Melanoma vaccine combined with immunomodulation.

Title
Combination of Alphavirus replicon particle-based vaccination with immunomodulatory antibodies: therapeutic activity in the B16 melanoma mouse model and immune correlates.

Authors and affiliations
Francesca Avogadri\textsuperscript{1}, Roberta Zappasodi\textsuperscript{1}, Arvin Yang\textsuperscript{1}, Sadna Budhu\textsuperscript{1}, Nicole Malandro\textsuperscript{1}, Daniel Hirshhorn-Cymerman\textsuperscript{1}, Shakuntala Tiwari\textsuperscript{1}, Maureen F. Maughan\textsuperscript{2}, Robert Olmsted\textsuperscript{2}, Jedd D Wolchok\textsuperscript{1}, Taha Merghoub\textsuperscript{1}.

\textsuperscript{1}Swim Across America Laboratory, Immunology Program, Sloan-Kettering Institute, New York, New York, United States of America.
\textsuperscript{2}AlphaVax, Inc., Research Triangle Park, North Carolina, United States of America.
Supplementary Figures

Supplementary Figure S1. Coat pigmentation after treatment with anti-GITR or anti-CTLA-4 mAb plus VRP-TRP2 vaccine. Mice received a first injection of VRP-TRP2 in combination with anti-GITR (αGITR) or anti-CTLA-4 mAb (αCTLA-4), or isotype control as described in Figure 1. Representative pictures of lower back (top) and front (bottom) showing the coat color of mice 3 months after receiving the indicated treatments.

Supplementary Figure S2. Modulation of the tumor immune infiltrate by anti-CTLA-4 or anti-GITR mAb. B16F10 tumor-bearing mice were vaccinated with control VRP-GFP (GFP, black) or VRP-TRP2 (TRP2, white) and then treated with anti-CTLA-4 (αCTLA-4) or anti-GITR mAb (αGITR), or isotype control as described in Figure 1. (A) Mean percentages ± SEM and representative examples of intra-tumor CD11c+/CD45+ T cells for 4-5 mice per group. (B) Mean percentages ± SEM of intra-tumor CD4+/CD45+ T cells for 3-4 mice per group. ** p<0.01; ns, not significant.

Supplementary Figure S3. PD-1 expression in circulating CD4+ T cells and intra-tumor CD8+ T cells, and CD4+Foxp3+PD-1+ T cell/Treg ratio at the tumor site. B16F10 melanoma-bearing mice were treated with VRP-TRP2 in combinations with anti-CTLA-4 (αCTLA-4) or anti-GITR (αGITR) mAb, or control isotype as shown in Figure 1. (A) Percentage of PD-1-expressing CD4+ T cells in the spleen or (B) intra-tumor CD8+ T cells, and (C) CD4+Foxp3+PD-1+ T cell/Treg ratio in the tumor from mice treated as indicated. ** p<0.01; ns, not significant.

Supplementary Figure S4. The increased CD4+PD-1+ T-cell tumor infiltrate induced by anti-CTLA-4 mAb is independent from the vaccine. B16F10 tumor-bearing mice were vaccinated with control VRP-GFP (GFP, black) or VRP-TRP2 (TRP2, white) and then treated with anti-CTLA-4 mAb (αCTLA4) or control isotype as shown in Figure 1. Mean percentages ± SEM of PD1+/CD4+ T cells within the tumor (left) or spleen (right) for 3-4 mice per group are reported. ** p<0.01, * p<0.05; ns, not significant.
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Supplementary Figure S1.

Supplementary Figure S2.
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Supplementary Figure S3.

A. CD4⁺ Spleen

B. CD8⁺ TILs

C. TILs

Supplementary Figure S4.
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