Supplemental Figure 1
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Supplemental Figure 1. TIGIT is upregulated on T cells and NK cells in melanoma patients and TIGIT expression is positively correlated with PD-1 expression on NY-ESO-1 CD8+ T cells. (A and B), Pooled data (n = 8) from the analysis of PBMCs of patients with advanced melanoma, correlating TIGIT and PD-1 expression in percentage (A) and in MFI (B) by NY-ESO-1–, Flu– and CMV–specific CD8+ T cells of patients; P values were obtained by Pearson tests. (C and D) Pooled data showing the frequency (C) and MFI (D) of TIGIT expression by different subsets of PBMCs from healthy donors (n = 13; NK cells: n =14) and melanoma patients (n = 8; NK cells: n =15). Data shown are representative of three independent experiments.
Supplemental Figure 2. TIGIT is upregulated on NY-ESO-1–specific CD8+ T cells upon stimulation with cognate antigen. (A and B) Pooled data (n = 6) of the expression of TIGIT in MFI on A2/NY-ESO-1 157-165 tet+ CD8+ T cells (A) and tet- CD8+ T cells (B) from PBMCs of melanoma patients at different time-points of a 6-d IVS in presence of cognate (NY-ESO-1 157-165) or irrelevant (HIV-pol 476-785) peptide. Data shown are from two independent experiments. Statistics are paired t-tests with *, $P < 0.05$; **, $P < 0.01$. Stars above bars indicate a significant difference of the time point compared to ex vivo. Stars above brackets indicate a significant difference between two time-points.
Supplemental Figure 3. TIGIT adds to PD-1 blockade to increase cytokine production by NY-ESO-1–specific CD8+ T cells. PBMCs of melanoma patients were incubated for 6 d in vitro in presence of NY-ESO-1 157-165 peptide and blocking mAbs against TIGIT (aTIGIT) and/or PD-1 (aPD-1) or isotype control mAbs (IgG) before evaluating intracellular cytokine production. (A and B) Pooled data (n = 9) showing the frequency among NY-ESO-1–specific CD8+ T cells and fold change of IFN-γ (A) and TNF- (B) producing A2/NY-ESO-1 157-165 tet+ CD8+ T cells according to indicated blocking mAbs treatment. P values were obtained by paired t tests and a Friedman test followed by Dunn’s multiple tests with: **, P < 0.01. Data are from two independent experiments performed in duplicates.
Supplemental Figure 4. TIGIT blockade has no effect on IL-10 production by PBMCs and the binding of TIGIT-Fc to its ligands has no effect on cytokine production by TA-specific CD8+ T cells. PBMCs of melanoma patients were incubated in vitro for 6 d with NY-ESO-1 157-165 peptide and blocking mAbs against PD-1 (aPD-1), TIGIT (aTIGIT), TIGIT-Fc or isotype control mAbs (IgG) before evaluating intracellular cytokine production of A2/NY-ESO-1 157-165 tet+ CD8+ T cells in response to cognate peptide. (A) Pooled data (n = 6) showing the frequency among CD8+ T cells of IFN-γ-, TNF- and IL-2–producing NY-ESO-1 157-165 tet+ CD8+ T cells from melanoma patients after incubation with TIGIT-Fc fusion protein. (B) Pooled data (n = 10) of the IL-10 concentration (pg) and fold change of IL-10 concentration in the supernatant of cultures after PD-1 blockade or TIGIT blockade. Paired t-tests were used to calculate P values; ns, non significant. Data are from two independent experiments performed in duplicates.
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(A) Representative flow cytometry analysis of TIGIT and PD-1 co-expression ex vivo by one CD8+ TILs isolated from metastatic melanoma. (B) Pooled data of the frequencies of HLA-DR, CD38 and CD57 CD45RA, CCR7, CD27 and CD28 ex vivo expression within the CD8+ TILs of metastatic melanomas (n = 9), and according to PD-1 and TIGIT expression. P values were obtained by a Friedman test followed by Dunn’s multiple tests (top right panel) and by repeated-measures ANOVA tests followed by Tukey’s multiple comparison tests (all other panels) with: *, P < 0.05; **, P < 0.01; ***, P < 0.001. Data are representative of two independent experiments.
Supplemental Figure 6. TIGIT blockade adds to PD-1 blockade to increase the frequencies of proliferating and degranulating TILs. (A) Representative dot plots of CD8+ TILs isolated from one metastatic melanoma showing IFN-γ-, TNF- and IL-2– production according to PD-1 and TIGIT expression after stimulation by PMA and ionomycin. (B and C) Representative flow cytometry analysis of the CFSE dilution (B) and CD107a expression (C) of CD8+ TILs after 5 d incubation with autologous non-CD3 cells isolated from metastatic melanoma, anti-CD3 mAbs and in the presence of blocking mAbs against PD-1 (aPD-1) and/or TIGIT (aTIGIT) or isotype control mAbs (IgG). Data are representative of two independent experiments.

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