Research Program on the Characterizing the Premalignant Lung Cancer Signature

Mary Reid, PhD

Professor of Oncology

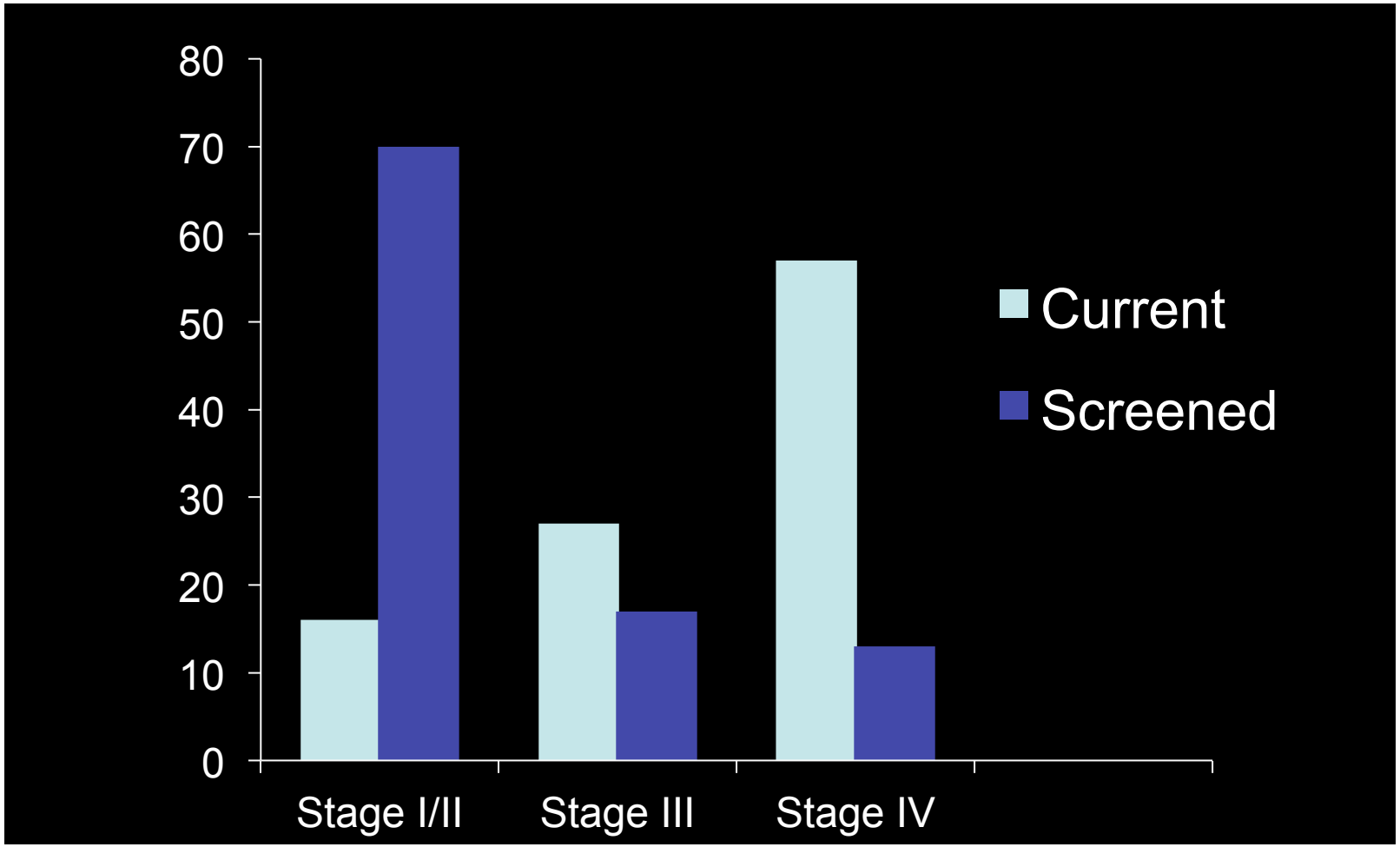
Director of Cancer Screening and Survivorship

PCGA: Premalignant Squamous Cell Lung Carcinoma Lesions Have Distinct Histologic Progression

Jennifer Beane[1], Sarah Mazzilli[1], Ania Tassinari[1], Josh Campbell[1], Christopher Moy[2], Michael Schaffer[2], Catalina Perdomo[1], David Jenkins[1], Mary Beth Pine[3], Gang Liu[1], Sherry Zhang[1], Hanqiao Liu[1], Jessica Vick[1], Evan Johnson[1], Suso Platero[2], Marc Lenburg[1], **Mary Reid[3]**, Samjot Dhillon[3], Avrum Spira[1]

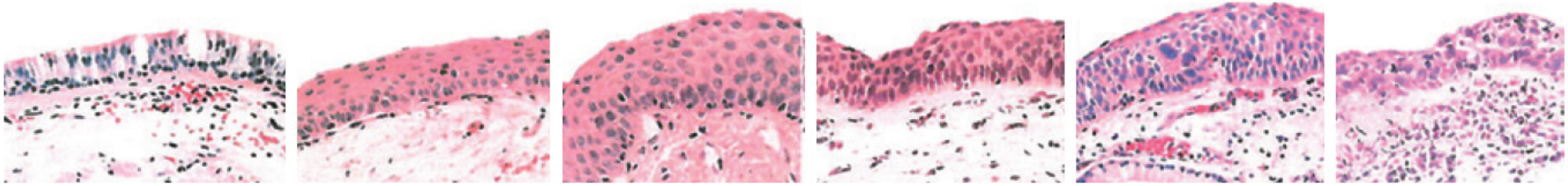
[1]Boston University School of Medicine, Boston, MA; [2]Janssen Pharmaceuticals, Inc., Springhouse, PA; [3]Roswell Park Cancer Institute, Buffalo, NY.

The Shift in Stage of Lung Cancer With LDCT Screening



Model Of Progression From A Bronchial Premalignant Lesion To Squamous Cell Carcinoma

Squamous Lung Carcinogenesis



Normal | Squamous metaplasia | Mild dysplasia | Moderate dysplasia | Severe dysplasia | Carcinoma *in situ*

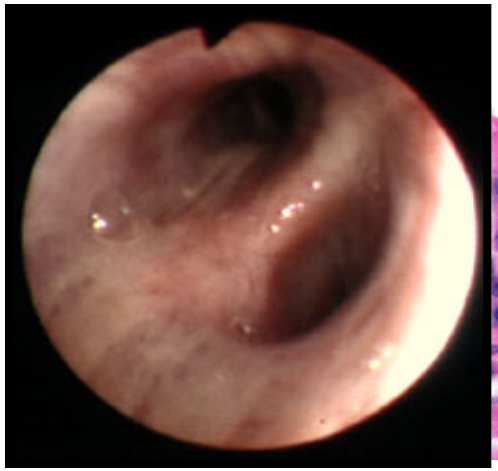
Only a subset of airway premalignant lesions progress to CIS/tumor

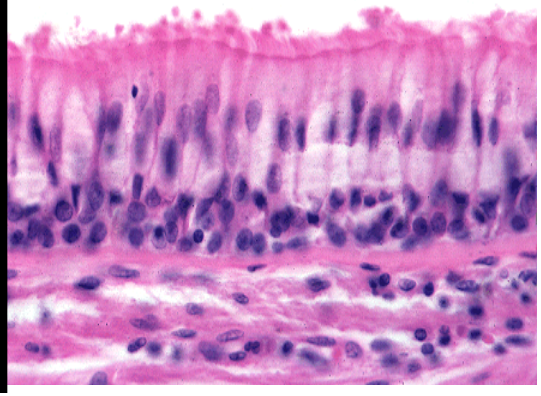
Many airway premalignant lesions will regress without intervention

Keith, R. L. & Miller, Y.E. *Nat. Rev. Clin. Oncol*. 2013
van Boerdonk R.A.A., *AJRCCM*, 2015
Merrick D., Can. Prev. Res. 2016

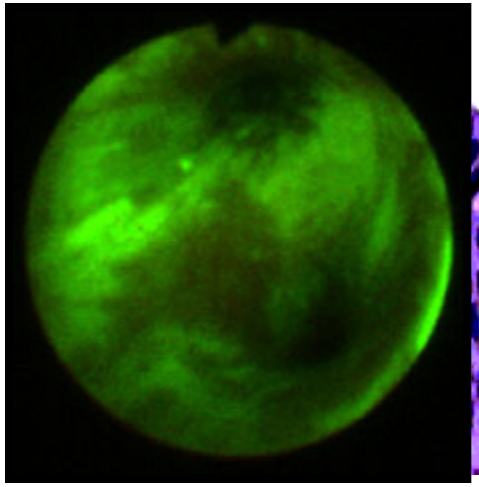
Bronchoscopy and Preinvasive Bronchial Epithelial Lesions

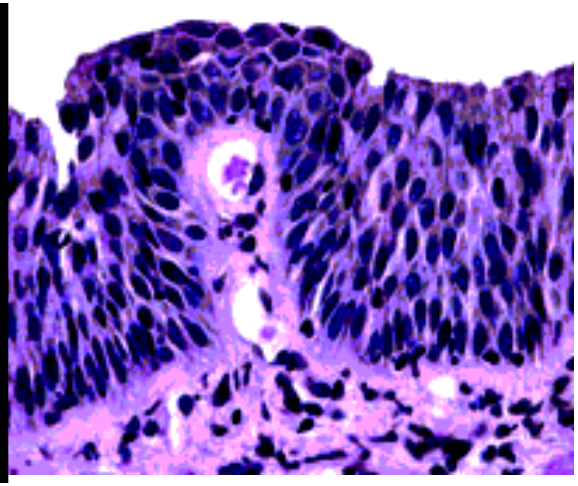
Normal epithelium: ciliated columnar cells, basal cells, and basement membrane



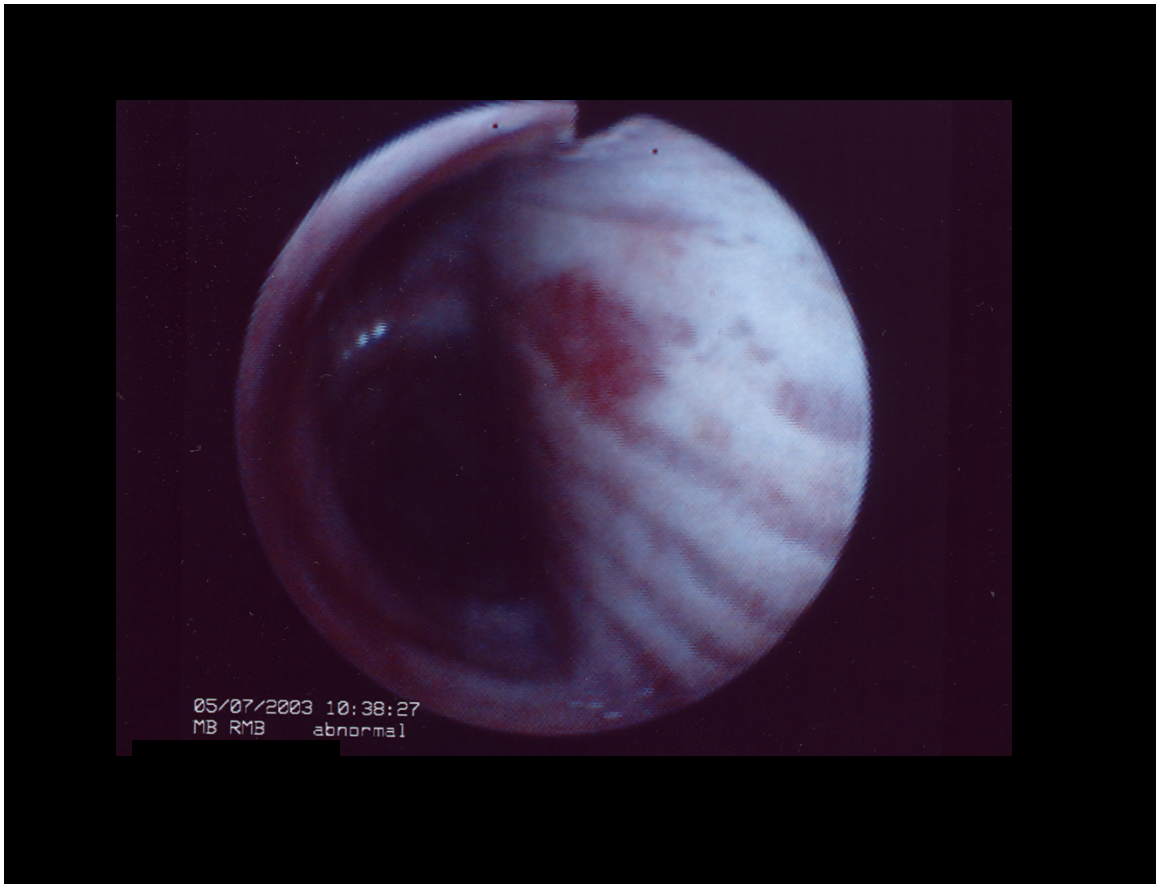


CIS/***Squamous dysplasia***: columnar size and shape altered; epithelial architecture regresses, Mitotic figures,

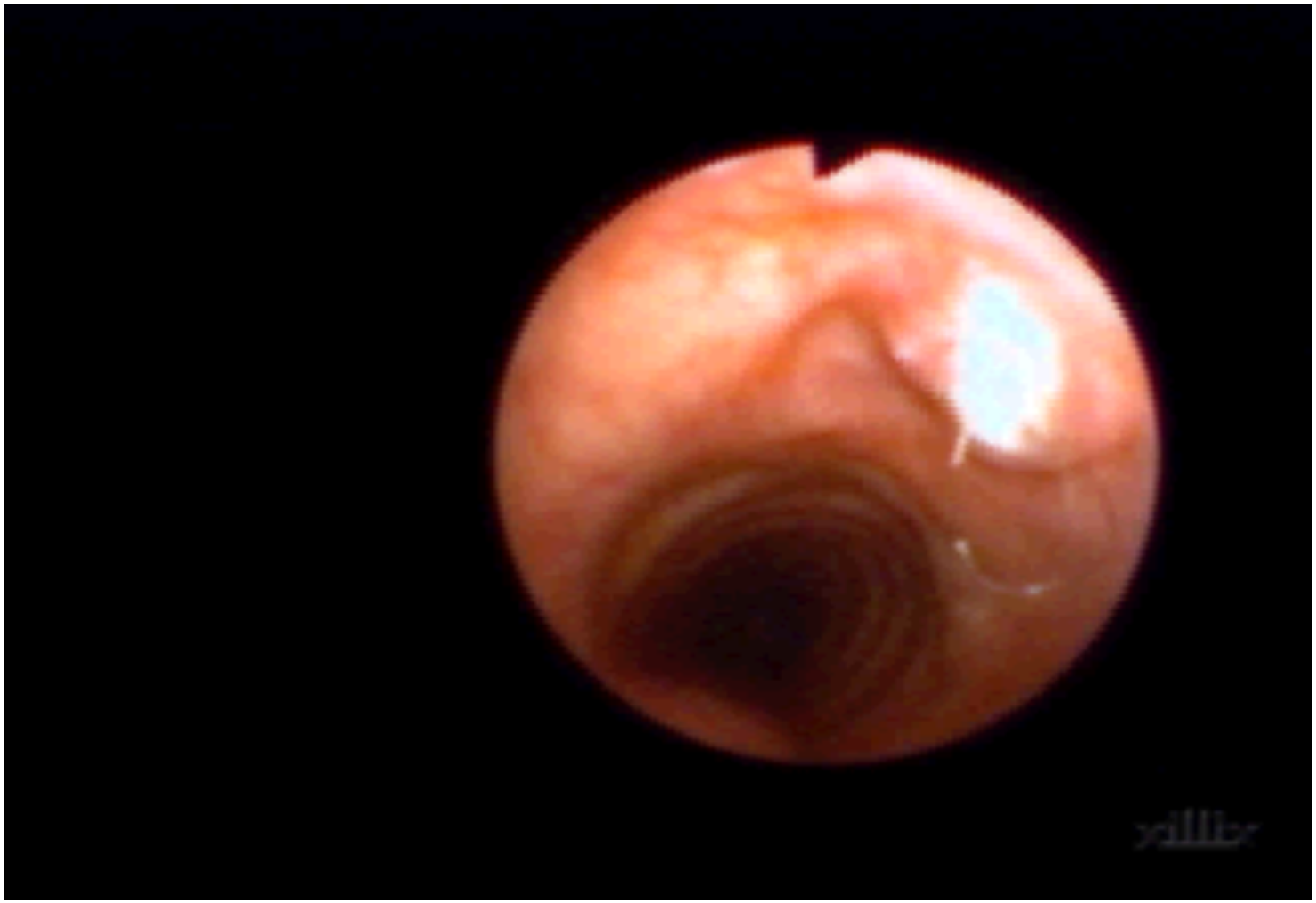




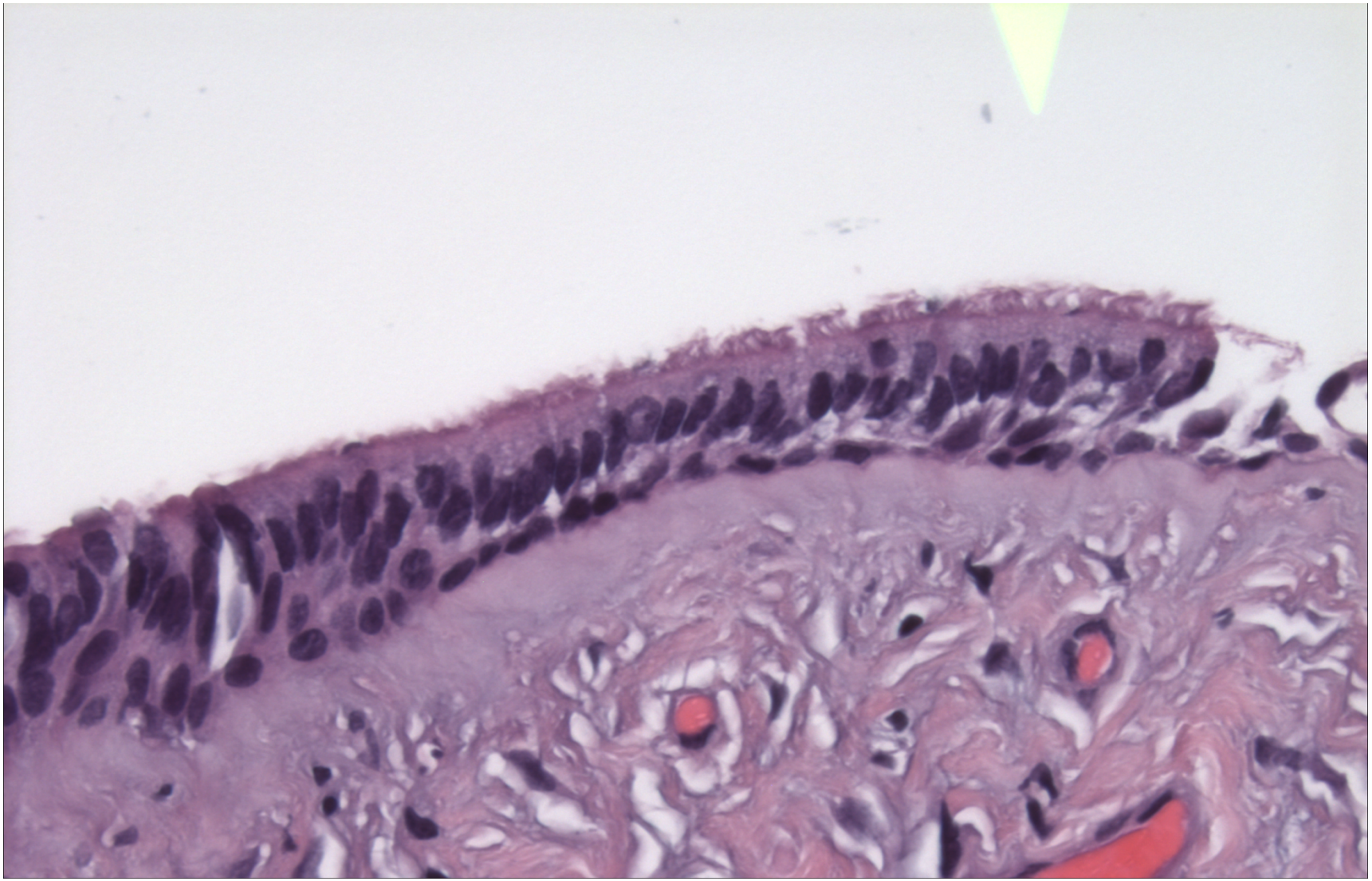
Abnormality Under White Light in Right Main Bronchus



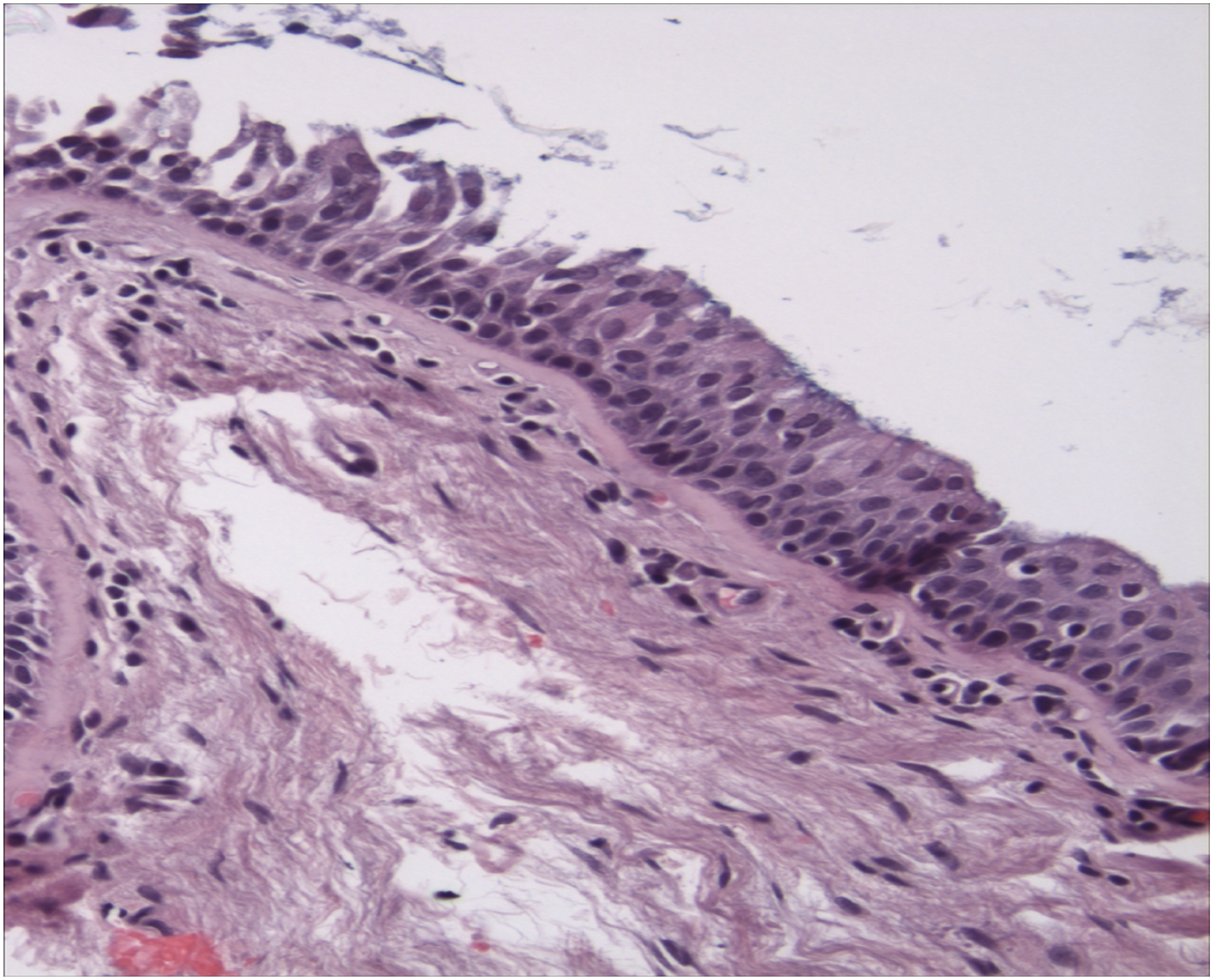
View of Carcinoma In-situ by Bronchoscopy



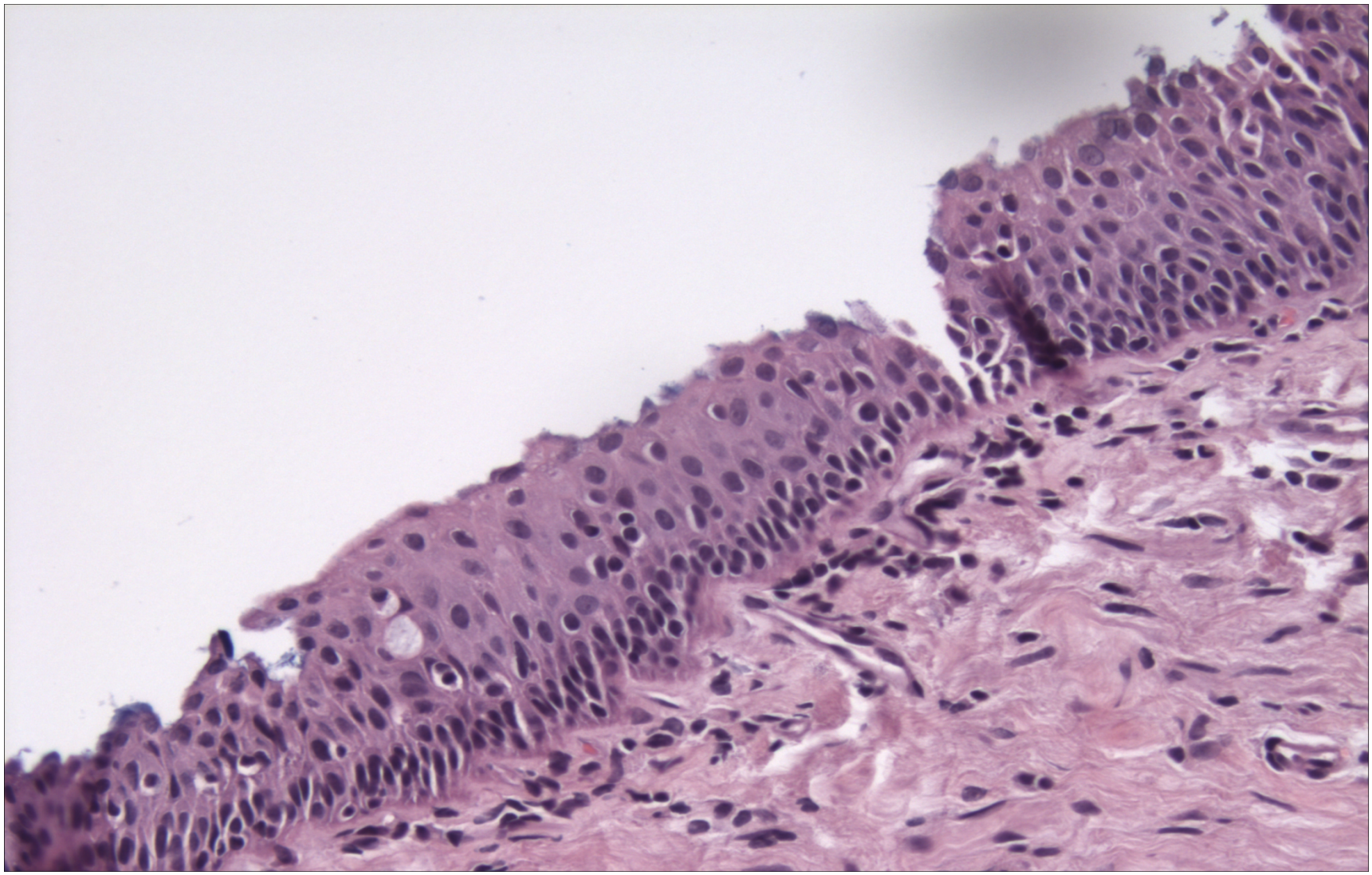
Normal Bronchoepithelium

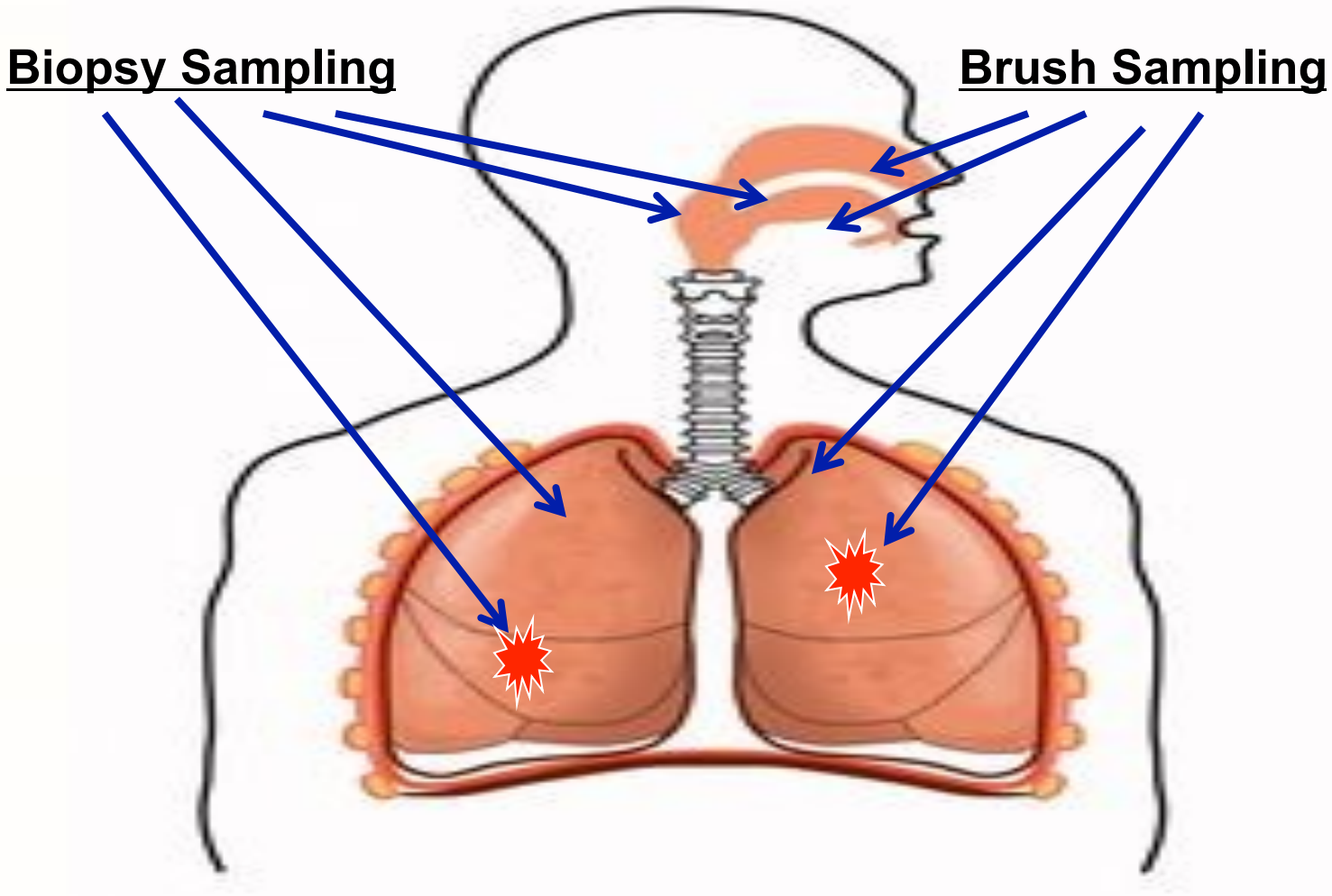


Hyperplasia



Squamous Metaplasia

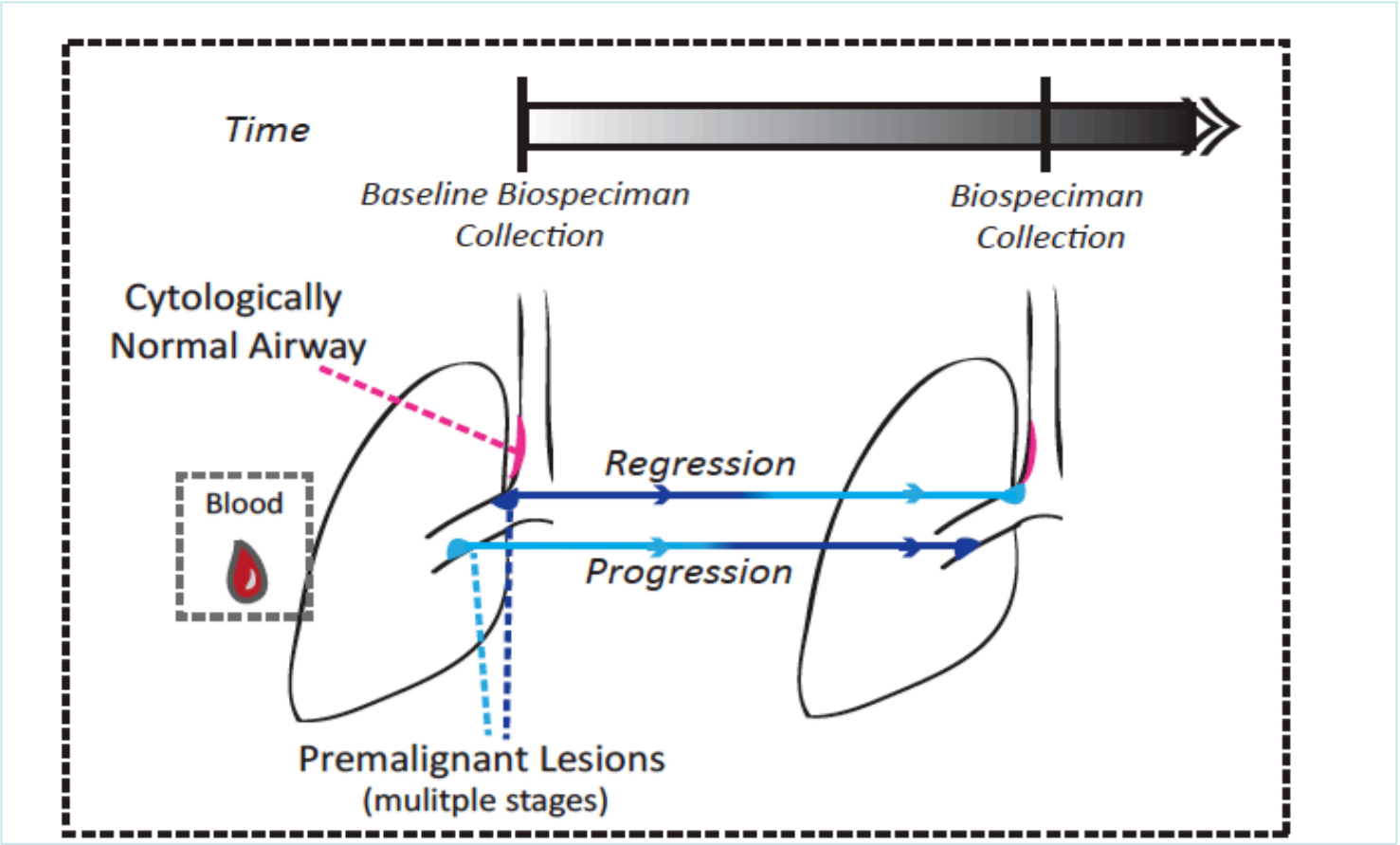




Blood Sampling: Biobanking, Paxgene

PCGA: Objectives and Study Design

- Molecular characterization of bronchial biopsies
- Identify molecular alterations associated with lesion progression



Subject Inclusion/Exclusion Criteria

High-risk subjects undergoing lung cancer screening at the Roswell Park Cancer Institute

- Group 1: Personal history of lung or upper aerodigestive cancer with no evidence of disease
- Group 2: No cancer history, age 50+, smoker with 20+ pack-years with one or more risk factors

↓

Select subjects with spatial and longitudinal samples

↓

Sample set 1
29 patients
Samples collected between 2010-2012

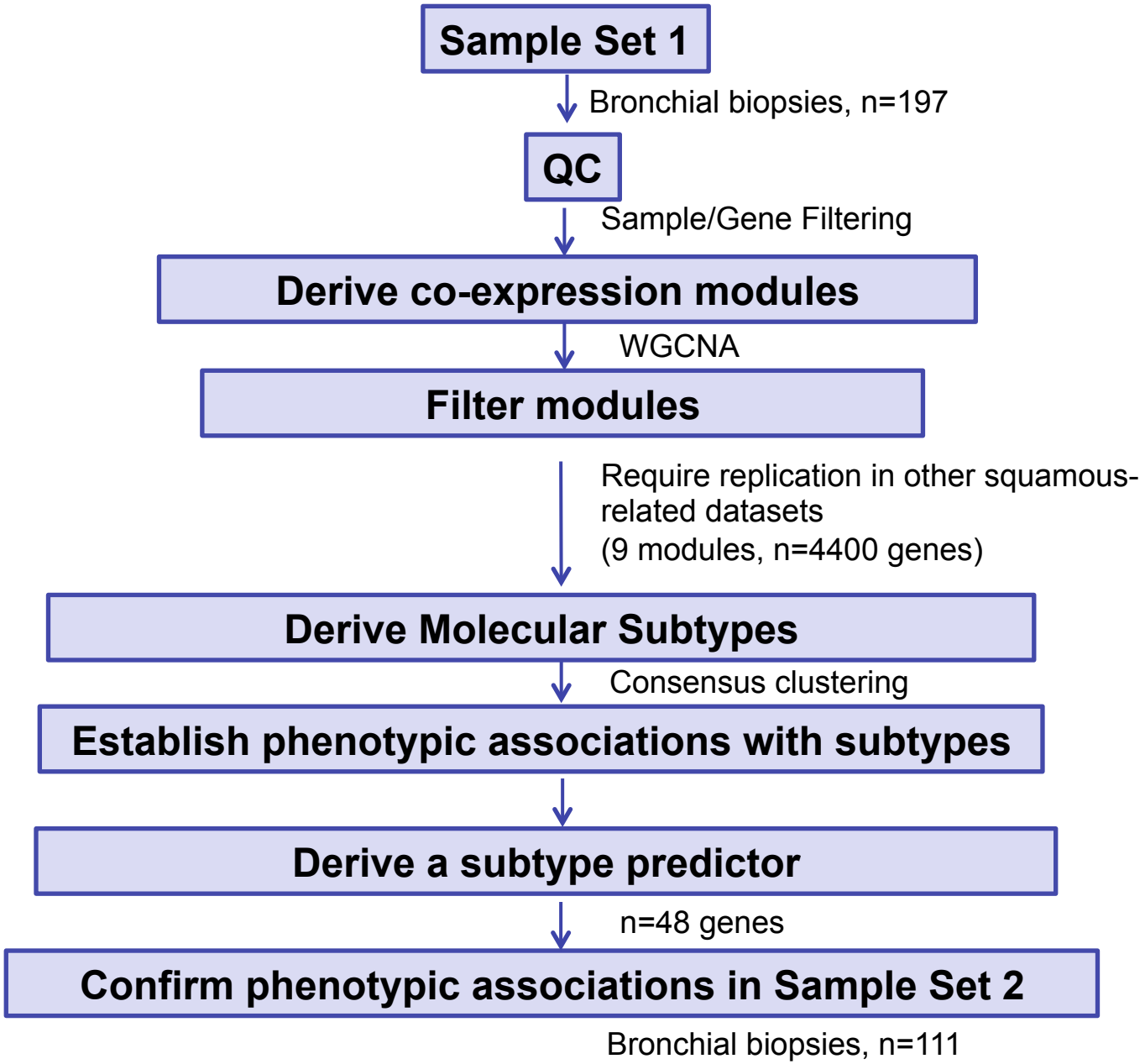
Sample set 2
20 patients
Samples collected between 2010-2015

Mary Reid, Samjot Dhillon, Mary Beth Pine

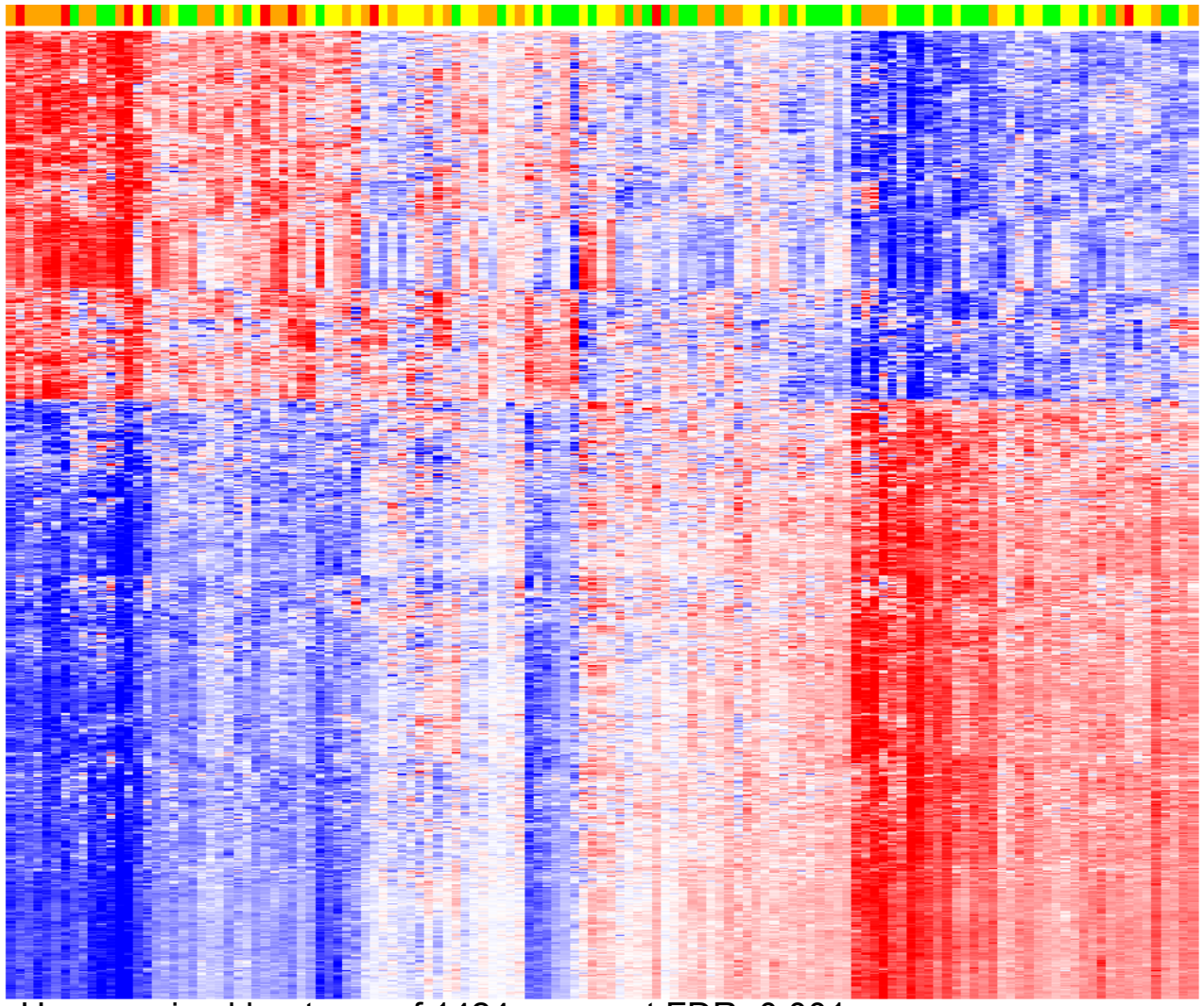
Subject/Sample Phenotypic Information

Subject	Sample Set 1	Sample Set 2
Gender	14 (M), 15 (F)	12 (M), 8 (F)
Age	59.4 (7.6)	58.7 (8.3)
Smoking Status	28 (Ever), 1 (Never)	19 (Ever), 1 (Never)
Prior History of Lung Cancer	20 (Yes), 9 (No)	12 (Yes), 8 (No)
COPD status	21 (Yes), 8 (Unknown)	11 (Yes), 8 (Unknown), 1 (No)
Sample		
Average # Biopies/Patient	6.8 (5.8)	5.5 (2.9)
Average # Bronchoscopies/Patient	2.4 (1.5)	2.4 (0.8)
Average Time Between Bronchoscopies (Days)	450 (419)	357 (434)
Total # Biopsies	197	111
Total # Brushes	91	49
Histology of Biopsies		
Normal	41	25
Hyperplasia	30	31
Metaplasia	47	15
Mild Dysplasia	23	13
Moderate Dysplasia	37	21
Severe Dysplasia	13	6
CIS	2	0
Unknown Histology	4	0

Brief methodology overview



Gene Expression Alterations Associated with Biopsy Histological Grade



Unsupervised heatmap of 1424 genes at FDR<0.001

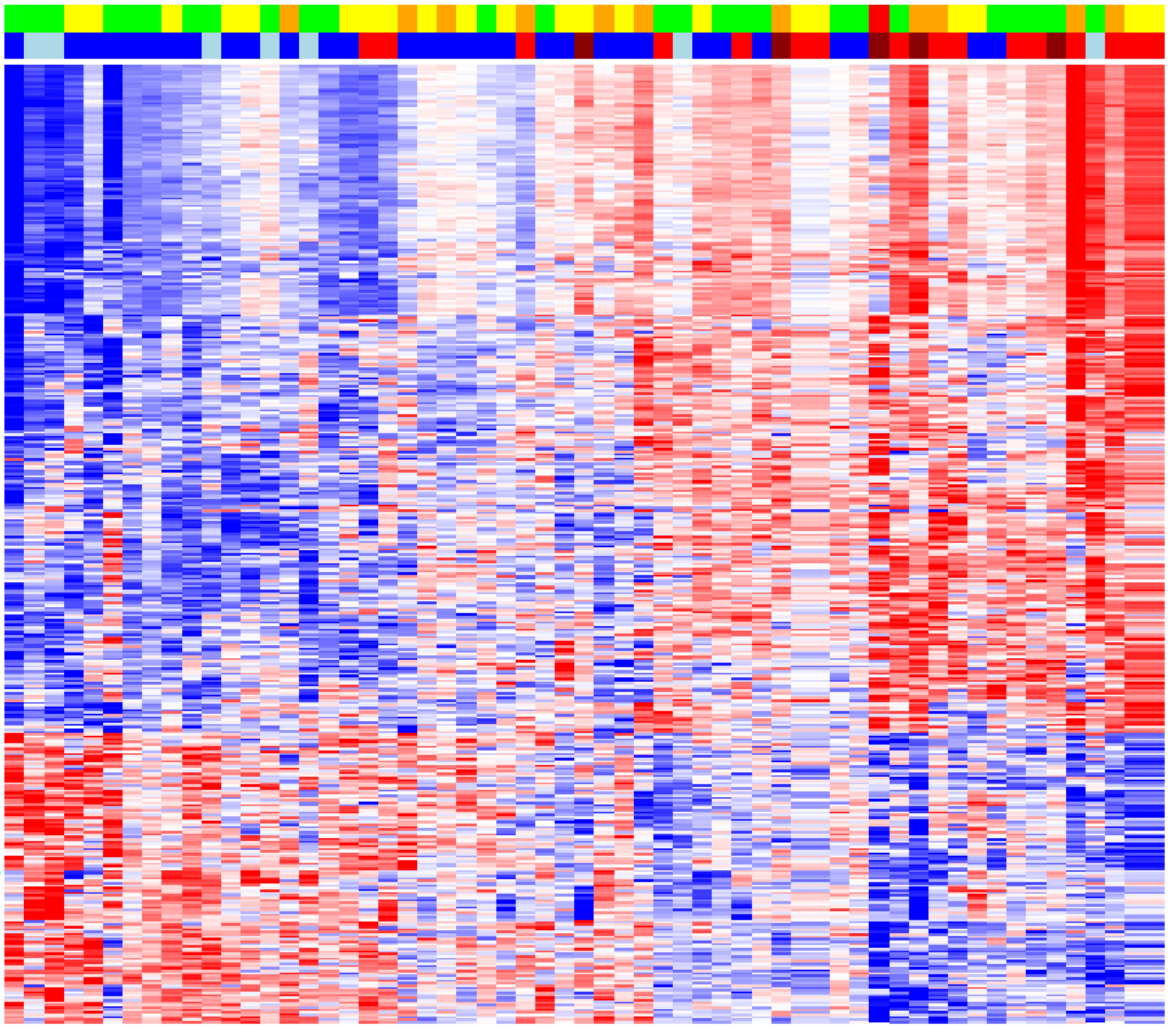
- Severe Dysplasia/CIS
- Mild/Moderate Dysplasia
- Metaplasia
- Normal/Hyperplasia

Pathways enriched at FDR<0.01

- **294 up-regulated genes**

- **Glycolysis**
- **Cell cycle**
- **Wnt signaling**
- **E2F pathway**
- **P53 signaling**
- **PI3K cascade**

Genes Associated with Premalignant Disease Progression within a Lesion Over Time



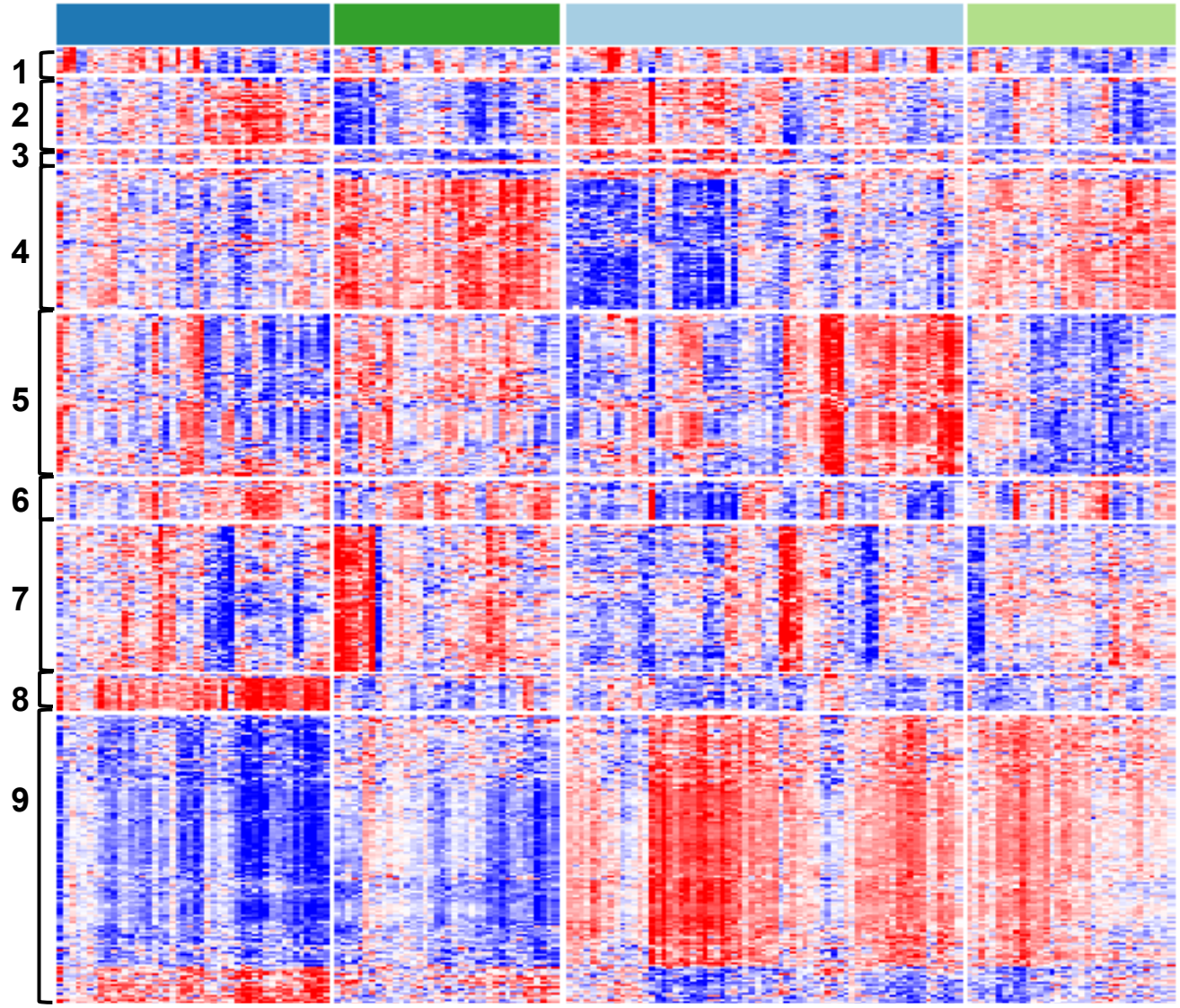
Pathways: 51 at p<0.01
42 up---regulated in stable/progressive
- Metabolic pathways
- DNA repair
- HIF1A
- IL12

9 down---regulated in stable/progressive
- Xenobiotics
- ARF6
- TRAF3/6-IRF
- Type 1 interferon
- JAK/STAT
- NO2-dependent IL-12 Pathway in NK cells

Progression

Regression

Discovery Of Four Molecular Subtypes of Premalignant Lesions



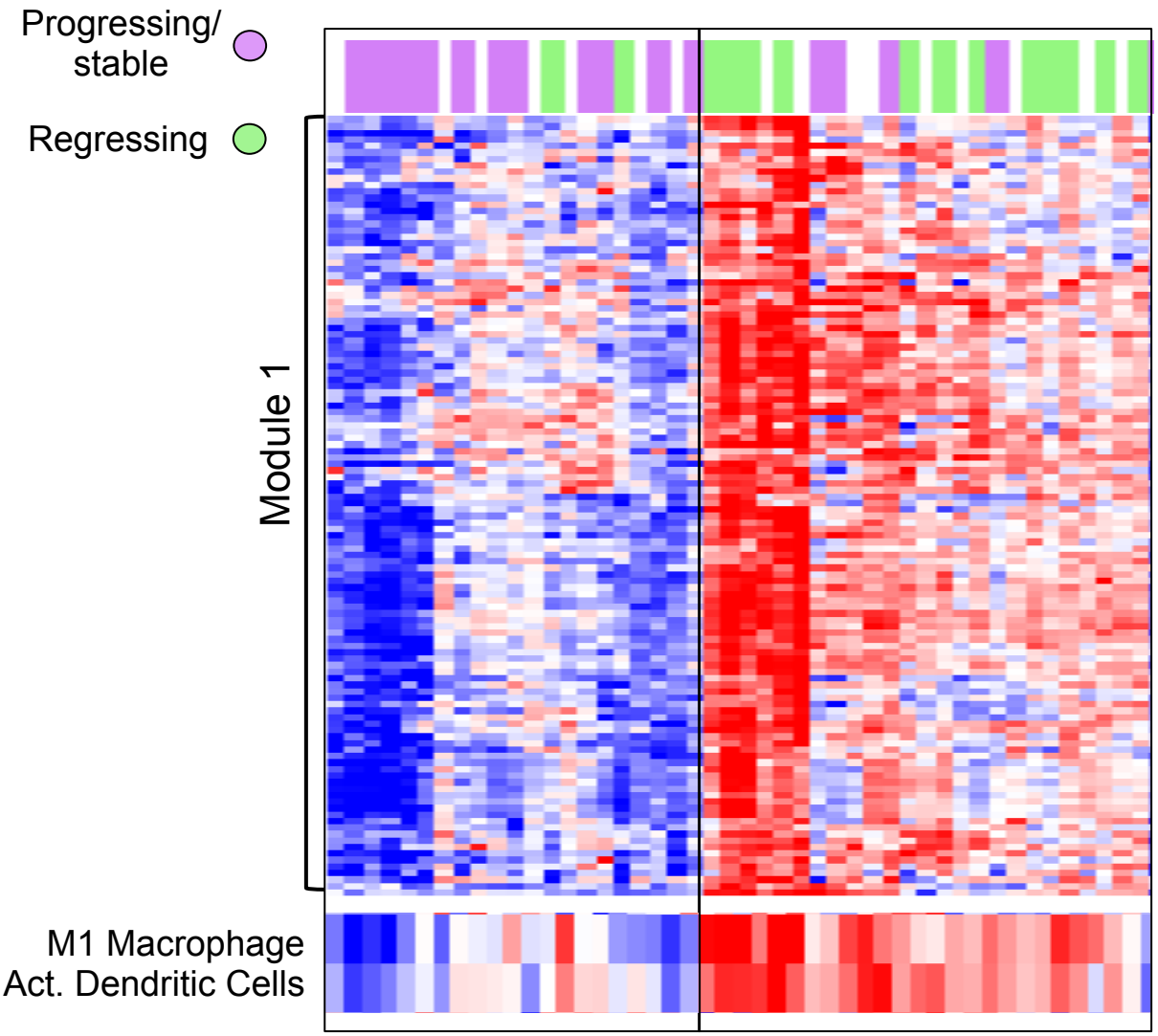
Subtype

1 INFγ signaling/T cell immunity (n=120)
2 OXPHOS, ETC (n=321)
3 TGFβ signaling (n=65)
4 Extracellular matrix (n=673)
5 Inflammatory Response (n=776)
6 Translation processes (n=182)
7 mRNA processing (n=701)
8 Cell cycle (n=170)
9 Cilium organization/assembly (n=1392)

Subtype discovery: consensus clustering with 80% resampling, pam clustering, and Pearson correlation across normalized median centered data

Wilkerson, M. *Bioinformatics.* 2010

Immune-associated module linked to lesion progression in high grade subtype



- 120 genes down-regulated in lesions with progression among high-grade subtype
- Genes are associated with INFγ signaling and T-cell mediated immunity:
  - HLA class 1 genes
  - Immunoproteosome
  - Transporters associated with antigen processing
  - Immune inhibitory signals (*PDL1, LAG3, IDO1*)

Newman A. *Nature*. 2015

Conclusions

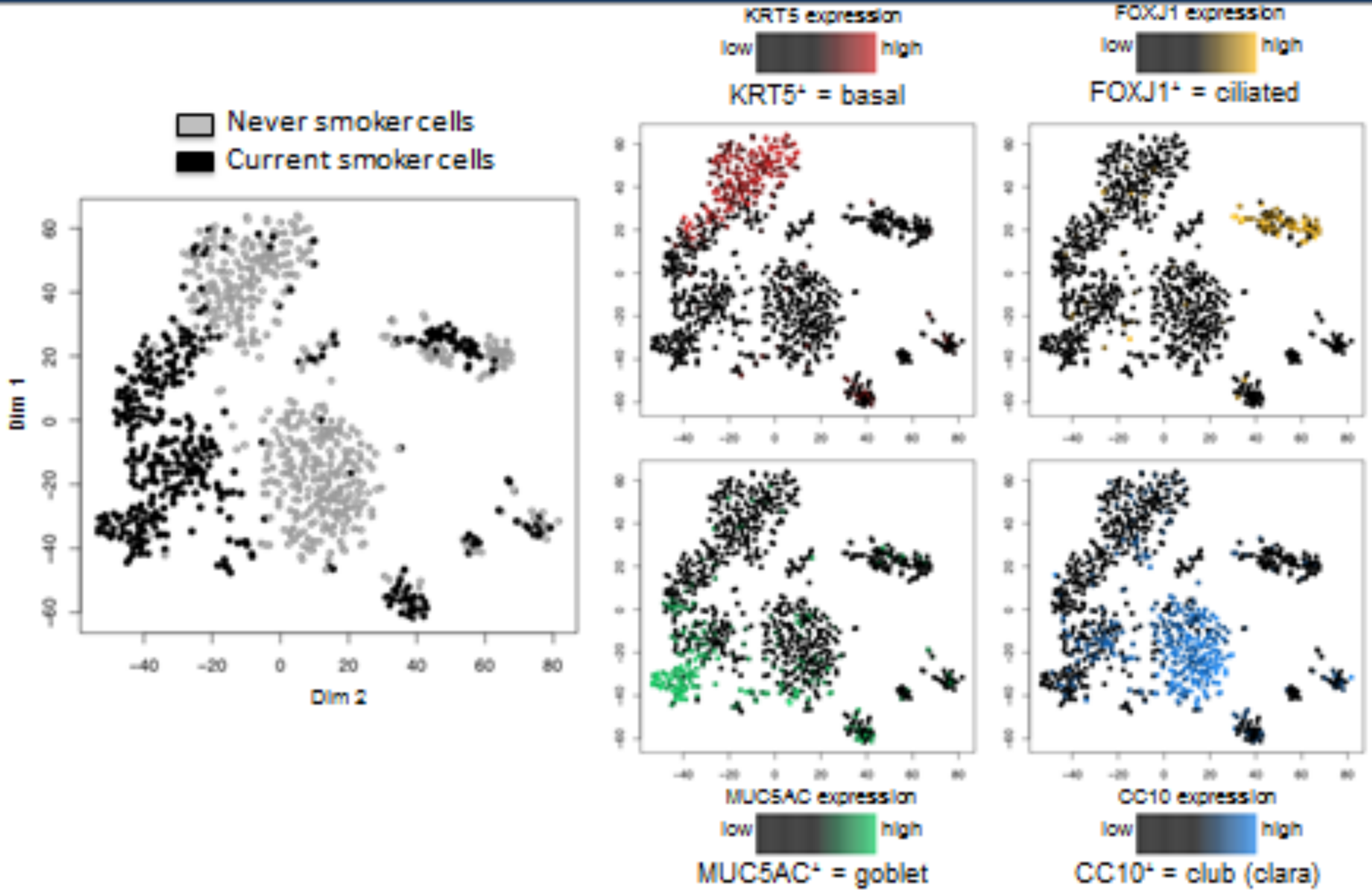
- mRNA-Seq profiling of bronchial biopsies identified four distinct molecular subtypes across genes expressed in the epithelium and the microenvironment
- The molecular subtypes are associated with histological grade, cell type, and previously defined SCC subtypes
- In the high-grade subtype, genes involved in interferon gamma signaling and T-cell mediated immunity are down-regulated with lesion progression

These results may lead to biomarkers of disease severity and progression as well as personalized therapies including immunoprevention

Future Directions

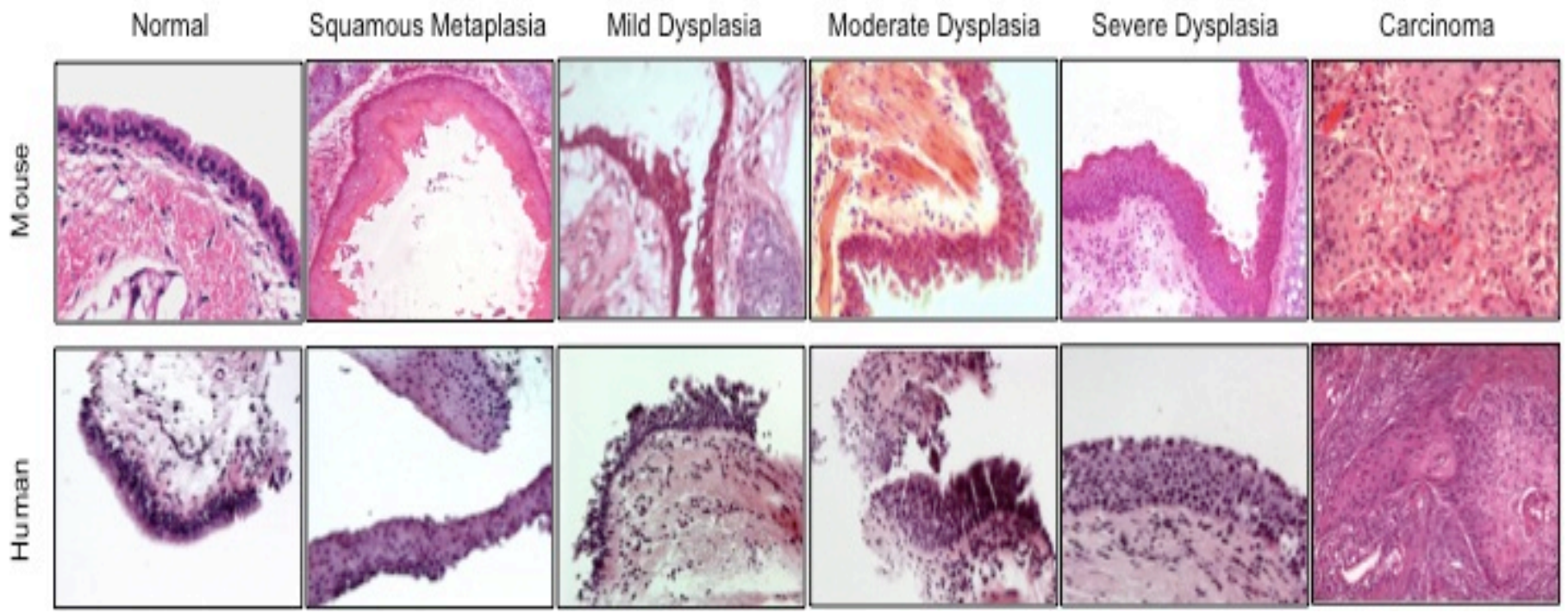
- Continue to sample patient cohort over time
- Single cell sequencing of the airways of high risk subjects
- Determining hallmarks of initiation and progression in different populations: AA versus CA, COPD, lung cancer survivors
- Use preclinical models to study the function of genes identified by the sequencing
- Use the sequencing data for other projects
  - RNA Editing with Santosh Patniak
  - Epigenetics with Joyce Ohm
- Targeting the EGFR Pathway with Cimavax

Epithelial subsets shift with smoking status



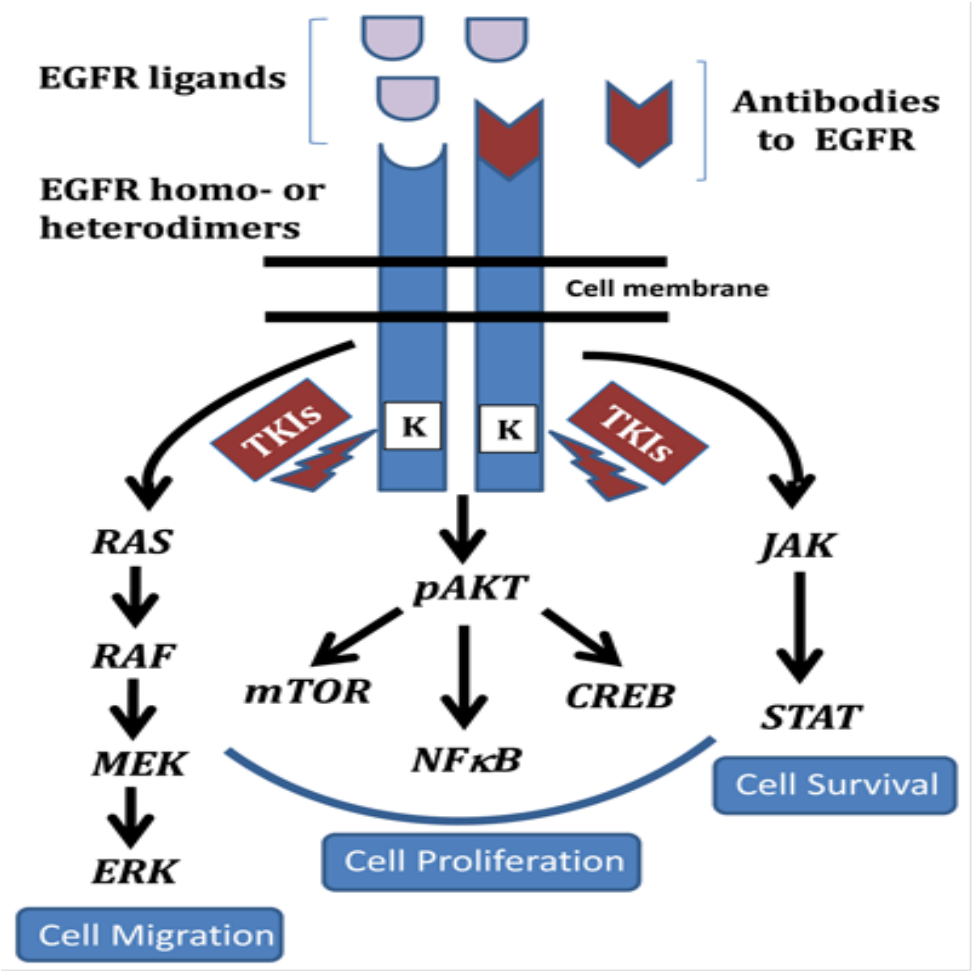
Histological Concordance Between the NTCU Mouse Model and Human Lesions Function of Differentially Expressed Genes

.



Sarah Mazzilli, BU

Targeting the EGFR Pathway



RPCI Collaborators

Pulmonology

Samjot Dhillon, MD

Manoj Mammon, MD (UBMD)

Medicine

Grace Dy, MD

Surgical Oncology

Saikrishna Yendamuri, MD, MS

Chukumere Nwogu, MD, PhD

Mark Hennon, MD

Lung Cancer Registry

Mary Beth Pine, MS

Cores

Prevention Lab, DBBR, Pathology

Translational Research

Pam Hershberger, PhD

Andrew Ray, PhD (UB)

Santosh Patniak, MD, PhD

Immunology

Scott Abrams, PhD

Pathology

Sara Pokharel, MD, PhD

Biostatistics

Alan Hutson, PhD

Support: Stacey Scott Lung Cancer Registry

Acknowledgements

Janssen Pharmaceuticals, Inc.

Matt Lorenzi
Suso Platero
Christopher Moy
Michael Schaffer

Boston University School of Medicine
Section of Computational Biomedicine

Jennifer Beane
Josh Campbell
Avrum Spira
Catalina Perdomo
Jake Kantrowitz
Yaron Gesthalter
Ania Tassinari
Gang Liu
Sherry Zhang
Hanqiao Liu
Sarah Mazzilli
Jake Kantrowitz
Liye Zhang
David Jenkins

Marc Lenburg
Stefano Monti
Evan Johnson
Jessica Vick

LUNGevity Foundation


THE
END