SUPPLEMENTAL DATA

Methods

CD4+ T cell response

CD4+ T cell response was measured via a standard CD4+ lymphocyte proliferation assay. Briefly, CD4 +T cells were purified from peripheral blood mononuclear cells (PBMC) by standard magnetic bead isolation using anti-CD4 monoclonal antibody (mAb) (Miltenyi Biotec, Bergisch Gladbach, Germany). The cells (1 x 10^5/well) were incubated in 200 ul/well of RPMI 1640 supplemented with 5% pooled autologous plasma (AP) in 96-well round-bottomed microtiter plates for 5 days, in the presence or absence of peptides. One µCi [^{3}H]-thymidine was added to each well and 20 hours later, the cells were harvested with a Harvester Mach IIIM (Tomtec, Hamden, CT) and counted in a 1450 MicroBeta TriLux (Wallac, Turku, Finland).

CD8+ response

In vitro immunization and human T cell cultures: To detect specific CD8+ T cell responses, we performed two rounds of stimulations of CD3+ T cells, *in vitro*. PBMC from patients were obtained by Ficoll density centrifugation and CD14+ monocytes were isolated by positive selection using mAb to human CD14 coupled with magnetic beads (Miltenyi Biotec) and part of the cells were used for the first stimulation of T cells at a ratio of 10: 1 (T: antigen presenting cells [APC]). The CD14 negative fraction of PBMC were used for isolation of CD3+ T cells, by negative immunomagnetic cell separation using a pan T cell isolation kit (Miltenyi Biotec). Purified CD3+ T cells were stimulated with immunizing peptides WT1-A1 or with the native peptide WT1-A (20 ug/ml) to expand the WT1-A specific CD8+ T cells. The cell cultures were carried out in RPMI 1640 supplemented with 5% autologous plasma, 1 µg/mL β2-microglobulin (β2-M; Sigma, St. Louis, MO), and 10 ng/mL IL-15 (R&D Systems, Minneapolis, MN) for 7 days. Monocyte-derived dendritic cells (DCs) were generated from remaining CD14+ cells, by culturing the cells in RPMI 1640 medium supplemented with 1% AP, 500 units/mL recombinant IL-4, and 1,000 units/mL GM-CSF. On days 2 and 4 of incubation, fresh

medium with IL-4 and GM-CSF was either added or replaced half of the culture medium. Maturation cytokine cocktail (IL-4, GM-CSF, 500 IU/mL IL-1, 1,000 IU/mL IL-6, 10 ng/ml TNF-α, and 1 ug/mL PGE-2), was added to all DC cultures on day 6. On day 7, mature DCs were used for secondary stimulation of CD3+T cells at a ratio of 1: 30, with the same condition for the first stimulation. Seven days later, CD8+ responses were then measured by IFN-γ secretion of the cells was examined by enzyme-linked immunospot (ELISPOT) assay and/or tetramer staining.

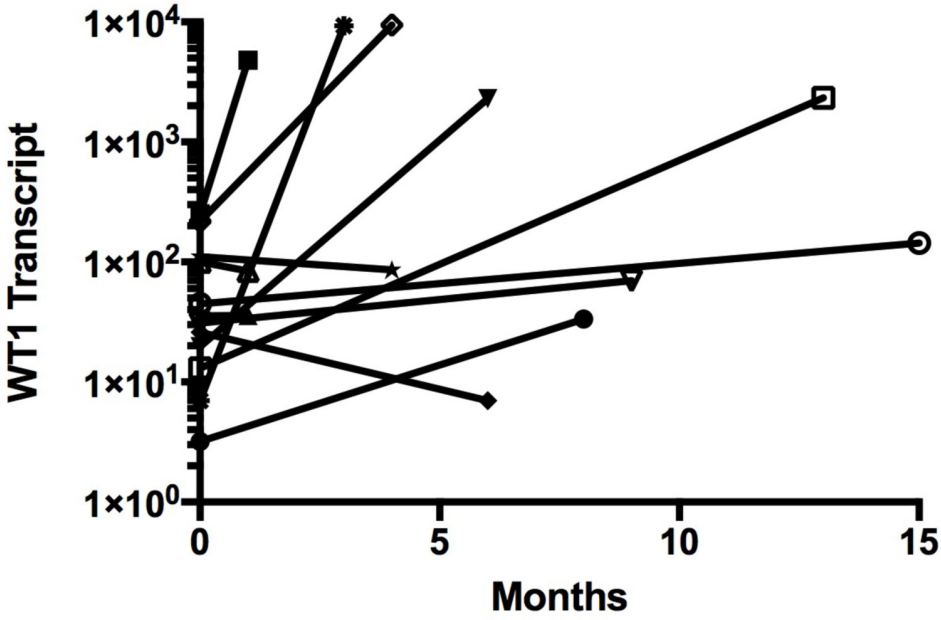
IFN-γ ELISPOT: HA-Multiscreen plates (Millipore, Burlington, MA) were coated with 100 μL of mouse anti-human IFN-γ antibody (10 μg/mL; clone1-D1K; Mabtech, Nacka Strand, Sweden) in PBS, incubated overnight at 4°C, washed with PBS to remove unbound antibody, and blocked with RPMI 1640/10% autologous plasma for 2 h at 37°C. T cells (10^5 cells) were incubated with autologous CD 14+ cells (10^4 cells) in the presence or absence of 20 μg/mL of the test peptides. All conditions were done in quadruplicate. The cells were incubated overnight at 37ºC and the plates are developed the next day using a secondary antibody. The spots were developed as described and spot numbers were automatically determined using a computer-assisted video image analyzer with KS ELISPOT 4.0 software (Carl Zeiss Vision, Ulm, Germany)[8,14,30].

Tetramer staining: WT1-A/A*02:01 tetramers conjugated with phycoerythrin were constructed by the Sloan-Kettering Institute Tetramer core facility. CD3+ T cells were stained with WT1-A/HLA-A*02:01 tetramer (1:50 dilution) and mAbs against CD3/ CD4/ CD8 or other markers, using a CYAN-ADP flow cytometer with Summit software (Dako Cytomation Inc., Carpinteria, CA). Analysis was performed using FloJo software (version 8.1; TreeStar, Inc, Ashland, OR).

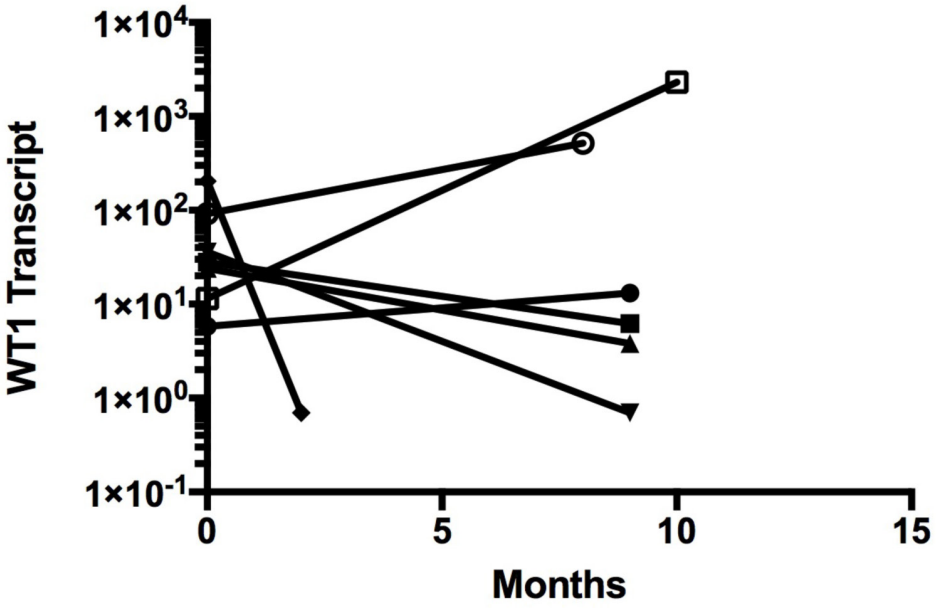
Results

Minimal Residual Disease

A



B



Supplemental Data Figure 1.

Caption: Change in WT1 transcript levels from baseline (time 0) in vaccinated patients. X axis: time in months after 1st GPS - the initial 6 doses of GPS were scheduled to be completed within 10 weeks (GPS 6-red line) while the subsequent 6 doses could be administered monthly. Therefore, for patients remaining in CR all vaccine therapy was completed in about 9 months (GPS 12- blue line).; Y axis: log scale WT1 transcript levels. A) Relapsed patients can show large increase in excess of 1-2 logs over baseline at the time of or shortly prior to clinical relapse. Pretreatment WT1 transcript levels were not a predictor of relapse. B Patients in continuous CR can have some variability in transcript levels but this occurs within a narrow (1 log) range. The one patient with a 2 log increase above baseline remains alive and without evidence of disease 54 months following diagnosis.

Flow Cytometry

Using a standard 4-6 color panel which includes myeloid, T and B markers, only 1/22 (4%) patients had a flow cytometric aberrancy detected c/w what would be considered CR MRD+ under ELN criteria. The WT1 transcript was detected by PCR but not markedly elevated at that time. He subsequently relapsed following the 7th vaccine.

Serial flow sampling was done in 21 of the 22 (95%) patients. Fourteen of the 22 (64%) of patients had some flow cytometric aberrancy detected while on study. Ten of the fourteen (71%) had this aberrancy detected at the time of clinical relapse. One other patient had an aberrancy which subsequently resolved with further testing. It is unclear if the vaccine had any role with this change. PCR did not increase > 1 log at that time. This patient remains in CR 58.5 months after achieving CR. Three others

had a flow aberrancy detected while in morphologic CR at completion of the vaccinations. All 3 eventually relapsed although only 1 of these had an increase in WT1 transcript > 2 logs.