

Supplementary Table: Adverse events. All adverse events by grade that were believed to be at least possibly related to treatment are shown. The numbers represent the number of total events experienced during the entire treatment period with respect to treatment arm.

Supplementary Figure 1: Patient allocation: Shown is a consort diagram depicting the patients randomized and analyzed.

Supplementary Figure 2: DNA immunization elicits PAP-specific cellular immune responses: Panel A: PBMC from a patient (#10) prior to treatment and after 12 weeks were labeled with a membrane intercalating dye (PKH26) and cultured for one week in the presence of PAP, PSA, tetanus toxoid, PHA, or media only. Cells were then stained by multiple cell surface markers and evaluated by flow cytometry. Shown are the CD4+ gated T cells with respect to PKH26 staining. Panel B: The number of cell divisions was determined by PKH26 dilution, to determine an antigen-specific CD4+ T cell precursor frequency (example shown is for tetanus response observed at 12 weeks). Panel C: With each immune time-point assessment, cryopreserved cells obtained pretreatment were concurrently assessed to evaluate the variability of response assessment over time. Shown are the IFN γ ELISPOT (solid circles) and granzyme B ELISPOT (open circles) data for the pretreatment samples (patient 9) re-assessed at the multiple time points.

Supplementary Figure 3: Immune responses to PSA and tetanus evaluated by intracellular cytokine staining. Intracellular cytokine staining was performed as in Figure 3 with PBMC samples collected pretreatment, after 12 weeks, and at one year (3 patients did not have one-year

samples). The frequency of CD8+ (left panels) or CD4+ (right panels) T cells expressing IFN γ , TNF α , IL-2, IL-4, IL-6, IL-10, or IL-17 was assessed following culture with tetanus toxoid (TET, top panels) or PSA (bottom panels).

Supplementary Figure 4: Immune responses to PAP, PSA and tetanus evaluated by cytokine secretion. PBMC samples collected pretreatment and at one year were cultured in the presence of PAP, PSA, tetanus, PHA (not shown) or media only for 72 hours. The presence of cells secreting IFN γ , granzyme B, IL-2, IL-4, IL-6, IL-10, IL-17, or TGF β was assessed by capture ELISA for each cytokine using culture supernatants, and the results are shown as change in optical density (Δ OD) for each antigen compared with media alone. Comparisons (paired t-test) from pre-treatment to post-treatment for which p values < 0.1 are shown.