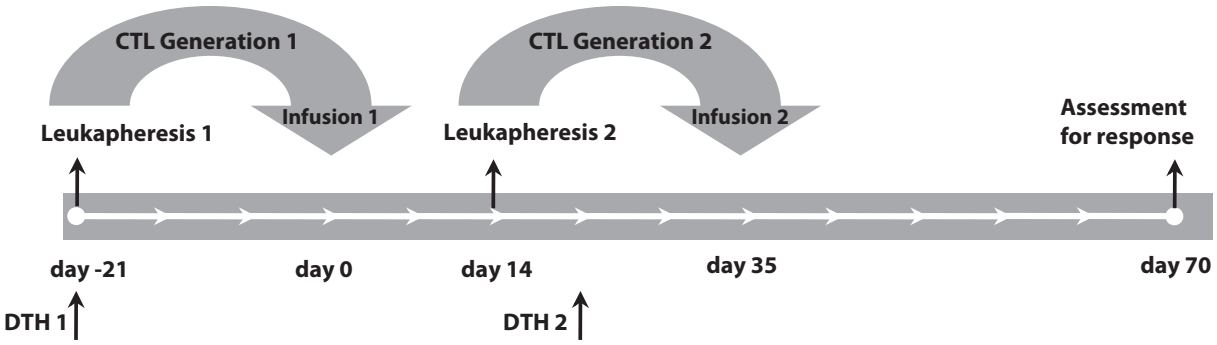

Fig. S1. Clinical protocol time line. Subjects underwent leukapheresis on day -21 to generate MART1-specific CTL. Three weeks later, autologous MART1-specific CTL were infused as graft 1 on day 0. Leukapheresis 2 was performed on day 14 in order to generate a second preparation of MART1 CTL which were then infused three weeks later on day 35. To assess recall responses to MART1 antigen, intradermal injections of MART1 peptide (50 μg) were administered pre-infusion on day -21 immediately after leukapheresis and on day 21. Skin biopsies of delayed-type hypersensitivity (DTH) reactions to injected peptides were performed 2 days after each injection. Assessment for response on day 70 was performed by physical examination and CT scans.

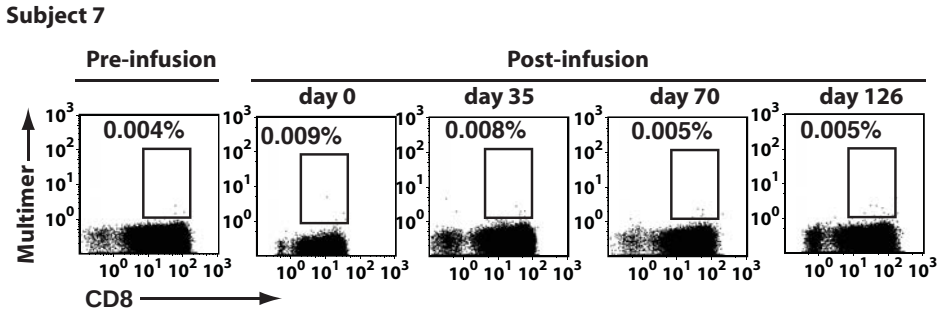

Fig. S2. Verification of HLA-A2/MART1 peptide multimer staining. In all experiments using HLA-A2 peptide multimer, samples were concurrently stained with HLA-A2/MART1 peptide multimer as well as HLA-A2/HIV pol peptide multimer (irrelevant control). Negative staining of samples used in Figure 2 by HLA-A2/HIV pol peptide multimer is shown as an example. The lack of staining for HIV pol T cells in these HIV negative patients verified the specificity of HLA-A2/MART1 peptide multimer staining.

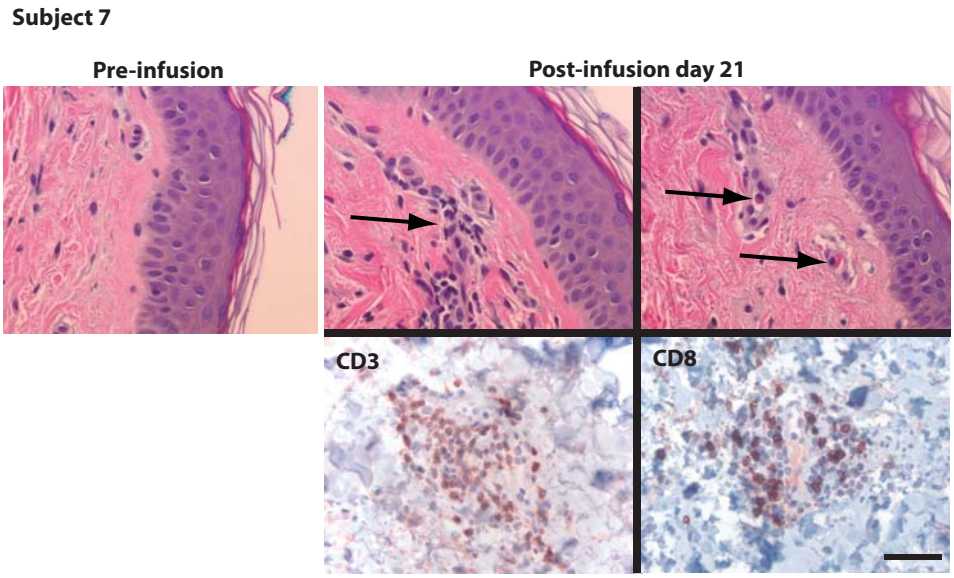

Fig. S3. Adoptive transfer increased delayed-type hypersensitivity reaction to injected MART1 peptide. Pre- and post-infusion skin biopsies of delayed-type hypersensitivity (DTH) reactions to MART1 peptide are shown for Subject 7. The pre-infusion biopsy showed a minimal DTH response. Post-infusion, a strong inflammatory infiltrate was observed in response to the injection of MART1 peptide (upper middle panel). Immunohistochemistry demonstrated that CD3+ CD8+ lymphocytes were recruited to the site and accumulated around blood vessels (lower panels). Recruitment of eosinophils was also observed (upper right), consistent with a typical DTH response. Scale bar, 50 μm, lower right, applies to all panels.

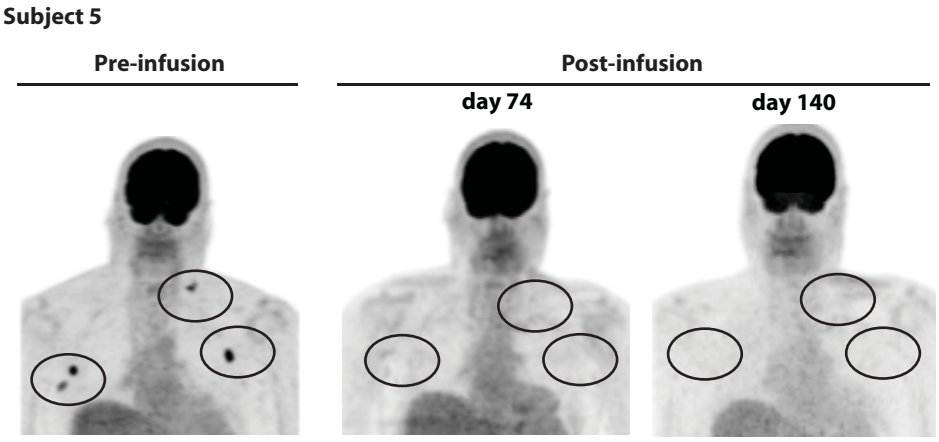

Fig. S4. Clinical response to adoptive transfer of MART1-specific T cells in Subject 5. Pre-infusion, positron-emission tomography (PET) images for Subject 5 revealed hypermetabolic lesions in left cervical and bilateral axillary lymph nodes which colocalized to enlarged nodes on CT scan. On post-infusion day 140, no evidence of disease was detected by PET. Uptake in the brain, liver, and spleen represents normal background signal and did not correspond to any lesions on CT scans.

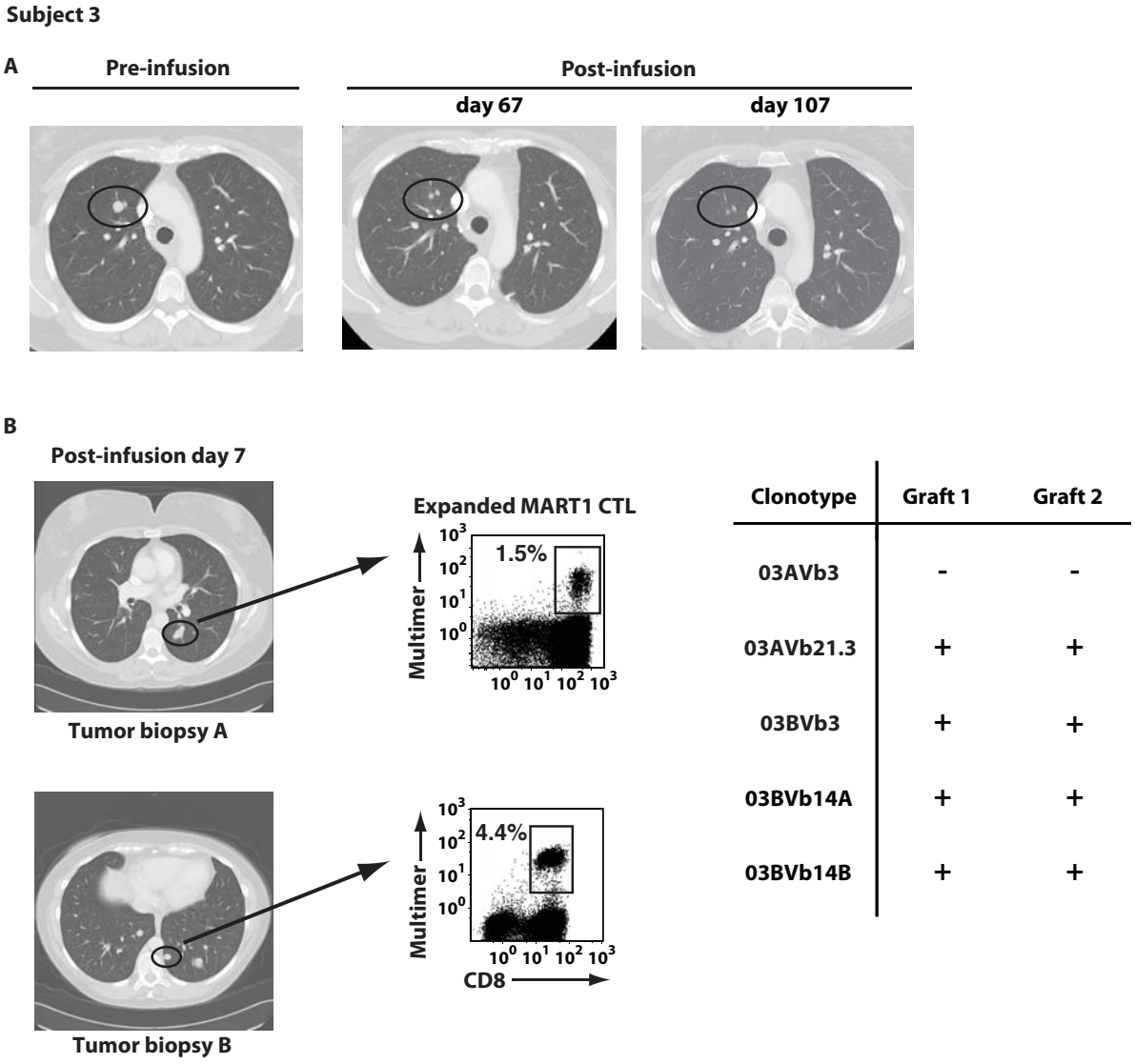

Fig. S5. Trafficking of transferred CTL to pulmonary lesions, Subject 3. (A) Subject 3, pre- and post-infusion CT scans demonstrated a mixed clinical response with regression of a pulmonary metastasis. (B) For Subject 3, two other pulmonary lesions (Tumor biopsies A and B) were resected 7 days after CTL infusion 1, and MART1-specific T cells were expanded in vitro from TIL using MART1 peptide pulsed aAPC (left and middle). Five MART1-specific clonotypes derived from TIL were identified, 2 from tumor biopsy A and 3 from tumor biopsy B. CDR3-specific clonotypic RT-PCR identified the presence of 4/5 of these MART1-specific T cell clonotypes in both CTL grafts (right).

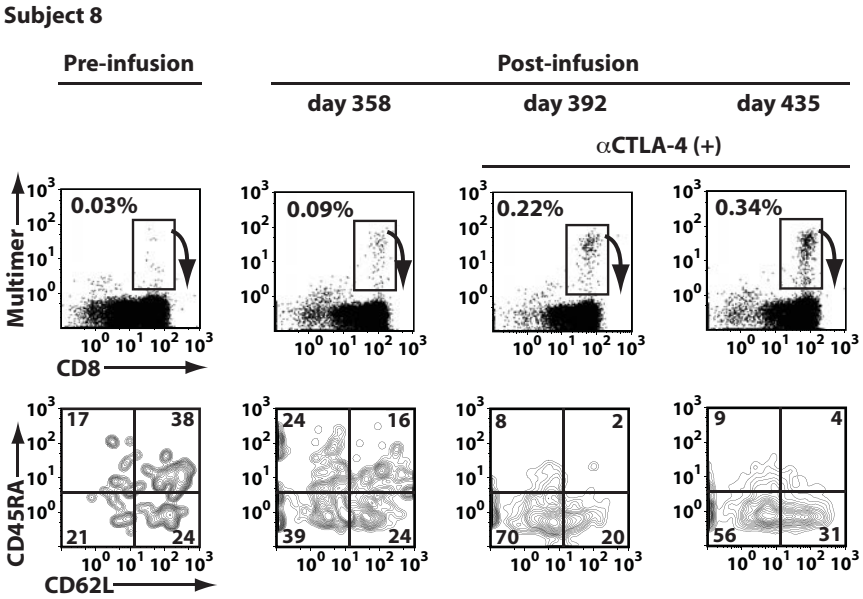

Fig. S6. Expansion of adoptively transferred MART1 CTL in Subject 8 treated with anti-CTLA-4 (ipilimumab) therapy. Following two infusions of MART1 CTL, Subject 8 began anti-CTLA-4 mAb therapy on post-infusion day 372. The frequency of peripheral MART1-specific CD8+ T cells, which was increased after adoptive transfer, was further increased with anti-CTLA-4 therapy. The CD45RA/CD62L phenotype of multimer positive cells is shown below the multimer stain for each time point and indicates that, pre-infusion, circulating MART1-specific T cells included a mixture of cells with naïve, memory, and terminally differentiated effector phenotypes (left). After CTL infusion, the percentage of MART1-specific CD8+ T cells increased to 0.09% on day 358 (middle). After the institution of anti-CTLA-4 therapy, further expansion of MART1 multimer+ T cells occurred on days 392 and 435 (right). After adoptive transfer and anti-CTLA-4 treatment, expanded MART1-specific T cells displayed a central memory and effector memory phenotype.