Session: The new WHO classification of digestive neuroendocrine neoplasms (NEN) and beyond

New insights from Lung NEN molecular data: new entities or new concepts?

International Agency for Research on Cancer
Lyon, France

Nicolas Alcala, Computational Biologist (GCS)

September 10, 2019

Disclosure Information

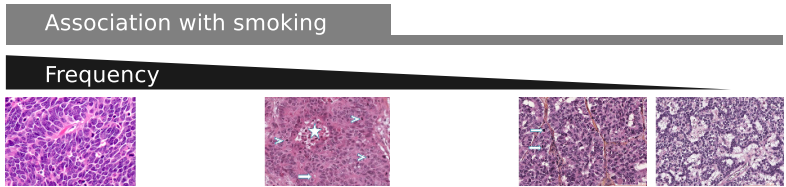
I hereby declare that I have had business or personal interests in the following industrial enterprises since 1 September 2018:

Name of the enterprise / Nature of the interest

Enterprise	Interest
None	

Lung neuroendocrine neoplasms

Understudied diseases, particularly atypical carcinoids



Published large multi-omic molecular studies

- **SCLC**: Peifer and Fernandez-Cuesta *et al., Nat Genet* 2012; Rudin *et al., Nat Genet* 2012; George *et al., Nature* 2015
- **LCNEC**: George *et al., Nat Commun* 2018
- **Typical carcinoids**: Fernandez-Cuesta *et al., Nat Commun* 2014

A common classification framework for neuroendocrine neoplasms: an International Agency for Research on Cancer (IARC) and World Health Organization (WHO) expert consensus proposal

Guido Rindi[1] · David S. Klimstra[2] · Behnoush Abedi-Ardekani[3] · Sylvia L. Asa[4] · Frederik T. Bosman[5] · Elisabeth Brambilla[6] · Klaus J. Busam[2] · Ronald R. de Krijger[7] · Manfred Dietel[8] · Adel K. El-Naggar[9] · Lynnette Fernandez-Cuesta[3] · Günter Klöppel[10] · W. Glenn McCluggage[11] · Holger Moch[12] · Hiroko Ohgaki[3] · Emad A. Rakha[13] · Nicholas S. Reed[14] · Brian A. Rous[15] · Hironobu Sasano[16] · Aldo Scarpa[17] · Jean-Yves Scoazec[18] · William D. Travis[2] · Giovanni Tallini[19] · Jacqueline Trouillas[20] · J. Han van Krieken[21] · Ian A. Cree[3]

Research needs identified by the expert group:

1. "Does a G3 category of lung NET exist comparable to that in the pancreas?"
2. "Studies on the separation between typical carcinoid and atypical carcinoid, and between these entities, SCLC and LCNEC"

Candidates for distinguishing NET G1 and G2, and NET and NEC

Marker of proliferation Ki-67

Ki-67 protein immune-reactivity has been suggested as a **marker of prognosis in LNENs as a whole**, and of differential diagnosis between carcinoids and SCLC, but it does not faithfully separate typical and atypical carcinoids

Orthopedia Homeobox Protein (OTP)

OTP expression suggested as a **marker of prognosis within pulmonary carcinoids** (Swarts, D.R. *et al., Clin Cancer Res* 2013; Papaxoinis, G. *et al., Endocr Pathol* 2017)

Aims

1. Provide the **first comprehensive multi-omic characterization of atypical pulmonary carcinoids**

2. **Unveil the molecular links between pulmonary carcinoids, LCNEC, and SCLC** through comparative genomic analyses of newly generated and previously published data

Methods

Through an **international consortium**, we have generated the 1st **multi-omic dataset** of pulmonary atypical carcinoids

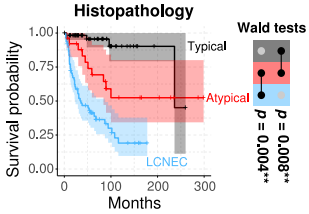
Data

- new data (genome, exome, transcriptome, and methylome) for 63 pulmonary carcinoids (including 27 atypical)
- integration with published data, to obtain a total of multi-omics data for 116 pulmonary carcinoids (including 35 atypical), 75 LCNEC, and 66 SCLC

Methods

- **Supervised multi-omic analyses**: **Teach a machine to predict histopathological type from multi-omic data** (Machine Learning--random forest algorithm--with leave-one-out cross validation)
- **Unsupervised multi-omic analyses**: **Discover new entities** (Group Factor Analysis--MOFA, Argelaguet *et al.*, *Mol Syst Biol* 2018--and consensus clustering)

Molecular classification refines the prognosis of histopathology



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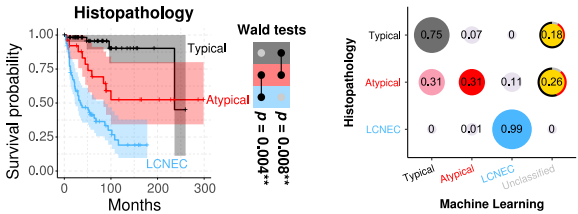

- Machine Learning splits atypical carcinoids into 2 groups: predicted typical and **predicted atypical**

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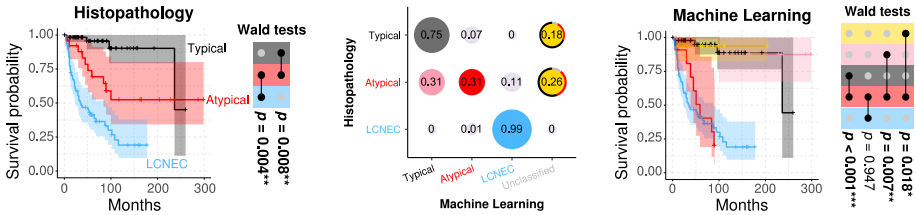

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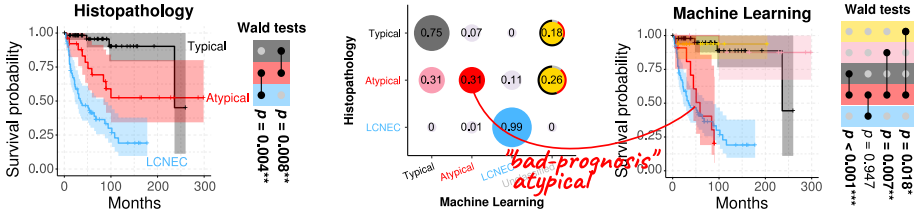

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- **Predicted atypical** have the same prognosis as LCNEC

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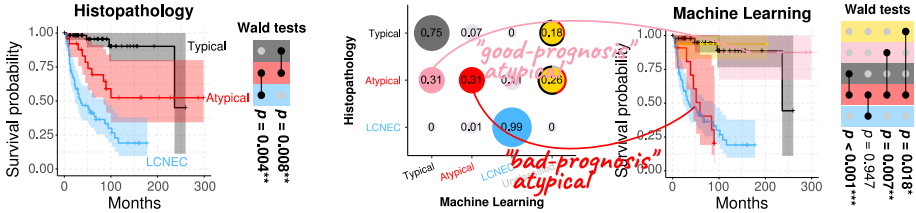

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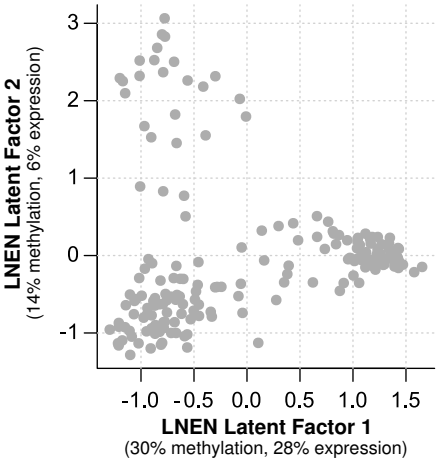
- **Predicted atypical** have the same prognosis as LCNEC
- Predicted typical have the same prognosis as **typical carcinoids**

"Bad prognosis" carcinoids have 2 distinct molecular profiles



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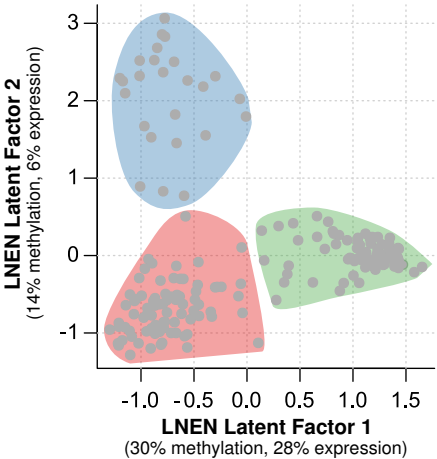

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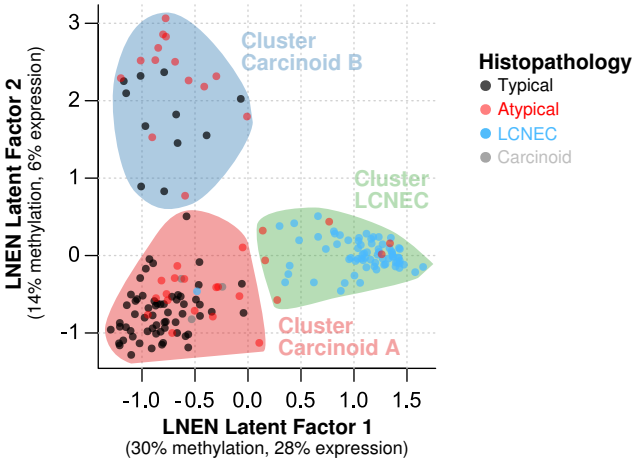

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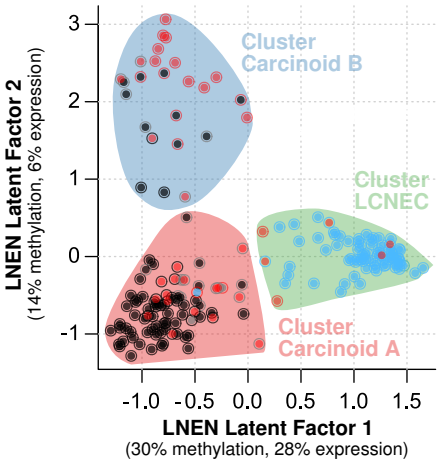

Histopathology
- Typical
- Atypical
- LCNEC
- Carcinoid

Machine Learning
- Typical
- Atypical
- LCNEC
- Unclassified

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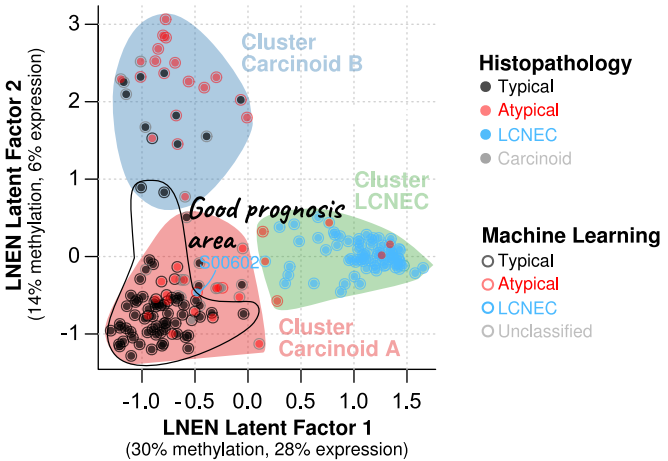

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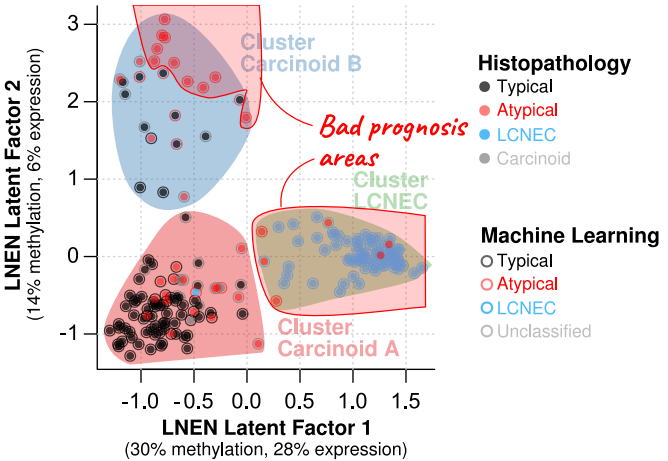

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- **Two bad prognosis areas**:

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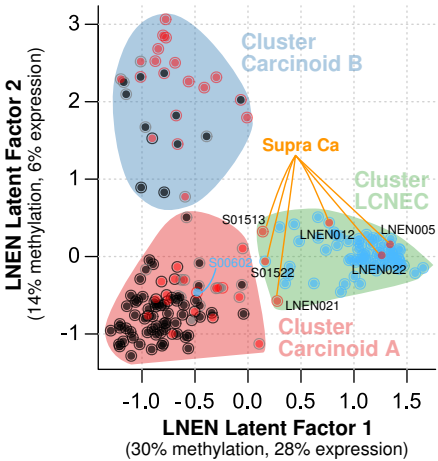

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  1. Cluster Carcinoid B

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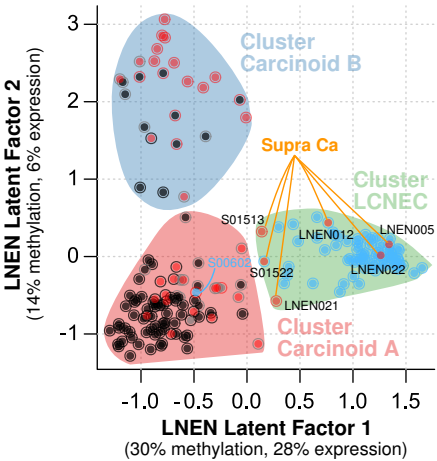

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  1. Cluster Carcinoid B
  2. Carcinoids in LCNEC cluster

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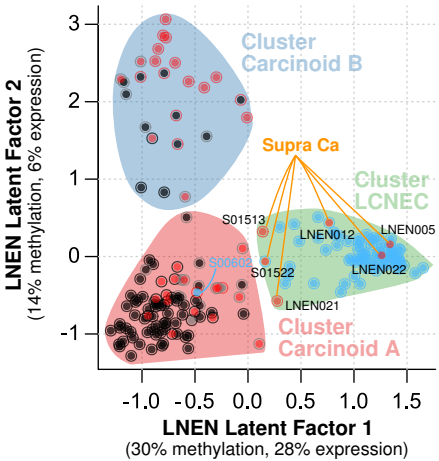

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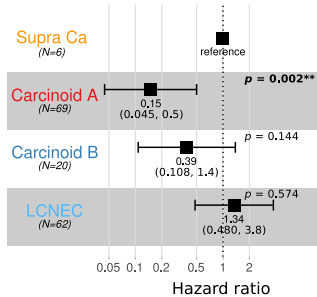

"Bad-prognosis" Cluster B has specific genomic and expression profile

- High expression of **retinoid and xenobiotic metabolism pathways**
- High expression of *OTP*
- Frequent mutations in **chromatine-remodeling gene** *MEN1*

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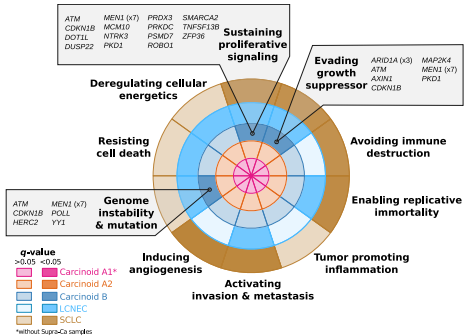
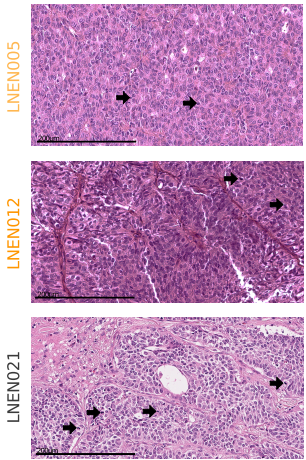


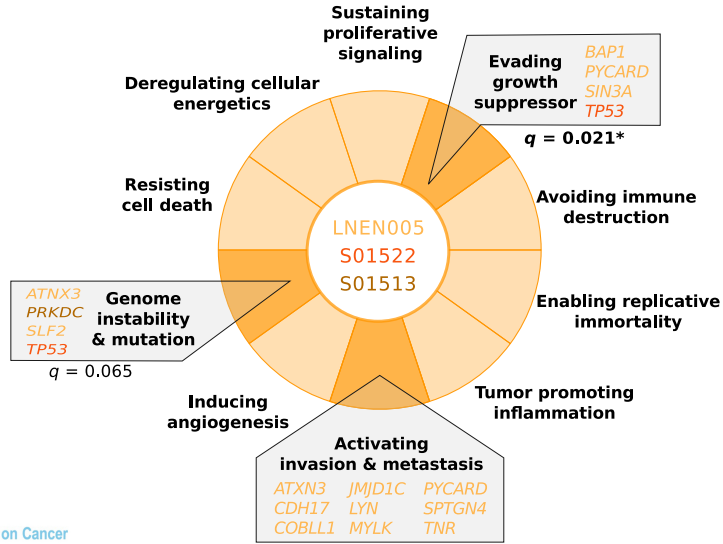
- Enrichment for mutations in **hallmarks of cancer**

Supra-carcinoids have carcinoid-like morphology yet molecular and clinical features of LCNEC

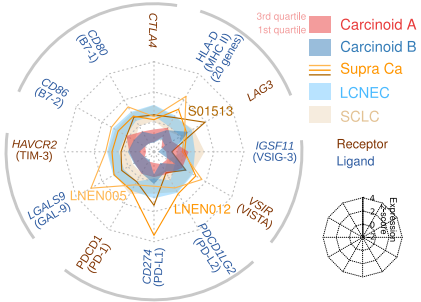


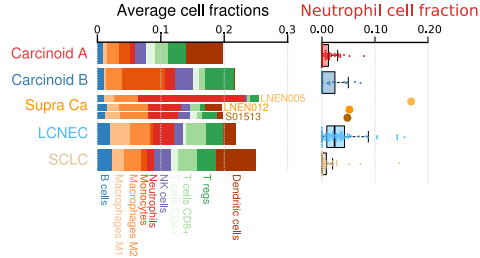
	LNEN005	LNEN012	LNEN021	LNEN022	S01513	S01522
CLASSIFICATION						
Histopathology	Atypical	Atypical	Atypical	Atypical	Atypical	Atypical
Morphological characteristics	carcinoid morph. 2 mitoses/2mm² No necrosis	carcinoid morph. 2 mitoses/2mm² No necrosis	LCNEC morph. 4 mitoses/2mm² No necrosis	NA	NA	NA
Machine learning	LCNEC	LCNEC	Atypical	LCNEC	Atypical	LCNEC
CLINICAL DATA						
Sex	M	F	F	F	M	M
Age at diagnosis	80	70	83	58	58	63
TNM Stage	IB	IIIC	IA1	IIB	IIIA	IV
Overall survival (months)	144.6	111.7	29.8	36.1	59	7
EPIDEMIOLOGY						
Smoking status	Former	NA	NA	NA	Never	Current
Other known exposure	Asbestos	NA	NA	NA	NA	NA
MULTI-OMICS DATA						
Data available	WES, RNAseq, Epic 850K	RNAseq	Epic 850K	Epic 850K	WGS, RNAseq	WES, Epic 850K
Mutated genes	*JMJD1C, KDM5C, BAP1*	NA	NA	NA	*DNAH17*	*TP53*
***MKI67* FPKM**	2.6	7.3	NA	NA	1.9	NA

Despite low mutation rate of carcinoids (≈ 0.4 muts/Mb), supra-carcinoids enriched for hallmarks of cancer mutations

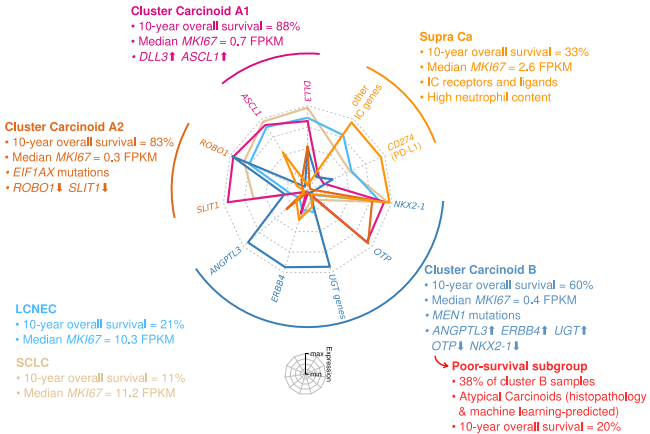


Supra-carcinoids show a particular immune pattern suggesting a therapeutic target





Summary



Published in Nature Communications

Integrative and comparative genomic analyses identify clinically relevant groups of pulmonary carcinoids and unveil the existence of supra-carcinoids

Alcala N[1*], Leblay N[1*], Gabriel AAG[1*], Mangiante L[1], Hervas D[2], Giffon T[1], Sertier AS[3], Ferrari A[3], Derks J[4], Ghantous A[5], Delhomme TM[1], Chabrier A[1], Cuenin C[5], Abedi-Ardekani B[1], Boland A[6], Olaso R[6], Meyer V[6], Altmuller J[7], Le Calvez-Kelm F[1], Durand G[1], Voegele C[1], Boyault S[8], Moonen L[4], Lemaitre N[9], Lorimier P[9], Toffart AC[9], Soltermann A[10], Clement JH[11], Saenger J[12], Field JK[13], Brevet M[14], Blanc-Fournier C[15], Galateau-Salle F[16], Le Stang N[16], Russell PA[17], Wright G[17], Sozzi G[18], Pastorino U[18], Lacomme S[19], Vignaud JM[19], Hofman V[20], Hofman P[20], Brustugun OT[21], Lund-Iversen M[22], Thomas de Montpreville V[23], Muscarella LA[24], Graziano P[24], Popper H[25], Stojsic J[26], Deleuze JF[6], Herceg Z[5], Viari A[3], Nuernberg P[7,27], Pelosi G[28], Dingemans AMC[4], Milione M[18], Roz L[18], Brcic L[25], Volante M[29], Papotti MG[29], Caux C[30], Sandoval J[2], Hernandez-Vargas H[31], Brambilla E[9], Speel EJM[4],

Discussion: new entities or new concepts?

- **2 molecular types of aggressive well-differentiated tumors**, both with prognosis possibly bad as that of G3 LCNEC:
  1. Carcinoid B, that correspond to current G1 or G2
  2. Supra-carcinoids, that correspond to a subset of current G2
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- With the discovery of carcinoid-like LCNECs (Rekhtman et al. Clin Cancer Res 2016; George et al. Nat Commun 2018), existence of supra-carcinoids suggest **links between well-differentiated and poorly-differentiated LNENs**
- Contrary to well-differentiated G3 gastrointestinal NENs, **supra-carcinoids do not have morphological features of poorly-differentiated G3**, only their molecular features

Study limitations

- Heterogeneous dataset
- Limited number of samples
- Limited information on some genomic characteristics: genomic rearrangements, mutational signatures, copy number changes
- Incomplete clinical and epidemiological characteristics: asbestos and smoking exposures

Biorepository

Frozen & FFPE material for
- 226 **typical carcinoids** T-N pairs
- 118 **atypical carcinoids** T-N pairs
  & 18 with multiple regions

Generate comprehensive, multidisciplinary, and multi-omics datasets for pulmonary carcinoids to:

1. **Validate and expand the discoveries**: DLL3, OTP, immune signatures, etc
2. **Characterize supra-carcinoids**
3. Model *in vitro* the **progression** PNEC → G1 (typical) → G2 (atypical) → supra-carcinoid
4. Identify **better markers** for the differential diagnosis of LNENs
5. Expand the **molecular knowledge** on these rare diseases: genomic analyses, ITH
6. **Identify the etiology** of these diseases: smoking and asbestos exposures

Detailed pathological review from 5 pathologists

Whole-genome, transcriptome, and methylome data

Well-annotated clinical and epidemiological databases

lungNENomics team

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Dr N Girard (coordinator clinical and epi data, Institute Curie, EURACAN)
Dr S Boyault (coordinator biorepository, CLB)
Dr T Dayton (coordinator in vitro studies, Clevers' lab, Utrecht)
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Dr J Derks
L Moonen

International Agency for Research on Cancer

World Health Organization

LungNEN network

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Prof P Hoffman
Dr LA Muscarella
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Dr F Le Calvez-Kelm
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INSTITUT NATIONAL DU CANCER

LA LIGUE CONTRE LE CANCER

NIH National Institutes of Health