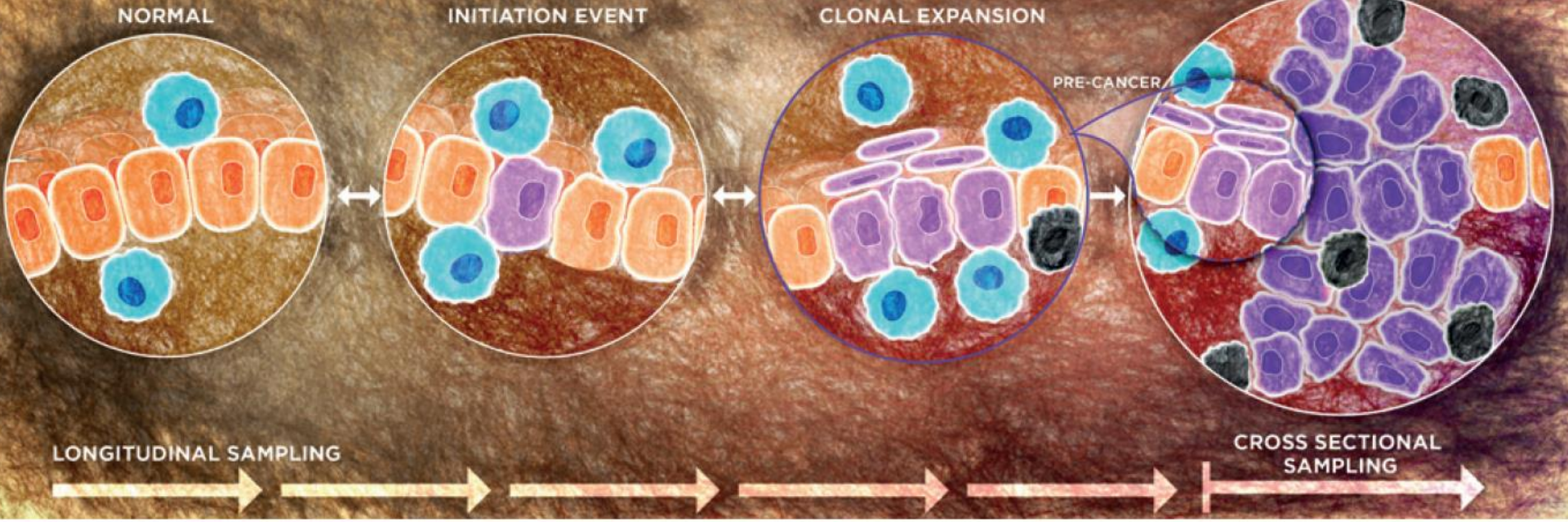

Lesiones Premalignas en Adenocarcinoma de Pulmón: Caracterización Patológica, Molecular e Immunológica.

THE UNIVERSITY OF TEXAS
MD Anderson Cancer Center
Making Cancer History®

XXIV Congress Sociedad Chilena de Anatomia Patologica (SCHAP)
November 11-13, 2020, Virtual Meeting, Santiago, Chile

Ignacio I. Wistuba, M.D
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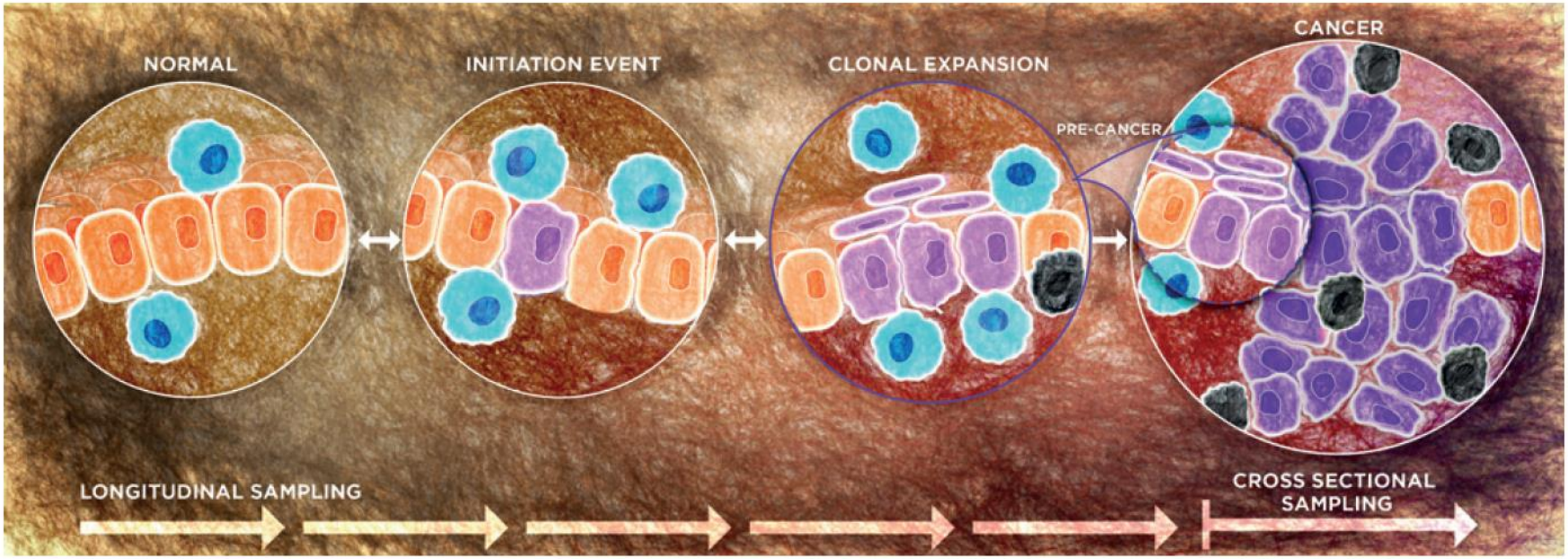
Disclosures

- **Advisory Board:** Genentech/Roche, Bayer, Bristol-Myers Squibb, Astra Zeneca/Medimmune, Pfizer, HTG Molecular, Asuragen, Merck, GlaxoSmithKline, Guardant Health, Oncocyte, and MSD.
- **Speaker**: Medscape, MSD, Genentech/Roche, Platform Health, Pfizer, AstraZeneca, Merck
- **Research support**: Genentech, Oncoplex, HTG Molecular, DepArray, Merck, Bristol-Myers Squibb, Medimmune, Adaptive, Adaptimmune, EMD Serono, Pfizer, Takeda, Amgen, Karus, Johnson & Johnson, Bayer, Iovance, 4D, Novartis, and Akoya.

Putting focus on Lung Pre-cancer Studies

Identification of individuals at highest risk for cancer

Early detection (improvement on clinical diagnostic methods)



(chemo/immune) Prevention

Modified from Campbell et al, Cancer Prev Res, 2016

Pivotal Descriptions - Field of Injury in the Lung

Bronchial Gene Expression Classifier (AEGIS-1 and -2)

Pre-Cancer Atlas (PCA)

2016

2015

2013

2008

Spatial – Localized FC (Genomic)

Spatial – Localized FC (Transcriptome)

Temporal Field of Cancerization (FC) (Transcriptome)

Trisomy 20 observed in the bronchial brushings from smokers and former uranium miners with and without lung cancer (6).

Alterations in anti-oxidant gene expression described in the bronchial epithelium of healthy smokers (34).

Smoking-induced bronchial gene expression changes are related to those in the nasal and oral epithelium (36).

pre-1996

1996

1997

1998

1999

2000

1944-1953: The first descriptions of field cancerization in patients with oral cancer (1-2).
1970: The first description of multifocal lung tumors in dogs exposed to cigarette smoke (42).

LOH and microsatellite alterations known to occur in lung tumors are described in dysplastic and histologically normal bronchial biopsies of smokers without lung cancer (15-16).
P53 point mutation is observed in the metaplastic and atypical bronchial epithelium of subjects without lung cancer (14).

Allelic loss is found in the bronchial epithelium of smokers undergoing diagnostic bronchoscopy for lung cancer (31).

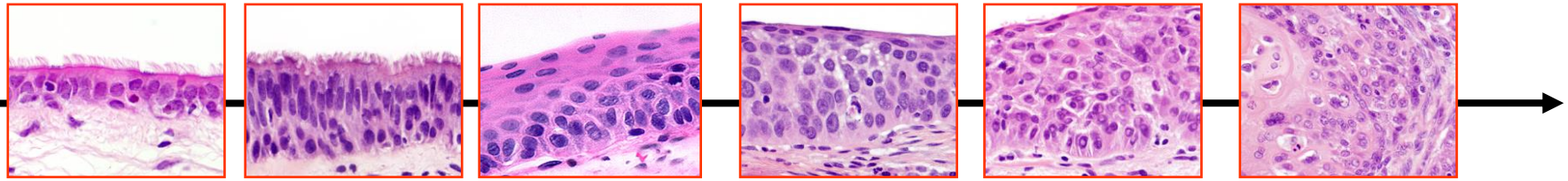
EGFR mutations are found more frequently in normal appearing bronchial epithelium within tumor than adjacent sites (17).
Increased telomerase expression is described in normal bronchial epithelum adjacent to tumor (21).

- *Revised from Steiling et ai Cancer Prev Res, 2010*
- *Kadara et al Cancer Prev Res, 2013; Kadara et al, J Natl Cancer Inst, 2015; Jakubek et al, Cancer Res, 2016*
- *Silvestri et al, New England J Med, 2015*
- *Kensler et al, Cancer Prev Res, 2016; Spira et al, PNAS, 2016*
- *Campbell et al, Cancer Prev Res, 2016*

Cancer

Sequential Pathogenesis of Lung Cancer

Central Compartment (bronchial structures)



Normal Epithelium

Basal Cell Hyperplasia

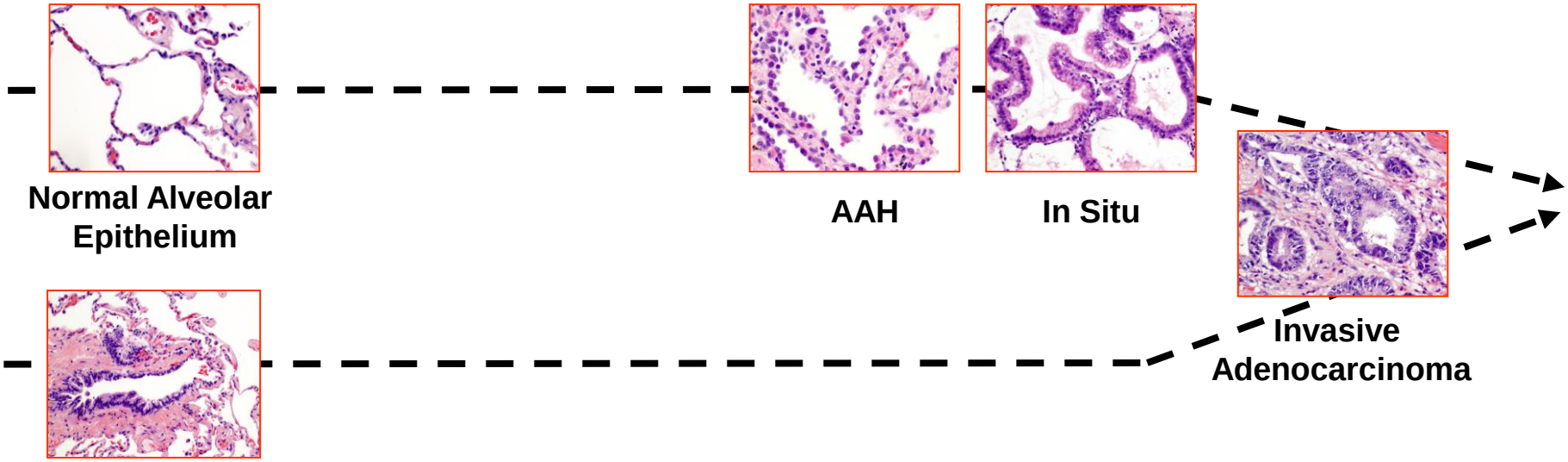
Squamous Metaplasia

Dysplasia

Carcinoma *In Situ*

Squamous Cell Carcinoma

Peripheral Compartment (bronchiolo-alveolar structures)



Normal Alveolar Epithelium

AAH

In Situ

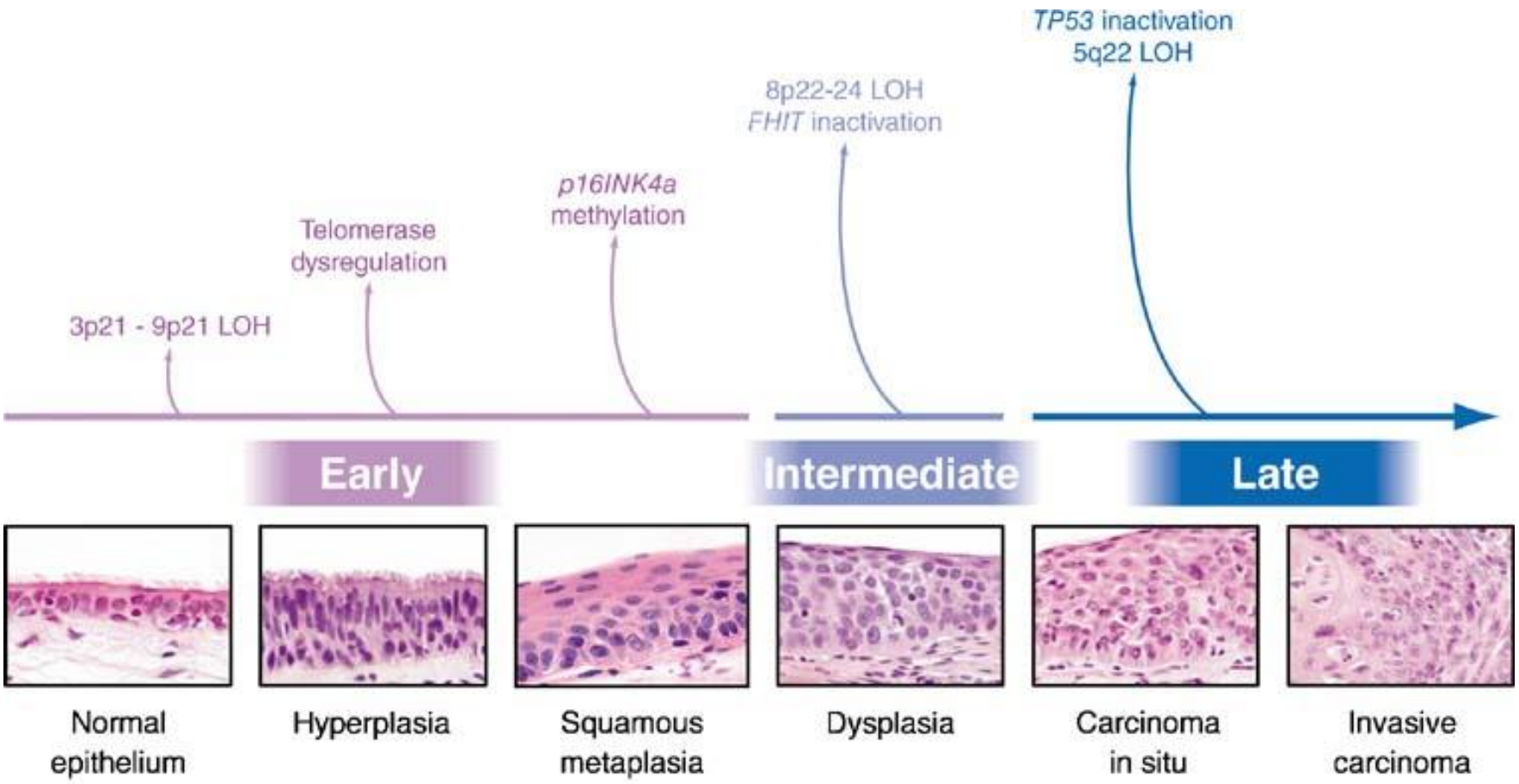
Invasive Adenocarcinoma

Normal Bronchiolar Epithelium

Cancer

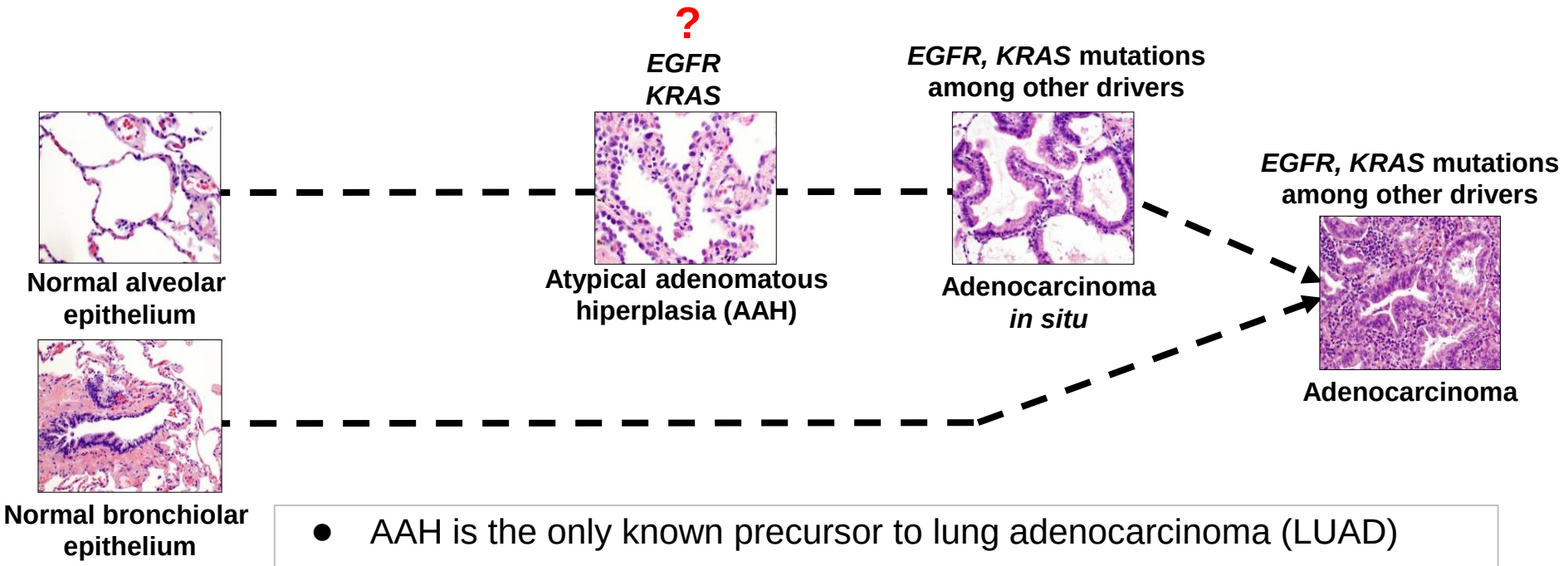
Wistuba, 2006 & 2014

Squamous Cell Lung Carcinoma Premalignacy Pathogenesis



Wistuba, Annu Rev Pathol Mech Dis, 2006

Lung Adenocarcinoma Premalignancy Pathogenesis



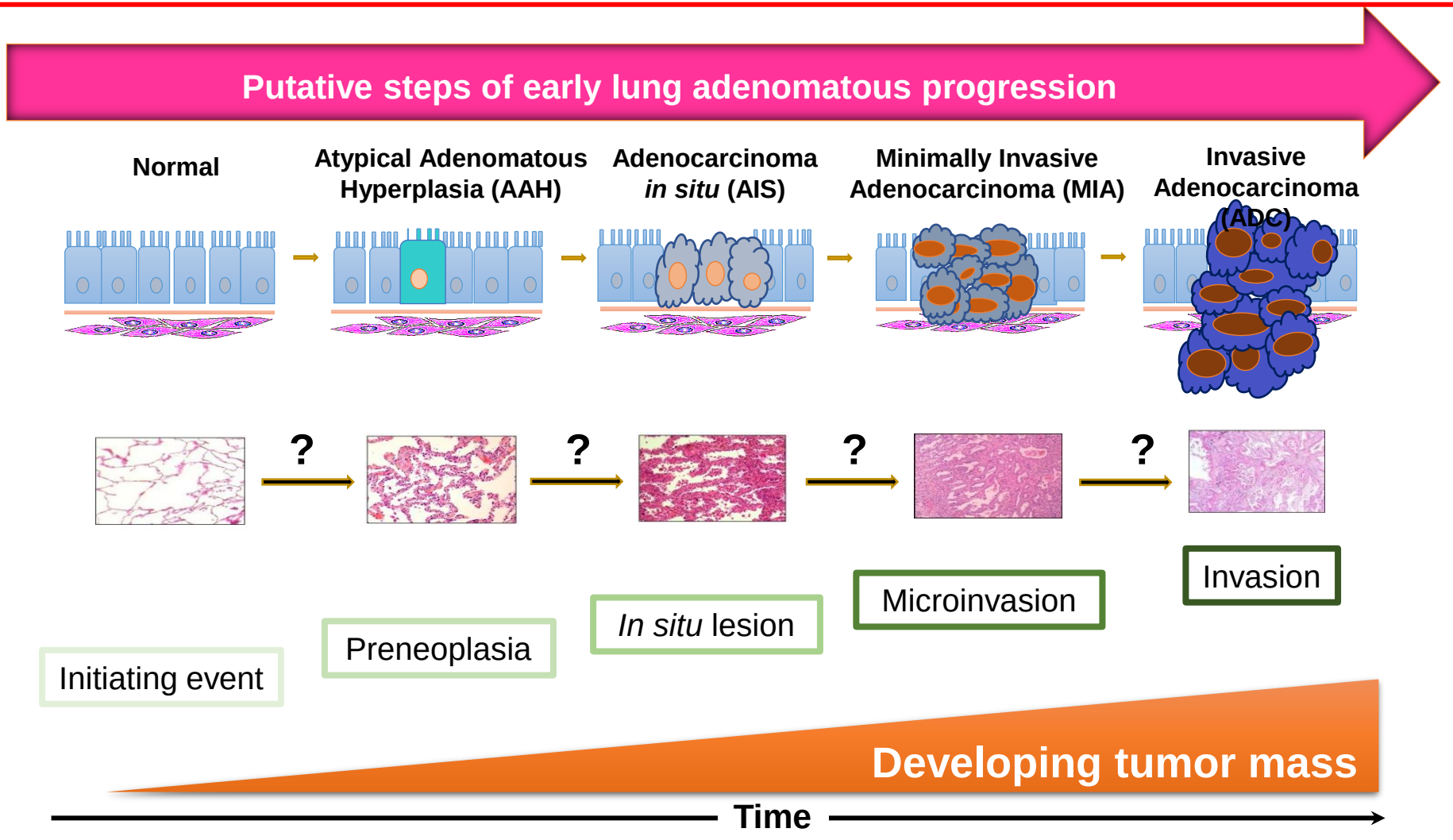
- AAH is the only known precursor to lung adenocarcinoma (LUAD)
- Molecular alterations in AAH
  - Mutations: *EGFR, KRAS*
  - LOH: 3p, 9p, 16p, 17q and 17p
  - Limited evidence on gene expression, epigenetic modification, and methylation

The role of AAH as precursor lesions to LUAD still remains poorly understood.

Cancer

Wistuba, Annu Rev Pathol Mech Dis, 2006

Rudimentary Knowledge in Lung Adenocarcinoma Carcinogenesis



Travis, et al. J Thorac Oncol (2016); Noguchi, Cancer Metastasis Rev (2010)

Lung Adenocarcinoma Premalignancy Outline of Presentation

- Lung field of cancerization
- Genomic evolution of premalignant lesions to malignancy
- Role of immune response in premalignancy to malignancy evolution

Lung Cancer Premalignancy Team

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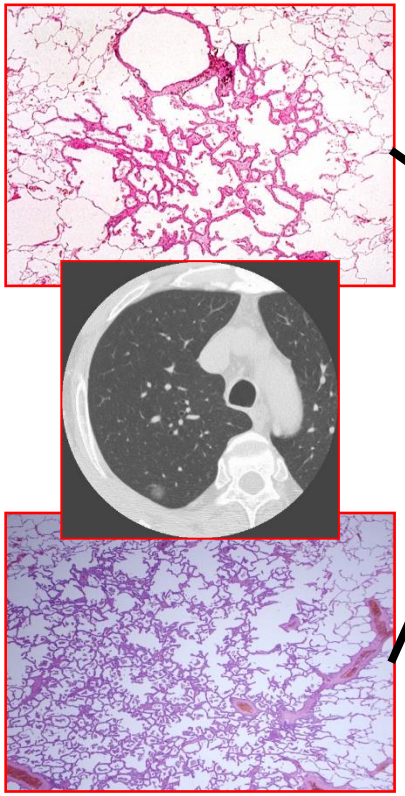
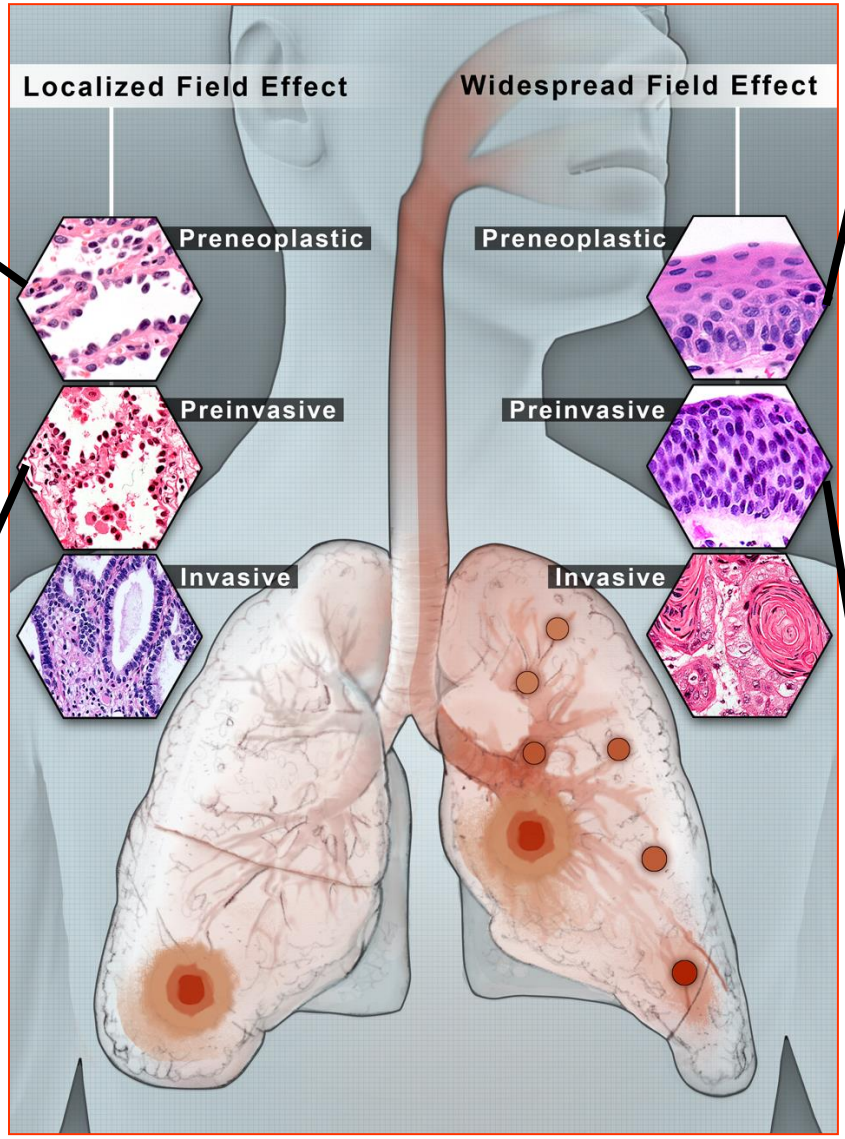
Cancer

Widespread and Localized Field Cancers in Lung Cancer Patients and Smokers

Adenocarcinoma

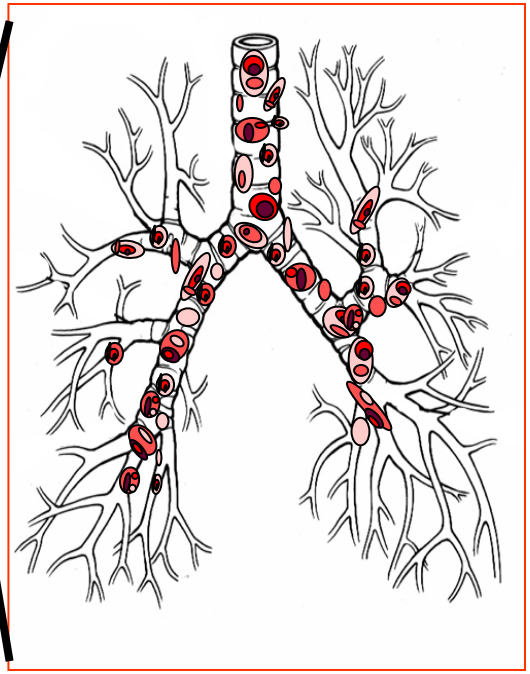


AAH in 23-35% of adenocarcinomas vs. 7-3% of squamous *(Nakanishi et al Br J Cancer, 1990; Chpaman et al, Br J Cancer, 2000)*



Modified from Gazdar et al, 2009

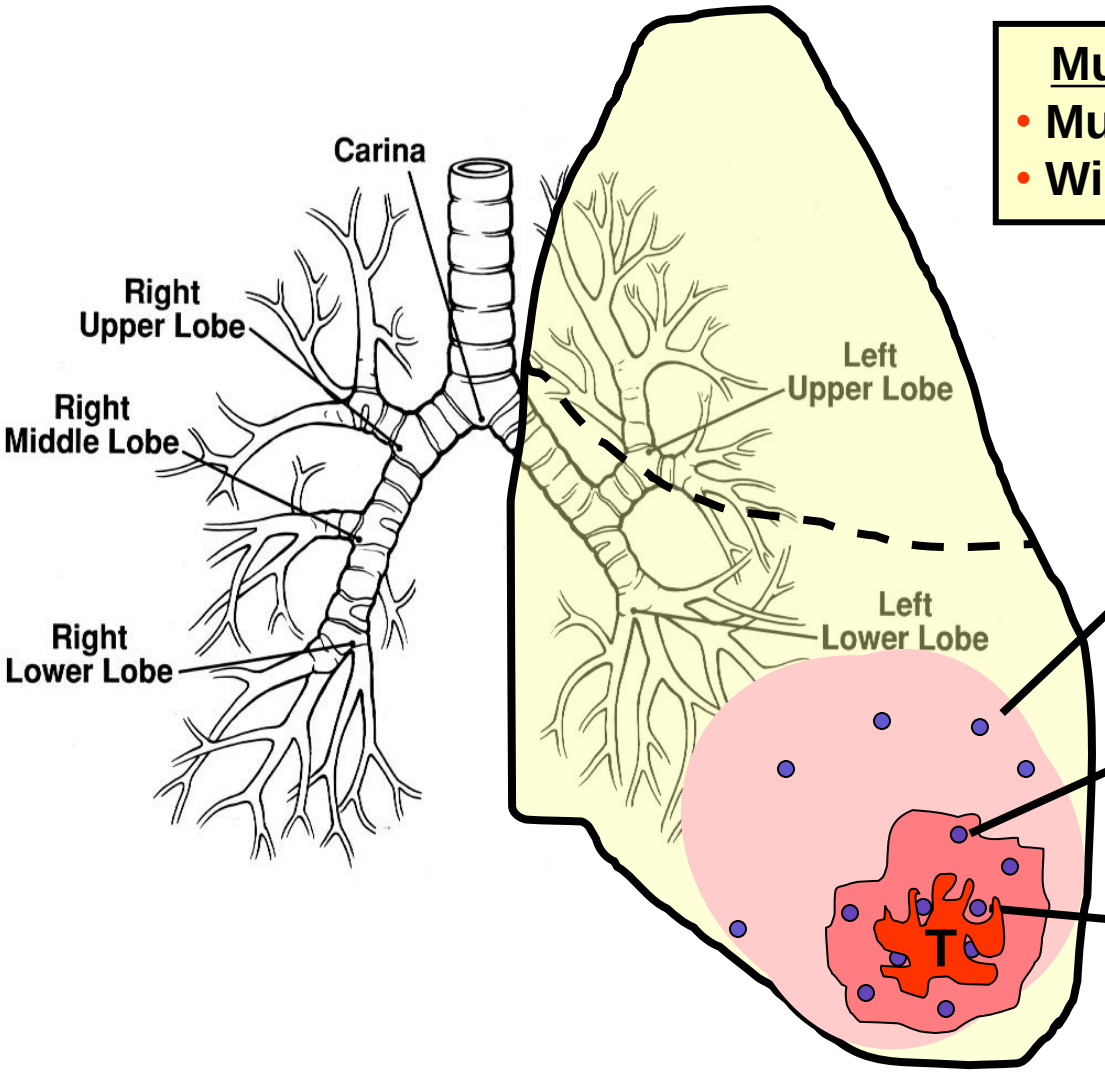
Squamous Cell Ca



Squamous dysplasia in central airway in 40% smokers *(Auerbach et al, 1962 & 1979)*

Cancer

Localized Field Effect in Lung Peripheral Airway *EGFR* Mutation Clonal Patches



Mutation in Normal Epithelium

- Mutant Tumors: 12/24 (50%)
- Wild-Type Tumors: 3/26 (12%)

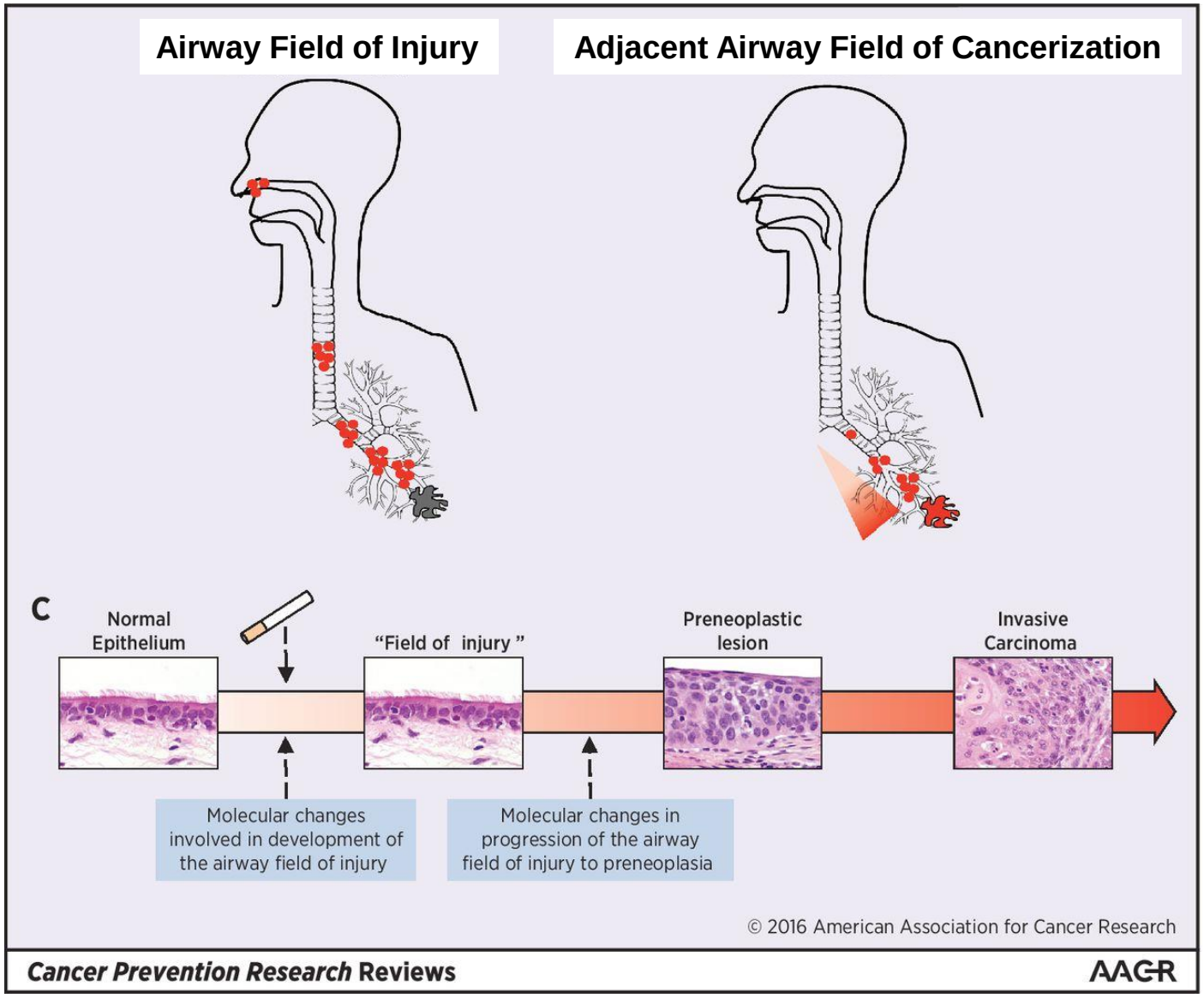
Distant
2/32 (6%)

Adjacent
13/63 (21%)

Inside
13/46 (28%)

Tang et al., Cancer Res 65: 7568, 2005
Tang et al., Cancer Prev Res, 1: 192, 2008

Premalignant Airway Fields in the Molecular Pathogenesis of Lung Cancer

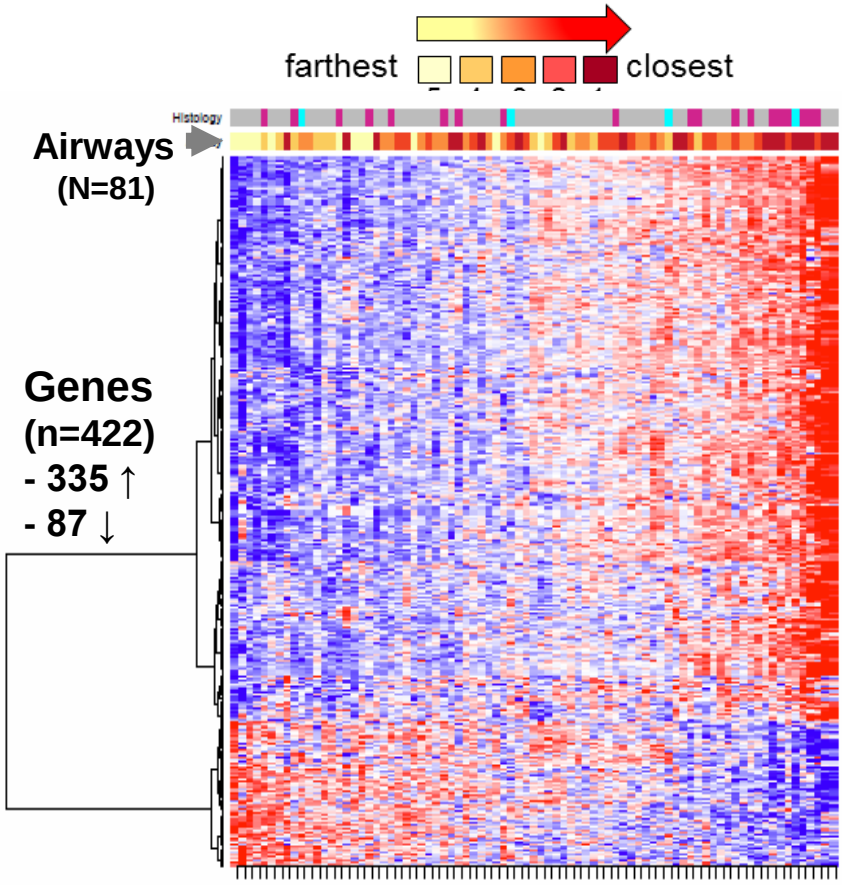


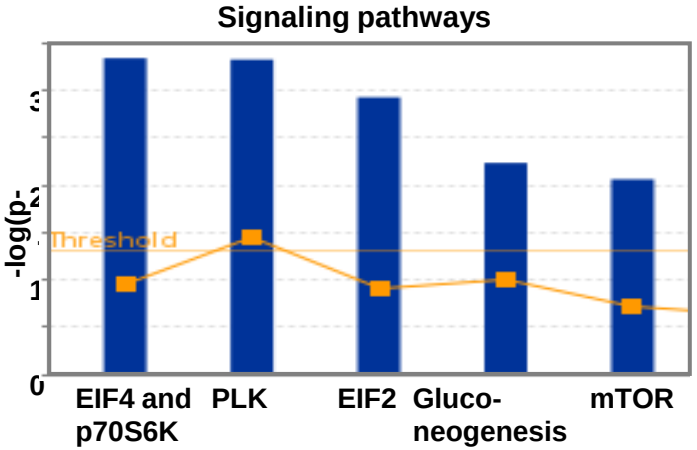
Modified from H. Kadara et al. Cancer Prev Res 2016;9:518-527

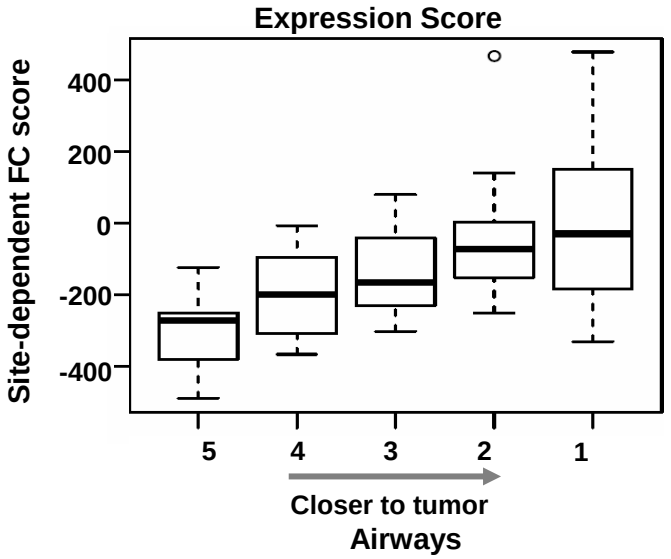
Transcriptome Architecture of the Field of Cancerization in Lung Cancer

NSCLC

Genes (n=422) modulated by distance from airway 5 (farthest) to airway 1 (closest)





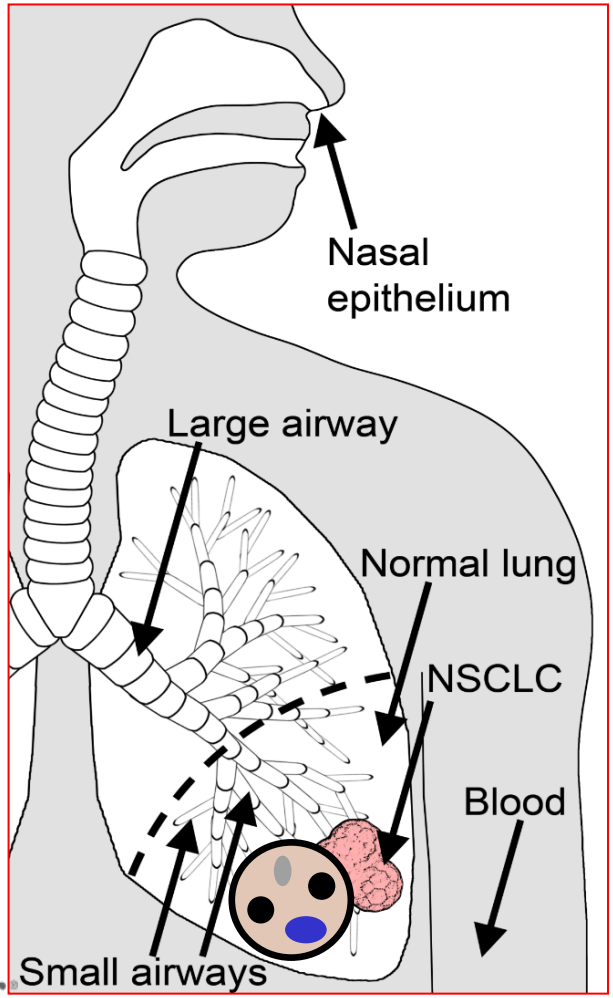


Cancer

Kadara et al, J Natl Cancer Inst, 2014

Comprehensive Profiling of the Airway Field in Early-stage NSCLC

Intra-tumoral and intra-field genomic architecture

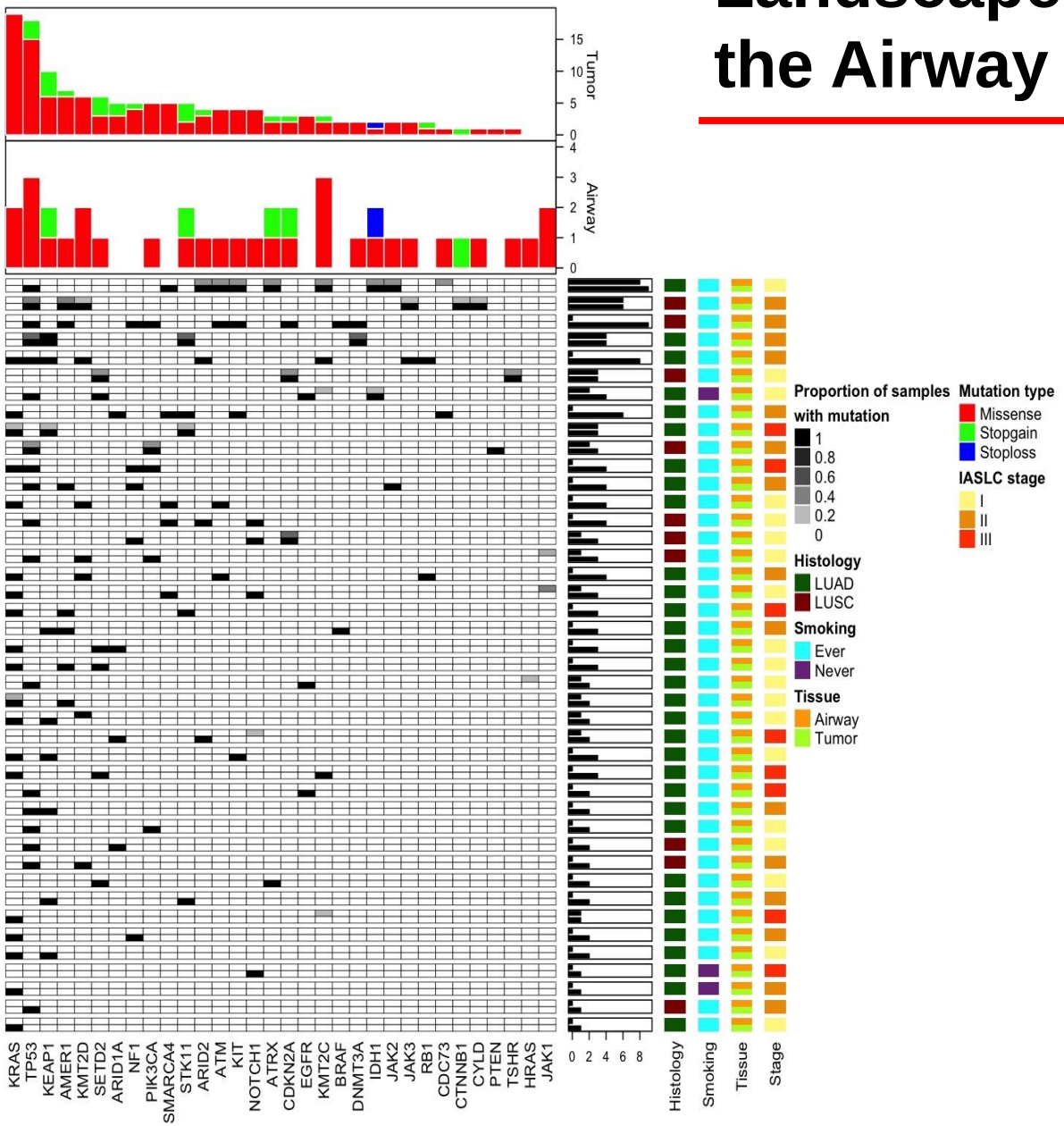


- 498 samples from 48 early-stage NSCLCs (37 LUADS and 11 LUSCs; 42 lifetime smokers, 6 non-smokers)
- Multiple spatially distributed airway samples
- Resected NSCLC sections (n = 47) and 3 – 8 multi-region lung tumor biopsies per case (tumor heterogeneity; n = 28)
- Normal white blood cells (n = 42) and uninvolved normal lung parenchyma

- Genome-wide SNP arrays profiling (Illumina)
- Ultra-deep (average depth ~1,200X) targeted sequencing of a panel of ~400 genes
- Genome-wide methylation profiling

Jakubek Y et al, Cancer Research, 2016

Landscape of Mutations in the Airway Field in NSCLC



Tissue	Cancer Genes*+
Small Airway	*AMER1*, *ARID2*, *ATM*, *ATRX*, *CDC73*, *CDKN2A*, *CTNNB1*, *CYLD*, *DNMT3A*, *HRAS*, *IDH1*, *JAK1*, *JAK2*, *JAK3*, *KEAP1*, *KIT*, *KMT2C*, *KMT2D*, *KRAS*, *NOTCH1*, *STK11*, *TP53*
Large Airway	*CDKN2A, PIK3CA, SETD2, TP53, TSHR*
Normal Lung	*RB1, RET, TSHR*
Nasal Epithelium	*AKT1*

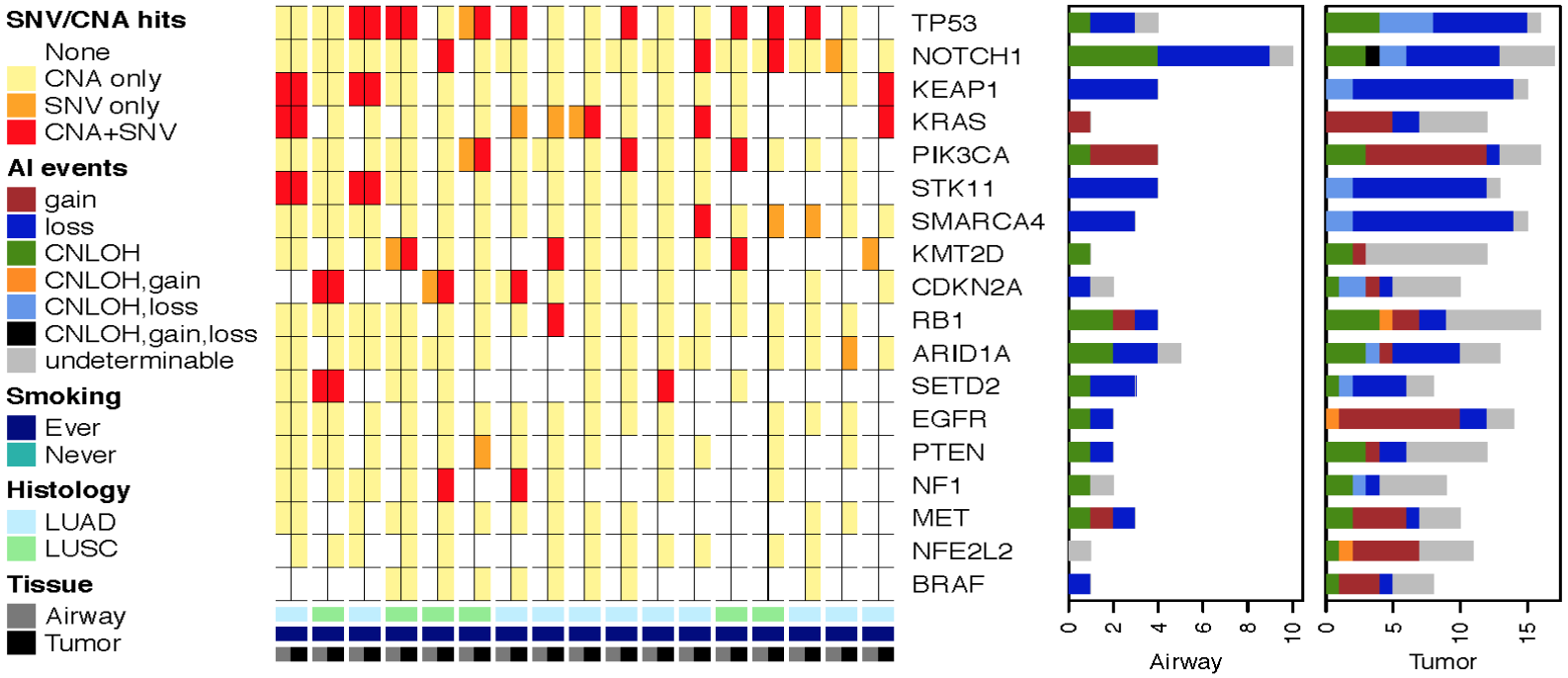
*TCGA LUAD, LUSC, putative cancer drivers
+Genes overlapping with corresponding tumor

There are shared driver mutations between tumors and adjacent/distant normal cells in the airway field of

Kadara H, Sivakumar S, Scheet P, American Journal of Respiratory and Critical Care Medicine, 2019

Cancer

Early “two-hit” Alterations in the Airway Field and NSCLC



Tissue	Two-hit cancer associated genes*+ in the airway field
Small Airway	*KEAP1*, *KRAS*, *STK11*, *TP53*
Large Airway	*CDKN2A*, *SETD2*, *TSHR*

*TCGA LUAD, LUSC
+Genes overlapping with corresponding tumor

First shared hit/second hit	Genes*+ with shared single hit acquiring second hit in matched tumors
SNV then AI	*CDKN2A*, *KRAS*, *PIK3CA*, *TP53*
AI then SNV	*NOTCH1*

*TCGA LUAD, LUSC
+Genes overlapping with corresponding tumor

Cancer

Kadara H, Sivakumar S, Scheet P, American Journal of Respiratory and Critical Care Medicine, 2019

Lung Adenocarcinoma Premalignancy
Lung field of cancerization - Summary

- Modern genomic technologies have confirmed previous studies, and better characterized a spatial filed of cancerization (*mutations, allelic imbalances, cancer-related gene expression signatures*) in the airway of patients with NSCLC.
- Shared mutations and allelic imbalances between non-malignant airway field and corresponding NSCLC tumors have been identified.
- Oncogenic “two-hit” alterations are detected in the airway field in patients with NSCLC.
- The field of injury in lung adenocarcinomas (peripheral) is different than in squamous cell carcinomas (central)

Collection of Lung Premalignant and Malignant Lesions (China/Japan)

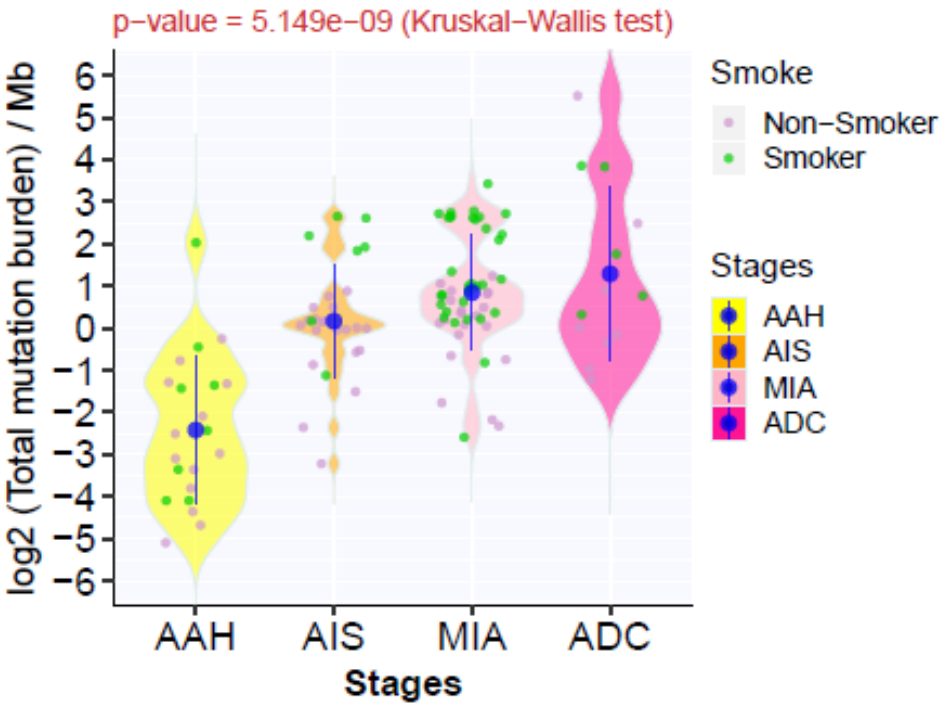
Lesions	Numbers of cases	Number of Lesions
AAH/ADC	3	6
AIS/ADC	3	8
MIA/ADC	3	4
AAH/AIS	3	9
AAH/MIA	3	9
AIS/MIA	2	7
AAH/AIS/MIA	1	8
AAH	5	8
AIS	7	15
MIA	20	42
Total	50	116

- 116 lesions from 53 patients (38 patients with multifocal diseases - 9 patients with invasive lung adenocarcinomas and pre-invasive neoplastic lesions; 9 patients with 2 or 3 types of pre-invasive neoplastic lesions and 20 patients with one type of pre-invasive lung lesions.

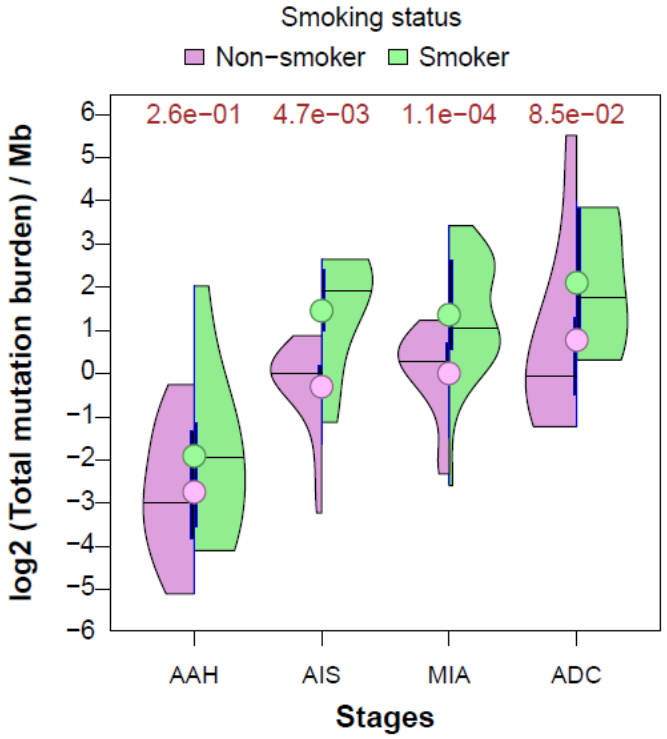
Hu et al, Nature Communications, 2019

Progressive Increase in Mutations from AAH to Invasive Lung Adenocarcinoma Whole Exome Sequencing (WES)

Mutation Burden



Mutation Burden By Smoking Status

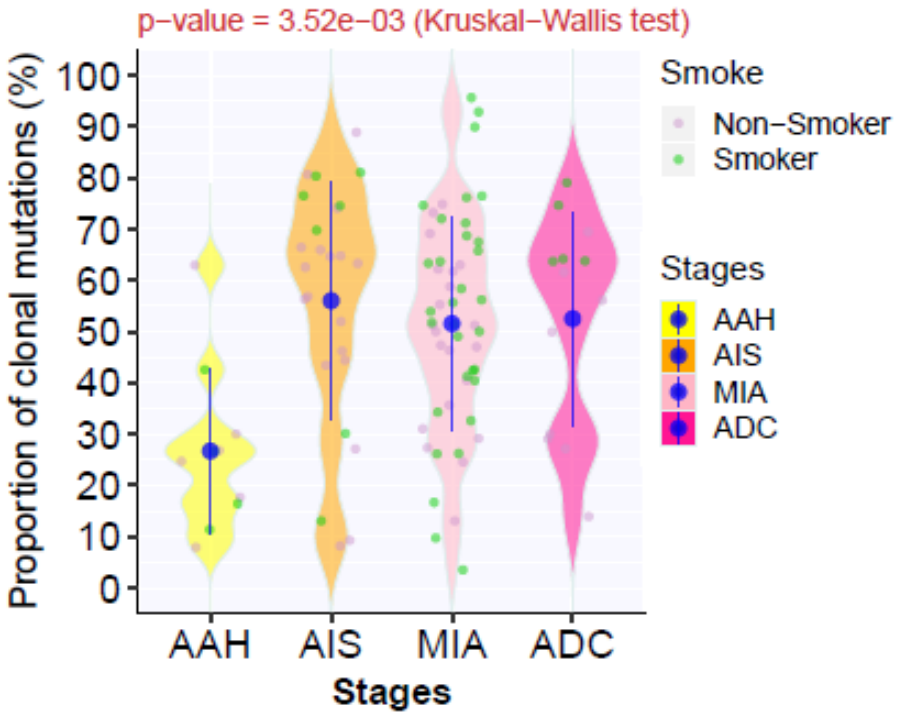


Cancer

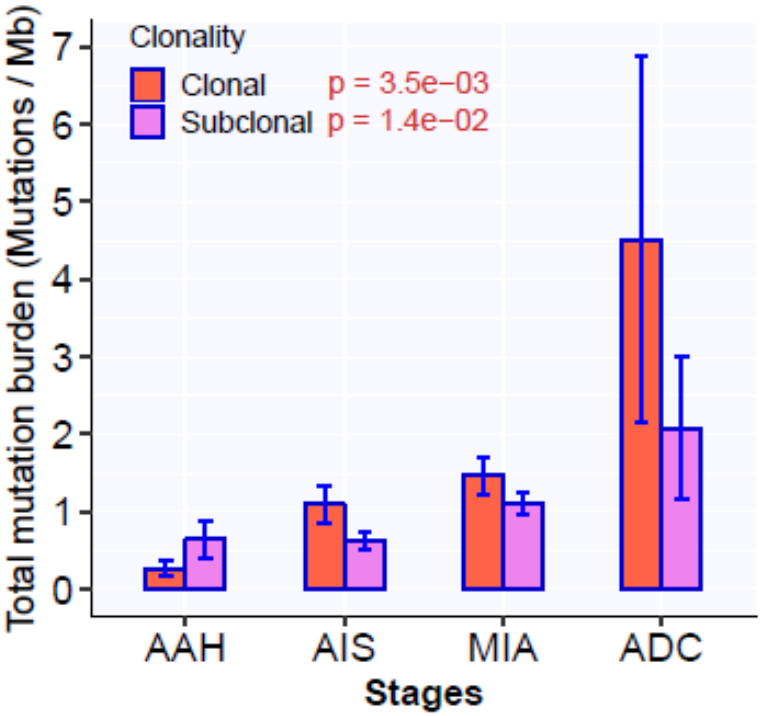
Hu et al, Nature Communications, 2019

Progressive Increase in Clonal and Subclonal Mutations From AAH to Invasive Adenocarcinoma

Proportion of Clonal Mutations



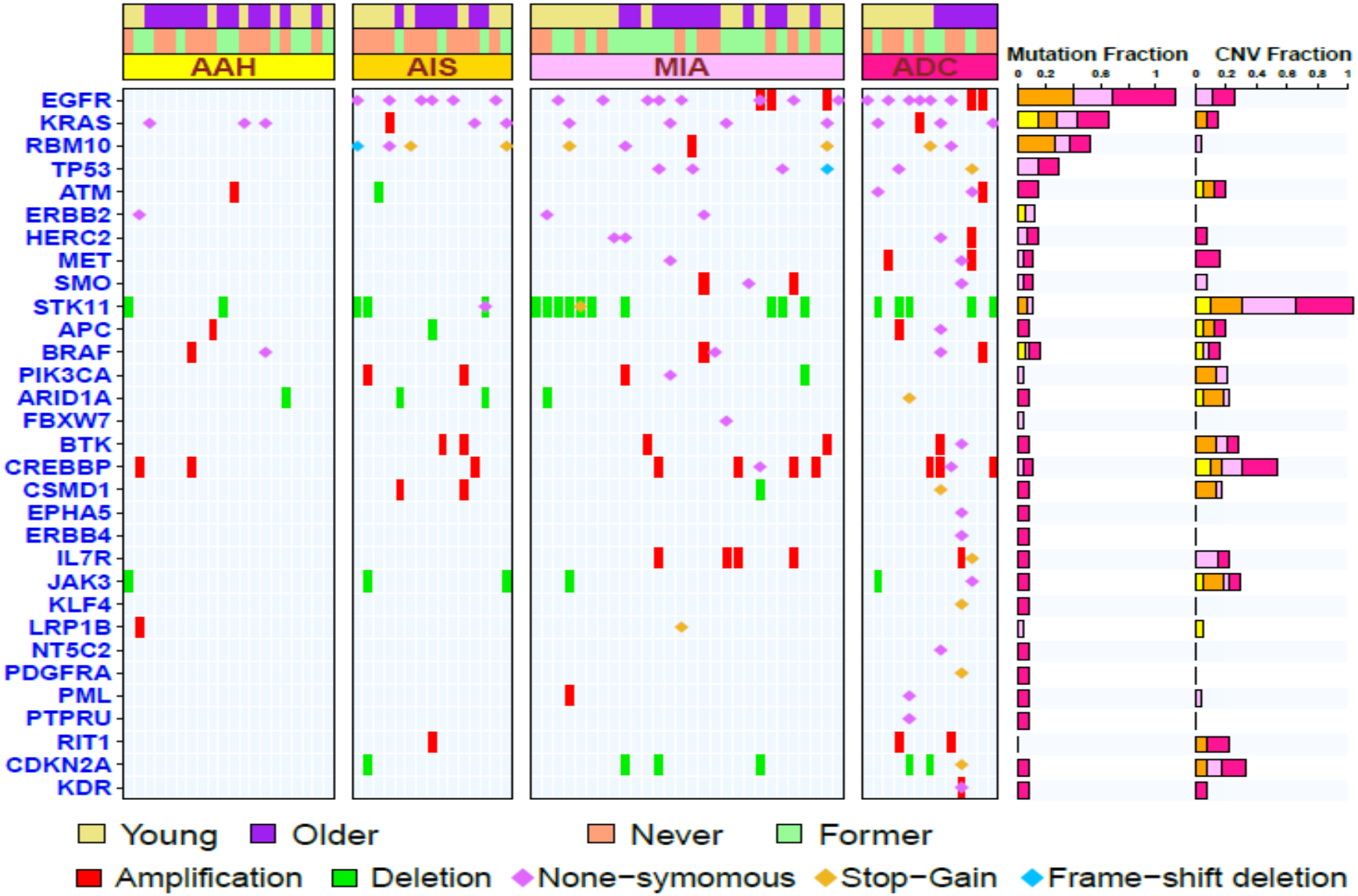
Clonal vs Sub-clonal Distribution



Cancer

Hu et al, Nature Communications, 2019

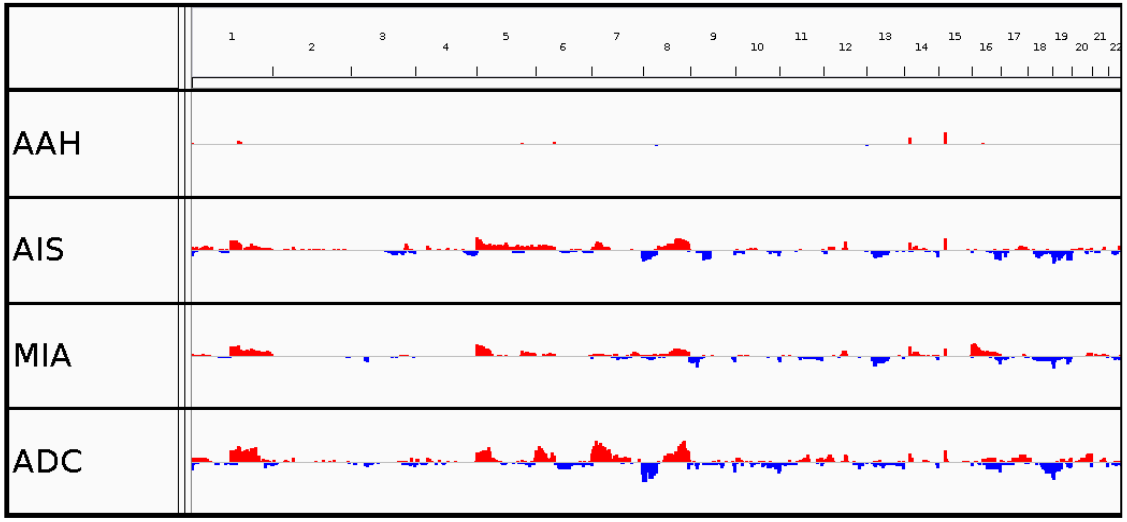
Commonly Mutated Cancer Genes - WES



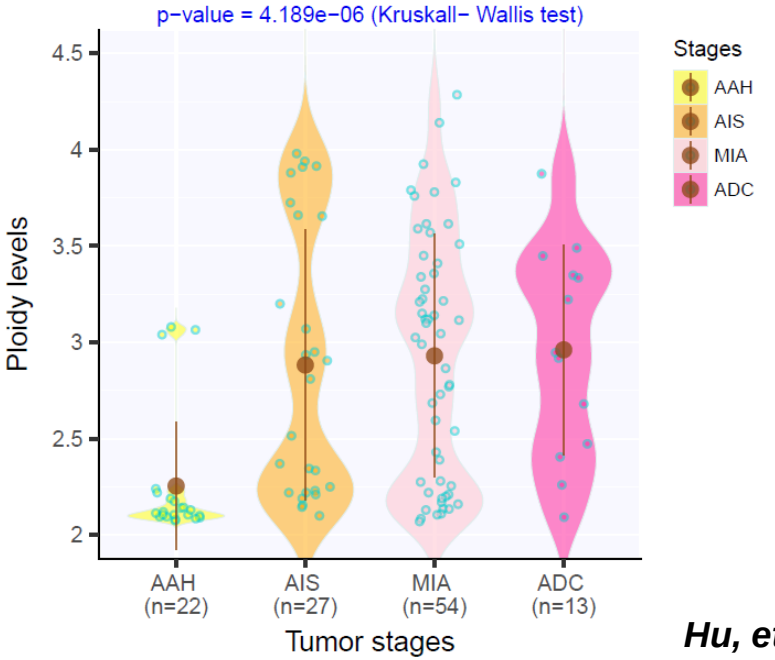
Cancer

Hu et al, Nature Communications, 2019

Ploidy Change at Transition from AAH to AIS - WES

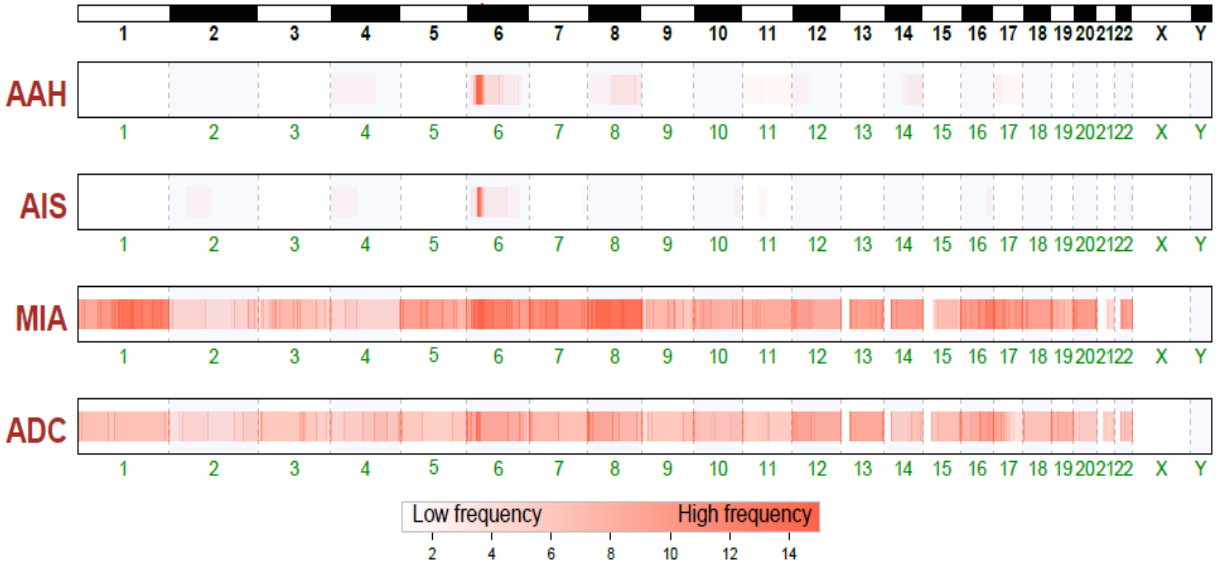


Ploidy Levels

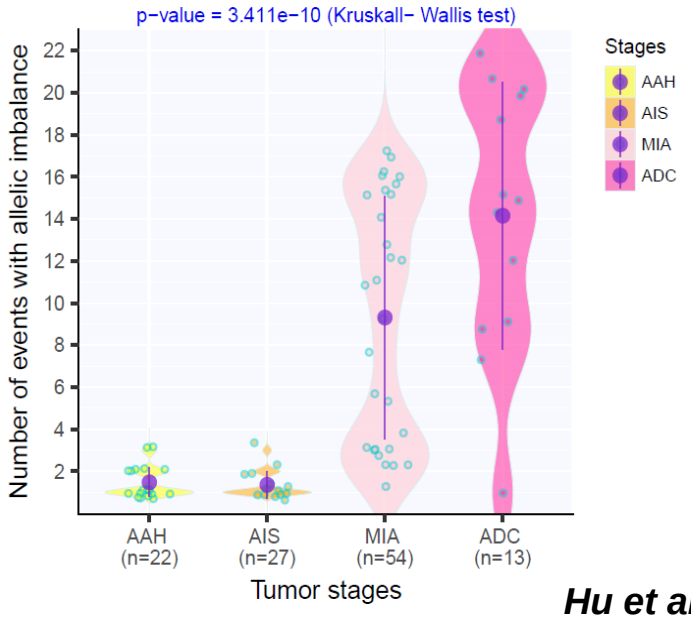


Hu, et al, Nature Communications, 2019

Allelic Imbalance Transition From AIS to MIA - WES



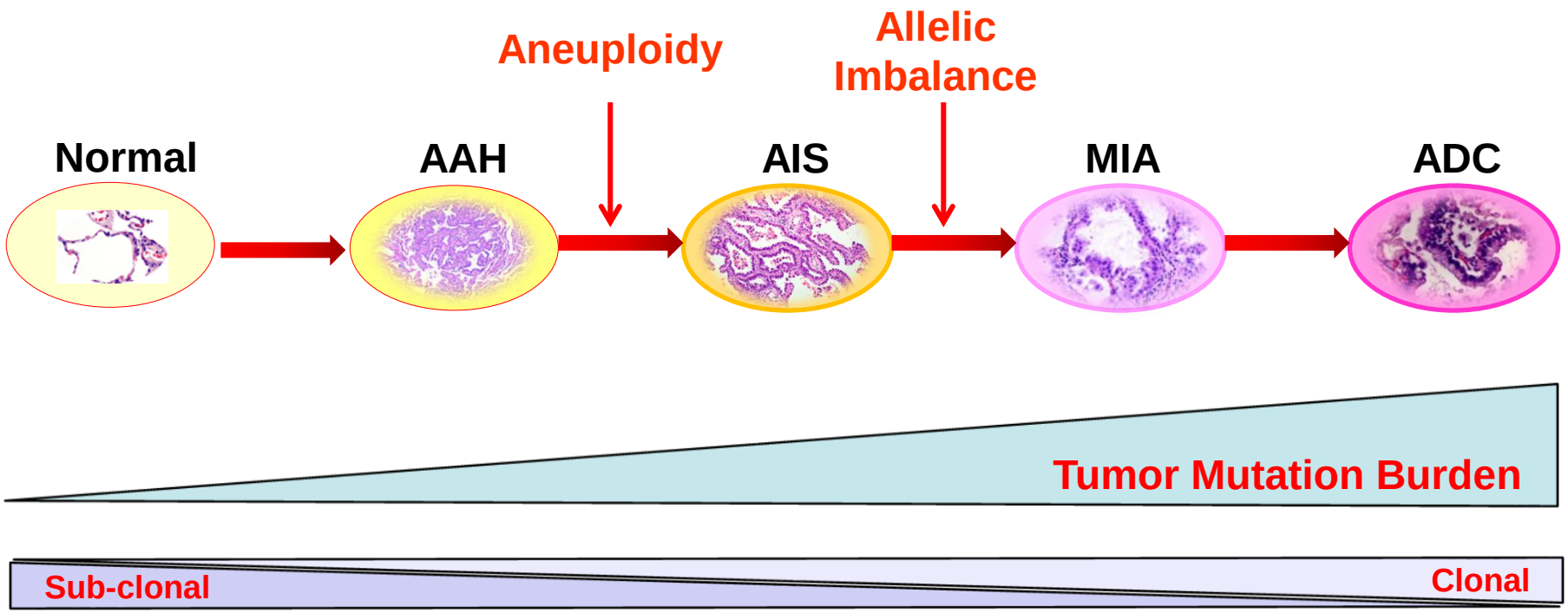
Number of Events with Allelic Imbalance



Cancer

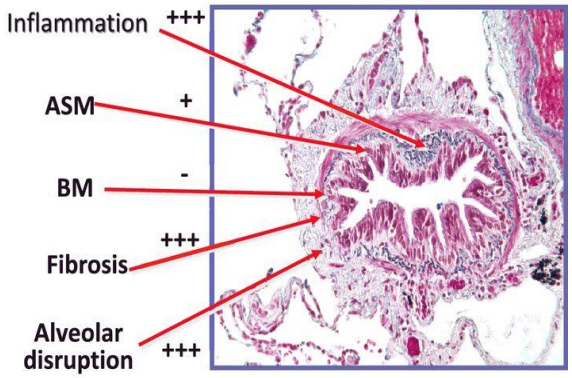
Hu et al, Nature Communications, 2019

Genomic Evolution Model from AAH to Invasive Adenocarcinoma



Modified from Hu et al, Nature Communications, 2019

Inflammation in COPD



Cigarette smoke (and other irritants)

Epithelial cells

Macrophage

CXCL9
CXCL10
CXCL11

TGF-β
FGF

IFNγ

TNF-α
IL-1β
IL-6

IL-23

CXCL1
CXCL8
CXCL5

CCL2

Fibroblast

CXCR3

CXCR2

CCR2

Monocyte

CTGF

Th1

Tc1

Th17

Neutrophil

Perforin

PROTEASES

Neutrophil elastase
MMP-9 (MMP12)

EGF
TGF-α

Fibrosis
(Small airways)

Alveolar wall destruction
(Emphysema)

Mucus hypersecretion
Chronic bronchitis

Cancer

Peter J. Barnes Clin. Sci. 2017;131:1541-1558

Comprehensive Immune and Genomic Profiling Lung Adenocarcinoma Pathogenesis

- Atypical Adenomatous Hyperplasia (AAH), n=9
- Adenocarcinoma In Situ (AIS), n=21
- Microinvasive Adenocarcinoma (MIA), n=21
- Invasive Adenocarcinoma (ADCA), n=6

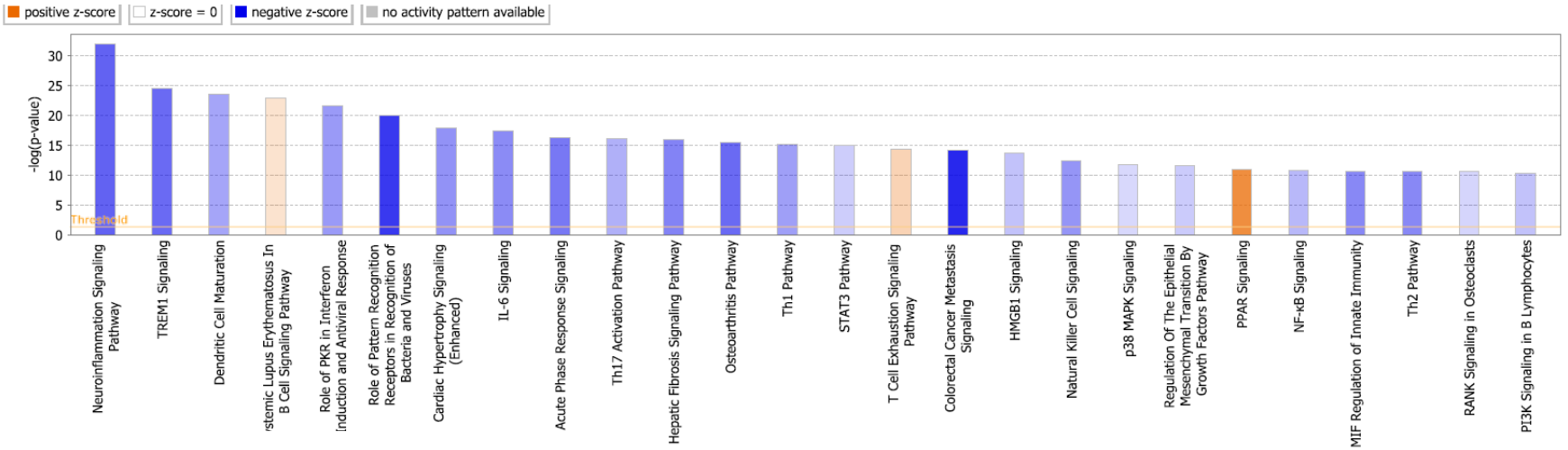
Cancer

H. Dejima, Xin Hu et al, MD Anderson Cancer Center (unpublished)

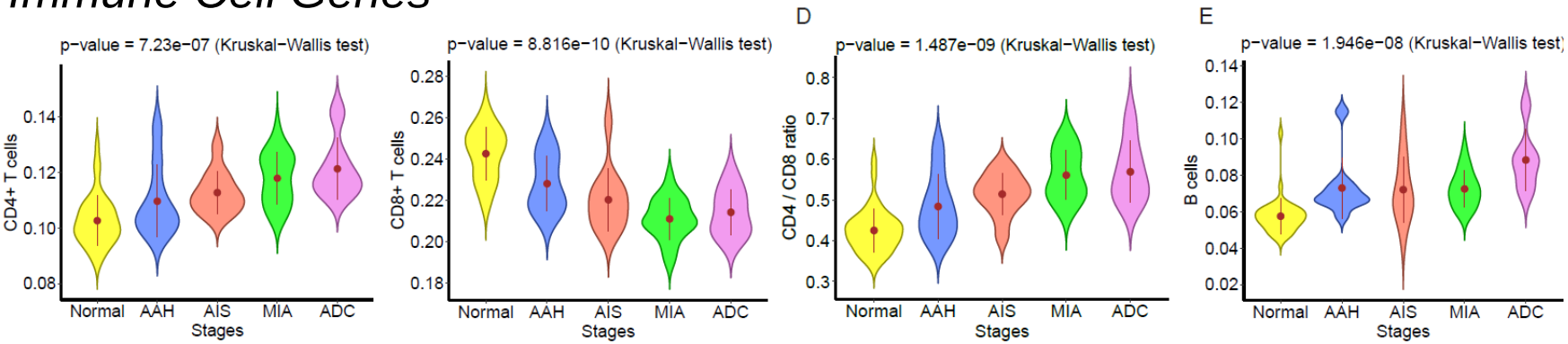
Immune Profiling
Lung Adenocarcinoma Pathogenesis

Nanostring Pan Cancer Immune Profiling Panel (n=770 genes)

Pathways



Immune Cell Genes

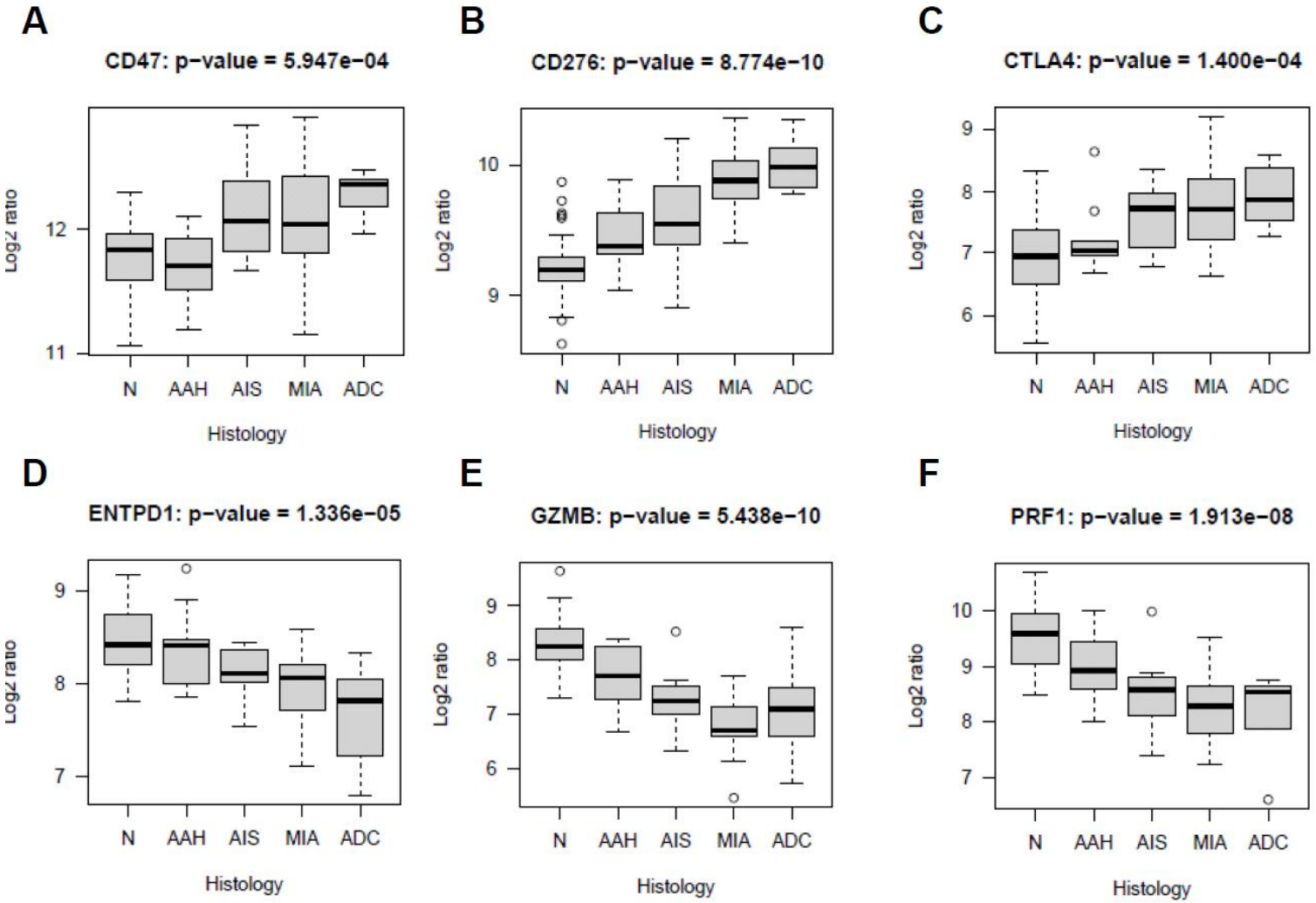


Cancer

H. Dejima, Xin Hu et al, MD Anderson Cancer Center (unpublished)

Immune Profiling
Lung Adenocarcinoma Pathogenesis

- Increase of immune suppressor genes
- Decrease of immune activation genes

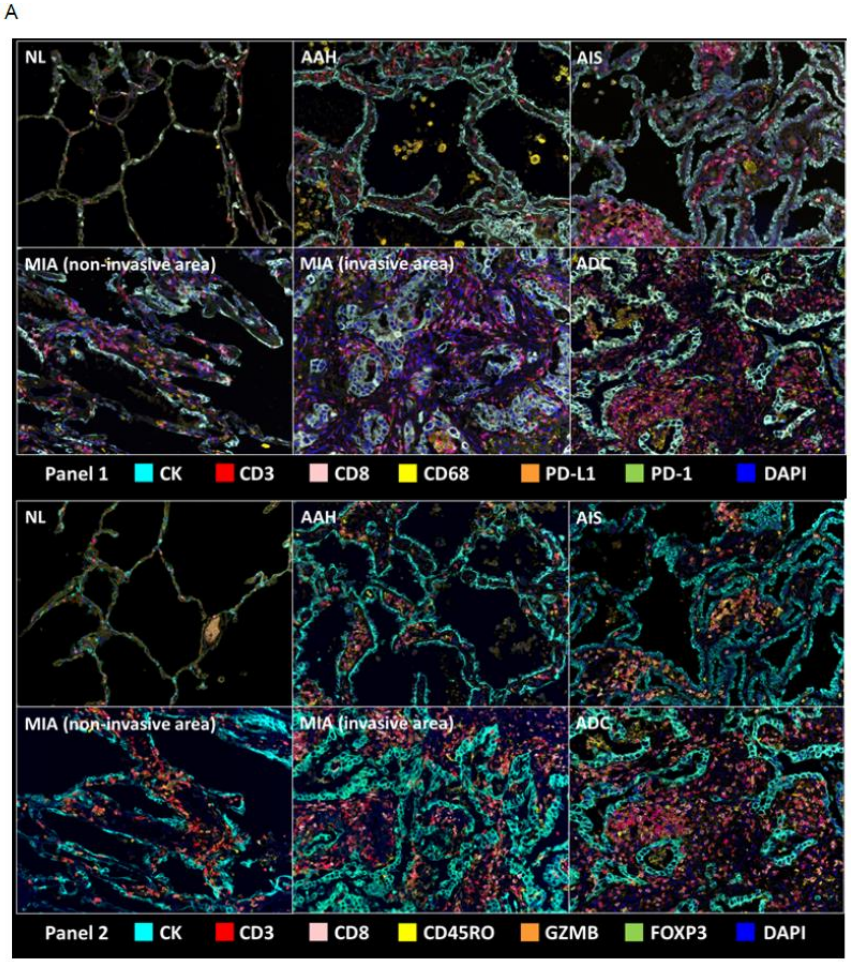


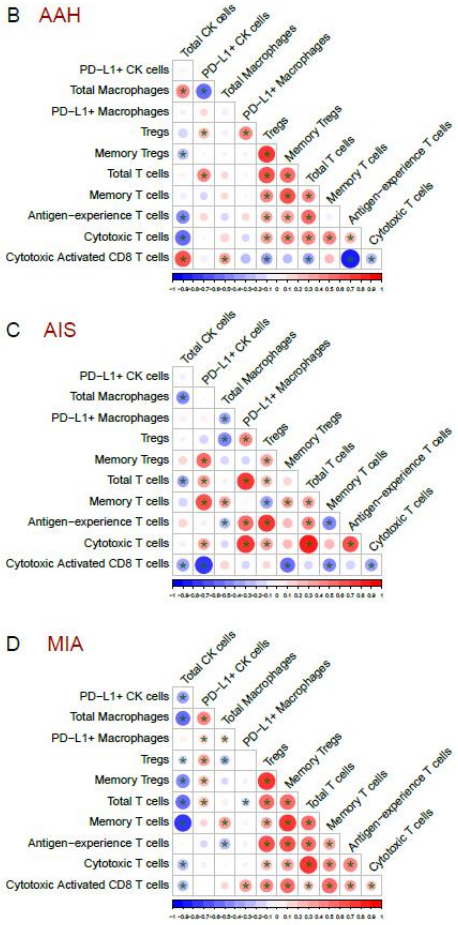
H. Dejima, Xin Hu et al, MD Anderson Cancer Center (unpublished)

Immune Profiling
Lung Adenocarcinoma Pathogenesis

mIF (Vextra/Polaris) Panels 1 and 2

Panel 1	Panel 2
PD-L1	CD3
PD-1	CD8
CD3	Granzyme B
CD8	CD45Ro
CD68	FOXP3
--	--
AE1/AE3	AE1/AE3
DAPI	DAPI

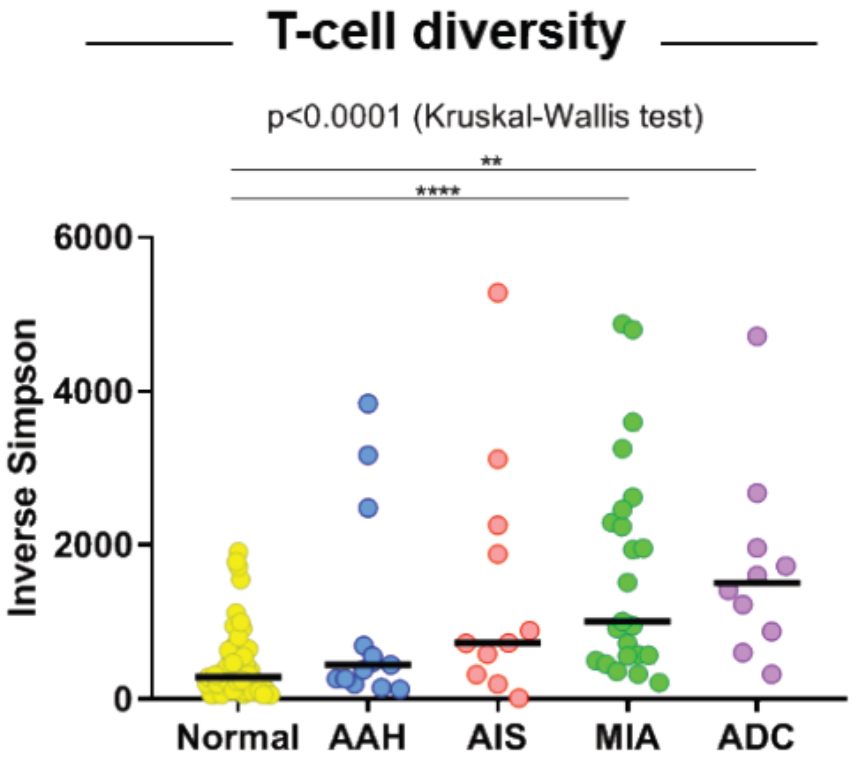


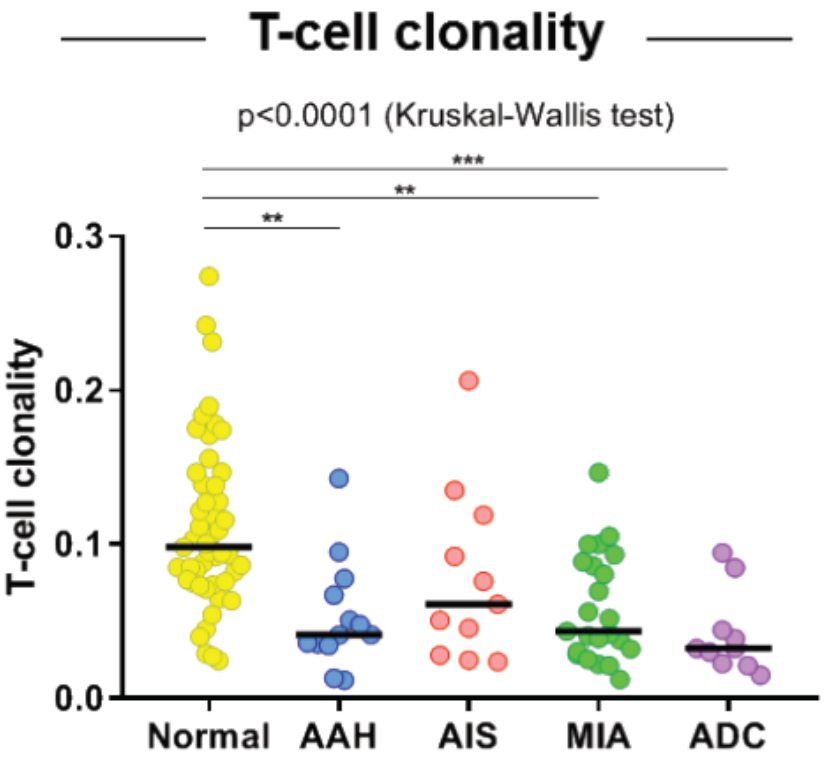


H. Dejima, Xin Hu et al, MD Anderson Cancer Center (unpublished)

Immune Profiling
Lung Adenocarcinoma Pathogenesis

TCR Sequencing (Adaptive)

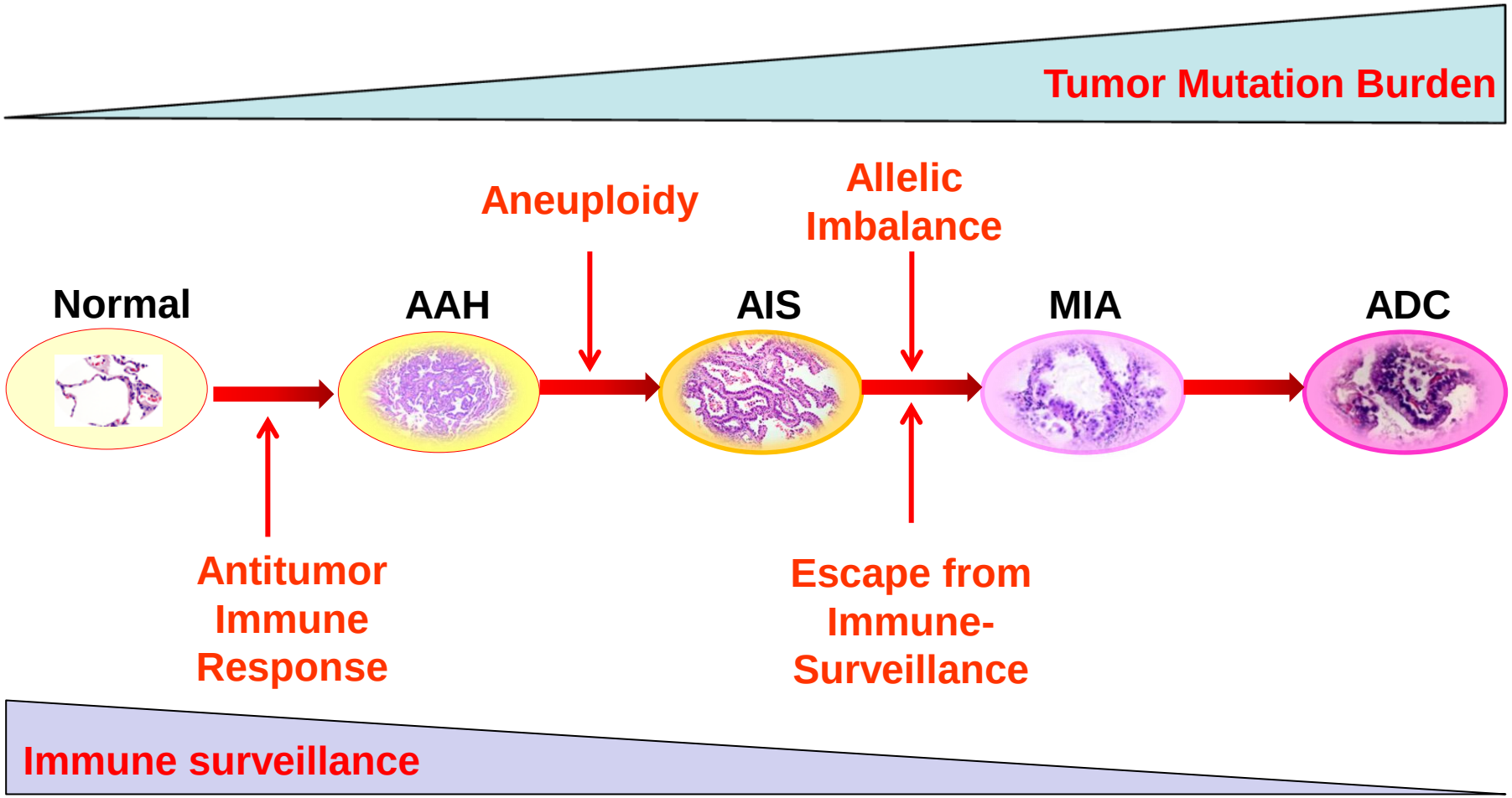




Cancer

H. Dejima, Xin Hu et al, MD Anderson Cancer Center (unpublished)

Genomic Evolution Model from AAH to Invasive Adenocarcinoma



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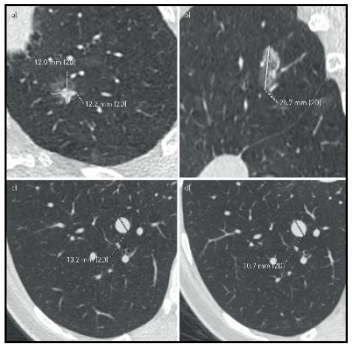
Modified from Hu et al, Nature Communications, 2019

IMPRINT-Lung trial

Randomized phase II trial of Immunotherapy with Pembrolizumab (PD-1) for the Prevention of Lung Cancer



N=81
Eligibility:
- Persistent IPNs with estimated cancer probability >15% if no history of lung cancer
- Persistent IPNs with estimated cancer probability >10% if positive for history of stage I-II NSCLC

Stratification:
- Prior history of lung cancer
- Biopsy

RANDOMIZATION 2:1

Observation N=27 → **CT chest at 3, 6, 12, 18 and 30 months**

Pembrolizumab 4 x 200 mg, Q3W N=54 → **CT chest at 1.5, 3, 6, 12, 18 and 30 months**

Primary Endpoint:
To determine whether immune checkpoint blockade eliminates high-risk IPNs at 6 months after treatment.

Cancer

Jay Zhang et al, MD Anderson Cancer Center, 2018

Lung Adenocarcinoma Premalignancy Summary

- Genomic abnormalities occur early in the multistage pathogenies of lung adenocarcinoma, starting with mutations (normal airway), followed by allelic imbalance and increase tumor mutational burden at invasive stages.
- Immune-response is an early phenomenon in lung adenocarcinoma pathogenesis (AAH), followed by escape from immune-surveillance at invasive stages.
- The discovery of genomic and immune-related abnormalities in the early pathogenesis of lung cancer may provide novel insights in the early diagnosis and interception of lung premalignancy.

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