

ANSWERS



Cancer & Evolution Symposium

RNA Whole-Transcriptome Sequencing in Cancer Diagnostics

Bonnie Anderson

Co-founder, Chairman and Chief Executive Officer

October 16, 2020



Developing clinically useful
genomic tests

That change what
happens next for
patients

... patients like Ashley



Five clinical indications

Addressing unmet
needs throughout
the care continuum

Early Detection

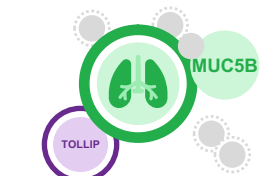
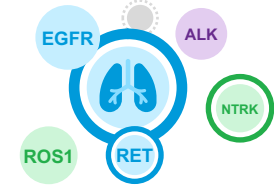
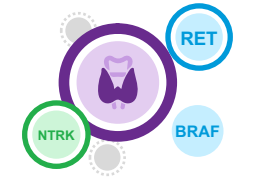


Nasal Swab
Classifier

Diagnosis & Prognosis



Inform Treatment



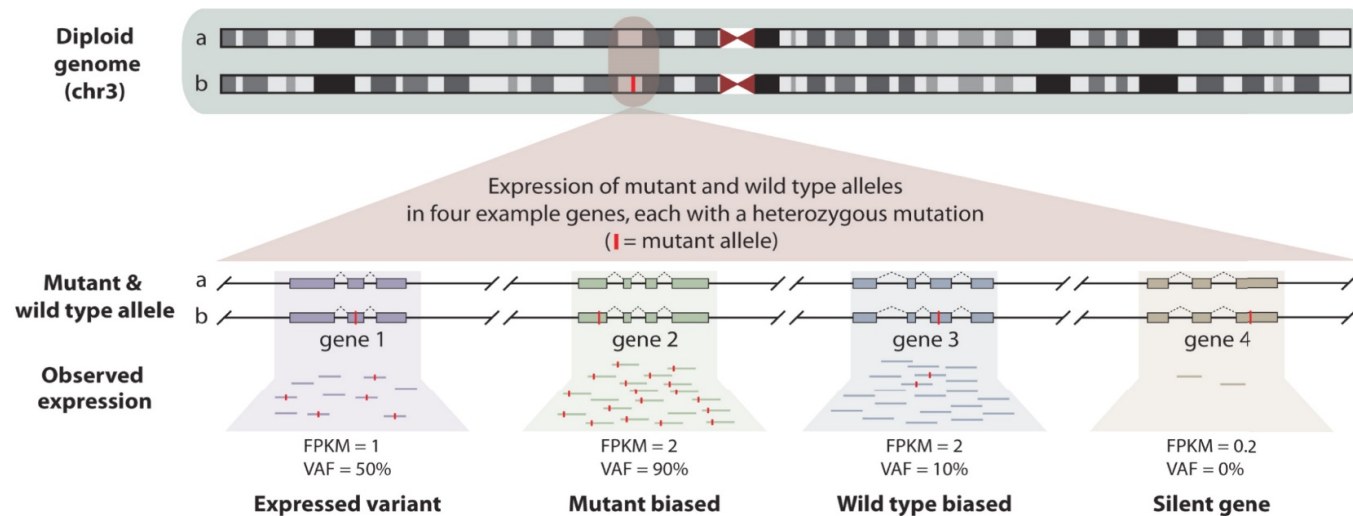
* Investigational product
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October 16, 2020

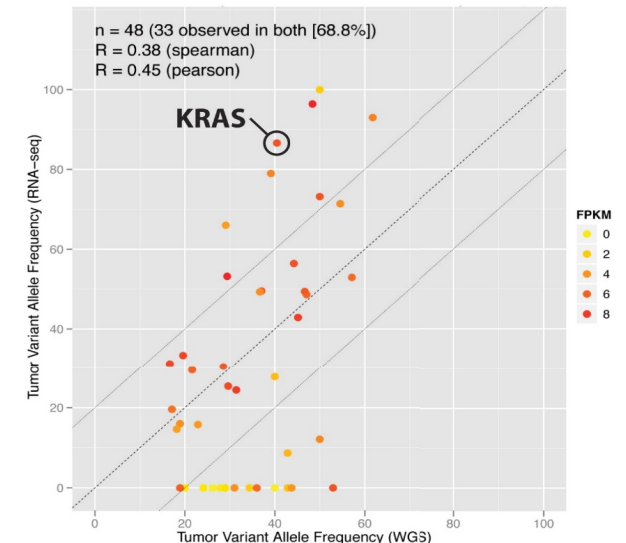
RNA = “The Living Genome”

- **Compare RNA-seq and DNA Whole Exome Seq (WES) in 17 lung cancer tissues**
 - Identify variants that are expressed or silent.
 - Also find allelic imbalance – alleles that are biased towards the variant or wild type allele
 - Somatic drivers like KRAS are found to be variant biased
 - Overall mutation rate of highly expressed genes is lower than non expressed genes (4 vs 14 mutations per Mbp), suggesting Transcription-coupled repair mechanism or other selection pressures

A Categories of variant expression in RNA-seq

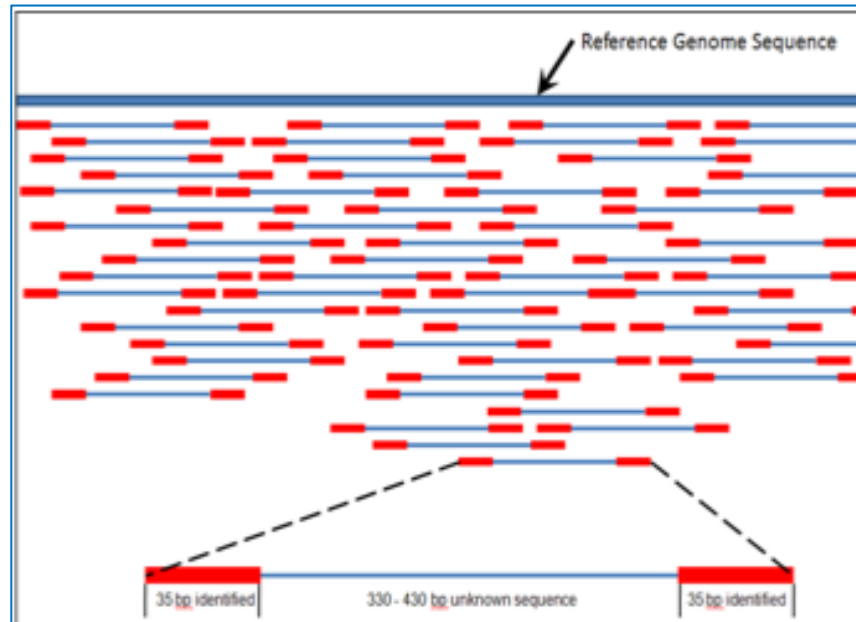


D WGS and RNA-seq variant allele frequencies



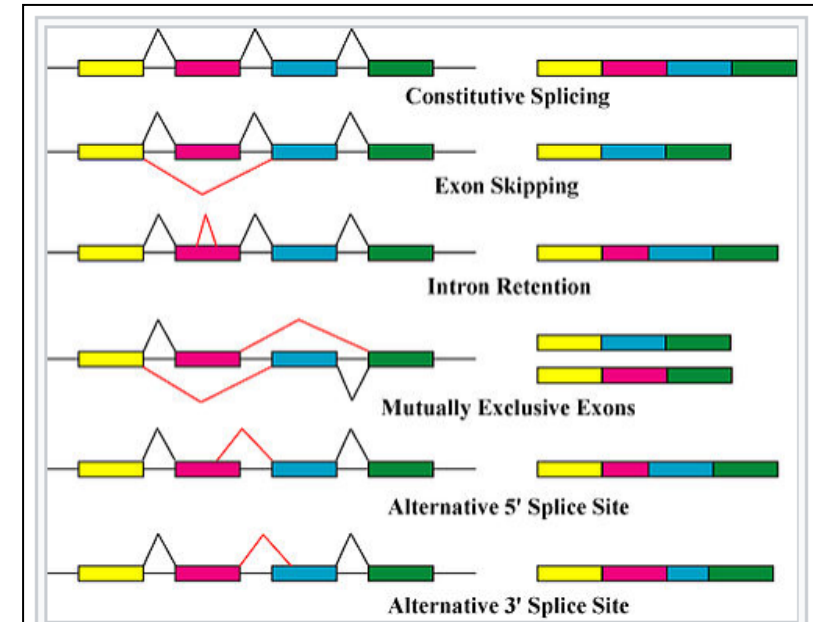
The “Unified Assay” RNA sequencing platform allows us to measure a rich source of transcript diversity

- Uses **425,437** enrichment probes to enable sequencing of **214,126** exons in **21,415** human genes
- The assay covers **98.3%** of the RNA Exome



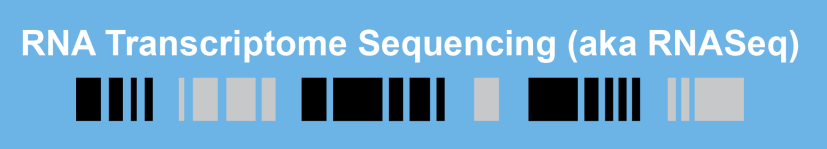
ONE ASSAY...ALL TRANSCRIPTS

AND



ONE GENE...MANY ISOFORMS

The power of RNA sequencing was needed in Afirma GSC



■ protein-coding
■ non-coding

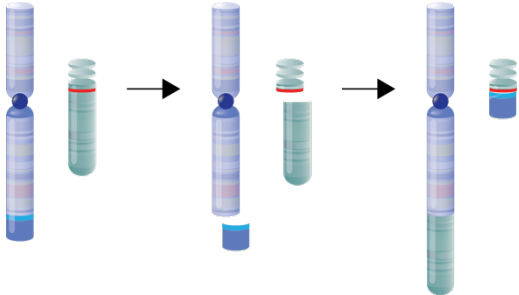
GENE EXPRESSION



SEQUENCE VARIANTS

AUUCGA UAC**A**GU CG**U**AAC

FUSIONS



MITOCHONDRIAL GENOME



LOSS OF HETEROZYGOSITY



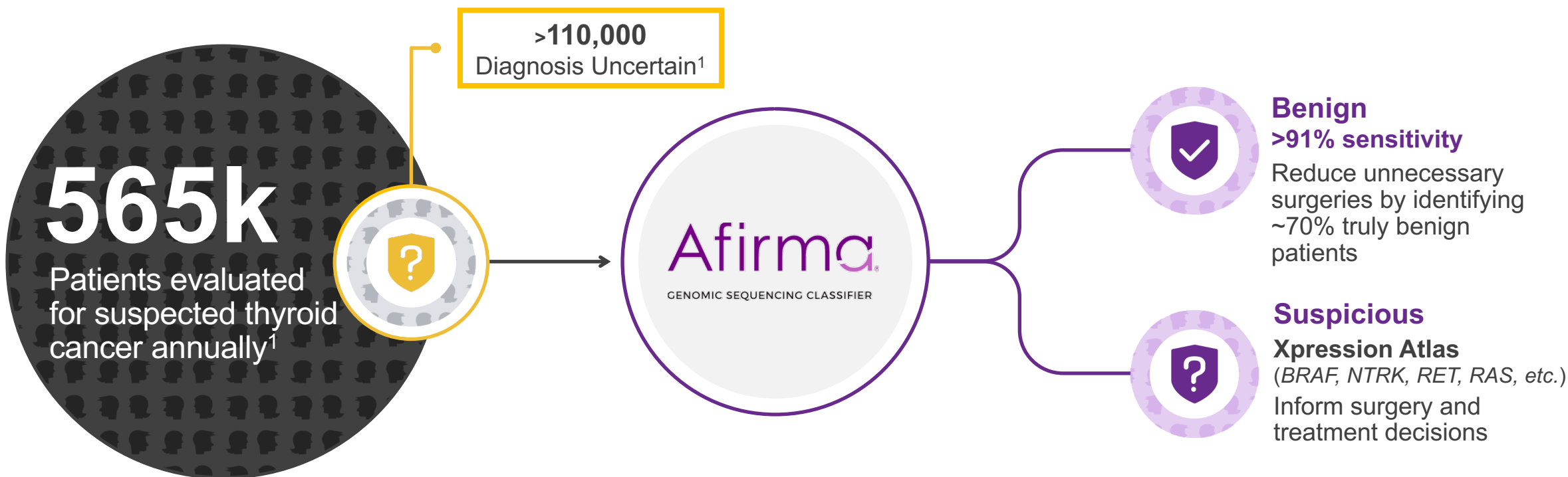
Normal
One copy from each
parental chromosome



Loss-Of-Heterozygosity (LOH)
One copy from each parental
chromosome

THYROID CANCER

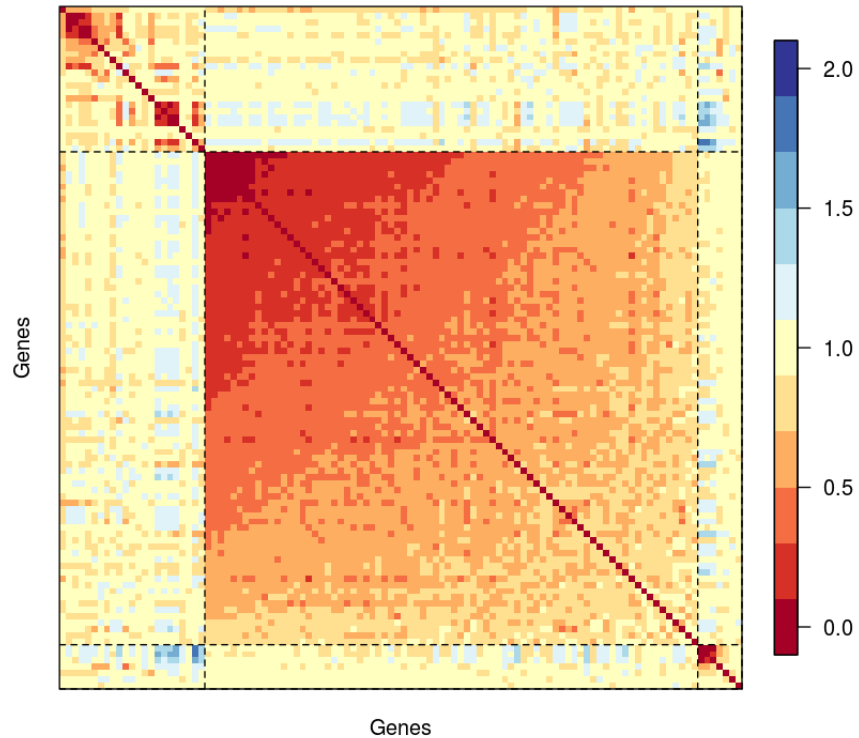
Improving patient outcomes from biological complexity



¹ Company estimates

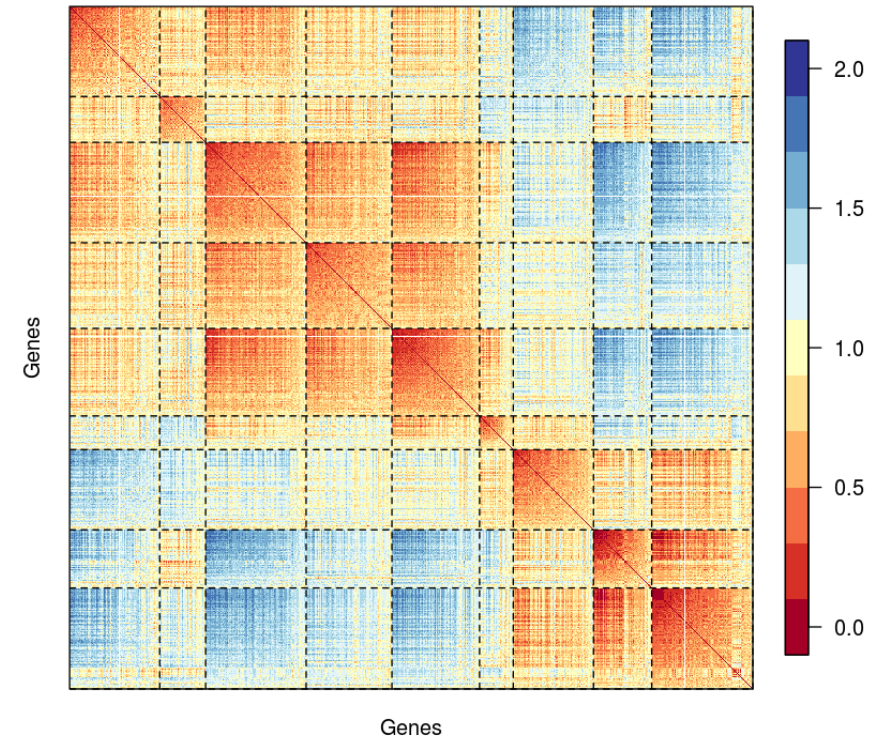
Algorithmic approach needs to fit the problem

Simple Biology



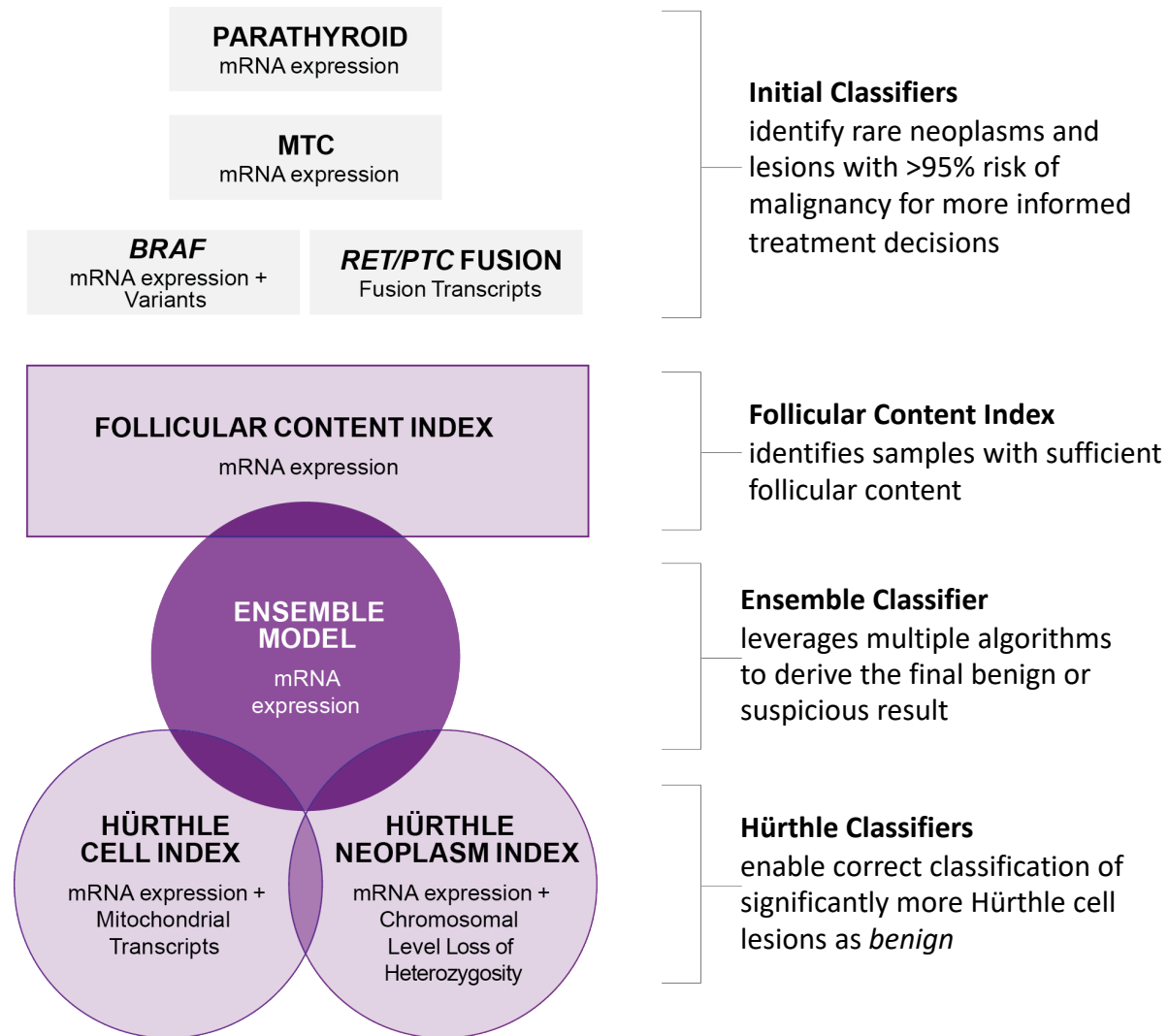
Strong, unique signature

Complex Biology



Diverse and complex signature

Afirma GSC utilizes >10,000 genes among multiple classifiers



Patel KN, et al. *JAMA Surg*, 2018

Afirma GSC was validated on 191 of the 210 samples with remaining RNA from Alexander EK, et al. *NEJM* 2012

Four key elements should be considered in clinical validation studies:



BLINDED



REPRESENTATIVE



MULTICENTER



PROSPECTIVE

Hao Y, et al. *BMC Systems Biology*. 2019

Clinical evidence is essential for adoption and reimbursement

THE NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Preoperative Diagnosis of Benign Thyroid Nodules with Indeterminate Cytology

Erik K. Alexander, M.D., Giulia C. Kennedy, Ph.D., Zubair W. Baloch, M.D., Ph.D., Edmund S. Cibas, M.D., Darya Chudova, Ph.D., James Diggans, Ph.D., Lyssa Friedman, R.N., M.P.A., Richard T. Kloos, M.D., Susan J. Mandel, M.D., M.P.H., Stephen H. Shah, M.D., David L. Steward, M.D., P. Sean Walsh, M.D., Martha A. Zeiger, M.D., Richard B. Lanman, M.D.

JAMA Surgery

Original Investigation

September 2018

Performance of a Genomic Sequencing Classifier for the Preoperative Diagnosis of Cytologically Indeterminate Thyroid Nodules

Kepal N. Patel, MD¹; Trevor E. Angell, MD²; Joshua Babiarz, PhD³; Neil M. Barth, MD^{4,5}; Thomas Blevins, MD⁶; Quan-Yang Duh, MD⁷; Ronald A. Grosse, MD⁸; R. Mack Harrell, MD^{9,10,11}; Jing Huang, PhD³; Giulia C. Kennedy, PhD³; Su Yeon Kim, PhD³; Richard T. Kloos, MD⁴; Virginia A. Livolsi, MD¹²; Gregory W. Randolph, MD¹³; Peter M. Sadow, MD, PhD¹⁴; Michael H. Shanik, MD¹⁵; Julie A. Sosa, MD¹⁶; S. Thomas Traweek, MD¹⁷; P. Sean Walsh, MPH³; Duncan Whitney, PhD³; Michael W. Yeh, MD¹⁸; Paul W. Ladenson, MD¹⁹

THE LANCET Respiratory Medicine

Use of a molecular classifier to identify usual interstitial pneumonia in conventional transbronchial lung biopsy samples: a prospective validation study

[Prof Ganesh Raghu, MD](#) • [Prof Kevin R Flaherty, MD](#) • [Prof David J Lederer, MD](#) • [Prof David A Lynch, MBChB](#) • [Prof Thomas V Colby, MD](#) • [Prof Jeffrey L Myers, MD](#) • et al. [Show all authors](#)

THE NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

A Bronchial Genomic Classifier for the Diagnostic Evaluation of Lung Cancer

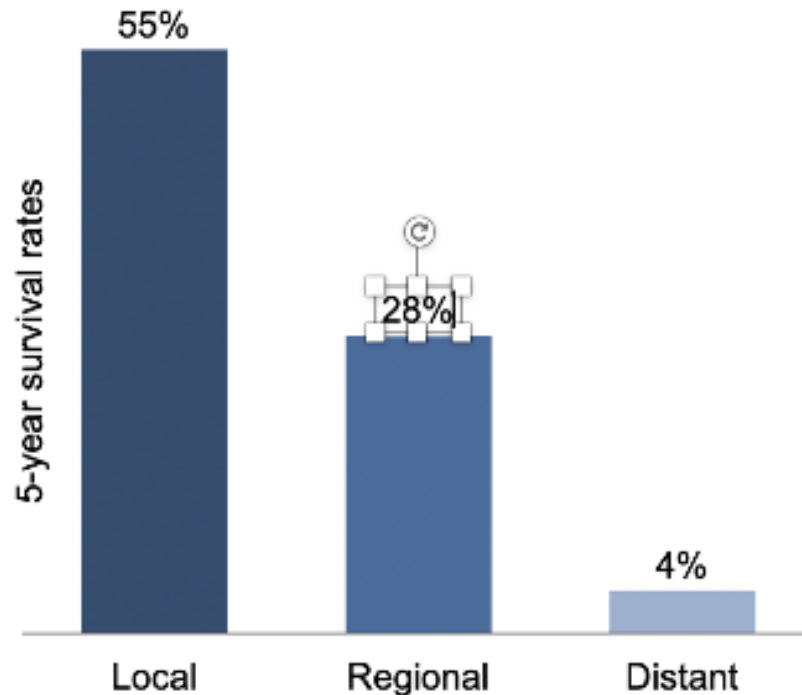
Gerard A. Silvestri, M.D., Anil Vachani, M.D., Duncan Whitney, Ph.D., Michael Elashoff, Ph.D., Kate Porta Smith, M.P.H., J. Scott Ferguson, M.D., Ed Parsons, Ph.D., Nandita Mitra, Ph.D., Jerome Brody, M.D., Marc E. Lenburg, Ph.D., and Avrum Spira, M.D., for the AEGIS Study Team*



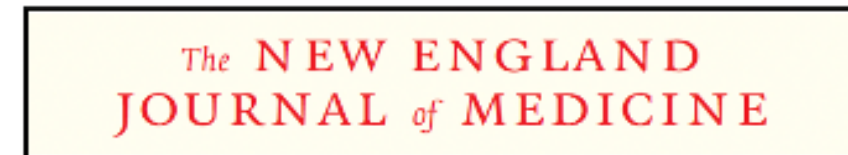
Applying the model to Lung Cancer

Early detection of lung cancer improves survival

5-YEAR SURVIVAL RATE SIGNIFICANTLY HIGHER WHEN LUNG CANCER DETECTED AT A LOCAL STAGE

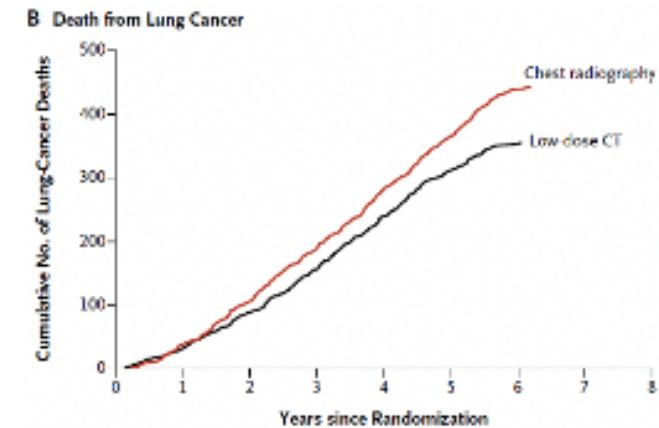


NATIONAL LUNG SCREENING TRIAL DEMONSTRATED 20% MORTALITY REDUCTION WITH CT SCREENING²



Reduced Lung-Cancer Mortality with Low-Dose Computed Tomographic Screening

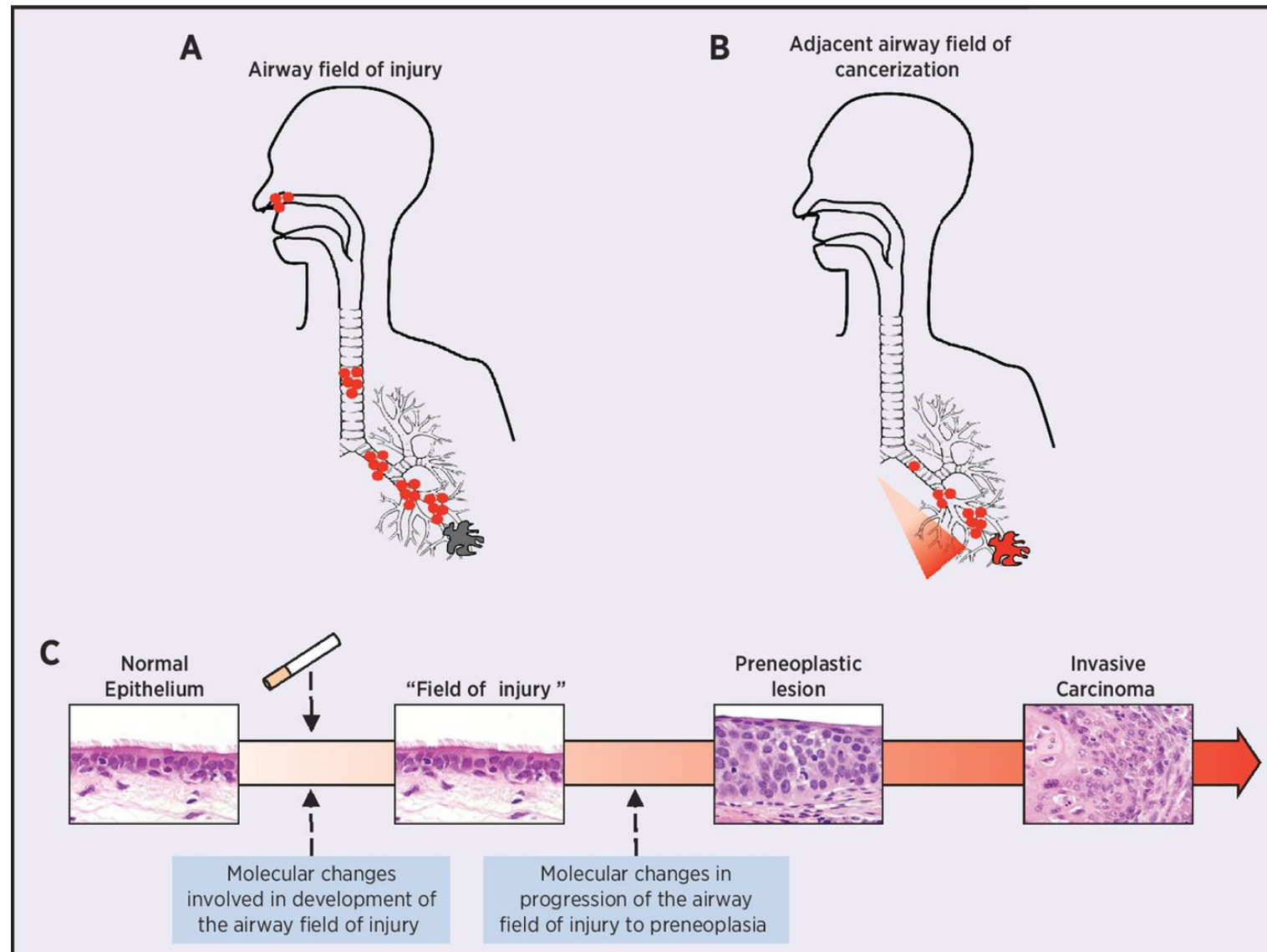
The National Lung Screening Trial Research Team*



1. American Cancer Society. Facts and Figures. 2017.

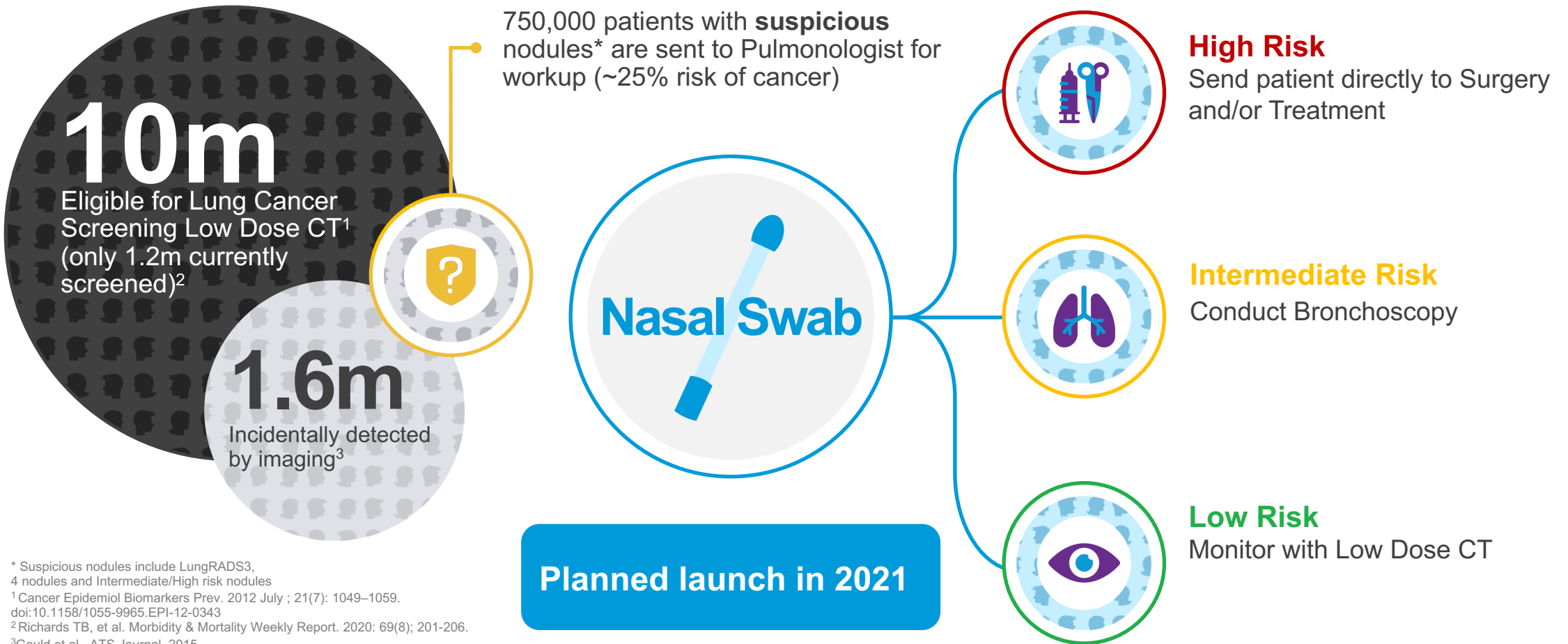
2. Aberle et al. NEJM 2011

Noninvasive nasal swab test based on “Field of Injury” science



NASAL SWAB CLASSIFIER

New risk assessment & diagnostic approach to improve standard of care



* Suspicious nodules include LungRADS3, 4 nodules and Intermediate/High risk nodules

¹ Cancer Epidemiol Biomarkers Prev. 2012 July ; 21(7): 1049–1059.
doi:10.1158/1055-9965.EPI-12-0343

² Richards TB, et al. Morbidity & Mortality Weekly Report. 2020; 69(8); 201–206.

³ Gould et al., ATS Journal, 2015

⁴ Lamb C, et al. American College of Chest Physicians (CHEST) Annual Meeting, Oct. 2019.

Training/Independent test set demographic information

Samples with $RIN \geq 3$

Training: 411 patients

Category	Sub-category	Benign 85	Malignant 326
Sex	Male	51	210
	Female	34	116
Age	Median	58	65
Smoking status	Current	25	146
	Former	60	180
Pack-year	Median	30	47
Nodule size	< 1	9	8
	1 to 2	16	52
	>2 to <3	9	45
	>=3	27	195
	Ill defined infiltrate	20	14
	Unknown	4	12
Nodule location	Central	22	120
	Peripheral	33	90
	Both	26	105
	Unknown	4	11
Histology	SCLC		42
	NSCLC		250
	Other		34
NSCLC type	Adenocarcinoma		106
	Squamous		100
	Large Cell		11
	Other		33

Independent Test Set: 261 patients

Category	Sub_category	Benign 57	Malignant 204
Sex	Male	38	131
	Female	19	73
Age	Median	57	66
Smoking status	Current	23	104
	Former	34	100
Pack-year	Median	20	48
Nodule size	< 1	7	6
	1 to 2	17	33
	>2 to <3	6	25
	>=3	14	125
	Ill defined infiltrate	10	10
	Unknown	3	5
Nodule location	Central	24	72
	Peripheral	21	61
	Both	8	65
	Unknown	4	6
Histology	SCLC		26
	NSCLC		161
	Other		17
NSCLC type	Adenocarcinoma		69
	Squamous		62
	Large Cell		10
	Other		20

Subsequent Independent Test Set Performance using Classifier with two cut-offs:

Sensitivity > 96% for Classification as Low Risk

Incoming Suspicious Nodules	Sensitivity	Specificity
All (N=261)	96.6% [93.1 – 98.6]	45.6% [32.4 – 59.3]

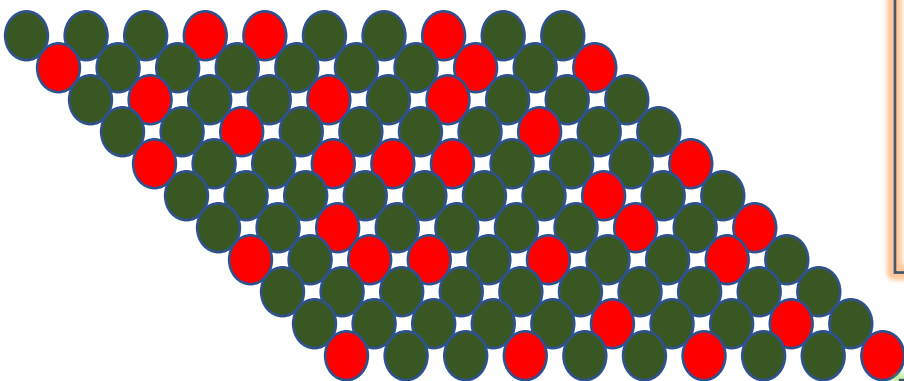
Specificity > 94% for Classification as High Risk

Incoming Suspicious Nodules	Sensitivity	Specificity
All (N=261)	50.0% [42.9– 57.1]	94.7% [85.4 – 98.9]

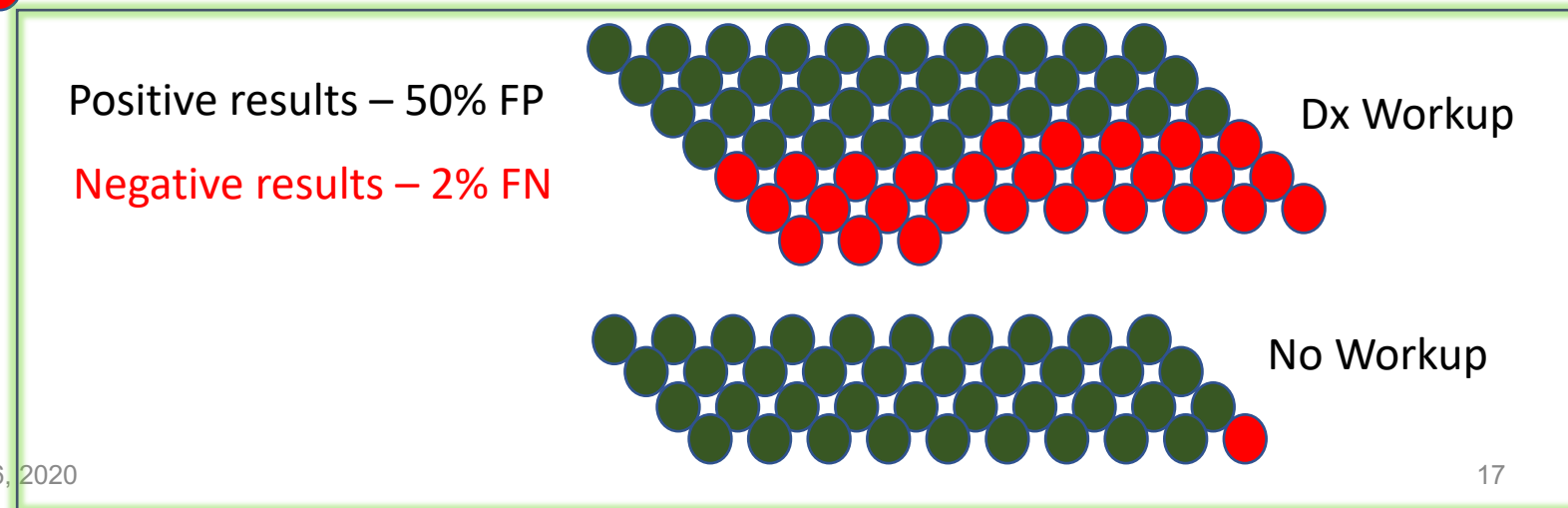
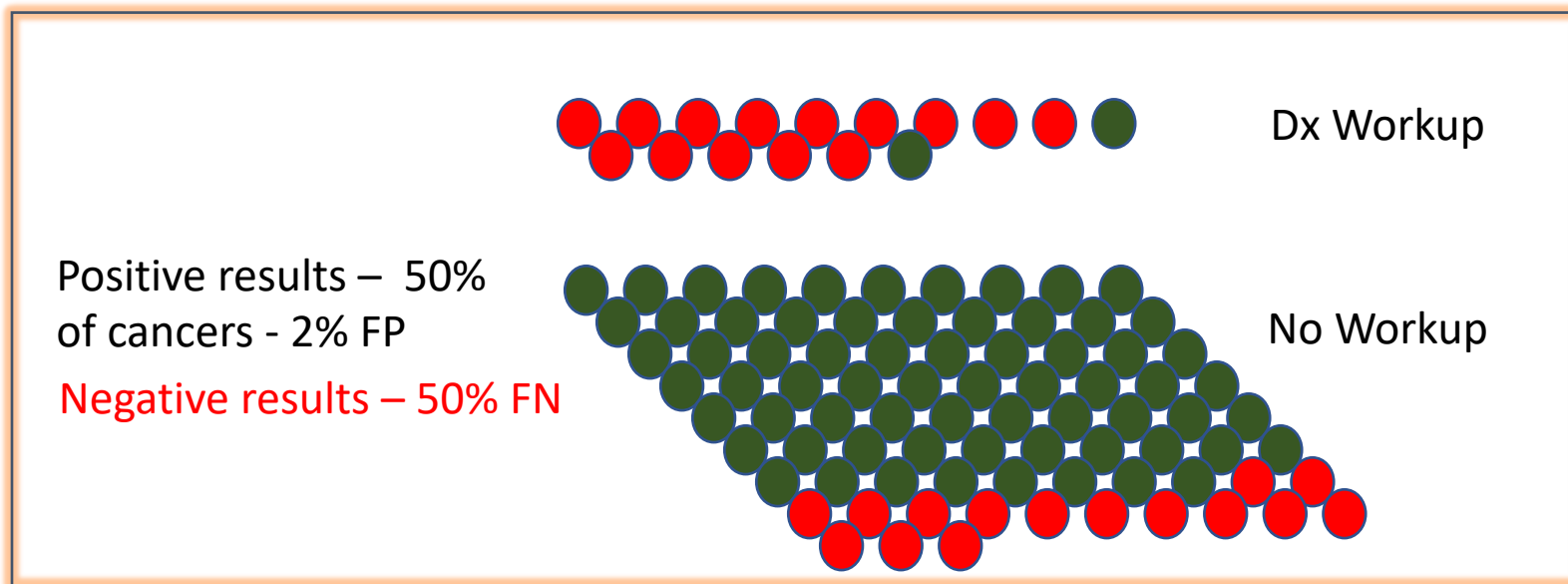
Test performance is optimized for desired outcome to avoid:

**Test with HIGH SPECIFICITY (98%)
But LOW SENSITIVITY (50%)**

30% Cancer prevalence
Post CT today

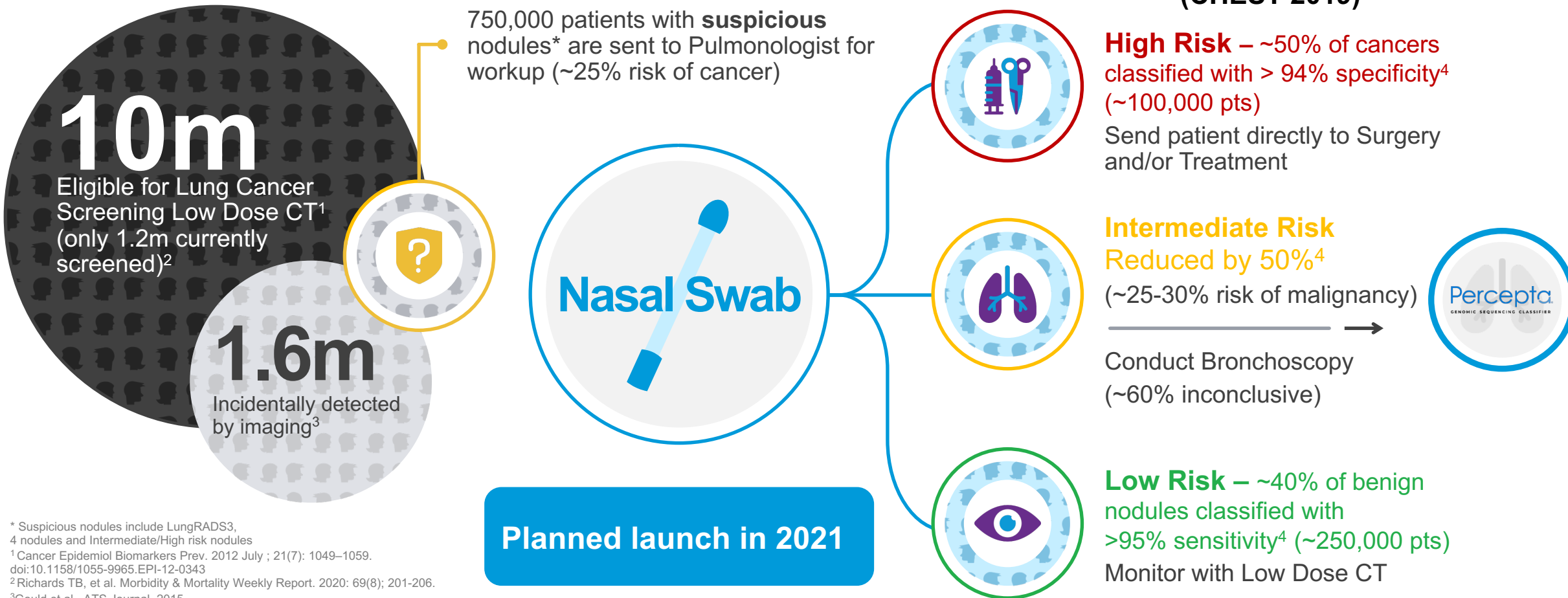


**Test with HIGH SENSITIVITY (98%)
But LOW SPECIFICITY (50%)**



NASAL SWAB CLASSIFIER

New risk assessment & diagnostic approach to improve standard of care



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