Time to GoInVivo™, validated checkpoint functional antibodies for cancer research

MIGUEL TAM, JASON WILLIAMS*, BINGGANG SUN, HONG ZHANG,
SALVADOR GARCIA-MOJICA, DZUNG NGUYEN, XIFENG YANG.
BioLegend, Inc. 9727 Pacific Heights Blvd, San Diego, CA 92121 | *University of Chicago, Chicago IL

Abstract

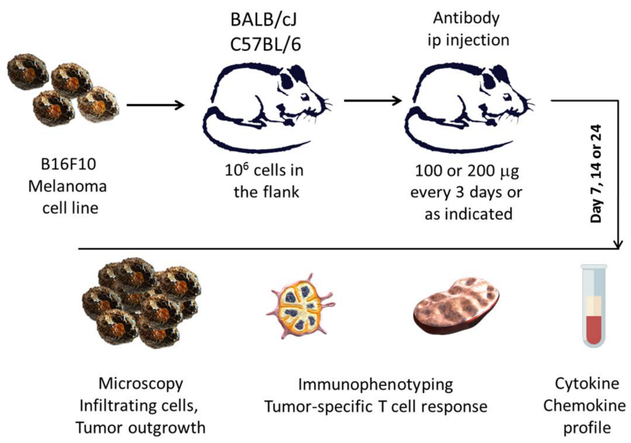
Immune checkpoints are molecules that have been described to control the immune response. Some important checkpoint molecules are gaining more and more attention as they regulate the balance between tumor elimination and tumor escape. Tumor cells communicate with these receptors to trick the immune response into suppression, allowing the tumor cell freedom to grow without immune cell intervention. To interfere with this process, some important checkpoint interactions can be manipulated with the use of bioactive antibodies.

Introduction

Immune checkpoint molecules control the fate of an immune response. Well-studied combinations of molecules include PD-1/PD-L1, CTLA-4/CD80 and CD86, LAG-3/MHC II, and Tim-3/Galectin 9. Our GoInVivo™ antibodies, against some of these immune checkpoint molecules, offer several advantages. They have been tested by flow cytometry and in vitro bioassays, are pathogen-free as tested by qPCR, and have excellent price for large sizes, among others. Here we present our results on the use of anti-mouse PD-L1 in the treatment of a mouse cancer model, melanoma. We characterize the phenotype and localization of T cells, in the tumor microenvironment and draining lymph nodes. We also study the cytokine profile in serum, as well as the antigen-specific T cell response.

Materials and Methods

Balb/cJ or C57BL/6J mice were injected with 10^6 B16F10 melanoma cells in the flank and treated with 100 or 200 µg of anti-PD-L1 at the time points indicated. Tissue samples and serum were collected and analyzed by flow cytometry, microscopy, or screened for cytokine content with LEGENDplex™.



Results

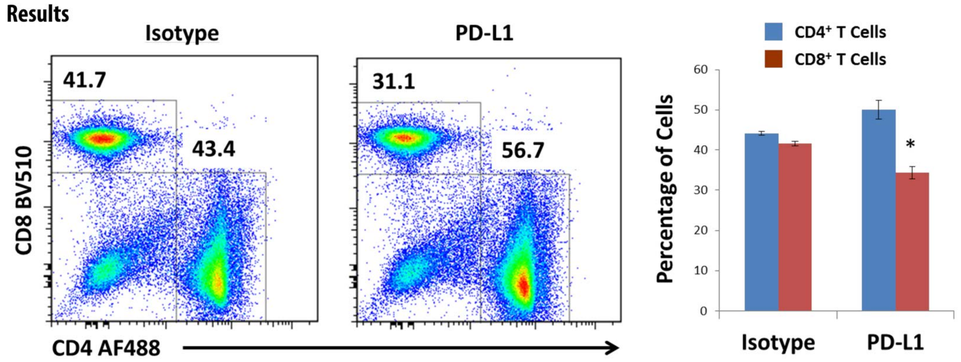


Figure 1. CD4 and CD8 T cells redistribute in the spleen. Animals were implanted for 14 days with B16 melanoma cells, after receiving 3 doses of anti-PD-L1 the CD8/CD4 T cell ratio increases.

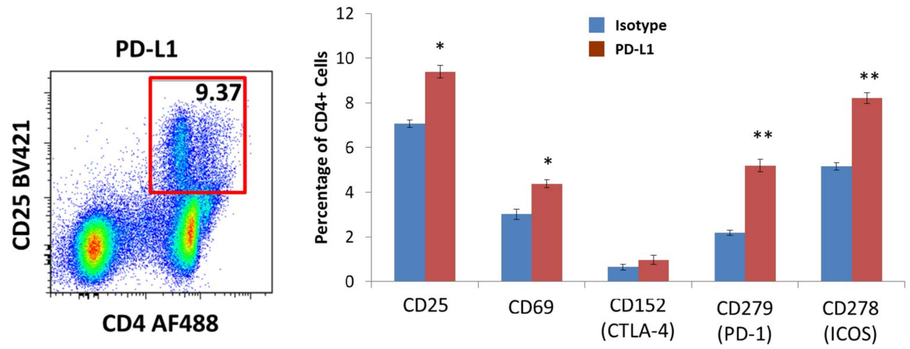


Figure 2. Splenic CD4 T cells show an activated phenotype. After tumor implant and antibody treatment, CD4 T cells show increased expression of CD25, CD69, CD278, and CD279. Similar results were observed in CD8 T cells (not shown).

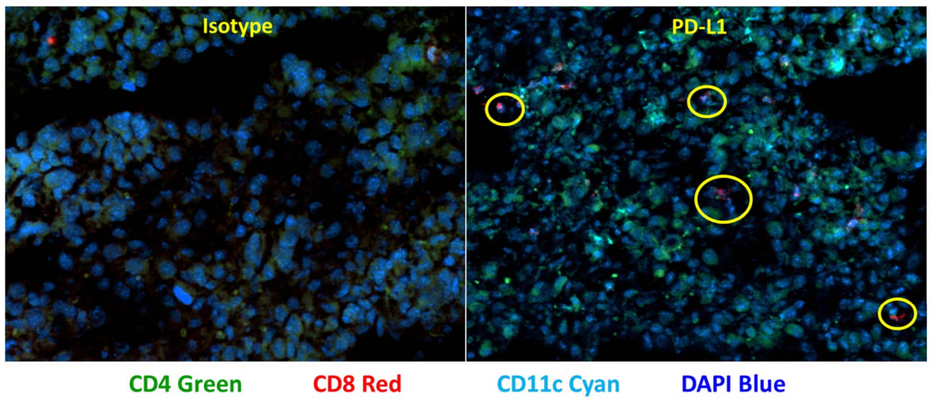


Figure 3. CD8⁺ cells infiltrate the tumor and co-localize with CD11c⁺ cells. After tumor implant and antibody treatment, the tumors were excised and analyzed by fluorescent microscopy. Pictures were taken with a 40X objective.

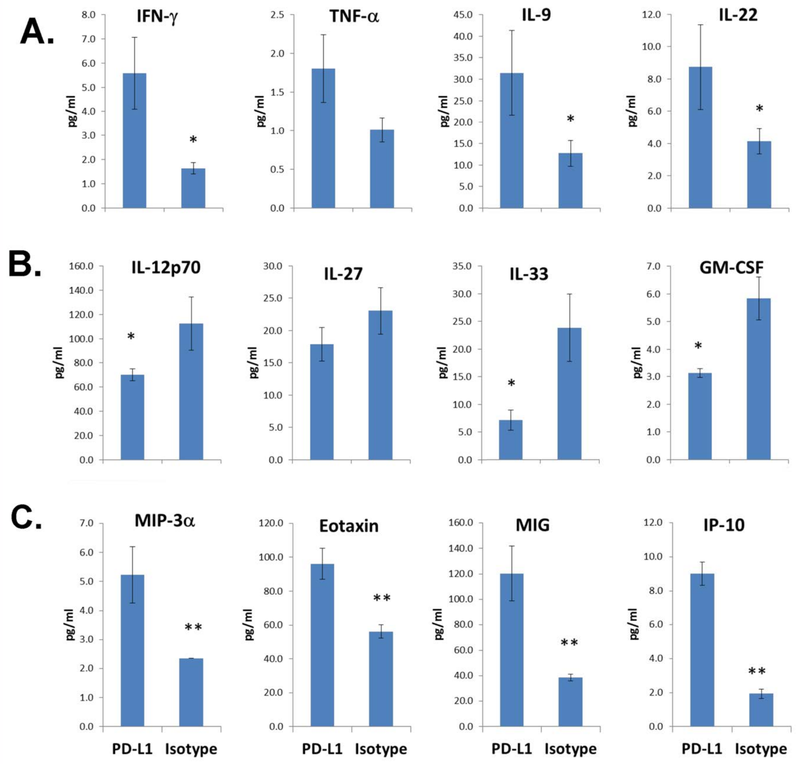


Figure 4. Cytokine and chemokine profile in serum. After tumor implant and antibody treatment, the analysis of the samples with LEGENDplex™ show A) Increased Th Cytokines. B) Decreased pro-inflammatory cytokines. C) Increased chemokine production.

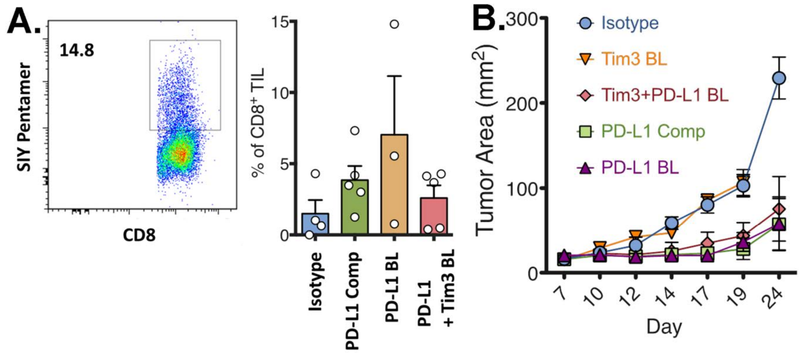


Figure 5. A) Tumor-specific infiltrating CD8⁺ T cells increase 24 days after treatment. Animals were implanted with SIYRYYGL-expressing B16 cells and 24 days after treatment the tumor was collected and analyzed. B) Anti-PD-L1 treatment reduces tumor growth.

Conclusions

Injection of anti-mouse PD-L1 in mice implanted with B16 melanoma cells:

1. Redistributes CD4 and CD8 T cell content in spleen and tumor, and induce activation of T cells
2. Stimulates production of Th cytokines and chemokines, while suppressing pro-inflammatory cytokine production
3. Increases tumor-specific T cells, as well as IFN-γ-producing cells (not shown)
4. Reduces tumor growth