# Of Mice and Men: Matched Observations of Lymphopenia, Splenic Retraction, and the Bone Marrow as Harbor for Lost T-cells in Mice and Patients with Glioblastoma

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## Introduction

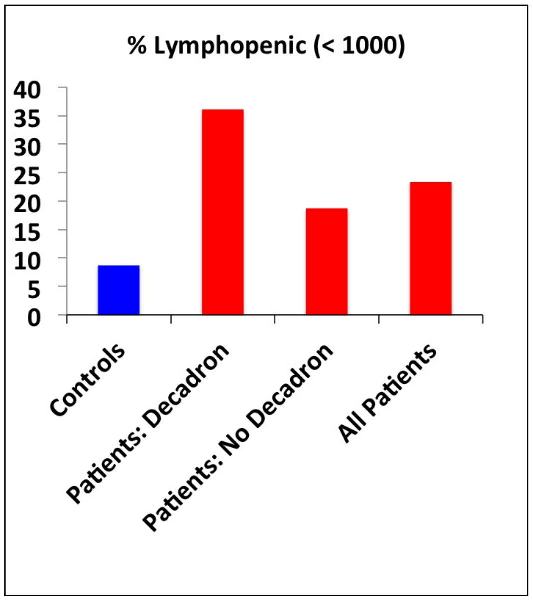
Glioblastoma (GBM) immunotherapy is hampered by poorly characterized patient lymphopenia and T-cell dysfunction. Murine glioma models reveal CD4+ T-lymphocyte disappearance from blood / lymph nodes / spleens, as well as marked splenic and thymic retraction. Meanwhile, the bone marrow of glioma-bearing mice reveals 6-fold expansion of CD4+ T-lymphocytes (1-3). We hypothesized that the blood, spleen, and bone marrow of patients with GBM would demonstrate similar alterations to T-cell homeostasis, and we addressed mechanistic questions in the mouse.

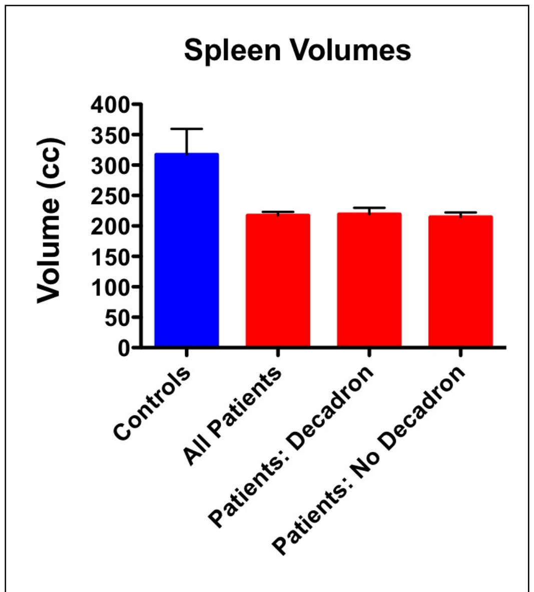
## Methods

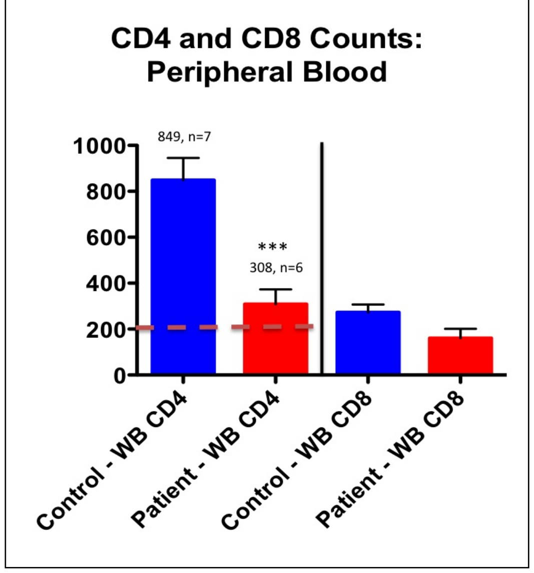
Samples are analyzed by 8-color flow cytometry, luminex, IHC, ELISA, and functional assays. Retrospective patient studies included complete blood counts and 3D spleen/tumor volumetric assessments in 284 treatment-naïve GBM patients and 46 age-matched controls. Ongoing prospective studies examine T-cells in blood, tumor, and bone marrow of 20 newly diagnosed GBM patients and 10 age-matched spinal fusion controls.

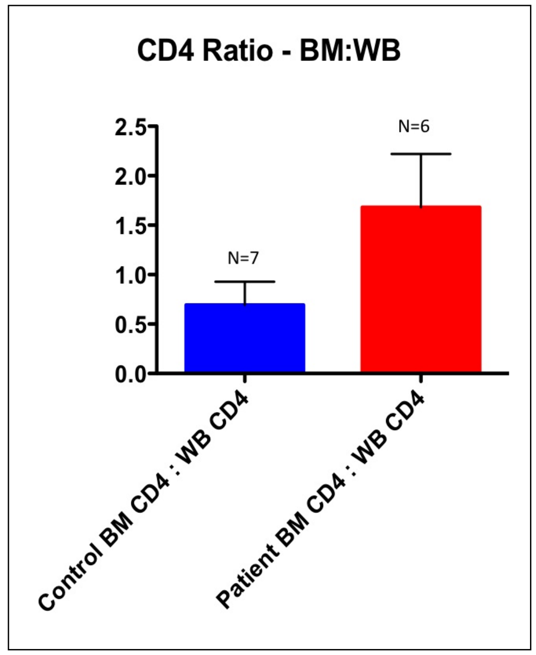
## Results

23% of treatment-naïve GBM patients prove lymphopenic (ct<1000 cells/ul) at presentation. Mean patient CD4 counts thus far are 308 (control 849, p<0.001). 50% of patients present with CD4 counts < 200 (AIDS demarcation in HIV). Patients demonstrate 32% reductions in spleen volume versus controls (P<0.0001), with 1/3 exhibiting greater than 50% volume reduction. Decadron exacerbates lymphopenia but does not affect spleen volume. Increased ratios of bone marrow to blood CD4 T-cells have similarly been detected in patients. In mice, this bone marrow CD4 accumulation proves to be brain-tumor specific and abrogated by anti-TGFß.




## Conclusions

Immunologically recapitulative murine glioma models demonstrate T-cell accumulation in bone marrow in association with disappearance from other immunologic compartments. Treatment-naïve GBM patients exhibit similar lymphopenia, dramatically low CD4 counts, shrunken spleens, and relative T-cell concentration in the bone marrow. In mice, these phenomena are glioma-specific and TGF-ß-influenced. This represents the first characterization of an unprecedented alteration to T-cell homeostasis in patients with GBM and has implications for GBM immunotherapy and immunology more broadly.