Breast Pathology Course 2017

Case 21

55 year old female presented with a locally advanced breast tumour with metastasis to the right lung and soft tissue of the left lumbar region.

The initial biopsy of the breast tumour diagnosed a malignant high-grade tumour (7 cm in the right breast) with a differential diagnosis of either spindle cell metaplastic carcinoma or malignant phyllodes tumour.

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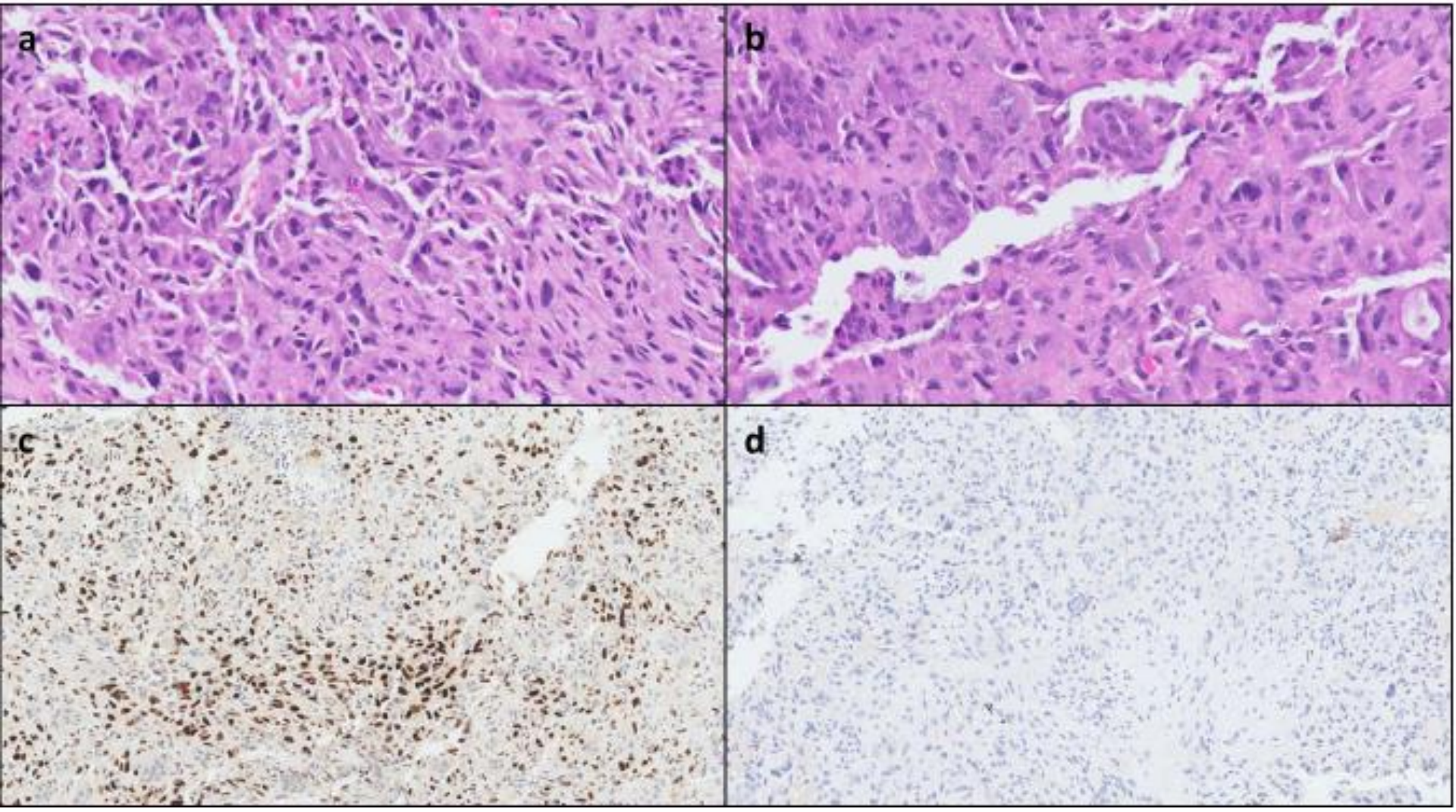
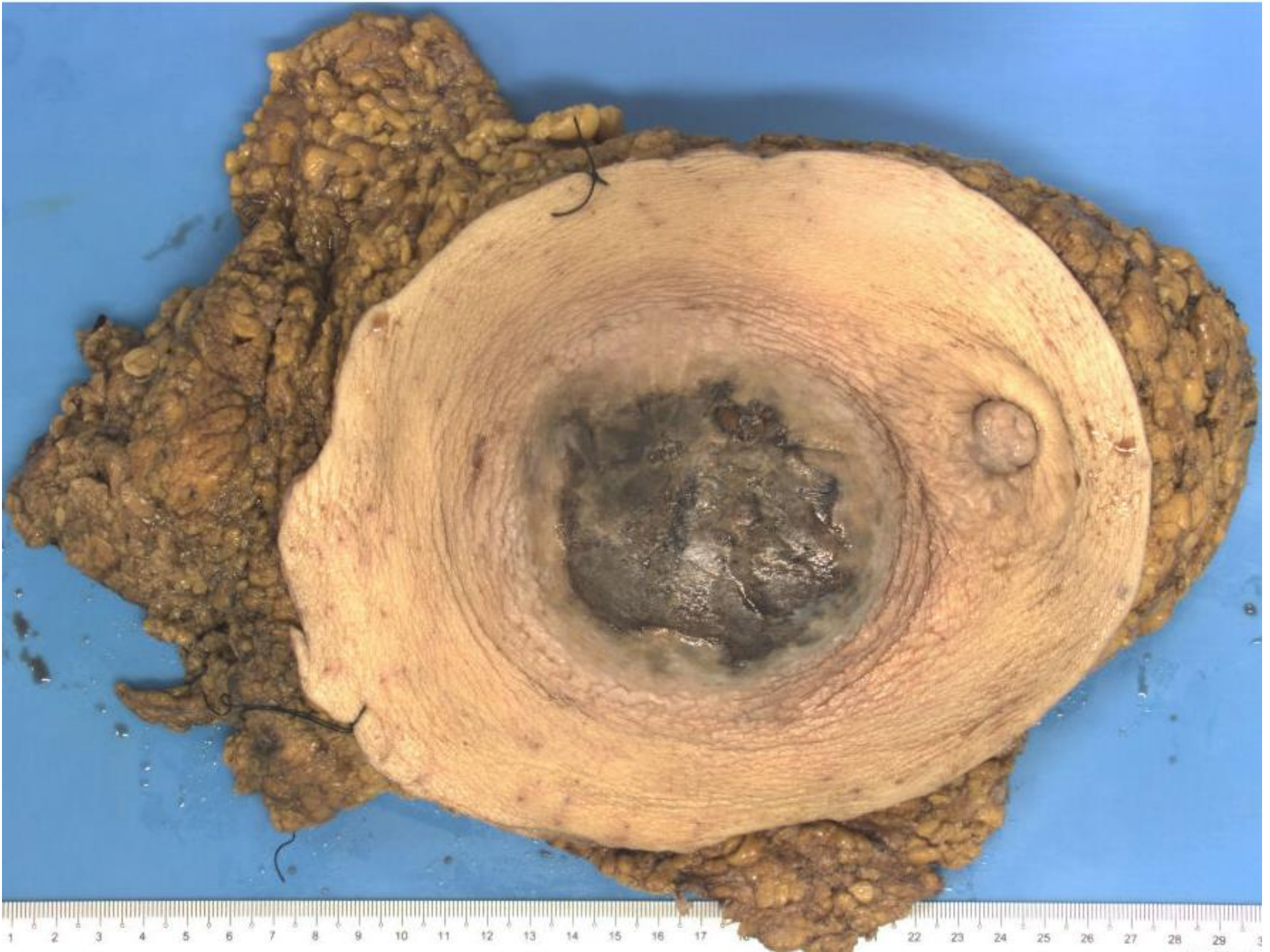
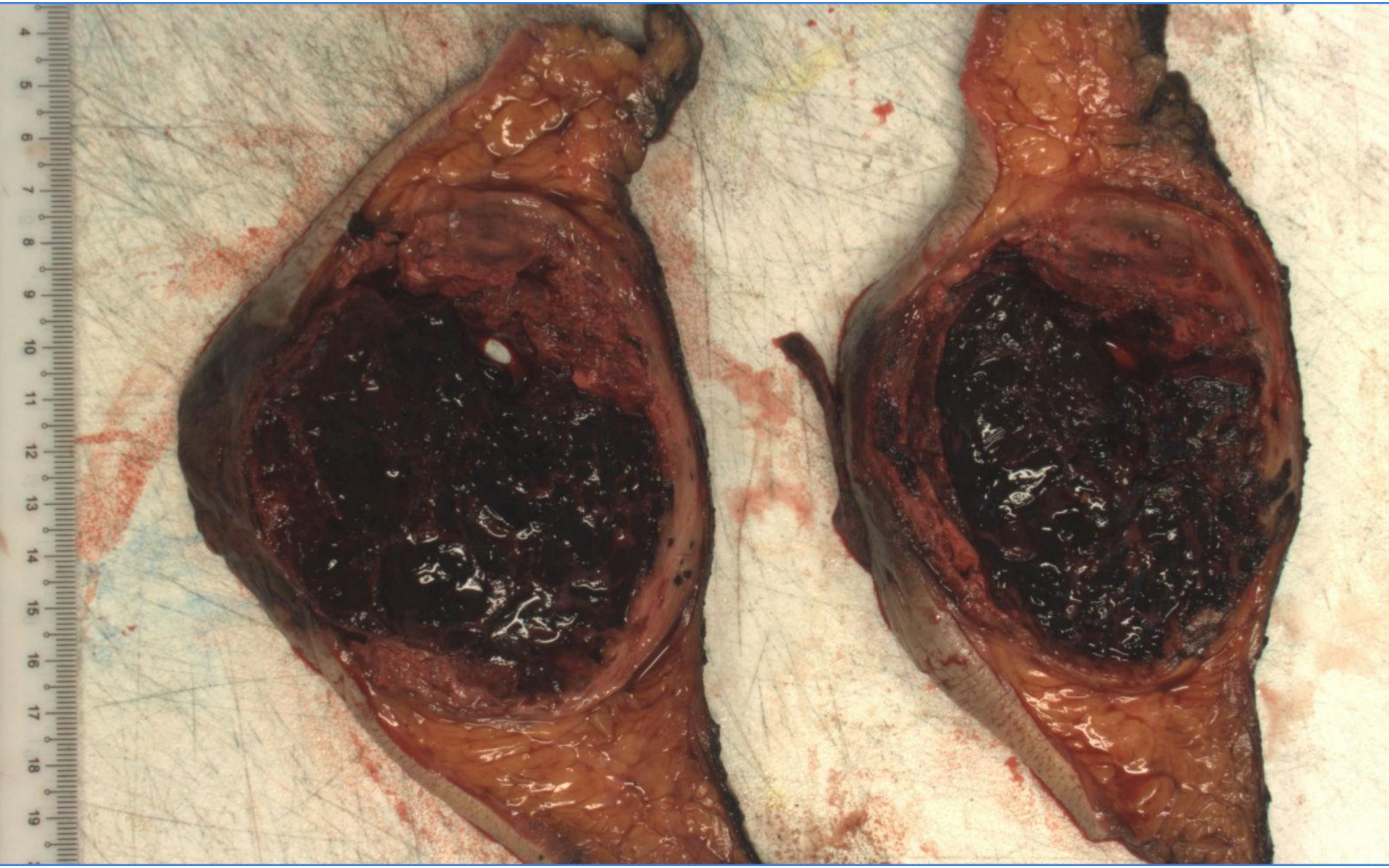
Pre-treatment core biopsy

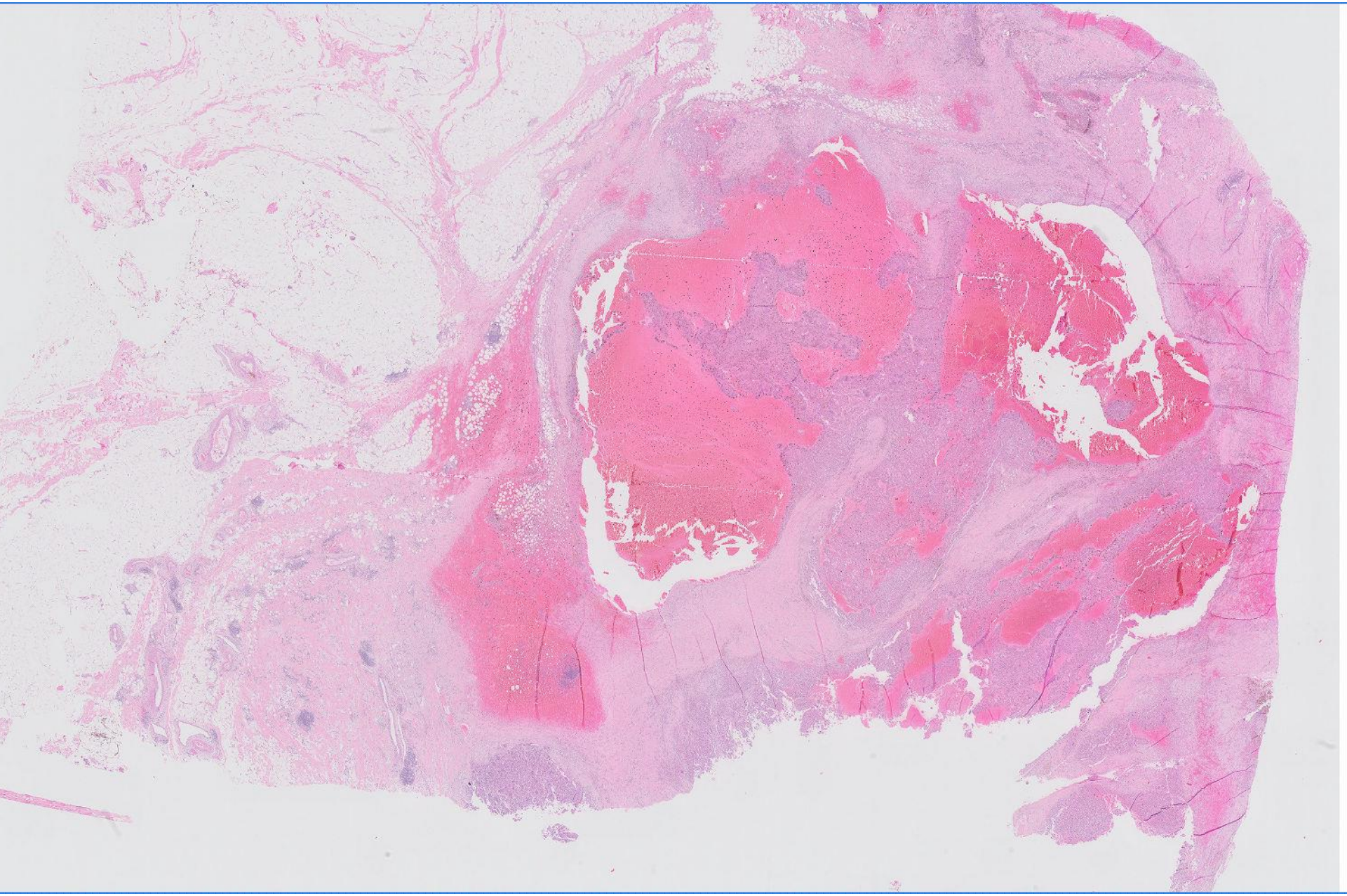


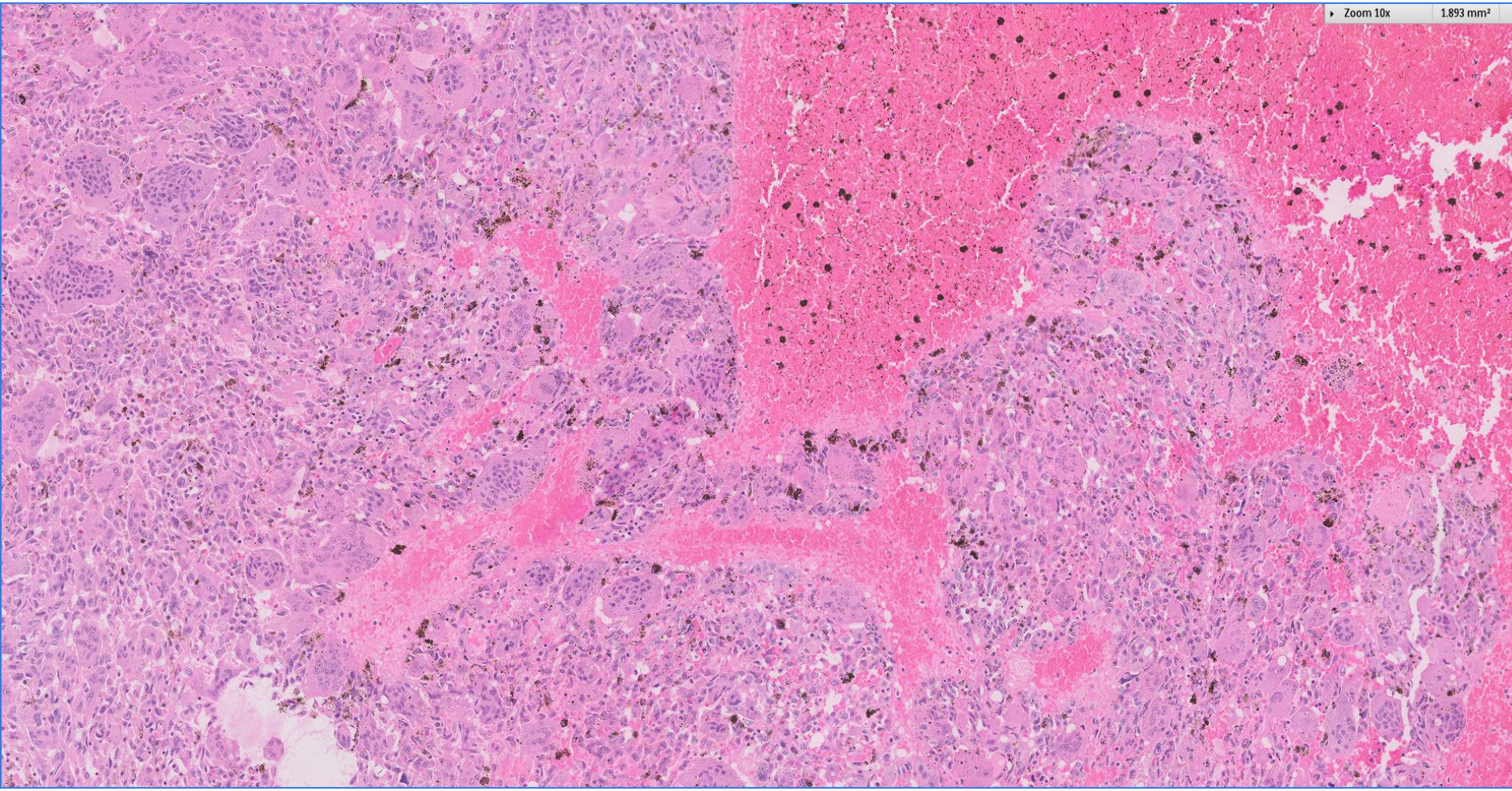
Pre-treatment biopsy (a-d). a and b) Haematoxylin and eosin staining of the tumour biopsy pre-treatment identified sheets of high grade malignant cells with an epithelioid/spindled morphology, marked nuclear pleomorphism and mitotic figures. Immunohistochemical staining of the tumour biopsy indicated c) patchy p63 reactivity and absent expression of d) 34βE12.

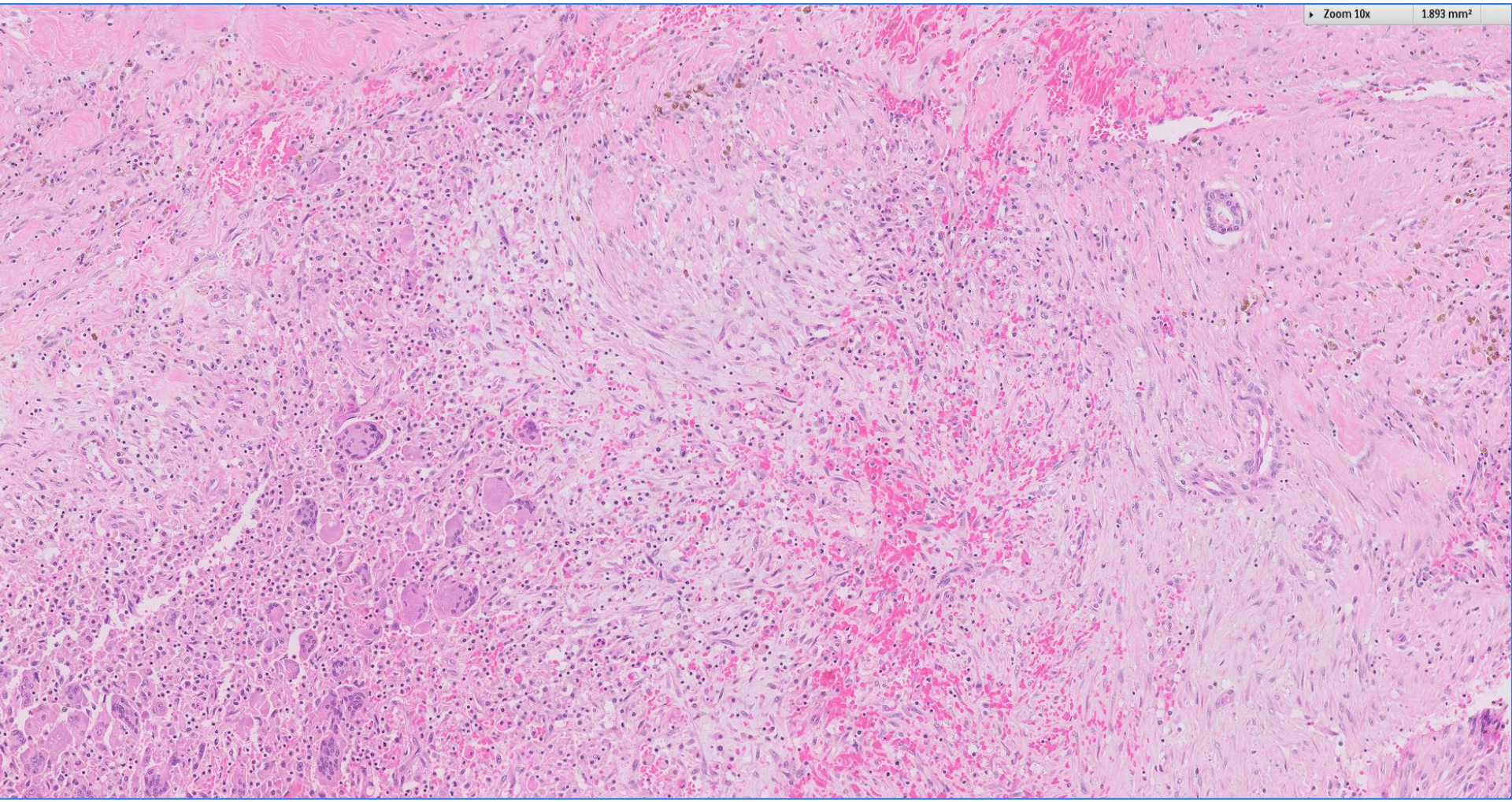
- The patient received nine cycles of paclitaxel-based first line systemic chemotherapy based on a presumptive diagnosis of metaplastic breast carcinoma.
- However, the disease progressed with an increase in the size of the breast tumour, lung nodule and lumbar metastasis, together with bleeding from the breast tumour and pain.
- A repeat biopsy followed by toilet right mastectomy with axillary clearance was performed, and similar microscopic features to the initial biopsy were observed with evidence of some treatment response.

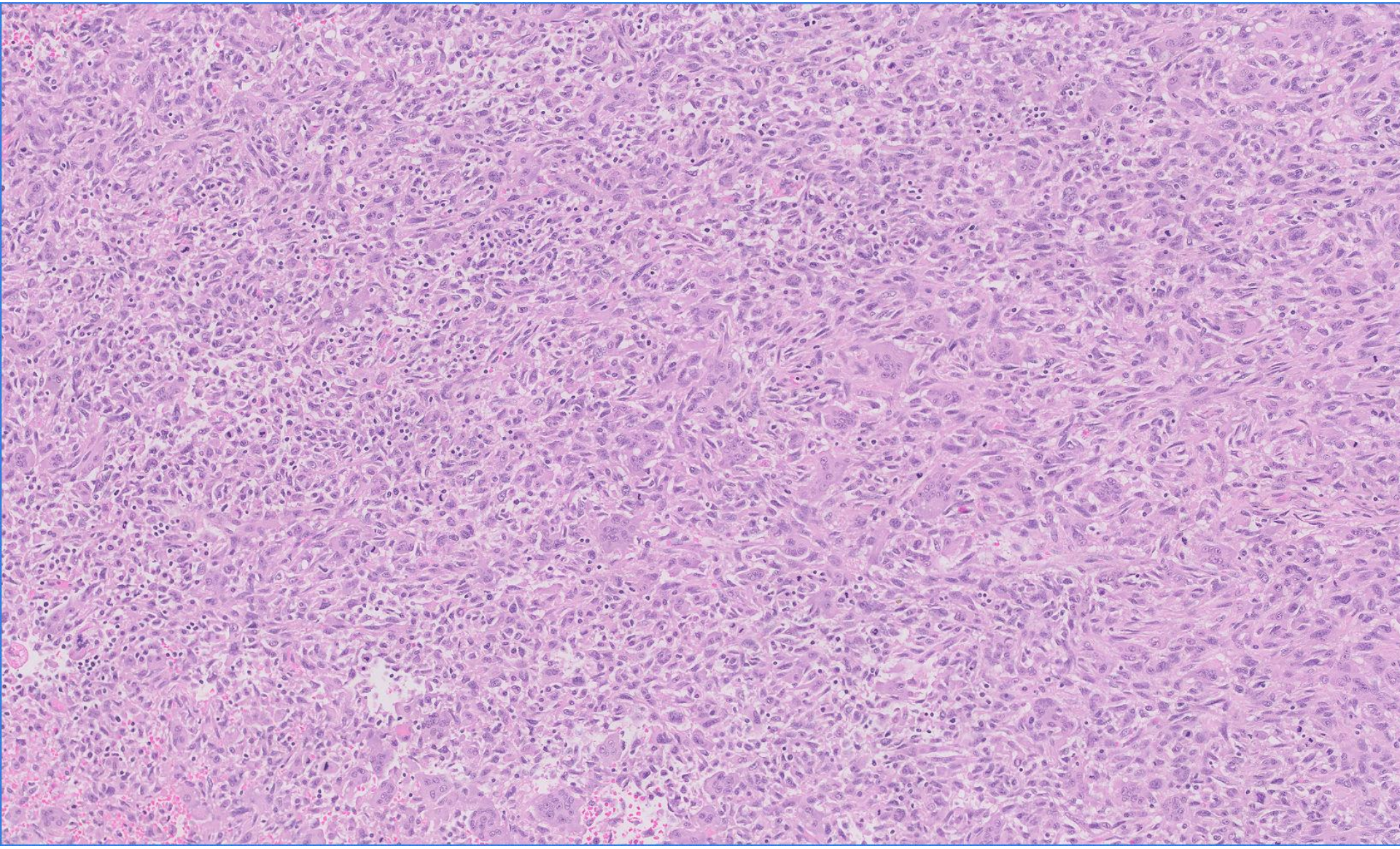


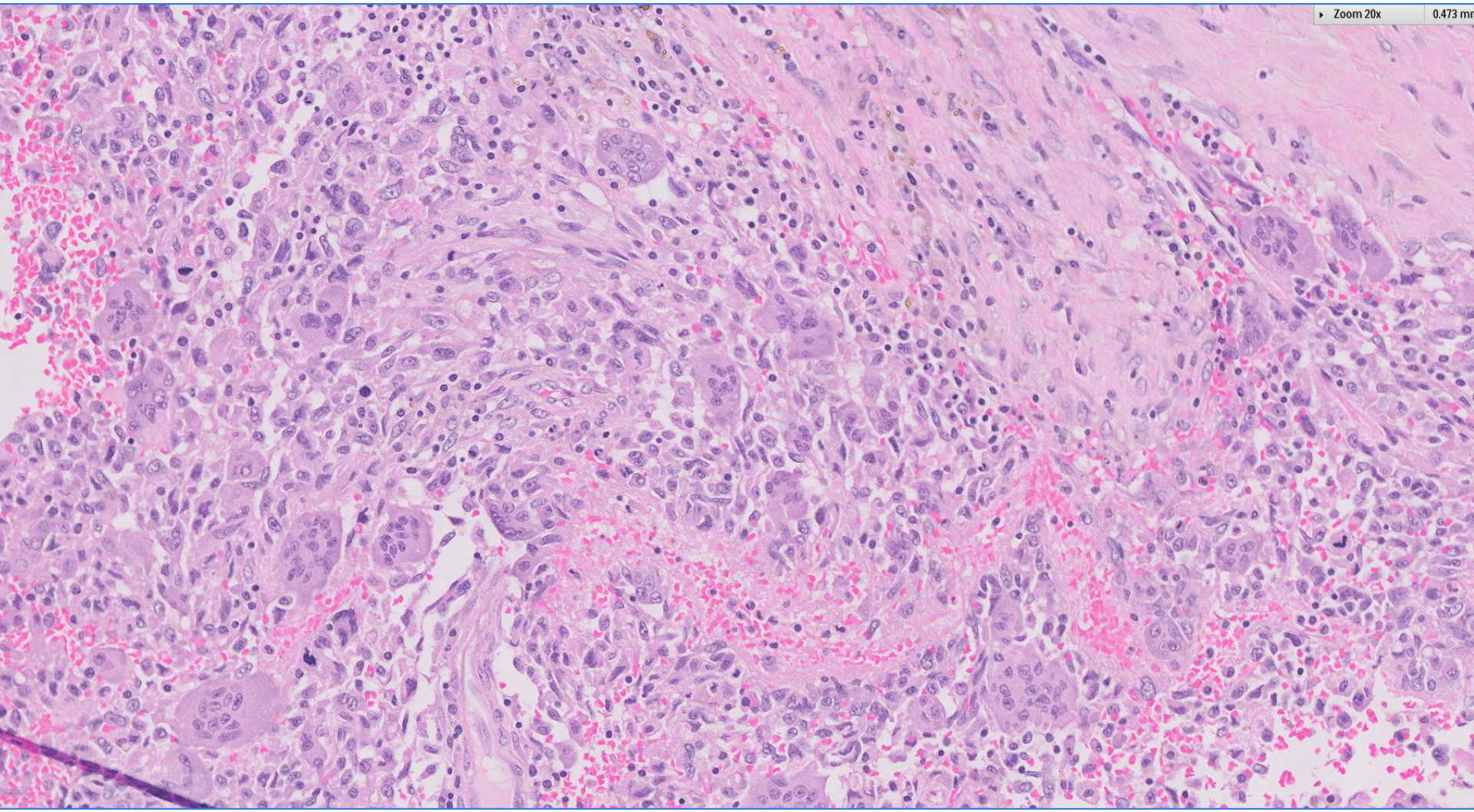




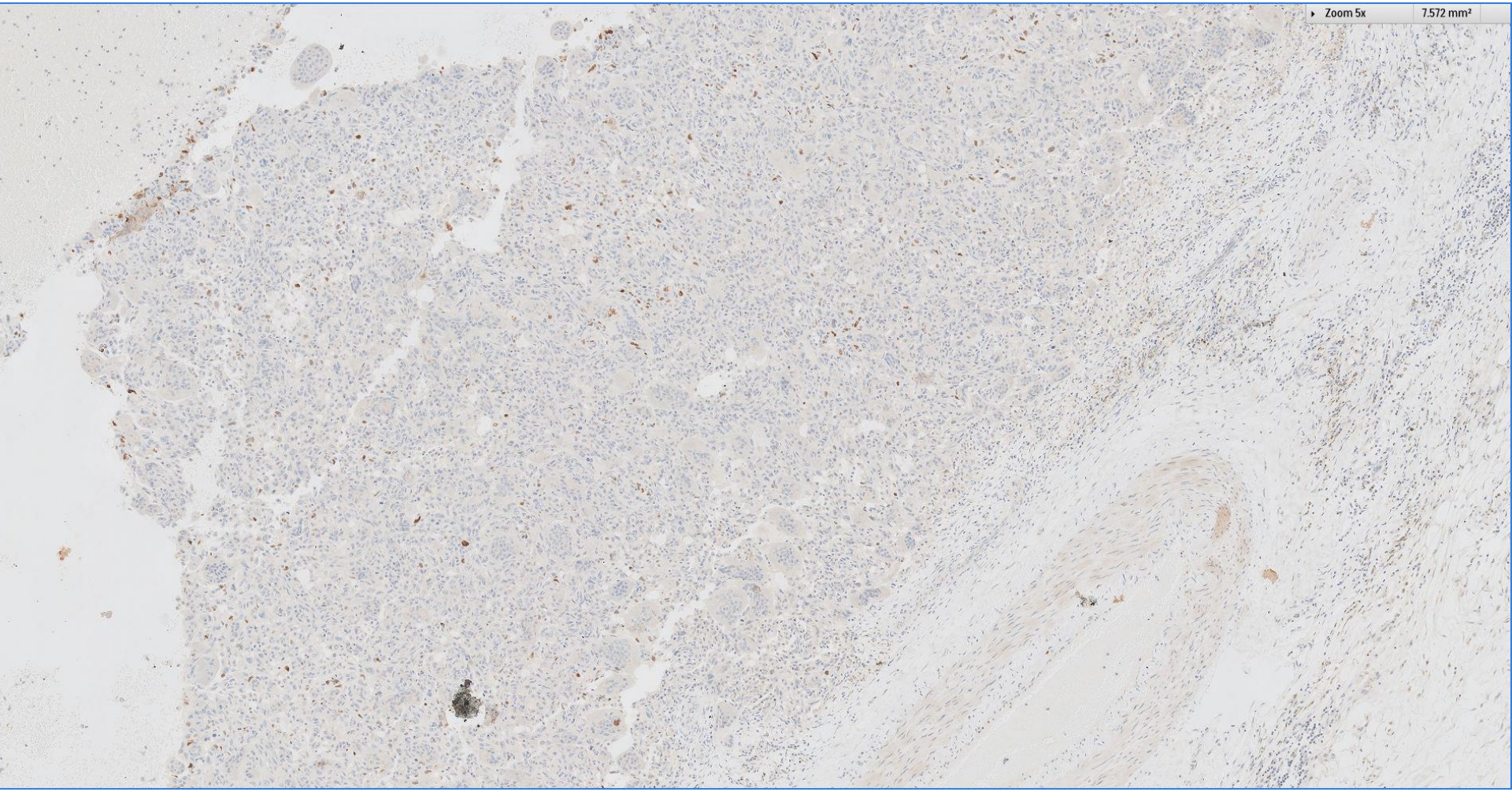

Zoom 10x
1.893 mm²


Zoom 10x
1.893 mm²

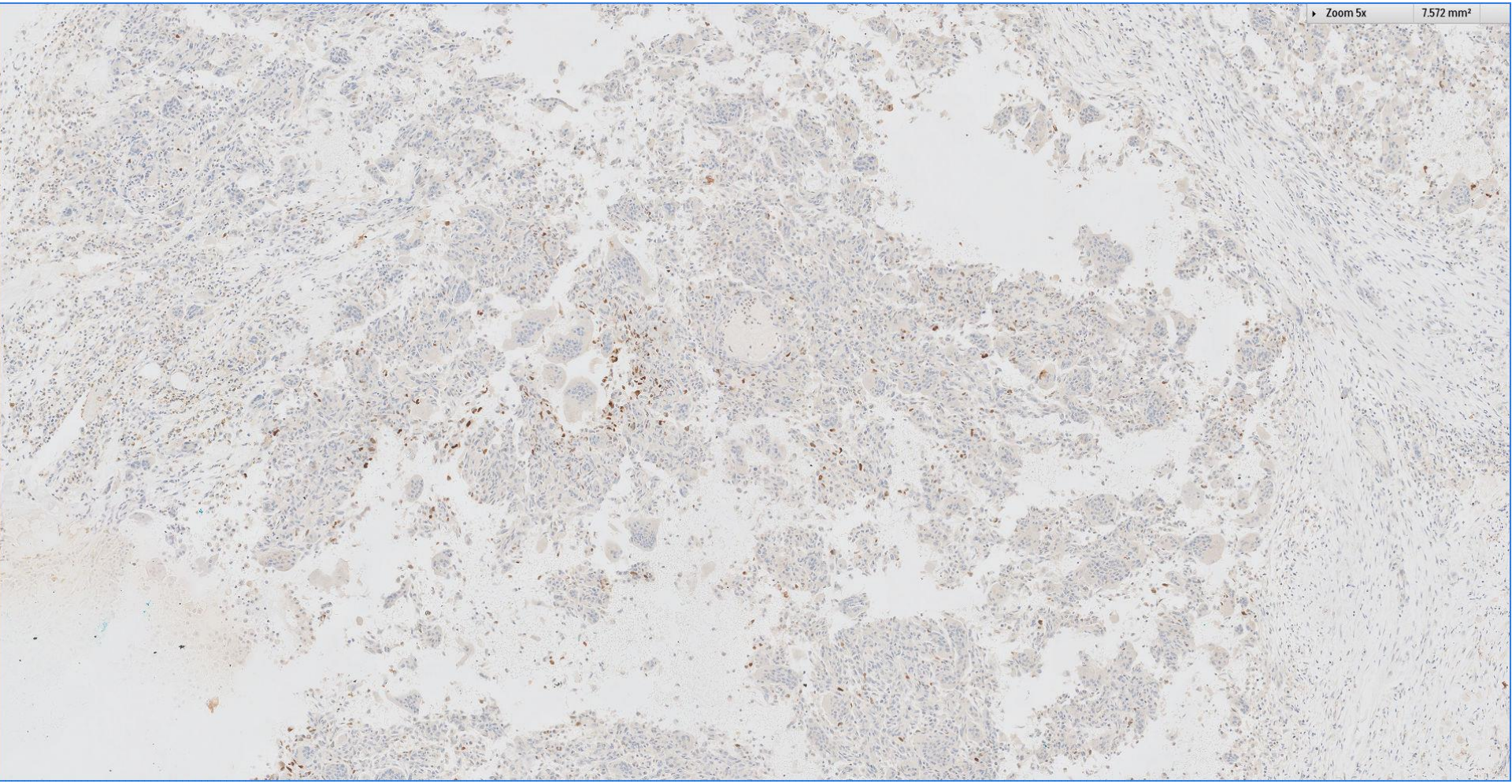



Zoom 20x
0.473 mm

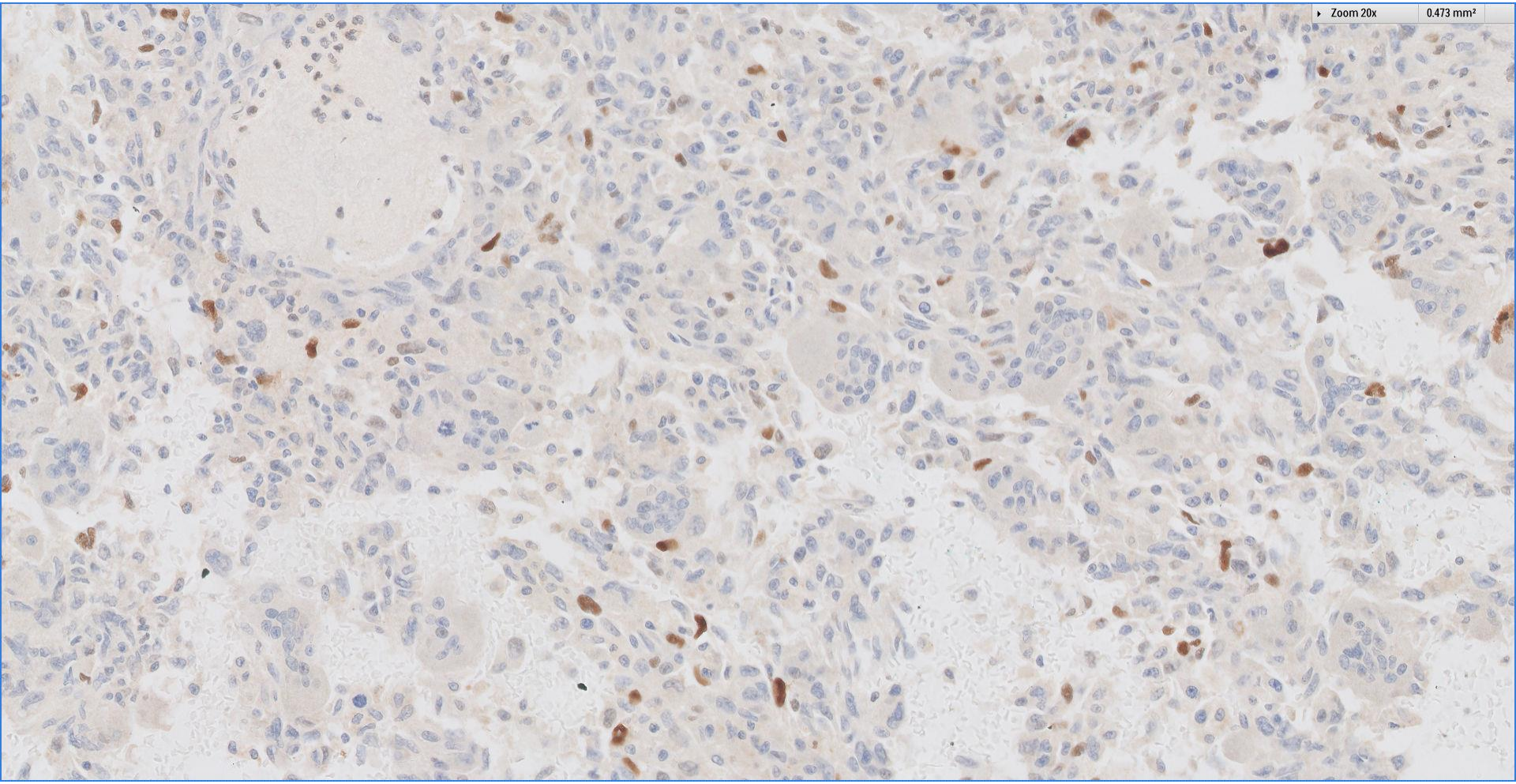
p63



p63

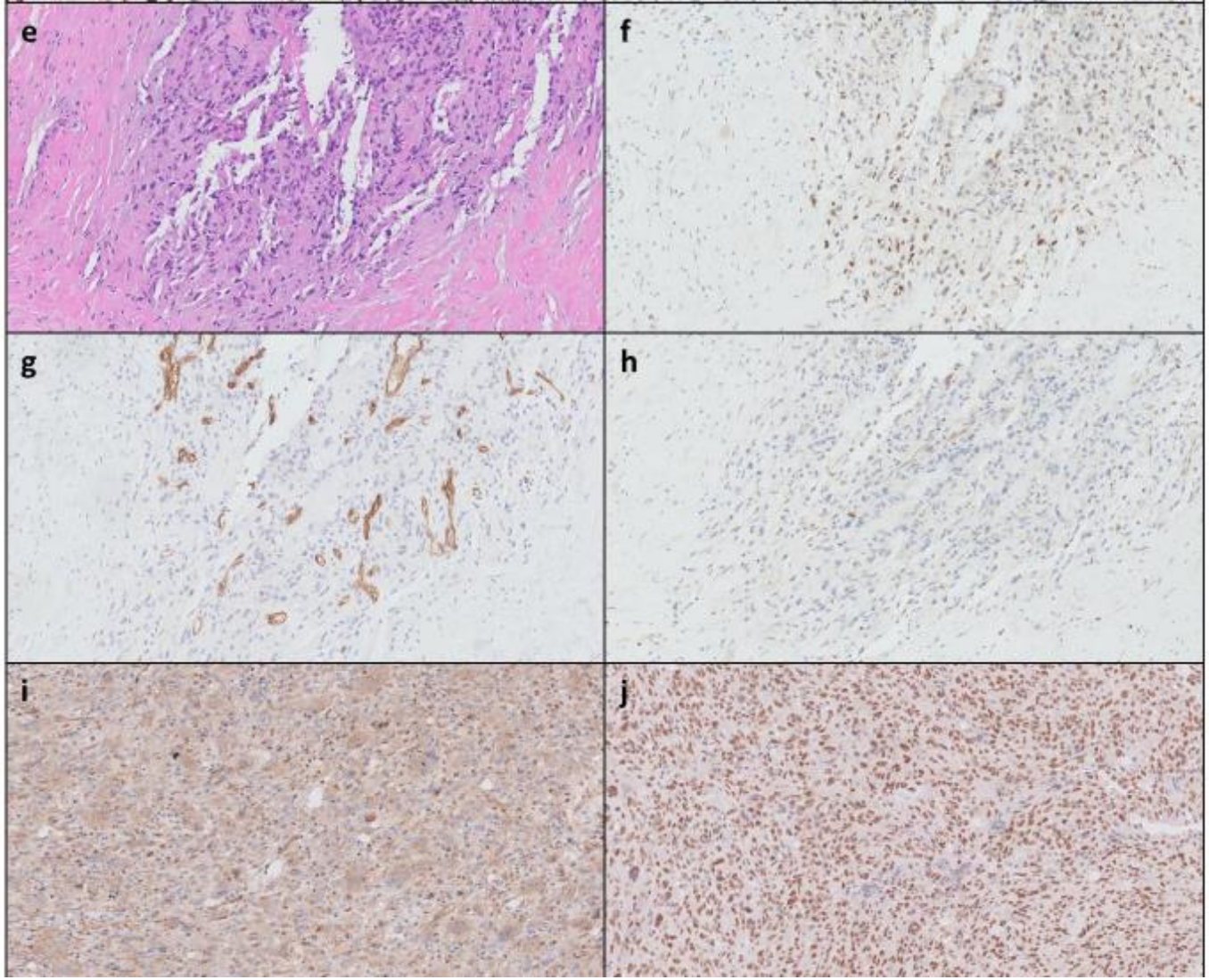


p63



Negative immunostains:

ER, PR, HER2, AE1/AE3, MNF116, CAM5.2, GATA3, CK5/6, CK14, EGFR, 34βE12, S100, HMB45, CD34 or Bcl2.



Post-treatment excision (e-j). e) Haematoxylin and eosin staining of the tumour resection post-treatment showed infarcted tumour with areas of high grade malignant spindle cells. Microscopic foci of viable tumour demonstrated malignant cells with pleomorphic, hyperchromatic and ovoid/spindled nuclei. Immunohistochemical staining showed f) patchy p63 staining and absent expression of g) CD34 and h) bcl-2 in the malignant spindle cells; cytoplasmic reactivity of i) β-catenin and nuclear staining of j) MED12 in tumour cells.

Diagnosis

- High grade malignant tumour, favour metaplastic spindle cell carcinoma
- Malignant phyllodes tumour not completely ruled out

- The patient opted for expectant management instead of further systemic chemotherapy.
- She was recently re-evaluated, remaining oligometastatic with stable metastases to the right lung and soft tissue of the left lumbar region.

- With informed patient consent, tumour was submitted for genomic studies using a 16 gene mutation panel.

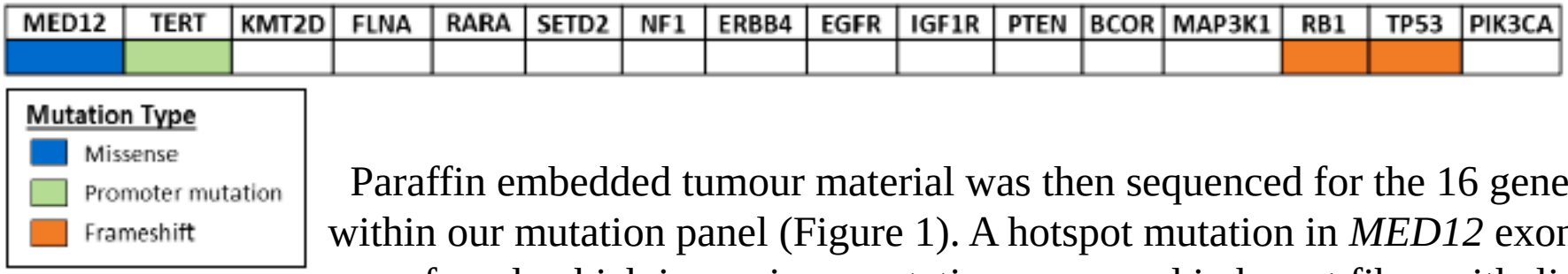
- **Figure 1. Workflow for the targeted sequencing of the 16 gene mutation panel in the fibroepithelial breast assay.** A) Formalin-fixed paraffin embedded samples were used for genomic DNA extraction using the QIAamp FFPE DNA tissue kit (Cat.no: 56404, Qiagen, Germany). The extracted FFPE DNA was subjected to an in-house multiplex PCR QC for quantity and quality. At least 50ng of QC-passed FFPE DNA was used for target enrichment. A custom panel of 16 genes was synthesized covering all exons*, apart from *TERT* where the amplicons spanned promoter positions chr5:1295228 and chr5:1295250 using the Qiagen Generead custom V2 platform (Cat no. 180941, Qiagen, Germany) and amplicon libraries were prepared according to the manufacturer's recommendation. Each nucleotide position was covered by a minimum of two overlapping amplicons. Sequencing was performed on an illumina Hiseq 4000 sequencer (illumina San Diego, CA). All samples were sequenced to a depth >300x of the target regions The variant calling for point mutations and indel detection were performed using Freebayes v0.9.20-16-g3e35e7210 and validated by visualization on IGV genome browser (Broad institute)(13).
- B) Somatic variants were identified using Freebayes and manually curated with IGV (integrated variant viewer v2.3). The variant frequencies are shown. The schematic diagram depicts the variant distribution for the sample using the 16 gene FEB panel.
- **Abbreviations:** *FFPE, formalin-fixed paraffin embedded; Lib prep, library preparation; QC, quality control.*
- * Assay format: 1 tube of multiplex primers covering exonic regions for 16 genes :
- *BCOR, EGFR, ERBB4, FLNA, IGF1R, KMT2D, MAP3K1, MED12,NF1, PIK3CA, PTEN, RARA, SETD2, TP53, RB1, TERT promoter 228, 250*

A

B Targeted-sequencing using the FEB assay for the patient

Gene	Transcript ID	Nucleotide (genomic)	Nucleotide (cDNA)	Amino Acid (Protein)	Variant Freq (%)	Mutation Type
RB1	NM_000321	g.chr13: 49030479 delA	c.1954delA	p.K652fs	11.32	Frameshift
TP53	NM_001126115	g.chr17: 7578240-7578241delCA	c.212_213delCA	p.V71fs	15.96	Frameshift
MED12	NM_005120	g.chrX: 70339254 G>T	c.G131T	p.G44V	15.10	Missense
TERT		g.chr5: 1295228 G>A			13.98	Promoter mutation

Schematic showing the mutations of the patient in the 16 genes panel



Paraffin embedded tumour material was then sequenced for the 16 genes within our mutation panel (Figure 1). A hotspot mutation in *MED12* exon 2 was found, which is a unique mutation expressed in breast fibroepithelial tumours. This finding supported the diagnosis of PT rather than SCMBC. Additional mutations in cancer-associated genes, including *RB1*, *TERT* and *TP53*, were found, corroborating the diagnosis of malignant PT. She is currently being worked up for potential curative metastatectomy of both metastatic lesions.

- To date, only 12 cases of SCMBC have been sequenced and reported, of which none harboured any mutations corresponding to those in our panel except TP53 and PIK3CA (Table 1).
- TP53 and PIK3CA are common mutations found in breast cancers and thus do not offer diagnostic value in the differential diagnosis of PT versus MBC.
- By whole-exome sequencing, Ng et al. identified multiple somatic genetic alterations in MBC, including spindle, squamous and chondroid subtypes, but mutations in MED12, RARA and TERT were not found.
- Reports of these 16 gene mutations in malignant PT are summarized in Table 1.

Table 1: Comparison of sequencing results of spindle cell metaplastic carcinomas with malignant phyllodes tumours of the breast, using the 16 gene panel.

	Ng *et al.* Clin Cancer Res 2017(8)	Ross *et al.* Arch Pathol Lab Med 2014(7)	Liu *et al.* Modern Path 2016(10)	Piscouglio et al. J Pathol 2016(11)	Cani *et al.* MCR 2015 (12)	Our data
Genes/tumour type	Spindle cell metaplastic carcinoma of the breast	Spindle cell metaplastic carcinoma of the breast	Malignant phyllodes tumour of the breast	Malignant phyllodes tumour of the breast	Malignant phyllodes tumour of the breast	Malignant phyllodes tumour of the breast
MED12	0/10 0%	0/2 0%	3/10 30%	4/13 31%	2/5 40%	8/19 42%
TERT	0/10 0%	0/2 0%	6/10 60%	8/13 62%	3/5 60%	11/19 58%
RARA	0/10 0%	0/2 0%	1/10 10%	1/13 8%	N.A	2/19 11%
PIK3CA	6/10 60%	0/2 0%	3/10 30%	1/13 8%	N.A	1/19 5%
PTEN	0/10 0%	0/2 0%	1/10 10%	1/13 8%	N.A	2/19 11%
KMT2D	0/10 0%	0/2 0%	2/10 20%	1/13 8%	N.A	5/19 26%
RB1	0/10 0%	0/2 0%	2/10 20%	5/13 38%	1/5 20%	2/19 11%
IGF1R	0/10 0%	0/2 0%	N.A	N.A	2/5 40%	0/19 0%
TP53	5/10 50%	0/2 0%	4/10 40%	6/13 46%	3/5 60%	3/19 16%
NF1	0/10 0%	0/2 0%	N.A	3/13 23%	1/5 20%	2/19 11%
ERBB4	0/10 0%	0/2 0%	N.A	0/13 0%	N.A	1/19 5%
SETD2	0/10 0%	0/2 0%	2/10 20%	3/13 23%	N.A	6/19 32%
MAP3K1	0/10 0%	0/2 0%	N.A	N.A	N.A	0/19 0%
EGFR	0/10 0%	0/2 0%	0/10 0%	4/13 31%	1/5 20%	2/19 11%
BCOR	0/10 0%	0/2 0%	1/10 10%	0/13 0%	N.A	3/19 16%
FLNA	0/10 0%	0/2 0%	N.A	N.A	N.A	2/19 11%

N.A=Not applicable

- Tumour resistance to first-line paclitaxel-based chemotherapy may be a clinical clue that the tumour was not a MBC.
- Management of malignant PT is typically based on sarcoma therapy principles rather than carcinoma treatment, but the efficacy of systemic chemotherapy against metastatic PT remains unknown.
- Further studies are urgently required to find new targets that will improve disease management and clinical outcomes of affected patients.
- Our previous work showed that compared to fibroadenoma or benign PT, borderline and malignant PTs typically exhibit additional mutations coupled with putative copy number alterations in NF1, RB1, TP53, PIK3CA, ERBB4 and EGFR, which are known oncogenes with transforming ability.
- Our 16 gene panel has the potential to differentiate between high-grade/malignant PT from its low-grade counterparts.

Summary

- Case of an advanced breast tumour composed of malignant spindle cells, in which a 16 gene mutation panel was used as an adjunctive tool to distinguish between malignant PT and MBC which were the two key histological differential diagnoses.
- Further studies are ongoing to validate this targeted mutation panel in an international breast fibroepithelial cohort.

Final diagnosis

Malignant phyllodes tumour

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CORRESPONDENCE

A genetic mutation panel for differentiating malignant phyllodes tumour from metaplastic breast carcinoma

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