The Development of an Anti-PD1 Mono-Specific Antibody (UMAB199) for Anatomic Pathology

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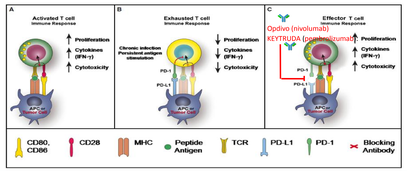
Introduction

◇ **Programmed death 1 (PD1) is up-regulated in activated T and B lymphocytes, also present on activated myeloid lineage cells such as monocytes, dendritic cells and NK cells.**
◇ **PD1 inhibits T-cell function upon binding to its ligands, PDL1 and PDL2, and it serves as a key checkpoint of the immune system.**
◇ **A high-quality monoclonal antibody is needed to evaluate PD1 protein levels in formalin-fixed paraffin-embedded (FFPE) tissue samples**
◇ **We utilized protein microarray technology to develop an anti-PD1 mono-specific antibody (UMAB199) for anatomic pathology application.**

Figure 1. PD-1 is the target of immunotherapy (*1*) .



Design & Methods

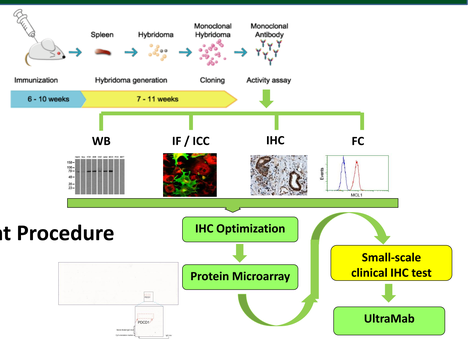
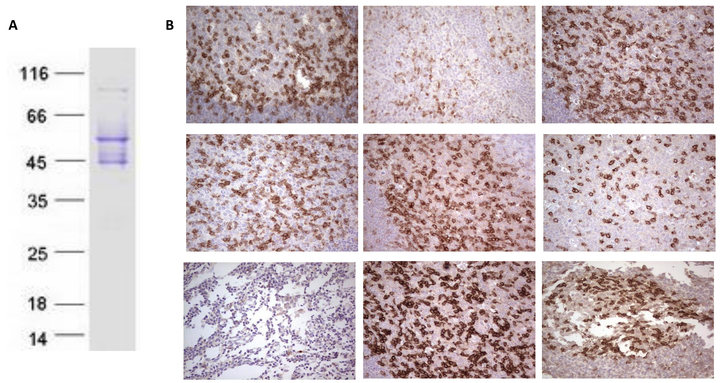


Figure 2. UltraMab Development Procedure

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 degree 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).

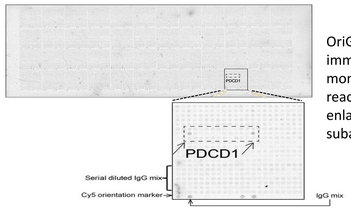
Results
Screening ultra-specific anti-PD1 IHC antibody (UMAB199)

Figure 3. Screen anti-PD1 antibodies by using FFPE tonsil tissues.



(A). Immunogen. SDS-PAGE gel and Coomossie Blue Staining of the purified full length human PD1 with C-terminal DDK tag expressed by human HEK293cells.
(B). Screening mouse anti-PD1 antibodies using FFPE human tonsil tissues by IHC.

Figure 4. Identify mono-specific anti-PD1 antibody UMAB199 by using 10k-Protein Microarray Technology.



OriGene over-expression protein microarray chip was immuno-stained with UltraMAB anti-PD1 mouse monoclonal antibody (UM800091, UMAB199). The positive reactive proteins are highlighted with two red arrows in the enlarged subarray. All the positive controls spotted in this subarray are also labeled for clarification.(10ug/ml)

Figure5. IHC condition Optimization in FFPE human tonsil tissues.

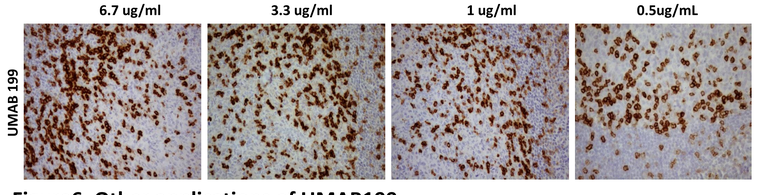
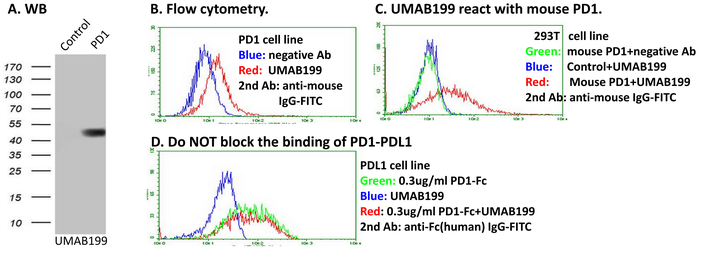


Figure6. Other applications of UMAB199.

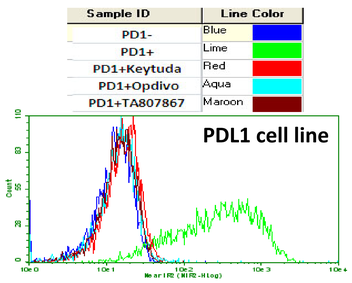


Results
Small-scale clinical IHC test of UMAB199

Table1. The evaluation of UMAB199's specificity on 148 FFPE human tissue samples by IHC.

Tissues	Positive/Total Samples				
Colon	1/5	Skin	0/3	Stomach carcinoma	0/3
Thyroid	2/5	Placenta	0/3	Kidney carcinoma	0/2
Lymph Node	6/6	Esophagus	0/3	Liver carcinoma	0/3
Tonsil	13/13	Stomach	0/3	Pancreas carcinoma	0/4
Cerebrum	0/3	Intestine	0/3	Esophagus carcinoma	0/3
Cerebellum	0/3	Mesothelium	0/3	Breast adenocarcinoma	0/2
Adrenal gland	0/3	Breast	0/2	Colon adenocarcinoma	0/3
Liver	0/5	Ovary	0/6	Prostate carcinoma	0/4
Kidney	0/5	Endometrium	0/6	Endometrium adenocarcinoma	0/3
Lung	0/6	Prostate	0/6	Ovary adenocarcinoma	0/6
Heart	0/3	Bladder	0/5	Bladder carcinoma	0/2
salivary glands	0/3	Melanoma	0/1	Thyroid carcinoma	0/2
Pancreas	0/2	Lymphoma	2/3	Lung carcinoma	1/3

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



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

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 PDL1.**

References

1. David F. McDermott et al. PD-1 as a potential target in cancer therapy. ***Cancer Medicine, 2013, 2(5): 662.***
2. Ma D, et al. Using protein microarray technology to screen anti-ERCC1 monoclonal antibodies for specificity and applications in pathology. ***BMC Biotechnology, 2012, 12: 88.***