

Case Study: BMS ROSE EBUS

An approach to rapid on-site evaluation

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Information required from the clinical team before procedure

- Is staging required?
 - No, diagnosis only.
- What is the pre-test positivity? (Likelihood of malignancy)
 - RUL mass seen on imaging. Patient has extensive mediastinal nodes and metastatic disease including retroperitoneal, adrenal and psoas.
 - High likelihood of malignancy. For low pre-test positivity, a discussion with the clinician beforehand may mean the procedure could stop with two passes demonstrating good nodal sampling only.
- Is there any relevant clinical history?
 - Patient has recent history of melanoma.
 - No previous BRAF testing has been performed
- Has this site previously been sampled?
 - No previous diagnosis from this lesion so sufficient material is required for all possible IHC and molecular testing depending on morphology.

Setup



Single Slide Assessment Process

1. Whole sample is expelled into a vial lid using a stylet



2. Mix sample



Macroscopic

Verbally feed back to clinician quantity of sample obtained for that pass

8. Transfer remaining sample into vial containing saline

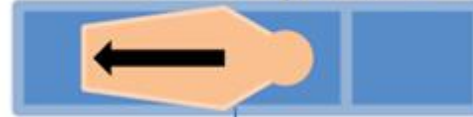
7. Needle flushed with saline into Vial

3. Place representative drop onto slide



Glass Microscope Slide

4. Direct spread



Verbally feed back to clinician adequacy for that pass

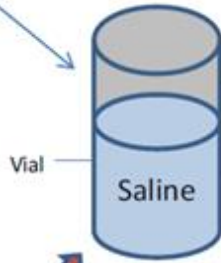
5. Stain air dried slide (Quick MGG)

6. Microscopy assessment

Microscopic

9. At the end of the procedure - Make aliquots for ancillary tests where required

10. Add Cytolyt preservative (Same amount of cytolyt to volume of sample)



Vial

Saline

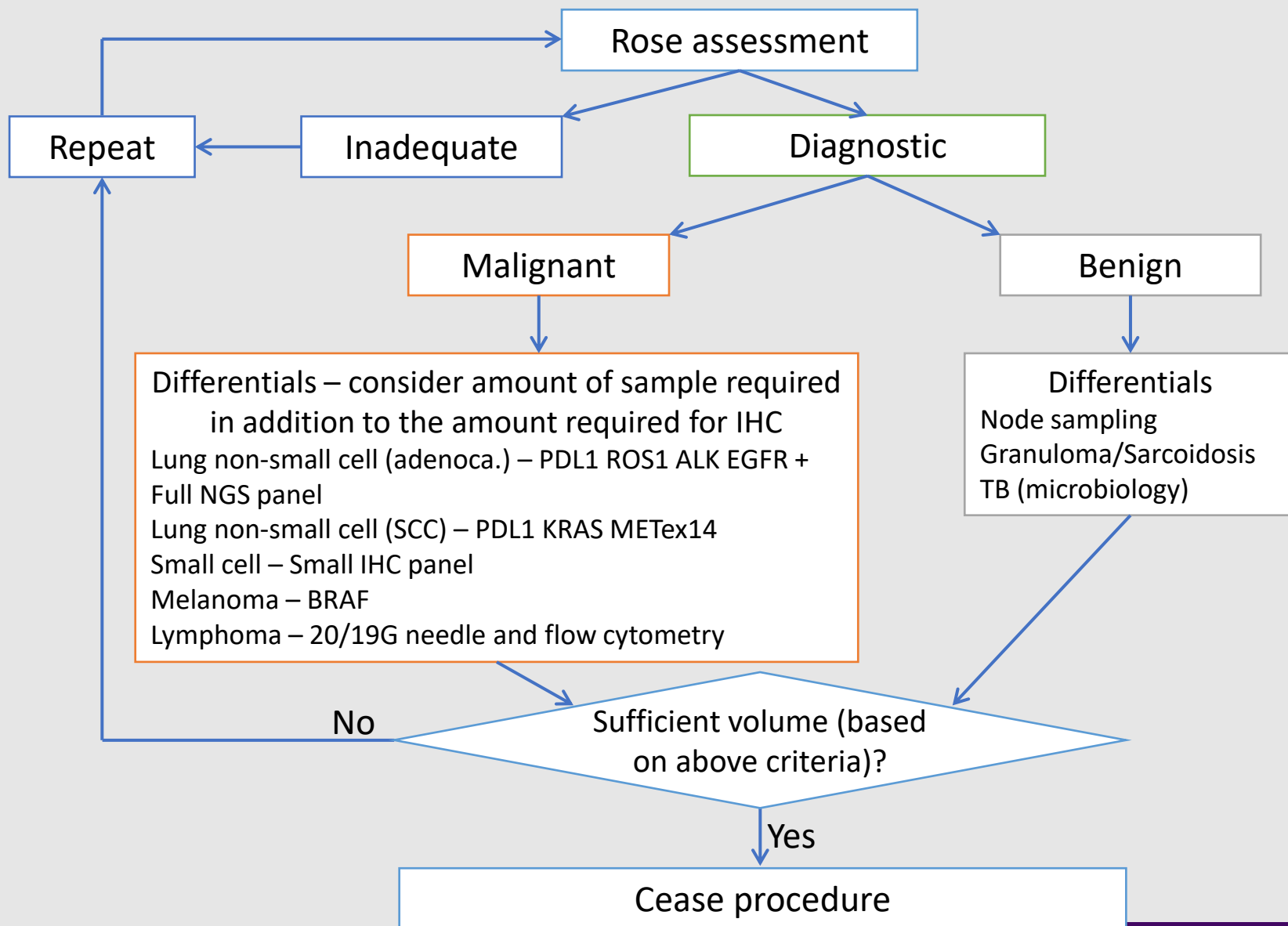
Flow Cytometry / microbiology



Saline

Glinski et al, 2018

ROSE decision tree

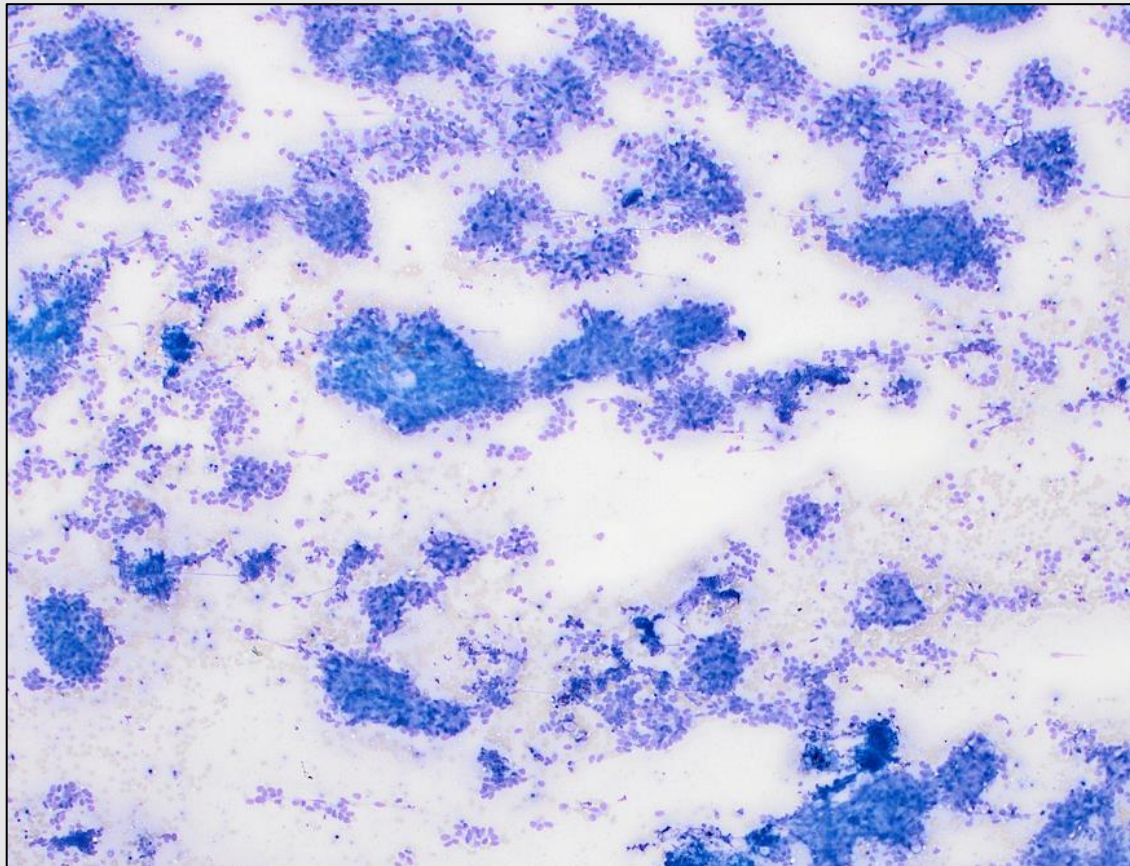


Pass 1

- Very small volume in pass 1.

Most of material on slide with very little remaining for needle washings

Slide has these appearances (x4):



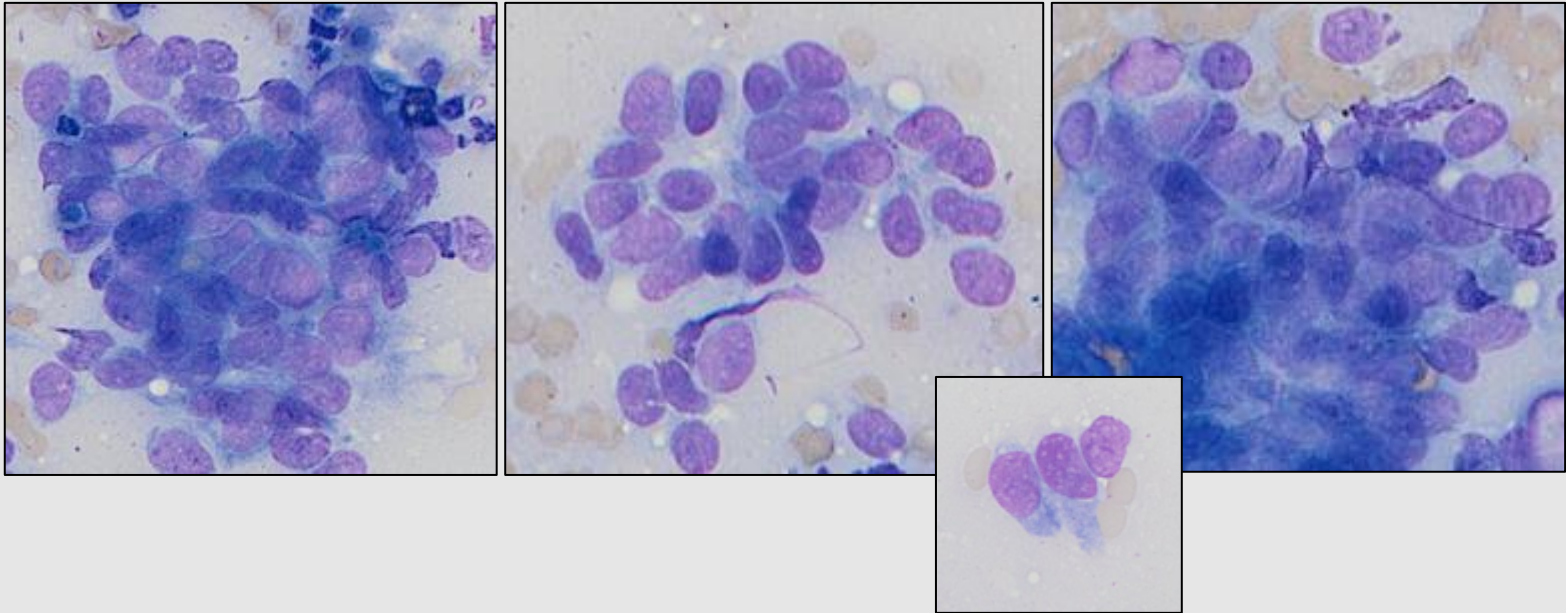
Pass 1 – ROSE thoughts and communication



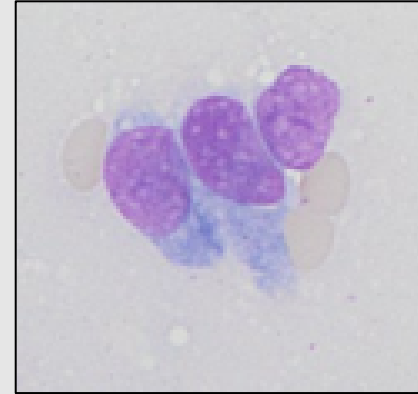
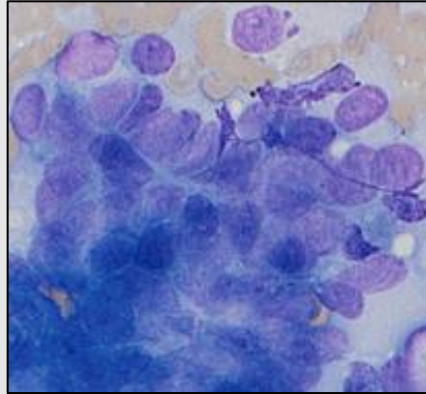
- Slide is cellular and diagnostic (material is not lymphoid or contaminant)
- Insufficient material is present in the washings for any ancillary testing
- Feedback to clinician – Small sample volume, diagnostic but insufficient. Attempt another pass, but if also low volume, consider using suction

Pass 2

- Higher volume sample but only occasional groups present (images at x20):



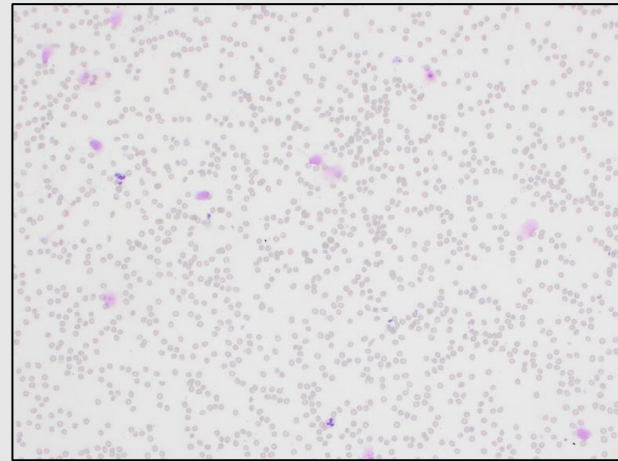
Pass 2 – ROSE thoughts and communication



- Cells are relatively small, and some groups appear to have cells moulding around each other
- However, in other areas, cells are more heaped and very occasional cells could be described as plasmacytoid.
- Although these features are most likely to represent small cell carcinoma, given the history of melanoma and the variety of presentations that melanoma can take, it is safest to ensure sufficient material for a melanoma and small cell IHC panel and subsequent BRAF testing, if required.
- Feedback to clinician – Good sample volume. Diagnostic cells present but still insufficient volume

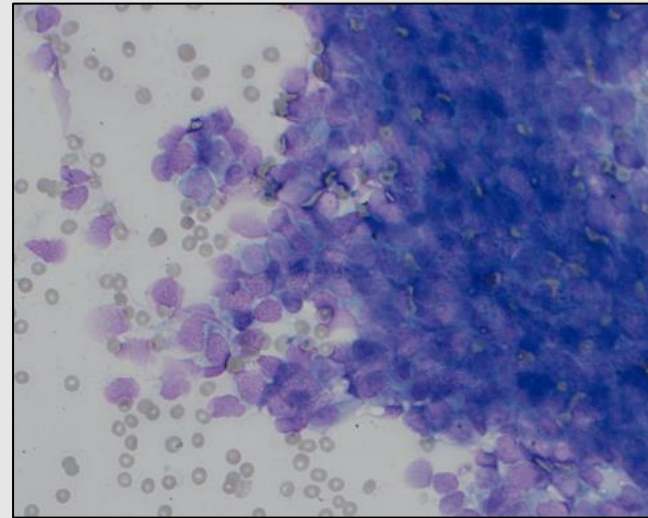
Pass 3

- Showed blood with sparse degenerate lymphocytes.
- A benefit of the single slide assessment process is that washings from these non-diagnostic passes can be placed into a separate pot to avoid diluting the diagnostic samples.
- Feedback to clinician – Non-diagnostic.



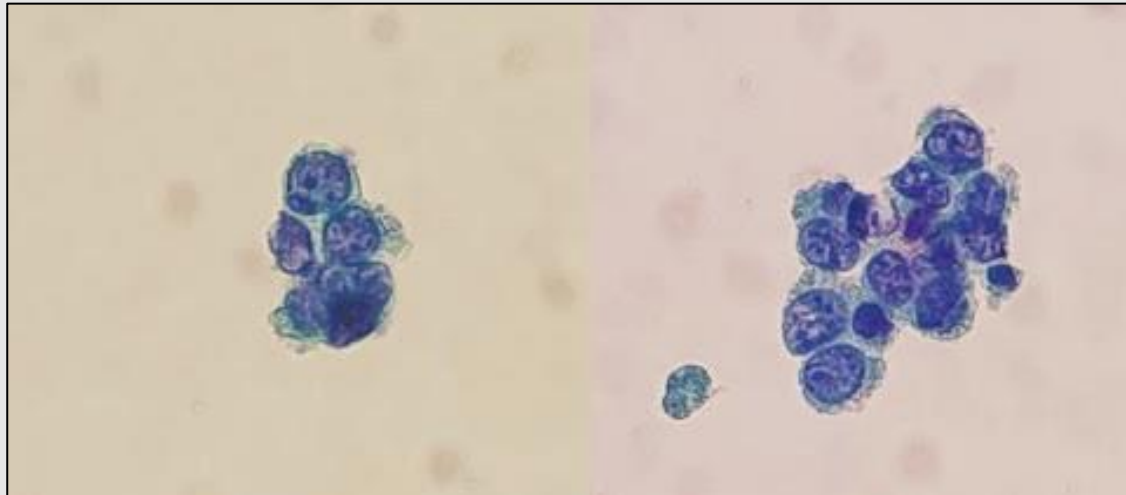
Pass 4

- A small proportion of the sample was used for the assessment leaving plenty in the washings for ancillary testing.
 - The slide contains numerous large groups of diagnostic cells similar to the first 2 passes.
 - Feedback to clinician – Diagnostic material present and sufficient
- The procedure was ceased.



Laboratory processing

- Following the procedure, the ROSE member of staff dictates how the sample should be processed in the laboratory.
- In this instance a pap stain is helpful as nuclear detail can help to visualise nuclear detail of small cell or any melanin pigment which may be present. A clot is essential as immunocytochemistry is required and BRAF testing should the sample represent melanoma.
- Request sent to laboratory for needle washings to be split with 1/3 for LBC preparation and 2/3 for clot
- Pap (x40) shown below.

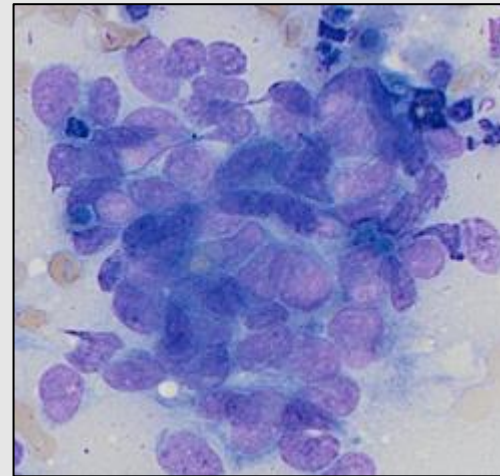
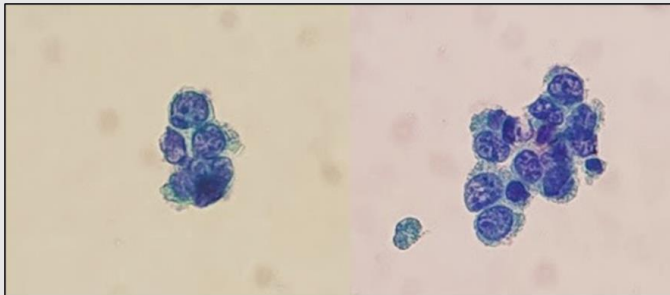


Features of small cell carcinoma

- High nucleocytoplasmic ratio
- Scant cytoplasm
- Granular “salt and pepper” chromatin
- Inconspicuous or absent nucleoli
- Intermediate size (2-3 times the size of a RBC) but can be smaller
- Necrosis and debris
- Moulding
- High proliferation (Ki67 >75%)
- Mitosis
- Streaks, clusters and single cells

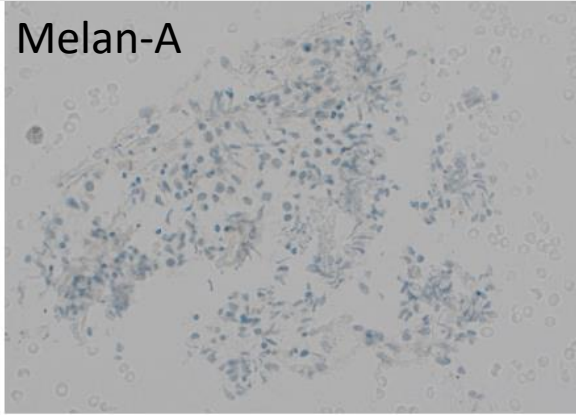
Morphology

The morphological features seen in this case are more likely to represent Small cell carcinoma however confirmatory immunocytochemistry is required.

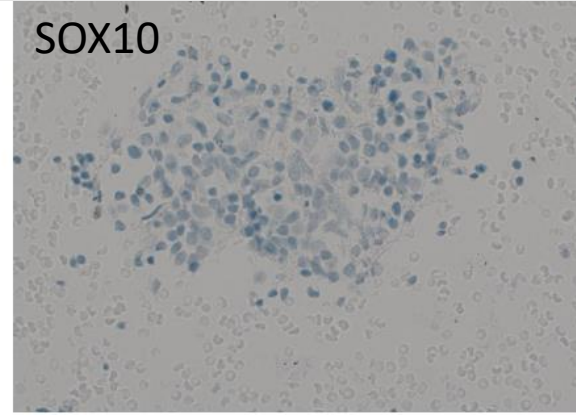


Immunohistochemistry

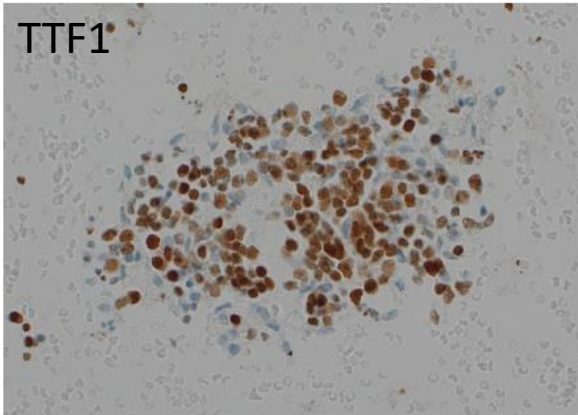
Melan-A



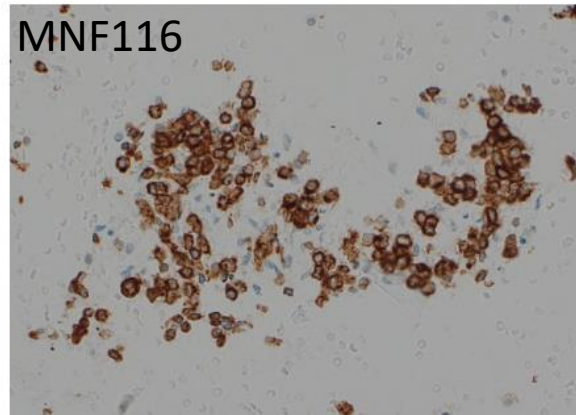
SOX10



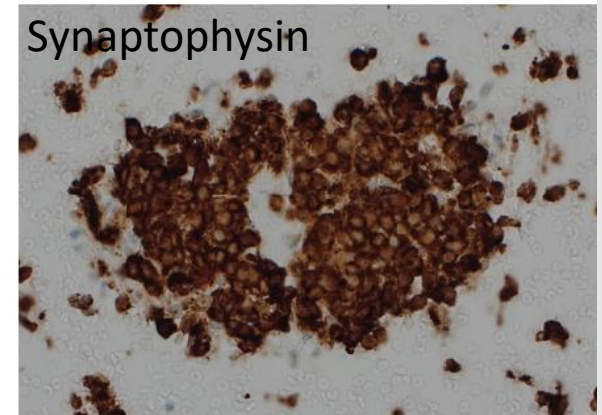
TTF1



MNF116



Synaptophysin



ICC confirmed small cell carcinoma

Benefits of ROSE

- Highly sensitive – particularly useful for smaller lesions (<3cm)
- Immediate feedback – Change of sample site
- Optimum preservation – Quality preparations
- Specimen management / triage
 - Immunocytochemistry
 - Molecular analysis
 - Microbiology
 - Flow cytometry
- Benefits to patients with co-morbidities and benign conditions (reduced complications)
- Best practice recommendations
- Insight into patient experience / obtain relevant clinical information
- Reduction in number of procedures
- Reduced referral to treatment times / waiting lists

