We developed more than 20 neutralizing anti-PD1 antibodies. Figure 7 showed one of them (TA807867) exhibited the similar neutralizing ability as Keytuda or Opdivo.

1. Antibody generation: Antibodies to PD1 were generated by immunizing Balb/c mice with a purified full-length human PD1 protein expressed in HEK293T. Enriched B cells from immune animals were fused to myeloma SP2/0 myeloma cells (ATCC) to generate hybridomas using standard techniques. Single clones were obtained by limiting dilution assays through 3 rounds screening and subcloning, and then screened with FFPE human tonsil tissue by IHC.

2. IHC: IHC staining was performed on 4-μm thick paraffin tissue sections. Briefly, de-paraffinized slides were treated in 1 mM EDTA (pH 8.0) antigen retrieval solution at 120 degrees for 2.5 min in a high pressure cooker. Slides were incubated in primary antibodies for 90 min at room temperature (RT) without serum blocking. ZSBio's PV9000 detection system was used in manual IHC assays.

3. Protein array and assays: Lysate protein chips were printed and protein assay were executed as described (2).

Table 1. The evaluation of UMAB199's specificity on 148 FFPE human tissue samples by IHC.

Figure 7. Other neutralizing anti-PD1 antibodies (e.g. TA807867).

Conclusions

1. We developed a monoclonal antibody UMAB199 with ultra-specificity against PD1 protein.
2. UMAB199 demonstrated superior performance on IHC application.
3. UMAB199 also works for flow cytometry & western blot, and can recognize mouse PD1. But it can not block the binding between PD1 and PD-L1.

References