NGS: A case based review

Next Generation Sequencing: Current Technology and Applications

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Objectives

- Present actual cases to illustrate principles of molecular analysis of tumors by next generation sequencing (NGS).
- What molecular abnormalities can NGS detect?
- What substrates/ tissue/ cell types can be reliably analyzed and what cannot?
- How does NGS aid in the current management of cancer patients?

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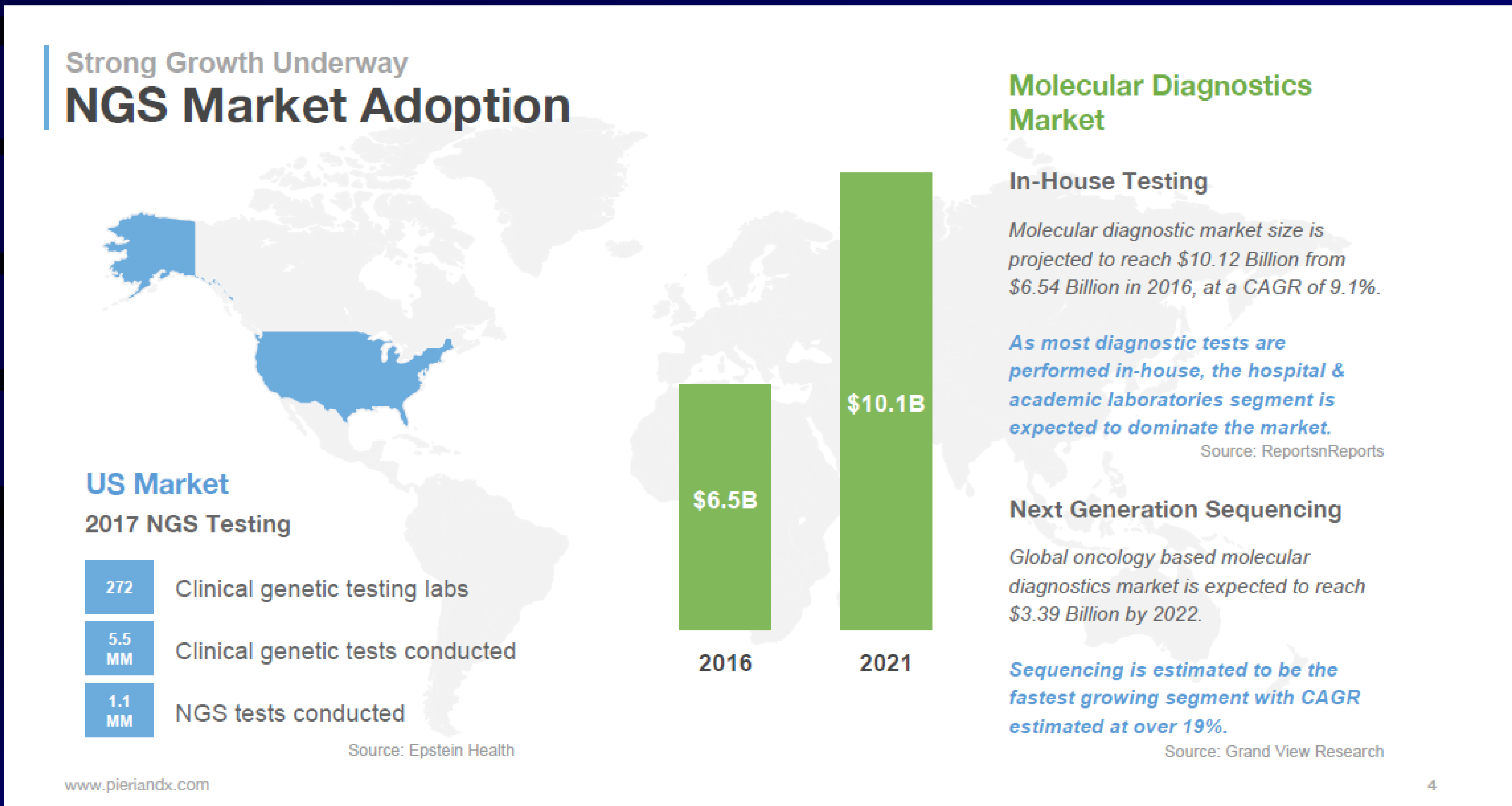
Next Generation Sequencing

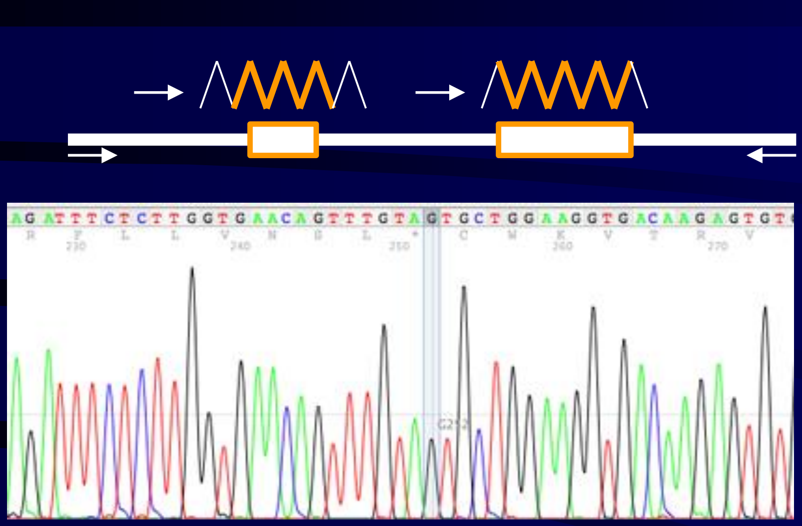
Also known as massive parallel sequencing



Courtesy of Dr. Eric Loo

Background of NGS

Sanger
(first generation sequencing)



NGS
(next generation sequencing

- NGS allows massive production of tens of millions of short sequencing fragments, high-throughput and low cost
- Dependent on the existence of a reference sequence and requires bioinformatics processing for alignment
- Enables testing of many genes in a single assay with very small amount sample nucleic acid

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Clinical Applications of NGS

- - Personalized/precision medicine
- - Inherited vs. acquired diseases
- - Tumor profiling
- - Choice of therapy

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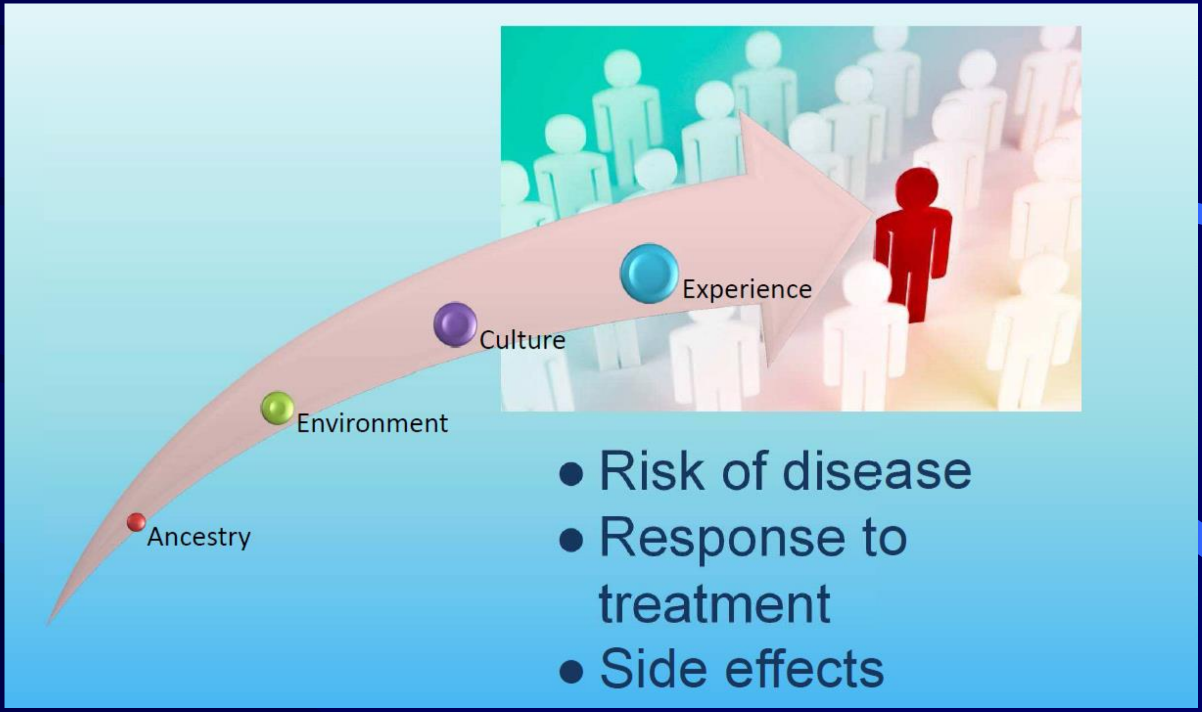
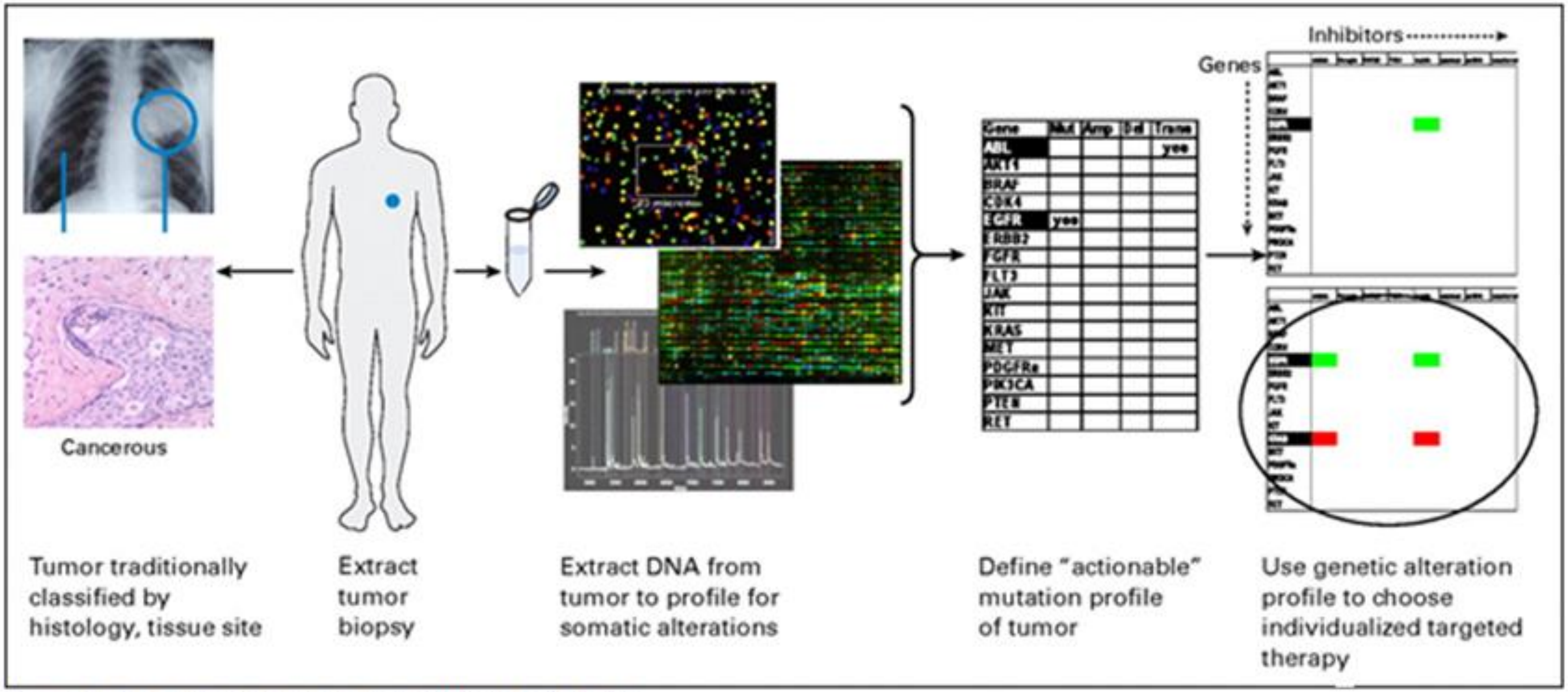
Personalized/Precision Medicine

Administer
- the right drug
- at the right dose
- to the right person
- at the right time

Individuality



Tumor Profiling



MacConaill L E , Garraway L A JCO 2010;28:5219-5228

(Comprehensive NGS test)

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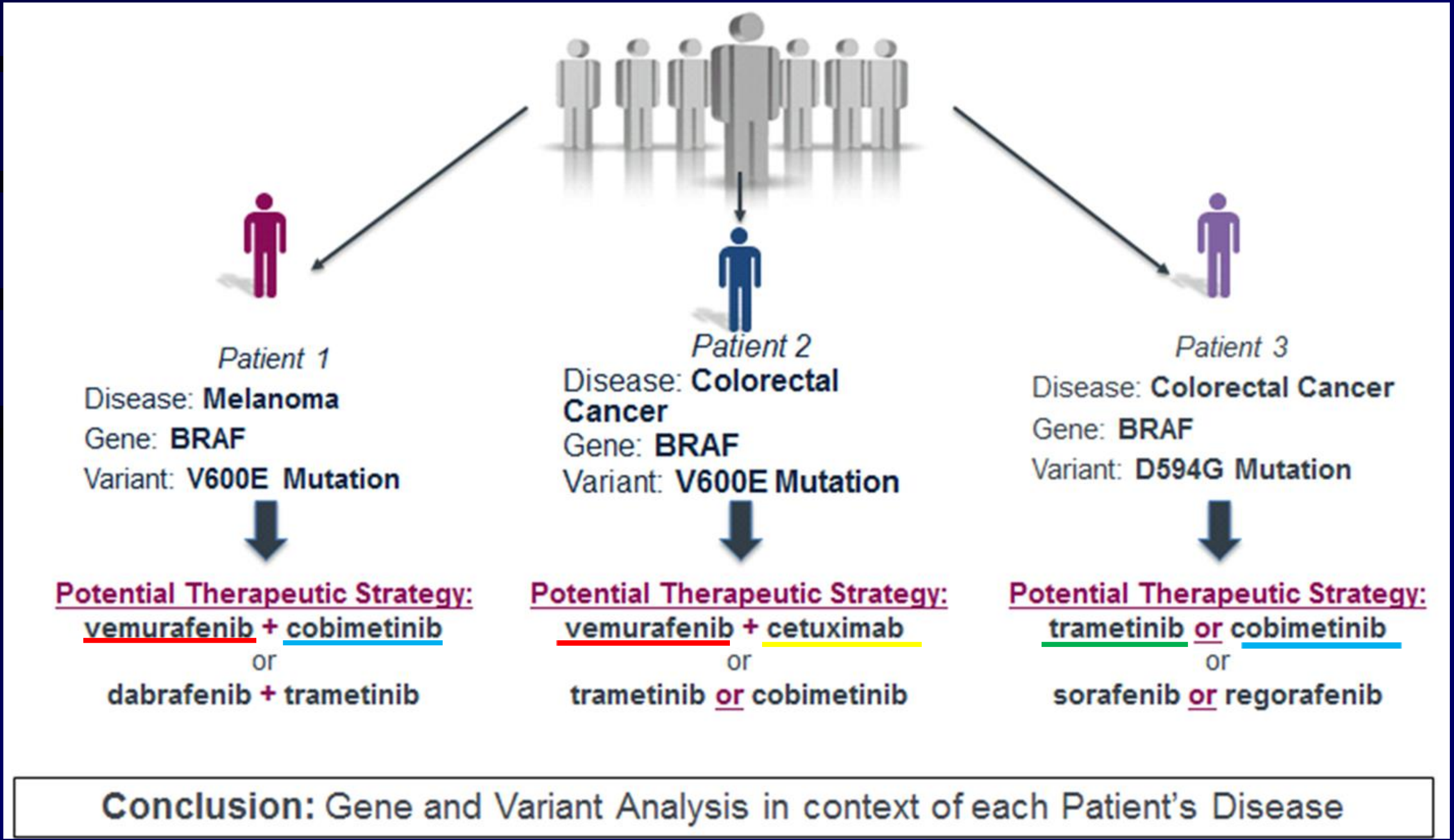
Choice of Therapy

- Matching the treatment to pathophysiology of disease
- Genetic variants that predict response
- Patterns of gene expression that reveal disease subtypes



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Landscape of Solid Tumor Oncology Biomarker Testing

Organ	Cancer	Biomarker	Assay	Drug
Breast	Breast	HER2	FISH, IHC	HER2: Trastuzumab, pertuzumab, Adotratuzumab emtansine
Gastro-intestinal	Colorectal	KRAS, NRAS, BRAF	PCR	EGFR: Cetuximab, panitumumab
	GIST	KIT	PCR	BCR/ABL: Imatinib
	Esophago-gastric adenocarcinoma	HER2	FISH, IHC	HER2: Trastuzumab
Lung	NSCLC	EGFR	Sequencing	EGFR: Erlotinib, gefitinib, afatinib
		ALK	FISH (IHC)	ALK: Crizotinib, certinib
		ROS1	FISH (IHC)	ROS1: Crizotinib
		BRAF	Sequencing	BRAF: Dabrafenib+trametinib
		PDL1	IHC	PDL1: Pembrolizumab
		KRAS	Sequencing	Identify pts not benefit for Mol.testing
	Lung adenocarcinoma	RET, BRAF, EGFR, HER2, KRAS, ALK, MET, ROS1	Multiplex sequencing	multiple drugs
Skin	Melanoma	BRAF	Sequencing	BRAF: Vemurafenib, dabrafenib

- Many biomarkers show mutually exclusive genetic alterations.
- Tests usually performed sequentially/ individually and therefore increases turnaround time to yield final results.
- Uses many slides instead of one or just a few.

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NGS is the Solution to Reduce TAT and Sample Size

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Breast	Breast	HER2	FISH, IHC	HER2: Trastuzumab, pertuzumab, Adotratuzumab emtansine
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		PDL1	IHC	PDL1: Pembrolizumab
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All these genetic alterations except for PDL1 can be tested by a single NGS assay with mutations, fusions and copy number variations, e.g., Oncomine Focus Assay (OFA)

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We Offer 4 Panels for Solid Tumor Tests

- Lung cancer (24 genes)
- Colorectal cancer (6 genes)
- Melanoma (11 genes)
- Complete tumor (52 genes)

Hotspot genes, n=35

AKT1	IDH2
ALK	JAK1
AR	JAK2
BRAF	JAK3
CDK4	KIT
CTNNB1	KRAS
DDR2	MAP2K1
EGFR	MAP2K2
ERBB2	MET
ERBB3	MTOR
ERBB4	NRAS
ESR1	PDGFRA
FGFR2	PIK3CA
FGFR3	RAF1
GNA11	RET
GNAQ	*ROS1*
HRAS	SMO
IDH1	

Copy Number Variants, n=19

ALK	FGFR3
AR	FGFR4
BRAF	KIT
CCND1	KRAS
CDK4	MET
CDK6	MYC
EGFR	MYCN
ERBB2	PDGFRA
FGFR1	PIK3CA
FGFR2	

DNA Panel

Fusion drivers, n=23

ABL1	FGFR2
AKT3	FGFR3
ALK	*MET*
AXL	NTRK1
BRAF	*NTRK2*
ERG	NTRK3
ETV1	PDGFRA
ETV4	PPARG
ETV5	RAF1
EGFR	RET
ERBB2	ROS1
FGFR1	

RNA Panel

Note: 52 genes total

Italic: *Not members of OFA for that aberration class*

Hotspot, CNV, Fusion, Hotspot + CNV, Hotspot + CNV + Fusion, **Hotspot + Fusion**, CNV+ Fusion

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Landscape of Current Myeloid Biomarker Testing

Disease	Biomarker	Assay	Drug
AML	FLT3	PCR, NGS	Midostaurin (Rydapt)
	IDH2	PCR, NGS	Enasidenib (Idhifa)
CML	BCR/ABL1	PCR, NGS	Imatinib (Gleevec)
			Dasatinib (Sprycel)
			Nilotinib (Tasigna)
			Bosutinib (Bosulif)
			Ponatinib (Iclusig)
APL	t(15;17)(q22;q12)/PML-RARA	PCR, FISH, karyotype, NGS	ATRA
			Arsenic trioxide
MPN	JAK2	PCR, NGS	Ruxolitinib

- NGS is the solution to reduce No. of Assays, TAT and sample size

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We Offer 3 Panels for Heme NGS Tests

- AML (55 genes)

- MPN (38 genes)

- Heme (74 genes)

Table 1. Oncomine Myeloid Research Assay gene targets.

Hotspot genes (23)		Full genes (17)		Fusion driver genes (29)			Expression genes (5)	Expression control genes (5)
ABL1	*KRAS*	*ASXL1*	*PRPF8*	*ABL1*	*HMGA2*	*NUP214*	*BAALC*	*EIF2B1*
BRAF	*MPL*	*BCOR*	*RB1*	*ALK*	*JAK2*	*PDGFRA*	*MECOM*	*FBXW2*
CBL	*MYD88*	*CALR*	*RUNX1*	*BCL2*	*KMT2A*	*PDGFRB*	*MYC*	*PSMB2*
CSF3R	*NPM1*	*CEBPA*	*SH2B3*	*BRAF*	*(MLL)*	*RARA*	*SMC1A*	*PUM1*
DNMT3A	*NRAS*	*ETV6*	*STAG2*	*CCND1*	*MECOM*	*RBM15*	*WT1*	*TRIM27*
FLT3	*PTPN11*	*EZH2*	*TET2*	*CREBBP*	*MET*	*RUNX1*		
GATA2	*SETBP1*	*IKZF1*	*TP53*	*EGFR*	*MLLT10*	*TCF3*		
HRAS	*SF3B1*	*NF1*	*ZRSR2*	*ETV6*	*MLLT3*	*TFE3*		
IDH1	*SRSF2*	*PHF6*		*FGFR1*	*MYBL1*			
IDH2	*U2AF1*			*FGFR2*	*MYH11*			
JAK2	*WT1*			*FUS*	*NTRK3*			
KIT								

Why Choose Oncomine Assays?

- Platform: Ion Torrent S5/Chef
- Panel: all genes with actionable mutations and fusions
- Small sample size: 10ng DNA/RNA from FFPE, blood or bone marrow
- Shorter TAT: Tumor 5-7 days; heme 3-5 days
- Single Workflow: process DNA/RNA sequencing simultaneously with automation workflow
- Reporting: Oncomine Knowledgebase Reporter

Summary of NGS Assays

- NGS has become the standard for molecular assays and harbors enormous potential value to the practice of oncology.

- Oncomine NGS assay was chosen because of low sample input, improved TAT, intelligent NGS content design, single workflow, and proven clinical utility.

- Guidelines for comprehensive biomarker analysis in solid tumors and hematological disorders are available.

- NGS results will change the paradigm of cancer therapy and facilitate precision medicine.

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Questions?

Case 1

- This 49 year old woman was transferred on 2/9/19 with WBC of 57,900, a Hgb of 5.3 and plts of 15K. Her O_2 saturation was 97.7% on nasal O_2 suppl.

- Her peripheral blood smear differential revealed 71% blasts/ promonocytes, 8% neutrophils, a Hgb of 5.3 and platelets of 15K. Normal counts were last reported on 6/25/18.

- SOB developed after 1 transfusion and chest X-ray disclosed diffuse bilateral infiltrates on 2/9/2019; hypotension requiring pressor support ensued.

- BAL, bronchial biopsy and cultures were negative for evidence of infection.

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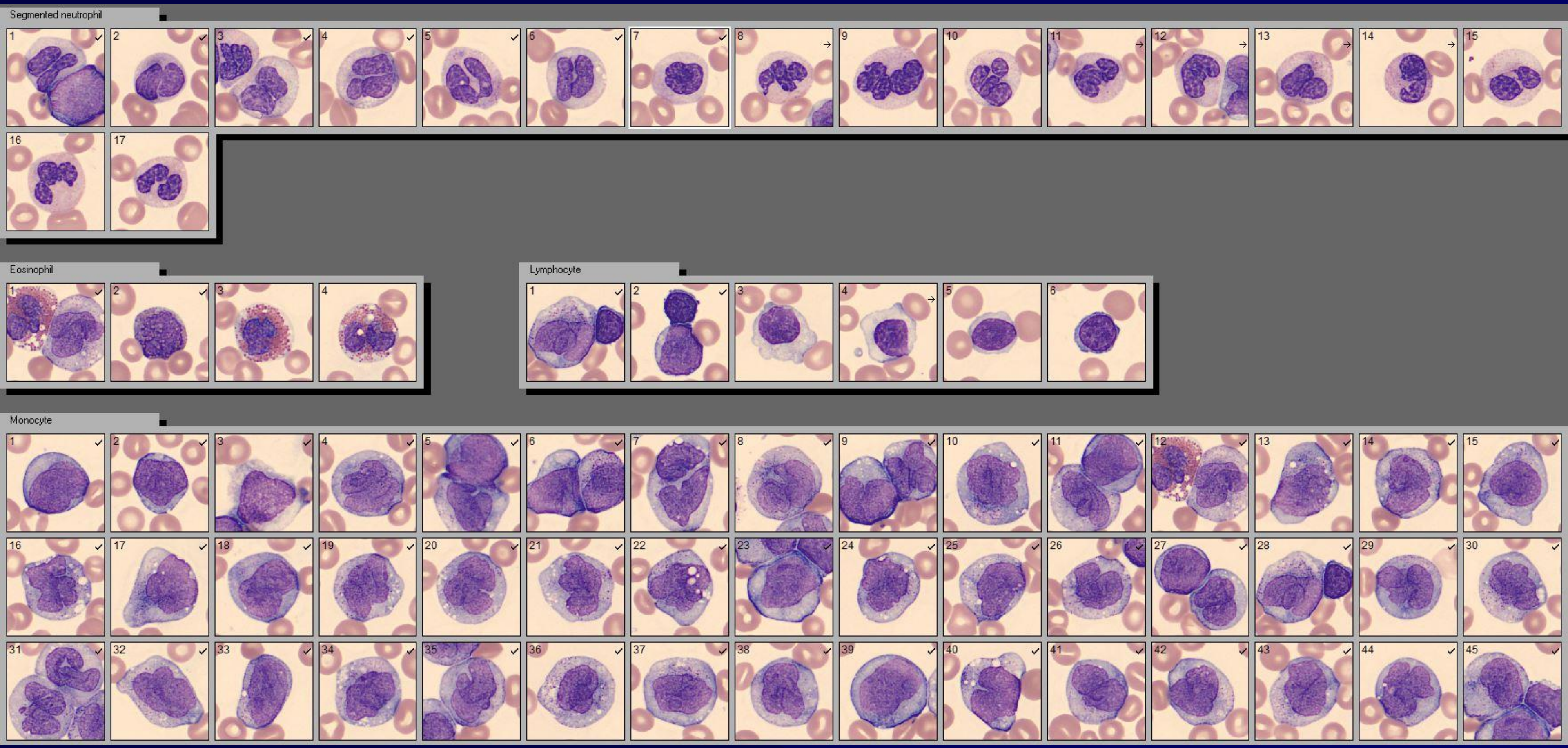
Case 1- peripheral blood



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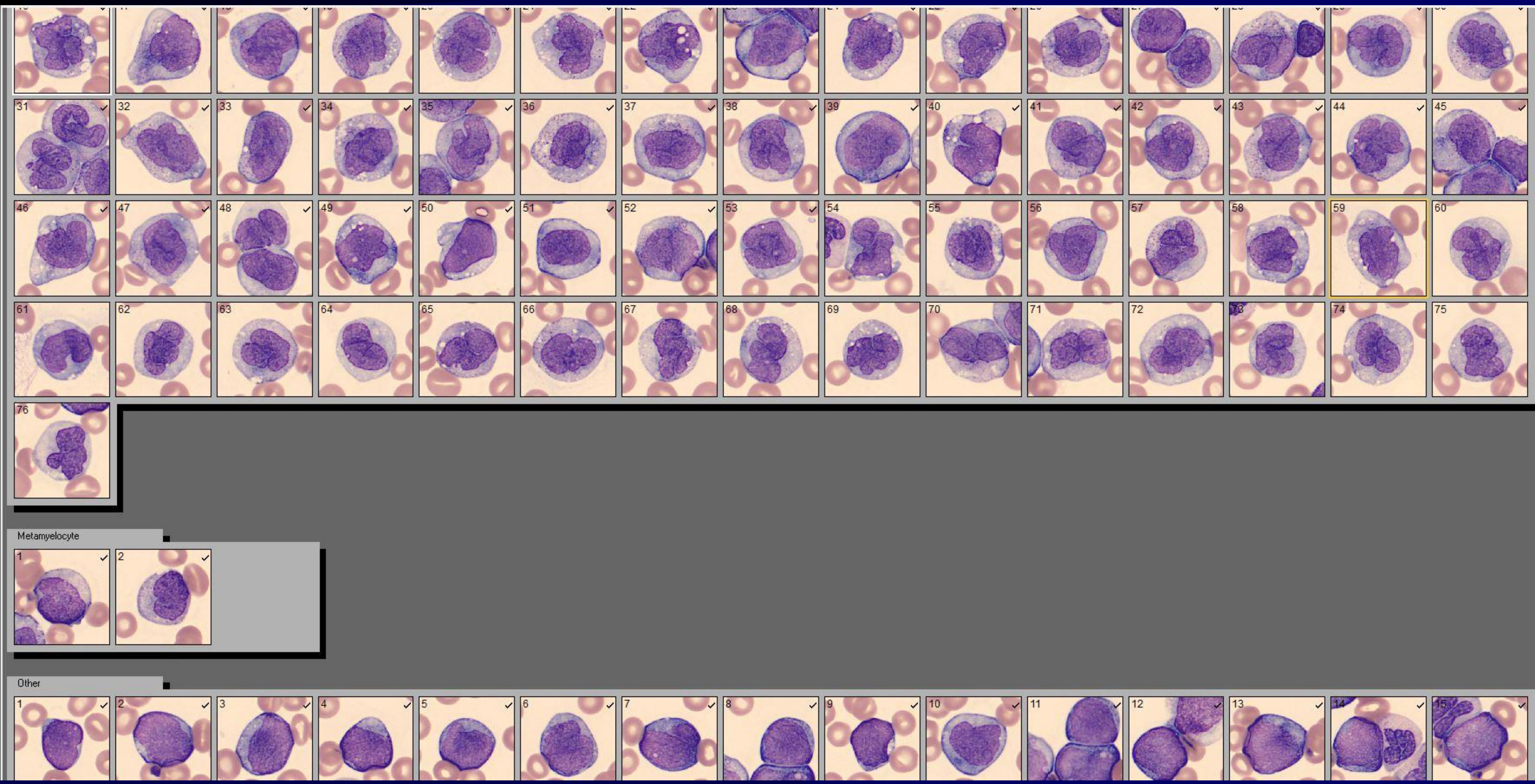
Case 1- peripheral blood



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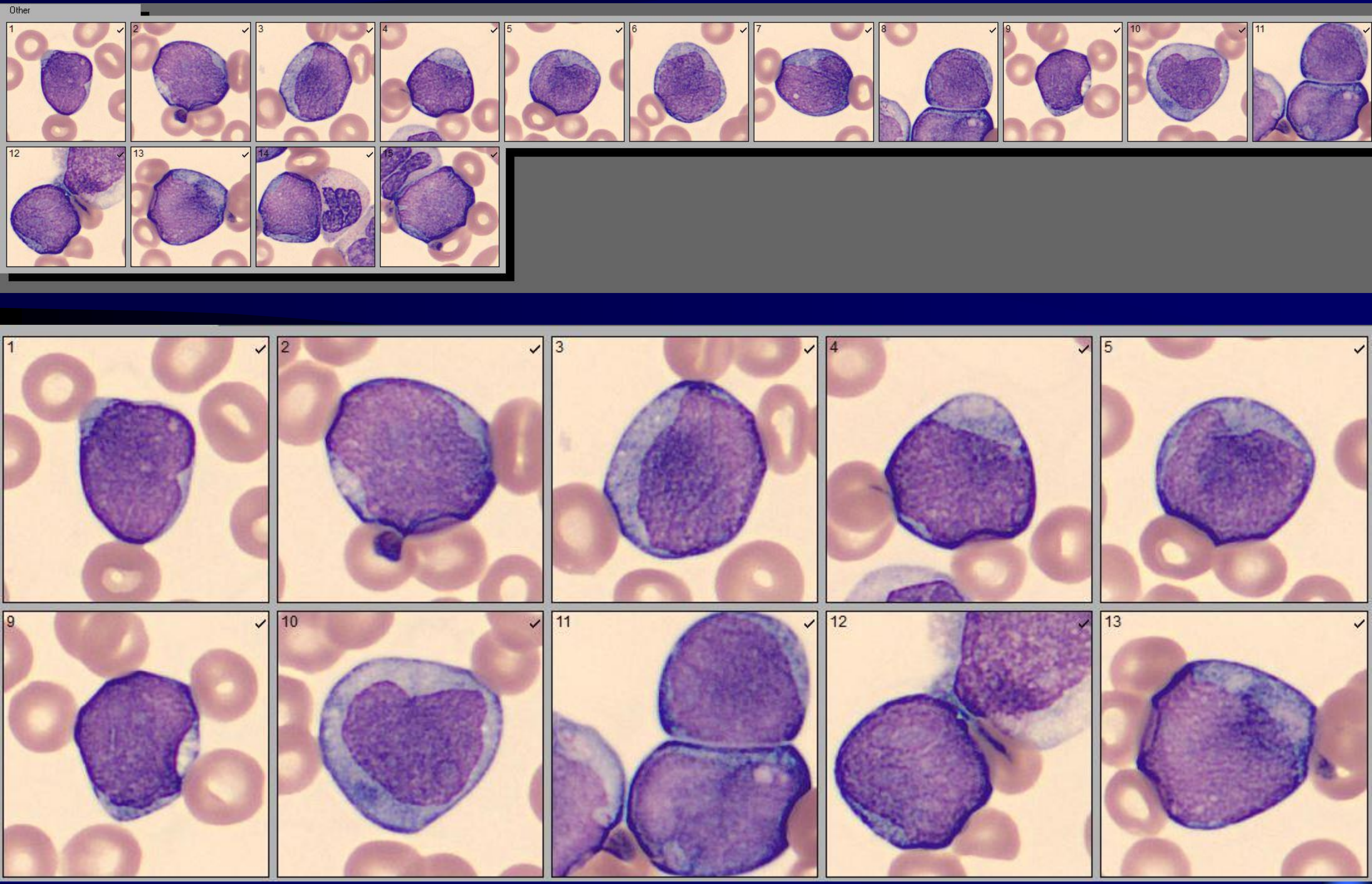
Case 1- peripheral blood



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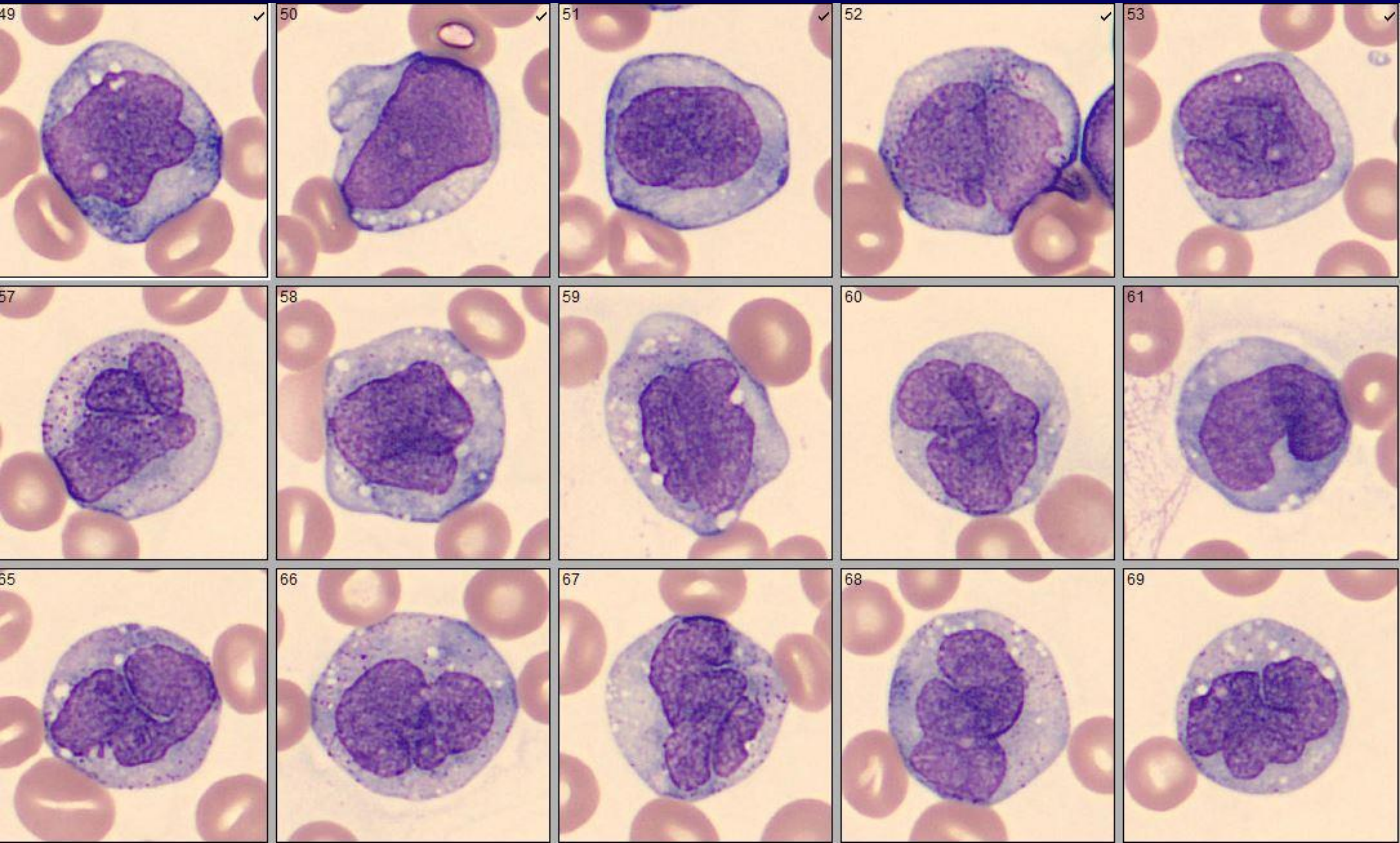
Case 1- peripheral blood



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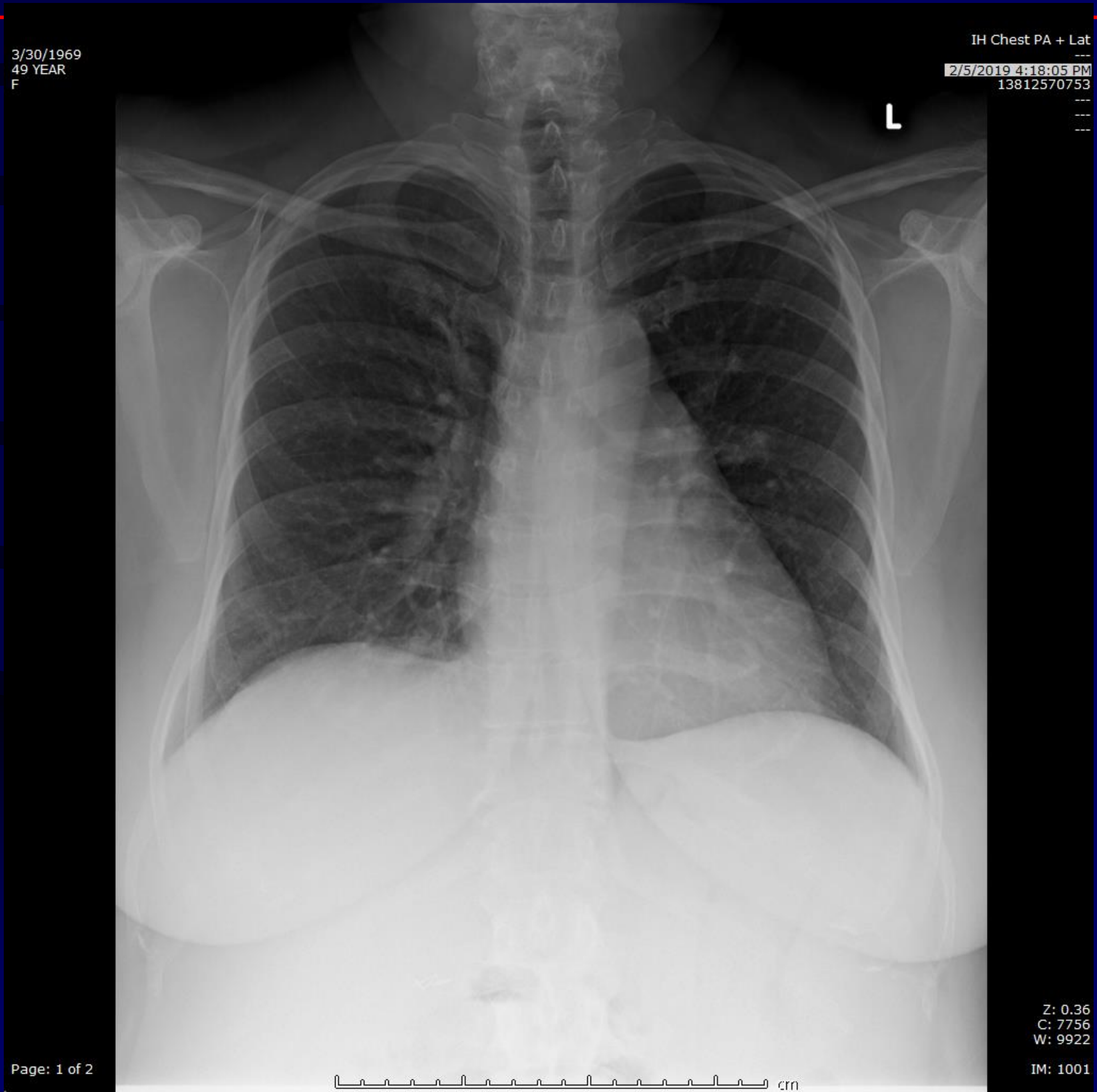
Case 1- Chest X-ray- 2/5/19



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Hospital Course

WBC increasd to 100K on 2/12. Initially treated with hydroxyurea, pending NGS analysis of peripheral blood, as she was too ill to undergo a bone marrow biopsy.

Respiratory status worsened with increasing pulmonary infiltrates, resulting in intubation and ventilator support.

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Hospital Course

NGS analysis resulted an AML defining mutation [inv 16/ CBFB/MYH11 fusion]; typical of acute myelomonotic leukemia (old FAB M4)

An additional mutation in the PTPN11 gene was also found with a 11% allele frequency (recently reported as adverse if >40%)

Negative for NPM1, CEBPA, FLT3 ITD/ TDK, and IDH1/2 mutations

Induction chemotherapy was initiated on 2/17. Respiratory status improved and extubated.

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NGS AML Molecular Profile

- 55 genes
- Hotspot genes: FLT3, CSF3R, DNMT3A, IDH1, IDH2, KIT, KRAS, NPM1, NRAS, PTPN11, RUNX1, SF3B1, SRSF2, U2AF1, WT1
- Full genes: ASXL1, BCOR, CEBPA, EZH2, PHF6, STAG2, TET2, ZRSR2
- Fusion drivers: ABL1, ALK, BCL2, BRAF, CCND1, CREBBP, EGFR, ETV6, FGFR1, FGFR2, FUS , HMGA2, JAK2, KMT2A, MECOM ,MET, MLLT10, MLLT3, MYBL1, MYH11, NTRK3, NUP214, PDGFRB, PDGFRA, RARA, RBM15, RUNX1, TCF3, TFE3
- Expression genes: BAALC, MECOM, MYC, SMC1A, WT1

Case 1: Hospital Course

WBC responded to nadir of 0.1 on 2/22 but did not recover through remainder of course.

Increased respiratory distress developed requiring re-intubation, pulmonary hemorrhage and by gram negative septic shock (Acinetobacter baumannii from BAL).

She unfortunately expired on 3/7/19.

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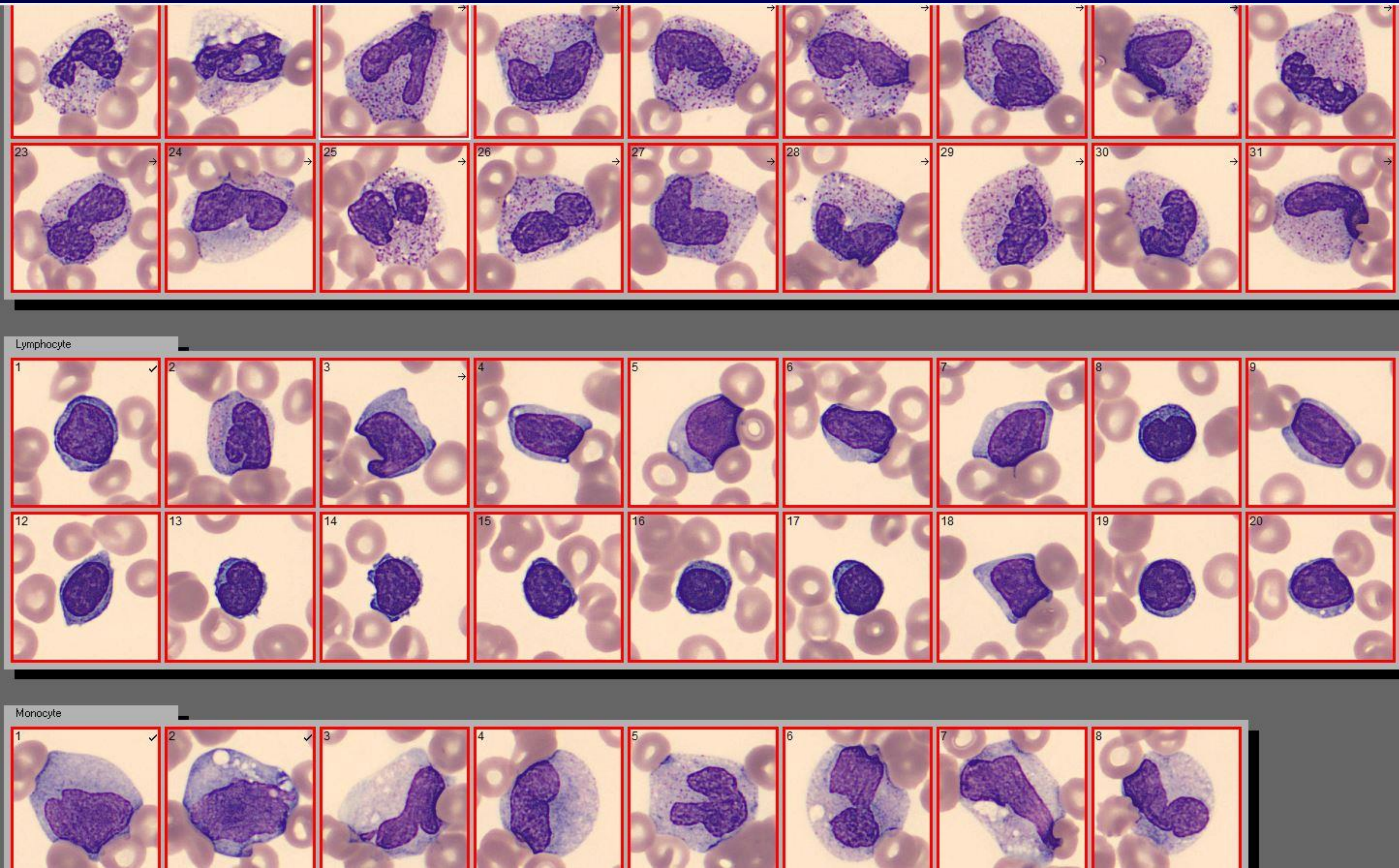
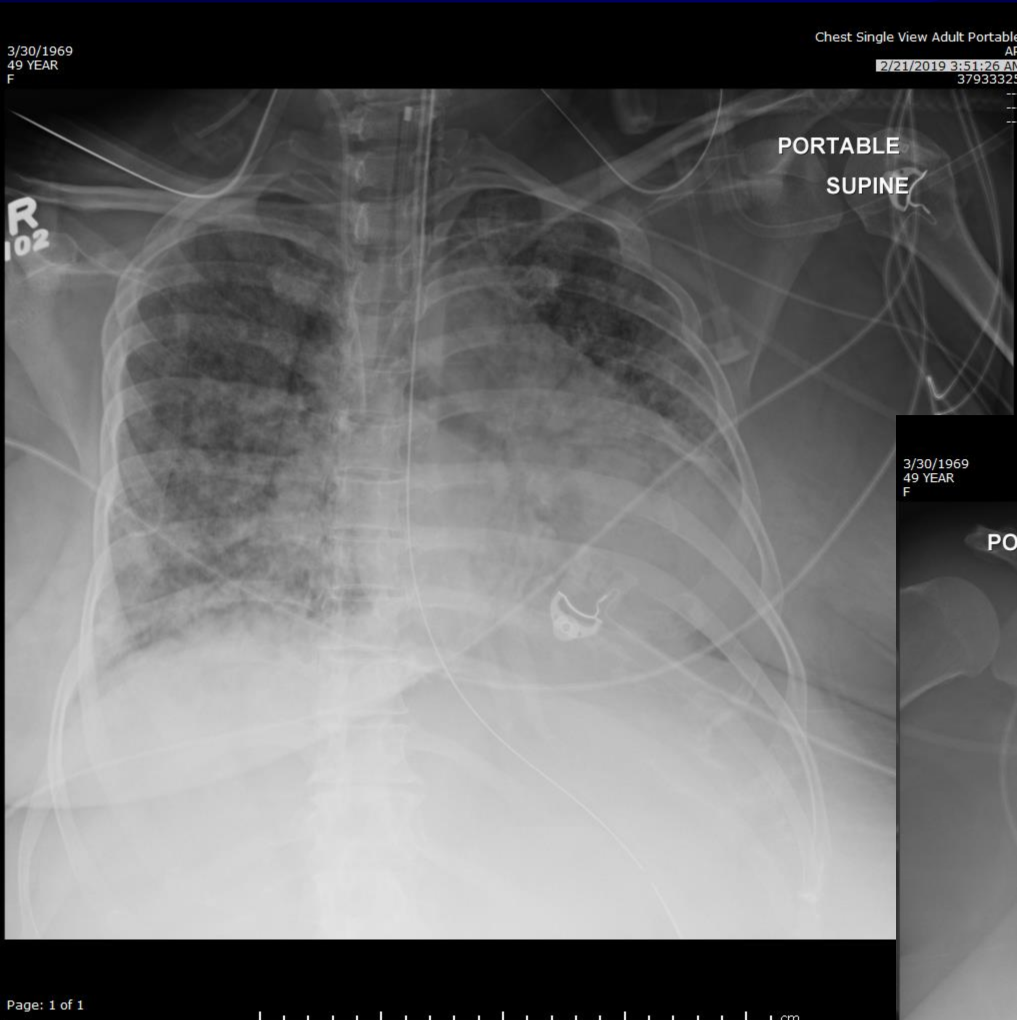
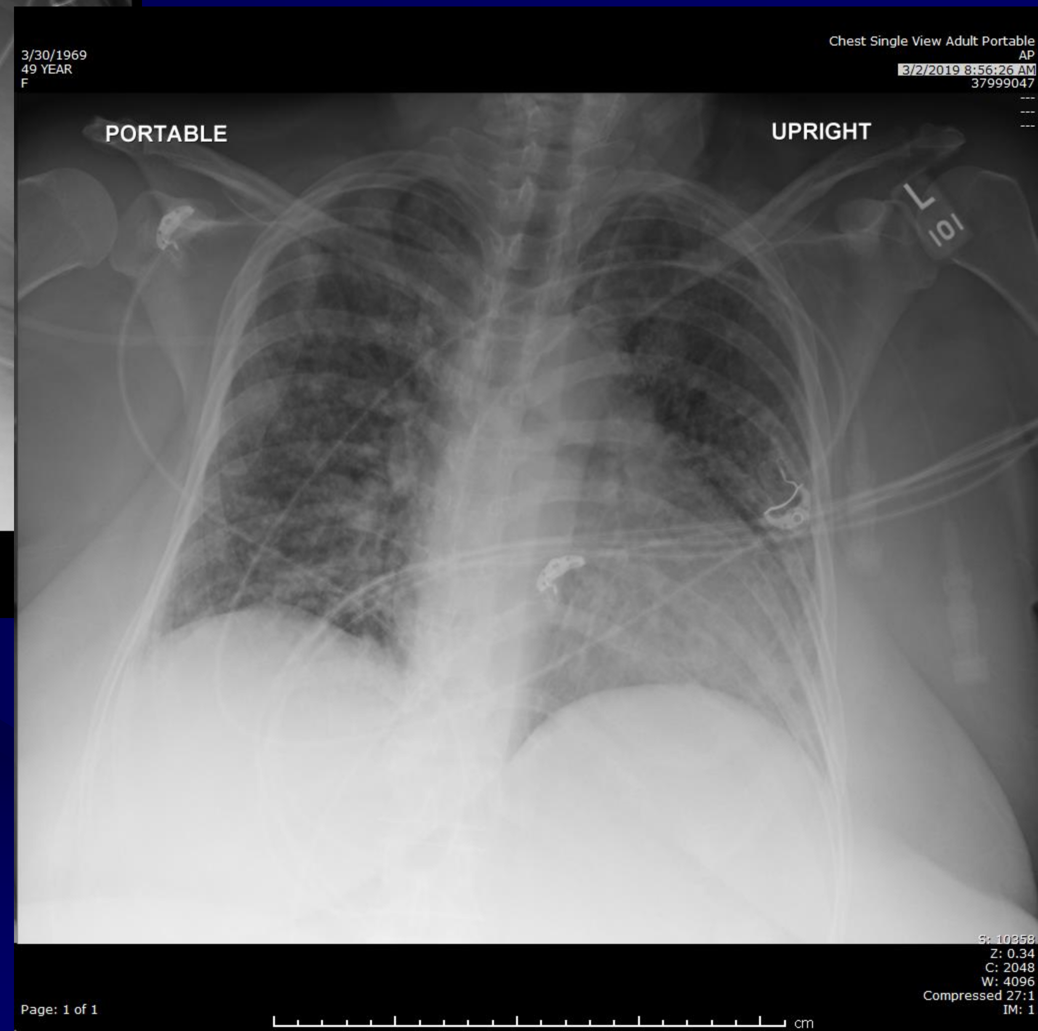
Case 1- peripheral blood post induction



CxR: 2/21/19





CxR: 3/2/19

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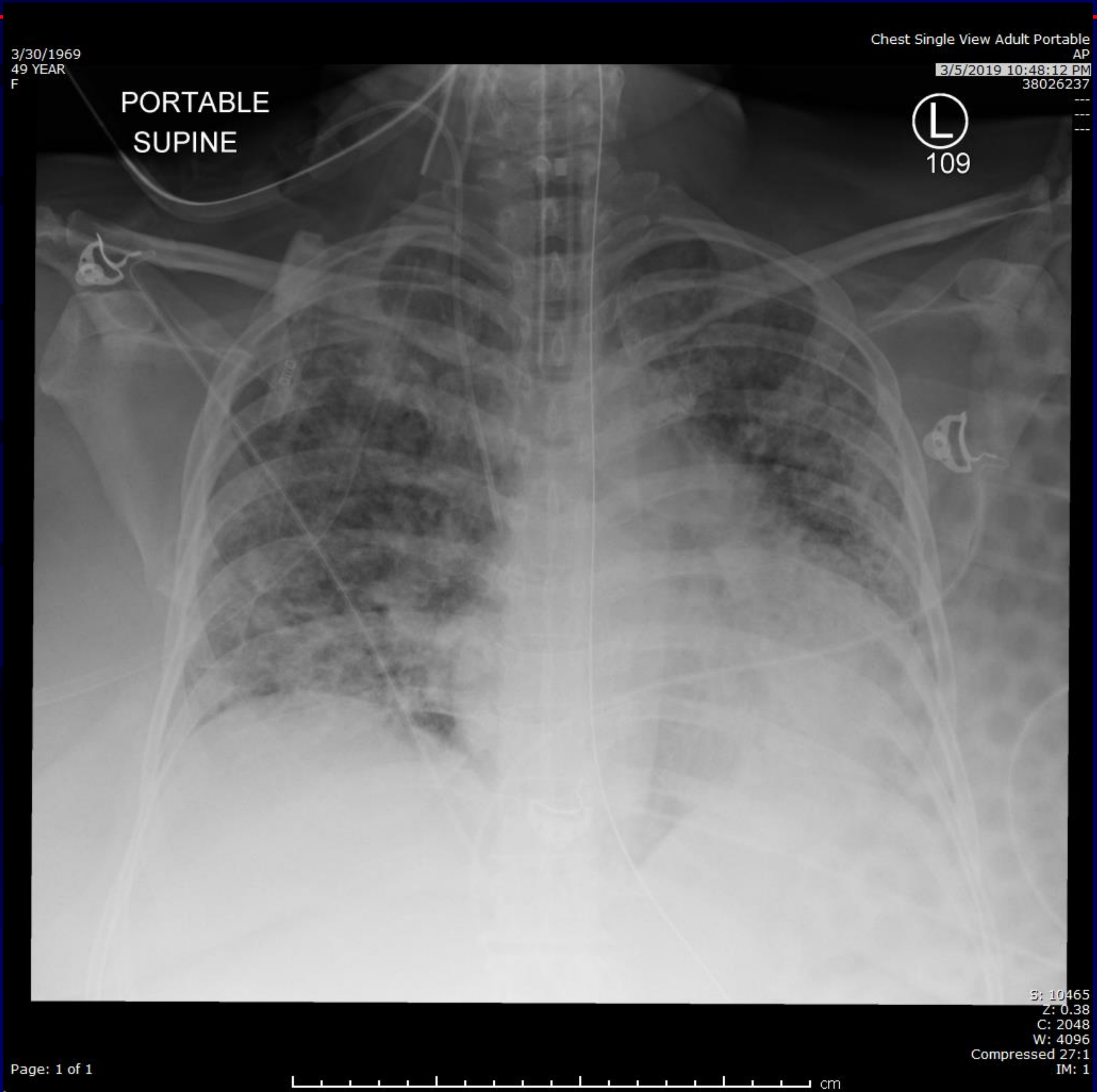
CxR: 3/5/19



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Case 1: Take home points

- NGS for hematopoietic neoplasms may be performed on peripheral blood, marrow aspirate or clot sections

- May detect mutations/ rearrangements and copy number variations, replacing some FISH and PCR based analyses.

- Turnaround equal to or more rapid than older assays and chromosome analysis.

- FISH still required/ desirable for monosomies, trisomies, large deletions (i.e. 5, 7), rapid detection of PML/RARA, other rearrangements.

Substrates for NGS analysis

- Peripheral blood or bone marrow aspirate
- Aspirate (marrow or any site) smears, including archived smears
- Formalin fixed, paraffin embedded, non-decalcified tissues (most common single source)
- Certain platforms can perform single or multiple gene analyses for solid tumors using circulating tumor cells/ DNA, i.e. T790M EGFR mutation.

Case 2: Clinical History

This 65 year old Vietnamese man presented in late June, 2015 with shortness of breath. A right pleural effusion and right middle lobe mass were discovered. Both were positive for adenocarcinoma.

CT scan of the head was negative for metastatic disease.

The biopsy tissue was submitted for single assay molecular analyses.

EGFR L858R exon 21 mutation identified.

ALK rearrangement negative.

Following the results, he was begun on single agent adjuvant therapy with erlotinib (Tarceva).

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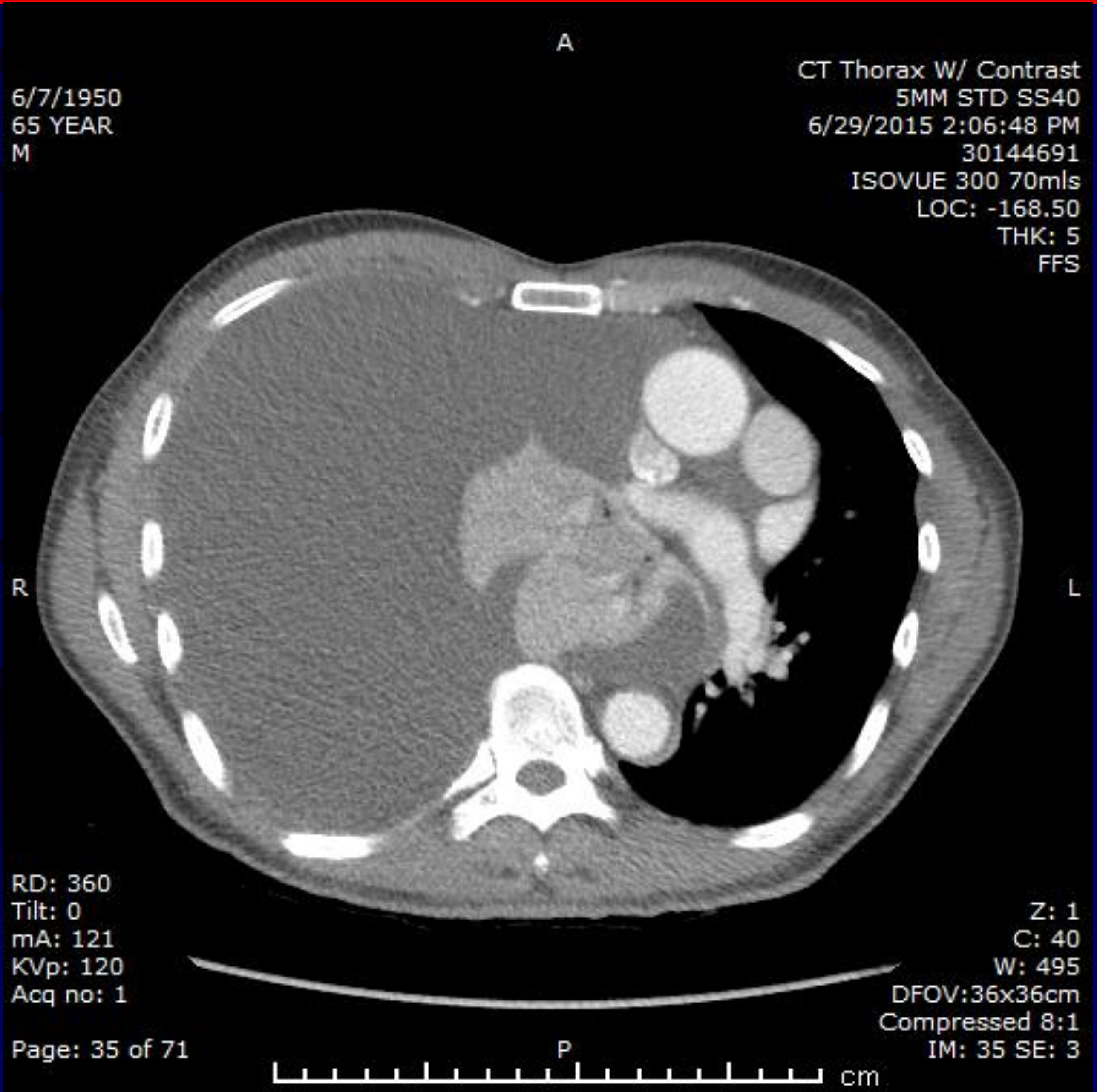
Case 2: imaging 6/29/2015



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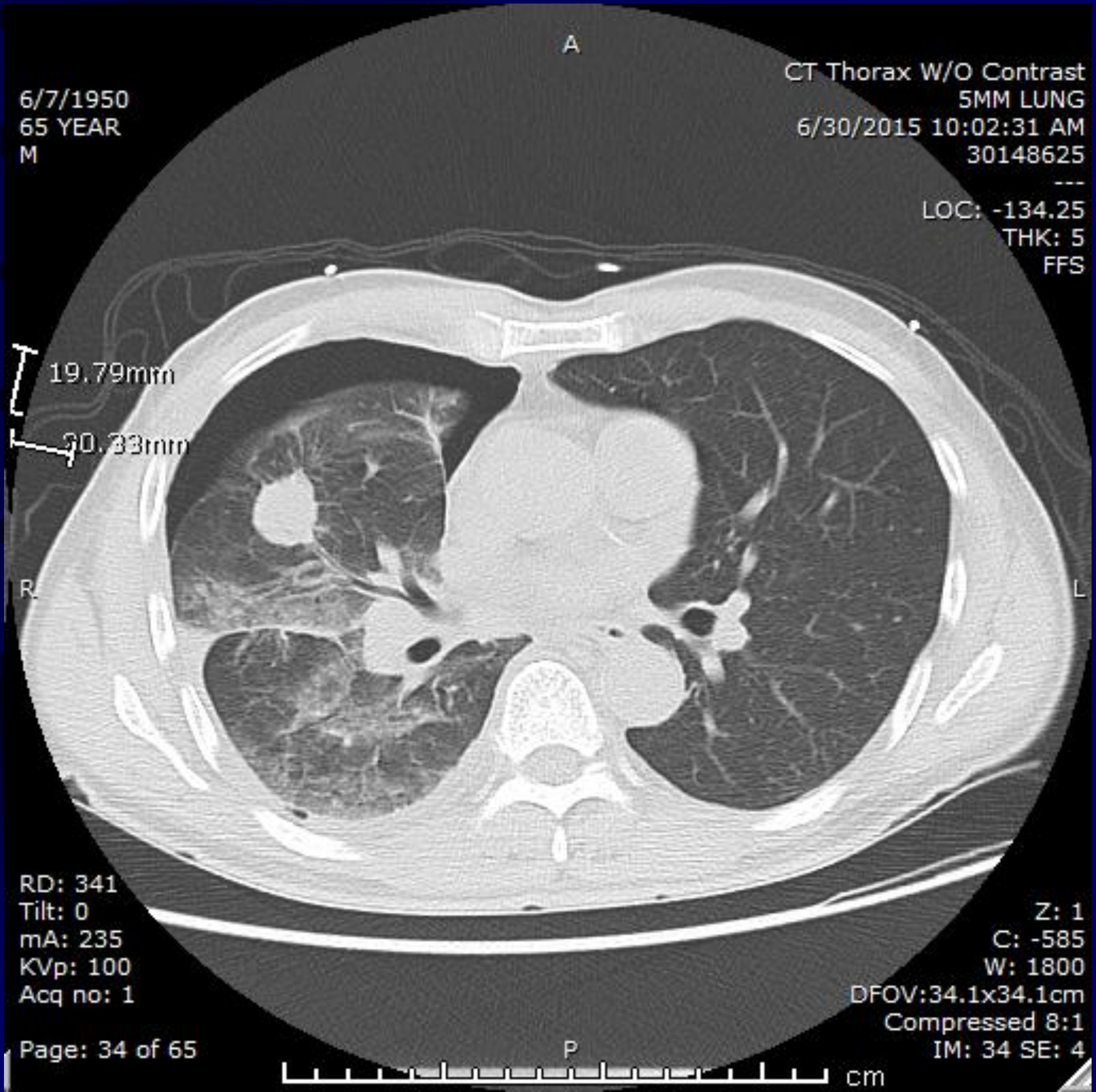
Case 2: 6/30/2015- post pleural drainage



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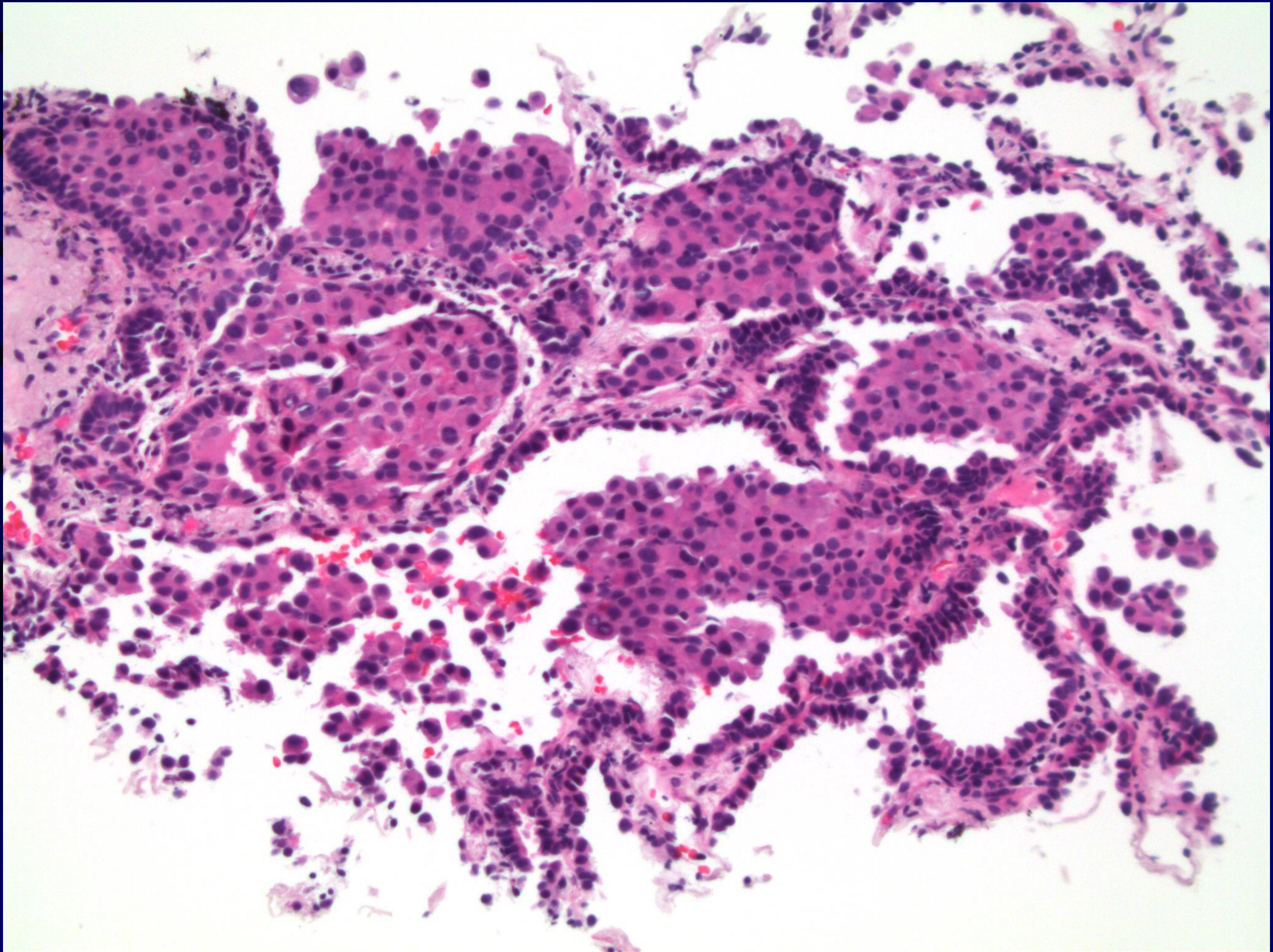
Case 2: CT guided biopsy



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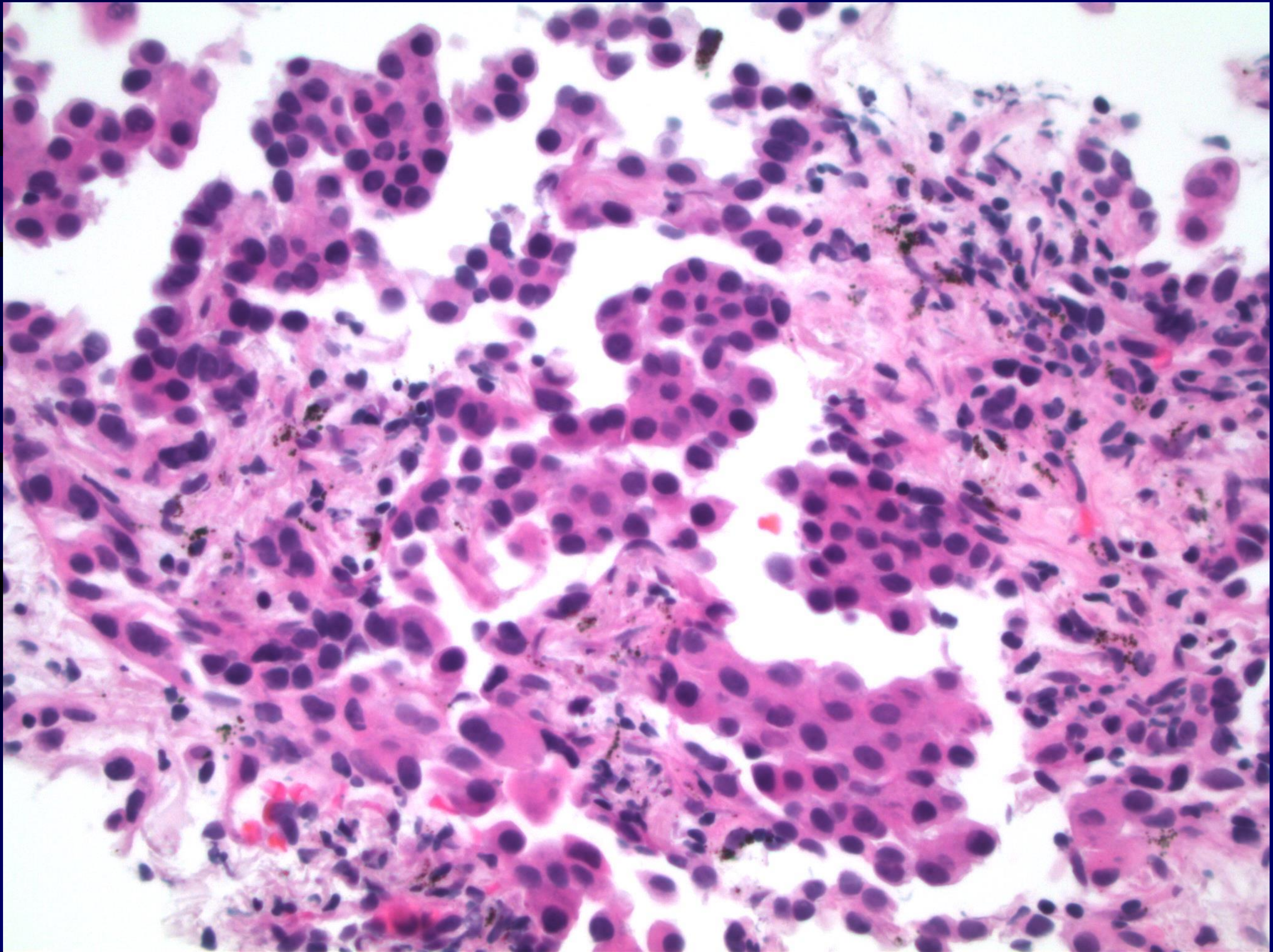
Case 2: CT guided biopsy



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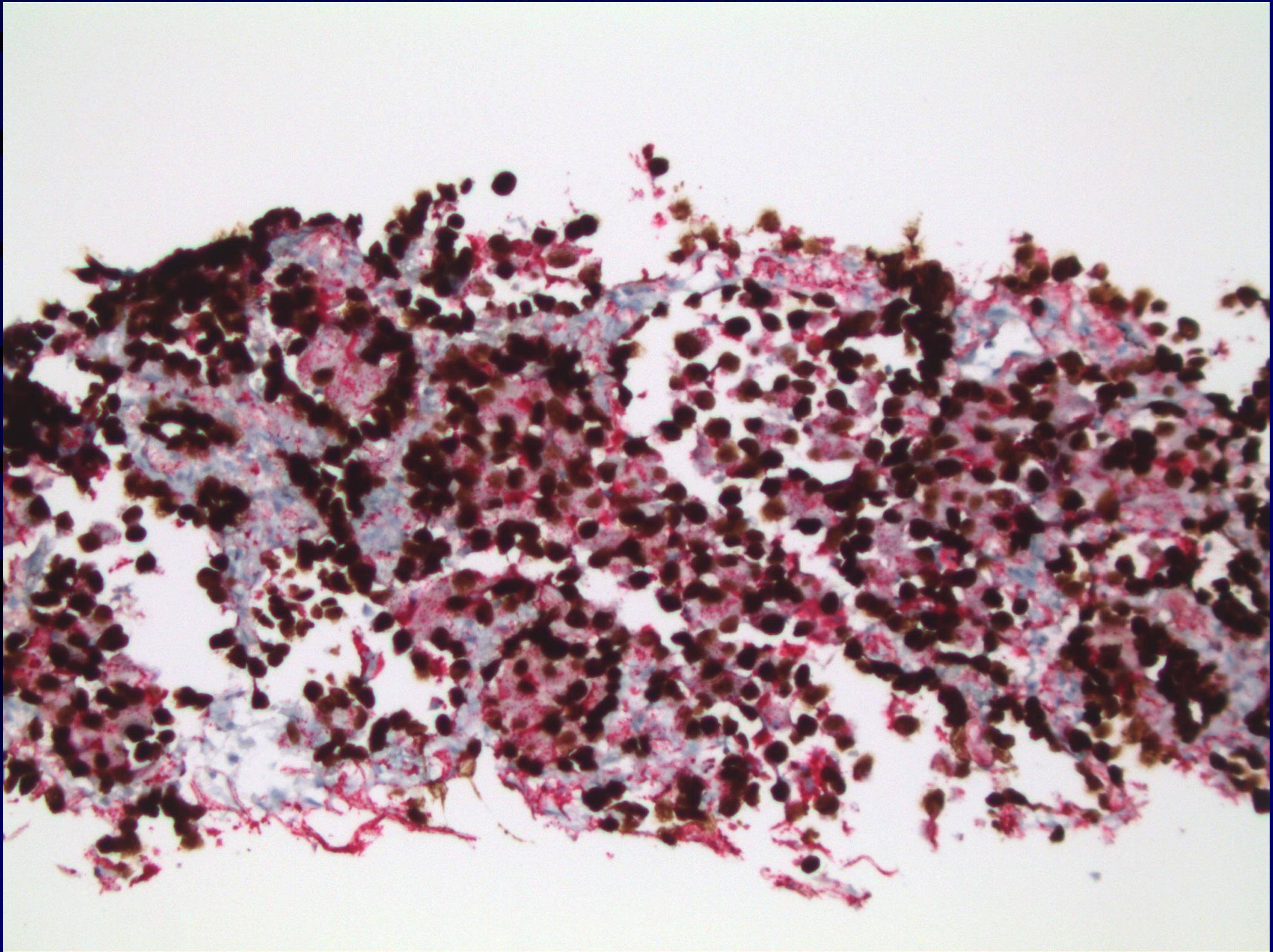
Case 2: TTF-1/ Napsin A



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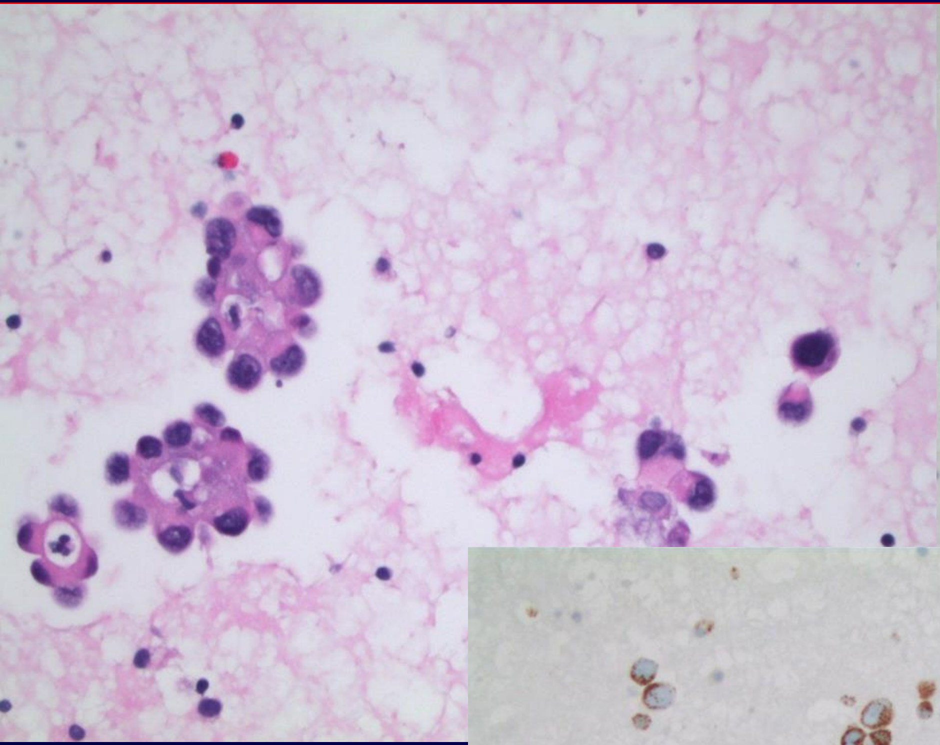
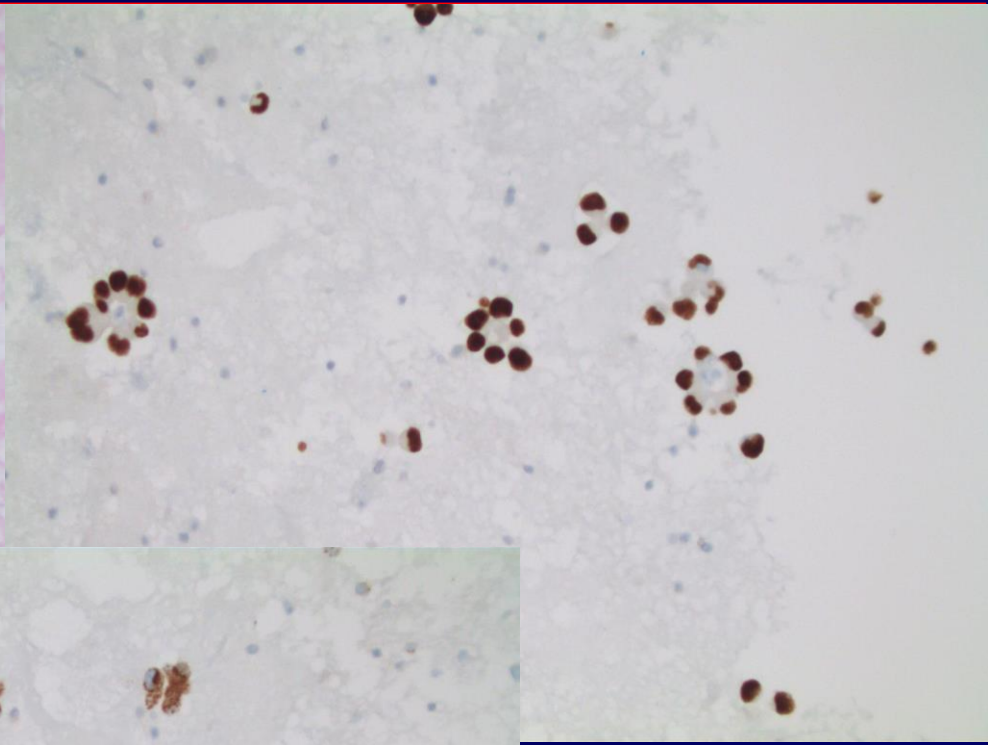
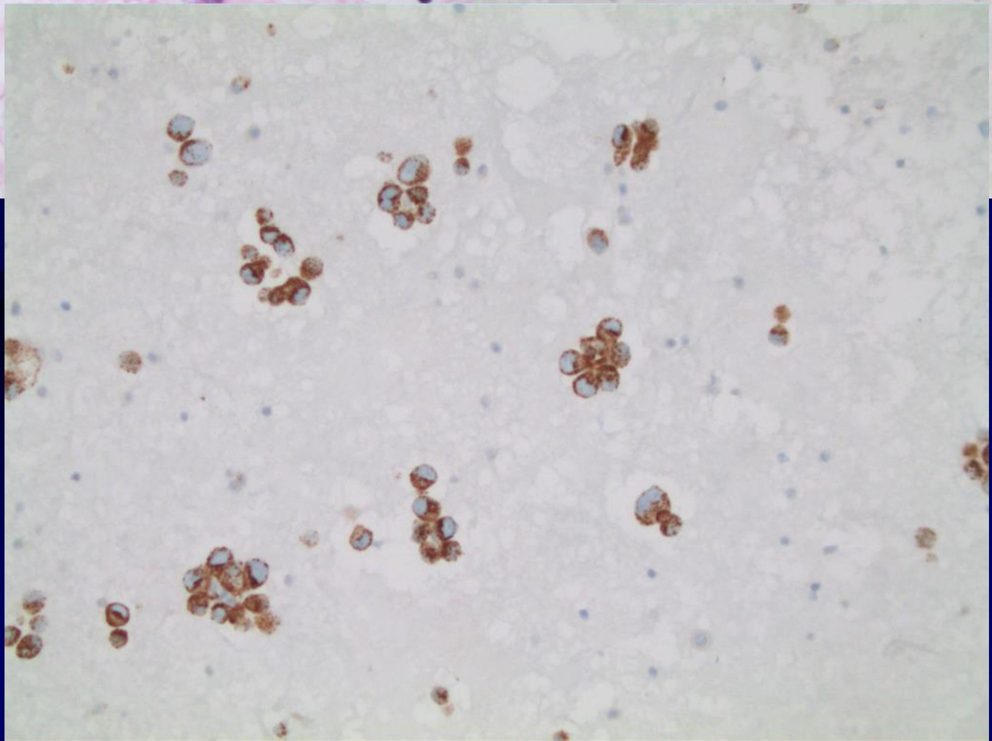
Case 2: pleural fluid





TTF-1



Napsin A

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Case 2: Clinical History (2)

Stable RUL nodule/ disease through 6/2016.

Radiation Rx administered to RUL and RML disease in Feb. and March of 2017.

No new disease through January 2018

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Case 2: Clinical History (continued)

In 7/2018, bilateral lung nodules were discovered, the largest 1.5 cm in the right lower lobe.

Biopsy and repeat molecular analysis were performed, now using the NGS Targeted Lung Profile.

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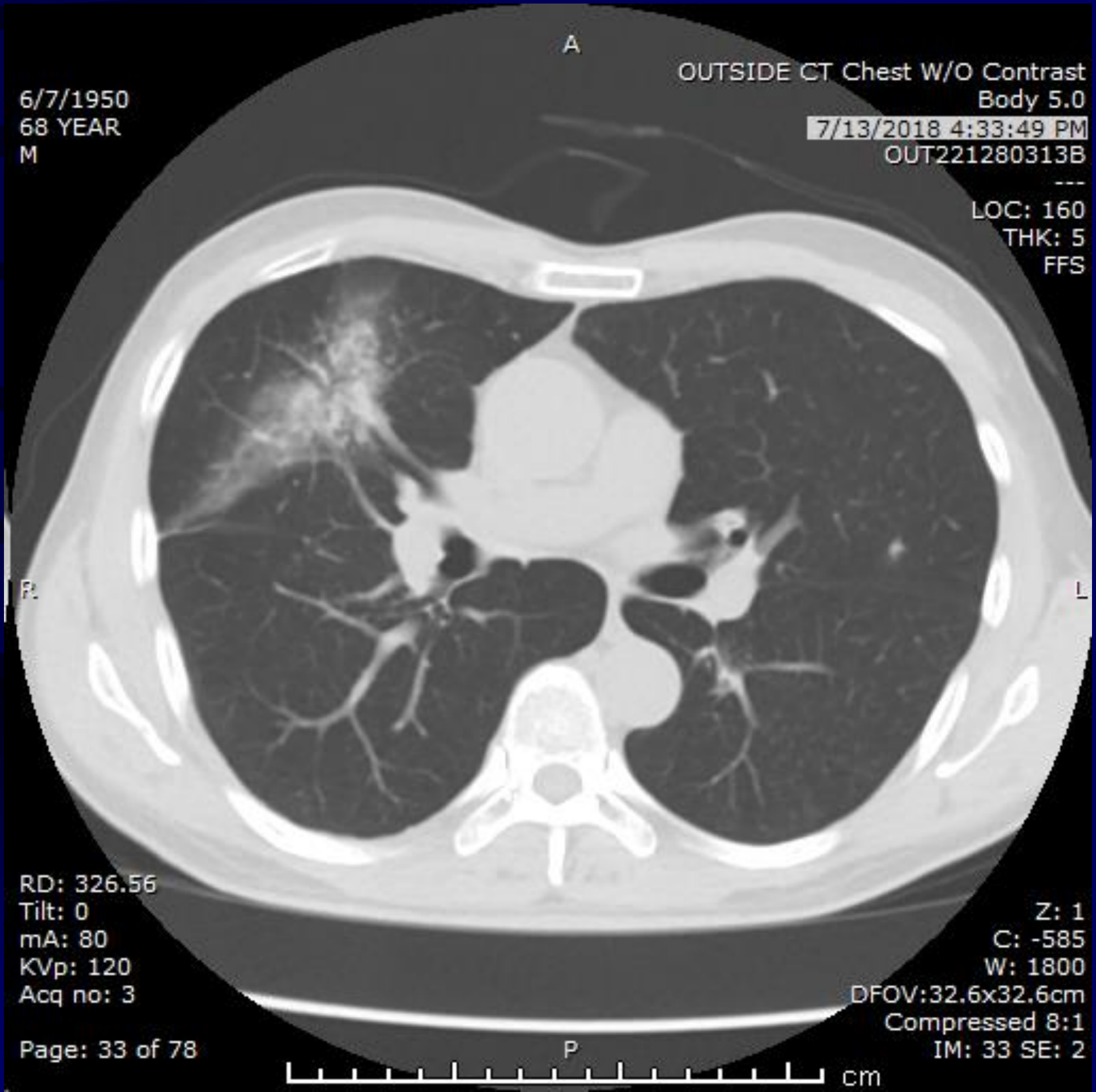
Case 2: imaging 7/2018- scarring RUL



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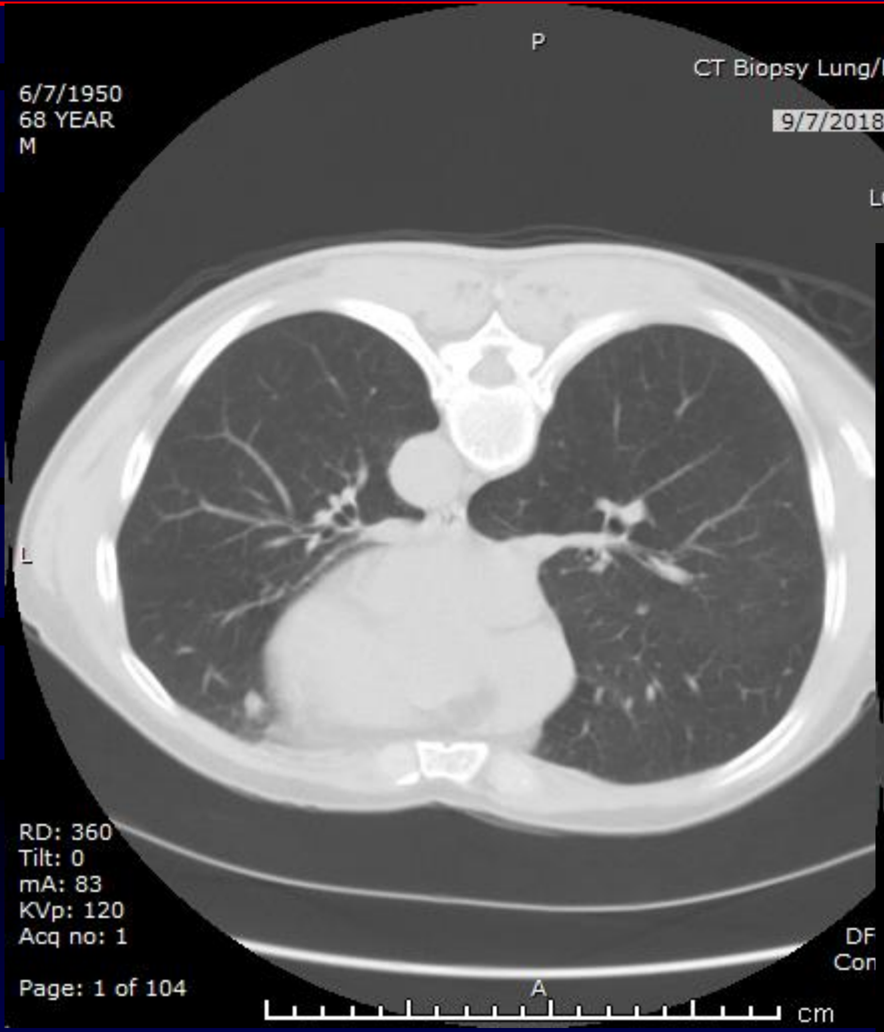
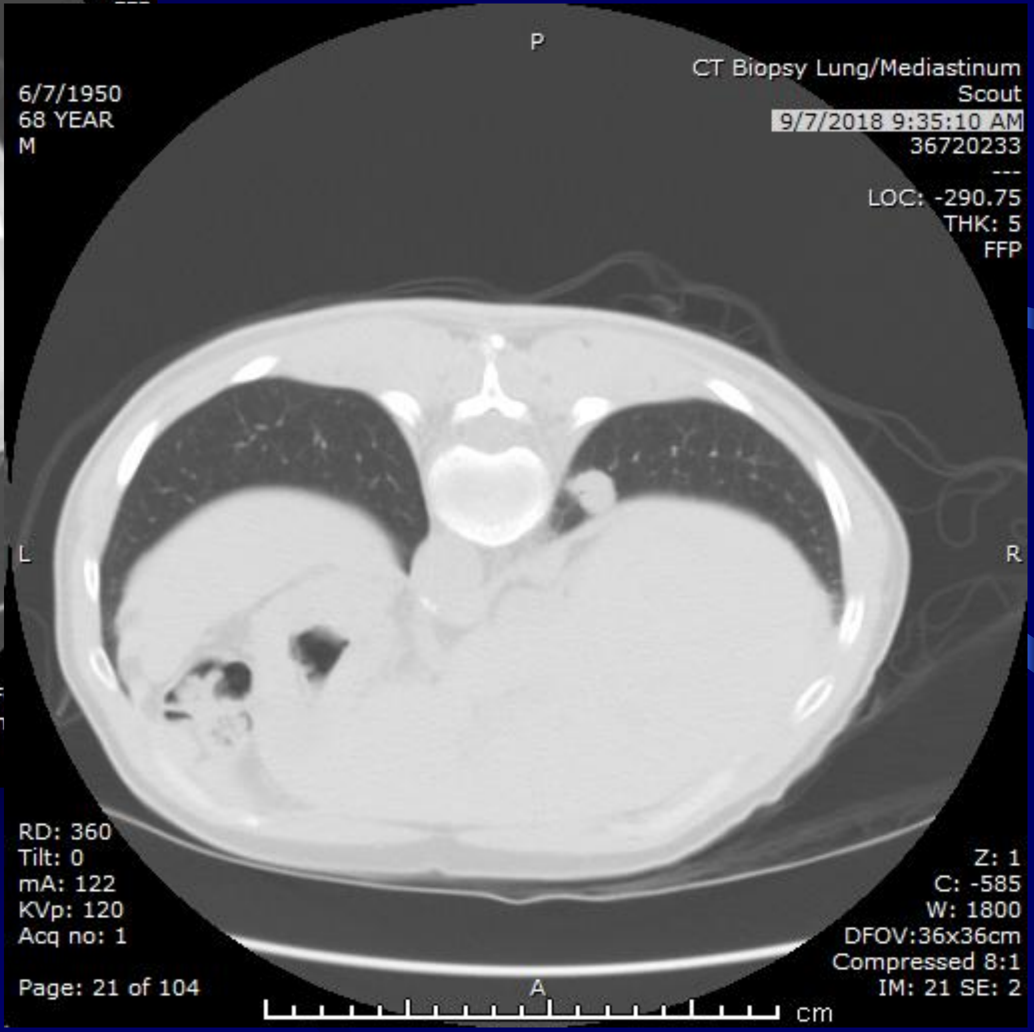
Case 2: imaging 9/7/2018- new lung nodules





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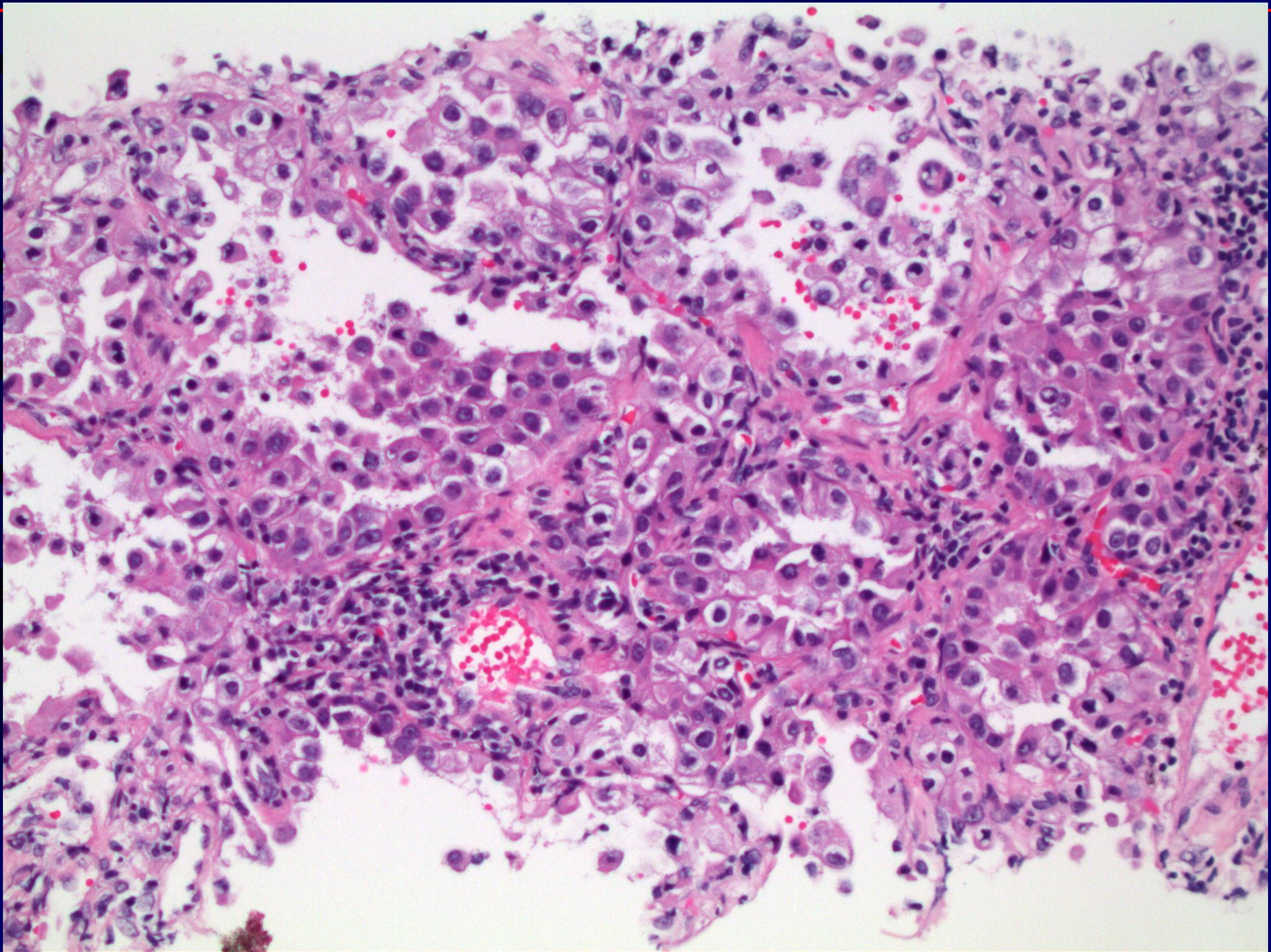
Case 2: CT-Lung biopsy- 9/7/2018



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NGS Target Gene Panel Lung Cancer

- 24 genes:
- Hotspot: AKT1, ALK, BRAF, CDK4, DDR2, EGFR, ERBB2, FGFR1, FGFR2, FGFR3, HRAS, JAK2, KRAS, MAP2K1, MET, MYC, NRAS, PDGFRA, RET, ROS1
- Copy Number Variation (CNV): CCND1, FGFR1, MET
- Fusion: ALK, MET, NTRK1, NTRK2, NTRK3, RET, ROS1

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Case 2: Clinical History (continued)

NGS testing disclosed the same exon 21 L858R mutation.

EGFR T790M mutation was also detected, conferring resistance to erlotinib, afatinib or gefinitib (EU) therapy.

Options for additional treatment: osimertinib (3rd generation EGFR TKI) or afatinib plus cetuximab.

Placed on osimertinib therapy with stable current stable disease.

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Acquired Resistance to TKI

- EGFR mutation positive tumors respond well to tyrosine kinase inhibitors (TKI's).
- The vast majority eventually develop resistance, 50-60% are due to secondary EGFR mutation T790M.
- Other mutations and transformation (small cell) account for another 20-25%; 15-20% unexplained.
- C797S mutation described for resistance to osimertinib.

Case 2: Take home points

- Re-testing of new localized or distant metastatic disease by NGS allows for comprehensive analysis for detection of new mutations such as EGFR T790M or C797S.
- The presence of EGFR T790M allows for changing therapy to osimertinib with likelihood of response.
- Resistance mutations continue to evolve with each generation of TKI.
- Similar approaches may be taken for kit mutations in the treatment of GIST or progression mutations in hematologic neoplasms.

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Case 3

- This 49 year old woman presents with a 2 month history of a painful lump over the left eye.
- She awoke on the morning of admission with left eyelid and cheek bone swelling and dizziness.
- Examination revealed left frontal and maxillary swelling.
- CT and MRI disclosed a 3-4 cm mass involving the frontal bone and adjacent soft tissues. A partial resection was undertaken by neurosurgery.

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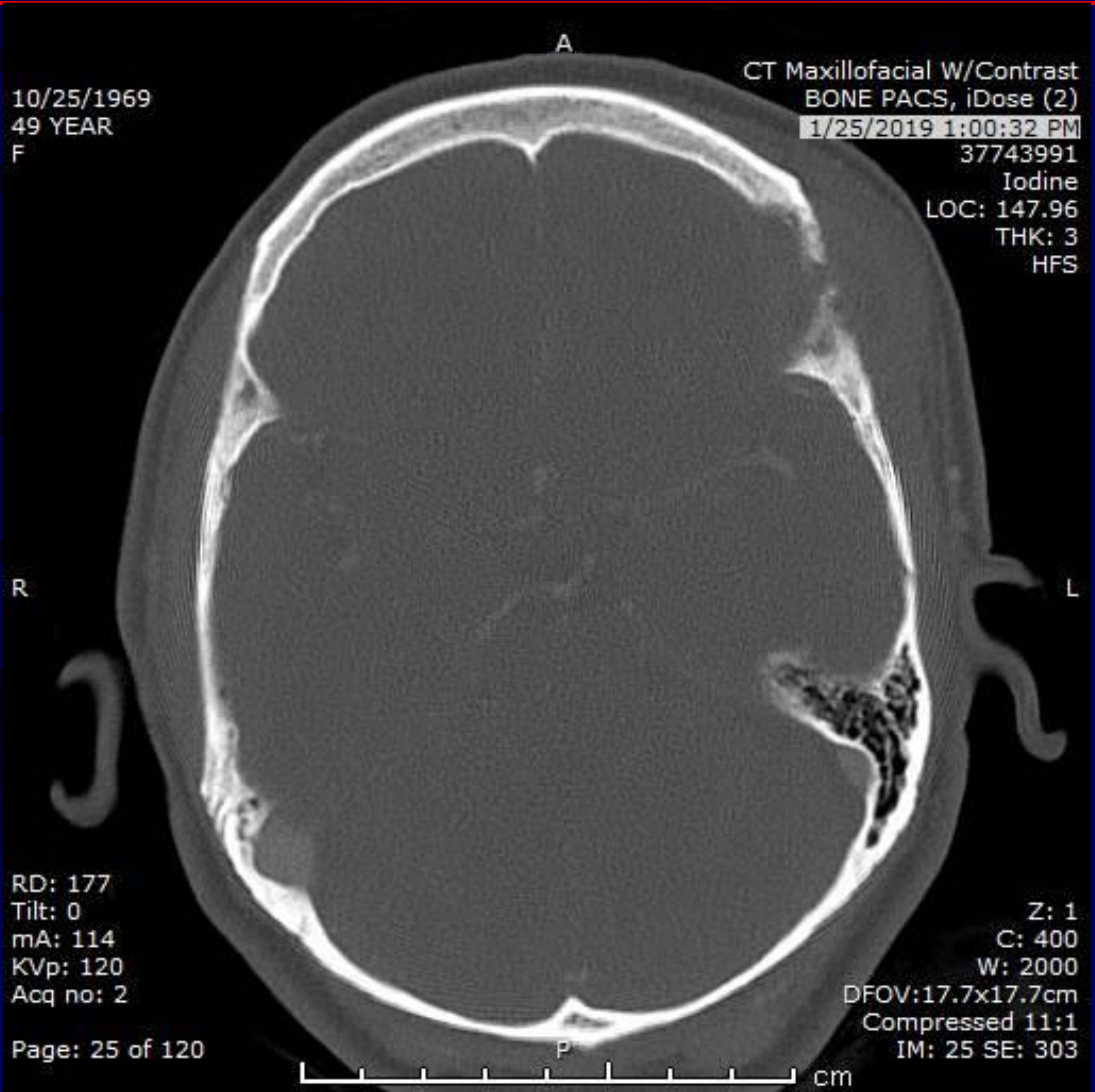
Case 3 CT



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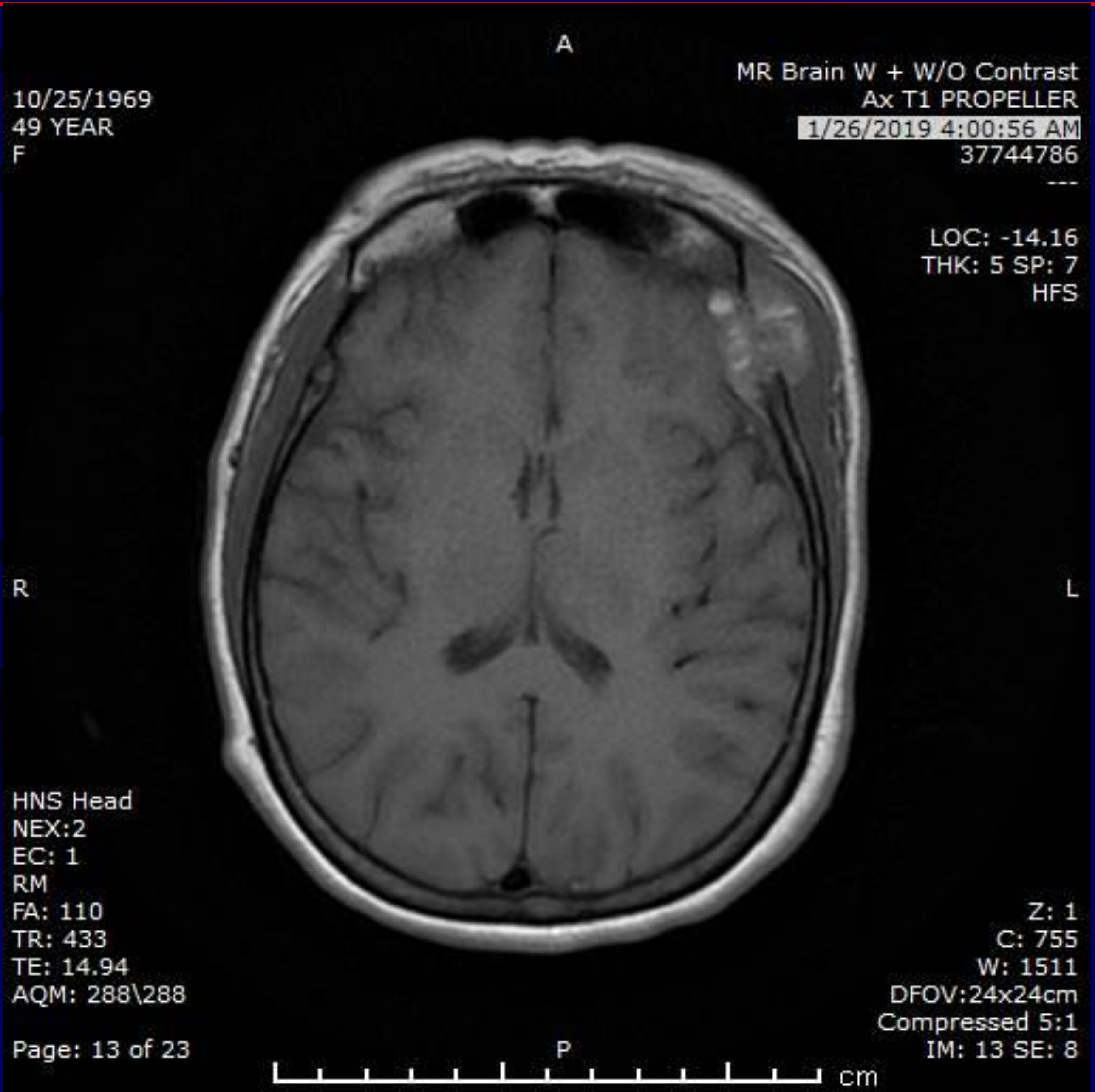
Case 3 MRI- Axial T1



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Case 3 MRI- post contrast

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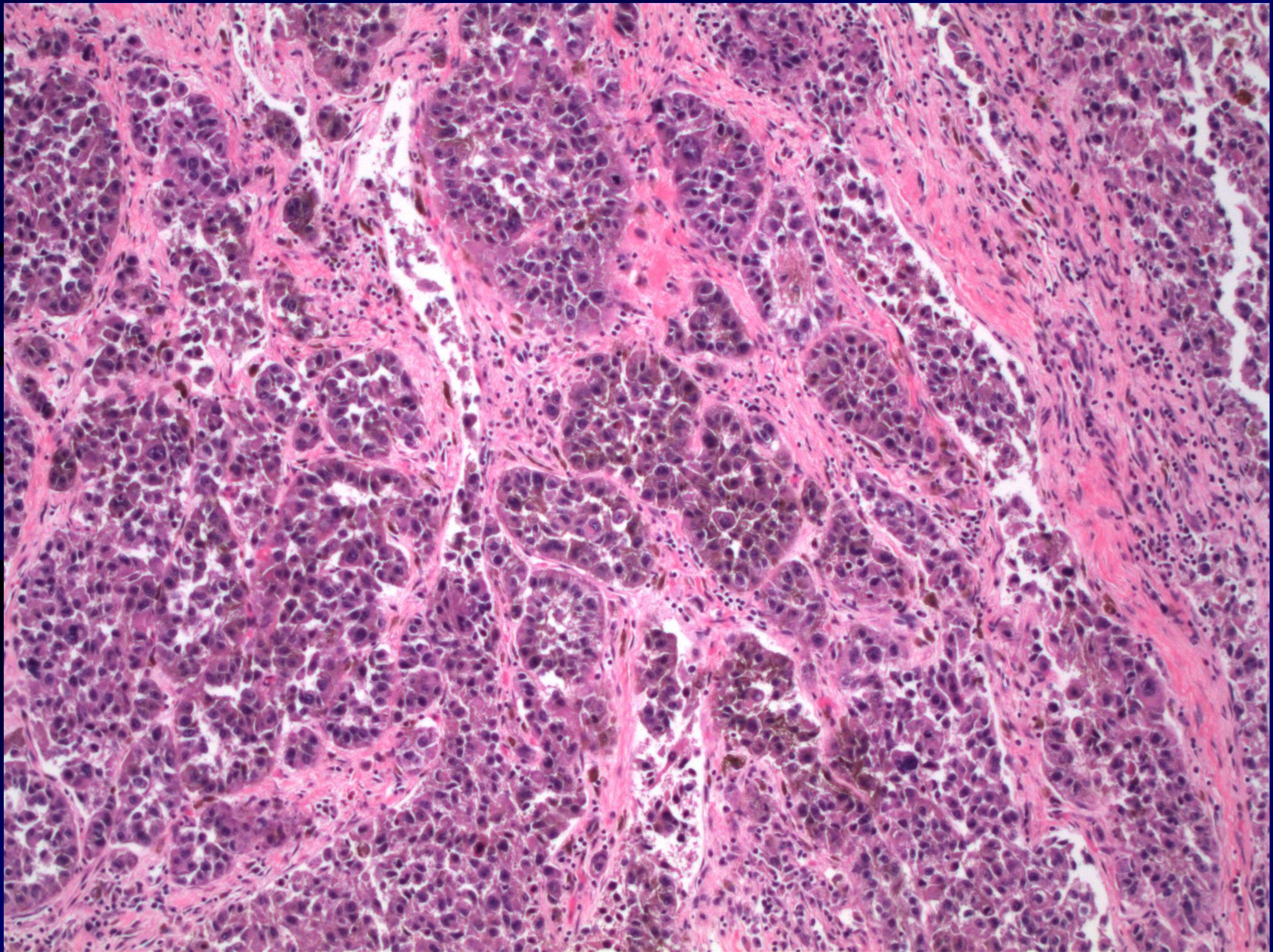
Case 3 Pathology



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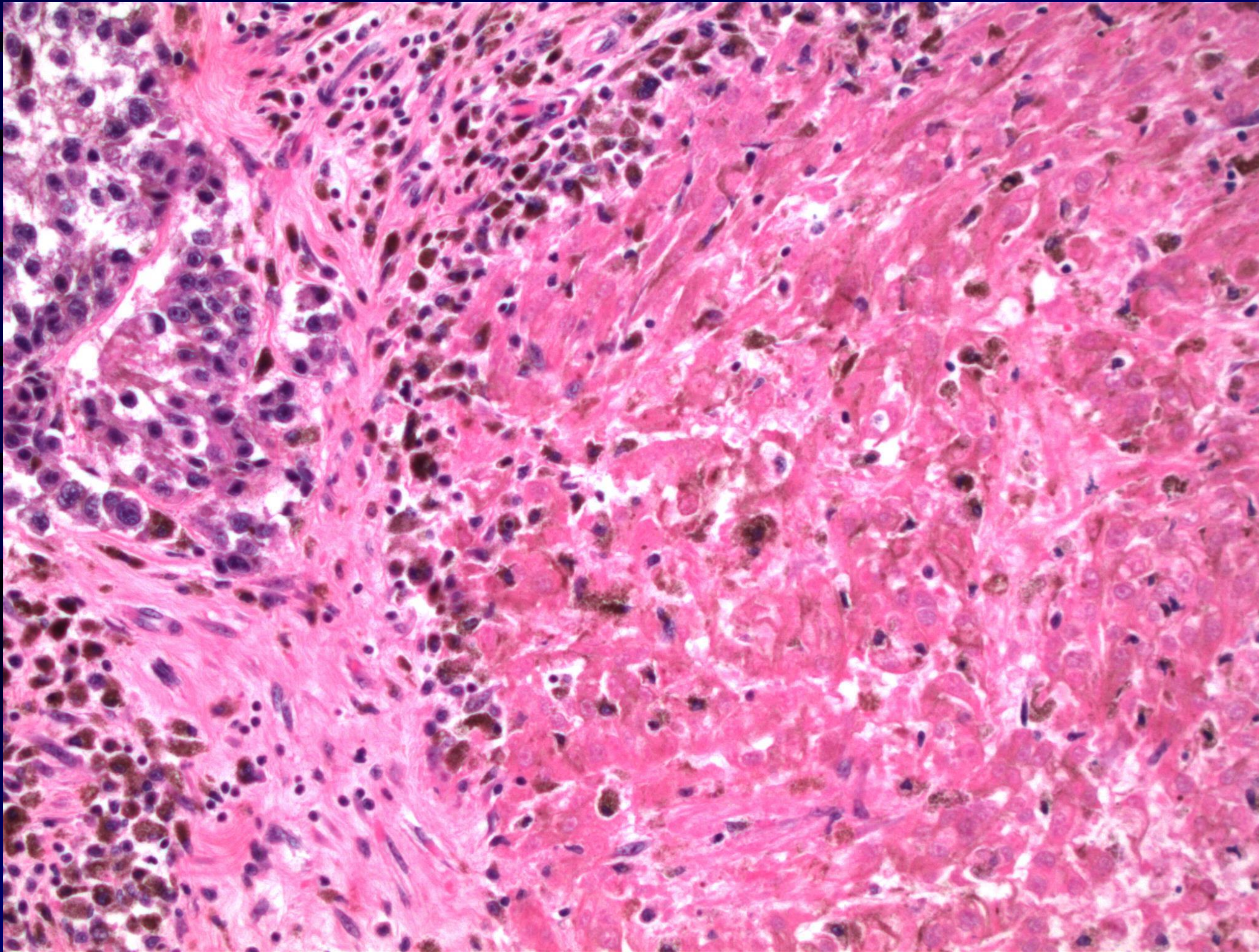

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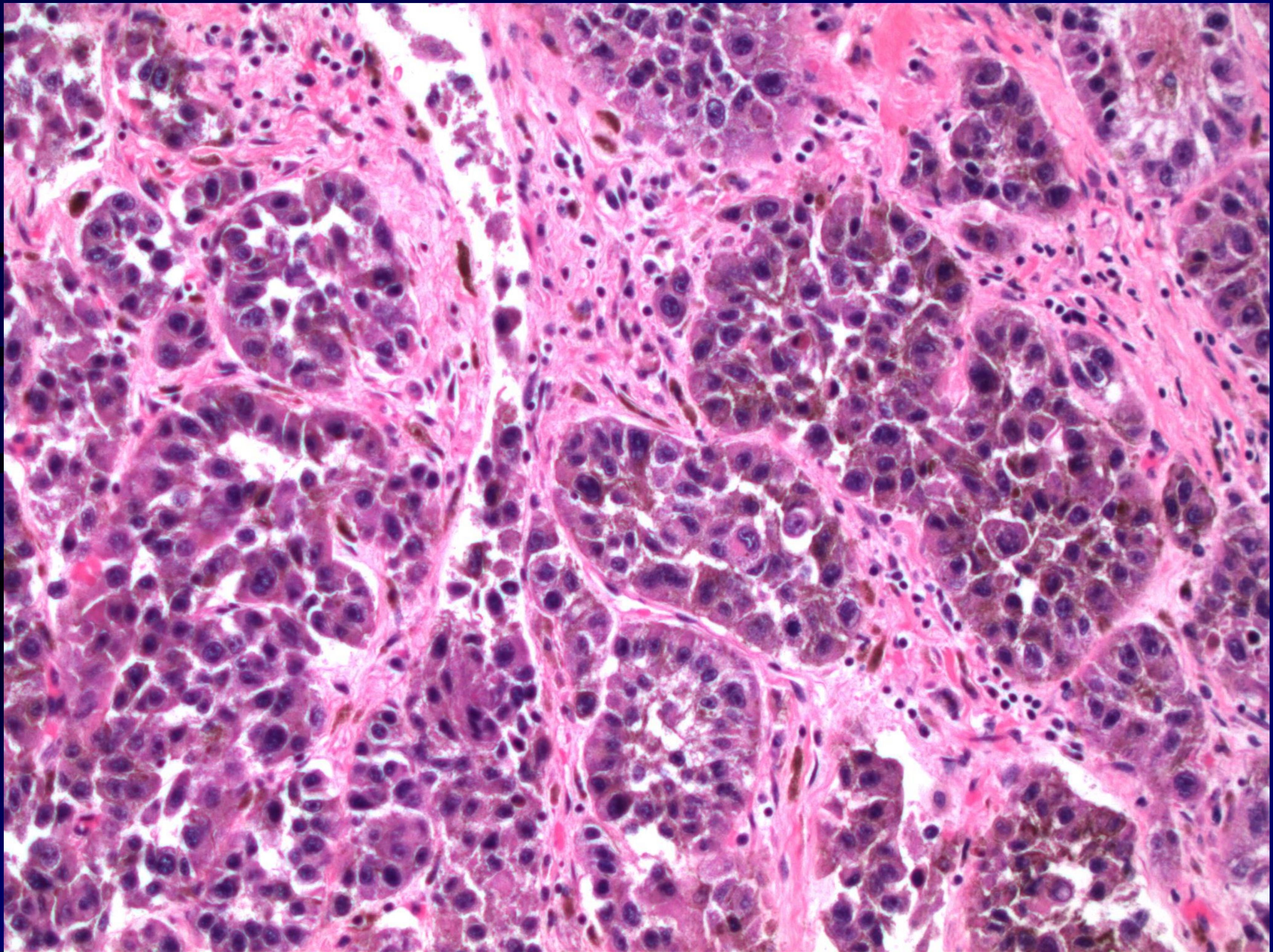

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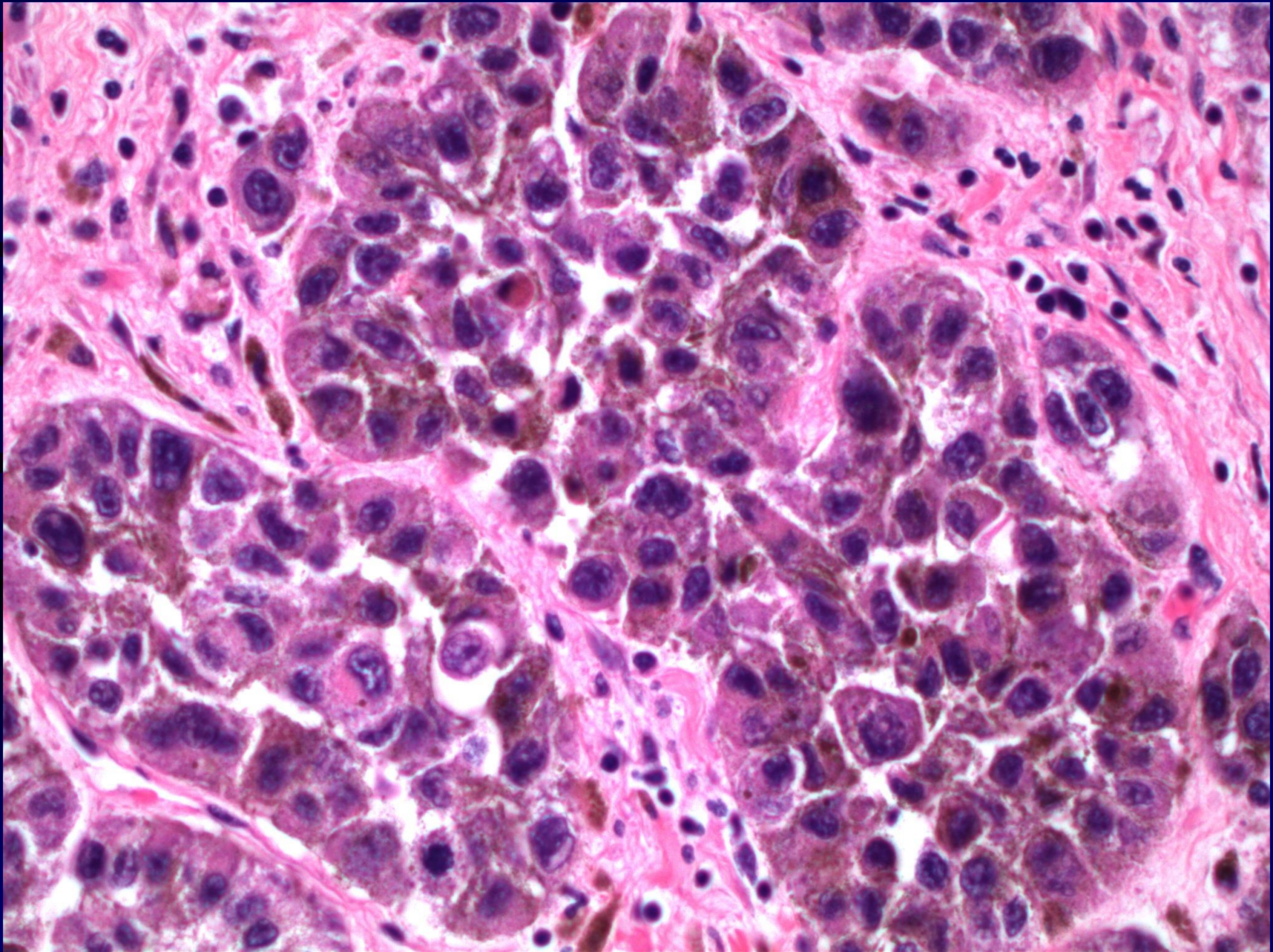

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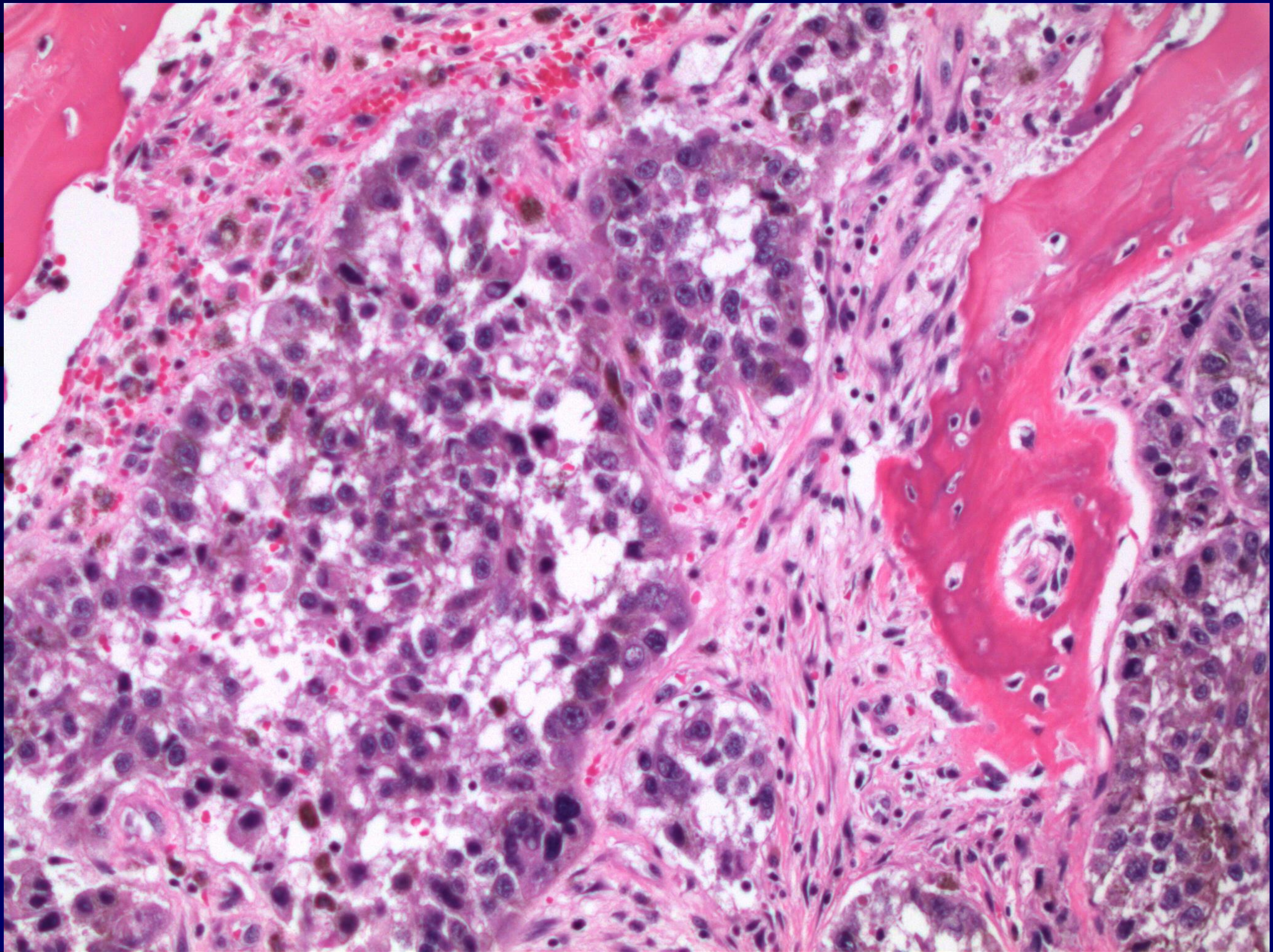
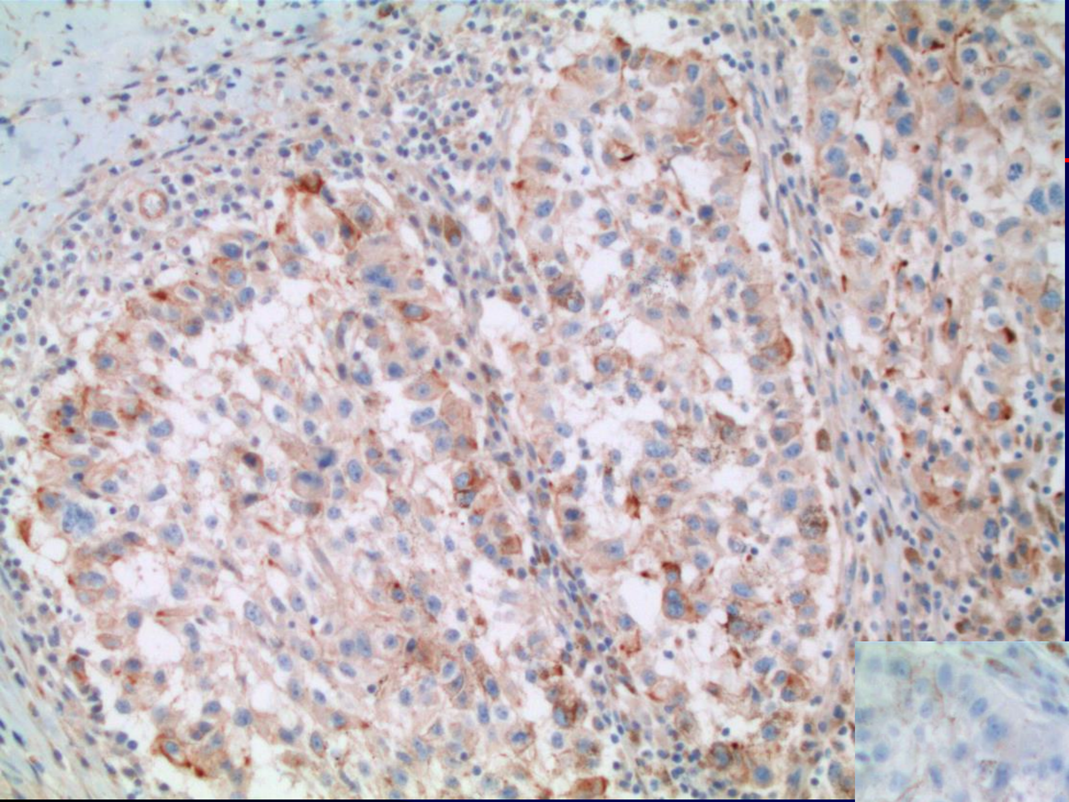
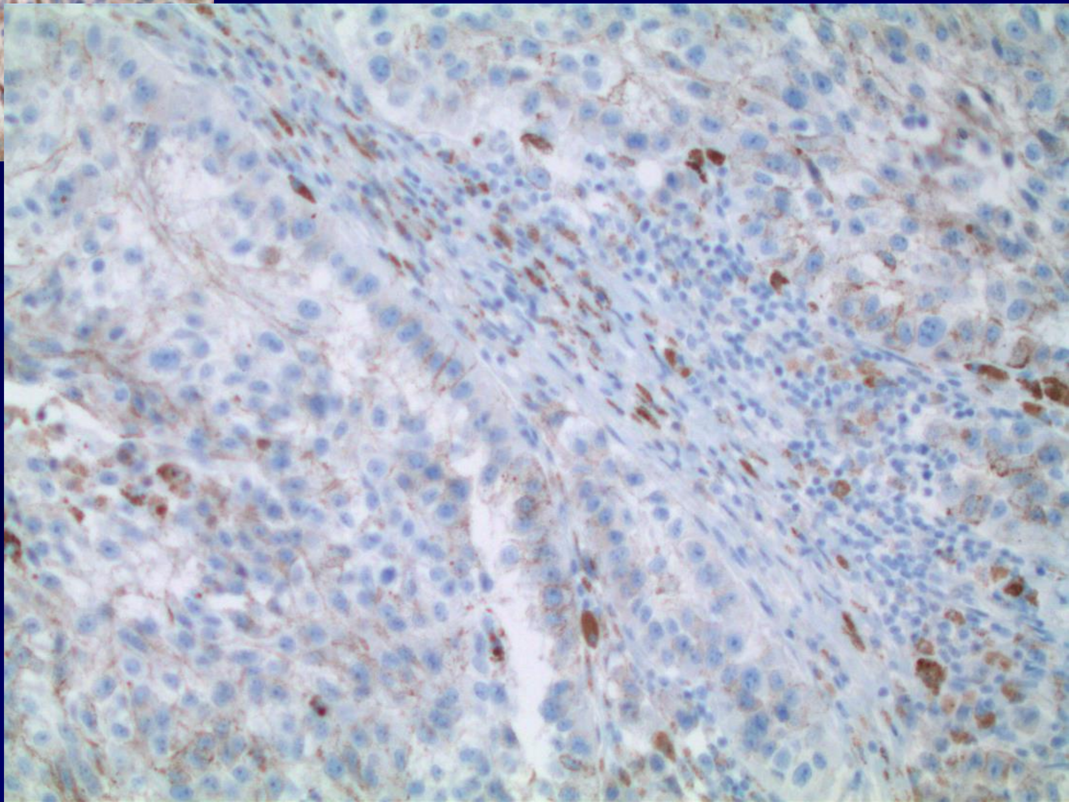
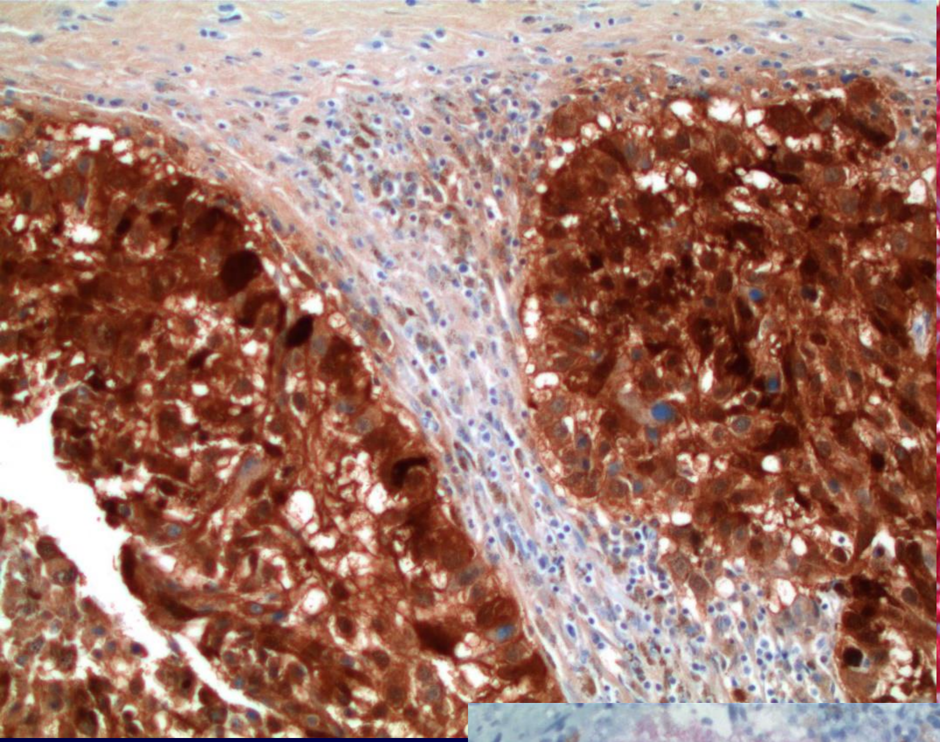




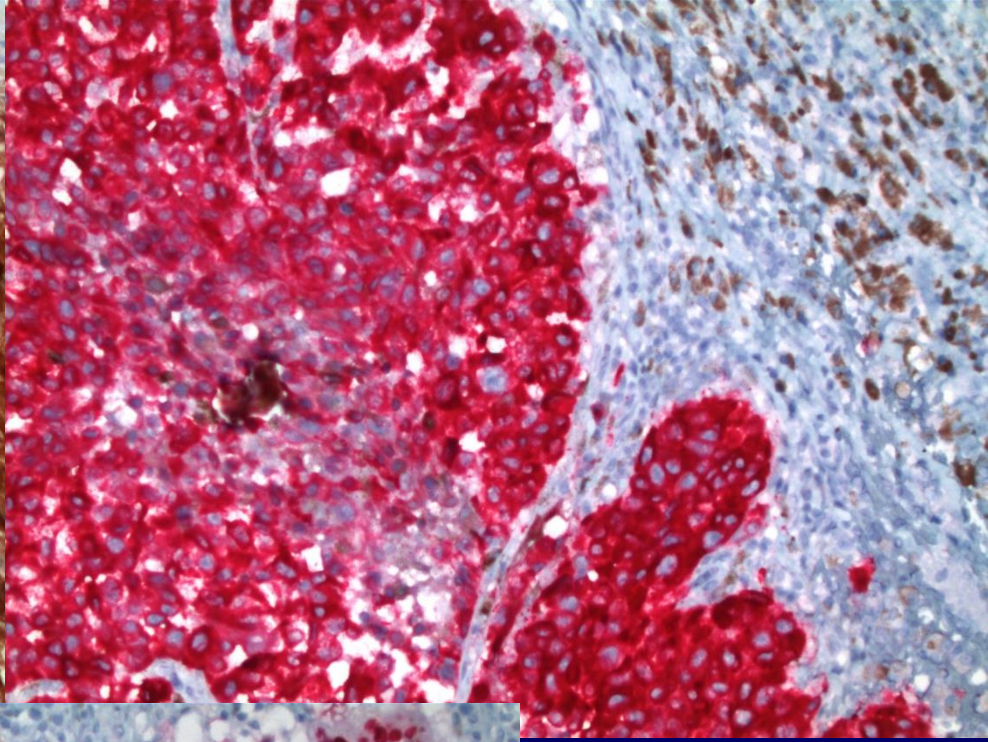
CK Oscar



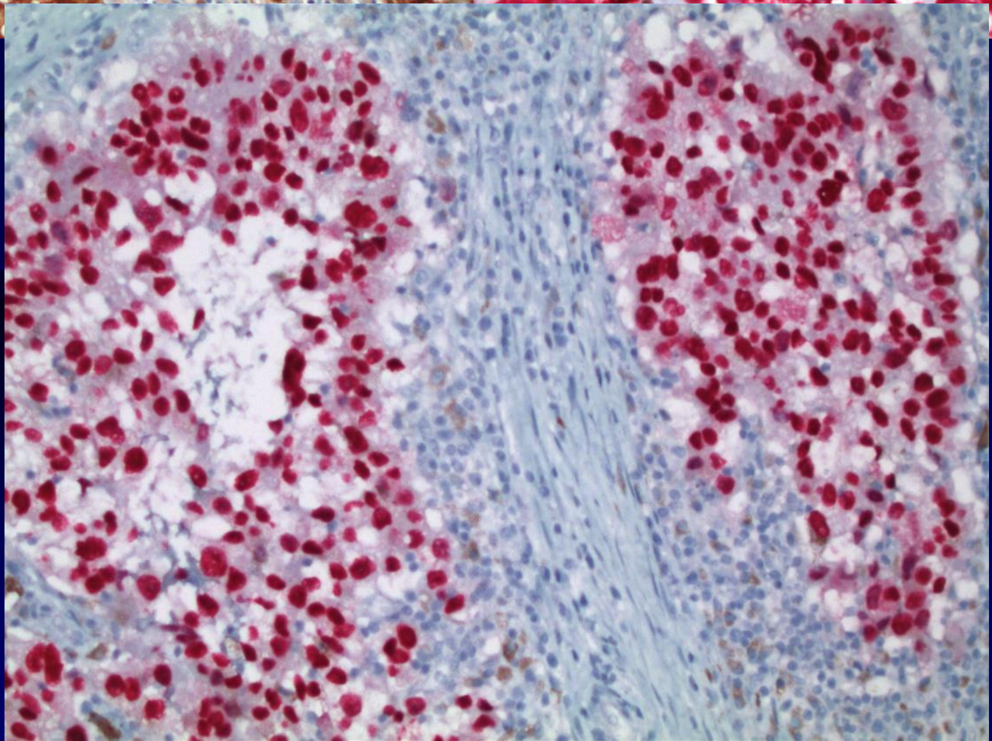
EMA



S-100



MelanA



Sox-10

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Case 3- Diagnosis

- Malignant melanoma, likely metastatic.
- Melanoma NGS analysis revealed BRAF V600K and V600M mutations.
- BRAF V600E mutation analysis by IHC (VE1 antibody) was negative.

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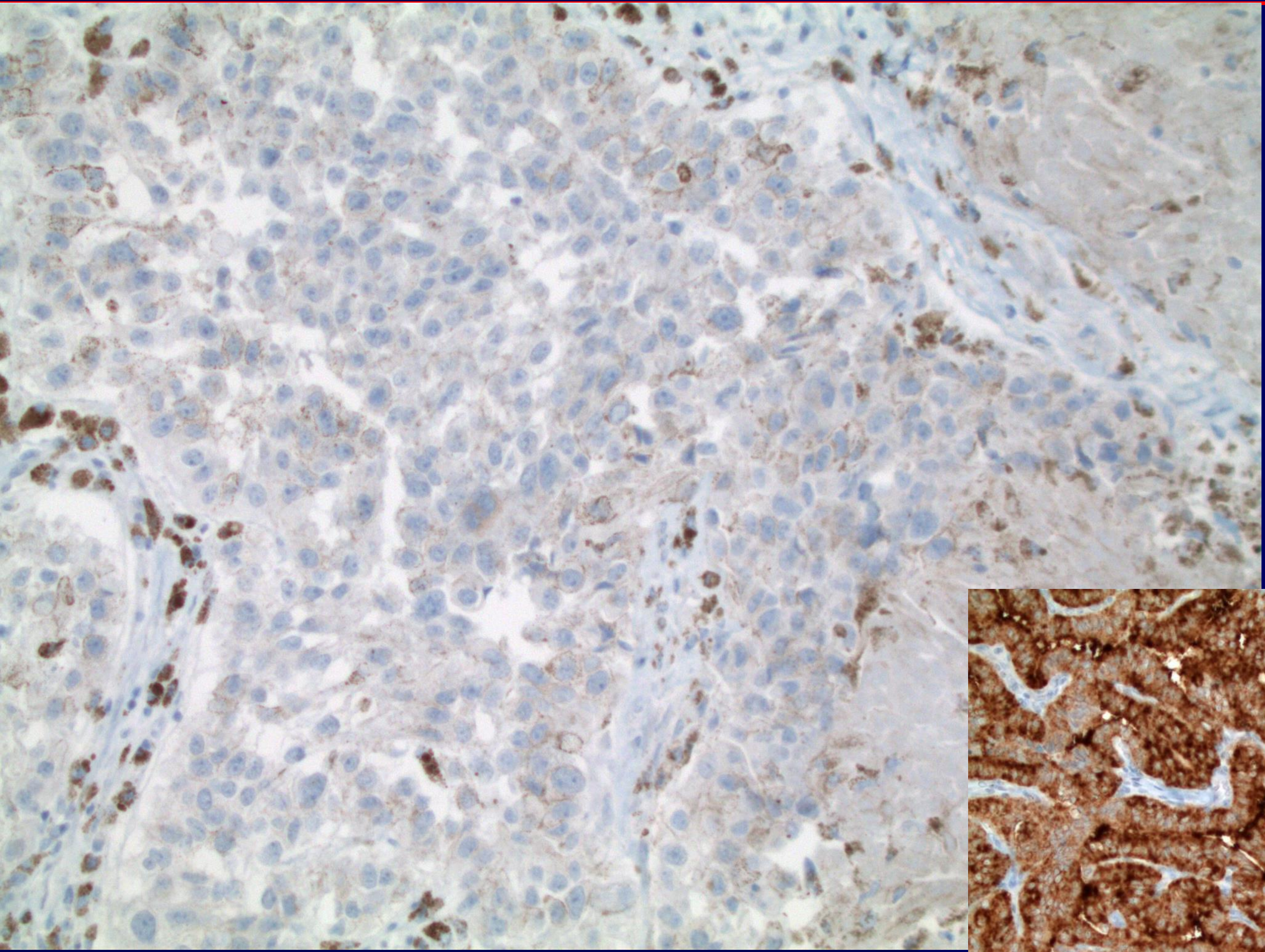
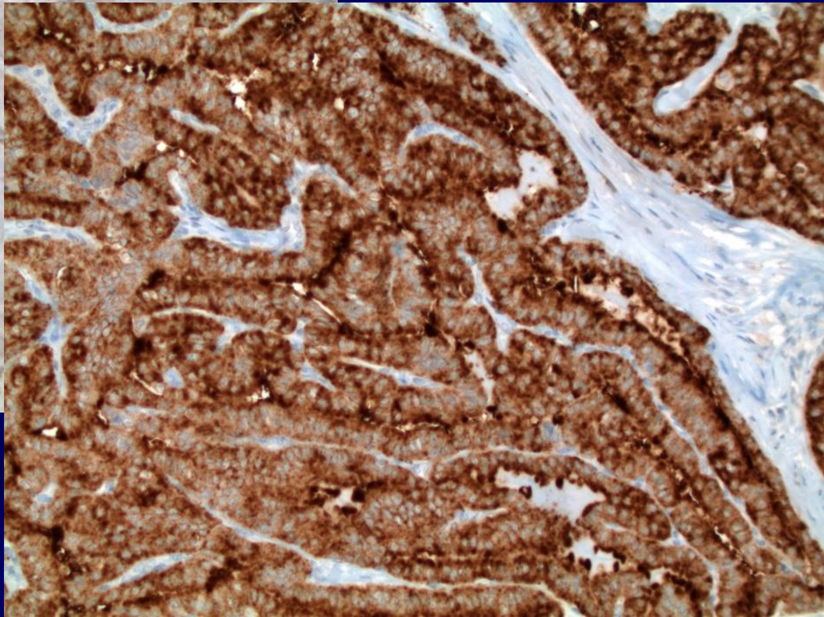
Case 3: VE1 (BRAF V600E mut. Ab): neg.



Case 3: melanoma negative



BRAF mutated Thyroid Papillary ca

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NGS Target Gene Panel Melanoma

- 11 genes:

- BRAF (40-50%, most common in cutaneous)
- NRAS (13%, 15-20% of cutaneous)
- MEK (6%); CTNNB1 (2-3%)
- KIT: 2-3% but in 15-40% of acral melanomas
- GNA11 (2%) and GNAQ (1%): 80% of uveal
- CCND1, CD4K, ERBB4, MAP2K1,

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Case 3- Take home points

- Current treatment of metastatic melanoma:

- Nivolumab (anti-PD-1) + ipilumimab (anti-CTLA-4) (+/- PD-L1 analysis with Dako 28.8 assay for Nivolumab) or Pembrolizumab (no specific assay required).

- Vemurafenib/ dabrafenib: response predicted by BRAF V600E, M or K mutations. MEK inhibitors (trametinib and cobimetinib) also used.
- Dabrafenib + trametinib vs vemurafenib + cobimetinib

- V600E IHC is predictive of BRAF mutation if strongly immunoreactive; if negative, mutation testing is required.
- Kit mutated tumors may respond to imatinib.

Case 4: Pulmonary Carcinoma

- This 73 year old was diagnosed with pulmonary adenocarcinoma, gr2 in 2011. Initially treated by LL lobectomy, Stage T1b N1.
- 1-7-13 to 1-10-13 SBRT for R lung local recurrence.
- 12-08-15 right lung nodule biopsy
- 12-18-15 PET CT: progression of disease with numerous pulmonary nodules, no FDG avid disease below the diaphragm
- 1-25-16 MRI Brain: no evidence of malignancy

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Case 4: Pulmonary Carcinoma (2)

- *1-29-16: Carboplatin and Pemetrexed 6 cycles (completed 5-13-16)*
- 2-22-16 Outside molecular testing: negative for EGFR, EML4-ALK, KRAS mutations;   Low PD-L1 (2-4%)
- 3-31-16 CT Chest: numerous bilateral metastases again seen, no lymphadenopathy, stable
- 2-23-17 CT Chest: interval increase in size of pulmonary nodules
- 3-07-17 MRI Brain: stable right parietal bone 1.2 cm enhancing osseous lesions, no intracranial metastases
- *3-10-17 changed to Nivolumab 240mg IV Q14*

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Case 4: Pulmonary Carcinoma (3)

- 8/31/17 CT chest: Mixed response. Overall stable disease.
- 11/27/17 CT chest: progression in lungs when compared with previous CTs.
- 12/7/2017: R lung met biopsy, consistent with lung adenocarcinoma.

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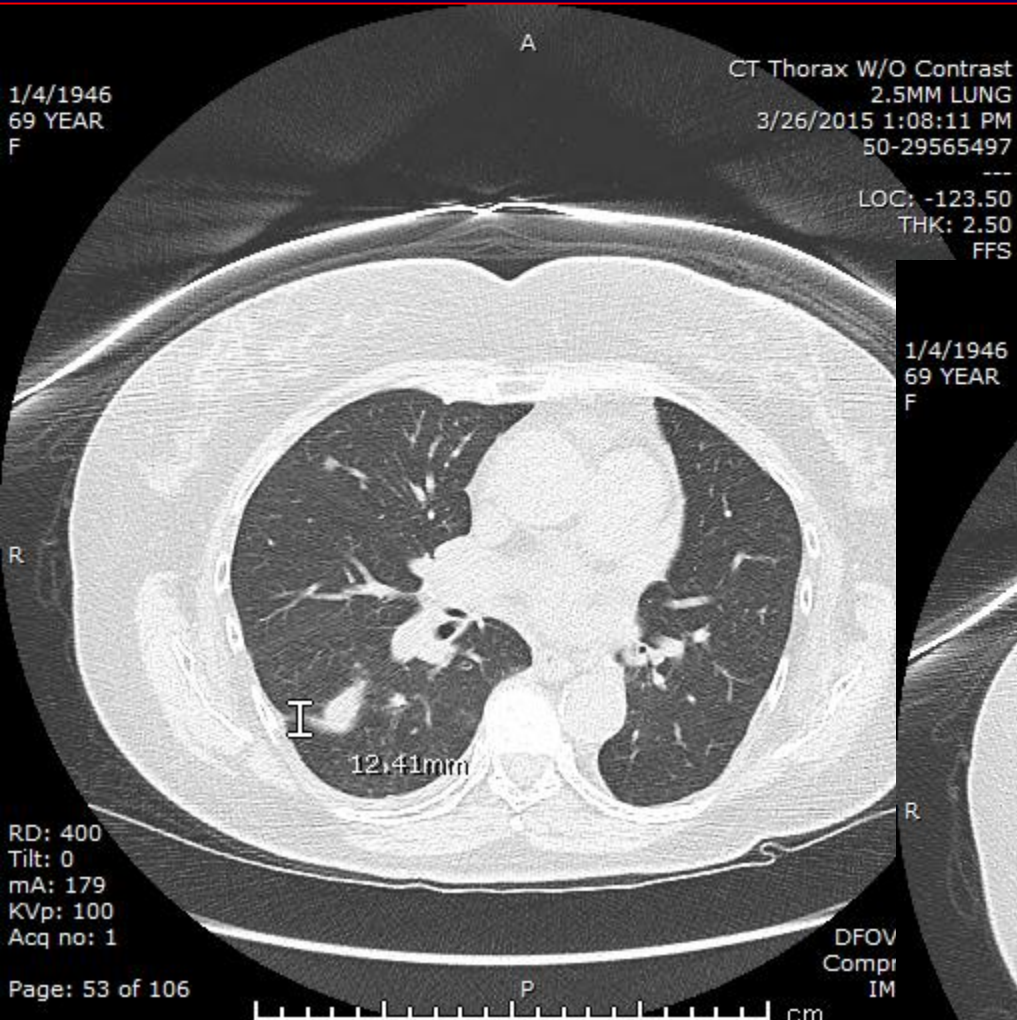
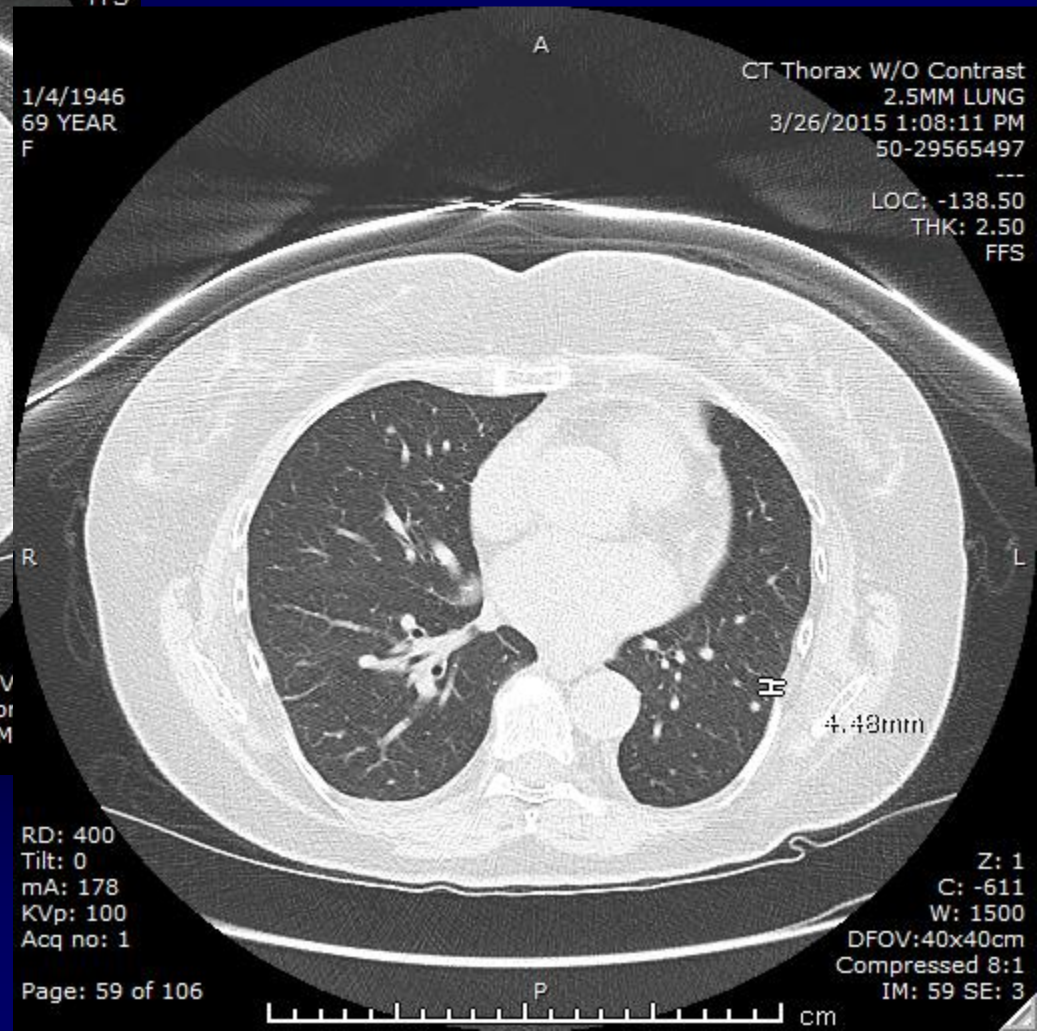
Case 4: 3/2015- bilateral nodules





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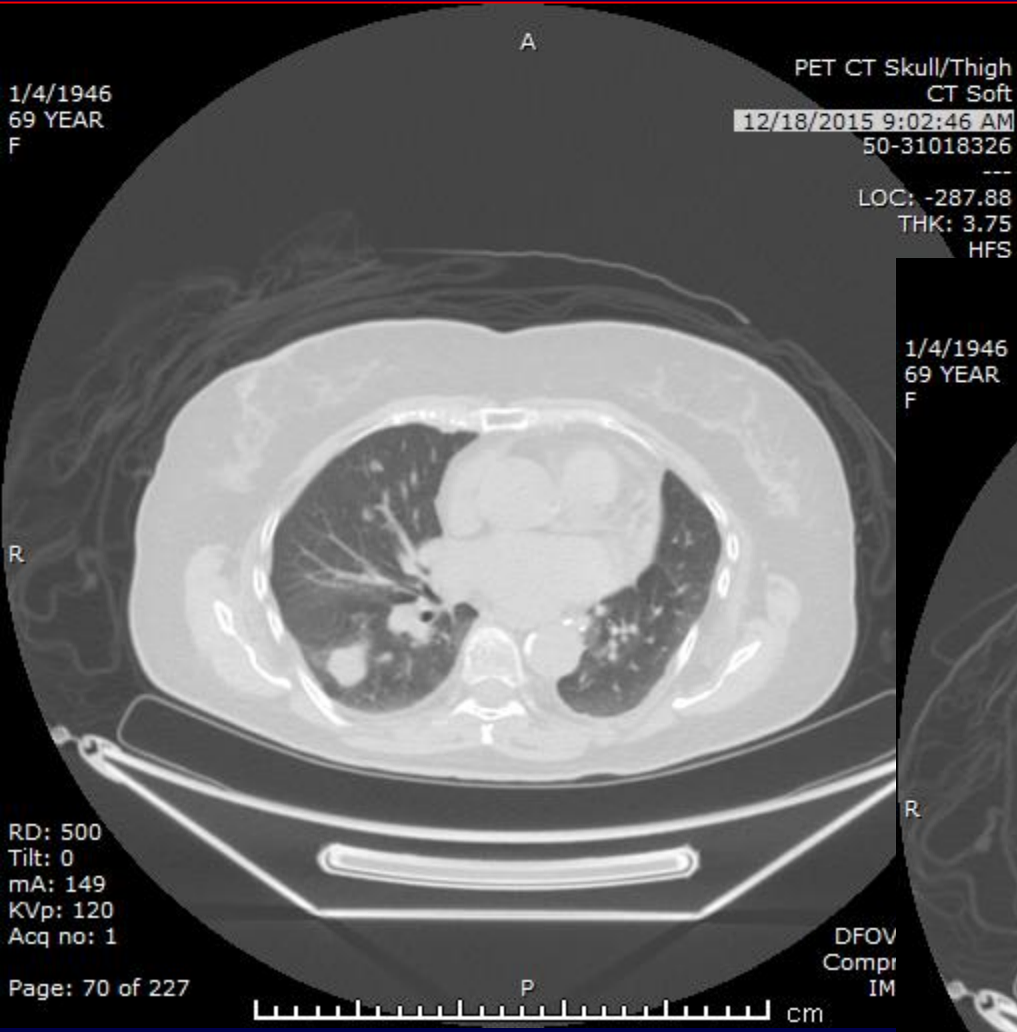
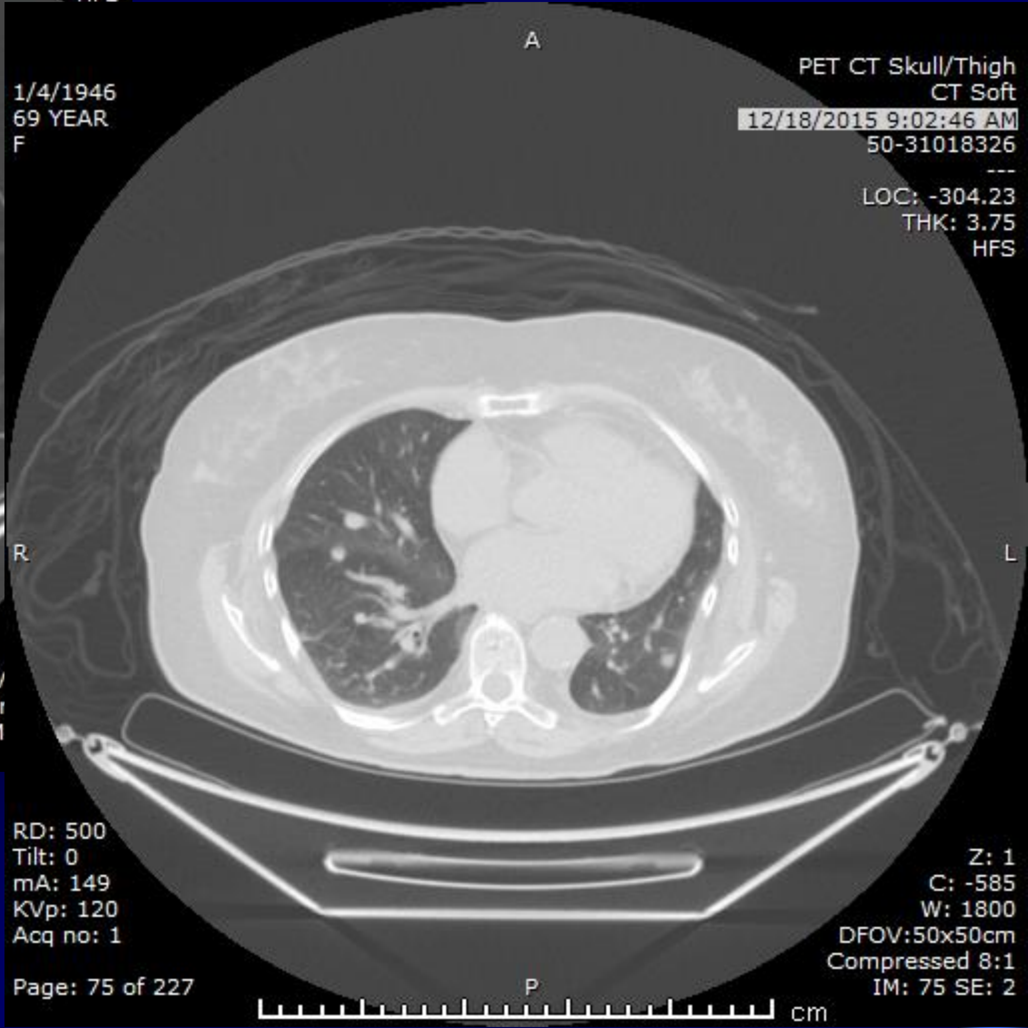
Case 4: Progression- bilateral nodules





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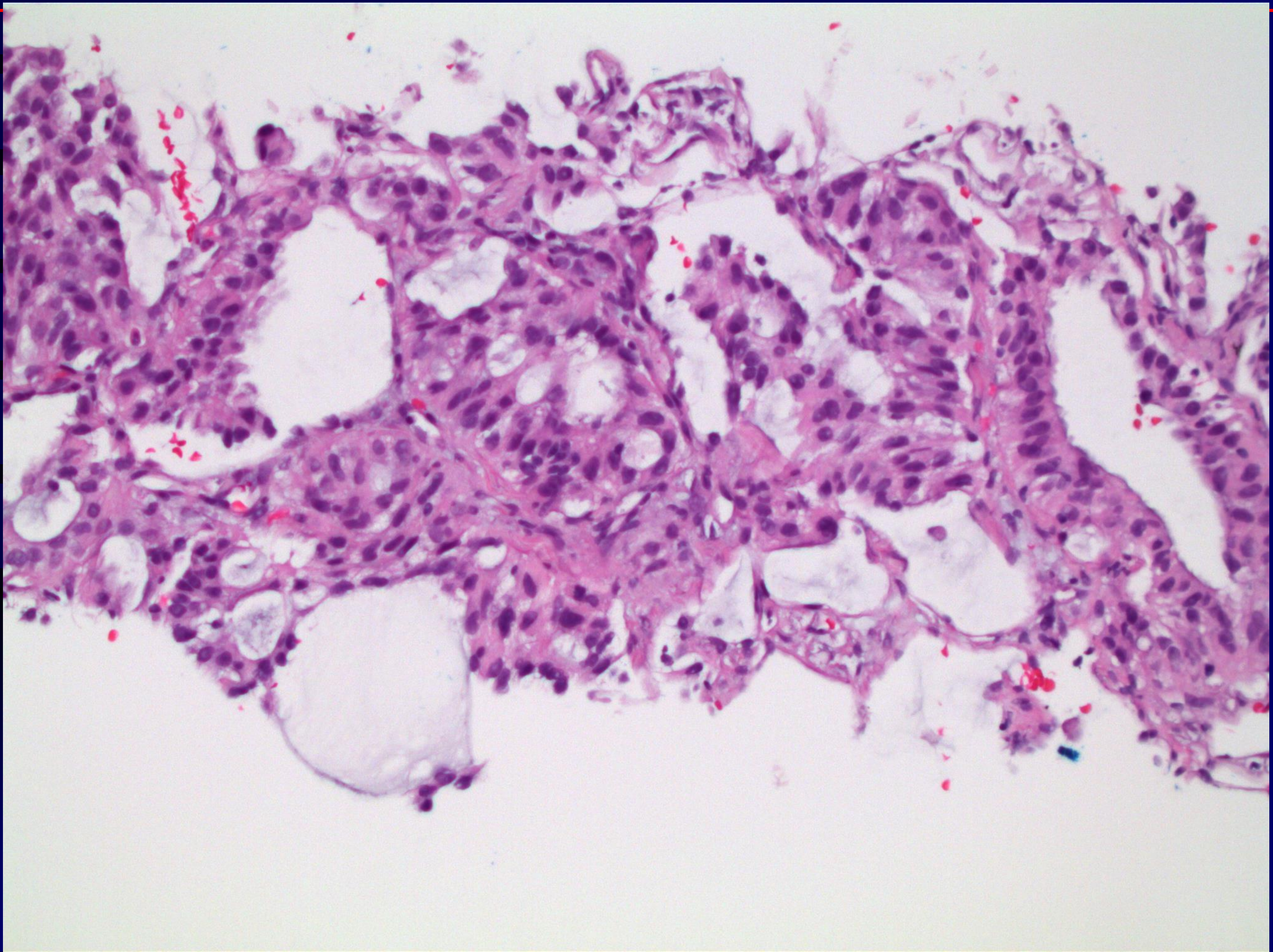
Case 4: Pathology- lung biopsy-1-2017



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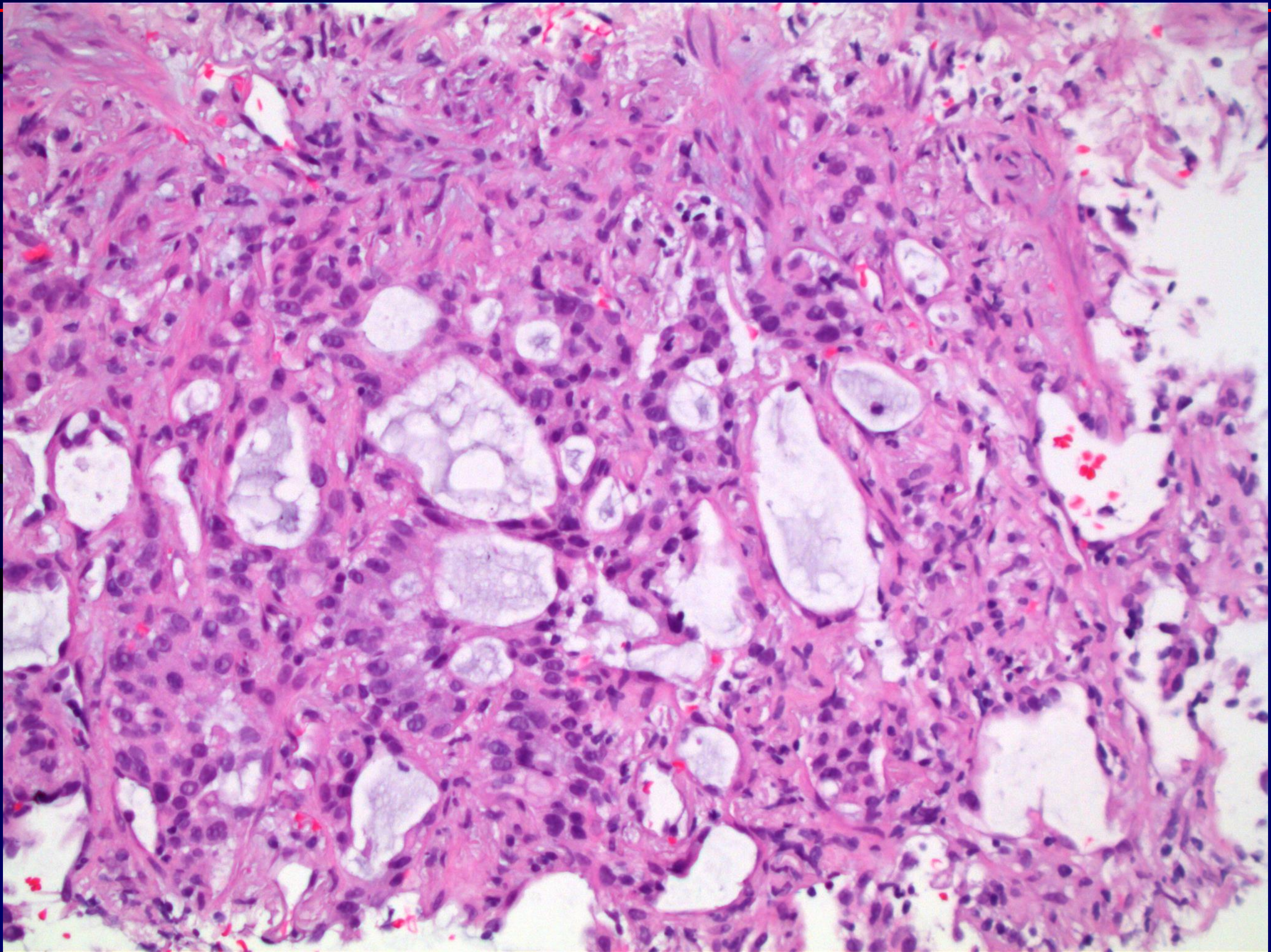
Case 4: Pathology- lung biopsy-1-2017



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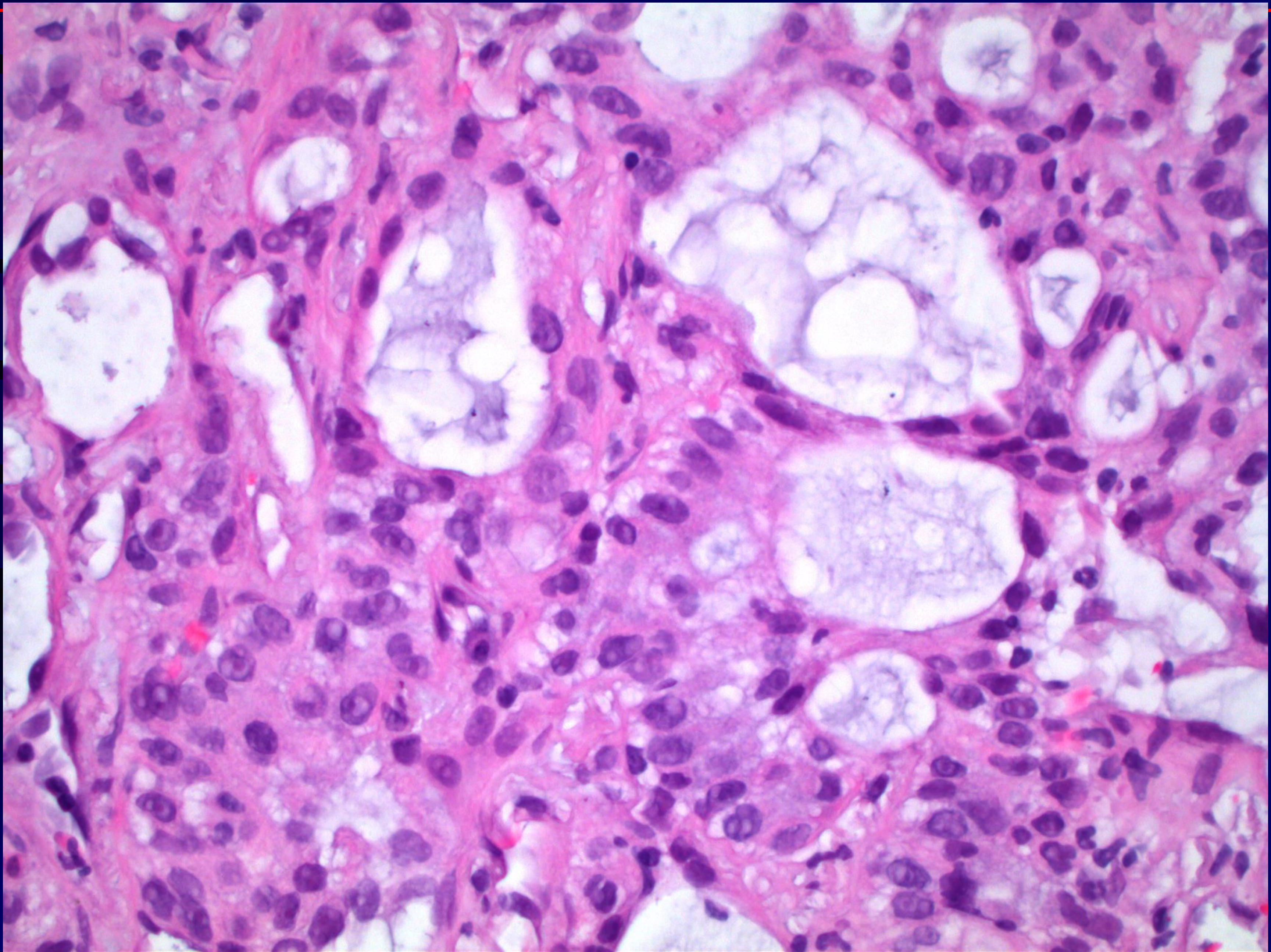
Case 4: Pathology- lung biopsy-1-2017



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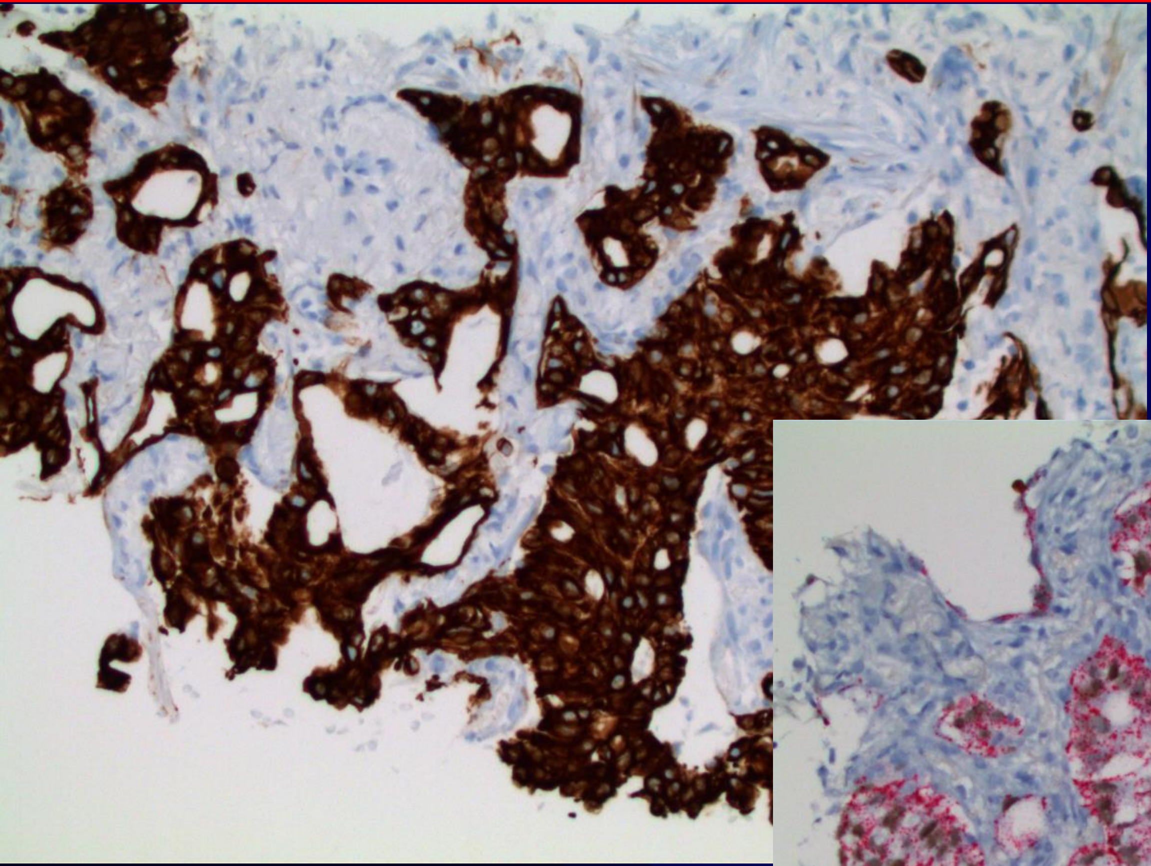
Case 4: Pathology- lung biopsy-1-2017



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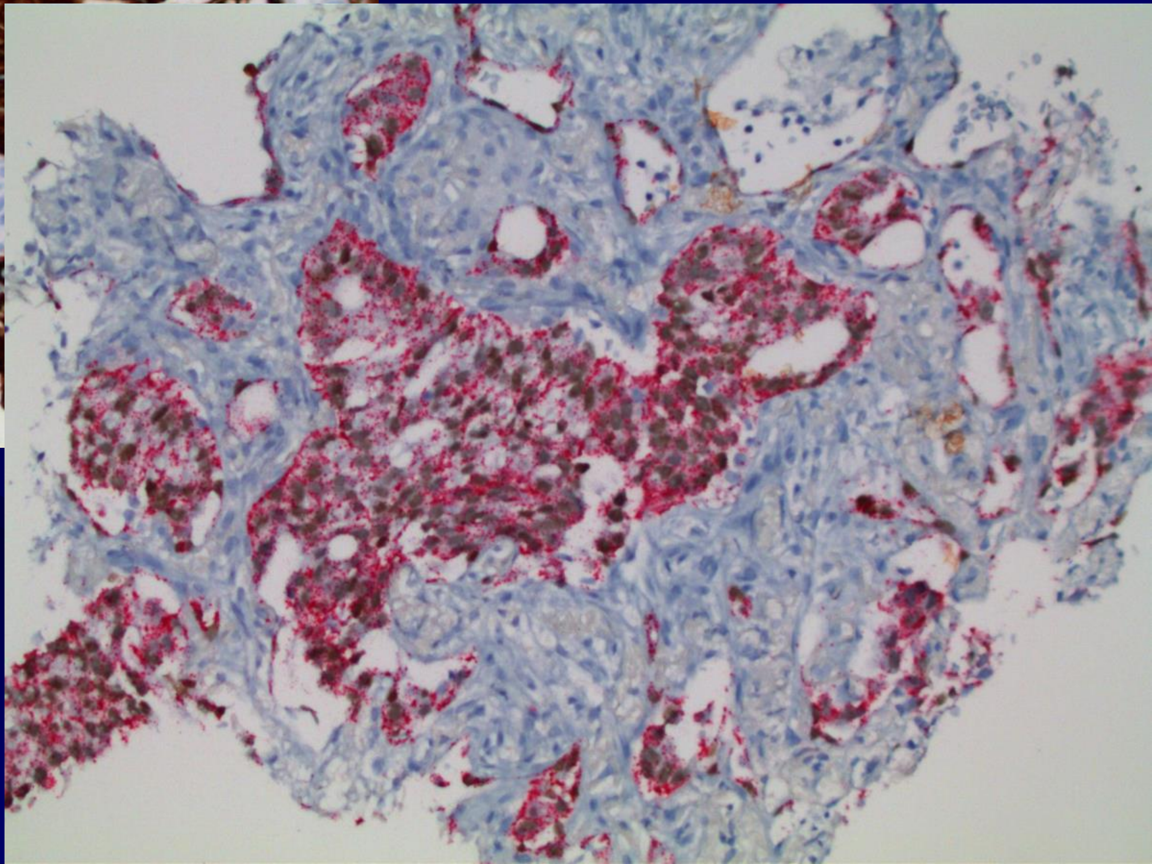

TTF-1/Napsin A

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Case 4: Molecular analysis by NGS

- *1/22/2018: NGS showed EML4-ALK- translocation.*
- *Low PD-L1 (1-4%).*
- *Alectinib 600 mg bid was started.*
- *Stable disease through 1/2019.*

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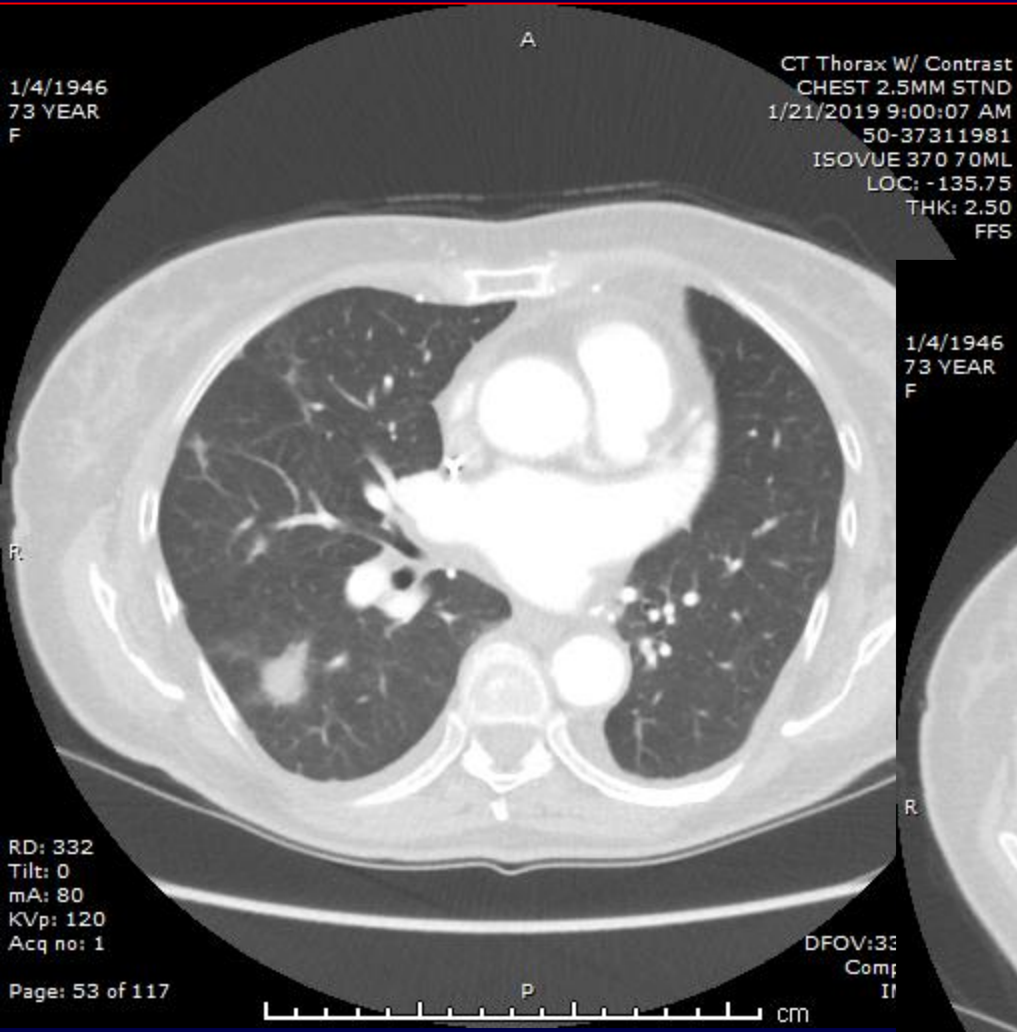
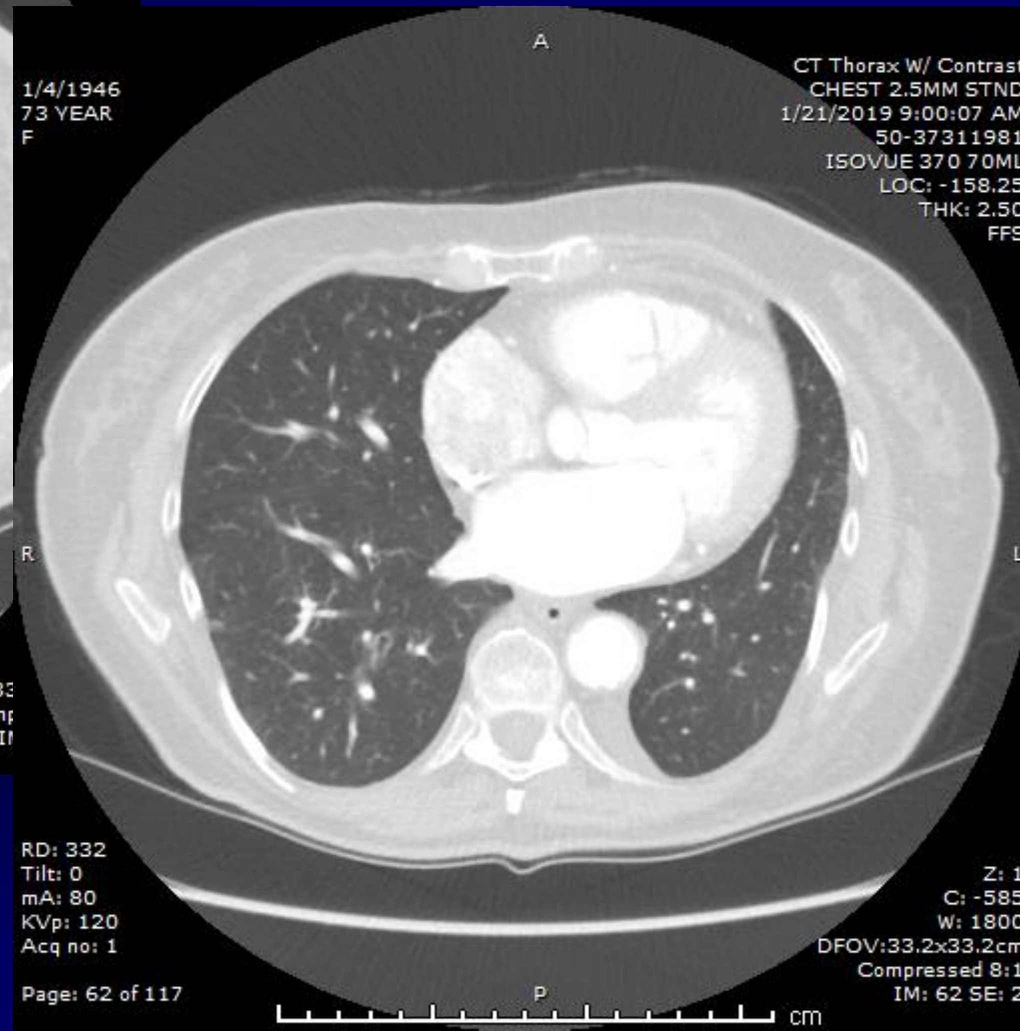
Case 4: 1-2019- stable disease





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Case 4: Take home points

- Targeted NGS for NSCLC detects the same gene fusions as FISH or PCR, with added sensitivity as compared to FISH (dependent on cell sampling, technical factors of the assay).

- Repeat testing with NGS covered a more broad panel than EGFR, ALK analysis from 3 years prior.

- Repeat testing with NGS uncovered the EML4-ALK fusion not detected by FISH at the time of primary diagnosis; allowing for directed single agent TKI therapeutic response.

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THE END