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ALK-positive histiocytosis and a clonally related chronic myelomonocytic leukemia

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Author contributions
GB and FS wrote the main manuscript and prepared Figures 2 and 3. RM prepared Figure 1 and wrote part of the manuscript. ER wrote part of the manuscript. All authors read and commented on the manuscript.

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The authors have nothing to disclose.

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The data that support the findings of this study are available from the corresponding author upon reasonable request.

Anaplastic Lymphoma Kinase (ALK)-positive histiocytosis is a non-Langerhans cell histiocytic neoplasm, resulting in the accumulation of histiocytes. This is a rare disease: the largest published cohort[1] includes 39 patients, both children and adults. Most of the remaining literature consists of case series or individual case reports. There is a broad spectrum of clinical manifestations, ranging from isolated lesions in a single organ to widespread, multi-organ involvement. While many organs can be affected, bone, liver and central nervous system are among the most commonly involved.[1] Recognizing this condition is clinically important, as patients typically show a robust response to ALK-inhibitors.
Pathogenetically, the disease is caused by *ALK* translocations, leading to ALK overexpression and, thereby, cell proliferation. In other tumor types, alternative mechanisms of *ALK* activation have been described, including partial deletion of *ALK*, resulting in a truncated protein with oncogenic potential.[2,3] Hitherto, this mechanism has not been described for ALK-positive histiocytosis.

For other types of histiocytic neoplasms, including Langerhans cell histiocytosis, Erdheim-Chester disease, and histiocytic sarcoma, there is an association with an underlying myeloid neoplasm, in particular with chronic myelomonocytic leukemia (CMML).[4,5] In these cases, the histiocytic neoplasm harbors the same driver mutations as the underlying myeloid neoplasm, with additional mutations that are typical for the histiocytic disorder at hand, classically in mitogen-activated protein kinase (MAPK)-pathway-related genes, such as *BRAF*, *KRAS*, *NRAS*, and *MAP2K1*. These mutations supposedly drive histiocytic differentiation of the myeloid precursor cells. However, for ALK-positive histiocytosis, an association with myeloid neoplasms has not been described.[1]
Of note, ALK-variants in myeloid neoplasms are rare and currently not used in the classification of these malignancies. In case reports, these have predominantly been described in acute myeloid leukemia.[6]

Here, we describe a case of an ALK-positive histiocytosis in the temporal bone that is clonally related to an underlying CMML.

An 80-year-old man (informed consent from the patient was obtained; this study does not fall under the scope of the Dutch Medical Research Involving Human Subjects Act) was referred to our tertiary hospital for chronic left-sided suppurative otitis media that persisted despite previous mastoidectomy and ventilation tube, prolonged topical, systemic antibiotics, and antifungal medication. CT and MRI of the temporal bone showed a residual mastoid cavity filled with soft tissue and erosion of the surrounding bone, including a defect in the posterior canal wall, sigmoid sinus, and mastoid tegmen (Figure 1A). MRI with diffusion-weighted imaging (DWI) showed areas of restricted diffusion within the lesion, suggestive of inflammatory tissue or cholesteatoma (Figure 1B). Revision surgery uncovered a dura-adherent mass in the mastoid, which was completely removed by piecemeal resection. Postoperative recovery was uneventful.
Histology showed a proliferation of monomorphic histiocytes with abundant, finely vacuolated cytoplasm (Figure 2A), immunohistochemically positive for CD68 (Figure 2B) and CD163, but negative for CD1a, Langerin, and S100. Remarkably, the histiocytes were diffusely immunoreactive to ALK (Figure 2C). *ALK* fluorescence in situ hybridization (FISH) analysis showed equivocal results with a single red signal in a subset of the cells (Figure 2D). Subsequently, whole transcriptome RNA sequencing (KAPA RNA Hyper Prep Kit with RiboErase, Roche; fusion calling was performed using StarFusion, version 1.8.0), showed an imbalance between the 5'-end and 3'-end of *ALK*, with overexpression of *ALK* exon 20-29 and hardly any expression of *ALK* exon 1-19. Together with the single red signal in FISH analysis and the loss of the green FISH probe spanning region, this pattern fits with an *ALK*

exon 1-19 deletion, confirming the diagnosis of ALK-positive histiocytosis. These RNA-sequencing findings are particularly relevant to the field, because exon 1-19 deletion has been described to lead to an active truncated protein for other ALK-driven tumors, but not yet for ALK-positive histiocytosis.[3,7] Also, a partial deletion may yield, as in our case, equivocal FISH results, and consequently, the diagnosis may be missed, while patients may benefit from ALK-inhibitory therapy.

In the same period that the ear complaints arose, the patient underwent evaluation for a thrombocytopenia (nadir 38×10^9/L) and leukopenia (nadir 2.6×10^9/L), which were identified during routine preoperative screening. Aside from otologic symptoms and notable ecchymoses, the patient exhibited no signs of active bleeding, recent infections, or B-symptoms. Hemoglobin levels were within normal range at the time. Initial work-up did not reveal an underlying etiology for the cytopenias: no vitamin deficiencies, no detectable platelet autoantibodies, and no evidence of splenomegaly on ultrasound.
The patient received platelet transfusions prior to invasive procedures, with satisfactory post-transfusion increments. Flow cytometry was performed on peripheral blood as part of further diagnostic work-up, prompted by a mild but persistent monocytosis (ranging from 0.8 to 1.9×10^9/L). This revealed that 25.6% of $CD45^+$ cells were monocytes, of which 94.6% were classical monocytes ($CD16^-$ $CD14^+$), which is suggestive of a CMML. Trephine biopsy showed a hypercellular (80% cellular) bone marrow with hyperplasia of the granulopoiesis (Figure 3A, B) without an increase in CD34-positive blasts. Immunohistochemically, no ALK-positive cells were present (Figure 3C) and hence we did not perform additional FISH analysis, as in the mastoid lesion, immunohistochemical staining proved to be a sensitive method for detection of the *ALK* aberration. Additional molecular analysis (Agilent, SureSelect DNA platform) of the bone marrow identified mutations in *ASXL1* [c.3754del, p.(Asp1252Thrfs*28), VAF44%], *SRSF2* [c.284C>T, p.(Pro95Leu), VAF44%], and *TET2* [c.3344_3345del, p.(Pro1115Hisfs*14), VAF43% and c.1263del,p.(Gly422Glufs*5), VAF43%] without cytogenetic abnormalities. This molecular profile confirmed the diagnosis of CMML as criteria for chronic myeloid leukemia, other myeloproliferative neoplasms, and myeloid/lymphoid neoplasms with eosinophilia and tyrosine kinase gene fusions were not met.
Together, these findings established the diagnosis of CMML-1. The CPSS-mol score was intermediate-1 based on the presence of an *ASXL1* mutation.[8,9]

In retrospect, we performed mutation analysis (Illumina, TSO500 NGS-panel) on the surgically removed ALK-positive histiocytosis and detected the same *TET2* mutations [c.3344_3345del, p.(Pro1115Hisfs*14), VAF17% and c.1263del, p.(Gly422Glufs*5), VAF21.1%] as well as the *ASXL1* mutation [c.3754del, p.(Asp1252Thrfs*28), VAF18.7%]. Although there was a relatively low coverage of *SRSF2* in TSO500-NGS data, analysis of the raw sequencing reads did show the presence of the same mutant reads in *SRSF2* [c.284C>T, p.(Pro95Leu), VAF25%] as in the bone marrow, making the presence of the identical *SRSF2* mutations in both processes likely.
The relatively high VAFs of the mutations and a high percentage of histiocytes on histology exclude the possibility that the mutations were detected in non-histiocyte, myeloid-derived elements.
Together, this proves that the ALK-positive histiocytosis is clonally related to the CMML.

In follow-up after surgical resection, a PET-CT scan showed no FDG uptake in the mastoid and no evidence of localizations of histiocytosis elsewhere in the body. The patient remains under regular follow-up by the ENT specialist, and until now, eight months after surgery, there are no signs of recurrent disease. If recurrence occurs and it is of clinical importance to

remove additional tissue, viably frozen cell suspensions could be obtained for further study (e.g. single-cell DNA sequencing). The patient's platelet count has remained stable at approximately 40×10^9/L, without evidence of bleeding or hematoma formation. Leukocyte counts have fluctuated but were within normal range at the most recent visit. He is currently in a watch-and-wait trajectory. Short courses of corticosteroids can be considered for thrombocytopenic CMML patients. However, given the side effects of glucocorticoids; the expected short duration of effects; the absence of bleeding; the patient's preference not to undergo treatment as long as he does not have symptoms; and the possibility to administer thrombocytes before invasive procedures, we decided not to treat him at this point. Blood counts will be monitored every six months.

In summary, we present an exceptional case with two novel, significant findings to the field. First, a partial non-kinase domain deletion of *ALK* (in our case deletion of exon 1-19), a for other malignancies already described mechanism of ALK-activation, also occurs as a pathogenic mechanism for ALK-positive histiocytosis. This is relevant because, even though immunohistochemistry points in our case toward the correct diagnosis, FISH analysis may cause confusion and might result in doubt or missing the diagnosis.
Second, we describe for the first time a clonal relation of ALK-positive histiocytosis with an underlying myeloid neoplasm. This is of importance for clinicians to consider when encountering an ALK-positive histiocytosis.

Thus, this case broadens our understanding of ALK-histiocytosis pathogenesis. Future research into the prevalence and optimal clinical management of such cases is needed.

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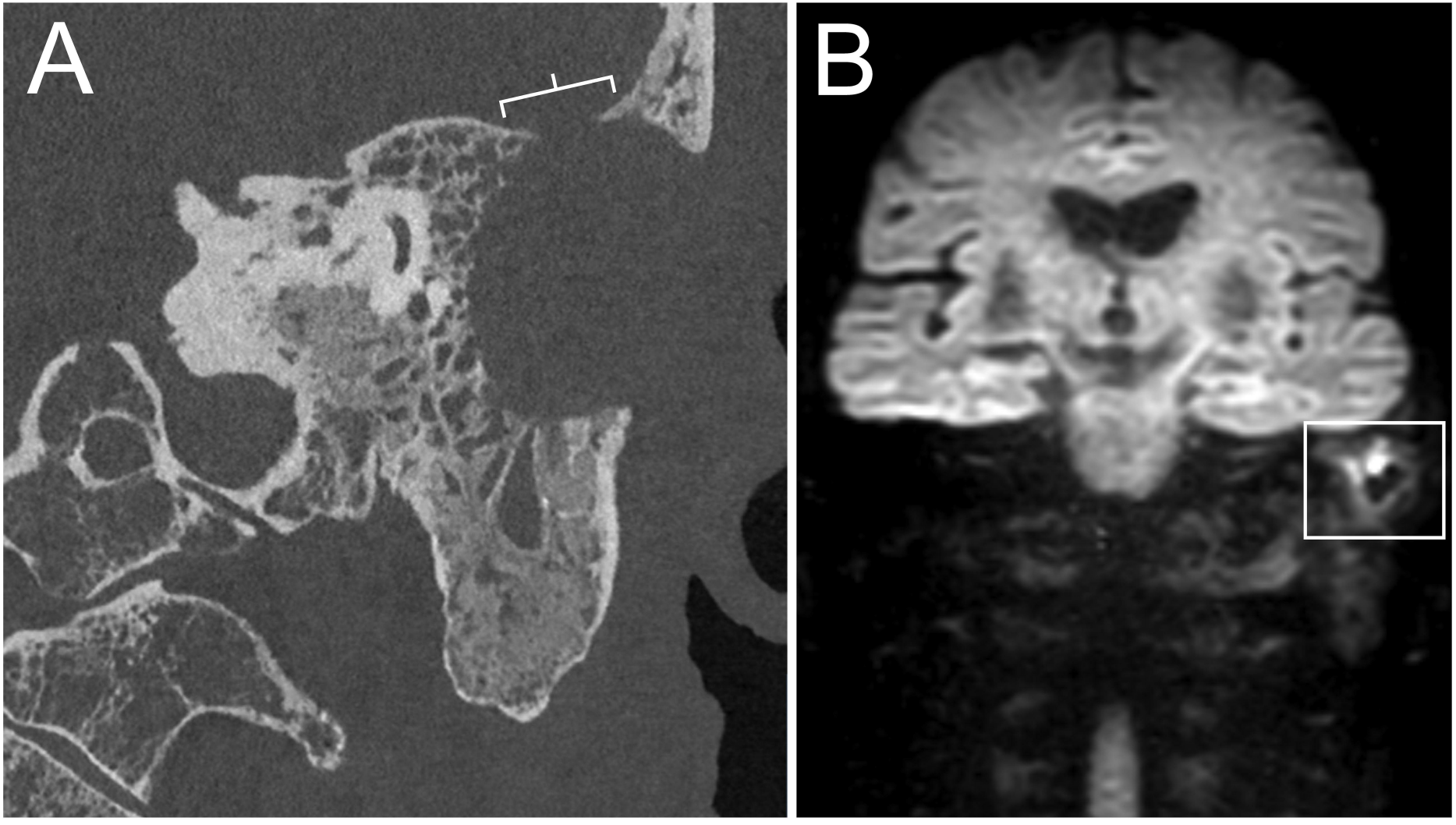
Figure legends

Figure 1 Radiology findings of the mastoid. A) Preoperative CT of the petrous bone (coronal plane) displaying the mastoid cavity on the left side with signs of chronic osteitis and bony erosion, resulting in a defect of the lateral skull base (*bracket*). B) Subsequent MRI DWI (coronal plane) showing areas of restricted diffusion within the corresponding mastoid cavity (*square*).

Figure 2 Histopathological findings of the mastoid lesion. A) H&E staining showing monomorphic histiocytic infiltrate. B) Immunohistochemical stain for CD68 is diffusely positive, confirming that the cells are histiocytes. C) Immunohistochemical stain for ALK is diffusely positive, including nuclear staining (arrow). D) Break-apart FISH for *ALK* (Cytocell LPS019) showing single red signals in a part of the cells. Scale bars A,B: 100 µm; C: 50 µm.

Figure 3 Trephine biopsy findings. A, B. H&E staining of the trephine biopsy showed hypercellularity and hyperplasia of the granulopoiesis. C. Immunohistochemical stain for ALK is negative. Scale bars A, C: 50 µm; B: 10 µm.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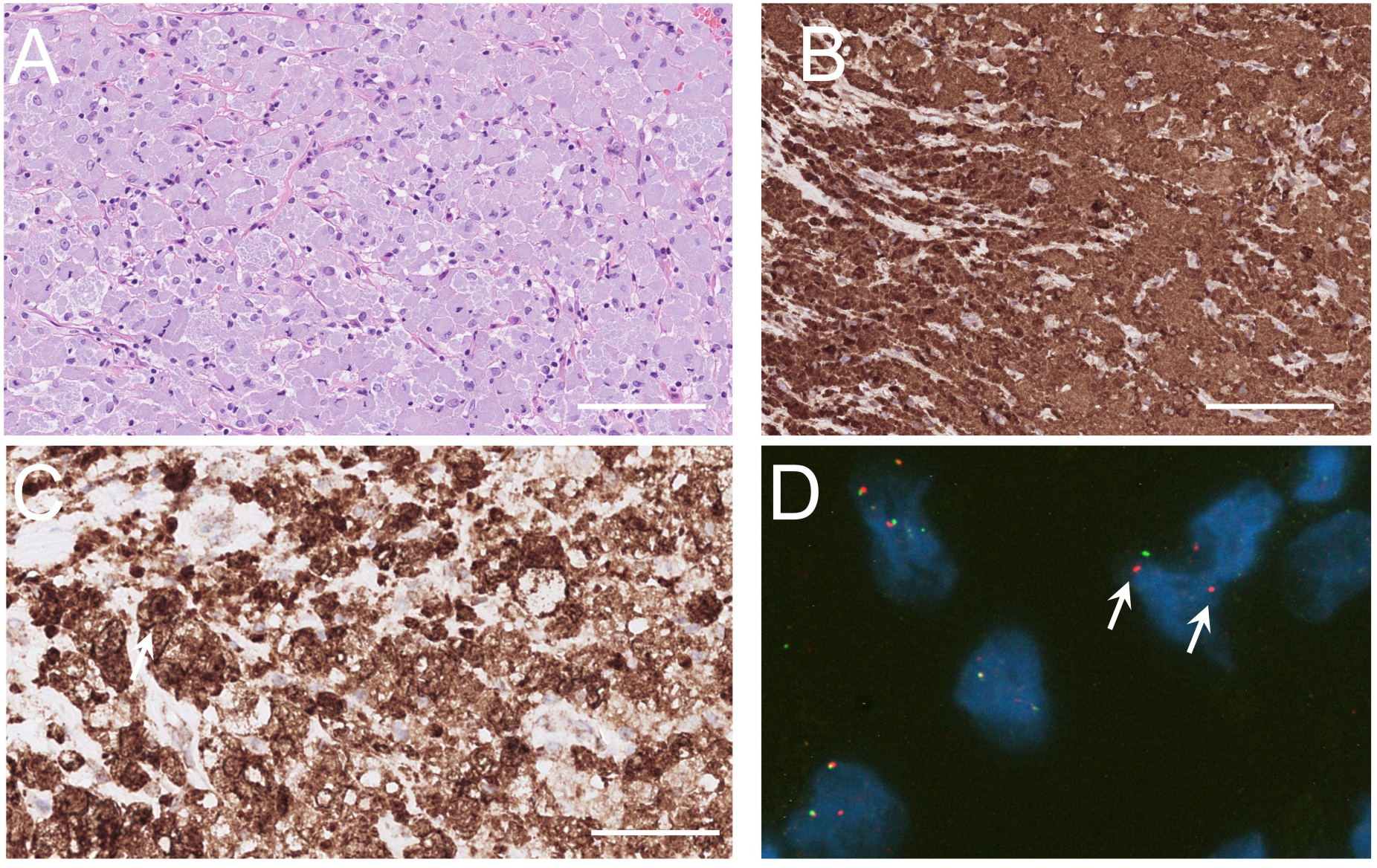
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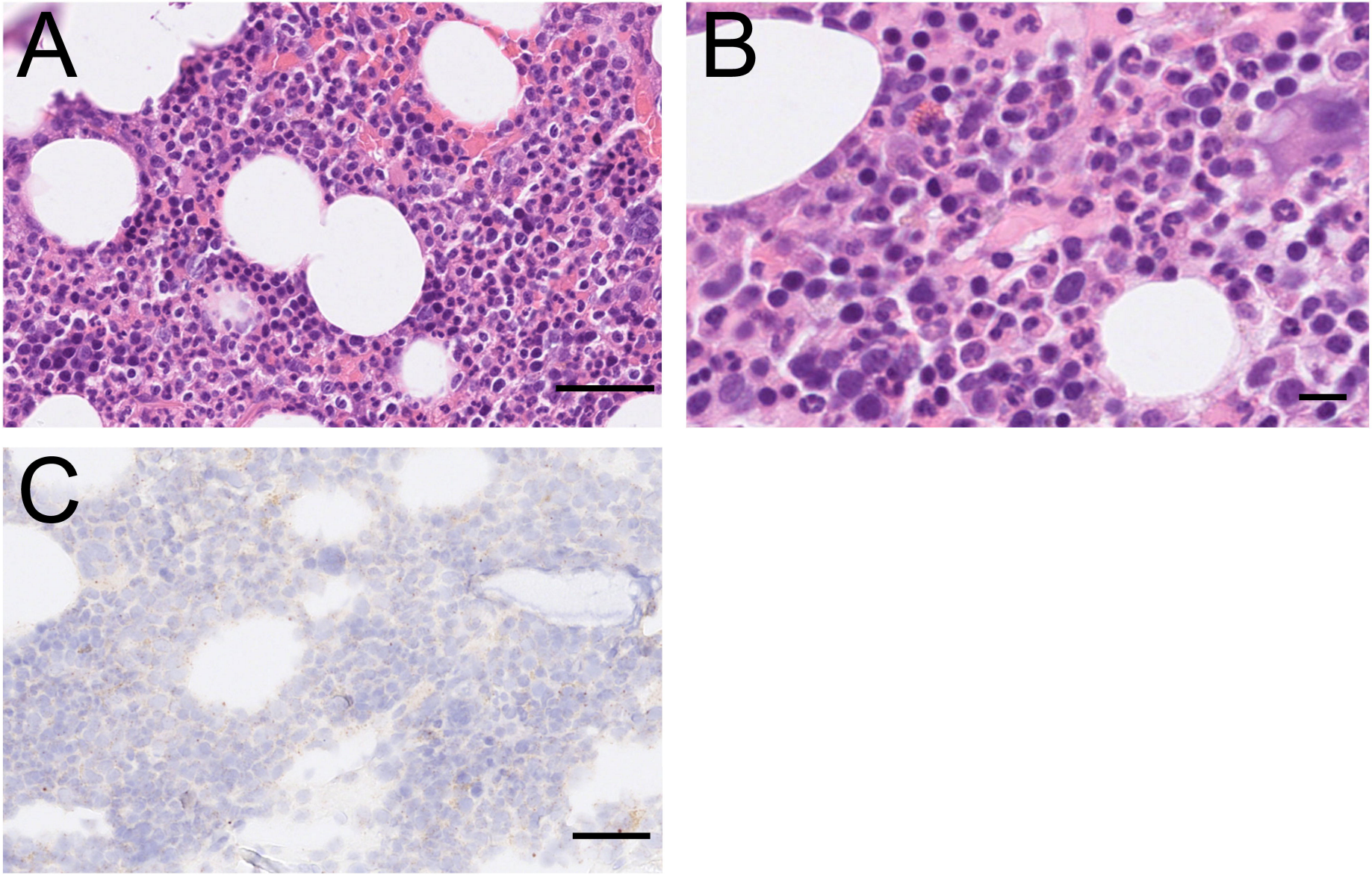
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