Pleural fluid and molecular analysis

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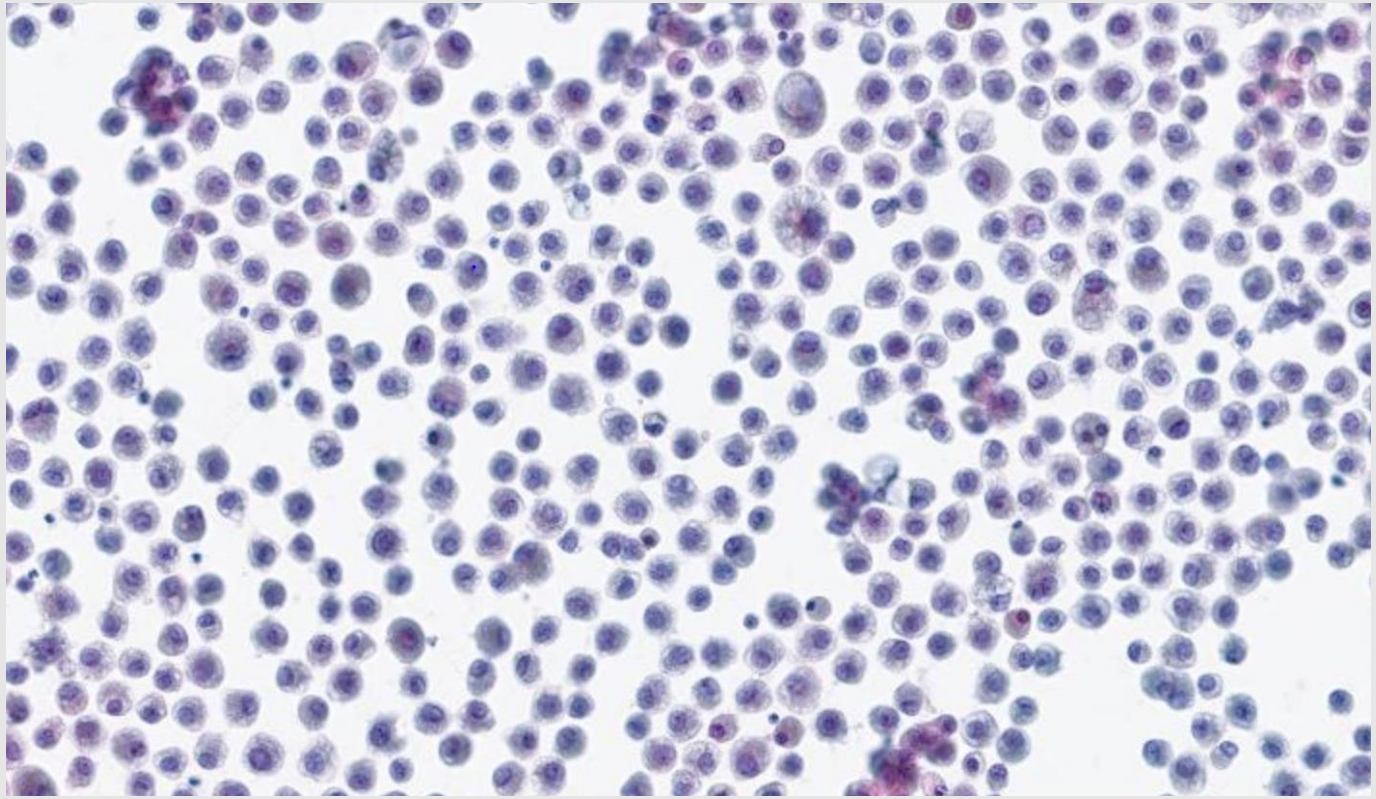
Clinical information

- Male, age 72.
- Smoker.
- No previous relevant medical history.
- Presented with dry cough and shortness of breath.
- Chest xray showed a massive pleural effusion.

Clinical information

- CT scan shows 3cm right upper lobe lung mass.
- Low density liver lesions and spinal lesions suspicious for metastatic deposits.
- Additionally a large intraluminal urinary bladder mass seen.
- Intercostal drain inserted and 75ml bloodstained fluid sent to cytology.

Pleural fluid cytology



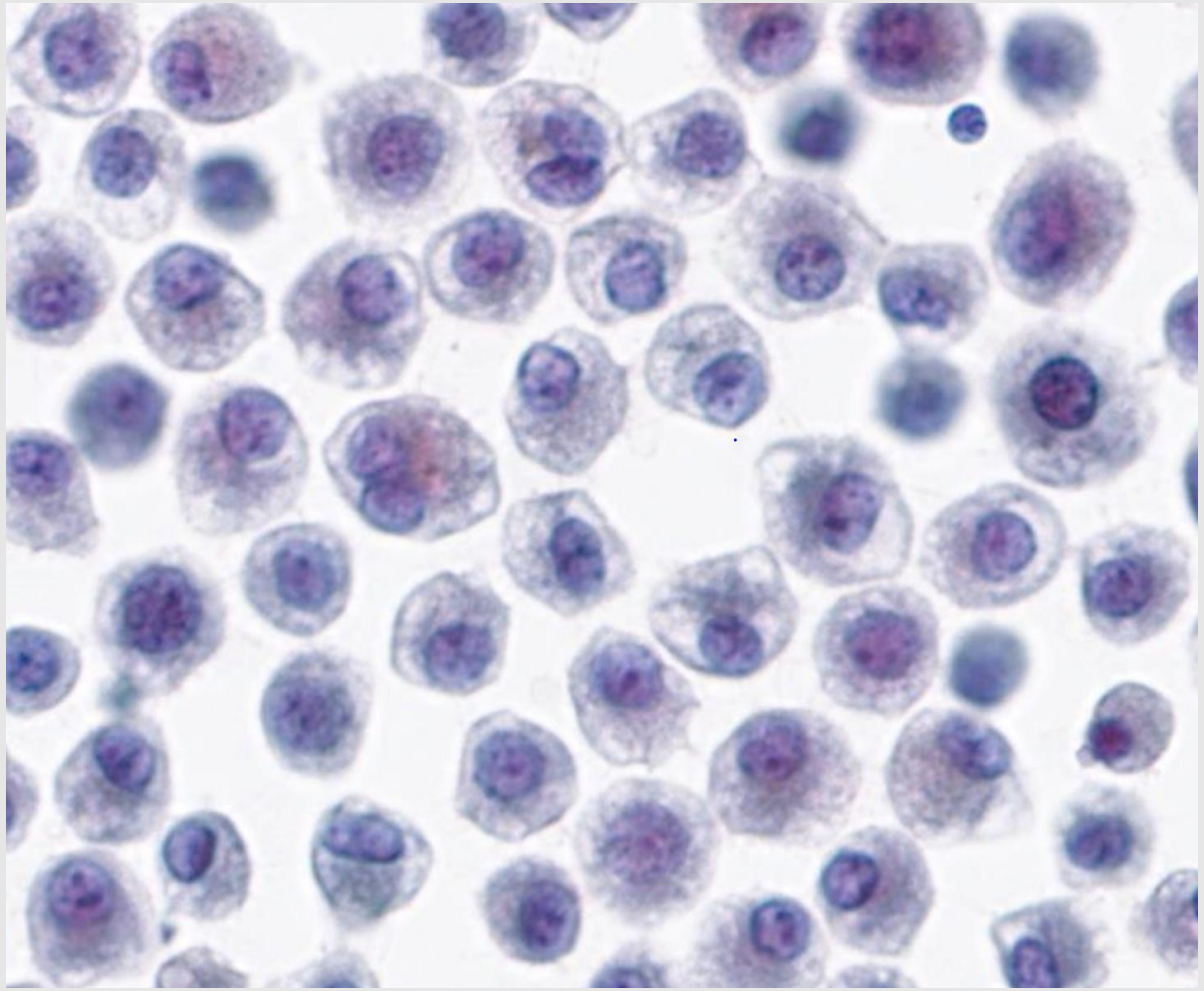
Papanicolaou X10 Magnification

Monomorphic appearance.
Highly cellular specimen.
Numerous singly dispersed malignant cells and a few malignant cell clusters.

Pleural fluid cytology

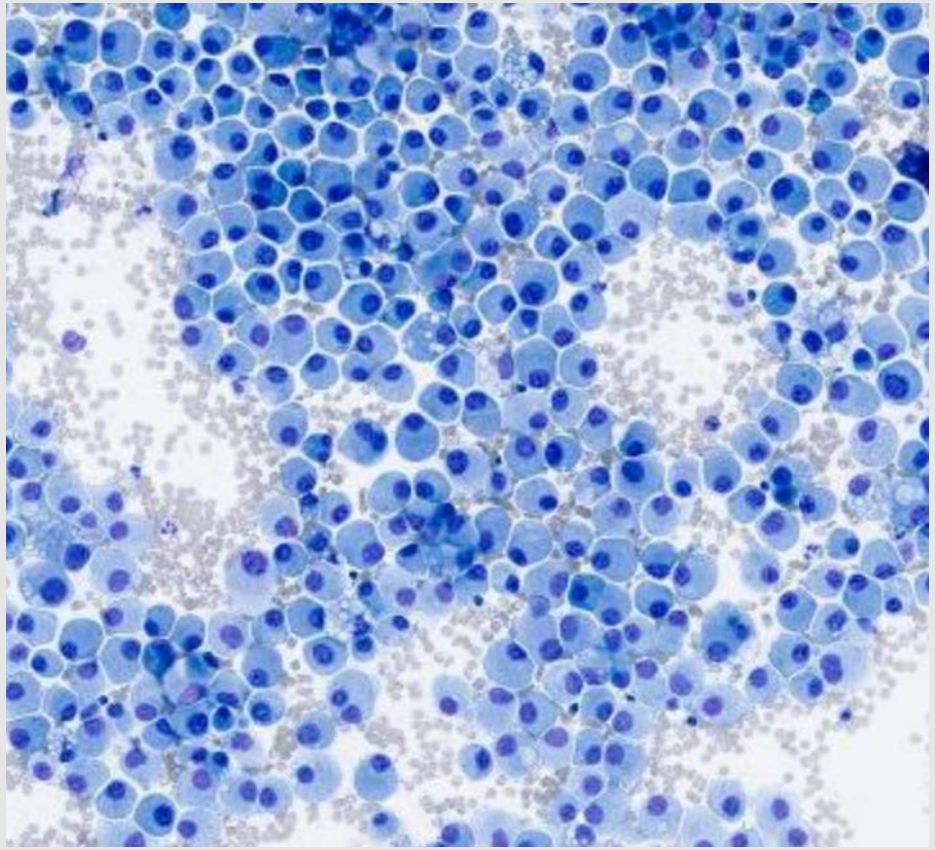
The malignant cells have enlarged eccentric nuclei and delicate cytoplasm. Many cells show small cytoplasmic vacuoles.

Individual cells could be misinterpreted as macrophages, but nuclei are round to oval and minimal background inflammatory cells.



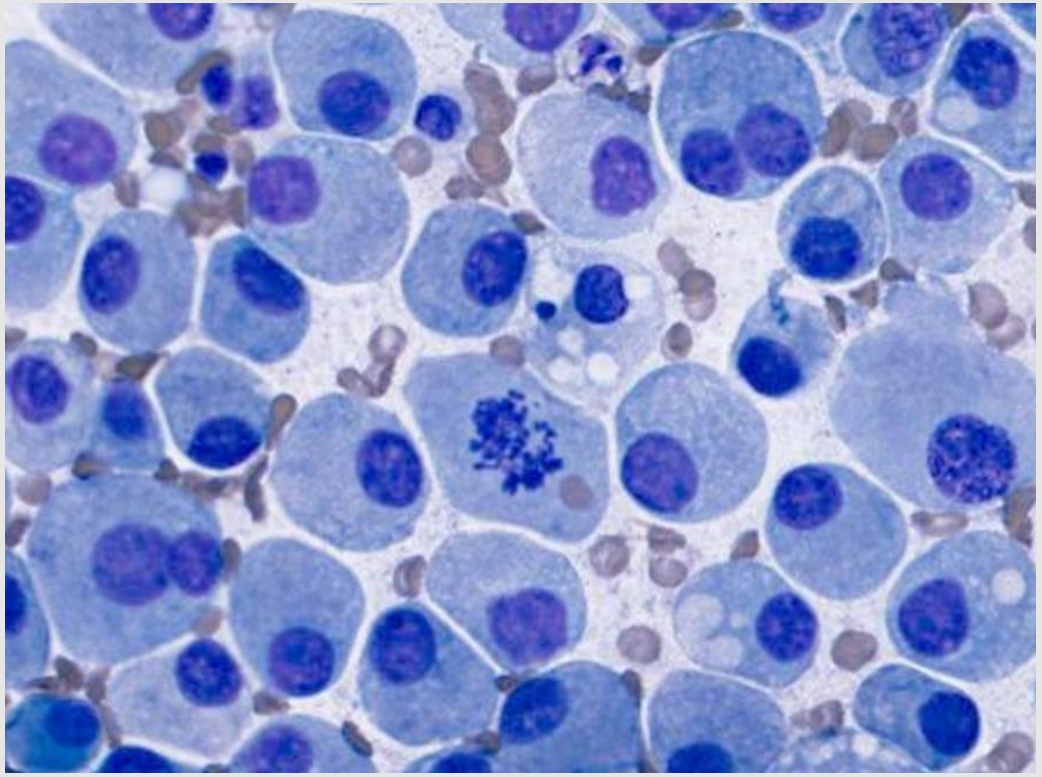
Papanicolaou X40 Magnification

Pleural fluid cytology



MGG X20 Magnification

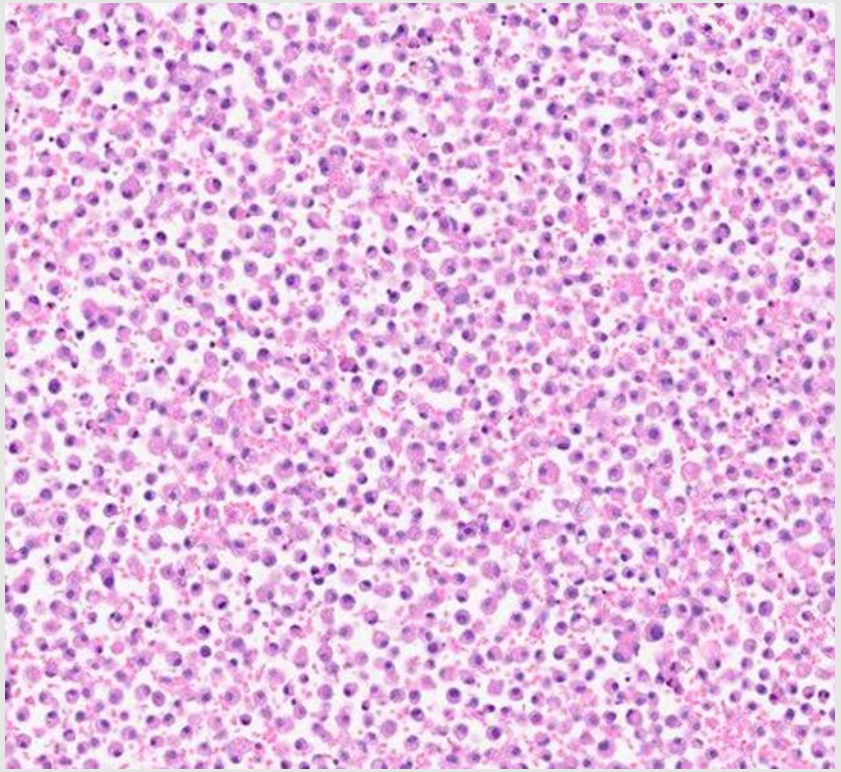
Bland appearing malignant cells. Mitotic figures seen.



MGG X40 Magnification

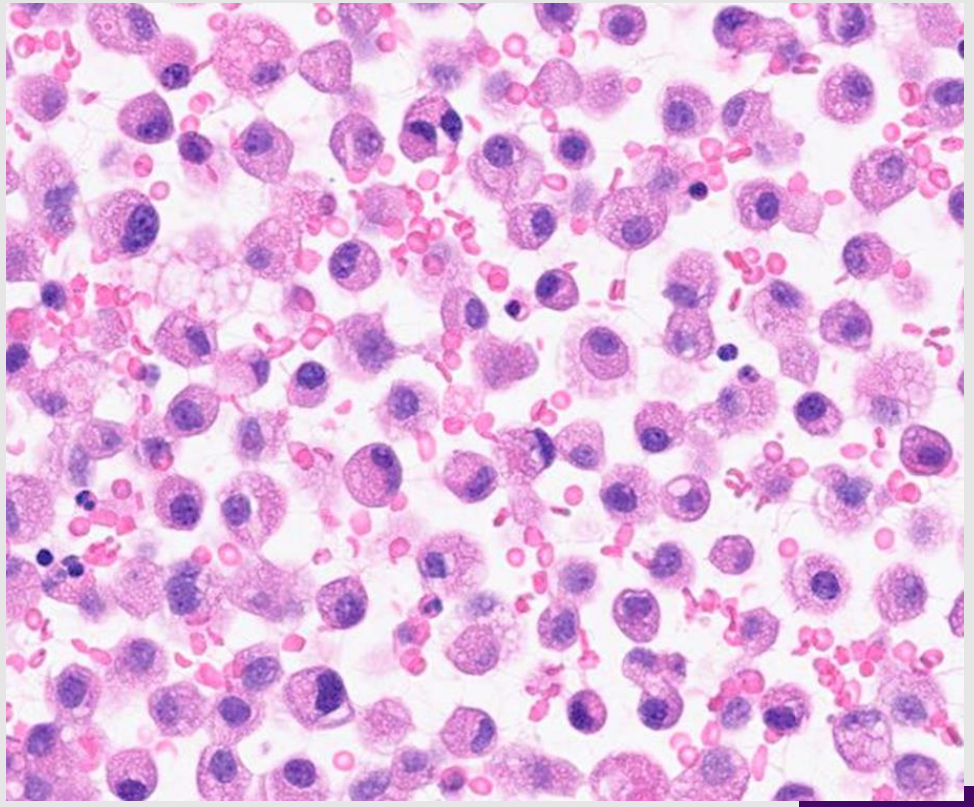
Mesothelial cells are inconspicuous or absent on morphological staining

Pleural fluid cell block



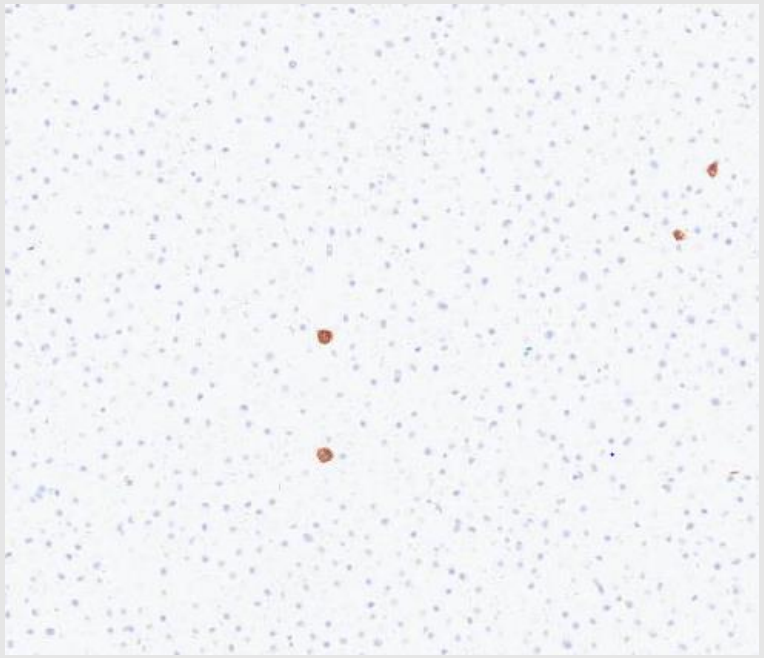
Cellular clot with numerous single cells

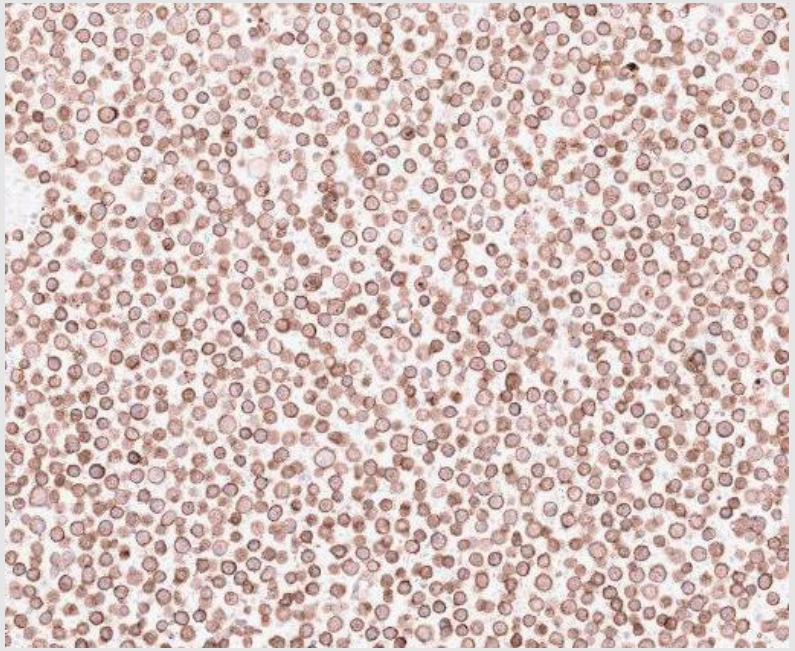
Discohesive cells with eccentric nuclei. Cytoplasm appears delicate and finely vacuolated.



Immunocytochemistry and Report

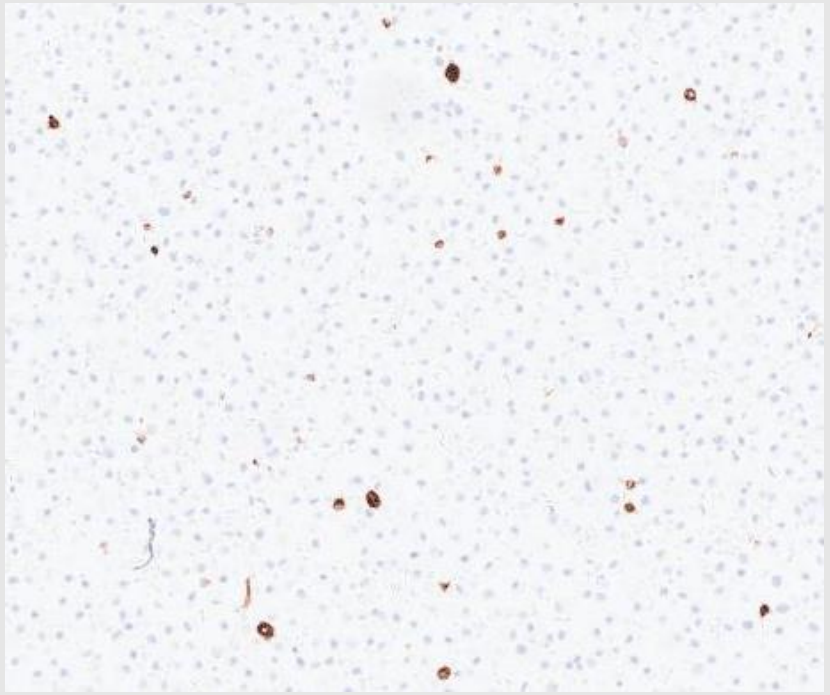
Rare calretinin positive mesothelial cells





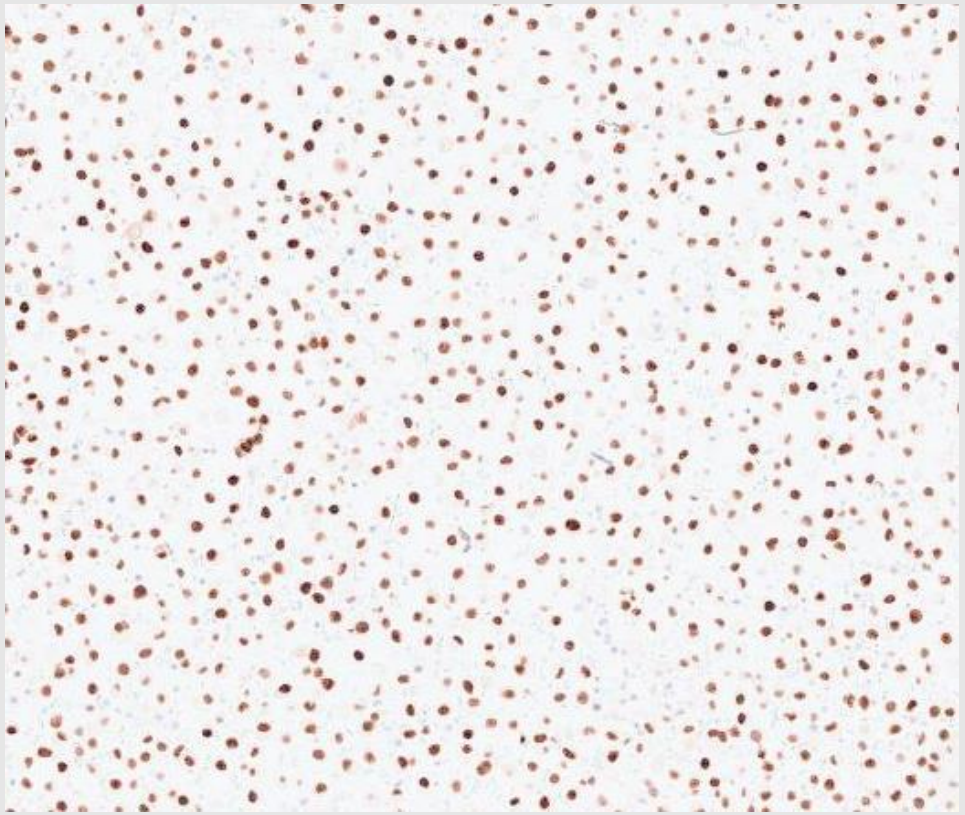
Large number of Claudin4 positive cells

Occasional CD68 positive macrophages

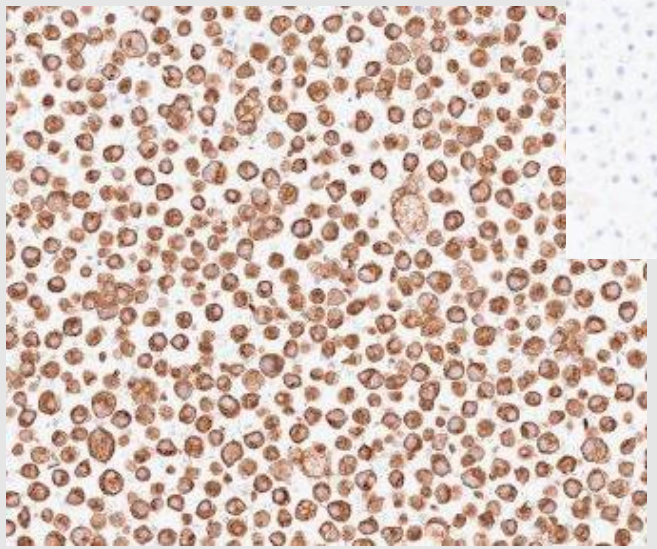


Immunocytochemistry and Report

Cells of interest show strong positive nuclear staining with TTF1.



Cytokeratin profile is CK7 +/CK20 -



Cytology report

- The cytological features are of malignant cells from a metastatic adenocarcinoma. A lung adenocarcinoma is the probable primary site. Mutation analysis performed, results as below:

- **EGFR Result: Mutation Present: Mutation: L858R** Protein: p.Leu858Arg Nucleotide Change: c.2573T greater than G,c.2573_2574delinsGT,c.2573_2574delinsG A
- KRAS, BRAF, NRAS Results: Mutations absent
- ALK, ROS1, RET Results: Rearrangements absent
- Met Exon 14 Skipping Result: Variant not identified
- PD-L1: Negative Less than 1% of tumour cells show membranous expression
- NTRK: Negative for NTRK translocation

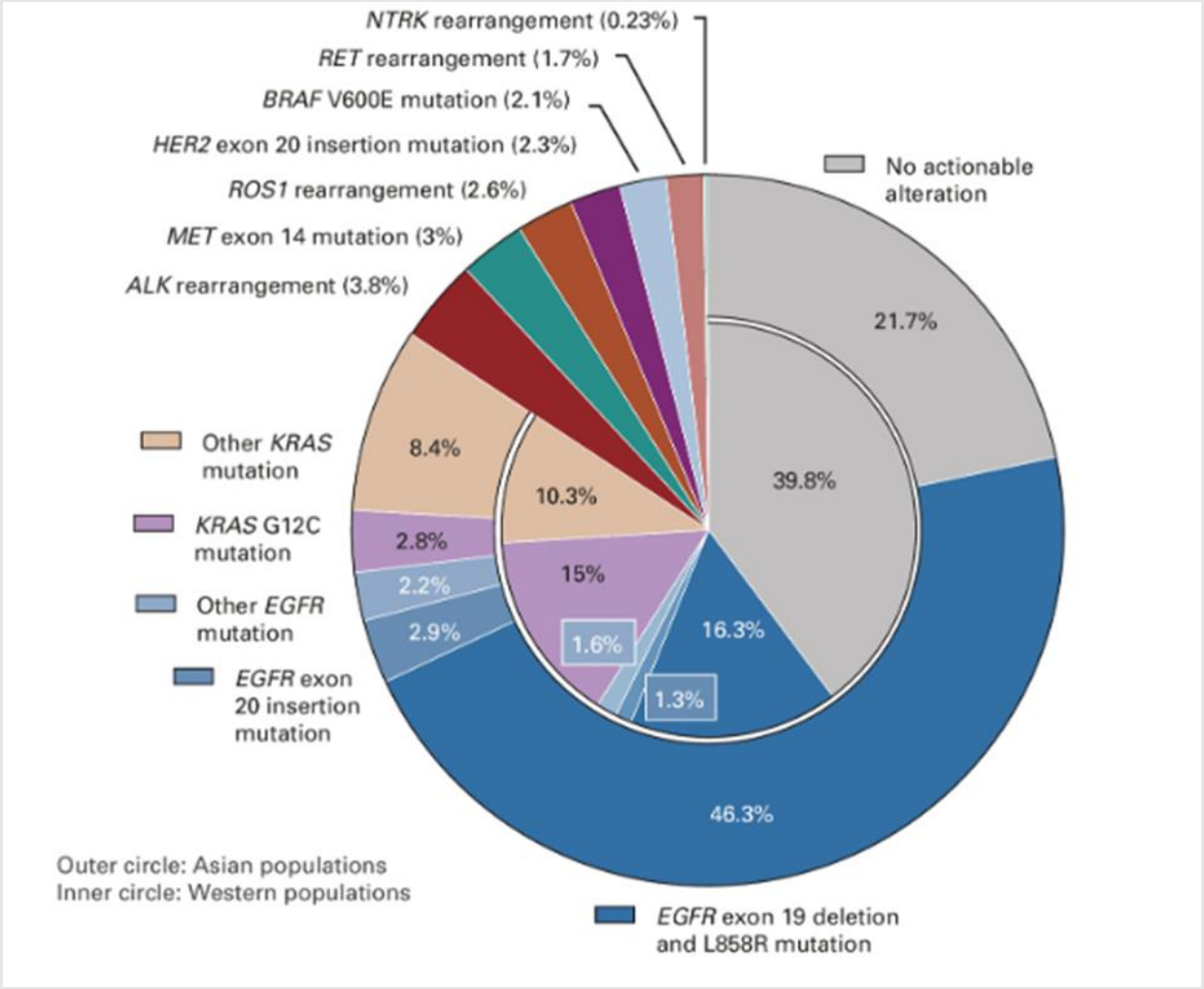
Outcome

- Patient underwent pleurodesis to control the re-accumulation of fluid in the pleural cavity. Pleurodesis can involve introducing a chemical irritant (e.g. talc) into the pleural space to induce inflammation and fibrosis and promoting adhesions between the pleural membranes.
- An USG liver biopsy confirmed metastatic adenocarcinoma from a lung primary.
- At MDT - no abdominal or pelvic lymphadenopathy – bladder mass likely coincidental primary and no treatment planned.
- Lung malignancy staged as T2 N2 M1c (liver & pleura).
- Patient referred to oncology – EGFR mutation indicates treatment options include tyrosine kinase inhibitors (TKIs).

Discussion

Mutation analysis in Non Small Cell Lung Carcinoma (NSCLC)

- Identifies activation mutations that confer sensitivity to targeted therapies or resistance mutations predicting lack of response to some therapies
- Common mutations and alterations in lung adenocarcinoma include EGFR and KRAS
- Some clinicopathologic features such as smoking and ethnicity are associated with increased likelihood of specific mutations



Frequency of oncogenic drivers in NSCLC - Image taken from Tan AC and Tan DSW Journal of Clinical Oncology 2022

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Discussion

Predictive markers in NSCLC

EGFR

- Increased prevalence in women, non smokers and young patients. More common in Asian populations
- Most common mutations are frame deletions in exon 19 and a point mutation (substitution) in exon 21 (L858R) which encompass 85-90% mutations. These mutations are sensitive to TKIs such as 1st generation TKIs erlotinib, gefitinib and 2nd generation afatinib
- Next most common are exon 20 insertions
- Mutations within genes 18 and 20 are less sensitive or resistant to TKIs
- Some patients develop drug resistance to 1st generation TKIs. Mechanisms include mutations in the TK domain (T790M), MET amplifications and RAS mutations.
- 3rd generation EGFR TKI can act against both sensitive and resistant TKI mutations such as EGFR T790M.

Discussion

KRAS

- KRAS mutations are more common in Western populations.
- Tend to be mutually exclusive with other targetable mutations such as EGFR.
- Common mutations include G12C, G12V and G12D and cause constitutional activation of the RAS oncoprotein
- Treatment for G21C mutations includes inhibitors of the RAS GTPase family

ALK

- Increased prevalence in relatively young, non smokers or light smokers.
- ALK alterations include rearrangements, amplifications and point mutations and lead to the constitutional expression and activation of the ALK protein
- EML4-ALK is the predominant ALK fusion
- ALK inhibitors (1st line therapy) includes crizotinib. Resistance can occur via new point mutations (e.g. L1196M) or by activation of KRAS or EGFR signalling pathways.
- 2nd generation drugs include alectinib and ceritinib

RET

- Rearrangements in this proto-oncogene occur mainly in adenocarcinoma, and in the European population occur in both smokers and non smokers.

Discussion

Ros1

- Rearrangement of the ROS1 gene involving many fusion partners.
- Found in young patients and non smokers
- Similar treatment options to ALK mutations

BRAF

- Mutations are more common in women. The most common mutation is the BRAF V600E which is thought to be mutually exclusive with KRAS and associated with female non smokers.
- Non V600E mutations may be harboured together with KRAS mutations and it is thought these may be associated with males with a smoking history.

MET

- Mutations increase the activity of the MET proto-oncogene and decrease the need for ligand activation causing overexpression
- MET exon 14 skipping mutations impact the downregulation of MET
- Treatment is with use of MET inhibitors

Discussion

PD-L1

- A checkpoint inhibitor protein, PD-L1 binds to PD-1 to suppress the immune response. Activation of this pathway by tumours helps evade the immune response.
- The tumour proportion score (TPS) is a method to analyse the level of expression of PD-L1 in the tumour
- At least 100 viable tumour cells are required. TPS is calculated by dividing the number of positive staining cells by the total number of tumour cells.
- Immunotherapy, such as Pembrolizumab targets the PD-L1/PD-1 interaction

Assessment of mutations present is recommended for all advanced stage NSCLC to best guide treatment decisions.

Malignant pleural effusion is a frequent complication of NSCLC and the importance of handling and processing these samples to facilitate the increased number of tests is paramount.

References

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2. Sutic M et al. Diagnostic, predictive and prognostic biomarkers in non-small cell lung cancer (NSCLCS) management J.Pers. Med. 2021,11;1102
3. Barbar J, et al. Emerging genetic biomarkers in lung adenocarcinoma. Open Med 2022 Oct 18; 10
4. Sholl LM et al. (eds.) IASLC Atlas of molecular testing for targeted therapy in lung cancer
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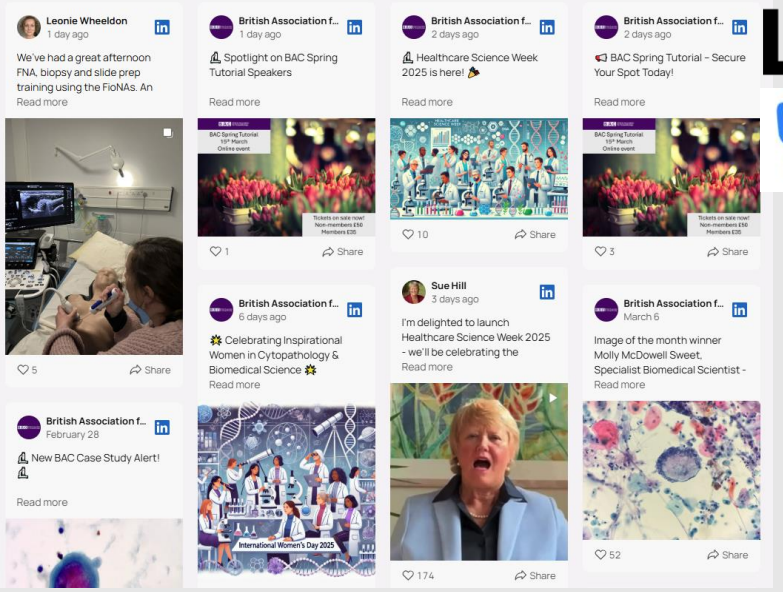

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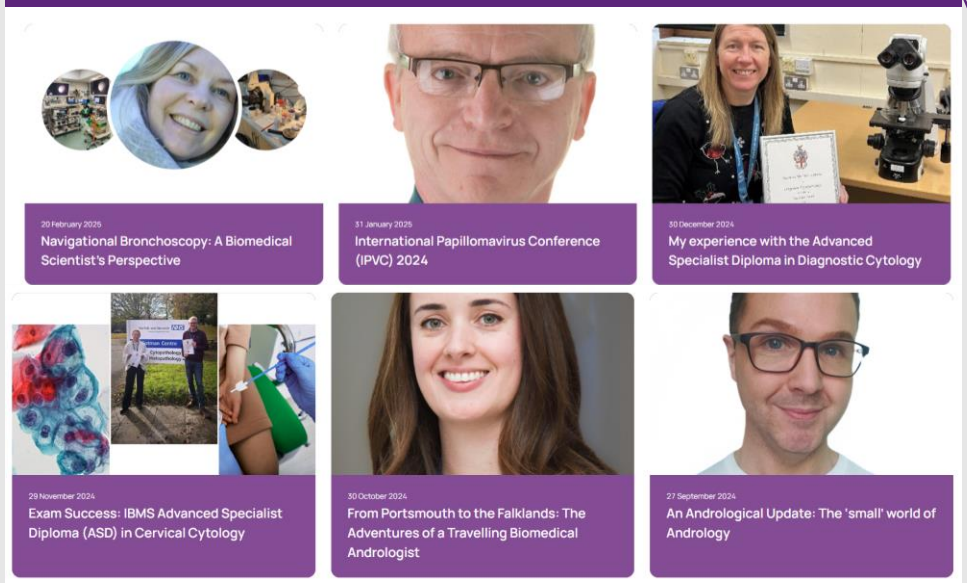

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