

Supplementary Materials and Methods

Clinical Histology

Initial evaluation of tumors and necropsies was performed at the University of Minnesota Comparative Pathology Shared Resource at the Masonic Cancer Center. Standard processing, embedding, and re-hydration methods were employed to generate tissue blocks. Histological subtype for study dogs was determined by standard H&E and vimentin staining, evaluated by veterinary pathologists, and confirmed by human pathologists at the University of Minnesota Medical School. Three human slides were taken from cases that had been previously de-identified.

Isolation of Peripheral Blood Mononuclear Cells and Interferon-Gamma ELISpot

Whole blood was collected in heparinized or EDTA-coated tubes and peripheral blood mononuclear cells (PBMCs) were isolated using density gradient centrifugation with lymphocyte separation medium. PBMCs were then cryopreserved in 93% heat-inactivated fetal bovine serum (FBS) and 7% DMSO. Cells were thawed and placed in culture overnight before ELISpot. Tumor-reactive interferon gamma production was measured by irradiation of 50,000 autologous or allogeneic tumor cells with 200 Gray and incubation with 200,000 PBMCs per well in 200 μ L RPMI 1640, in a pre-made canine interferon-gamma ELISpot plate (R&D systems). PBMCs from a normal dog, pre-vaccination, and 3 months post-vaccination were used, and Counts were quantified in an automated fashion using a CTL ImmunoSpot® ELISpot reader.