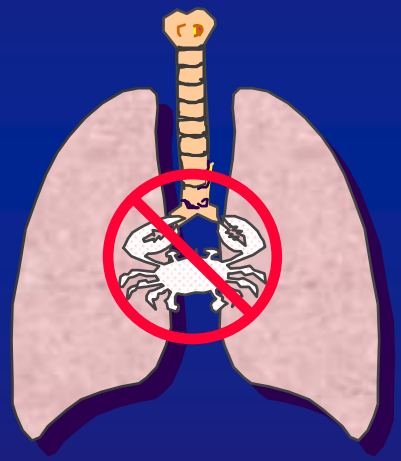
Lung Cancer Chemoprevention



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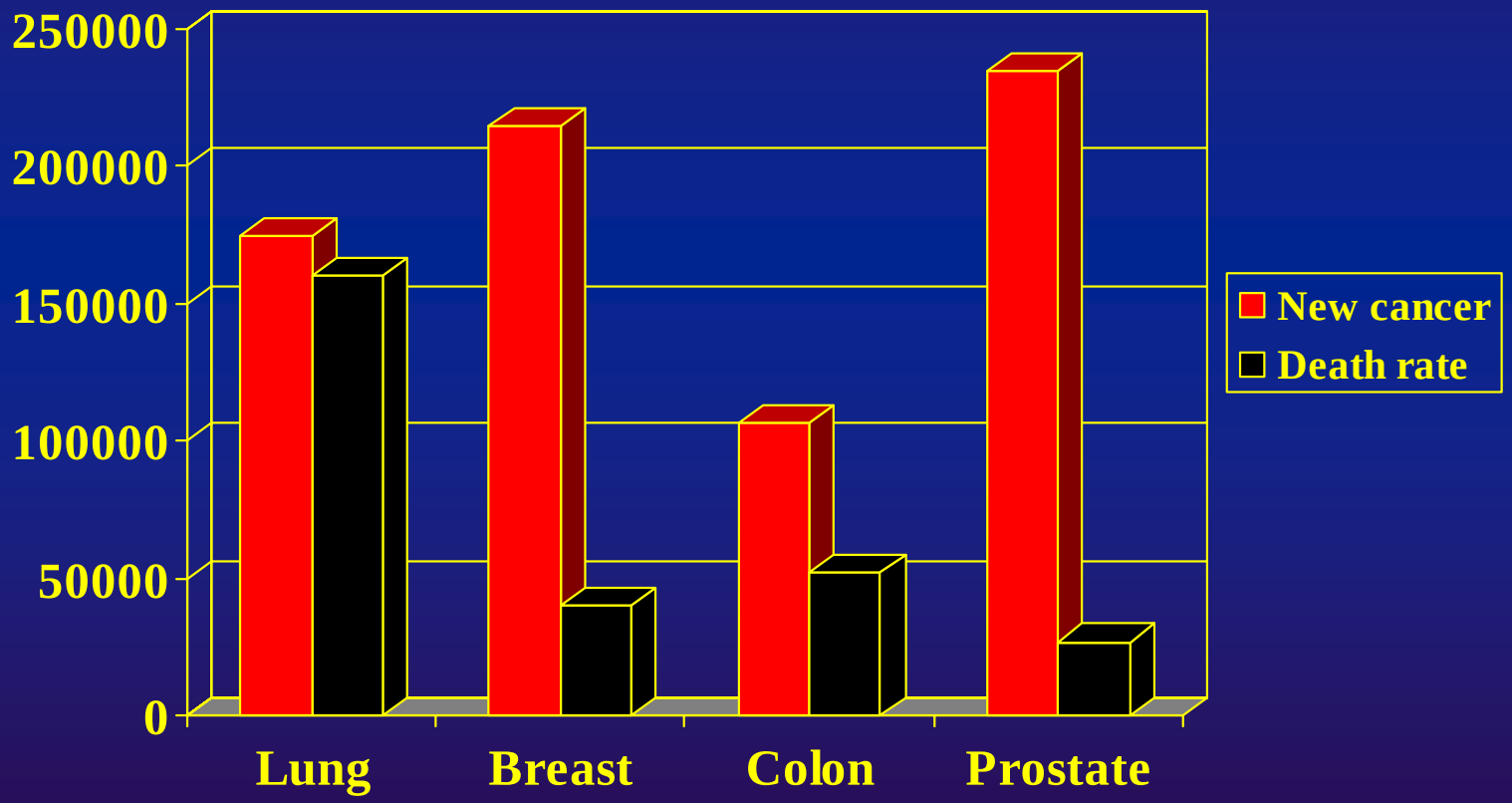
Lung Cancer Statistics

- **Leading cause of cancer death in the world.**

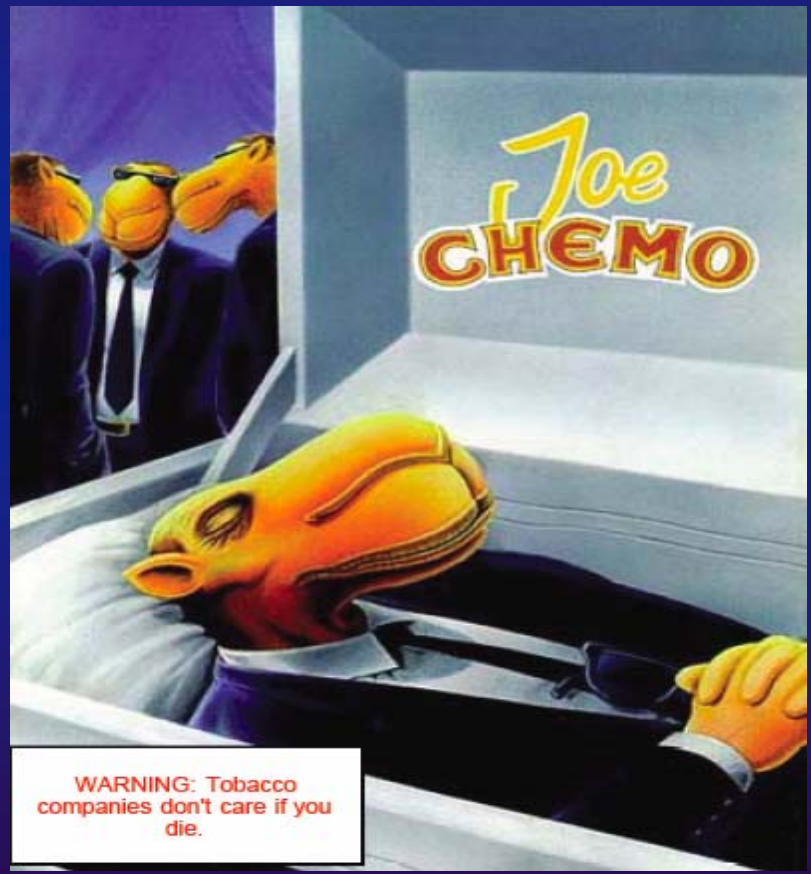


American Cancer Society Estimates, 2007

Lung Cancer Epidemiology

- Over 90% caused by smoking.
- Today, more cancer diagnosed in former smoker than in current smoker.
- 5 year survival rate: ~15% because most cases are diagnosed at a late invasive stage.

- **The lack of effective therapy underscores the imminent need to explore new frontiers of management.**



JoeChemo.org and Adbusters magazine

Chemoprevention

Definition

- **Chemoprevention is the use of natural or synthetic agents to reverse, suppress, or prevent the carcinogenic process to invasive cancer.**

--Michael Sporn, M.D., 1976.

- **The concept is similar to the use of antihypertensive and lipid lowering medications to prevent heart disease or stroke.**

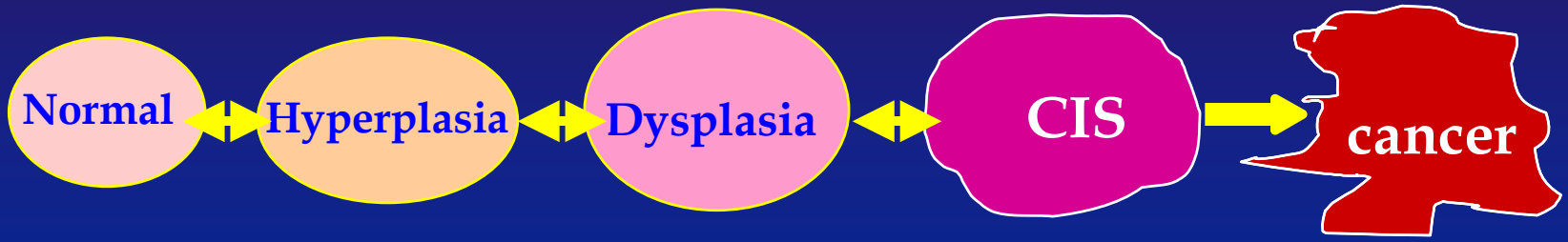
Lung Carcinogenesis

- Lung cancer results from a lengthy, complex interaction between
  - genetic predisposition
  - environmental influences.
- Initiation - genetic instability
- Promotion

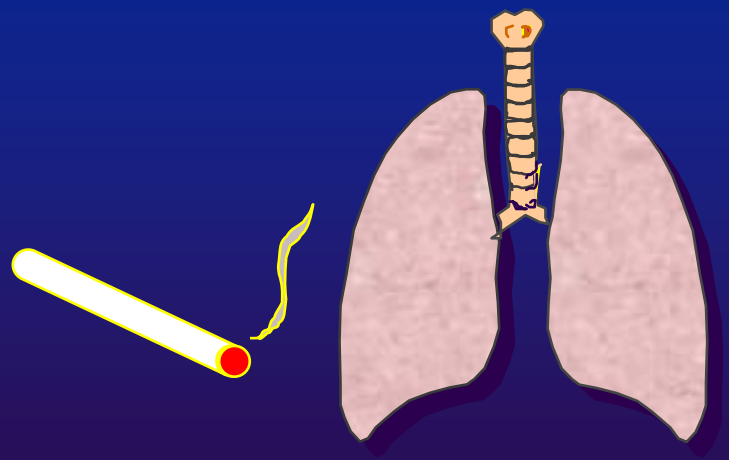
Major Molecular Abnormalities in the Pathogenesis of Lung Cancer

- **Growth factors, receptors, and activation of oncogenes**
  - GRP/BN and their receptors, IGF, HGF and its receptor MET, EGFR
  - Her2/neu
  - RAS mutations
  - MYC amplification and deregulated expression
  - Aberrant BCL-2 expression
  - Cyclin D1 expression
- **Loss of function of tumor suppressor genes**
  - p53, RB, p16, FHIT, RASSF1A, APC
- **Loss of DNA repair mechanisms**
- **Aberrant methylation resulting in loss of gene expression**
  - APC, CDH13, RARb, FHIT, RASSF1A, TIMP-3, P16, MGMT, DAPK
- **Expression of telomerase activity and cellular immortality**
- **Resistance to apoptosis**
- **Activation of tumor-stimulated angiogenesis**
- **Suppression of anti-tumor immunity**

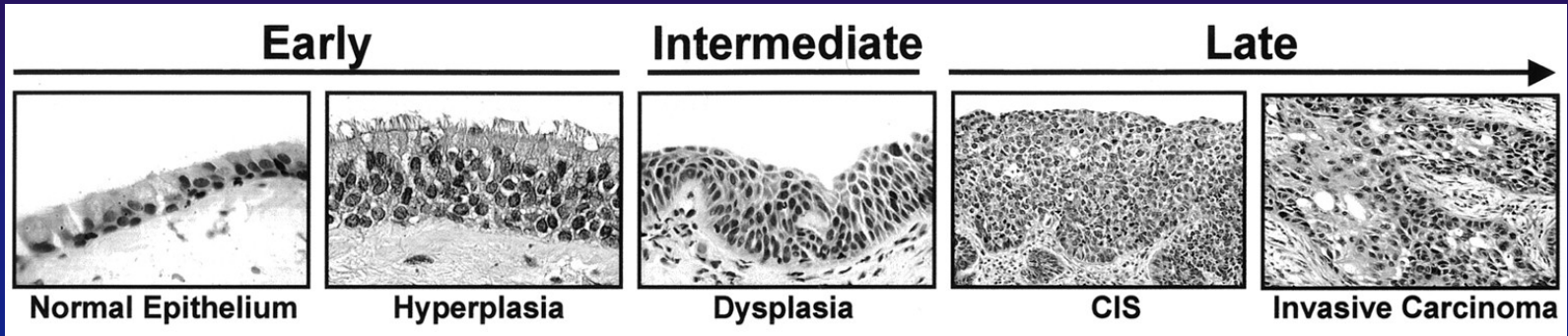
I. Multi-step carcinogenesis



II. Field Carcinogenesis



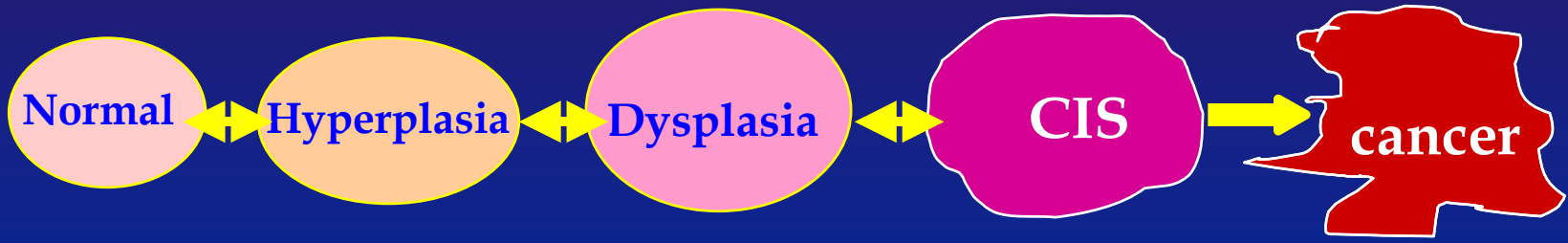
Sequential Changes During Lung Carcinogenesis



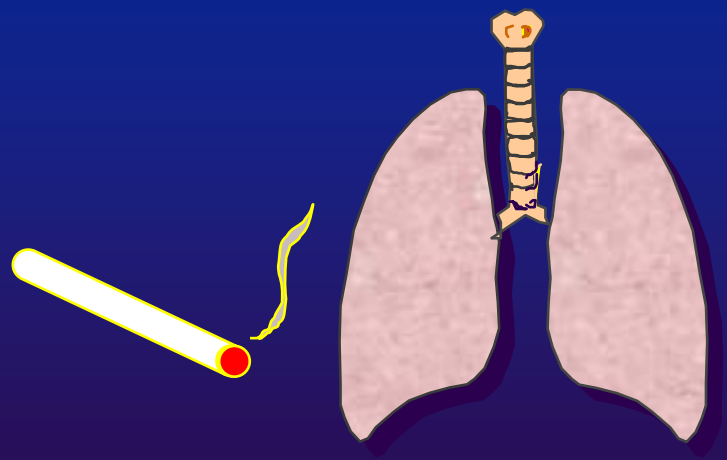
3p LOH/small Telomeric deletion Deletions	3p LOH/Contiguous	~80%
Microsatellite Alterations		~ 50%
9p21 LOH		~ 70%
Telomerase Dysregulation Upregulation	Telomerase	~ 80%
MYC Over-expression		~ 60%
8p21-23 LOH		~ 80%
Neoangiogenesis		~ 40%
Loss of Fhit Immunostaining		~ 40%
P53 LOH	*TP53* Mutations	~ 70%
Aneuploidy		~ 80%
Methylation		~ 100%
5q21 APC-MCC LOH		~ 30%
K-ras Mutation		~ 20%

Hirsch, F et al, 2001, Clin Cancer Res.

I. Multi-step carcinogenesis



II. Field Carcinogenesis



Goals of Chemoprevention

- At the cellular level, inhibit the mechanisms that may lead to or facilitate malignant transformation.
- At the tissue level, prevent and/or reverse the development or progression of preneoplasia.
- At the clinical level, reduce the incidence of cancer.

Key components to the design of Lung cancer chemoprevention trials

- **Identification of efficacious agent used at pharmacologically appropriate doses.**
- **The safety profile of the chemopreventive agent.**
- **Selection of appropriate high risk cohorts**
  - **Primary:healthy high-risk (smokers)**
  - **Secondary: premalignancy**
  - **Tertiary: prevention of second primary**
- **Defining primary study endpoints that are predictive of reduced cancer incidence.**

Chemoprevention of Epithelial Cancer

Investigators	Agents	Cancer Type	Outcome
Hong 1990	13-*cis* RA	Nead & Neck	(+)
Fisher 1998	Tamoxifen	Breast	(+)
Steinbach	Celecoxib	Colorectal in FAP	(+)
Arber 2006	Celecoxib	Colon	(+)
Bertagnolli 2006	Celecoxib	Colon	(+)

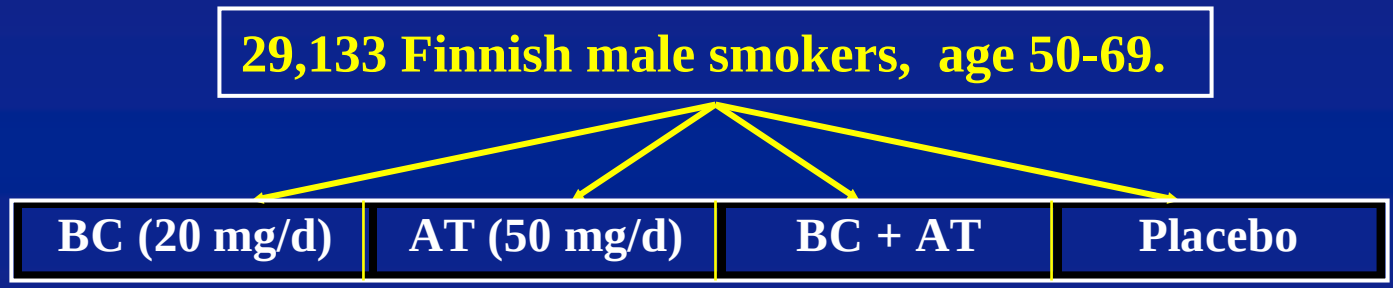
RANDOMIZED LUNG CANCER CHEMOPREVENTION TRIALS

Investigator	Agents	Endpoint	Outcome
Heimburger 1988	Folate and Vit B12	Sq. Metaplasia	(-)
Arnold 1992	Etretinate	Sputum atypia	(-)
Pastorino 1993	Retinyl palmitate	Second primaries (not on 5 year survival)	(+)
Lee 1994	13cRA	Metaplasia	(-)
McLarty 1995	β-carotene/retinol	Sputum atypia	(-)
Kurie 2000	4-HPR	Sq. Metaplasia	(-)
Lam 2002	ADT	Dysplasia	(+)
Lam 2004	Budesinide	Dysplasia ↓ P53 & BCL2	(-)
Van den Berg 2007	Fluticasone	Metaplasia, Ki 67, telomerase expression	(-)
Finnish 1994	β-carotene/vitamin E	Lung cancer	(-)
Omenn 1996	β-carotene/retinal	Lung cancer	(-)
Physician's Health 1996	β-carotene	Lung Cancer	(-)
Intergroup 2000	13cRA	Second primaries	(-)
EUROSCAN 2000	Retinol/NAC	Second primaries	(-)
Linxian 2006	4 diff combinations of Vitamins/minerals	Lung cancer mortality	(-)

Phase III Lung Cancer Prevention Trials

Primary

- The Alpha-Tocopherol, Beta-Carotene (ATBC) Study



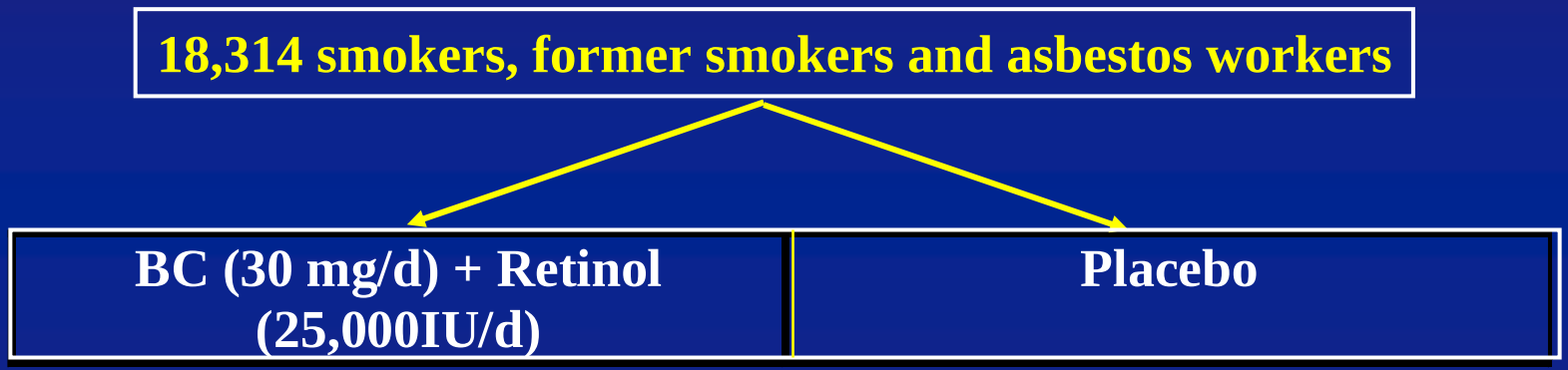
- Treatment duration: 5-8 yrs.
- Lung cancer incidence: 876 new cases.
- BC group: 18% increase in lung cancer incidence and 8% in total mortality.

NEJM 1994. 330(15):1029-35

Phase III Lung Cancer Prevention Trials

Primary

- **The Beta-Carotene and Retinol Efficacy Trial (CARET)**



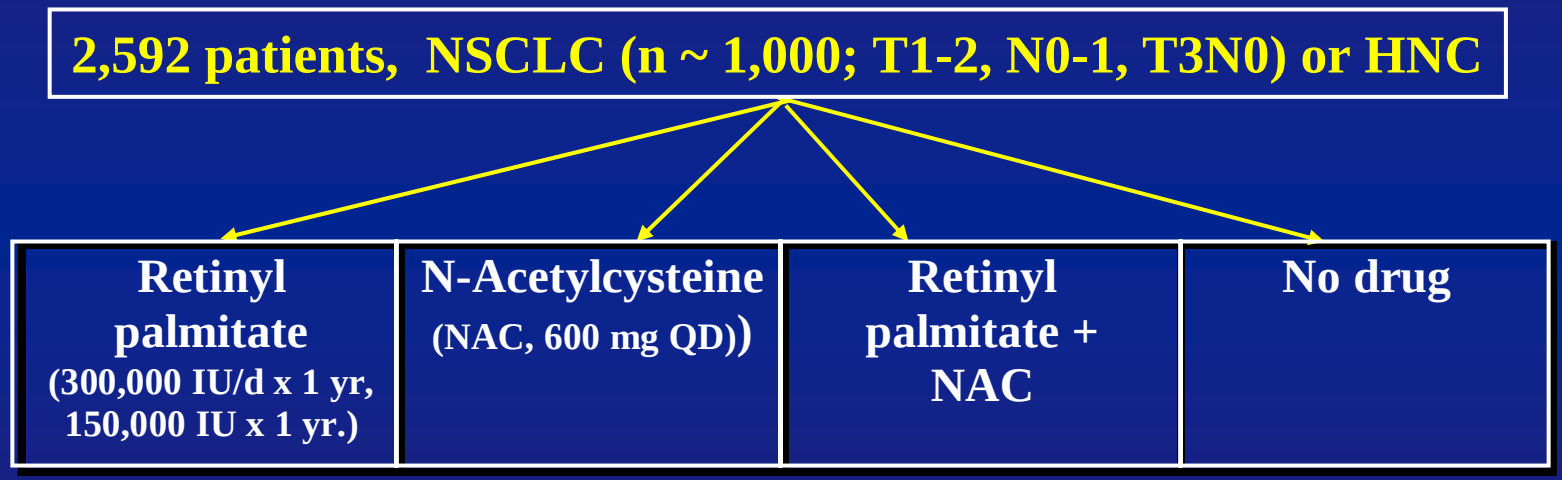
- Mean treatment duration: 4 years.
- Interim analysis: active treatment group had 28 % increase in lung cancer incidence, 17% increase in total mortality (46% increase of death from lung cancer and 26% from cardiovascular disease).

Omenn GS, et al. NEJM 1996. 334(18):1150

Phase III Lung Cancer Prevention Trials

Tertiary

- Euroscan



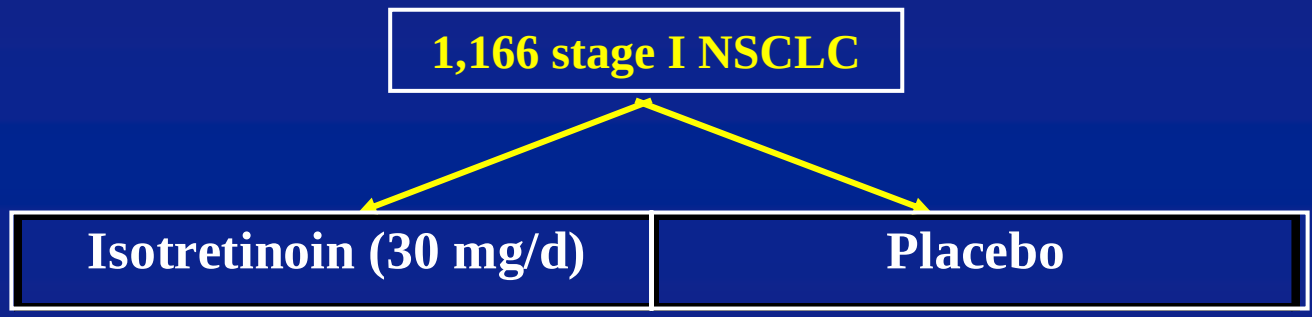
- Treatment duration 2 years
- Median follow up 49 months.
- 916 reported event (recurrence, SPT, or death)
  no difference in overall and/or event free survival.

Van Zandwijk N, JNCI, 92(12): 977-986

Phase III Lung Cancer Prevention Trials

Tertiary

- The phase III Lung Intergroup Trial



- Median follow up: 3.5 years.
- No difference in overall rates of SPT, recurrences or mortality.
- 2^0 multivariate analysis: harmful in current smokers and may be beneficial in never smokers. (HR:4.39)

Lippman SM, et al. JNCI. 2001. 93(8) 605-618

Phase III Lung Cancer Prevention Trials

Primary

- **Linxian Lung Cancer Chemoprevention Study**
  - 29,584 Healthy adults 40-69 years (Linxian, China)
  - Double-blind, a partial factorial design

    A: retinol and zinc
    B: riboflavin and niacin
    C: ascorbic acid and molybdenum
    D: β-carotene, α-tocopherol and selenium
  - Intervention from 1986-1991; Follow-up 1991-2001
  - No significant differences in Lung Cancer Death rates
  - No significant interactions for age, gender or smoking

Kamangar et al, Cancer Epidemiol Biomarkers Prev 2006

Phase III Lung Cancer Prevention Trials

- Lessons learned:

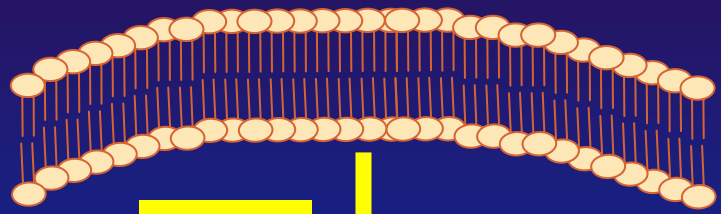
  Most of the phase III trials have been conducted based on epidemiologic or preclinical findings without systematic evaluations with phase IIa and b pilot studies.

  Phase II feasibility trials should be conducted prior to lauching into costly and time consuming phase III trials.

Candidate Lung Cancer Chemopreventive Agents

- **COX-2 inhibitors**
  (Phase II, UCLA; M.D. Anderson, primary)
- **Prostacyclin analogs**
  (Phase II, LCBCC, U. of Colorado, secondary)
- **Lipoxygenase inhibitors**
  (Phase II, Karmanos Cancer Institute, secondary)
- **Selenium**
  (Phase III, multicenter, tertiary)
- **ACAPHA**
  (Phase II, BCCA, secondary)
- **Green Tea extract**
  (Phase IIB, secondary)
- **Sulindac**
  (Multi-center)

Eicosanoid Signaling Pathway



PLA2

Lipoxygenases (LOX)

cytp450

Arachidonic Acid

Epoxyarachidonic Acids

Leukotrienes

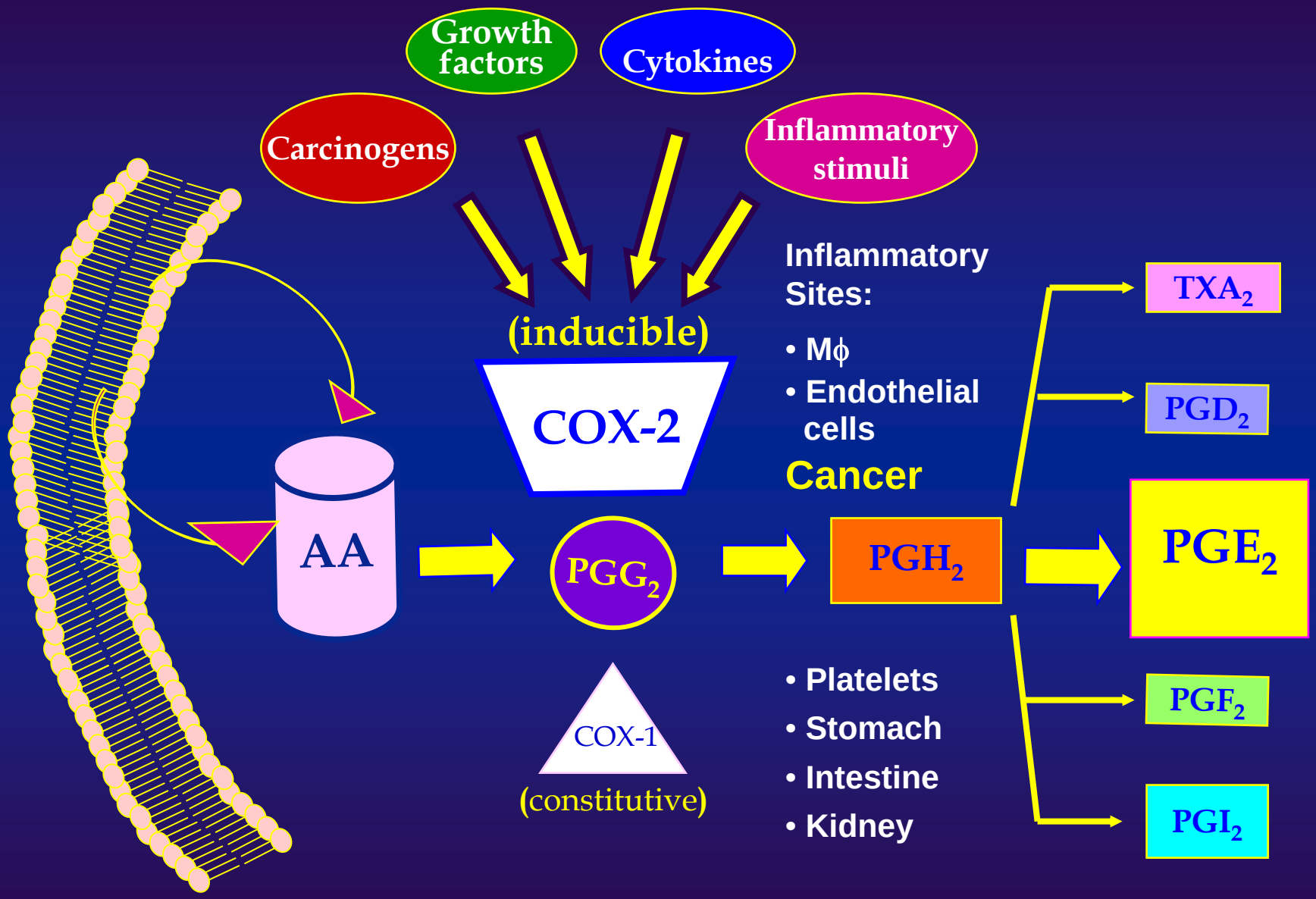
HETEs

HPETEs

Cyclooxygenases (COX)

Prostaglandins

Thromboxane A2


Growth factors
Cytokines
Carcinogens
Inflammatory stimuli
(inducible)
COX-2
Inflammatory Sites:
• Mϕ
• Endothelial cells
Cancer
AA
PGG2
PGH2
TXA2
PGD2
PGE2
PGF2
PGI2
COX-1
(constitutive)
• Platelets
• Stomach
• Intestine
• Kidney

RATIONALE FOR COX-2 INHIBITION

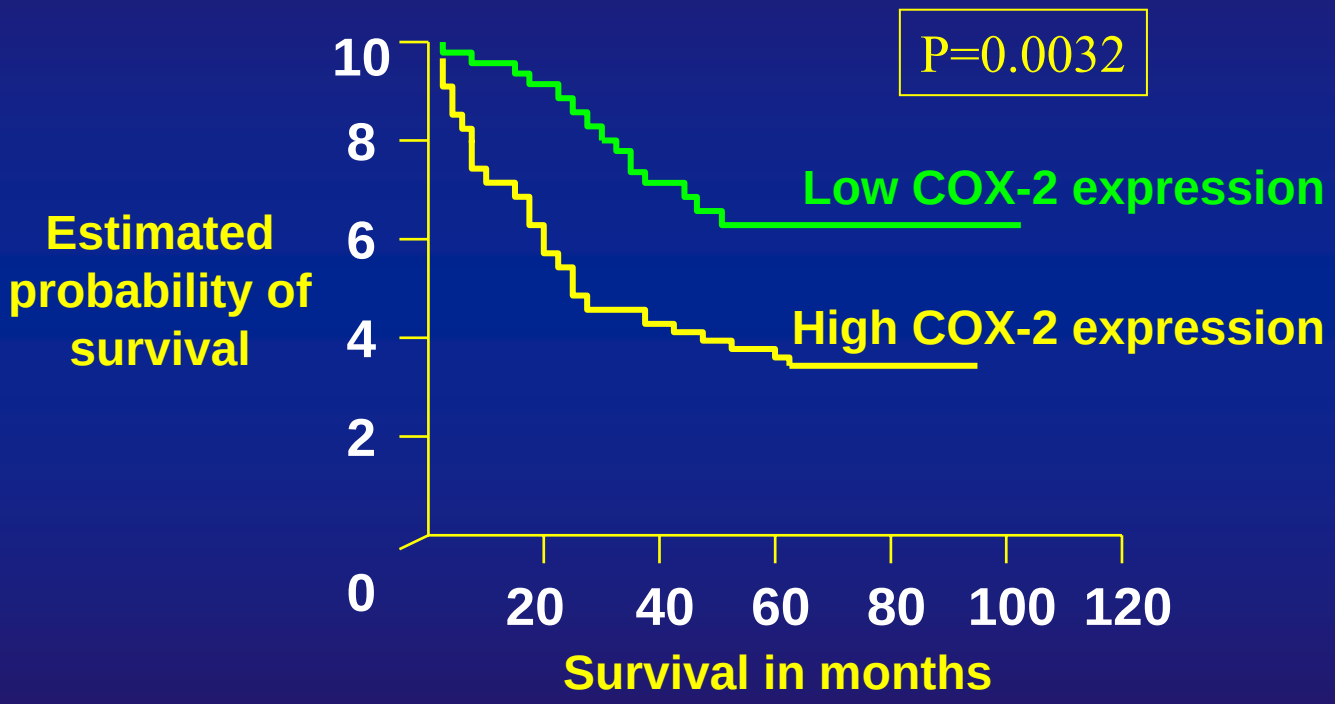
Overproduction of PGE_2 is associated with a variety of carcinogenic mechanisms.

- **Abnormal expression of epithelial growth factors**
- **Suppression of antitumor immunity**
- **Enhancement of angiogenesis**
- **Increase tumor invasiveness**
- **Resistance to apoptosis**

RATIONALE FOR COX-2 INHIBITION

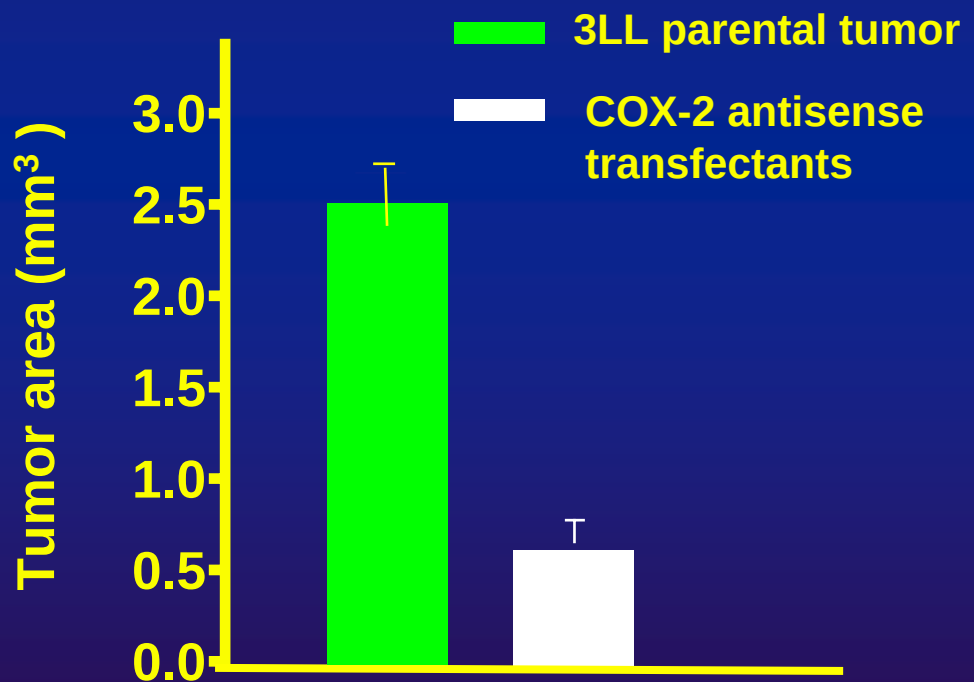
- **Epidemiologic studies : NSAIDs use is associated with reduced cancer risk** (NHNES I Schreinemachers DM, Everson RB. Epidemiology. 1994. 5:138-146, Muscat JE etal. Cancer 2003, 97:1732-6)
- **FDA approved for chemoprevention of Colorectal cancer in FAP patients.**
- **Overexpressed in NSCLC and preneoplasia** (Huang M, ca Res 1998; Wardlaw S, Carcinogenesis 2000; Hosomi Y, Lung Ca 2000)
- **COX-2 expression may be a prognostic indicator for NSCLC.** (Khuri F et al Clin Ca Res 2001, Achiwa H et al Clin Ca Res 1999)

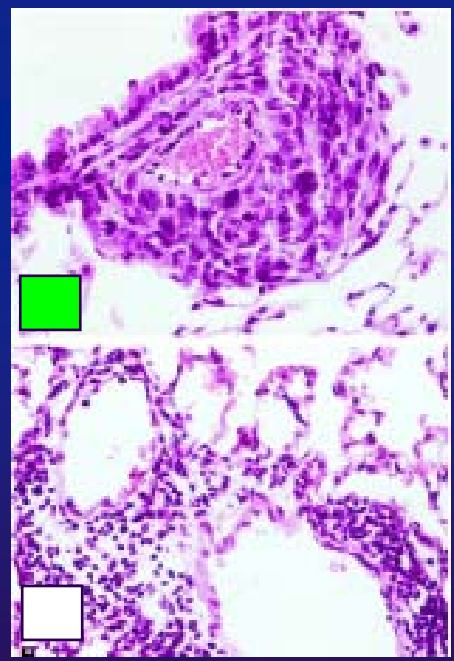
RATIONALE FOR COX-2 INHIBITION



Brabender et al. Ann of Surg 2002

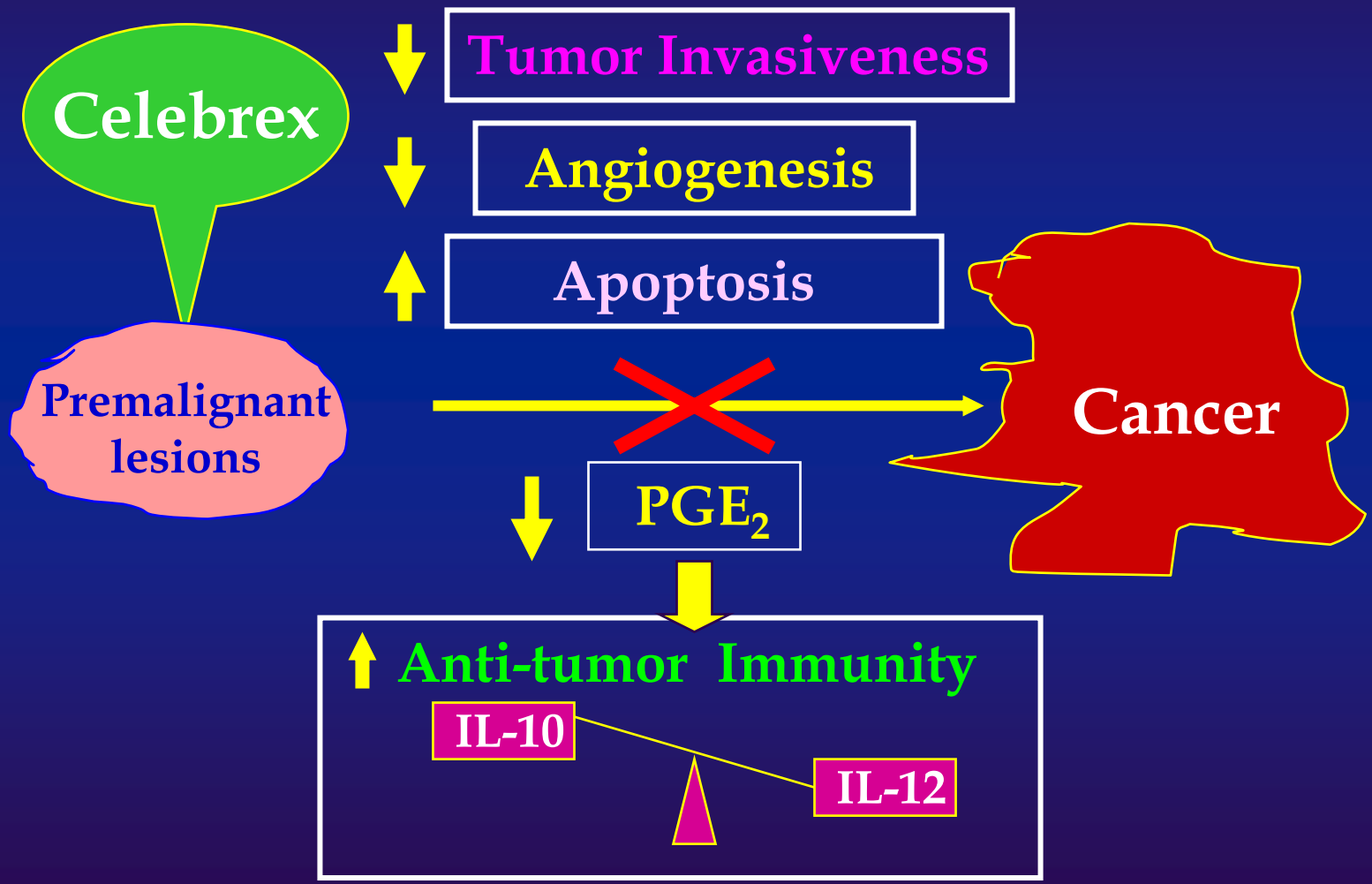
- **COX-2 inhibition is capable of inhibiting lung tumorigenesis *in vivo* in murine models.** (Stolina M, J Immunol 2000; Roux N, Ca Res 1998; Masferrer J, Ca Res 2000; Williams C, JCI 2000)



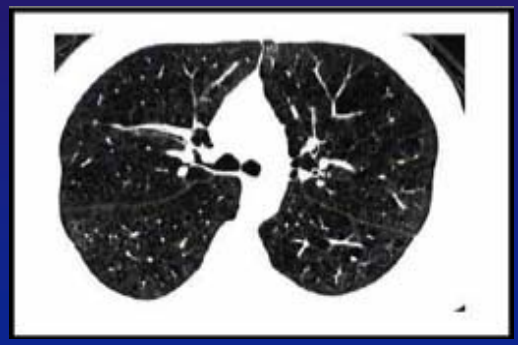


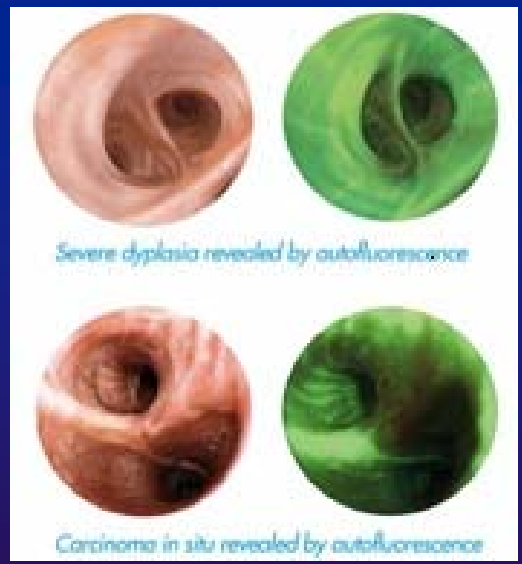
Stolina M, J Immunol 2000

RATIONALE FOR COX-2 INHIBITION

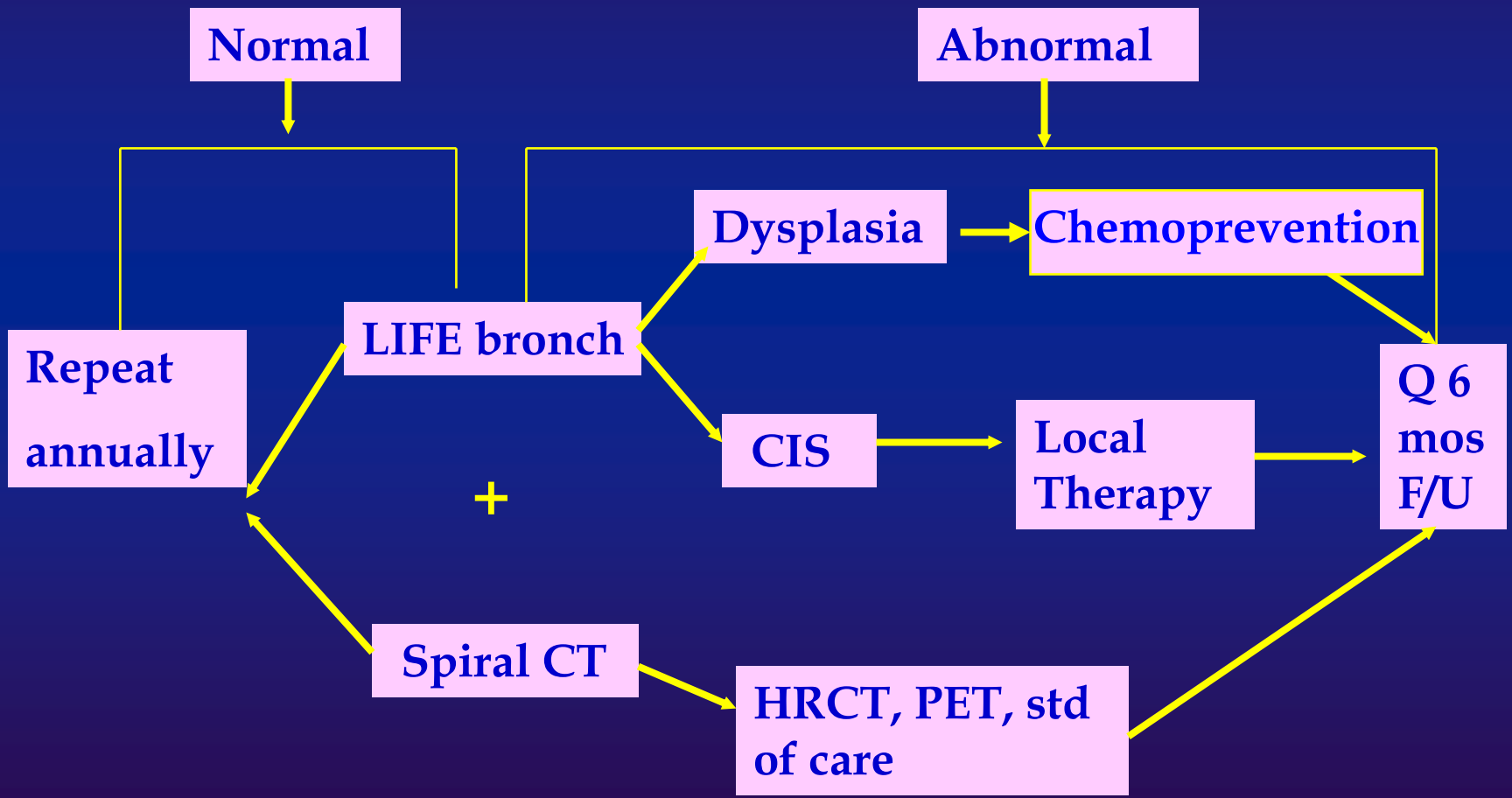


Early Lung Cancer Detection Tool



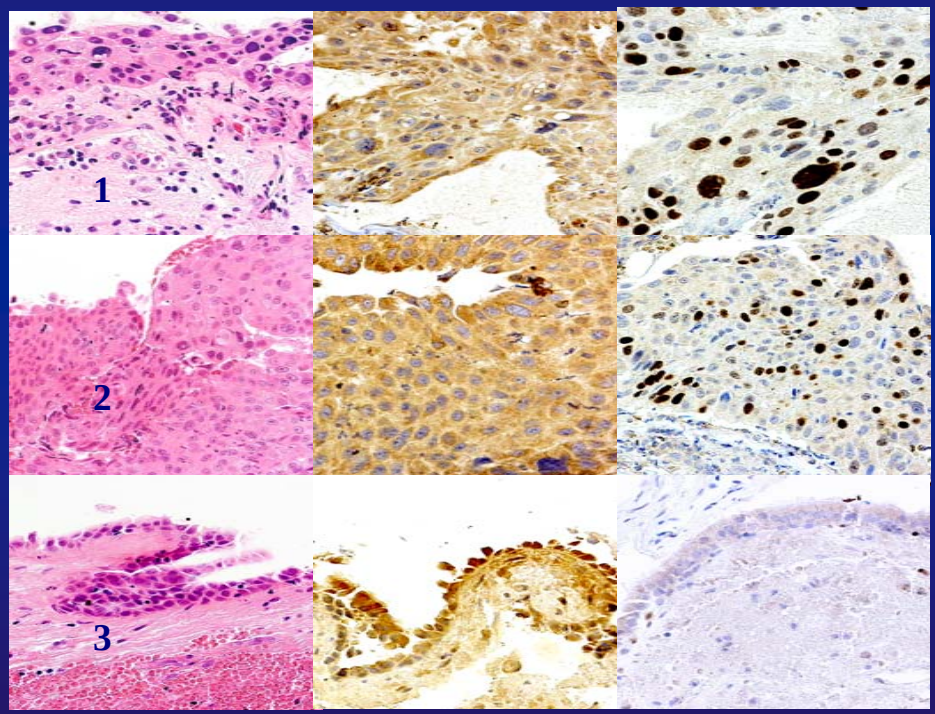


Surveillance and Chemoprevention of Second Lung Cancer

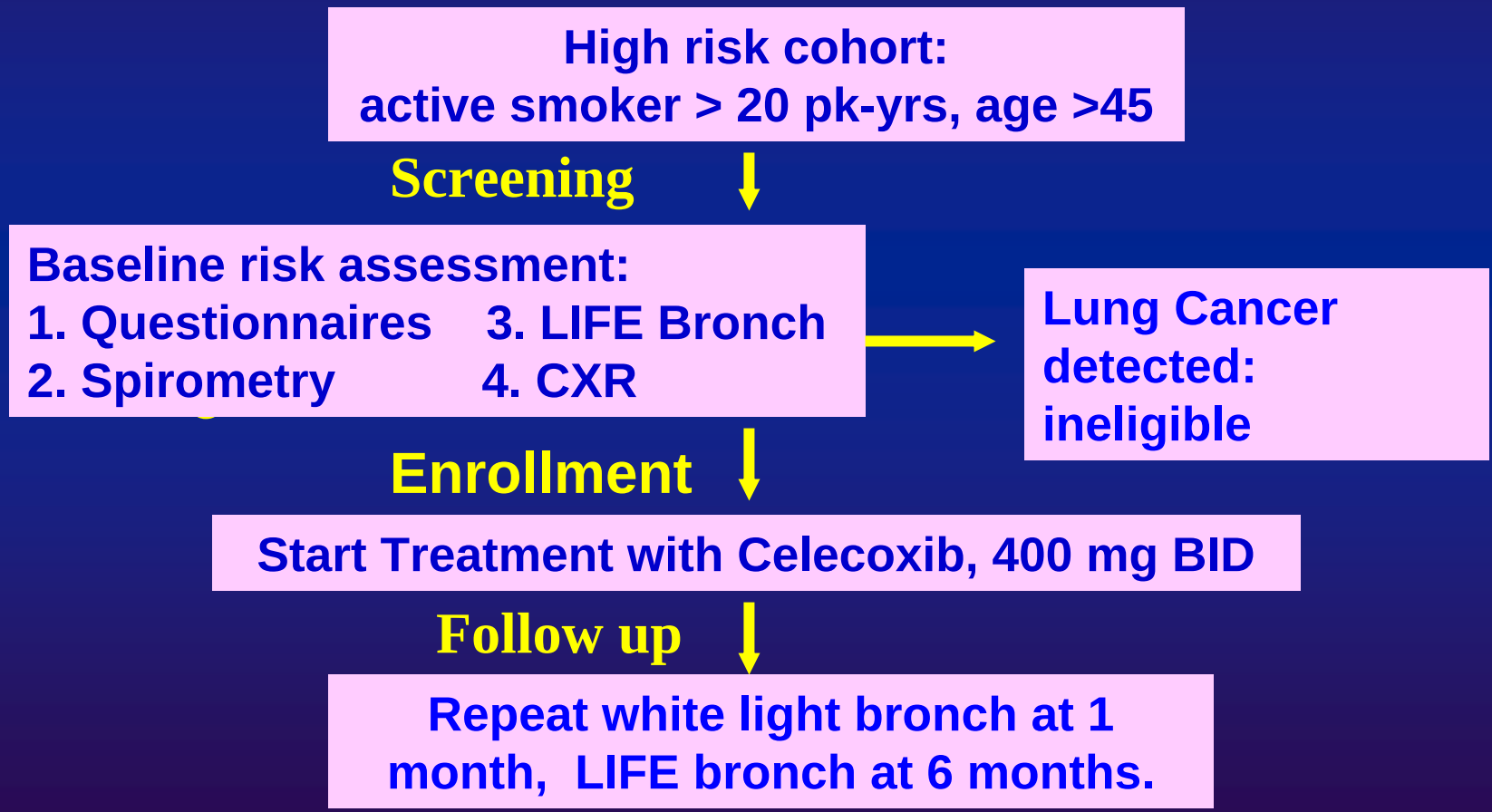


Case report

H &E COX-2 Ki-67



I. Celecoxib for Chemoprevention of Primary Lung Cancer in Heavy Smokers



I. Celecoxib for Chemoprevention of Primary Lung Cancer

Baseline Subject Characteristics

	Mean	Range
Age, yrs	54	47 - 7
Gender, M/F	9/11	-
Smoking hx (pky)	42	20 -159
Ethnicity	A/B/C/H	1/2/15/2
Family history	5	
COPD	10/20	

I. Celecoxib for Chemoprevention of Primary Lung Cancer in Heavy Smoker

Outcome Measures

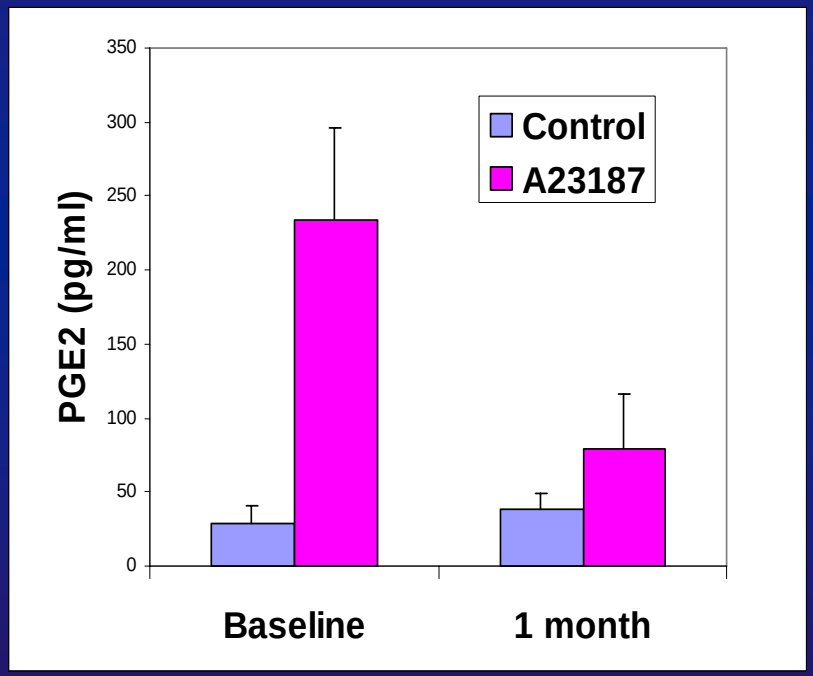
I. Modulation of Intra-pulmonary PGE2 production.

II. BAL cells functional analysis. balance of IL-10 and IL-12 in the lung microenvironment.

II. SEBM: Ki-67 (cellular proliferation), Histopathology.

RESULTS

Oral administration of Celecoxib inhibits PGE2 synthesis by A23187-stimulated BAL cells

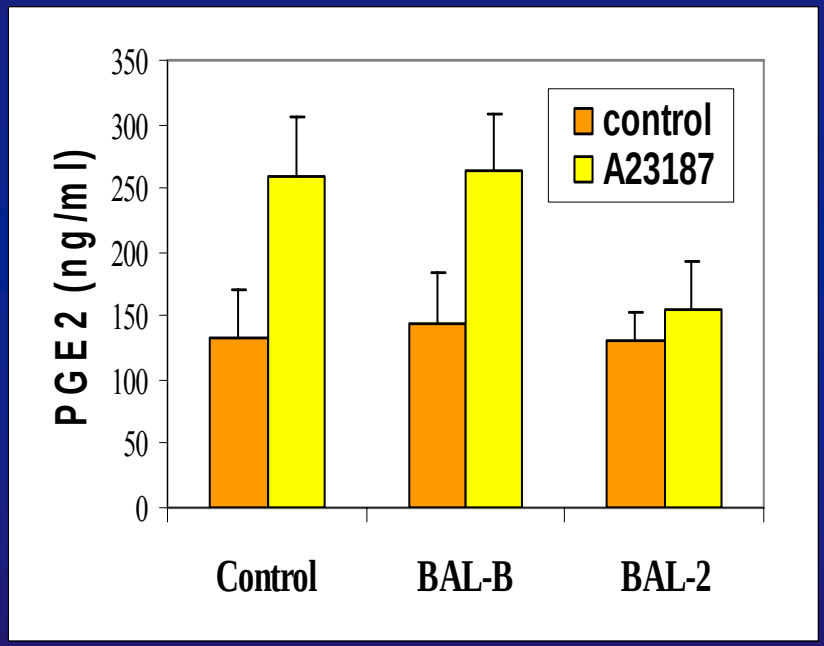


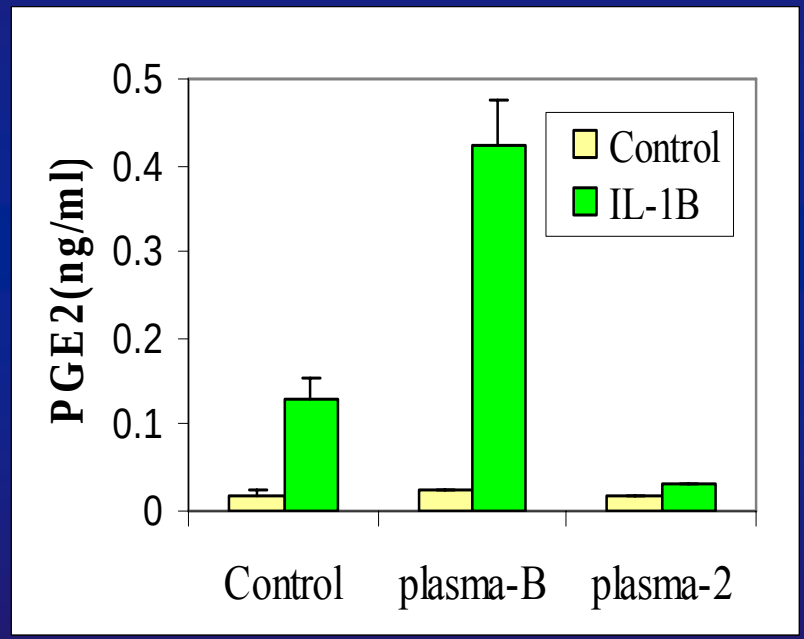
Freshly isolated BAL cells before and after 1 month Celecoxib treatment were stimulated with A23187 for 30 minutes. Celecoxib significantly inhibited the A23187-induced PGE2 synthesis. ($p < 0.01$, $n = 6$).

Mao J, et al, Clin Cancer Res. 2003

RESULTS

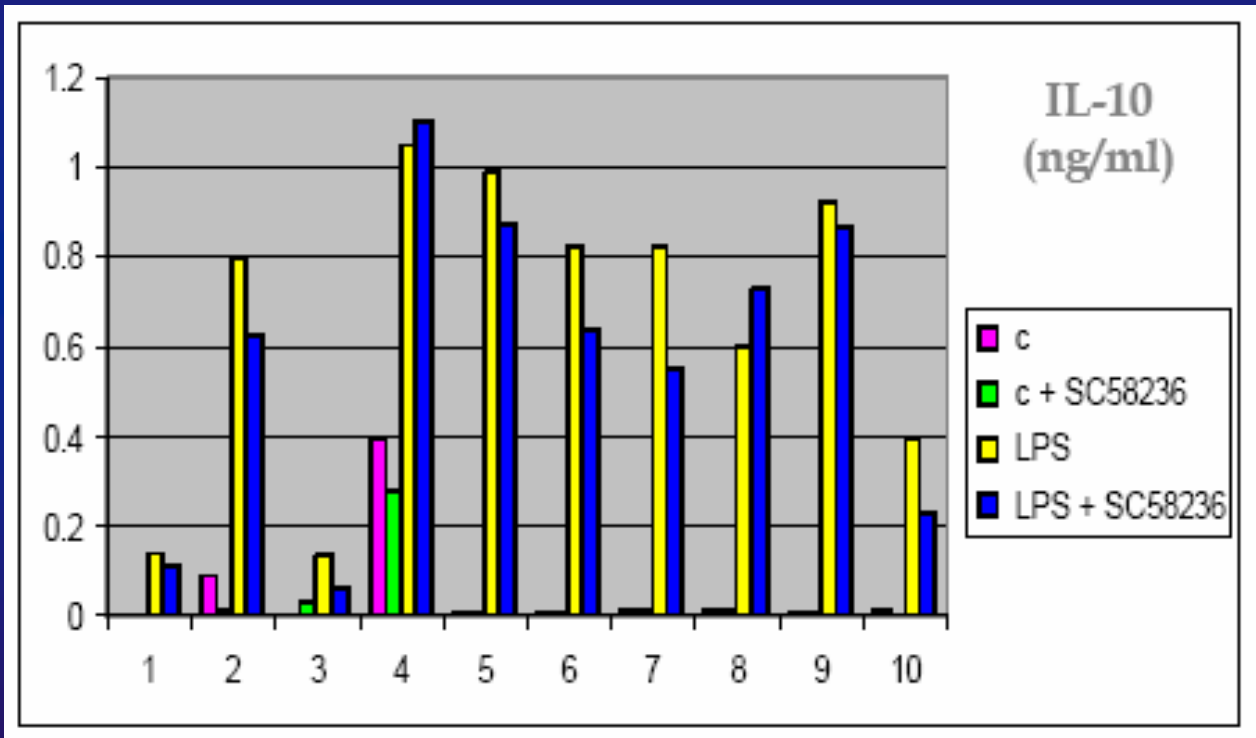
Post-treatment BAL fluid and plasma abrogated PGE2 production by stimulated NSCLC cells (A549)





Mao J, et al, Clin Cancer Res. 2003

Inhibition of COX-2 decreased the LPS-induced, up-regulation of IL-10 by BAL cells collected from smokers.



Mao J, et al, Clin Cancer Res. 2003

Effects of Celecoxib on histopathology in smokers

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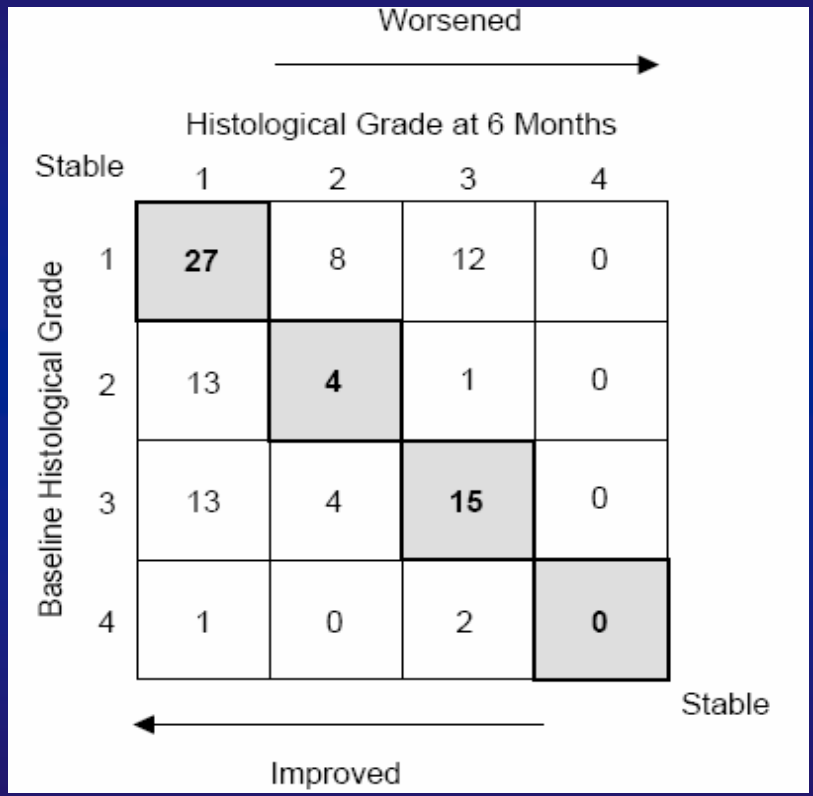


Grade

1 - Normal

2 - Hyperplasia

3 - Squamous metaplasia

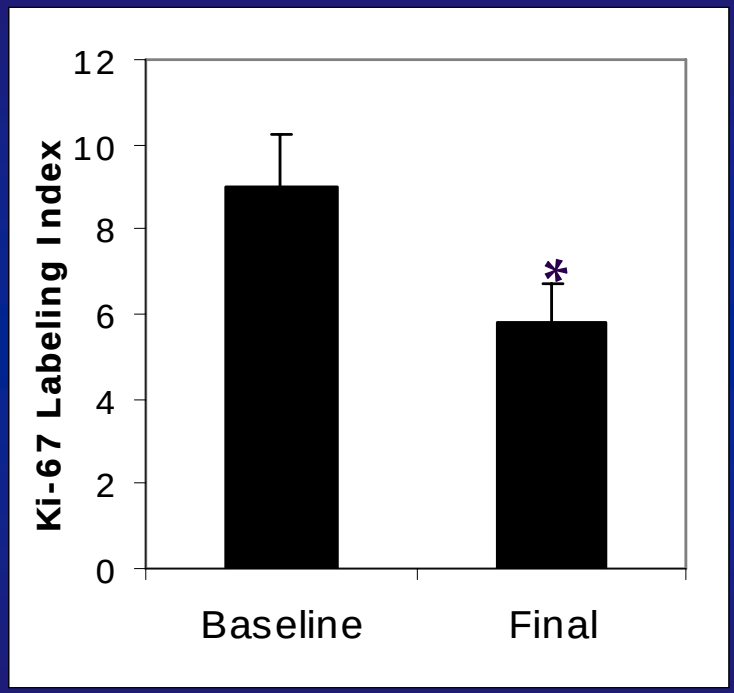
4 - Mild Dysplasia

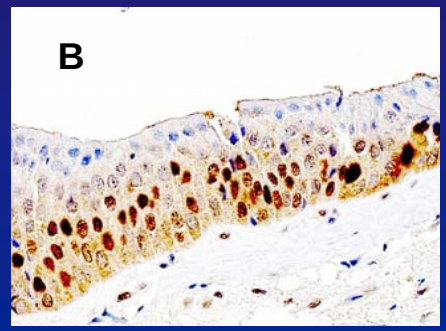
Mao J, et al, Clin Cancer Res. 2006

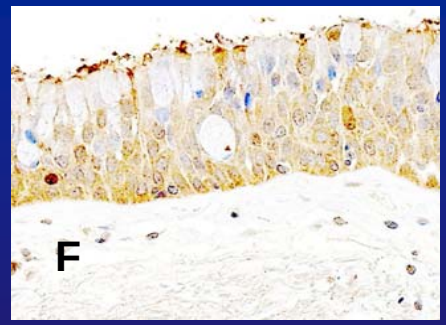
Ki-67

- Ki-67 is a proliferation marker expressed in all phases of the cell cycle except in resting cells.
- Abnormal epithelial proliferation is a hallmark of tumorigenesis.
- Elevated Ki-67 expression is associated with poor prognosis.
- Elevated Ki-67 levels can be detected in areas where squamous metaplasia is lacking.
- Ki-67 may be a useful marker for lung cancer risk.

6 months of Celecoxib reduced Ki-67 LI by 35%.







Mao J, et al, Clin Cancer Res. 2006

Summary

- Oral Celecoxib blocked the capacity of PGE2 production by smokers' BAL cells.
- Plasma and BAL fluid obtained from treated subjects blocked PGE2 production by stimulated NSCLC cells A549 *in vitro.*
- Inhibition of COX-2 blocked the release of IL-10 by LPS stimulated BAL cells from smokers, may restore anti-tumor immunity.
- Oral Celecoxib decreased Ki-67 LI in bronchial biopsies, indicating that celecoxib may be capable of favorably modulating the proliferation indices in bronchial tissue of active smokers.
- These findings support the continued investigation of COX-2 inhibiton in lung cancer chemoprevention.

II. Lung Cancer Chemoprevention with Celecoxib in Ex-Smokers

Overall Objectives

- **To determine the feasibility of Celecoxib for chemoprevention of lung cancer in high risk ex-smokers. Celecoxib will be evaluated for its impact on cellular and molecular events associated with lung carcinogenesis:**
  - **1) modulation of a panel of biomarkers of field cancerization,**
  - **2) regulation of arachidonic acid metabolism,**
  - **3) antitumor immunity**
  - **4) angiogenesis in the lung microenvironment.**

II. Lung Cancer Chemoprevention with Celecoxib in Ex-Smokers

Former smokers, > 30 pk-yrs, age >45;
Stage I NSCLC post curative resection

Screening

Baseline risk assessment:

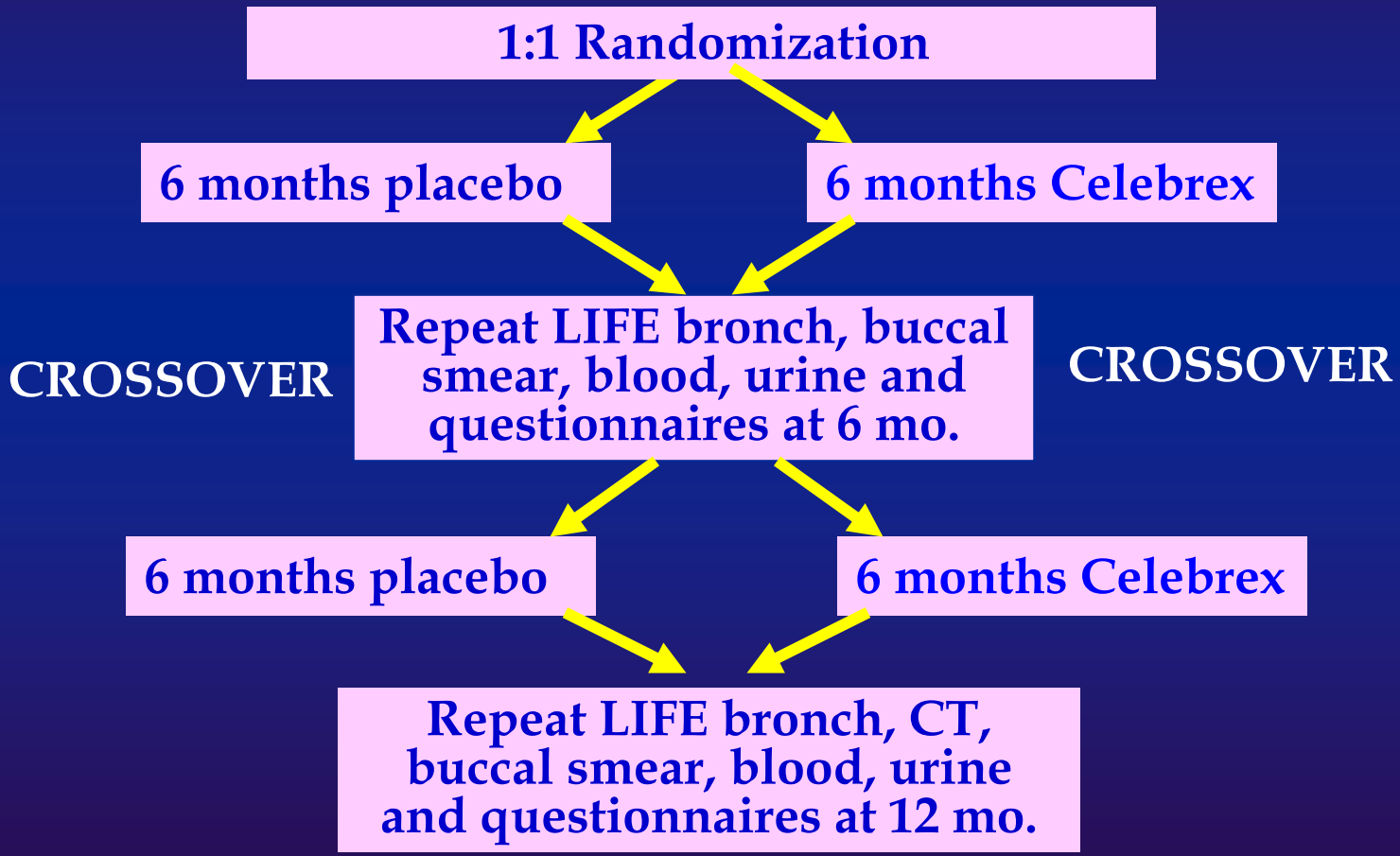
1. Questionnaires
2. Spirometry
3. Sputum induction
4. LIFE Bronch
5. Spiral CT
6. Buccal smear
7. Blood
8. Urine collection.

→ Lung Cancer detected: ineligible

Enrollment

1:1 Randomization
Stratification: 1. Prior stage I NSCLC.
2. preneoplasia

II. Lung Cancer Chemoprevention with Celecoxib in Ex-Smokers



Rationale for Crossover Design

- Each subject will act as their own statistical control, eliminating problems with inter-subject variability.
- All patients will eventually receive active treatment for 6 months, maximizing sample size, help pt. recruitment and retention.
- the primary analyses will take place at 6 months --essentially a parallel group comparison.
- The arm that receives active drug first will allow us to ascertain whether or not the chemopreventive effect is sustained after cessation of therapy.

Laboratory Studies and SEBM

1. Bronchial biospsies

Immunostaining: Ki-67, Cox-2, EGFR, p16, p27, cyclin D1 & E, bcl-2, p53, CD44,

Histopathology

Frozen biopsy

DNA analysis

2. BAL

Fluid: PGE2, IL10, IL-12, VGEF, CXC chemokines, MMP, TIMP-1, LTB4

Cytology

Alveolar Macrophages:

1.Functional analysis.

2. RNA

3. Sputum:

Cytology

4. Blood

Plasma

Buffy coat

5. Buccal Smear

DNA analysis

6. Urine

7. Primary Tumor:

Cox-2

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