Detection failure of BRAF V600E by Oncomine Dx Target Test in Lung Cancer

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Objective

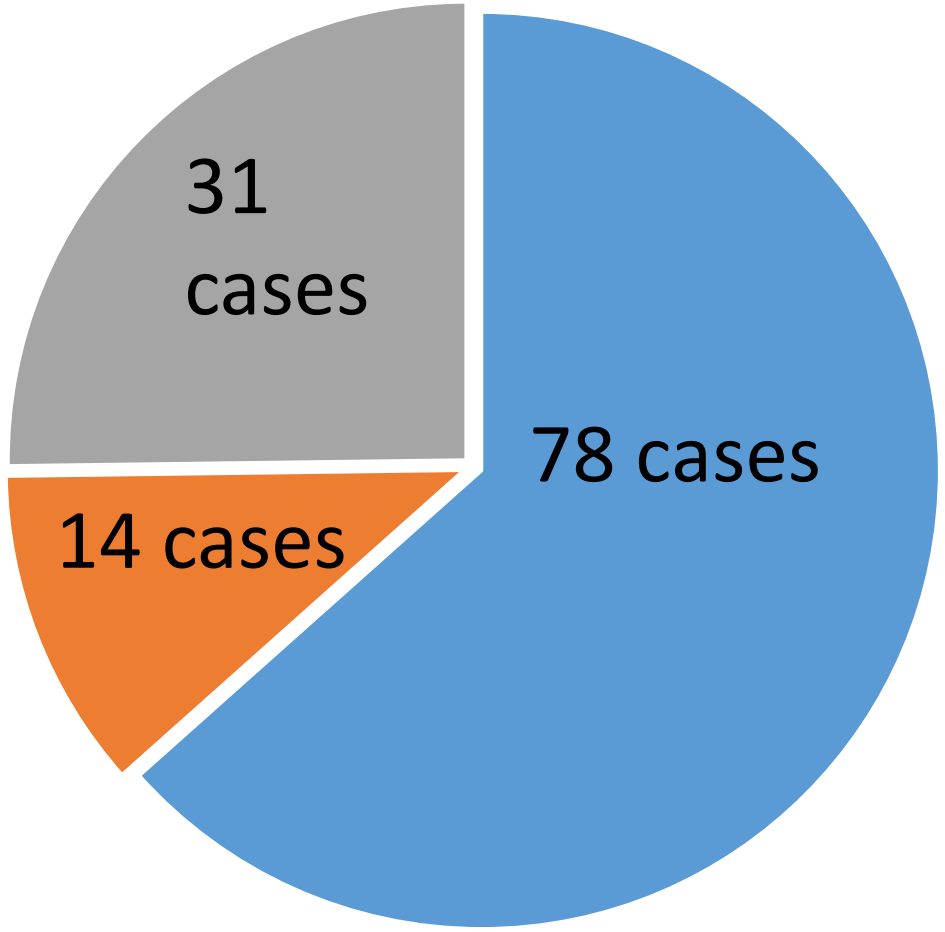
- To identify the causes and countermeasures for BRAF V600E test failures with the Oncomine Dx Target Test CDx system (ODxTT).

Subject and Method

- The study included 123 cases of ODxTT in 2019-2020, especially those that were not determinated.

Failed tests were 44 of 123 cases (36.6%).

The results of the single-plex BRAFV600E test in 123 patients with non-small cell lung cancer at Tsuchiura Kyodo General Hospital in 2019-2020.

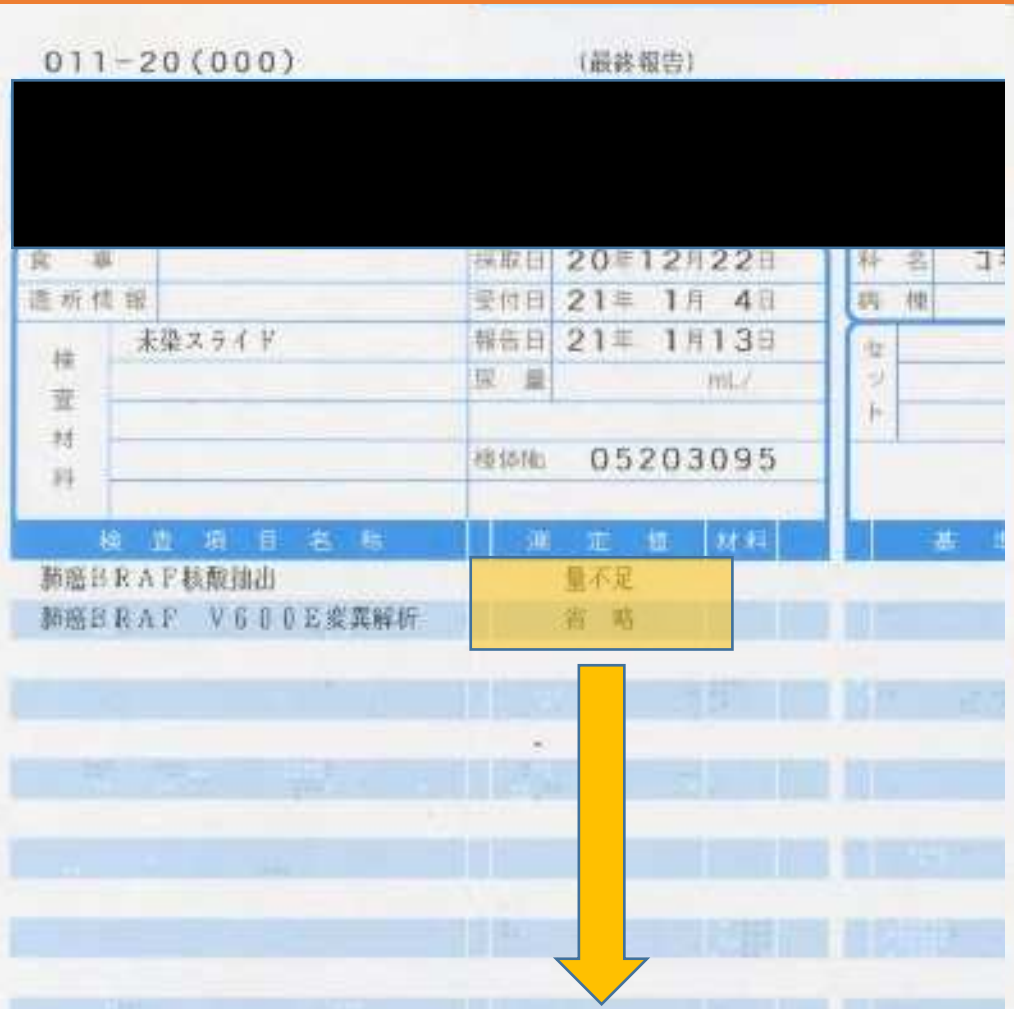


*Not the results submitted as multi-plex ODxTT

Insufficient volume and not determinated, 36.6%, and no positive cases.
When submitting in single-plex at our hospital, for example, EGFR/ PD-L1 →ALK→ROS1/MET →BRAF→、thin slices are made in consideration of frequency and other factors.
It is estimated that in many cases, 51 thin slices were routinely performed for the above 6 tests, and the 41st-51st thin slice was submitted to BRAF.

Specimens were not being sent to BRAF V600E, which has a low frequency.

BRAF V600E test failures include insufficient volume and not determinated



011-20(000)　(最終報告)

[redacted]

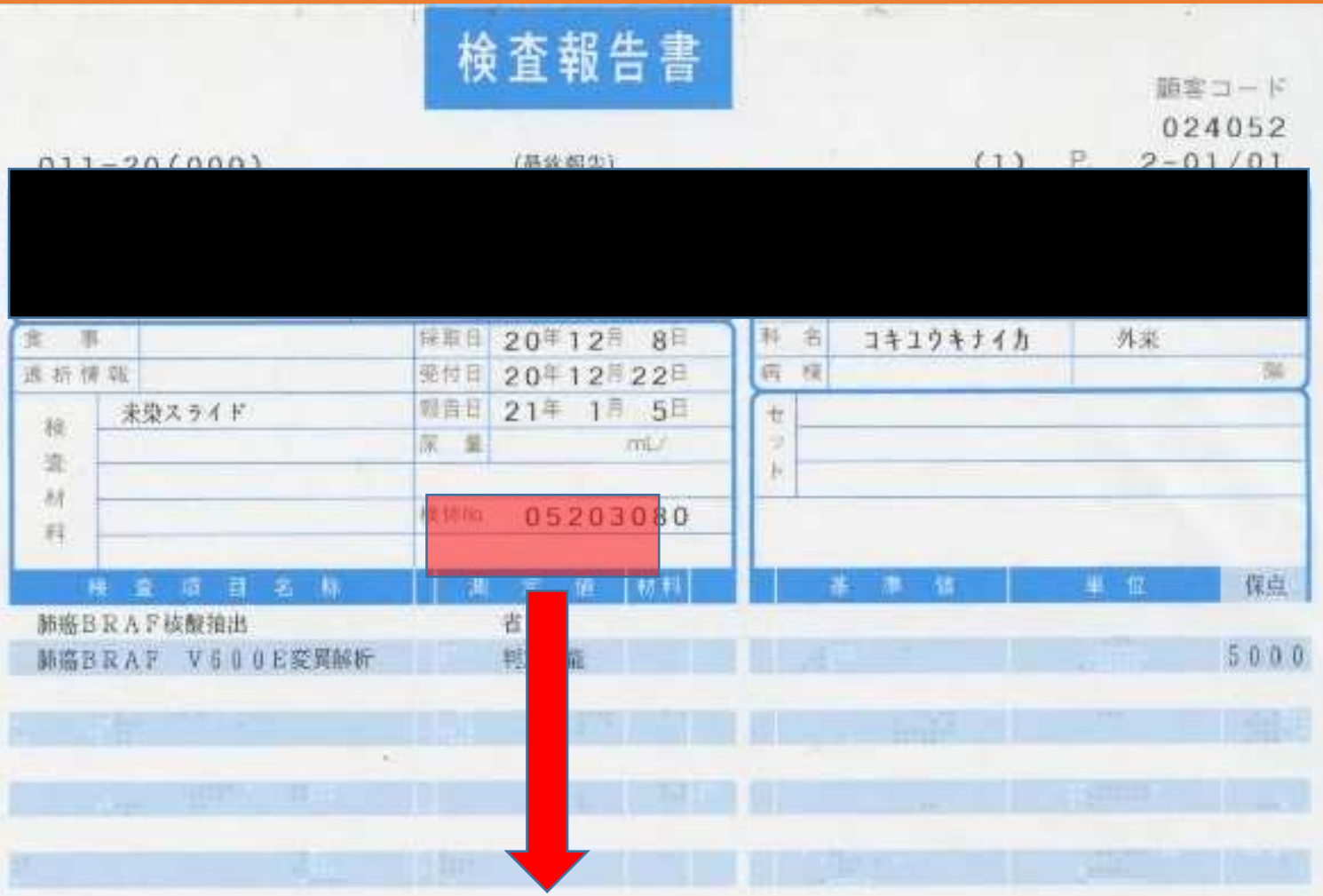
食事	採取日	20年12月22日	科名
送所情報	受付日	21年 1月 4日	病棟
検査材料 未染スライド	報告日	21年 1月13日	セット
	尿量	mL/	
	検体No.	05203095	

検査項目名称	測定値	材料	基
肺癌BRAF核酸抽出	量不足		
肺癌BRAF V600E変異解析	省略		

Unable to extract specified amount of DNA/RNA.

Insufficient volume.

Insufficient volume= Insufficient nucleic acids (quantity problem)



検査報告書

顧客コード 024052

011-20(000)　(最終報告)　(1)　P　2-01/01

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食事	採取日	20年12月 8日	科名	コキュウキナイカ 外来
送所情報	受付日	20年12月22日	病棟	
検査材料 未染スライド	報告日	21年 1月 5日	セット	
	尿量	mL/		
	検体No.	05203080		

検査項目名称	測定値	材料	基準値	単位	保点
肺癌BRAF核酸抽出	省				
肺癌BRAF V600E変異解析	判				5000

DNA and RNA are extracted in specified quantities

DNA fragmentation and RNA degradation

not determinated= poor quality of nucleic acid（quality problem)

- Characteristics of 14 cases of BRAF non-determinable submitted as single-plex at our hospital
- All specimens were biopsied.
- Although not directly related to not determinated, some specimens had tumor cell content of less than 10%.
- Gross size of specimens at initial HE
- >2x2 mm (6 cases), <1x1 mm (3 cases), and in between (5 cases)

- 7 specimens with necrosis and contusion (7/14)
- 4 specimens with hemorrhage (actually small specimens)
- One case of adenocarcinoma with preserved airspace (actually a small specimen)
  →In fact, there were 8 cases of specimens that were close to less than 1x1 mm at the time of the first thin slice (8/14).

- In all 14 cases, the reason for the failure of not determinated (small size ≒ few tumor cells ≒ few nucleic acids, necrosis, or contusion) was inferred.

- Five of the 14 cases were positive for other driver genes.
- 3 EGFR positive (2 L858R, 1 Deletions/exon19), 1 ROS1 positive, 1 ALK positive.
- 8 of 14 patients were PD-L1 positive.

In next slides, four of the specimens, about 2x2 mm gross, are presented.

Case 1

A 56-year-old man visited his local doctor with a chief complaint of cough. He was referred to a doctor because a CT scan of the chest showed a mass shadow on the right lung.

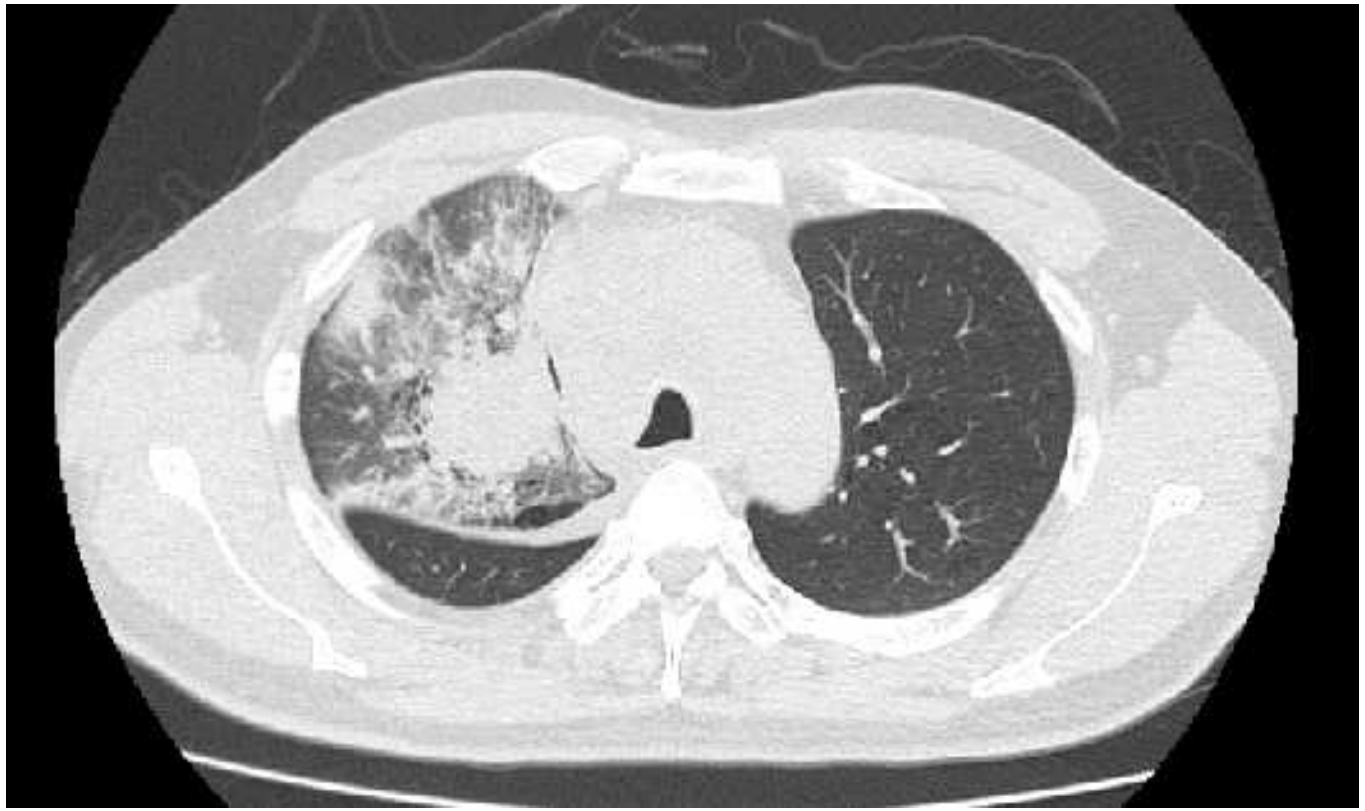
Smoked 30 cigarettes a day until the day of consultation.

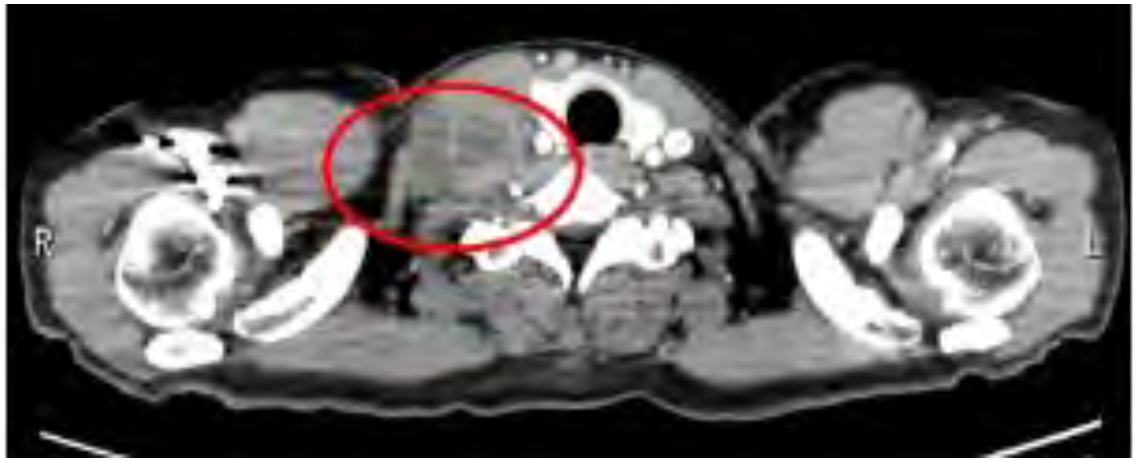
Occupational history: Sales of car parts, history of dust inhalation (metal polishing, organic solvent)

History of hypertension, hyperuricemia, ureteral stone, tonsillectomy, scoliosis

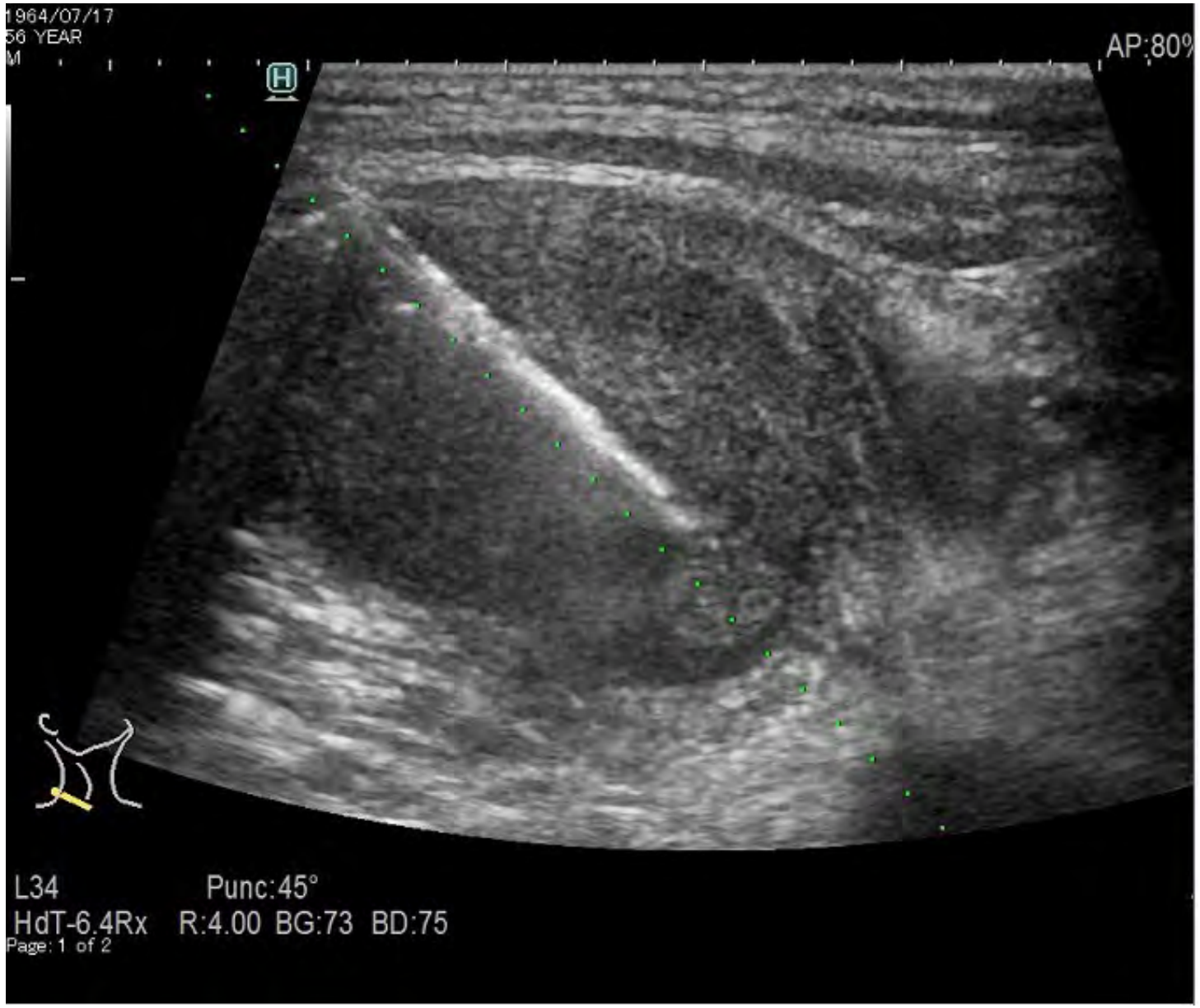
cT4N3(right neck,#7, #4R, right pulmonary hilar)M1b(ADR,OSS) cStage IVB

A needle biopsy was performed from the right cervical lymph node.





Echographic images at the time of biopsy

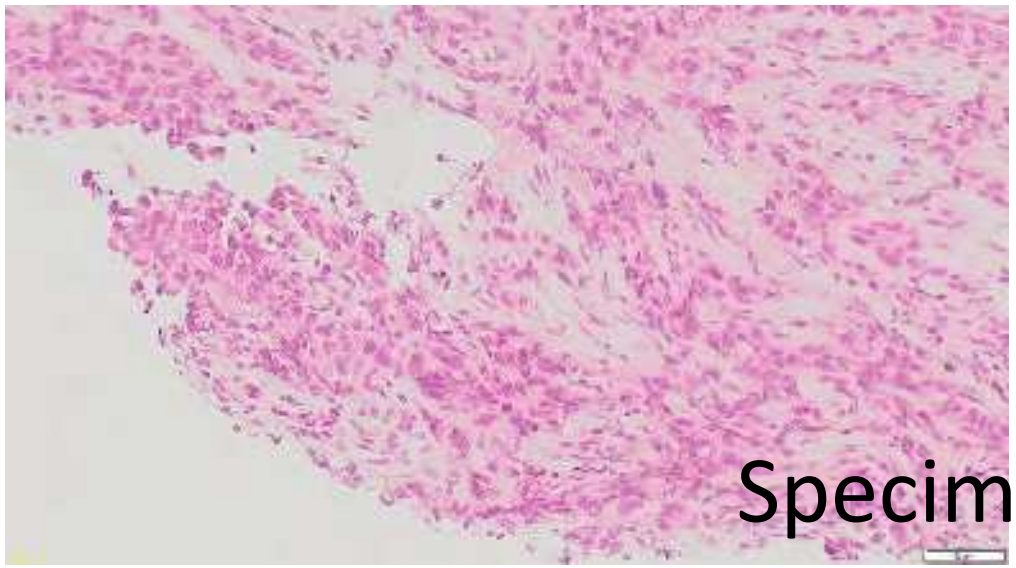


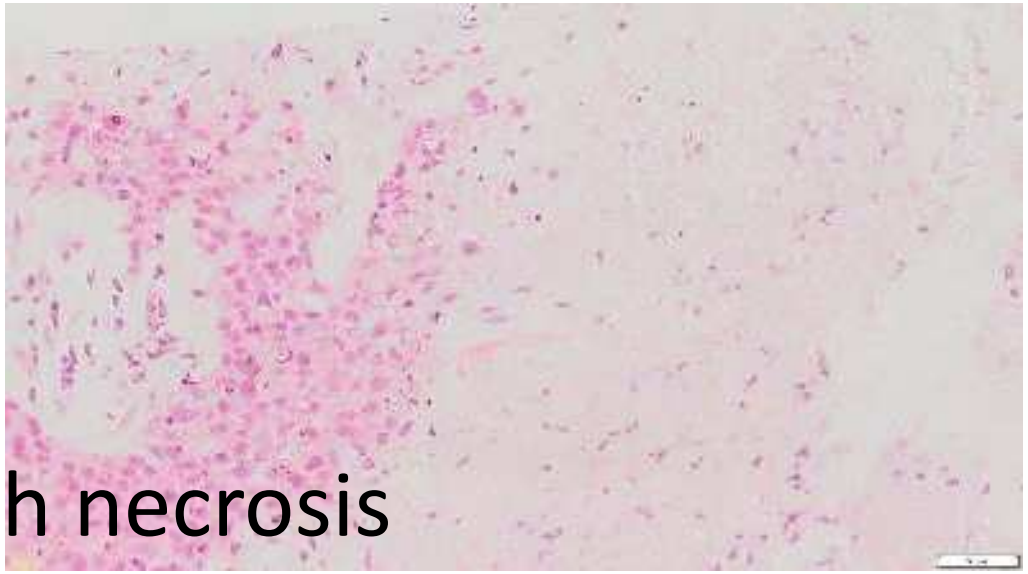
Biopsy from 4 times with 18G 22 mm strokes
Specimen was terminated due to sufficient specimen volume.

The specimen collection was described with confidence that the specimen collection was successful.

Contrast-enhanced CT suggests Low, necrosis.

Right cervical lymph node, needle biopsy specimen no EGFR mutation, ALK(iAEP) score 0, ROS1 (-), ArcherMET(CDx) (-), PD-L1 TPS 25-49%, BRAFV600E not determinated





Specimens with necrosis

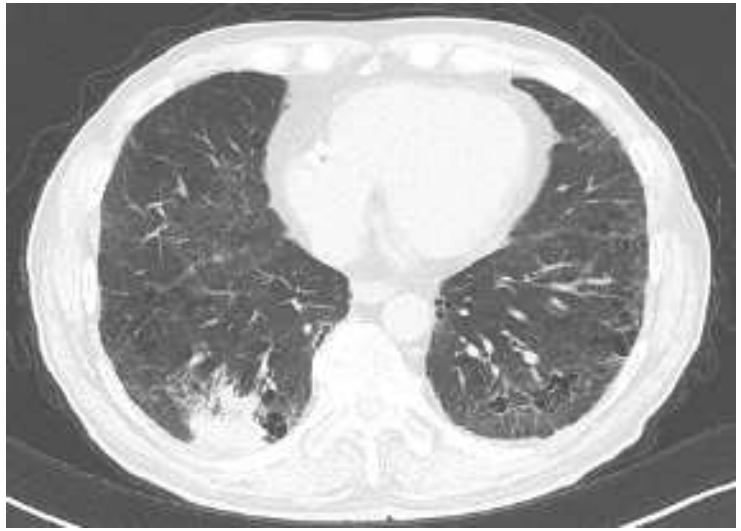
About 70% of the area was necrotic. The number of tumor cells is sufficient. The possibility that the test was performed including the necrotic area cannot be ruled out. No markings were left on the specimen when the lung cancer gene test was ordered (untraceable). →We believe that discussion was necessary when it was found that the decision was not possible.Pembrolizumab (q3w) is being administered.

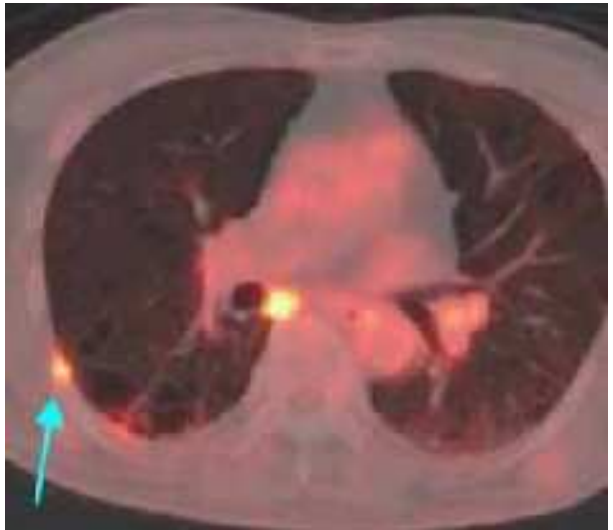
Case 2

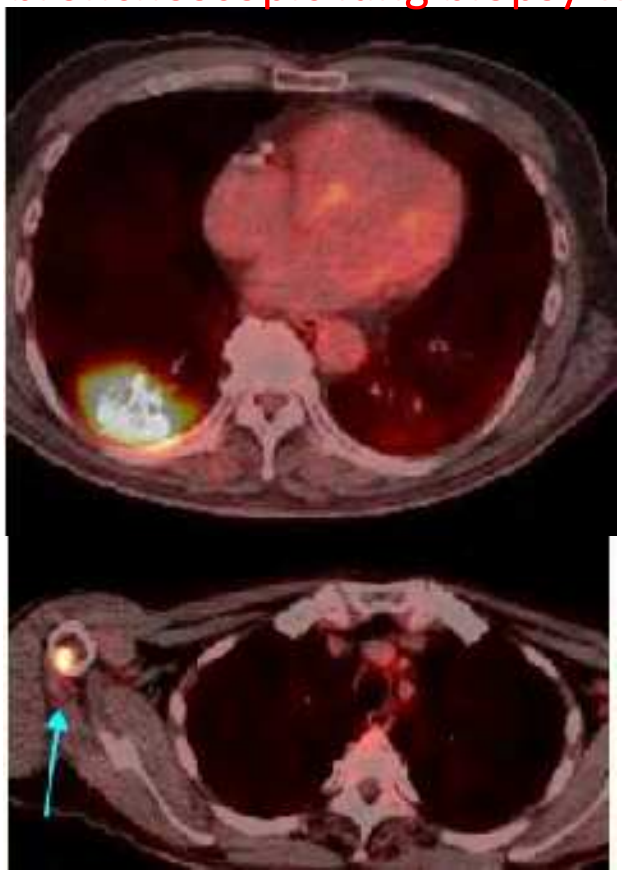
68-year-old male cT2aN2M1b(OSS,PLE) cStage IVA

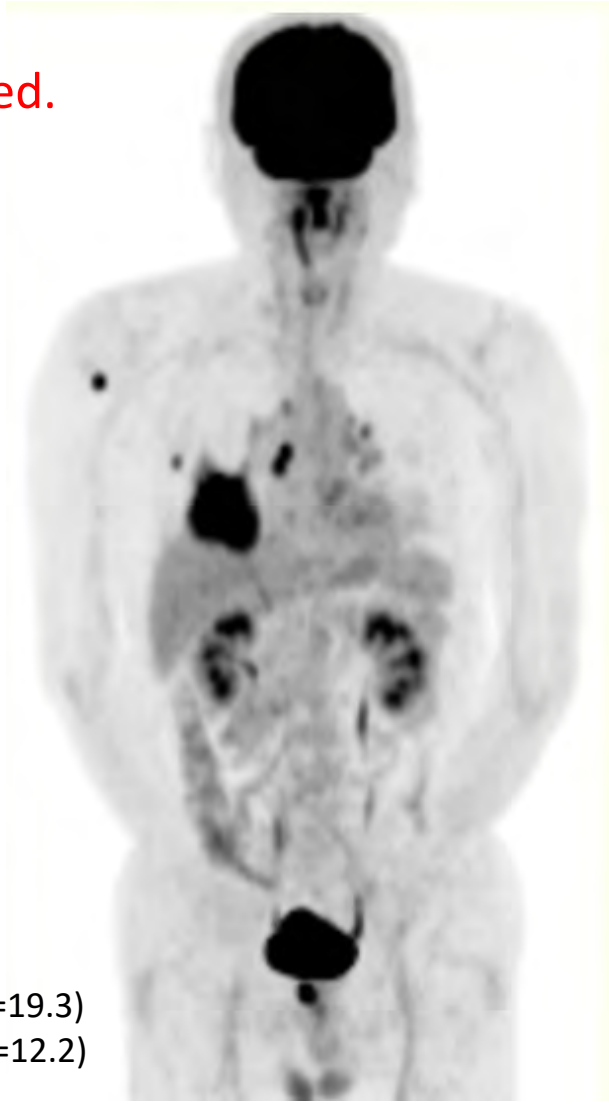
While attending a cardiologist for unstable angina pectoris, he was referred to a respiratory physician for a suspected lung cancer on CT scan.

A bronchoscopic lung biopsy was performed.









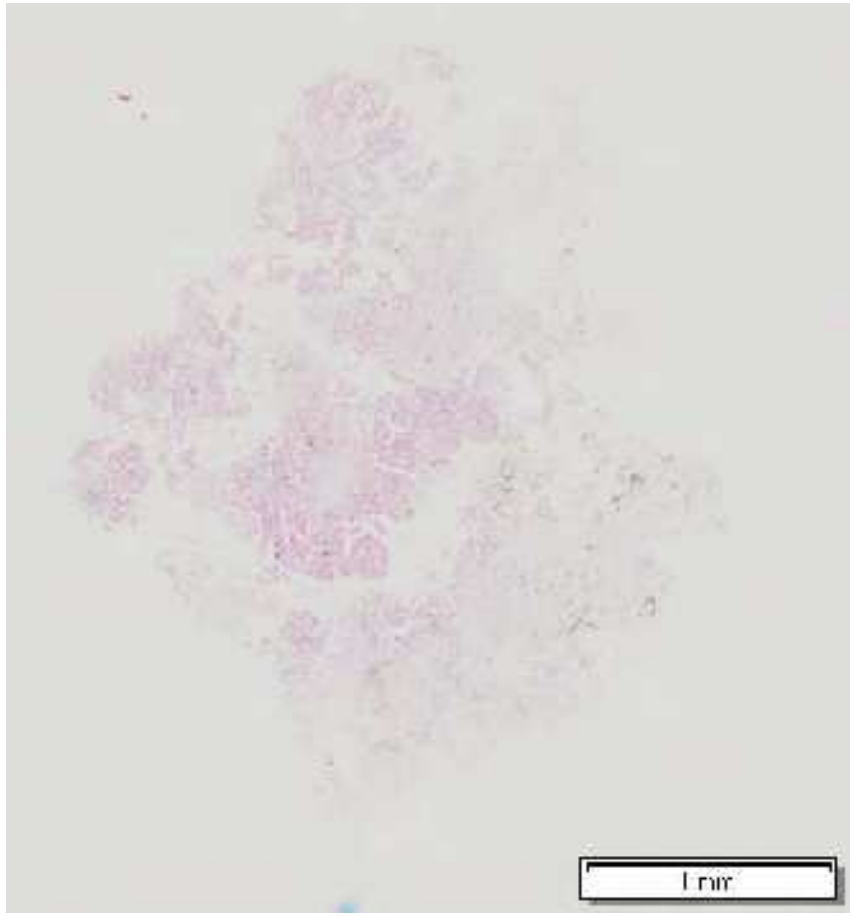
- 4 cm large mass in lower lobe of right lung (SUVmax=19.3)
- FDG accumulation in right lobe of prostate (SUVmax=12.2)
- FDG accumulation in the humerus and pleura.
- Suspected right lower lobe lung cancer and prostate cancer

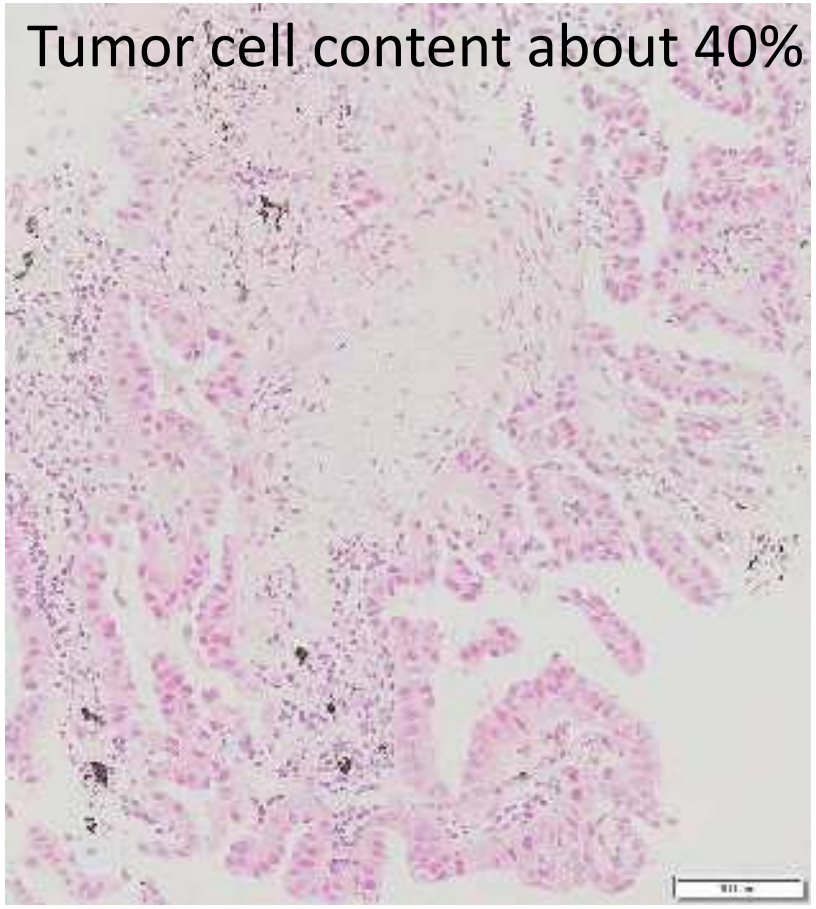
Right lung TBB specimen

No EGFR mutation, ALK(iAEP) score 0, ROS1 (-), PD-L1 TPS <1%,

BRAF V600E not determinated

1st line: CBDCA+nab+PTX PD





Virtually 1x1 mm

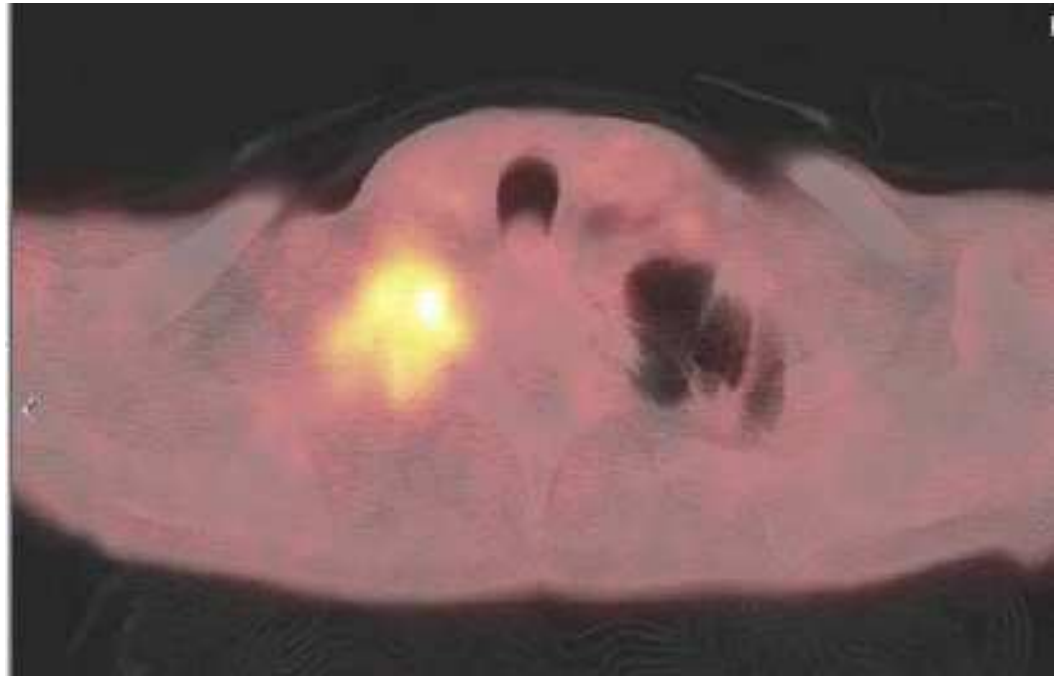
Less than 1x1 mm

Specimens?

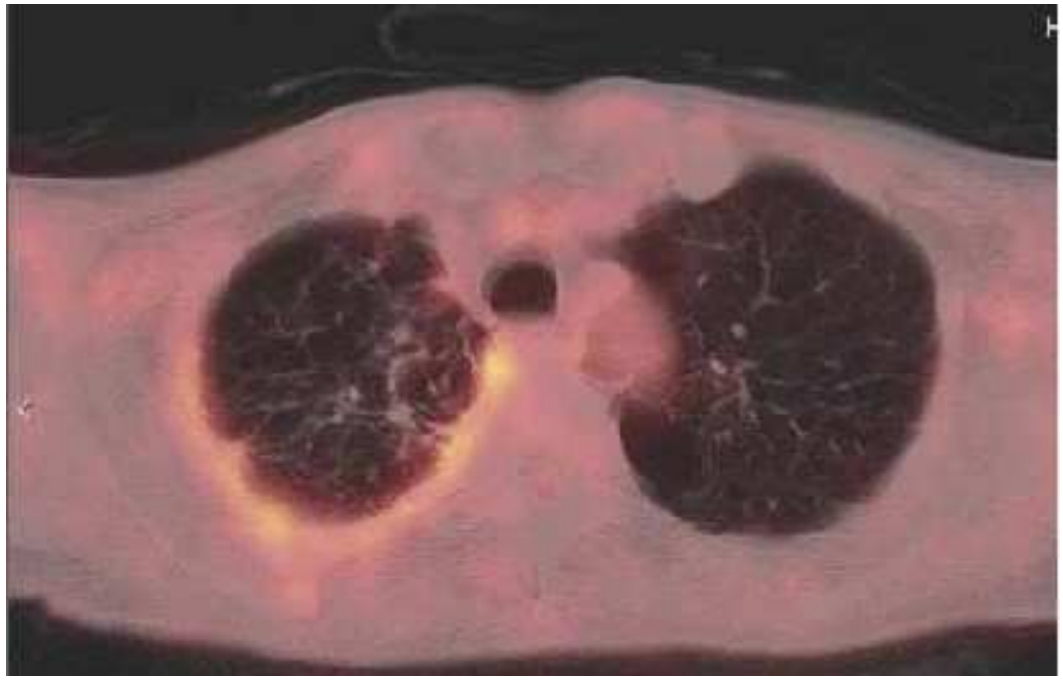
The area from which the specimen was taken remained an air space, the specimen area was therefore smaller, and there were fewer tumor cells. Also, the specimen may have been small due to multiple thin slices, but this is not verifiable because there is no HE remaining corresponding to the area where it was submitted.

Case3

71-year-old male, right pleural biopsy, T1cN0M1a stage IVA.
PET-CT showed increased FDG accumulation in the right lung apex and right pleura, and pleural biopsy revealed non-small cell carcinoma.

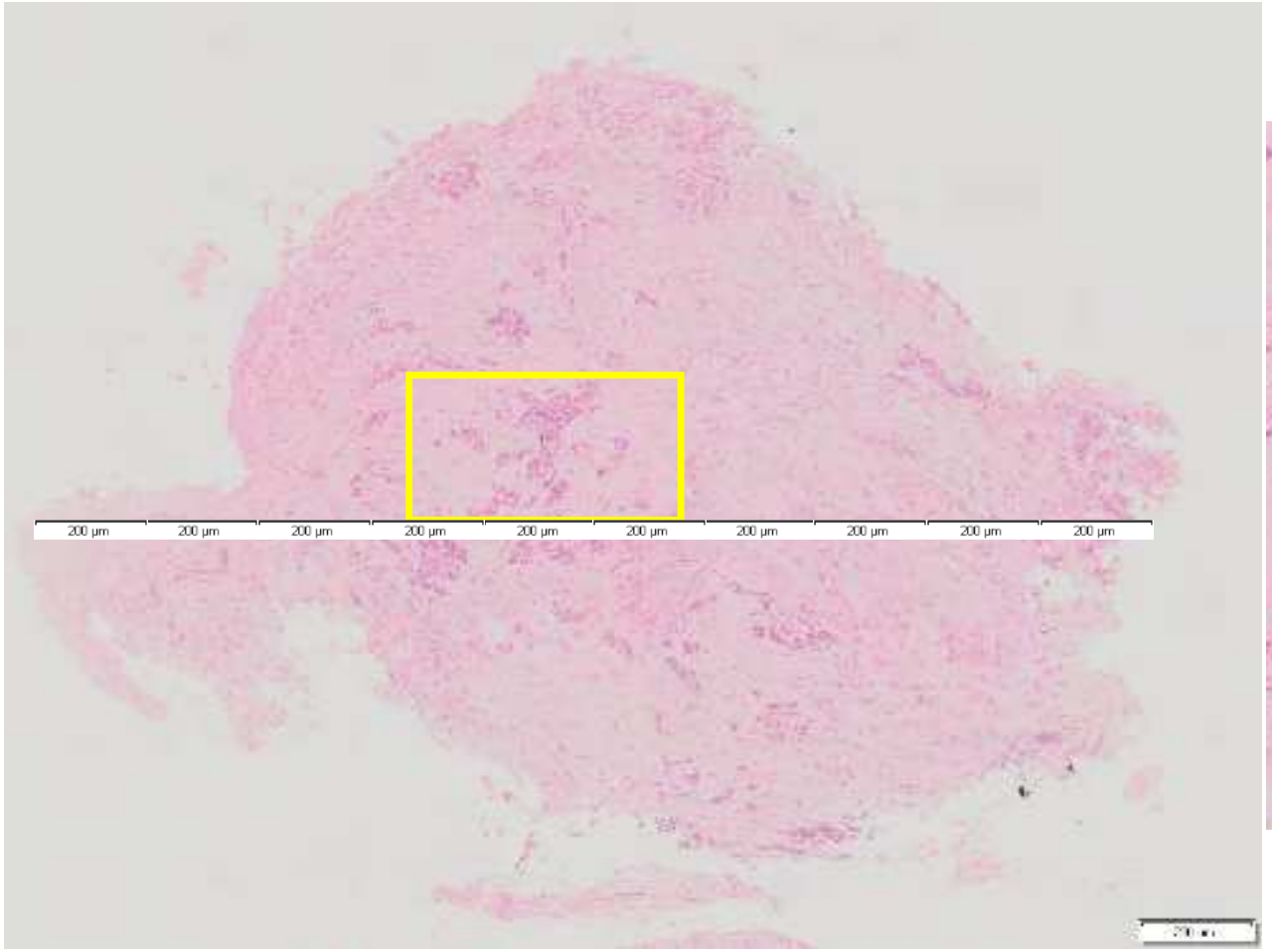


FDG accumulation in the right lung (SUVmax=6.0)

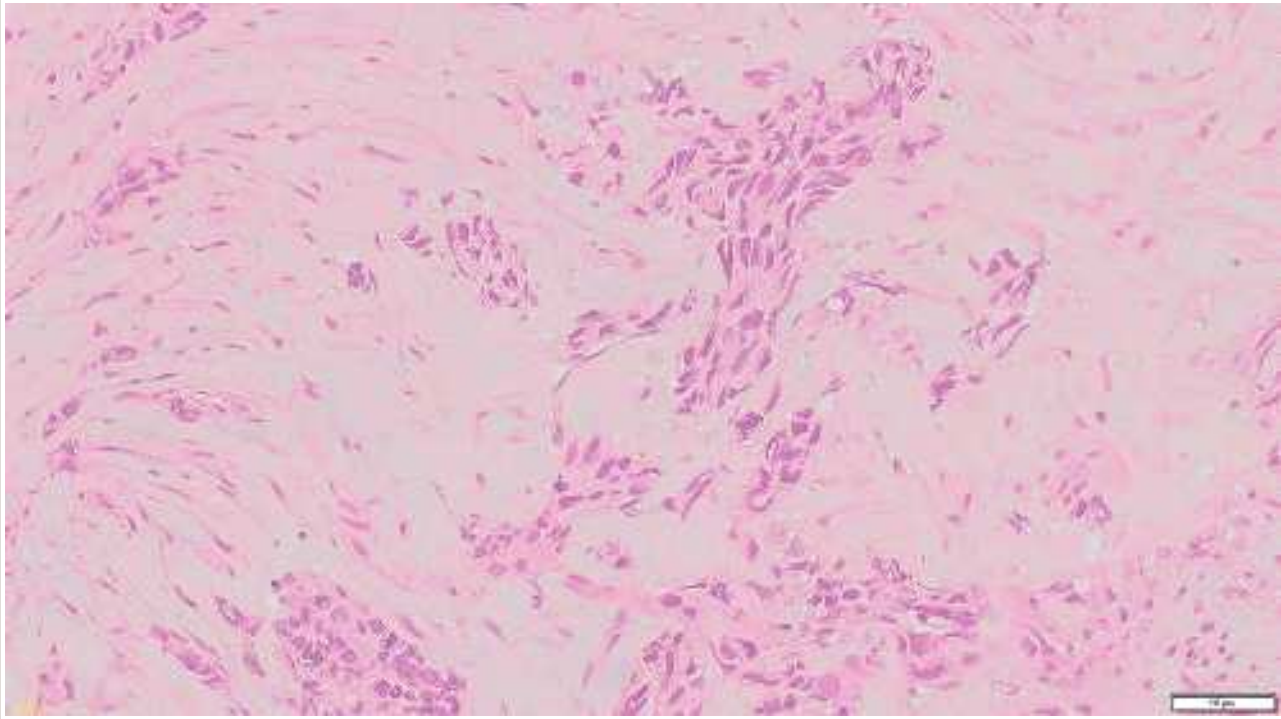


FDG accumulation in right pleura(SUVmax=5.6)

No EGFR mutation, ALK(iAEP) score 0, ROS1 (insufficient or fragmented RNA, possible false negative), BRAF V600E not determinated.
1st line: CBDCA + PEM + pembrolizumab → pembrolizumab alone



Tumor cell content 50-60%.

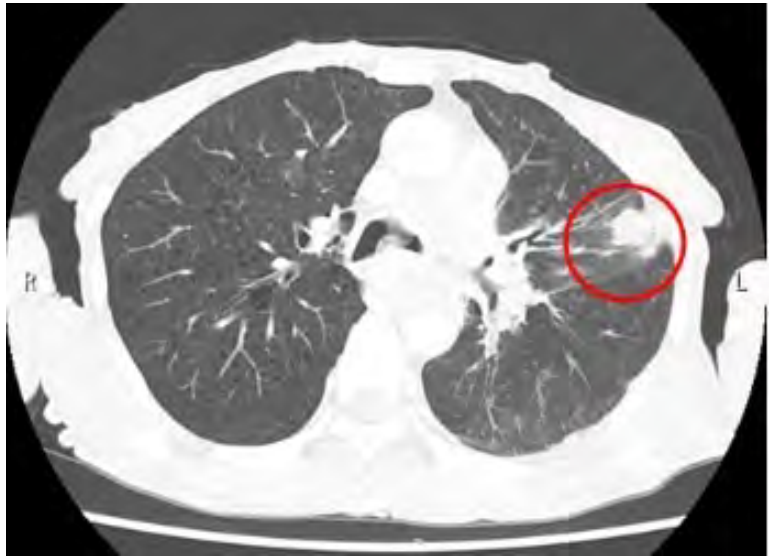
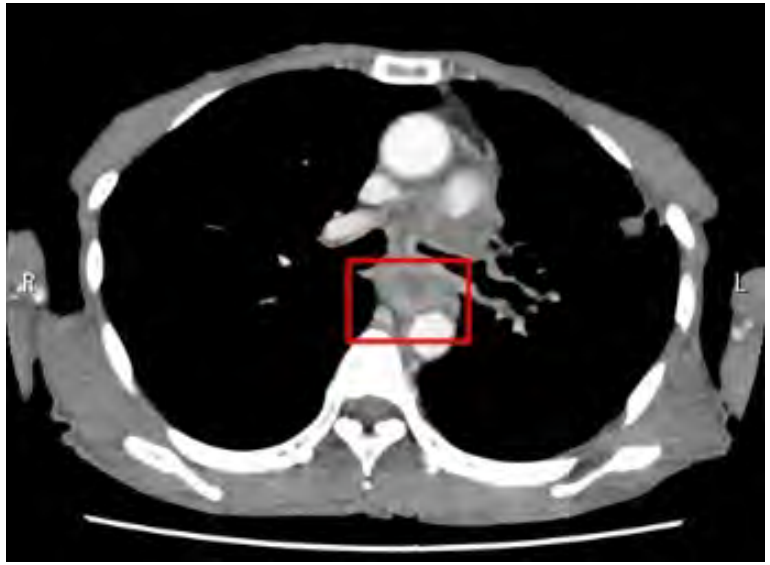
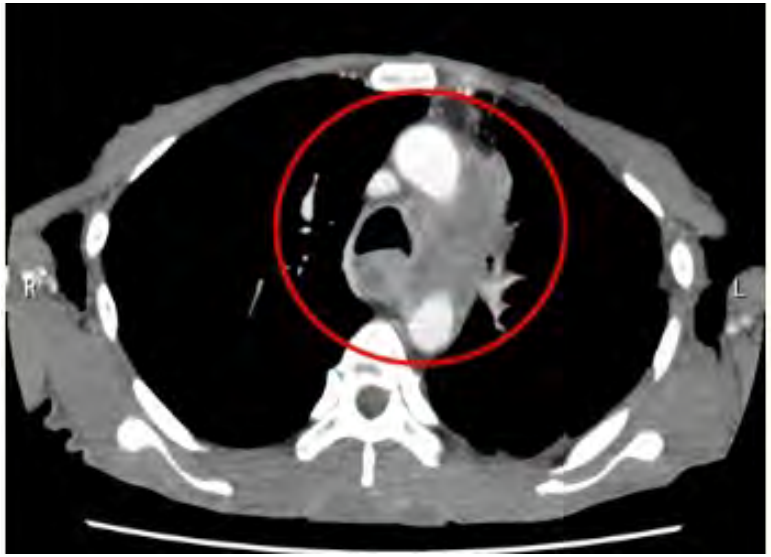


crushing specimens

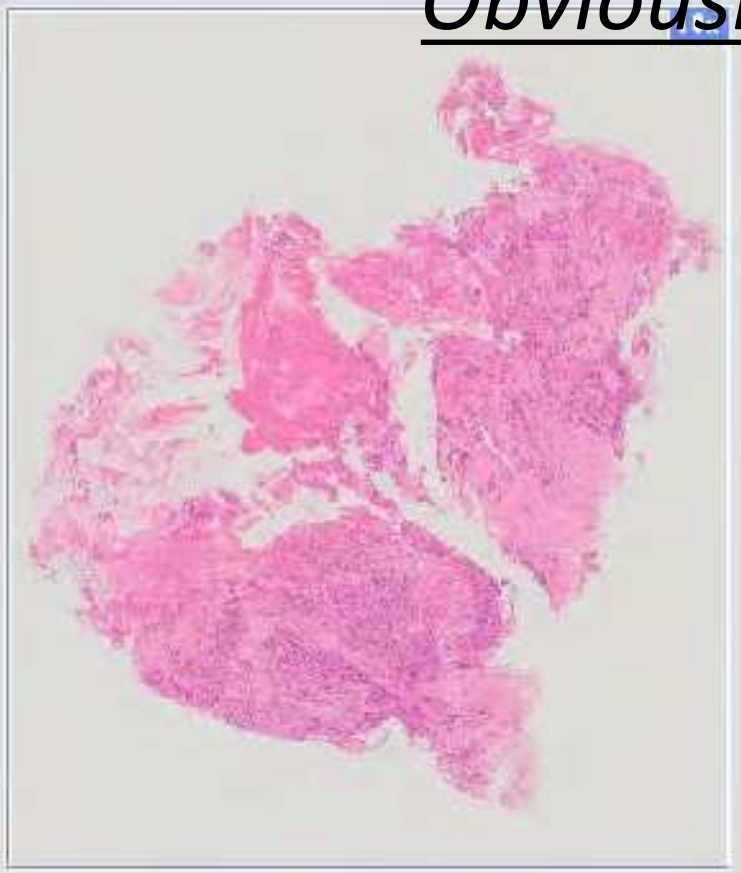
Sufficient tumor tissue at about 2 mm width

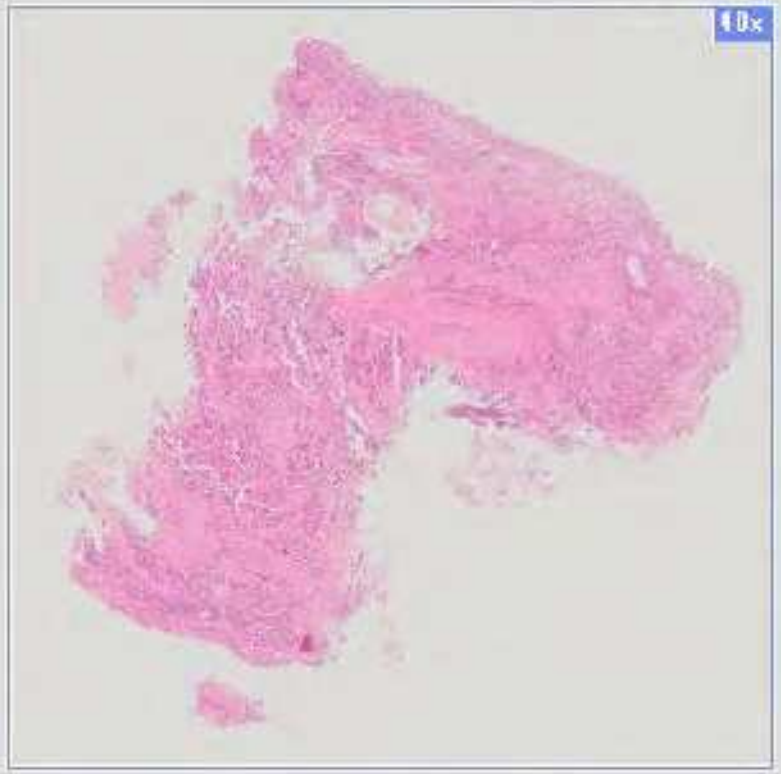
Casa4

63 years old male, smoked 20 cigarettes/day for 40 years (20-60 years old).
History of von Recklinghausen's disease
He was referred to our otorhinolaryngology department after a visit to his local doctor for yellow sputum. He was referred to the Department of Otorhinolaryngology. Contrast-enhanced CT showed a nodule shadow (15 mm) in the left upper lobe of the lung and a mass in the mediastinum from the pulmonary hilum to the mediastinum.
Bronchoscopic lung biopsy was performed.
No EGFR mutation, ALK(iAEP) score 0, ROS1 (-), BRAF V600E not determinated, PD-L1 TPS1-24%.
1st line: CBDCA+nabPTX+pembrolizumab →pembrolizumab maintenance

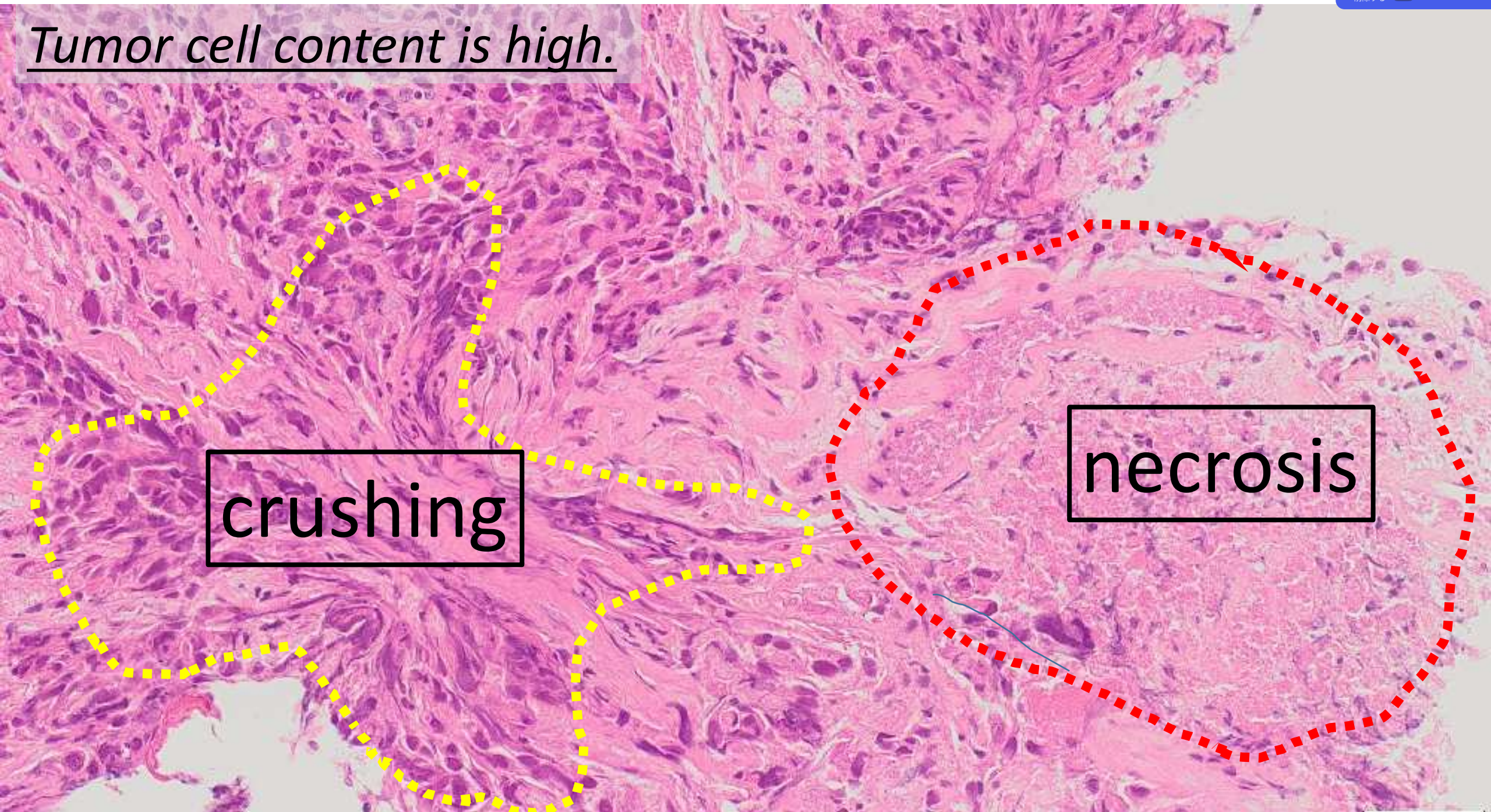




Obviously larger than 2x2 mm in size





Tumor cell content is high.

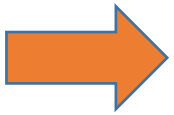


Contact LSI Medience regarding 8 cases of BRAF V600E non-determination in 2020

- PCR amplification was poor in 6 of 8 cases
- 2 out of 8 cases had uneven amplicon reads.

→The cause was DNA fragmentation or chemical modification.

- Insufficient formalin fixation (<3 hours) of biopsy specimens submitted in the afternoon
- Over-fixation (more than 6 hours) of biopsy specimens in formalin



For biopsy specimens, there is no record of "time of start of formalin fixation" and "time of removal from formalin" at our hospital, so they are untraceable.

Conclusion

- Of the 123 non-small cell lung cancer cases submitted by our hospital from January 1, 2019 to December 31, 2020 for oncomine single-plex BRAF V600E testing, 34 cases had insufficient sample volume and 1 case was inconclusive in which DNA and RNA were extracted in specified amounts but were not testable. Four cases existed.

- According to LSI Medience, the eight cases of inconclusive results in 2020 consisted of six cases of poor PCR amplification and two cases of uneven amplicon reads.

- The nucleic acid status was considered to be the cause of inadequate formalin fixation or over-fixation with formalin, but the formalin fixation time of biopsy specimens was not controlled and could not be verified.

- The specimens were not controlled for formalin fixation time, and thus could not be verified. ・Regarding insufficient volume, some specimens had a specimen volume of 2x2 mm or more at the first HE, and whether the specimen volume was insufficient due to thinning could not be verified because there were no HE specimens close to the submission surface.

Initiatives for Cancer Genome Medicine

- June 2017 Changed to 10% neutral buffered formalin solution.
- Sept. 2018 To prevent formalin overfixation, Friday biopsy specimens are processed on Saturday.
- When there are 3 or more consecutive holidays, surgical specimens are cut out on the first day of the holiday.
- June 2020 Started noting the start time of formalin fixation of surgical specimens.

Future Tasks

- Control formalin fixation time and prevent under- or over-fixation with formalin
  Reduction of time from collection of biopsy specimen to start of formalin fixation.
  Management of fixation start time.
- Effective use of specimens
  Storage of Class V cytology LBC specimens in cell blocks and their use for testing
- Closer cooperation and exchange of opinions with clinicians when submitting specimens for cancer genome testing.
  In case of insufficient volume or inability to determine the test results, discussion between clinicians and pathologists is necessary.
- Monitoring of test results for accuracy control (to see if there is any deviation between the predicted frequency and the results at our hospital).
  It is easier if paper slips are kept. Retrieval from the pathology system is labor intensive and difficult to review. Scattered cases where results are not captured in the pathology system.