Assessment of the current and emerging criteria for the histopathological classification of lung neuroendocrine tumours in the lungNENomics project

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ENETS EUROPEAN NEUROENDOCRINE TUMOR SOCIETY

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Background:

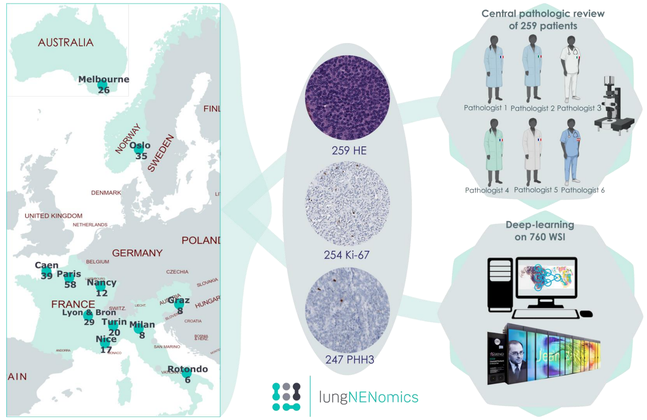


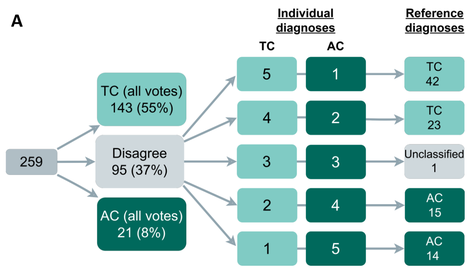
Figure 1: Six thoracic pathologists diagnosed 259 LNETs per 2021 WHO criteria, distinguishing ACs from TCs by focal necrosis and/or 2-10 mitoses per 2 mm².

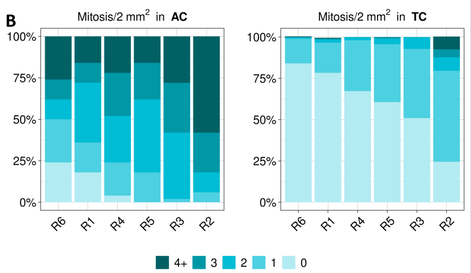
The **lungNENomics cohort** presents a **unique opportunity** to assess the **strengths** and **limitations** of **current WHO classification** and to evaluate the **utility** of **emerging markers**

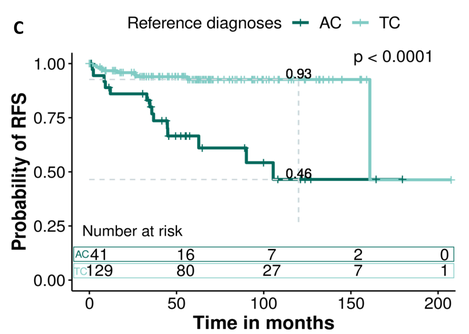
Methods:

- Tumour proliferative activity: **Ki-67 index** and **phospho-histone H3** (PHH3) protein expression => both quantified by **each pathologist** in hot-spot areas + **automatically** by the Pathonet [Negahbani SCI REP 2021] **deep learning** models at the **whole slide image** (WSI) scale.
- **Hypothetical classification** systems generated **for each marker** and nine cut-off values, **replacing mitotic count**. Prognostic values of resulting groups were assessed based on RFS.
- The **unsupervised Barlow twins** [Zbontar PMLR 2021, Quiros Arxiv 2022] **deep learning** model was used to **identify** potential **new morphological features.**

Result 1: Limitations of the current morphological criteria







- The **current classification** of LNETs is :
- **Moderately reproducible => AC** more prone to **misdiagnosis** than **TC (Figure 2A)** mainly due to **mitotic figure counting variability (Figure 2B)**.
- **Imperfect prognostic value (Figure 2C) =>** Up to **11% TCs relapse** within first year post-resection; up to **71% ACs don't** (individual pathologist diagnoses).

Figure 2: A) Flow diagram of the pathological review leading to reference diagnoses. **B)** Distributions of the number of mitoses by pathologist. **C)** Kaplan-Meier curves of recurrence-free survival (RFS).

In addition to 2021 WHO criteria distinguishing AC from TC, **we propose adding Ki67 and PHH3 IHC:**

- **To guide mitotic count,** considering tumor heterogeneity and high proliferation areas;
- **To identify potential NET-G3** with carcinoid morphology (>10 mitoses/2mm² & Ki-67 > 20%);
- **To ensure consistency of mitotic count with PHH3 staining** (~2x more PHH3 positive-cells expected than the number of mitotic figures on HE);
- **To detect possible AC when Ki67 reaches 5%** either on biopsy or **when the mitotic count is not feasible**;

Perspectives:. **Multi-omics** data analysis reveals **robust molecular groups** with varying **morphological features** using unsupervised deep-learning methods, **paving the way for morpho-molecular classification.**

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Result 2: Added value of Ki-67 and PHH3 in the assessment of proliferative activitv

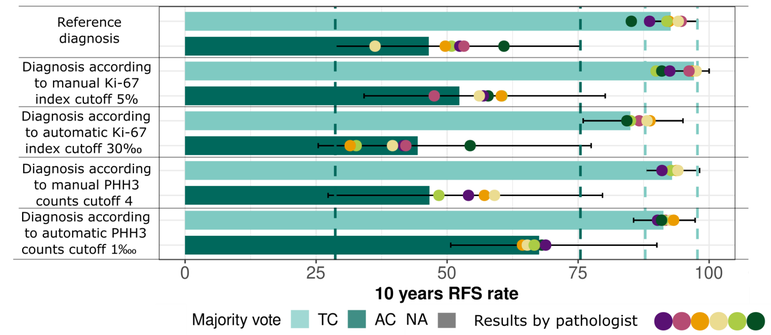

Figure 3: (**Left**) For each classification system, the coloured bar corresponds to the 10- year RFS rate based on the diagnoses resulting from majority voting.

- **A wide range of thresholds** applied to **pathologists' estimates** of **Ki-67** and **PHH3** expression enable LNETs to be **divided** into **two groups** with **similar prognostic** values **to those defined by the WHO criteria (Figure 3)**.
- **Ki-67 improves high-risk TC detection** but **not AC specificity**.
- **PHH3 speeds up mitotic** counting but does **not** significantly **reduce inter-observer variability.**
- **Automatic** evaluation **of Ki-67 and PHH3 reach the performance of expert pathologists (Figure 3)**.

Result 3: Deep learning to uncover novel clinically relevant morphological features

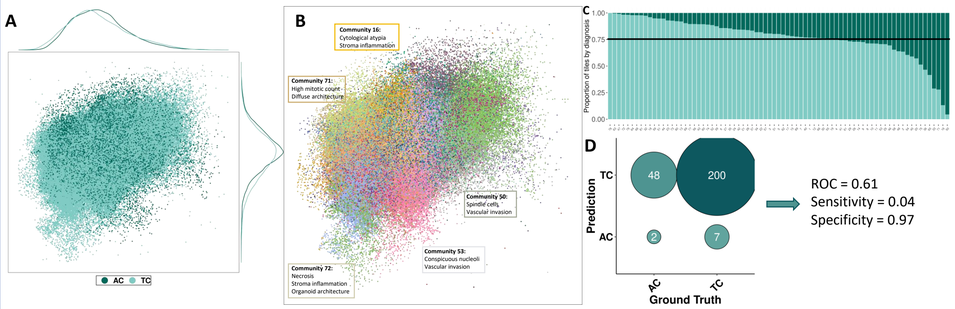

Figure 4: Unsupervised deep-learning experiment on HE/HES WSI. **A)** 2D morphological map color-coded by patient diagnosis. **B)** Leiden communities annotated for morphological enrichment. **C)** Tile proportions per Leiden community by tumor type. **D)** Confusion matrix & performance metrics.

- **State-of-the-art deep learning** on WSI **doesn't aid TC vs. AC distinction** due to histological similarity **(Figure 4)**, **hinting at morphology's limitations**.

Future Directions for Research:

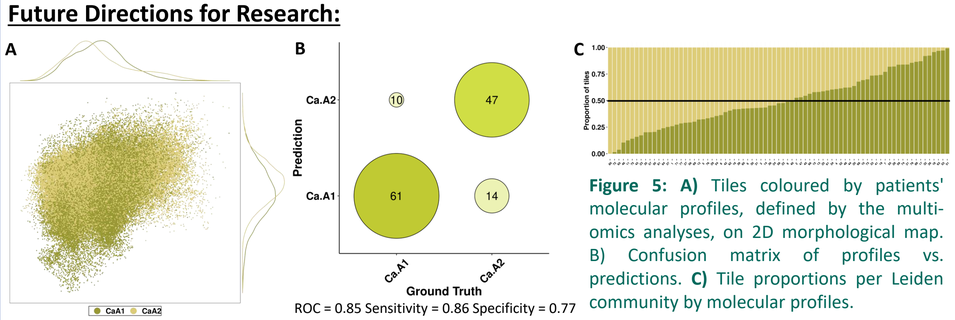

Figure 5: A) Tiles coloured by patients' molecular profiles, defined by the multi-omics analyses, on 2D morphological map. B) Confusion matrix of profiles vs. predictions. **C)** Tile proportions per Leiden community by molecular profiles.

- **Barlow-Twins outperforms** in **classifying** tumours by **molecular profiles** [Alcala NAT COM 2019] compared to their histological types **(Figure 5)**, **opening new perspectives for more-molecular classification.**