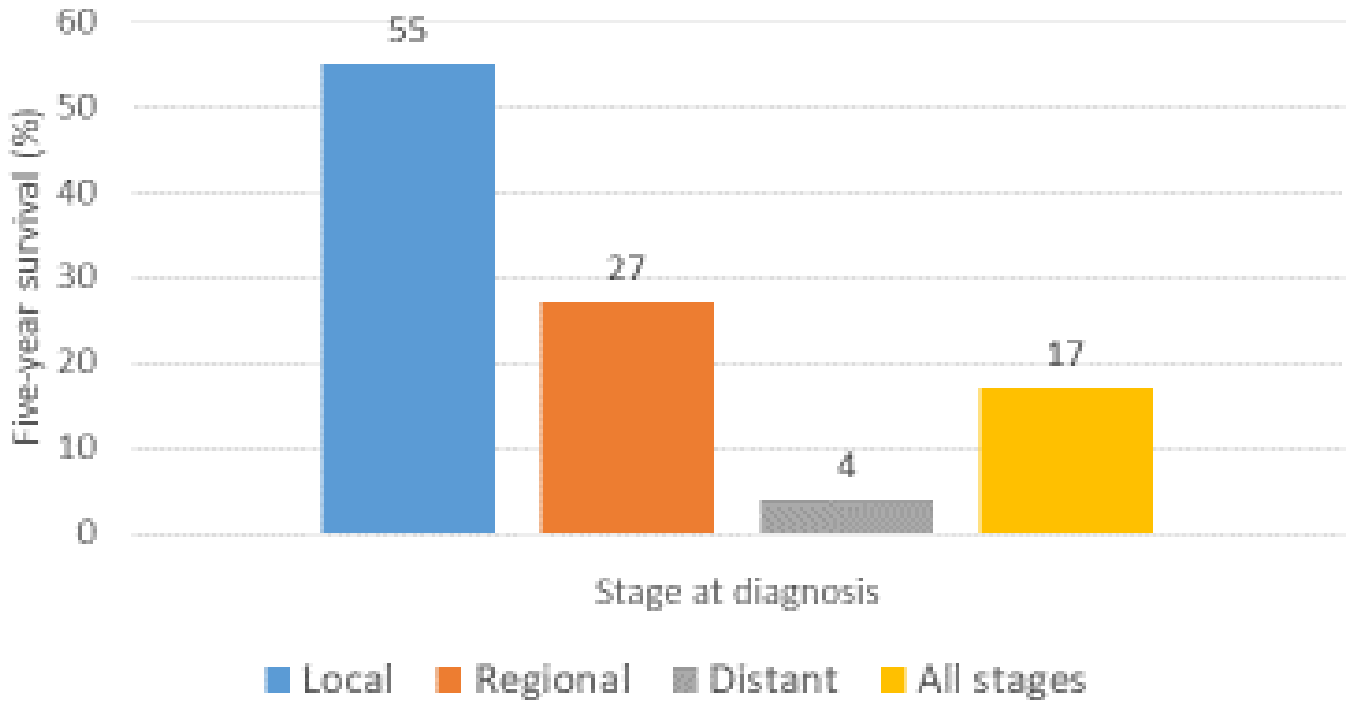
ROLE OF BREATHPRINTING AND CfDNA IN EARLY DETECTION OF LUNG CANCER

Kajal Arora

20.11.20

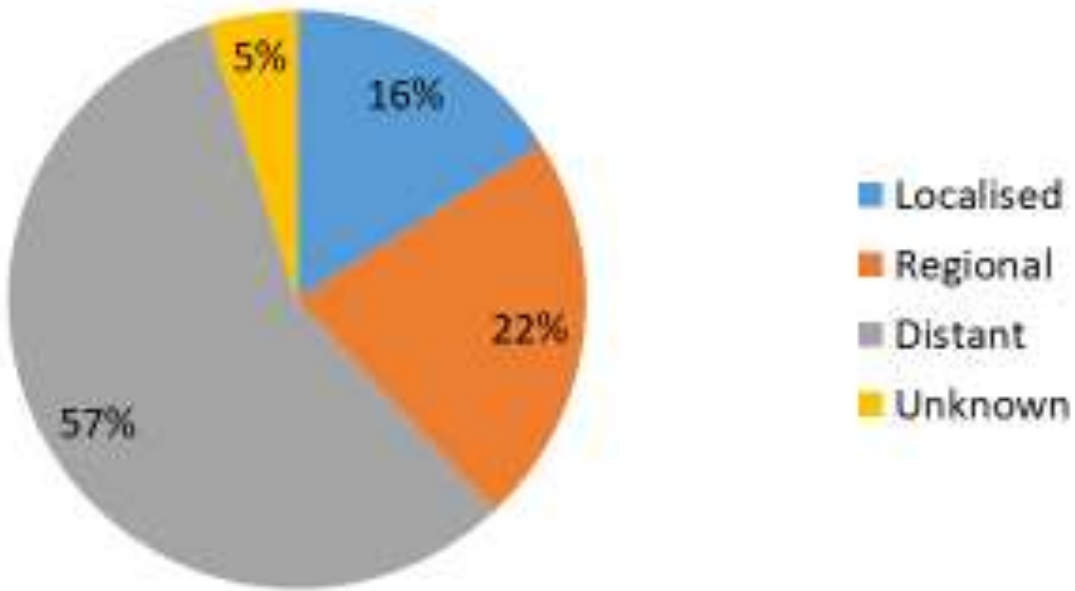
WHY IS THERE NEED FOR NON INVASIVE MARKERS

Lung cancer survival by stage at diagnosis



American Cancer Society. Cancer Facts & Figures 2016

Lung cancer: Stage at diagnosis



SEER (U.S. NCI) cancer statistics 2007-2013

	NLST trial	NELSON trial
Screening design	Annual LDCT X 3 yrs. vs. Annual CXR X 3 yrs.	LDCT at 0, 1, 3, 5.5 yrs. vs. No screening
Enrolled participants	53,454	15,822
Positive result	Nodule diameter ≥4 mm	Volume >500 mm^3 OR Volume 50-500 mm^3 + Volume doubling time <400 days
Entry criteria		
Age in years	55-74	50-75
Smoking history	≥30 pack-years	≥15/d for >25 years or ≥10/d for >30 years
Results		
Lung cancer detection rate	2.4%	3.2%
Proportion of stage I cancers	50% vs. 31%	40% vs. 14%
Reduction in rate of lung cancer related deaths	20%	24%

Courtesy Dr. K. T. Prasad

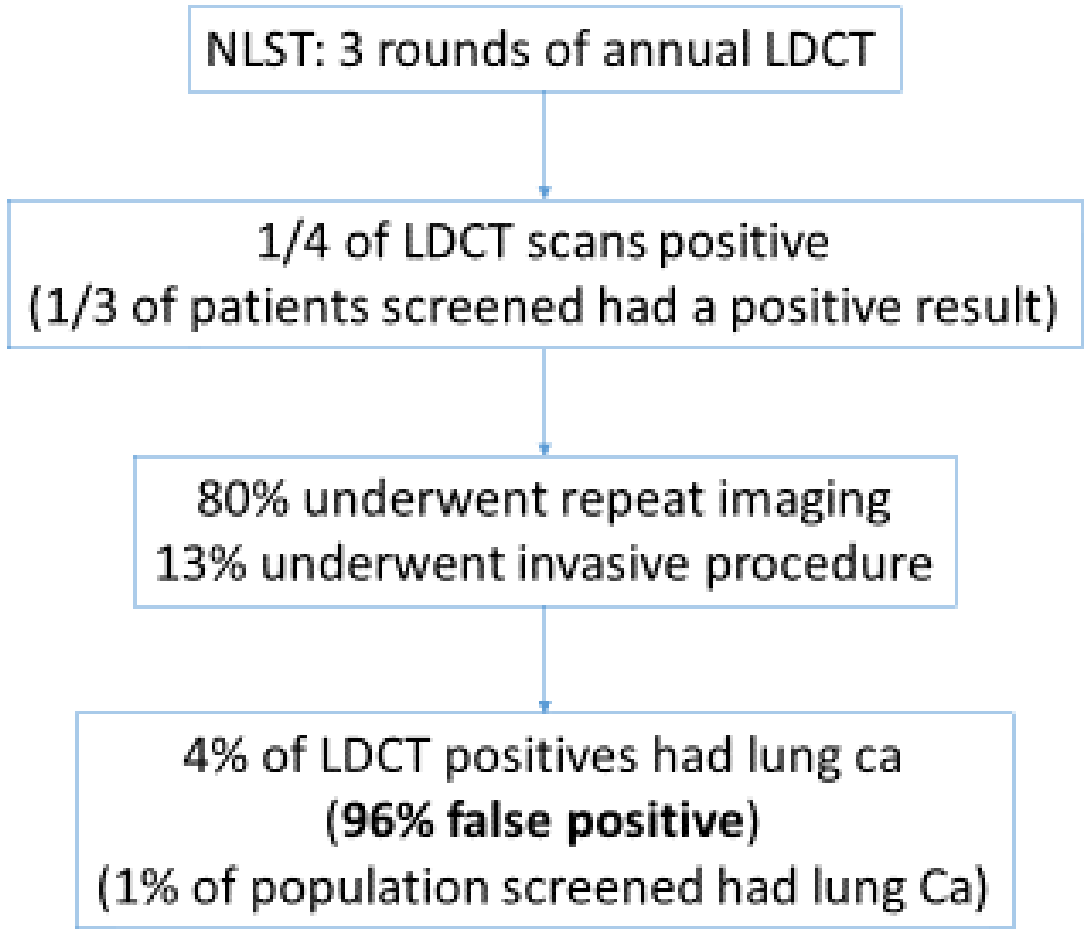
Issue 1: Radiation risk

- In the NLST trial, the average radiation received by the entire screened population (over 3 years) was about 8 mSv (including additional radiological procedures done for LDCT-positive subjects)
- Approximately 1 cancer death may be caused by this amount of radiation for every 2500 persons screened
- However, this radiation risk usually manifests 10-20 years later
- Risk may outweigh benefits in younger subjects, especially if screening is continued beyond 3 years

JAMA 2012;307(22)

Courtesy Dr. K. T. Prasad

Issue 2: False positives



Courtesy Dr. K. T. Prasad

ISSUES 3

- Final results of the NLST represented 83 averted deaths among 26 722 participants
- On the other side of the ledger, the screening caused 16 iatrogenic deaths from diagnostic workups, which included 10 246 imaging studies, 322 percutaneous biopsies, 671 bronchoscopies, 713 surgical procedures, and 228 complications (86 classified as major)

Woolf et.al. *JAMA Intern Med*. 2014;174(12):2019-2022

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Selection Criteria for Lung-Cancer Screening

Martin C. Tammemägi, Ph.D., Hormuzd A. Katki, Ph.D., William G. Hocking, M.D., Timothy R. Church, Ph.D., Neil Caporaso, M.D., Paul A. Kvale, M.D., Anil K. Chaturvedi, Ph.D., Gerard A. Silvestri, M.D., Tom L. Riley, B.Sc., John Commins, B.Sc., and Christine D. Berg, M.D.

PLCO$_{M2012}$

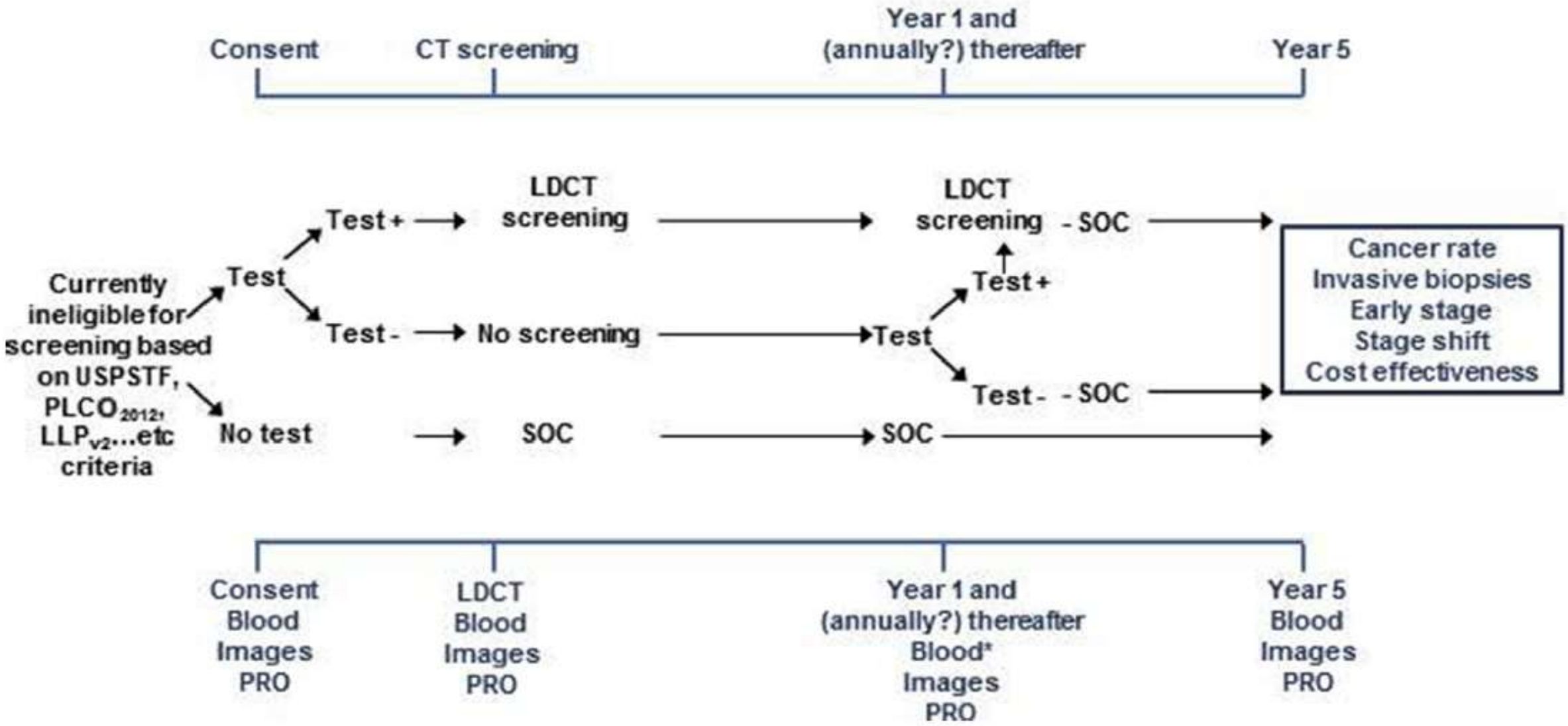
Variable	Odds Ratio (95% CI)	P Value
Age, per 1–yr increase†	1.081 (1.057–1.105)	<0.001
Race or ethnic group‡		
White	1.000	
Black	1.484 (1.083–2.033)	0.01
Hispanic	0.475 (0.195–1.160)	0.10
Asian	0.627 (0.332–1.185)	0.15
American Indian or Alaskan Native	1	
Native Hawaiian or Pacific Islander	2.793 (0.992–7.862)	0.05
Education, per increase of 1 level†§	0.922 (0.874–0.972)	0.003
Body-mass index, per 1-unit increase†	0.973 (0.955–0.991)	0.003
Chronic obstructive pulmonary disease (yes vs. no)	1.427 (1.162–1.751)	0.001
Personal history of cancer (yes vs. no)	1.582 (1.172–2.128)	0.003
Family history of lung cancer (yes vs. no)	1.799 (1.471–2.200)	<0.001
Smoking status (current vs. former)	1.297 (1.047–1.605)	0.02
Smoking intensity¶		
Duration of smoking, per 1-yr increase†	1.032 (1.014–1.051)	0.001
Smoking quit time, per 1-yr increase†	0.970 (0.950–0.990)	0.003
Model constant		

Table 4. Accuracy of Lung-Cancer Classification According to Alternative Criteria in the PLCO Intervention-Group Smokers.*

Criteria†	Participants with Lung Cancer (N = 678)	Participants without Lung Cancer (N = 36,654)	Total Participants (N = 37,332)	Predictive Value
NLST				
Criteria positive	482 TP (3.4%)	13,662 FP (96.6%)	14,144	PPV, 3.4%
Criteria negative	196 FN (0.8%)	22,992 TN (99.2%)	23,188	NPV, 99.2%
Sensitivity	71.1%			
Specificity		62.7%		
$PLCO_{M2012}$ ‡				
Criteria positive	563 TP (4.0%)	13,581 FP (96%)	14,144	PPV, 4.0%
Criteria negative	115 FN (0.5%)	23,073 TN (99.5%)	23,188	NPV, 99.5%
Sensitivity	83.0%			
Specificity		62.9%		

CONCLUSION

- As compared to NLST criteria, $PLCO_{M2012}$ criteria improved sensitivity (83% vs 71.1%, $p<0.001$) and positive predictive value (4% vs 3.4%, $p=0.01$) without loss of specificity (62.9% vs 62.7%, respectively, $p=0.54$)



Seijo et al Journal of Thoracic Oncology Sept 2018

STUDY	COHORT	INTERVENTION	OUTCOME
Sozzi et.al. Retrospective study Smokers enrolled in MILD trial	939 participants 69 lung cancer 870 disease free (n=652, LDCT arm; n=287, observation arm)	microRNA signature classifier	Negative predictive value of 99% and 99.86% for detection and death because of disease Combination of both MSC and LDCT lead to 5 times reduction of LDCT false positive rates to 3.7%
BIOMILD: On going trial			

Sozzi et.al. *J Clin Oncol 32:768-773*

BIOMILD

- Plasma microRNA Profiling as First Line Screening Test for Lung Cancer Detection: a Prospective Study
- Risk stratification according to miRNA levels and, therefore, selection of dedicated LDCT follow-up strategy, either in 1 or 3 years
- Number of LDCT scans reduced to one third in subjects with low risk
- Subjects at higher risk maintained on yearly LDCT follow-up
- ClinicalTrials.gov Identifier: NCT02247453

ORIGIN OF CONCEPT

Quantitative Analysis of Urine Vapor and Breath by Gas–Liquid Partition Chromatography

(orthomolecular medicine/vitamins/controlled diet)

LINUS PAULING et.al.

- Method use - Gas chromatography
- Quantitative determination of about 250 substances in sample of breath and of about 280 substances in sample of urine vapor

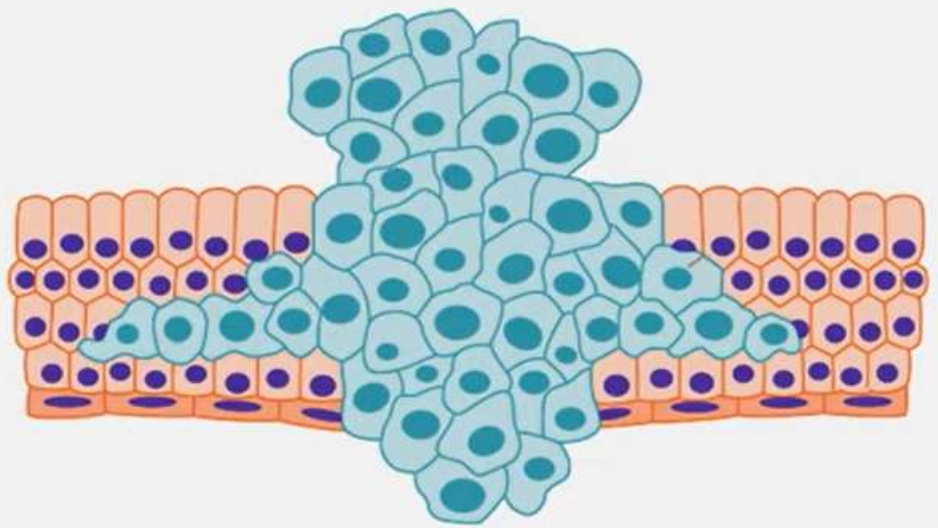
Proc. Nat. Acad. Sci. USA, Analysis of Urine Vapor, 1971

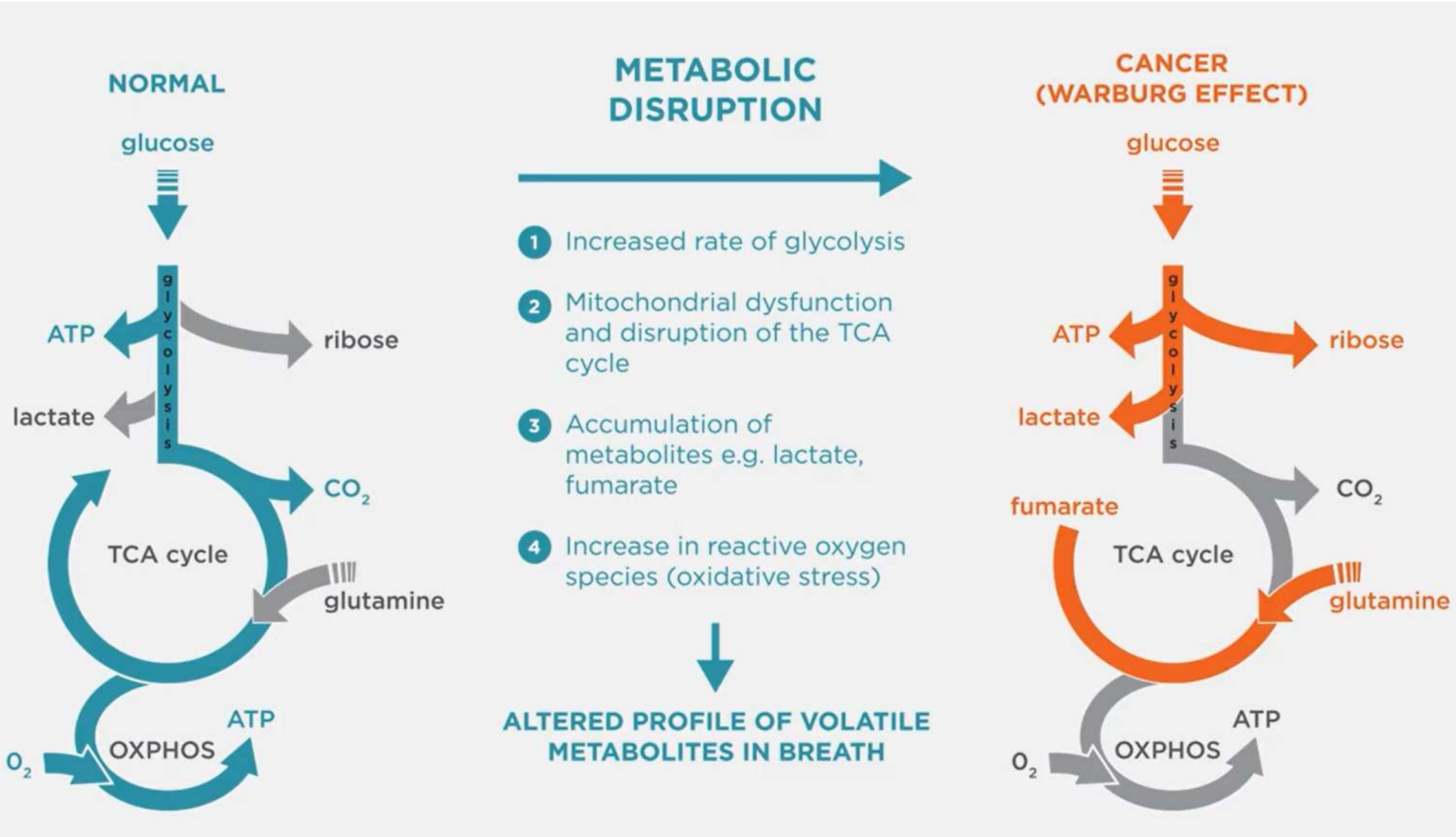
ENVIRONMENT

ONCOGENES

MITOCHONDRIAL DYSFUNCTION

TUMOUR INITIATION AND PROGRESSION



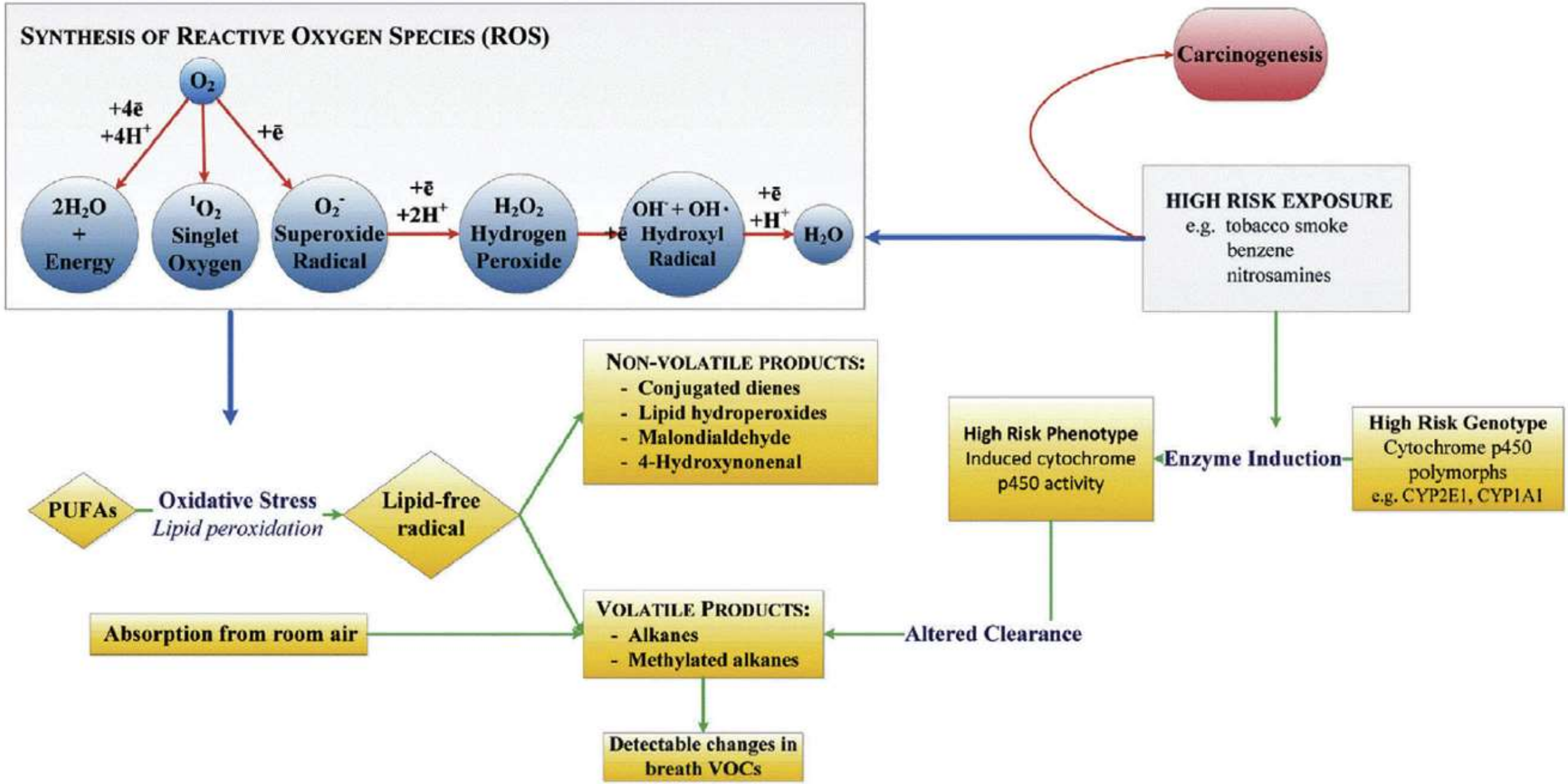

NORMAL
glucose
glycolysis
ATP
ribose
lactate
CO_2
TCA cycle
glutamine
ATP
O_2
OXPHOS
METABOLIC DISRUPTION
1 Increased rate of glycolysis
2 Mitochondrial dysfunction and disruption of the TCA cycle
3 Accumulation of metabolites e.g. lactate, fumarate
4 Increase in reactive oxygen species (oxidative stress)
ALTERED PROFILE OF VOLATILE METABOLITES IN BREATH
CANCER (WARBURG EFFECT)
glucose
glycolysis
ATP
ribose
lactate
CO_2
fumarate
TCA cycle
glutamine
ATP
O_2
OXPHOS

WARBURG'S EFFECT

- Fundamental to cancer cell survival
- Altered metabolic intermediates functioning as building blocks for new cells
- Favor survival in oxygen deprived environment
- VOCs excellent candidate biomarkers for early detection of lung cancer

Compounds	Possible Endogenous Source	Main Products and/or Derivatives	Exogenous Origin
Alkanes alkenes	Oxidative stress (PUFA peroxidation)	Ethane Pentane Heptane Octane Decane Undecane Dodecane Nonadecane Isoprene 2,2,4-Trimethylexane Propane Eicosane	Natural, plastics or petrol/fuels
Alcohols	Hydrocarbon metabolism Absorbed through GI tract	Propanol Butanol 2-Ethyl-1-hexanol 4-Penten-2-ol Ethanol Methanol Heptadecanol	Natural, diet or disinfectants
Aldehydes	Metabolism of alcohols Lipid peroxidation	Propanal Butanal Pentanal Hexanal Heptanal Octanal Nonanal Formaldehyde Acetaldehyde	Natural, diet or waste products Smoking

Ketones	Fatty acid oxidation Protein metabolism	Acetone Butanone Pentanone Hexanone Heptanone Benzophenone Hendecanone Pentadecanone Heptadecanone	Natural, diet, waste products or drugs/ fragrances/paint
Carboxylic acids	Metabolism of amino acids	Benzoic acid Propanoic acid Acetic acid	Food preservatives, solvents, polymers
Esters	Metabolic pathway of alcohols and acids	Ethanoate Propanoate Acetate	Fatty oils, natural wax, fruit essential oils
Nitriles	—	Acetonitrile Azulencarbonitrile	Smoking
Aromatic compounds	—	Benzene Toluene Styrene 2,5 Dimethylfuran Anthracene Dimethylnaphtalene	Petrol, smoking, natural (styrene),tar, oil
Terpens	—	Limonene Camphor	Natural or cosmetics


SYNTHESIS OF REACTIVE OXYGEN SPECIES (ROS)
O_2
+4ē
+4H+
+ē
2H2O + Energy
1O2 Singlet Oxygen
O2- Superoxide Radical
+ē
+2H+
H2O2 Hydrogen Peroxide
+ē
OH- + OH· Hydroxyl Radical
+ē
+H+
H2O
Carcinogenesis
HIGH RISK EXPOSURE
e.g. tobacco smoke
benzene
nitrosamines
NON-VOLATILE PRODUCTS:
- Conjugated dienes
- Lipid hydroperoxides
- Malondialdehyde
- 4-Hydroxynonenal
High Risk Phenotype
Induced cytochrome p450 activity
Enzyme Induction
High Risk Genotype
Cytochrome p450 polymorphs
e.g. CYP2E1, CYP1A1
PUFAs
Oxidative Stress
Lipid peroxidation
Lipid-free radical
Absorption from room air
VOLATILE PRODUCTS:
- Alkanes
- Methylated alkanes
Altered Clearance
Detectable changes in breath VOCs

CANINE MODEL OF BREATH ANALYSIS

Canine scent detection in the diagnosis of lung cancer: revisiting a puzzling phenomenon

R. EHMANN ET AL.

- Prospective, blinded trial
- From December 2009 to April 2010
- Breath samples from patients with COPD (n-50) or suspected lung cancer(n-84) and from healthy individuals (n-110)
- Lung cancer detected with overall sensitivity of 71% and specificity of 93%
- Lung cancer detection independent from COPD and presence of tobacco smoke and food odours

R. Ehmann et al. Eur Respir J 2012; 39: 669–676

Applied methods for breath testing and sampling

- Glass tube used for breath sampling
- Lumen filled with polypropylene fleece
- Participants exhaled 5 times into tubes
- Randomisation of cancer samples
- Dogs trained to indicate positive test tube by lying on floor in front of tube with muzzle touching test tube

Diagnostic Accuracy of Canine Scent Detection in Early- and Late-Stage Lung and Breast Cancers

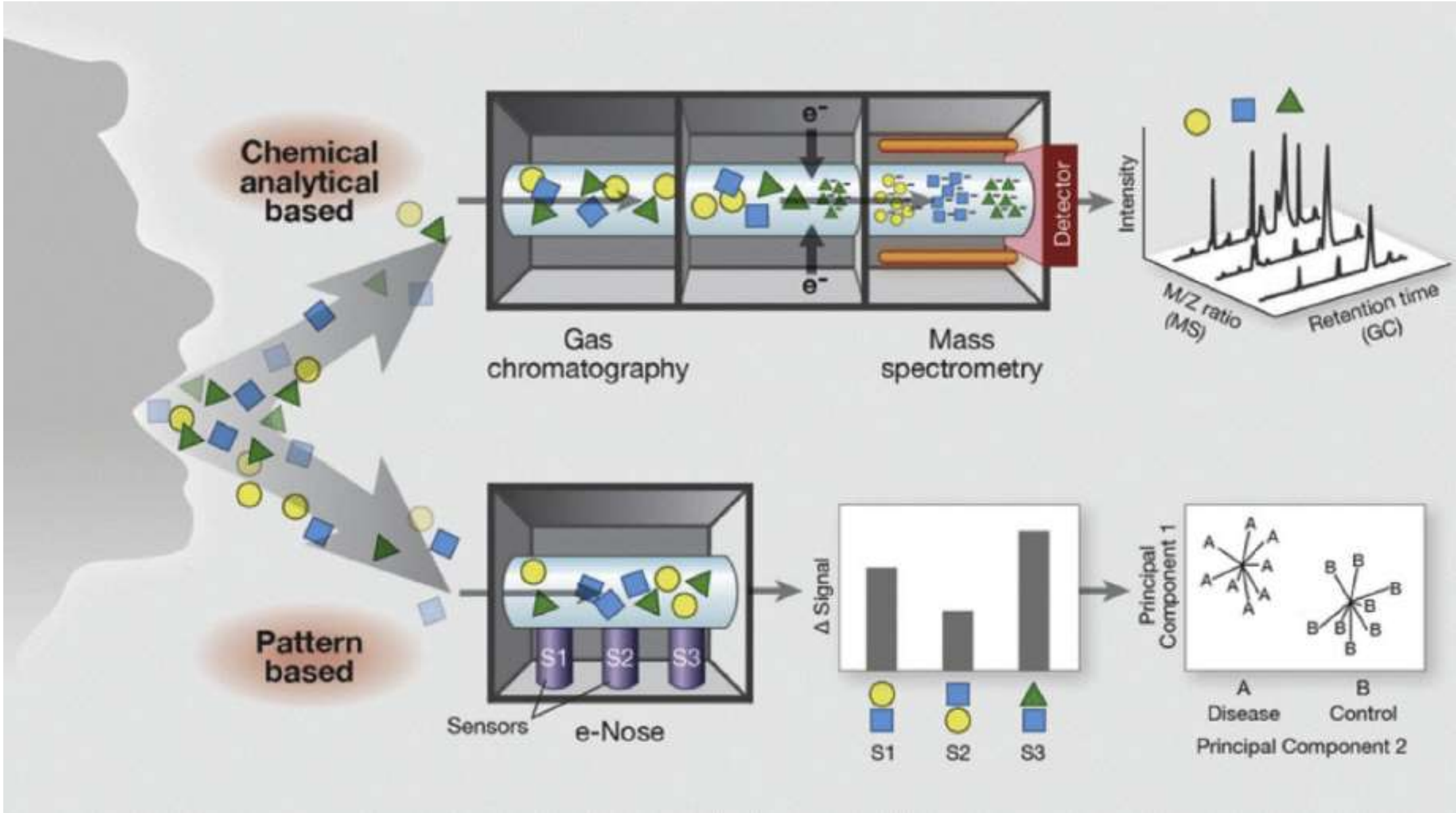
McCulloch et. al.

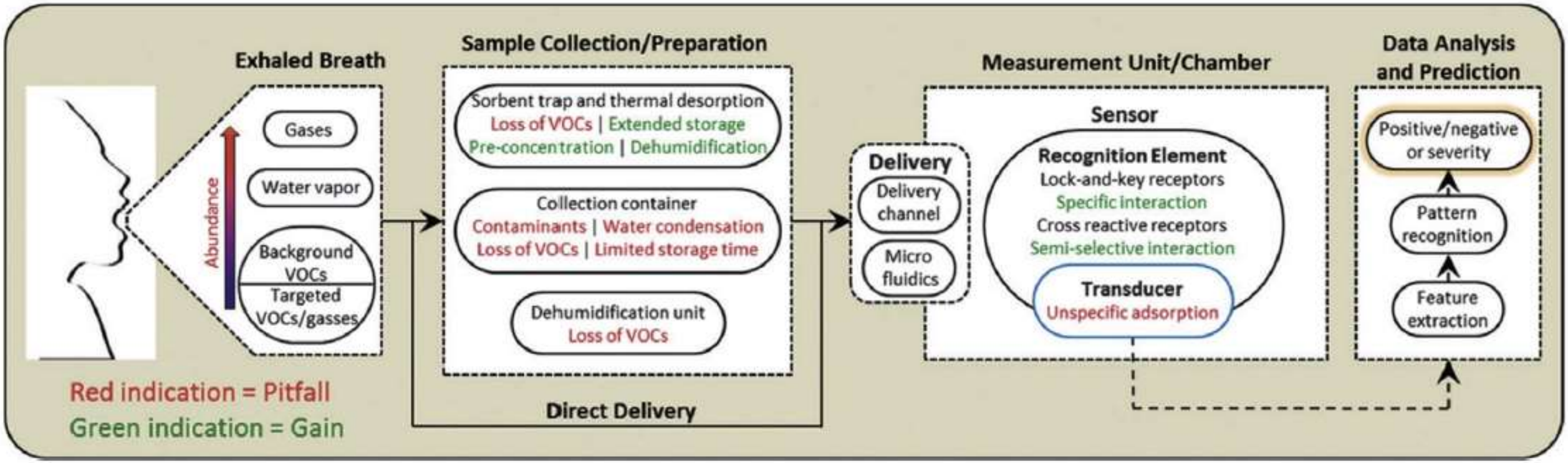
- Prospective blinded trial
- Food reward based method for 5 household dogs
- Cohort – 55 lung, 31 breast cancers and 83 healthy controls
- Among lung cancers overall sensitivity for canine scent detection compared to biopsy confirmed conventional diagnosis – 0.99 and overall specifity – 0.99
- Among breast cancer patients, sensitivity – 0.88 and specificity 0.99

McCulloch et.al. INTEGRATIVE CANCER THERAPIES 5(1); 2006

RATIONALE FOR ANIMAL STUDIES

- Novel concept linking canine scent detection to pattern recognition-based models of currently available e-nose device
- Canine olfactory receptors can be used as biophysical template to structure e-noses


Chemical analytical based
e⁻
e⁻
Detector
Intensity
M/Z ratio (MS)
Retention time (GC)
Gas chromatography
Mass spectrometry
Pattern based
S1
S2
S3
Sensors
e-Nose
Δ Signal
S1
S2
S3
Principal Component 1
A
A
A
A
A
A
A
B
B
B
B
B
B
B
A
Disease
B
Control
Principal Component 2

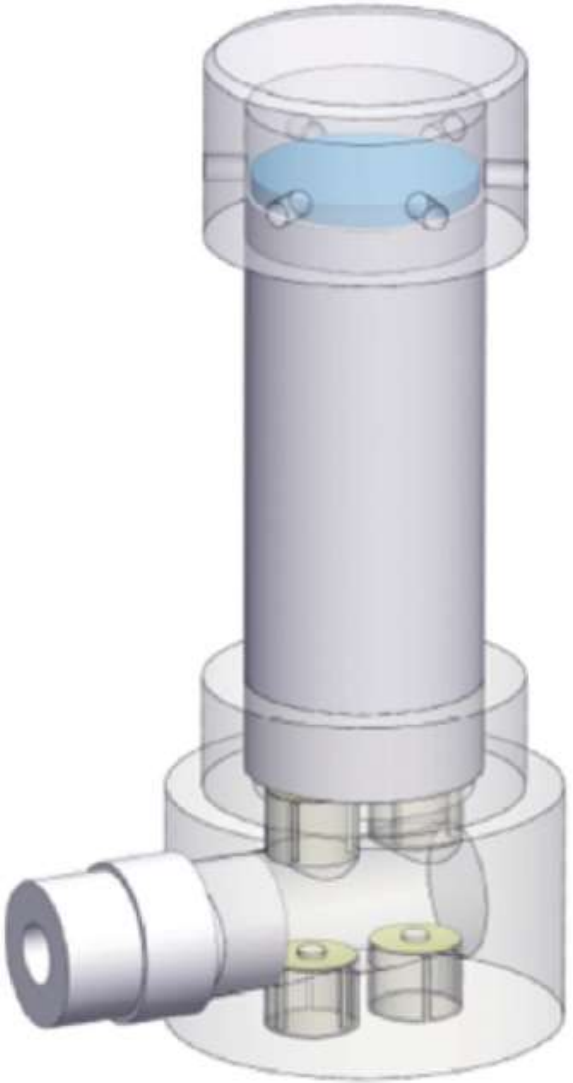


STEPWISE PROCESS OF BREATH ANALYSIS

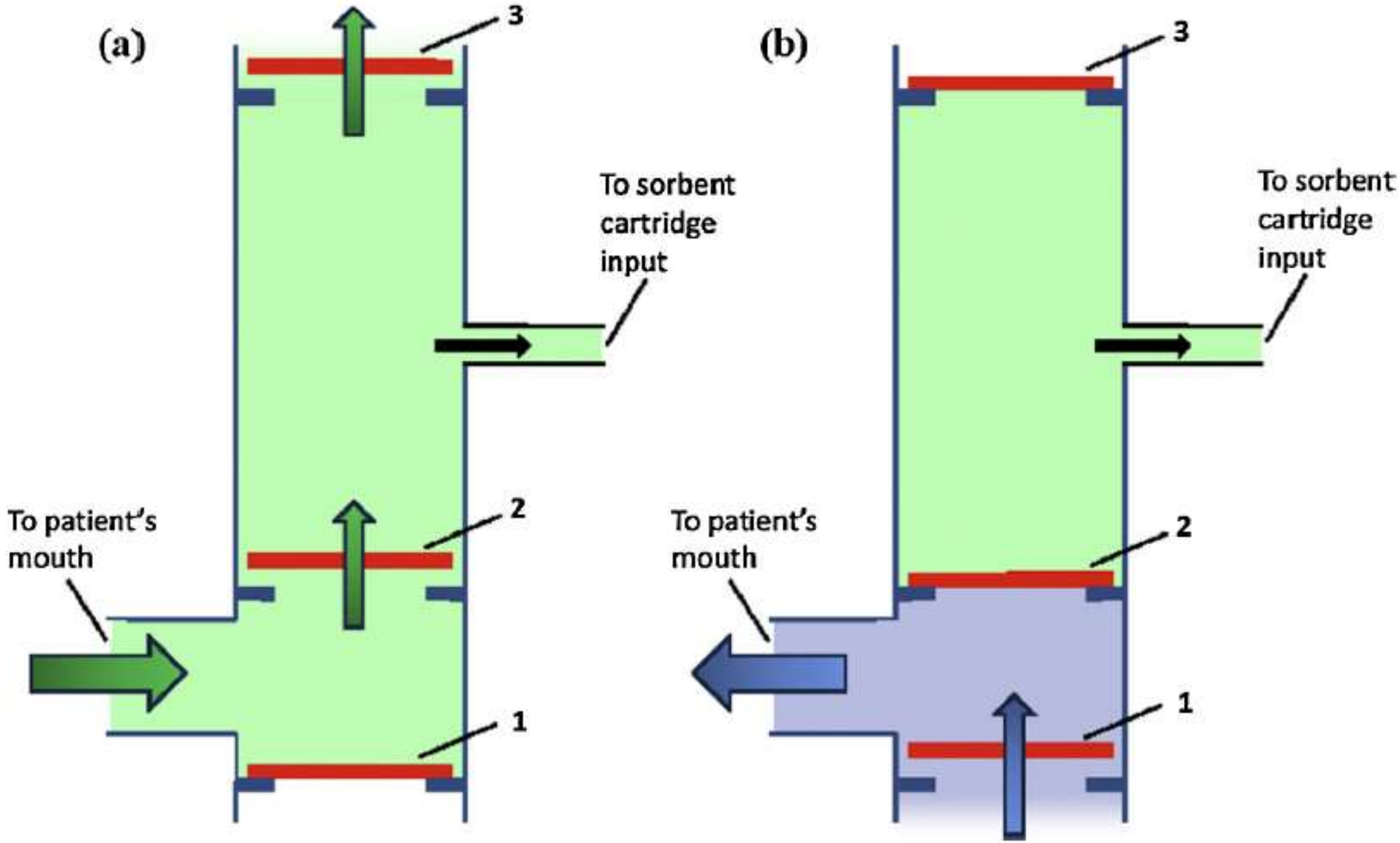
BEST STANDARDS

- Device to collect exhaled breath onto cartridge which can be transported and stored
- An instrument for desorbing exhaled breath into sensor cell
- Versatile sensor system including several nonselective sensing elements able to measure biological exhalates

EXHALED BREATH SAMPLING - PNEUMOPIPE



Rocco et.al. European Journal of Cardio-Thoracic Surgery 49 (2016)

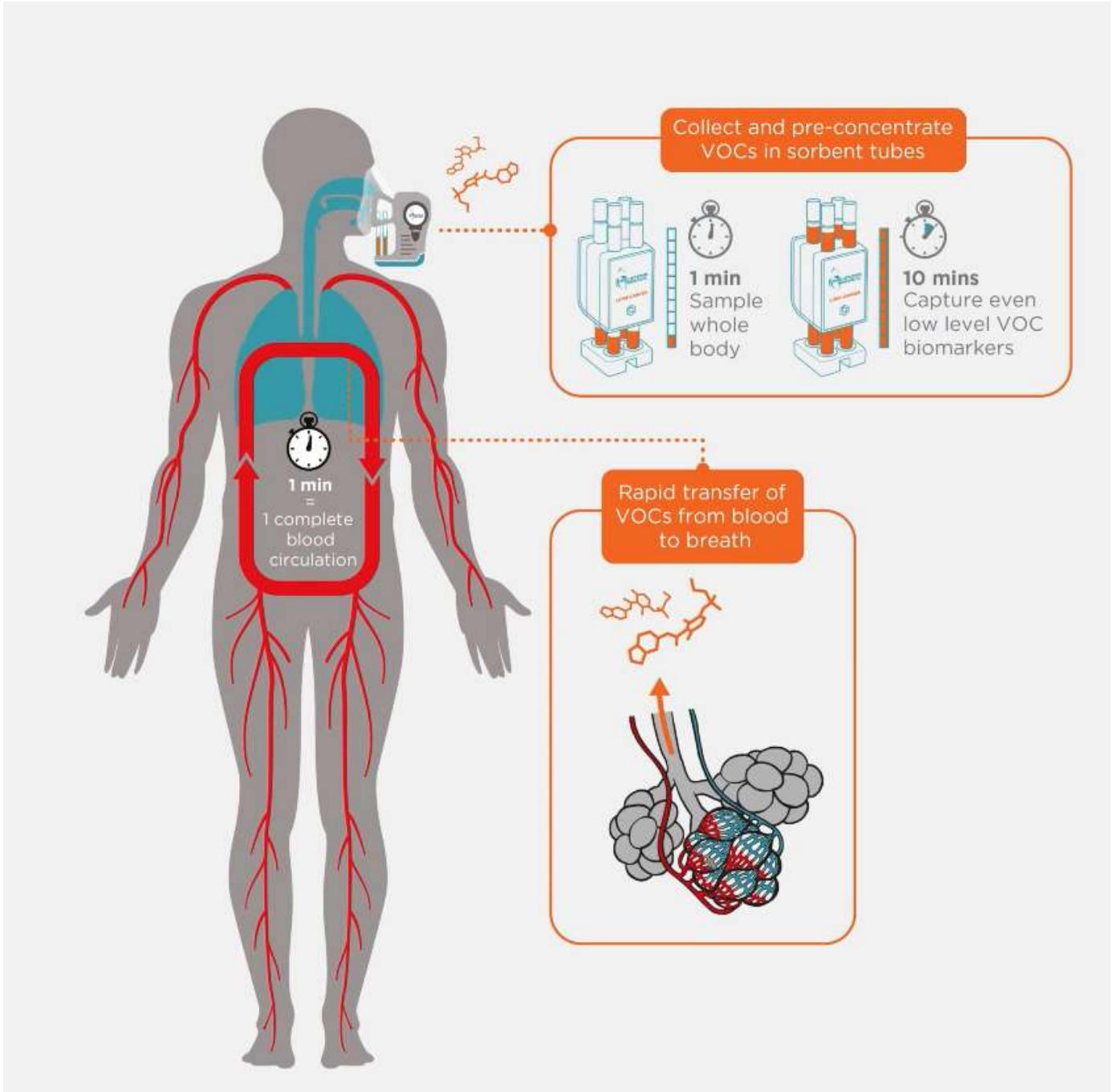

(a)
3
To sorbent cartridge input
To patient's mouth
2
1
(b)
3
To sorbent cartridge input
To patient's mouth
2
1

BENEFITS

- Feasible for respiratory impaired subjects
- Permits continuous sampling of exhaled air even during inhalation
- Transportability of cartridge containing sample suitable for remote analysis, point of care systems
- Thermal desorption into sensor chamber augments sensor performance in terms of resolution (via preconcentration procedures) and of discrimination (via thermal separation of VOCs mixture)

Rocco et.al. European Journal of Cardio-Thoracic Surgery 49 (2016)

EXHALED BREATH SAMPLING - ReCIVA



Increase in sensitivity for detection of VOCs implicated in lung cancer

BIONOTE

- Designed and fabricated at the Laboratory of Electronics for sensor Systems of the Campus Biomedico University of Rome
- Enables simultaneous analysis of vapour and liquid phase of same sample
- Gas sensor array composed of 7 quartz crystals with resonance frequency of 200MHz

BIONOTE

- Quartz microbalances functionalized with anthrocyanins extracted from 3 different plant tissues: red rose, red cabbage and blue hortensia
- Detect mass changes induced by VOCs on surface sensors
- Mass changes induce alterations in oscillation frequency of quartz wafer
- Lung cancer signatures detected based on breath pattern demonstrated by oscillation frequency

Cyanose 320

Cyanose 320 (Smiths Detection Inc.)

- Portable e-nose system consisting of 32 polymer composite sensors
- Once gas mixture passes through sensor array, its chemical components induce reversible changes in electrical resistance of sensors
- Sensors are made cross responsive, detection of particular VOC is based on 32-dimensional response pattern of array rather than single sensor

Cyanose 320

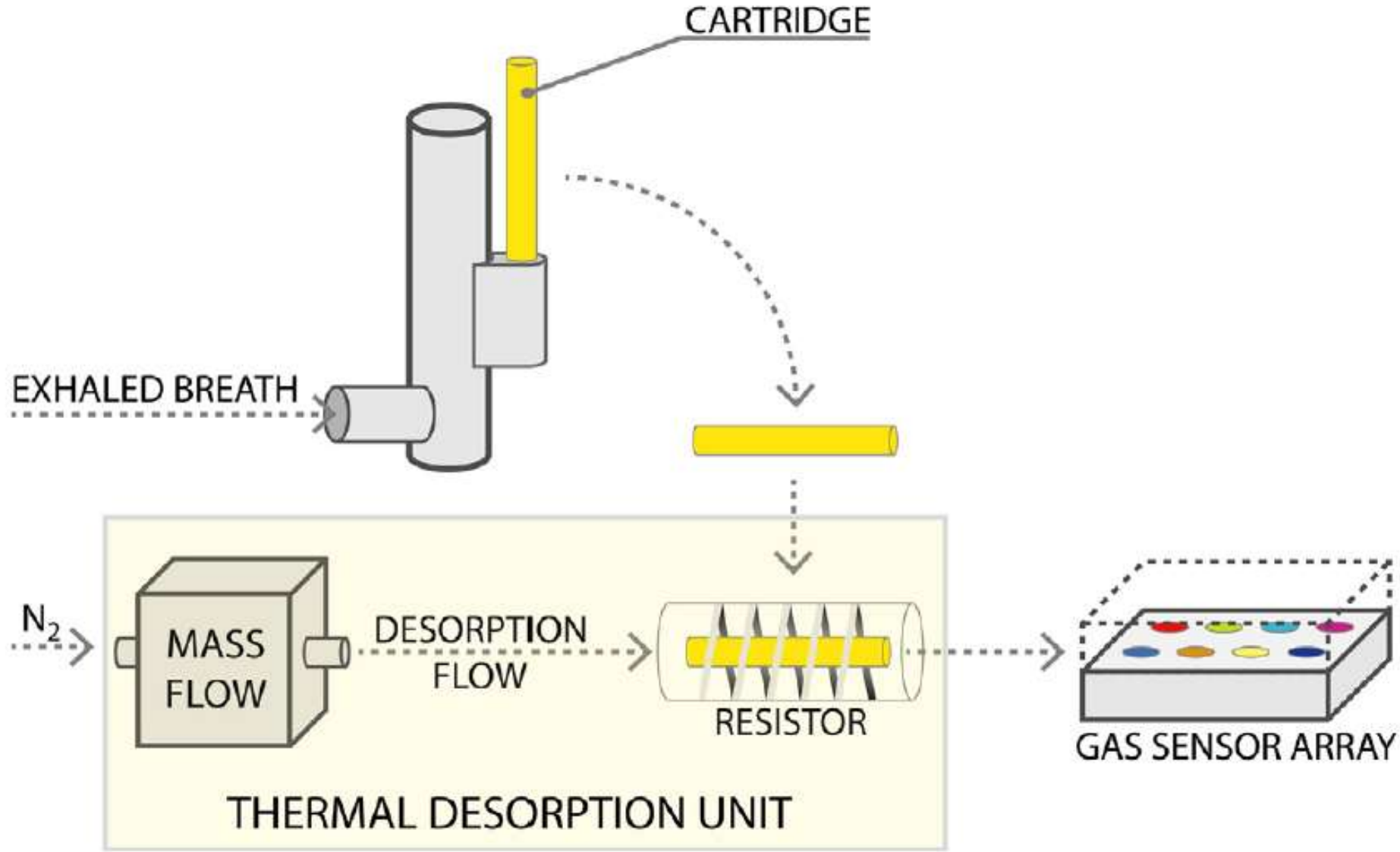
Cyanose 320 (Smiths Detection Inc.)

- According to chemical diversity of array material, resistance changes in 32 sensors results in unique pattern of array rather than single sensor – SMELL PRINTS

Characteristics	Cyranose®	BIONOTE
Working principle	Conductometric sensors	Acoustic-mass sensors
Sensing material	Conducting polymers	Anthocyanins
Array composition	32 different polymers	28 different responses obtained by 7 sensors operating at four different temperatures
Selectivity	Non-selectivity of the individual sensors and the mechanisms of interaction with VOCs	
Performance at low concentrations for standard compounds such as ethanol and hexane	Comparable	

DESORBING UNIT

- Interface apparatus for cartridge desorption into sensor chamber
- Goal to obtain uniform heating of tube from 50°C to 200°C and finally cleaning cartridge, holding temperature at 300°C for five minutes
- Final fingerprint – sequence of 4 n-dimentional gas sensor array at 4 temperatures (50°C-100°C-150°C-200°C)
- At these temperature step tenax cartridge content is desorbed into sensor cell
- Each sensor gives four responses one for each temperature


CARTRIDGE
EXHALED BREATH
N_2
MASS FLOW
DESORPTION FLOW
RESISTOR
GAS SENSOR ARRAY
THERMAL DESORPTION UNIT

50°C	100°C	150°C	200°C	250°C
Propane	Hexane	Octane	Dodecane	Octadecane
Butane	Heptane	Nonane	Tetradecane	Eicosane
Pentane	1-Propanol	Decane	Hexadecane	
Methanol	2-Butanol	Hexanol	Biphenyl	
Ethanol	1-Butanol	Hexanal		
2-Propanol	1-Pentanol	4-Heptanone		
Acetone	2-Methyl Propanal	2-Heptanone		
Methylenechloride	2-Butanone	Heptanal		
	2-Pentanone	Octanal		
	3-Methyl Butanal	Nonanal		
	3-Pentanone	1,3-Dichlorobenzene		
	Tetrachloroethylene	1,4-Dichlorobenzene		
	Benzene	1,2-Dichlorobenzene		
	Toluene	Naphthalene		
		Phenol		
		Ethylbenzene		
		Limonene		
		P-Xylene		
		m-Xylene		
		o-Xylene		
		1,3,5-Trimethylbenzene		
		Benzaldehyde		

	LIMITATIONS	ADVANTAGES	REMEDY
Sampling	- Dilution of VOC s in dead space - Lack of standardization (pCO2 sample/pCO2 end tidal) - VOCs stability in bag/cartridge - Storage time - VOCs concentration - Contamination (i.e. diet)	For patient: easy and non invasive	• Semiselective sorbent material with preconcentration of VOCs • Thermal desorption • Standardisation with cartridges/ containers
Pre-concentration	Thermal desorption can cause VOCs degeneration	Resolution power enhancement Exhaled breath separation in VOCs or VOCs families	Microfluidics (i.e. extremely low volumes i.e. nanolitres) management of water component of VOCs
GC-MS	Specialized personnel Expensive equipment Sampling techniques	Provides VOC library as reference	Portable GC-MS

	LIMITATIONS	ADVANTAGES	REMEDY
Optical and non optical spectroscopic devices	Photo-bleaching Low portability	High sensitivity Adjustable selectivity for specific VOCs identification Multiplicity of data sources (changes in intensity, fluorescence, wavelength and spectral	Portable spectrometer Pretreatment for sample preservation

	LIMITATIONS	ADVANTAGES	REMEDY
Colorimetric e-nose devices Acoustic based e-nose devices Conductometric based e-nose devices	No VOCs identification Many different working principles (not univocal definition of e-nose) Time consuming calibrations Sample collection crucial Alterations of electrode surface coating Changes in humidity/temperature yield Necessity of micro-heater	Provides exhaled breath fingerprint Used in outpatient or bedside Cost effective Relatively inexpensive Temperature based selectivity Enhance surface coating chemosensitivity with improved material (i.e. carbon nanoparticles)	Provide pattern recognition of VOCs

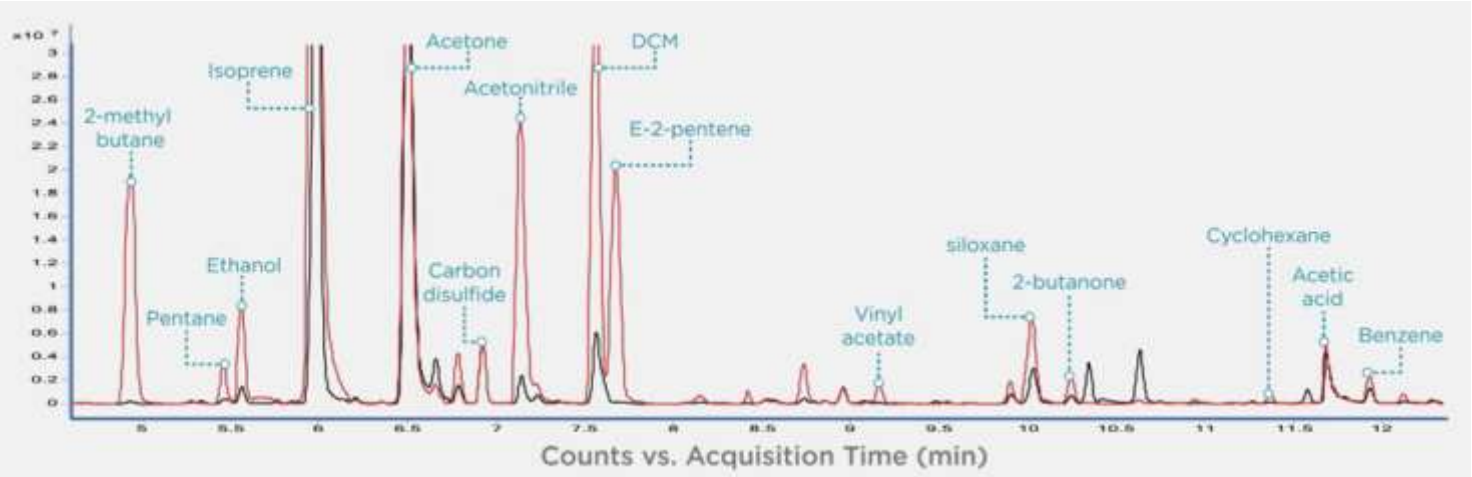
LONESTAR

Less expensive and user friendly

FAIMS technology used by Lonestar

Produces information regarding chemical spectrum and

unique features associated with each VOC

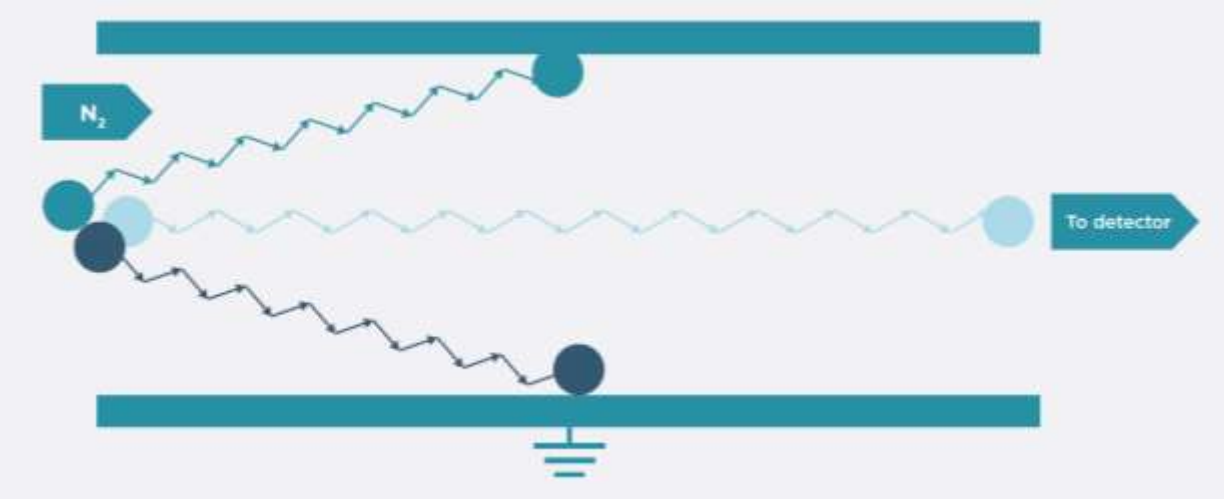


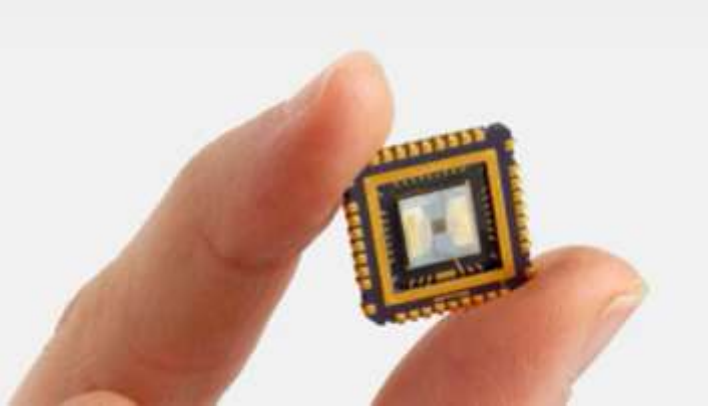
FIELDS ASYMMETRIC ION MOBILITY SPECTROMETRY (FAIMS)

Ion mobility spectrometry separates molecules according to speed at which they move through gas under influence of electric field.

Uses asymmetric alternating voltage to separate molecules according to how their shape changes in high electric fields

Unlike MS does not require molecule to move through vacuum avoiding need for vacuum generator and smaller foot print





LUCID TRIAL

- LuCID (lung cancer indicator detection)
- International multi-centre prospective case control cohort study
- Recruitment based on clinical suspicion of lung cancer based on symptomatology or incidental imaging finding
- Clinical trial ID NCT02612532

LUCID TRIAL

- Two arms:

1. Early detection of lung cancer with aim to increase cases diagnosed at Stages 1 and 2
2. Differences in breath profiles pre and post surgery with patient acting as their own control

STUDY	COHORT	INTERVENTION	OUTCOME
Barash et.al. 2011		Gold nanoparticles GC-MS	Discrimination done between lung carcinoma and healthy cells 96% sensitivity, 86% specificity, and 93% accuracy
McWilliams et.al. 2014	25 lung cancer 166 high risk smoker control without cancer	Cyranose 320 (Smiths Detection Inc.)	• Correctly differentiate high-risk smokers/ex-smokers from subjects with lung cancer (accuracy between 75%-85%) • Smell-prints of high-risk smokers significantly distinct from those diagnosed with lung cancer
Rocco et.al. 2017	100 patients	BIONOTE	Sensitivity and specificity – 86% and 95% for differentiating patients and healthy adults
Tirzïte et.al. 2018	252 cancer patients 223 patients without cancer	Cyronose 320	• Sensitivity 95.8% for smokers and 96.2% for non smokers • Specificity 90.6% for non smokers and 92.3% for non smokers

Barash et.al. Nanomedicine: NBM2012
Williams et.al. IEEE Trans Biomed Eng. 2015;62:2044-2054
Rocco et.al. European Journal of Cardio-Thoracic Surgery 49 (2016
Tirzïte et al 2019 J. Breath Res

STUDY	COHORT	INTERVENTION	OUTCOME
Pled et.al. 2012	53 malignant patients and 19 benign	Nanoarray	Accuracy of 88% differentiating between benign and malignant
Shlaomi et.al. 2017	119 patients 30 with benign nodules 89 with LC with or without EGFR mutation	Nanomaterial based sensor array	• Discrimination of early LC from benign nodules accuracy 87%, PPV and NPV – 87.7% and 87.5% respectively • Positive breath printing for EGFR testing can be used in treatment plan if tissue is not adequate for diagnosis
Kort et.al. 2018	107 with NSCLC and 200 healthy subjects	Aeronose (eNOSE company)	• Sensitivity 78% and specificity 57%

Shlomi et.al. Journal of Thoracic Oncology 2017
Peled et.al. *J Thorac Oncol.* 2012
Kort et.al. ERJ 2018

Table 2. Sensitivity and Specificity Outcomes From Different Models of Breath Analysis

Method of Breath Analysis	Sensitivity (%)	Specificity (%)	Accuracy	Accuracy[a] Adca	Accuracy[a] Squam	Accuracy[a] Stage I
Canine	71-99	82-99	–	–	–	–
GC-MS	51-90	67-100	69-97	88	88	88
E-nose	70-93	73-100	80-100	83	86	85
E-nose + GC-MS	100	80-96	88-94	–	–	–

Mazzone et.al. J Thorac Oncol.2012
Peled et.al. J Thorac Oncol. 2012

ROLE OF CELL FREE DNA

- In 30% of NSCLC cases, tissue sample not available even for biomarker testing, either at diagnosis or at disease progression
- Generated by the specific fragmentation pattern of ctDNA(160bp), indicative of a nuclease-dependent degradation
- In lung cancer, the levels of cfDNA have been found to correlate with variety of clinical and pathological features, including volume, extent of necrosis, and histology of the tumor

nature medicine

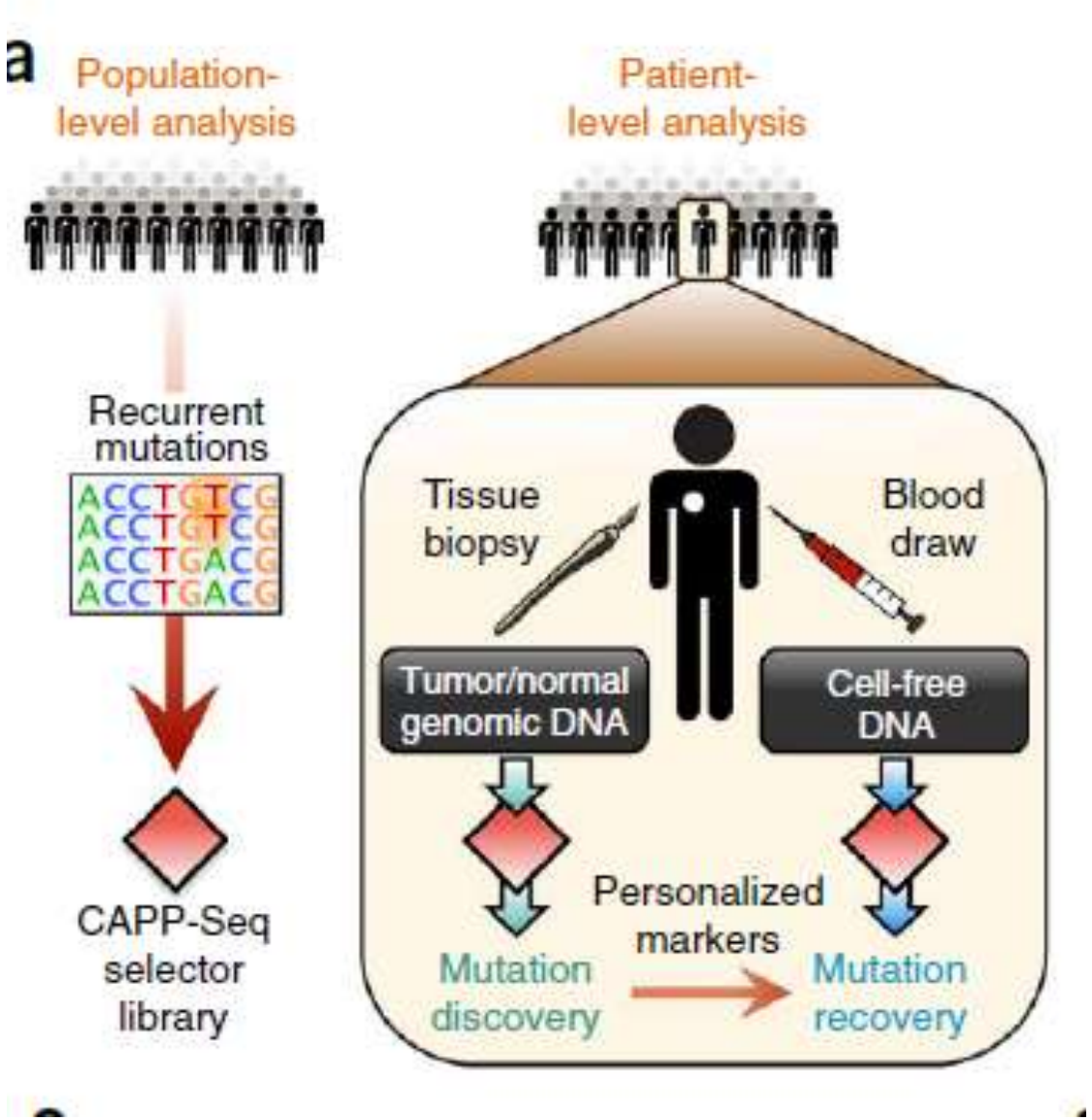
An ultrasensitive method for quantitating circulating tumor DNA with broad patient coverage

Newman et.al.

- Cancer personalized profiling by deep sequencing (CAPP-Seq), CAPP-Seq for non–small-cell lung cancer (NSCLC) with design covering multiple classes of somatic alterations that identified mutations in >95% of tumors

- ctDNA in 100% of patients with stage II–IV NSCLC and in 50% of patients with stage I, with 96% specificity for mutant allele fractions down to ~0.02%

Newman et.al. Nature Medicine May 2014



CAPP-Seq, combines optimized library preparation methods for low DNA input masses with multiphase bioinformatics approach to design a ‘selector’ consisting of biotinylated DNA oligonucleotides that target recurrently mutated regions in the cancer of interest

CAPP-Seq is the first NGS-based method for ctDNA analysis that achieves both an ultralow detection limit

Newman et.al. Nature Medicine May 2014

nature medicine

An ultrasensitive method for quantitating circulating tumor DNA with broad patient coverage

Newman et.al.

- Levels of ctDNA highly correlated with tumor volume and distinguished between residual disease and treatment-related imaging changes, and measurement of ctDNA levels allowed for earlier response assessment than radiographic approaches

Newman et.al. Nature Medicine May 2014

TECHNIQUES

integrated Digital Error Suppression (iDES)

Requirement:

- Low quantities of cell-free DNA (cfDNA) in the blood
- Sequencing artifacts limit analytical sensitivity

Newman et al. Nat Biotechnol. 2016 May

iDES

Combines two techniques for the efficient recovery of cfDNA molecules

- In silico elimination of highly stereotypical background artifacts

- Molecular barcoding strategy - tagging individual DNA molecules with unique identifiers

- Barcodes enable the precise tracking of individual molecules
- Making it possible to distinguish authentic somatic mutations arising in vivo from artifacts introduced ex vivo

Newman et al. Nat Biotechnol. 2016 May

iDES

- Improve the sensitivity of CAPP-Seq by ~3 fold
- iDES-enhanced CAPP-Seq facilitates noninvasive variant detection across hundreds of kilobases
- Biopsy-free profiling of EGFR kinase domain mutations with 92% sensitivity and 96% specificity and detection of ctDNA down to 4 in 105 cfDNA molecules

Newman et al. Nat Biotechnol. 2016 May

RESULTS

- iDES-enhanced CAPP-Seq could detect tumor-derived DNA 10-fold below original description of CAPP-Seq
- When iDES was applied to pretreatment plasma, ctDNA was significantly detectable in 93% of patients, including 3 of 3 stage I tumors
- ctDNA was significantly detectable in 73% of pre- and post-treatment plasma samples (n=86), with a specificity of 100%

Newman et al. Nat Biotechnol. 2016 May

Targeted error correction sequencing (TEC-Seq)

- Allows ultrasensitive direct evaluation of sequence changes in circulating cell-free DNA using massively parallel sequencing
- Based on targeted capture of multiple regions of the genome and deep sequencing (~30,000×) of DNA fragments

Phallen et al., Sci. Transl. Med. 9, eaan2415 (2017)

RESULTS

- Examined 58 cancer-related genes encompassing 81 kb
- Analysis of plasma from 44 healthy individuals identified genomic changes related to clonal hematopoiesis in 16% of asymptomatic individuals but no alterations in driver genes related to solid cancers
- Evaluation of 200 patients with colorectal, breast, lung, or ovarian cancer detected somatic mutations in the plasma of 71, 59, 59, and 68% respectively, of patients with stage I or II disease

Phallen et al., Sci. Transl. Med. 9, eaan2415 (2017)

Table 2. Cancer patients detected using TEC-Seq. NA, not applicable.

Cancer type	Patients (*n*)	Patients with ctDNA alterations (*n*)	Fraction of patients with ctDNA alterations (%)
Lung			
I	29	13	45
II	32	23	72
III	4	3	75
IV	6	5	83
I–IV	71	44	62

More than three quarters of patients with advanced disease (stages III and IV) and 62% of patients with localized disease (stages I and II) detected

Phallen et al., Sci. Transl. Med. 9, eaan2415 (2017)

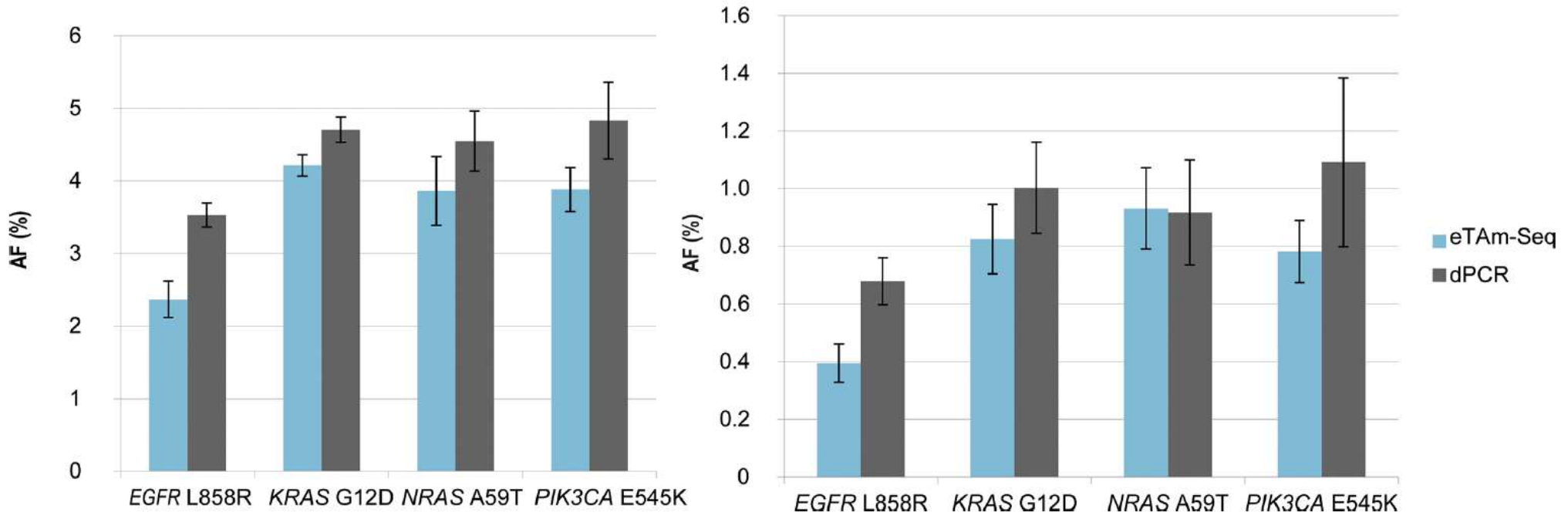
Tam-Seq™

- InVision™ liquid biopsy platform uses enhanced TAm-Seq™ (eTAm-Seq™) technology
- Tam-Seq™ technology - Amplicon-based next generation sequencing method
- Gale et.al. compared quantitative performance of eTAm-Seq technology for analysis of single nucleotide variants in clinically-relevant genes as compared to digital PCR (dPCR)

Gale et.al. PLoS ONE 13(3):e0194630

RESULTS

- Assay detected mutant alleles down to 0.02% allele frequency, with high per-base specificity of 99.9997%



Gale et.al. PLoS ONE 13(3):e0194630

Osimertinib benefit in *EGFR*-mutant NSCLC patients with *T790M*-mutation detected by circulating tumour DNA

Remon et.al.

- The T790M positivity in ctDNA reported in 24 out of 48 (50%) NSCLC patients
- For 9 of 24 patients with ctDNA T790M-positivity, T790M AF was lower than 0.5% in the liquid biopsy
- Conclusion: ctDNA from liquid biopsy can be used as a surrogate marker for T790M in tumour tissue.

Remon et.al. Annals of Oncology 28: 784–790, 2017

CONCLUSION

- Only handful of studies evaluating the role of breathprining and particularly CfDNA in early detection of lung cancer
- The technology has potential but needs large scale studies for establishing clinical significance