

APPENDIX

Appendix 1: Luminex Analysis

19 cytokines (IL-1b, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IL-17, G-CSF, GM-CSF, IFN-g, TNF-a, TGF-b1, TGF-b2, TGF-b3, and IFN-a) and 11 chemokines: MCP-1, RANTES, IP-10, MCP-1, MIP-1a, MIP-1b, MCP-2, MCP-3, MIP-3a, MIP-3b and SCYB16. Markers were measured using Luminex bead-based assays Cytokine 17-plex (M5000031YV: G-CSF, GM-CSF, IFN-g, IL-1a, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IL-17, MCP-1, MIP1a, TNF-a) with RANTES (171B5025M). Cytokine IFN-a2 (171B6010M) using cytokine group II standards (171D6001). Chemokine IP-10 (171BK34MR2), MCP-1 (171NK35MR2), MIP-1a (171BK44MR2), MCP-3 (171BK38MR2), MCP-2 (171BK37MR2), MIP-3a (171BK47MR2), MIP-3b (171BK48MR2), SCYB16 (171BK51MR2) with chemokine standard (171DK0001) (BioRad, Hercules, CA) and TGFb 1, 2, 3, kit (TGFBNAG-64K-03) (EMD Millipore, Billerica, MA) according to the manufacturer's protocols. Concentrations were calculated using a five-parameter standard curve. Serum samples were assayed in duplicate, and averaged to calculate final concentrations.

Appendix 2: Antibodies for Flow Cytometric Analysis

The T cell panel included the following antibodies: BV605-conjugated anti-CD8a (clone RPA-T8, diluted 1:125), BV711-conjugated anti-CD4 (clone OKT4, diluted 1:125), FITC-conjugated anti-CD62L (clone DRED-56, diluted 1:5), PerCp-Cy5.5-conjugated anti-ICOS (clone C398.4A, diluted 1:100), APC-conjugated anti-CD25 (clone BC96, diluted 1:50), AF700-conjugated anti-CD45RA (clone HI100, diluted 1:20), APC-eFluor 780-conjugated anti-CD127 (1:50), PE-conjugated anti-PD-1 (1:20), and PE-Cy7-conjugated anti-PD-L1 (1:20), and BV421-conjugated anti-Foxp3 (1:20). The NK cell panel included the following antibodies: BV421-conjugated anti-Tim3 (clone F38-2E2, diluted 1:10), BV510-conjugated anti-CD83 (clone HB15e, BD Biosciences, San Diego, CA, USA; diluted 1:100), BV605-conjugated anti-CD56 (clone HCD56, diluted 1:50), FITC-conjugated anti-CD3 (clone HIT3a, diluted 1:125), AF700-conjugated anti-CD16 (clone 3G8, diluted 1:50), PE-conjugated anti-PD-1 (clone RMP1-30, diluted 1:20), and PE-Cy7-conjugated anti-PD-L1 (clone 29E.2A3, diluted 1:20).

Supplemental Figure Legend

Supplemental Figure 1: Percent Natural killer cells of Total Lymphocytes in PBMC of individual patients, by site irradiated: **A.** Parenchymal sites. **B.** Bone. **C.** Brain.

Supplemental Figure 2: Percent Memory CD4⁺ T cells of Total Lymphocytes in PBMC of individual patients, by site irradiated: **A.** Parenchymal sites. **B.** Bone. **C.** Brain.

Supplemental Figure 3: No changes in PD-1⁺ CD4⁺ T cells after SAR to any site. **A.** Percent PD-1⁺ CD4⁺ T cells after SAR to all sites. **B.** Percent PD-1⁺ CD4⁺ T cells after SAR to parenchymal sites alone.

Supplemental Figure 4: No changes to CD4⁺ CD25⁺ Foxp3⁺ T regulatory cells after SAR to any site. **A.** Percent CD4⁺ CD25⁺ Foxp3⁺ CD4⁺ T cells after SAR to all sites. **B.** Percent CD4⁺ CD25⁺ Foxp3⁺ T cells after SAR to parenchymal sites alone.