Figure S1. Prostate GVAX/ipilimumab treatment induces CD4<sup>+</sup> and CD8<sup>+</sup> T cell differentiation. Levels of circulating differentiated CD4<sup>+</sup> and CD8<sup>+</sup> T cells were determined before (w0), during (w4, w8, w12, w16, w20, w24) and after (follow-up (fu)) Prostate GVAX/ipilimumab treatment by whole blood flowcytometric analysis. Naive CD4<sup>+</sup> T cells were defined as CD4<sup>+</sup>CD45RO<sup>-</sup> cells and non-naive CD4<sup>+</sup> T cells as CD4<sup>+</sup>CD45RO<sup>+</sup> cells. Naive CD8<sup>+</sup> T cells were defined as CD27<sup>-</sup>CD45RA<sup>+</sup> and non-naive CD8<sup>+</sup> T cell population as the sum of effector (CD27<sup>-</sup>CD45RA<sup>+</sup>), effector memory (CD27<sup>-</sup>CD45RA<sup>-</sup>) and central memory T cells (CD27<sup>+</sup>CD45RA<sup>-</sup>). 

A) Mean percentage ± SEM of non-naive CD4<sup>+</sup> T cells over follow-up for all 28 patients. B) Kaplan Meier curve for treatment-induced increases in non-naive CD4<sup>+</sup> T cells. Number of patients and corresponding median survival for each group are given. C) Mean percentage ± SEM of non-naive CD8<sup>+</sup> T cells over follow-up for all 28 patients. D) Kaplan Meier curve for treatment-induced increases in non-naive CD8<sup>+</sup> T cells. Number of patients and corresponding median survival for each group are given. Differences between pre- and on- or post-treatment were analyzed with the repeated measures ANOVA with a post-hoc Dunnett’s multiple comparisons test. Differences were considered significant when p<0.05, as indicated with an asterisk (* p<0.05, ** p<0.01).
Figure S2. Prostate GVAX/ipilimumab treatment induces CD4+ T cell activation. Numbers of circulating CD4+HLA-DR+, CD4+ICOS+, CD4+CD25intFoxP3+, CD4+CTLA-4+ and CD4+PD-1+ cells were determined before (w0), during (w4, w8, w12, w16, w20, w24) and after (fu) Prostate GVAX/ipilimumab treatment by flowcytometric analysis on whole blood or isolated PBMC. Percentage of marker+ cells over follow-up are given for A) CD4+HLA-DR+ T cells, B) CD4+ICOS+ T cells, C) CD4+CD25intFoxP3+ T cells, D) CD4+CTLA-4+ T cells and E) CD4+PD-1+ T cells. Differences between pre- and on- or post-treatment were analyzed with the repeated measures ANOVA with a post-hoc Dunnett’s multiple comparisons test. Differences were considered significant when p<0.05, as indicated with an asterisk (* p<0.05, ** p<0.01).
Figure S3. High pre-treatment frequencies of total CD4⁺CTLA4⁺ T cells are associated with survival benefit after Prostate GVAX/ipilimumab therapy. Pre-treatment frequencies of circulating CD4⁺CTLA-4⁺ cells were determined by flow cytometry. A) A representative CD4⁺CTLA-4⁺ analysis is shown (pre-gated on CD3 positivity). B) Kaplan Meier curve for high pre-treatment values of CTLA-4⁺ cells in total CD4⁺ T cells. Number of patients and corresponding median survival for each group is given. Statistical analyses were performed by log rank testing and differences were considered significant when p<0.05.