Anti-Telomerase CD4+ Th1 Immunity and Monocytic-Myeloid-Derived-Suppressor Cells are Associated with Long-Term Efficacy Achieved by Docetaxel, Cisplatin, and 5-Fluorouracil (DCF) in Advanced Anal Squamous Cell Carcinoma: Translational Study of Epitopes-HPV01 and 02 Trials

**Figure S1.** The overall effect of DCF treatment on the antigen-specific T-cell responses in SCCA patients. PBMC from SCCA patients whose samples were available before and after DCF chemotherapy (n = 65 for E6 and hTERT, n = 64 for E7 and n = 59 for CEF) were analyzed for antigen-specific T-cell responses by IFN, ELISpot assay. (A–D) The linking individual plot was used to follow the intensity of HPV16-E6 (A), HPV16-E7 (B), hTERT (C) and antiviral T-cell responses (D) in SCCA patients before and after treatment. Increase specific-immune responses are represented by red gray points, decrease specific-immune responses by blue points and absence of immune responses by black points.
Figure S2. Gating strategy for flow cytometry analyses. The figure shows the gating strategy to analyze immune checkpoints (A), Treg (B) or M-MDSC (C) populations. After exclusion of doublets and death cells, CD4 and CD8 T-cell populations were sectioned. The expression of CTLA-4 (or OX40), 4-1BB, PD-1 and TIM-3 were analyzed on CD4 and CD8 T-cells (A). Frequencies of Treg cells were observed in CD4 T-cell population. Expression of CD226 and TIGIT were analyzed on CD4, Treg and CD8 T-cells (B). M-MDSC and monocyte populations were analyzed after exclusion of lineage (CD3, CD56, CD19) and among PBMC (C).
Figure S3. Determining thresholds for Treg and M-MDSC populations. The thresholds of Treg (A) and M-MDSC (C) populations after DCF treatment were chosen with maximizing of the log-rank test (maxstat cutoff). The relationship between overall survival and Treg (B) and M-MDSC (D) distributions was also investigated using the restricted cubic splines method with graphical evaluation. The selected thresholds were used to analyze the association of Treg and M-MDSC high subpopulations with immune responses and survival of SSCA patients.
Figure S4. M-MDSC levels and not Treg levels are associated with the clinical outcomes of SCCA patients. PBMC from 19 healthy donors and SCCA patients before ($n = 82$) and after ($n = 69$) DCF chemotherapy were analyzed for Treg and M-MDSC populations by flow cytometry. A Kaplan-Meier OS curve in SCCA patients before and after DCF chemotherapy according to Treg levels. B Kaplan-Meier OS curve in SCCA patients before and after DCF chemotherapy according to M-MDSC levels. Log-rank test, where ** $p < 0.01$. 
Figure S5. Monocyte levels are not associated with the clinical outcomes of SCCA patients. PBMC from 19 healthy donors and SCCA patients before ($n=82$) and after ($n=69$) DCF chemotherapy were analyzed for monocytes populations by flow cytometry. (A) Frequencies (%) of monocytes. We selected a median at the threshold (16.1%) to separate into 2 groups our patients. (B) Kaplan-Meier survival curve in SCCA patients before and after DCF chemotherapy. (C) Kaplan-Meier OS curve in SCCA patients before and after DCF chemotherapy according to monocytes levels.
Figure S6. Flowchart. DCF = docetaxel, cisplatin and 5-fluorouracil.