

Evaluation Platform for Bispecific Antibodies



WuXi Biology, Oncology & Immunology Unit



2023.06

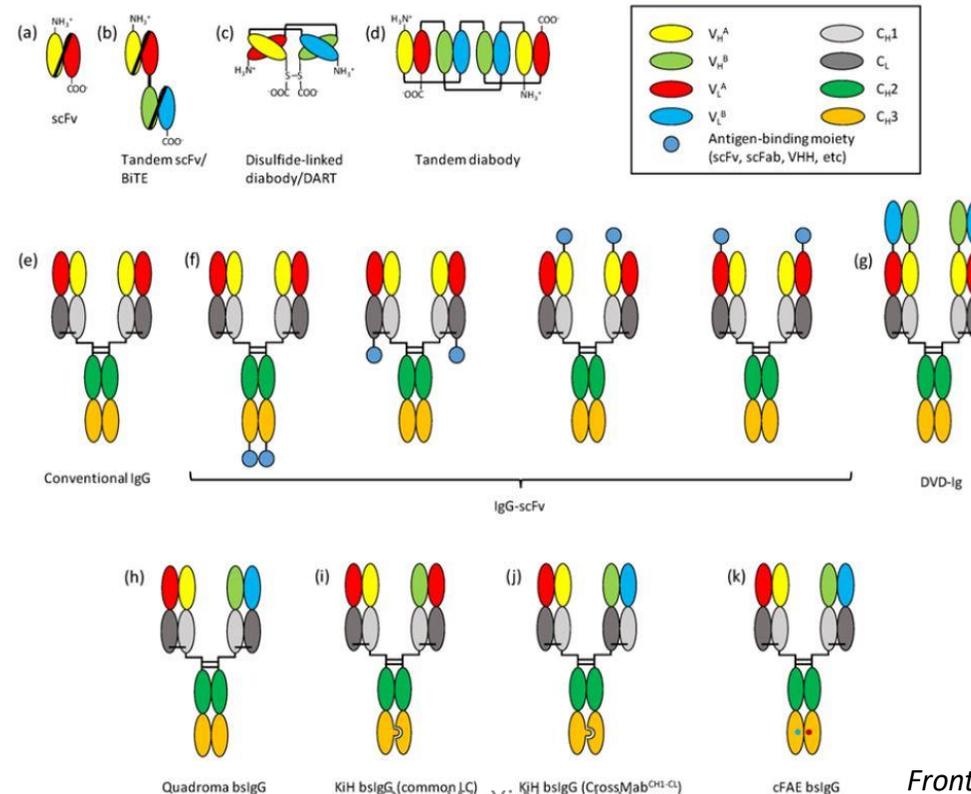
OncoWuXi Newsletter

Outline

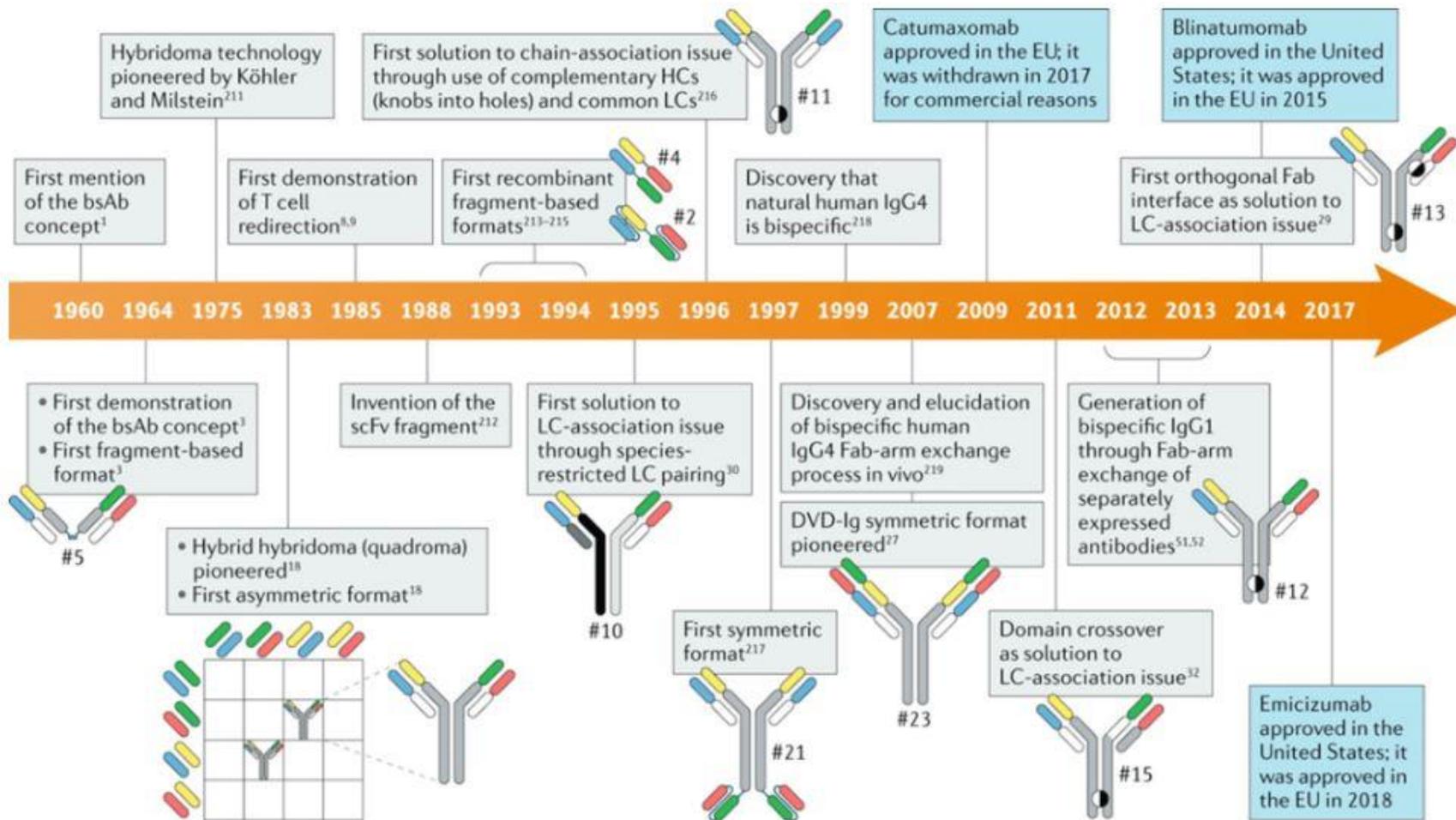
- Introduction of bispecific antibodies
- Bispecific antibody platform in market
- Solutions for bispecific antibody evaluation
- Case studies
 - T cell engager (CD3-CD19 bispecific antibody)
 - T cell engager (CD3-CLDN 18.2 bispecific antibody)
 - Immune checkpoint (PD1-CTLA4 bispecific antibody)

Introduction of bispecific antibodies (BsAbs)

- Bispecific antibodies (BsAbs) address 2 different antigens or epitopes by binding to 2 different targets associated with cancer process.
- Three different components are required for the generation of a BsAb: the binding domains, the “multimerization core” that allows homo- or hetero-multimer formation, and the linkers connecting the other blocks.
- The mechanisms of action can be categorized into the following 4 groups: 1) Targeting tumor-associated antigens (TAAs), 2) targeting TAAs with payload, 3) Targeting immune checkpoints and 4) Engaging immune cells.

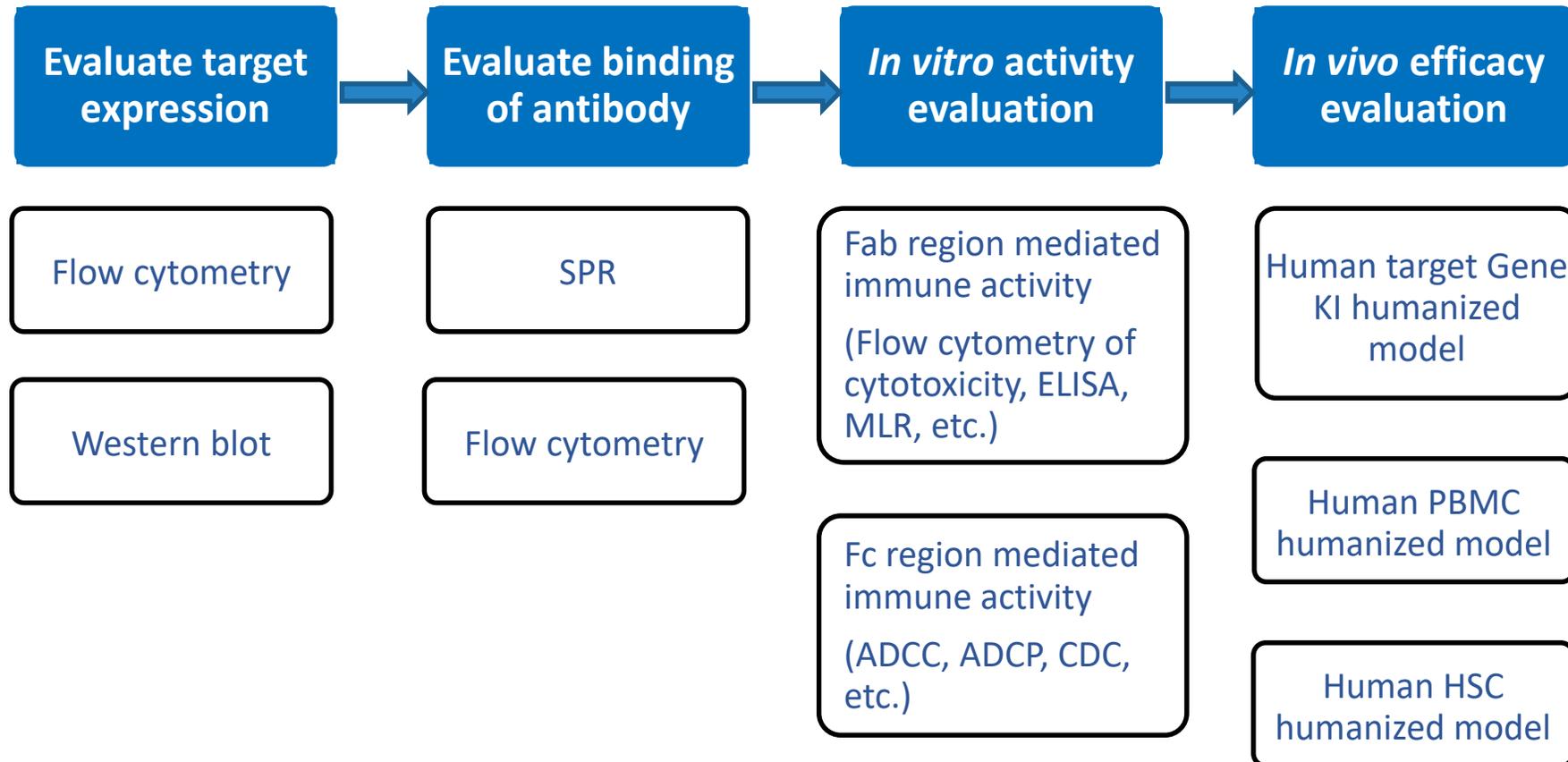


Introduction of bispecific antibodies (BsAbs)



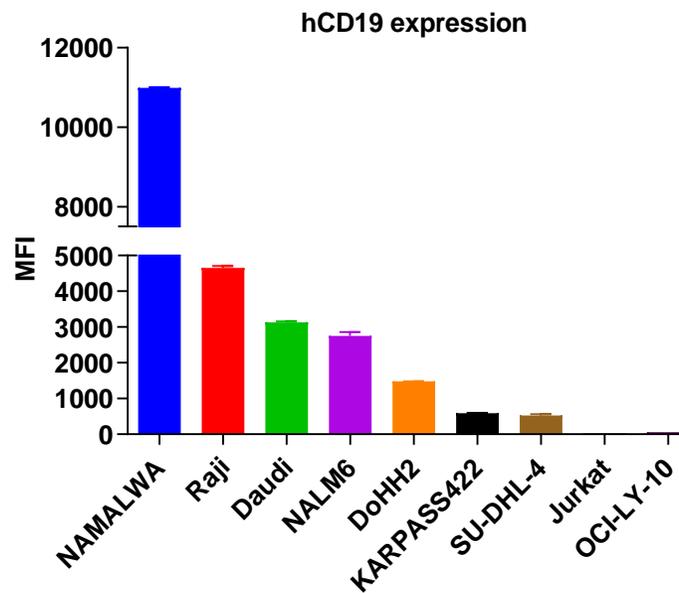
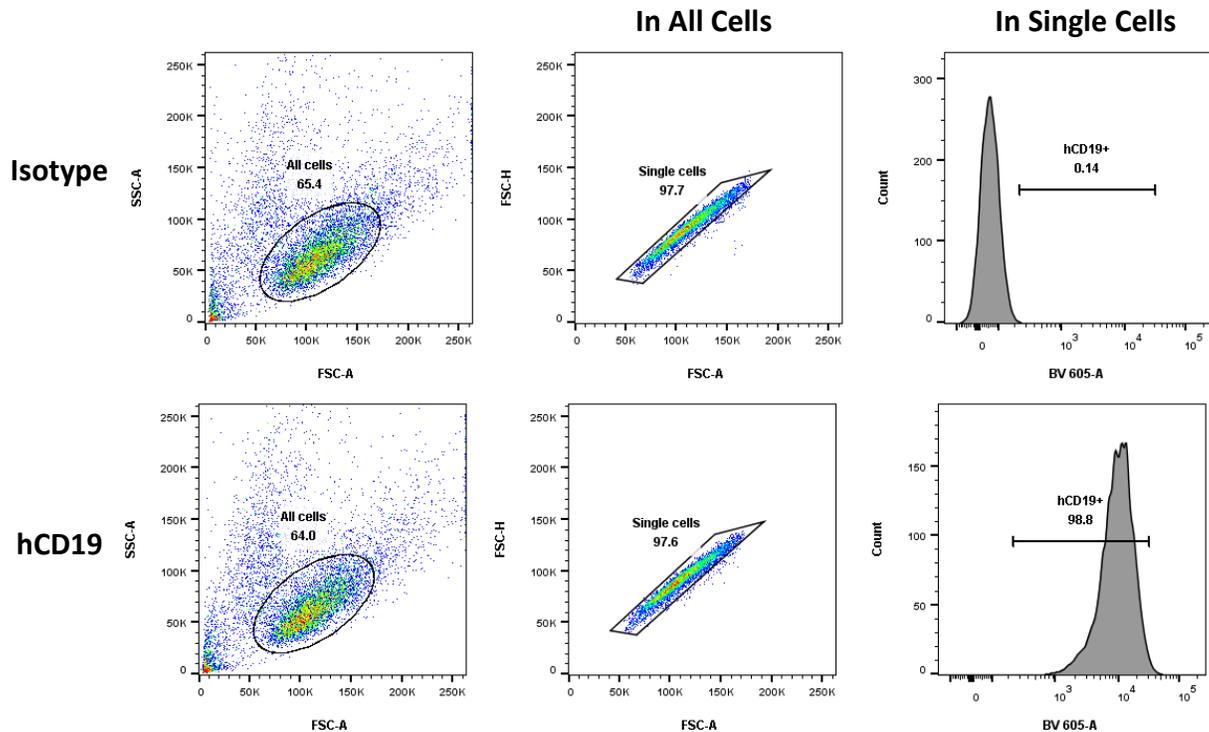
Nat Rev Drug Discovery. 2021

Solutions for bispecific antibody evaluation



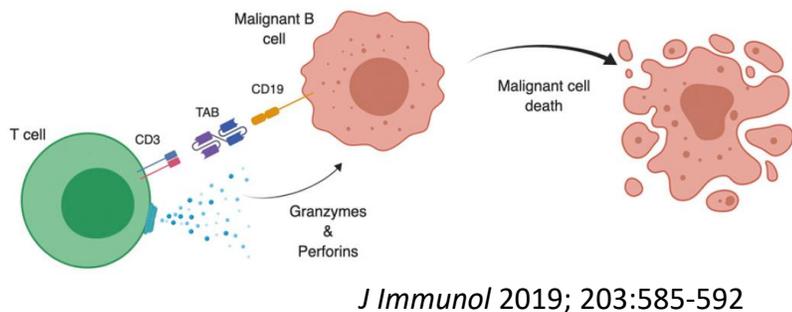
Case study: anti-CD19/CD3 BsAb

Target expression validation by flow cytometry

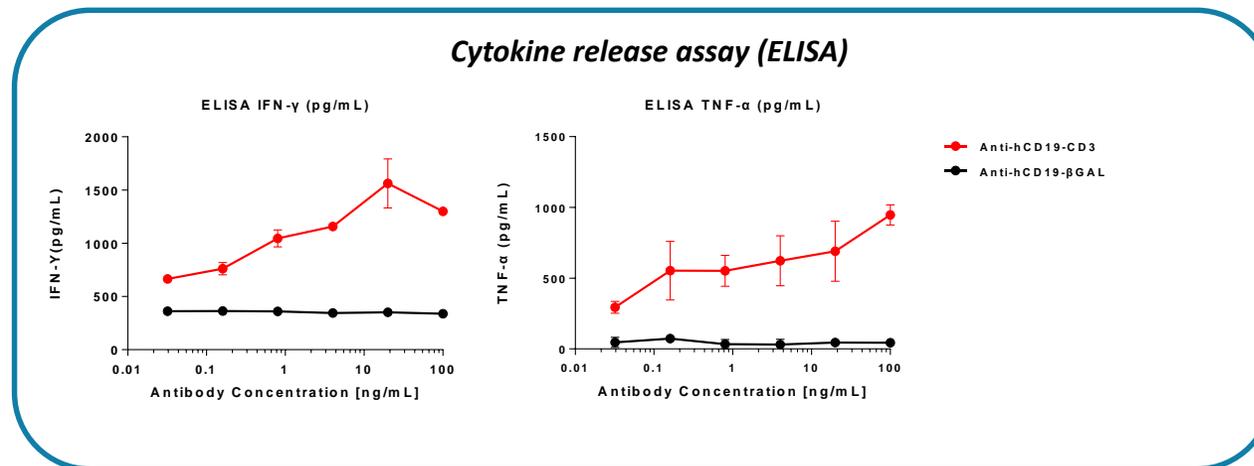
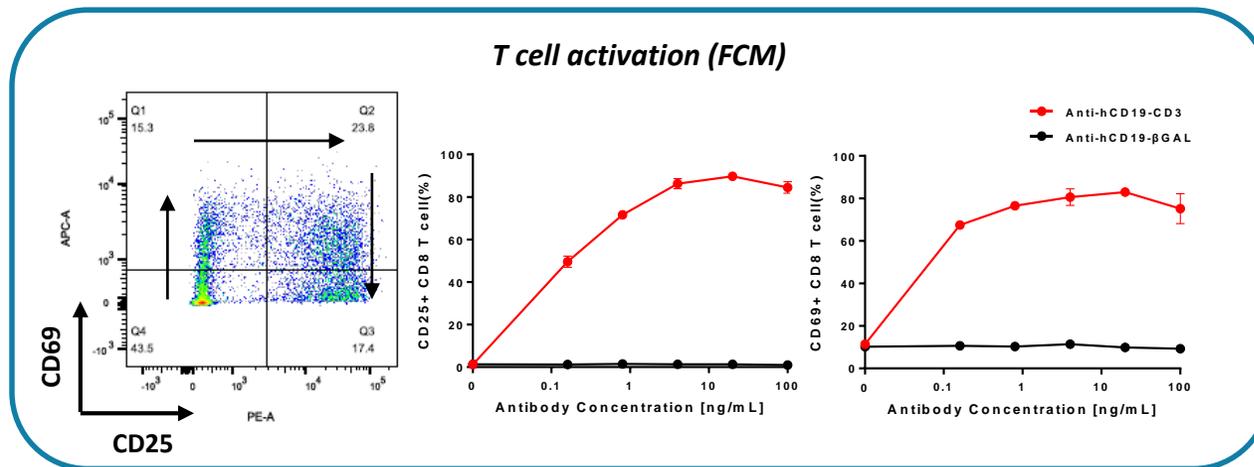


Case study: anti-CD19/CD3 BsAb

T cell activation assay

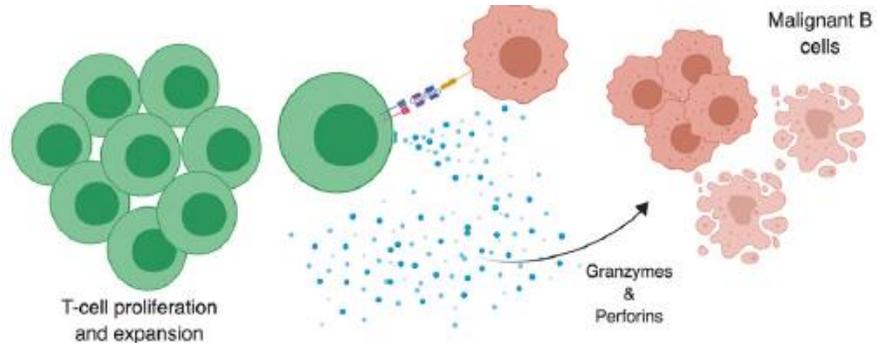


- Regular ELISA
- MSD-Multiplex ELISA
- Luminex multiplex system
- FCM analysis

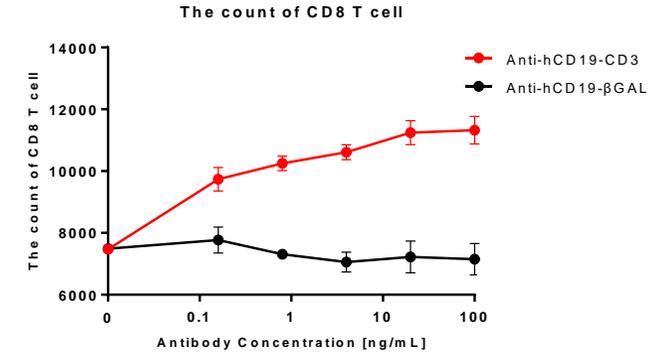
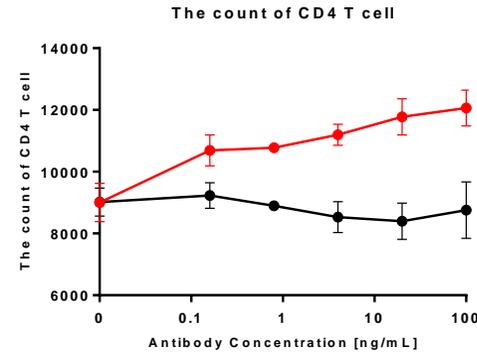


Case study: anti-CD19/CD3 BsAb

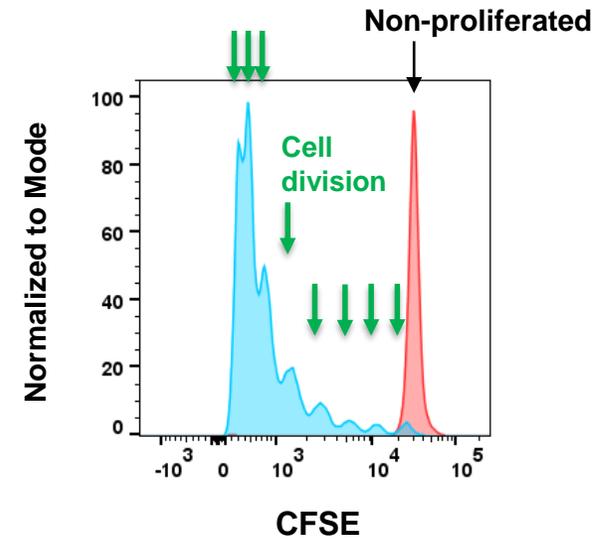
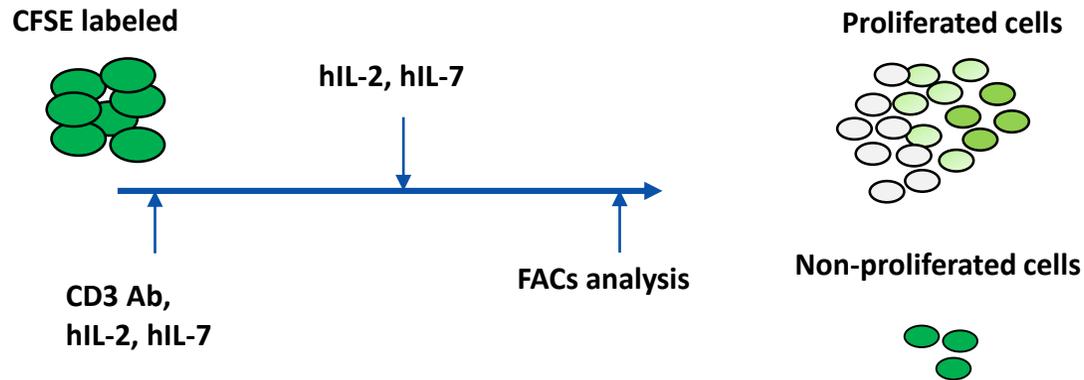
T cell proliferation assay



J Immunol 2019; 203:585-592



CFSE T cell proliferation assay



Anti-CD3/CD19 bispecific antibody promotes the proliferation of T cells

Case study: anti-CD19/CD3 BsAb

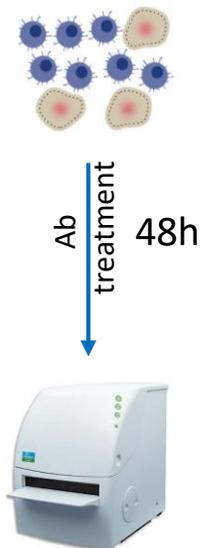
T cell killing assay

Work flow

PBMCs/T cell co-culture with target cells stained by Celltrace/Calcein (Day0)

Treat with BsAb (Day0)

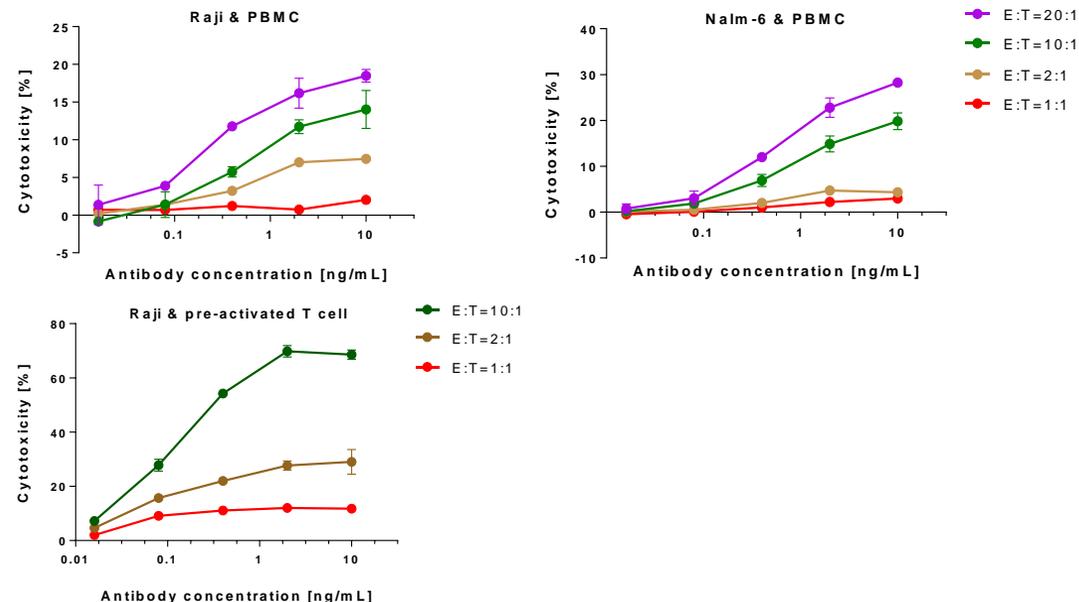
Collect cell pellet for analysis (Day2)



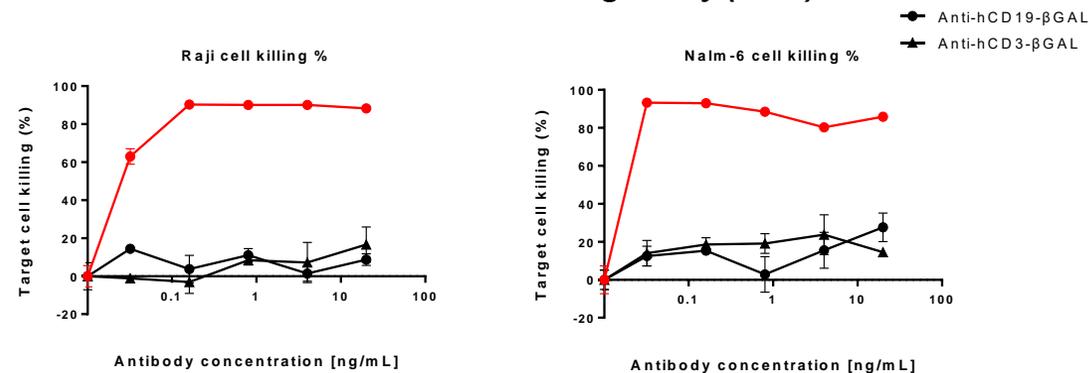
T cell mediated killing assay

- Calcein-release assay
- FCM analysis
- Lactate dehydrogenase test
- Real-time cell monitoring system

E:T ratio screening (Calcein-release assay)



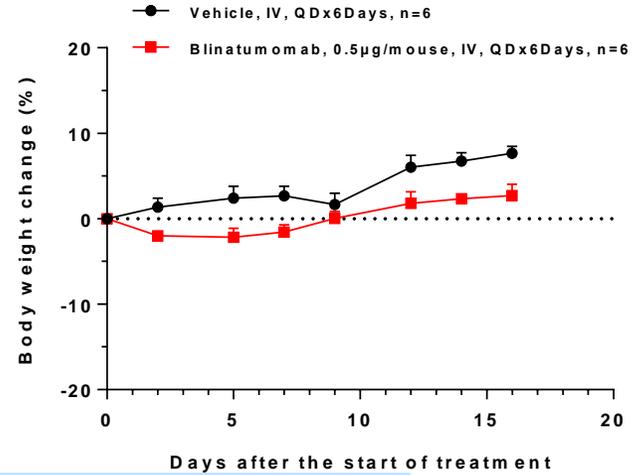
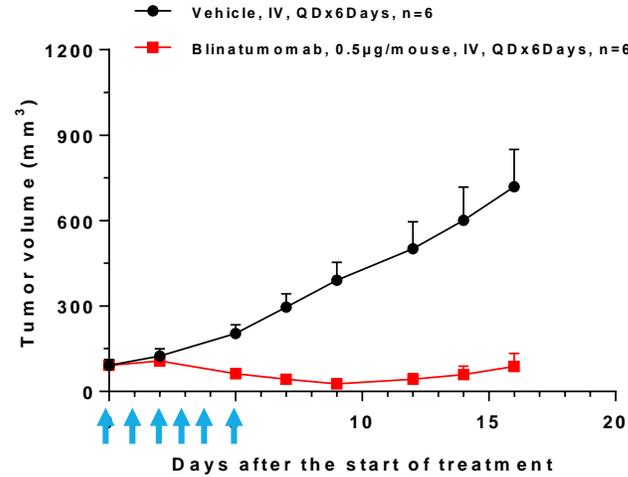
T cell-mediated killing assay (FCM)



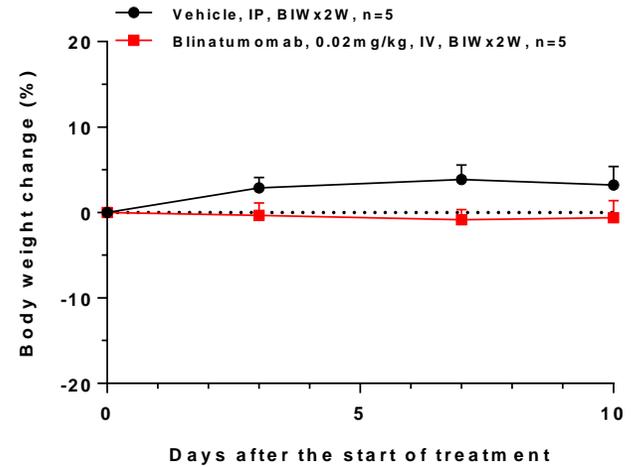
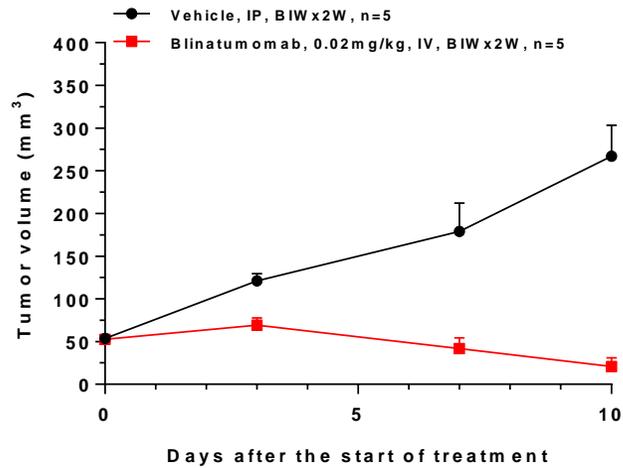
Case study: anti-CD19/CD3 BsAb

In vivo evaluation

Raji/PBMC-NOG-dKO humanized model, IV injection



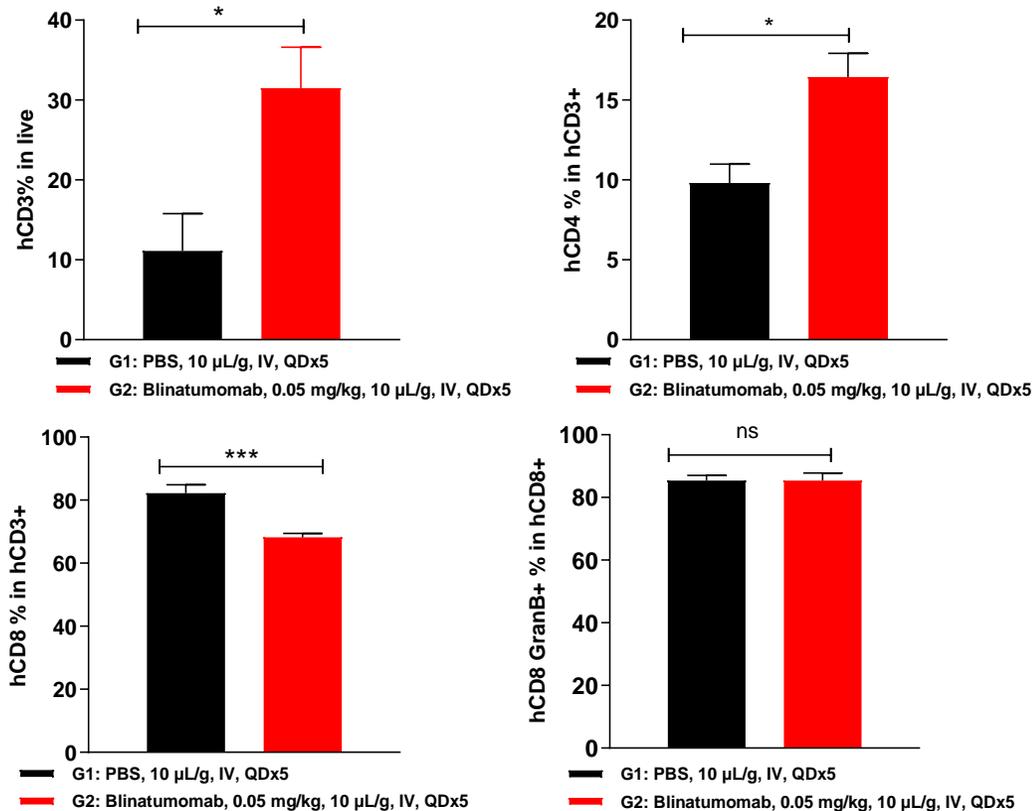
Raji/PBMC-NOG humanized model, co-inoculation



Case study: anti-CD19/CD3 BsAb

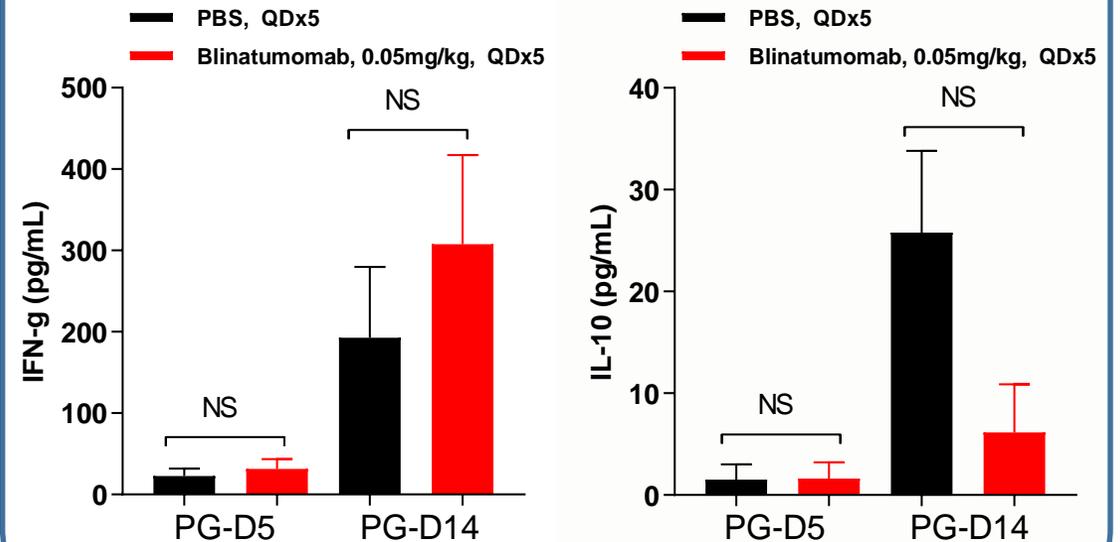
TIL analysis and cytokine measurement on samples from *in vivo* study

TIL analysis



Anti-CD3/CD19 BsAb increases the proportion of tumor infiltrating T cells *in vivo*

Cytokine release assay (ELISA)

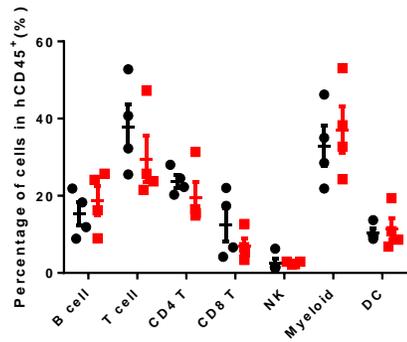
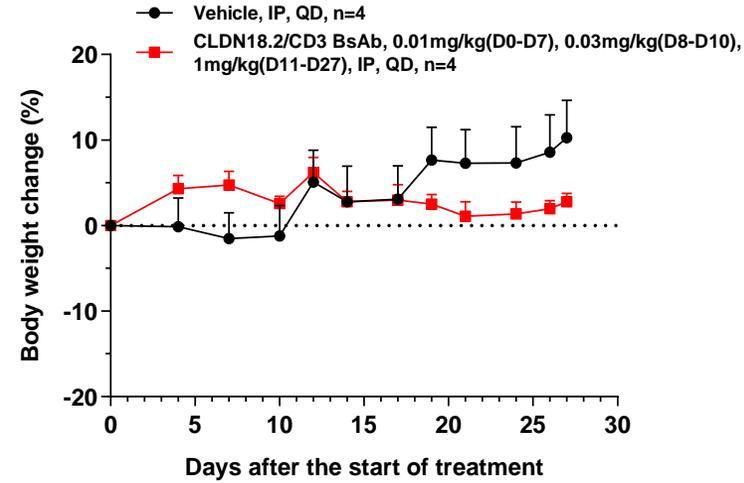
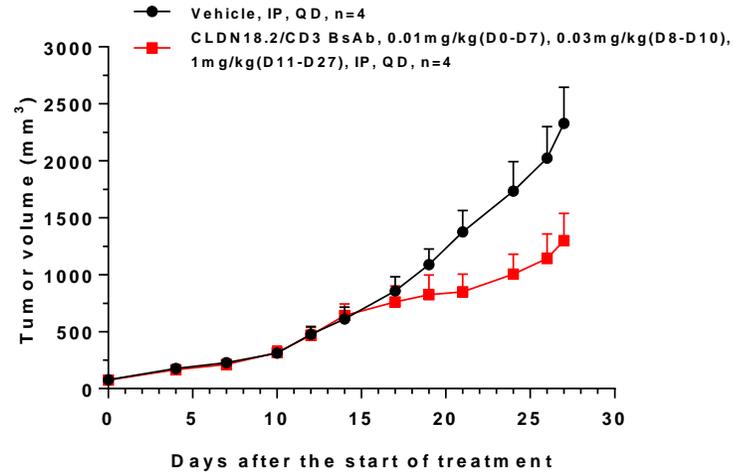


Anti-CD3/CD19 BsAb didn't trigger cytokine release syndrome *in vivo*

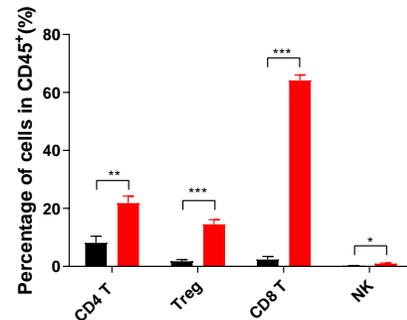
Case study: anti-CLDN18.2/CD3 BsAb

In vivo evaluation

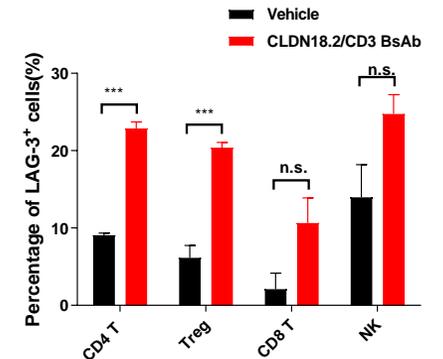
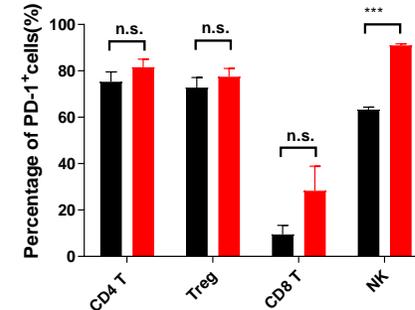
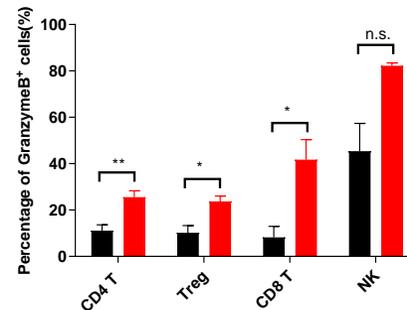
hCLDN18.2-Mia PaCa 2/HSC-NOG-EXL humanized model



Blood

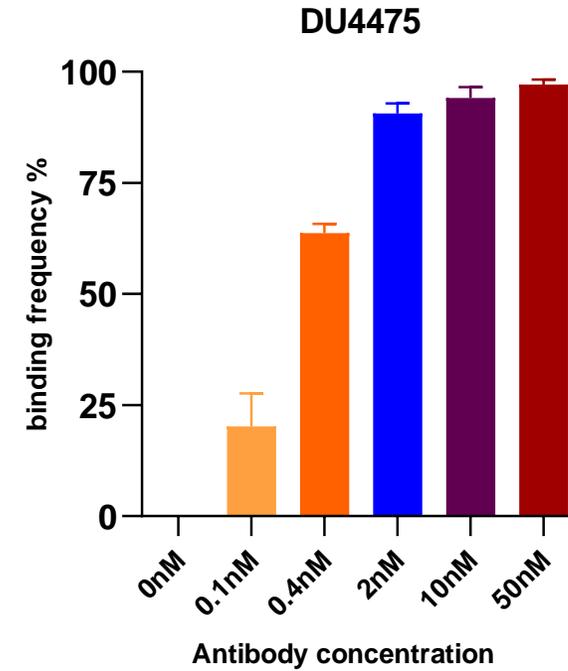
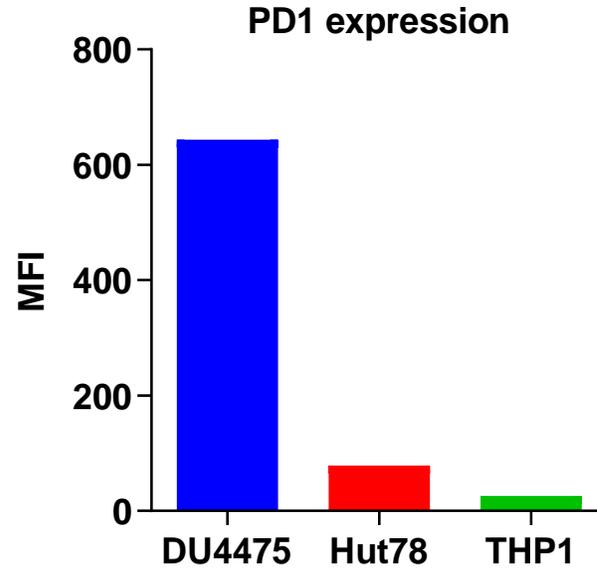
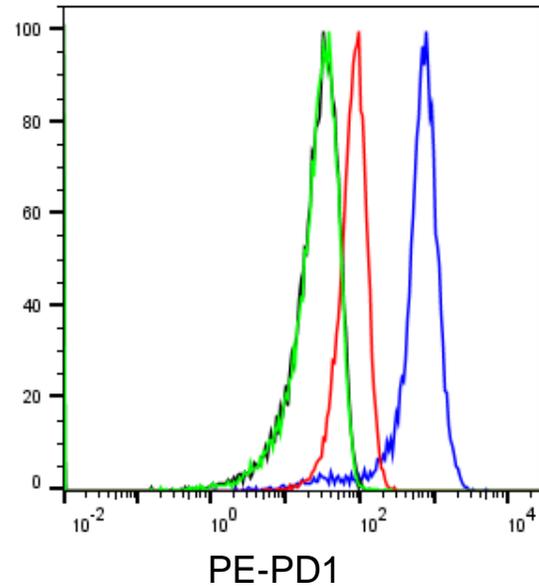


Tumor



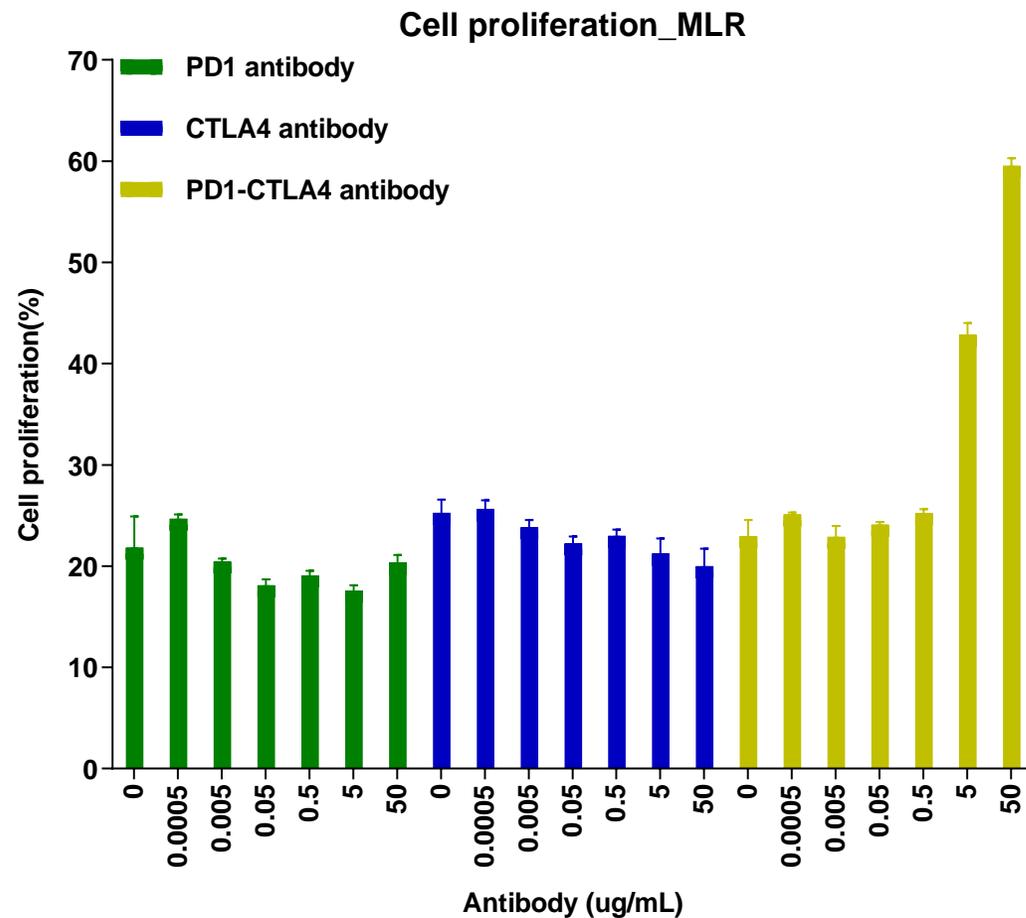
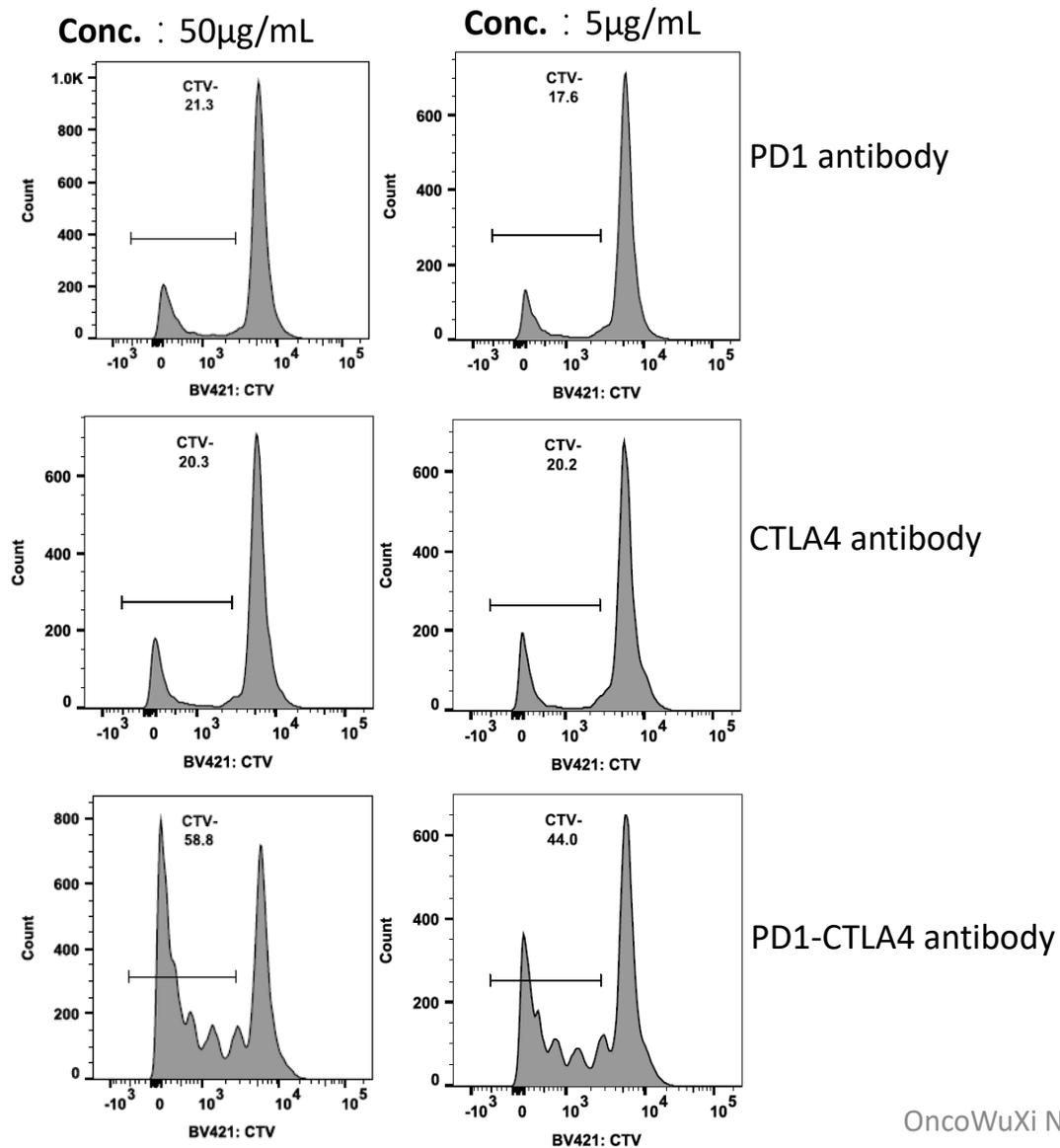
Case study: anti-PD1/CTLA4 BsAb

Binding analysis of antibody by flow cytometry



Case study: anti-PD1/CTLA4 BsAb

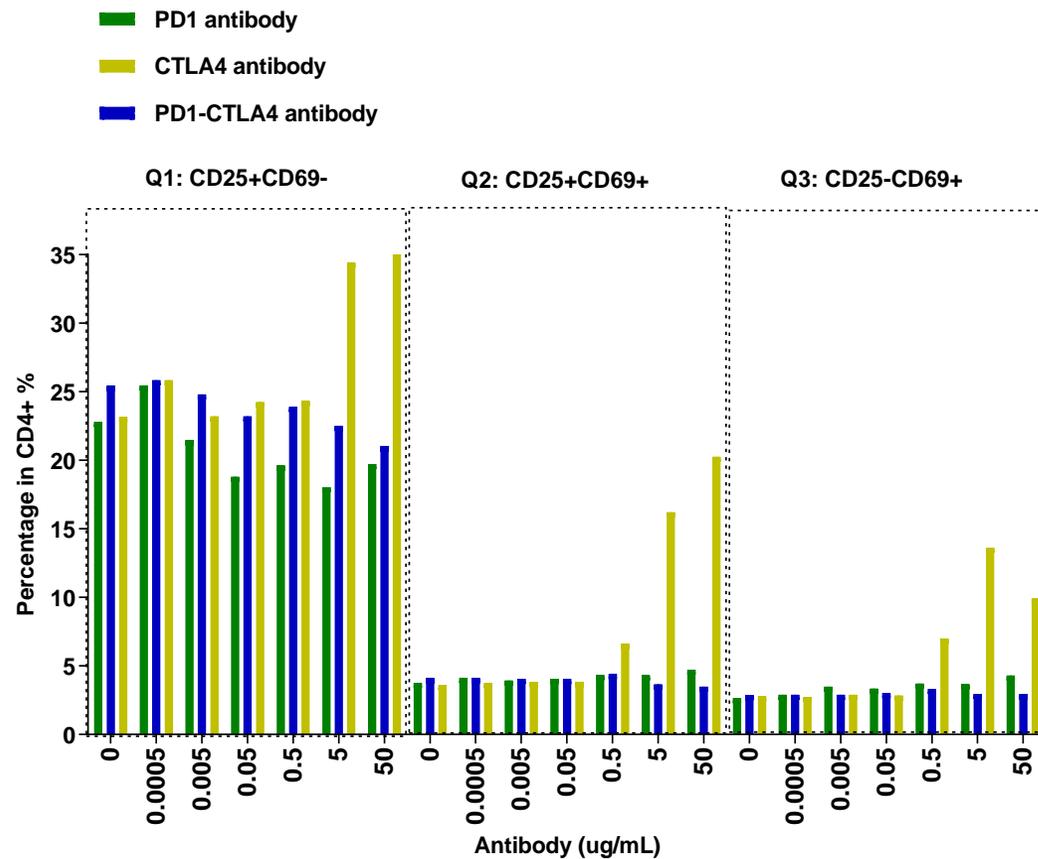
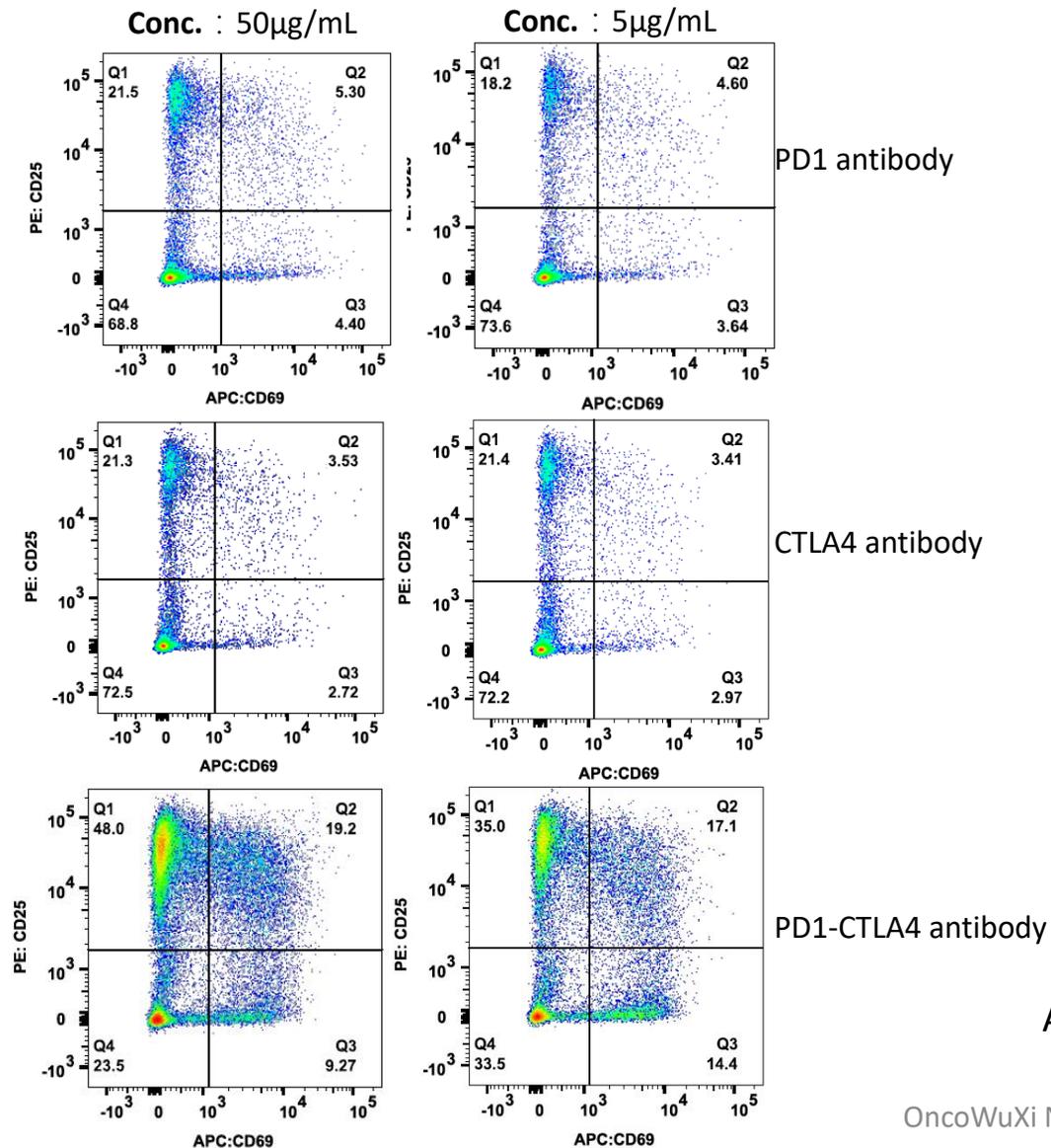
Proliferation analysis by *in vitro* MLR assay



Anti-PD1/CTLA4 BsAb promotes the proliferation of CD4⁺ T cells in MLR system

Case study: anti-PD1/CTLA4 BsAb

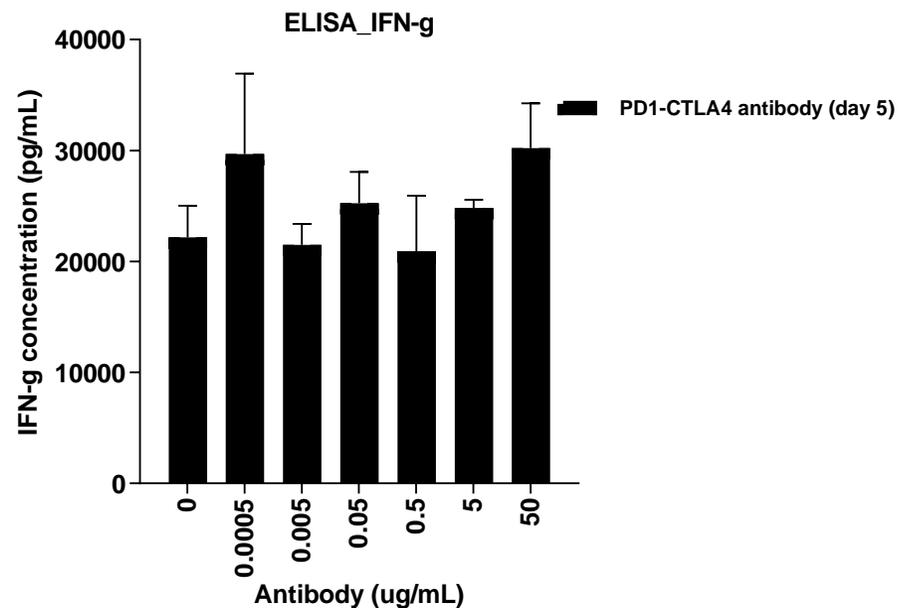
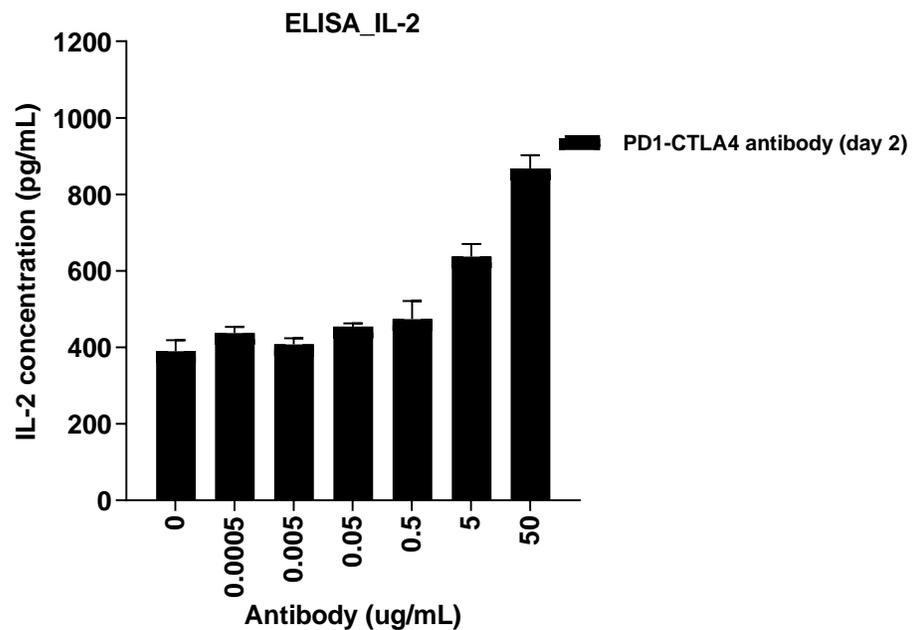
Activation analysis by *in vitro* MLR assay



Anti-PD1/CTLA4 BsAb promotes the activation of CD4⁺ T cells in MLR system

Case study: anti-PD1/CTLA4 BsAb

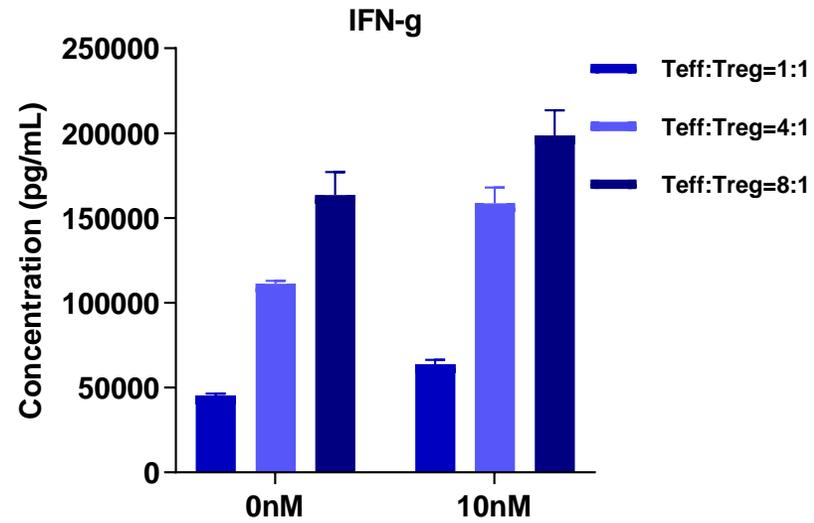
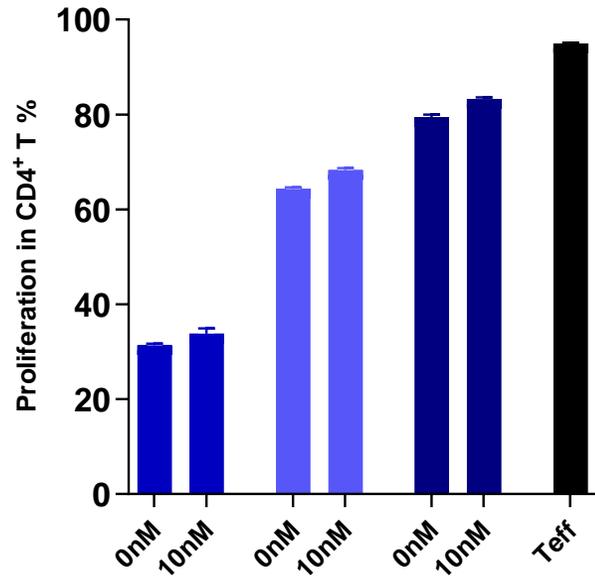
ELISA analysis in MLR assay



Anti-PD1/CTLA4 BsAb promotes the activation of CD4⁺ T cells in MLR system

Case study: anti-PD1/CTLA4 BsAb

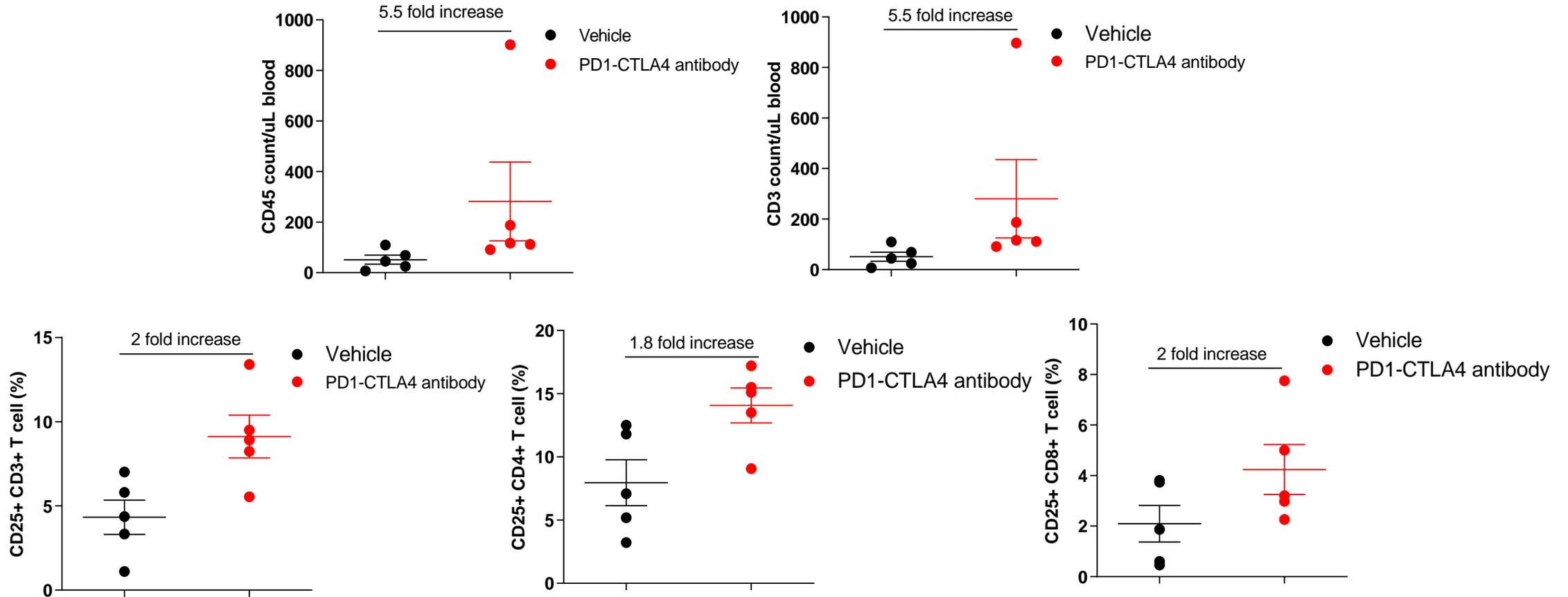
In vitro Treg suppression assay



Anti-PD1/CTLA4 BsAb reverses the suppressive effect of Treg cells *in vitro*

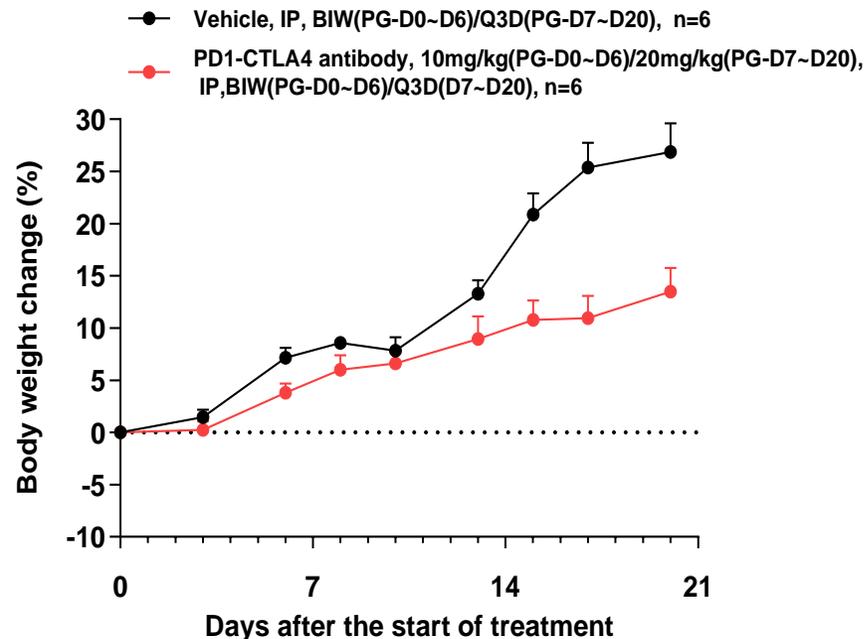
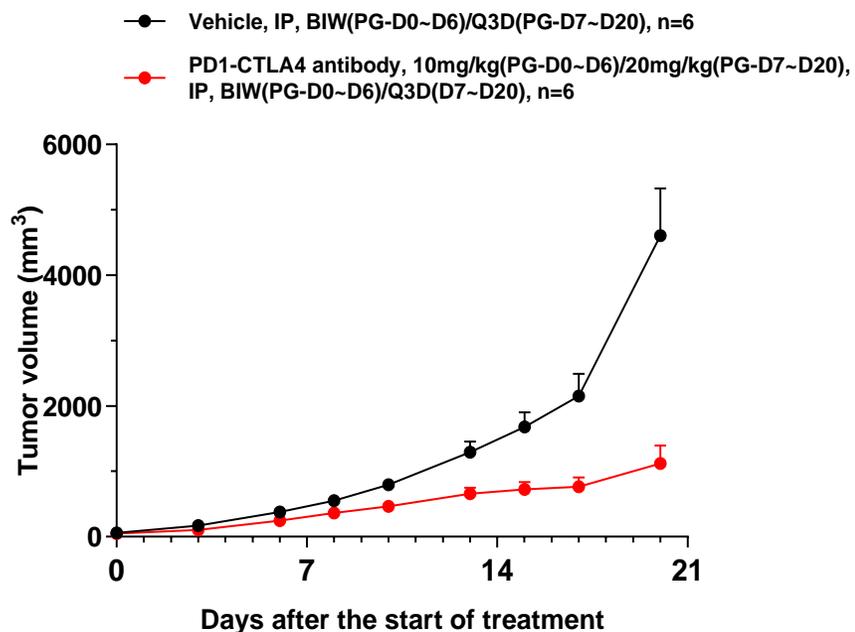
Case study: anti-PD1/CTLA4 BsAb

In vivo analysis of T cell activation and proliferation stimulated by antibody



Anti-PD1/CTLA4 BsAb was injected i.p. to PBMC humanized mice

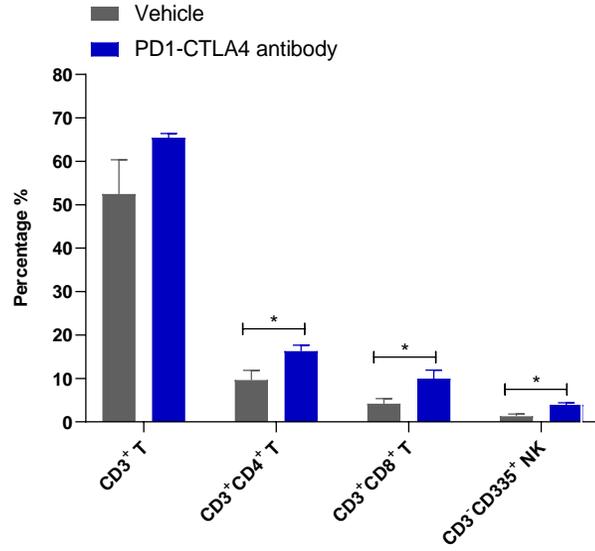
MC38 subcutaneous model in hPD1/hCTLA4 KI mice



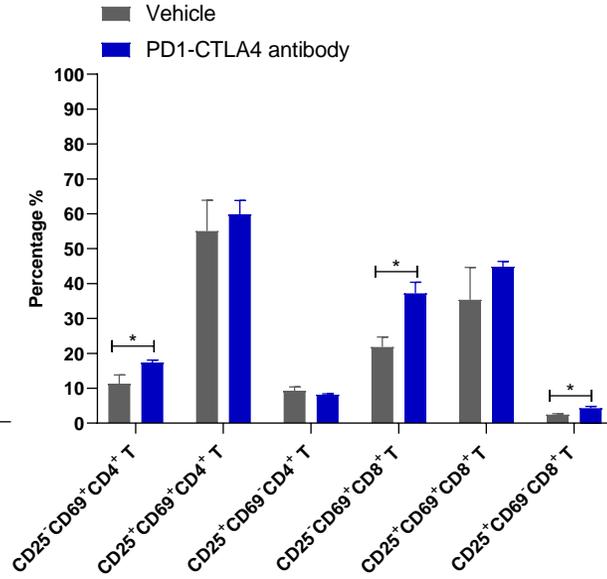
Anti-PD1/CTLA4 BsAb significantly inhibits the tumor growth *in vivo*

Case study: anti-PD1/CTLA4 BsAb

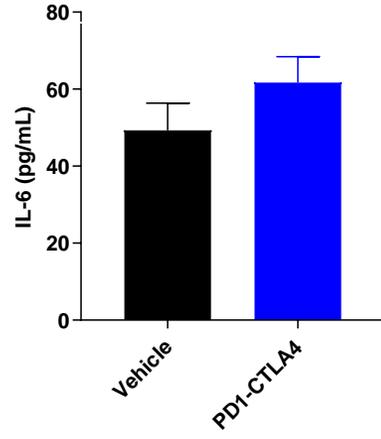
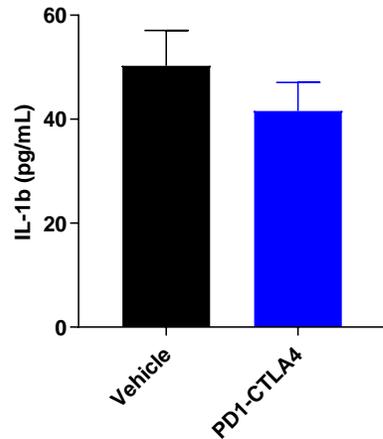
TIL and cytokine analysis on samples from *In vivo* study



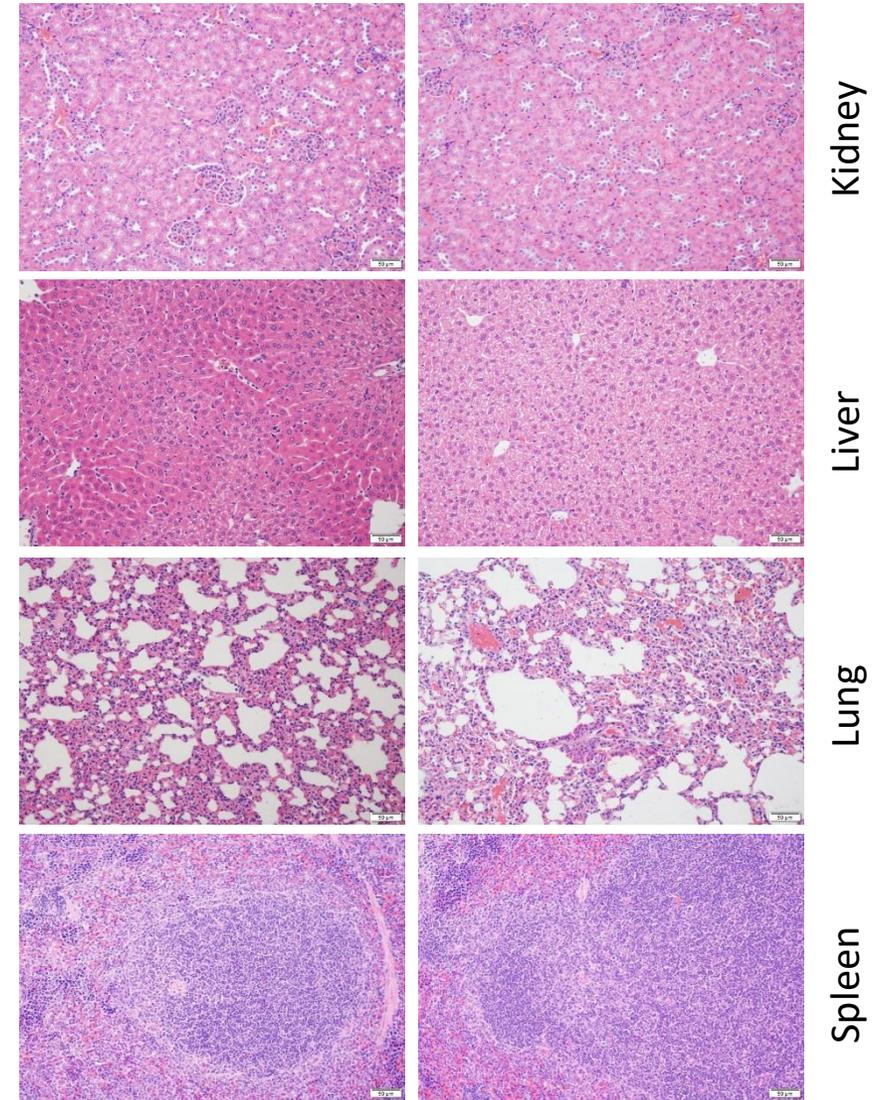
cell phenotype



cell activation



Anti-PD1/CTLA4 BsAb is safe *in vivo*



Vehicle

PD1-CTLA4 antibody



OUR COMMITMENT

Improving Health. Making a Difference.

For questions and requests, please email to OIU-BD-Translation@wuxiapptec.com



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