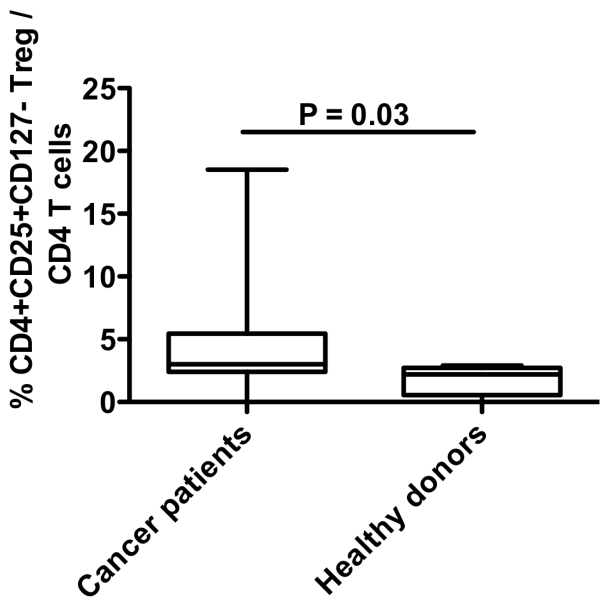
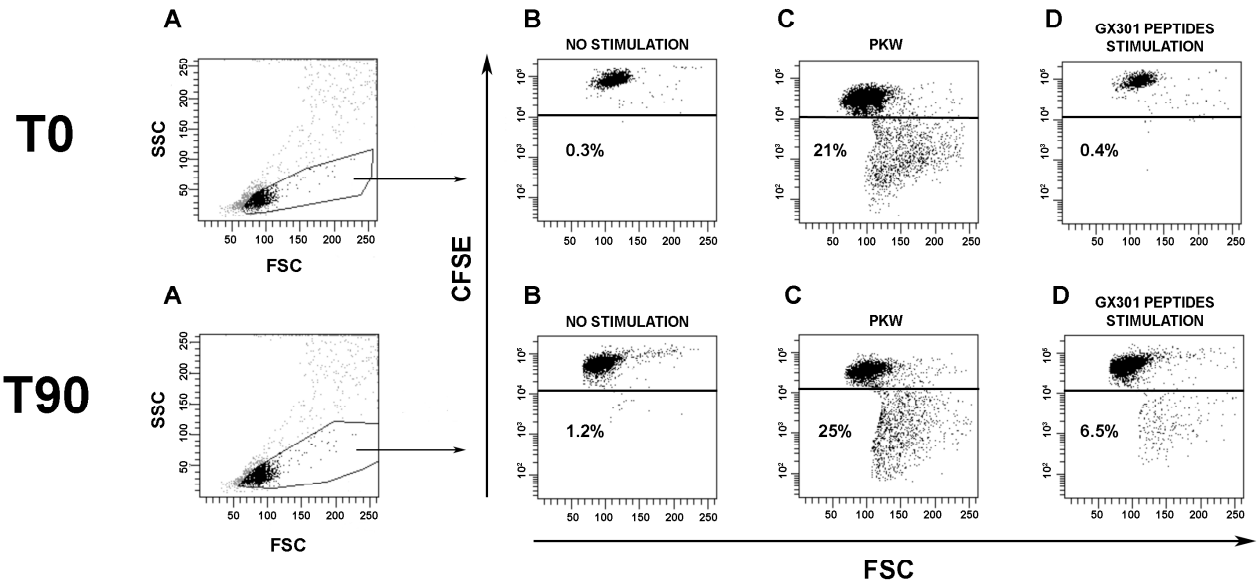
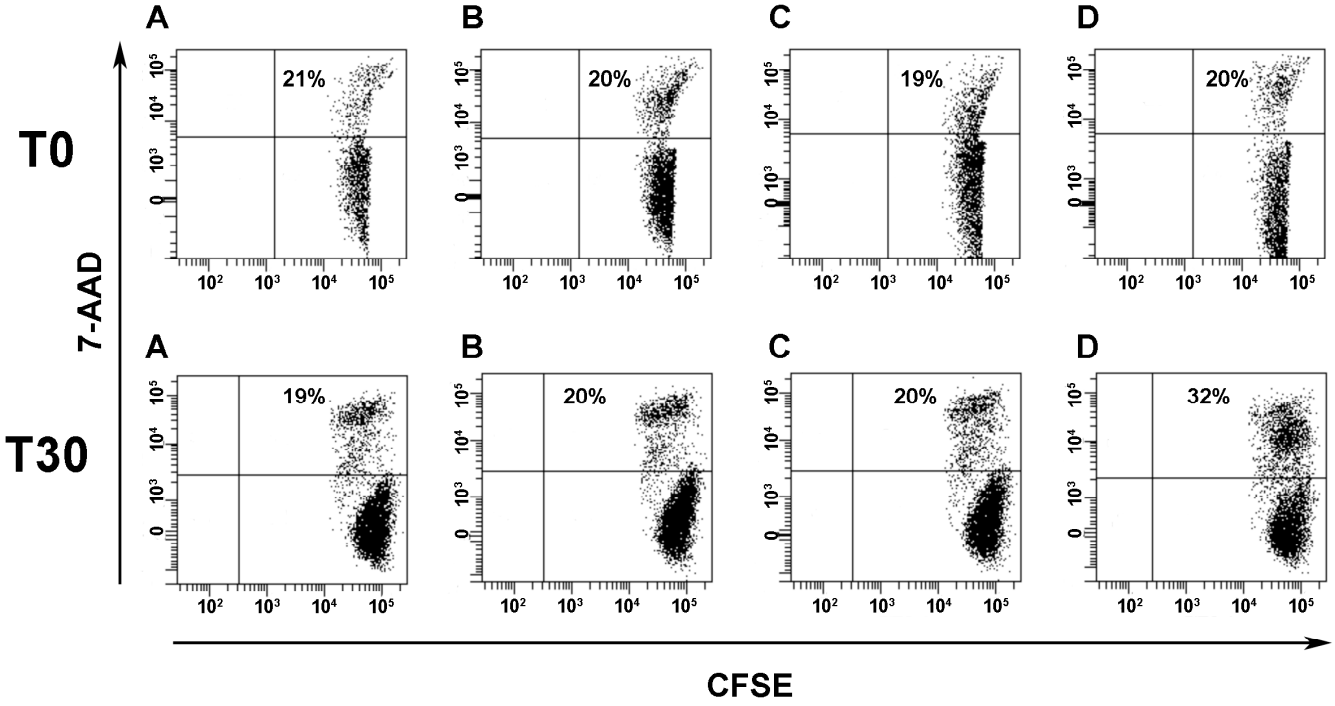


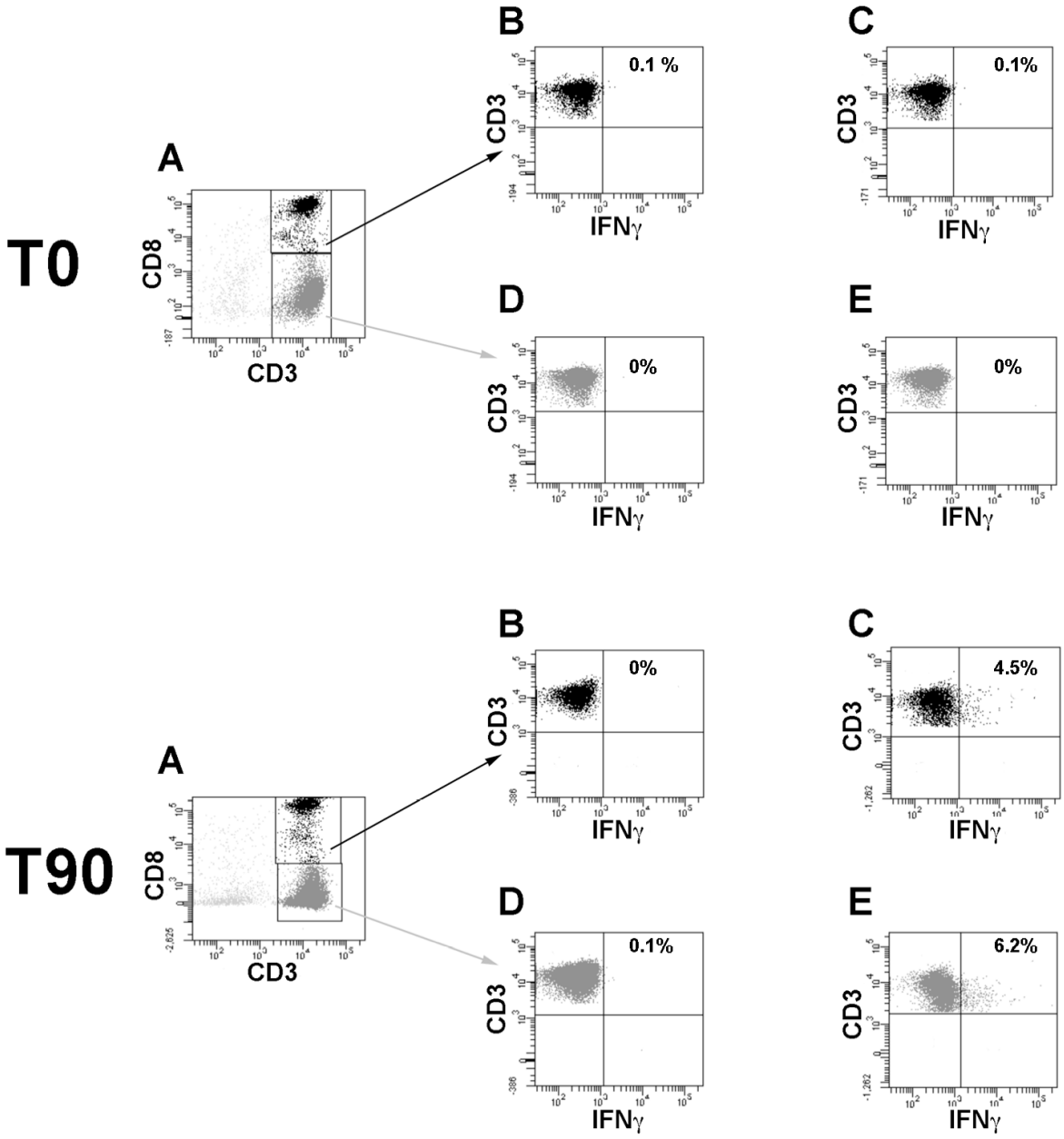
Supplementary Fig. S1. Frequency of circulating CD4+CD25+CD127- Treg in GX301 treated cancer patients at baseline and 10 healthy donors. The existence of a statistically significant difference between the two groups was analyzed by the Mann-Whitney test for non-parametric values.



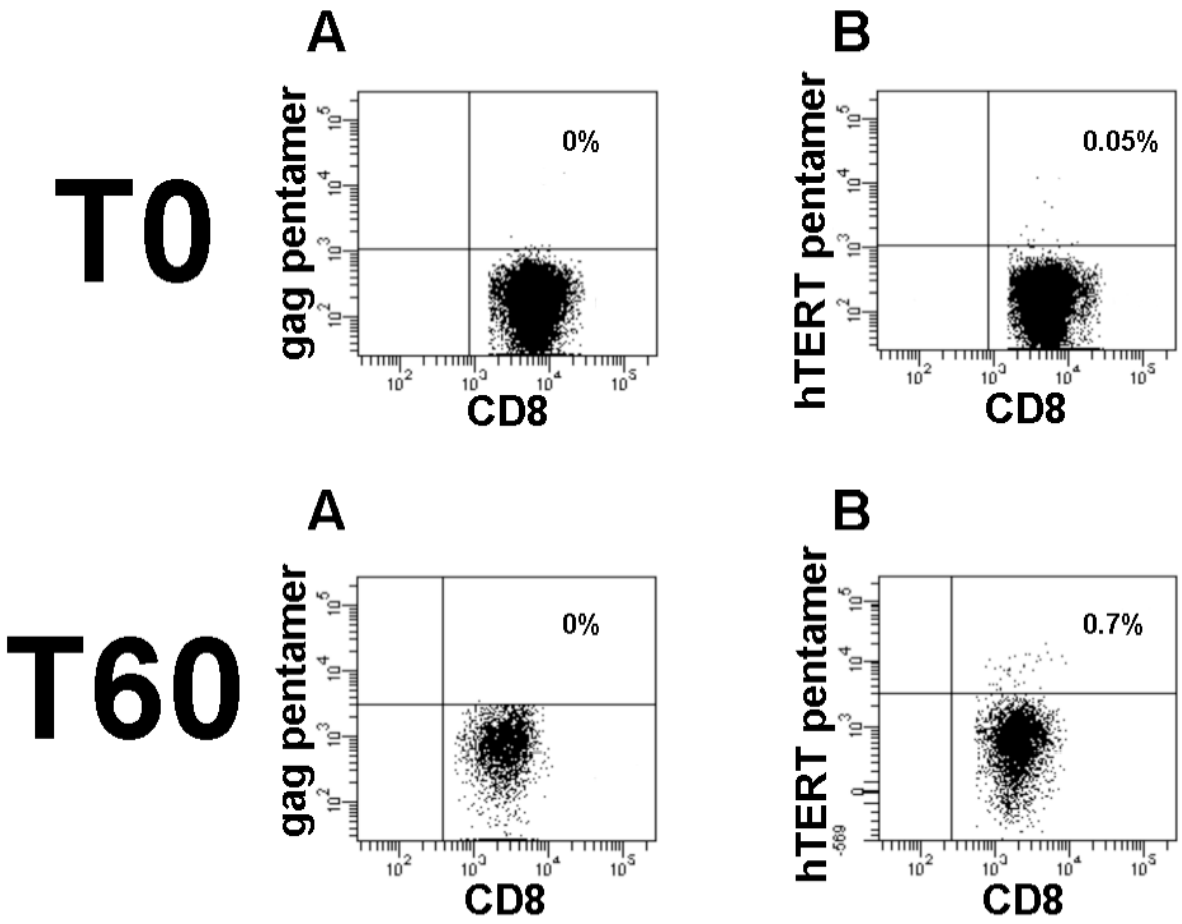
Supplementary Fig. S2. Representative proliferation assay with PBMC from patient TEL11R. A cytometric gate was set on total lymphocytes based on their physical parameters in a forwards/side scatter (A). Patient's PBMC were cultured alone (B) or in the presence of either PKW (1 μg/ml) (C) or GX301 peptides (20 μg/ml each) (D). The upper (T0) and lower (T90) rows refer to tests performed at baseline and after 90 days from the first vaccination, respectively. The analysis shows that the stimulation index of peptide-stimulated PBMC was 5 folds higher than that of unstimulated PBMC only in the tests performed after vaccination.



Supplementary Fig. S3. Representative cytotoxic assay with PBMC from patient TEL14P. A cytometric gate was set on CFDA-SE positive T2 target cells. T2 cell lysis was assessed in cultures performed with: A) unpulsed T2 cells; B) T2 cells pulsed with GX301 peptides; C) unpulsed T2 cells plus patient's PBMC; D) T2 cells pulsed with GX301 peptides plus patient's PBMC. The upper (T0) and lower (T30) rows refer to tests performed at baseline and after 30 days from the first vaccination, respectively. The analysis shows cell lysis percentages higher in the culture containing T2 cells pulsed with GX301 peptides plus patient's PBMC (panel D) than in the negative control cultures (panels A, B, C) only in the tests performed after vaccination (lower row). The percentage of specific lysis (15%) was then normalized for the percentage of CD8+ T cells present in patient's PBMC (detected by immunofluorescence analysis) in order to achieve data on the real cytotoxic potential of patient's CD8+ T cells and to normalize the measurements among the different patients.



Supplementary Fig. S4. Representative intracellular staining for IFNg on PBMC from patient TEL07P. PBMC were cultured for 10 days in the presence of GX301 peptides (20 µg/ml each) before analysis. The analysis was performed on both $CD3^+CD8^+$ and $CD3^+CD8^-$ (corresponding to $CD4^+$) T cells after cell staining with an anti-CD3-PerCPCy5.5 mAb and an anti-CD8-APC mAb (panels A). (B) and (C) panels show the percentages of IFNγ+ T cells in $CD3^+CD8^+$ T cells, while panels (D) and (E) show the percentages of $IFN\gamma^+$ T cells in $CD3^+CD8^-$ T cells. T0 and T90 refer to tests performed at baseline and after 90 days from the first vaccination, respectively.



Supplementary Fig. S5. Representative analysis of frequency of $CD8^+$ T cells binding a HLA-$A2^+$ pentamer loaded with the $hTERT_{540\text{-}548}$ peptide in PBMC from patient TEL09P. A cytometric gate was set on CD8+ T cells based on their stain with an anti-CD3-PerCPCy5.5 mAb and an anti-CD8-APC mAb. A) Unspecific binding to control HLA-$A2^+$ pentamer loaded with the HIV-1 gag p24 $peptide_{19\text{-}27}$; B) Binding to the HLA-$A2^+$ pentamer loaded with the $hTERT_{540\text{-}548}$ peptide. Upper (T0) and lower (T60) rows refer to tests performed at baseline and after 60 days from the first vaccination, respectively.