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Antimicrobials

During his hospital stay, the patient was treated with oral tecovirimat (Day of Illness 22-Day of Illness 36/Hospital Day 11 and Hospital Day 12-Hospital Day 27), bictegravir/emtricitabine/tenofovir alafenamide (Hospital Day 11-Hospital Day 24), atazanavir/ritonavir/dolutegravir/tenofovir alafenamide (Hospital Day 25-Hospital Day 27), ceftriaxone (Hospital Day 4-Hospita Day 25), metronidazole (Hospital Day 4-Hospital Day 15; Hospital Day 17-Hospital Day 19; Hospital Day 23-Hospital Day 25), meropenem (Hospital Day 25-Hospital Day 27), vancomycin (oral: Hospital Day 2-Hospital Day 4; IV: Hospital Day 4-Hospital Day 27), fluconazole (Hospital Day 1-Hospital day 18), micafungin (Hospital Day 23-Hospital Day 27), and trimethoprim/sulfamethoxazole (Hospital Day 2-Hospital Day 12; Hospital Day 24-Hospital Day 27).

Methods

Specimen collection

Autopsy was performed at the Los Angeles County Department of Medical Examiner-Coroner by Virchow dissection at a negative pressure room. The Medical Examiner-Coroner had received the ACAM2000 vaccine prior to autopsy. The Medical Examiner Fellow and the tech assisting with the autopsy received the JYNNEOS vaccine after the autopsy was completed.

Clinical specimens for orthopoxvirus diagnostic testing were collected per the U.S. Centers for Disease Control and Prevention (CDC) guidelines[1]. Lesion swabs in viral transport media and fresh, unfixed tissue from major organs were submitted for real-time Polymerase Chain Reaction (PCR) diagnostic testing. Formalin-fixed tissues from major organs were used to prepare paraffin embedded tissue blocks for histology. This activity was reviewed by CDC and was conducted consistent with applicable federal law and CDC policy (45 C.F.R. part 46.102(l)(2), 21 C.F.R. part 56; 42 U.S.C. §241(d); 5 U.S.C. §552a; 44 U.S.C. §3501 et seq.). The decedent's family consented to the autopsy and was aware of the possible publication of this manuscript.

Diagnostic testing

DNA was extracted using the QIAGEN QIAamp DSP DNA Blood Mini Kit Tissue Kit (Germantown, MD) and tested using the 510(k) cleared non-variola Orthopoxvirus real-time PCR assay following CDC Laboratory Response Network (LRN) protocols[2,3].

Histology slides prepared from sampled organs were stained with hematoxylin and eosin (H&E), scanned, and interpreted using a VENTANA DP 200 slide scanner (Roche Diagnostics, Rotkreuz, Switzerland). Mpox immunochemistry stain was not available, no immunochemistry was performed to identify virus in tissues.

Whole genome sequencing and genomic analysis of mpox virus specimens

Libraries were generated from extracted DNA using a tiling-amplicon based approach[10] and prepared for sequencing with the Illumina DNA prep kit (Illumina, San Diego, California). Pooled libraries were sequenced on the Illumina MiSeq v. 2 (paired-end 150), with 0.5-1 million reads per library. Primers were trimmed and consensus genomes generated at a minimum depth of coverage of 5X via the TheiaCoV_Illumina_PE Workflow Series on Terra.bio[11]. Consensus genomes were analyzed with Nextclade[12] to determine clade, lineage, and mutations present relative to the lineage B.1 reference genome. Allele frequencies for tecovirimat mutations were determined using Geneious version 2022.2.2 (https://www.geneious.com/). Consensus genomes of sufficient quality were submitted to GISAID (Global Initiative on Sharing Avian Influenza Data); raw reads were submitted to SRA (Sequence Read Archive), both are public data repositories. Accession numbers are provided.

Tecovirimat resistance assay

Tissue samples were homogenized and inoculated on confluent African Green monkey kidney BSC-40 cells. At 4+ (75%-100%) cytopathic effect, the cell monolayer and supernatant were harvested. Virus titers were determined by serial dilution plaque assay in the same cell line. For tecovirimat resistance, the drug was added to confluent Vero E-6 cells in 96-well plates in a staggered 10-fold dilution ranging from 0.0015 – 5.0 μM drug concentration and incubated 1 hour at 37° C in 6% CO_2. Plates were then infected with 0.01 – 0.02 multiplicity of infection (MOI) of virus and incubated 3 days at 35° C in 6% CO_2. After incubation, cell plates were fixed with crystal violet stain containing formalin (F.C. v/v > 4%) to visualize intact cells. A PerkinElmer EnSpire multimode plate reader (PerkinElmer, Waltham, MA) was used to measure absorbance at 570 nm. GraphPad Prism (GraphPad Software, San Diego, CA) was used to

calculate the half maximal effective concentration (EC50) for tecovirimat. Extended concentration tecovirimat resistance assays up to 500 μM drug concentration were subsequently set up to determine antiviral endpoint.

Whole genome sequencing and tecovirimat resistance results

Whole genome sequencing confirmed mpox Clade IIb lineage B.1.2 (Nextclade) on fifteen post-mortem specimens. Six known mutations linked with high-level tecovirimat resistance in vaccinia virus, originally reported in laboratory cell culture, were identified in the OPG057 gene (F13L) encoding the VP37 protein[4,5]. At least one previously described mutation was identified in each specimen, with several specimens having multiple mutations. Mutation P243S with unknown phenotypic effect was also identified. All mutations were present as heterogenous populations. Viable virus was successfully cultured from 12 of 15 tissue specimens received for resistance testing; liver, brain and adrenal tissue could not be cultured due to destruction of the cell monolayer one day post inoculation likely caused by contamination or due to cytotoxic effect of the tissues themselves. Tecovirimat resistance was confirmed by phenotypic testing on all twelve tissues. Resistance was observed in some tissues at a minimum EC_{50} ~ 0.5 µM tecovirimat and at a maximum in some tissues > 500 µM. Compared to a reference strain (mpox Clade IIa, 2003, US) these isolates were 1.5 $\log_{10}$ to > 4.5 $\log_{10}$ more resistant.

References:

1. Lab Advisory: CDC Specimen Collection Guidelines for Monkeypox. Centers for Disease Control and Prevention (CDC). https://www.cdc.gov/locs/2022/05-16-2022-lab-advisory-cdc_specimen_collection_guidelines_monkeypox_virus.html Accessed September 26, 2022.
2. Centers for Disease Control and Prevention – Laboratory Response Network. Extraction of Orthopoxvirus DNA using QIAGEN QIAamp DSP DNA Blood Mini Kit for Subsequent Testing by a Real-time PCR Assay. Protocol #1130-R4.
3. Centers for Disease Control and Prevention – Laboratory Response Network. Detection of Non-variola Orthopoxvirus DNA by Fluorogenic 5' Nuclease Assay using the Applied Biosystems® 7500 Fast Dx Real-time PCR Instrument. Protocol #1108-R9.
4. Duraffour S, Lorenzo MM, Zöller G, et al. ST-246 is a key antiviral to inhibit the viral F13L phospholipase, one of the essential proteins for orthopoxvirus wrapping. J Antimicrob Chemother. 2015 May;70(5):1367-80.
5. FDA Monkeypox Response. https://www.fda.gov/emergency-preparedness-and-response/mcm-issues/fda-monkeypox-response Accessed September 19, 2022

Table 1. Allele frequencies of VP37 mutations detected in different tissue specimens

Specimen Source	Allele Frequencies (Max = 1)							Cycle Threshold Values	Accession Numbers	
	P243S	N267D*	A288P*	A290V*	D294V*	A295E*	I372N*		GISAID‡	SRA‡
Skin†	-	-	0.9	-	0.4	-	-	13.98	EPI_ISL_15120471	SRS15247069
Skin†	-	0.14	0.33	0.18	0.21	-	-	13.76	EPI_ISL_15120472	SRS15247070
Spleen†	-	-	0.29	0.14	0.17	0.13	-	14.21	EPI_ISL_15120473	SRS15247071
Lymph†	-	0.16	0.45	0.11	0.15	-	-	13.52	EPI_ISL_15120474	SRS15247072
Liver	-	0.11	0.28	0.15	0.16	-	-	15.04	EPI_ISL_15120475	SRS15247073
Lung†	0.24	0.11	0.25	0.14	0.14	-	-	17.28	EPI_ISL_15120476	SRS15247074
Kidney†	0.17	0.12	0.21	0.15	0.2	-	-	20.87	EPI_ISL_15120477	SRS15247075
Heart†	0.32		0.14	0.15	0.11	-	-	22.35	EPI_ISL_15120478	SRS15247086
Bone Marrow†	-	-	0.37	0.21	-	-	-	17.2	-	-
Adrenal	-	-	0.33	0.13	0.24	-	-	15.73	EPI_ISL_15120479	SRS15247085
Brain	-	-	0.13	0.24		0.12	-	21.88	EPI_ISL_15120480	SRS15247087
GI Tract†	-	-	0.1	0.13	0.14	0.6	-	13.32	EPI_ISL_15120481	SRS15247088
Pleural Fluid†	-	-	0.17	-	-	0.1	-	22.62	EPI_ISL_15120482	SRS15247089
Facial Lesion†	-	-		0.96	-	-	-	20.09	EPI_ISL_15120483	SRS15247090
Testicular†	-	-		0.2	-	-	0.52	19.46	EPI_ISL_15120484	SRS15247091

*Associated with tecovirimat resistance in vaccinia virus †Confirmed phenotypic resistance ‡GAIS: Global Initiative on Sharing Avian Influenza Data; SRA:

Table 2. Clinical Laboratory Results

Measure	Reference range	Illness Day 26, Hospital Day 2	Illness Day 32, Hospital Day 8	Illness Day 41, Hospital Day 17	Illness Day 43, Hospital Day 19	Illness Day 47, Hospital Day 21	Illness Day 48, Hospital Day 24	Illness Day 50, Hospital Day 26
White-cell count (per µl)	4.5 - 10.0	31.5*	10.9*	13.6*	28.3*	68.9*	80*	126*
Red-cell count (per µl)	4.40 - 5.60	-	-	-	-	-	3.48†	-
Platelet count (per µl)	160 - 360	181	185	404*	386*	163	114†	plts clumped
Hemoglobin (g/dl)	13.5 - 16.5	15.2	12.3†	10.2†	9.9†	9.3†	8.4†	8†
Hematocrit (%)	40 - 49	46.4	37.2	30.7†	30†	27.8†	26.5†	25.9†
Sodium (mmol/liter)	135 - 145	121†	132†	131†	134†	129†	128†	128†
Potassium (mmol/liter)	3.5 - 5.1	5.3*	4.4	4.1	4.4	6.3*	4.4	5.4*
Chloride (mmol/liter)	100 - 110	86†	97†	99†	101	98†	97†	97
Carbon dioxide (mmol/liter)	20 - 30	22	24	21	17†	21	19†	16†
Anion gap (mmol/liter)	10 - 20	-	-	-	-	-	16	-
Glucose (mmol/liter)	65 - 99	103*	89	100*	108*	98	111*	-
Blood urea nitrogen (mmol/liter)	8 - 22	64*	17	6	9	17	24*	-
Creatinine (mg/dl)	0.50 - 1.30	1.28	0.59	0.45†	0.49	0.83	0.94	2.69*
Total protein (g/dl)	6 - 8	-	-	-	-	-	4.9†	4.9†
Albumin (g/dl)	3.5 - 5.0	-	-	-	-	-	1.4†	-
Total bilirubin (mg/dl)	<1	-	-	-	-	-	0.2	-
Procalcitonin (ng/ml)	<0.25	-	-	0.33*	-	-	-	14.7‡
Alanine aminotransferase (U/liter)	10 - 50	-	-	-	-	-	11	-
Aspartate aminotransferase (U/liter)	10 - 50	-	-	-	-	-	84*	-
Alkaline phosphatase (U/liter)	40 - 129	-	-	-	-	-	376*	-
Prothrombin time (sec)	11.8 - 14.4	-	-	-	-	-	15.7*	25*
International normalized ratio	0.88 - 1.14	-	-	-	-	-	1.27*	2.32*
Venous lactate (mmol/liter)	0.5-1.6	-	-	-	-	1.6	-	-
Calcium (mg/dl)	8.5 - 10.3	8.7	8.8	-	7.6	6.7†	6.9†	6.9†
Magnesium (mg/dl)	1.6 - 2.6	2.9*	1.8	-	1.8	3.2*	2	2.8
Osmolality (mOsm/kg)	275 - 295	280	-	-	-	-	-	-
Phosphorus (mg/dl)	2.5 - 4.5	-	-	-	-	-	3.6	-
Uric Acid (mg/dl)	4 - 8.1	11.1*	-	-	-	-	-	-
CRP (mg/l)	<1	-	184.2*	-	-	-	-	-
Ferritin (ng/ml)	30 - 330	-	-	-	-	-	3334*	-
GGT (U/l)	8 - 61	-	-	-	-	-	126*	-
Haptoglobin (mg/dl)	30 - 200	-	-	-	-	-	306*	-
CD4+ (cells/mm^3)	512 – 1,499	<35†	-	-	-	-	-	-
HIV Viral Load (copies/mL)	<20	26,500*	-	-	-	-	-	-

*Above reference range

† Below reference range

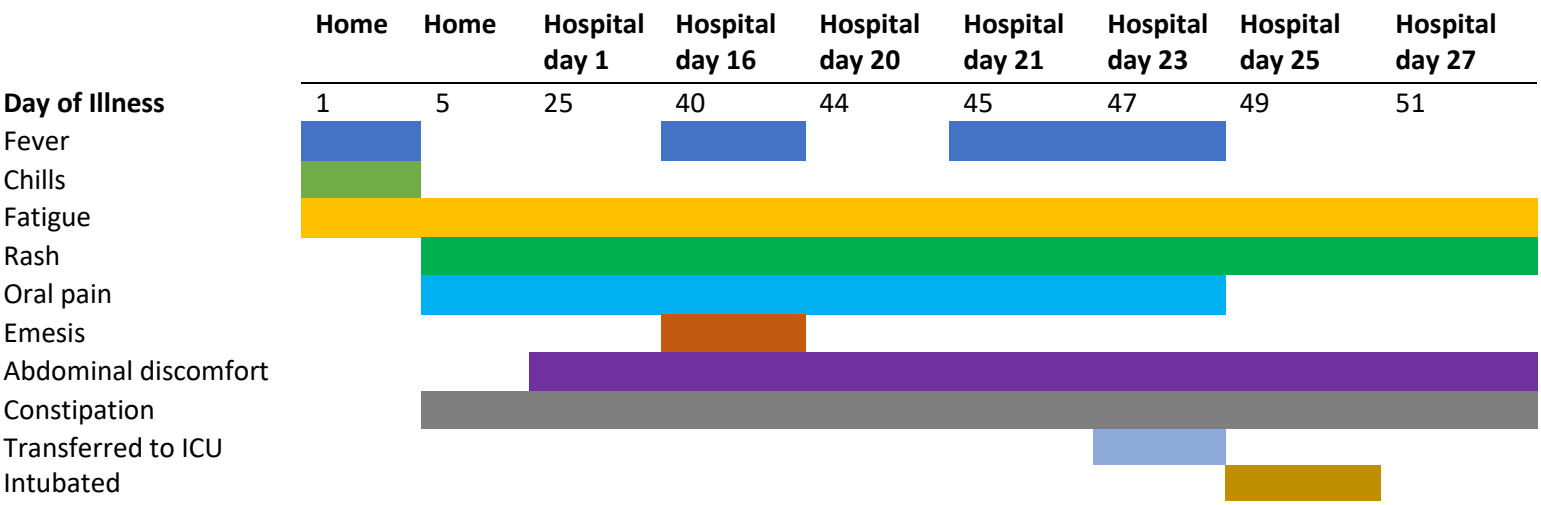
‡ Data from Illness Day 49, Hospital Day 25

Table 3. Diagnostic Test Results				
Pathogen/Target	**Test**	**Type of Sample**	**Date of Collection**	**Result**
Covid	PCR*	Nasopharyngeal Swab	Hospital day 1	Negative
Gonorrhea	PCR*	Rectal Swab	Hospital day 1	Negative
Chlamydia	PCR*	Rectal Swab	Hospital day 1	Negative
Cryptococcus	Antigen Enzyme Immunoassay	Blood	Hospital Day 2	Negative
Syphilis	Antibody	Blood	Hospital Day 2	Positive
Syphilis	RPR Titter	Blood	Hospital Day 2	Negative
Bacteria	Culture	Blood	Hospital Day 2	No growth at 5 days
Bacteria	Culture	Blood	Hospital Day 2	No growth at 5 days
Clostridium difficil	PCR*	Stool	Hospital Day 7	Negative
Giardia	Antigen Enzyme Immunoassay	Stool	Hospital Day 9	Negative
Cryptosporidium	Antigen Enzyme Immunoassay	Stool	Hospital Day 9	Negative
Cytomegalovirus IgG	Antibody	Blood	Hospital Day 9	Positive
Cytomegalovirus IgM	Antibody	Blood	Hospital Day 9	Negative
Acid Fast Bacilli	Acid Fast Smear	Stool	Hospital Day 10	None observed at 0, 1, 7 and 14 days
Acid Fast Bacilli	Culture	Stool	Hospital Day 10	None isolated at 1, 7 and 14 days
Cryptococcus	Antigen Enzyme Immunoassay	Blood	Hospital Day 11	Negative
Salmonella	Culture	Stool	Hospital Day 11	Negative
Shigella	Culture	Stool	Hospital Day 11	Negative
Yersinia	Culture	Stool	Hospital Day 11	Negative
Campylobacter	Culture	Stool	Hospital Day 11	Negative
E. Coli O157:H7	Culture	Stool	Hospital Day 11	Negative
Herpes Simplex Virus 1 and 2	PCR*	Rectal Swab	Hospital Day 13	Negative
Ova and Parasites	Microscopy	Stool	Hospital day 15	Negative
Bacteria	Culture	Blood	Hospital Day 18	Staphylococcus epidermidis
Bacteria	Culture	Blood	Hospital Day 18	Staphylococcus epidermidis
Bacteria	Culture	Pleural Fluid	Hospital Day 18	No growth at 5 days
Bacteria	Gram Stain	Pleural Fluid	Hospital Day 18	Rare PMNs observed
Adeno Deaminase	Chemistry	Pleural Fluid	Hospital Day 18	9.5 U/L
Protein	Chemistry	Pleural Fluid	Hospital Day 18	5.4 g/dL

Triglycerides	Chemistry	Pleural Fluid	Hospital Day 18	59 mg/dl
pH	Chemistry	Pleural Fluid	Hospital Day 18	7.97 pH
LDH	Chemistry	Pleural Fluid	Hospital Day 18	584 U/L
Cholesterol	Chemistry	Pleural Fluid	Hospital Day 18	33 mg/dL
Nucleated Cell Count	Chemistry	Pleural Fluid	Hospital Day 18	752 /cumm
Segmented Neutrophils	Chemistry	Pleural Fluid	Hospital Day 18	75%
Lymph	Chemistry	Pleural Fluid	Hospital Day 18	22%
Monocytes/Histiocytes	Chemistry	Pleural Fluid	Hospital Day 18	3%
MRSA	Culture	Sputum induced	Hospital day 19	None isolated
Pseudomonas aeruginosa	Culture	Sputum induced	Hospital day 19	None isolated
Bacteria	Gram Stain	Sputum induced	Hospital day 19	No PMNs† observed, 2+ GN Coccobacilli, 1+ GN Rods
Coccidiomycosis	Antibody	Blood	Hospital day 19	Negative
Acid Fast Bacilli	Acid Fast Smear	Pleural Fluid	Hospital day 19	None observed
Acid Fast Bacilli	Culture	Pleural Fluid	Hospital day 19	None isolated at 1 week
Bacteria	Culture	Blood	Hospital day 19	No growth at 5 days
Acid Fast Bacilli	Acid Fast Smear	Sputum Induced	Hospital Day 20	None observed
Acid Fast Bacilli	Culture	Sputum Induced	Hospital Day 20	None isolated at 1 week
Bacteria	Culture	Blood	Hospital Day 20	No growth at 5 days
Bacteria	Culture	Pleural Fluid	Hospital Day 20	No growth at 5 days
Bacteria	Gram Stain	Pleural Fluid	Hospital Day 20	Rare PMNs† observed
Acid Fast Bacilli	Acid Fast Smear	Sputum Induced	Hospital Day 21	None observed
Acid Fast Bacilli	Culture	Sputum Induced	Hospital Day 21	None isolated at 1 week
Bacteria	Culture	Blood	Hospital Day 23	No growth at 5 days
Fungitell (1-3)-beta-D-Glucan	Fungitell	Blood	Hospital Day 23	Negative
Clostridium difficil	PCR*	Stool	Hospital Day 24	Negative
Histoplasmosis	Antigen Enzyme Immunoassay	Urine	Hospital day 24	Negative
Pneumocystis Pneumonia (PCP)	Direct Fluorescent Antibody	Sputum Induced	Hospital day 25	Negative
Fungal	Culture	Blood	Hospital Day 25	No growth at 6 days
MRSA	Culture	Sputum induced	Hospital day 25	None isolated
Pseudomonas aeruginosa	Culture	Sputum induced	Hospital day 25	None isolated
Bacteria	Gram Stain	Sputum induced	Hospital day 25	3+ PMNs† observed

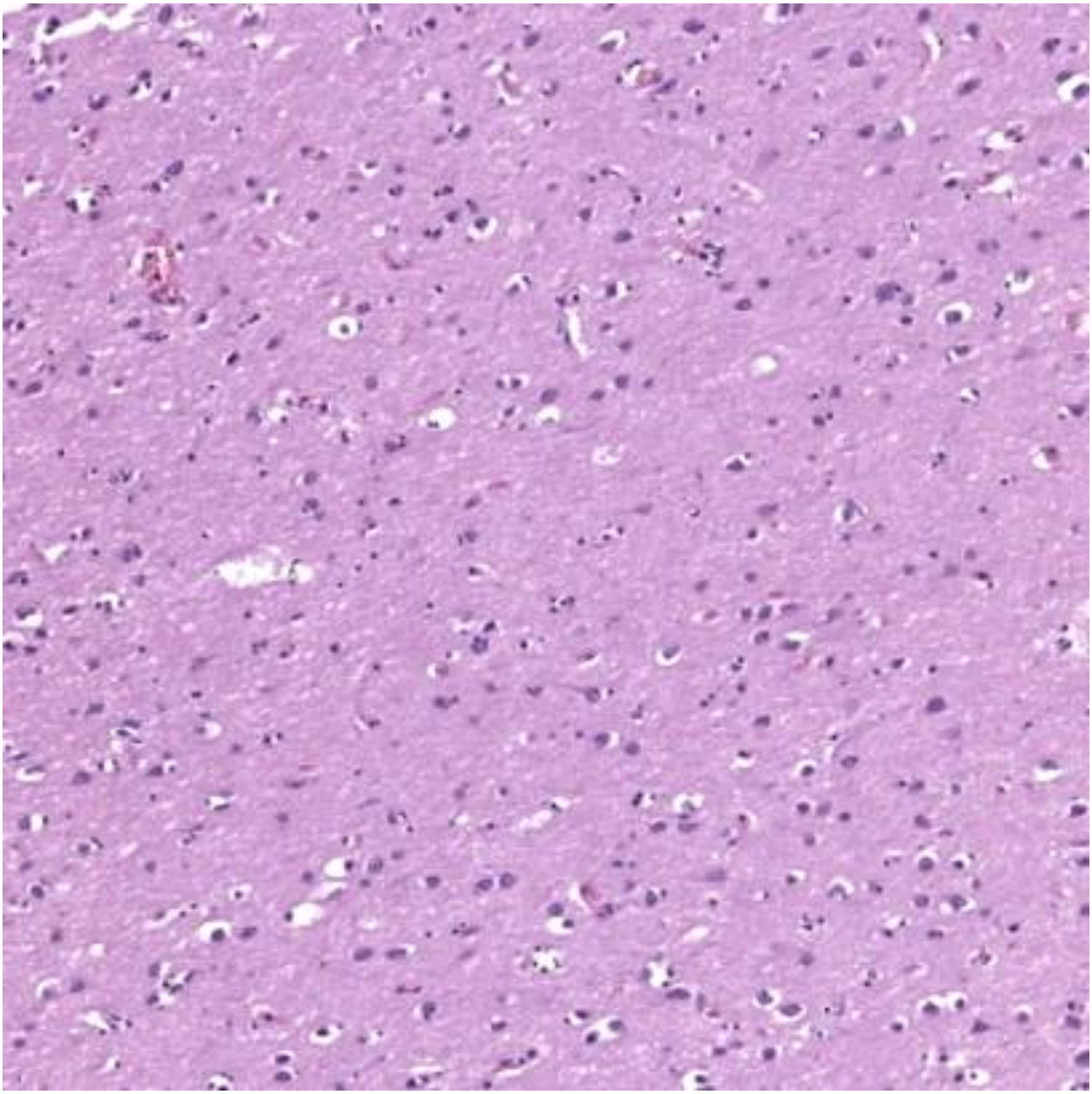
Bacteria	Culture	Blood	Hospital Day 27	No growth at 3 days
Bacteria	Culture	Urine	Hospital Day 26	Negative
Orthopoxvirus	PCR*	Brain	Autopsy	Positive
Orthopoxvirus	PCR*	Bone Marrow	Autopsy	Positive
Orthopoxvirus	PCR*	Lung Lymph Node	Autopsy	Positive
Orthopoxvirus	PCR*	Inguinal Lymph Node	Autopsy	Positive
Orthopoxvirus	PCR*	Lung	Autopsy	Positive
Orthopoxvirus	PCR*	Heart	Autopsy	Positive
Orthopoxvirus	PCR*	Liver	Autopsy	Positive
Orthopoxvirus	PCR*	Spleen	Autopsy	Positive
Orthopoxvirus	PCR*	Adrenal Glands	Autopsy	Positive
Orthopoxvirus	PCR*	Kidney	Autopsy	Positive
Orthopoxvirus	PCR*	Gastrointestinal tract	Autopsy	Positive
Orthopoxvirus	PCR*	Testicle	Autopsy	Positive
Orthopoxvirus	PCR*	Skin Lesion 1	Autopsy	Positive
Orthopoxvirus	PCR*	Skin Lesion 2	Autopsy	Positive
Orthopoxvirus	PCR*	Facial Skin Lesion	Autopsy	Positive
Orthopoxvirus	PCR*	Pleural Fluid	Autopsy	Positive

*Polimerase Chain Reaction
†Polymorphonuclear Neutrophils

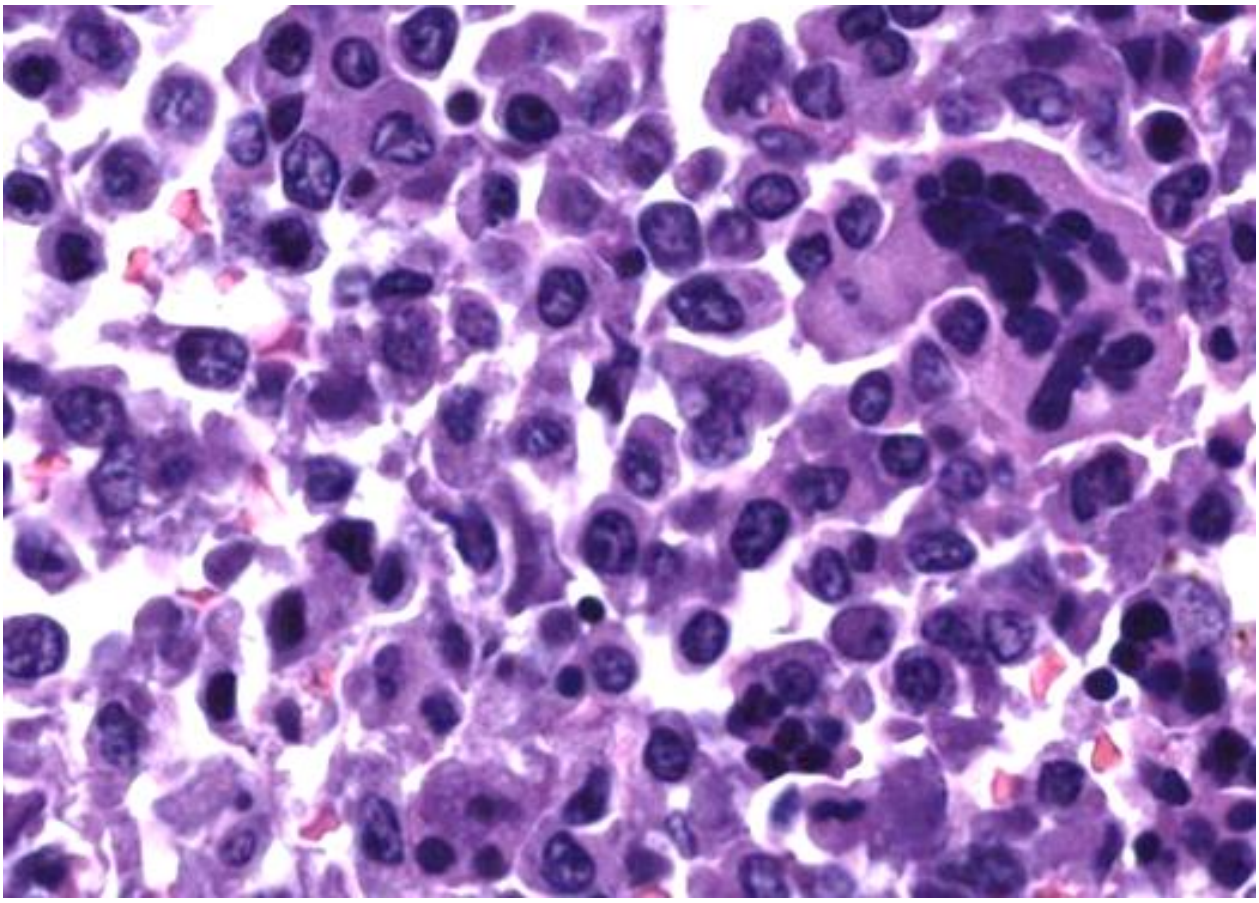


Supplemental Figure 1. Major event and symptoms time-line

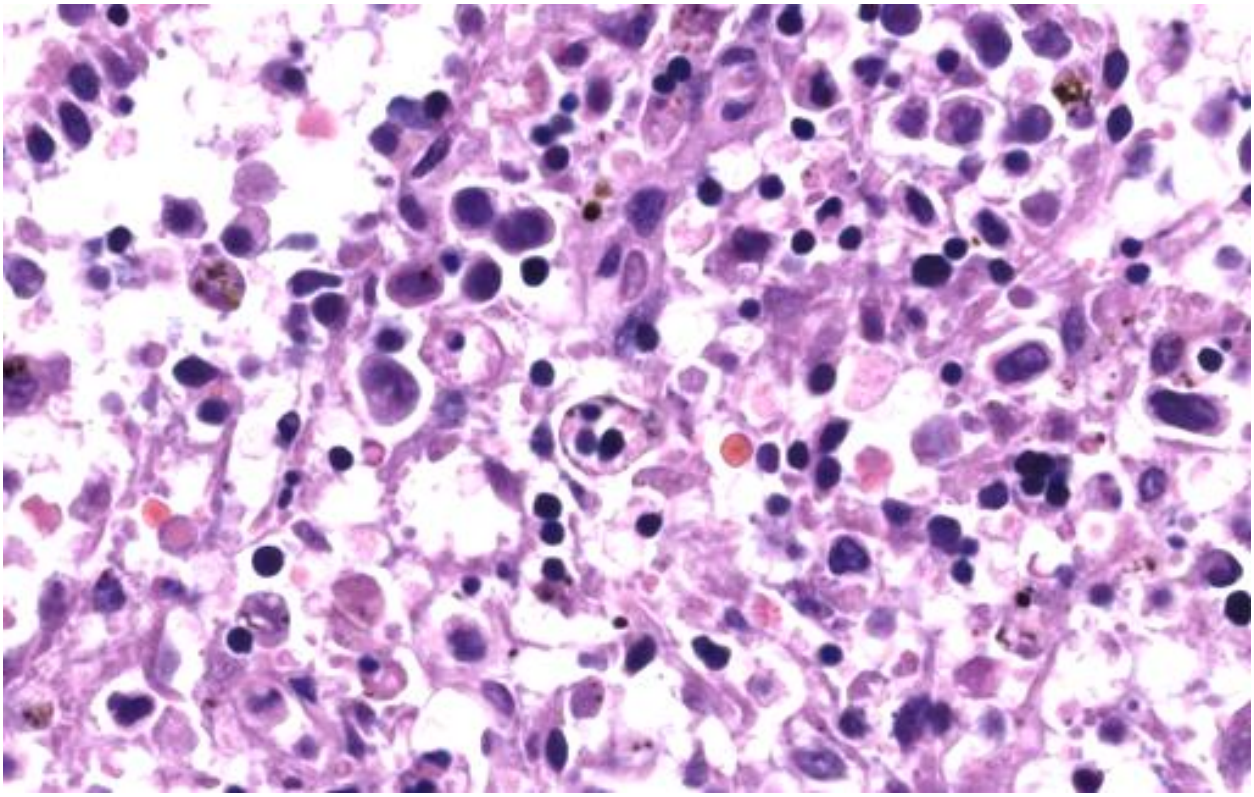
Supplemental Figure 2A. Mpox lesions across the epiglotis.



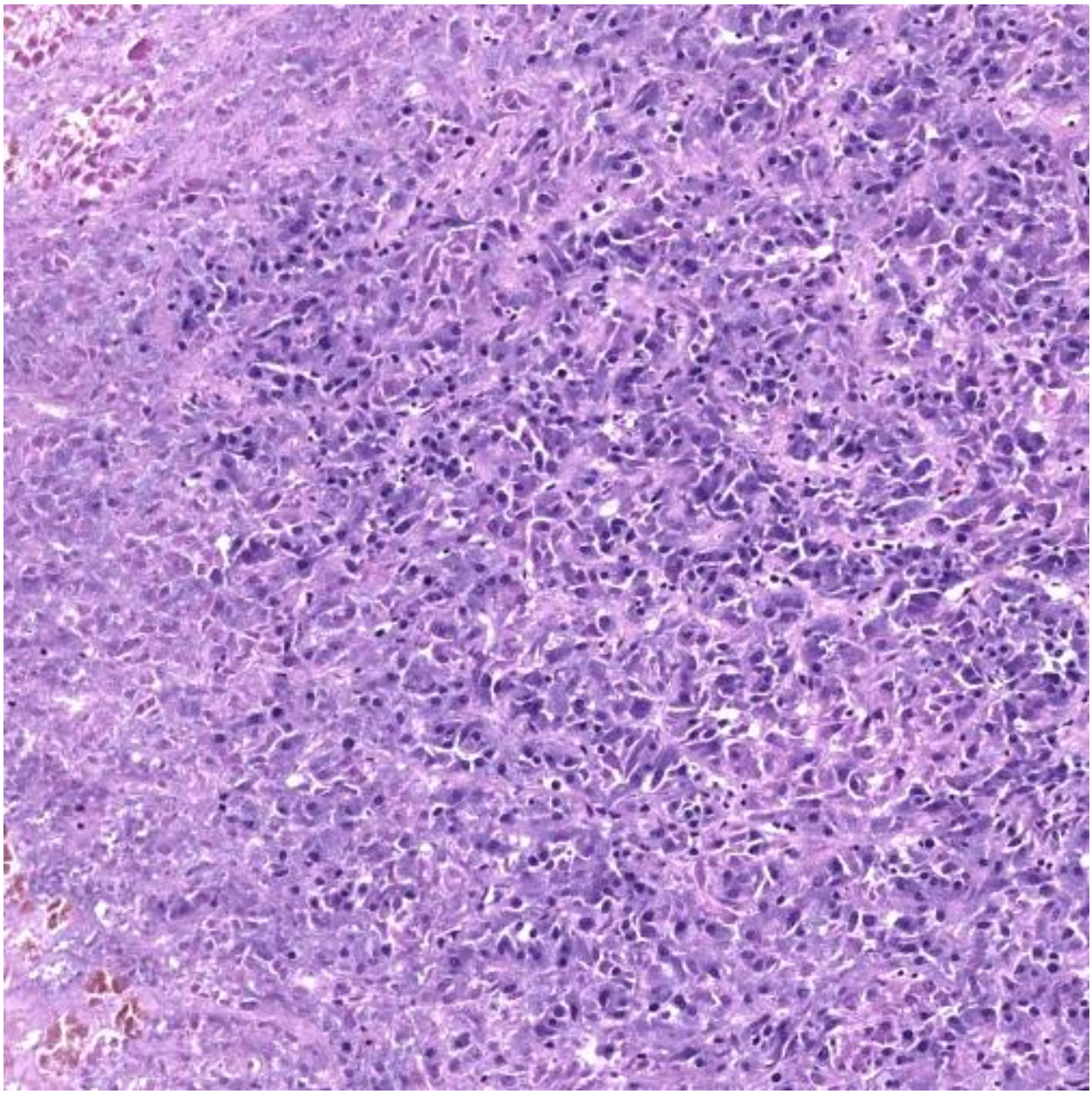
Supplemental Figure 2B. The brain (H&E 20X) is seen with no significant inflammation, the layers of the cerebral cortex are well-formed, triangular neurons and glial cells are not increased in number and are scattered in a background of pink neuropil.



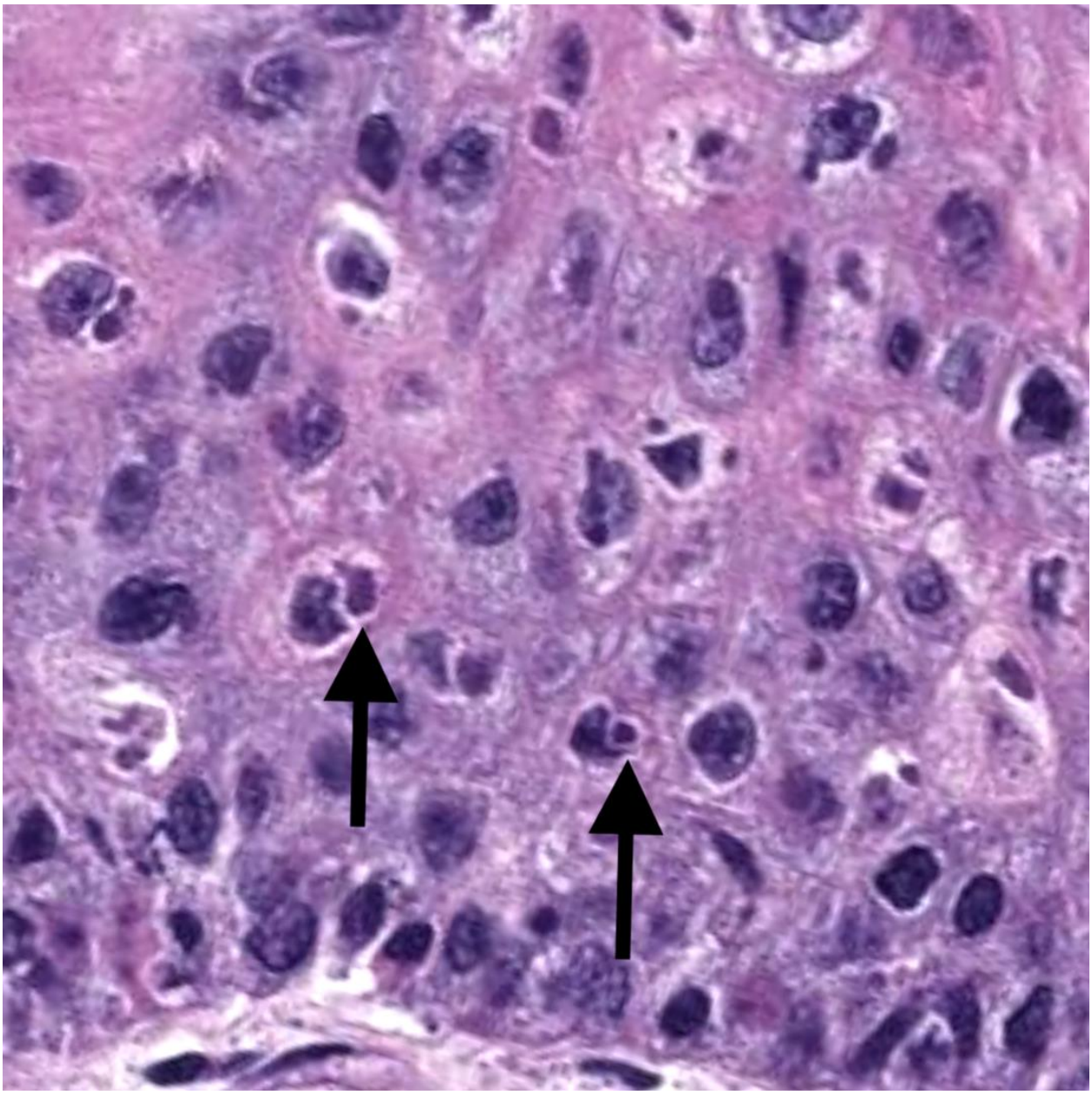
Supplemental Figure 2C. Markedly hypercellular bone marrow (99% cellular) (H&E 20X) with expanded trilineage hematopoiesis, including myeloid cell hyperplasia and hyperlobated megakaryocytes, occurring in a background of necrotic debris and high cell turnover. Occasional bone marrow macrophages demonstrate evidence of hemophagocytic lymphohistiocytosis (HLH).



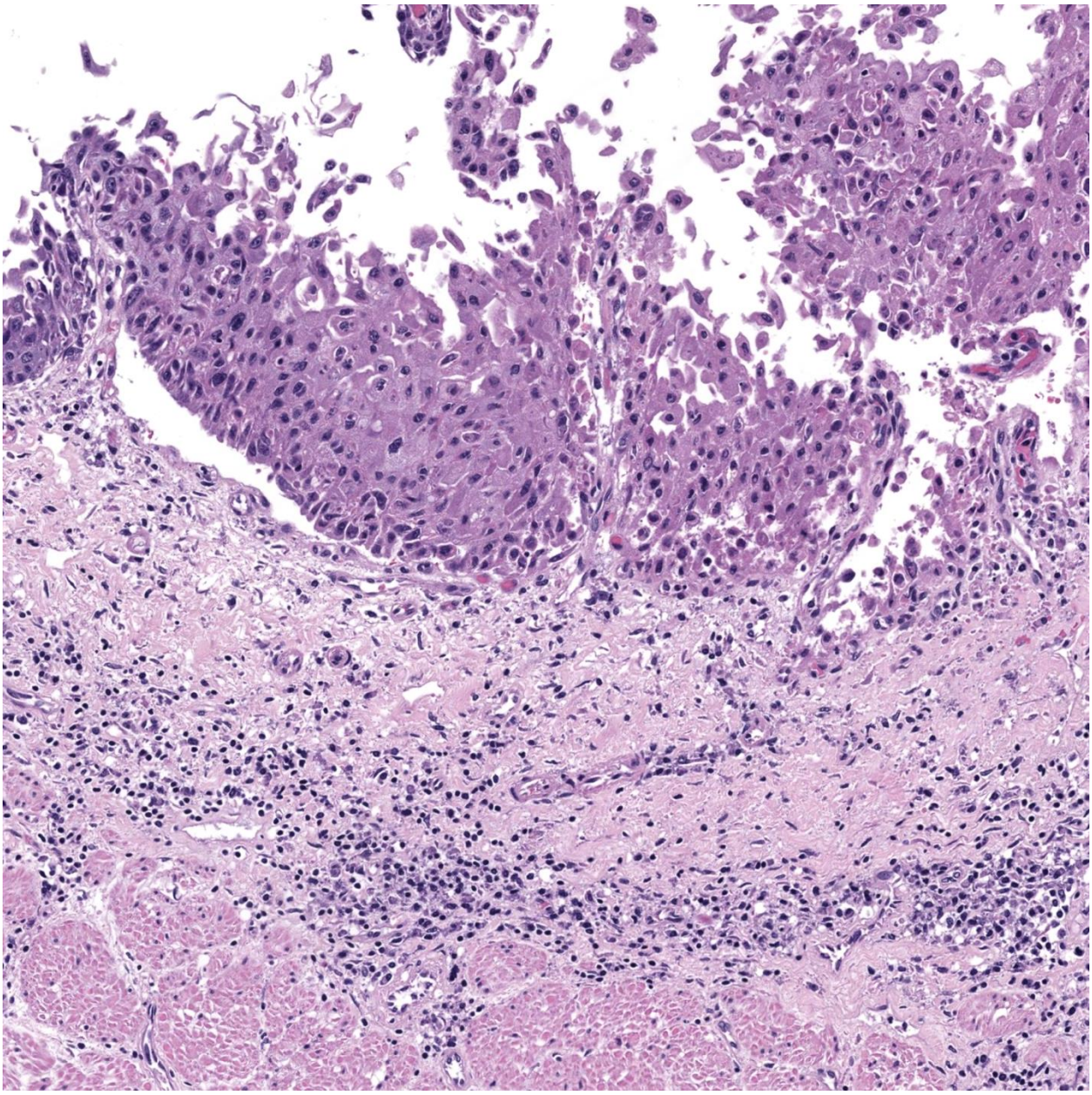
Supplemental Figure 2D. The spleen (H&E 20X) is notable for white pulp expansion by reactive lymphocytes, plasma cells, and histiocytes featuring prominent hemophagocytic lymphohistiocytosis (HLH).



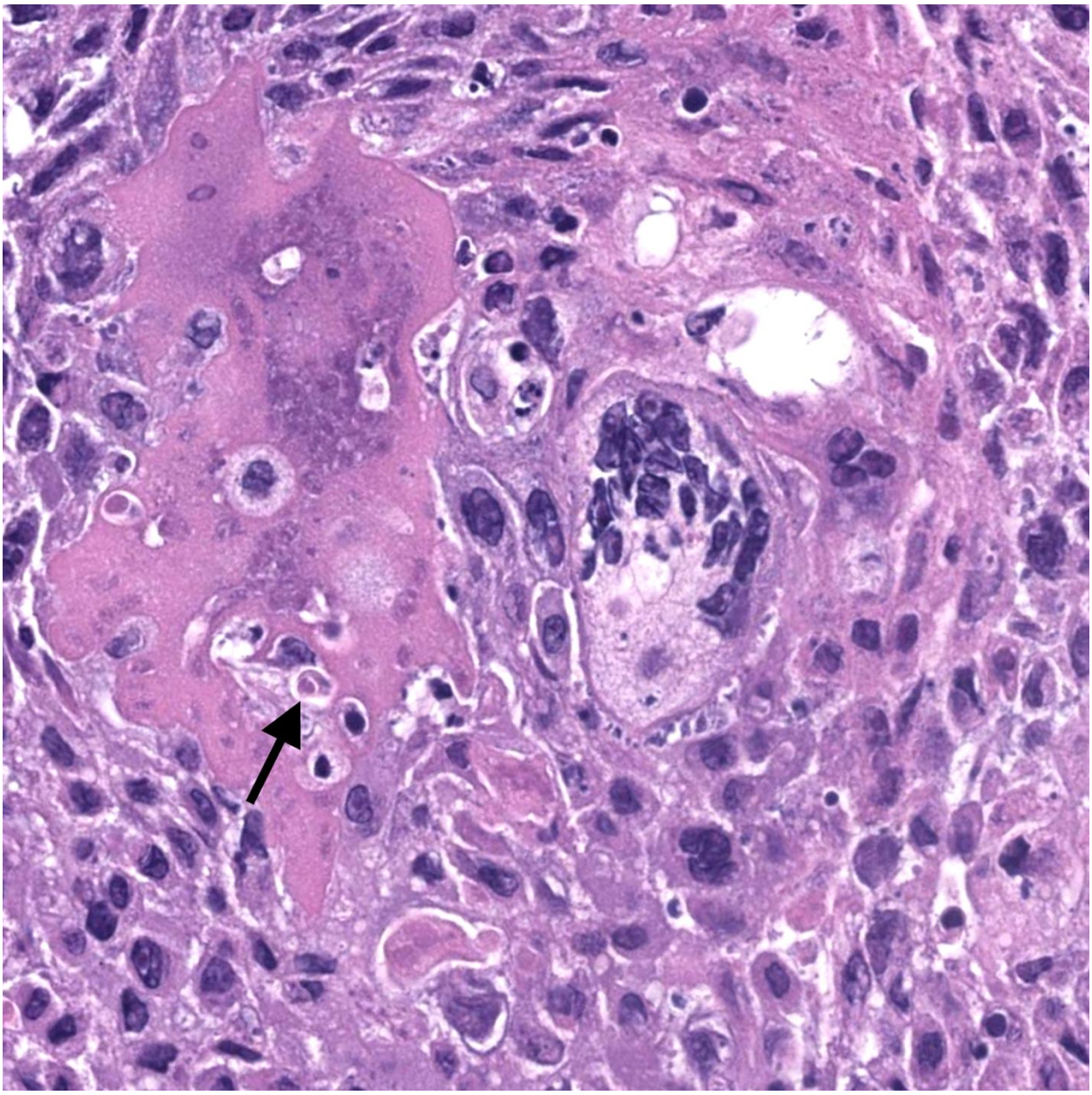
Supplemental Figure 2E. The adrenal gland (H&E 10X) demonstrates diffuse autolyzed parenchyma commonly seen in the post-mortem setting; some of the soft tissue around the right adrenal gland demonstrates mild chronic inflammation. There is no histomorphologic evidence of viral changes.



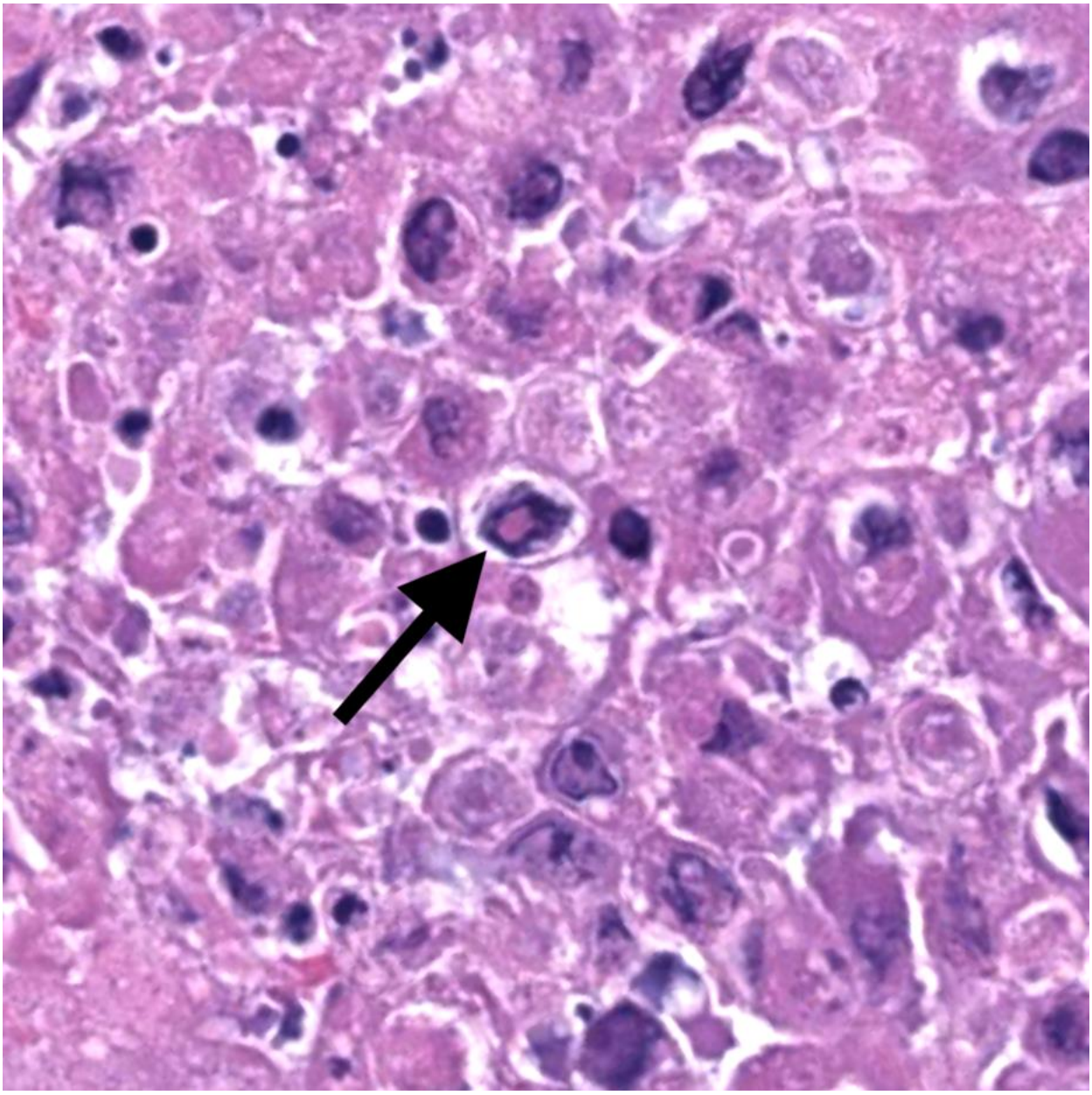
Supplemental Figure 2F. Epiglottis (H&E 40x): High power view of the epiglottis mucosa demonstrates scattered epithelial cells exhibiting eosinophilic intracellular inclusions, consistent with Guarnieri bodies (arrows).



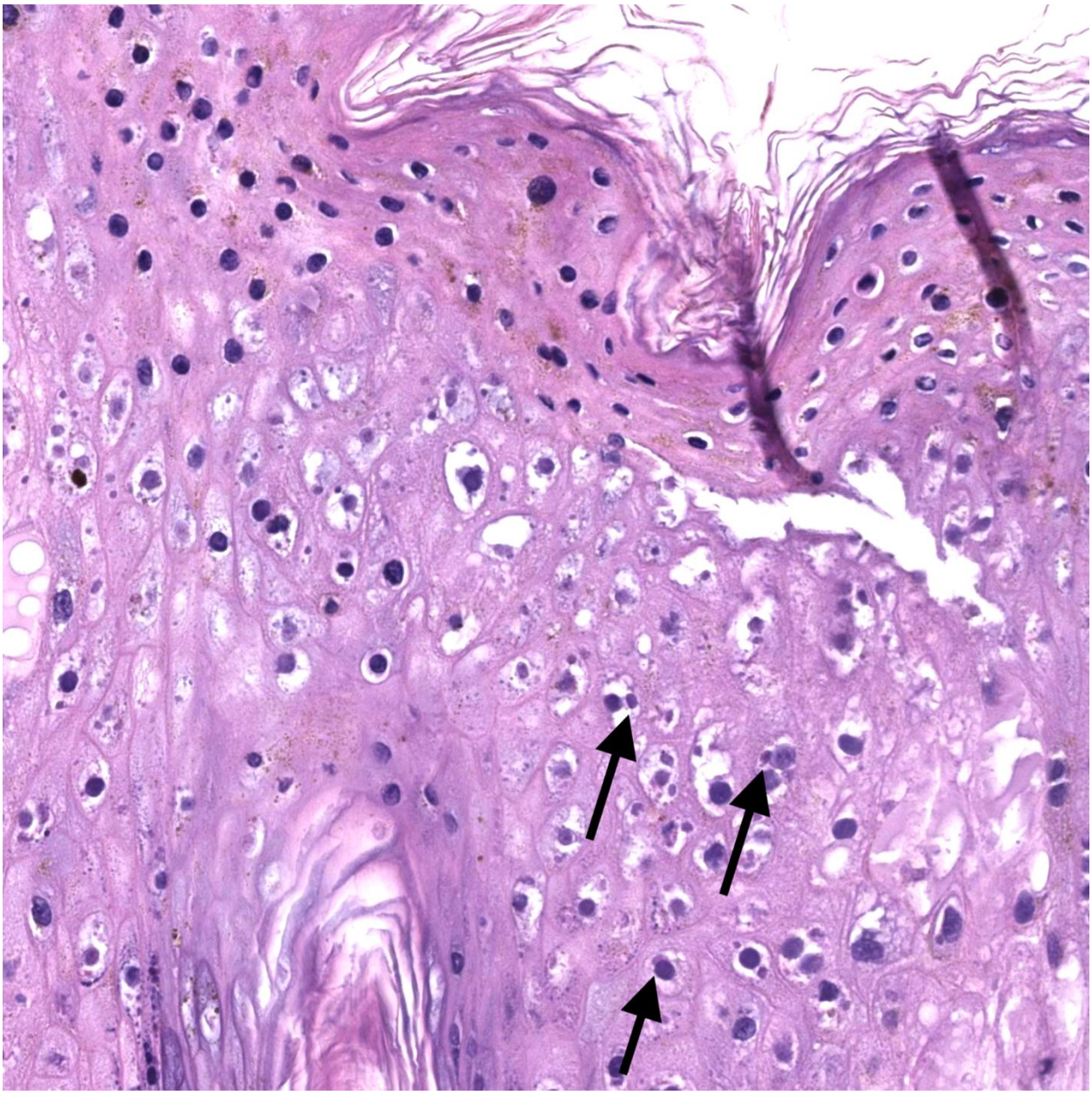
Supplemental Figure 2G. Esophagus (H&E 20x): The esophagus demonstrates large areas of mucosal ulceration and necrosis, with scattered large multinucleated cells with cleared cytoplasm. Some epithelial cells exhibit eosinophilic intracellular inclusions, consistent with Guarnieri bodies.



Supplemental Figure 2H. Tongue (H&E 20X): The ulcerated lesion of the tongue shows multinucleated cells with nuclear enlargement, nuclear grooves, hyperchromasia as well as some red nucleoli; there are some cells which demonstrate chromatin margination and intracellular eosinophilic inclusions consistent with Guarnieri bodies (arrow).



Supplemental Figure 2I. Tongue (H&E 40X): High magnification field of tongue showing epithelial cell with an eosinophilic inclusion consistent with Guarnieri body (arrow).



Supplemental Figure 2J. Skin bulla (H&E 20X): Higher magnification of the pustular stage skin bulla shows ballooning degeneration of keratinocytes with scattered eosinophilic inclusions, consistent with Guarnieri bodies (arrows).

Supplemental Figure 2K. Perianal lesions, necrotic tissue, ulcerations noted.

Supplemental Figure 2L. Rectum with polyp (clear arrow) and multiple ulcers (filled arrows).

Supplemental Figure 2M. Pustular skin lesions with central necrotic ulceration.