Multiple Bispecific Checkpoint Combinations Promote T cell activation

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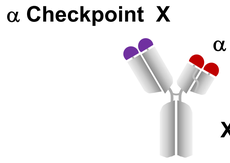
Introduction

- Tumor infiltrating lymphocytes (TILs) co-express multiple checkpoint receptors, in contrast to lymphocytes found in the periphery (Matsuzaki et al PNAS 2010, Fourcade et al Cancer Res 2012, Gros et al JCI 2014)
- TILs that co-express multiple checkpoint receptors may be resistant to single-checkpoint blockade
- We produced PD-1 x CTLA-4, PD-1 x LAG-3, CTLA-4 x LAG-3, and PD-1 x BTLA bispecific antibodies and characterized their T cell activation activity *in vitro* and *in vivo* (huPBMC-engrafted NSG mice)

Summary

- Bispecific antibodies selectively target T cells co-expressing multiple checkpoint receptors
- All bispecific antibody pairs enhanced IL-2 production in an *in vitro* SEB stimulation assay relative to control, indicating functional checkpoint blockade
- Bispecific antibodies promote human T cell proliferation in mice engrafted with human PBMCs
- CTLA-4 x LAG-3 bispecific combines productively with anti-PD-1 to promote triple checkpoint blockade and strong T cell stimulation

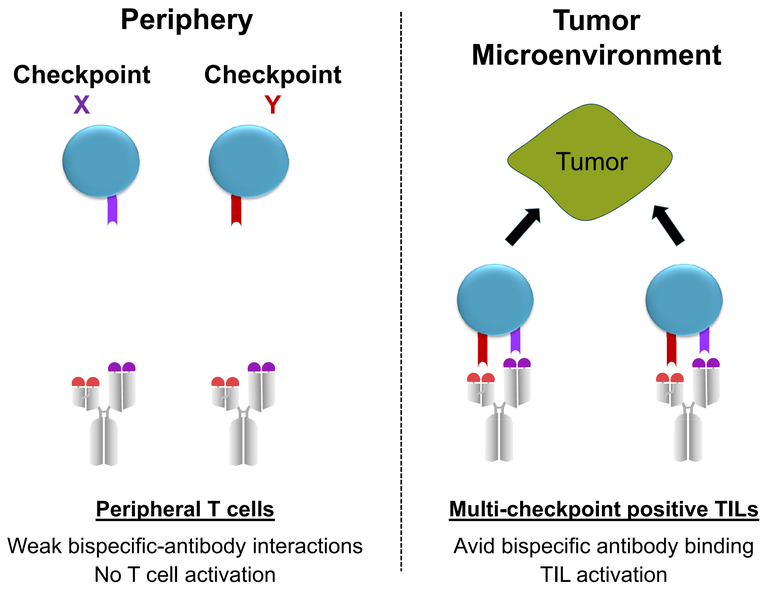
Dual-checkpoint bispecific antibody design



- High yielding stable cell lines
- Protein A + Ion exchange chromatography

- Modified Fc domain eliminates FcγR interactions
- Modified Fc domain with Xtend technology to promote long half-life
- Fc substitutions promote heterodimer formation and facilitate purification by standard methods
- Optimized checkpoint receptor antibodies were plugged into the platform without further reformatting

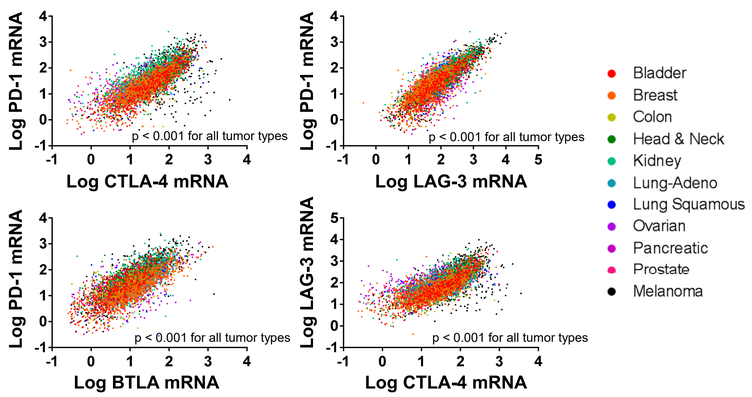
TIL activation with bispecific antibodies



Peripheral T cells
Weak bispecific-antibody interactions
No T cell activation

Multi-checkpoint positive TILs
Avid bispecific antibody binding
TIL activation

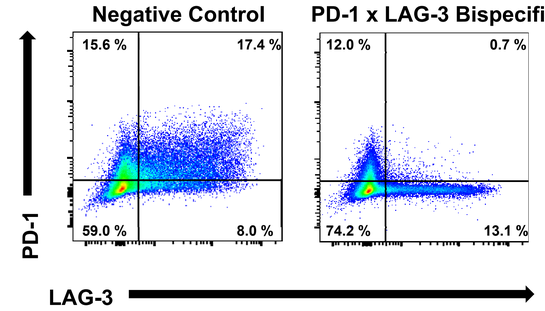
TILs co-express checkpoint receptors



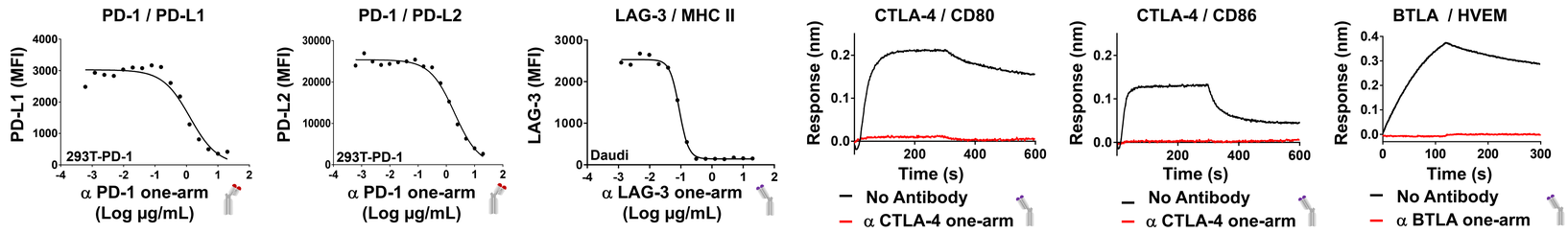
The results shown are based upon data generated by the TCGA Research Network: http://cancergenome.nih.gov/

Bispecific antibodies selectively target dual-checkpoint positive T cells

Receptor occupancy of PD-1 and LAG-3 on human CD3⁺ T cells stimulated with SEB

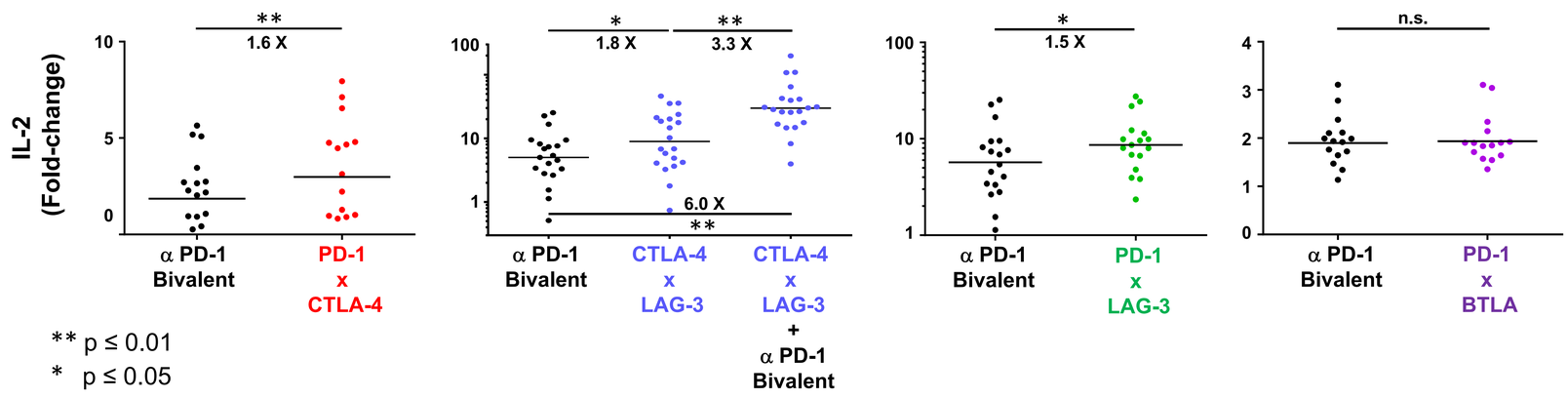


Component antibody domains block checkpoint receptor / ligand interactions



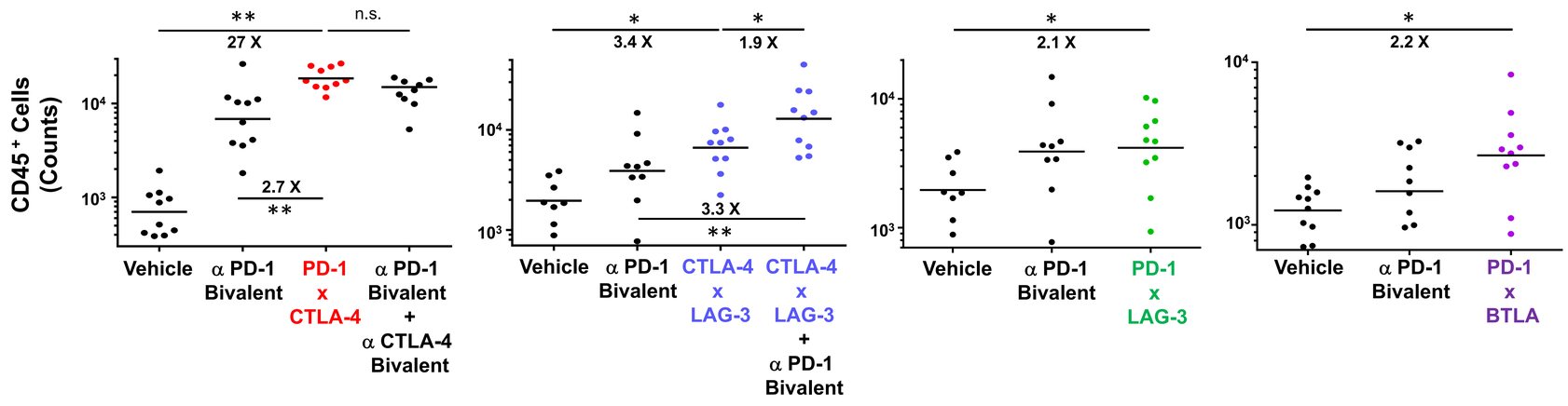
Bispecific checkpoint blockade promotes T cell activation *in vitro*

SEB-stimulated human lymphocytes (multiple healthy donors)



** p ≤ 0.01
* p ≤ 0.05

Bispecific checkpoint blockade promotes human T cell proliferation in huPBMC-NSG mice



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