# Supplemental Material

## Intratumoral Immunomodulation at a Single Site

## Cures Disseminated Disease Including in the Brain

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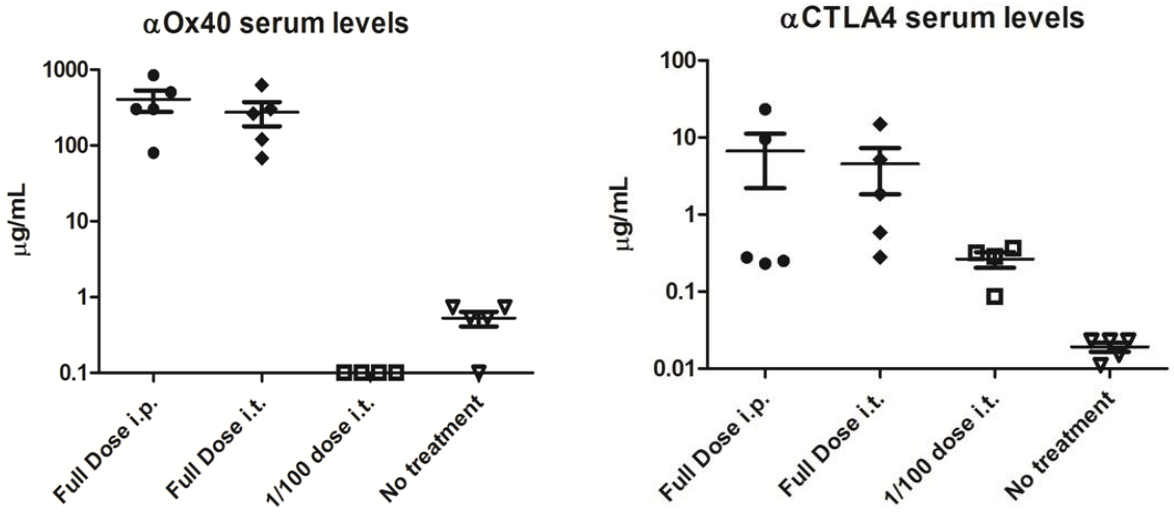
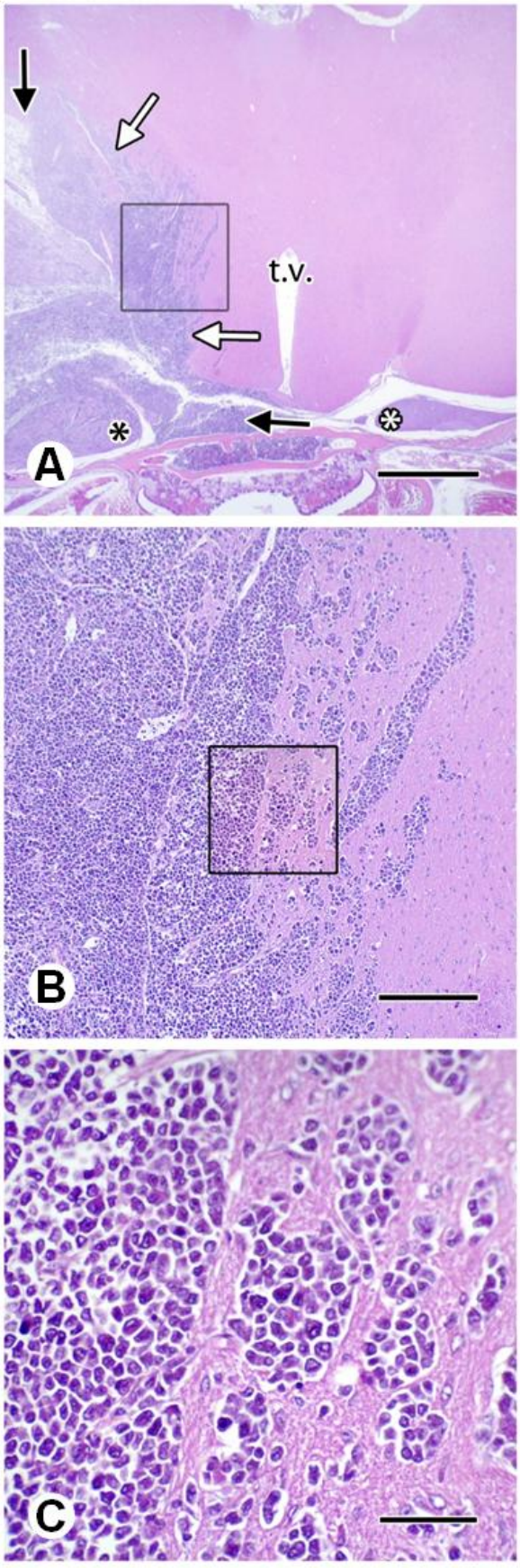
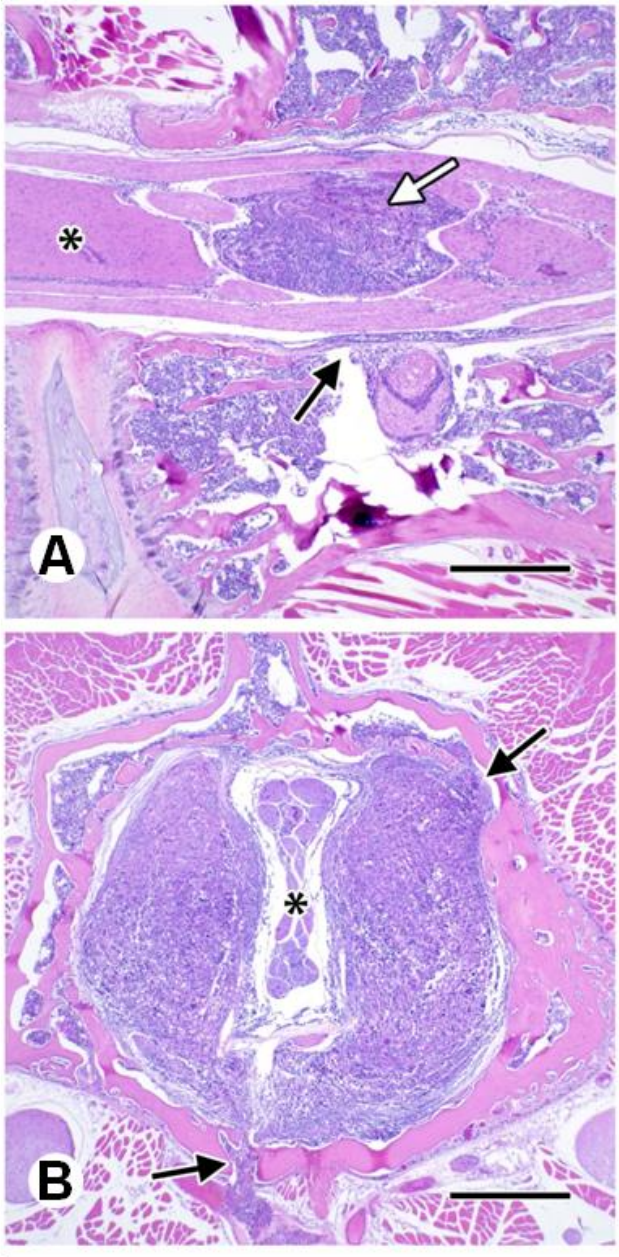


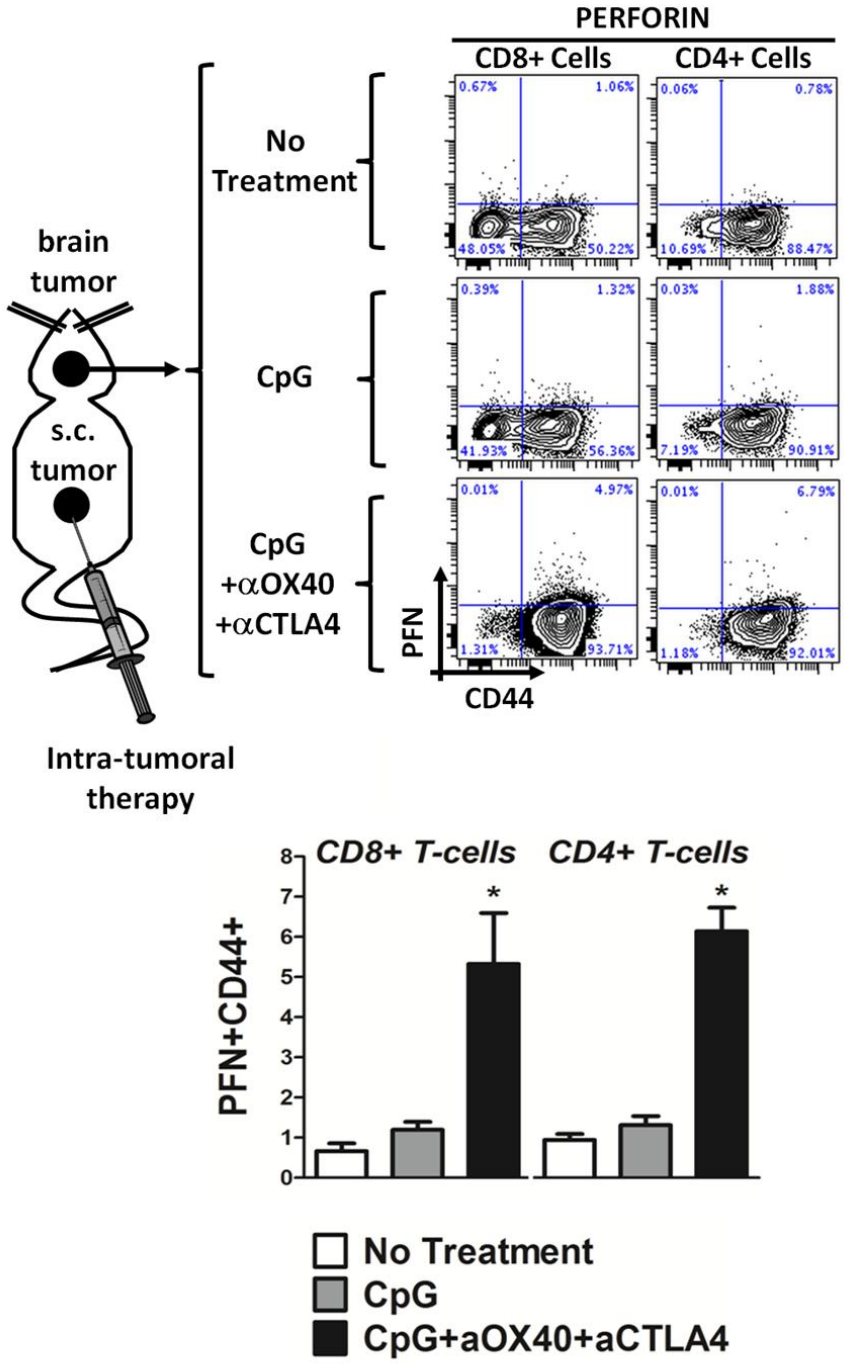

**Supplemental Figure 1:** Serum levels of anti-OX40 and anti-CTLA4 mAbs after intra-tumoral low dose injections. Mice were challenged with $5x10^6$ A20 tumor cells in two separate s.c. sites. Seven days later, mice received the 5 days course of CpG + low doses of aOX40/aCTLA4 into one of the s.c. tumors. On day 3 after completion of the treatment, blood samples were collected and serum concentrations of anti-OX40 and anti-CTLA4 were measured by ELISA.




**Supplemental Figure 2:** (**A**) H&E staining of in situ brain from a mouse with CNS lymphoma. Low magnification shows marked asymmetric invasion of neoplastic lymphocytes in the meninges (black arrows), and the brain parenchyma of the subjacent thalamus and hypothalamus (white arrows). The third ventricle (t.v.) is deviated due to the unilateral involvement of the brain, and the increased size of one of the oculomotor nerves (black asterisk) due to invasion by neoplastic lymphocytes, compared to the size of the normal contralateral nerve (white asterisk). Bar = 1000 microns. (**B**) Inset from panel (A), demonstrating the invasion of the neoplastic lymphocytes into the subjacent parenchyma of the brain. Bar = 500 microns. (**C**) Inset from panel (B), revealing the neoplastic lymphocytes to be organized into groups or sheets of cells with no visible stroma. The cells are round to polygonal with delineated cell borders enclosing scant eosinophilic cytoplasm. The nuclei are round to ovoid with an average of one inconspicuous nucleolus embedded within clumped chromatin. Mitotic figures are absent in this photomicrograph. Bar = 50 microns.




**Supplemental Figure 3:** (**A**) H&E staining of in situ spinal cord from mice with brain lymphoma. Sagittal sections of sacral spinal cord and caudal equina (black asterisk) reveal marked infiltration of the meninges (white arrow) by neoplastic lymphocytes, which are similar in morphology to those described in the brain. Note the invasion and osteolysis (black arrow) of the neoplastic lymphocytes through the cortical bone of the adjacent vertebra, and into the bone marrow. Bar = 200 microns. (**B**) Transverse sections of sacral spinal cord and caudal equina (black asterisk) reveal massive infiltration of the meninges by neoplastic lymphocytes, which are similar in morphology to those described in Figure 1. Note the invasion and osteolysis (black arrow) of the neoplastic lymphocytes through the cortical bone of the adjacent vertebra, and into the bone marrow and hypaxial skeletal musculature. Bar = 200 microns.




**Supplemental figure 4:** Brains from CNS lymphoma bearing mice were collected on day 8 of s.c therapy and re-exposed overnight to irradiated A20 tumor cells. T-cells were subsequently stained for surface CD44 and intracellular Perforin (PFN). Illustrating FACS plot (upper panels) and median values from 5 mice are shown (*p<0.0001 for both CD8+ and CD4+ cells).