**Supplementary data**

**Long-term Survival in Glioblastoma with *Cytomegalovirus* pp65-targeted Vaccination**

Kristen A. Batich1,2, Elizabeth A. Reap1, Gary E. Archer1,3,Luis Sanchez-Perez1, Smita K. Nair4, Robert J. Schmittling1, Pam Norberg1, Weihua Xie1, James E. Herndon II5, Patrick Healy5, Roger E. McLendon2,3, Allan H. Friedman1,3, Henry S. Friedman1,3,Darell Bigner1,2,3, Gordana Vlahovic1,3,Duane A. Mitchell1,2,3\*ⱡ, and John H. Sampson1,2,3,6,7\*ⱡ

1Department of Neurosurgery; 2Department of Pathology; 3Preston Robert Tisch Brain Tumor Center; 4Division of Surgical Sciences, Department of Surgery; 5Department of Biostatistics and Bioinformatics; 6Department of Immunology; 7Department of Radiation Oncology, Duke University Medical Center, Durham, NC

\*D.A.M. and J.H.S. contributed equally to this work as co-senior authors.



**Supplementary Figure S1.** Kinetics of pp65 responses in long-term survivors. A, Patient 2 with an OS of 64 months at the time of analysis showed an increase in pp65 responses following Vaccines-1 to 3. Responses then diminished following DI-TMZ cycles 2 to 4. DI-TMZ cycles 5 to 9 were held due to thrombocytopenia. B, Patient 3 with an OS of 61.9 months at the time of analysis showed a similar increase in pp65 responses following three vaccines with pp65-DCs. DI-TMZ cycles 2 to 9 with monthly pp65-DCs showed fluctuating pp65 responses with overall decline. C, Patient 4 with an OS of 60.7 months at the time of analysis demonstrated the steady increase in pp65 responses once DI-TMZ was held, with a decline following DI-TMZ cycle 2 similar to the other long-term survivors, and an increase in pp65 reactivity later on following Vaccine-9. D, Patient 5 with an OS of 59 months at the time of analysis corroborated the same increase in pp65 responses following Vaccines 1-3, with the same decline once monthly DI-TMZ cycles were resumed. DI-TMZ cycle 8 was held due to thrombocytopenia.



**Supplementary Figure S2.** pp65 tetramer-positive CD8+ T-cells in sampled patients. pp65 tetramer-positive CD8+ T-cells in the peripheral blood of six patients for available HLA types B35 (patient 4 and 10), B07 (patient 5, 8, and 10), A01 (patient 6) and A02 (patient 6 and 8), and A24 (patient 7 and 8). Tetramer-positive cells increased following DI-TMZ cycle 1, likely due to reactive homeostatic expansion following lymphodepletion. Tetramer positivity did remain elevated after three DC vaccinations to Pheresis-2. Similarly to pp65 functional responses, tetramer-positive CD8+ T cells diminished at time point Vaccine-4 after reinitiating DI-TMZ with cycle 2.