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.


- 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

I. Multi-step carcinogenesis

- Normal
- Hyperplasia
- Dysplasia
- CIS
- cancer

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

<table>
<thead>
<tr>
<th>Investigators</th>
<th>Agents</th>
<th>Cancer Type</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hong 1990</td>
<td>13-cis RA</td>
<td>Nead &amp; Neck</td>
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<td>Fisher 1998</td>
<td>Tamoxifen</td>
<td>Breast</td>
<td>(+)</td>
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<td>Steinbach</td>
<td>Celecoxib</td>
<td>Colorectal in FAP</td>
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<td>Arber 2006</td>
<td>Celecoxib</td>
<td>Colon</td>
<td>(+)</td>
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<tr>
<td>Bertagnolli 2006</td>
<td>Celecoxib</td>
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<td>(+)</td>
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<tr>
<td>Investigator</td>
<td>Agents</td>
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<tr>
<td>Heimburger 1988</td>
<td>Folate and Vit B12</td>
<td>Sq. Metaplasia</td>
<td>(-)</td>
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<tr>
<td>Arnold 1992</td>
<td>Etretinate</td>
<td>Sputum atypia</td>
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<tr>
<td>Pastorino 1993</td>
<td>Retinyl palmitate</td>
<td>Second primaries</td>
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<tr>
<td></td>
<td></td>
<td>(not on 5 year survival)</td>
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<tr>
<td>Lee 1994</td>
<td>13cRA</td>
<td>Metaplasia</td>
<td>(-)</td>
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<tr>
<td>McLarty 1995</td>
<td>β-carotene/retinol</td>
<td>Sputum atypia</td>
<td>(-)</td>
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<tr>
<td>Kurie 2000</td>
<td>4-HPR</td>
<td>Sq. Metaplasia</td>
<td>(-)</td>
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<tr>
<td>Lam 2002</td>
<td>ADT</td>
<td>Dysplasia</td>
<td>(+)</td>
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<tr>
<td>Lam 2004</td>
<td>Budesinide</td>
<td>Dysplasia</td>
<td>(-)</td>
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<tr>
<td>Van den Berg 2007</td>
<td>Fluticasone</td>
<td>Metaplasia, Ki 67,</td>
<td>(-)</td>
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<tr>
<td></td>
<td></td>
<td>telomerase expression</td>
<td></td>
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<tr>
<td>Finnish 1994</td>
<td>β-carotene/vitamin E</td>
<td>Lung cancer</td>
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<td>Omenn 1996</td>
<td>β-carotene/retinal</td>
<td>Lung cancer</td>
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<td>Second primaries</td>
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<td>EUROSCAN 2000</td>
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<td>Second primaries</td>
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<td>Linxian 2006</td>
<td>4 diff combinations of Vitamins/minerals</td>
<td>Lung cancer mortality</td>
<td>(-)</td>
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</table>
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.

29,133 Finnish male smokers, age 50-69.

Phase III Lung Cancer Prevention Trials

Primary

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

18,314 smokers, former smokers and asbestos workers

BC (30 mg/d) + Retinol (25,000IU/d)  Placebo

- 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).

Phase III Lung Cancer Prevention Trials

Tertiary

- Euroscan

2,592 patients, NSCLC (n ~ 1,000; T1-2, N0-1, T3N0) or HNC

- Retinyl palmitate (300,000 IU/d x 1 yr, 150,000 IU x 1 yr.)
- N-Acetylcysteine (NAC, 600 mg QD)
- Retinyl palmitate + NAC
- No drug

- 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

1,166 stage I NSCLC

- 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
  
  - 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 launching 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

**Arachidonic Acid**

- Lipoxygenases (LOX)
- Cytochrome P450 (cytp450)
- Cyclooxygenases (COX)

**Products**

- Leukotrienes
  - HETEs
  - HPETEs
- Prostaglandins
  - Thromboxane A2
- Epoxyarachidonic Acids

**Regulatory Enzymes**

- PLA2
Carcinogens, Growth factors, Cytokines, and Inflammatory stimuli lead to the induction of COX-2, resulting in the production of PGG2, which is then converted to PGH2. PGH2 can further be converted to TXA2, PGD2, PGE2, PGF2, and PGI2. Inflammatory Sites include Mφ, Endothelial cells, Cancer, Platelets, Stomach, Intestine, and Kidney. Growth factors and Cytokines are constitutive, while COX-2 is inducible.
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 (NHINES I Schreinemachers DM, Everson RB. Epidemiology. 1994. 5:138-146, Muscat JE et al. Cancer 2003, 97:1732-6)

- **FDA approved for chemoprevention of Colorectal cancer in FAP patients.**


The figure shows a Kaplan-Meier survival curve comparing survival in months between patients with low COX-2 expression (green line) and high COX-2 expression (yellow line). The estimated probability of survival is lower for patients with high COX-2 expression, with a statistically significant difference indicated by $P=0.0032$. The survival in months is plotted on the x-axis, and the estimated probability of survival on the y-axis.

RATIONALE FOR COX-2 INHIBITION

Celebrex

Premalignant lesions

Tumor Invasiveness

Angiogenesis

Apoptosis

PGE$_2$

Cancer

↑ Anti-tumor Immunity

IL-10

IL-12
Early Lung Cancer Detection Tool
Surveillance and Chemoprevention of Second Lung Cancer

- **Normal**: Repeat annually
- **Abnormal**: Dysplasia → Chemoprevention  
  - Abnormal: Chemoprevention
  - Normal: Local Therapy → Q 6 mos F/U
  - Spiral CT → HRCT, PET, std of care

- LIFE bronch
- CIS
Case report

H &E  COX-2  Ki-67

1

2

3
I. Celecoxib for Chemoprevention of Primary Lung Cancer in Heavy Smokers

High risk cohort:
active smoker > 20 pk-yrs, age >45

Screening

Baseline risk assessment:
1. Questionnaires  3. LIFE Bronch
2. Spirometry       4. CXR

Lung Cancer detected: ineligible

Enrollment

Start Treatment with Celecoxib, 400 mg BID

Follow up

Repeat white light bronch at 1 month, LIFE bronch at 6 months.
## I. Celecoxib for Chemoprevention of Primary Lung Cancer

### Baseline Subject Characteristics

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

RESULTS

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

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

Effects of Celecoxib on histopathology in smokers


<table>
<thead>
<tr>
<th>Baseline Histological Grade</th>
<th>Histological Grade at 6 Months</th>
<th>Stable</th>
<th>Improved</th>
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<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>27</td>
<td>8</td>
</tr>
<tr>
<td></td>
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<td>8</td>
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<td>4</td>
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</table>

**Grade**

1 - Normal
2 - Hyperplasia
3 - Squamous metaplasia
4 - Mild Dysplasia
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%.

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 inhibition 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

1:1 Randomization

- 6 months placebo
- 6 months Celebrex

CROSSOVER

Repeat LIFE bronch, buccal smear, blood, urine and questionnaires at 6 mo.

CROSSOVER

- 6 months placebo
- 6 months Celebrex

Repeat LIFE bronch, CT, buccal smear, blood, urine and questionnaires at 12 mo.
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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