

1 **Title**

2 **Ameloblastic fibrosarcoma of the maxilla with EGFR exon 20 insertions: Relevance of**
3 **whole-exome sequencing in molecular understanding and therapeutic proposals for rare**
4 **cancers**

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6

Abstract (189 words [<200 words])

Ameloblastic fibrosarcoma (AFS) is the most common odontogenic sarcoma, but the incidence is relatively low, and its molecular biology is poorly understood. We experienced a young female patient with a rapidly growing soft tissue tumor of the left maxilla, which eventually occupied the left side of the oral cavity. Histologically, the tumor mainly consisted of a proliferation of atypical spindle to polygonal cells without any specific differentiation, but a small number of benign odontogenic epithelial foci mainly in the tumor periphery were also noted; thus, a diagnosis of AFS was made. We performed whole-exome sequencing (WES) on the tumor to investigate its molecular features and identify therapeutic options. We found that the tumor harbored EGFR exon 20 insertions and MDM2 amplification; the former may be a target for newly developed tyrosine kinase inhibitors in case of recurrence. To the best of our knowledge, this is the first case of AFS for which WES was performed and with EGFR mutation. Our case provides new genetic information on AFS and suggests that comprehensive genetic analysis can clarify the molecular biology in rare cancers, potentially leading to the proposal of therapeutic strategies.

Keywords

Ameloblastic fibroma, Ameloblastic fibrosarcoma, EGFR exon20 insertion, MDM2 amplification, Whole-exome sequencing

26 **Main (1640 words)**

27 **Introduction**

28 Although ameloblastic fibrosarcoma (AFS) is the most common type of odontogenic sarcoma,
29 the incidence is relatively low, and only approximately 100 cases have been reported to date
30 (1). Its genetics is poorly understood, and personalized treatment for the patients has not been
31 realized. We experienced a case of AFS in a young female patient and investigated its
32 molecular features with whole-exome sequencing (WES), which clarified that the tumor, for
33 the first time reported in AFS, harbored EGFR exon 20 insertions and MDM2 amplification.

34

35 **Case report**

36 The patient was a 21-year-old female who had been treated for asthma in her childhood. She
37 had no history of smoking. She had noticed swelling in her left upper gingiva one month
38 earlier and had consulted a physician. She underwent a biopsy for the lesion with a clinical
39 diagnosis of epulis, but the histology suggested myofibroblastic tumor. After the first biopsy,
40 the tumor regrew, and the patient consulted a second physician. The patient underwent a
41 second biopsy, and a solitary fibrous tumor was suspected. Imaging analyses showed that the
42 tumor was located in the left upper gingiva to the maxilla, was 2.3 cm × 1.6 cm in size, and
43 had accompanying destruction of the maxilla.

44 She was admitted to our hospital for further investigation and treatment. After the

second biopsy, the tumor grew more rapidly and extended into the maxillary sinus with enlargement of the cervical lymph nodes (Figure 1). The third biopsy in our hospital revealed that the tumor was sarcoma, but a final diagnosis could not be made because it did not exhibit any features of differentiation. The tumor grew further, and eventually occupied the oral cavity with hemorrhage and reached the left orbital floor (Figure 1). Because preoperative chemotherapy was not considered for such an undifferentiated sarcoma, the patient underwent a left-sided subtotal maxillectomy with elective neck dissection, and the defect was reconstructed with an anterolateral thigh (ALT) free flap.

Macroscopically, the tumor was poorly circumscribed (9.0 cm × 6.0 cm × 5.0 cm in size) and had invaded the left side of the maxilla, maxillary sinus, periodontal region, and infratemporal fossa. The oral mucosa was ulcerated, and this was associated with the tumor invasion. In the cut sections, the tumor was whitish to tan in color with hemorrhage and contained some gelatinous areas. An impacted tooth was observed within the tumor (it was already indicated in imaging analyses), suggesting a relationship to the tumor histogenesis. (Figure S1).

Microscopically, the tumor was mainly composed of diffuse proliferation of spindle to polygonal cells with variegated cellular density in an edematous, myxoid, or fibrous background with occasional hemorrhage. The tumor cells sometimes aggregated, especially around vessels with epithelioid appearance. Consistent with the macroscopic findings, the

tumor destroyed the maxilla and alveolar bone, but the invasion pattern was not destructive or permeative, and the pre-existing trabeculae were relatively reserved (Figure 2). No osteoid or chondroid matrix was observed, and necrosis was not evident.

The tumor cells had large nuclei and amphophilic to eosinophilic, or vacuolated cytoplasm. Mitoses were observed at 2 to 3/10 high power fields (HPFs). In addition to the mesenchymal cell proliferation, the tumor contained a small number of odontogenic epithelial cell foci without significant atypia, mainly in the periphery (Figure 2). The surgical margins were negative for the tumor, and no lymph node metastasis was identified.

Immunohistochemistry (IHC) revealed that the mesenchymal tumor cells were positive for BCOR, CDK4, and MDM2 (Figure 3), and only focally positive for aSMA (5%). H3K27me3 and INI1 expressions were retained. The cells were negative for BRAF V600E, CCNB3, CD31, CD34, CD99, cytokeratin (clone AE1/AE3 and CAM5.2), desmin, ERG, myogenin, NKX2.2, S-100 protein, SATB2, STAT6, SYT-SSX, and WT-1 (clone 6F-H2). The Ki-67 labeling index was approximately 20% in the hot spot (Figure 3); as with the morphology, no apparent signs of differentiation were found in IHC. Consistent with positivity for MDM2, dual-color fluorescent *in situ* hybridization (DISH) revealed MDM2 amplification in the mesenchymal component (Figure 3). Altogether, a diagnosis of AFS was made.

We next investigated the molecular findings of the tumor using WES with

representative formalin-fixed paraffin-embedded (FFPE) tissues to better understand the tumor biology, speculate on its aggressiveness, and hopefully provide therapeutic options. The results showed that the putative driver mutation was EGFR exon 20 insertions (p.D770delinsDNPH) (Figure 4). We performed IHC for EGFR and confirmed that most tumor cells (approximately 80%) showed a membranous expression of EGFR (Figure 3). The total mutation load was relatively low, with 20 mutations (mut.)/exome (0.61 mut./MB). The copy number analysis identified lesions affecting 33.7% of the entire genome and focal amplification peaks of the murine double minute 2 (MDM2) on chromosome 12. Focal amplifications of chromosomes 6 and 8 were also noted, but these peaks spanned no putative oncogenes (Figure 4).

After the surgery, the patient received postoperative proton therapy at 66 Gy (RBE)/33fr and has remained disease-free for approximately six months.

Discussion

Although rare, AFS is the most common odontogenic sarcoma. It mainly develops in young patients (median age: 27.3 years) with slight male predominance (M:F = 1.5:1). Approximately 80% of the tumors arise from the mandible, and the remaining cases occur in the maxilla. The tumor is considered the malignant counterpart of ameloblastic fibroma (AF), a representative benign mixed epithelial and mesenchymal odontogenic tumor, and

approximately half of AFSs occur from pre-existing AF; this was not evident in our case, however [1, 2].

Histologically, the tumor comprises two components, the epithelial and the mesenchymal component. The epithelial component is odontogenic and cytologically benign and forms lamina-like strands or large islands with peripheral palisading. The mesenchymal component comprises malignant spindle to polygonal cells showing cytologically low- to intermediate-grade malignancy, with variable nuclear atypia and mitotic activity. The epithelial-mesenchymal ratio is variable but often low because the latter component overgrows the former as the tumor progresses. Immunohistochemically, no specific or diagnostic markers for AFS are known so far [1].

Radical resection is the standard treatment strategy. Although the recurrence rate is not low (approximately 1/3 of cases), distant metastasis is observed in <5% of patients, and the overall mortality rate is approximately 25% [1].

Due to its rarity, the molecular findings of AFS are poorly understood. The only reliable and reproducible finding seems to be its recently discovered frequent BRAF mutation. Agaimy et al. reported that five among seven cases of AFS harbored BRAF V600E, and another possessed NRAS Q61K [3]. Coura et al. reported that two among three cases of AFS harbored BRAF V600E mutation, and that four among ten AFs also harbored the same mutation [4]. These results strongly indicate that BRAF mutation is one of the drivers

commonly observed in AFS and AF.

Here, we performed WES for AFS for the first time, which enabled us to investigate the genes thoroughly. We found that the tumor harbored EGFR mutation together with MDM2 amplification. Because EGFR is also a gene belonging to the MAPK pathway as with BRAF and NRAS, our case emphasizes the relevance of the cell-autonomous activation of the MAPK pathway in AFS. EGFR exon 20 insertions are known to occur in a small proportion of pulmonary adenocarcinomas [5]. Although tumors with this mutation are generally resistant to current tyrosine kinase inhibitors, new drugs, such as Poriotinib, show a relatively good response for those with the mutation [6], suggesting that it could be druggable soon, at least in lung cancers. Interestingly, EGFR exon 20 insertions are also known to be the driver mutations of fibrous hamartoma of infancy (FHI), a complete benign mesenchymal tumor [7]. This finding may suggest that the influence of this mutation in tumor behavior might differ among cell types, particularly between epithelial and mesenchymal tumors.

MDM2 amplification is also a well-known genetic abnormality observed in malignancies, including sarcomas [1, 8, 9]. Because no benign tumors with recurrent MDM2 amplification are known, it may be a feature differentiating AFS from AF. Targeting therapies for MDM2 are also under investigation [10] and are hoped to be feasible shortly.

Generally, sarcoma shows high levels of copy number alteration (CNA) whereas low levels of mutation (it is consistent with our case) and affected regions or genes in CNA are

correlated with (or different among) histological subtypes [9]. Together with a clinical suspicion of high-grade malignancy, radiologically and pathologically, the most important differential diagnosis in our case was conventional osteosarcoma (OS) (fibroblastic OS) although, together with the benign epithelial islands, several morphological features, such as osseous invasion preserving pre-existing trabeculae, were not typical for conventional OS. Several studies demonstrated that OS has complex karyotypes, with structural and numerical aberrations frequently influenced by chromothripsis. *TP53* is almost always affected, and *RBI*, *ATRX*, and *DLG2* are also commonly mutated (29–53%) [1, 11-13]. These features were rather different from those observed in our case. Thus, a comprehensive genetic analysis would also contribute to an accurate pathological diagnosis.

EGFR mutation is rare in sarcoma although its overexpression is sometimes observed [14], and the sensitivity of EGFR-mutated sarcoma for tyrosine kinase inhibitors seems inconclusive. Considering the seemingly strong EGFR dependency and membranous EGFR expression in our case, however, we think that new tyrosine kinase inhibitors could be considered in case of recurrence with careful monitoring.

Because the tumor in our patient proliferated rapidly, high-grade malignancy was clinically suspected, and the undifferentiated histology of the tumor observed in small biopsy specimens also suggested such a possibility. The detailed histological observation of the resected specimen detected a small number of benign odontogenic epitheliums, however,

which led to the diagnosis of AFS, generally low to intermediate malignancy. WES for the tumor revealed a potentially druggable EGFR mutation and several CNA events, including MDM2 amplification, but the alteration pattern was not as complex as often seen in OS. Our case underscores the relevance of the detailed genetic analysis to manage rare cancers for which standard treatment strategies have not yet been established.

Disclosure Statement

No conflicts of interest to disclose

Ethical Statement

Not applicable, not a research

Figure legends

Figure 1. Macroscopic and imaging findings of the tumor

A reddish tumor was seen on the left upper gingiva (a). Within one month, the tumor grew rapidly and eventually occupied the oral cavity with hemorrhage. The upper left teeth shifted inside due to the expansion of the tumor (b). Imaging analyses revealed a left maxillary tumor extending into the oral cavity and subcutaneous tissues (c) and reaching the left orbital floor (d) (contrast-enhanced fat-saturated T1-weighted MRI: c, coronal, d, sagittal)

Figure 2. Microscopic findings of the tumor

The tumor mainly consisted of a diffuse proliferation of spindle to polygonal cells (a). The mesenchymal cells sometimes aggregated, especially around vessels, and had an epithelioid appearance (b). They had large round to oval nuclei, and amphophilic or slightly eosinophilic cytoplasm. The mitoses were observed at 2–3/10 HPFs (c). The mesenchymal component showed osseous invasion, but the pre-existing trabeculae were relatively reserved (d). Also, a small number of epithelial islands were also observed within the tumor (e). The epithelial foci showed no significant atypia (f). (a-f: H&E staining).

Figure 3. Immunohistochemical and dual-color *in situ* hybridization (DISH) findings of the tumor.

The mesenchymal tumor cells were positive for CDK4 (a) and MDM2 (b), and the Ki-67 labeling index was around 20% in the hotspot (c). MDM2 amplification was confirmed by DISH analysis (b, inset). The tumor also expressed membranous EGFR (d).

Figure 4. Whole-exome sequencing for the mesenchymal component of the tumor

The tumor harbored 20 mutations per exome (0.61 mutations/MB), one of which was EGFR exon 20 insertions (p.D770delinsDNPH) (a). The copy number analysis identified lesions affecting 33.7% of the entire genome and focal amplification peaks of the murine double minute 2 (MDM2) on chromosome 12. Focal amplifications of chromosomes 6 and 8 were also noted, but these peaks spanned no putative oncogenes (b) (red and blue lines depict each allele).

204 **Supplementary information**

205 **Figure S1. Macroscopic findings of the tumor**

206 The tumor was white to tan in color, containing some gelatinous areas (a). One impacted
207 tooth (in the white circle) was observed within the tumor (b).

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References

1. El-Naggar AK, Chan JKC, Grandis JR, Takata T, Slootweg PJ (Eds): WHO Classification of Head and Neck Tumours (4th edition). IARC: Lyon 2017.
2. Muller S, Parker DC, Kapadia SB, Budnick SD, Barnes EL. Ameloblastic fibrosarcoma of the jaws. A clinicopathologic and DNA analysis of five cases and review of the literature with discussion of its relationship to ameloblastic fibroma. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 1995;79(4):469-77.
3. Agaimy A, Skalova A, Franchi A, Alshagroud R, Gill AJ, Stoehr R, et al. Ameloblastic fibrosarcoma: clinicopathological and molecular analysis of seven cases highlighting frequent BRAF and occasional NRAS mutations. *Histopathology.* 2020;76(6):814-21.
4. Coura BP, Bernardes VF, de Sousa SF, Diniz MG, Moreira RG, de Andrade BAB, et al. Targeted next-generation sequencing and allele-specific quantitative PCR of laser capture microdissected samples uncover molecular differences in mixed odontogenic tumors. *J Mol Diagn.* 2020;22(12):1393-9.
5. Network CGAR. Comprehensive molecular profiling of lung adenocarcinoma. *Nature.* 2014;511(7511):543-50.
6. Remon J, Hendriks LEL, Cardona AF, Besse B. EGFR exon 20 insertions in advanced non-small cell lung cancer: A new history begins. *Cancer Treat Rev.* 2020;90:102105.
7. Park JY, Cohen C, Lopez D, Ramos E, Wagenfuehr J, Rakheja D. EGFR exon 20 insertion/duplication mutations characterize fibrous hamartoma of infancy. *Am J Surg Pathol.* 2016;40(12):1713-8.
8. WHO Classification of Tumours Editorial Board. Soft tissue and bone tumours. Lyon (France): International Agency for Research on Cancer; 2020.
9. elizabeth.demicco@sinaihhealthsystem.ca CGARNEa, Network CGAR. Comprehensive and integrated genomic characterization of adult soft tissue sarcomas. *Cell.* 2017;171(4):950-65.e28.
10. Ray-Coquard I, Blay JY, Italiano A, Le Cesne A, Penel N, Zhi J, et al. Effect of the MDM2 antagonist RG7112 on the P53 pathway in patients with MDM2-amplified, well-differentiated or dedifferentiated liposarcoma: an exploratory proof-of-mechanism study. *Lancet Oncol.* 2012;13(11):1133-40.
11. Chen X, Bahrami A, Pappo A, Easton J, Dalton J, Hedlund E, et al. Recurrent somatic structural variations contribute to tumorigenesis in pediatric osteosarcoma. *Cell Rep.* 2014;7(1):104-12.
12. Cheng L, Pandya PH, Liu E, Chandra P, Wang L, Murray ME, et al. Integration of genomic copy number variations and chemotherapy-response biomarkers in pediatric sarcoma. *BMC Med Genomics.* 2019;12(Suppl 1):23.
13. Smida J, Xu H, Zhang Y, Baumhoer D, Ribi S, Kovac M, et al. Genome-wide analysis of somatic copy number alterations and chromosomal breakages in osteosarcoma. *Int J Cancer.* 2017;141(4):816-28.
14. Ren W, Korchin B, Zhu QS, Wei C, Dicker A, Heymach J, et al. Epidermal growth factor receptor blockade in combination with conventional chemotherapy inhibits soft tissue sarcoma cell growth in vitro and in vivo. *Clin Cancer Res.* 2008;14(9):2785-95.

Figure 1

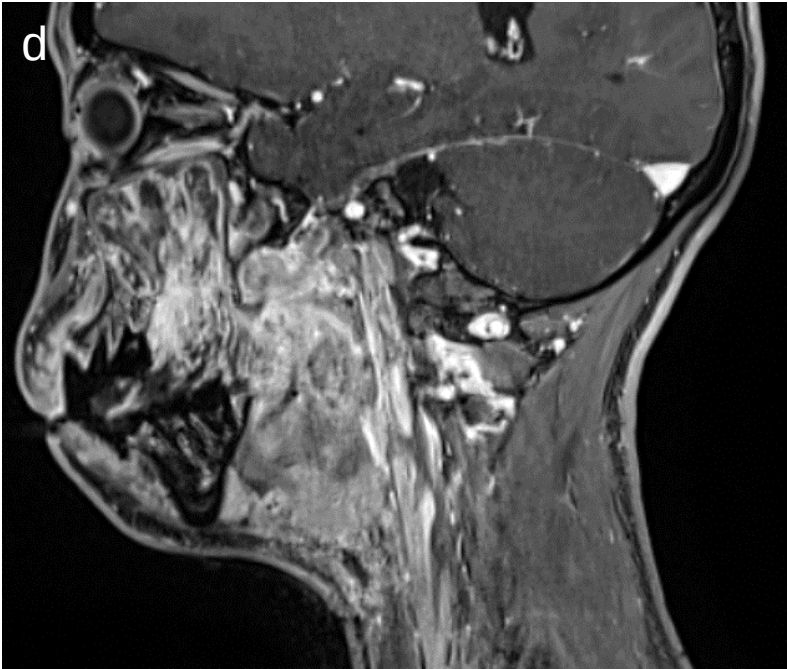
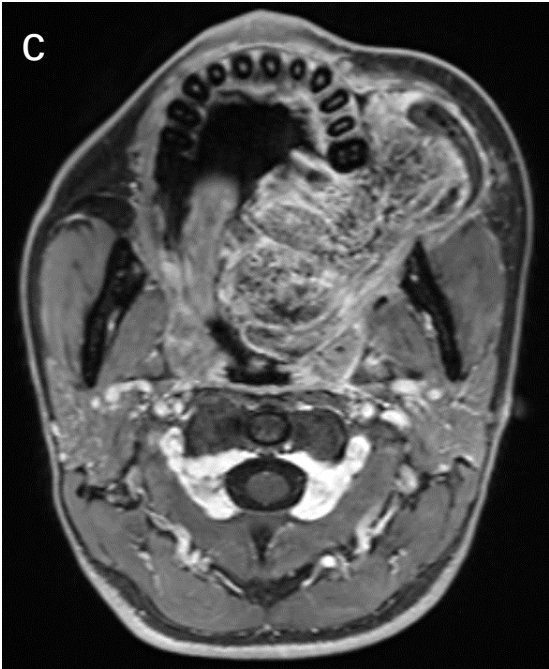
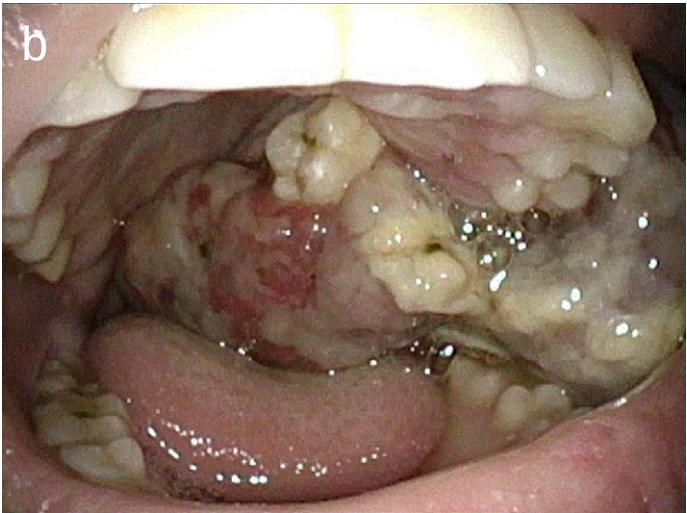
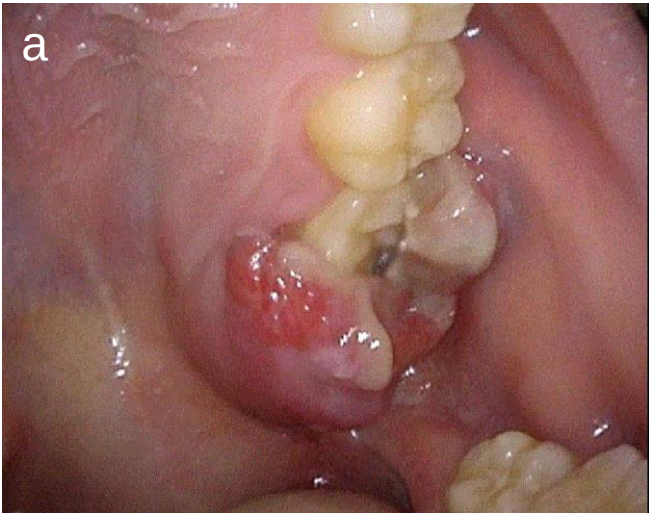


Figure 2

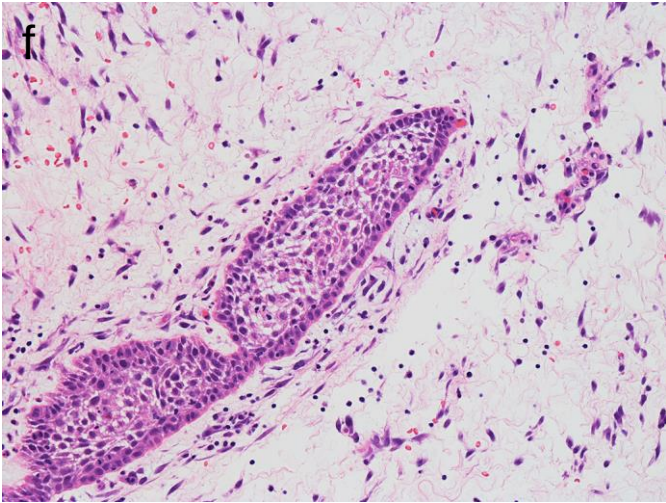
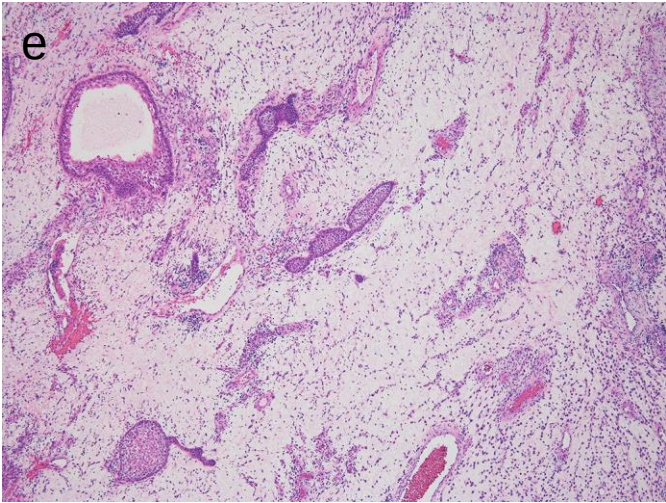
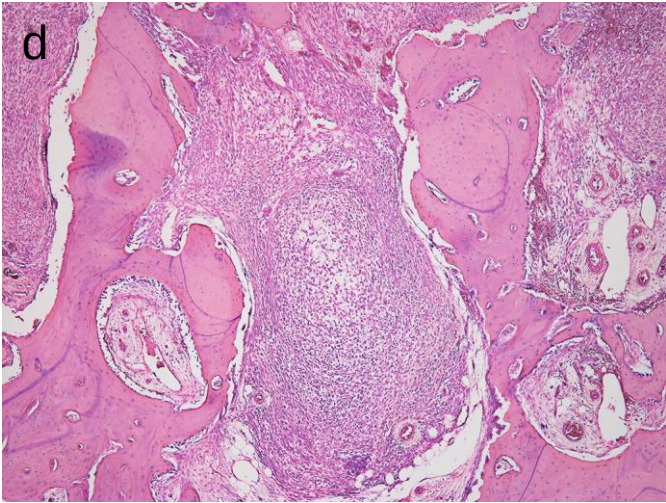
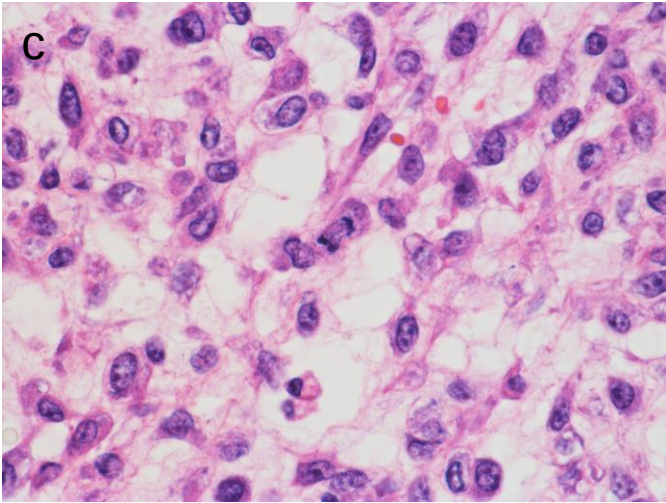
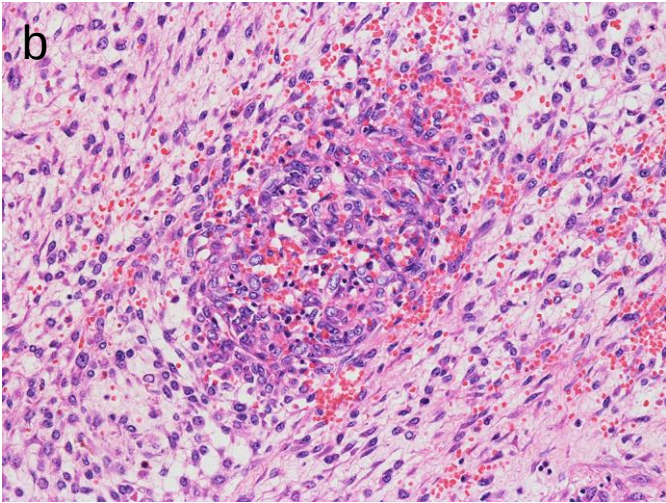
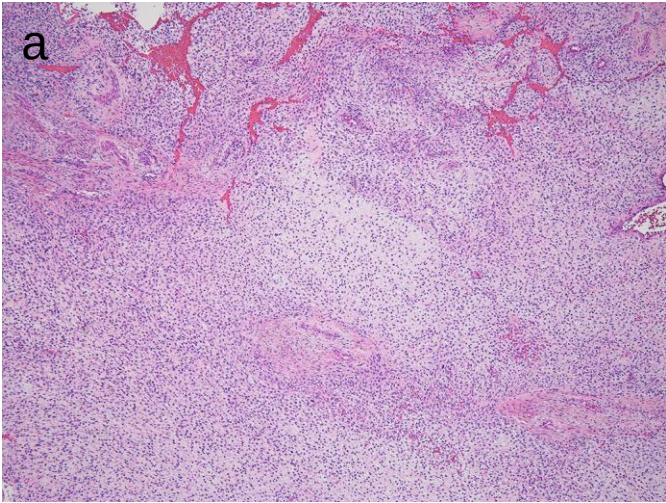


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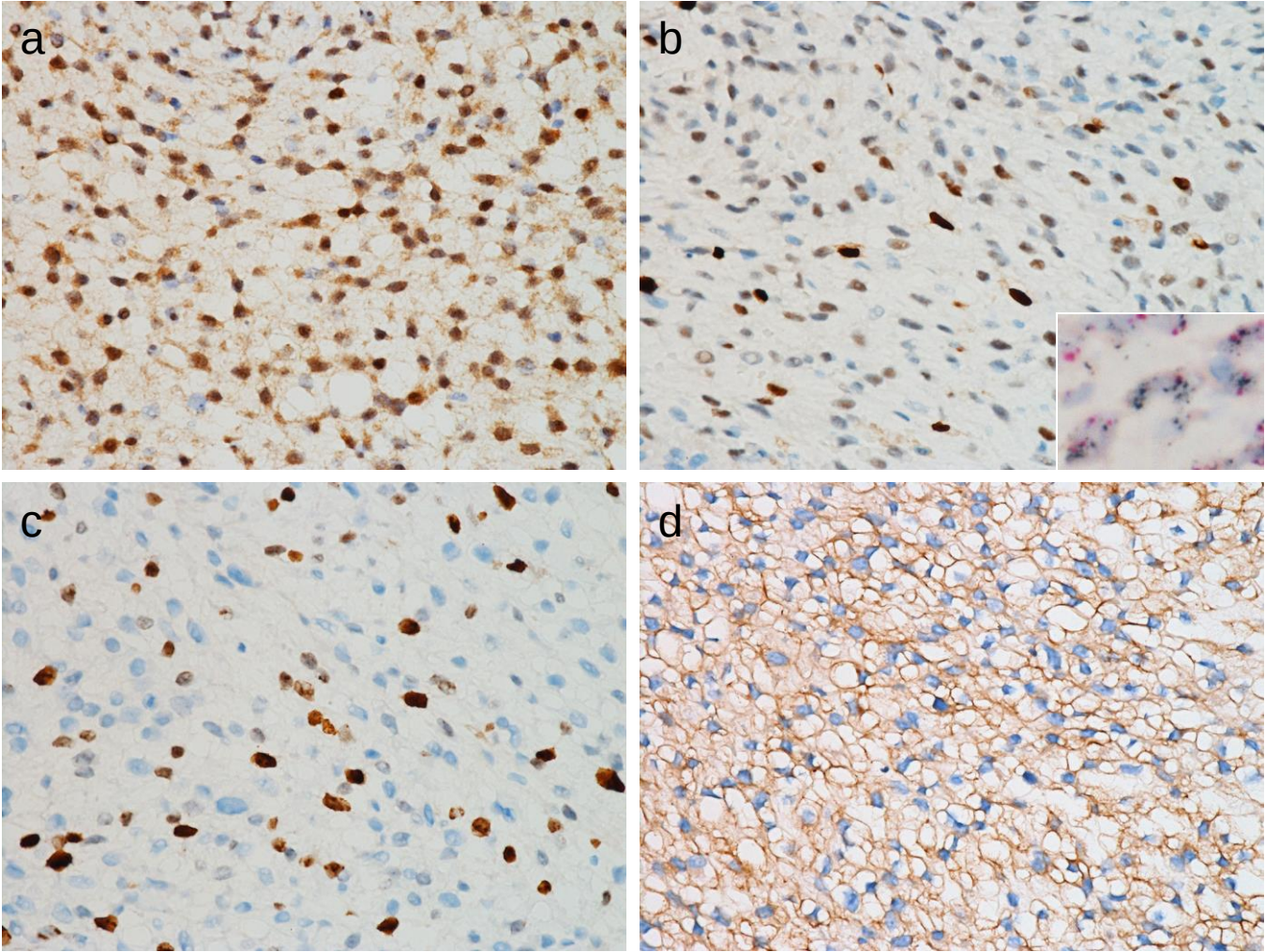
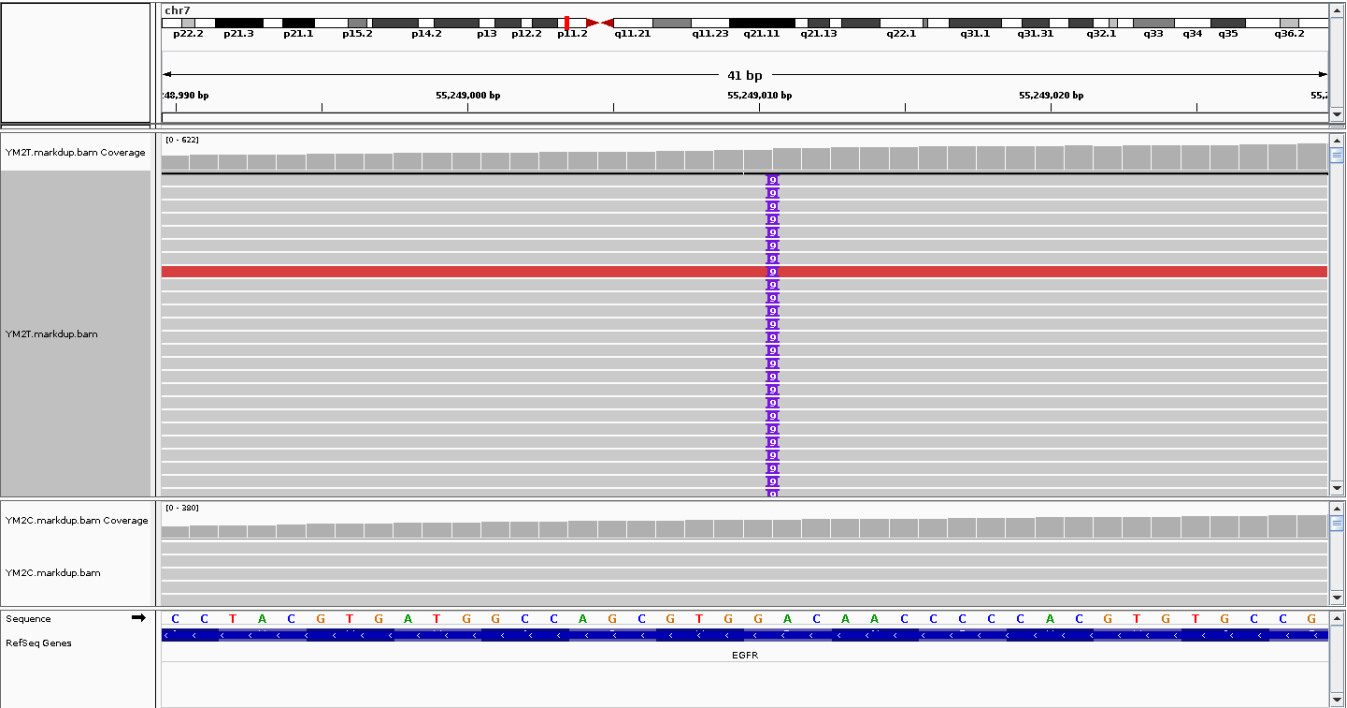


Figure 4

a



b

