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:

<table>
<thead>
<tr>
<th>Name of the enterprise / Nature of the interest</th>
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<td>None</td>
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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

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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

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

- Predicted atypical have the same prognosis as LCNEC.
- Predicted typical have the same prognosis as typical carcinoids.
Molecular classification refines the prognosis of histopathology

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

- LNEN Latent Factor 1
  - 30% methylation, 28% expression
- LNEN Latent Factor 2
  - 14% methylation, 6% expression

- Good prognosis area: NET (G1 and G2) predicted as G1 by ML
- Two bad prognosis areas:
  1. Cluster Carcinoid B
  2. Carcinoids in LCNEC cluster

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"Bad prognosis" carcinoids have 2 distinct molecular profiles

- **Cluster Carcinoid A**
- **Cluster Carcinoid B**
- **Cluster LCNEC**

### Histopathology
- **Typical**
- **Atypical**
- **LCNEC**
- **Carcinoid**

- **Good prognosis area**: NET (G1 and G2) predicted as G1 by ML
- **Two bad prognosis areas**: Carcinoids in LCNEC cluster

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"Bad prognosis" carcinoids have 2 distinct molecular profiles

Histopathology
- Typical
- Atypical
- LCNEC
- Carcinoid

Machine Learning
- Typical
- Atypical
- LCNEC
- Unclassified
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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 chromatin-remodeling gene MEN1
"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**

Enrichment for mutations in **hallmarks of cancer**
Supra-carcinoids have carcinoid-like morphology yet molecular and clinical features of LCNEC

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<th>LNEN005</th>
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Despite low mutation rate of carcinoids ($\approx 0.4$ muts/Mb), supra-carcinoids enriched for hallmarks of cancer mutations

- Sustaining proliferative signaling
- Evading growth suppressor
- Avoiding immune destruction
- Enabling replicative immortality
- Tumor promoting inflammation
- Activating invasion & metastasis
- Inducing angiogenesis
- Resisting cell death
- Deregulating cellular energetics
- Genome instability & mutation

$q = 0.021^*$
Supra-carcinoids show a particular immune pattern suggesting a therapeutic target
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 L1, Hervas D1, Giffon T1, Sertier AS1, Ferrari A1, Derks J1, Ghantous A2, Delhomme TM1, Chabrier A1, Cuenin C3, Abedi-Ardekani B1, Boland A1, Olaso R1, Meyer V1, Altmuller J1, Le Calvez-Kelm F1, Durand G1, Voegele C1, Boyault S5, Moonen L5, Lemaître N9, Lorimier P1, Toftart AC1, Soltermann A19, Clement JH13, Saenger J15, Field JK13, Brevet M14, Blanc-Fournier C15, Galateau-Salle F16, Le Stang N16, Russell PA17, Wright G17, Sozzi G18, Pastorino U18, Lacomme S19, Vignaud JM19, Hofman V20, Hofman P20, Brustugun OT21, Lund-Iversen M22, Thomas de Montpreville V23, Muscarella LA23, Graziano P24, Popper H25, Stojic JS26, Deleuze JP26, Herceg Z2, Viari A2, Nuernberg P27, Pelosi G26, Dingemans AMC, Milione M18, Roz L18, Bricic L25, Volante M28, Papotti MG29, Caux C29, Sandoval J1, Hernandez-Vargas H31, Brambilla E3, Speel EJM31.
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
  3. This challenges the idea that G1<G2<G3 in terms of aggressiveness
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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
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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
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

- Dr L Fernandez-Cuesta (PI, GCS)
- Dr M Foll (coordinator computational biology, GCS)
- Prof S Lantuejoul (coordinator pathology, CLB)
- 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)
- Dr N Alcala (postdoc, GCS)
- N Leblay (PhD student, GCS)
- A Gabriel (PhD student, GCS)
- L Mangiante (PhD student, GCS)

### IARC

- Dr I Cree
- Dr Z Herceg
- Dr A Ghantous
- C Cuenin

### CRCL

- Prof C Caux
- Dr H Hernandez-Vargas

### CLB

- Anne-Sophie Sertier
- Anthony Ferrari

### LungNEN network

- Prof M Brevet
- Prof V Thomas de Montpreville
- Prof F Galateau-Salle
- Prof JM Vignaud
- Prof P Hoffman
- Dr LA Muscarella
- Prof M Papotti
- Dr M Volante
- Dr L Roz
- Dr OT Brustugun
- Dr GM Wright
- Prof O Popper
- Prof E Brambilla
- Prof C Blanc-Fournier
- Prof G Pelosi
- Prof M Milione
- Dr L Brcic

### Maastricht Uni Hospital

- Prof EJ Speel
- Dr AM Dingenmans
- Dr J Derks
- L Moonen

### GCS

- Dr J McKay
- Dr B Abedi-Ardekani
- TM Delhomme
- A Chabrier
- Dr C Voegele
- Dr F Le Calvez-Kelm
- G Durand

### Funding

- CLB
  - Anne-Sophie Sertier
  - Anthony Ferrari

- GCS
  - Dr J McKay
  - Dr B Abedi-Ardekani
  - TM Delhomme
  - A Chabrier
  - Dr C Voegele
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  - Dr A Ghantous
  - C Cuenin

- CRCL
  - Prof C Caux
  - Dr H Hernandez-Vargas

- National Institutes of Health
  - INSTITUT NATIONAL DU CANCER
  - LA LIGUE CONTRE LE CANCER

- World Health Organization
  - International Agency for Research on Cancer