Dissection des étapes de la carcinogenèse bronchique précoce: rôle du microenvironnement

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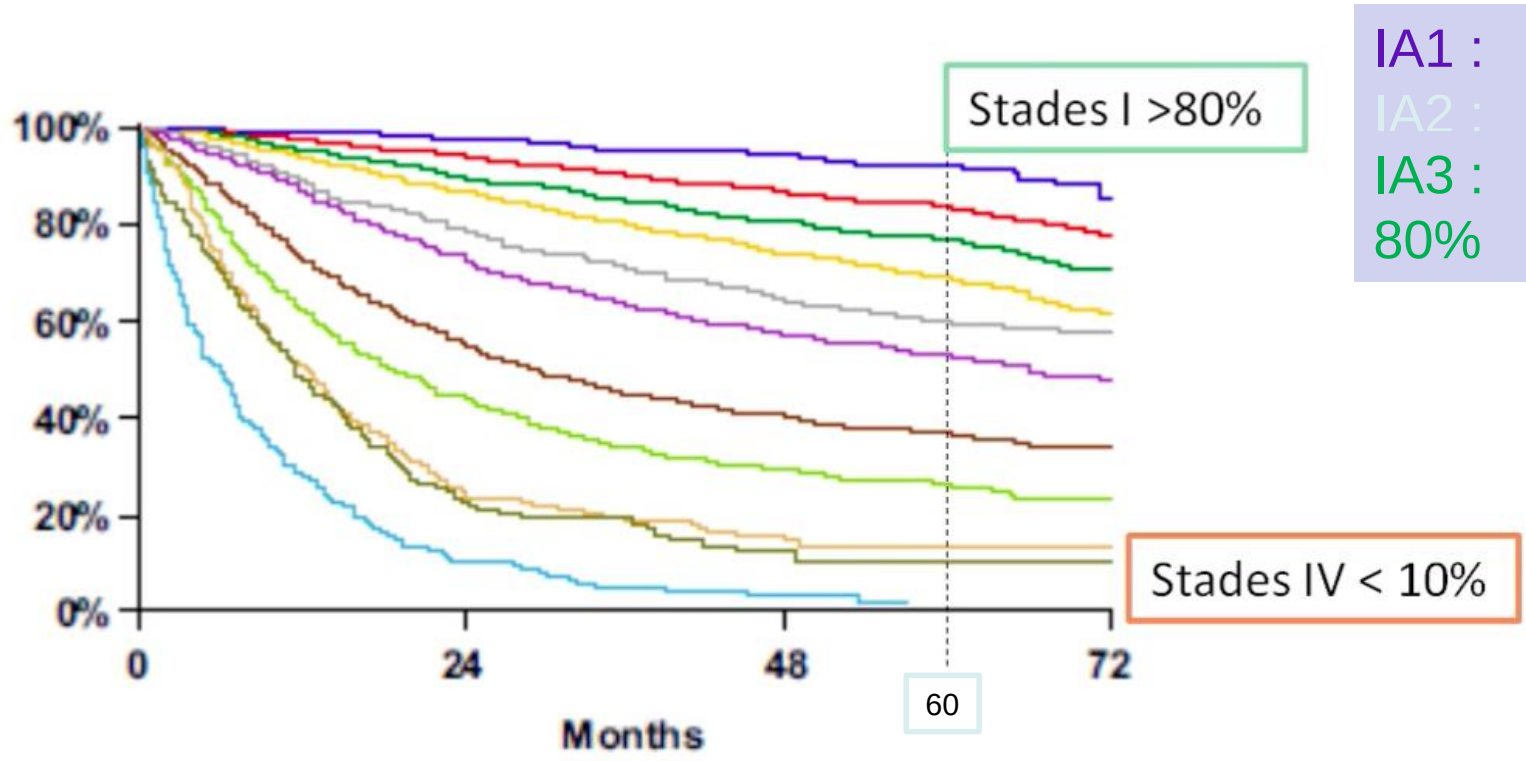
Agir précocement = meilleure chance de guérison



Goldstraw P et al J, Thorac Oncol 2016; 11 : 39-51

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Comment agir précocement sur le cancer ?

To Accurately identify patients with pre-malignancies and treat these pre-malignancies *before* they progresses towards carcinogenesis and localization

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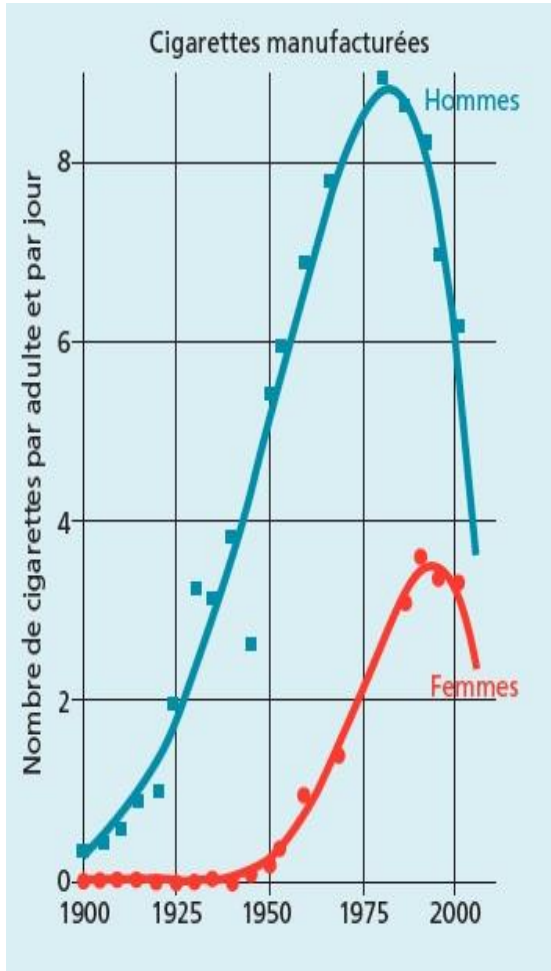
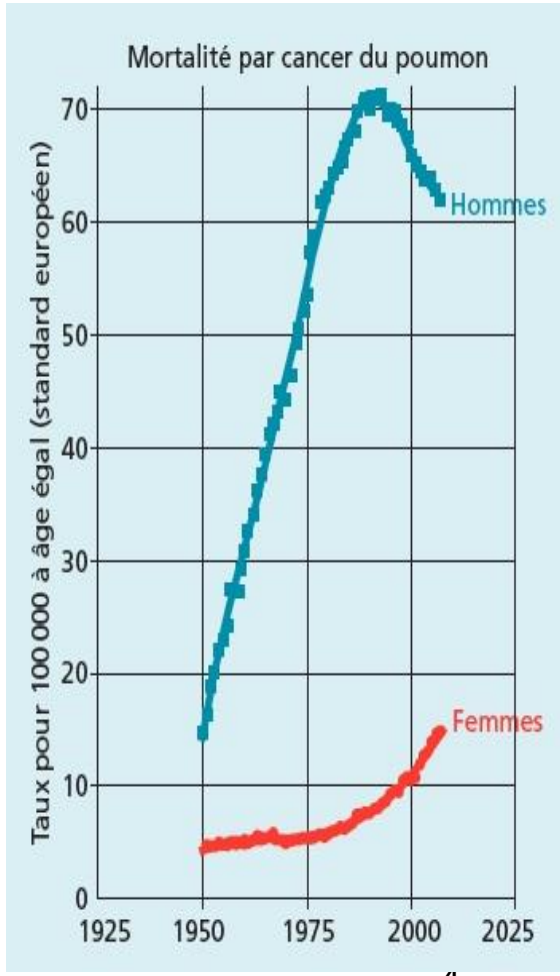
Main cause of lung cancer: tobacco

Deaths by lung cancer/100 000			
Non smokers	Current smokers		
	1-14	15-24	≥ 25
10	78	127	251





Comment agir précocement sur le cancer ?

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EDITORIAL

Mortality Reduction with Low-Dose CT Screening for Lung Cancer

Stephen W. Duffy, M.Sc., and John K. Field, Ph.D., F.R.C.Path.

With the NELSON results, the efficacy of low-dose CT screening for lung cancer is confirmed. Our job is no longer to assess whether low-dose CT screening for lung cancer works: it does. Our job is to identify the target population in which it will be acceptable and cost-effective.

ORIGINAL ARTICLE

Reduced Lung-Cancer Mortality with Volume CT Screening in a Randomized Trial

H.J. de Koning, C.M. van der Aalst, P.A. de Jong, E.T. Scholten, K. Nackaerts, M.A. Heuvelmans, J.-W.J. Lammers, C. Weenink, U. Yousaf-Khan, N. Horeweg, S. van 't Westeinde, M. Prokop, W.P. Mali, F.A.A. Mohamed Hoesein, P.M.A. van Ooijen, J.G.J.V. Aerts, M.A. den Bakker, E. Thunnissen, J. Verschakelen, R. Vliegenthart, J.E. Walter, K. ten Haaf, H.J.M. Groen, and M. Oudkerk

ABSTRACT

BACKGROUND

There are limited data from randomized trials regarding whether volume-based, low-dose computed tomographic (CT) screening can reduce lung-cancer mortality among male former and current smokers.

METHODS

A total of 13,195 men (primary analysis) and 2594 women (subgroup analyses) between the ages of 50 and 74 were randomly assigned to undergo CT screening at T0 (baseline), year 1, year 3, and year 5.5 or no screening. We obtained data on cancer diagnosis and the date and cause of death through linkages with national registries in the Netherlands and Belgium, and a review committee confirmed lung cancer as the cause of death when possible. A minimum follow-up of 10 years until December 31, 2015, was completed for all participants.

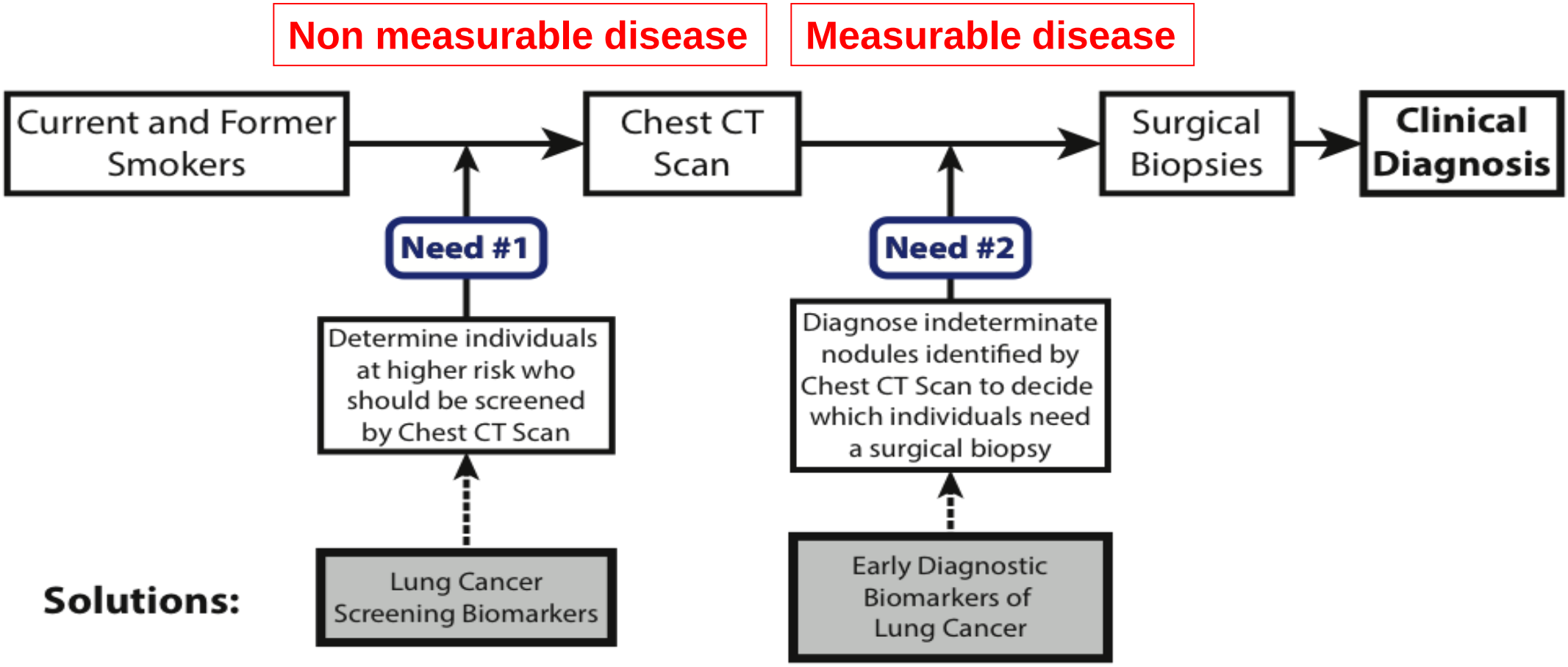
RESULTS

Among men, the average adherence to CT screening was 90.0%. On average, 9.2% of the screened participants underwent at least one additional CT scan (initially indeterminate). The overall referral rate for suspicious nodules was 2.1%. At 10 years of follow-up, the incidence of lung cancer was 5.58 cases per 1000 person-years in the screening group and 4.91 cases per 1000 person-years in the control group; lung-cancer mortality was 2.50 deaths per 1000 person-years and 3.30 deaths per 1000 person-years, respectively. The cumulative rate ratio for death from lung cancer at 10 years was 0.76 (95% confidence interval [CI], 0.61 to 0.94; P=0.01) in the screening group as compared with the control group, similar to the values at years 8 and 9. Among women, the rate ratio was 0.67 (95% CI, 0.38 to 1.14) at 10 years of follow-up, with values of 0.41 to 0.52 in years 7 through 9.

CONCLUSIONS

In this trial involving high-risk persons, lung-cancer mortality was significantly lower among those who underwent volume CT screening than among those who underwent no screening. There were low rates of follow-up procedures for results suggestive of lung cancer. (Funded by the Netherlands Organization of Health Research and Development and others; NELSON Netherlands Trial Register number, NL580.)

Role of biomarkers in lung cancer screening/detection



J. BrothersII, K. Hijazi, C. Mascaux, El-Zein RA, Sptiz MR, Spira A; BMC Medicine, 2013

Comment agir précocement sur le cancer ?

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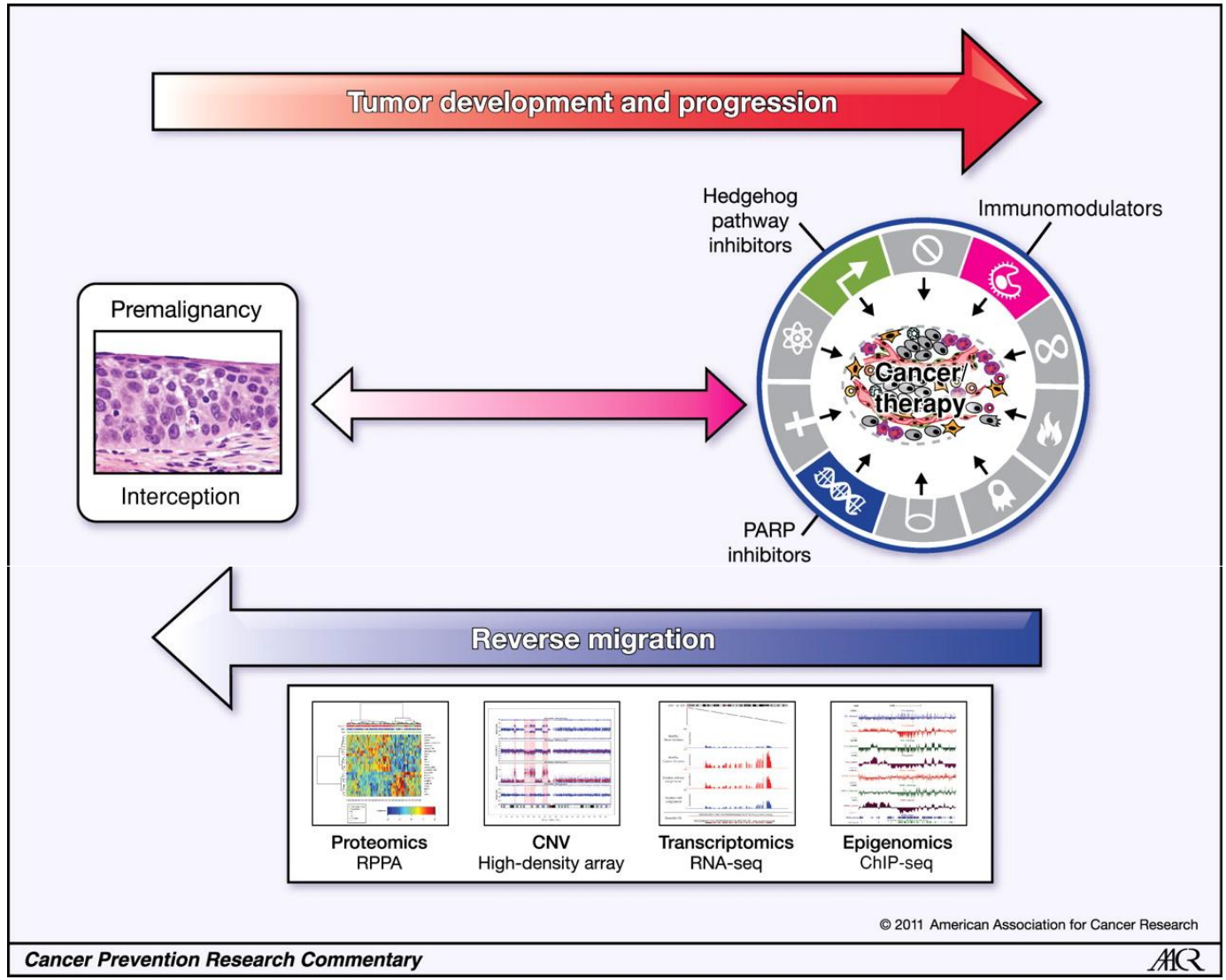
Cancer Interception Through Reverse Migration

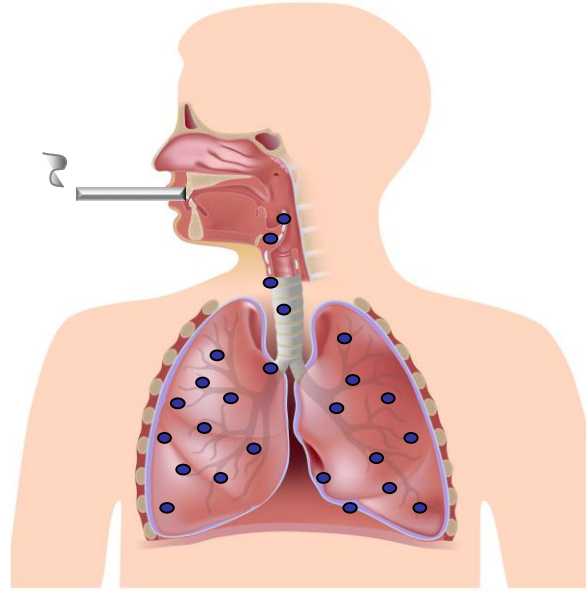


Blackburn EH, Cancer Prev Res 2011;4:787-792

THE AIRWAY EPITHELIAL "FIELD OF INJURY" **HYPOTHESIS**

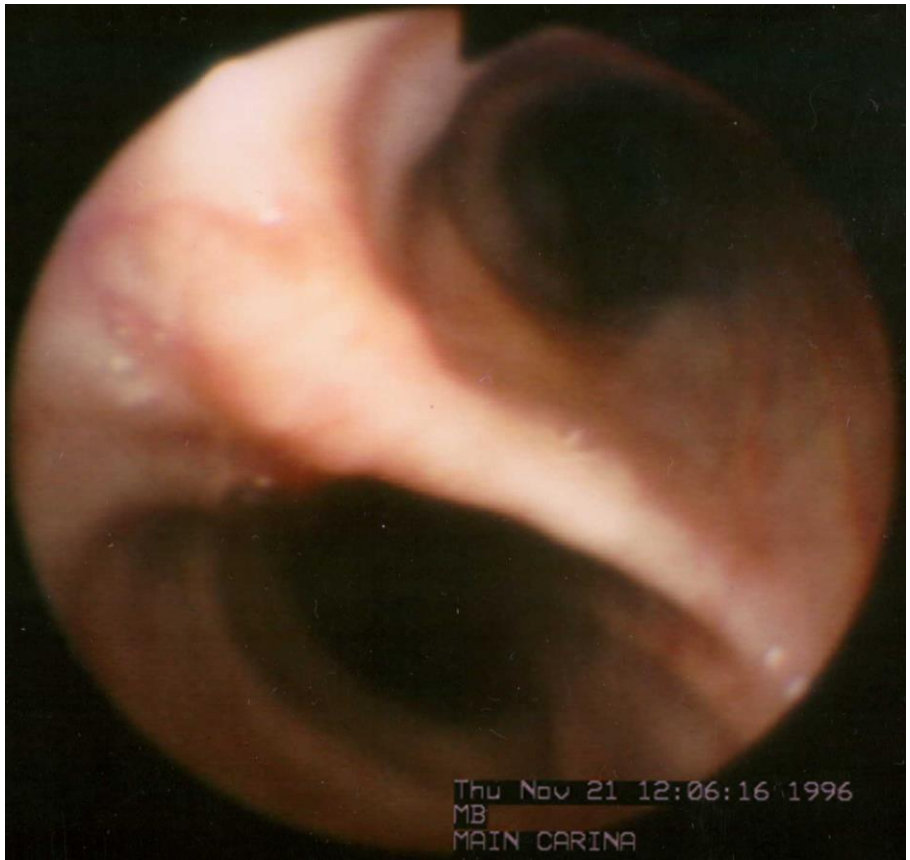
- Cigarette smoking causes molecular damages throughout the lungs and respiratory tract

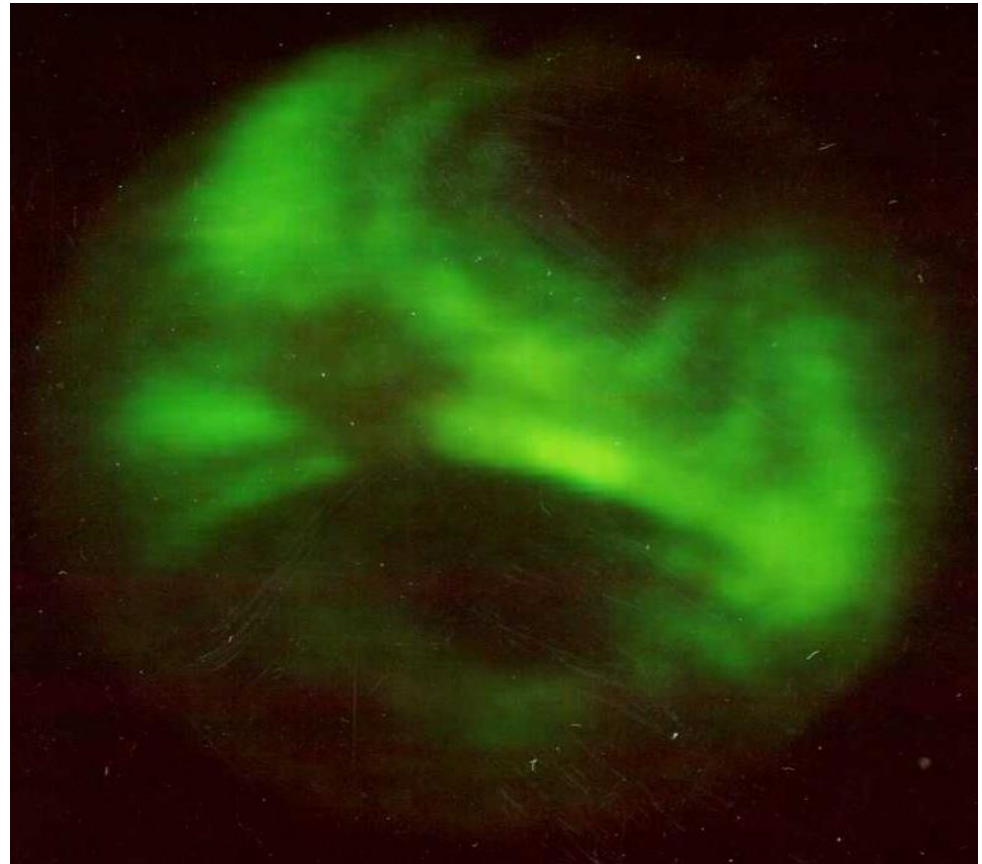
- Epithelial cells reflect the genomic response to smoking and risk for smoking-associated lung disease



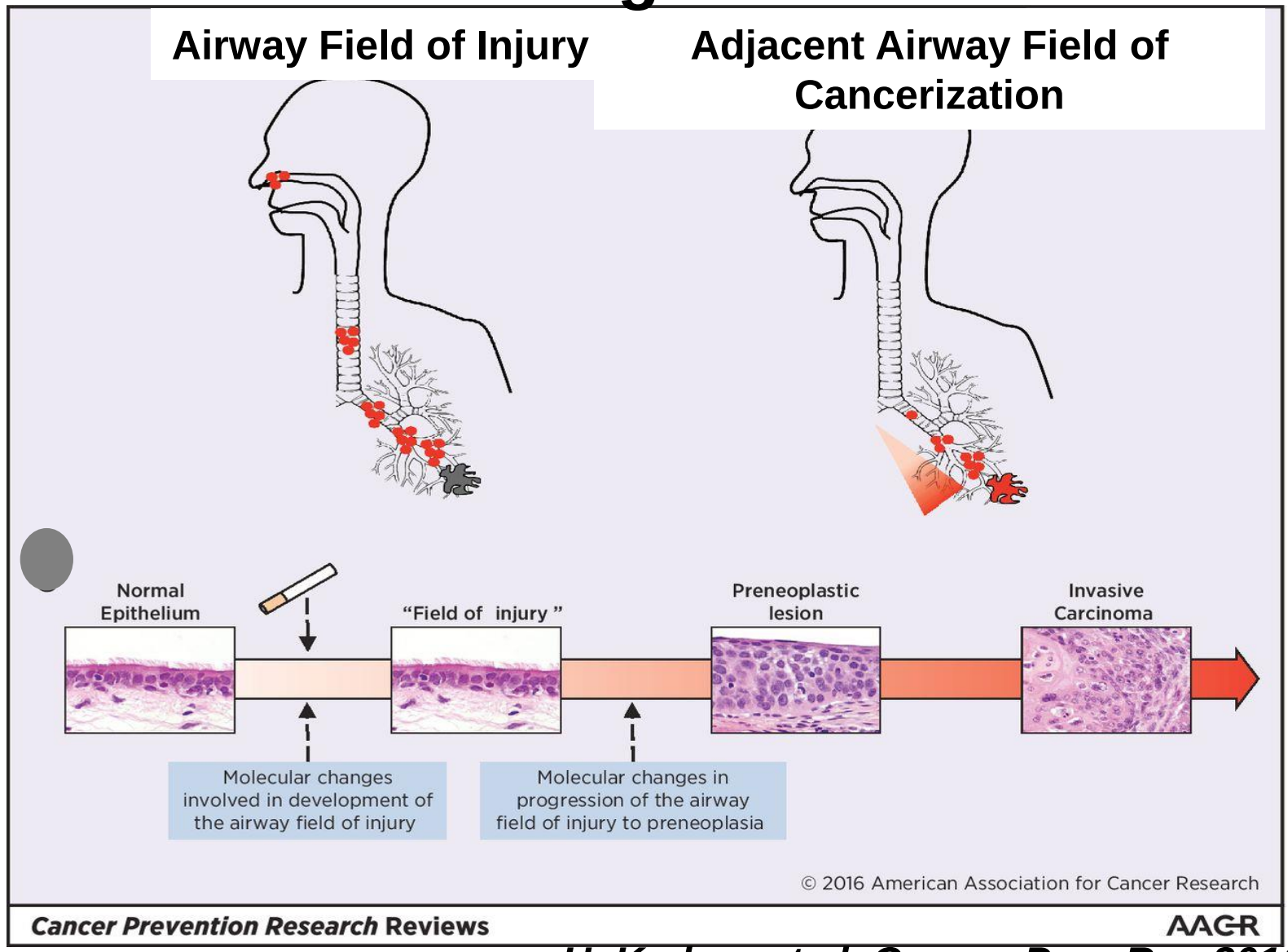
Field cancerisation

- The whole respiratory epithelium is targetted by carcinogens that are in tobacco smoke
- Smokers develop genetic and morphologic abnormalities diffusely in their airways

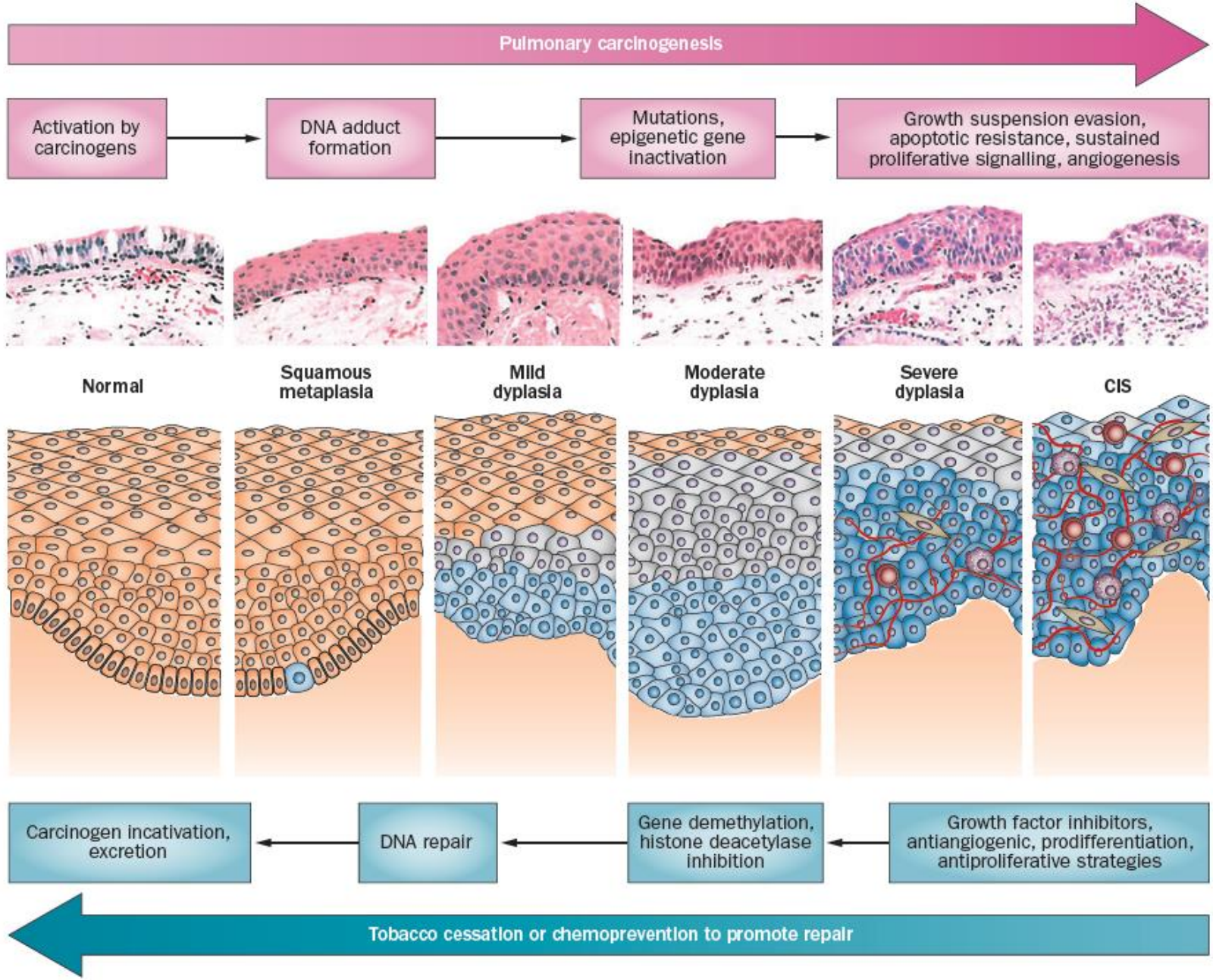




THE AIRWAY EPITHELIAL "FIELD OF INJURY" : **Premalignant Airway Fields and Molecular Pathogenesis**



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



Robert Keith and York Miller, Nat Rev, 2013

Hypothesis

- Characterization of the evolution of molecular abnormalities in lung carcinogenesis would allow:

  - **Better understanding of the mechanisms of the genesis and development of squamous cell carcinoma**
  - **Identification of new biomarkers for the early detection of lung cancer**
  - **Targets for chemoprevention**

Molecular Changes Reported for Lung Squamous Carcinogenesis

Normal	Hyperplasia	Metaplasia	Mild dysplasia	Moderate dysplasia	Severe dysplasia	In situ carcin	SCC
3p21	PAM	RARβ	mutations p53	NPM	p14^{arf}	CA IX	Rb
3p22-24	telomerases	keratins 4, 8,	FHIT		EGFR	MMP-3	5p
3p25	MMP-9	17 et 18	Bax, Bcl-2		Ki-67	c-ets1	5q
9p21	TIMP-1	Cyclin D1	aneuploidy		COX-2		c-erbB-2
PCNA	TIMP-2	Cyclin E	involucrine		53BP1		
protein p53	NF-κB	3p14.2	keratins 6, 10, 13, 14		ATM		
		3p 14-21	involucrin		CHK2		
		methyl p16	MDM2		H2AX		
		p21^{waf}	microvessels				
		c-JUN	MMP-11				
		MMP-1	μ-PA				
		CD44	protein p16				
		histogroups A et B	hnRNP				
		survivin	17p				
		Hps 10 et 60	VEGF				
		maspin	Activated Caspase 3				
			E cadherin				
			catenins				
			S100A2				

Molecular Changes Previously Reported for Lung Squamous Carcinogenesis

Normal	Hyperplasia	Metaplasia	Mild dysplasia	Moderate dysplasia	Severe dysplasia	In situ carcin	SCC
3p21 3p22-24 3p25 9p21 PCNA protein p53	PAM telomerases MMP-9 TIMP-1 TIMP-2 NF-κB	RARβ keratins 4, 8, 17 et 18 Cyclin D1 Cyclin E 3p14.2 3p 14-21 methyl p16 $p21^{waf}$ c-JUN MMP-1 CD44 histogroups A et B survivin Hps 10 et 60 maspin	mutations p53 FHIT Bax, Bcl-2 aneuploidy involucrine keratins 6, 10, 13, 14 involucrin MDM2 microvessels MMP-11 μ-PA protein p16 hnRNP 17p VEGF Activated Caspase 3 E cadherin catenins S100A2	NPM	$p14^{arf}$ EGFR Ki-67 COX-2 53BP1 ATM CK2	CA IX MMP-3 c-ets1 c-erbB-2	Rb 5p 5q

Eur Respir J 2008; 32: 678–686
DOI: 10.1183/09031936.00008408

The role of NPM, p14arf and MDM2 in precursors of bronchial squamous cell carcinoma

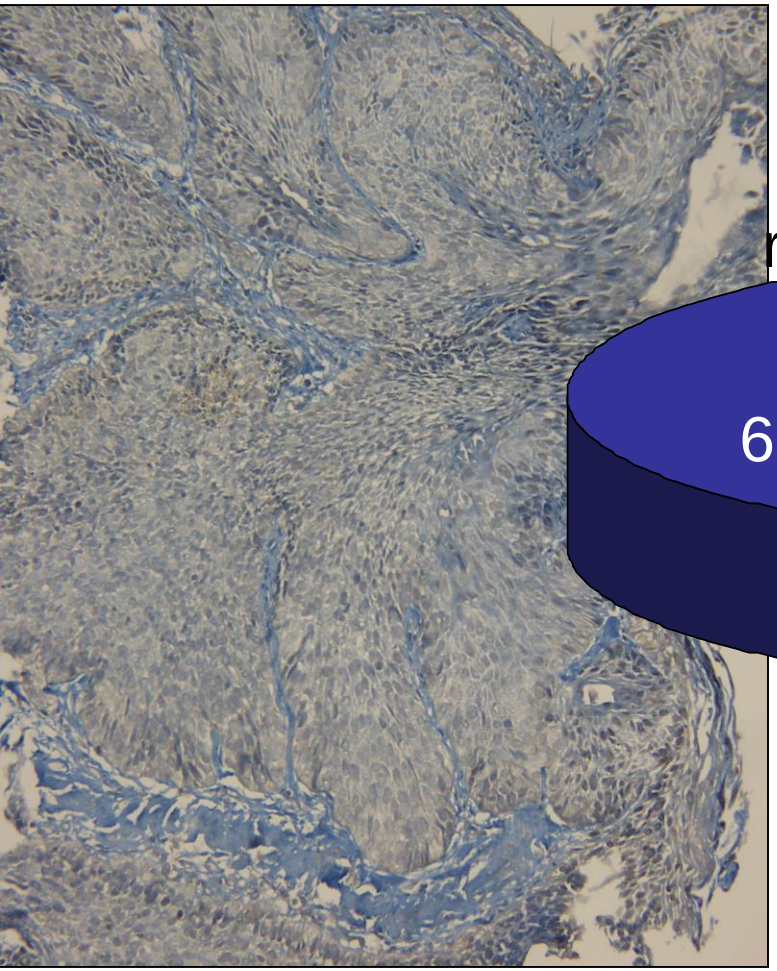
C. Mascaux*, F. Bex#, B. Martin*, A. Burny¶, A. Haller+, M. Paesmans§, K. Willard-Gallo[f], V. Ninane and J-P. Sculier***

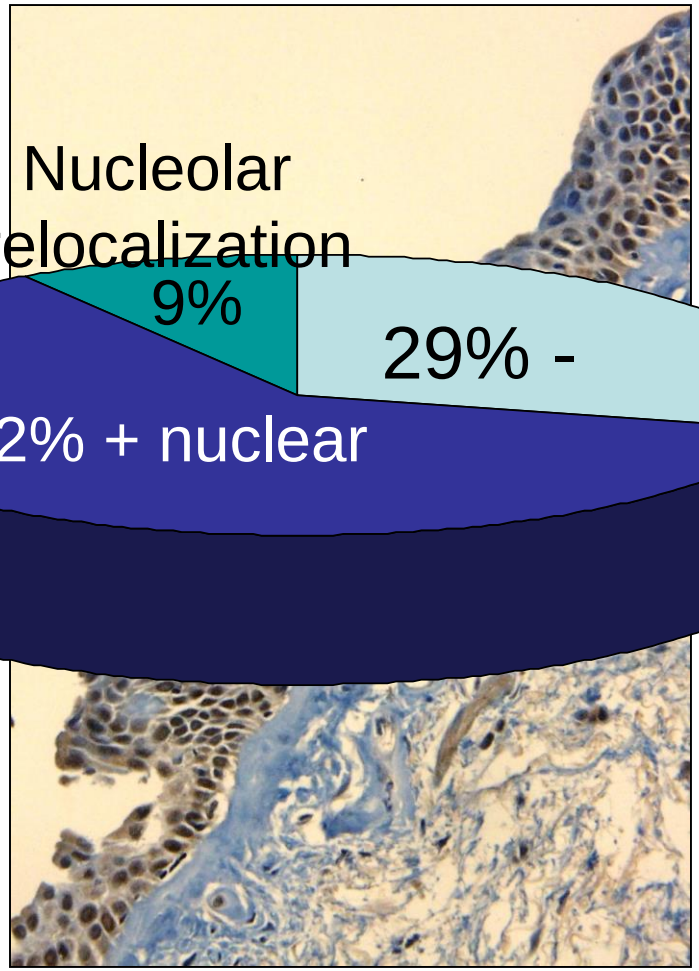
$p14^{ARF}$: 144 biopsies

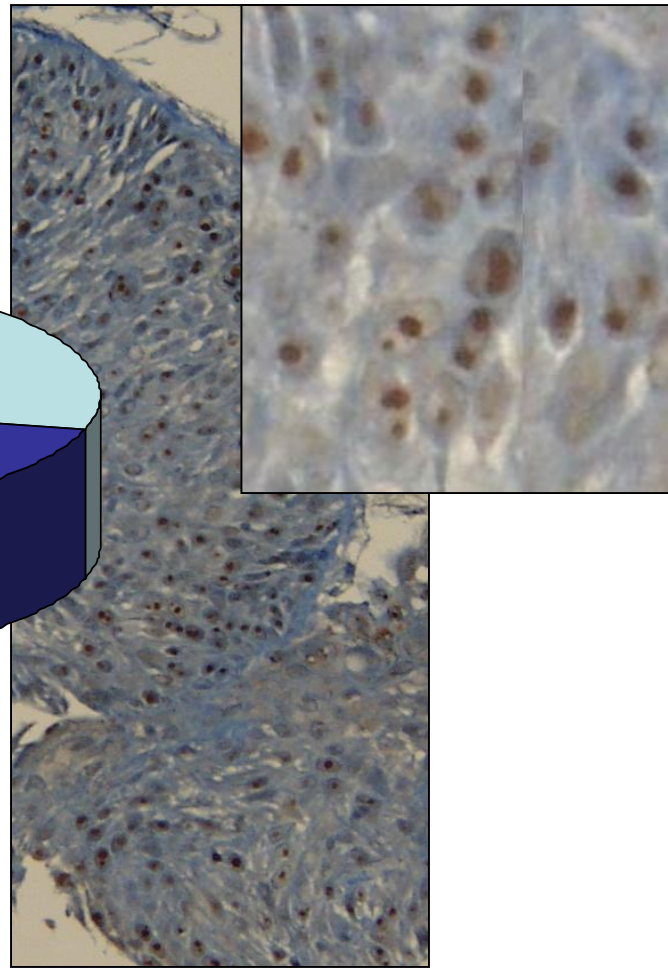
Negative

Diffuse nuclear

Localized in the nucleoli



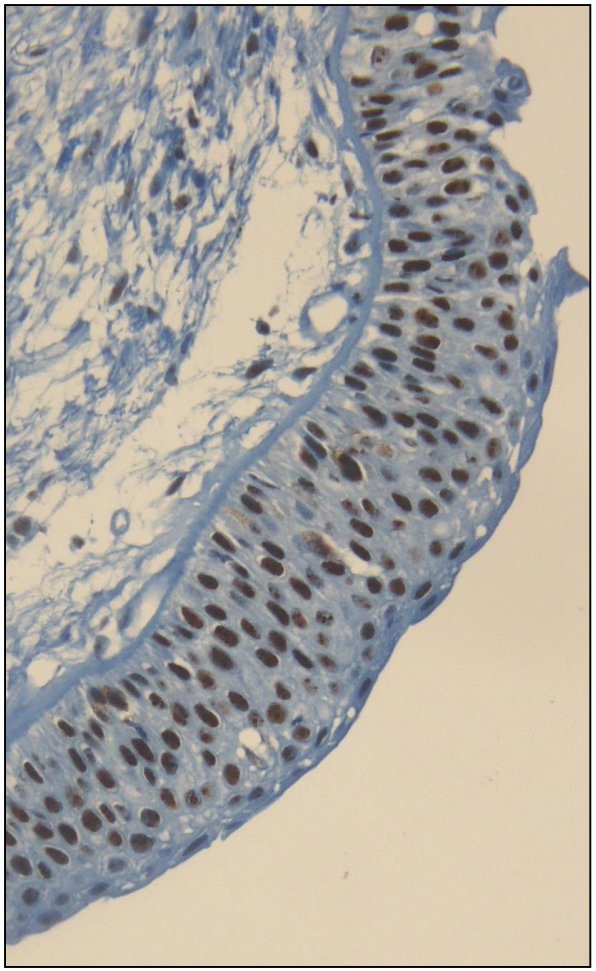




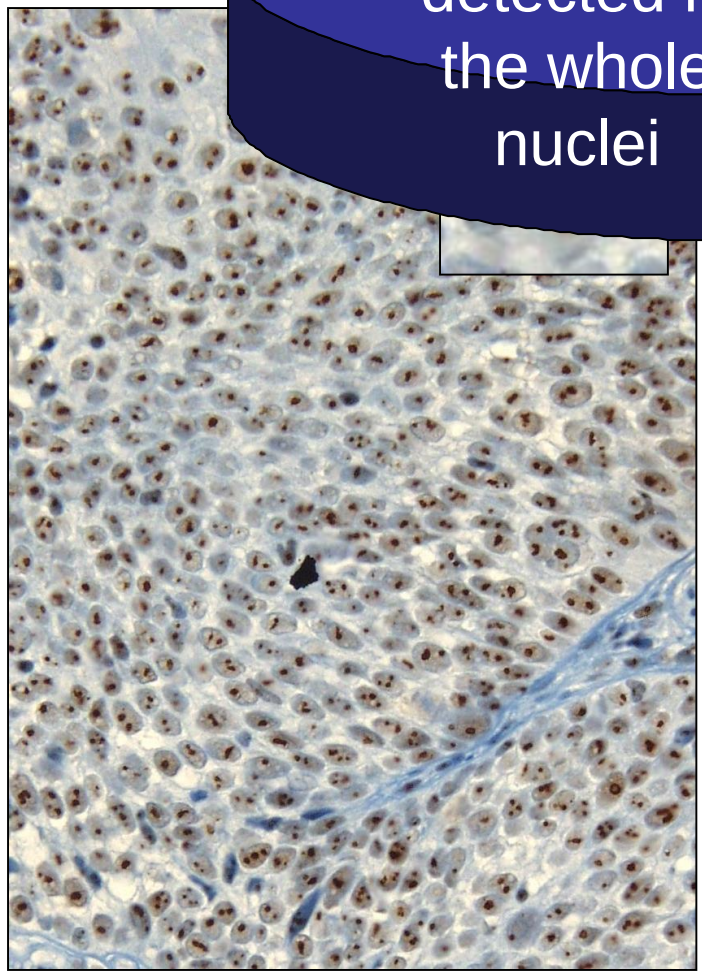
Mascaux et al, Eur Resp J, 20

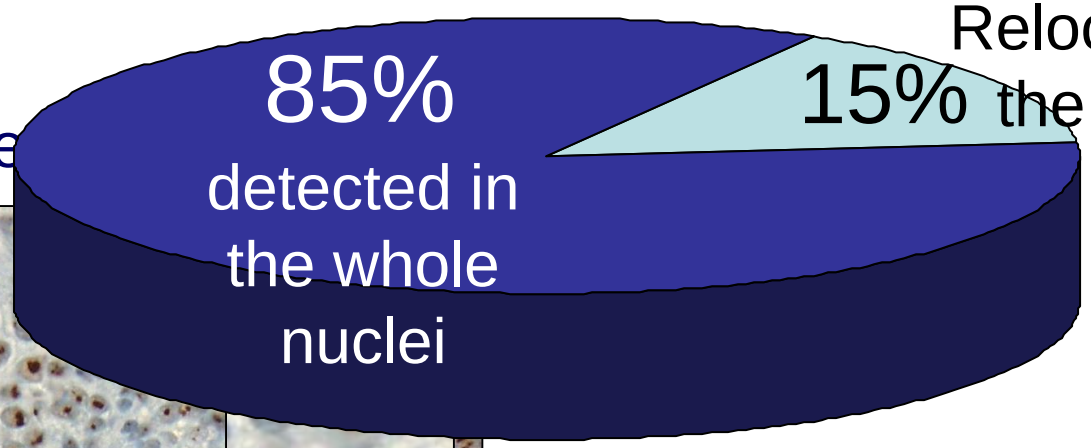
NPM: 123 biopsies

Nuclear diffuse



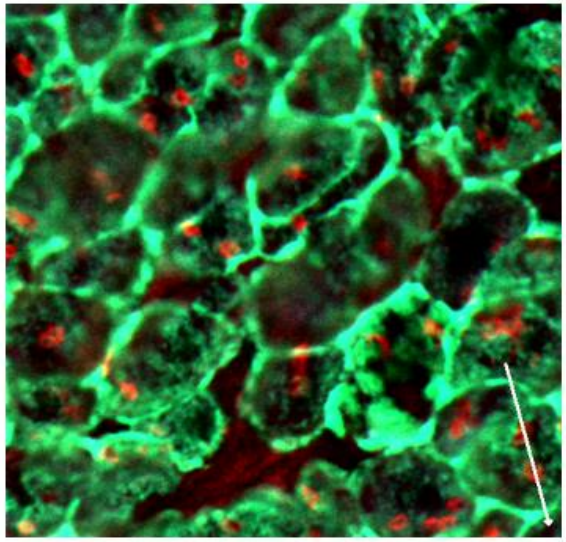
Nucle



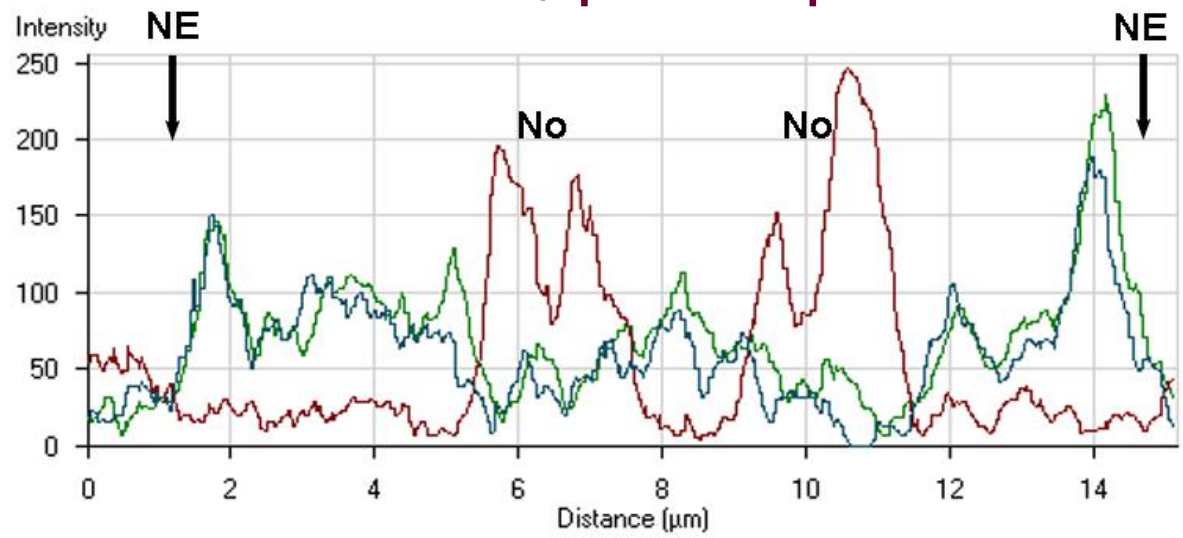


Mascaux et al. Eur Resp J

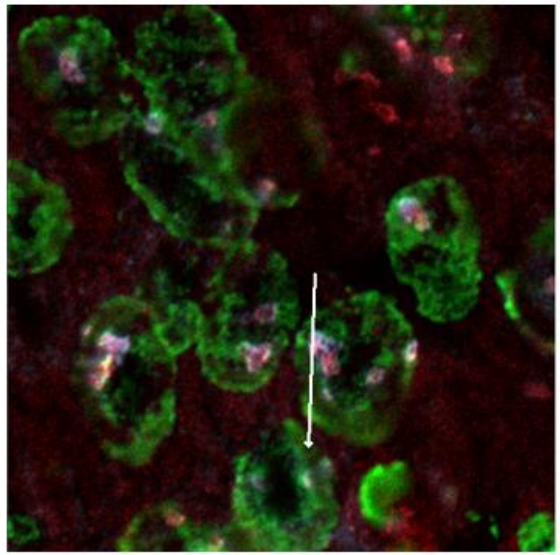
MDM2, p53 et p14^{arf}

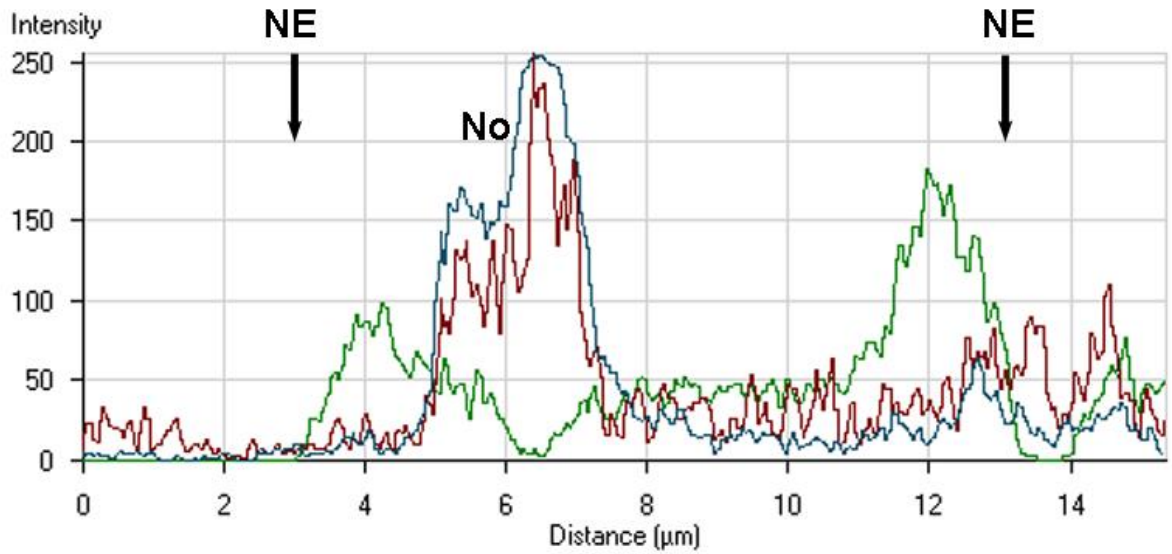


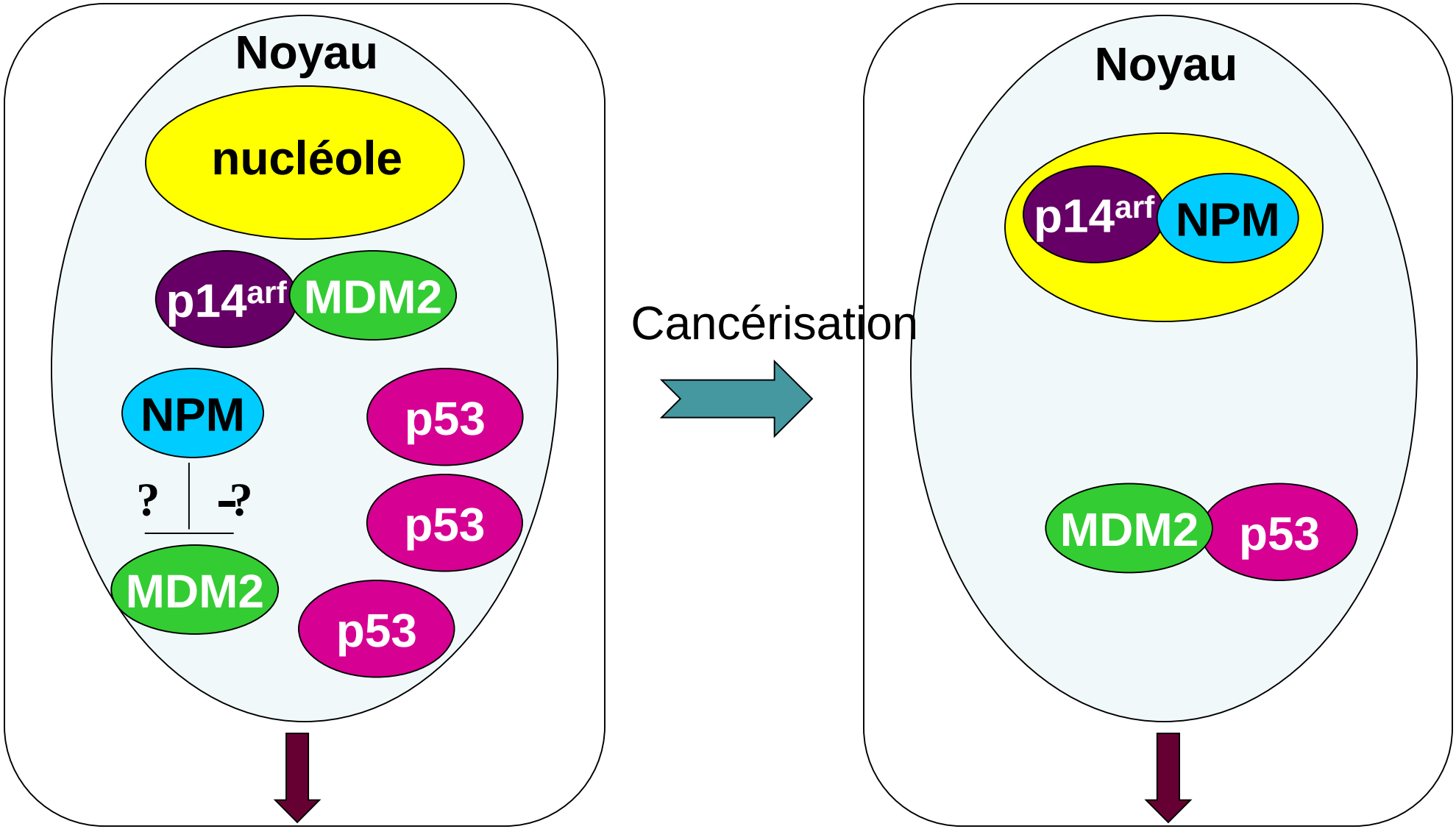
10 µm



NPM, p14^{arf} et MDM2





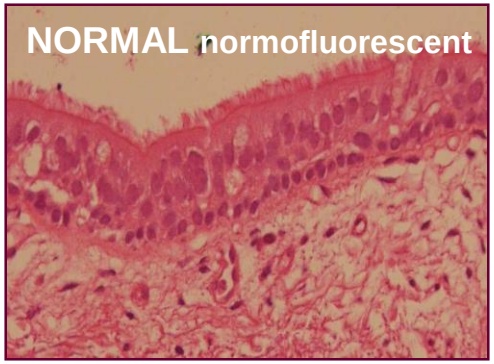

Noyau
nucléole
p14arf
MDM2
NPM
?
–?
MDM2
p53
p53
p53
Cancérisation
Noyau
p14arf
NPM
MDM2
p53
Arrêt du cycle cellulaire
et apoptose
Prolifération
cellulaire

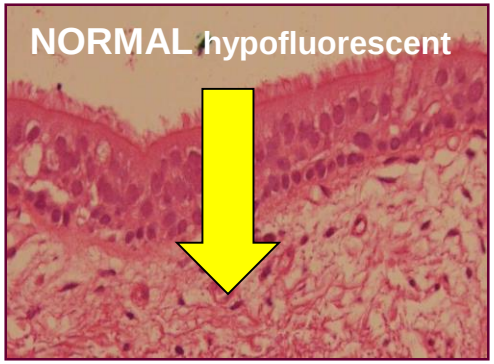
Eur Respir J 2009; 33: 352–359
DOI: 10.1183/09031936.00084108

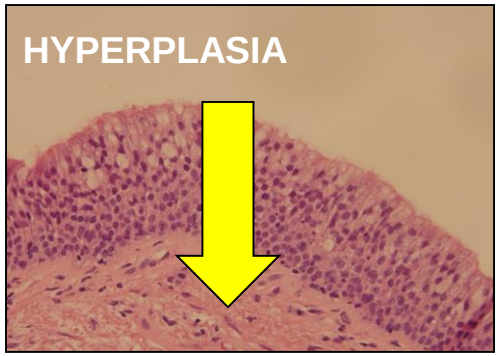
Evolution of microRNA expression during human bronchial squamous carcinogenesis

C. Mascaux*,f, J.F. Laes#,f, G. Anthoine*, A. Haller¶, V. Ninane+, A. Burny§ and J.P. Sculier*

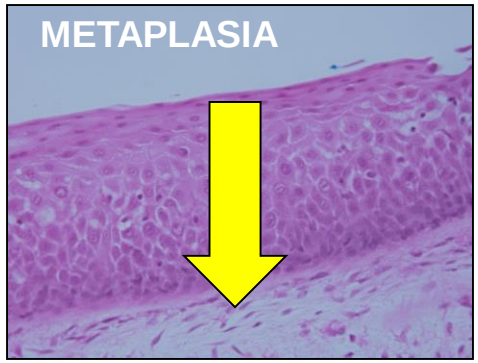
Group A

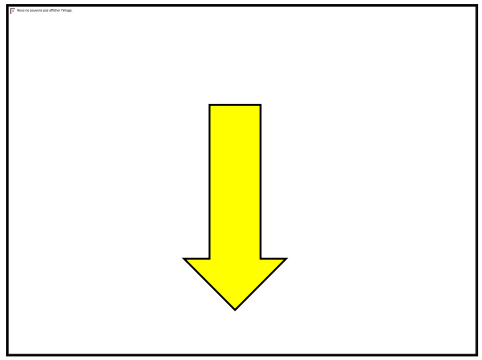


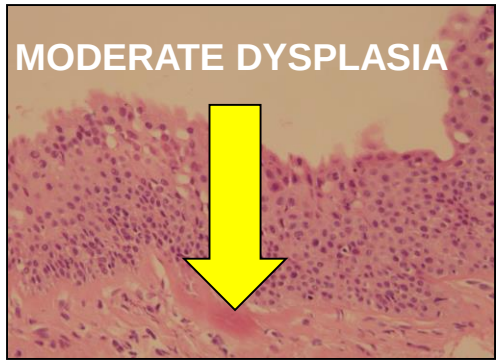




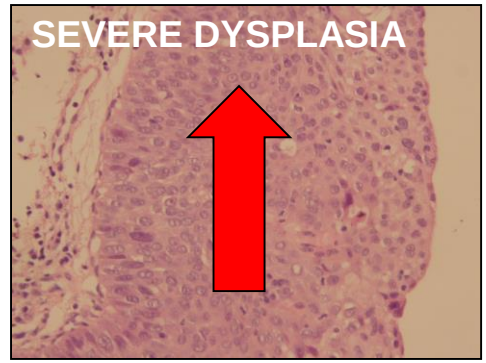
Group B

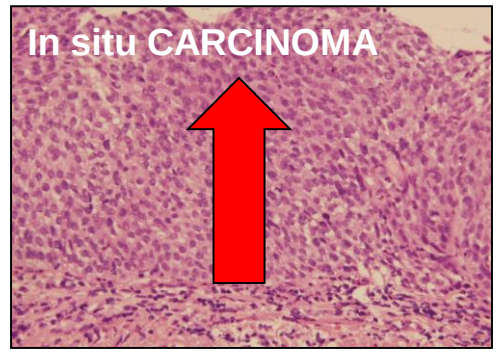




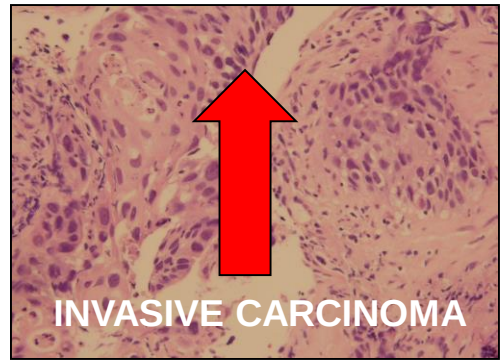


Group C





GROUP C1



GROUP C2

Quels miARNs?

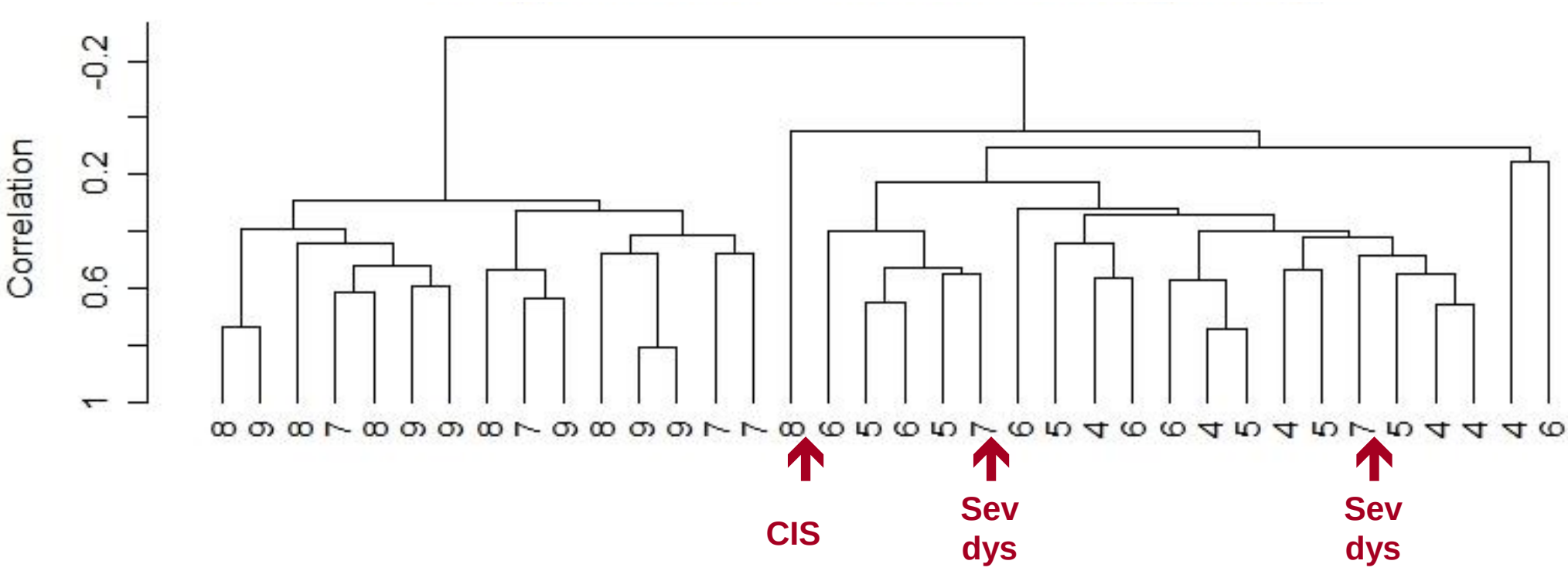
- Des miARNs décrits dans l'embryogenèse pulmonaire et qui évoluent de manière inverse dans la carcinogenèse

  = perte de la différenciation cellulaire épithéliale bronchique normale pour l'acquisition d'une autre différenciation, métaplasique, pluristratifiée et malpighienne

  - miR-34c, miR-15a: ↓
  - miR-99a, miR-142-3p, miR-142-5p, miR-214, miR 301 : ↓ puis ↑

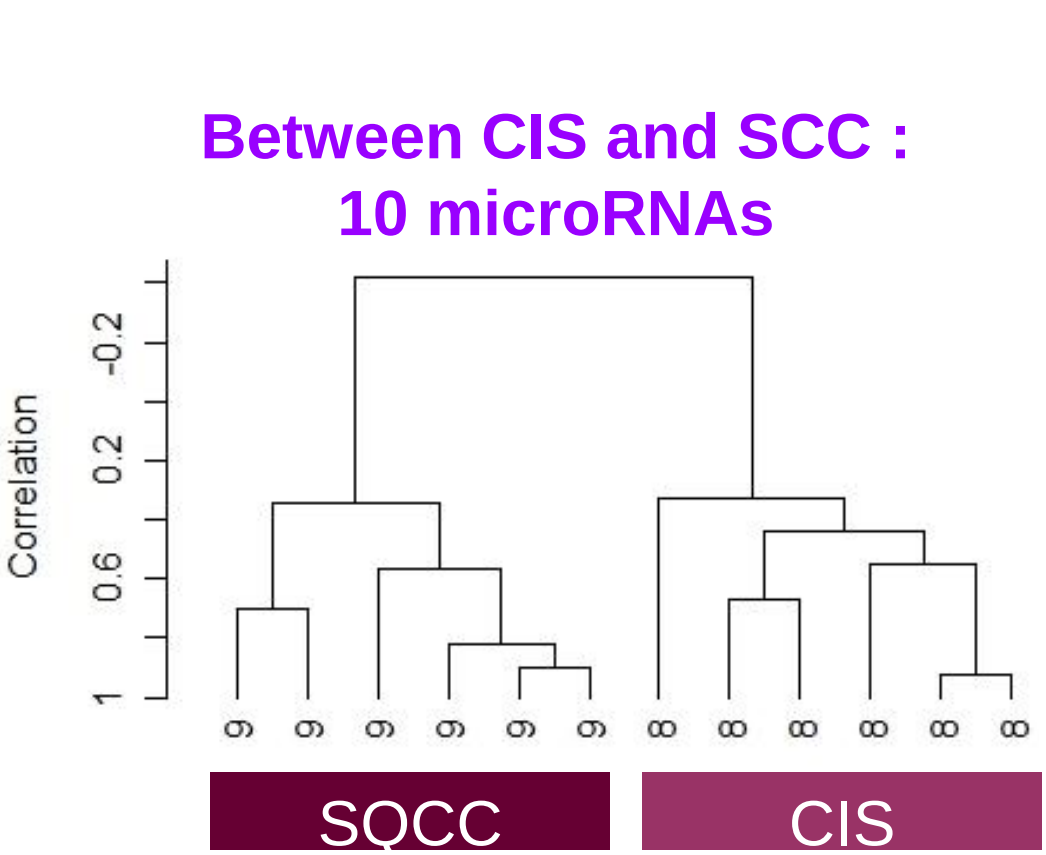
Discriminating potential of miRNAs expression profiles

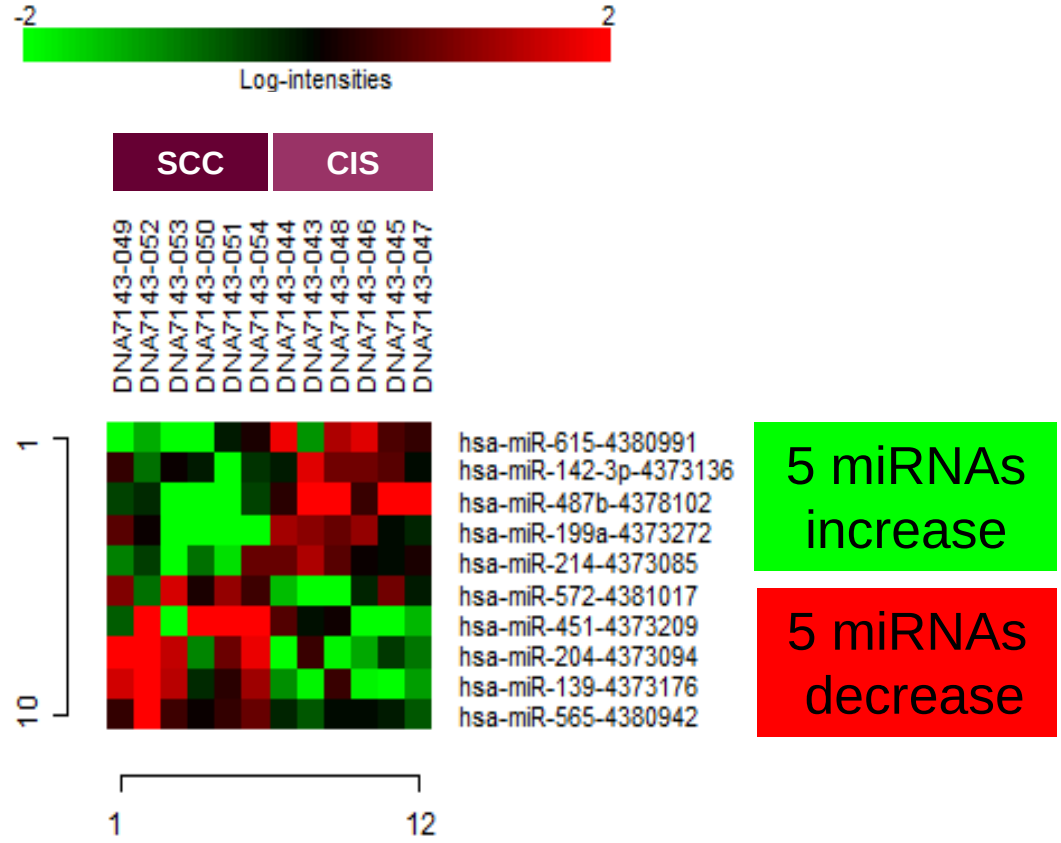
Between groups B and C: 30 miRNAs



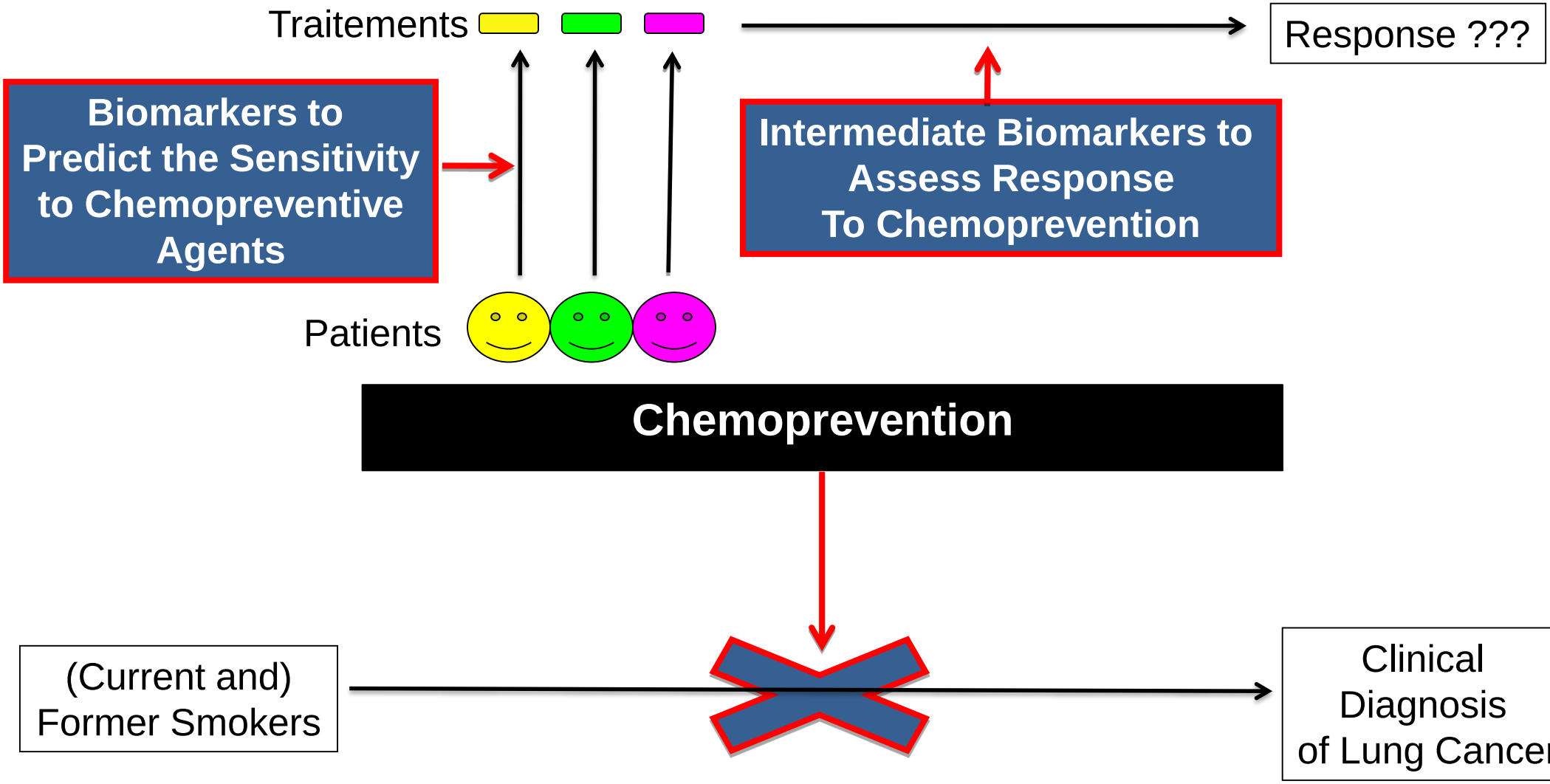
Discriminating potential of microRNAs expression profiles

Between CIS and SCC : 10 microRNAs





Mascaux et al, Eur Resp J, 2009


Traitements
Response ???
Biomarkers to Predict the Sensitivity to Chemopreventive Agents
Intermediate Biomarkers to Assess Response To Chemoprevention
Patients
Chemoprevention
(Current and) Former Smokers
Clinical Diagnosis of Lung Cancer

No phase III trial testing chemopreventive agents for lung cancer has shown benefit

Table 1 | Phase III chemoprevention trials

Intervention	Chemoprevention setting	*n*	Results
Aspirin	Primary	5,139 22,071 39,876	Negative[39] Negative[37] Negative[38]
Beta carotene	Primary	29,133 22,071	Harmful (RR = 1.18)[42] Negative[15]
Beta carotene and retinol	Primary	18,314	Harmful (RR = 1.28)[122]
Multivitamins and minerals	Primary	29,584	Negative[43]
Vitamin E	Primary	29,133	Negative[42]
Retinyl palmitate	Tertiary	2,592	Negative[40]
13-*cis*-retinoic acid	Tertiary	1,166	Negative[41]
N-acetyl cysteine	Tertiary	2,592	Negative[40]
Selenium	Tertiary	1,772	Negative[44]

Abbreviation: RR, relative risk.

Robert Keith and York Miller, Nat Rev, 2013

No agent is recommended for chemoprevention of lung cance

Cancer Prevention Research

Oral Iloprost Improves Endobronchial Dysplasia in Former Smokers

Robert L. Keith, Patrick J. Blatchford, John Kittelson, et al.

Cancer Prev Res 2011;4:793-802. Published online June 1, 2011.

The University of Colorado in Collaboration with other Lung SPORE investigators developed a Phase II, placebo-controlled multicenter chemoprevention trial comparing the prostacyclin analog, Iloprost, to placebo. This trial is the first to meet a primary endpoint of improvement in endobronchial histology.

Iloprost Chemoprevention Trial: Results

Table 2: Treatment effect on bronchial histology

	Iloprost			Placebo			Treatment Effect[1]		
	Baseline	6-month	Change	Baseline	6-month	Change	Difference	(95% CI)	P-Value
All Completers									
Average	2.64	2.41	-0.23	2.56	2.54	-0.02	-0.15	(-0.39, 0.09)	0.21
Worst	4.25	3.85	-0.40	3.91	4.14	0.23	-0.43	(-0.84, -0.03)	0.038
Dysplasia Index	35.0	27.3	-7.70	34.2	33.3	-0.92	-5.97	(-13.3, 1.33)	0.11
Response Proportion[2]	24/60 (0.40)			13/65 (0.20)			0.20	(0.04, 0.36)	0.014
Former Smokers									
Average	2.12	1.73	-0.39	2.07	2.11	0.04	-0.41	(-0.71, -0.11)	0.010
Worst	3.59	2.83	-0.76	3.11	3.68	0.57	-1.10	(-1.76, -0.45)	0.002
Dysplasia Index	20.8	10.9	-9.91	22.9	24.6	1.70	-12.4	(-21.0, -3.92)	0.006
Response Proportion[2]	14/29 (0.48)			4/28 (0.14)			0.34	(0.10, 0.58)	0.006
Current Smokers									
Average	3.13	3.05	-0.07	2.93	2.87	-0.06	0.06	(-0.30, 0.42)	0.74
Worst	4.87	4.81	-0.06	4.51	4.49	-0.03	0.12	(-0.36, 0.60)	0.62
Dysplasia Index	48.2	42.6	-5.64	42.8	39.9	-2.90	-0.33	(-11.7, 11.0)	0.96
Response Proportion[2]	10/31 (0.32)			9/37 (0.24)			0.08	(-0.13, 0.29)	0.47

- Response defined as ≥1 point reduction in maximum histology

(similar magnitude of change as seen comparing current to former smokers)

Population

- In the 152 patients randomized in the Iloprost trial, paired and same site baseline and FU biopsies were available in 125 patients:

- 29/31 current/former smokers in the Iloprost arm
- 28/37 current/former smokers in the placebo arm

- We planned to analyze 500 biopsies: 4 biopsies from each of the 125 patients:

- 2 biopsies at baseline (worst and best diagnosis)
- 2 biopsies at FU at the same site after six months

MicroRNAs selection

- MicroRNA to be tested have been selected from the list of 69 miRNAs identified in bronchial human biopsies as being differentially expressed during lung squamous carcinogenesis (Mascaux et al, Eur Respir J 2009; 33: 352–359)

  - 14 microRNAs were selected based on their expression change in high-grade lesions and/or in inflammation

Changes of miRNA expression in FU samples compared to baseline samples

miRNA	Significant changes in miRNA expression in FU versus baseline samples (t test)						
	All	Any smokers status		Current smokers		Former smokers	
		Iloprost	Placebo	Iloprost	Placebo	Iloprost	Placebo
miR-34-c	No change						
miR-9	Down P<0.0001 FDR	Down P=0.0007 FDR	Down P=0.001 FDR	Down P=0.0022 FDR	Down P=0.047	Down (P=0.0868)	Down P=0.0071 FDR

miR-9 was significantly down-regulated in FU biopsies as compared with baseline biopsies, but was not correlated with histology changes.

Correlation between miRNA expression changes and histological changes at FU

miRNA	Baseline samples	Paired samples: FU expression compared to baseline				
	Correlation between miRNA expression and histology grade (low to high)	Correlation between miRNA expression changes and histology change (up-grading) at FU compared to baseline				
		All	Current smokers		Former smokers	
			Iloprost	Placebo	Iloprost	Placebo
miR-34-c	Down R=0.36 P<0.0001, FDR	Down R=0.23 P=0.0003, FDR	Down R=0.26 P=0.041	Down R=0.23 P=0.0466	Down R=0.24 (P=0.0666)	Down R=0.14 NS
miR-9	No Change					

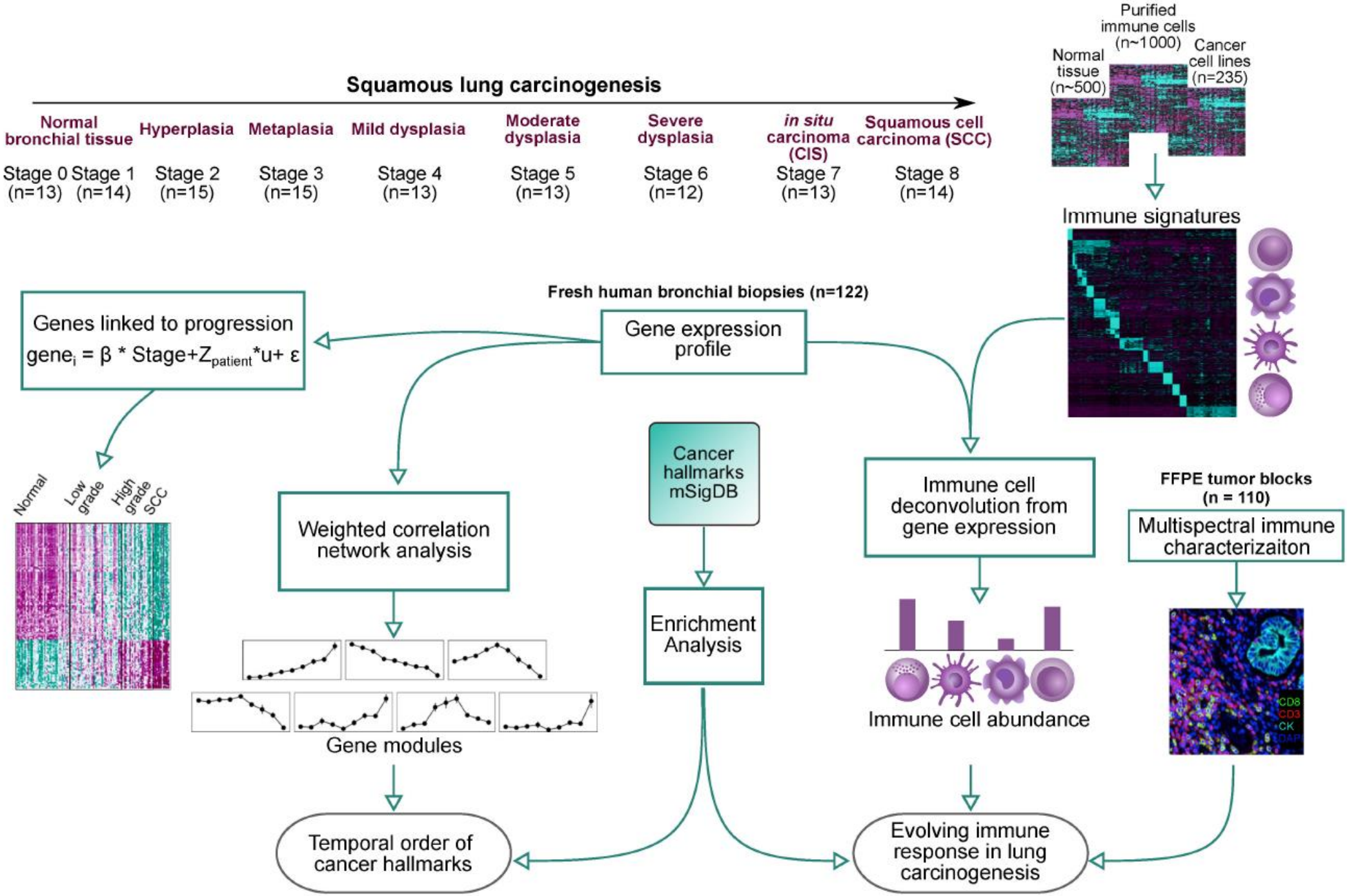
miR-34c is significantly down-regulated in samples at FU that are up-graded and inversely

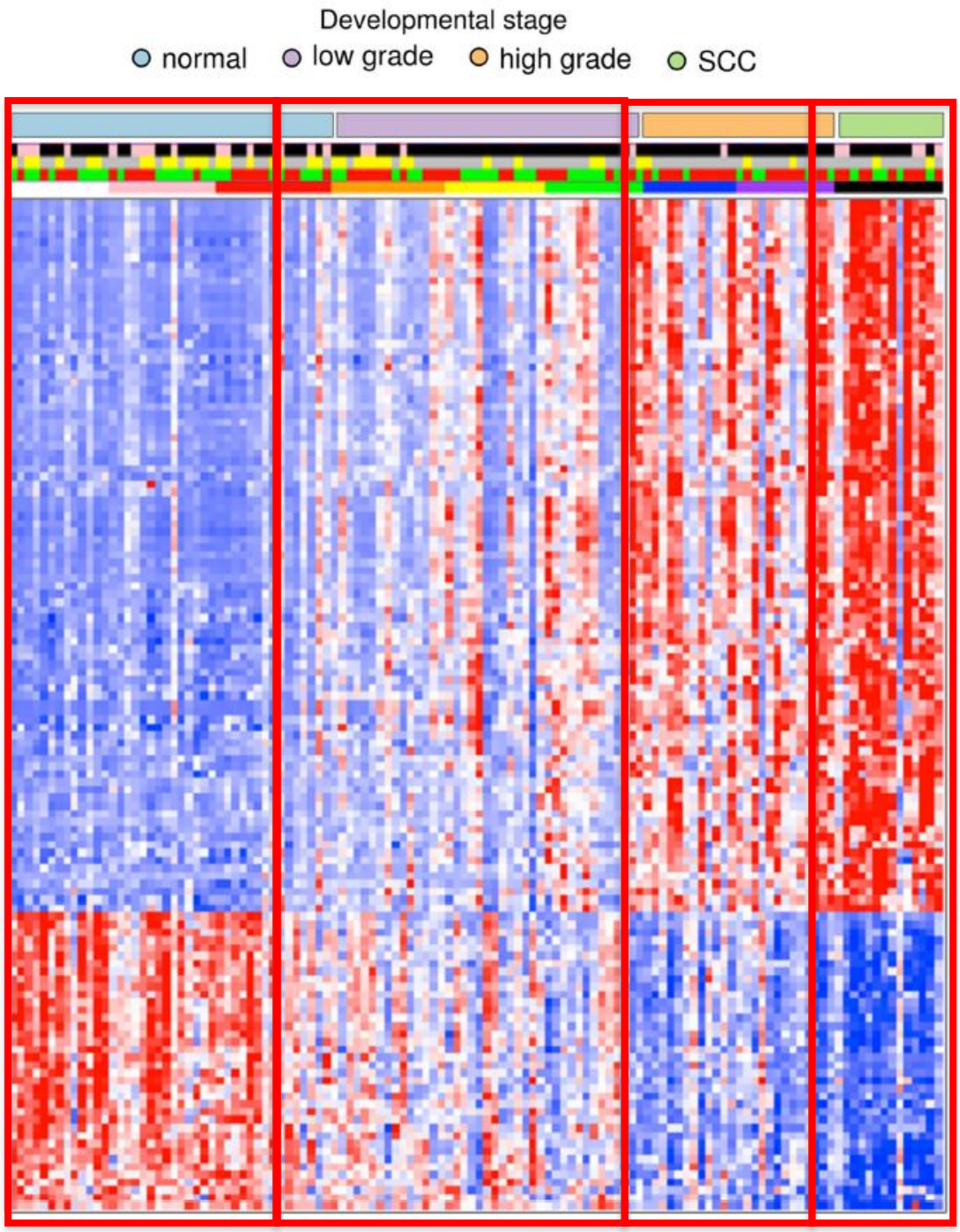
LETTER

https://doi.org/10.1038/s41586-019-1330-0

Immune evasion before tumour invasion in early lung squamous carcinogenesis

Céline Mascaux[1,2,3,4,14,15,18]*, Mihaela Angelova[5,6,7,8,16,18], Angela Vasaturo[5,6,7,8], Jennifer Beane[2], Kahkeshan Hijazi[2], Geraldine Anthoine[1], Bénédicte Buttard[5,6,7,8], Françoise Rothe[9], Karen Willard-Gallo[10], Annick Haller[11,17], Vincent Ninane[12], Arsène Burny[13], Jean-Paul Sculier[1], Avi Spira[2] & Jérôme Galon[5,6,7,8]*


Squamous lung carcinogenesis
Normal bronchial tissue
Hyperplasia
Metaplasia
Mild dysplasia
Moderate dysplasia
Severe dysplasia
in situ carcinoma (CIS)
Squamous cell carcinoma (SCC)
Stage 0 (n=13)
Stage 1 (n=14)
Stage 2 (n=15)
Stage 3 (n=15)
Stage 4 (n=13)
Stage 5 (n=13)
Stage 6 (n=12)
Stage 7 (n=13)
Stage 8 (n=14)
Purified immune cells (n~1000)
Normal tissue (n~500)
Cancer cell lines (n=235)
Immune signatures
Fresh human bronchial biopsies (n=122)
Genes linked to progression
gene_i = β * Stage+Z_patient*u+ ε
Gene expression profile
Normal
Low grade
High grade
SCC
Weighted correlation network analysis
Gene modules
Cancer hallmarks mSigDB
Enrichment Analysis
Immune cell deconvolution from gene expression
Immune cell abundance
FFPE tumor blocks (n = 110)
Multispectral immune characterizaiton
CD8
CD3
CK
DAPI
Temporal order of cancer hallmarks
Evolving immune response in lung carcinogenesis



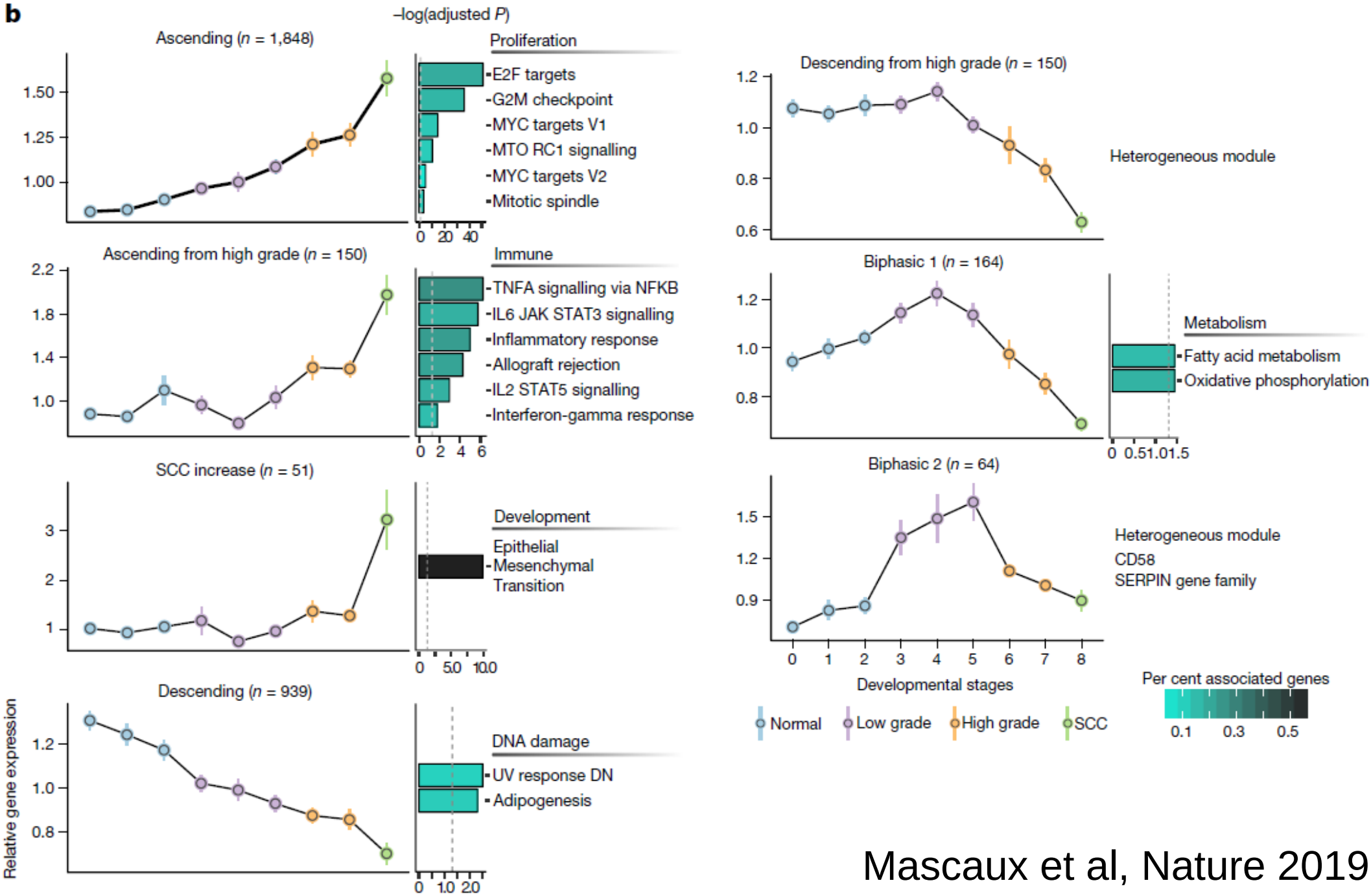
Cancer history
- No
- Yes

Smoking history
- Current
- Former

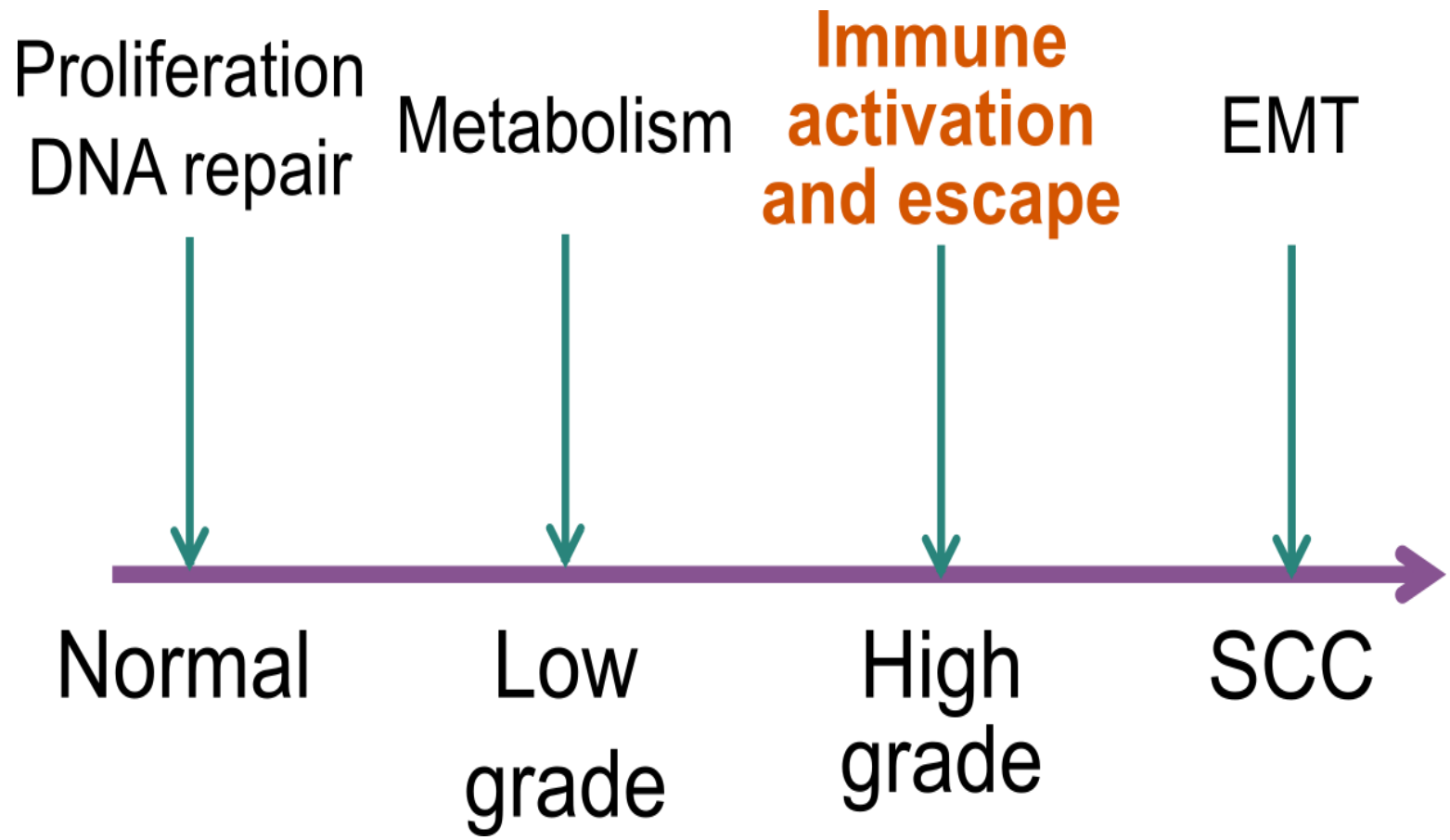
Gender
- Male
- Female

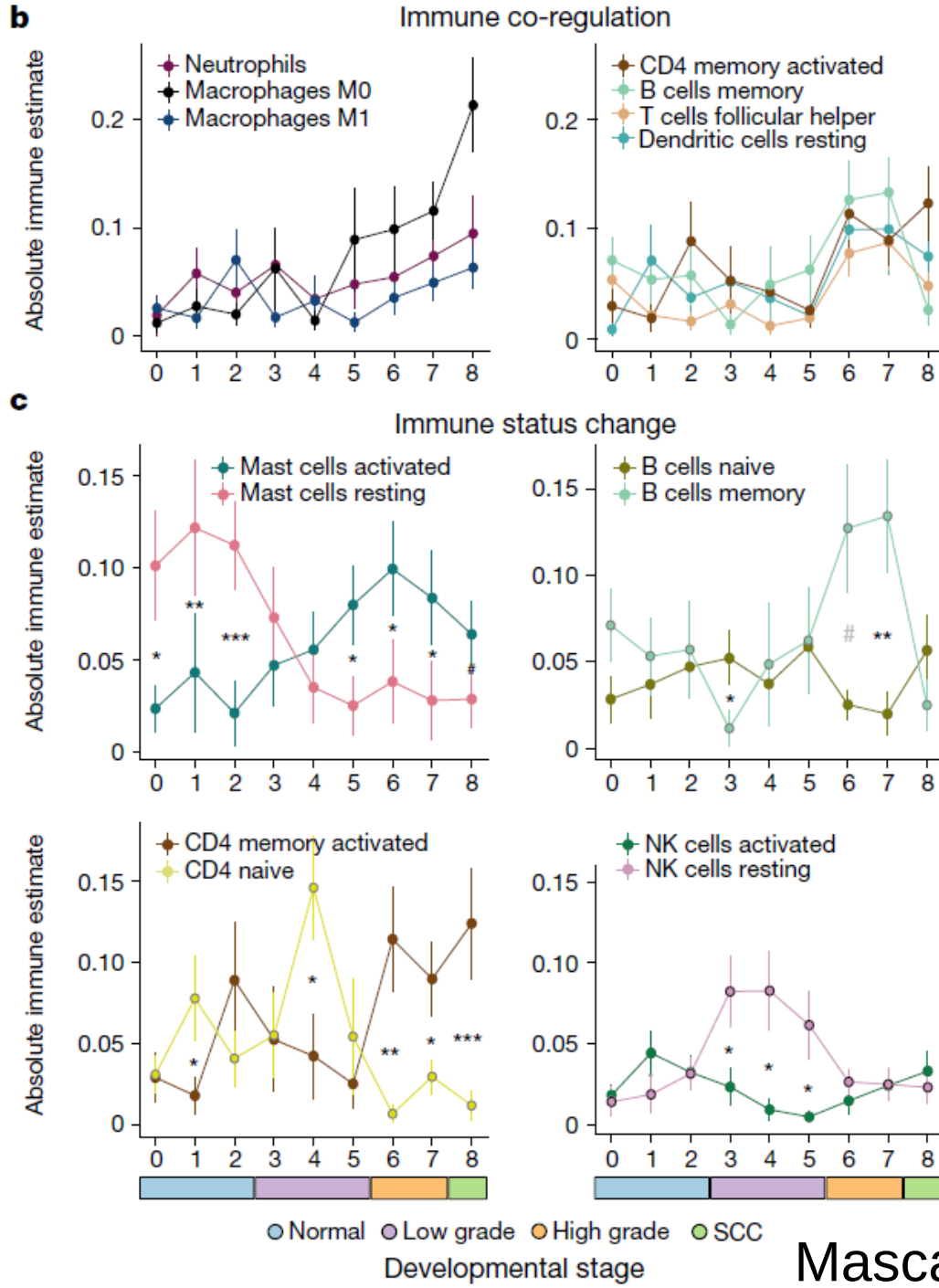
Developmental stage
- 0
- 1
- 2
- 3
- 4
- 5
- 6
- 7
- 8

Mascaux et al, Nature 2019

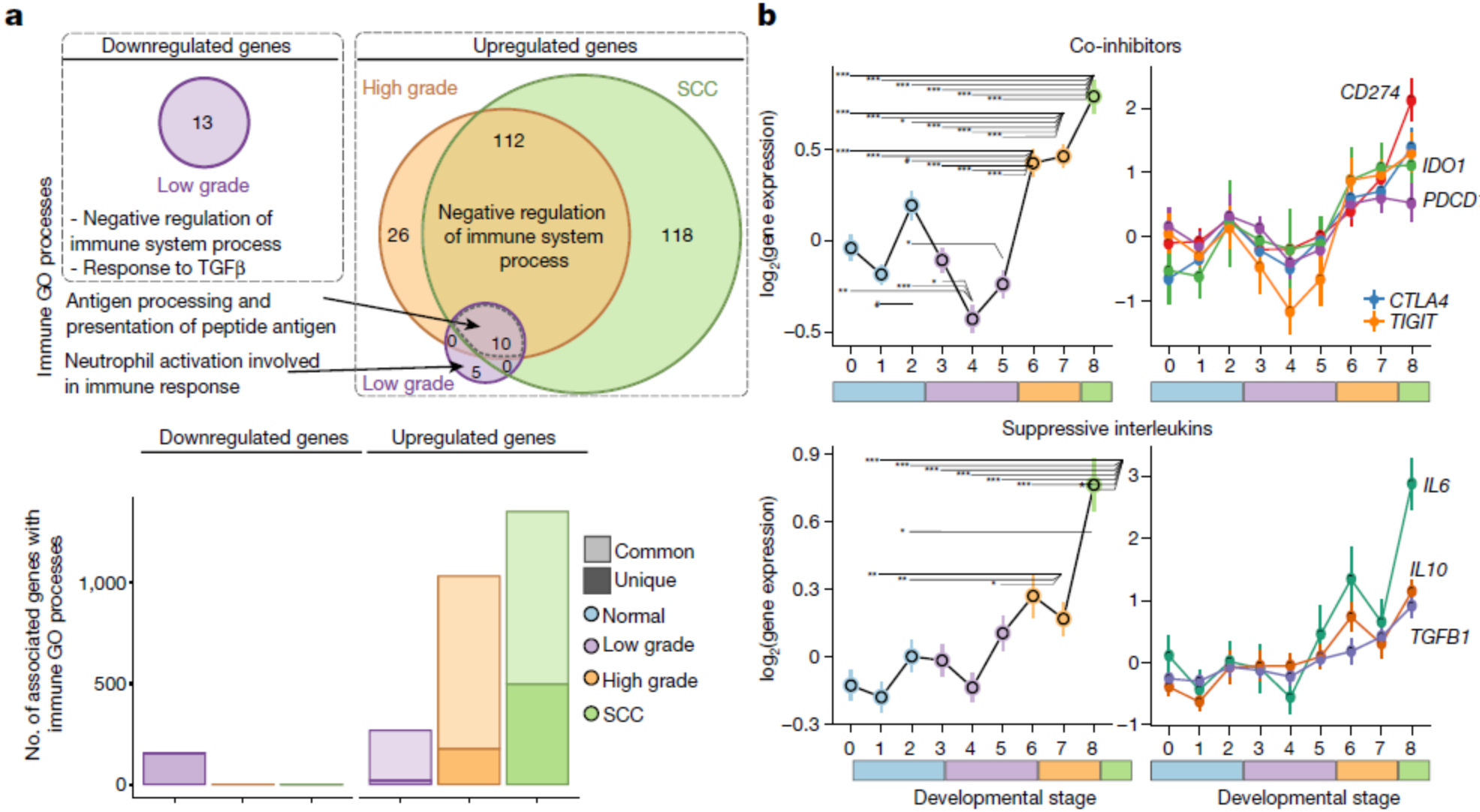


Mascaux et al, Nature 2019

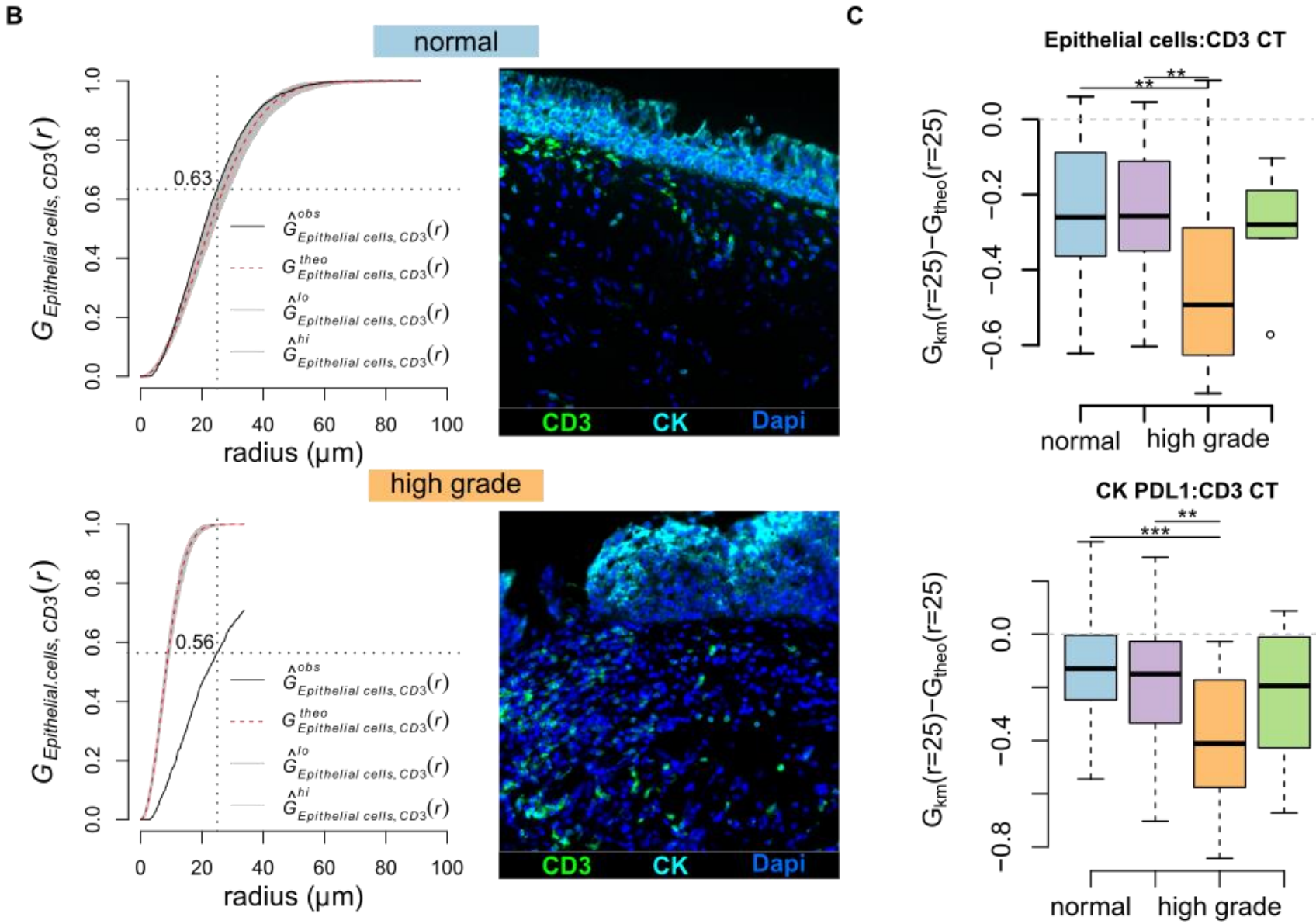

Proliferation
DNA repair
Metabolism
Immune
activation
and escape
EMT
Normal
Low
grade
High
grade
SCC

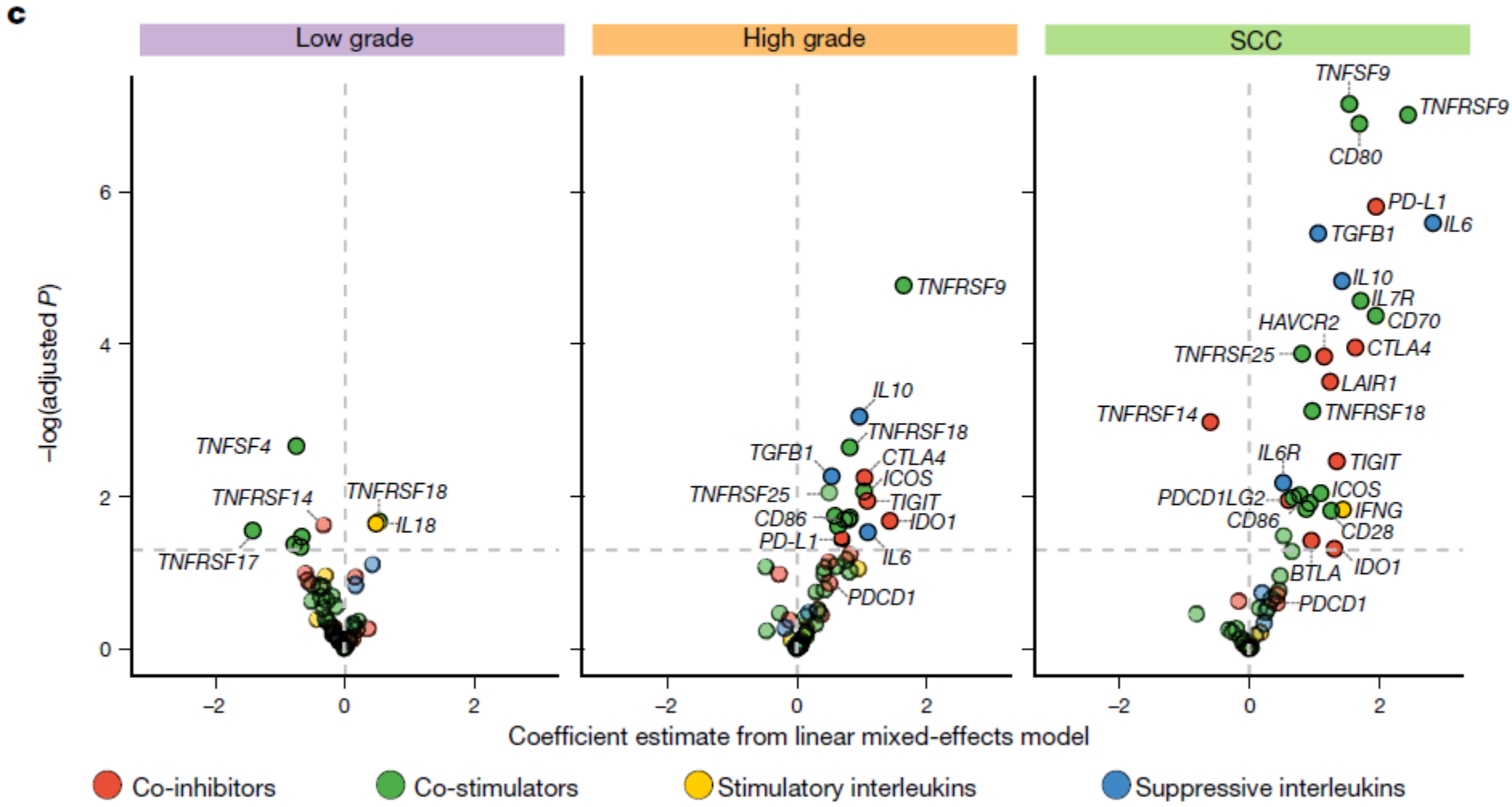


Mascaux et al, Nature 2019

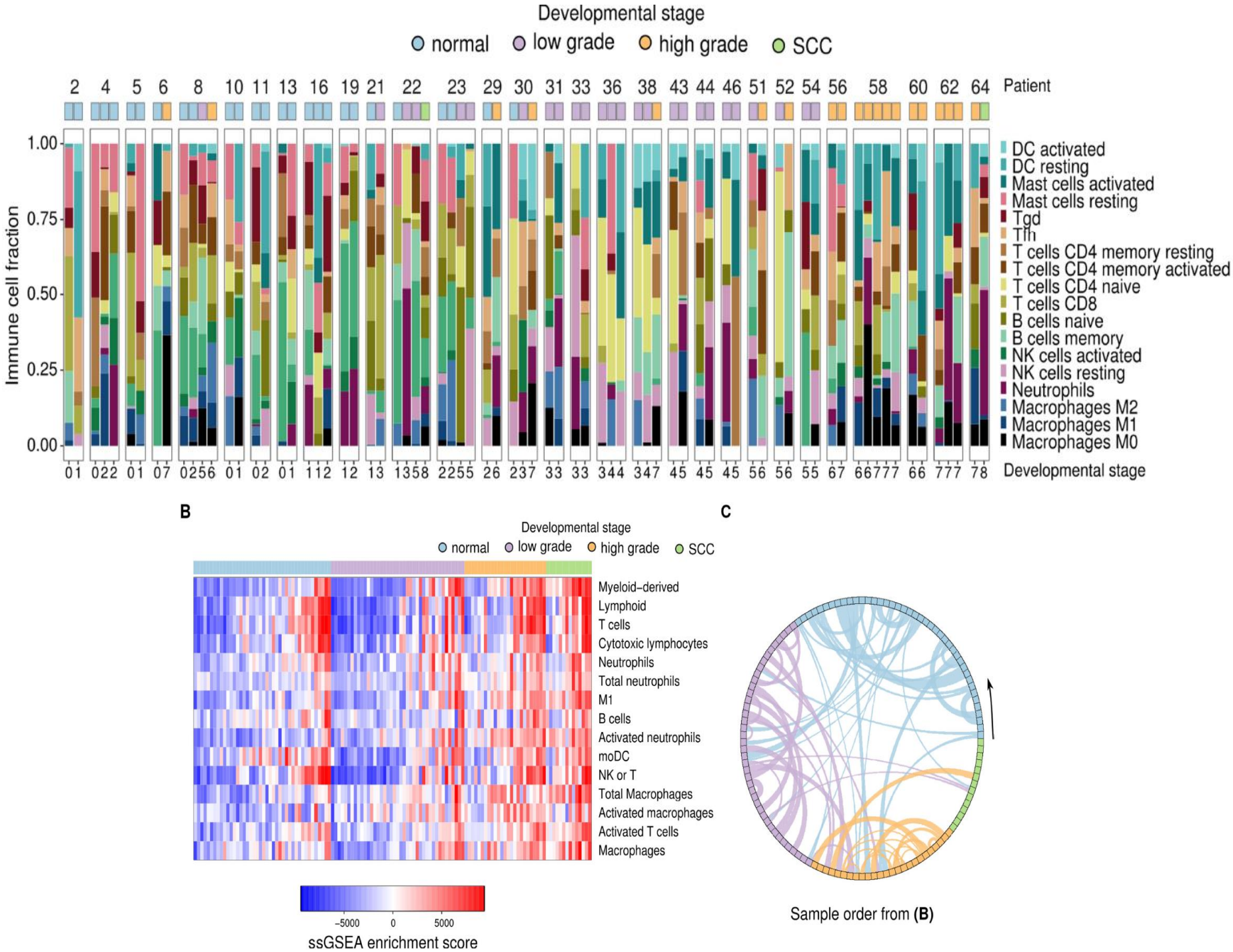


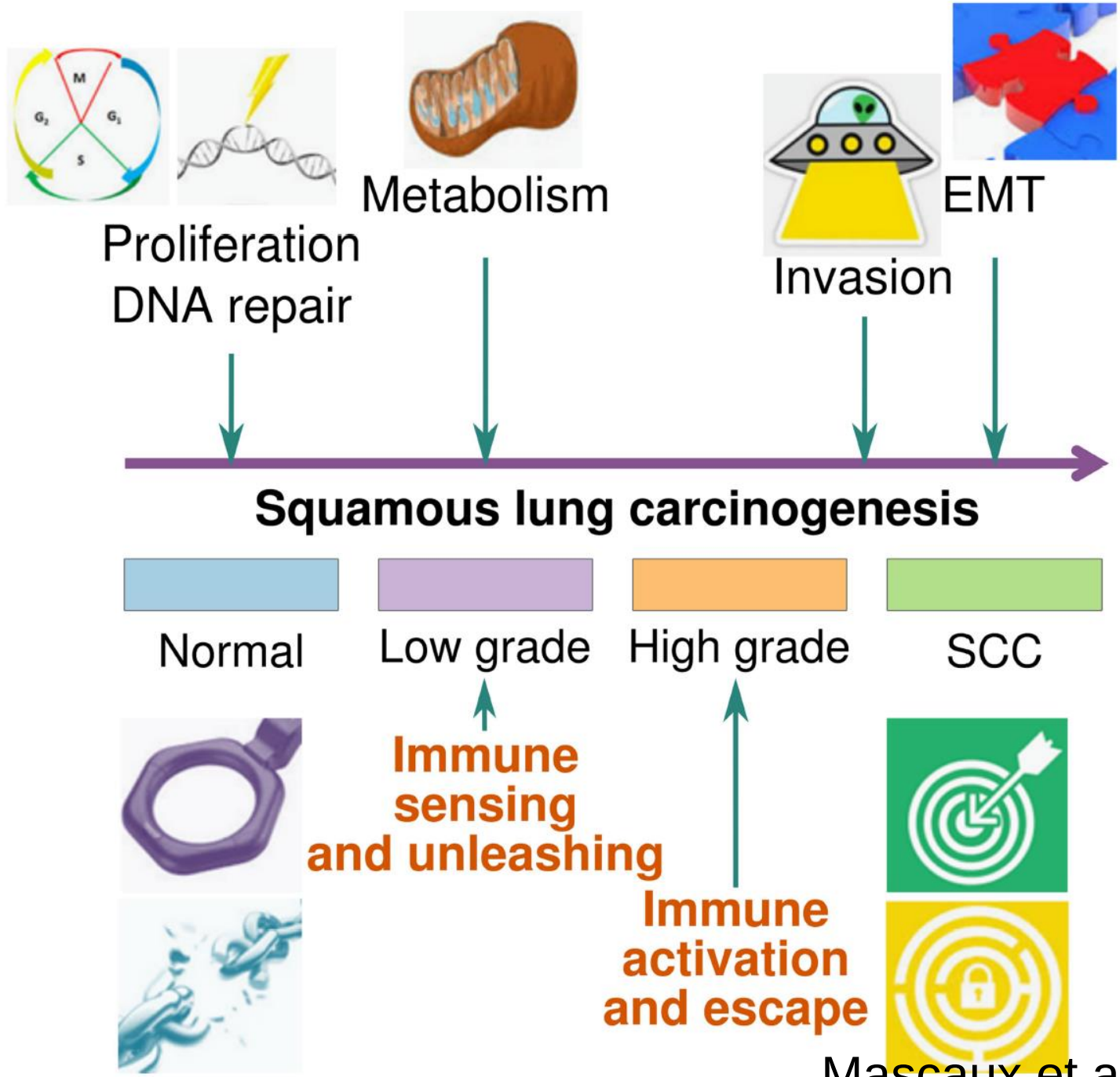
Mascaux et al, Nature 2019


B
normal
high grade
0.63
0.56
radius (μm)
CD3
CK
Dapi
C
Epithelial cells:CD3 CT
CK PDL1:CD3 CT
normal
high grade



Mascaux et al, Nature 2019


Developmental stage
normal
low grade
high grade
SCC
Patient
2 4 5 6 8 10 11 13 16 19 21 22 23 29 30 31 33 36 38 43 44 46 51 52 54 56 58 60 62 64
Immune cell fraction
1.00
0.75
0.50
0.25
0.00
DC activated
DC resting
Mast cells activated
Mast cells resting
Tgd
Tfh
T cells CD4 memory resting
T cells CD4 memory activated
T cells CD4 naive
T cells CD8
B cells naive
B cells memory
NK cells activated
NK cells resting
Neutrophils
Macrophages M2
Macrophages M1
Macrophages M0
01 022 01 07 0256 01 02 01 112 12 13 1358 2255 26 237 33 33 344 347 45 45 45 56 56 55 67 66777 66 777 78
Developmental stage
B
Developmental stage
normal
low grade
high grade
SCC
Myeloid−derived
Lymphoid
T cells
Cytotoxic lymphocytes
Neutrophils
Total neutrophils
M1
B cells
Activated neutrophils
moDC
NK or T
Total Macrophages
Activated macrophages
Activated T cells
Macrophages
−5000
0
5000
ssGSEA enrichment score
C
Sample order from (B)



Mascaux et al, Nature 2019

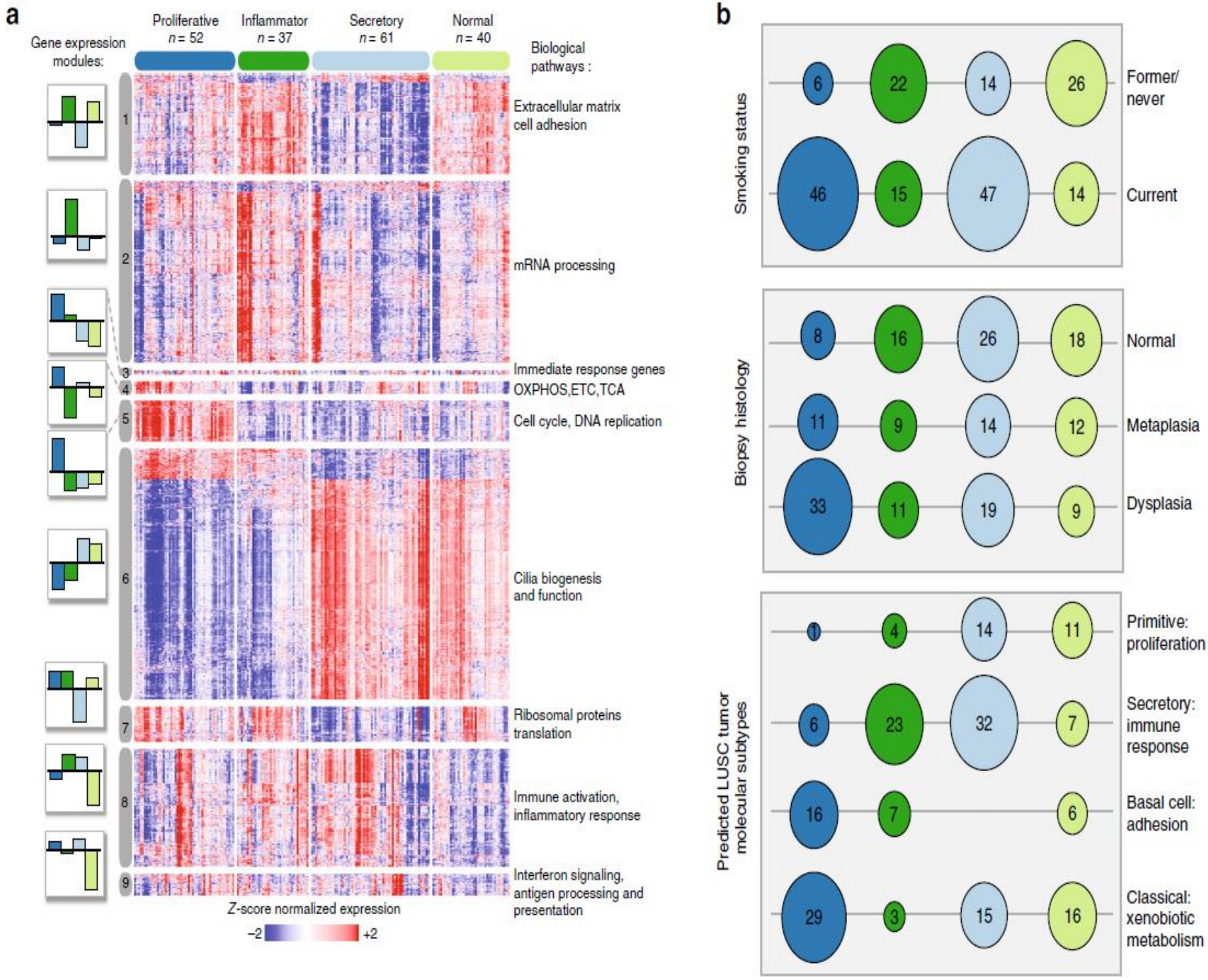
nature COMMUNICATIONS

ARTICLE

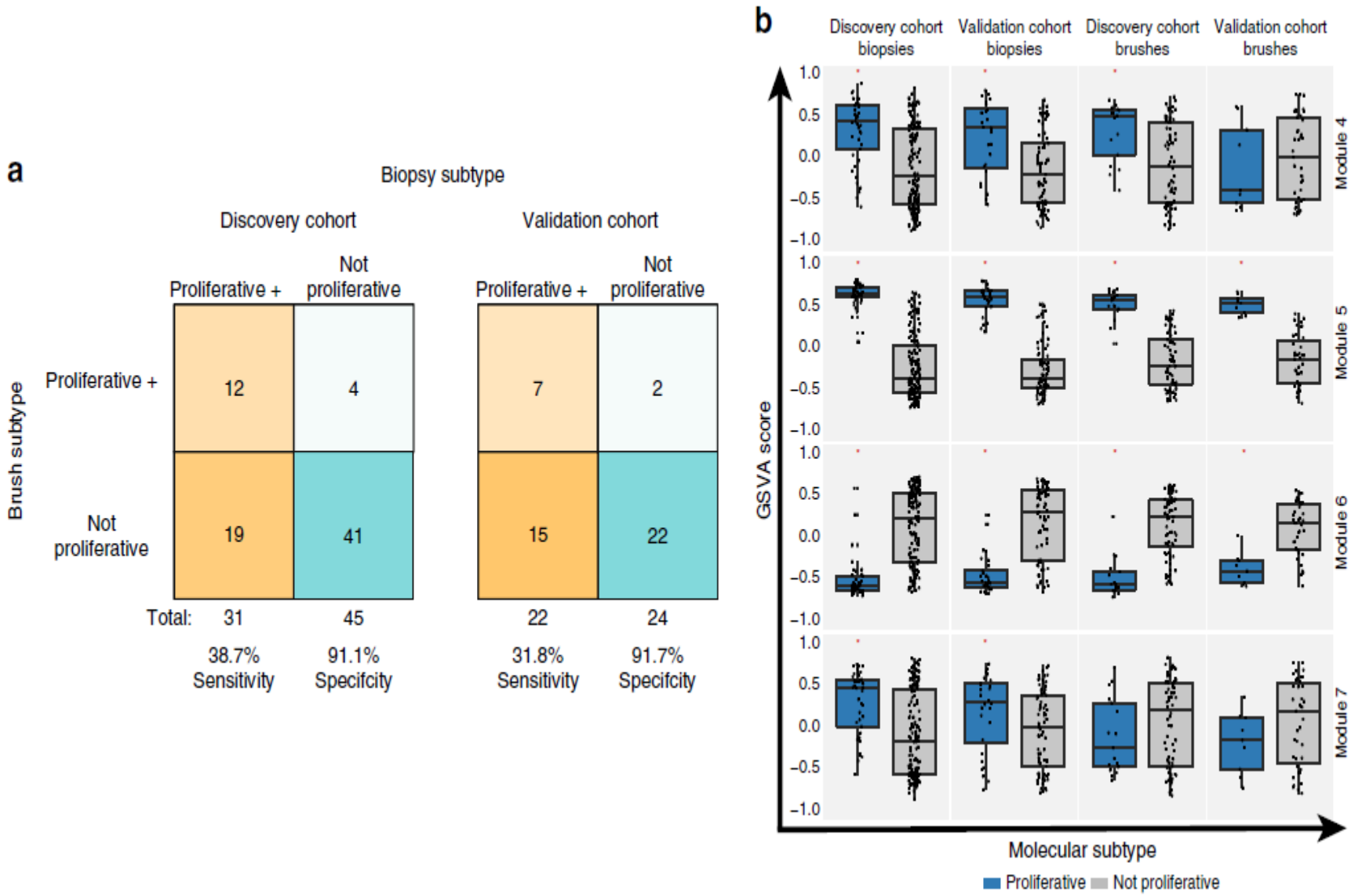
https://doi.org/10.1038/s41467-019-09834-2 OPEN

Molecular subtyping reveals immune alterations associated with progression of bronchial premalignant lesions

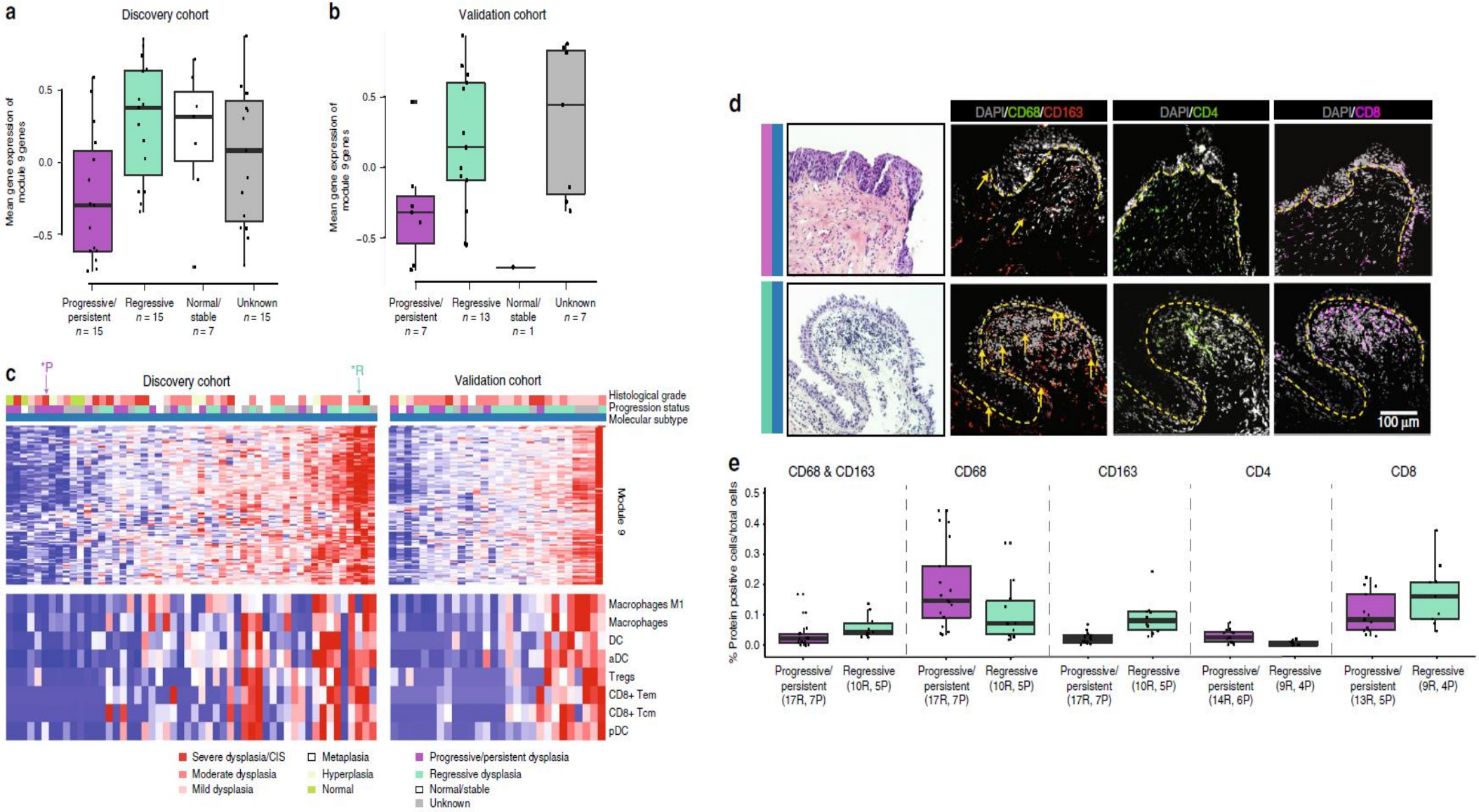
Jennifer E. Beane[1], Sarah A. Mazzilli[1], Joshua D. Campbell[1], Grant Duclos[1], Kostyantyn Krysan[2], Christopher Moy[3], Catalina Perdomo[4], Michael Schaffer[3], Gang Liu[1], Sherry Zhang[1], Hanqiao Liu[1], Jessica Vick[1], Samjot S. Dhillon[5], Suso J. Platero[6], Steven M. Dubinett[2], Christopher Stevenson[7], Mary E. Reid[8], Marc E. Lenburg[1] & Avrum E. Spira[1,3]

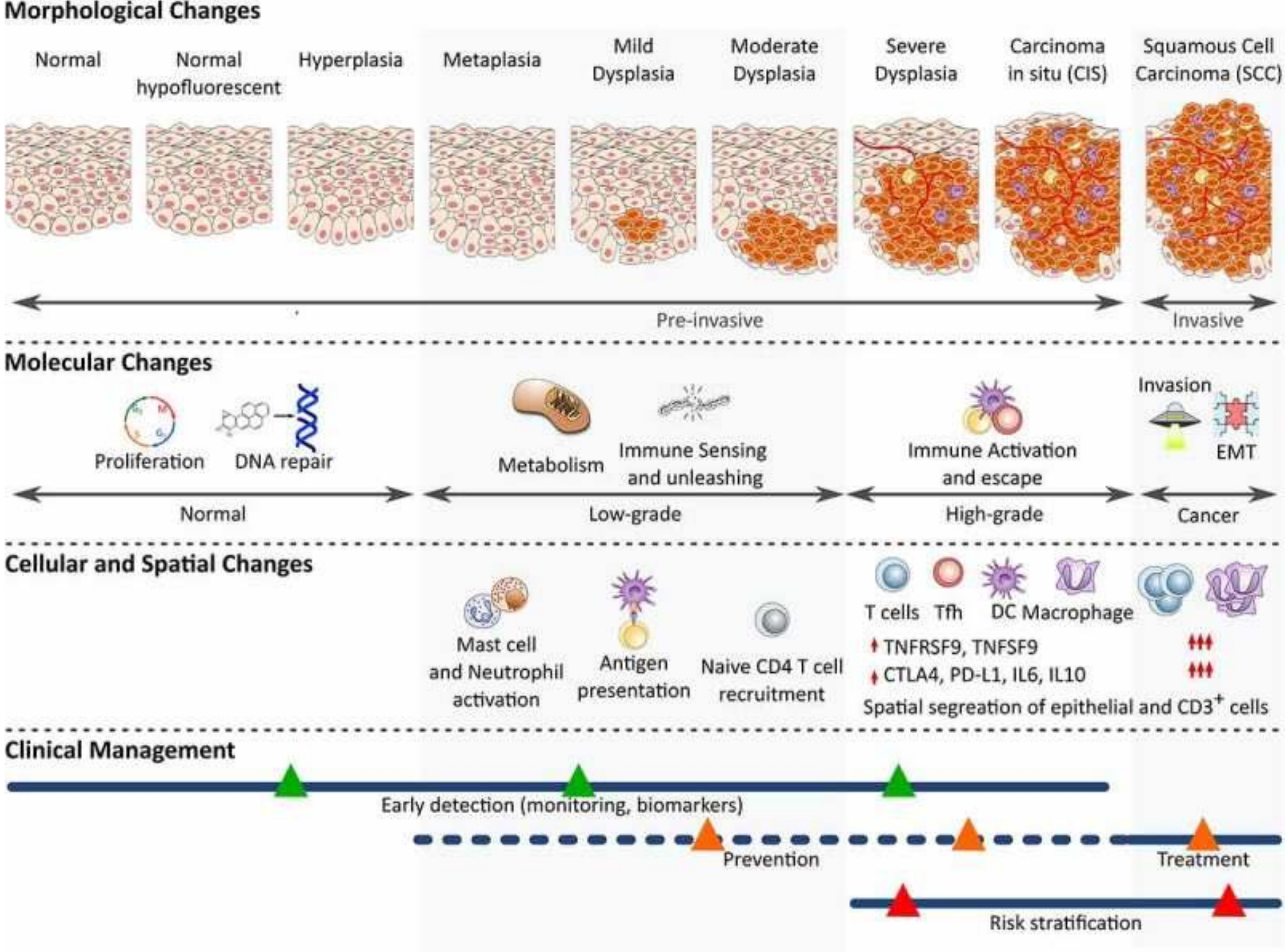

a
Proliferative n = 52
Inflammator n = 37
Secretory n = 61
Normal n = 40
Gene expression modules:
Biological pathways :
1
Extracellular matrix cell adhesion
2
mRNA processing
3
Immediate response genes
4
OXPHOS,ETC,TCA
5
Cell cycle, DNA replication
6
Cilia biogenesis and function
7
Ribosomal proteins translation
8
Immune activation, inflammatory response
9
Interferon signaling, antigen processing and presentation
Z-score normalized expression
−2
+2
b
Smoking status
6
22
14
26
Former/ never
46
15
47
14
Current
Biopsy histology
8
16
26
18
Normal
11
9
14
12
Metaplasia
33
11
19
9
Dysplasia
Predicted LUSC tumor molecular subtypes
1
4
14
11
Primitive: proliferation
6
23
32
7
Secretory: immune response
16
7
6
Basal cell: adhesion
29
3
15
16
Classical: xenobiotic metabolism

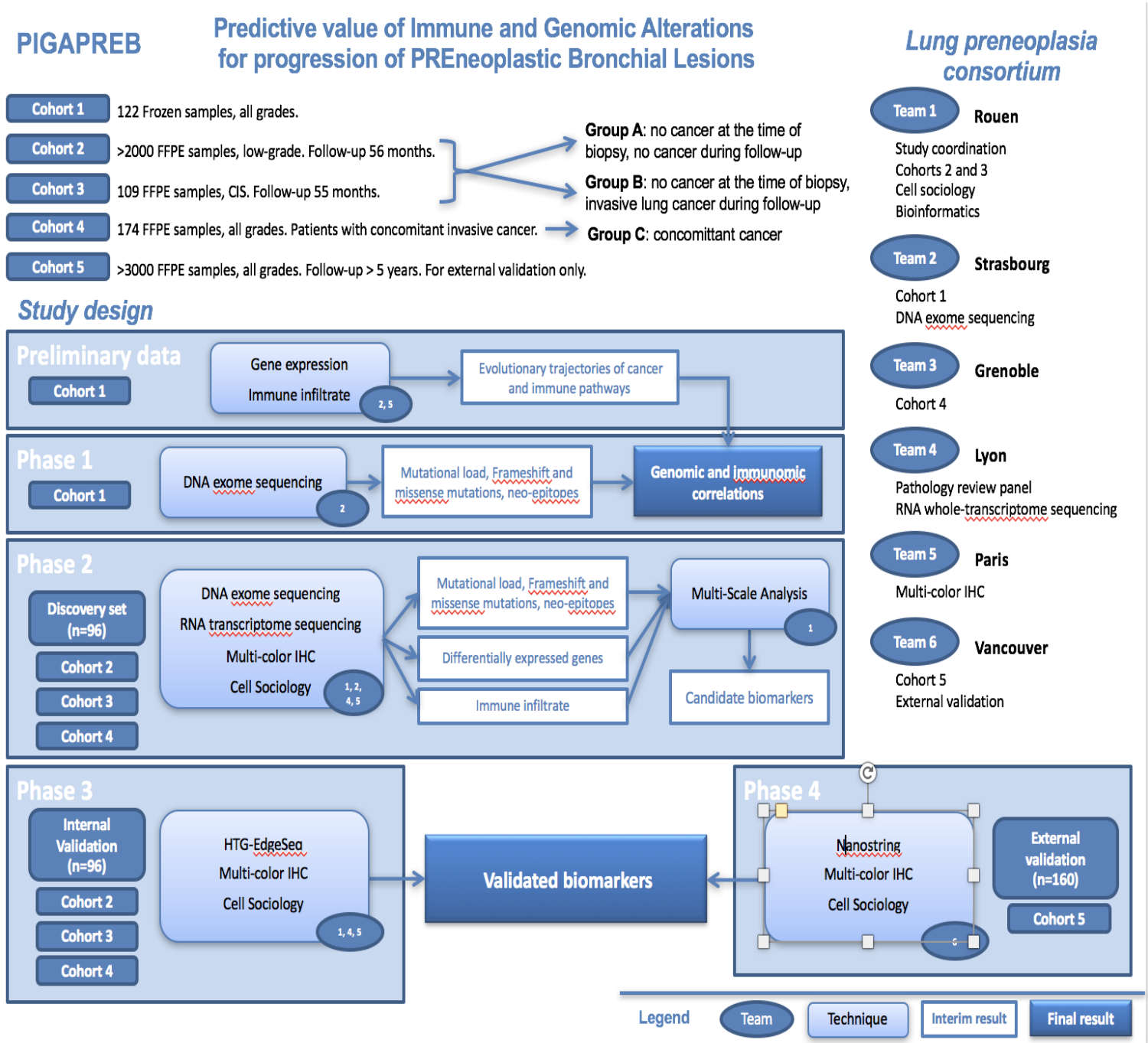
Normal appearing brushing predicts the presence of proliferative lesions



Immune alteration associated with lesion outcome in the proliferative subtype




Morphological Changes
Normal
Normal hypofluorescent
Hyperplasia
Metaplasia
Mild Dysplasia
Moderate Dysplasia
Severe Dysplasia
Carcinoma in situ (CIS)
Squamous Cell Carcinoma (SCC)
Pre-invasive
Invasive
Molecular Changes
Proliferation
DNA repair
Metabolism
Immune Sensing and unleashing
Immune Activation and escape
Invasion
EMT
Normal
Low-grade
High-grade
Cancer
Cellular and Spatial Changes
Mast cell and Neutrophil activation
Antigen presentation
Naive CD4 T cell recruitment
T cells
Tfh
DC
Macrophage
TNFRSF9, TNFSF9
CTLA4, PD-L1, IL6, IL10
Spatial segreation of epithelial and CD3+ cells
Clinical Management
Early detection (monitoring, biomarkers)
Prevention
Treatment
Risk stratification



O cancer 2020-2022

Conclusions

- Au plus tôt l'on agit sur le cancer, meilleures sont les chances de guérir

- Les lésions prénéoplasiques sont: des étapes intermédiaires entre le tissu normal et le cancer et sont associées avec des anomalies histologiques

- La prédiction de la progression des lésions prénéoplasiques est possible
- L'interception du cancer en prévenant sa progression serait déterminante pour réduire la mortalité par cancer
- Des données récentes indiquent un rôle essentiel pour le microenvironnement immum à ces stades les plus précoces de la carcinogenèse
- Ces données suggèrent un rôle pour l'immunothérapie dans la chimioprévention et l'interception du cancer