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**City of Hope...where the power of knowledge saves lives.**



Duarte, California (Los Angeles)

# **Breast Cancer Prevention with Phytochemicals in Grape Juice**

Shiuan Chen

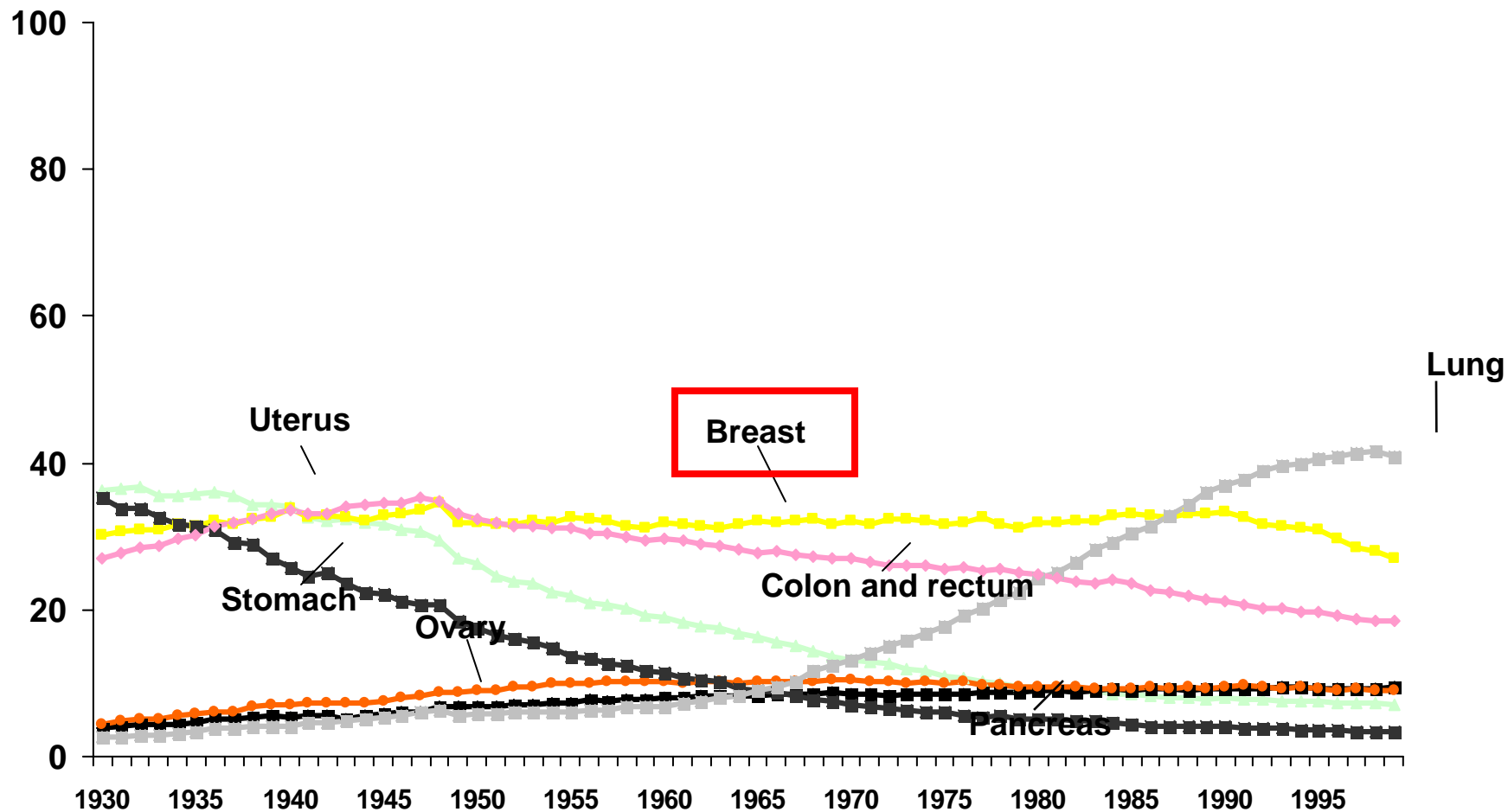
schen@coh.org

City of Hope/Beckman Res. Institute

Duarte, CA 91006

# Cancer Death Rates\*, for Women, US, 1930-1999

Rate Per 100,000

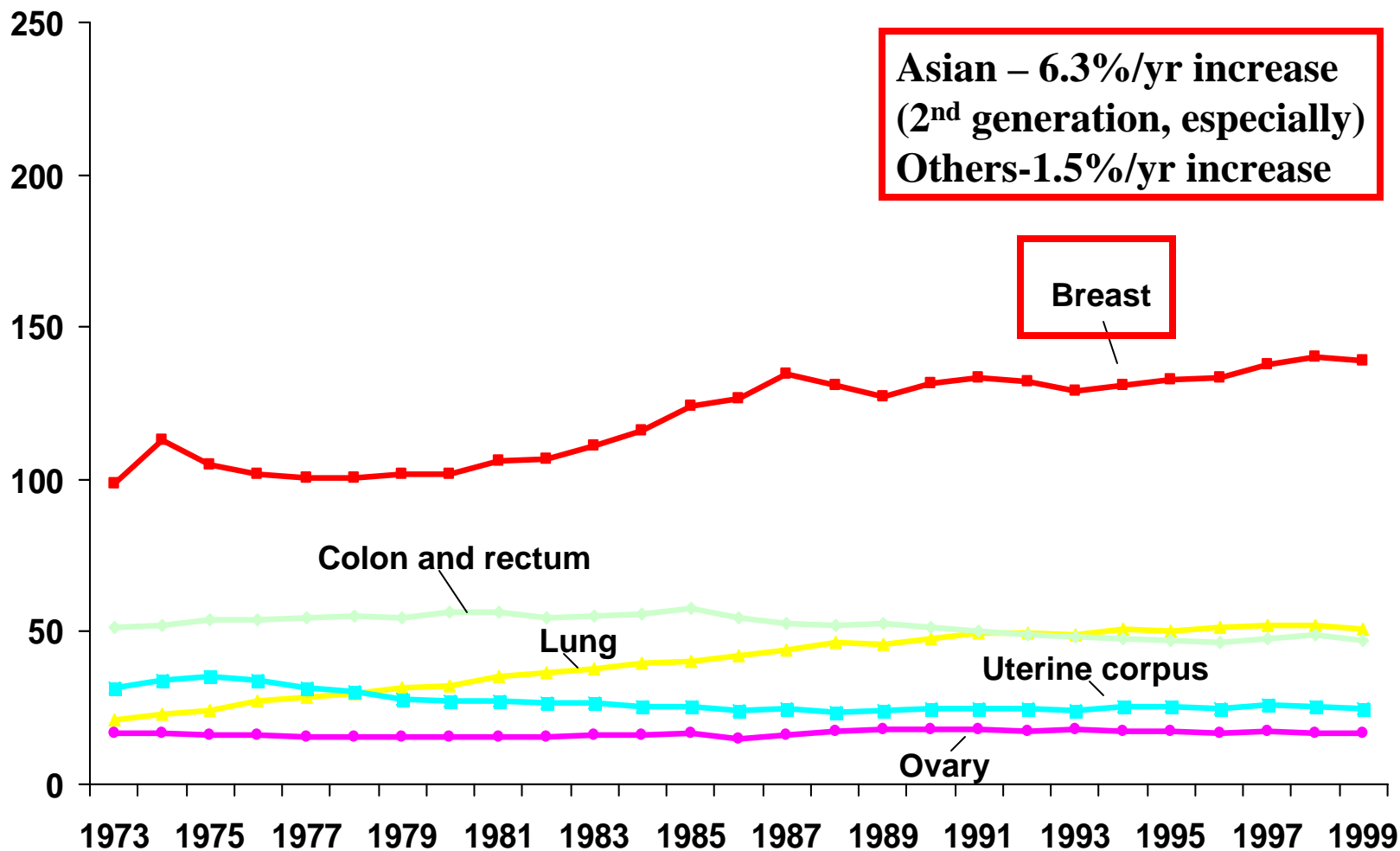


\*Age-adjusted to the 2000 US standard population.

Source: US Mortality Public Use Data Tapes 1960-1999, US Mortality Volumes 1930-1959,  
National Center for Health Statistics, Centers for Disease Control and Prevention, 2002.

# Cancer Incidence Rates\* for Women, US, 1973-1999

Rate Per 100,000



\*Age-adjusted to the 2000 US standard population.

Source: Surveillance, Epidemiology, and End Results Program, 1973-1999, Division of Cancer Control and Population Sciences, National Cancer Institute, 2002.

# What Is Breast Cancer?

- Breast cancer is a malignant (cancerous) tumor that develops from cells in the breast.
- Most breast lumps are benign (not cancerous).
- Early detection is very important because the cancer can spread if not treated at its earliest stages.

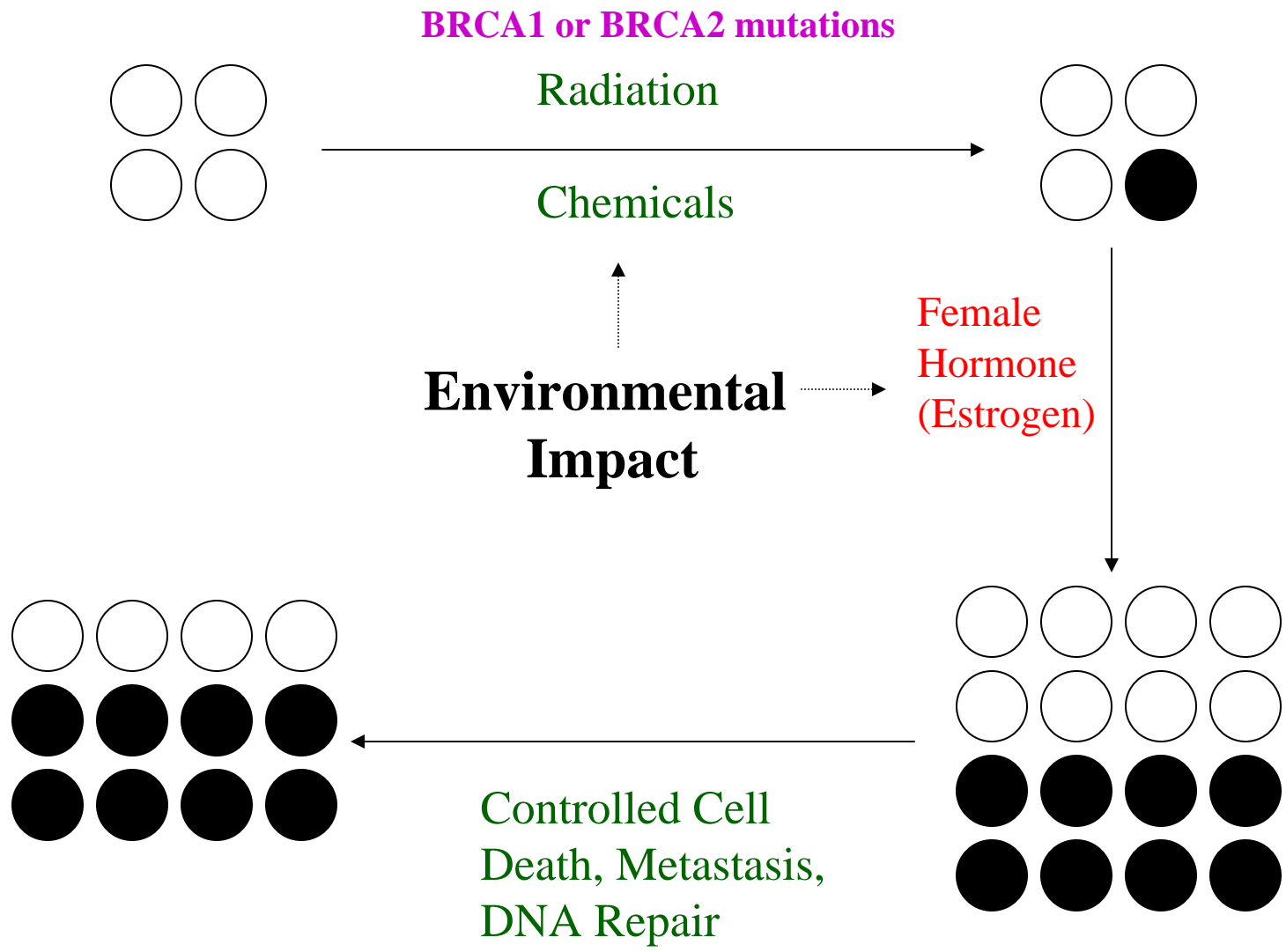
# Symptoms

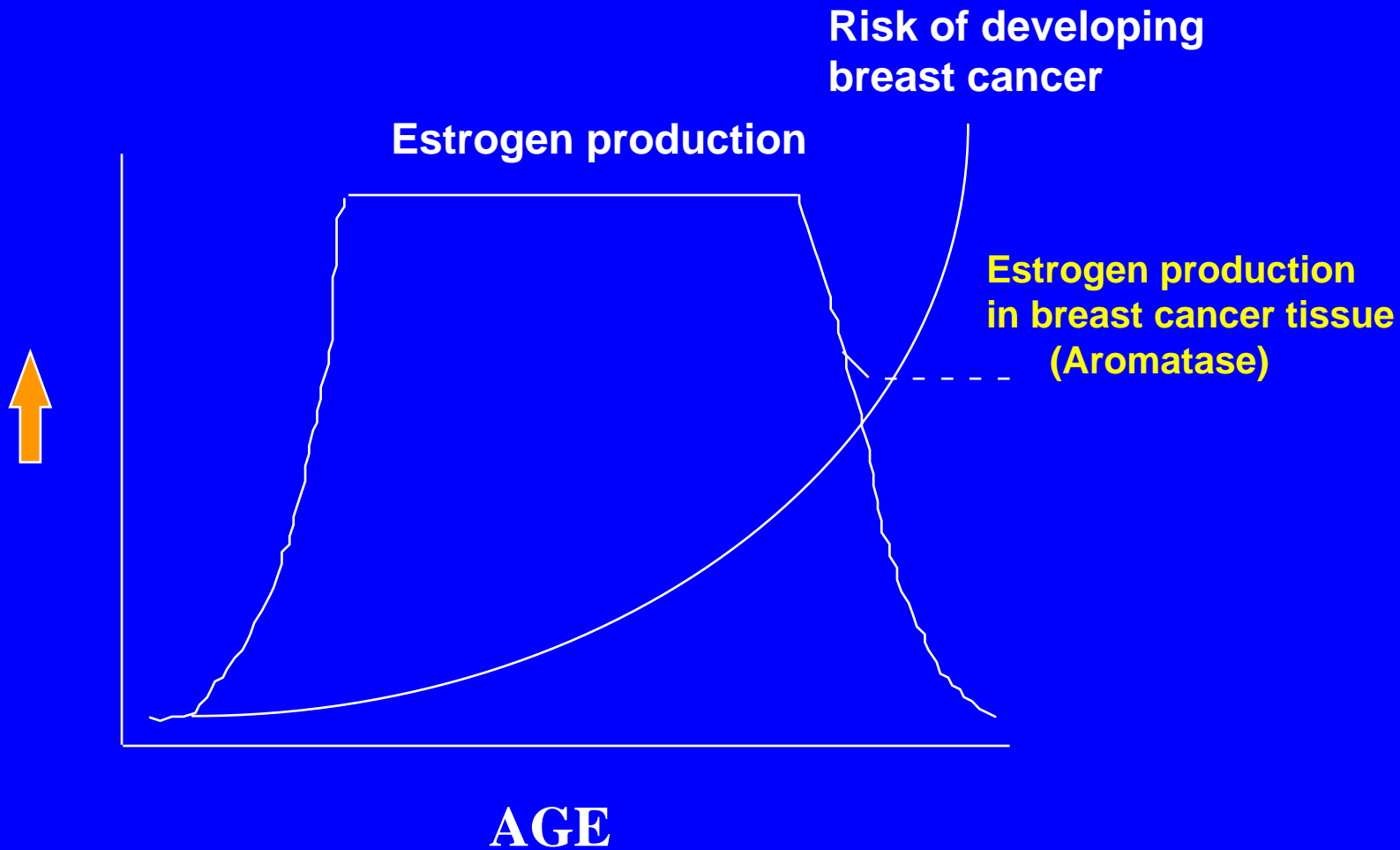
- The most common sign of breast cancer is a new lump or mass.
- Other signs include:
  - Generalized swelling of part of a breast (even if no distinct lump is felt).
  - Skin irritation or dimpling.
  - Nipple pain or retraction (turning inward).
  - Redness or scaliness of the nipple or breast skin.
  - Discharge other than breast milk.

# Risk factors for breast cancer in females

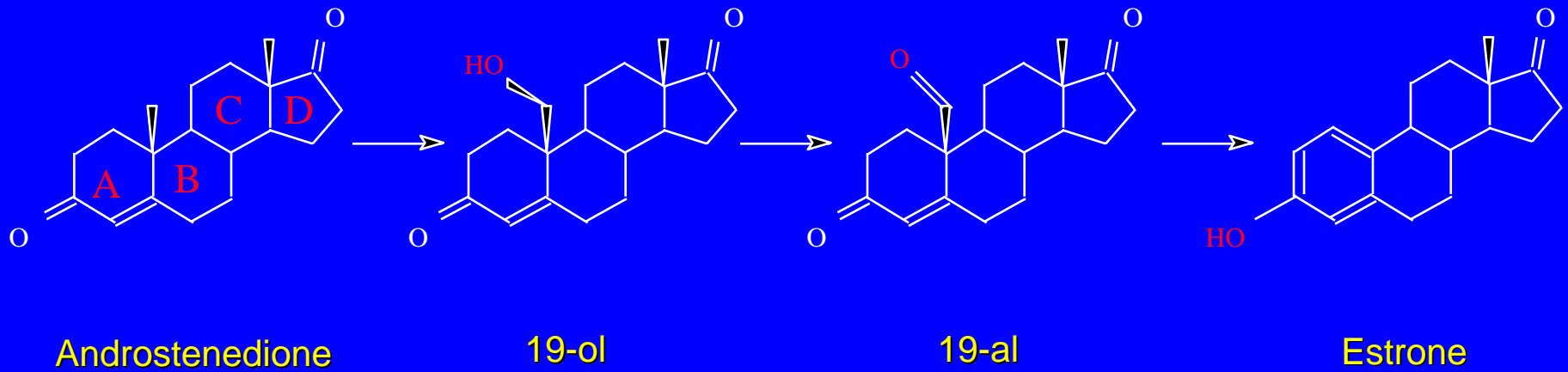
- A family history of the disease.
- Early onset of menstruation.
- Late menopause.
- A first child after age 30.
- Never had children.
- (Estrogen Replacement Therapy)
- (Obesity)

**“Prevention!!!”**

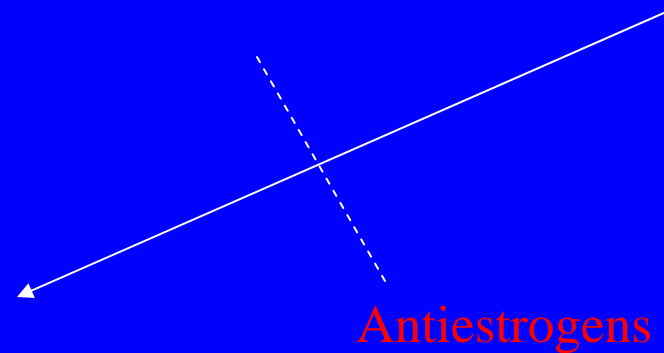
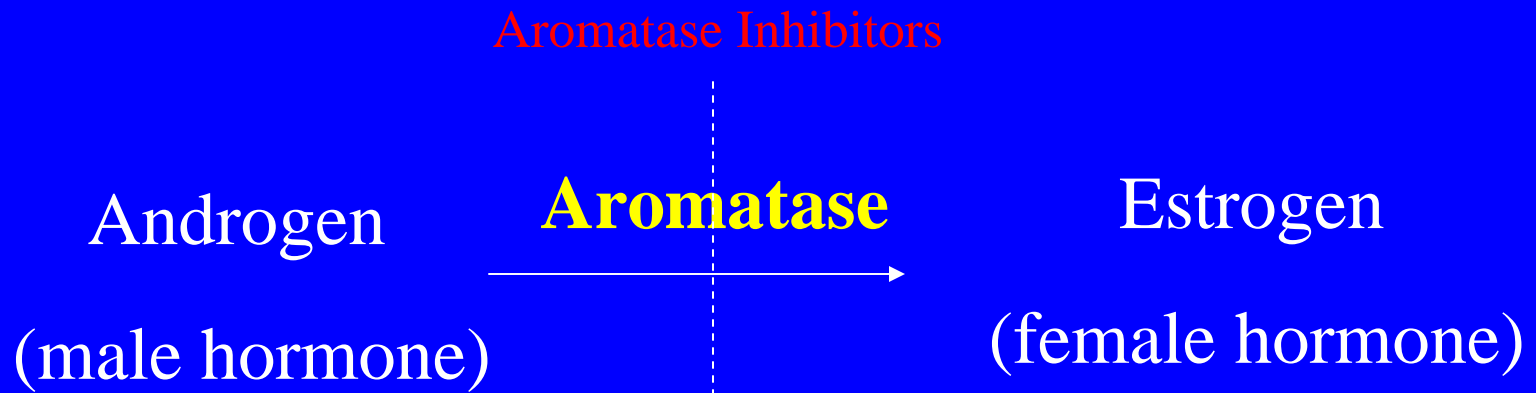




**Over expression of aromatase in the breast tissue of postmenopausal women is thought to be a risk factor for breast cancer.**

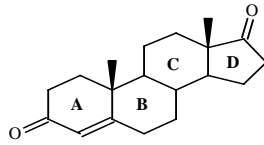


**Aromatase converts androgens to estrogens**

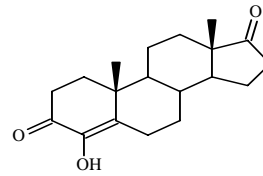


**Estrogen Receptor  
Protein**

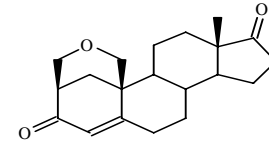
Fig. 3 Structures of androstenedione and nine aromatase inhibitors



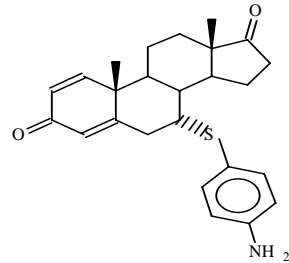
Androstenedione



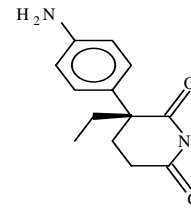
4-Hydroxyandrostenedione  
(4-OHA, Formestane, Lentaron)



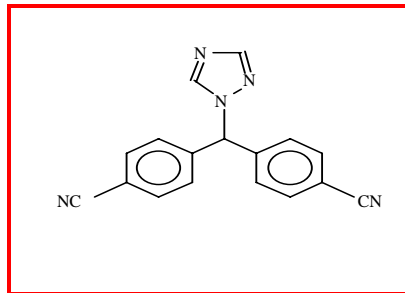
MDL 101,103



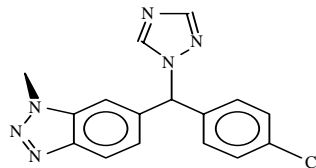
7 $\alpha$ -APTADD



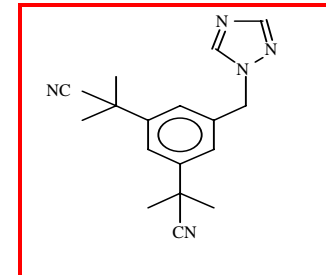
Aminoglutethimide  
(AG, Orimeten)



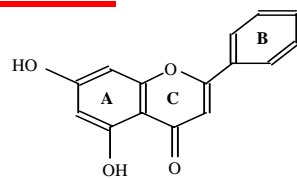
CGS 20267  
(Letrozole)



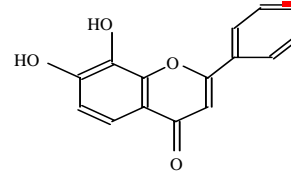
Vorozole



ICI D1033  
(Anastrozole, Arimidex)



Chrysin



7,8-Dihydroxyflavone

Winer, E.P., Hudis, C., Burstein, H.J., Chlebowski, R.T. *et al.* American Society of Clinical Oncology technology assessment of the use of aromatase inhibitors as adjuvant therapy for women with hormone receptor-positive breast cancer: Status report 2002. *J. Clin Oncol.* 20, 3317-3327 (2002).

# The ATAC Trial

- The trial is a large, multinational, double-blind, placebo-controlled, randomized trial comparing standard adjuvant therapy of 5 years of tamoxifen versus 5 years of anastrozole versus 5 years of both agents given in combination in postmenopausal patients.
- 9,366 postmenopausal women with hormone receptor-positive or unknown tumors.
- July 1996-March 2000, results were first reported at the 2001 San Antonio Breast Conference.
- 381 centers, 21 countries

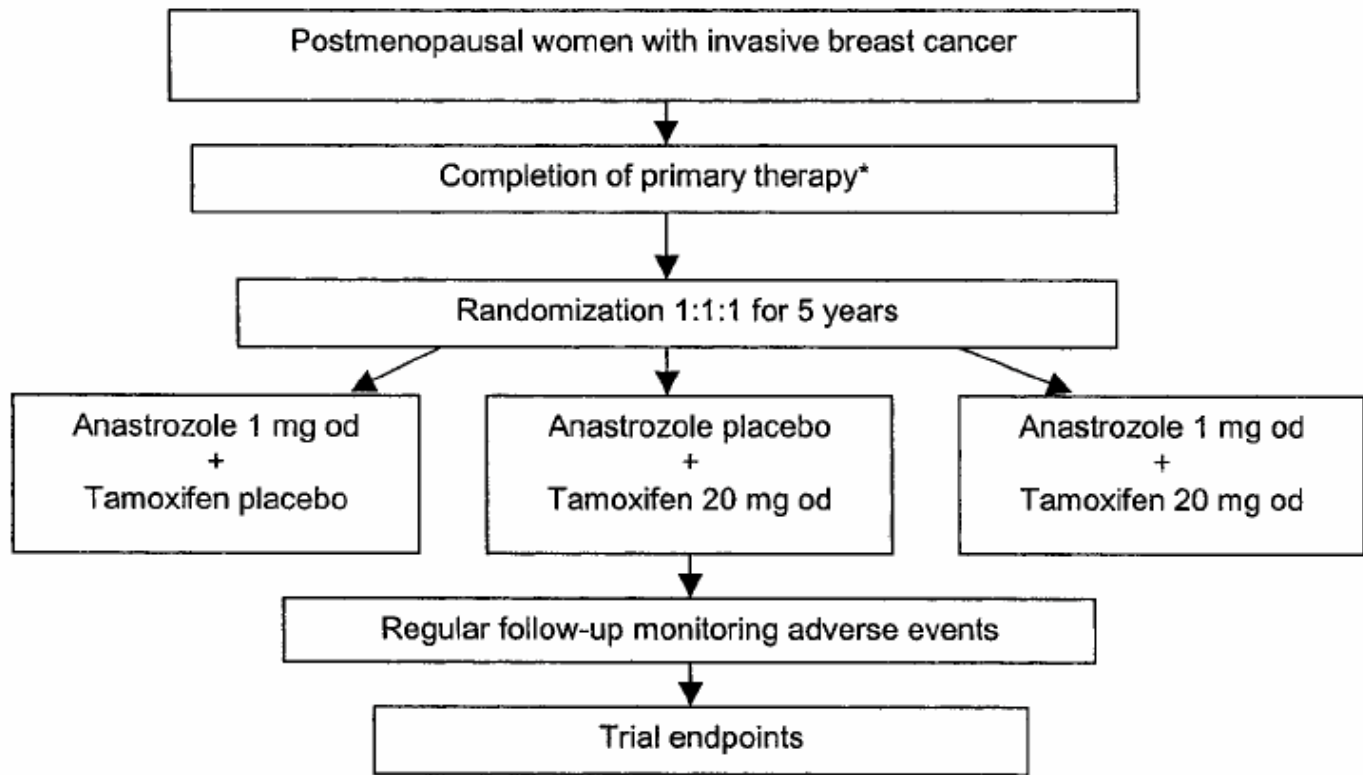


Fig 1. ATAC trial design. \*Surgery ± radiotherapy ± chemotherapy. (Patients may start trial therapy while still receiving radiotherapy.)

**Disease-free survival (defined as locoregional recurrence, distant recurrence, new primary breast cancer, or death from any cause) and safety/tolerability.**

**Secondary end points included the incidence of second non-breast cancer primaries, time to distant recurrence, and overall survival.**

**Table 1. Table of First Events in ITT Population (Based on December 2001 presentation)**

	No. of Patients		
	Anastrozole (n = 3,125)	Tamoxifen (n = 3,116)	Combination (n = 3,125)
First event	317	379	383
Recurrence			
Locoregional	67	83	81
Distant	156	181	202
Contralateral			
Invasive	9	30	23
DCIS	5	3	5
Deaths*	–	–	–

\*Approximately 600 total deaths across the three arms, of which approximately 60% are because of breast cancer. A formal survival analysis has not been performed (J. Purvis, personal communication, April 2002).

**Table 2. Adverse Events**

	% of Patients		<i>P</i>
	Anastrozole	Tamoxifen	
Hot flashes	34.3	39.7	< .0001
Musculoskeletal disorders	27.8	21.3	< .0001
Fatigue/tiredness (asthenia)	15.6	15.1	.5415
Mood disturbances	15.5	15.2	.6900
Nausea and vomiting	10.5	10.2	.7005
Weight gain*	9.2	11.0	.0207
Fractures	5.9	3.7	< .0001
In spine, hip, wrist	2.2	1.5	.0299
Vaginal bleeding	4.5	8.2	< .0001
Vaginal discharge	2.8	11.4	< .0001
Endometrial cancer	0.1	0.5	.0267
Cataracts	3.5	3.7	.5427
Ischemic cardiovascular disease	2.5	1.9	.1391
Ischemic cerebrovascular events	1.0	2.1	.0006
Venous thromboembolic events	2.1	3.5	.0006
DVT events	1.0	1.7	.0183

Abbreviation: DVT, deep venous thrombosis.

\*Ten percent gain in body weight from baseline to year 2 (non-pre-defined adverse event).

## **Letrozole Is More Effective Neoadjuvant Endocrine Therapy Than Tamoxifen for ErbB-1– and/or ErbB-2–Positive, Estrogen Receptor–Positive Primary Breast Cancer: Evidence From a Phase III Randomized Trial**

*Ellis et al., J. Clin Oncol, 19: 3808-3816, 2001*

**RESULTS:** For study biopsy-confirmed ER+ and/or PgR+ cases that received letrozole, 60% responded and 48% underwent successful breast-conserving surgery. The response to tamoxifen was inferior (41%,  $P = .004$ ), and fewer patients underwent breast conservation (36%,  $P = .036$ ). Differences in response rates between letrozole and tamoxifen were most marked for tumors that were positive for ErbB-1 and/or ErbB-2 and ER (88% v 21%,  $P = .0004$ ).

**CONCLUSION:** ER+, ErbB-1+, and/or ErbB-2+ primary breast cancer responded well to letrozole, but responses to tamoxifen were infrequent. This suggests that ErbB-1 and ErbB-2 signaling through ER is ligand-dependent and that the growth-promoting effects of these receptor tyrosine kinases on ER+ breast cancer can be inhibited by potent estrogen deprivation therapy.

# Letrozole/Neoadjuvant Endocrine Therapy: Phase III Randomized Trial

- A double-blinded, randomized phase III neoadjuvant endocrine therapy trial to compare 4 months of letrozole 2.5 mg daily with tamoxifen 20 mg daily for postmenopausal women with hormone receptor-positive breast cancer who are ineligible for breast-conserving surgery.
- March 1998 - August 1999
- 324 patients; 154 study subjects received letrozole and 170 tamoxifen. From 55 centers in 16 countries.
- At months 2 and 3, breast ultrasound; at month 4, surgical assessment, a final ultrasound and mammogram.

**Table 1. Treatment Response Based on Clinical Measurement According to Study Biopsy Assignment of ER and PgR Expression in the Two Arms of the Study**

	Marker Category	No. of Responders/Total	Response Rate (%)	Odds Ratio for Response	95% CI	P
Letrozole	ER+	72/120	<u>60</u>	6.50	1.76-24.03	.005
	ER-	3/16	19			
	PgR+	56/89	63	2.41	1.17-4.99	.018
	PgR-	19/46	41			
	ER+, PgR+	54/85	64	1.55	0.69-3.46	.287
	ER+, PgR-	18/34	53			
	ER-, PgR+	2/4	50		—	—
	ER-, PgR-	1/12	8			
Tamoxifen	ER+	49/123	<u>40</u>	5.30	1.17-24.07	.031
	ER-	2/18	11			
	PgR+	36/84	43	1.92	0.93-3.95	.076
	PgR-	16/57	28			
	ER+, PgR+	33/81	41	1.07	0.50-2.32	.855
	ER+, PgR-	16/41	39			
	ER-, PgR+	2/2	100		—	—
	ER-, PgR-	0/16	0			

NOTE. The odds ratio for response (marker-positive v marker-negative) was calculated by unadjusted logistic regression. Abbreviation: CI, confidence interval.

**Table 3. Treatment Response Based on Clinical Measurements by ErbB-1 and ErbB-2 Category in the Two Arms of the Study**

	Marker Category	No. of Responders/Total	Response Rate (%)	Odds Ratio for Response	95% CI	P
Letrozole	ErbB-2+	11/16	69	1.93	0.63-5.88	.250
	ErbB-2-	64/120	53			
	ErbB-1+	5/12	42	0.57	0.17-1.89	.355
	ErbB-1-	68/122	56			
	ErbB1/2+	15/26	58	1.18	0.50-2.79	.714
	ErbB1-2-	58/108	54			
	ErbB1/2+ and ER+	15/17	88	6.27	1.36-28.87	.018
	ErbB1-2- and ER+	55/101	54			
Tamoxifen	ErbB1/2+ and ER-	0/9	0		—	—
	ErbB-2+	4/23	17	0.31	0.10-0.97	.045
	ErbB-2-	48/119	40			
	ErbB-1+	0/7	0		—	.051*
	ErbB-1-	49/131	37			
	ErbB1/2+	4/29	14	0.23	0.07-0.70	.010
	ErbB1-2-	45/109	41			
	ErbB1/2+ and ER+	4/19	21	0.37	0.11-1.19	.095
	ErbB1-2- and ER+	42/100	42			
ErbB1/2+ and ER-	0/10	0		—	—	

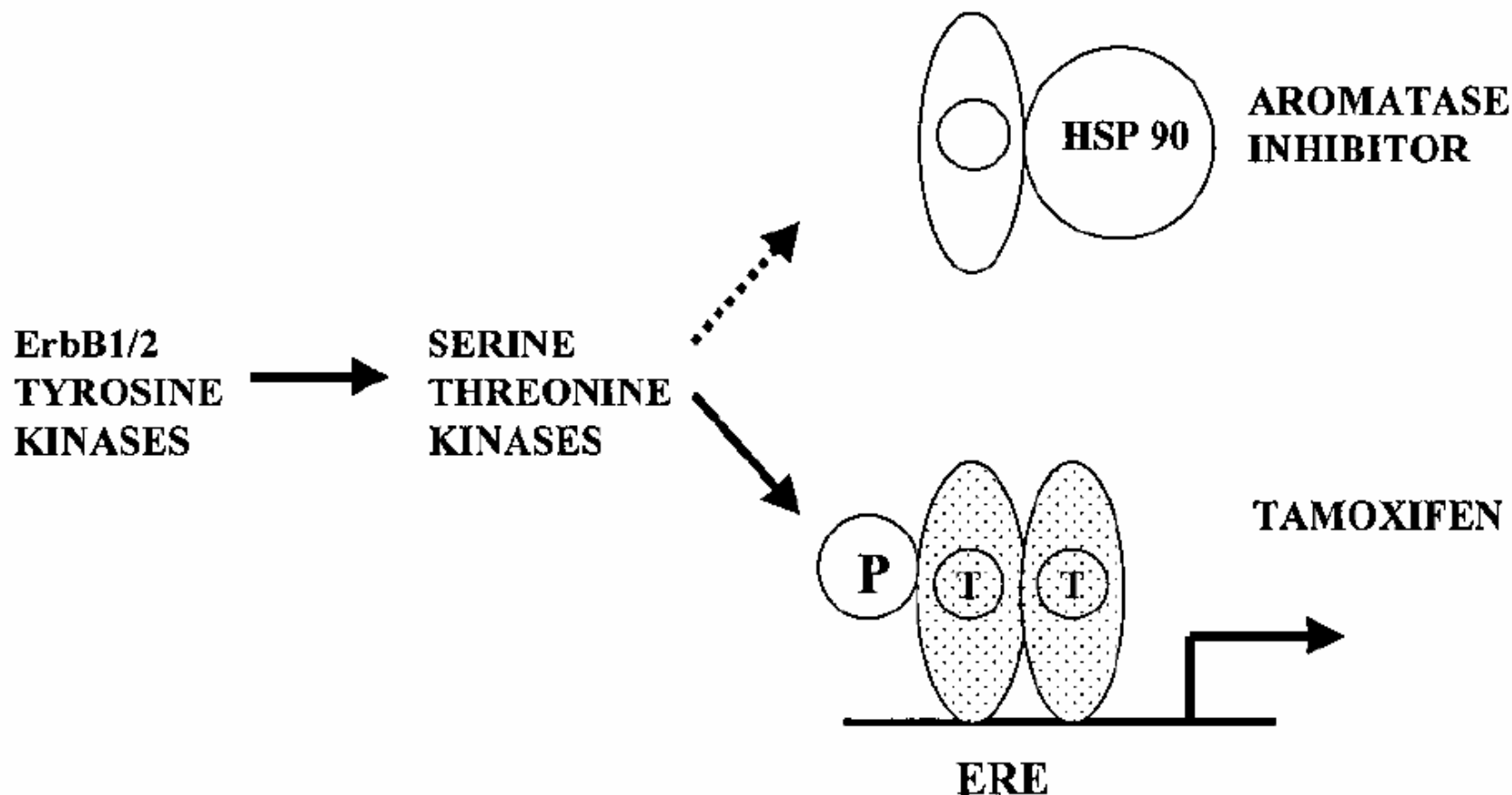
NOTE. The odds ratio for response (marker-positive v marker-negative) was calculated by unadjusted logistic regression. ErbB1/2+, ErbB-1 and/or ErbB-2 expressed; ErbB1-2-, both receptors not expressed.

\*By Fisher's exact test.

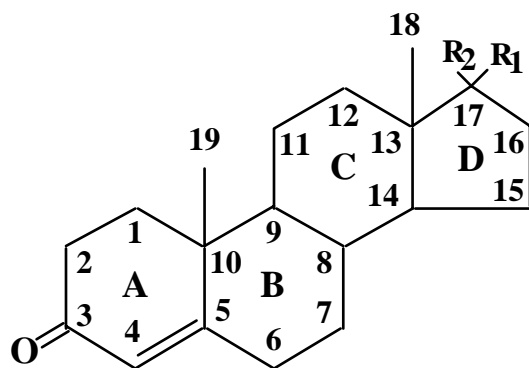
**Table 4. Calculation of Odds Ratio of Clinical Response, Letrozole Versus Tamoxifen, in Subgroups of Patients With Tumors That Were Either ErbB-1+ and/or ErbB-2+ (ErbB-1/2) and ER+ or ErbB-1- and ErbB-2- (ErbB-1-2-) and ER+**

Category	Letrozole		Tamoxifen		Odds Ratio (letrozole v tamoxifen)	95% CI	P
	No. of Responders/ Total	%	No. of Responders/ Total	%			
ErbB-1/2+ ER+	15/17	88	4/19	21	28	4.5-177	.0004
ErbB-1-2- ER+	55/101	54	42/100	42	1.7	0.9-2.9	.0780

NOTE. This analysis ignored PgR status because none of the ErbB-1/2+ cases was ER-, PgR+.

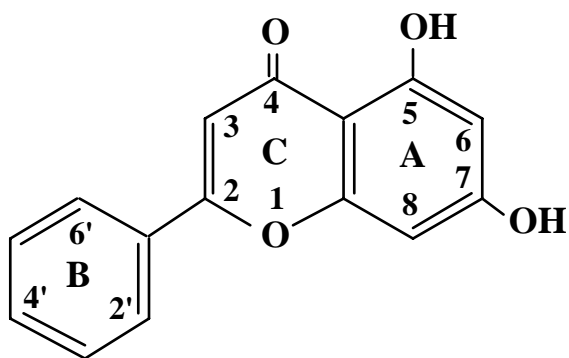


**Fig 3. A simple model to explain the superior efficacy of letrozole when ER is coexpressed with ErbB-1 and/or ErbB-2. These tumors are estrogen-dependent and therefore sensitive to estrogen deprivation. However, ErbB-1/2-dependent ER phosphorylation (P) prevents tamoxifen (T) from acting as an antagonist, compromising effectiveness.**

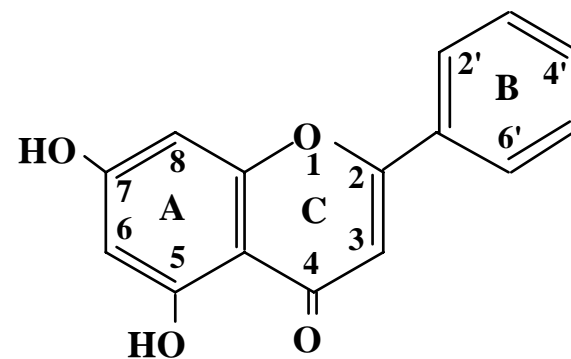


R<sub>1</sub>, R<sub>2</sub> = O : androst-4-ene-3,17-dione

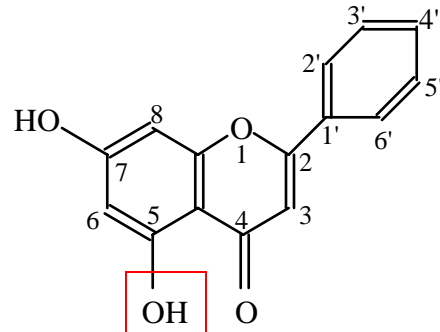
R<sub>1</sub> = H ; R<sub>2</sub> = OH : testosterone



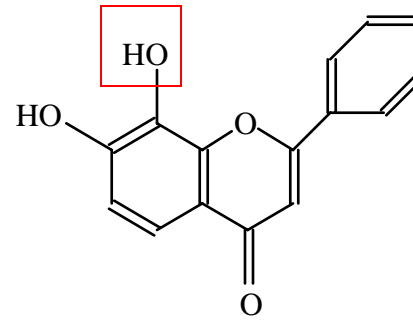
chrysin (A)



chrysin (B)



**Chrysin**  
(5,7-Dihydroxyflavone)



**7,8-Dihydroxyflavone**

Ki (μM)

<u>WT</u>	2.6 ± 0.1	10 ± 1
<u>I133Y</u>	0.3 ± 0.1	86 ± 25
<u>I395F</u>	0.6 ± 0.1	1.3 ± 0.2
<u>I474Y</u>	0.1 ± 0.0	0.3 ± 0.1
<u>P308F</u>	3.2 ± 0.3	13 ± 2
<u>D309A</u>	13 ± 3	40 ± 21
<u>T310S</u>	1.4 ± 0.2	1.7 ± 0.5

**Kao, Y.-C., Zhou, C., Sherman, M., Laughton, C. A., and Chen, S.** Molecular basis of the inhibition of human aromatase by flavone and isoflavone phytoestrogens. A site-directed mutagenesis study. *Environmental Health Perspectives*, 106:85-92, 1998.

**Chrysin**

**Biochanin A**

**Genistein**

**I133**

*4-keto*

*No side chain*

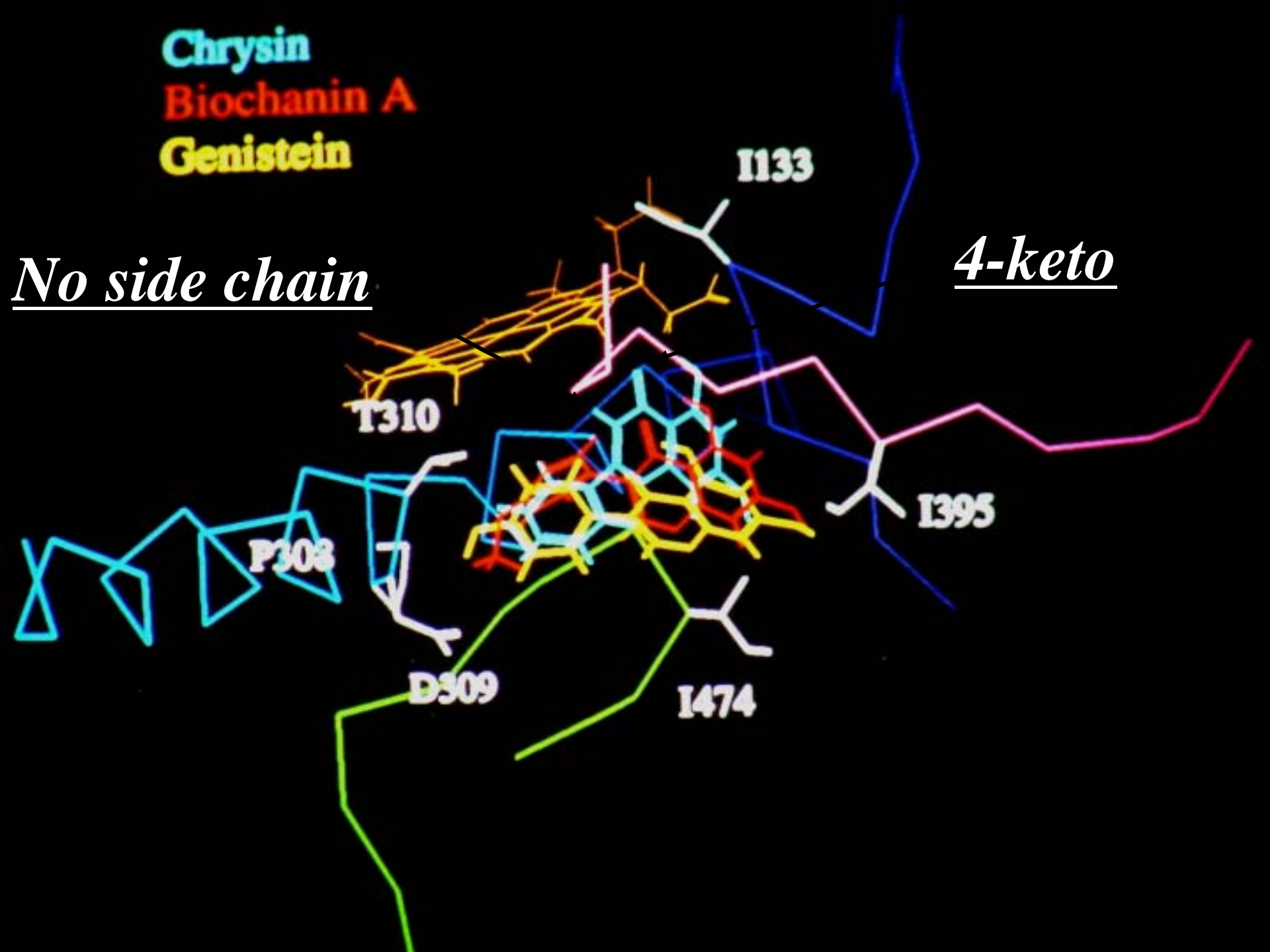
**T310**

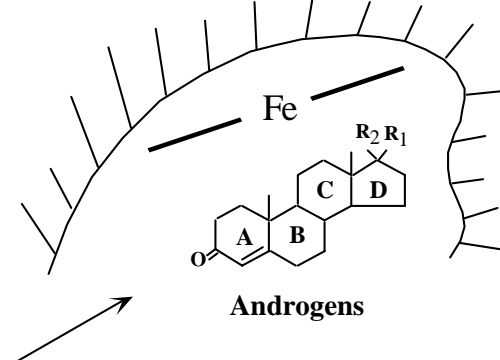
**I395**

**P308**

**D509**

**I474**

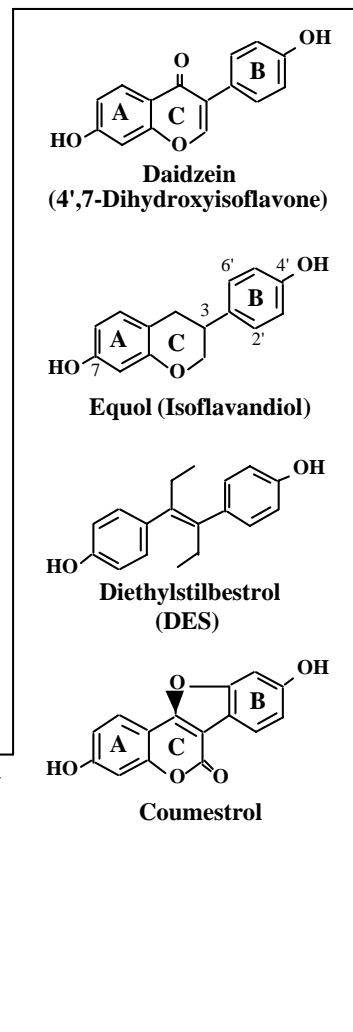




ER

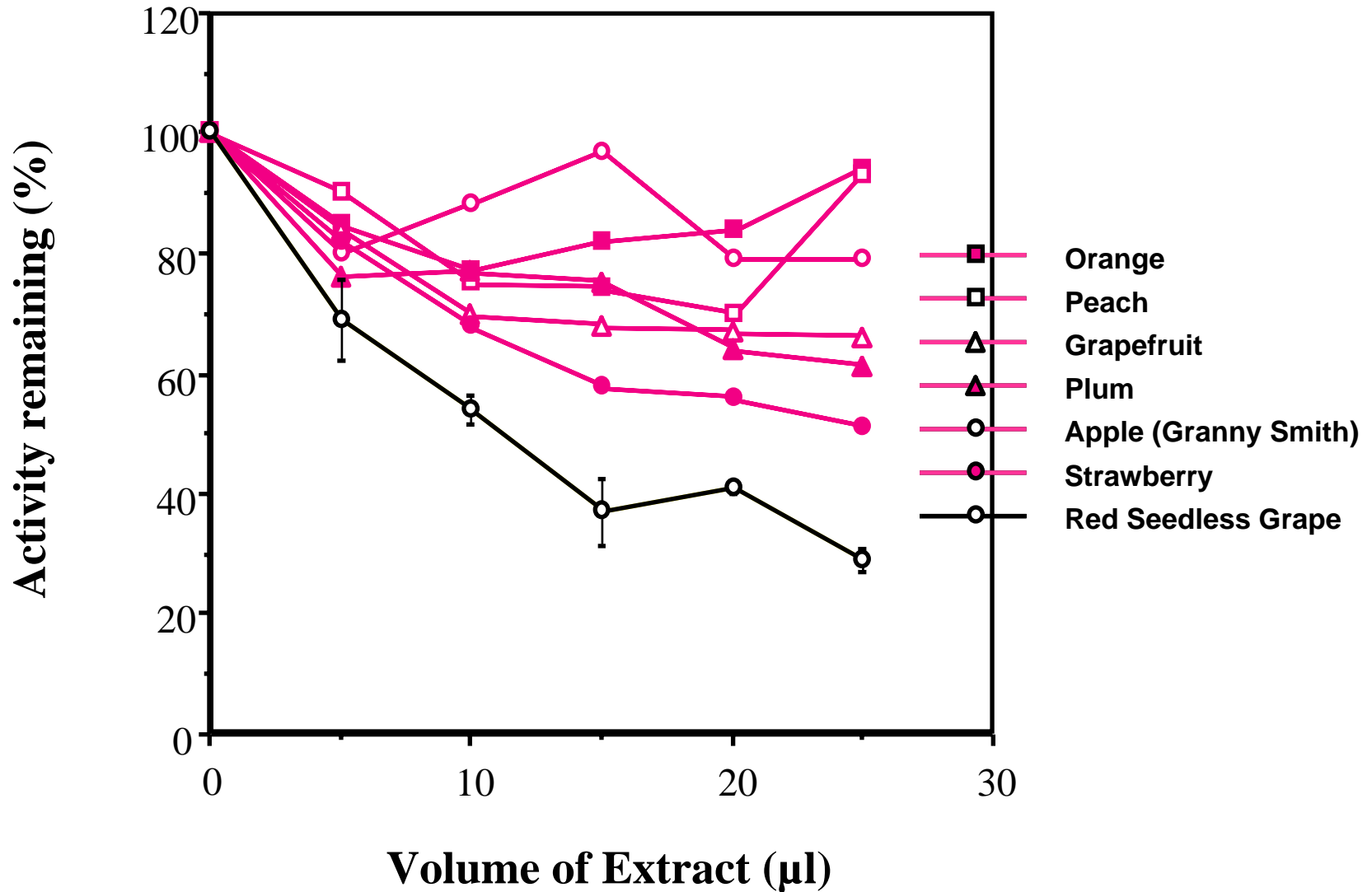


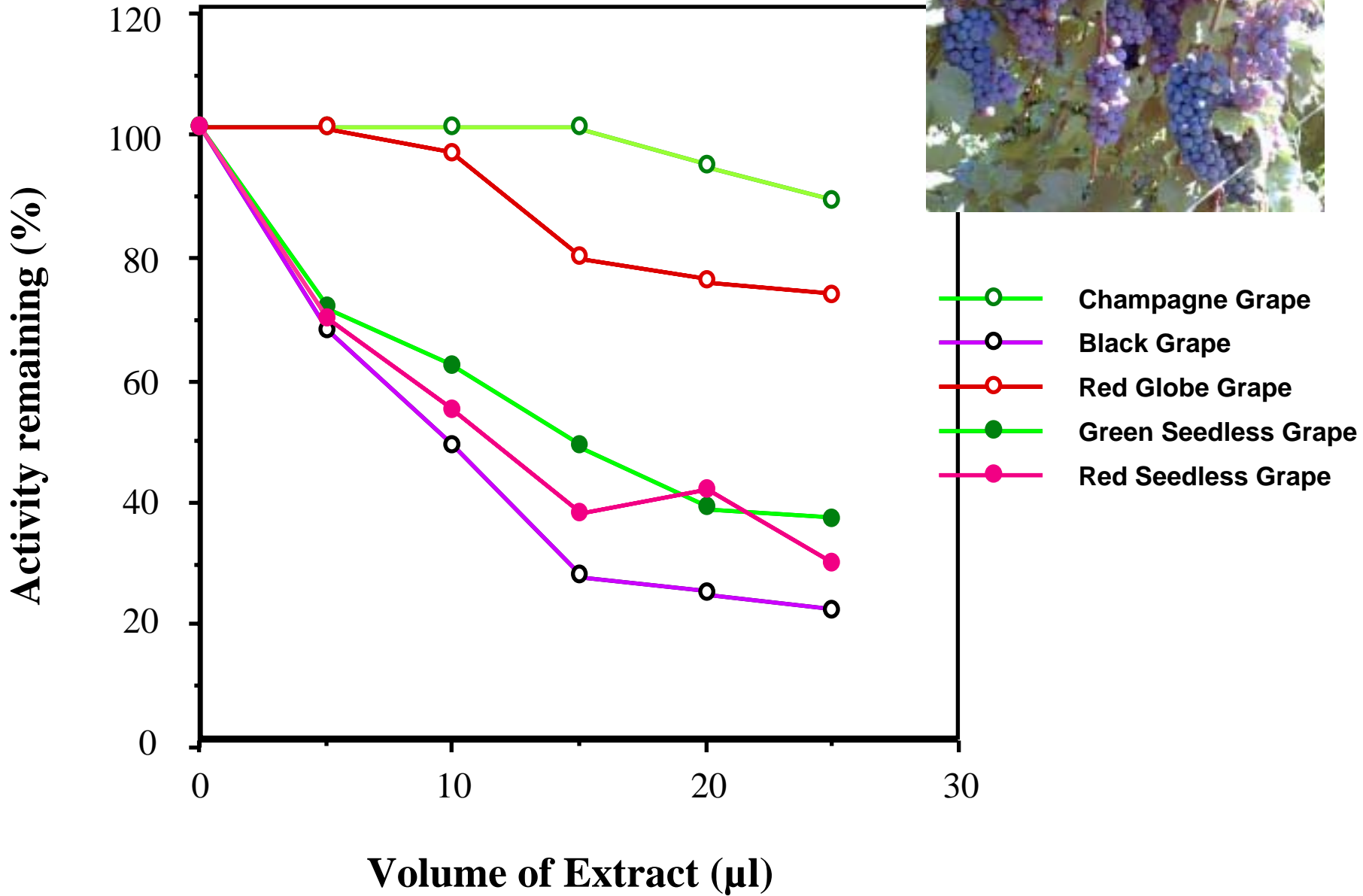
Flavones	
Inhibitor	Non-inhibitor
<p><b>Chrysin</b> (5,7-Dihydroxyflavone)</p>	<p><b>Baicalein</b> (5,6,7-Trihydroxyflavone)</p>
<p><b>Flavone</b></p>	<p><b>6-Hydroxyflavone</b></p>



Isoflavones	
Inhibitor (weak)	
<p><b>Genistein</b></p>	<p><b>Genistein</b></p>

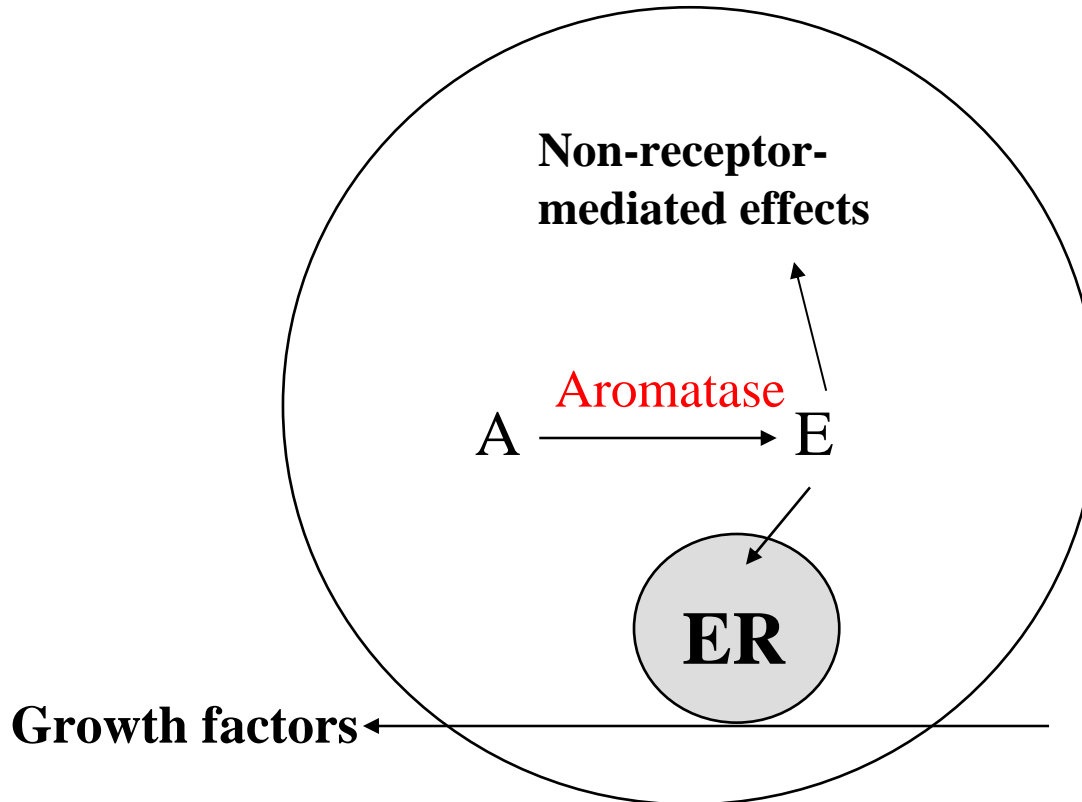
Chen, S., Sun, X.-Z., Kao, Y.-C., Kwon, A., Zhou, D., and Eng, E. Suppression of breast cancer cell growth with grape juice. *Pharmaceutical Biol.*, 36:53-61, 1998.





# Cell culture studies

## MCF-7aro - Breast cancer cell line



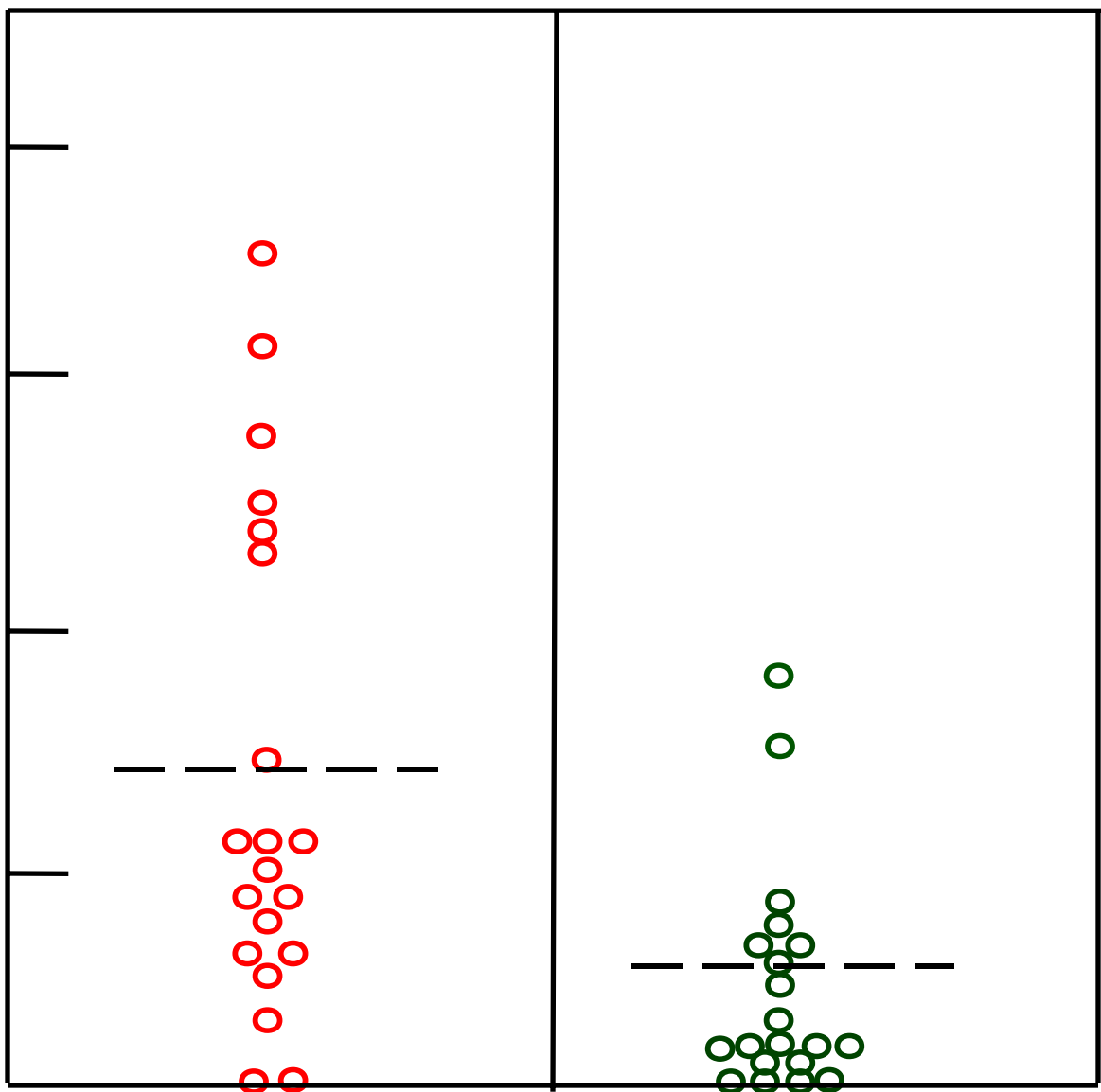
Zhou, D., Pompon, D., and Chen, S.: Stable expression of human aromatase cDNA in mammalian cells - A useful system for aromatase inhibitor screening. *Cancer Res.* 50, 6949-6954 (1990).

**Tumor Weight (mg)**

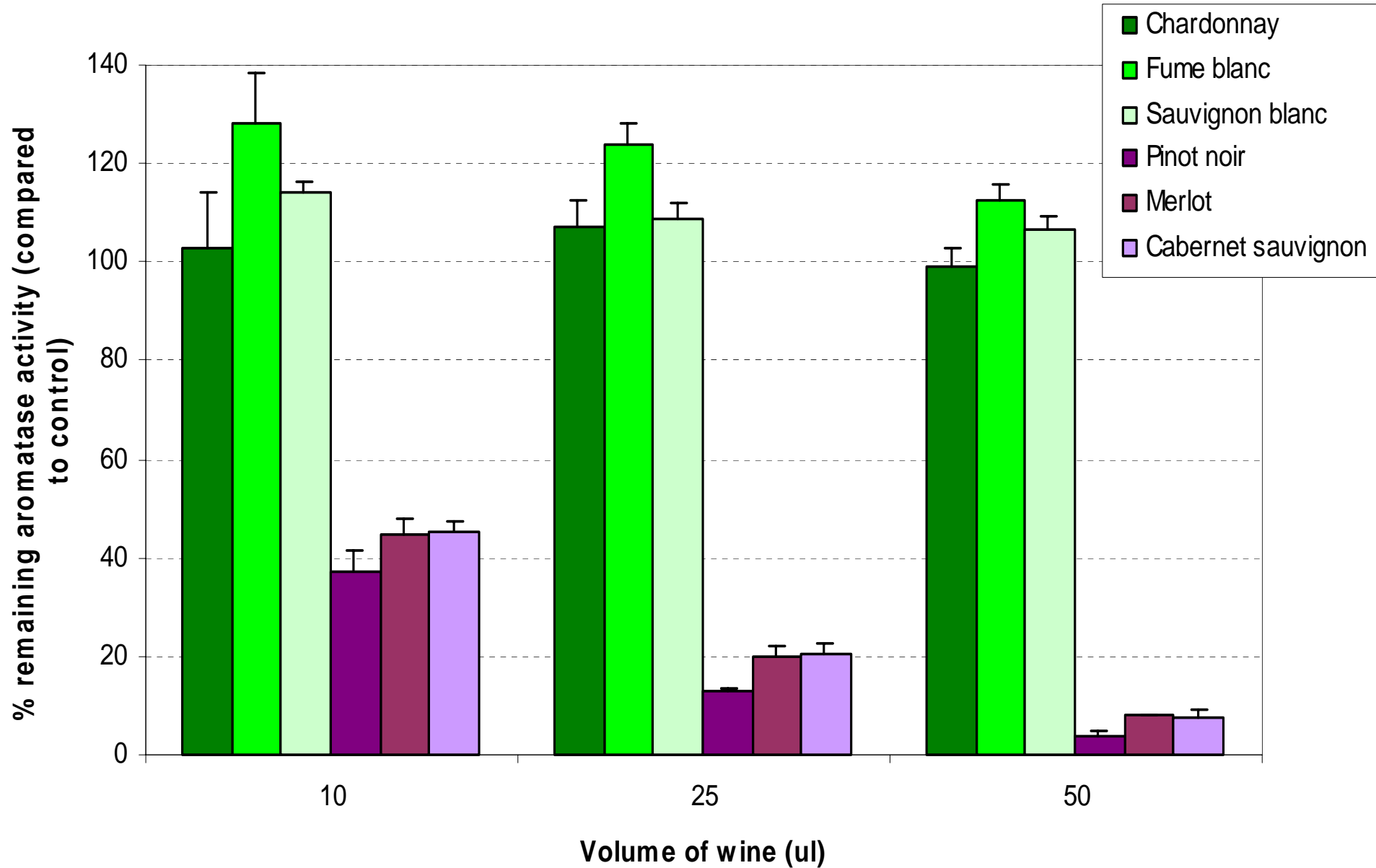
40  
30  
20  
10  
0

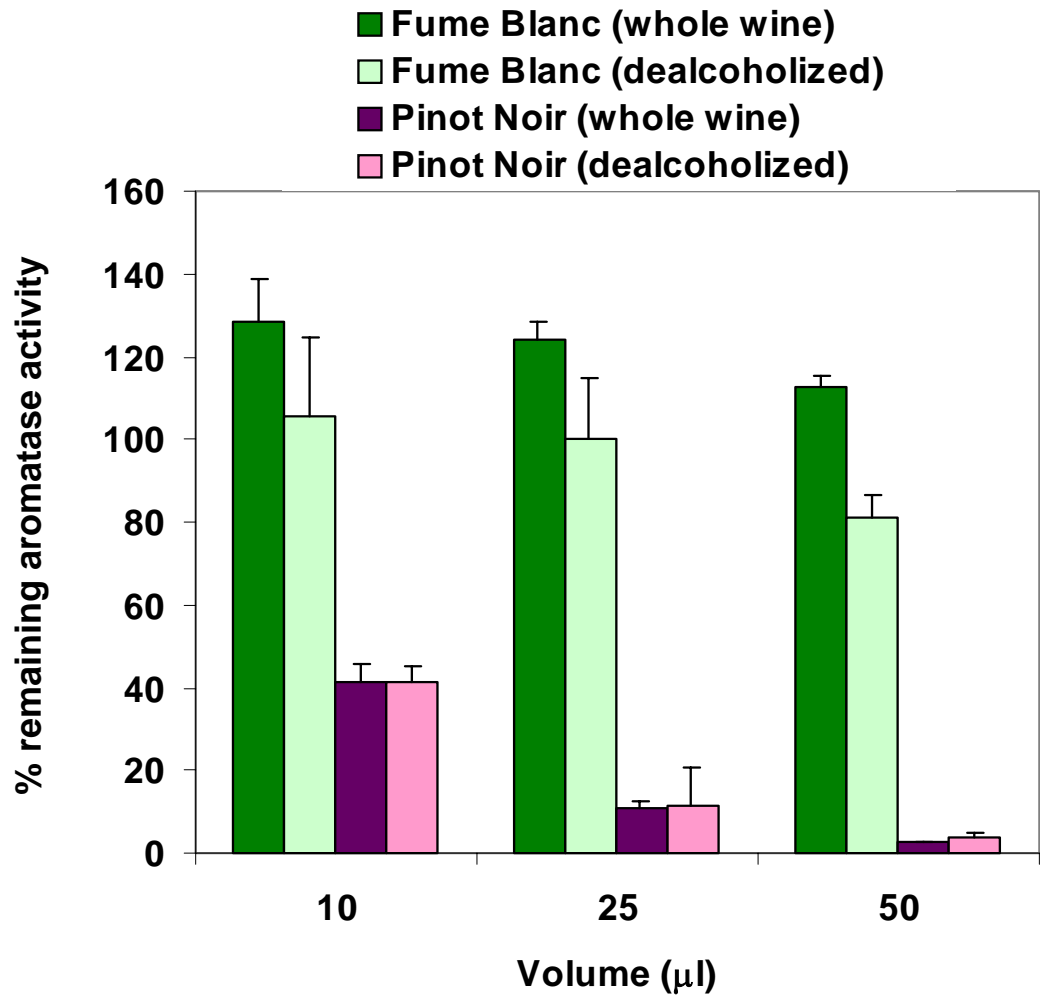
**Control**

**Grape Juice fed**

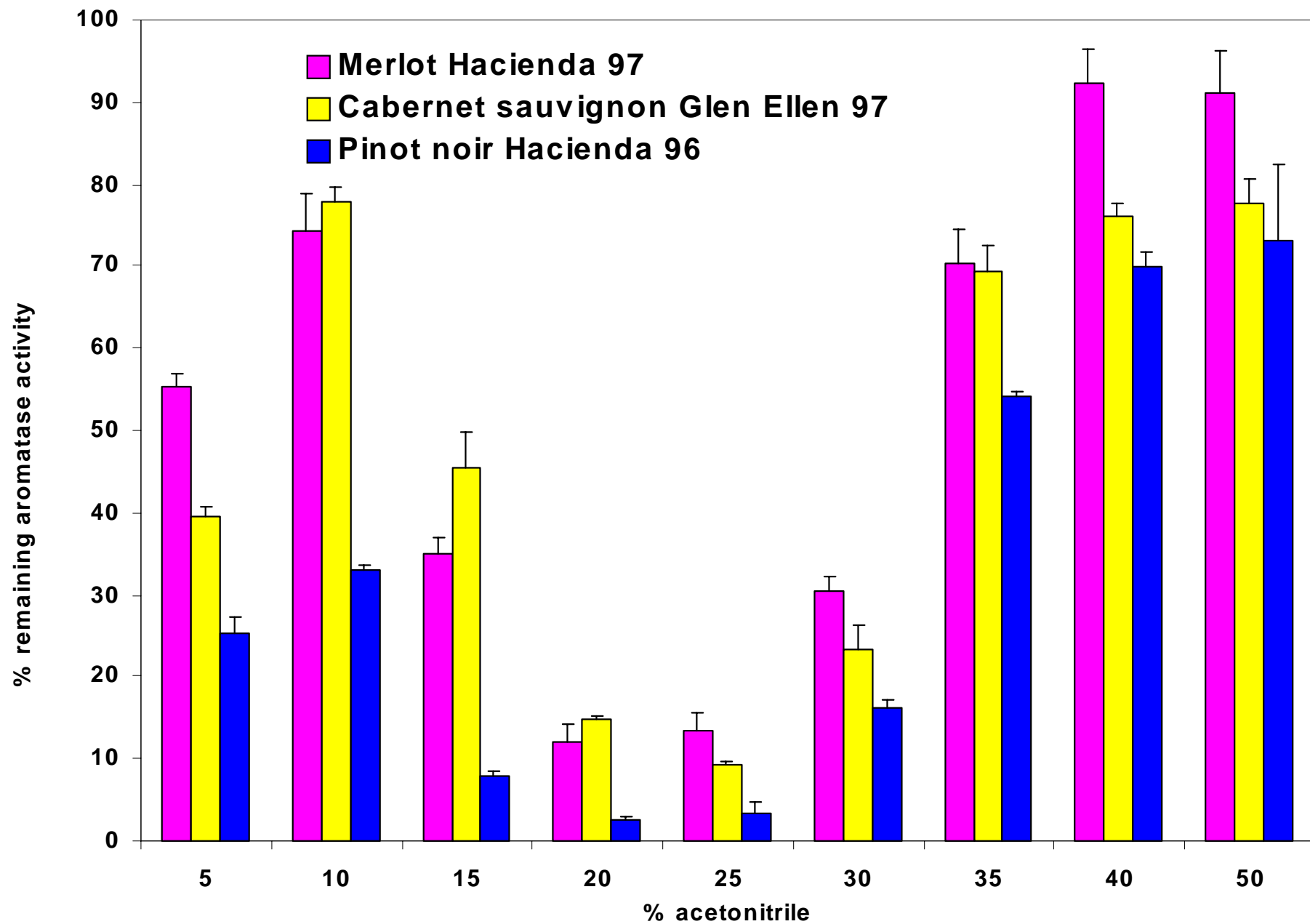


# Human Placental Microsome Assay Aromatase Inhibition with White and Red Whole Wines





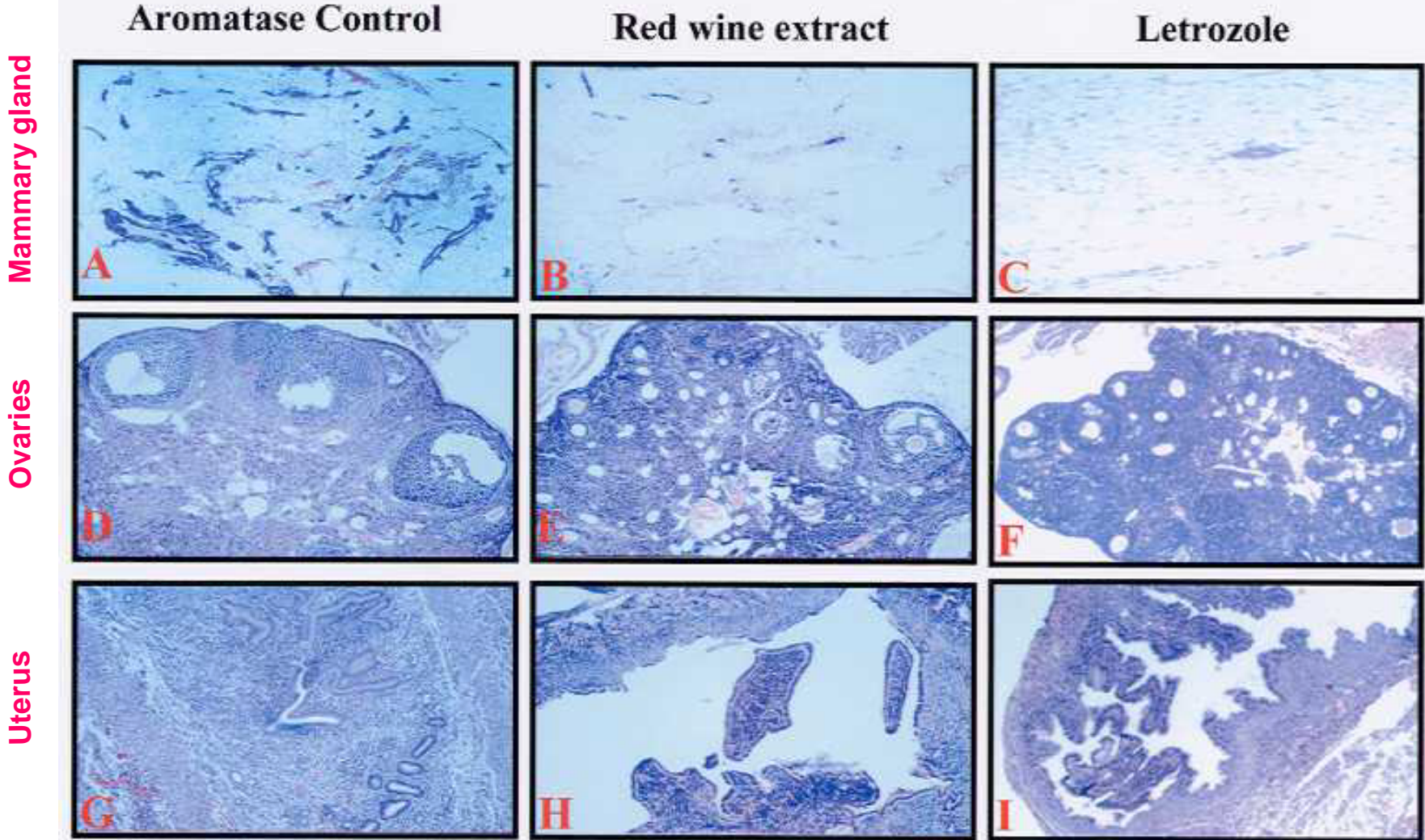
Eng, E.T., Williams, D., Mandava, U., Kirma, N., Tekmal, R.R., and Chen, S. Suppression of aromatase (estrogen synthetase) by red wine phytochemicals. Breast Cancer Research and Treatment, 67: 133-146, 2001.

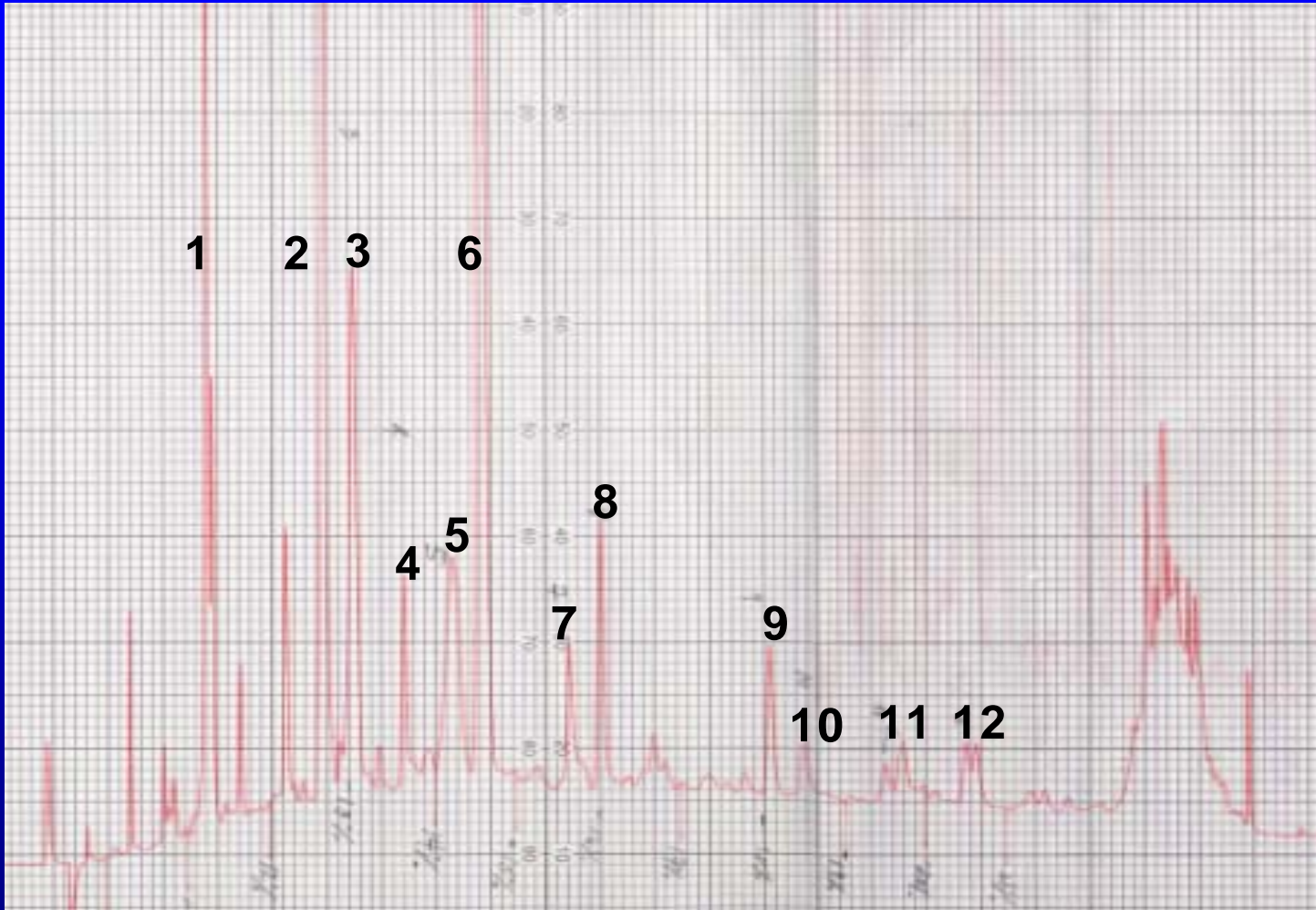


## *In vivo* experiments

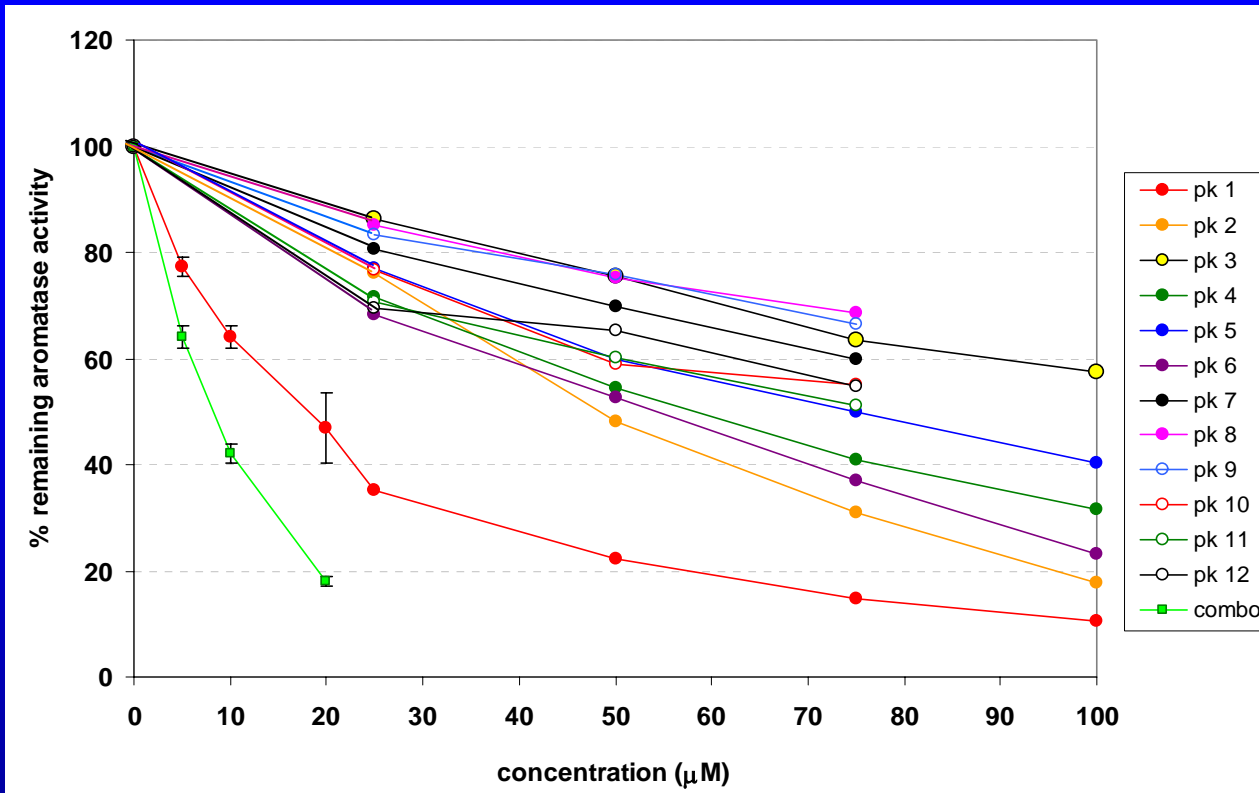
- Transgenic mouse
- Over- expression of aromatase in mammary tissue
- Three week treatment
- Daily gavage red wine 20% MeCN extract

# Histological sections of mammary gland, ovary and uterus of aromatase transgenic mice.



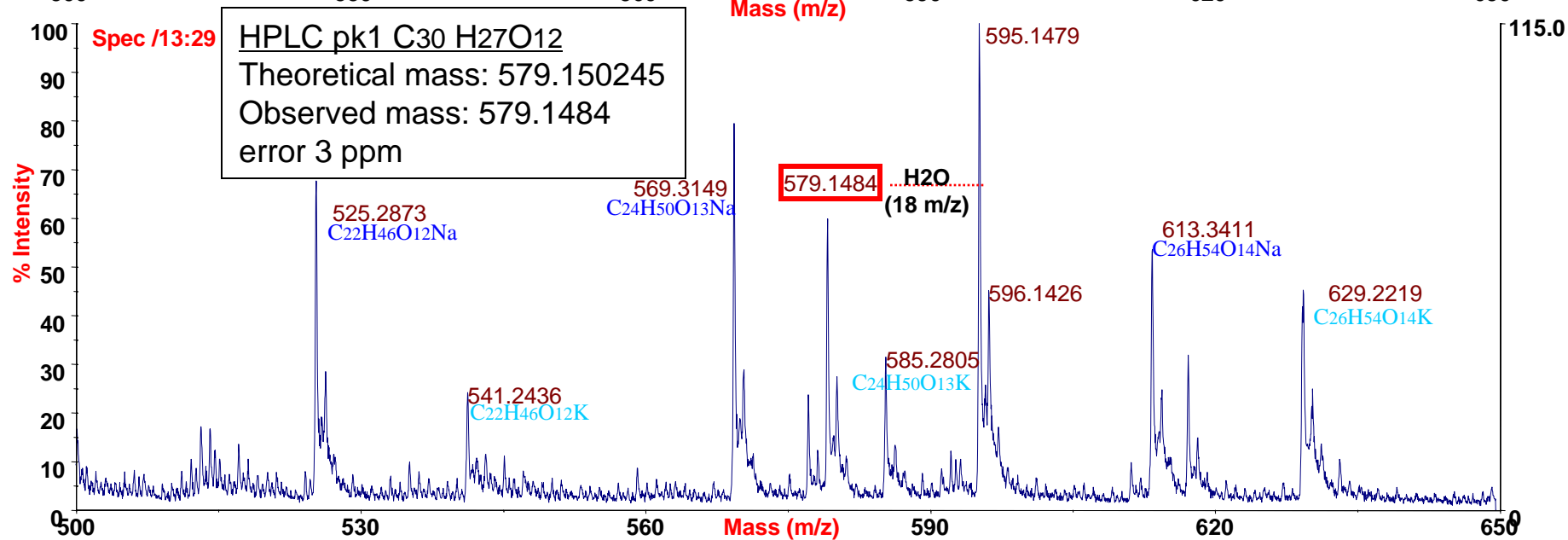
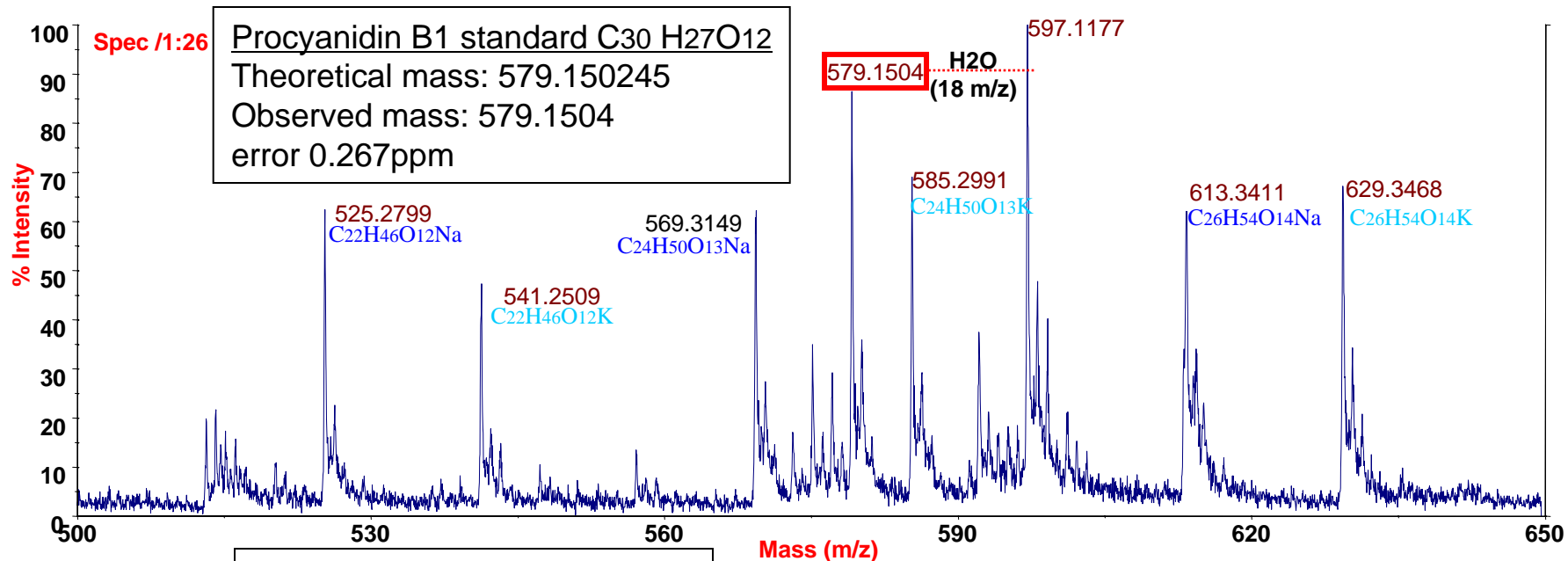


**HPLC (High Performance Liquid Chromatography)**



Eng, E. T., Ye, J., Williams, D., Phung, S., Moore, R. E., Young, M. K., Gruntmanis, U., Braunstein, G., and Chen, S. Suppression of estrogen biosynthesis by procyanidin dimmers in red wine and grape seeds. *Cancer Res.*, 63: 8516-8522, 2003.

# Mass Spectrometry- Accurate Mass Determination

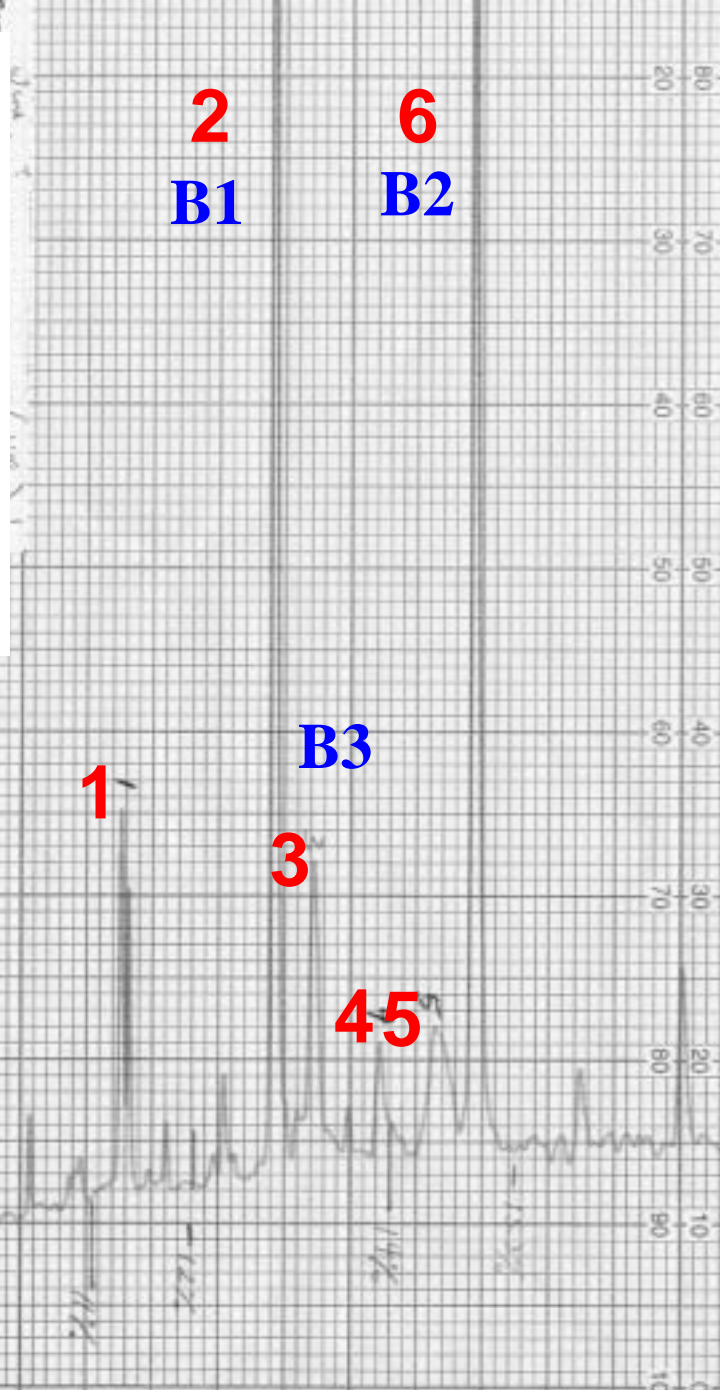


## Mass spectrometer

- Ion trap mass spec
- Electrospray ion source: positive ion mode
- Spray induced 900 volts
- Capillary temp 130 °C
- Samples diluted 1:1 in
  - 90% acetonitrile
  - 10% water
  - 2% acetic acid

	<u>MS</u>	<u>MS<sup>2</sup></u>
<b>Peak 1</b>	<b>579</b>	<b>301</b>
<b>Peak 2</b>	<b>579</b>	<b>427</b>
<b>Peak 3</b>	<b>579</b>	<b>427</b>
<b>Peak 4</b>	<b>149</b>	
<b>Peak 5</b>	<b>579</b>	<b>301</b>
<b>Peak 6</b>	<b>579</b>	<b>427</b>
<b>B1</b>	<b>579</b>	<b>427</b>
<b>B2</b>	<b>579</b>	<b>427</b>
<b>B3</b>	<b>579</b>	<b>427</b>

Start  
Min  
Min  
Min  
C  
Start



sample: Wine Polyamide 70% MeOH  
C1 8HPLC  
gradient: 2-10%-5min, 10-30%-60 min,  
30-98%-15 min

1

2  
B1

3

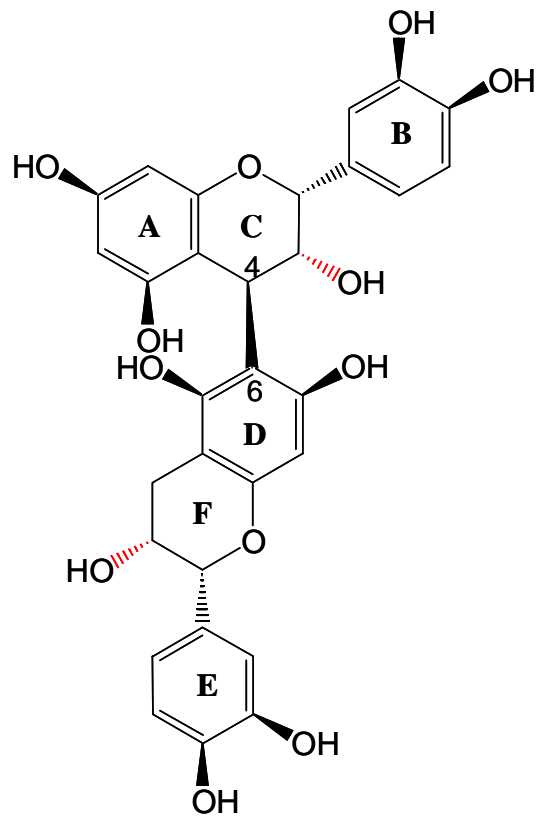
B3

4  
5

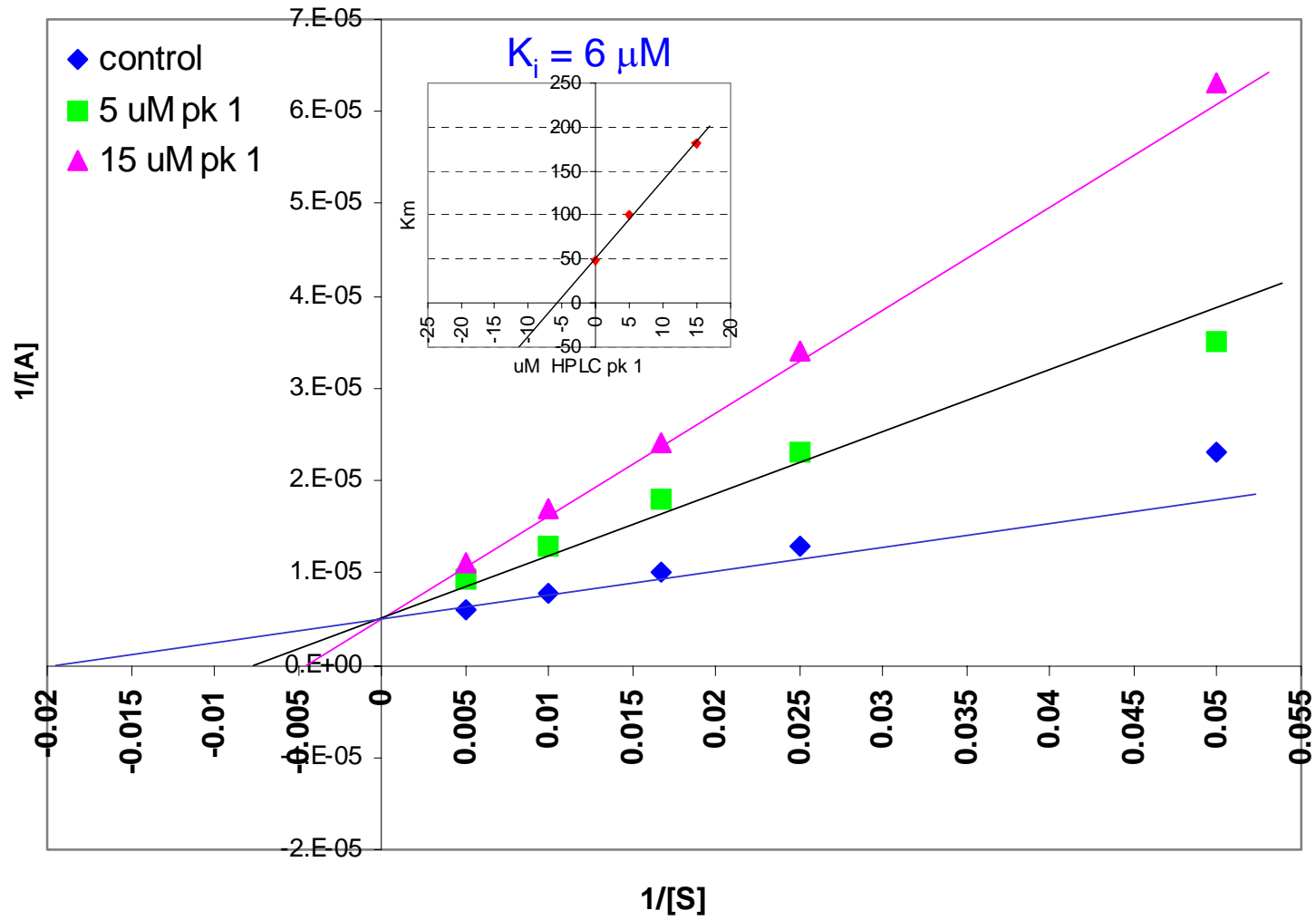
6  
B2

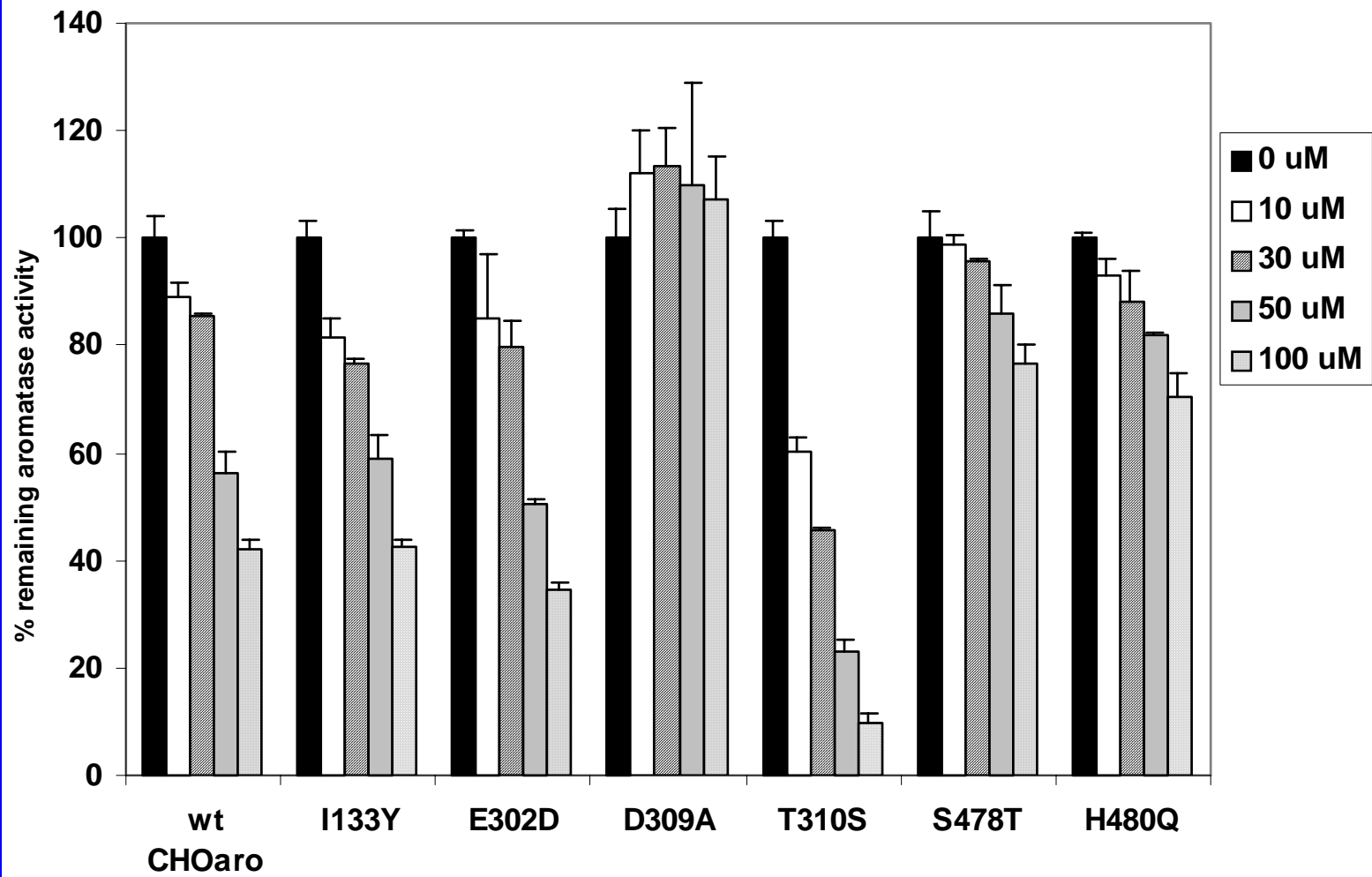
10%  
20%  
30%  
40%  
50%

20  
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40  
50  
60  
70  
80  
90  
100

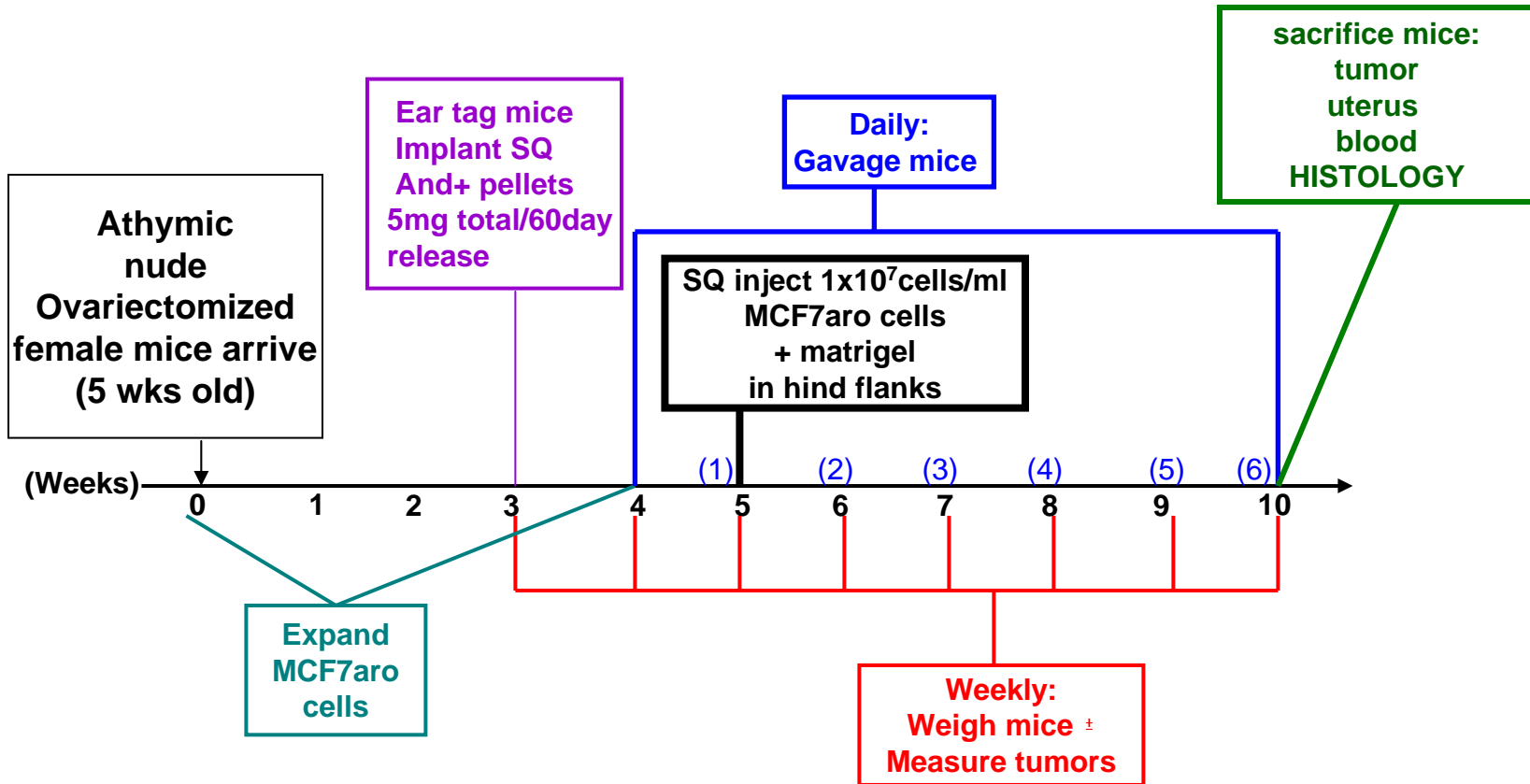


**Procyanidin B5**  
 $C_{30}H_{26}O_{12}$   
Mol. Wt.: 578.5





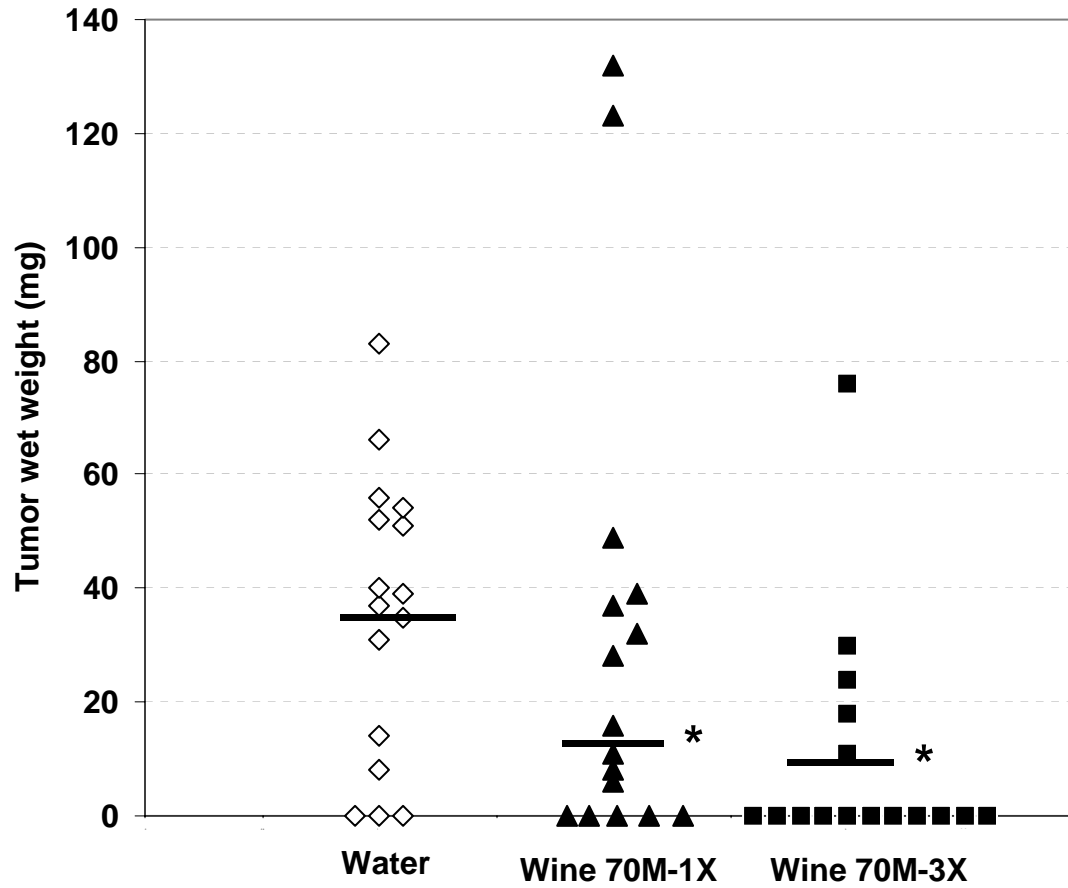
# Animal Protocol Chemoprevention Model



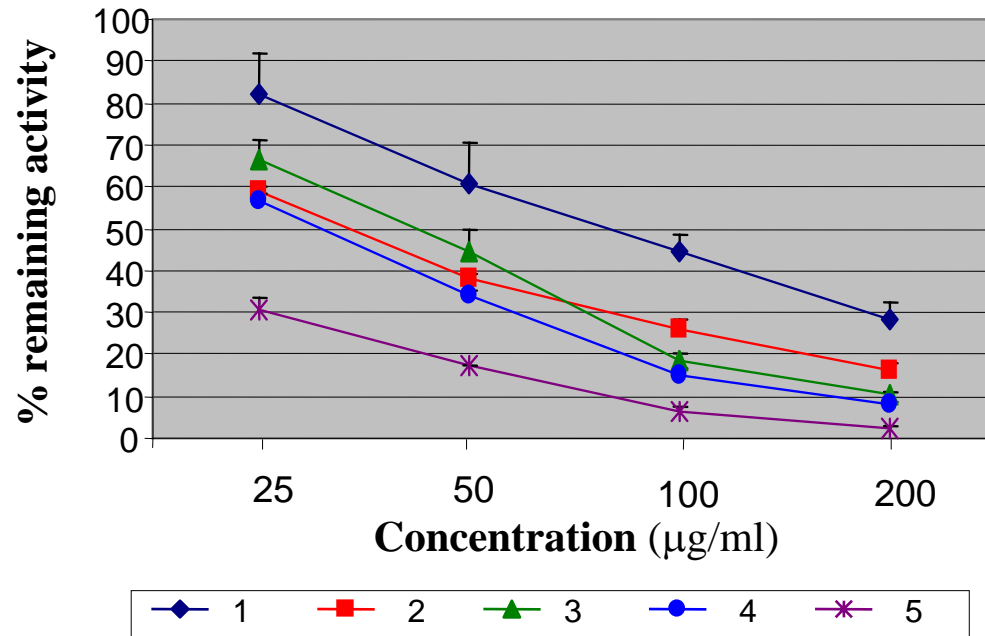
Mice: Charles River Laboratories, Wilmington, MA

Androstenedione pellets: Innovative Research of America, Sarasota, FL

Matrigel: Collaborative Biomedical Products, Franklin Lakes, NJ



## Aromatase inhibition activity of wine marc extract *in vitro*



No	Name	Extraction yield*
1	2002 Chardonnay Pomace	100
2	2002 NV CAB	22
3	2002 Zinfandel Pomace	34
4	2002 Cab Sauvignon Pomace	50
5	wine seed	

\* (mg/g of fresh marc)

