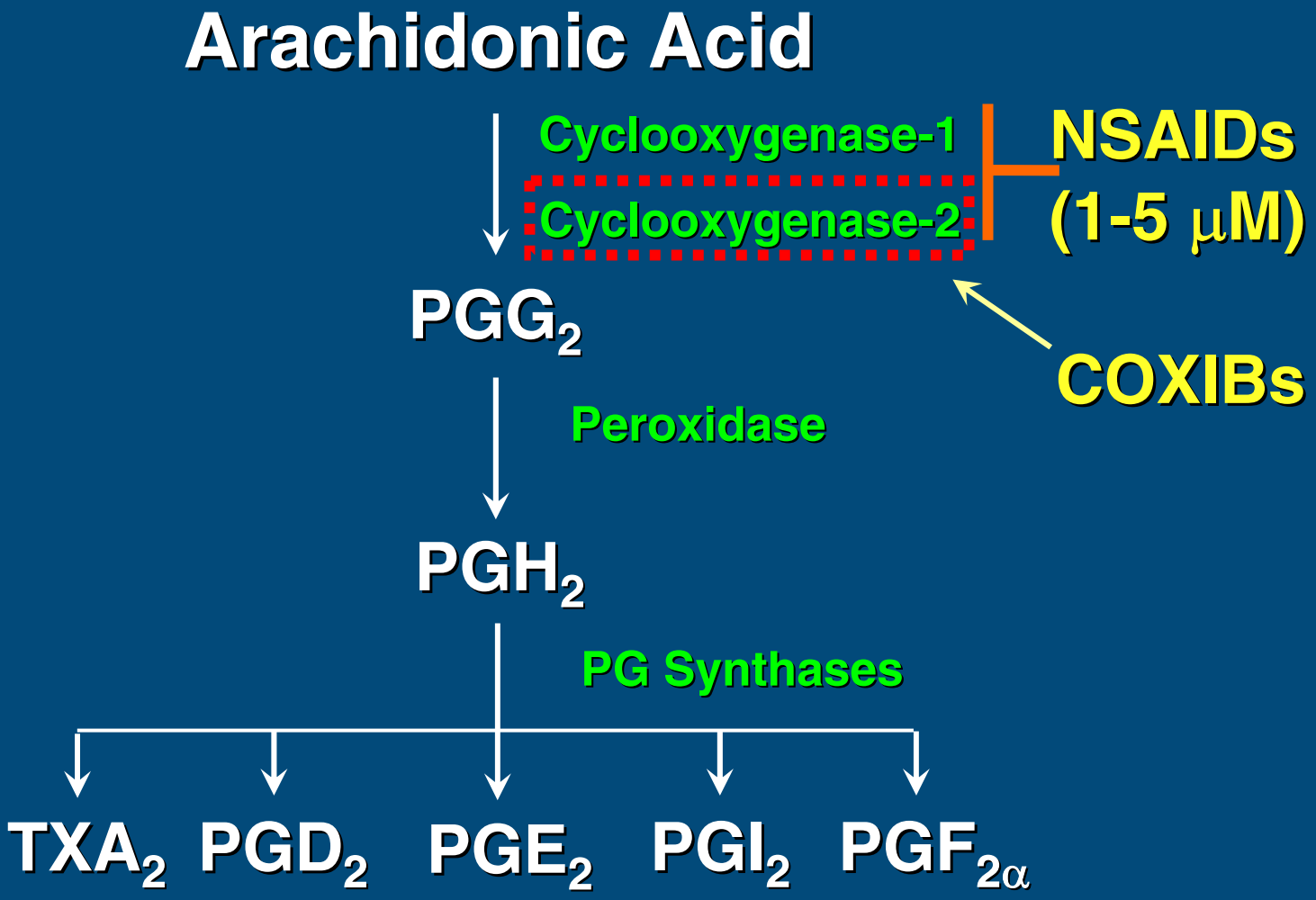
COX-2-dependent regulation of cytokine balance and apoptosis resistance in non-small cell lung cancer

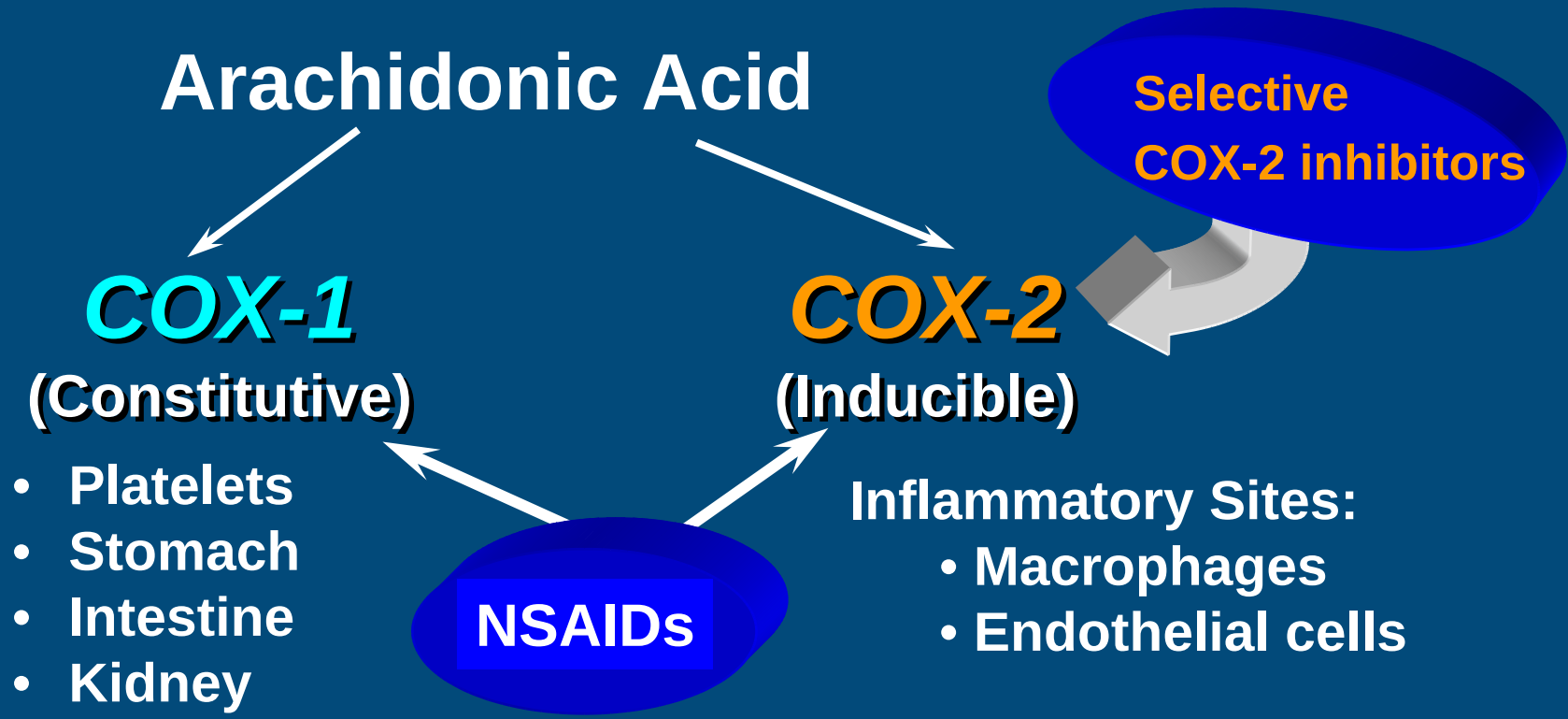
iSBTc 19th Annual Meeting
San Francisco
November 6, 2004

Steven Dubinett, M.D.
UCLA Lung Cancer Research Program

Prostaglandin (PG) Pathway



COX-1 Versus COX-2



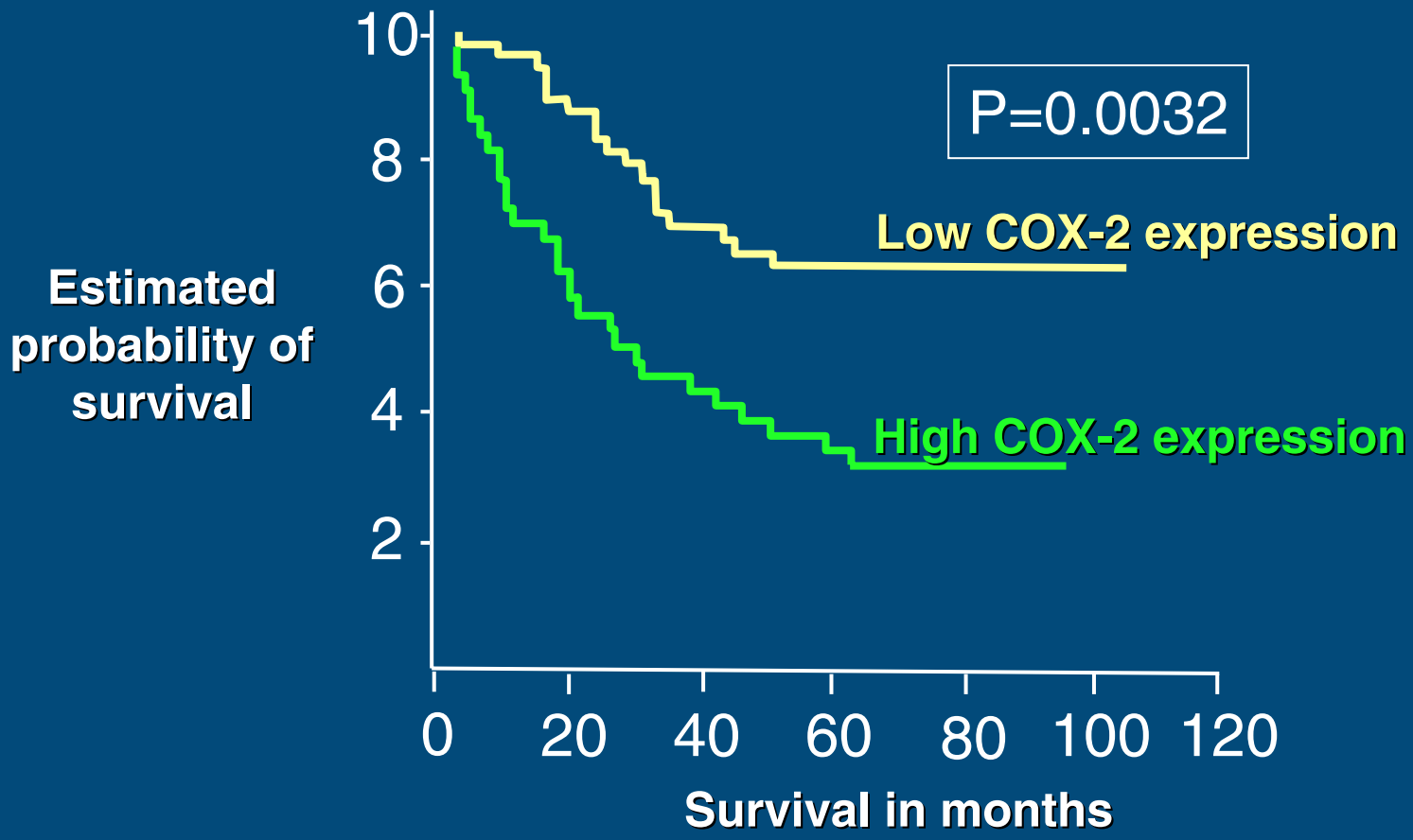
COX-2 in Lung Cancer

- **Overexpressed in NSCLC and pre-neoplasia**
  Huang M, Ca Res 1998; Wardlaw S, Carcinogenesis 2000; Hosomi Y, Lung Ca 2000)
- **A marker of poor prognosis in stage I NSCLC**
  (Khuri F, Clin Ca Res 2001; Achiwa H, Clin Ca Res 1999)
- **Induced by tobacco carcinogens**
  (Yan Z, JBC 2000; Rioux N, Inflam Res 1999)
- **Preclinical studies of COX-2 inhibitors suggest antitumor and chemopreventive efficacy** (Stolina M, J. Immunol 2000; Roux N, Ca Res 1998, Masferrer J, Ca Res 2000, Williams C, JCI 2000)
- **Epidemiologic data suggest subjects who routinely use NSAIDs have decreased lung cancer risk**
  (ie Akhmedkhanov Brit J Cancer 2002)

Rationale for COX-2 Inhibition in Lung Cancer

- **Induces apoptosis and enhances cytotoxicity of anticancer agents** (Hida T, Clin Cancer Res 2000)
- **Induces antiangiogenic effects in lung cancer models** (Masferrer J, Ca Res 2000)
- **Restores antitumor immunity**
  (Stolina M, J. Immunol 2000; Huang M, Cancer Res 1998)
- **Decreases tumor invasiveness**
  (Dohadwala M, J. Biol Chem 2001 & 2002)

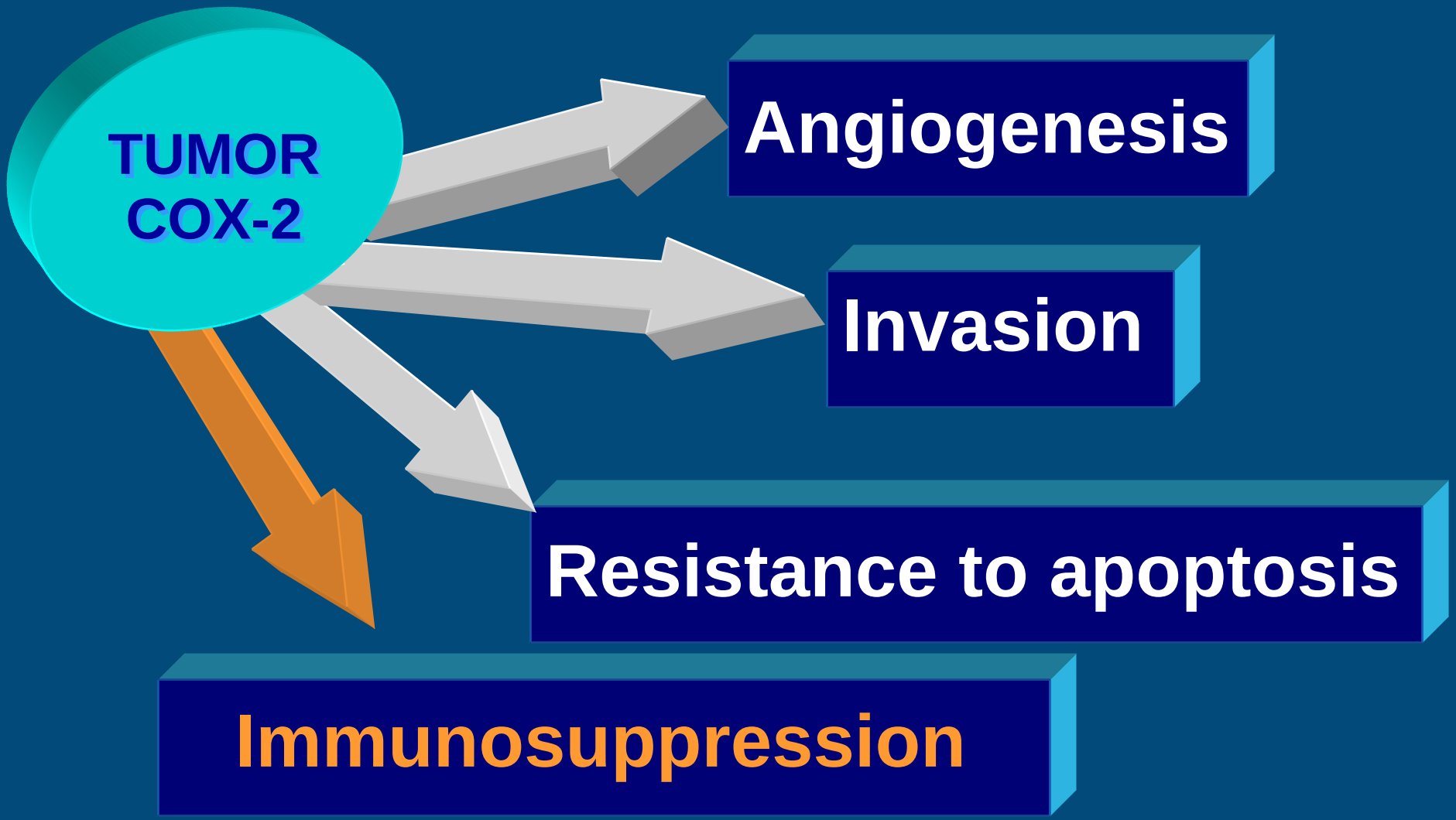
Survival of Patients with NSCLC



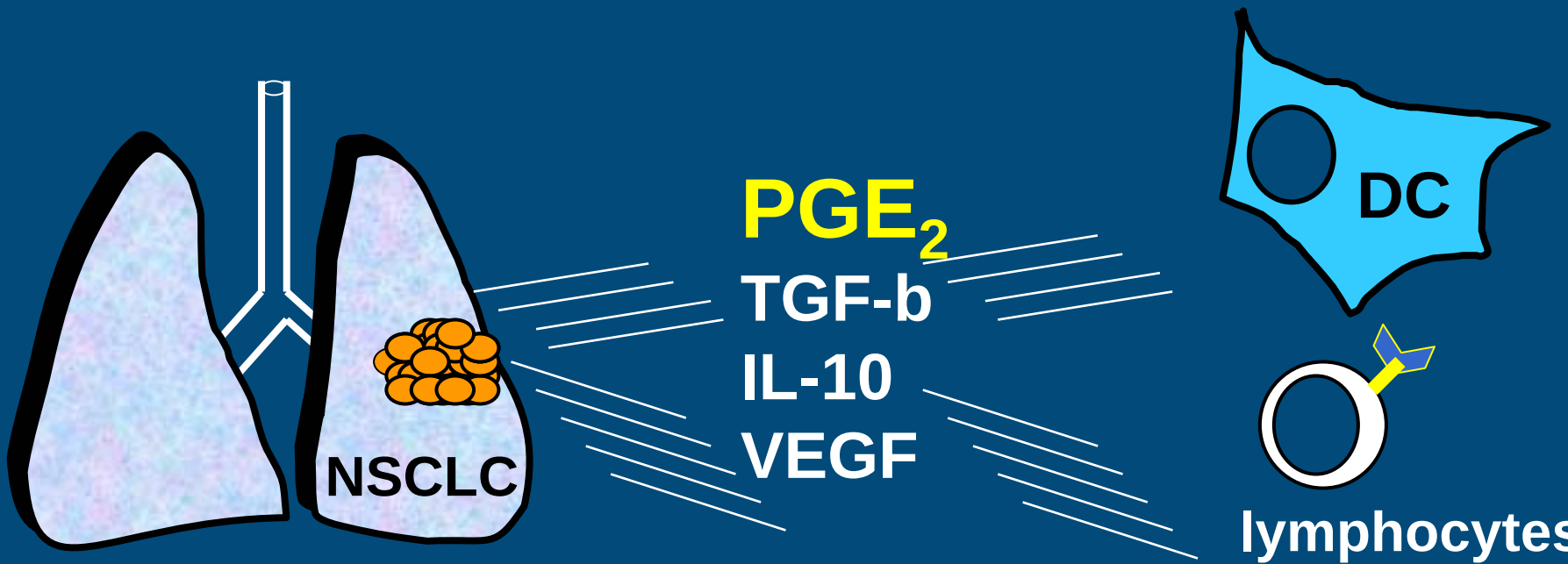
Brabender et al. *Ann of Surg* 2002;235:440

How does COX-2 regulate the malignant phenotype in non-small cell lung cancer?

COX-2-dependent Modulation of the Malignant Phenotype



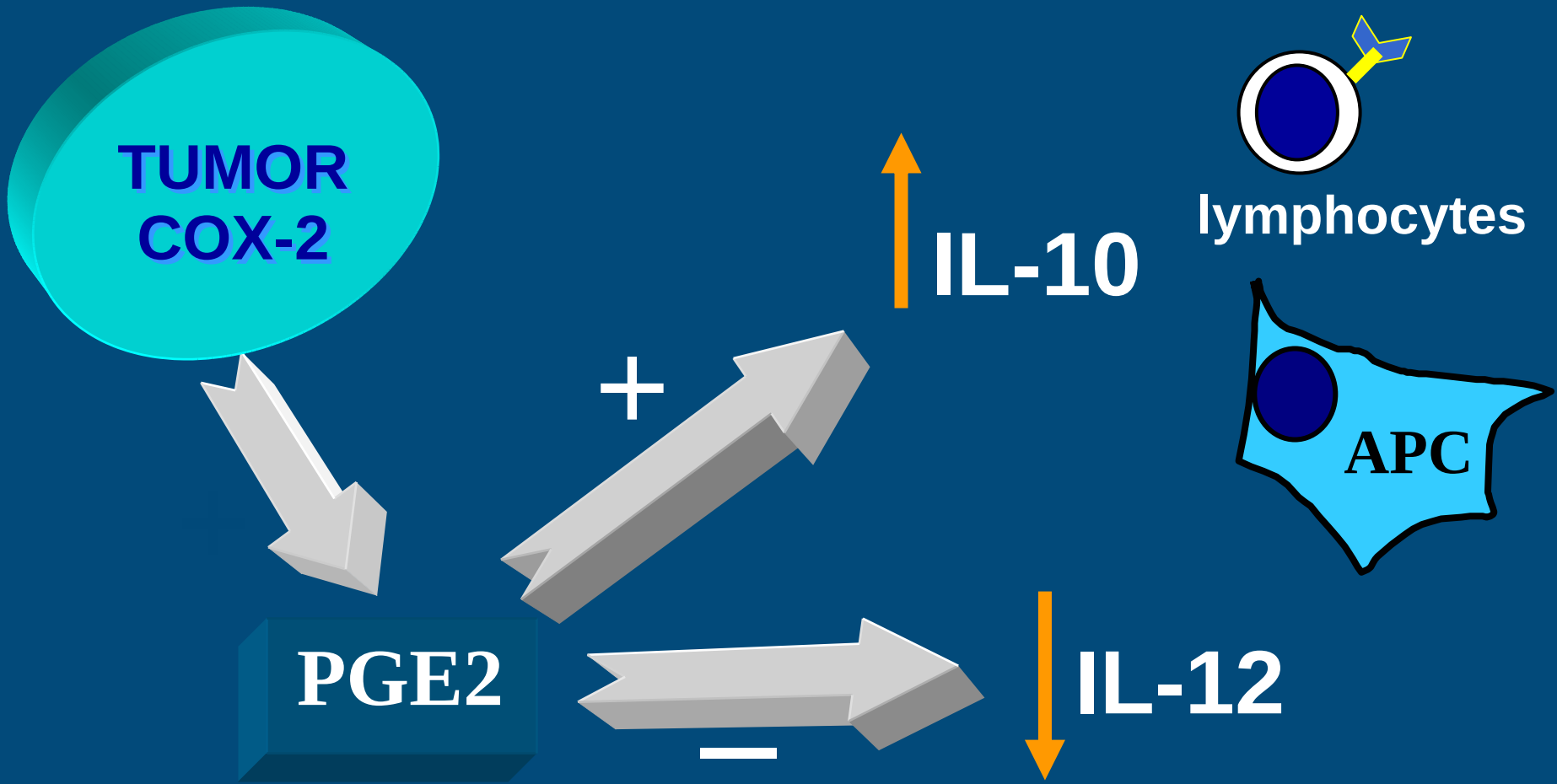
Lung Cancer-mediated Inhibition of Antitumor Immunity



- Lack of costimulatory molecules
- Production of immune inhibitory mediators

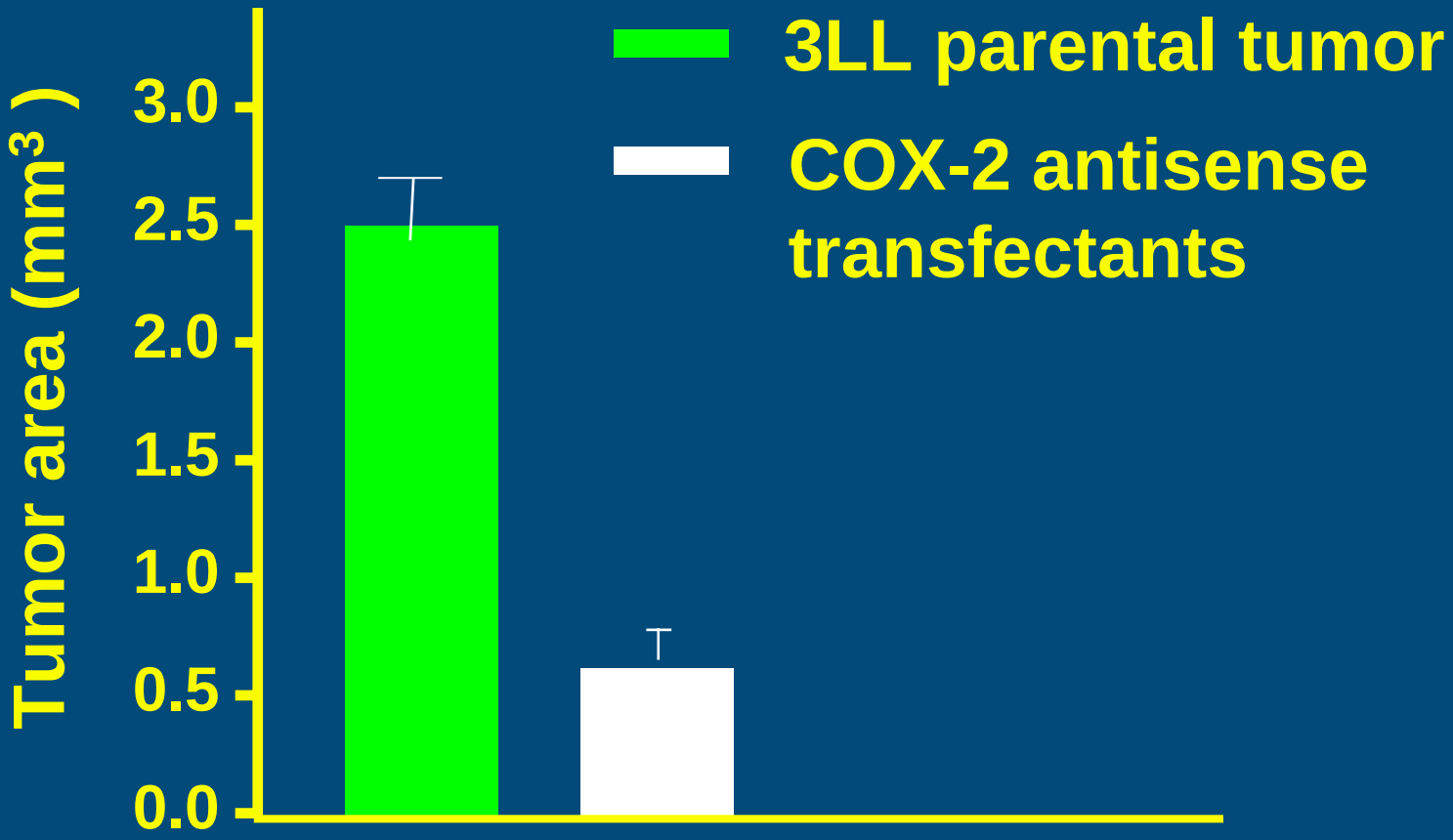
ANERGY

COX-2-mediated inhibition of antitumor immunity in NSCLC

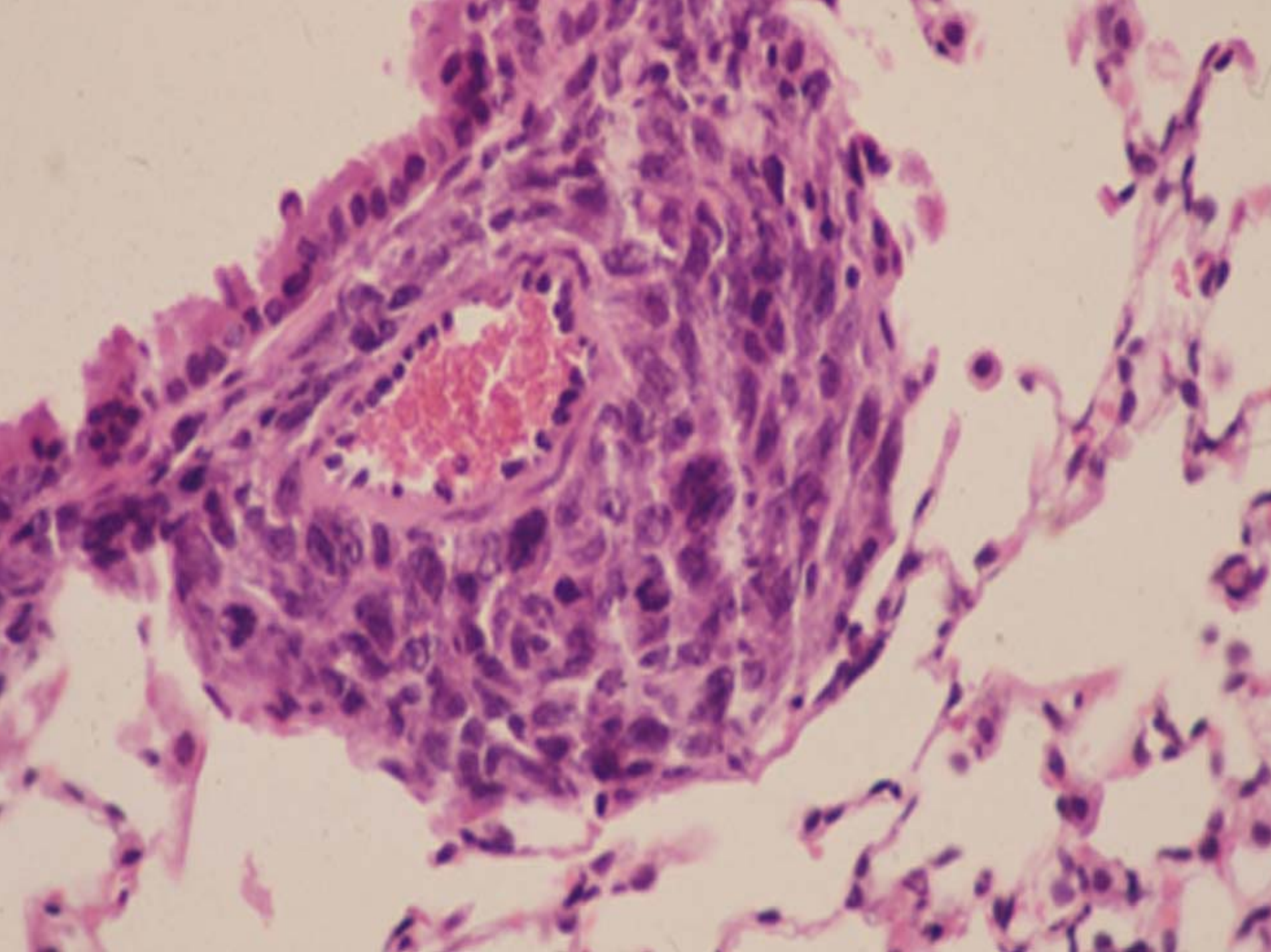


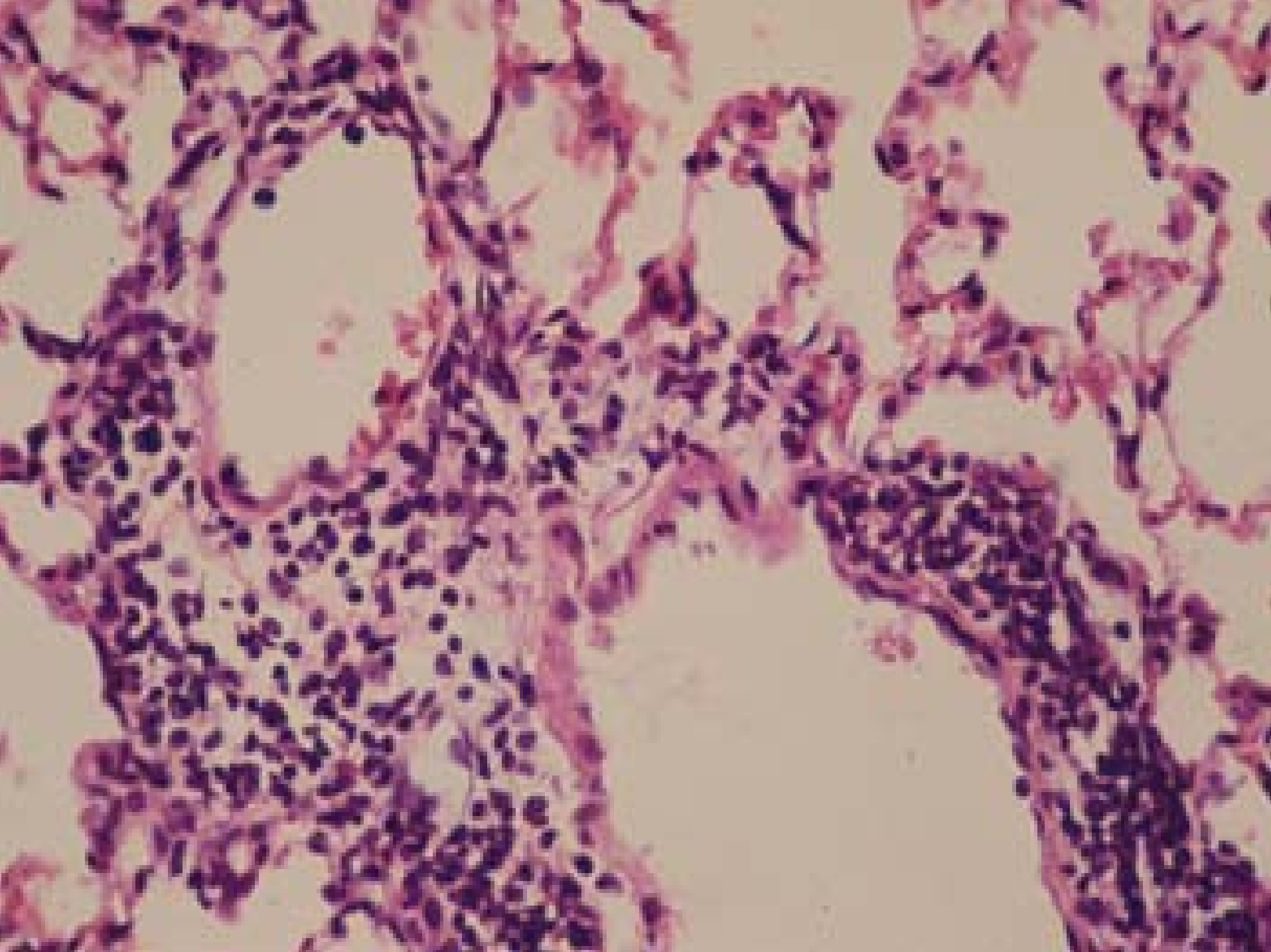
Huang et al,Cancer Research 1998; 58:1208
Stolina et al, Journal of Immunology 2000; 164: 361

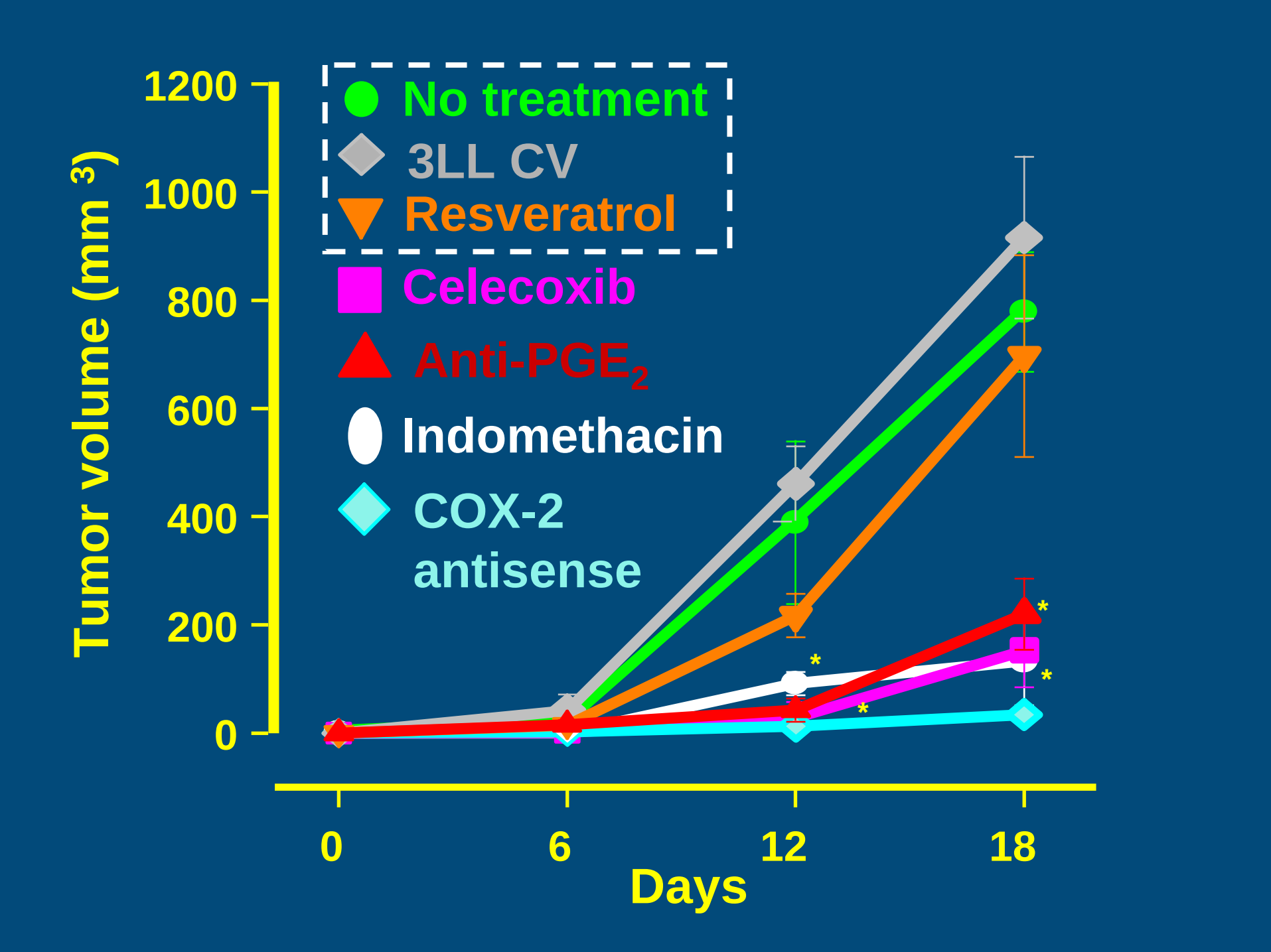
Specific Inhibition of Tumor COX-2 Limits Tumor Growth *in vivo*



Stolina et al J Immunology 2000





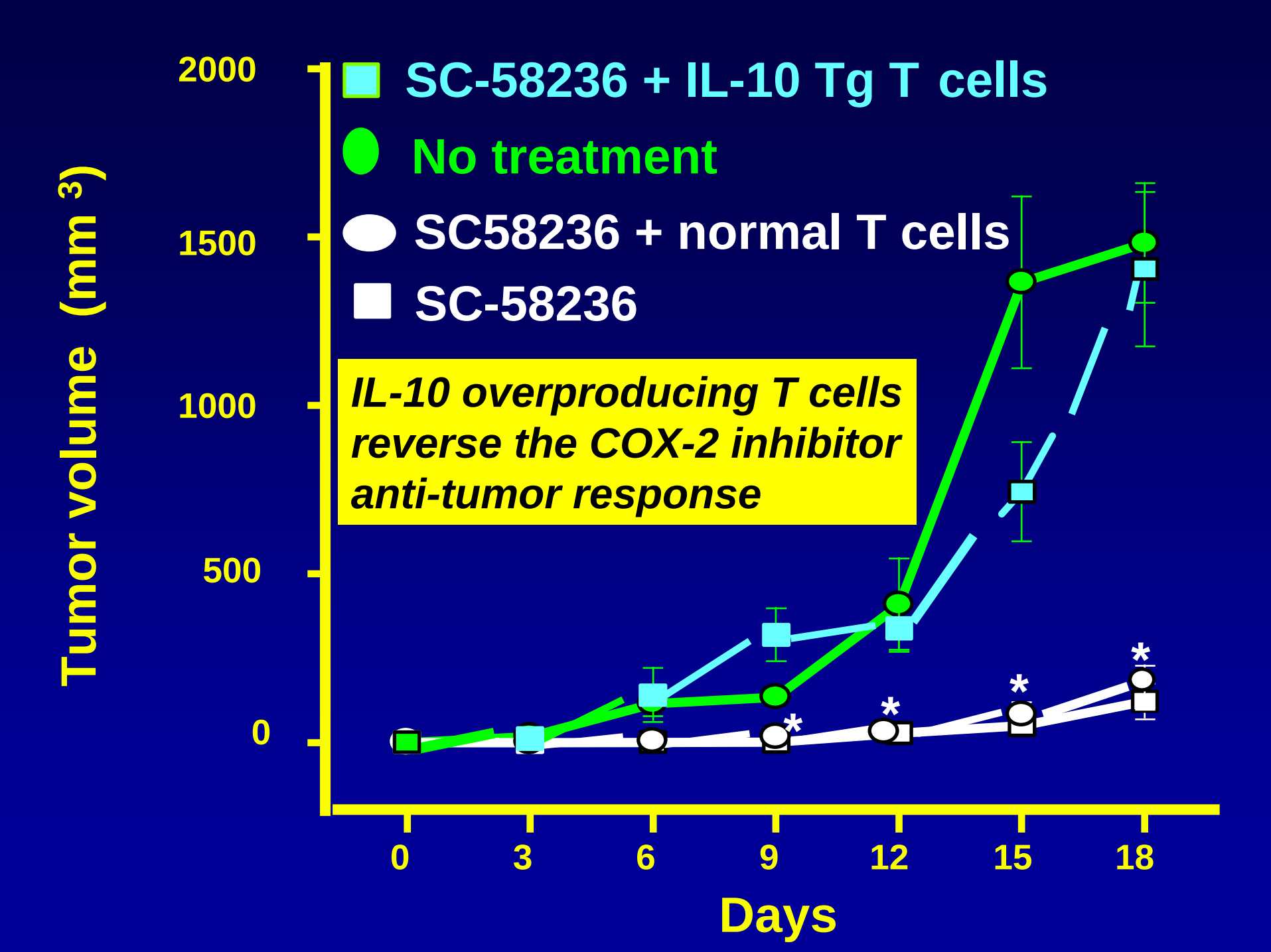

No treatment
3LL CV
Resveratrol
Celecoxib
Anti-PGE2
Indomethacin
COX-2
antisense
Tumor volume (mm 3)
1200
1000
800
600
400
200
0
0
6
12
18
Days
*
*
*
*

COX-2 Inhibition in vivo Reduces PGE_2 and IL-10 Production by *Tumor Tissues*

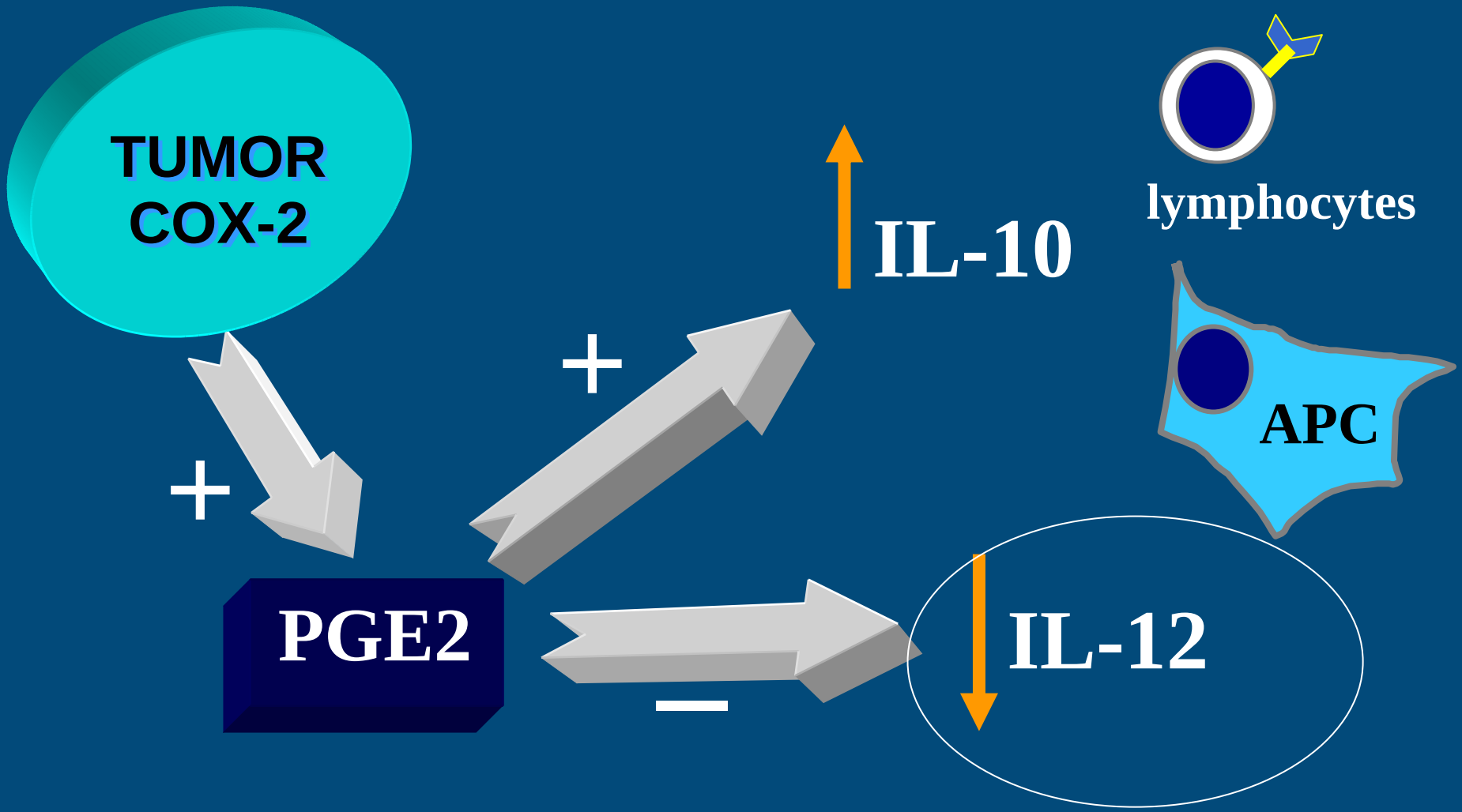
(ng/ml/g of tumor tissue)

3LL tumors from:	IL-10	PGE_2
Untreated mice	734 ± 74	27 ± 2
COX-2 Antisense	184 ± 27*	11 ± 2*
Celecoxib-treated	398 ± 38*	17 ± 1*
Indomethacin-treated	268 ± 60*	12 ± 1*

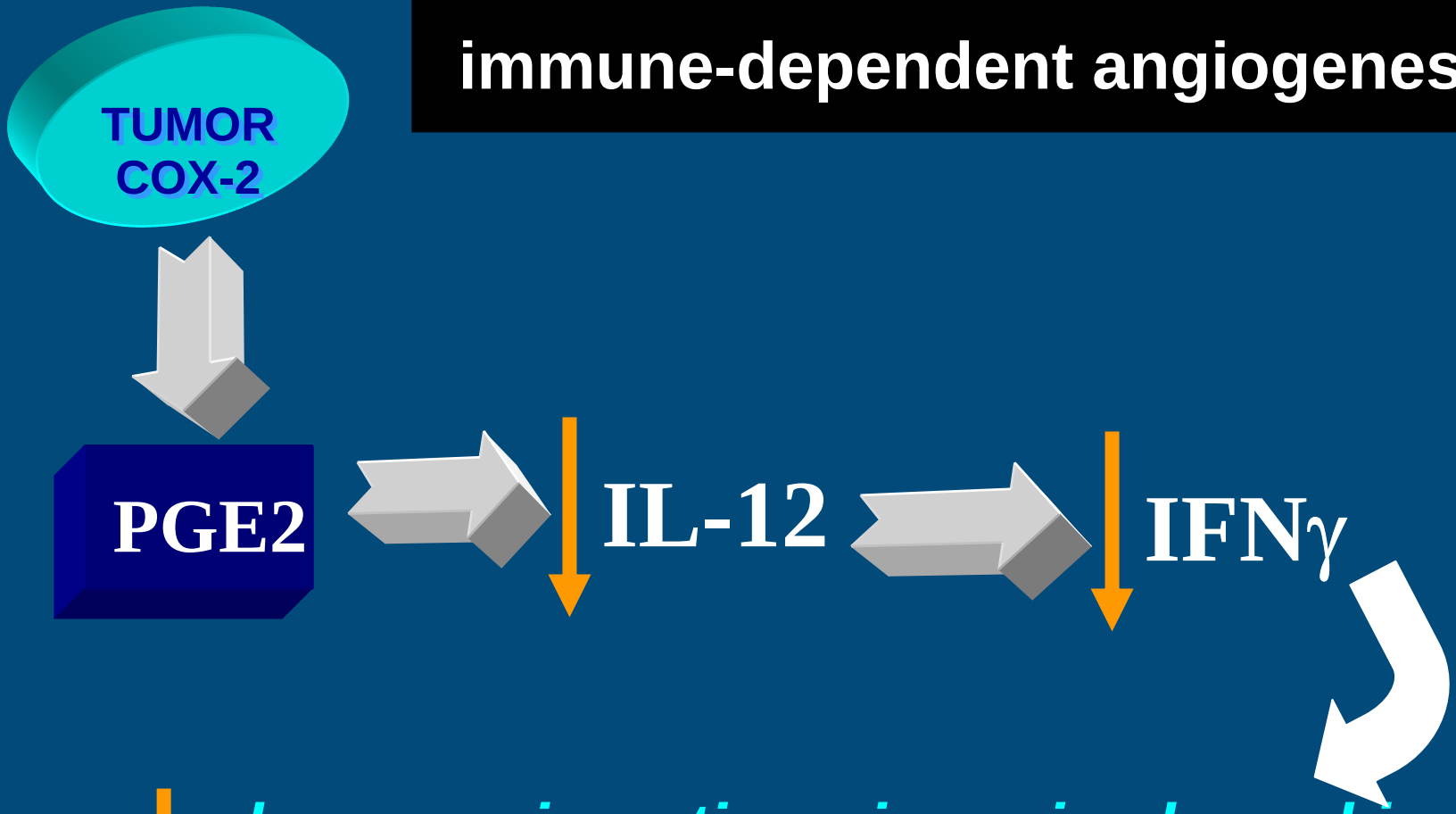
* $p<0.05$ compared to untreated tumor bearing mice.


SC-58236 + IL-10 Tg T cells
No treatment
SC58236 + normal T cells
SC-58236
IL-10 overproducing T cells reverse the COX-2 inhibitor anti-tumor response
Tumor volume (mm 3)
2000
1500
1000
500
0
0
3
6
9
12
15
18
Days
*
*
*
*

COX-2-mediated inhibition of antitumor immunity in NSCLC



COX-2-mediated inhibition of immune-dependent angiogenesis

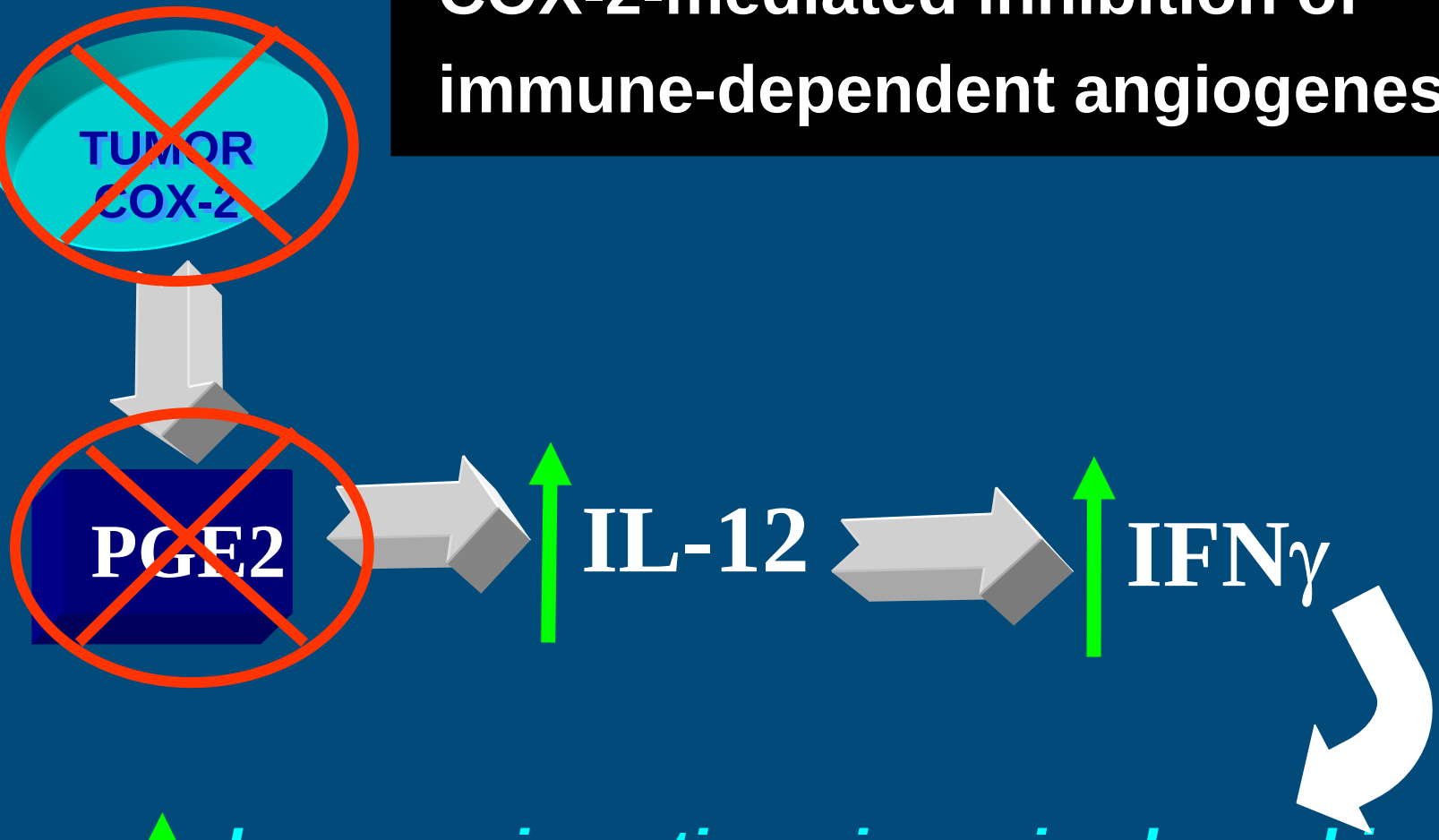


decrease in anti-angiogenic chemokines

Interferon –inducible protein 10 (IP-10)

Monokine induced by IFNγ (MIG)

COX-2-mediated inhibition of immune-dependent angiogenesis



decrease in anti-angiogenic chemokines

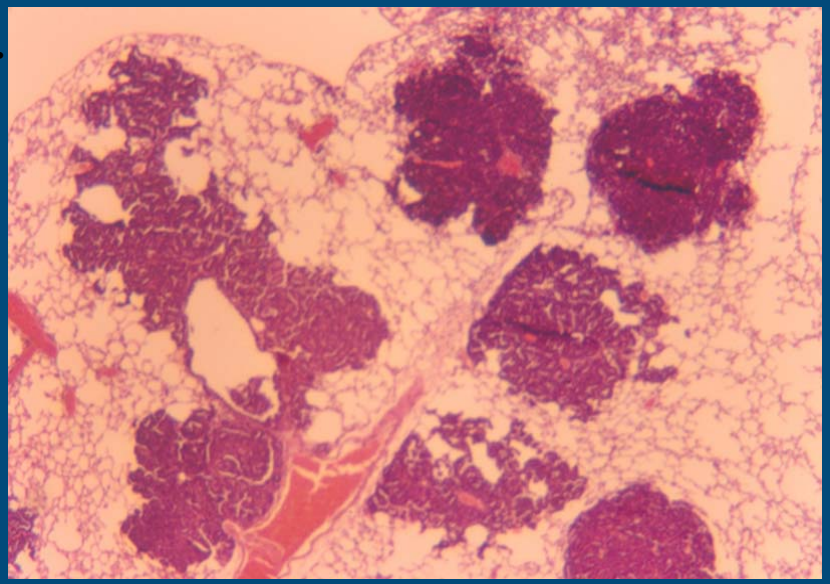
Interferon –inducible protein 10 (IP-10)

Monokine induced by IFNγ (MIG)

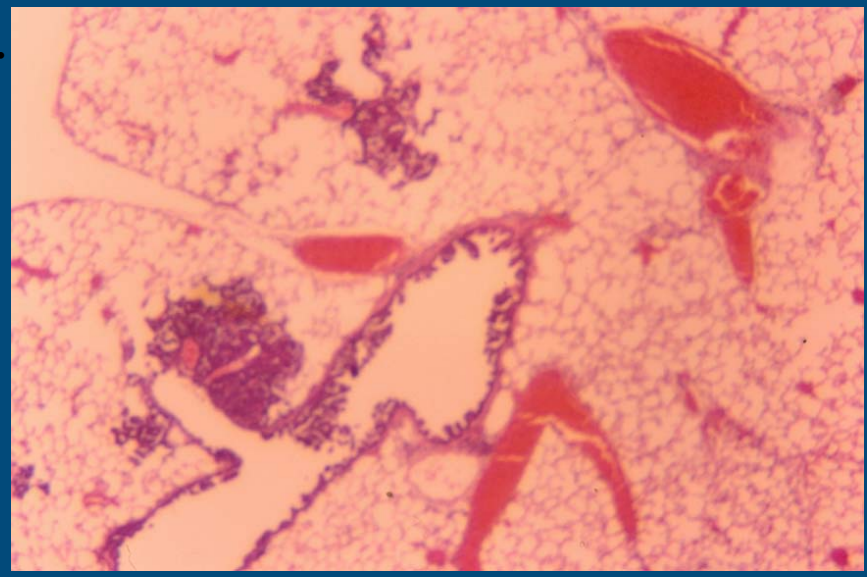
Control

Systemic COX-2 Inhibition

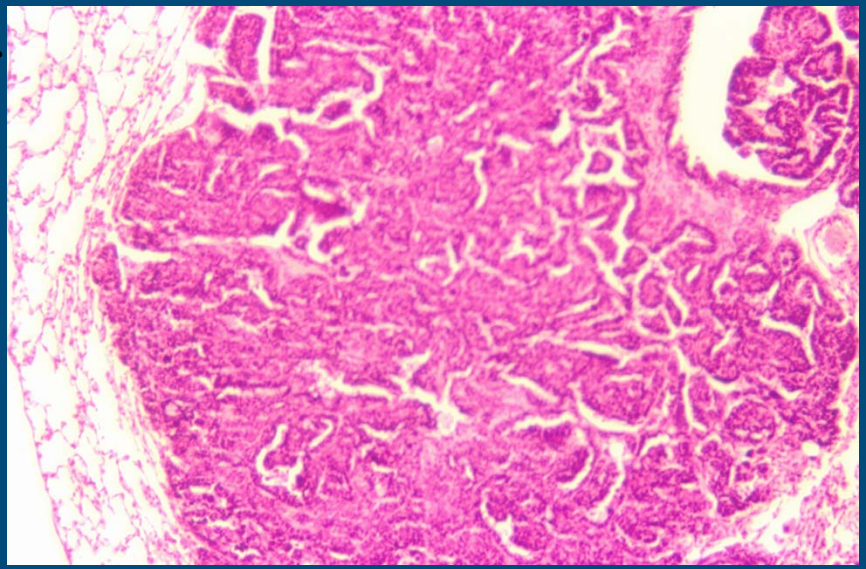
A.



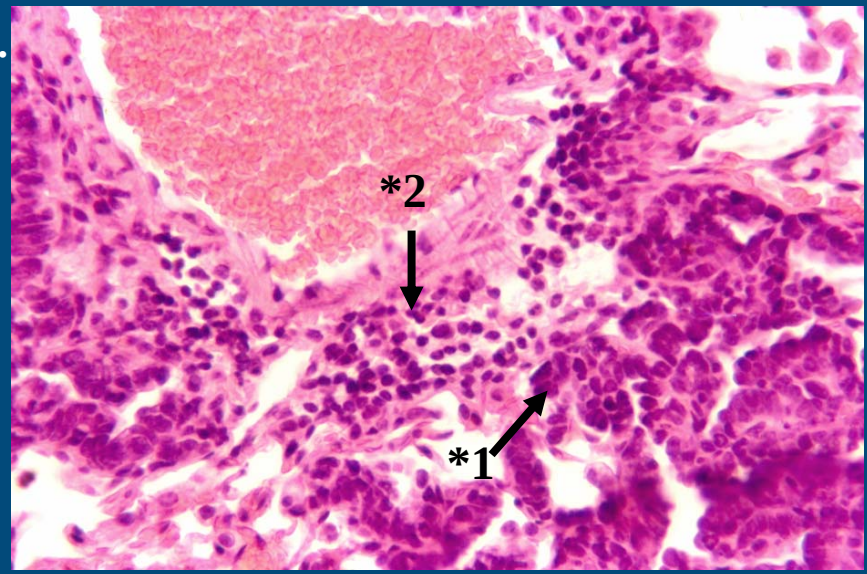
B.

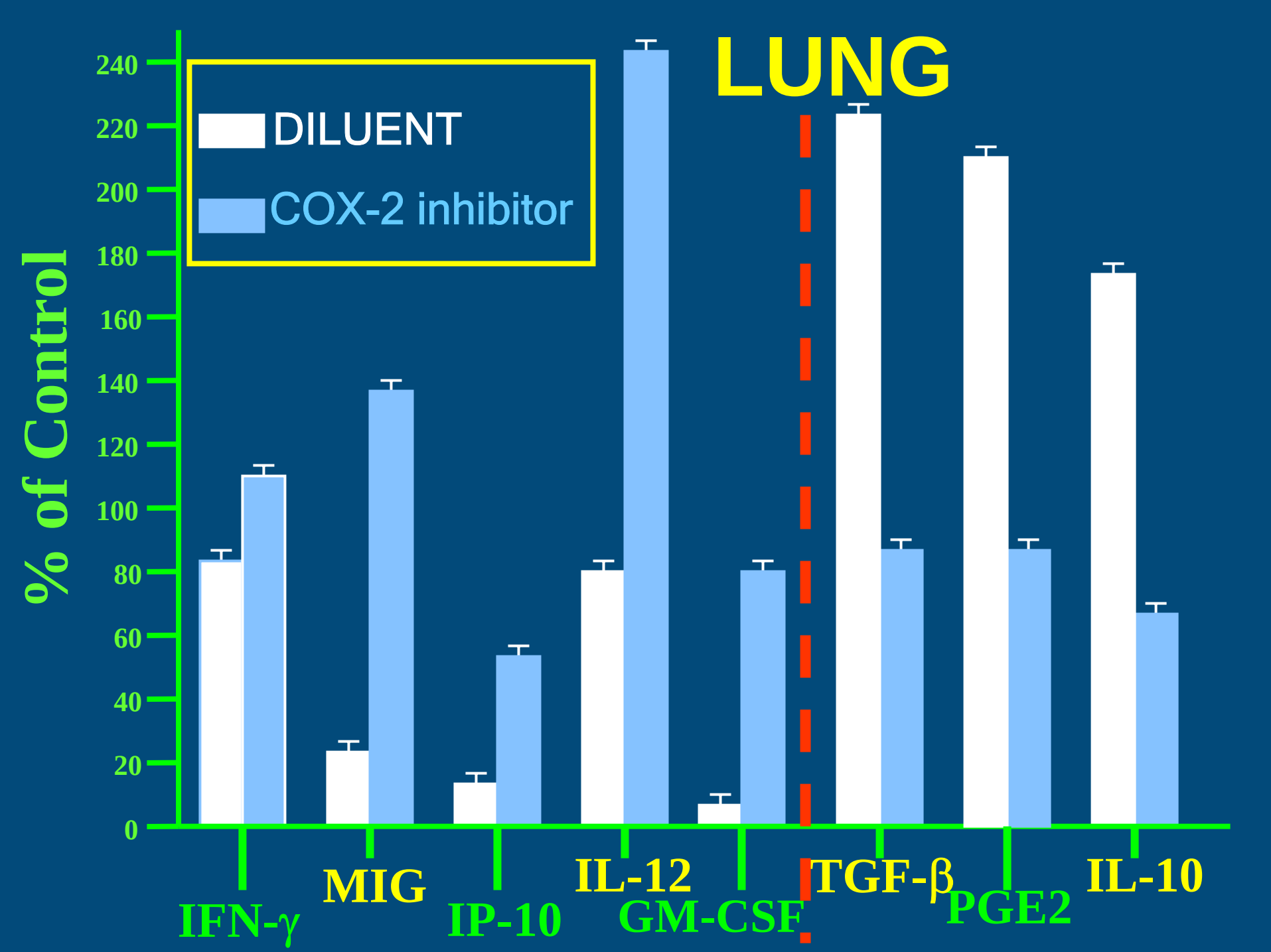


C.



D.




LUNG
DILUENT
COX-2 inhibitor
% of Control
240
220
200
180
160
140
120
100
80
60
40
20
0
IFN-γ
MIG
IP-10
IL-12
GM-CSF
TGF-β
PGE2
IL-10

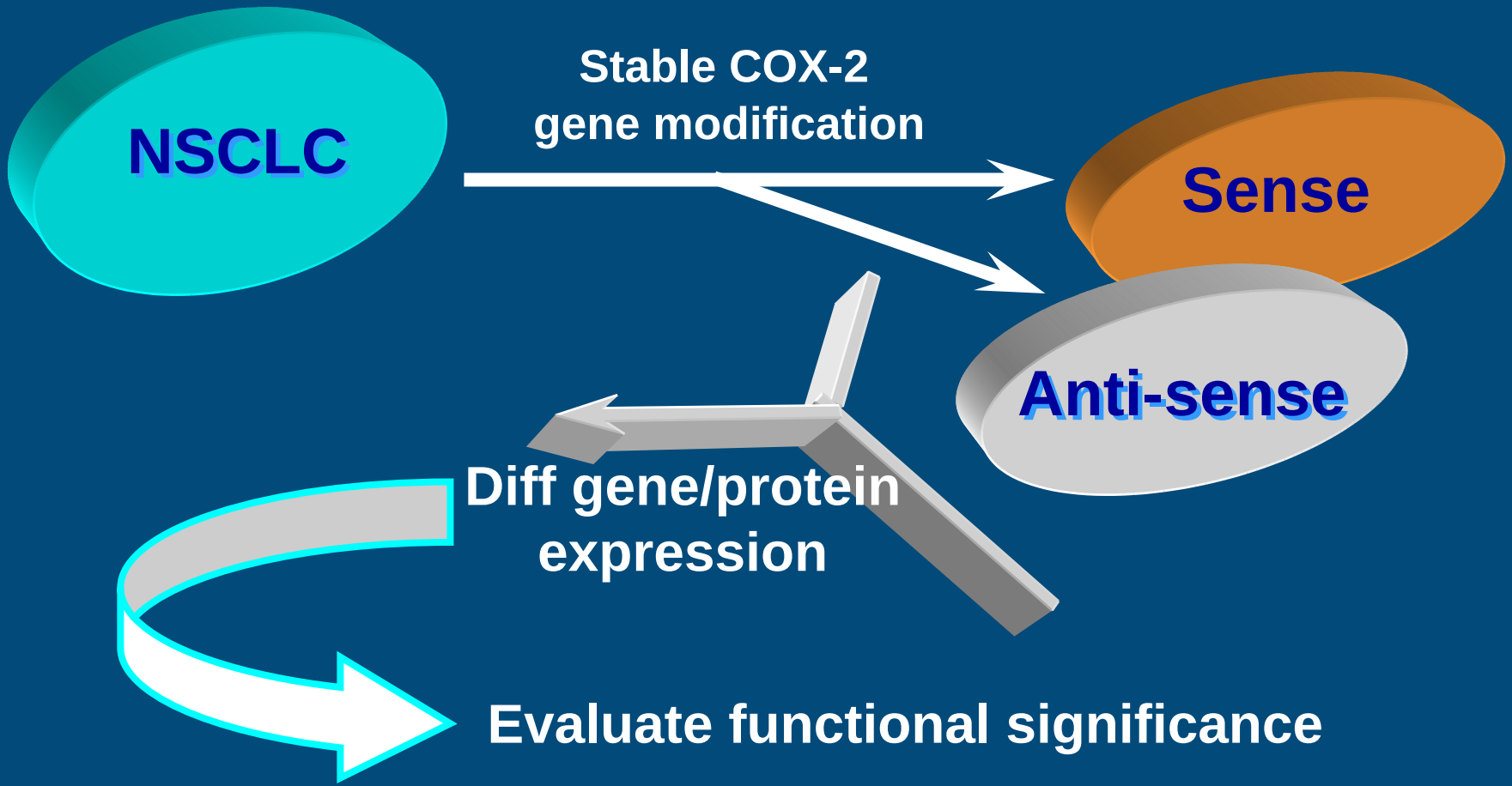
Biology of COX-2 Inhibition in Cancer

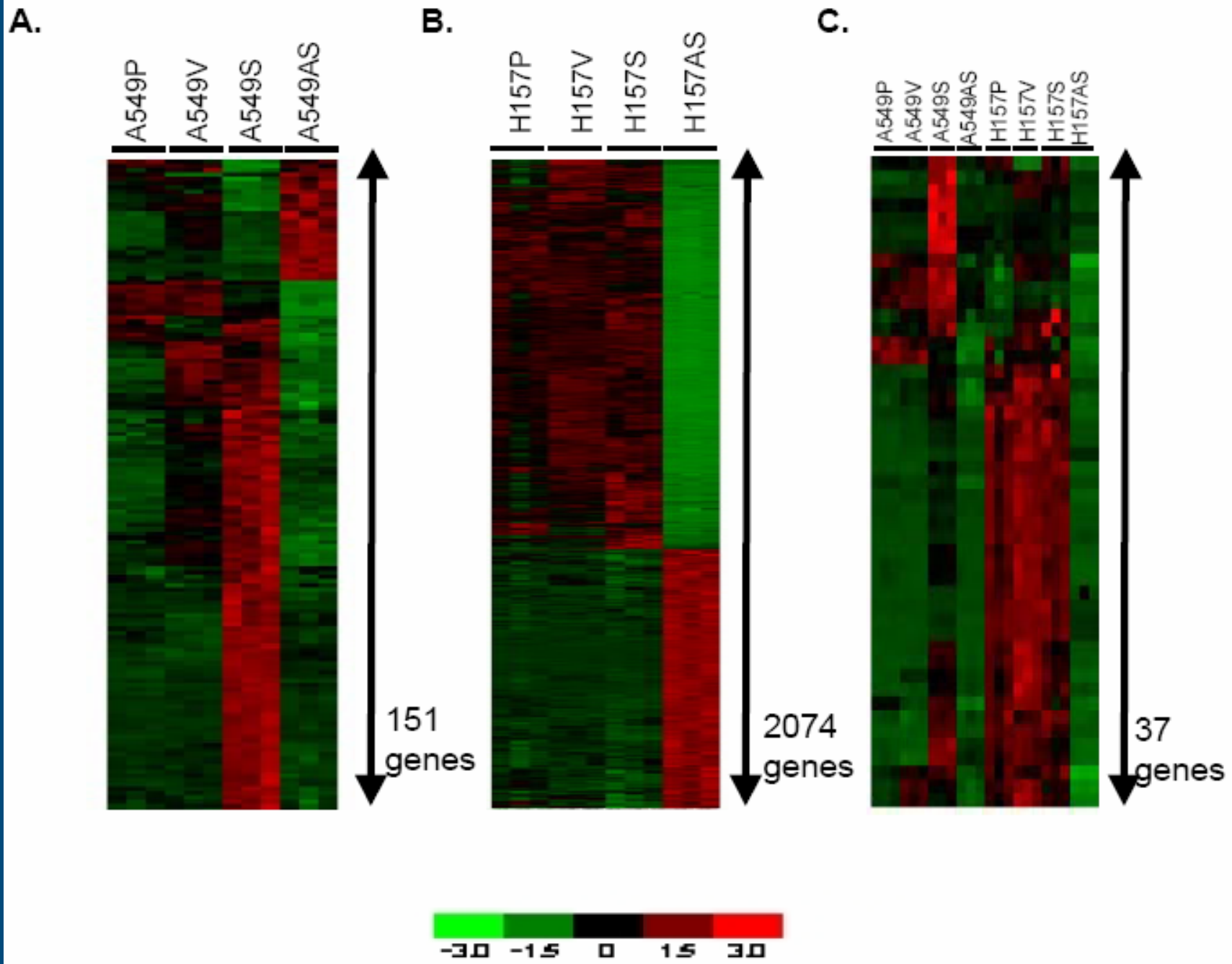
- How can we further our understanding of the impact of COX-2 expression in human lung cancer?

- What biomarkers should we monitor in trials that utilize COX-2 inhibitors?

Note: trials reported by David Johnson (ASCO 2003), Nasser Altorki (JCO 2003), and Jenny Mao (Clin Cancer Res 2003) demonstrating decreased PGE_2 following celecoxib in NSCLC patients

What COX-2-dependent Genes/Proteins Mediate the Malignant Phenotype in NSCLC?




A.
A549P
A549V
A549S
A549AS
151 genes
B.
H157P
H157V
H157S
H157AS
2074 genes
C.
A549P
A549V
A549S
A549AS
H157P
H157V
H157S
H157AS
37 genes
-3.0
-1.5
0
1.5
3.0

What COX-2-dependent Genes/Proteins Mediate the Malignant Phenotype in NSCLC?

Tumor invasion: CD44 and MMP2

Dohadwala et al, JBC 2001, 276: 20809 &
JBC 2002, 277:50828

Immune regulation: IL-10 and IL-12

Stolina et al, J. Immunol 2000,164:361 &
Huang et al, Cancer Res 1998, 58:1208

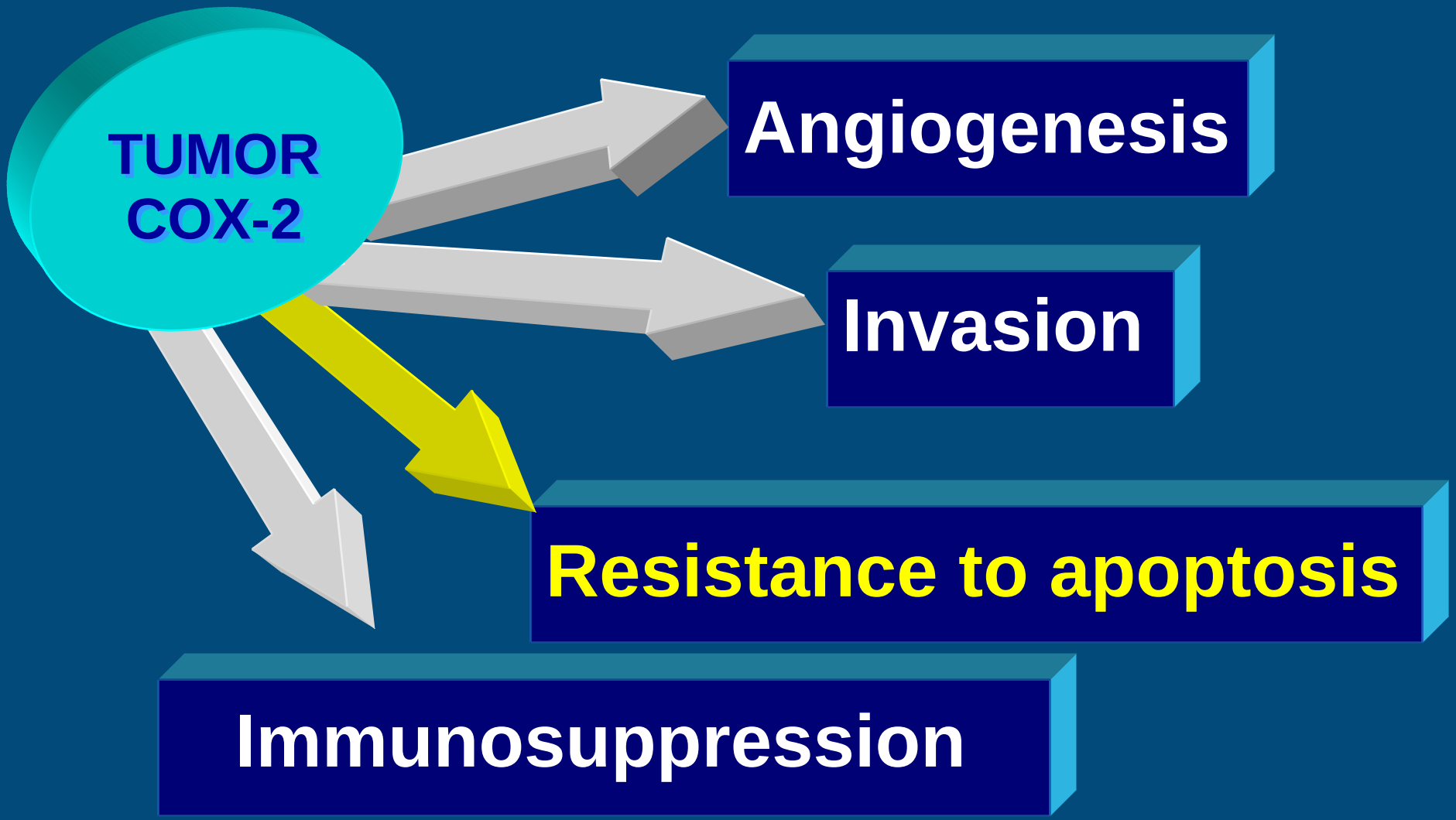
Angiogenesis: CXCL5, CXCL8, VEGF

Pold et al Cancer Research 2004, 64(5):1853-60

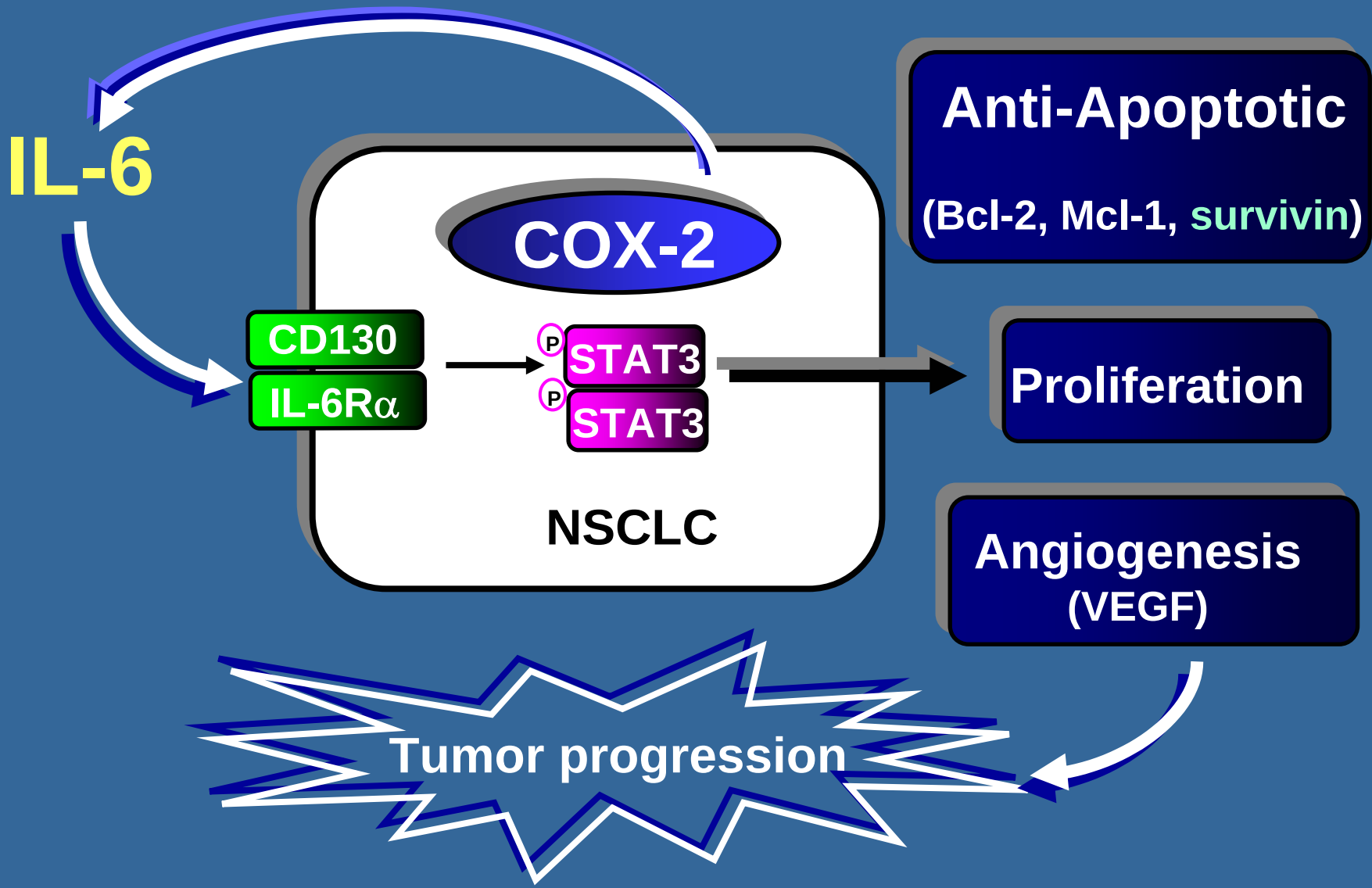
Apoptosis: survivin, IGF-BP-3, IL-6

Krysan et al, FASEB J Jan. 2004 & Ca Res 2004, 64: 6359
Pold et al Cancer Res 2004, 64: 6549

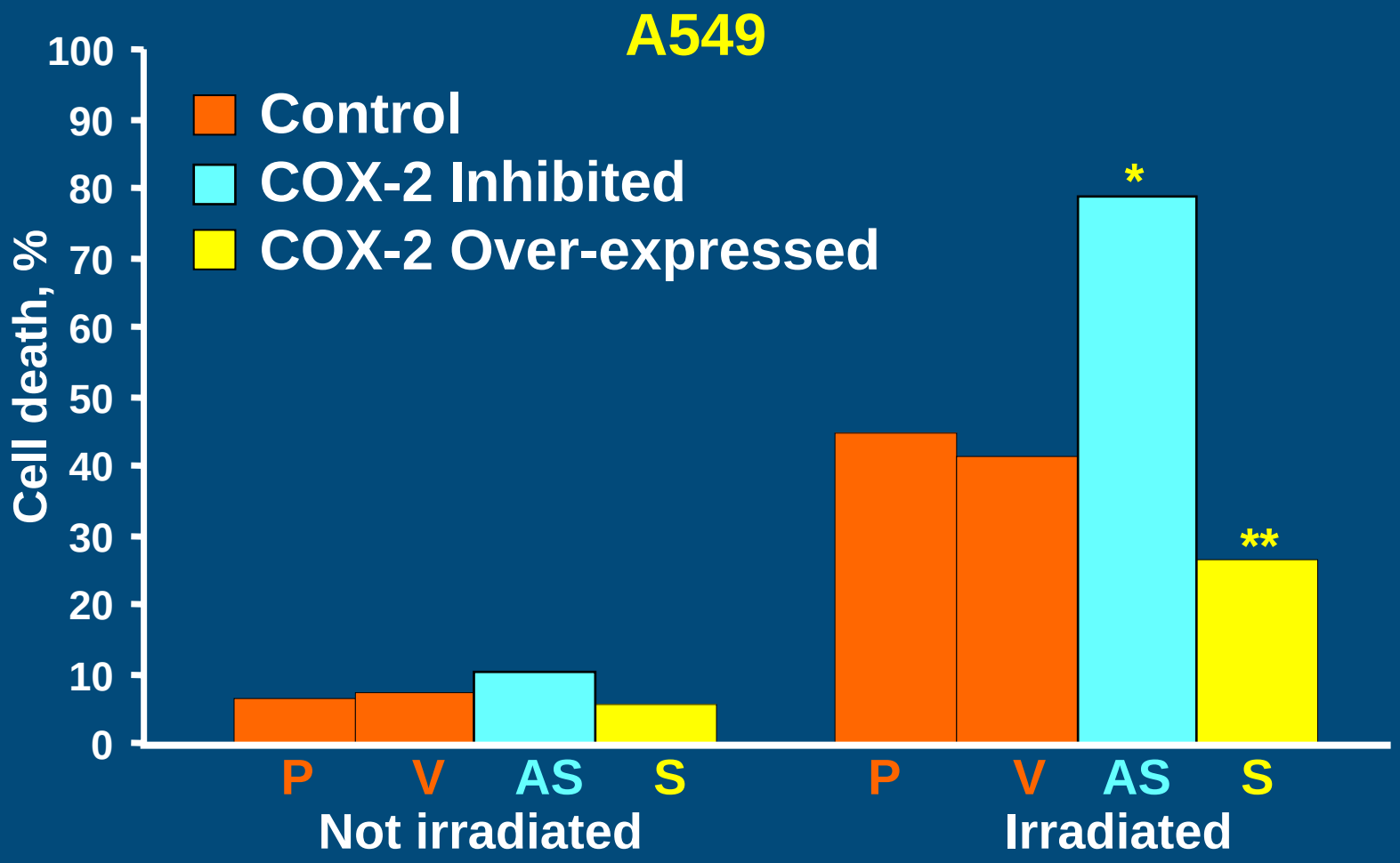
NSCLC COX-2-dependent Modulation of the Malignant Phenotype



COX-2 and STAT3 in NSCLC



COX-2-dependent Apoptosis Resistance in NSCLC Cells



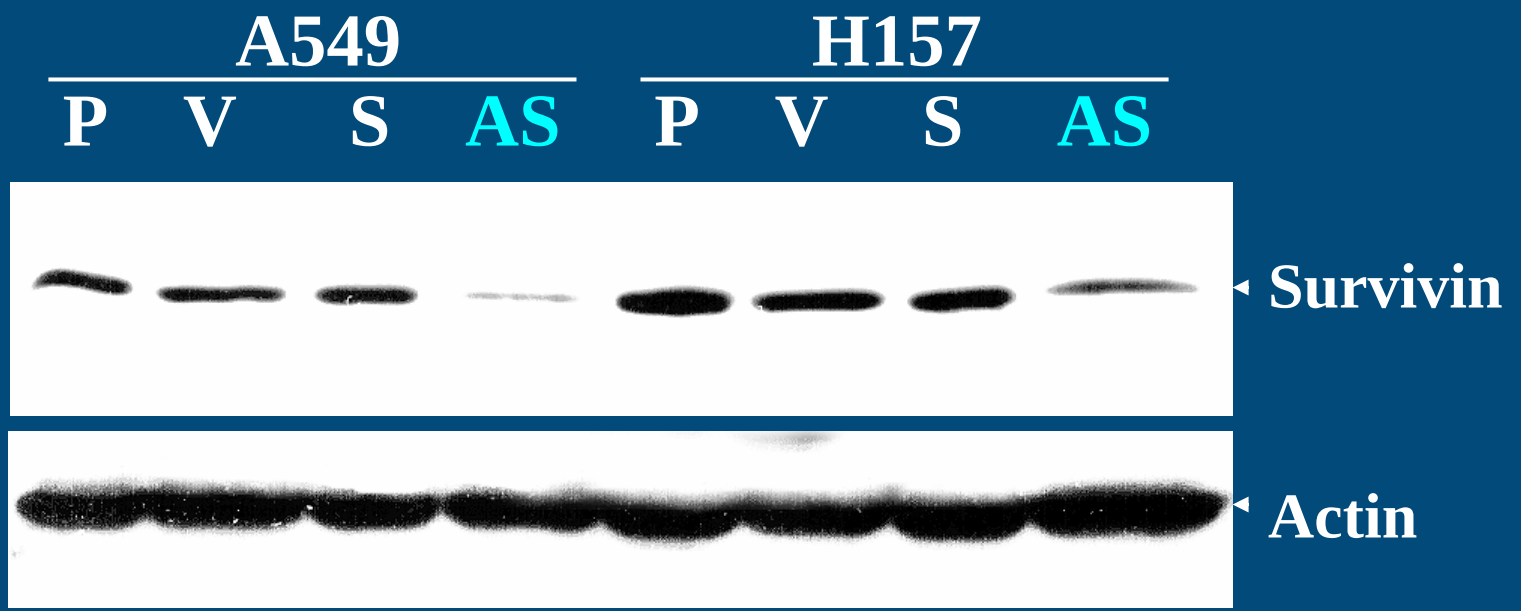
* $p<0.0001$, ** $p<0.005$ vs control

Krysan et al FASEB Journal Jan 2004

Survivin

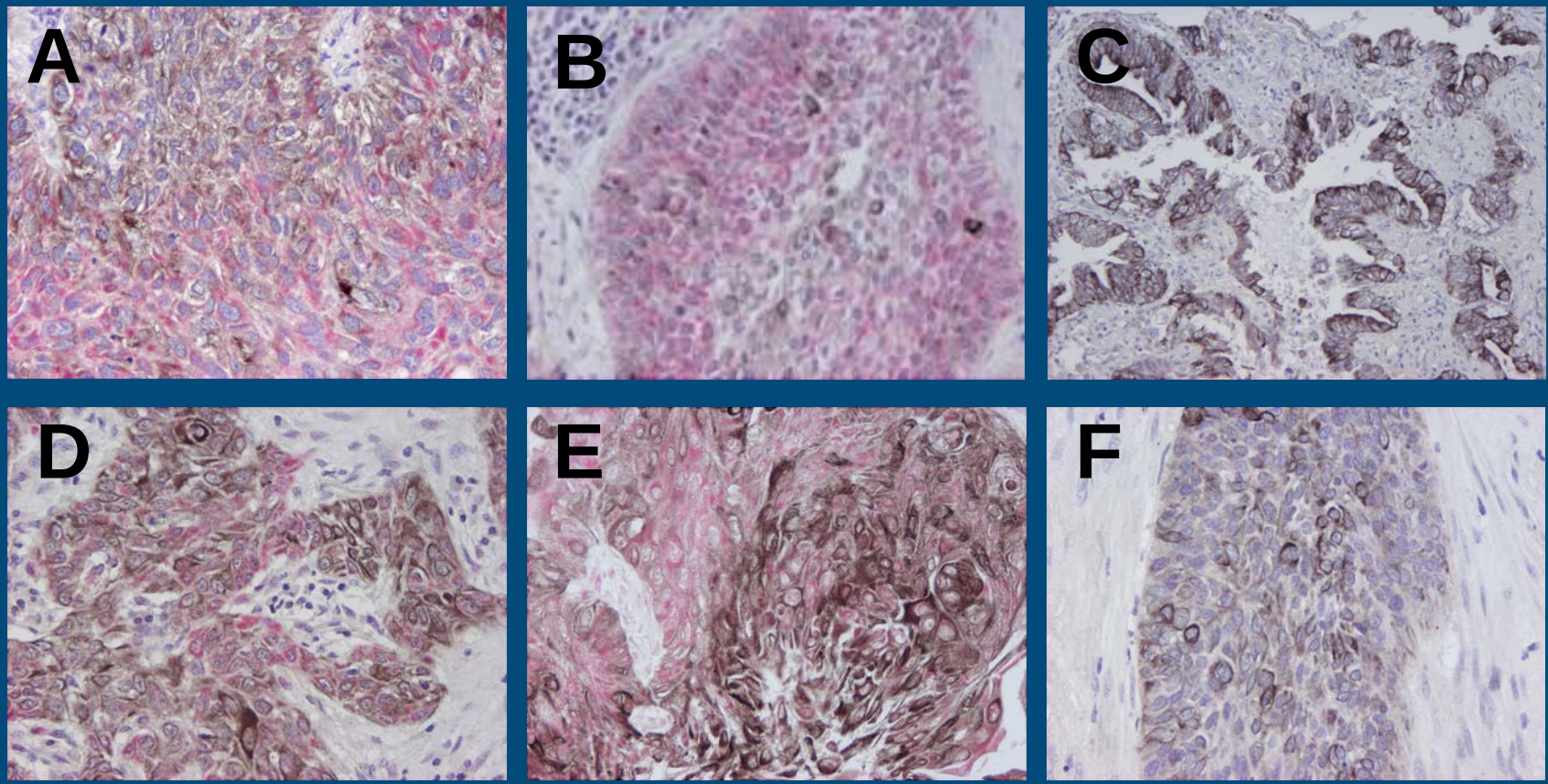
- A member of Inhibitor of Apoptosis Proteins (IAP) family
- Binds caspases
- Frequently over-expressed in human malignancies
- Over-expression is associated with poor prognosis in NSCLC

COX-2-dependent survivin expression in COX-2 gene-modified NSCLC cells



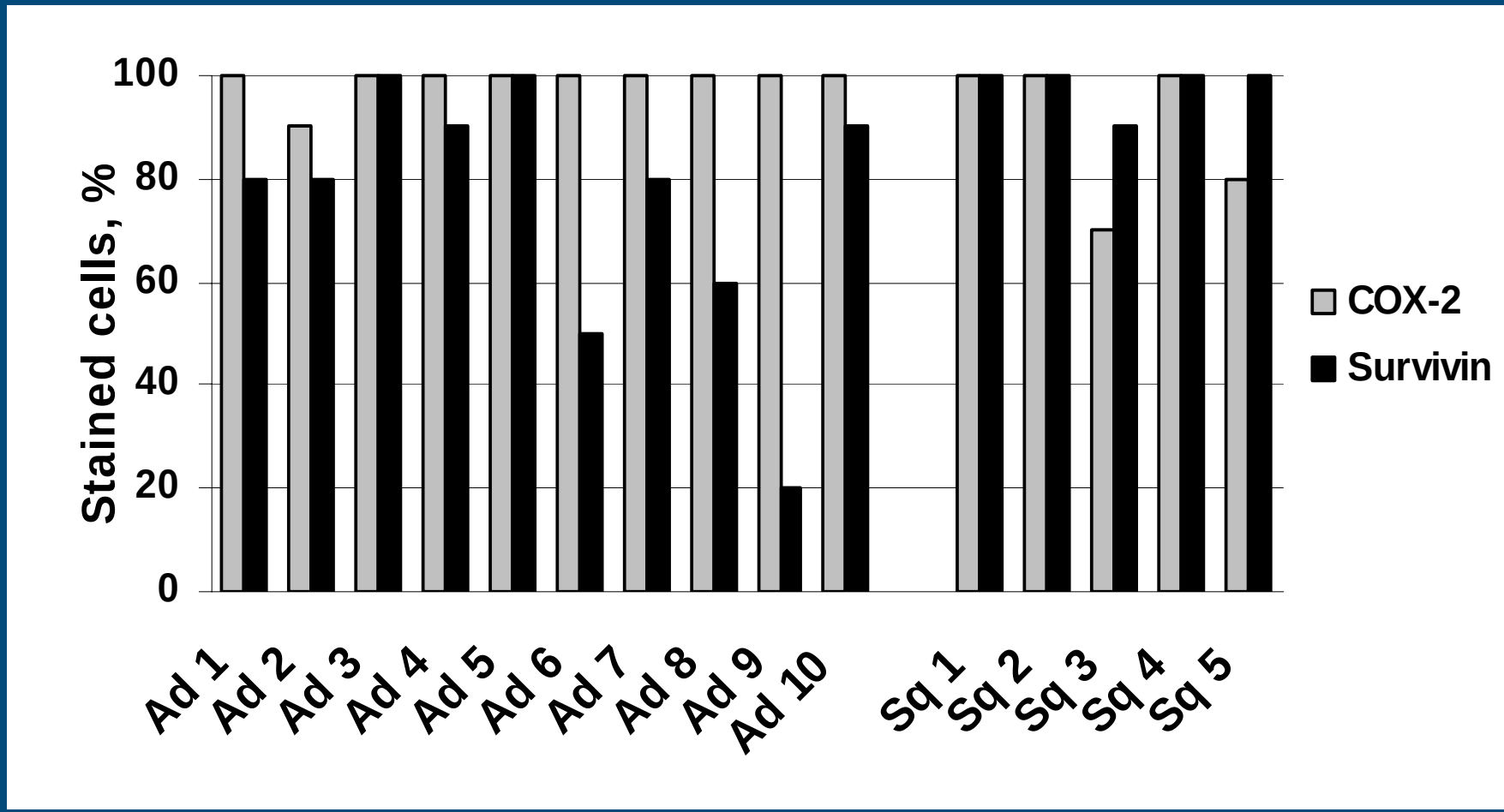
Krysan et al FASEB Journal Jan 2004

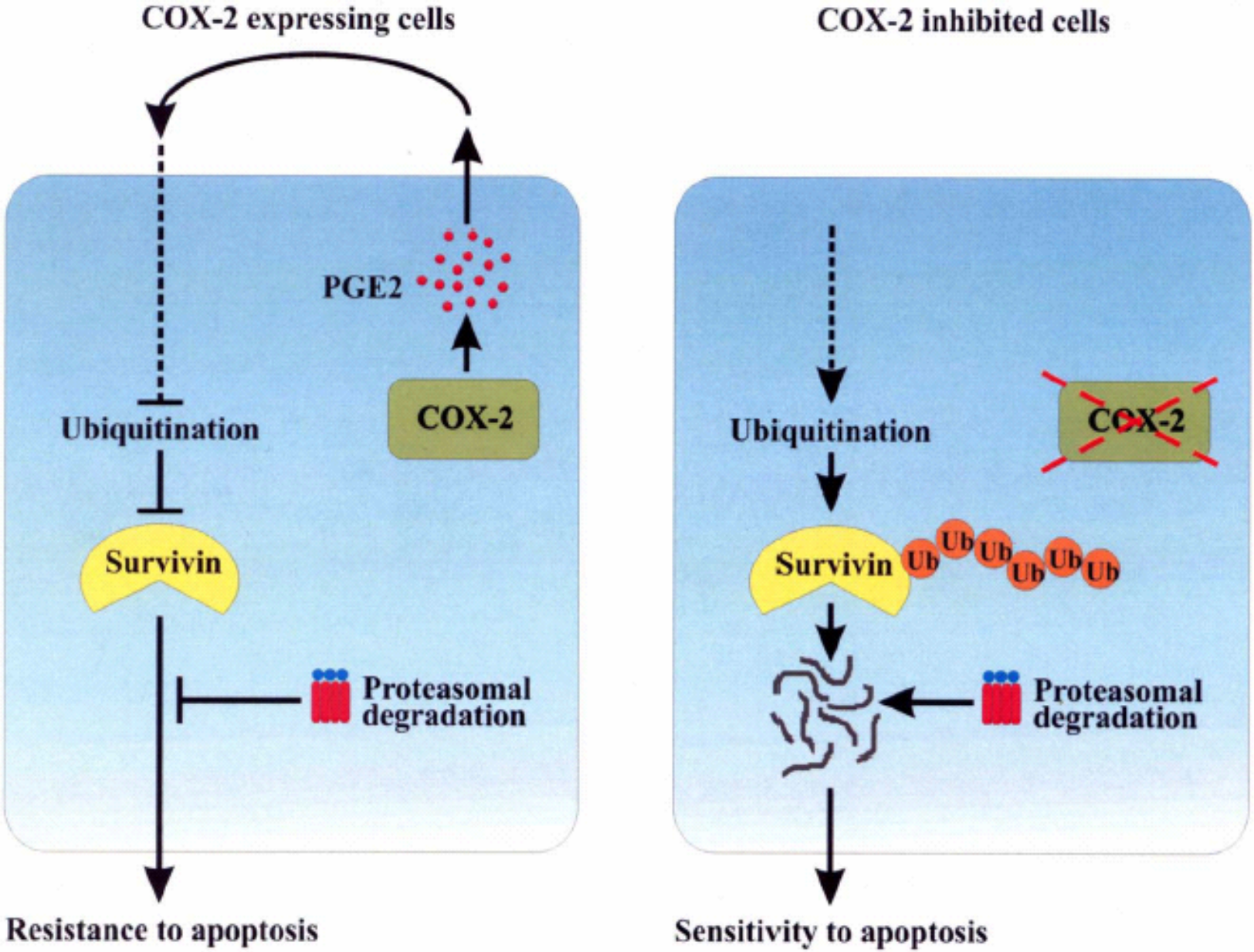
Co-expression of COX-2 and Survivin in Lung Adenocarcinoma (A, B, C) and Squamous Cell Carcinoma (D, E, F)



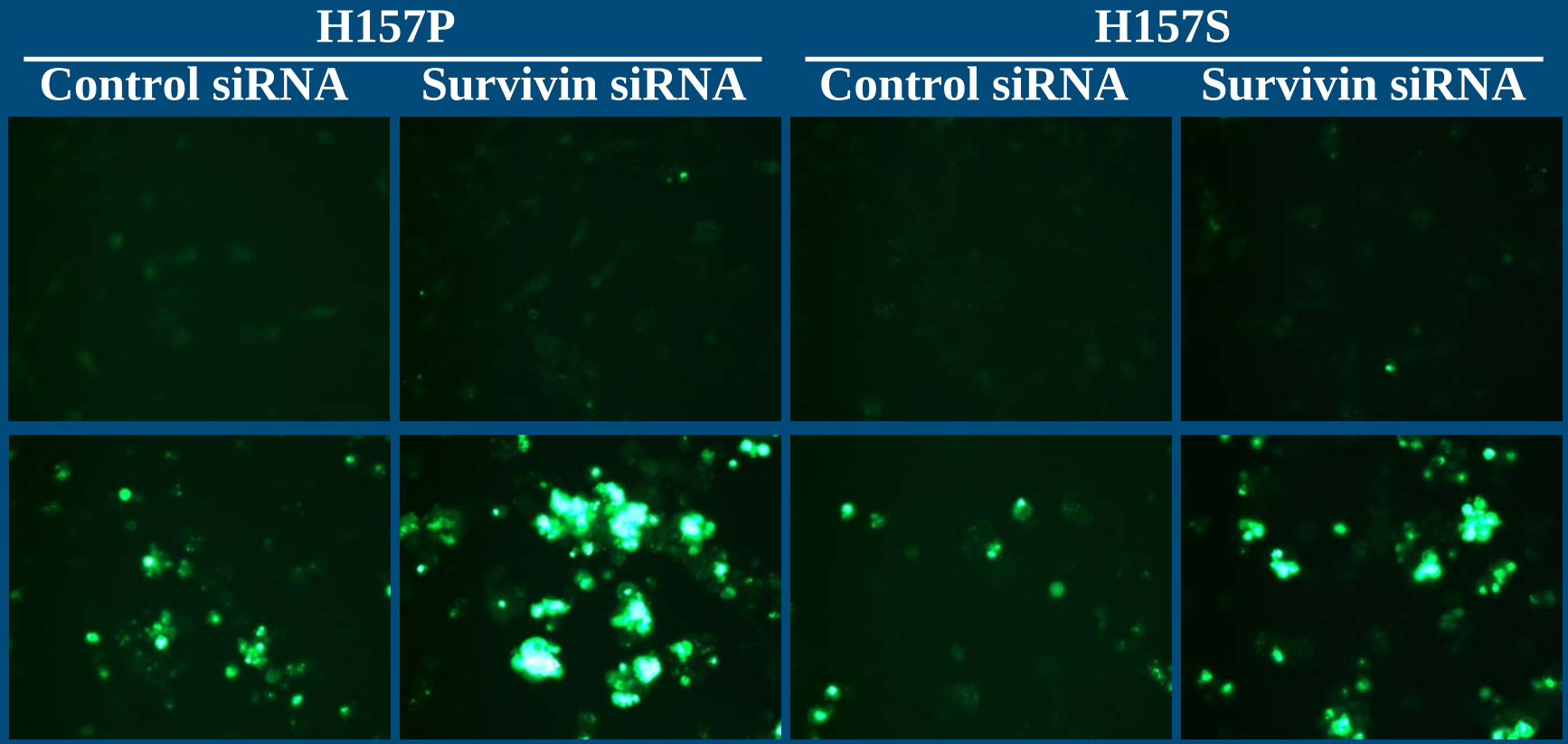
Krysan et al FASEB Journal Jan 2004

Co-expression of COX-2 and Survivin in Lung Adenocarcinoma and Squamous Cell Carcinoma




COX-2 expressing cells
PGE2
COX-2
Ubiquitination
Survivin
Proteasomal degradation
Resistance to apoptosis
COX-2 inhibited cells
Ubiquitination
COX-2
Survivin
Ub
Ub
Ub
Ub
Ub
Ub
Proteasomal degradation
Sensitivity to apoptosis

Inhibition of survivin expression with siRNA significantly reduces apoptosis resistance of H157S NSCLC cells



Upper sections — no apoptosis induction

Lower sections — 6 μg/ml Camptothecin~18 hours

Survivin Conclusions

1. Overexpression of COX-2 as well as treatment with PGE2 significantly increases the apoptosis resistance of NSCLC cells.

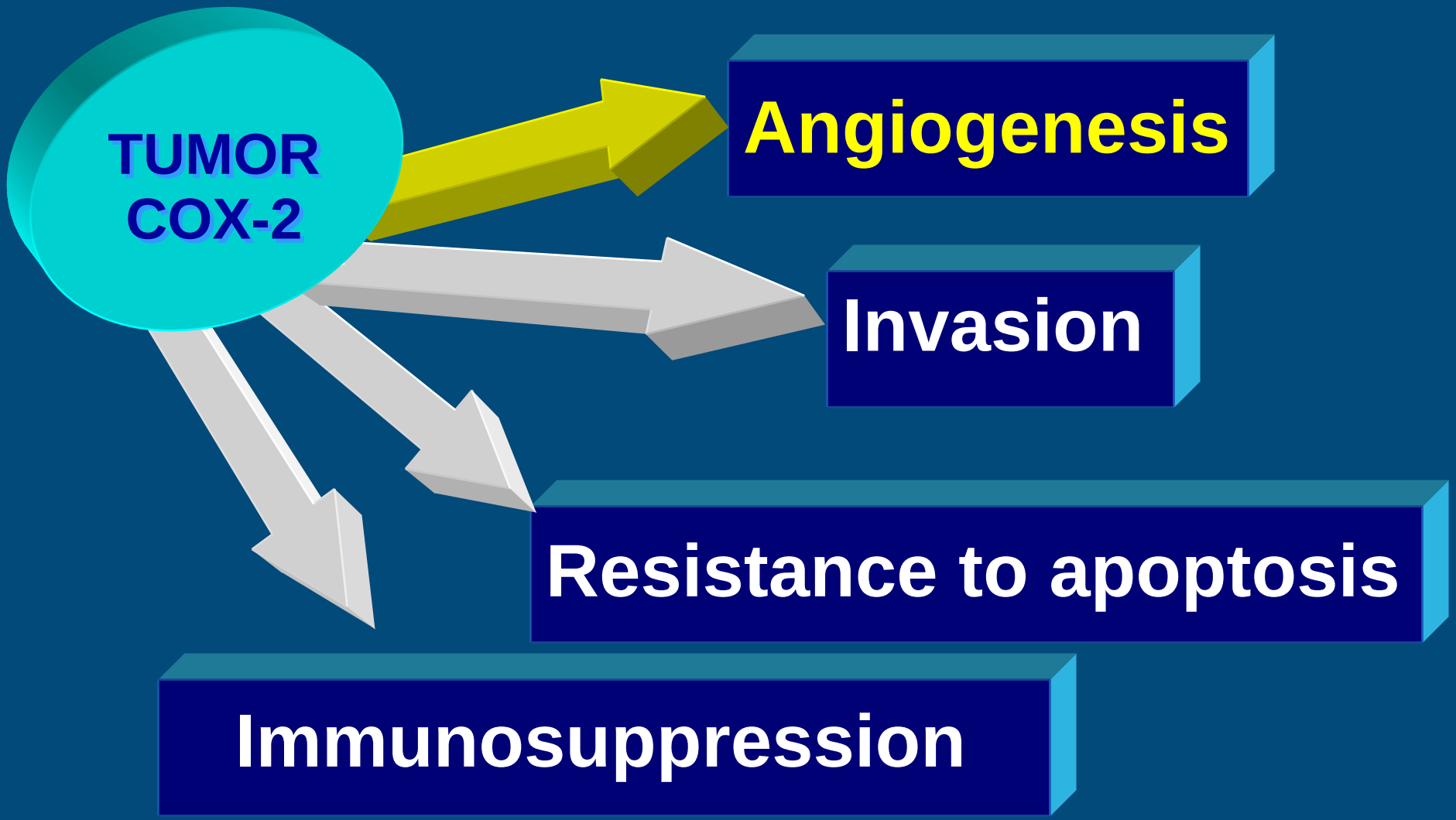
2. Survivin expression is COX-2-dependent in NSCLC cells and its level is lowered by COX-2 specific inhibitors.

3. Ubiquitination of survivin is blocked by high levels of COX-2 and PGE2.

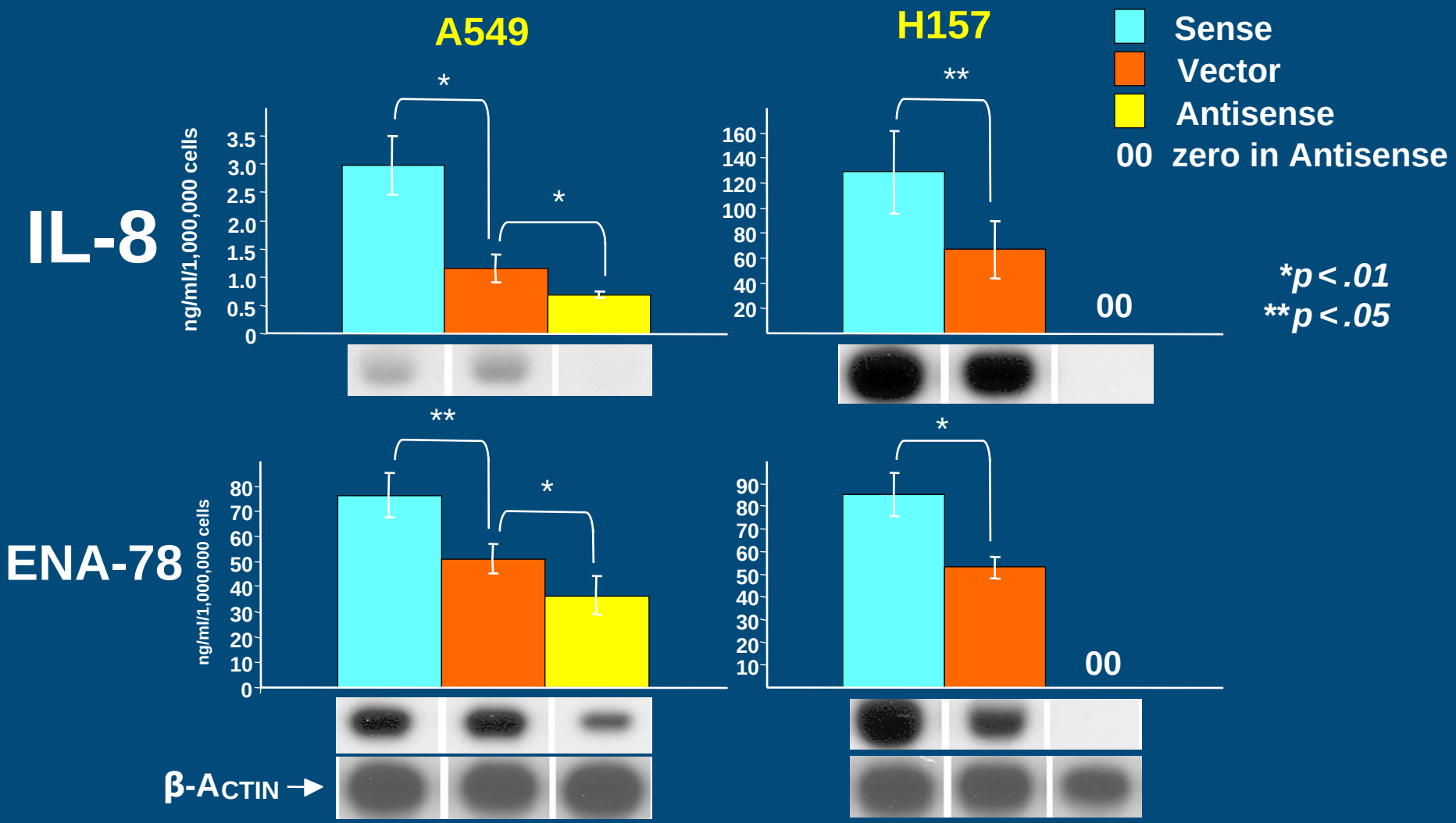
4. Survivin and COX-2 are frequently co-expressed in human NSCLC.

Krysan et al FASEB Journal Jan 2004

NSCLC COX-2-dependent Modulation of the Malignant Phenotype

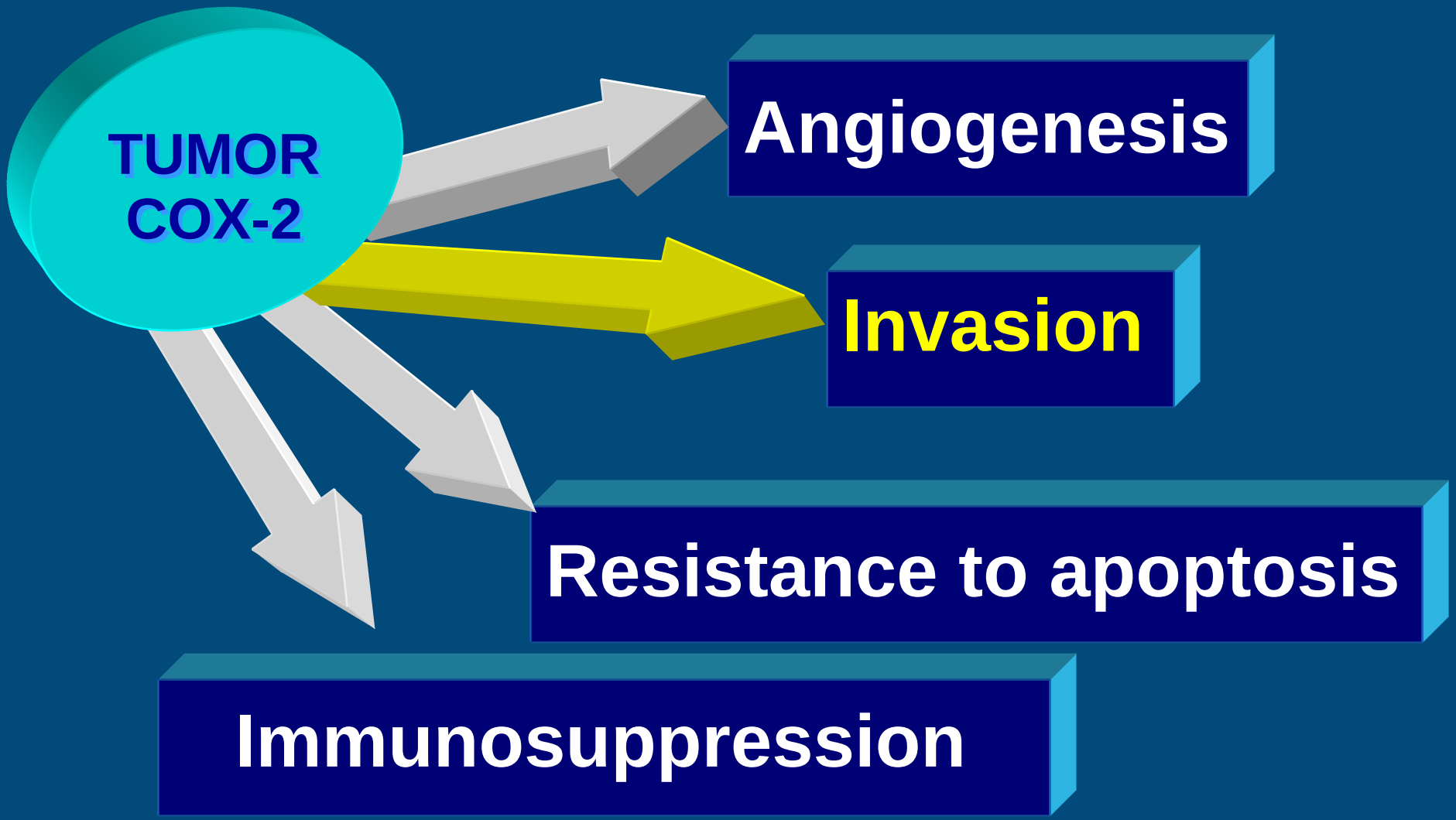


COX-2 Regulates Pro-angiogenic Chemokine Expression in NSCLC

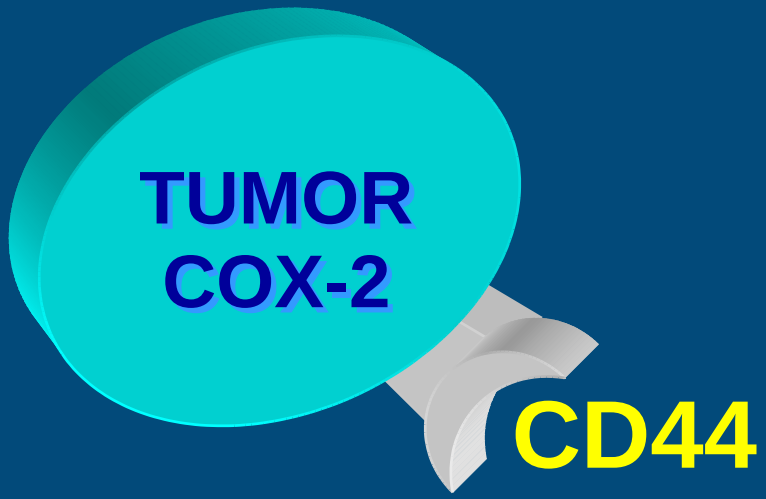


Pold, M., *et al.* (2004) *Cancer Res*. 64(5):1853-60

NSCLC COX-2-dependent Modulation of the Malignant Phenotype



COX-2-mediated Invasion by CD44

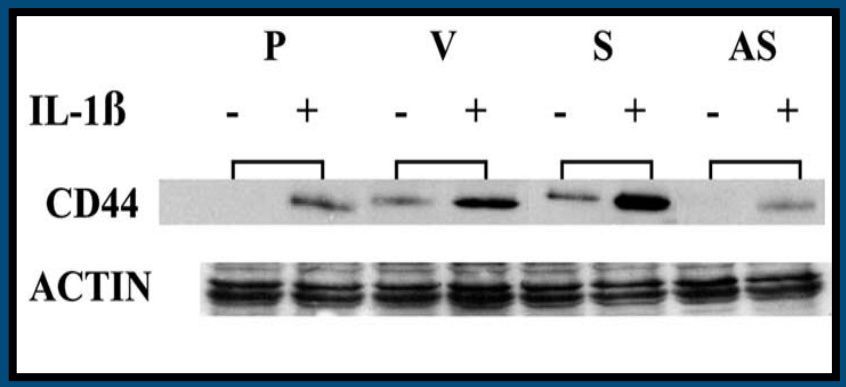


The receptor for hyaluronate, plays an important role in regulating tumor growth and metastases because it mediates adhesion to ECM

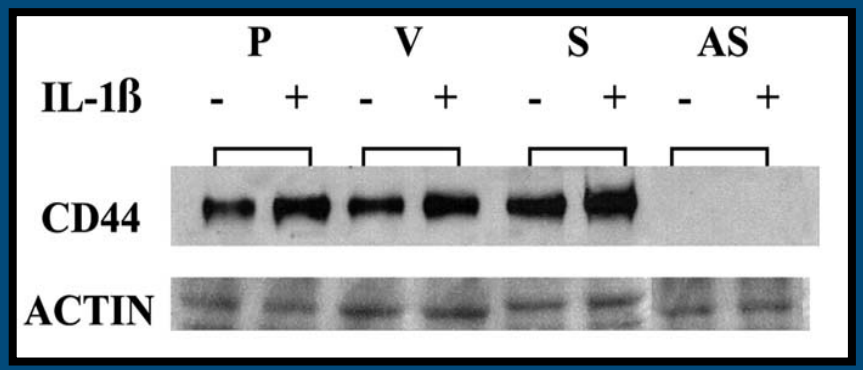
Dohadwala et al, J. Biol Chem. 2001, 276: 20809
J. Biol Chem. 2002, 277: 50828

COX-2-dependent Invasion of NSCLC is Mediated by CD44 Expression

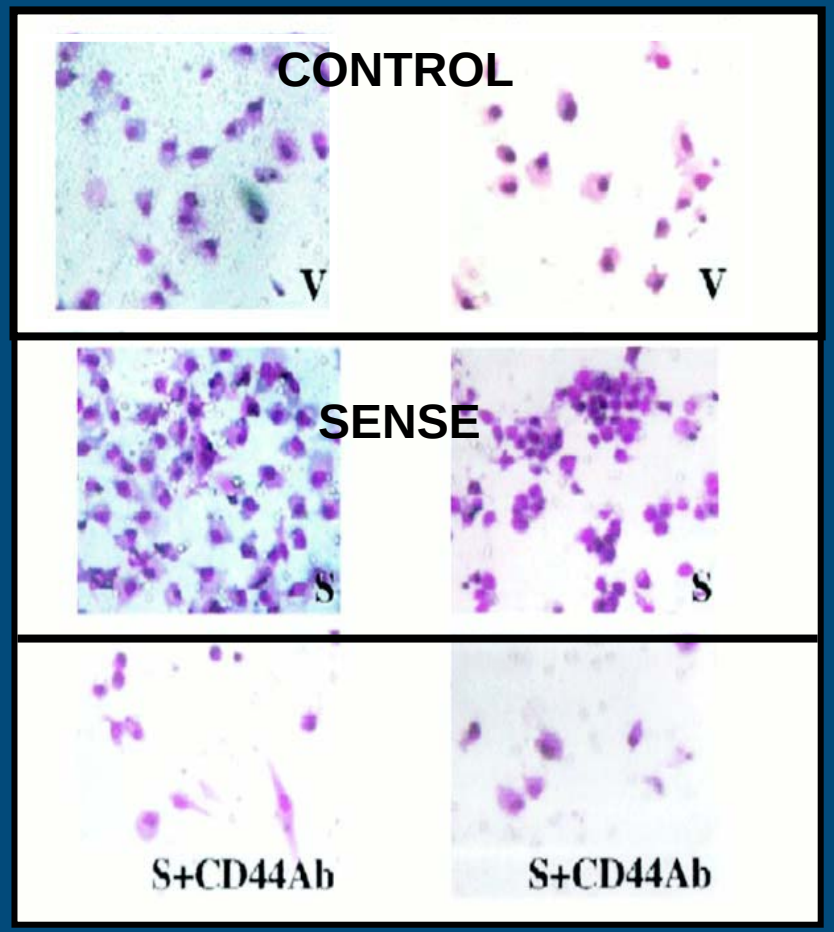
A549



H157

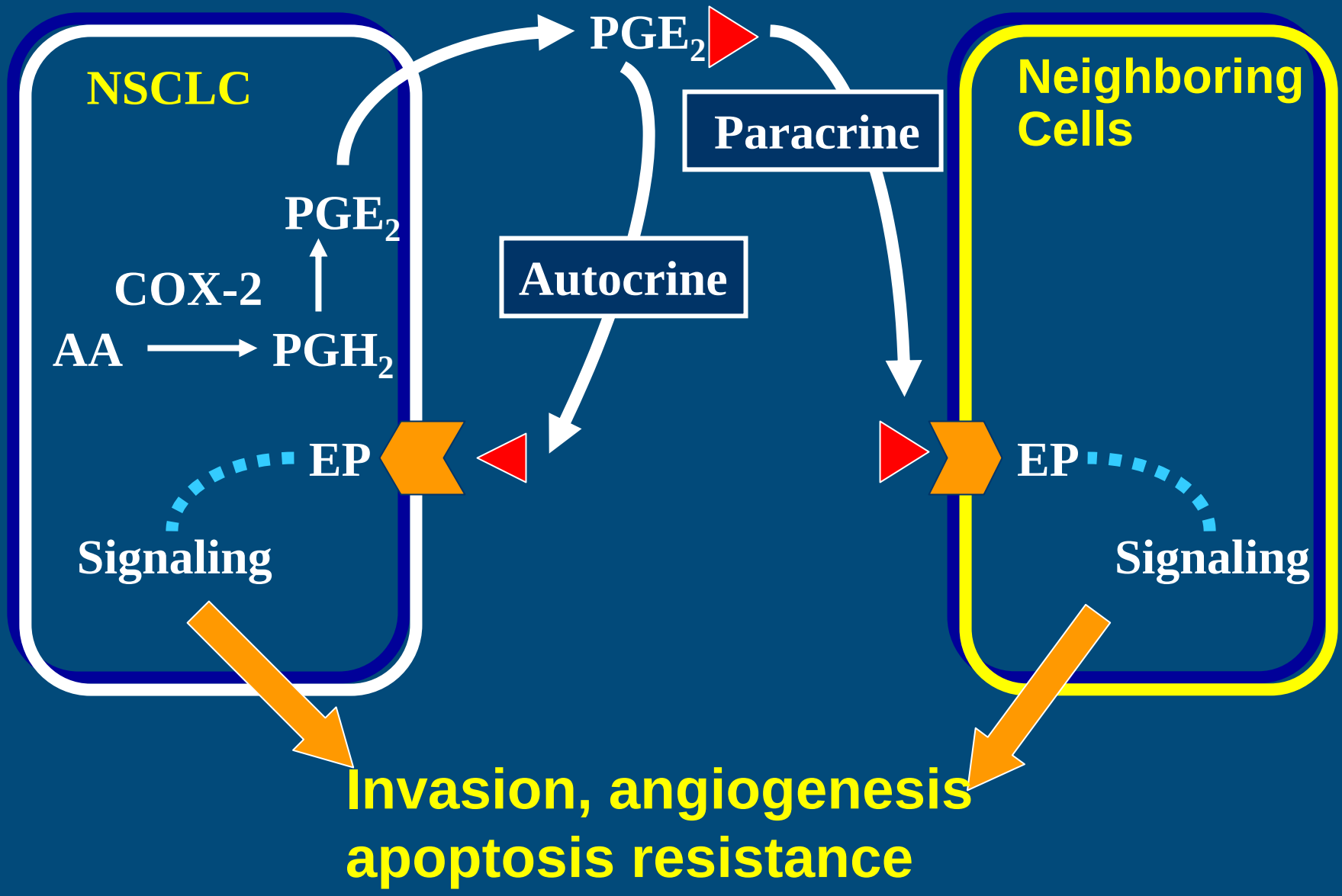


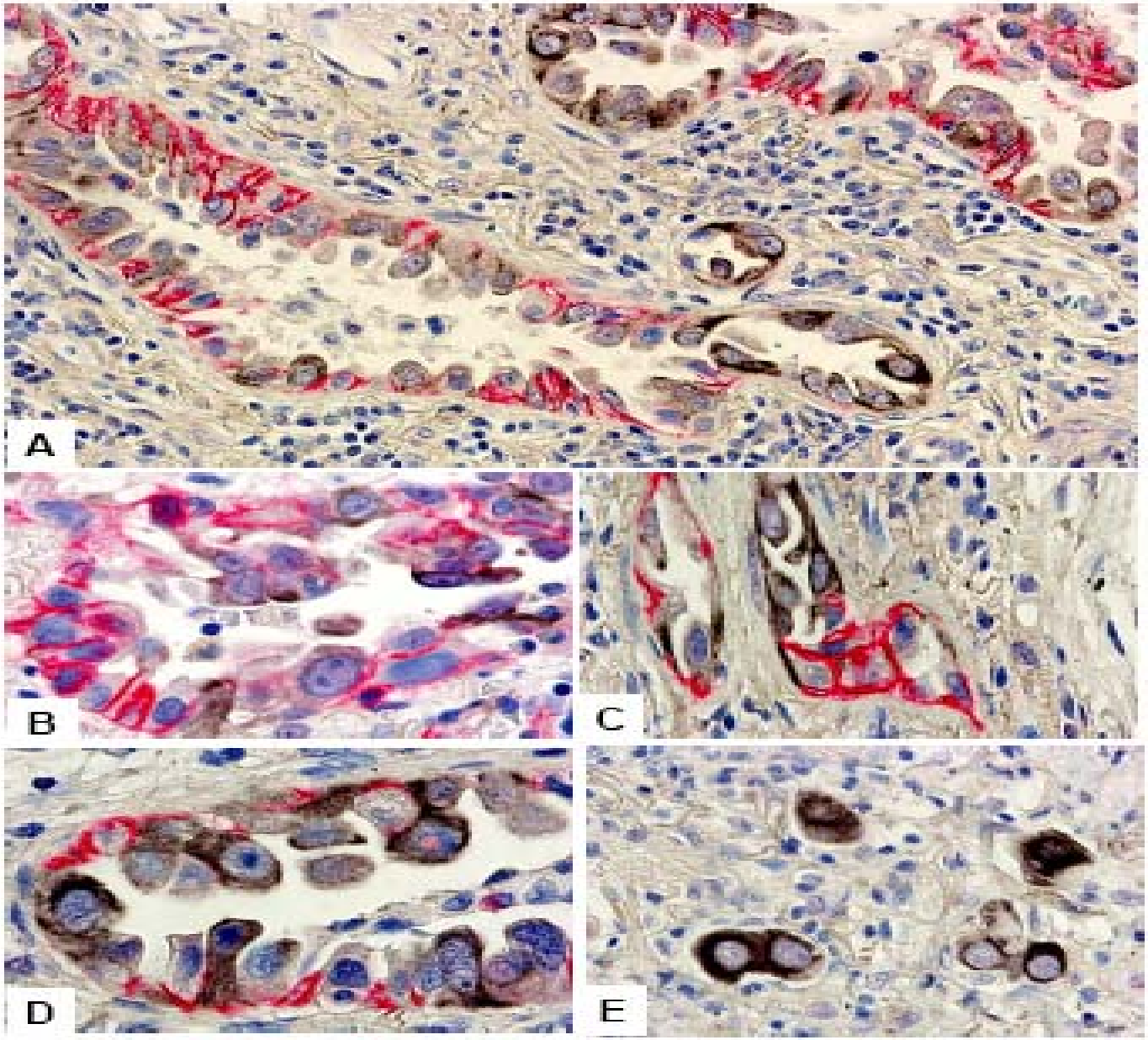
A549 H157



Dohadwala *et al.* JBC (2001) 276:20809 and JBC (2002) 277:50828

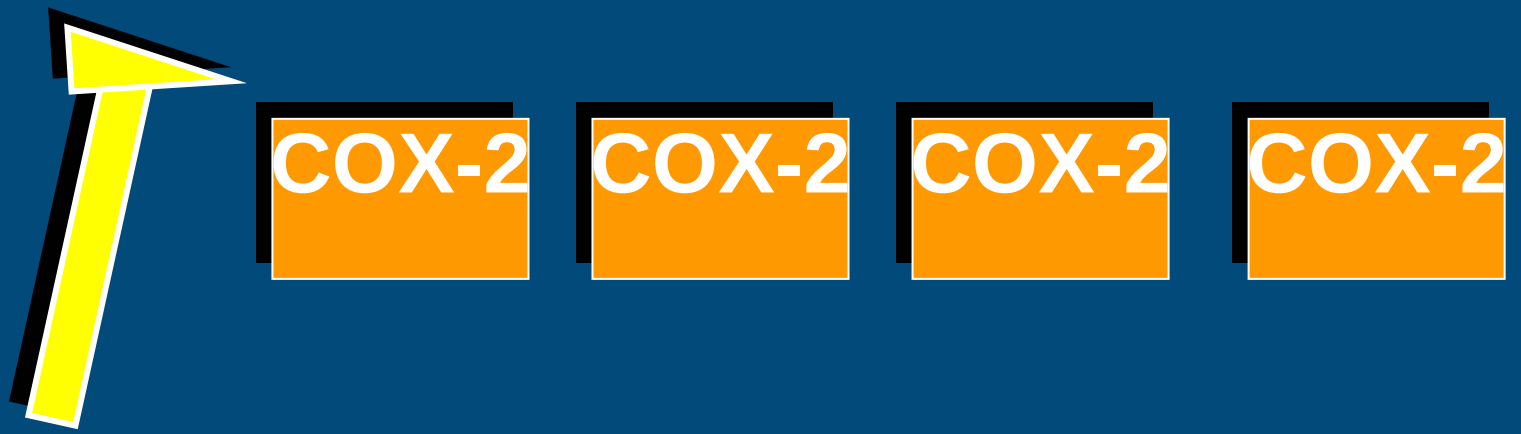
PGE2 mediated autocrine/paracrine signaling





E-cadherin stains red; COX-2 brown

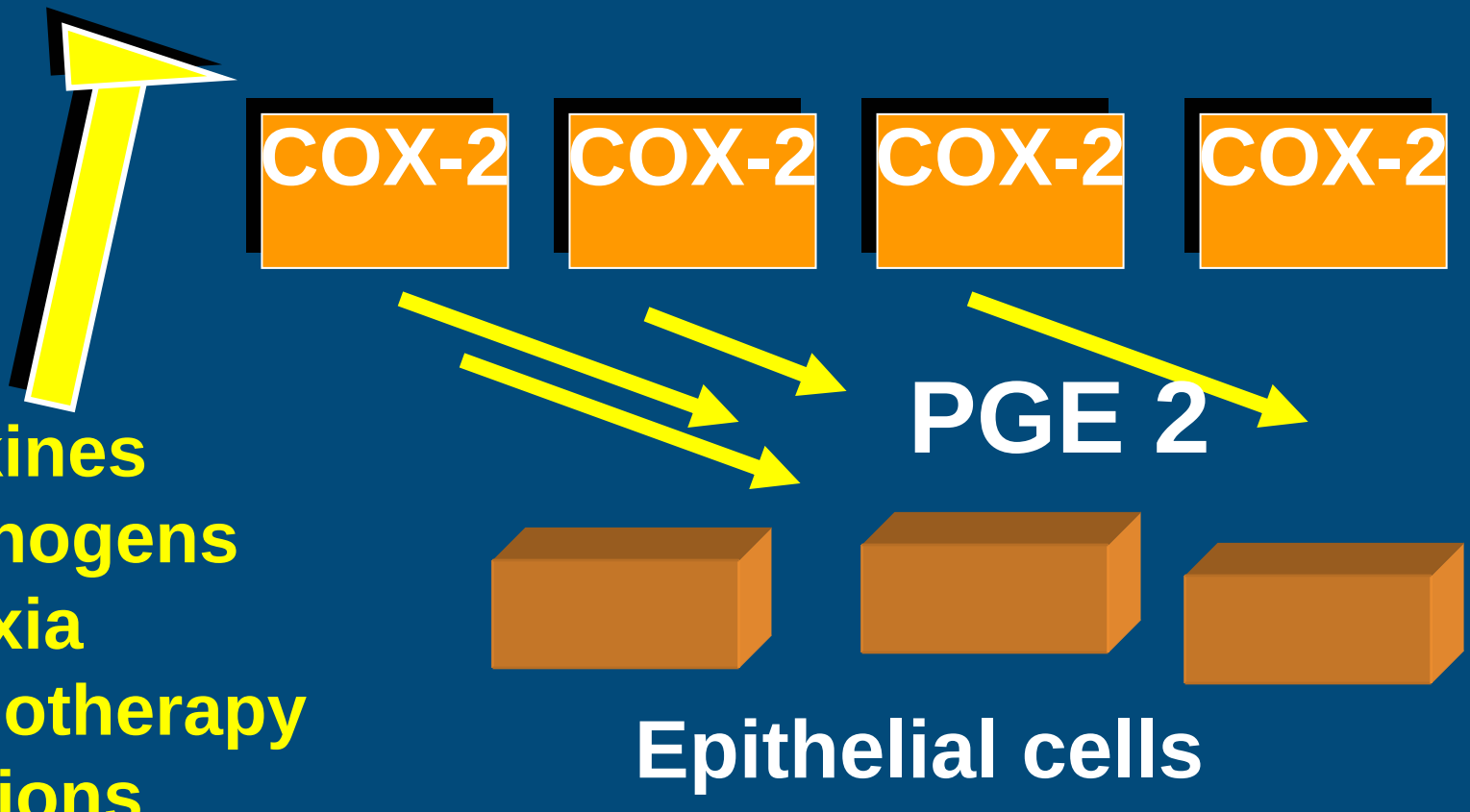
COX-2
An Injury Response in Lung Epithelium



Cytokines
Carcinogens
Hypoxia
Chemotherapy
Mutations (K-ras)

Epithelial cells

COX-2
An Injury Response in Lung Epithelium



Cytokines
Carcinogens
Hypoxia
Chemotherapy
Mutations

UCLA Lung Cancer Research Laboratory

Faculty

Sherven Sharma
Raj Batra
Mehis Pold
Min Huang
Jenny Mao
Karen Riedl

Trainees and Staff

Mariam Dohadwala
Felicita Baratelli
Ming Liu
Kostyantyn Krysan
Nathalie Heuze-Vrouch
Li Zhu
Brian Gardner
Jie Luo
Ying Lin
Ling Zhang
Seok-Chul Yang
Lawrence Hsu
Xiaoyan Cui
Wen Mao
Marina Stolina
Harnisha Dalwadi

Collaborators

Robert Strieter
Mitchell Kronenberg
Robert Figlin
Lee Goodglick
Michael Fishbein
Brian Escuardro
Jeff Lin
Min Qin
Saswati Hazra

Lung Cancer SPORE
NIH P50 CA90388