

Novel techniques for classification of NENs, including carcinoids, LCNEC and SCLC

APP Summer Meeting

The Hague

20th June 2025

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Classifying and nomenclature

“Its not atypical carcinoid, it’s a typical carcinoid”

Classifying and nomenclature

- Classification needs to be relevant to patient management
- “**atypical carcinoid**” or “**grade 2 neuroendocrine tumour**”
 - Not a huge difference to the patient
- “carcinoid **with metastatic potential**”
 - Big difference to the patient
- We know these exist but current classification does not capture or indicate this

However....

- *novel techniques* for classification rather than novel classifications
- What does classification mean anyway?
 - Formal diagnostic category
 - Significant prognostic/behavioural differences
 - Management
 - Requires reliable morphology
- Risk of over subtyping via morphology and molecular
- Each tumour is probably molecularly unique
- Clinical relevance

What are our molecular tools?

- 'Traditional' NGS
 - Finding specific aberrations and classifying by presence and type
 - NSCLC – very familiar with EGFR
 - EGFR wild-type – candidate for immunotherapy
 - EGFR aberration – sensitising, resistant, unknown

Molecular classification

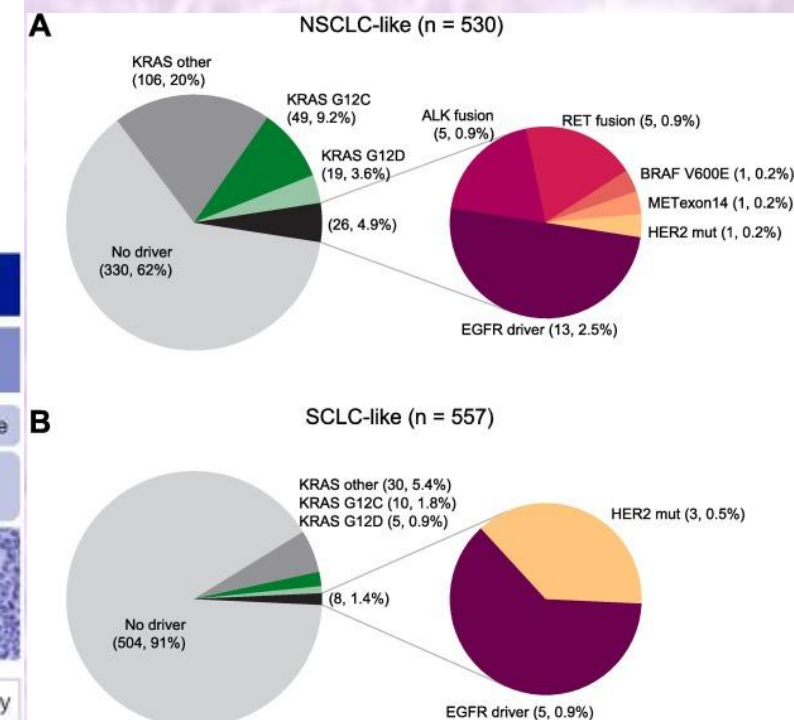
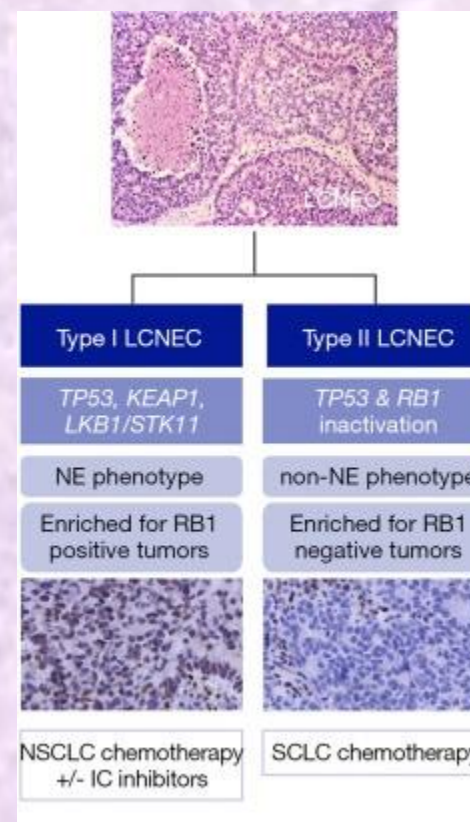
- Large cell neuroendocrine carcinoma
- Heterogenous disease
 - Morphological diversity
 - Behavioural diversity
 - Molecular diversity
 - SCLC-like (p53/Rb1)
 - NSCLC-like (STK11, KRAS, KEAP1)
 - Carcinoid-like (MEN1)
 - Other classifiers (ASCL1, DLL3, NOTCH)



CONSENSUS STATEMENT

Large cell neuroendocrine lung carcinoma: consensus statement from The British Thoracic Oncology Group and the Association of Pulmonary Pathologists

Colin R. Lindsay^{1,2,3}, Emily C. Shaw⁴, David A. Moore^{3,5}, Doris Rassl⁶, Mariam Jamal-Hanjani^{3,7}, Nicola Steele⁸, Salma Naheed⁹, Craig Dick¹⁰, Fiona Taylor¹¹, Helen Adderley⁷, Fiona Black¹², Yvonne Summers², Matt Evans¹³, Alexandra Rice¹⁴, Aurelie Fabre¹⁵, William A. Wallace^{16,17}, Siobhan Nicholson¹⁸, Alex Haragan¹⁹, Phillipe Tanriere²⁰, Andrew G. Nicholson^{14,21}, Gavin Laing²², Judith Cave⁹, Martin D. Forster^{3,23}, Fiona Blackhall^{1,2,3}, John Gosney¹⁹, Sanjay Popat^{24,25} and Keith M. Kerr²²

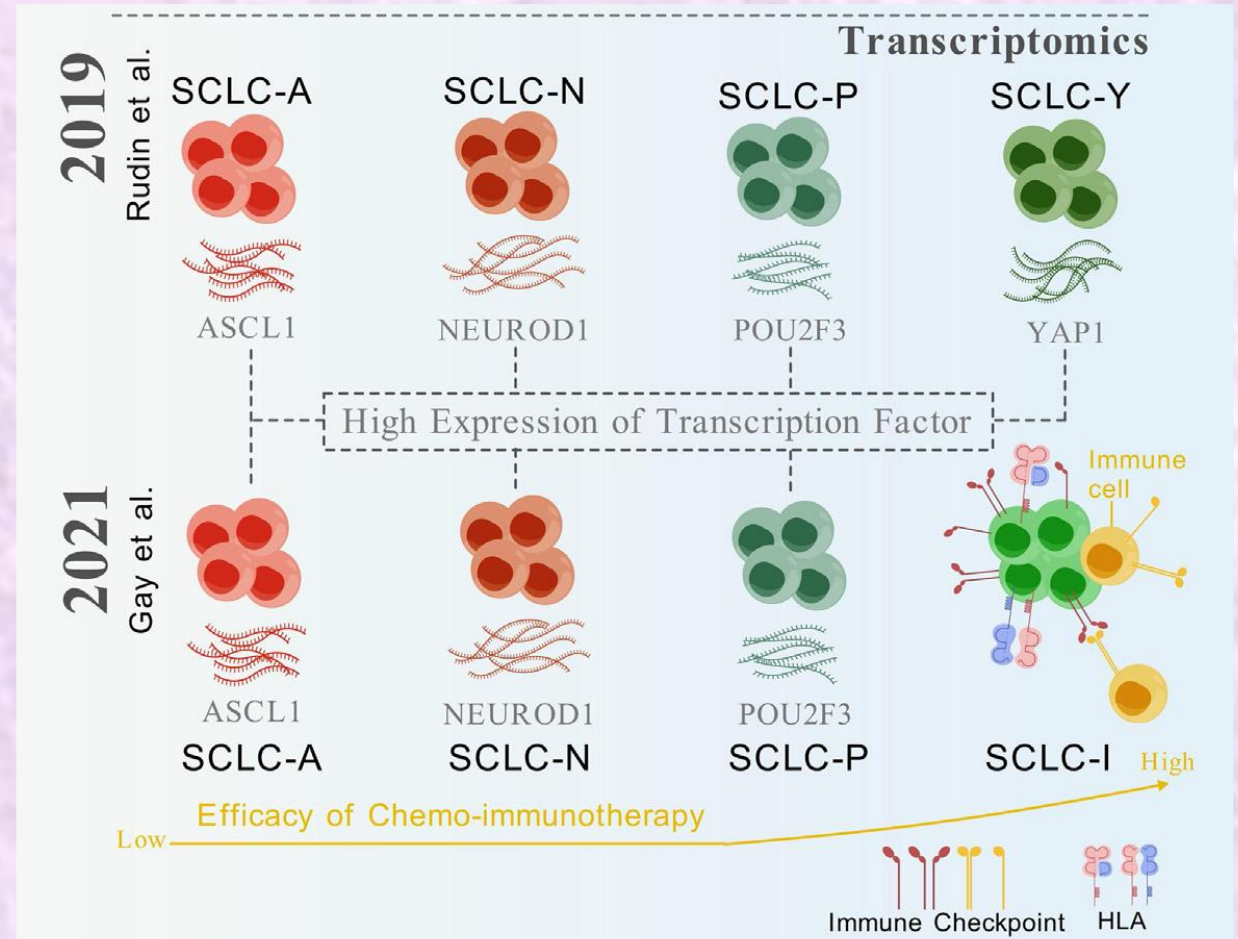


What are our molecular tools?

- ‘Traditional’ NGS
- ‘-omics’
 - WGS/WES, transcriptomics, methylomics, proteomics

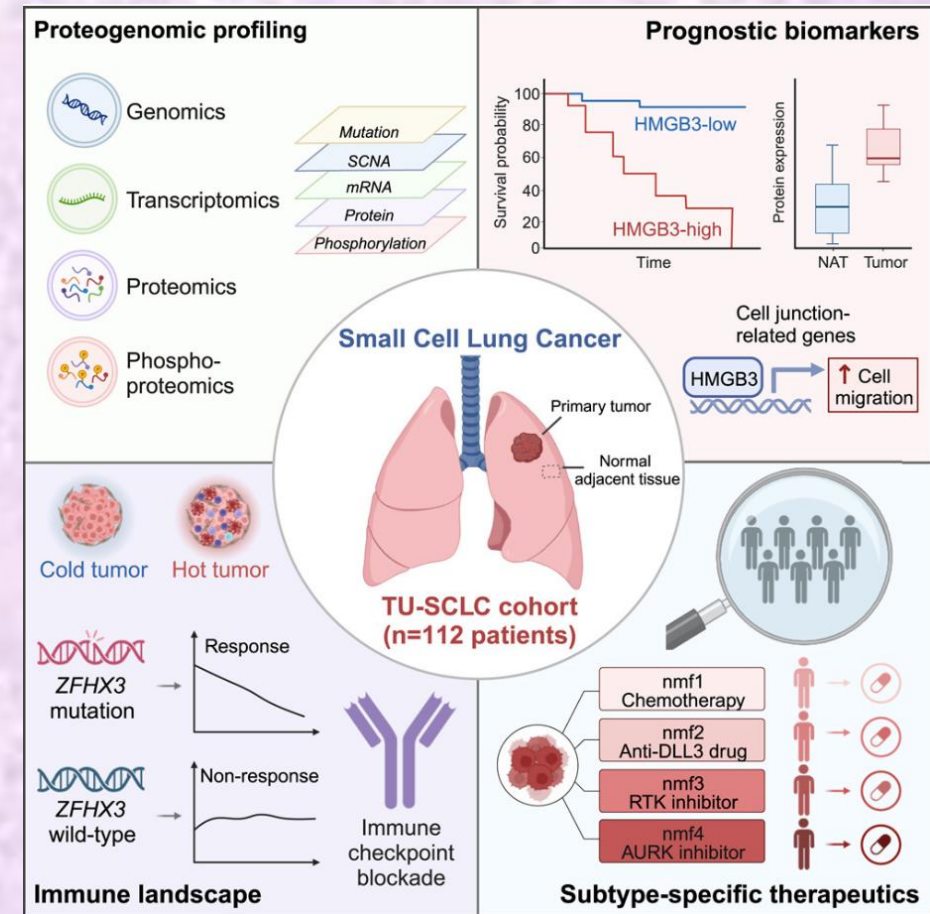
Molecular classification - SCLC

- Traditionally:
 - Morphologically homogenous
 - Behaviourally homogenous (terrible prognosis)
- Genomics
 - Molecularly homogenous (p53/RB1 loss of function)
- Transcriptomics
 - Reveal subgroups based on transcription factor expression
 - Survival data hard!

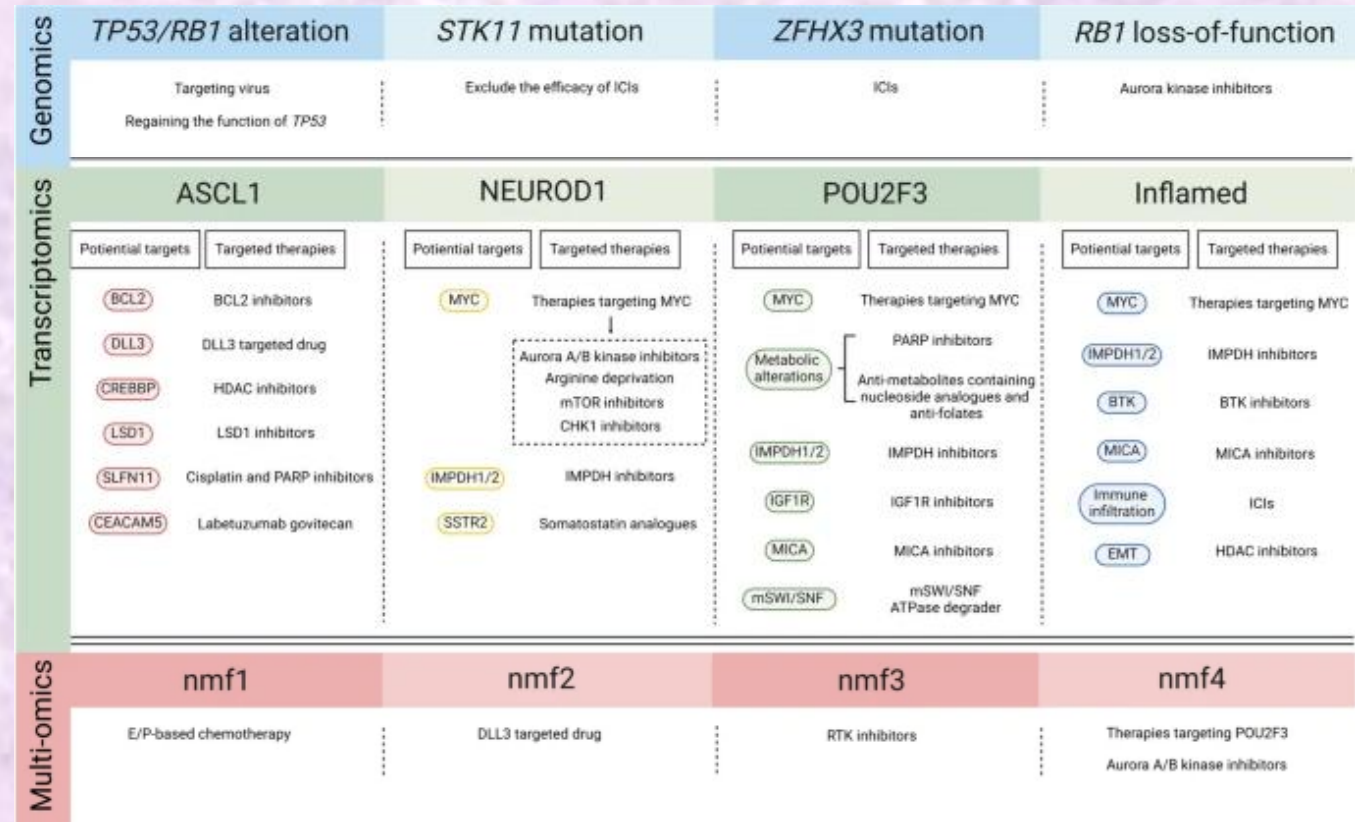


Molecular classification - SCLC

- Multi-omics
 - Genomics, transcriptomics, proteomics, phosphoproteomics
 - Further subclassification with even more clinical relevance



- Genomics = simple
 - Wrong!
 - Many SCLC may require “second hit”
 - In large SCLC study of 3600 cases:
 - ~21% were Rb1 -/-
 - ~3% were p53 -/-
 - ~5.5% were p53 *and* Rb1 -/-
 - Had KRAS, BRAP and KEAP1 enrichment
 - Therapeutics guidance is key
-
- The diagram illustrates a multi-omics approach to SCLC treatment guidance. It is organized into three main horizontal sections: Genomics (top, blue), Transcriptomics (middle, green), and Multi-omics (bottom, red). The Genomics section focuses on TP53/RB1 alterations, showing a targeting virus and the goal of regaining TP53 function. The Transcriptomics section, which is the largest, lists potential targets (BCL2, DLL3, CREBBP, LSD1, SLFN11, CEACAM5) and their corresponding targeted therapies (BCL2 inhibitors, DLL3 targeted drug, HDAC inhibitors, LSD1 inhibitors, Cisplatin and PARP inhibitors, and Labetuzumab/govitecan). The Multi-omics section focuses on nmf1 and E/P-based chemotherapy. Vertical labels on the left indicate the data type for each section: Genomics, Transcriptomics, and Multi-omics.
- | Genomics | TP53/RB1 alteration | |
|----------|--------------------------------|--|
| | Targeting virus | |
| | Regaining the function of TP53 | |
-
- | Transcriptomics | ASCL1 | |
|-----------------|-------------------|-------------------------------|
| | Potential targets | Targeted therapies |
| | BCL2 | BCL2 inhibitors |
| | DLL3 | DLL3 targeted drug |
| | CREBBP | HDAC inhibitors |
| | LSD1 | LSD1 inhibitors |
| | SLFN11 | Cisplatin and PARP inhibitors |
| | CEACAM5 | Labetuzumab/govitecan |
-
- | Multi-omics | nmf1 |
|-------------|------------------------|
| | E/P-based chemotherapy |

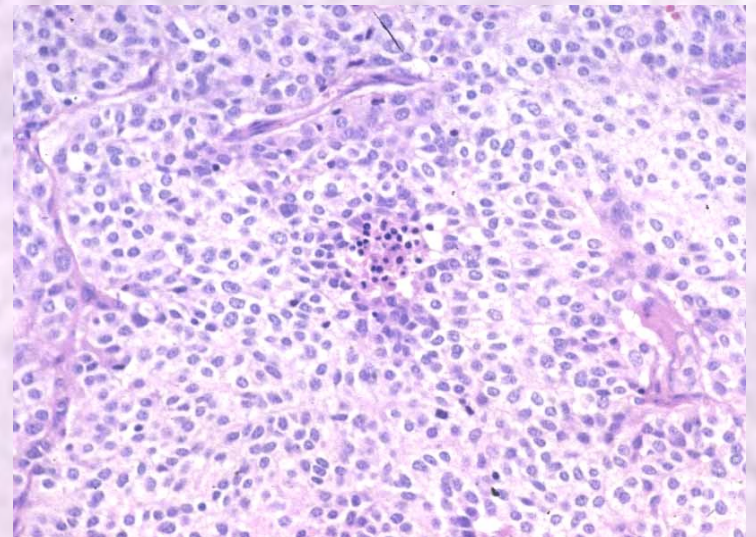
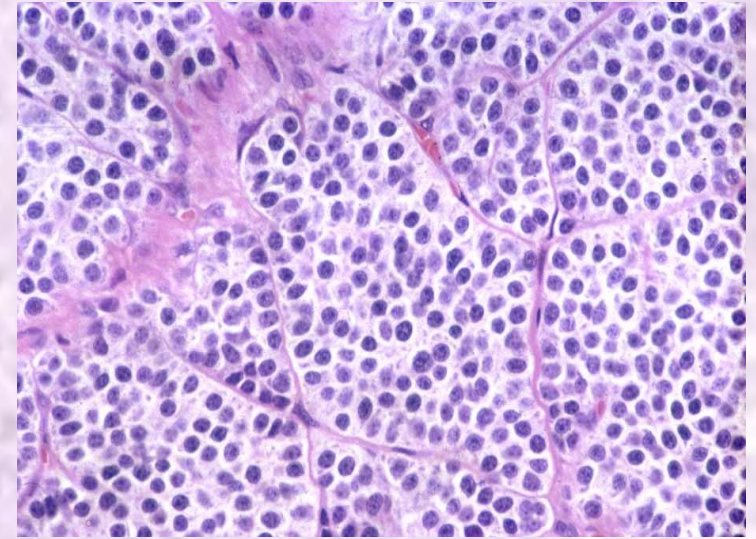


What are our molecular tools?

- 'Traditional' NGS
 - Deliverable in routine clinical setting
- Complex -omics
 - Not so deliverable in routine clinical setting
- What can we do about that?

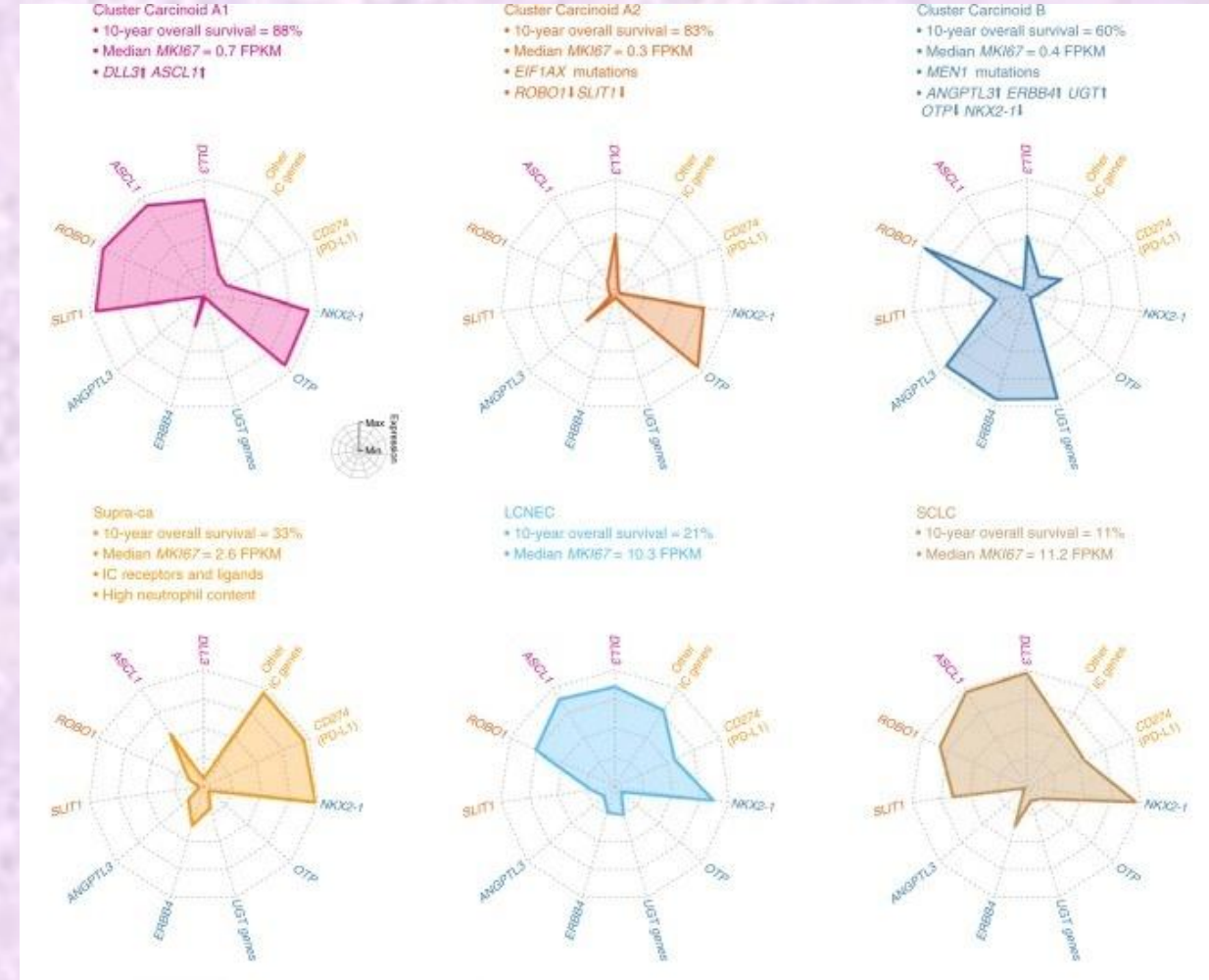
Molecular classification - Carcinoids

- Typical carcinoid
 - <2 mitoses/ 2mm^2
 - No necrosis
- Atypical carcinoid
 - $2-10$ mitoses/ 2mm^2
 - Foci of necrosis (punctate)
- Carcinoid morphology
- Behavioural outliers
 - Prognostic markers e.g. CD44, TOP, ASCL1
- Complex molecularly

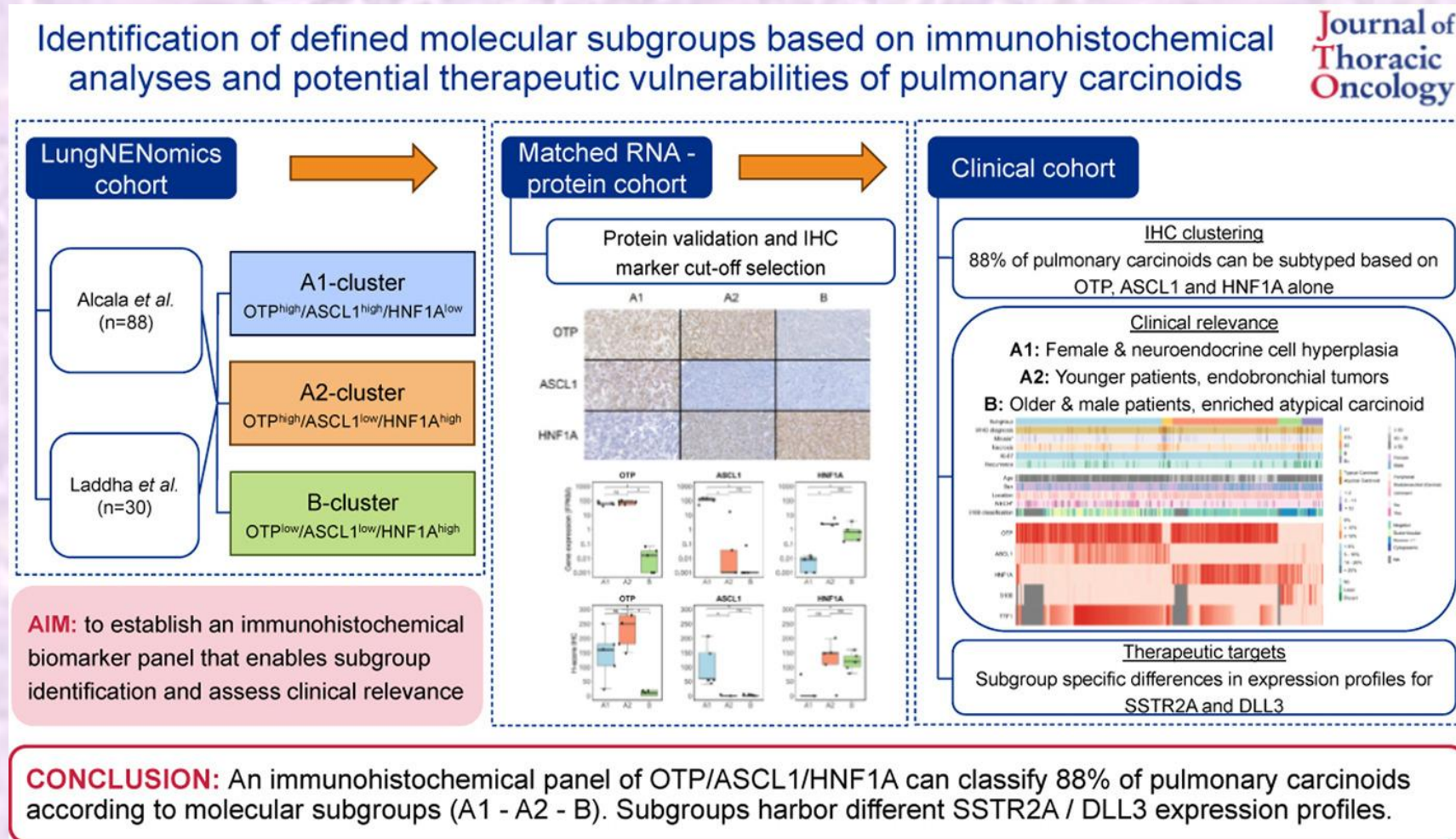


Carcinoids

- Alcala *et al.* 2019, Laddha *et al.* 2019
- Gabriel *et al.* 2020
 - Transcriptome and methylome data
 - ML and multi-omics factor analyses
 - A1/LC1 – strong immune activity
 - A2/LC3
 - B/LC2 – weak/no immune activity
 - Demographic and anatomical correlations
 - **Supra-carcinoid**
 - Morphologically carcinoid
 - Molecularly LCNEC

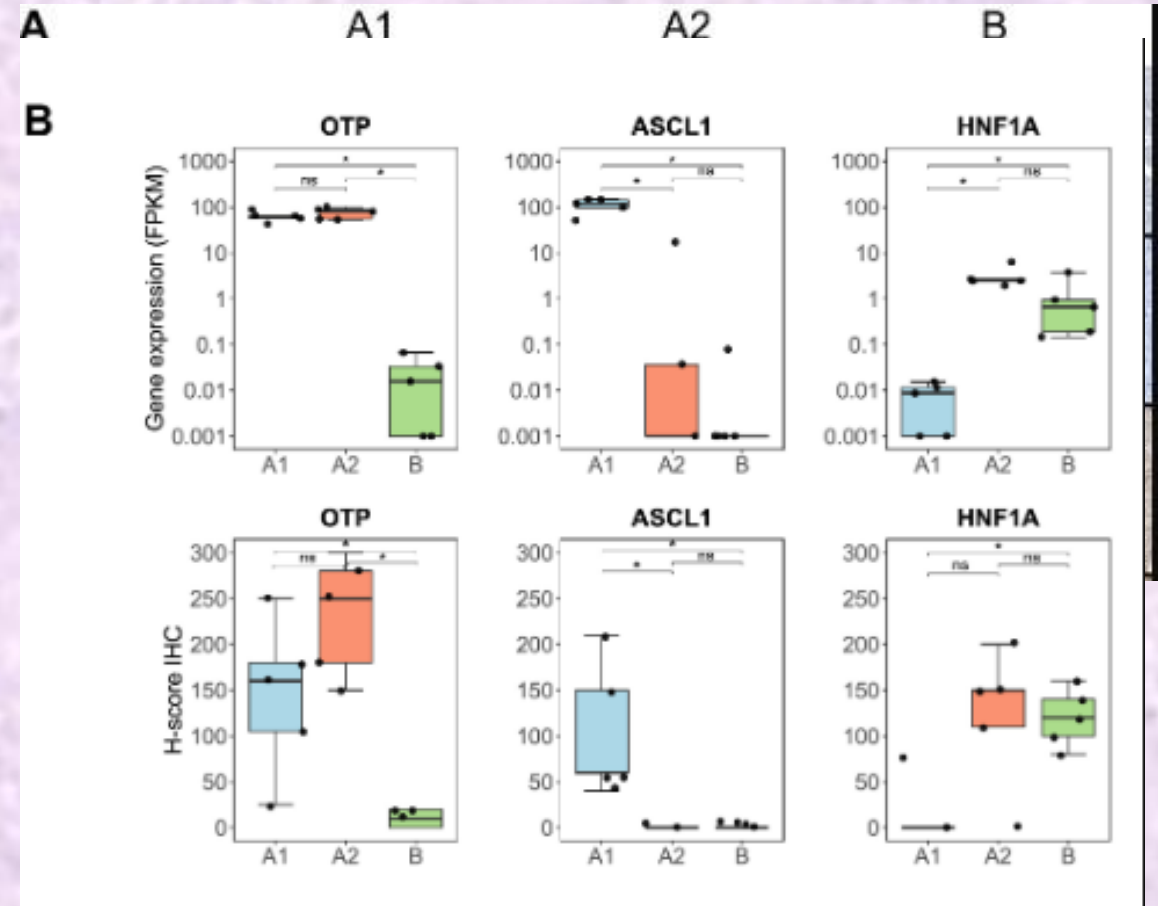


Immunohistochemistry.... Again?



Translating complex molecular data

- RNA of interest based on transcriptomic data
 - e.g (OTP, ASCL1, TTF1, HNF1A, S100, ANGPTL3)
- Protein expression
 - There is never a perfect correlation!
- H-score developed
 - (albeit on small numbers)



Translating complex molecular data

	OTP	ASCL1	HNF1A	Gender	Age	Site	SSTR2A	DLL3
A1	+	+	-	Female	Older	Peripheral		+
A2	+	-	+		Younger	Central	+	
B	-	-	+	Male		Atypical/higher recurrence		

- 88% of the carcinoids could be classified in this manner

- A1x

- TTF1 high / S100 negative
- ASCL1, DLL3, female
- OTP lost, necrosis, atypical, worse prognosis

- Bx

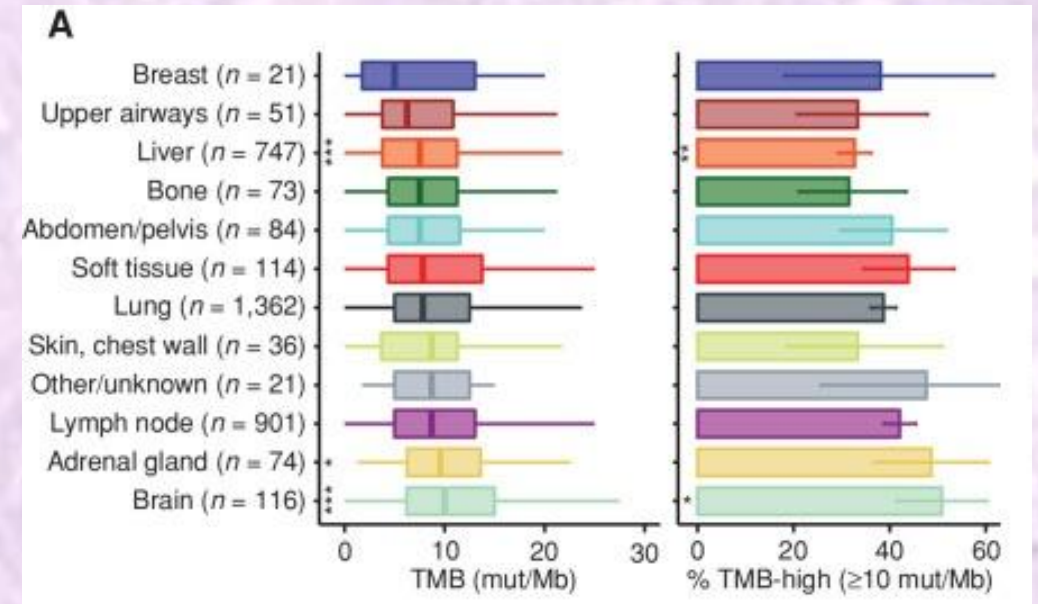
- TTF1 low / S100 enriched
- Low HNF1A, ASCL1, DLL3

What are our molecular tools?

- 'Traditional' NGS
- Complex -omics
- Immunochemistry
- Anything else?

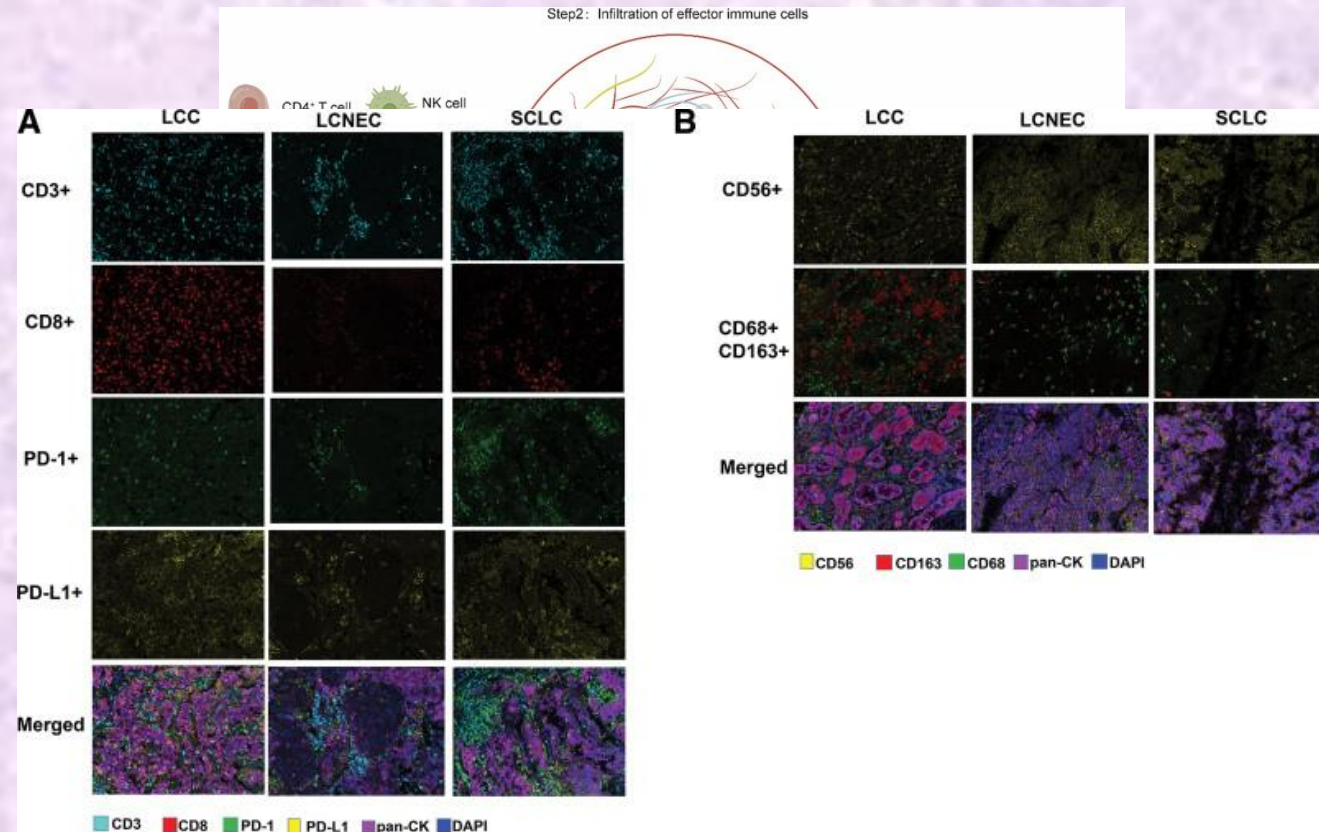
TMB (Tumour mutational burden)

- Immunotherapy
- Not widely implemented
 - Defining TMB-H, panel/platform variability, etc.
- SCLC
 - Potential behavioural biomarker
 - Associated with site specific sampling
 - (e.g. highest seen in brain metastases)
- A “free” biomarker?



The TME (Tumour Microenvironment)

- Focus has been on the tumour cells
- Data shows variation in TME between NENs
- Possibility of sub-classification
 - Based on therapeutic intent
- Dependent on familiar techniques
- Other proteomic approaches (e.g. MS) unlikely in the day-to-day
- Classification or prediction



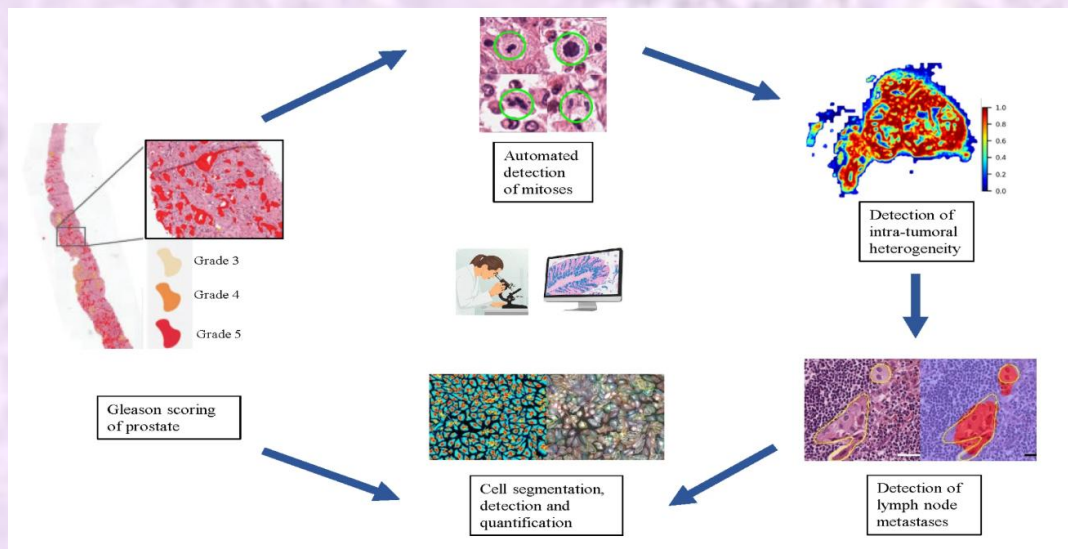
What are our molecular tools?

- 'Traditional' NGS
 - +/- TMB
- Immunochemistry
 - +/- Immunofluorescence
 - Multiplex
- Complex -omics
- Spatial mass-spectrometry

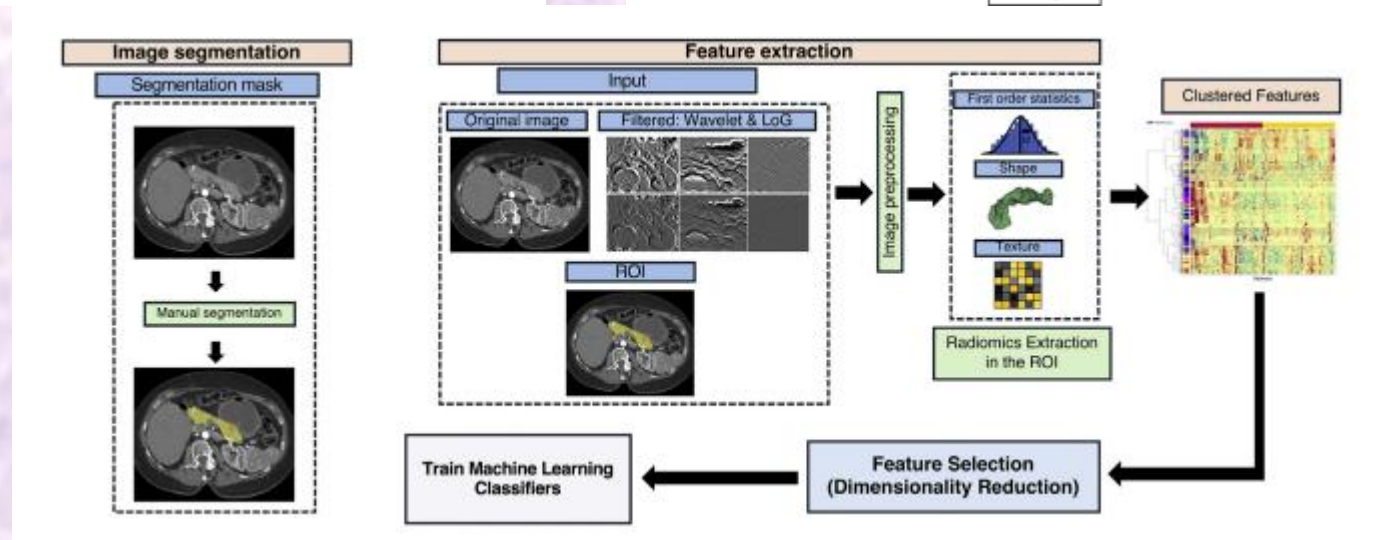
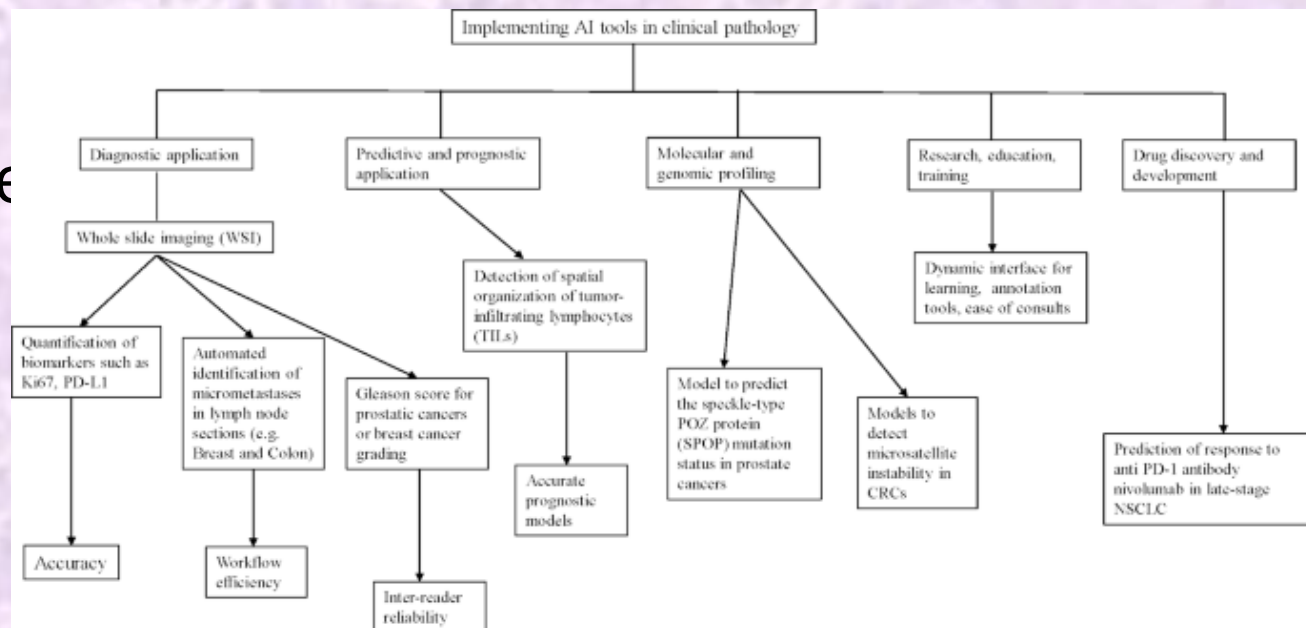
Thinking beyond molecular pathology

- Radiology ('radio-omics')
- Blood biomarkers (paraneoplastic syndrome e.g. adrenalin, serotonin)
- DIPNECH
- Other factors
 - Demographics, smoking history, etc
- What can help with all this complex data?

AI (Artificial intelligence)



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AI for multi-modal data

- This is where it gets exciting

Rubbish in = rubbish out

Conclusion

- Many 'novel' techniques in research setting
- In clinical setting
 - NGS
 - Immunochemistry
- Molecular classification of SCLC, LCNEC and carcinoids all exist
 - Showing clinical utility is the hope
- Large data with AI is long-promised
 - Little specific to pulmonary NENs
- The future is exciting!