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Fig. S15. Anti-melanoma therapeutic activity is impaired in ICAM-1 deficient mice.

Table S1. Clinical trials of CTLA-4 and PD-1 checkpoint blockade combined with a cancer vaccine.
Supplementary Fig. 1. Anti-CTLA-4 therapeutic activity is impaired by addition of OVA/IFA vaccination in thymoma tumor model.

(A) Experimental scheme. Mice bearing 7-day E.G7.OVA tumors were vaccinated with OVA/IFA s.c. or control/IFA s.c on day 7 or were left unvaccinated (untreated) or anti-CTLA-4 therapy on days 7, 9, 11 and 13 after tumor injection. (B) Tumor burden measurement on individual mice. Number in parenthesis represents the proportion of mice with tumor free survival. (C) Kaplan Meier survival. All data shown are representative of two experiments, each with n = 10-15 mice per group. * P < 0.05, Log-rank test.
Supplementary Fig. 2. OT-I Teff therapeutic activity is abrogated by the addition of gp100/IFA vaccination.

(A) Experimental scheme. Mice bearing 7-day old B16.OVA received OVA-specific OT-I T cells (1x10^6) i.v., gp100-specific pmel-1 T cells (1x10^6) i.v. and VSV.OVA (1x10^7) i.v. or gp100/IFA or control/IFA. (B) Expression of proliferation, inhibitory cell surface markers in PBMC 8 d after vaccination. (C) Tumor burden measurement on individual mice, data right panel represents the mean ± SEM. Data shown are representative of two experiments, each with (n= 10 mice per group). * P < 0.05 determined by unpaired two-tailed t test.
Supplementary Fig. 3. Gating strategy for pmel-1 and non-pmel-1 CD8+ Teff.

Mice received naive gp100-specific CD90.1+ pmel-1 T cells i.v., followed by immunization i.v. with VSV.gp100 on day 0 and anti-CTLA-4 + Gvax therapy on days 0, 3 and 6. Pmel-1 and non-pmel-1 Teff gating strategy in PBMC 7 days after vaccination. (A) CD44 and CD11a surface expression (B) IFN-γ secretion in response to hgp100 peptide stimulation.
Supplementary Fig. 4. CD8+ Teff localize to sites of vaccination with non-cognate antigen.

Mice bearing 3 day B16-BL6 melanoma were injected with pmel-1 T cells and received early anti-CTLA-4 + Gvax therapy and vaccinated with saline/IFA or hgp100/IFA or combination or left untreated. (A) p15E-specific IFN-γ+ (B) non-pmel-1 endogenous gp100-specific IFN-γ+ T cells quantified at tumor and vaccination site 9 days after vaccination. (n = 8 mice per group). Right panels indicate tumor/vaccination site fold increase. Data represent the mean ± SEM. (C) CD8+ TRP-2 pentamer specific T cells in VdLN, spleen, tumor and vaccination site 9 days after gp100/IFA vaccination. Two independent experiments, each with (n = 5 mice per group). * P < 0.05 determined by unpaired two-tailed t test. Bar represents the mean ± SEM. Bar represents the mean ± SEM of individual mice (left panel).
Supplementary Fig. 4 (D-E). CD8+ Teff localize to sites of vaccination with non-cognate antigen.

Mice are treated as in 4A. Plots show (D) T-bet and Eomes expression by naïve, non-pme-1 Teff and pmel-1 Teff in PBMC, VdLN and vaccination site. (E) IFN-γ secretion by non pmel-1 and pmel-1 Teff from vaccination site 9 days after vaccination following incubation with P15E or gp100 peptide. Two independent experiments, each with \( n = 5 \) mice per group. * \( P < 0.05 \) determined by unpaired two-tailed t test. Data represent the mean ± SEM.
Supplementary Fig. 4 (F-G). CD8+ Teff localize to sites of vaccination with non-cognate antigen.

Mice were treated with standard anti-CTLA-4, Gvax and gp100/IFA. (F) CD45+ leukocytes PD-L1 and FasL expressions in tumor and vaccination site were analyzed 9 days after vaccination. Bars mean ± SEM, right panel, show frequency of leukocyte subset as a percent of total CD45+ cells. (G) Bcl-2, Bcl-xL, and Bim expression on naïve CD8+ T cells, non-pmel-1 CD8+ Teff and pmel-1 CD8+ Teff at VdLN, spleen and vaccination site 9 d after gp100/IFA vaccination. Two independent experiments, each with (n=5 mice per group). * P < 0.05 determined by unpaired two-tailed t test. Data represent the mean ± SEM.
Supplementary Fig. 5. Chemokine receptor and ligand expressions in tumor and at vaccination site.

Mice were injected with B16-BL6 cells s.c. and gp100-specific pmel-1 T cells i.v. on day 0, followed by vaccination with hgp100/IFA s.c. or control/IFA s.c. (control vaccination) on day 3 or anti-CTLA-4 and Gvax on days 3, 6 and 9 or untreated or combination. 9 d after vaccination (A) Chemokine receptor expression in naive, pmel-1 and non-pmel-1 Teff in PBMC (B) Chemokine ligand repertoire at tumor and vaccination site 9 d after vaccination. Data are representative of two experiments, each with (n = 5 mice per group). * P < 0.05 determined by unpaired two-tailed t test. Bars represent the mean ± SEM.
Supplementary Fig. 6. CXCL11, IFN-γ and VCAM-1 mRNA expressions and overall patient survival obtained from public TCGA repositories.

(A) CXCL11, (B) IFN-γ and (C) VCAM-1. Right panel, CD8 mRNA level (mean ± SEM) determined by paired two-tailed t test. (https://tcga-data.nci.nih.gov and http://gdac.broadinstitute.org/).
Supplementary Fig. 7. Quantification of non-pmel-1 and pmel-1 Teff levels by immunofluorescence imaging.

Mice bearing B16-BL6 were injected with pmel-1 T cells i.v. and immunized with hgp100 in IFA or control/IFA on day 3 and received anti-CTLA-4 on days 3, 6 and 9 and/or IFN-γ on days 3, 5, 7, 9 and 11 after tumor injection. Non-pmel-1 and pmel-1 Teff frequency at tumor and vaccination site 9 d after vaccination, (n=25-50 data points per group). * P < 0.05 determined by nonparametric Kruskal-Wallis test. Data represent the mean ± SEM.
Supplementary Fig. 8. ICAM-1 and VCAM-1 expressions on CD31+ vasculature at vaccination site.

Mice bearing B16-BL6 were injected with pmel-1 T cells i.v. and immunized with hgp100 in IFA on day 3 and/or received anti-CTLA-4 on days 3, 6 and 9 and/or IFN-γ neutralization on days 3, 5, 7, 9 and 11 after tumor injection or left untreated. ICAM-1 and VCAM-1 expression on CD31+ vasculature at the vaccination site 9 days after vaccination, (n=25-50 data points per group). * \( P < 0.05 \) determined by nonparametric Kruskal-Wallis test. Data represent the mean ± SEM.
Supplementary Fig. 9. CXCR3 blockade reduces inflammatory reaction at the vaccine injection site.

Mice bearing 3 day B16-BL6 melanoma were injected with pmel-1 T cells and received hgp100 in IFA on day 0 and early anti-CTLA-4 + Gvax on days 3, 6 and 9 after tumor injection. (A) Distribution of CD11b+ leukocyte subsets and their CXCR3 expression at vaccination site. (B) Mice treated as in A received IgG control or CXCR3 blockade on days 3, 5, 7, 9 and 11 after tumor injection. Pictures show skin inflammatory reaction at the vaccine injection site 9 d after vaccination. Data shown are representative of two experiments, each with (n=5 mice per group).
Supplementary Fig. 10. IFN-γ from CD8+ Teff induces early iMo localization to the vaccination site.

Mice were injected s.c. with PBS, IFA or IFA + recombinant murine IFN-γ. Bars show mean ± SEM CD11b+ myeloid cells sampled from injection site on days 1, 2 and 3 after treatment, (n=5 mice per group) * P < 0.05 determined by unpaired two-tailed t test.
Supplementary Fig. 11. Anti-CTLA-4 therapeutic activity is enhanced by addition of gp100 peptide pulsed DCs.

(A) Experimental scheme. Mice bearing 3-day B16 tumors received gp100-specific CD90.1+ pmel-1 T cells i.v., followed by transfer of OVA peptide (control) or gp100 peptide pulsed DC i.v. on day 0 and/or anti-CTLA-4 and Gvax therapy on days 0, 3 and 6 or left untreated. (B) Tumor burden measurement on individual mice. Number in parenthesis represents the proportion of mice with tumor free survival. (C) Kaplan Meier survival curves. Data shown are representative of two experiments, each with (n = 8 – 15 mice per group). * P < 0.05, Log-rank test.
Supplementary Fig. 12. Anti-melanoma therapeutic activity is impaired by CXCR3 blockade.

(A) Experimental scheme. Mice bearing 3 day B16-BL6 melanoma were injected with pmel-1 T cells, immunized with VSV.gp100 i.v. and received early anti-CTLA-4 therapy + Gvax on days 3, 6 and 9 and/or IgG control i.p. or anti-CXCR3 blockade i.p. on days 3, 5, 7, 9 and 11 after tumor injection. (B) Kaplan-Meier survival curve, (n=15 mice per group) * P < 0.05, Log-rank test. (C) TRP-2 –specific and pmel-1 Teff level in tumor and vaccination site 9 d after vaccination. Data representative of two independent experiments (n= 5 mice per group). * P < 0.05 determined by unpaired two-tailed t test. Bars show mean ± SEM.
Supplementary Fig. 13. Frequency and distribution of pmel-1 T cells in tissues following different vaccination strategies.

Mice bearing B16 tumor were injected with naive pmel-1 T cells, received anti-CTLA-4 + Gvax therapy on days 6, 9 and 12 and and/or vaccination with gp100 peptide in IFA or hgp100 in saline or IFA with covax or VSV.gp100 on day 6 after tumor injection. CD8+ pmel-1 T cell as % of total CD8+ in PBMC, VdLN, tumor and vaccination site 7 d after vaccination, representative of two experiments, each with (n = 5 mice per group). Data shown are mean ± SEM.
Supplementary Fig. 14. Gp100/IFA vaccination progressively reduces proliferation, trafficking and increases dysfunction of gp100-specific T cells.

(A) Wild type C57BL/6 were challenged with B16-BL6 cells and received late anti-CTLA-4 + Gvax therapy on days 7, 10 and 13 and vaccination with VSV.gp100 i.v. and/or gp100/IFA on day 7. PBMC were analyzed on days 9 and 17 after vaccination. Data shown are non-pmel-1 and pmel-1 Teff (A) Ki67+ (B) CXCR3+ (C) IFN-γ+ (D) T cell level. Data shown are representative of (mean ± SEM, n = 5 mice per group). * P < 0.05 determined by unpaired two-tailed t test.
Supplementary Fig. 15. Anti-melanoma therapeutic activity is impaired in ICAM-1 deficient mice.

(A) Experimental scheme. Wild type C57BL/6 versus ICAM-1 KO mice were challenged with B16-BL6 cells and received late anti-CTLA-4 + Gvax therapy on days 7, 10 and 13 and vaccination with VSV.gp100 i.v. and/or gp100/IFA on day 7. (B) Kaplan Meier survival curves of two independent experiments, each with n=10 mice per group, * P < 0.05, Log-rank test. (C-D) Wild type C57BL/6 treated as in B received IgG control i.p. or anti-ICAM-1 i.p. on days 7, 9, 11, 13 and 15 after tumor injection. (C) pmel-1 Teff level in tumor and PBMC (D) IFN-γ+ TNF-α+ pmel-1 T cells in PBMC, 7 d after vaccination, representative of two experiments, each with (n = 5 mice per group). * P < 0.05 determined by unpaired two-tailed t test. Bars show mean ± SEM.
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25 Jonsson Comprehensive Cancer Center PD-1 NY-ESO-1-specific T cells + DC vaccine Solid tumors Phase 1 NCT02775292
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32 Sidney Kimmel Comprehensive Cancer Center CTLA-4 Viral vaccine (PROSTVAC) Prostate Cancer Phase 2 NCT00113984
33 Sidney Kimmel Comprehensive Cancer Center CTLA-4 DNA vaccine Pancreatic Cancer Phase 1 NCT00836407

A ClinicalTrials.gov was accessed on 07/25/2016.