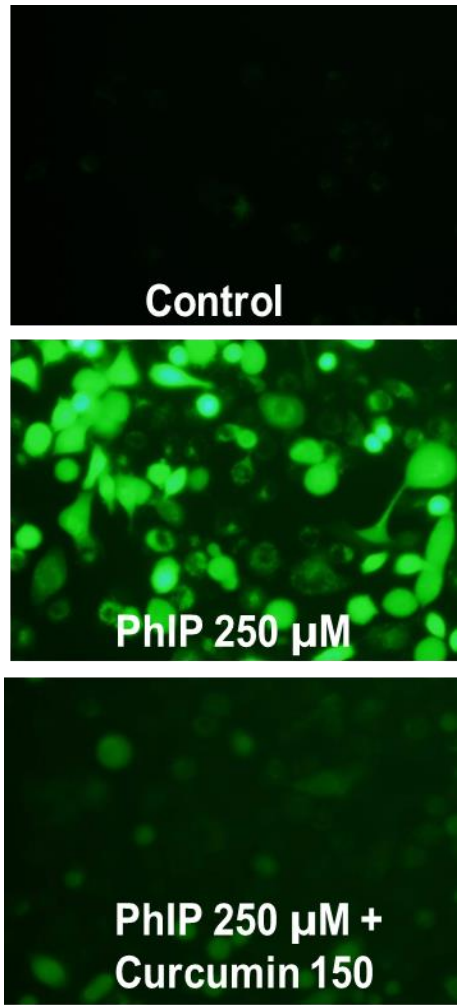


Effect of various doses of Curcumin on 250 µM PhIP induced toxicity in MCF-10A cells.

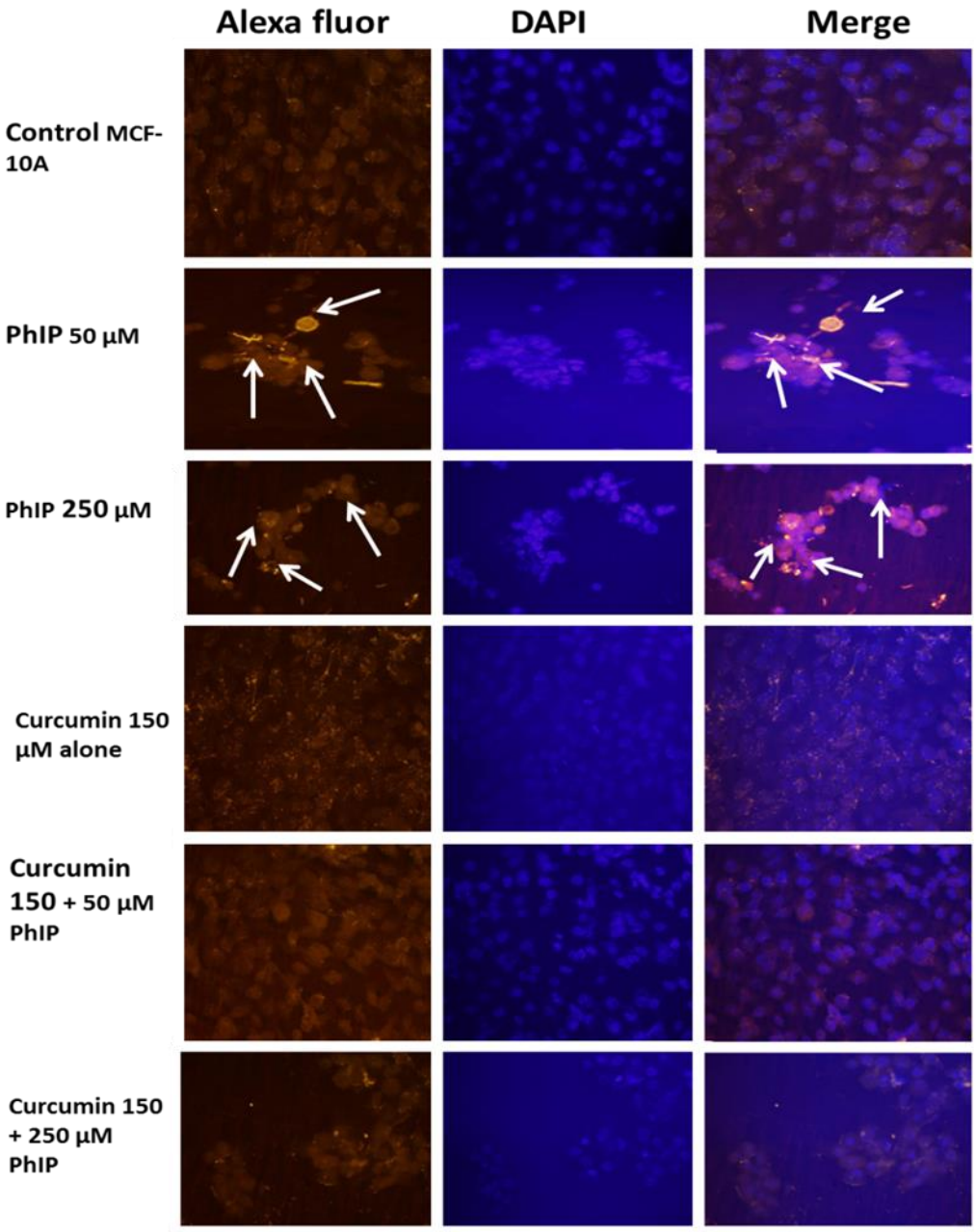
PhIP metabolism induces production of ROS [Sato, K. *et al.* **Evidence of Direct Generation of oxygen free radicals from heterocyclic amines by NADPH/cytochrome P-450 reductase in vitro**. Jpn. J. Cancer Res., 83 (1992), pp 1204-1209



Quantitative fluorescence intensity analysis of ROS production in PhIP-treated MCF-10A cells upon co-treatment with curcumin

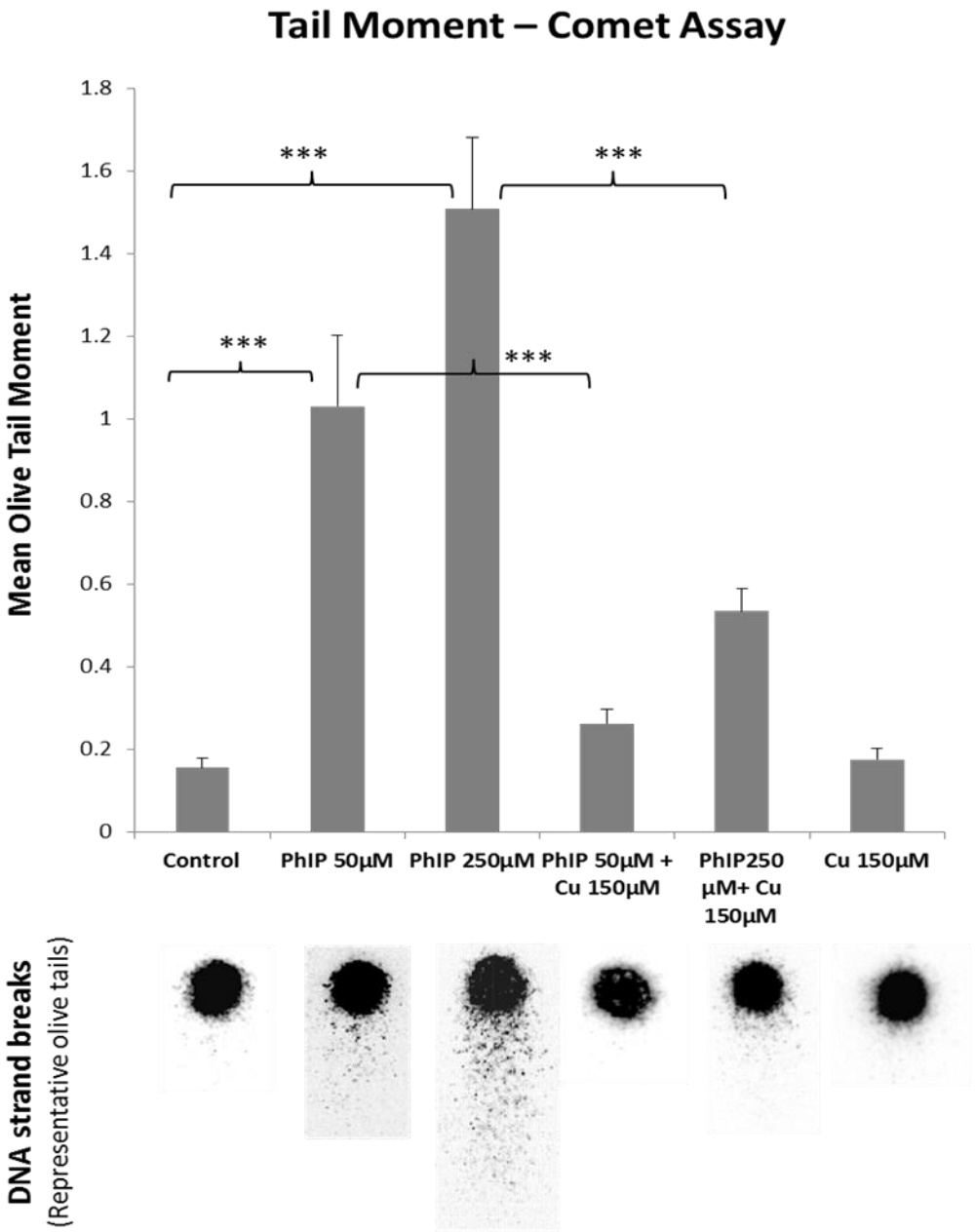


Relative DCF-DA fluorescence emission from cells treated with PhIP ± curcumin

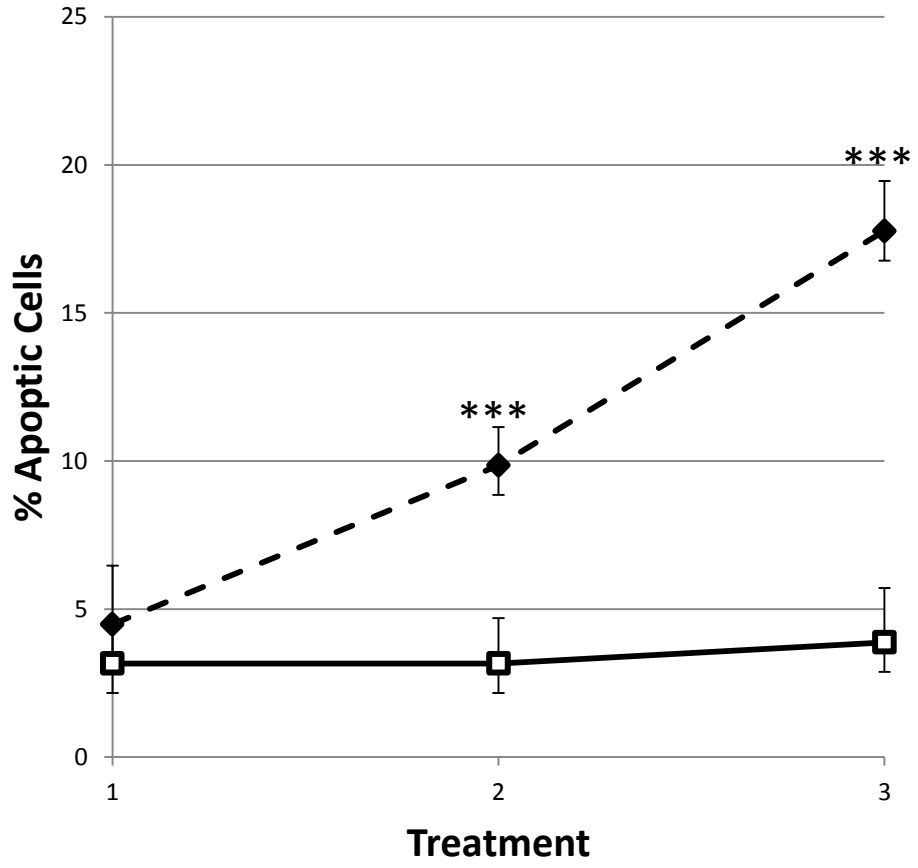


Immunofluorescence assay of DNA adduct formation using an anti-DNA adduct antibody

DNA Damage studies with Curcumin



PRESENEC OF Curcumin SUPPRESS APOPTOSIS



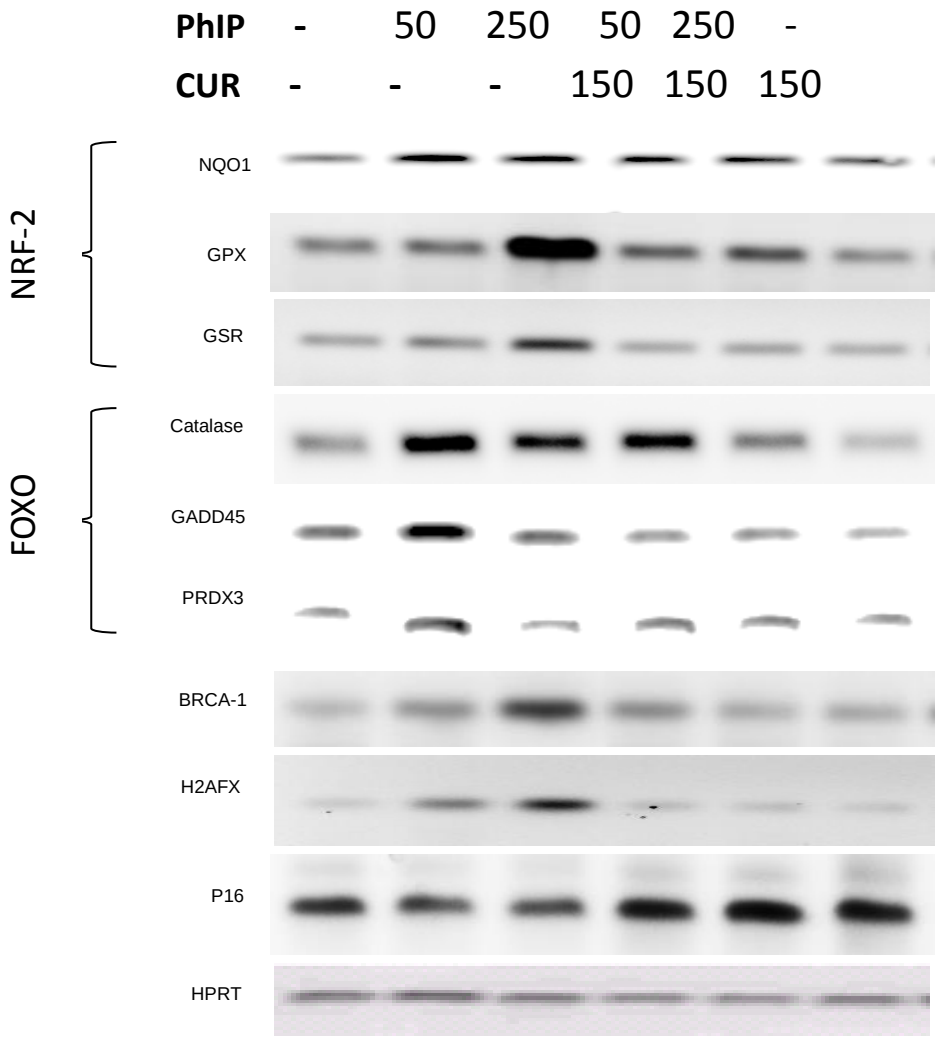
Series1

1. Control
2. PhIP 50 µM
3. PhIP 250 µM

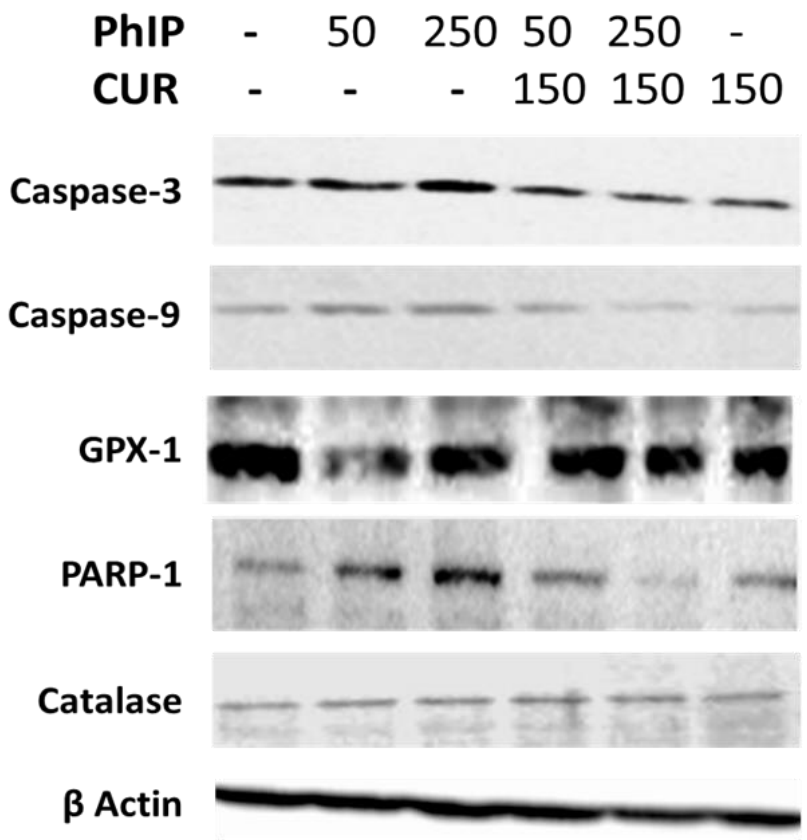
Series2

1. Curcumin 150 µM
2. PhIP 50 µM + Curcumin 150 µM
3. PhIP 250 µM + Curcumin 150 µM

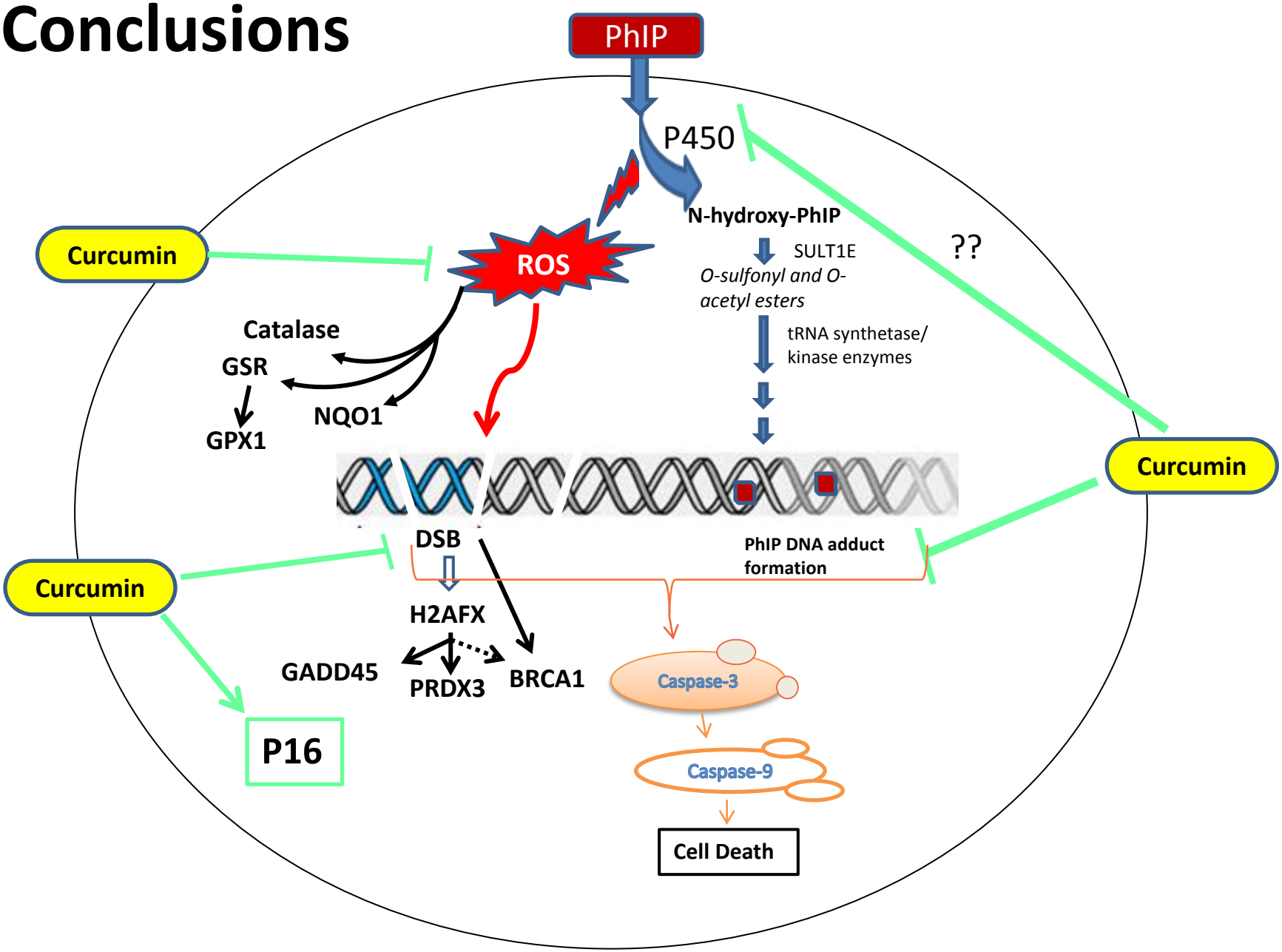
Effect of PhIP and curcumin alone and in combination on Nrf2, FOXO, BRCA-1, H2AFX and P16 signaling pathways, with HPRT used as a normalization control



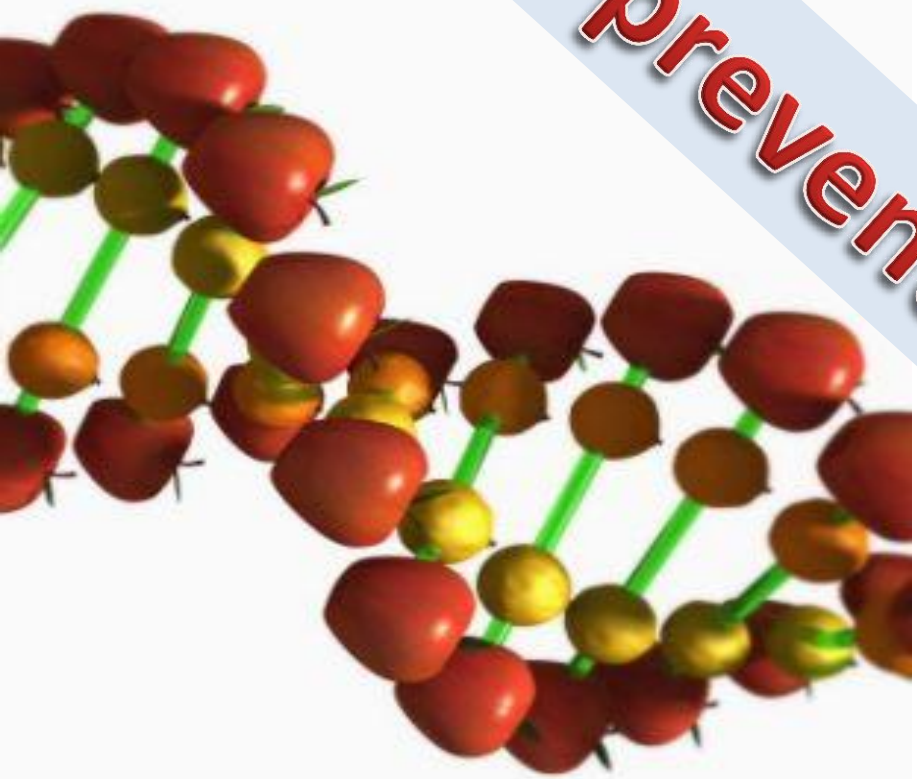
Western blot analysis results of PhIP-treated MCF-10A cells with and without co-treatment with curcumin.



Conclusions



Why not prevent it?



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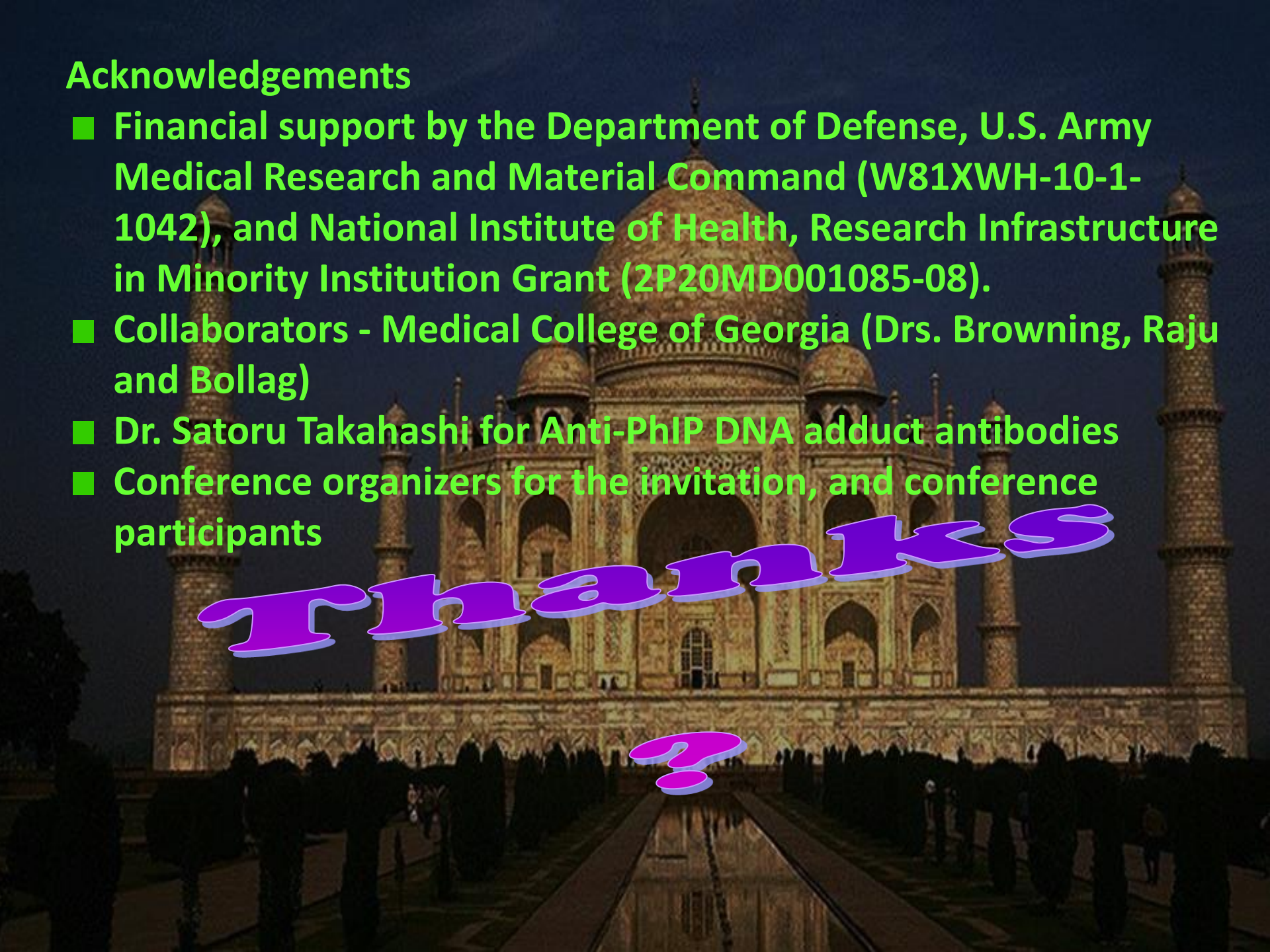
- Collaborators - Medical College of Georgia (Drs. Browning, Raju and Bollag)
- Dr. Satoru Takahashi for Anti-PhIP DNA adduct antibodies
- Conference organizers for the invitation, and conference participants



Histone modifications

Tomatoes
(Lycopene)

Turmeric
(Curcumin)

Cinnamon
(Coumaric acid)

Cashew nuts
(Anacardic acid)

Apples
(Phloretin)

Soybean
(Genistein)

Tea
(EGCG)

Grapes
(Resveratrol)

Citrus
(Hesperidin)

Coffea
(Caffeic acid)

Broccoli
(Isothiocyanates)

Garlic
(Allyl mercaptan)

DNA methylation

Author of the *New York Times* Bestseller
EAT RIGHT 4 YOUR TYPE

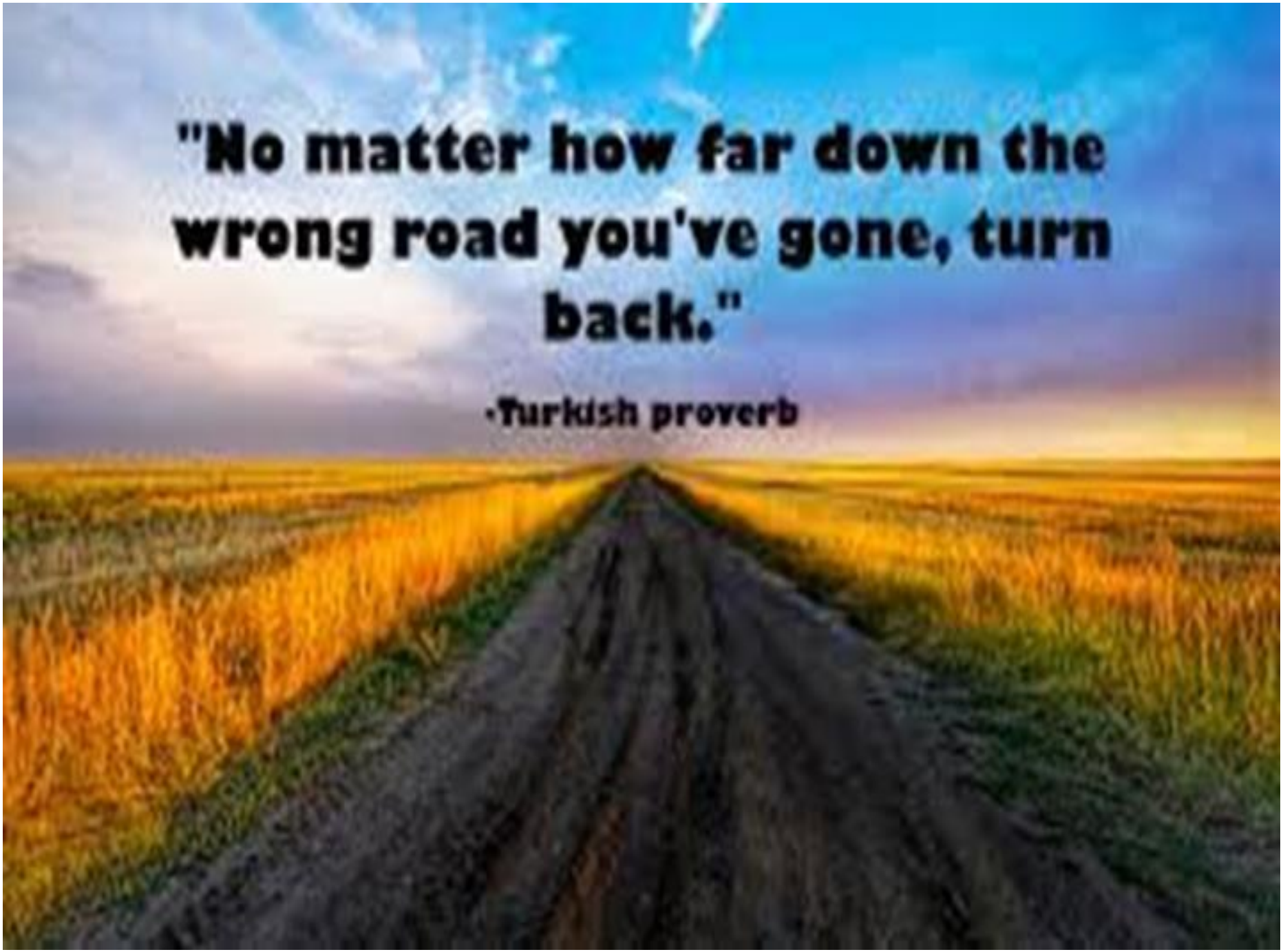
CHANGE YOUR GENETIC DESTINY

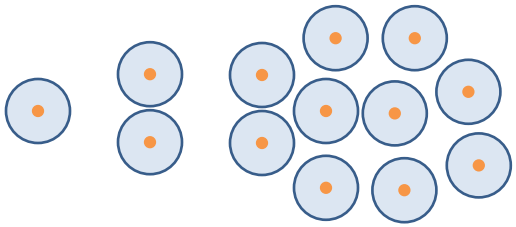
Previously titled *The GenoType Diet™*

THE REVOLUTIONARY GENOTYPE DIET

YOUR PERSONALIZED PLAN TO
LOSE WEIGHT, BOOST ENERGY,
AND IMPROVE IMMUNITY

Dr. Peter J. D'Adamo
with Catherine Whitney


"No matter how far down the wrong road you've gone, turn back."
-Turkish proverb



2015 US Cancer Cases*

Men 848,200		Women 810,170	
Prostate	26%	29%	Breast
Lung and bronchus	14%	13%	Lung and bronchus
Colon & rectum	8%	8%	Colon & rectum
Urinary bladder	7%	7%	Uterine corpus
Melanoma of skin	5%	6%	Thyroid
Non-Hoc lymp			in na
Kidney &			of skin
Oral cavity & pharynx	4%	3%	Pancreas
Leukemia	4%	3%	Leukemia
Liver and intrahepatic bile duct	3%	3%	Kidney & renal pelvis
All Other Sites	21%	21%	All Other Sites

BREAST CANCER IS THE MOST PREVELANT CANCER IN WOMEN

***Excludes basal and squamous cell skin cancers and in situ carcinomas except urinary bladder.**
Source: American Cancer Society, 2015.

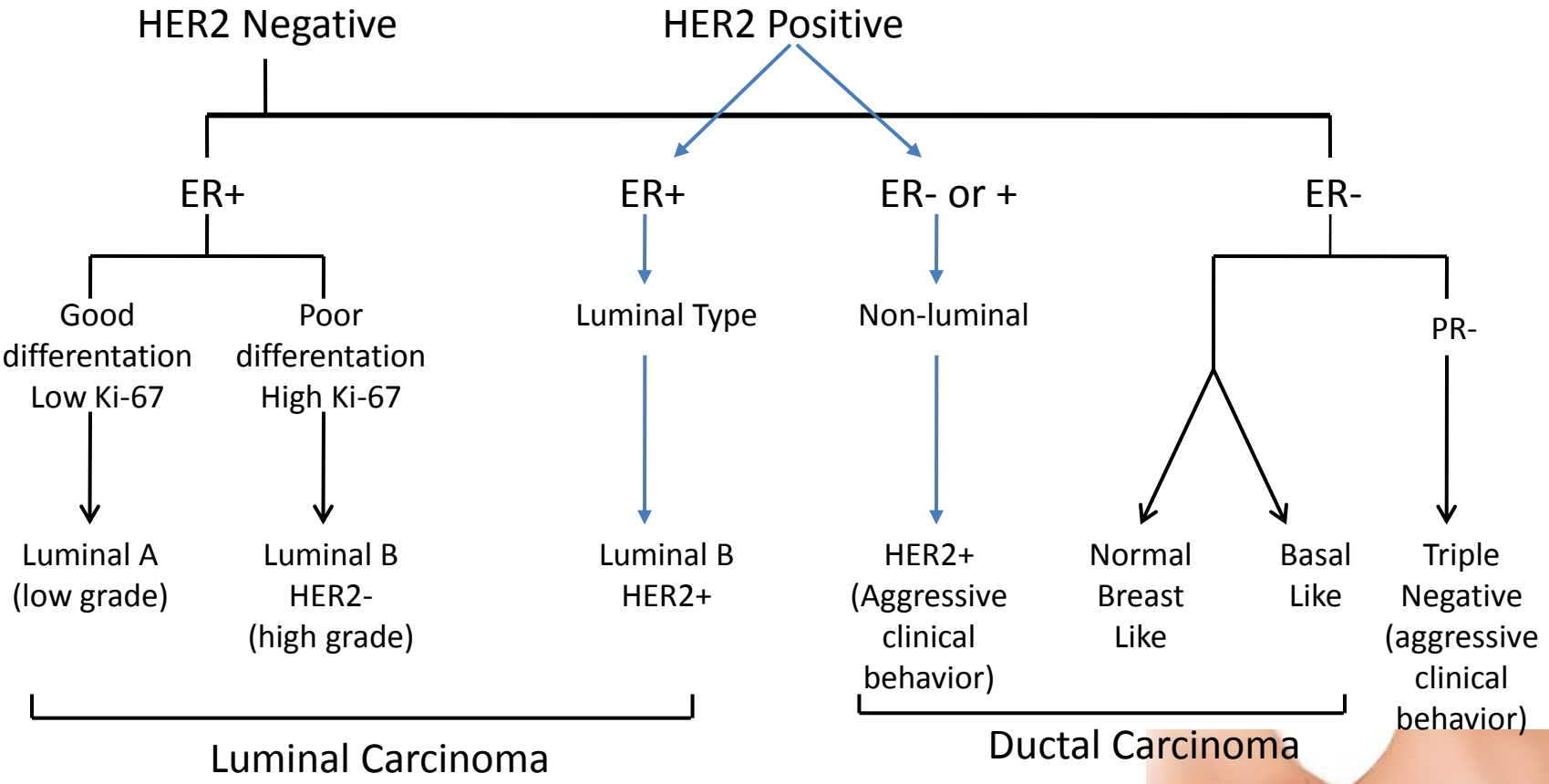
Estimated Cancer Deaths in the US in 2015

Men			Women	
Lung & bronchus	28%		26%	Lung & bronchus
Prostate	9%		15%	Breast
Colon & rectum	8%		9%	Colon & rectum
Pancreas	7%		7%	Pancreas
Liver & intrahepatic bile duct	5%		5%	Ovary
Leukemia	5%		4%	Leukemia
Esophagus	4%			e corpus
Urinary bladde	4%			Hodgkin phoma
Non-Hodgkin lymphoma	4%		3%	Liver & intrahepatic bile duct
Kidney & renal pelvis	3%		2%	Brain & other nervous system
All other sites	24%		23%	All other sites

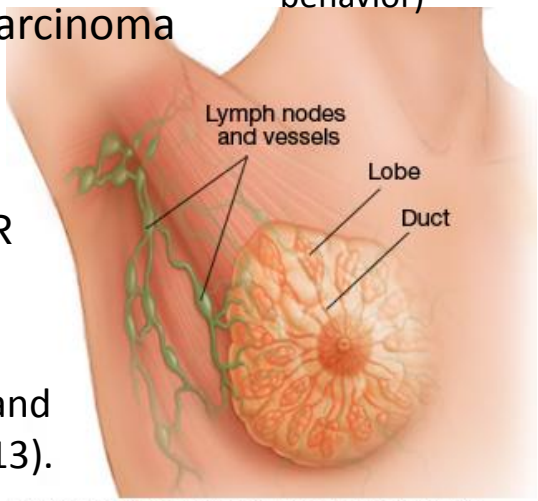
Breast Cancer is the second leading cause of deaths among women

Source: American Cancer Society 2015

Complexity of Breast Cancer sub-type



Molecular classification and clinicopathological features of breast cancer. HER2 - Human epidermal growth factor 2: determined by immunohistochemical (IHC) analysis; ER – Estrogen receptor; PR - Progesterone receptor; Ki-67 – Nuclear antigen Ki-67.



Reference: Jain, A. **Targeting Breast Cancer**, In: Signaling Gene Regulation and Cancer. Singh S. R. and Mishra M. K. (ed). Nova Science Publishers, Inc. (2013).